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Factors affecting the measurement of stability and safety of cosmetic products

by

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Abstract

Factors affecting the measurement of stability and safety of cosmetic products

There are a large number of products that come under the heading ‘cosmetic product’. Each is required, according to the EU Cosmetic Regulation, to demonstrate formulation stability to a EUROTOX Safety Assessor before being placed on the market. The regulation places a requirement on the Safety Assessor to take into account the long-term stability of the product in question but does not specify any protocol by which to obtain this data. Various guideline documents have been written, most notably by ISO 18811:2018 and Cosmetics Europe (Colipa), which use elevated temperature stress testing to accelerate reactions, and using the Arrhenius model to extrapolate duration of shelf lives from the results. More specifically the assumption is made that any reaction observed displayed 1st order rate kinetics with respect to temperature and that that behaviour can be quantified as each 10ºC increase in temperature doubles the rate of reaction (or Q10=2).

This research challenged the accuracy of the recommended accelerated stability tests with regard to emulsions. To do this, 65 emulsions were made on the laboratory scale which altered by emulsifier type and concentration; oil phase ratio and work done during emulsification. These emulsions were tested according to the recommended protocols of accelerated testing given in the guidance documents and put on long-term ambient temperature test for direct comparison with accelerated results. Three new parameters were introduced to measure the accuracy, precision and predictive threshold of the accelerated tests.

It was found that for the emulsions studied, four measurement parameters out of the five tested showed that the assumptions made for elevated testing were both inaccurate and imprecise for the prediction of long-term stability. Indeed, in three of these parameters: viscosity; appearance and colour; the predictive threshold did not extend beyond the extent of the accelerated testing time, 16 weeks, let alone up to the 96-week+ shelf-life of a cosmetic product. It was also demonstrated, however, that one parameter, pH, which is more aligned to the original Arrhenius studies had a good adherence to the accelerated testing extrapolation, showing a predictive threshold beyond the 96-week target for the formulations tested. This showed that the parameters of measure need to be more critically considered before being subjected to accelerated stability extrapolations.

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Author's Declaration

I confirm that all aspects of this thesis are my own work.

Talks and Presentations

In-Cosmetics Global Summit 2016 – 'The Science behind Stability'

The University of Sunderland – Lecturing on Colloidal Chemistry and Emulsion Theory for the Cosmetic Science Degree Course.

Society of Cosmetic Scientists, Principles and Practises of Cosmetic Science Course 2018 – ''Emulsion Theory'' and ''Skin Care Products''.

Bournemouth University Post Graduate Conference 2017 – 'Critical examination of the measurement of stability in Cosmetic Products', winning prize for best presentation.

Table of Definitions

Term	Definition
Accelerated Stability Model (ASM) or Accelerated Stability Evaluation	A study designed to speed up naturally occurring destabilization processes due to intrinsic or extrinsic factors and which predicts the behaviour over the long term. (ISO 18811:2018)
Accuracy	The degree to which a measurement conforms to the correct value.
Ambient Temperature (°C)	The non-controlled storage temperature of a cosmetic product once on the market.
Average Prediction Error	The mean of a formulation's Prediction Error for a given parameter. It is an indication of the predictive accuracy.
Coalescence	The process that leads to the fusion of smaller particles into larger ones as a result of particle collisions.
Colloid	A microscopic dispersion of one substance throughout another.
Cosmetic Product	Any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours (EC No. 1223/2009).
Creaming	The rising of dispersed phase particles from a colloid due to a discrepancy in density between the continuous and internal phase.
Durability	The amount of time a substance that stays within a defined physical and chemical specification.
DVLO Theory	Collective theory used to describe the behaviour of approaching charged particles in a liquid medium, put forward by (Derjaguin and Landau 1941; Verwey and Overbeek 1948)
Emulsion	A mixture of two immiscible liquids.
External or Continuous Phase	The medium in which an internal phase is suspended.
False-Fail	A formulation that fails accelerated testing conditions but passes long-term real-time testing.

False-Pass	A formulation that passes accelerated testing conditions but fails long-term real-time testing.
First order rate Kinetics	A reaction that proceeds at a rate that depends linearly with respect to one experimental variant e.g. reactant concentration or experimental condition.
Flocculation	The mechanism where internal phase particles in a colloid clump together to form aggregates or flocs.
Internal or Dispersed Phase	The particles or droplets within the Continuous Phase.
Long-term Stability	The monitoring of a substance's durability at a controlled ambient temperature for the duration of that substance's shelf-life.
No-Observed-Adverse-Effect-Level (NOAEL)	the maximum concentration of a substance that is found to have no adverse effects upon the test subjects.
Oswald Ripening	The phenomenon of dispersed phase molecules migrating from smaller dispersed droplets through the continuous phase to larger dispersed droplets.
Precision	The closeness of two or more measurements to each other.
Prediction Error	The difference between the result of an accelerated test and the real-time result it was predicting.
Prediction Error Range	The difference between the highest prediction error and the lowest prediction error for a given data set. It is a indication of predictive precision.
Sedimentation	The settling of dispersed phase particles from a colloid due to a discrepancy in density between the continuous and internal phases.
Shelf-life	The declaration made on a cosmetic product as to its durability on the market.
Stability	The physical or chemical change of a substance over time.

Chapter 1 Introduction

From as early as 10,000BC, cosmetics have been used by humans to alter the appearance of their skin and hair. Early men and women used scented oils and ointments to clean and soften the skin; and dyes and paints to decorate skin and hair. Ancient Egyptians used essential oils as perfumes; mixed clays to use as sunblock for lips and cheeks; and chewed on tamarisk leaves to freshen breath. Romans used animal or vegetable oil mixed with water, lime powder and perfume to create a cleansing cream to use instead of soap during bathing; and fine coloured powders were used as make up to increase attraction (Chaudhri and Jain 2014). Most of these products were home made to recipes, much like cooking, and no two batches were ever identical. While there is evidence of monasteries creating and selling perfumed waters through the bubonic plague, and centres of science and guild associations investigating the healing powers of creams and ointments throughout the middle-ages, a recognisable cosmetics industry was not seen until the eighteenth century. By 1791 many small, high-end, independent perfumery shops had opened in Paris and London (Martin 1999), and in the nineteenth century some of the names still recognised today began to dominate the market: Eugène Rimmel started shops in Paris and London in 1834, William Colgate opened a business in New York in 1806, and William Yardely, who purchased a perfumery company from the Clever family, in London in 1823 (Geoffrey 2010).

During the early 20th century, industrialisation of production of cosmetic products meant that they began to become more affordable to the general public and the industry grew rapidly. In response to the industry growth, various legislations were passed to regulate the cosmetics markets in local authority areas. For example, in the USA the FDA passed the Food, Drug and Cosmetics act in 1938, and the EU passed the first Cosmetics Directive in 1976. These regulations focussed the development of cosmetics on the safety to the public, but it was not until the latest legislation passed in the European Union, in 2009, that the safety and stability of a cosmetic product were linked. This section introduces the demands placed on a cosmetic product from the latest legislation and the short comings of that demand. It will then explain the concepts involved in the stability of colloids as a basis for the experimental design and results discussion.

1.1 Cosmetic Regulation

In Europe the European Union regulates the cosmetics industry under EU 1223/2009. It defines a Cosmetic Product in Article 2.1.a as:

> 'any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours.'

Introduced in 2009 and fully enforced in July 2013, EU 1223/2009 (Recast) (European Union 2009) is the first European-wide cosmetic legislation. Before it, each member state, including the United Kingdom, had their own legislation to regulate cosmetics within their own regions which were all superseded by the regulation.

The scope of the regulation EU1223/2009 is stated in Article 1 to -

> '....establish[es] rules to be complied with by any cosmetic product made available on the market, in order to ensure the functioning of the internal market and a high level of protection of human health.'

As such, it put the protection of human health as central to the regulation itself. Safety of cosmetic products is assured by each product receiving a Safety Assessment, which -

> '.....shall be carried out by a person in possession of a diploma or other evidence of formal qualifications awarded on completion of a university course of theoretical and practical study in pharmacy, toxicology, medicine or a similar discipline, or a course recognised as equivalent by a Member State.' (Article 10.2)

A Safety Assessor should therefore perform a bespoke safety assessment for each new product introduced to market and take all factors of that product into consideration, including stability of that product over time.

1.1.1 Stability as Part of Safety of Cosmetic Products

A Safety Assessor takes two main criteria into consideration when assessing the safety of a product – formulation and stability. For the formulation, the safety of a product is calculated by assessing the dermal toxicity of each individual component by reference to published safety literature for topical application of each material's highest No Observed Adverse Effect Level (NOAEL). NOAEL is achieved by calculating the amount of the cosmetic product that will be exposed to the skin during the product's use and ensuring the consumer is not exposed to a component material at a level that may have an adverse effect. The Safety Assessor is also required to calculate a 'Margin of Safety' for the use of a cosmetic product (European Union 2009). There is no limit stipulated in EU 1223/2009 for how large the margin of safety must be for the cosmetic product to be declared safe. However, it is common to see safety margins in the region of 1×10^2, meaning the NOAEL would not be reached for any individual raw material until the customer used one hundred times more of the product than the Safety Assessor calculated would normally be used.

One of the main principles that a Safety Assessor must consider is the uniformity of the cosmetic product being assessed. An assessor must be given evidence that the formulation is uniform throughout the cosmetic product itself and that this remains constant throughout its shelf life. This is so that the exposure and skin loading of the product's individual raw materials can be accurately calculated and assessed.

The Safety Assessor is required to take many aspects of the cosmetic product into account, including, in Annex 1 Part A 2 (European Union 2009)–

> 'The stability of the cosmetics product under reasonably foreseeable storage conditions.'

and, under Annex 1 Part B 3 (European Union 2009), must comment on -

> 'Impacts of the stability on the safety of the cosmetic product'

Instability can take many forms but can be categorised into chemical changes (such as pH, colour and odour) and physical changes (such as particle size, viscosity or separation of materials). This is an important distinction because a chemical change would mean that the formulation contained a chemical that was not present when the materials were first mixed. Whereas, any physical changes would mean the

product was not necessarily uniform and had a potential increase in concentration of materials in certain areas of the cosmetic product. Any changes would demonstrate that the formulation had changed away from that determined as safe by the Safety Assessor, and so invalidating the Safety Assessment and potentially putting the public at risk.

Article 19 of the cosmetic regulation EU 1223/2009 stipulates that a product's label must have information on the durability of the product with respect to its safety, as defined in Article 3. This means that the label must state for how long the product is considered to be safe once on the market, known as its shelf life. Article 19 states that the product label must have (European Union 2009) -

> ''the date until which the cosmetic product, stored under appropriate conditions, will continue to fulfil its initial function and, in particular, will remain in conformity with Article 3 ('date of minimum durability').
>
> The date itself or details of where it appears on the packaging shall be preceded by the symbol shown in point 3 of Annex VII or the words: 'best used before the end of'.
>
> The date of minimum durability shall be clearly expressed and shall consist of either the month and year or the day, month and year, in that order. If necessary, this information shall be supplemented by an indication of the conditions which must be satisfied to guarantee the stated durability.
>
> Indication of the date of minimum durability shall not be mandatory for cosmetic products with a minimum durability of more than 30 months. For such products, there shall be an indication of the period of time after opening for which the product is safe and can be used without any harm to the consumer. This information shall be indicated, except where the concept of durability after opening is not relevant, by the symbol shown in point 2 of Annex VII followed by the period (in months and/or years)''

For clarity, the 'period of time after opening' declaration means the product has been assessed as safe for 30 months plus the declared period after opening, which can be up to 24 months, totalling a declaration of safety for 54 months or 4.5 years.

In this section the general principles of the assessment of safety for cosmetic products have been described as outlined in the cosmetic regulation EU 1223/2009.

However, there are some large omissions from the cosmetic regulation EU 1223/2009, and strong commercial market forces, that could cause problems with the assessment of safety:

- Whilst this is the first cosmetic regulation or directive to mention stability at all as part of the assessment of safety, it does not describe a standard protocol for stability testing. It relies instead on each manufacturer to set and justify their own protocols.
- The cosmetic regulation stipulates a 'reasonably foreseeable storage condition', without specifying what that means.
- It is commercially attractive to get products to market as quickly as possible, with as long a declaration of durability as possible, to return a company's investment in product development. Therefore there is commercial pressure to justify the minimising of the testing time for new products.

The numerous stability protocols that have been adopted are based on the application of Kinetic Theory. This application will be described in the following sections.

1.2 Factors Effecting the Stability of Colloids

This section introduces the concepts involved in the stability of colloids, which will be used to explain the experimental design and discuss the results obtained.

1.2.1 Types of Colloid

A colloid is a microscopic dispersion of two substances: the dispersed phase; inside a continuous phase. The dispersed phase takes the form of particles or droplets, which commonly have one dimension in the region of 1 - 1000 nm (Dunne 1987). The physical state of the two phases describes the nature of the colloid, for example, gases dispersed in a liquid are called foams, and solids dispersed in a liquid are called sols (solid suspensions). When two immiscible liquids mix together, it is called an emulsion and is illustrated in Figure 1-1. In cosmetic products, colloids are used extensively to give a wide range of product types, from creams with high

phase ratios, to fractional amounts of fragrance dispersed in a wash product, or solids dispersed in liquids to make clays and gels. In emulsions, the two phases are usually an aqueous, polar phase and a lipid, non-polar phase, although there are examples of silicones (non-lipid, non-polar) and glycols (non-aqueous, polar) being used as one of the phases as well. When he was extending his work on diffusion of gases to liquids, Thomas Graham found that some mixtures can be separated by filtration or osmosis (colloids) and some cannot (solutions) (Graham 1861). This was the first recorded observation of interface and colloid science.

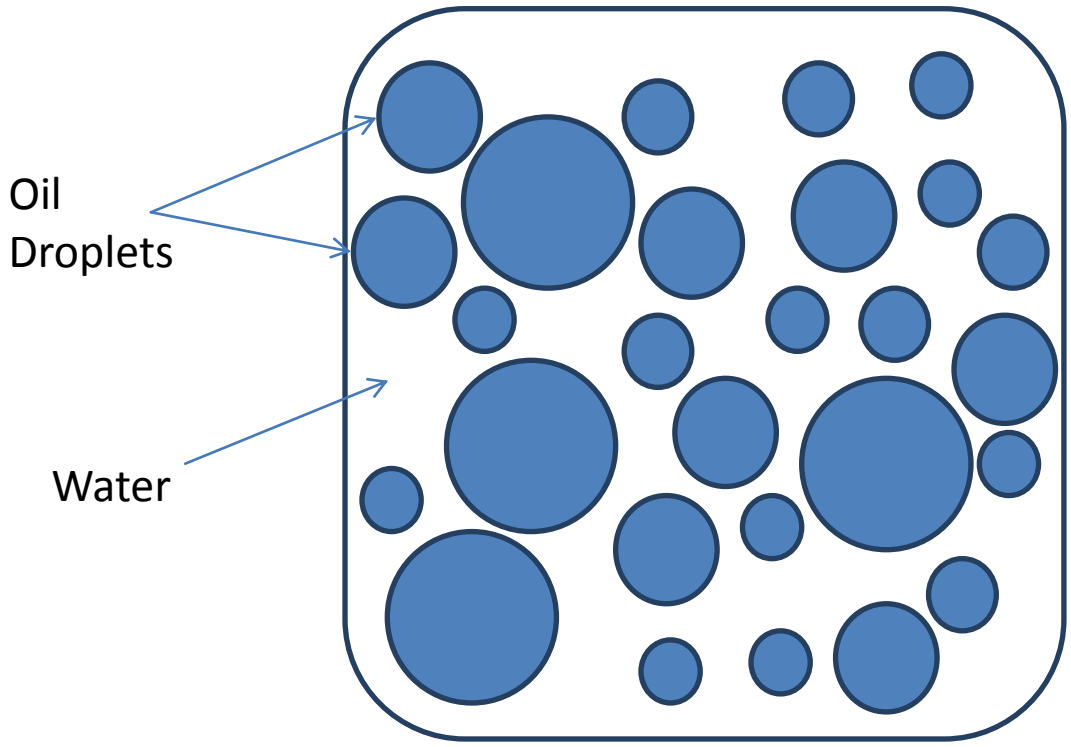


Figure 1-1 Illustration of an Oil in Water Emulsion.

After Graham's description of colloids, investigations into the thermodynamic and kinetic behaviour of these systems were carried out.

1.2.2 Thermodynamic Stability of Colloids

Taking thermodynamics first, the formation of a colloid could be described by the second law of thermodynamics (Wagner 1976):

$$\Delta G_{form} = \gamma_{AB}\,\Delta A - T\Delta S$$

Equation 1-1 Second Law of Thermodynamics

where ΔG_{form} is the free energy of formation, T is the temperature and ΔS is the change in entropy of the system. γ_{AB} is the interfacial tension between the two phases with the unit of mN m^{-1} and ΔA is the change in interfacial surface area that has been created during the process with units of m^2. Where a change to the system is being considered, if the free energy of the system (ΔG_{form}) becomes positive, it shows that the change requires an energy input to take place, for example mixing and/or heating. If ΔG_{form} is negative, it shows the change will take place spontaneously with no energy input required.

Together, $\gamma_{AB}\,\Delta A$ is the interfacial energy or work done (W), to create the new interfacial surface, usually expressed in J.

$$W = \gamma_{AB}\,\Delta A$$

Equation 1-2 Work required to mix two liquids together (SCS 2009)

The magnitude of the Interfacial Tension (γ_{AB}) is driven by the difference in polarity between the two phases. The larger the difference in polarity between the phases the higher the interfacial tension between them and the higher the energy barrier to mix them together, hence the work needed to mix them (W) also increases.

The formation of a colloid requires an increase in interfacial surface area, as shown in Figure 1-2 demonstration of interfacial surface area increase with dispersed particle diameter decrease (VertuTek 2014), hence ΔA is large and positive for this process. The smaller the resulting droplets become, the higher the surface area becomes, and ΔA increases along with work needed (W).

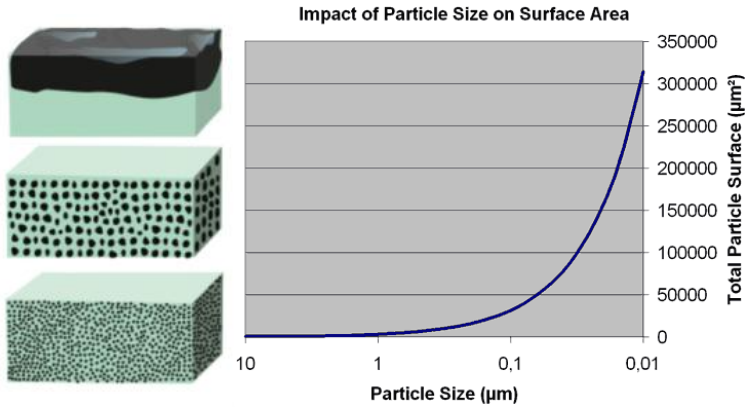


Figure 1-2 demonstration of interfacial surface area increase with dispersed particle diameter decrease (VertuTek 2014)

Interfacial tension can be small between two phases but is never negative because at zero interfacial tension the two phases can freely mix at a molecular level. In the

majority of dispersing processes, where there is some interfacial tension between phases, $\gamma_{\mathrm{AB}}\,\Delta\mathrm{A}$ must be larger than $\mathrm{T}\Delta S$, and therefore $\Delta\mathrm{G}_{\mathrm{form}}$ is always large and positive. Hence, the formation of colloids is not spontaneous and always requires an input of energy. This is the reason why high shear mixers (for example Silverson homogenisers) are needed to form emulsions with a relatively small particle size.

To allow some feel of magnitude, when trying to emulsify 10 ml of oil into water to produce a droplet size of 0.2 µm, the increase in surface area is of order 10^6. If the interfacial tension between the water and oil is 52 mN m^{-1}(as it is for hydrocarbon liquid) (SCS 2009), the work required will be in the order of 2 J.

The collapse of a colloid back into two discrete phases represents a large decrease in the interfacial surface area, hence ΔA becomes large and negative. As the interfacial tension (γ_{AB}) between the phases can change in magnitude but is never negative, $\gamma_{\mathrm{AB}}\,\Delta\mathrm{A}$ or W is always negative, leading to $\Delta\mathrm{G}_{\mathrm{form}}$ being negative. Hence, the collapse of a colloid does not require energy input and occurs spontaneously. Therefore, all colloids are thermodynamically unstable and will separate into their discrete phases given enough time.

In the practical example of the hydrocarbon being emulsified into water, the 2 J of work to make the emulsion remains in the system as potential energy; the system is inherently thermodynamically unstable and rapidly undergoes whatever transformations are possible to minimise that energy, in this case, by reducing the interfacial area.

If the interfacial tension was reduced, to 1 mN m^{-1}, either by addition of some other material or change in oil phase, the work required to make the emulsion would be 0.3 J. Whilst that is a significant decrease in W and advantageous to industrial processes, the system remains thermodynamically unstable as there is still an increased level of potential energy, and so the system will again transform itself to reduce this.

Arising from the thermodynamic instability of all colloids, emulsions separate into their immiscible liquid phases over time. Strictly, once separation has occurred, it is no longer an emulsion at all, as the dispersed phase is no longer dispersed in the continuous phase.

Although, as has been demonstrated, all emulsions are thermodynamically unstable, the thermodynamic descriptions do not give any indication of rate of transformation.

The rate of transformation is the realm of kinetics, which will be reviewed in the following passages.

1.2.3 Kinetic Stability of Colloids

In order to increase marketability, cosmetic products should have as long a stability profile as possible. Therefore, cosmetic formulators have focussed on kinetically stabilising emulsions to create suitable products. To assess the kinetic stability of emulsions, the mechanisms of instability need to be understood. There are five mechanisms of instability, and hence kinetic descriptions, for emulsions: creaming; sedimentation; coalescence; flocculation and disproportionation (Tadros 2013).

- Creaming occurs when dispersed particles are less dense than the continuous phase and therefore tend to rise to the surface. The rate depends on the physical properties of the continuous phase, for example rheology.
- Sedimentation is similar to creaming except the dispersed phase has a higher density compared to the continuous phase. The particles therefore tend to settle to the bottom of the container under gravity and remain as discrete entities. The sedimentation rate also depends on the physical characteristics of the continuous phase.
- Coalescence is the process that leads to the fusion of smaller particles into larger ones because of particle collisions. The continuous phase in between the dispersed phase droplets thins until it collapses and the droplets fuse together. The most important feature of this process is the reduction in the interfacial area that occurs when the particles amalgamate, which makes it thermodynamically favourable.
- Flocculation is the mechanism whereby particles clump together to form aggregates or flocs. The particles remain as distinct entities and do not fuse together to form larger ones. There are two sub-categories to flocculation: Brownian Flocculation, when droplets collide due to random Brownian motion; and Sedimentation Flocculation, where droplets collide due to movement in a vertically linear manner due to difference in density with the dispersed phase. The most important aspect of this process is that there is no overall change in the surface area of the dispersed particles.

- Disproportionation or Ostwald Ripening is the phenomenon of the dispersed phase molecules migrating from smaller dispersed droplets through the continuous phase to larger dispersed droplets. This is driven by the higher internal pressure of a small droplet compared to a larger droplet, creating a diffusion gradient. When this occurs, the large droplets get larger and the smaller droplets get smaller, eventually destabilising the system.

These five processes do not occur discretely and often occur at the same time or as a direct result of each other. For example, an emulsion may exhibit creaming, which forces the dispersed phase droplets together into flocs, which thins the continuous phase barrier, triggering coalescence and eventually complete phase separation. They all, however, lead to total phase separation of the system as illustrated in Figure 1-3.

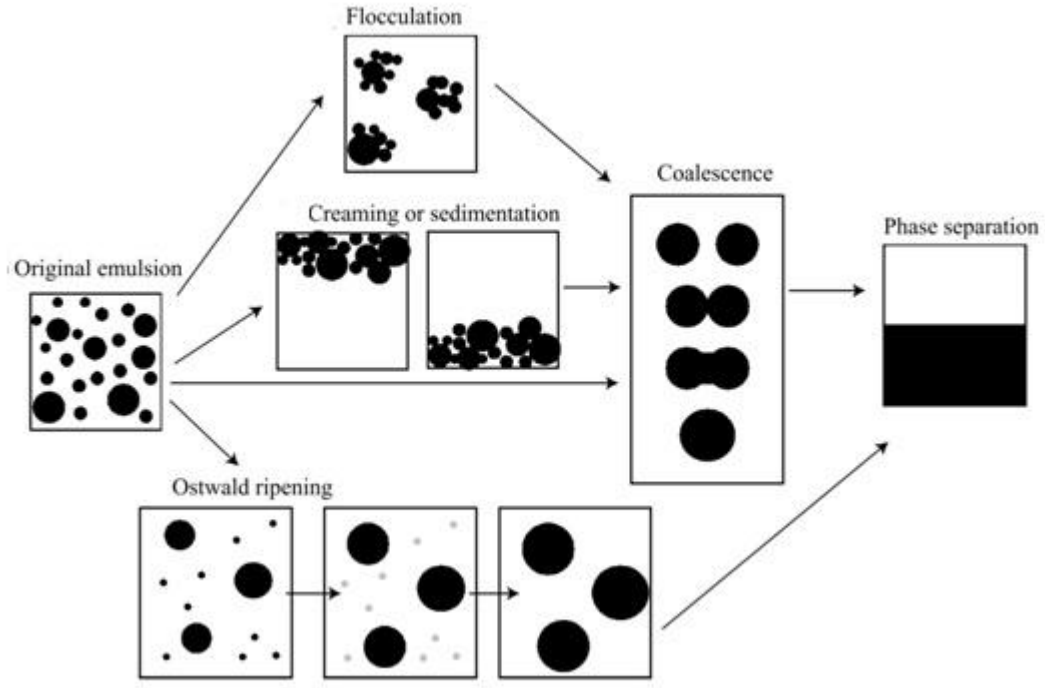


Figure 1-3 Schematic of possible paths of emulsion instability mechanisms leading to phase separation (isalama.files.wordpress.com 2015).

The kinetic descriptions of these processes are complex as the margin between one process stopping and the next starting is difficult to determine. However, there are some general principles and mathematical descriptions for the main factors affecting the rate of each process.

1.2.3.1 Coalescence and Flocculation

Coalescence and flocculation occur due to collisions of the dispersed phase droplets. These collisions can result in: repulsion, when droplets move apart again with no change to the colloidal state; coalescence, where the droplets join to make a larger droplet, decreasing the overall surface area of the colloid; or flocculation, when the droplets do not move apart but associate and move together through the colloid but overall surface area remains the same.

Collisions occur due to Brownian diffusion, gravitational sedimentation and intermolecular interactions in the system. Rates of collisions of liquid droplets within an immiscible liquid were described by Zhang and Davis (1991), when they analysed the movement of droplets in a system due to Brownian diffusion, gravitational sedimentation and some intermolecular attractions. This work is flawed as they disregarded any repulsive intermolecular forces between droplets which are clearly present as detailed by (Israelachvili and McGuiggan 1988) and further evaluated by (Dagastine et al. 2006). Nevertheless, they showed that collisions, or at least droplet approaches, due to Brownian motion and gravitational sedimentation have many factors, one of which is temperature, a point which will be examined more closely in a later section. As the Zhang and Davis (1991) paper showed, Brownian movement leads to particles approaching each other but it is intermolecular forces between the particles that play a more significant role as the particles begin to interact. The intermolecular forces that play the most significant role are Van der Waals attractive forces, and electrostatic and steric repulsion forces.

1.2.3.1.1 Van der Waals Forces

Van der Waals forces are attractive forces between particles and come in three classifications: London dispersion forces (induced dipole interaction), Keesom forces (permanent dipole interactions) and Debye interactions (permanent-induced dipole interactions). These will be explained in turn in the following section.

London dispersion forces occur due to the constant flux of electron clouds around a molecule. In any molecule, areas of electron deficient or rich areas arise, which

cause momentary electrostatic charges or dipoles on the molecule. These momentary charges will induce opposite charges on neighbouring molecules (an induced dipole), resulting in a mutually attractive interaction. In small molecules, this interaction is weak and can collapse as quickly as it forms, but as the molecule or particle gets bigger, the London dispersion forces amplify. In the simplest geometry of a sphere these interactions can be modelled as described in Equation 1-3 Increase in London dispersion forces with molecular/particle size (Oversteegen and Lekkerkerker 2003):

$$F_{LD} = \frac{A_H R}{12\,H}$$

Equation 1-3 Increase in London dispersion forces with molecular/particle size (Oversteegen and Lekkerkerker 2003)

where F_{LD} is the London dispersion force of attraction, A_H is the Hanmaker constant, R is the radius of the spherical particles and H is the distance between the particles. Equation 1-3 Increase in London dispersion forces with molecular/particle size (Oversteegen and Lekkerkerker 2003) is a model for two spherical particles in a vacuum. In reality, the dispersed phase is not in a vacuum but in the continuous phase and there will be some molecular interaction between the dispersed and continuous phase molecules. Although, of course, as the distance between the spheres decreases ($H \rightarrow 0$ and/or $R \gg H$) the intermolecular interactions between the continuous phase and dispersed phase become less important and Equation 1-3 becomes more accurate.

Equation 1-3 Increase in London dispersion forces with molecular/particle size (Oversteegen and Lekkerkerker 2003) shows two important factors concerning emulsion stability. Firstly, as the distance between two droplets decreases, the London Dispersion force of attraction increases, leading to the conclusion that the more closely the droplets approach each other, the more likely they are to collide. Secondly, as the size of droplet increases, the London Dispersion also increases, showing that the bigger the droplets, the higher the attraction and again more likely a collision. This also leads to a secondary conclusion that, once coalescence has started it is more likely to continue and gain speed as the London forces of attraction increase as droplet size in the system increases.

Keesom forces are intermolecular forces that occur when a molecule has a permanent area of electron richness or poorness in its structure, creating a permanent dipole. A permanent dipole arises when one of the molecule constituents

has a highly electronegative nucleus compared to the rest of the molecule. This means it has a small, highly charged nucleus with minimal electron shielding, therefore a stronger attractive interaction to electrons in the outer bonding shells than neighbouring nucleic centres. As the highly electronegative area draws electrons to it, it creates a negative charge around it and a positive charge elsewhere in the molecule, creating two permanent dipoles. When two molecules with permanent dipole centres approach each other, the negative dipole of one is attracted to the positive dipole of the other, leading to an attractive force. One of the best known and extreme cases of Keesom interaction is hydrogen bonding in water. The oxygen nucleic centre is very small (atomic weight of 16 with 8 protons) and its outer shell of electrons is almost full (electron configuration $1S^2\ 2S^2\ 2P^4$). As p-shell electrons are poor at electron shielding of the nucleus, the positive charge of the nucleus extends beyond the outer shell of its own electrons and on to any others nearby. This is the fundamental concept of electronegativity - the oxygen centre will pull electrons towards it, creating a permanent negative electron cloud around it, whilst also creating an electron deficiency (positive charge) elsewhere in the molecule. In the case of water, the hydrogen nucleic centre is also very small but it does not hold such a big charge (one proton) and hence does not have as large an attractive force on its electron as the oxygen nucleic centre does. In a water molecule, where two hydrogen atoms and one oxygen atom are bonded covalently, the bonding pair of electrons in each bond are attracted much more to the oxygen nucleic centre than the two hydrogen centres. This leads to a concentrated electron cloud around the oxygen centre (permanent negative dipole, δ^-) and an electron deficiency around the hydrogen centres (permanent positive dipole, δ^+). The effect is exaggerated by the shape of a water molecule which, instead of being linear (like CO_2), is bent with the four pairs of outer shell electrons (two bonding and two lone pairs) adopting a tetrahedral shape.

When two water molecules approach each other, the oppositely charged dipoles attract, making the molecules orientate to maximise the favourable interaction, leading to exceptionally strong intermolecular forces.

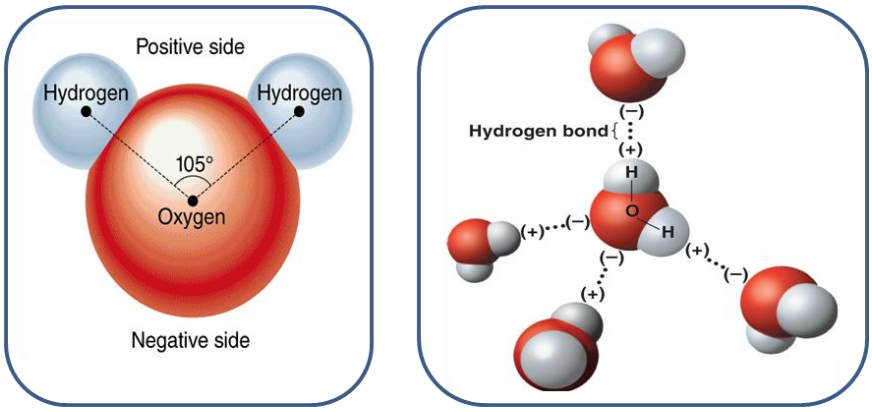


Figure 1-4 Illustration of strong Keesom intermolecular forces in water (StudyBlue 2011)

Indeed, the hydrogen bonding is so strong in water that the molecules keep their favourable interactions despite addition of high energy levels, which is the reason water has such a high boiling point in relation to the other hydrides of the group six atoms. It is also quite an elegant way of showing why water and non-polar oils are immiscible. The water molecules are attracted far more strongly to each other than to the non-polar oil. The water effectively squeezes the non-polar oil out of the mixture so that the water molecules can maximise their attractive intermolecular interactions between one another.

In relation to emulsion stability, it can be easily seen that molecules or particles of an internal phase that have a permanent dipole, when free to move in a non-interactive solution, will have a high level of attraction, and therefore high chance of collision. Equally, if the molecules or particles of an external phase have strong attractive intermolecular forces, it causes the internal phase to be squeezed out of dispersion, which also leads to a destabilised emulsion and phase separation.

In conflict with these Van der Waal attractive intermolecular forces are two repulsive forces, known as electrostatic repulsion forces and steric repulsion forces. These will be discussed in turn in the following section.

1.2.3.1.2 Electrostatic Repulsion Forces

Electrostatic repulsion forces occur when the surface of the internal phase droplets acquires a common charge, resulting in an electrostatic repulsion as the droplets approach, and hence becomes a barrier to coalescence and stabilises the system.

These forces are relevant to systems where the continuous phase is polar, like water, as there is an abundance of dissociated ions with which the surface can interact. A droplet surface becoming charged in a non-polar continuous phase would increase surface tension and destabilise the colloid. A droplet can acquire a charge through several mechanisms; the two most relevant to cosmetic emulsions are (Myers 1999):

- Ionisation - Groups on the surface of a colloidal particle ionise as they interact with the continuous phase, resulting in the particle acquiring a surface charge. The net surface charge acquired by the particle is strongly influenced by the pH of the solution. For example, it is possible for certain classes of compounds adsorbed on the surface of the particle to acquire either a positive or negative charge. For example, carboxylic groups attached to long carbon chains which are lipid soluble, can interact with the continuous phase at the particle surface and result in either a positive charge at low pH (abundance of H^+ in solution and at the surface) or a negative charge at high pH (abundance of OH^- in solution and at the surface). The pH where the net charge is zero is called the isoelectric point.
- Ion adsorption -The particle acquires a net surface charge as a result of ions adsorbing from the bulk continuous phase onto the surface of the internal phase droplet. The charge can be positive or negative depending on the nature of the adsorbed ion. In order to be an effective stabiliser, the adsorbed ion has to migrate to the surface of the droplet from the continuous phase and adsorb strongly. These types of molecules are called ionic surfactants (surface active molecules) and in emulsions are specifically called emulsifiers. They result in a net charge on the internal phase droplets, and they will be covered in some detail in the next section.

Once a droplet has acquired a surface charge, counter ions from the continuous phase are attracted to the surface and create a tight layer of ions close to the droplet surface - this is called the Stern layer. It is characterised by a linear decrease in electrostatic potential through the layer. However, the counter ions cannot aggregate with enough density to offset the surface charge and the electrostatic effects of the surface charge are observed beyond the Stern layer into the continuous phase. Thus, a second layer of both positive and negative ions, but a higher concentration of counter ions, called the diffuse layer, aggregate around the

droplet. The diffuse layer is characterised by an exponential drop in electrostatic potential across the layer to the point where the surface potential has been offset by the increased counter ion concentration. The continuous phase beyond the diffuse layer has equal amounts of positive and negative ions. This system of Stern layer and diffuse layer is commonly referred to as an Electrical Double layer.

It has been shown (Sennett and Olivier 1965a) that part of the double layer around the particle is stationary in relation to the particle itself, which means the particle and part of its double layer move through the external bulk phase together. The distance from the particle surface at which the electrical double layer stops moving with the particle is called the Slipping or Shear Plane. The shear plane can be found experimentally by applying an electrical current across the bulk system and is found at the point at which the double layer and the external bulk phase move in opposite directions. The shear plane is not necessarily the point at which the surface charge of the particle is offset by the counter ions in the diffuse layer. This means that the double layer system still has some electrostatic potential energy beyond its shear plane. This electrostatic potential energy is commonly referred to as Zeta potential and is an important concept in emulsion theory and illustrated in Figure 1-5.

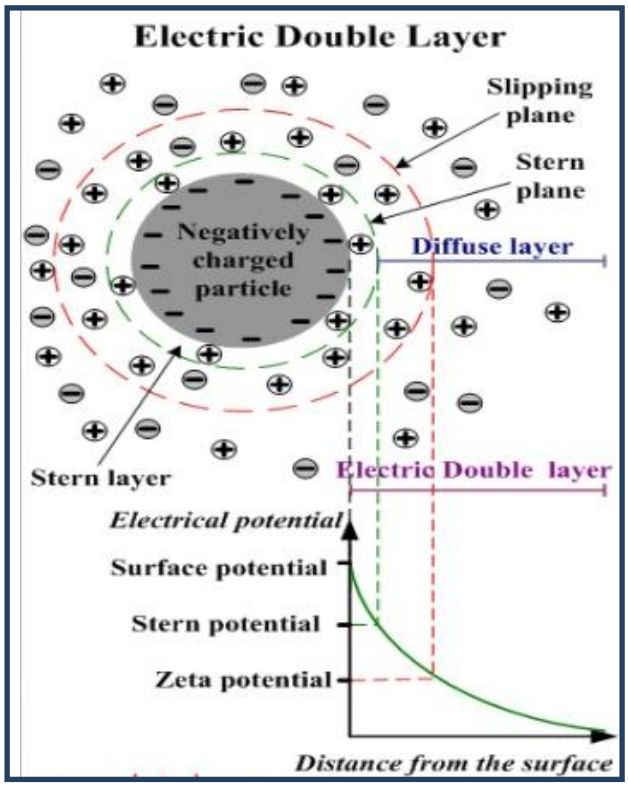


Figure 1-5 Electronic double layer and potential energy change with distance from charged surface (Kopeliovich 2001).

In practice, the measurement of Zeta potential is the energy required to shear the droplet and its associated double layer away from the continuous phase. It is found experimentally by measuring the velocity at which the particles move towards a charged electrode in relation to the voltage of the electrode and viscosity of the external phase.

It can be seen that the higher the Zeta potential the more electrostatic repulsive force is exerted on approaching droplets, and the more likely the emulsion is to remain stable. As a generalisation a ±30 mV is often cited as the threshold of colloidal stability (Stubenrauch 2006) - above ±30 mV, particles repel each other enough to maintain colloidal stability, and below the repulsion is not enough to prevent particle collision.

Zeta potential is related to the charge density of the ions that are absorbed onto the particle surface, the packing structure of the ions at the surface and the ion content of the external phase. Hence, if the external phase was anhydrous, the zeta potential would be zero because there are no ions in the external phase to set up the electronic double layer. It is not directly related to the particle size until the point where the particle size directly affects any of the three above mentioned variables.

1.2.3.1.3 Steric Repulsion Forces

Steric Repulsion Forces are found when macromolecules are adsorbed at the interface and provide a physical barrier to coalescence. These macromolecules are usually polymers, but can be natural macromolecules like proteins and gums, which have areas of polarity and non-polarity along their carbon or silicone chain that migrate to the polar and non-polar phases respectively (Bobin et al. 1999). These macromolecules act as a physical barrier to the internal phase droplets approaching each other as the long chains of these molecules entangle and prevent the droplets ever contacting as visualised in Figure 1-6 below.

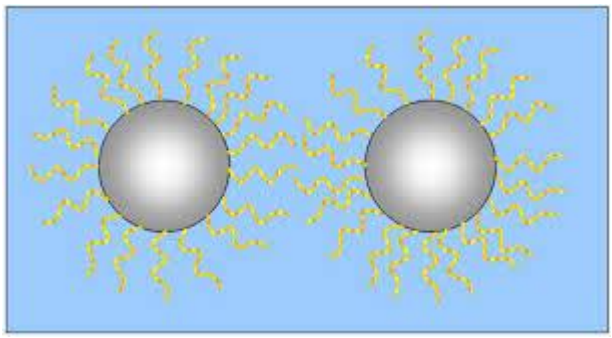


Figure 1-6 Illustration of macromolecular entanglement preventing droplet coalescence (Zeroday 2015)

Steric Repulsion Forces are short range repulsion forces and therefore become more important as the droplets become closer to one another. They are the primary repulsion forces in systems where the external phase is non-polar as there is no long range electrostatic repulsion in such systems.

The molecular weight of the macromolecules is extremely important in Steric Repulsion Force's ability to stabilise emulsions. If too low compared to the internal phase droplet size, they do not form a large enough physical barrier, but if too large, the macromolecules can bind to more than one droplet, which has the effect of aiding flocculation and coalescence of the droplets (so-called bridging flocculation) as illustrated by Figure 1-7.

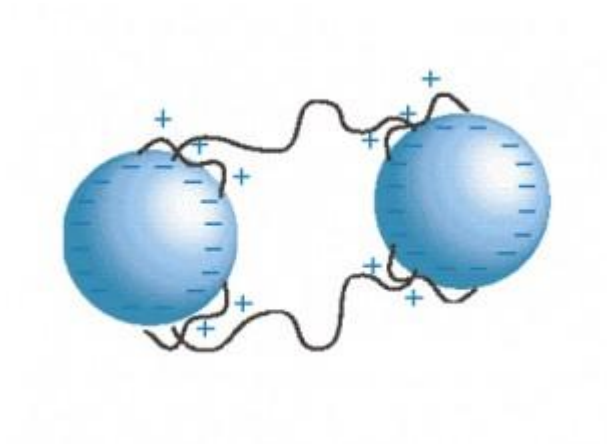


Figure 1-7 Illustration of bridging flocculation destabilising emulsion (Zeroday 2015)

1.2.3.2 DVLO Theory

It is the balance between these attractive and repulsive forces that determines what happens when dispersed phase droplets approach. The relative strengths of the

attractive Van der Vaal forces and the electrostatic and steric repulsive forces are combined into what is known as the DVLO theory. Named after the scientists that studied the effects, (Derjaguin and Landau 1941) and (Verwey and Overbeek 1948) who developed the theories on electrostatic repulsive forces and Van der Waals attractive forces independently, but came to the same conclusions. The DVLO theory attempts to explain certain colloidal behaviour by plotting the total force acting on the particles against distance of approach between two droplets. At any given distance the total energy potential can be described as the sum of the energy potential of attraction and repulsion. This is shown in equation 1-4 where V_T is total energy potential between particles; V_A is the attraction between particles due to van der Waals, mostly London Forces; V_R is the repulsion between particles due to the electrical double layer of co-ions and counter ions at the surface of a particle and steric repulsion forces of particles adsorbed at the droplet surface.

$$V_T = V_A + V_R$$

Equation 1-4 – DVLO Theory equation

If the repulsive forces are stronger, the total potential energy is positive and the particles move apart and the colloidal state is maintained. If the attractive forces are stronger, the total potential energy becomes negative and the droplets move closer together leading to coalescence and a decrease the overall surface area of the internal phase, eventually leading to phase separation.

At long distances, beyond the diffuse layer, the repulsive force is low as the surface charge of the droplet is completely offset by the counter ions in the diffuse layer. As the surfaces approach to within the diffuse layer the repulsive forces increase quickly to a maximum where the surfaces almost touch and the stern layers interact or steric effects are seen from molecules absorbed at the surface. The specific shape of this curve is dependent on the surface charge of the internal phase particle, charge of the counter ions and concentration of the counter ions (Israelachvili and McGuiggan 1988).

Van der Waals forces of attraction, as described by equation 1-3 Derjaguin approximation, become stronger as the droplets approach each other to a maximum as the surfaces almost touch. The strength of attraction, also shown by the Derjaguin approximation, is dependent on the size of the droplets approaching (Wiese and Healy 1970); and on the nature of the molecules within the internal phase (Kabalnov 1998).

When V_T is calculated over a range of distances we can see a V_T curve, as illustrated in figure 1-8. It can be seen that in the example plot of V_T, at long ranges the two surfaces have zero interaction, but as they approach, the attractive Van der Vaal forces become stronger than the repulsive forces and the surfaces begin to attract. This attractive state peaks at an area called the secondary minimum, and then, at a distance comparable to the diffuse layer, the repulsive electrostatic forces begin to become more significant and overwhelm the attractive forces as the distance between surfaces continues to decrease. This overall repulsive force peaks at the area called the primary maximum or V_{max}. It is also known as the energy barrier as this is the kinetic energy two particles on a collision course must overcome with their mass or velocity in order to agglomerate (Trefalt and Borkovec 2014). As the surfaces come closer still the attractive forces begin to become more significant again and overpower the repulsive forces, leading to a large force of attraction peak close to the surfaces touching – known at the primary minimum.

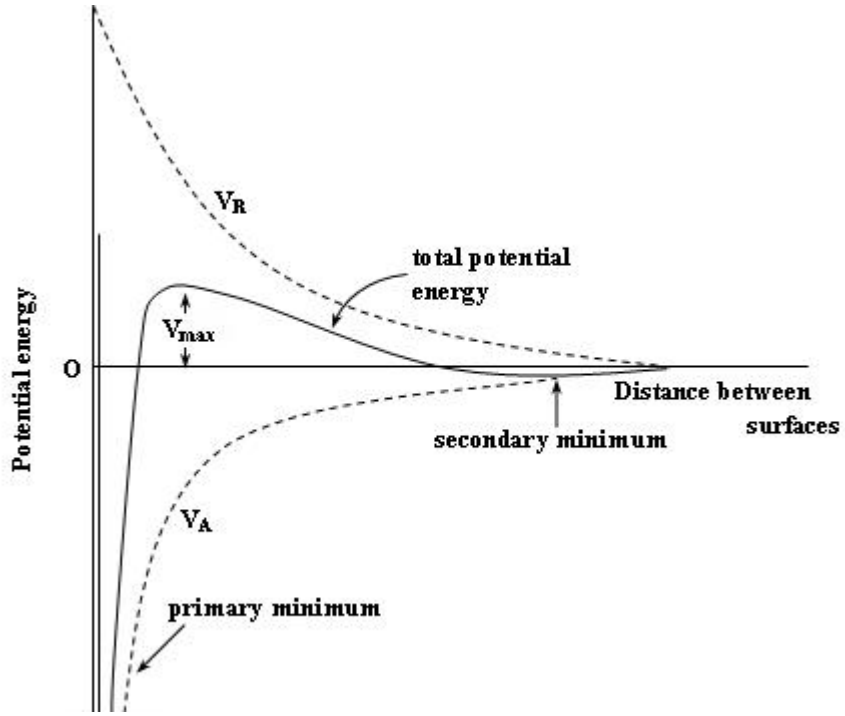


Figure 1-8 DVLO theory graph of energy potential against distance of separation

At very short distances, in the order molecular lengths, the repulsive forces become very strong either due to direct stern layer interactions or steric repulsion which become dominant and is often referred to as hard sphere repulsion (Wu et al. 1998).

Understanding these V_T curves helps explain colloidal stability and behaviour. For example, two particles that cannot overcome the energy barrier move apart but can get caught in the secondary minimum range where there is a weak overall attraction. They then move together through the bulk continuous phase but are easily separated again with mechanical work like stirring – this explains flocculation behaviour. The V_T curve can be affected by an emulsion formulator by adjusting variables like salt content (counter ion concentration), salt type (counter ion strength) and surfactant type (surface charge) which all affect the diffuse layer thickness; and surface and zeta potential. By adjusting these parameters the V_T curve can be manipulated to enhance stability, for example, using a surfactant with a higher ionic charge will increase the primary maximum/energy barrier and therefore increase stability; or by increasing electrolyte content (salt concentration) the electric double layer contracts as the surface charge it shielded more, which decreases the primary maximum and deepens the secondary minimum, encouraging flocculation (García-García et al. 2007), this would be observed as a decrease in zeta potential.

These concepts of mechanisms and pathways of emulsion behaviour are important and are used to explain observed stability behaviour. It is possible to measure the rate of coalescence and flocculation by close examination of the internal phase droplets. As coalescence is the merging of two droplets, the overall droplet size increases. This can be seen by measuring droplet size of the internal phase at various time points and observing the rate of increase over time. Flocculation is more difficult to measure as, by definition, the size of the droplets does not change, but instead forms aggregates that move together through the continuous phase. As flocculation can be a stepping stone on the pathway to coalescence, the same particle size measurements often show flocculation has taken place.

1.2.3.3 Sedimentation and Creaming

Sedimentation and creaming occur by a different mechanism to flocculation and coalescence, and happens because of a large difference in density between the internal and continuous phase. Due to gravity, the lower density oil phase will migrate above the water phase resulting in an increase in concentration at the surface (creaming) or bottom (sedimentation).

The rate of sedimentation and creaming is described by Stokes Law (Tadros 2013):

$$V = \frac{2r^2(p_{sphere} - p_{fluid})g}{9\mu}$$

Equation 1-5 Stokes Law

where V is the velocity of dispersed phase particle (that is rate of sedimentation and creaming), r is the radius of the particle and g is acceleration due to gravity. μ is the viscosity of the continuous phase, ρ_{sphere} is the density of the internal phase and ρ_{fluid} is the density of the external phase.

Analysis of Stokes Law shows that the rate of sedimentation or creaming is dependent on –

- The size of internal phase particle – as particle size increases, rate of sedimentation or creaming increases as well. Hence, any flocculation or coalescence will have an effect on rate of sedimentation or creaming.
- The differential between the density of the internal and external phases - if there is a large differential the rate of creaming and sedimentation will also be large.
- The viscosity of the continuous phase, which it is inversely proportional to. As viscosity increases, the rate of creaming or sedimentation decreases. Viscosity is dependent on many things, including for most fluids, temperature.

1.2.3.4 Disproportionation

Disproportionation is a process, often referred to as Ostwald Ripening that is dependent on the diffusion of disperse phase molecules from smaller to larger droplets through the continuous phase. The pressure of dispersed material is greater for smaller droplets than larger droplets, as shown by the Laplace equation (Sennett and Olivier 1965b):

$$P = 2\gamma/r$$

Equation 1-6 Laplace Equation

where P is the Laplace pressure, γ is the surface tension and r is the droplet radius.

This pressure differential between small and large droplets constitutes the driving force for diffusion, but the rate of diffusion depends on the solubility of the dispersed phase in the continuous phase. The higher the disperse phase volume, the greater its relative vapour pressure (and thus solubility), as given by the Kelvin equation (Myers 1999):

$$\ln\left[\frac{P_o}{P}\right] = \frac{2\gamma V_m}{rRT}$$

Equation 1-7 Kelvin Equation

where P is the vapour pressure of the liquid droplet, Po is the vapour pressure of the bulk liquid, γ is the surface tension, r is the droplet radius, V is the molar volume of the disperse phase, R is the gas constant and T is the temperature.

The diffusion rate is also impacted directly by the viscosity of the continuous phase as described by the Stokes-Einstein equation (Mason 1999):

$$D = \frac{k_b\, T}{6\pi\eta \mathrm{r}}$$

Equation 1-8 The Stokes-Einstein Equation

where D is the diffusion coefficient of a droplet, η is the continuous phase viscosity and K_b is Boltzmann's constant.

The measurement of both the droplet size and size distribution of emulsions is critical in the measurement of the rate of Ostwald Ripening.

A variety of sizing techniques is available, including laser diffraction and light scattering spectroscopy, but the most widely used is microscopy image analysis (for regular emulsions), as the droplets are relatively easy for edge-finding software to identify and size.

1.2.4 Surfactants – Emulsifiers

The purpose of emulsifiers is to aid in the formation of, and kinetically stabilise, an emulsion. It does this by two mechanisms:

- lowering the energy requirement for droplet formation (by decreasing the interfacial tension (γ_{AB})), and
- decreasing the rate of droplet reversion back to the discrete phases.

Emulsifiers are a group of molecules that have some solubility in both polar and non-polar media. This characteristic arises because of their chemical structure which has long, non-polar carbon chain, which is lipophilic/hydrophobic, along with polar functional groups at one end, which are lipophobic/hydrophilic, as illustrated in figure 1-8.

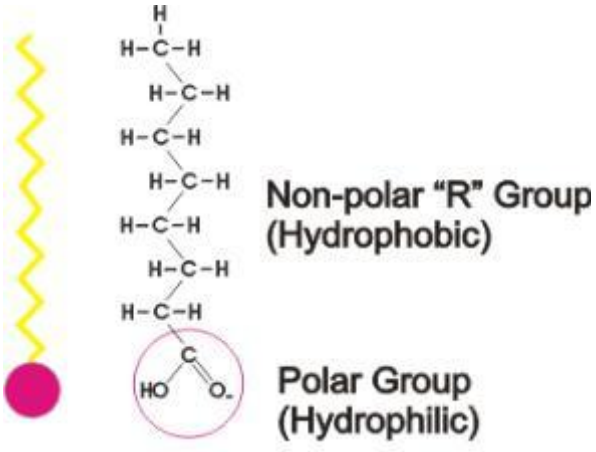


Figure 1-9: Example of a surfactant structure. (D Foam Inc 2005)

This hydrophilic and hydrophobic property within the same molecule allows the molecule to position itself at the boundary between oil and water, or the surface of the droplet as shown if figure 1-9. This behaviour is termed surface active and thus the molecules are called surfactants.

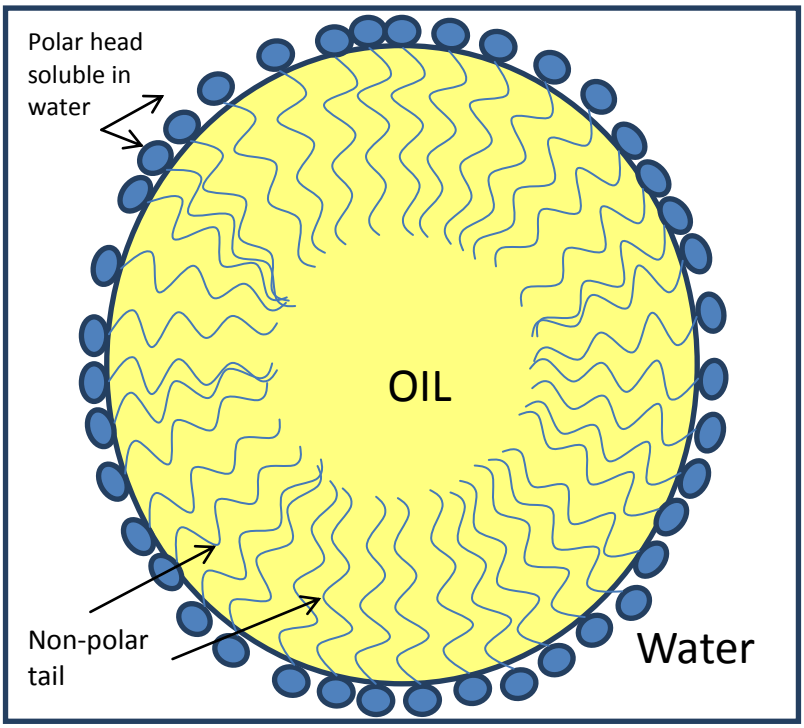


Figure 1-10 Surfactant molecule migrating to phase boundary

This behaviour results in a layer being created at the polar/non-polar surface which decreases the overall interfacial tension (γ_{AB}) between the two phases, and therefore (from Equation 1-2) decreases the work (W) needed to increase the overall surface area and aids droplet formation.

Once at the phase boundary layer, the surfactant molecule can aid colloidal stability in two ways. If it holds an overall charge on its polar head group, it can use electrostatic repulsive forces to decrease the likelihood of a droplet collision (only possible if the continuous phase has polar ions present) or it can form a physical barrier using steric repulsion forces to stop droplet collision and coalescence. Both processes are described in section 1.2.3.

There are four main groups of emulsifier: anionic, cationic, non-ionic and amphoteric as described by Rhein and Rieger (1997). They will each be described briefly in the following passages.

1.2.4.1 Anionic Surfactant

An anionic surfactant is characterised by its polar head having a negative charge after dissociation in water. They are often a carboxylic acid, sulphate or sulphonic acid group on the end of a long hydrocarbon chain. The carboxylic acid group

dissociates in water to leave a negative charge and the long non-polar chain adheres to the least polar region it can find, either an oil region or into the air at the surface of the system. Common examples are stearic acid show in Figure 1-10:

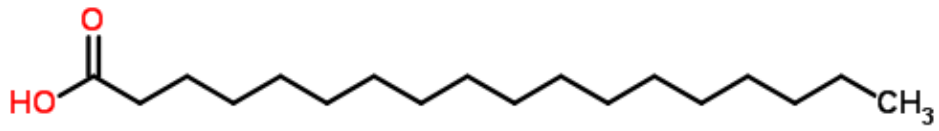


Figure 1-11 Stearic Acid (C18 Carboxylic Acid) molecular structure

and Sodium Lauryl Sulphate shown in Figure 1-11.

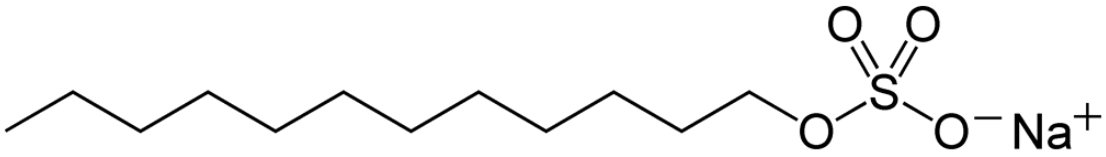


Figure 1-12 Sodium Lauryl Sulphate molecular structure

1.2.4.2 Cationic Surfactants

Cationic surfactants are characterised by their positive charge associated to their polar head group. They are mostly seen in hair care formulations because areas of damaged hair hold a negative charge. They are commonly quaternary ammonium compounds such as cetrimonium chloride and benzalkimonium chloride. There structures are shown in Figure 1-12 and 1-13.

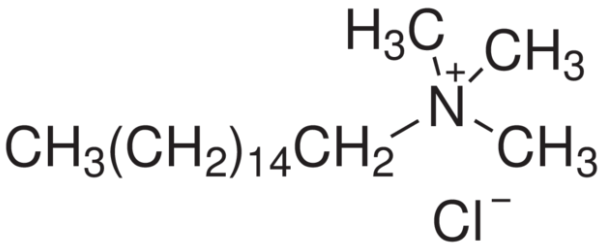


Figure 1-13 Cetrimonium chloride molecular structure

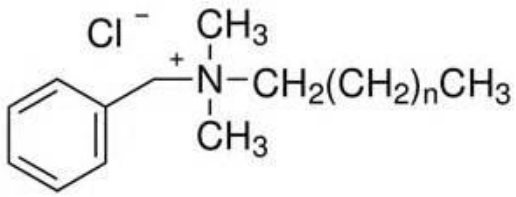


Figure 1-14 Benzalkmonium chloride molecular structure

1.2.4.3 Amphoteric Surfactants

Amphoteric, or Zwitterionic, surfactants are characterised by having both a positive and a negative centre on a carbon chain. Their behaviour is complex, as it depends on the pH of the aqueous phase they are in - they behave as cationic in acidic media and anionic in alkaline media. They are compatible with either cationic or anionic surfactants and are therefore used as versatile co-surfactants. A common amphoteric surfactant found in many detergent systems is cocoamidopropyl betain, its structure is given in figure 1-15.

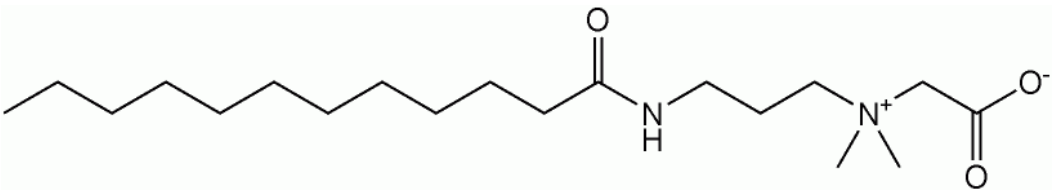


Figure 1-15 Cocoamidopropyl Betain molecular structure

1.2.4.4 Non-ionic Surfactant

Non-ionic surfactants are the most common type of surfactant used in cosmetic products. They are characterised by having no charge on their polar head group on dissolution in water, meaning they have no ionic charge in water. Instead, their surfactant properties arise from hydrophilic functional groups on a carbon chain. The degree of polarity and the length of the hydrophobic carbon chain give a great variety of surfactant strength and efficacy. For example, most fatty alcohols show some surfactant properties but the alcohol group is small compared to the long carbon chain, hence they are not soluble in water and are found almost completely

in the oil phase. A typical example is cetyl alcohol which has a carbon chain length of 16 carbon centres; its structure is given in figure 1-16.

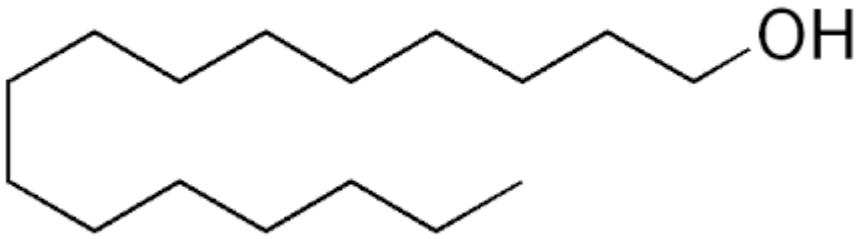


Figure 1-16 Cetyl (C16) alcohol molecular structure

The surfactant strength of a fatty alcohol can be increased by the addition of more polar functional groups such as ethylene oxide. A number of ethylene oxide groups can replace the alcohol group in a polymerisation reaction. The more ethylene oxide groups that are added, the stronger the polar head becomes and the smaller the carbon chain becomes in proportion. By controlling the number of ethylene oxide groups added to the carbon chain, the properties of the surfactant are tailored to any desired efficacy, as shown by the polymerisation reaction shown in Figure 1-17.

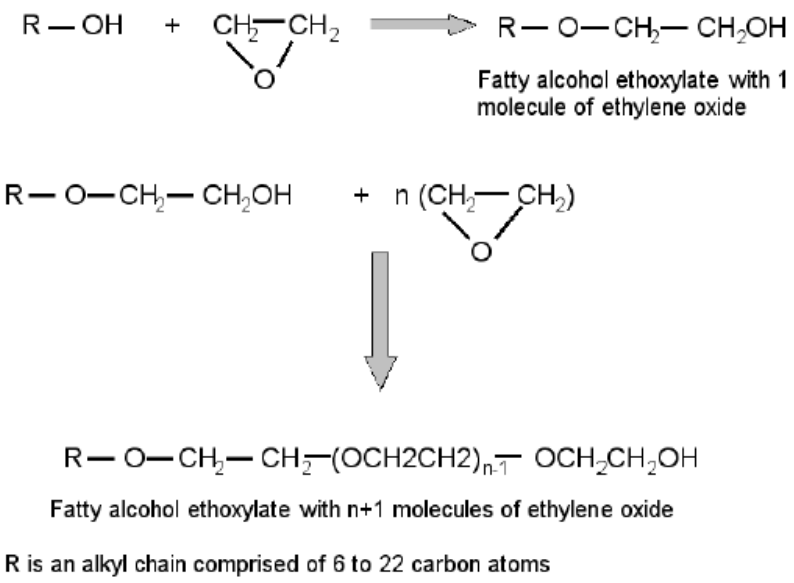


Figure 1-17 Polymerisation reaction of ethylene oxide and fatty alcohol (SCS 2009)

The concept of ‘strength’ of a non-ionic surfactant has resulted in the advent of an arbitrary scale of 0-20, called the hydrophilic-lipophilic balance (HLB value) it was introduced by William C. Griffin at a public meeting in Chicago 1949, and later that year published in the Journal of the Society of Cosmetic Chemists (Griffin 1949). In this paper, Griffin suggested that the nature of a non-ionic surfactant can be described by the portion of the emulsifier molecule that absorbs into the water phase

as compared to the oils phase, and suggested an experimental procedure of how to obtain a substance's HLB value experimentally. In later papers he also described how to calculate these value by the chemical structure (Griffin 1954), although he observed that for some functional groups this was not accurate and therefore should be checked experimentally. The HLB value gives an estimation of the type of surfactant behaviour the surfactant will have:

Table 1-1 HLB value and Surfactant behaviour

HLB Value		Surfactant Function
1-5		Water in Oil Emulsifier
5-8		Water in Oil Emulsifier/Wetting agent
8-12		Oil in Water Emulsifier/Wetting Agent
12-15		Oil in Water Emulsifier/Detergent
15-20		Oil in Water Emulsifier/Detergent and Solubiliser

Table 1-1 shows that changing the ratio of water-soluble to oil-soluble portions of the emulsifier changes its behaviour and the emulsion made. At low HLB, there is a high ratio of oil-soluble portions in the molecule, meaning the majority of the molecule sits in the oil phase at the interface. This encourages the water to be the internal phase, due to steric hindrance of the non-polar chains in the lipid phase and the polar heads trying to maximise their interaction with the water phase. Conversely, the higher the HLB value, the higher the ratio of water-soluble portions to oil-soluble, meaning most of the molecule is found in the water phase. This forces the droplet to form around the oil rather than the water, creating oil in water emulsion. As the HLB continues to rise, the emulsifier becomes almost completely soluble in water and makes the oil droplets smaller and smaller. This allows for detergency (removal of lipid soil from a solid surface) and solubilisation (incorporation of lipids into an aqueous system that remains transparent, for example fragrance into a wash product to create a micro-emulsion appearing as a clear gel).

Davis (1973) extended the use of HLB values by taking the calculation beyond whole molecule, non-ionic surfactants and assigned HLB values to specific functional groups to calculate overall HLB value for all surfactants as shown in Table 1-2.

Table 1-2 The Davis HLB Group numbers for various functional groups

Hydrophilic Groups	Lipophilic Groups

Functional Group	HLB Value	Functional Group	HLB Value
R-SO_4Na	35.7	R-CF_3	-0.87
R-CO_2K	21.1	R-F_2-	-0.87
R-CO_2Na	19.1	R-CH_3	-0.475
R-N (tertiary amine)	9.4	R-CH_2-	-0.475
Ester (sorbitan ring)	6.3	R-CH-	-0.475
Ester (free)	2.4	R-CH(X)-	-0.475
R-CO_2H	2.1	R-$CH_2CH_2CH_2O$-	-0.15
R-OH (free)	1.9		
R-O-	1.3		
R-OH	0.5		
R-CH_2CH_2O-	0.33		

This took the possible values beyond the original 0-20.

$$HLB = 7 + \Sigma \text{ (hydrophilic group numbers)} - \Sigma \text{ (lipophilic group numbers)}$$

Equation 1-9 Davis Equation for HLB value

Hence, all emulsifiers can be assigned an HLB value to assess their behaviour and strength. The HLB value has been taken into consideration when selecting which emulsifiers to test in the experimental design.

1.3 Literature Review

This section focuses on types of instability and the various techniques published in literature to predict the stability of systems over time. This is then used to justify the experimental design and methods used in this research.

1.3.1 Accelerated Stability Evaluation

Defined in the British Standards Institute – Standards Publication on Cosmetic Stability Testing as a study designed to speed up naturally occurring destabilization processes due to intrinsic or extrinsic factors and which predicts the behaviour over the long term (The British Standards Institution 2018). Protocols can be designed to

induce physiochemical changes or test microbiological resilience. These protocols are important to the cosmetics industry because they enable the development cycles to be as short as possible, getting products to market quickly and generating revenue.

1.3.1.1 Physiochemical Stability

With no enforced protocol of stability testing stipulated in the current, or any previous, legislation, the cosmetic industry has created many test protocols depending on each individual research company's standard operating procedures. Many follow stability guidelines documents such as ISO18811:2018 (The British Standards Institution 2018), the Brazilian ANVISA guidelines (National Health Surveillance Agency 2004), the American PCPC guidelines (Personal Care Products Council 2011) or Cosmetics Europe (Colipa) guidelines from 2004 (Cosmetics Europe 2004). Each of these guideline documents refers to the pharmaceutical industry protocol, which has a prescriptive route of stability declaration (ICH Harmonised Tripartite 2003). However, it should be acknowledged that the primary purpose of a pharmaceutical stability test is to ensure that the active pharmaceutical ingredient (API) in the product is still active and at the desired concentration throughout its shelf-life. Hence, the focus is on the degradation of that material and not necessarily on the other attributes of the whole pharmaceutical formulation (such as colour, viscosity or odour) (Waterman and Adami 2005).

Pharmaceutical stability protocols were laid out by the International Conference on Harmonisation (ICH Harmonised Tripartite 2003) and were adopted by the European Agency for Evaluation of Medicinal and Health Products (EMEA) in 2003, and most recently by the World Health Organisation (WHO), in 2009 (World Health Organisation 2009). Although there are some minor differences between the guidelines (Henal et al. 2011), they all use the principle of stress testing to accelerate processes that may be seen at ambient conditions to build up a body of evidence for declared shelf-life. This is based on the theory that increasing temperature increases the rate of a reaction (Waterman and Adami 2005), although the guidelines are very careful not to quantify the acceleration ratio, and shelf-life cannot be declared on accelerated data alone. The protocols instead insist on long-term, controlled ambient conditions of at least 24 months to declare a shelf-life. A summary of the ICH testing protocols is given in the Henal et al. (2011) article and is

summarised in Table 1-3 It is a useful demonstration of the level of detail the ICH prescribes for such testing.

Table 1-3 Physical Stability Requirements of Pharmaceutical products according to ICH guidelines (ICH Harmonised Tripartite 2003)

Parameter	Pharmaceutical Formulation
Batches to test	Data provided on at least three primary batches. Two should be at least pilot scale batches.
Container Closure System	Container closure system for testing should be the same as that proposed for marketing, including secondary packaging
Specification	The list of tests and proposed acceptance criteria which all test points should meet
Testing frequency	Long Term studies: 0, 3, 6, 12, 18, 24 months and annually through the proposed re-test period. Intermediate: 0, 6, 9, 12 months Accelerated: 0, 3, 6 months.
Storage Conditions	Long Term: 25°C +/- 2C/60% RH +/- 5% RH or 30°C +/- 2C/65% RH +/- 5% RH. Intermediate: 30°C +/- 2°C/65% RH +/- 5% RH. Accelerated: 40°C +/- 2°C/75% RH +/- 5% RH

Stability Commitment	If the data does not cover the proposed shelf life granted at the time of approval, a commitment should be made to continue the long-term studies through the proposed shelf-life and the accelerated studies for six months post approval
Evaluation	Based on the evaluation of the data, shelf life should be established.
Statement/Labelling	A storage statement should be established based on the stability evaluation of the drug substance.

Although the Henal et al. (2011) article is a basic comparison of four international guidelines with no analysis or opinion of which guideline is most appropriate, it does highlight some important parameters in which all the guidelines agree. Most notably:

- stability testing must be carried out on at least three primary batches, two of which should be pilot batch size.
- a pharmaceutical product becomes out of specification once 90% of the declared API content can no longer be recovered, i.e. 10% has degraded.
- acknowledgment of the different climactic zone in which the drug product is being distributed, the difference in ambient temperature in those zones and the effect this will have on shelf-life.
- long-term studies should be carried out for the time of declared shelf-life and for a minimum of 24 months.

These are stringent and structured protocols that each pharmaceutical product must declare results to the Regulatory body in the region of sale before placement on market. They acknowledge that accelerated stability data is useful as a guide to real-time stability but insist on real-time testing for verification.

The principle of subjecting a product to stress conditions and using the results to extrapolate what happens at ambient conditions is used in many industries, including paper (Havermans and Porck 2002) and food (Singh et al. 2012). The

relationship between accelerated and real-time data will be explored in the following passages.

In a summary paper on pharmaceutical stability testing, Bajaj et al. (2012) cited the origin of the accelerated test in the Arrhenius Equation (Arrhenius 1889), which described the relationship between temperature and reaction rate (referred to in the article as degradation rates).

$$lnK = lnA + \frac{Ea}{RT} \quad \text{or} \quad K = A\, e^{-\frac{Ea}{RT}}$$

Equation 1-10 Arrhenius Equation (Arrhenius 1889)

where K is the rate of reaction (or degradation), A is the frequency factor (molecular collisions with enough energy and correct orientation to react per second), Ea is the activation energy (J/mol), T is absolute temperature (K) and R is the gas constant (8.31 J/K/mol).

Bajaj et al. (2012) stated in their article that if activation energy, frequency factor and temperature were known for two temperature points, then degradation rate at low temperature could be extrapolated from those observed at stress temperatures. This also assumes that the rate of degradation followed first order rate kinetics with respect to temperature, meaning a linear or constant change of rate of reaction with change of temperature.

Extrapolations are made by plotting *ln(K)* vs. $\frac{1}{T}$, which is effectively plotting rate of reaction against temperature at which that reaction occured (Fan and Zhang 2014). If first order kinetics is indeed true, this plot is linear with the slope equal to $-\frac{E_a}{R}$ and the true Y-intercept is *ln(A).* Using this plot, the rate of reaction can be extrapolated for any given temperature, an example of which is shown in Figure 1-18.

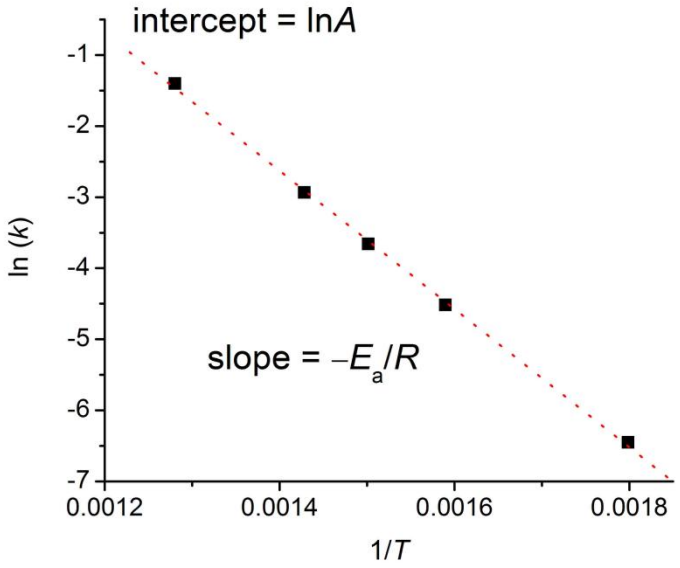


Figure 1-18 Example of an Arrhenius plot

More recent studies by Waterman et al. (2014) considered degradation of pharmaceutical actives by different rate orders, that is second and third rate reactions, but these have yet to be validated or adopted by any regulatory body.

Despite the similarities between the two industries, the cosmetic industry has not adopted the ICH guidelines for introduction of new products onto the market into its literature. Instead the industry relies on a series of guidelines and each development company to develop its own accelerated testing protocols and justifications for shelf life determination of their specific products. All of these protocols are based on the principle of stress testing to extrapolate real time behaviour. These principles were first suggested in a cosmetic context by Cannell (1985) in an article in the International Journal of Cosmetic Science, and is still one of the only papers concerning stability testing of cosmetic products in that journal. Around the same time, similar protocols were being outlined by (Idson 1988) and again in a later paper (Idson 1993) in the American Journal – Drug and Cosmetic Industry. These papers were a direct contributor to the International Federation of Cosmetic Chemists (IFSCC) monograph on Fundamentals of Stability Testing. The IFSCC monograph and the Cannell paper are both cited in all of the cosmetic stability guideline documents; ISO18811:2018 (The British Standards Institution 2018), the Brazilian ANVISA guidelines (National Health Surveillance Agency 2004), the American PCPC guidelines (Personal Care Products Council 2011) or Cosmetics Europe (Colipa) guidelines from 2004 (Cosmetics Europe 2004). They are cited in

the context of appropriate extrapolation techniques of real-time data from accelerated results. Indeed in the ISO18811:2018 (The British Standards Institution 2018) this context of the citation is:

“Accelerated test conditions may vary and should be established based on correlations to real time storage conditions for the specific region or market. References to commonly used accelerated test conditions for testing cosmetic products are provided in the Bibliography.”

The Bibliography entry in ISO 18811:2018, contains all of the guidance documents mentioned above.

In both the Cannell (1985) article and IFSCC monograph, the Arrhenius Equation is cited as the most relevant way of extrapolating real-time data from accelerated testing results. It also quantifies an appropriate extrapolation as a 10ºC rise in temperature doubles the rate of reaction. This generalisation is referred to as the ‘Q rule’ (Anderson and Scott 1991) and states that a rate of reaction decreases by a constant factor (Q_{10}) when the storage temperature decreases by 10ºC. The value of Q_{10} is typically set at 2, 3 or 4 with Q = 2 as the most conservative assumption and Q = 4 more speculative. The theoretical activation energies for each value of Q is calculated and compared to the experimental true activation energy with the most appropriate value of Q then applied to the accelerated data.

The most conservative assumption of Q=2 is the basis of the cosmetic Accelerated Stability Model’s assertion that the rate of reaction doubles for each 10ºC jump in storage temperature.

Table 1-4 Table to show the correspondence between accelerated data and the real-time data if Q=2

	Initial	1 week	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
4ºC	0	Standard for Comparison					
25ºC		Real time testing					
40ºC		4 weeks	8 weeks	16 weeks	32 weeks	48 weeks	64 weeks
45ºC		6 weeks	12 weeks	24 weeks	48 weeks	72 weeks	96 weeks

The Cannell (1985) paper itself acknowledges that this extrapolation is crude at best, as illustrated at the limits of the testing. The choice of Q value makes a huge difference to how far into the future the testing represents. For example tables 1-5 and 1-6 show the time points in the future that are represented if Q=3 or Q=4:

Table 1-5 Table to show the correspondence between accelerated data and the real-time data if Q=3

	Initial	1 week	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
4ºC	0	Standard for Comparison					
25ºC		Real time testing					
40ºC		9 weeks	18 weeks	36 weeks	72 weeks	108 weeks	144 weeks
45ºC		13.5 weeks	27 weeks	54 weeks	108 weeks	162 weeks	216 weeks

Table 1-6 Table to show the correspondence between accelerated data and the real-time data if Q=4

	Initial	1 week	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
4ºC	0	Standard for Comparison					
25ºC		Real time testing					
40ºC		16 weeks	32 weeks	64 weeks	128 weeks	192 weeks	256 weeks
45ºC		32 weeks	64 weeks	128 weeks	256 weeks	384 weeks	512 weeks

However, if Q has a value less than 2, the time points in the future that are represented are significantly shorter, as shown in table 1-7 which assumes Q=1.5:

Table 1-7 Table to show the correspondence between accelerated data and the real-time data if Q=1.5

	Initial	1 week	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
4ºC	0	Standard for Comparison					
25ºC		Real time testing					
40ºC		2.25 weeks	4.5 weeks	9 weeks	18 weeks	27 weeks	36 weeks
45ºC		2.8 weeks	5.63 weeks	11.25 weeks	22.5 weeks	33.75 weeks	45 weeks

Cannell also noted that the extrapolation is only appropriate for assay data form analytically obtained results (pH viscosity, ingredient concentration etc), and may not be appropriate for the subjective aspects of cosmetic products (including colour, odour, texture etc.) which are difficult to treat mathematically.

Both the Anderson and Scott (1991) and Bajaj et al (2012) papers highlighted that the Q-rule is only an approximation and both state that the model falsely assumes that Q_{10} will remain constant over all temperature ranges, instead suggesting that Q_{10} changes depending on which 10ºC jump is being studied. Both papers recommend using the Q rule only as a tool for early indication of which components are viable candidates to progress to full-scale testing and not a true reflection of long-term stability performance. Indeed Anderson and Scott (1991) studied the degradation of a drug over time, and found that applying a Q rule of Q=2 to an accelerated test yielded an approximate shelf life of 2.3 years with a theoretical

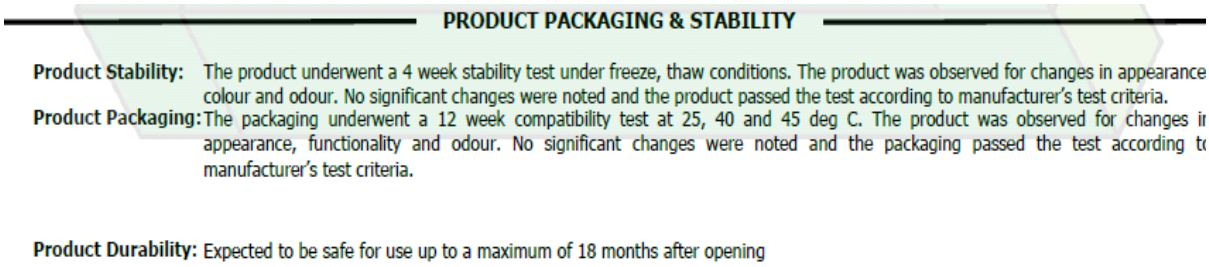
activation energy of 12.2 Kcal/mol and application of Q=3 gave 17 years with theoretical activation energy of 19.4 Kcal/mol. However, the true activation energy was 18Kcal/mol, so the Q rule model would predict that the Q=3 estimation of 17 years would be fairly accurate, whereas the actual real-time testing showed a shelf life of just 10 years.

The pharmaceutical industry is not the only industry that has questioned the use of Arrhenius equation kinetics for shelf life prediction. A paper published in Food Science and Nutrition (Peleg et al. 2012) criticised the use of the model to predict enzymatic reactions, microbial growth (activation and inactivation) and vitamin degradation. (Peleg et al. 2012) also pointed out the mathematical errors involved in applying the Arrhenius principles to non-molecular processes. As stated in the paper, the Arrhenius equation has units of 'moles' in its solution through the universal gas constant, 'what is a ''mole'' of mayonnaise, orange juice concentrate, or Ketchup?'. Indeed this argument can be extended to detergents, creams and fragrances of the cosmetics industry, which are mixtures of many chemicals and structures.

To their credit, most of the guidelines set out by the various standards organisations recommend that a real time testing sample be kept in ambient conditions for the duration of the shelf life. However, there is no requirement, or indeed recommendation, to revisit the safety assessment once this data is collected, or inform the end user that there is a difference between the a product that has not completed real-time testing and a product that has. With strong commercial pressure to get products to market with the minimum development time as possible, and with the guidelines non enforceable, many cosmetic protocols apply the Q=2 assumption to elevated temperature conditions as a true reflection of long-term stability, which decreases the development time from the shelf life declaration, 3 - 4.5 years, to just 3-6 months. Justifying the application by pointing to the fact the Q=2 is the most conservative estimation in the Q rule model in pharmaceutical applications.

Products are able to obtain a Safety Assessment from a Safety Assessor after as little as eight weeks, although more commonly 12 weeks, of elevated and real-time temperature testing. There is no requirement for the manufacturer to declare in which countries the product is to be distributed; hence there is no commitment to any specific 'ambient' temperature. Additionally, there is no requirement to stability test pilot or full-scale manufactured batches to verify scale-up from laboratory to

large scale manufacturing, nor for the manufacturer to commit to completing any long-term, real-time tests to validate declared shelf-life. A sample of a Safety Assessment is given in figure 1-19 below.



PRODUCT PACKAGING & STABILITY

Product Stability: The product underwent a 4 week stability test under freeze, thaw conditions. The product was observed for changes in appearance colour and odour. No significant changes were noted and the product passed the test according to manufacturer's test criteria.

Product Packaging: The packaging underwent a 12 week compatibility test at 25, 40 and 45 deg C. The product was observed for changes in appearance, functionality and odour. No significant changes were noted and the packaging passed the test according to manufacturer's test criteria.

Product Durability: Expected to be safe for use up to a maximum of 18 months after opening

Figure 1-19 Excerpt from a Safety Assessment of product currently on market

These assumptions on physical stability form a significant focus of investigation of this body of research.

1.3.1.2 Microbiological Stability

As well as the physical stability of a cosmetic product, the microbiological stability of a product also has to be considered by a Safety Assessor. Most cosmetic formulations are susceptible to microbiological contamination (Hitchins 1991), and should be protected from such contamination. Indeed, in the Hitchins (1991) paper, the danger is highlighted by case studies of *Pseudomonas aeruginosa* eye infections associated with mascara contamination.

Microbiological contamination comes from a variety of organisms – bacteria (gram positive and gram negative), mould, yeast, fungi and viruses. The contamination itself can come from a variety of sources, including unclean manufacturing vessels, contaminated water supply at the manufacturing site, contaminated packaging at the filling site and air-bound microbes/spores settling on a product's surface (Campana et al. 2006). However, the most common source of contamination is human contact by the end user, especially if the packaging is an open jar into which the end user dips a finger. Hence, where and how much contamination a cosmetic product may receive is beyond the manufacturer's control. Therefore, each cosmetic product must be able to protect itself against any microbiological contamination that it may encounter. Whilst some products are not microbiologically susceptible (Ghalleb et al. 2015) due to absence of free water or presence of aggressive solvents like ethanol, most need to employ preservatives to ensure absence of microbiological contamination.

Again, the cosmetic regulation EU 1223/2009 does not specify a standard test criterion for assuring preservative efficacy, stating only:

> 'Microbiological quality; the microbiological specifications of the substance or mixture and the cosmetic product. Particular attention should be paid to cosmetics used around the eyes, on mucous membranes in general, on damaged skin, on children under the age of three, and on elderly people or those showing compromised immune responses. The results of preservation challenge testing should also be included.'

A 'preservation challenge' test is the common name for Preservative Efficacy Testing for topical products as outlined by many regulatory guidelines, including the European Pharmacopeia (Ph. Eur.), US Pharmacopeia (USP), CTFA Microbiology Guidelines for Cosmetics (M-3 and M-4), ASEAN Cosmetic Harmonised Testing Method Association of Southeast Asian Nations, and most recently ISO standard 11930 - Evaluation of the antimicrobial protection of a cosmetic product. These test methods differ slightly but are all built upon the principles of aggressive inoculation and monitoring of microbes in the consumable product. The method requires that any microbiologically susceptible product be inoculated by a known level of five specific organisms and the rate of decrease be monitored over time. The results are compared to a specification criterion to assess the product's preservative efficacy. In an article (SIEGERT 2013) detailed the differences in the various methods, including microbes, inoculation levels and reduction criteria. An example of the differences is given in table 1-4 below, showing the different reduction criteria for the various methods.

Table 1-8 Specification Criteria for microbe reduction following inoculation (Siegert 2013)

Criteria		**Species**	**Required Log Reduction**						
			2d	7d	14d	21d	28d	35d	42d
Ph. Eur	A	Bacteria	>2	>3	_	_	NI	_	_
	B		_	_	>3	_	NI	_	_

USP <51>			–	–	>2	–	NI	–	–
CTFA M-3			–	>2	NI	NI	NI	–	–
CTFA M-4			–	>3	CR	CR	CR	–	–
ASEAN			–	>3	NI	NI	NI	–	–
KoKo	A		–	>4	>4	>4	>4	>4	>4
	B		–	>3	>3	>3	>3	>3	>3
ISO 11930	A		–	>3	NI	–	NI	–	–
	B		–	–	>3	–	NI	–	–
Ph. Eur	A	Fungi	–	–	>2	–	NI	–	–
	B		–	–	>1	–	NI	–	–
USP <51>			–	–	NI	–	NI	–	–

CTFA M-3			_	>1	NI	NI	NI	_	_
CTFA M-4			_	>1	CR	CR	CR	_	_
ASEAN			_	NI	NI	NI	>1	_	_
KoKo	A		_	>3	>3	>3	>3	>3	>3
	B		_	>2	>2	>2	>2	>2	>2
ISO 11930	A	Yeast	_	>1	NI	_	NI	_	_
	B		_	_	>1	_	NI	_	_
ISO 11930	A	Mould	_	_	>0	_	>1	_	_
	B		_	_	>0	_	NI	_	_
Table Key		_	No Test						
		NI	No Increase						
		CR	Continued Reduction						

As can be seen from the number of validated test methods and specifications, Preservative Efficacy Testing as a proof of microbiological stability has been

extensively researched and corroborated. The selection of microbes, inoculation criteria and reduction criteria have all been extensively tested and risk assessment flow charts are available (ISO 11930) to apply the experimental results to the safety of the end user. Almost all of the Cosmetic Industry has adopted one of the protocols stated above, the most common being the European Pharmacopeia or the ISO 11930 protocols. Unlike the physiochemical testing, the protocols from the pharmaceutical or ISO standards have not been compromised and hence retain their validation. Therefore, the investigation of the Preservative Efficacy Test will not be a main aim of this research, which will instead concentrate on the knowledge gap of the physical stability testing.

1.4 Knowledge Gap and Scope of Research

Although there is significant data being collected daily from various cosmetic manufacturers on the stability of their cosmetic products, due to product confidentiality and a lack of co-operation between manufacturers, this data is only ever viewed in the context of that one manufacturer's results. There is no opportunity to view a wider range of results and challenge the Accelerated Stability Model's accuracy. There is also significant market pressure to maintain the status quo as it means minimal testing time and expense for companies trying to enter the cosmetics market. As a result this research aims to construct a robust test of the stability model currently in place and the assumptions it makes.

It will do this by asking four questions:

- Using empirical data from experimentation of multiple cosmetic products that undergo both accelerated and real-time testing, does the industry standard Accelerated Stability Model deliver a reasonably accurate prediction of real time stability?
- Does an evaluation of the Arrhenius equation's terms and solutions support or oppose its applicability to cosmetics products support or oppose the use of accelerated stability models in cosmetic products?
- Are there more appropriate or accurate tests that could be performed on these formulations?
- Is there any action the industry can take to make the testing protocols more accurate or relevant?

In order to answer these questions this study has a series of objectives;

- Create a body of formulations that can be measured under accelerated and real-time conditions for direct comparison.
- Create a parameter that will allow for a quantification of accuracy and precision of the accelerated stability model.
- With the above parameter as evidence, ascertain whether there are some experimental measurable that are modelled better than others by the accelerated stability models.
- Critically examine the Arrhenius equation terms and possible outcomes in the context of cosmetic formulations.
- Draw conclusions from the body of formulation results and assess the overall adherence of real-time data to the accelerated stability data.
- Compose a series of recommendations to industry based on the findings of this study.

By answering these research questions and achieving the objectives above, this study aims to contribute to cosmetic science knowledge by publishing a set of data that directly compares accelerated data to real time data and evaluate the results. In doing so it highlights the need for a standardised stability protocol for specific formulation types and treatment of results within the Cosmetic Regulations.

Chapter 2 Experimental Design

The aim of this research was to test the theory that Accelerated Stability Models were representative of the true behaviour of cosmetic products over time. To test this empirically, a comparison was made between changes a product exhibited while it experienced accelerated testing conditions and changes it exhibited in real-time testing. To do this, a series of cosmetic formulations were created and subjected to accelerated storage conditions and controlled ambient storage conditions. Their physical and chemical characteristics were tested at specific time points during accelerated stability. These results were then directly compared to results of the samples held at ambient temperature for the corresponding amount of time the Accelerated Stability Model suggests that the results were comparable. The accuracy of the model against real-time data was analysed across many formulations and conclusions drawn as to the accuracy of the accelerated stability model. The formulations' specifications, storage conditions, methods of analysis and testing time points are detailed in the following sections.

2.1 Formulations

There are many different formulation types used in the cosmetics industry, from emulsions to hydro-alcoholic solutions to detergent blends. Due to the length of the real time testing (96-weeks) and the research study time (three years), there was a 17 week time period where formulations had to be made and start testing. With one formulation made a day, and one day a week for a testing, this allowed 68 formulations to be made. The decisions was made that rather than do a study of limited sample size of 5-10 formulations on each product type, this study would focus on the most common cosmetic formulations – oil-in-water emulsions and have a larger sample to draw conclusions from. This larger sample size also allowed a more detailed look at formulation variations of emulsions, including emulsifier type, emulsifier inclusion level, oil phase ratio and work done during emulsification.

The emulsions were formulated to encompass as many of the variables of emulsion production as possible. In order to do this, a typical oil-in-water emulsion 'base' was

kept constant and four variables altered between each formulation. The oil in water cream base was given in Table 2-1:

Table 2-1 Oil-in-water emulsion base

Phase	Material	Concentration %ww	Function
Water	Water	QS to 100%	Solvent
Water	Glycerine	5.0	Humectant
Water	Phenoxyethanol	0.9	Preservative
Water	Ethylhexyl Glycerine	0.1	Preservative
Oil	Cetyl alcohol	10% of Oil Phase	Wax Thickener
Oil	Capric/caprylic Triglyceride	90% of Oil Phase	Emollient Oil
Oil	Emulsifier	**Variable**	Emulsification
Oil	Fragrance - PERFUME ALFONSO MANGO 411357 (Fragrance Oils Ltd)	0.5	Fragrance

Firstly, the emulsifiers used to make the emulsions were chosen to represent different ionic types and HLB values, as well as a polymeric emulsifier, commonly used in the cosmetic industry. The six different emulsifiers selected were two anionic emulsifiers, two non-ionic emulsifiers, one cationic emulsifier, and one polymeric emulsifier:

- Anionic 1 - Sodium Stearoyl Glutamate (Trade name: Eumulgin SG, BASF) - anionic emulsifier with an HLB value of 23.
- Anionic 2 - Glyceryl Stearate and potassium stearate, ingredient name glyceryl stearate SE (Trade name: Cutina GMS SE, BASF) – anionic emulsifier with an HLB value of 18.

- Non-Ionic 1 - Blend of Cetearyl Glucoside and Cetearyl Alcohol (Trade name: TegoCare CG90, Evonik Industries AG Personal Care) – non-ionic emulsifier with an HLB value of 11.
- Non-Ionic 2 - Blend of PEG-100 Stearate and Glyceryl Stearate (Trade name: Lexamul 561, Inolex) – non-ionic emulsifier with a HLB value of 19.
- Cationic 1 - Behentrimonium Methosulfate (Trade name: Incroquat Behenyl TMS-50, Croda Chemicals) – cationic emulsifier with an HLB value of 15.
- Polymeric 1 - Sodium Polyacrylate (Trade name: Cosmedia SP, BASF) – polymeric emulsifier.

Secondly, the amount of emulsifier added was varied depending on each emulsifier used. Each emulsifier had a recommended usage ranging from the manufacturer to create a viable emulsion product when used as the primary emulsifier. Each emulsifier was therefore used at two concentrations - the middle of the recommended range and the lowest recommended level:

1.	Sodium Stearoyl Glutamate	1% and 2.5%.
2.	Glyceryl Stearate SE	1% and 3%.
3.	Blend of Cetearyl Glucoside and Cetearyl Alcohol	2% and 4%.
4.	Blend of PEG-100 Stearate and Glyceryl Stearate	2% and 4%.
5.	Behentrimonium Methosulfate	2% and 4%.
6.	Sodium Polyacrylate	1% and 2%.

Thirdly, the size of the internal oil phase was varied to represent a cross section of possible sizes in a cosmetic product. The larger the internal phase, the higher the surface area created when emulsions form and the more emulsifier needed to stabilise the system (Myers 1999).

Table 2-2 Table of emulsions for each emulsifier

		Emulsifier	
phase ratio (W:O)		conc. 1	conc. 2
70	30	1	3
		2	4
60	40		5
			6

Increasing the internal phase to external phase ratio, while keeping the emulsifier concentration constant, should introduce instability to the system as detailed in Table 2-2. In addition, the two anionic emulsifiers had more phase ratios investigated to give more insight into whether there is a phase ratio beyond which an emulsion is unstable for a given emulsifier concentration as detailed in Table 2-3.

Table 2-3 Table of emulsion for the two anionic emulsifiers

		Anionic Emulsifier	
phase ratio (W:O)		conc. 1	conc. 2
80	20	1	11
		2	12
75	25	3	13
		4	14
70	30	5	15
		6	16
65	35	7	17
		8	18
60	40	9	19
		10	20

Finally, the energy input during emulsification was varied to see if the amount of energy put into the emulsification process affects stability. This was an investigation of the two thermodynamic equations given in the Literature Review, (**Error! Reference source not found.** and Equation 1-2 Work required to mix two liquids together (SCS 2009)). These equations demonstrate that the more energy placed into the emulsification stage, the greater the change in interfacial surface area and the smaller the internal phase droplets. Furthermore, as shown in Equation 1-3 Increase in London dispersion forces with molecular/particle size (Oversteegen and

Lekkerkerker 2003). Hence, the smaller the particle size, the slower the rate of coalescence and the more stable the system should be.

Particle size can be controlled at emulsification stage by adjusting the amount of shear energy put into the system. To create these emulsions the same Silverson Benchtop High Shear Mixer (L4 series) was used to create the shear energy needed to form the emulsions. It uses the 'rotor sator' type mixing to create a shearing effect.

Figure 2-1 Picture of Silverson L4 series homogeniser used for emulsion preparation

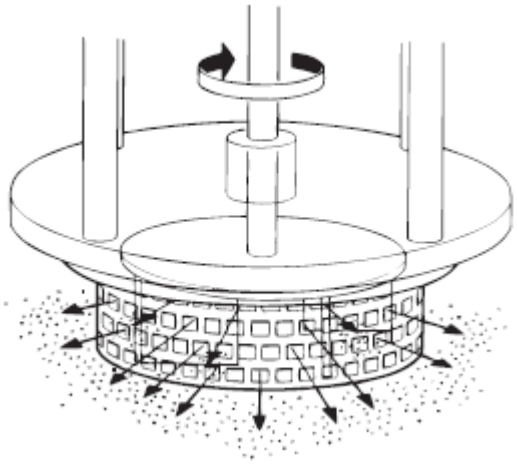


Figure 2-2 Illustration of rotor sator type high shear mixing

Shear rate is given by the equation:

$$Shear\ rate = tip\ speed\ \div distance\ between\ rotor\ and\ sator$$

Equation 2-1 Calculation of Shear Rate from tip speed

And tip speed is given by the equation:

$$Tip\ speed = rotation\ rate\ of\ rotor\ \times rotor\ circumference$$

Equation 2-2 Calculation for tip speed from rotor circumference and rotation speed

Therefore, as the same mixer was used for all preparations, the circumference of the mixer and distance between the rotor and sator screen were constant, the only variable available to adjust was the rotation speed. As described in Equation 2-1 Calculation of Shear Rate from tip speed and Equation 2-2 Calculation for tip speed from rotor circumference and rotation speed, rotation speed is directly proportional to shear rate. Hence, each formulation was made twice with the same high-speed homogeniser, applied for 30 seconds during the emulsification step, once set to 3000 rpm and once set to 6000 rpm which doubles the shear rate.

In total, 65 samples were made, and each was given a unique reference number as detailed in Table 2-4 below:

Table 2-4 Full table of test emulsions

Emulsifier type				Anionic				Cationic		Non-ionic				Polymeric	
Emulsifier				Sodium Stearoyl Glutamate		Glyceryl Stearate SE		Behentrimonium Methosulfate		PEG-100 Stearate and Glyceryl		Cetearyl Glucoside + Cetearyl alcohol		Sodium Polyacrylate	
Percentage				1	2.5	1	3	2	4	2	4	2	4	0.75	1.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Formulation numbers											
phase ratio (W:O)															
80	20	3000	30	1.01	2.01	19.01	20.01								
		6000	30	1.02	2.02	19.02	20.02								
75	25	3000	30	3.01	4.01	17.01	18.01	23.01	24.01						
		6000	30	3.02	4.02	17.02	18.02	23.02	24.02						
70	30	3000	30	5.01	6.01	15.01	16.01		26.01	45.01	46.01	55.01	56.01	75.01	76.01
		6000	30	5.02	6.02	15.02	16.02		26.02	45.02	46.02	55.02	56.02	75.02	76.02
65	35	3000	30	7.01	8.01	13.03	14.03								
		6000	30	7.02	8.02	13.04	14.04								
60	40	3000	30	9.01	10.01	11.03	12.01				50.01		60.01		80.01
		6000	30	9.02	10.02	11.04	12.04		30.02		50.02		60.02		80.02

A 1 kg batch of each formulation was made and used for both the accelerated and real-time tests. Once completed, each batch was split into five 100g glass jars, and one put in each respective storage condition for testing. This was to ensure the formulations tested were comparable at the start of the tests.

2.2 Storage Conditions and Duration of Testing

The choice of storage conditions was selected based on the recommendations to industry from the Cosmetics Europe (Colipa) guidelines from 2004:

“Tests are often performed at 37°C, 40°C or 45°C during 1, 2, 3… months but the temperature used and the duration will depend on the product type.” (Cosmetics Europe 2004)

which were later repeated in the ISO18811:2018 cosmetic stability guidelines:

“Cosmetic stability guidelines list various storage conditions and durations for accelerated stability testing:

— (30 ± 2) °C;

— (37 ± 2) °C;

— (40 ± 2) °C;

— (45 ± 2) °C;

— (50 ± 2) °C.

Durations range from one week to three months" (The British Standards Institution 2018).

Both of which take the suggested temperature points from the Pharmaceutical ICH guidelines of accelerated and intermediate testing conditions as highlighted in Table 1-3 Physical Stability Requirements of Pharmaceutical products according to ICH guidelines.

2.2.1 Accelerated Storage Conditions and Time Test Points

All of the products were placed in accelerated and real-time storage conditions, and tested at the time points indicated below. All samples were equilibrated to 25ºC for 24 hours before testing at each time point. The testing schedule for the elevated storage conditions were shown in Table 2-5 below -

Table 2-5 Table of conditions and testing time points of accelerated testing

	Initial	1 week	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
4ºC							
25ºC							
40ºC							
45ºC							

2.2.2 Real time Storage Conditions and Time Test Points

The testing schedule for the real-time, controlled ambient storage conditions were given in Table 2-6:

Table 2-6 Table of conditions and testing time points of real time testing

	Initial	24 weeks	32 weeks	48 weeks	64 weeks	72 weeks	96 weeks
25ºC							

As discussed in the literature review, these time points were selected because they represent the points at which the accelerated models should be equivalent to real-time testing results if Q_{10}=2. This is the value cited in the IFSCC Monologue and the (Cannell 1985) that may be appropriate for cosmetic products, and hence it is these time comparisons that the main discussions and conclusions are drawn. These time

points are shown in Table 1-4 Table to show the correspondence between accelerated data and the real-time data if Q=2.

However, some discussion will be given to the possibility of Q_{10} being equivalent to some other value. It is important to understand the importance of the value of Q if it is indeed not equal to 2. A value higher than 2 would mean that the changes seen on accelerated temperature points are not seen in real time results until later than predicted as illustrated in Table 1-5 Table to show the correspondence between accelerated data and the real-time data if Q=3. This creates the situation where a product may fall outside of pass criteria during accelerated testing but remains within specification during the real time testing – the so-called false fail result. Although this result, if seen regularly on an industry scale, represents a waste of developmental resource, it does not raise any safety concerns as the formulation would never be placed on the market.

However, Q may be smaller than 2, as shown in Table 1-7 Table to show the correspondence between accelerated data and the real-time data if Q=1.5. If Q has a value below 2 it could create a situation where the changes seen on accelerated temperature points are seen much sooner in real time results than predicted if Q=2 is assumed. The consequence would be that a product may stay within the pass criteria during accelerated testing but fall outside of specification during the real time storage – the so-called false pass result. This result, if seen regularly on an industry scale, could be very damaging for the cosmetics industry because the product would be behaving differently to prediction. In the best case this may just be a quality issue, in the worst case it could represent a significant safety risk to the general public and financial liability for the brand concerned.

2.3 Methods of Analysis

As this study was measuring the accuracy of the common accelerated stability testing, the methods of analysis are the same as those suggested by the stability testing guideline documents form Colipa in 2004 and ISO 18811:2018. In these guidelines, it is suggested to the designer of the tests to consider the type of formulation being tested before choosing the methods of analysis for both physical and chemical changes. In the case of all products including emulsions, organoleptic

changes (appearance, colour and odour) are most obvious to a product consumer. These are included in the analysis for this study, taking note of the ISO 18811:2018 recommendation:

“In addition, the product may be examined for changes in odour/taste and colour, as these are indicative of chemical changes. A grading system (either numerical or descriptive) may be devised to more objectively characterize the degree of these changes.” (The British Standards Institution 2018)

- Appearance – Formulations were observed through the glass container and on metal spatula. Any changes from initial description were noted. Any changes in texture or consistency were noted including separation, which was described (looking for creaming, sedimentation or coalescence). Changes in appearance were placed on an arbitrary scale of 1-5, one being a slight change and 5 a significant change. Detailed procedure can be seen in Chapter 4 – Colour, Odour and Appearance, Methodology section.
- Colour – Formulations were compared to a Pantone reference book and 4ºC standard sample to specify colour change. The procedure was performed in a calibrated light box to control ambient light. Colour change was placed on an arbitrary scale of 1-5, one being a slight change and 5 a significant change. Detailed procedure can be seen in Chapter 4 – Colour, Odour and Appearance, Methodology section.
- Odour – Formulations were checked for odour change, which could indicate rancidity of vegetable oils and/or fragrance change to the added fragrance. All formulations were compared to the 4ºC standard sample as its odour should not change significantly. Odour change were also placed on an arbitrary scale of 1-5, one being a slight change and 5 a significant change. Detailed procedure can be seen in Chapter 4 – Colour, Odour and Appearance, Methodology section.

The guidance documents also recommend performing specific tests for the type of products being tested and the possible destabilising mechanisms that could be seen with that type of product. Therefore, four further parameters were also measured which provided more information on what is happening to the emulsion structure before it is seen on the macro scale – pH, viscosity, droplet size (by digital microscope) and zeta potential.

- pH – Formulations were checked at each test point for pH using a pH probe. The probe was calibrated daily using standardised buffer solutions. A change in pH indicates a chemical change within the sample which can destabilize an emulsion (Hunt and Dalgleish 1994). Detailed procedure can be seen in Chapter 5 – pH, Methodology Section
- Viscosity (or resistance to flow) - this was measured using a calibrated, rotational flow viscometer (Brookfield). Viscosity will be taken as a 'single point' reading as this is the standard industry test protocol. Multi-point viscosity profiles can be made to describe the behaviour of substances under different shear stresses and rates, however, this is beyond the scope of this research, which is investigating the industry standard test and their relation to safety. A significant change in viscosity indicates a change in texture of a formulation and can be an early indication of a change in interaction between its two phases and is indirectly proportional to the rate of creaming and sedimentation as shown by Stoke's equation (Sherman 1983). Detailed procedure can be seen in Chapter 6 – Viscosity, Methodology Section.
- Digital Optical Microscopy – Formulations were observed under 500x and/or 1000x magnification to obtain droplet size and dispersion. A minimum of 100 droplets was measured per sample, and average droplet size, maximum/minimum droplet size and standard deviation were calculated from the images obtained. Changes in particle size can be an early indication of coalescence, flocculation or disproportionation (Wiese and Healy 1970) Detailed procedure can be found in Chapter 7 – Additional Tests Performed, Microscopy – Digital Optical Microscope, Methodology Section.
- Zeta Potential – All formulations were subjected to analysis by Laser Diffraction (Malvern Zeta Sizer Nano ZS90) and micro-rheology measurements to obtain the Zeta potential of the emulsions formed. This measurement was taken once as the Zeta potential of a given system is related to the emulsifier, the emulsifier packing at the droplet surface and the amount of ions present in the external phase - it does not change with particle size. Zeta potential is a direct measurement of inter-particle repulsion and therefore a measure of an emulsions tendency to coalesce or flocculate (Sennett and Olivier 1965a). Detailed procedure can be found in Chapter 7 – Additional Tests Performed, Zeta Potential, Methodology Section.

The full test schedule for each formulation created is given in Table 2-7:

Table 2-7 Full test schedule for a test formulation

Tests	0 days	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16	Week 24	Week 32	Week 48	Week 64	Week 72	Week 96
Appearance 40°C													
Appearance 45°C													
Appearance Dark 25°C													
Appearance Fridge													
Colour 40°C													
Colour Dark 25°C													
Colour 45°C													
Odour 40°C													
Odour 45°C													
Odour Dark 25°C													
Odour Fridge													
pH 40°C													
pH 45°C													
pH Dark 25°C													
pH Fridge													
visc 40°C													
visc Dark 25°C													
visc Fridge													
visc 45°C													
microscopy 40°C													
microscopy Dark 25°C													
microscopy Fridge													
microscopy 45°C													

2.4 Layout of Methodology, Results and Discussion

As there are multiple parameters being measured across multiple formulations, assessing all the results at once in a traditional Methodology, Results, Discussion and Evaluation section would be confusing and unnecessarily complex.

Therefore the following Methodology, Results, Discussion and Evaluation sections are separated out into Chapters of the parameters being measured: Organoleptic Measurements (Colour, Odour and Appearance); Viscosity; pH and Additional Tests. This enables the findings and discussion points on each parameter to be laid out and followed more easily for conclusions to be drawn. A Chapter will also be included to look at the effect the built-in variation of the emulsion formulations had on the stability results and accuracy of the Accelerated Stability Model.

A final Chapter is included after those detailed above to bring the individual measurement parameters and formulation variations together to assess the overall accuracy of the Accelerated Stability Model and answer the research questions detailed in the Introduction Section.

Chapter 3 Mathematical Observations

3.1 Mathematical Observations of the Arrhenius Equation

The Arrhenius Equation (Equation 1-10 Arrhenius Equation (Arrhenius 1889)) given in the literature review section, describes the factors affecting the rate of reaction (K). K can be found experimentally by monitoring a reaction over time and plotting the change in reaction variable over time, to yield a rate of reaction line of best fit. If this line is linear, it shows that the rate of reaction remains constant over the time measured for that temperature. The same experiment performed over a range of temperatures will yield a different K value for each temperature used. This range of K values for a reaction can be applied to the rearranged form of the Arrhenius equation:

$$\ln K = \ln A - \frac{Ea}{RT}$$

Equation 3-1 Rearranged Arrhenius equation

This means the experimentally found K values for each temperature can be plotted on a graph of ln K vs 1/T, which, if the model fits, yields a straight line of slope $-E_a/R$. As R is constant, this allows calculation of E_a from experimental data. The y-intercept will give ln(A) which allows calculation of the frequency factor.

The Q-rule was designed to give an approximation of the effect of changing temperature on rate of reaction (Bajaj et al. 2012). It is used in the pharmaceutical industry as a guide to the change of degradation rates of a drug stored at various temperatures, such that when the storage temperature decreases by 10ºC, the degradation rate decreases by a constant factor (Q_{10}). An assumed value of two for Q_{10} is considered conservative, whereas a value of four for Q_{10} is considered speculative (Bajaj et al. 2012). A common practice is to assign Q_{10} the value of two (doubles the rate), three (triples the rate) or four (quadruples the rate) and to work back through the Arrhenius equation to theoretically calculate activation energy which can then be checked against experimental data and an Arrhenius plot:

$$\log\left(\frac{k_2}{k_1}\right) = \frac{-E_a}{2.303R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

Equation 3-2 Change in rate of reaction over two temperatures, derived from the Arrhenius equation

As long as $T_2 - T_1 = 10^oC$, then:

$$Q_{10} = \frac{k_2}{k_1}$$

Equation 3-3 The Q-rule

The Q number with most accurate theoretical activation energy to the true value is then applied to accelerated data and theoretical shelf-life prescribed.

There is no requirement for any cosmetic product to calculate activation energy of a particular reaction, or to obtain real-time data at ambient conditions. Without calculation of activation energy, there is no way of assigning a theoretical value of Q. Instead, Q is always assumed to be two, as this is the most conservative value and therefore the real-time reaction is at least underestimated.

3.1.1 Cosmetic Application of Q-rule

As demonstrated by the real example given in Anderson and Scott (1991) which studied the application of accelerated stability models in the pharmaceutical industry detailed in the Literature Review, the Q- rule can be inaccurate and needs verification by real-time data. In the example given in their paper, the application of the Q-rule to specific drug degradation gave a theoretical shelf life of around 17 years, whereas real-time testing showed the true shelf life was 10 years. Without real-time experimental data to check theoretical data against, adoption of the Q-rule is not advised since it can lead to the extrapolation of poor conclusions.

There is no requirement within the Cosmetic Regulation to check the application of the Q-rule against real-time data to justify and validate the approximation of Q. In fact there is no requirement to detail that the Q rule is being applied at all, even though all of the accelerated stability evaluations are based around it.

Moreover, when assessing accelerated data, there is no requirement to create Arrhenius plots for K against 1/T using the cosmetic accelerated data to check if the resultant plot is a straight line. A straight line plot would at least support that the rate of reaction changes linearly over changes in temperature, and therefore, whether the Accelerated Stability Testing is applicable. Without this exercise being performed for each formulation reaction, there is no justification for applying the Q-

rule to any formulation to extrapolate results of stability and safety of cosmetic products.

3.1.2 Activation Energy

With no requirement to analyse accelerated data to see if the Arrhenius plot is linear, there is also no analysis done on Activation Energy of the given reaction. Reactions with a higher E_a have a steeper slope ($-E_a/R$) within their Arrhenius plots, showing that their rate is more susceptible to change with changes in temperature. Thus their rate of reaction will increase more with an increase of temperature than a reaction of lower activation energy as demonstrated by Figure 3-1.

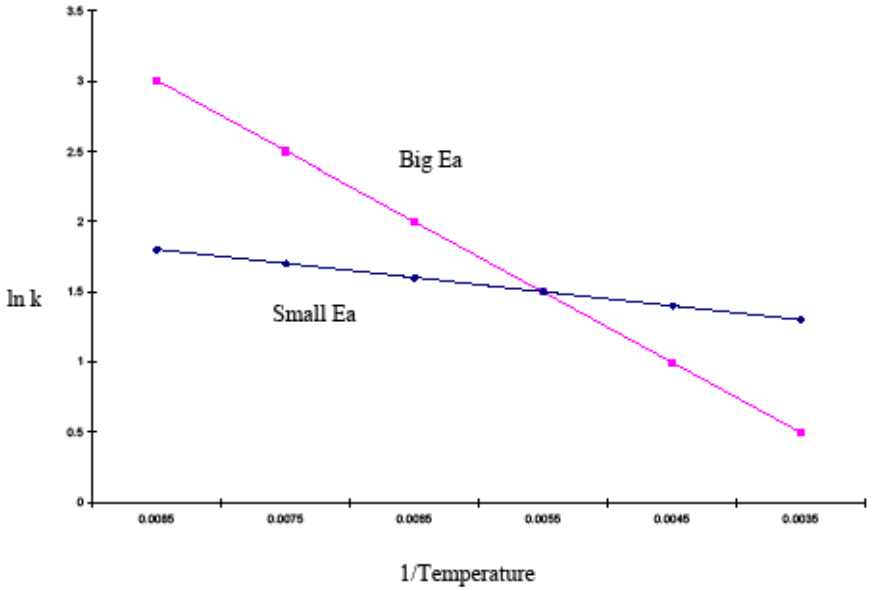


Figure 3-1 Affect E_a has on Arrhenius plot

This statement which can be extrapolated from the integrated Arrhenius equation (Equation 3-1 Rearranged Arrhenius equation) and is shown graphically above, contradicts the assumption made in cosmetics that increasing temperature increases rate of reaction consistently for all systems i.e. Q always = 2. There is no requirement for E_a to be calculated from experimental data for a given reaction, therefore there is no validation that the Q=2 assumption is accurate. Hence, the cosmetic Accelerated Stability Evaluations may be making unjustifiable assumptions about the susceptibility of reactions to temperature, and using those assumptions to justify the stability and safety of cosmetic products on the market.

3.1.3 The Units of the Arrhenius Equation solutions

Activation energy in the Arrhenius equation has the unit of KJ mol^{-1}. As emulsion and colloidal systems are not molecular systems it is difficult to apply the measurement of a mol. Particles and droplets have different molecular weights depending on their size, so it is impossible to determine how much or how many droplets constitute a 'mol'.

This questions the applicability of an equation that was originally deduced from eight sets of data from reactions where molecules are reacting or decomposing to form new products (Logan 1982). It is worth noting that, as Cannell highlight himself (Cannell 1985), while there are molecular changes occurring within a cosmetic formulation which should be monitored, the physical stability of a colloid is not a chemical reaction but rearrangement of molecules within a system. Hence, the Arrhenius equation should not be applied to the macro changes in emulsion/colloidal structure such as coalescence, creaming, sedimentation, Oswald Ripening or viscosity change. This leads to the assertion that the Accelerated Stability Models may be inappropriate for modelling macro scale processes such as appearance changes and phase separation.

3.1.4 The use of Absolute Temperature (K)

The linearity of an Arrhenius plot has long been thought of as proof that the Arrhenius equation is valid, as it shows that the rate of change in rate of reaction stays constant over a range of temperatures.

However, describing temperature in Kelvin means that the temperature scale is compressed and therefore a change in temperature is diluted. As Pointed out by (Peleg et al. 2012), if T/K was replaced by T/ºC +b (an arbitrary constant number) in Equation 3-1 Rearranged Arrhenius equation, then as b becomes larger changes in T/ºC become less significant. If b is significantly larger than T/ºC this phrase becomes near constant and then plotting Ln K vs 1/(T+b) would become near linear. At the temperature accelerated stability tests 20-45ºC, T/ºC is indeed much less significant than b (+273.16ºC) which is used to calculate T/K. Essentially this always makes the plot of ln K vs 1/T near linear for the range of accelerated stability temperatures. However, the linearity of the Arrhenius plot is just an output of the

properties of the equation itself, and only in the more extremes of temperature affecting rate of reaction would it not yield a straight line.

Similarly as R is a constant, 8.31 J/K/mol, the phrase RT (in Kelvin) in the Arrhenius equation has a value of 2434 J/mol for 20ºC to 2642 J/mol for 45ºC. This means that every E_a is reduced by a factor of around 2500x before it is treated to the exponential factor e. This decrease is, of course, larger for large activation energies and smaller for small activation energies. This has the effect of decreasing the importance of the activation energy magnitude in the Arrhenius equation and forces all values of K closer together, compressing the scale. Hence, unless the activation energy has a very large change with temperature (large enough to still be significant after a 2500x reduction) the Arrhenius plots will always yield a near-linear plot that can be fitted with a straight line extrapolation.

This combination of the properties of the Arrhenius equation and the temperature ranges of the Accelerated Stability Model creates a false impression that the Arrhenius plot is linear for all cosmetic systems. In order to prove that the Arrhenius plot is genuinely linear, a wider range of temperatures would need to be studied for a given reaction.

This further calls into question the Arrhenius equation's ability to extrapolate the long-term stability of cosmetic products over time, given the current common stability protocols.

3.1.5 Temperature Range's Effect on Ea

As the Accelerated Stability Models uses temperatures of 40ºC and 45ºC to model ambient temperature, only T values of 293-318 K (20^0 - 45^0 C) can be applied to the Arrhenius model. This effectively makes the $\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$ term constant in *Equation 3-2 Change in rate of reaction over two temperatures, derived from the Arrhenius equation*. If Q_{10} has the value of two, as the Accelerated Stability Models requires, the only activation energy that allows the equation to balance is 50kJ mol^{-1} (if A is assumed to be constant). At 50kJ mol^{-1} rate of reaction at 293K is 1.12 x 10^{-9} and at 303K it is 2.38 x 10^{-9}, a rough doubling of rate of reaction for the 10ºC rise. However, if activation energy is in fact 25 KJ mol^{-1}: then K ranges from 3.47 x 10^{-5}

for 293K to 4.8 x 10^{-5} for 303K – an increase by a factor of 1.383 as opposed to 2.125.

This means that in order for the Accelerated Stability Model to be accurate, any reaction that occurs in a cosmetic product has to have an activation energy of close to 50KJ/mol. There is, of course, no evidence that this is the case for the reactions that occur within cosmetic formulations, and the premise should be verified by analysis of the experimental data. This again casts doubt on whether the current stability extrapolations from the Accelerated Stability Model are fit for the purpose of deducing long-term stability and safety.

3.2 Mathematical Observations of Stokes Law

3.2.1 External Phase Viscosity

As discussed in the Background Section on colloidal chemistry, rates of sedimentation and creaming is described by Stokes Law Equation 1-5 Stokes Law (Tadros 2013). It was highlighted that the viscosity of the continuous phase is indirectly proportional to the rate of sedimentation and creaming.

There are many models, including the Arrhenius equation, that describe the relationship between viscosity of liquids and temperature. There is no universal model and the relationship depends on the exact system that is being studied but most models show an exponential relationship between temperature and viscosity. This holds true for the viscosity of water, which makes up the majority of oil-in-water emulsions' continuous phases. The equation 3-5 below is accurate to within 2.5% from 0 °C to 370 °C (Kestin et al. 1978):

$$\mu(T) = 2.414 \times 10^{-5} \times 10^{247.8/(T-140)}$$

Equation 3-4 Exponential behaviour of viscosity of water with temperature (Kestin et al. 1978)

where T has units of Kelvin, and μ has units of kg/ms.

Oil-in-water emulsions will have different rheological properties depending on their individual compositions however, it is generally true that the viscosity of creams decreases with increasing temperature (Sherman 1983). The nature of this

relationship is different for each system and can be exponential, as shown by Bakshi and Smith (1984) in their study on the viscosity of milk products.

If the rate of change in viscosity is exponential with regard to temperature, then so is the rate of sedimentation and creaming over that change in temperature as the two are inversely proportional to each other, as described by Stokes Law.

This shows that the assumptions in the Accelerated Stability Models that changes in rate of reaction over temperature occur in a linear manner are inaccurate in regard to the processes of sedimentation and creaming, and should not be used to assign stability for these processes.

3.2.2 Internal Phase Viscosity

Cosmetic products like emulsions are commonly designed to be spreadable on the skin at skin temperature. Hence, cosmetic formulators often attempt to make oil phases with melting points around skin temperature, so that the product slips across the skin giving a pleasant skin feel. It is known that when a substance is approaching and reaches its melting temperature, its viscosity decreases non-linearly with respect to temperature until the transition to liquid has been achieved (Elert).

If accelerated stability data is obtained at 40ºC and 45ºC, well above common skin temperature, it may be that the internal oil phase is in a different physical state to that of the same oil phase at 25ºC. Hence, reactions and interactions that take place at 40ºC and 45ºC may be a poor reflection of what occurs at ambient temperature.

3.2.3 Density Changes with Temperature

The relationship between density and absolute temperature is described by the observations of thermal expansion. As the temperature of a substance rises the kinetic energy within that material rises causing increased molecular energy which move faster. In a gas this creates either an increase in volume or in pressure if there is no room to expand inside the containing vessel. The same effects are seen in a liquid, as heat increases, volume also increases which decreases density (Kell 1975) and (Hepler 1969). Thermal expansion occurs at different rates for different liquids depending on each liquids heat capacity (Barron and White 2012).

It is demonstrated in the Stokes equation (Equation 1-5 Stokes Law) that the differential in the densities of the internal and external phases is directly proportional to the rate of sedimentation and creaming. Therefore, increasing the temperature of storage of an emulsion will change the density differential phrase of the equation and make the system behave differently that the system at ambient conditions. This shows that the assumptions of the accelerated stability testing; that changes in rate of reaction over temperature occur in a linear manner, are inaccurate with regard to the processes of sedimentation and creaming, and should not be used to assign stability for these processes.

3.3 Conclusions

Due to the nature of cosmetic product design, the current Accelerated Stability Model assumptions and procedures and the temperatures at which the products were tested, there appeared to be no stage of the implementation of the Arrhenius model to Cosmetic Accelerated data that was justifiable or appropriate.

The only possible exception to this assertion was the chemical reactions taking place on the molecular scale, and even these would have needed validation from real-time data to show valid extrapolations - all reactions that took place on a macro scale were not applicable to the Arrhenius model.

Thus it has been shown that the current method for declaring long-term stability of cosmetic products was inappropriate with regard to the mathematical implementation of the Arrhenius model to Accelerated Stability data.

Chapter 4 Organoleptic Parameters - Appearance, Colour, Odour

The organoleptic parameters are the sensorial characteristics of a formulation. As such, they are most likely to be perceived by the consumer of the cosmetic product when on market. Therefore, they are paramount when considering the perceived quality of the product in use. For prospective products, any changes in the organoleptic parameters need to be accurately predicted so costly complaints or recalls for poor quality are avoided. This chapter will outline the method used to test the organoleptic parameters as well as detail the results and conclusions of the predictive capacity of the Accelerated Stability Model.

4.1 Organoleptic Methods

4.1.1 Sample Preparation of Colour and Appearance Measurement

- The sample temperature was checked to be 25ºC +/- 1ºC
- The sample was identified as either a liquid or a solid.
- The sample was inspected for extraneous substances (e.g. undispersed materials, contaminants)
- The sample was placed in the same type of container as the standard sample and filled to the same depth of product
- The standard, unless otherwise stated was the 4ºC sample.

4.1.2 Sample Testing Colour and Appearance Measurement

- The sample was compared to the standard for ‘Appearance’ under the four parameters of: uniformity, texture, opacity and skin feel. Any changes were given a value on a scale of 1-5, 1 being a slight difference to standard and 5 being a severe difference to standard.
- The sample was compared to the standard for colour (unless otherwise indicated on the specification), clarity and general appearance within the

specified parameters. The pantone standard colour reference book can also be noted for comparison.

- For colour comparison the sample was placed in the light cabinet and the light set at ‘D65’ (artificial daylight bulb) & ‘F’ (artificial store light bulb).
- Results were recorded on the sample testing form and added to the ‘Coptis’ software testing database which was used to record all results.

4.1.3 Sample Preparation of Odour Measurement

- The sample temperature must be checked to be 25ºC +/- 1ºC.
- The sample was placed in the same type of container as the standard sample and filled to the same depth of product
- The standard, unless otherwise stated, was the 4ºC sample.

4.1.4 Sample Testing of Odour Measurement

- Ensure hands were odour free.
- Any warnings involving inhalation of the sample were noted. (Looked up on the Material Safety Data Sheet to check all hazards).
- Samples were smelled in an odour-free area and note any difference in the odour.
- Samples were smelled both immediately after lid removal (head space) and after the lid had been removed from the sample for 1 minute (bulk odour).
- If there was no difference identified, then a ‘0 - as initial’ result is given. If there was a difference noted, the difference was placed on an arbitrary scale of 1-5, 1 being a slight change and 5 being a severe change.
- Results were recorded on the sample testing form and added to the ‘Coptis’ testing database.

4.1.5 Measurement of Parameters

To reflect the common practice of industry, the assessment of these fairly broad parameters of colour, odour and appearance was done by judging the severity of any change seen rather than a measurement of the parameter itself as recommended by ISO 18811:2018 (The British Standards Institution 2018). For

example, rather than measure the texture of each sample, the difference in texture of the test sample to the standard is ranked on a scale of 0 – 5:

0 – No Change, all attributes are the same as the initial standard sample.

1 – Minimal Change, only noticeable by direct comparison to standard.

2 – Slight Change, may be noticed by someone familiar with the formulation.

3 – Noticeable Change, can be identified without the need for direct comparison with standard, likely to be picked up by consumer. Not necessarily detrimental to performance.

4 – Significant Change, obvious change to the formulation which may be detrimental to product performance, perceived quality or safety.

5 – Severe Change, a critical change to the product's attribute which is detrimental to the product's performance, perceived quality or safety.

This type of assessment is used quite widely in industry stability tests of subjective parameters, though different scales can be used, for example the 'Boots GR10:2008 – guidelines for cosmetic product stability testing' document uses a scale of 0-4, but the same principle applies.

4.1.6 Pass/Fail Criteria

The pass/fail criteria for a cosmetic product's appearance, colour or odour was dependent on the ability of the consumer to be able to notice a difference, either during use of a pack of product or when buying a new pack, and whether that change is detrimental to the product. Hence, in industry, any change observed as ranked 3 or higher is classed as a fail and can only be conceded with justification from a qualified person. Therefore, for the purposes of this study any change observed as ranked 3 or higher is classed as a fail.

To assess the Accelerated Stability Models accuracy within this arbitrary 0-5 scale, two new parameters have been developed, designated the Average Predictive Error and Prediction Error Range. These were calculated by comparison of the values given by the Accelerated Stability Model and the real-time values that they predicted. For example, if the Accelerated Stability Model is accurate the results obtained at 1 week at 40ºC should be the same as the results obtained at 20ºC after

4 weeks; the results at 2 weeks at 40ºC should be the same as the results obtained at 20ºC after 8 weeks, and so on. This comparison of equivalent results has been illustrated in Table 4-1:

Table 4-1 Results table and accuracy parameters

weeks	0	1	2	4	8	12	16	24	32	48	96
Average Appearance 40°C		A	B	D	F	G					
Average Appearance 45°C			C	E			H				
Average Appearance 25°C				A1	B1	C1	D1	E1	F1	G1	H1
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				A-A1	B-B1	C-C1	D-D1	E-E1	F-F1	G-G1	H-H1
Average ASM Prediction Error										Average Prediction Error	

The difference in the equivalent results was then calculated to give the prediction error at each time point. The difference from the largest and smallest prediction error value is the prediction error range, and gives an indication of the Accelerated Stability Model's precision. The average of the prediction errors can be calculated to give the average prediction error, and gives an indication of the Accelerated Stability Model's accuracy. This gives two indicators of how well the Accelerated Stability Model predicts the long term stability of a product, with a lower value showing a better predictive capacity of parameter changes. These values can be given for individual formulations' or a group of formulations' averaged results, to give a broader quantification of the Accelerated Stability Method's accuracy and precision.

A plot of Accelerated Stability Model's Prediction Error against the time in weeks that it is predicting will also render a graph which shows when the Accelerated Stability Model becomes inaccurate. This will show at what time point the predictive data become inaccurate when compared to real-time data, designated the Accurate Prediction Threshold. For justification of use to assure cosmetic products long term stability, the cosmetics industry requires the Accurate Prediction Threshold to be equal to, or greater than, 96 weeks.

For the purposes of this study, given the range of the scale, an Average Prediction Error of less than one will be considered an accurate prediction and a Prediction Error Range of less than 1.25 considered to be a precise prediction of stability.

4.2 Organoleptic Results

In this section, the analysis of the changes in Appearance, Colour and Odour will be taken in turn. To view all the results in full, refer to Appendix 1 – Organoleptic Results.

4.2.1 Appearance

Of the 65 emulsions made, there were eight that were so unstable that they did not create an emulsion at all. These formulations were so fundamentally unstable that once the high shear mixing was removed from the system the oil and water phases immediately divided into their discrete phases. This meant that they were not stable long enough to be put in any storage conditions to begin testing. They were all in the Anionic emulsifier 2 section (Glyceryl Stearate & Potassium Stearate blend) and were related in that they were all the lowest recommended use of this emulsifier. These formulations are highlighted in red in Table 4-2 below:

Table 4-2 Table highlighting formulations too unstable to start testing

Emulsfier type				Anionic				Cationic		Non-Ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
percentage				1	2.5	1	3	2	4	2	4	2	4	1	2
Secondary variables		Mechanical Work	Time (secs)												
80	20	3000	30	1.01	2.01	19.01	20.01								
		6000	30	1.02	2.02	19.02	20.02								
75	25	3000	30	3.01	4.01	17.01	18.01	23.01	24.01						
		6000	30	3.02	4.02	17.02	18.02	23.02	24.02						
70	30	3000	30	5.01	6.01	15.01	16.01		26.01	45.01	46.01	55.01	56.01	75.01	76.01
		6000	30	5.02	6.02	15.02	16.02		26.02	45.02	46.02	55.02	56.02	75.02	76.02
65	35	3000	30	7.01	8.01	13.03	14.03								
		6000	30	7.02	8.02	13.04	14.04								
60	40	3000	30	9.01	10.01	11.01	12.03				50.01		60.01		80.01
		6000	30	9.02	10.02	11.04	12.04		30.02		50.02		60.02		80.02

These formulations do not appear in the results section as no data could be gathered on them. They will be discounted from all analysis.

In addition to the above there were a further eight formulations that were stable enough to be put on test but all storage conditions had separated by the time the week 1 measurements were due to be taken. Essentially the appearance results for these formulations were all 5 (severe change) after 1 week. For example formulation 15.01 results are given in Table 4-3:

Table 4-3 Typical results of a formulation too unstable to reach first test point

Formulation No.	15.01						
Week	0	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16
Appearance 40°C		SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance 45°C		SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance 20°C	White cream	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance Fridge		SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)

These formulations are highlighted in Table 4-4 in yellow:

Table 4-4 Table to highlight formulations that were too unstable to reach first test point

Emulsfier type				Anionic				Cationic		Non-Ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
percentage				1	2.5	1	3	2	4	2	4	2	4	1	2
Secondary variables		Mechanical Work	Time (secs)												
80	20	3000	30	1.01	2.01	19.01	20.01								
		6000	30	1.02	2.02	19.02	20.02								
75	25	3000	30	3.01	4.01	17.01	18.01	23.01	24.01						
		6000	30	3.02	4.02	17.02	18.02	23.02	24.02						
70	30	3000	30	5.01	6.01	15.01	16.01		26.01	45.01	46.01	55.01	56.01	75.01	76.01
		6000	30	5.02	6.02	15.02	16.02		26.02	45.02	46.02	55.02	56.02	75.02	76.02
65	35	3000	30	7.01	8.01	13.01	14.03								
		6000	30	7.02	8.02	13.02	14.04								
60	40	3000	30	9.01	10.01	11.01	12.01				50.01		60.01		80.01
		6000	30	9.02	10.02	11.04	12.04		30.02		50.02		60.02		80.02

As these formulations have no accelerated stability or indeed long-term stability data to compare they will be removed from all future analysis although they do appear in the Results section.

Of the 49 formulations remaining after the removal of the formulations that were too unstable to test, there was only one observable change in appearance: emulsion splitting. This was observed in 8 of the formulations. The other 41 formulations were stable with regard to appearance on both long-term and accelerated storage conditions and achieved a test ‘pass’ for all storage conditions. This indicated that the Accelerated Stability Model would have correctly assigned a stability test ‘pass’ to these formulations.

The eight formulations that showed a change in appearance were 2.01, 2.02, 12.01, 12.04, 16.01, 20.01, 50.01 and 46.01. Six of these formulations only saw a change in appearance in the raised temperature storage conditions, with no change being seen in the corresponding long-term ambient storage conditions. This meant that, in industry, these formulations would have been failed according to the Accelerated Stability Model but, as the long term ambient conditions showed no change in appearance, the Accelerated Stability Model’s predictions were inaccurate. An example data set is given in Table 4-5, which shows formulation 2.01 appearance results:

Table 4-5 Formulation 2.01 Appearance results

Formulation No.	2.01										
Tests	0 days	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16	Week 24	Week 32	Week 48	Week 96
Appearance 40°C		AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	SPLITTING (4)	SPLITTING (4)				
Appearance 45°C		AS INITIAL	SPLITTING (1)	SPLITTING (1)	SPLITTING (2)	SPLITTING (4)	SPLITTING (4)				
Appearance 20°C	Thin, off white lotion	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL
Appearance Fridge		AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL				

The other two formulations 12.04, results shown in table 4-6, and 16.01 saw both the elevated temperature and the long-term ambient storage condition samples change in appearance to such a degree that they are both classed as failed tests. This indicates that in industry, the Accelerated Stability Model would have correctly failed these formulations for stability.

Table 4-6 Formulation 12.04 Appearance results

Formulation No.	12.04										
Tests	0 days	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16	Week 24	Week 32	Week 48	Week 96
Appearance 40°C		SPLITTING (2)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)				
Appearance 45°C		SPLITTING (5)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)				
Appearance 20°C	White cream	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	SPLITTING (3)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)	SPLITTING (5)
Appearance Fridge		AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL	AS INITIAL				

These results will be looked at in more detail in the Discussion section, to assess the accuracy of the Accelerated Stability Model when applied to this accelerated and real-time data.

4.2.2 Colour

Of the 49 formulations that survived past the first week's testing, 18 showed a colour change in at least one of the elevated temperature storage conditions. All of these observations were for yellowing of the formulation and were all significant enough to have resulted in a failed test under the Accelerated Stability Model. These tests were 1.01, 1.02, 2.01, 2.02 3.01, 3.02, 4.01, 4.02, 5.01, 5.02, 6.01, 6.02, 7.01, 7.02, 8.01, 10.02, 23.01 and 23.02.

However, of these formulations, only four showed a colour change in the long-term ambient storage condition: 10.02, 8.01, 6.02, 2.01. These four had a significant enough colour change to have been classed as a failed test for a detrimental change to the product i.e. a change classed as 3 or higher. For example formulation 10.02 results are given in table 4-7.

Table 4-7 Example of colour change detected in both elevated storage conditions and ambient storage

Formulation No	10.02										
weeks	0	1	2	4	8	12	16	24	32	48	96
Colour 40°C	0	2	2	3	3	4	4				
Colour 20°C	0	0	0	0	0	0	0	2	2	3	3
Colour 45°C	0	3	3	3	3	4	4				

This indicates that the other 14 formulations that would have been failed for instability for colour change under the Accelerated Stability Model did not in fact, see

the same changes taking place over the corresponding long-term storage condition. For example table 4-8 shows formulation 5.01 results.

Table 4-8 Example of Colour change detected in elevated temperatures not seen at ambient storage

Formulation No	5.01										
weeks	0	1	2	4	8	12	16	24	32	48	96
Colour 40°C	0	2	3	3	3	4	4				
Colour 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	3	3	3	3	4	4				

It is also worth noting that the majority of the formulations that changed colour are part of the same Anionic emulsifier set, suggesting that either the emulsifier itself is yellowing with heat or age, or that there is a reaction between the emulsifier and another constituent of the formulations, perhaps the fragrance or preservative, that may only occur at higher temperatures.

These results will be looked at in more detail in the Discussion section to assess the accuracy of the Accelerated Stability Model when applied to this accelerated and real-time data.

4.2.3 Odour

Of the 49 formulations that were subjected to long term and accelerated stability testing, only two formulations showed a change in odour: 7.02 and 4.02. Both of these were for a loss of odour and in both cases the loss of odour was only seen in the elevated temperature storage conditions, where the changes were significant enough to be classed as a noticeable change (3) and would therefore have resulted in a ‘fail’ result. Neither formulation showed an odour loss in the long-term ambient temperature storage condition, showing that the loss of odour in the accelerated conditions was a false fail or that Q_{10}<2.

Table 4-9 Formulation 7.02 and 4.02 odour results

Formulation No	7.02										
weeks	0	1	2	4	8	12	16	24	32	48	96
Odour 40°C	0	0	0	0	0	2	3				
Odour 45°C	0	0	0	0	0	3	3				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Formulation No	4.02										
weeks	0	1	2	4	8	12	16	24	32	48	96
Odour 40°C	0	2	2	2	2	3	3				
Odour 45°C	0	2	2	2	3	3	3				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0

It is worth noting that both of these formulations are found in the same anionic emulsifier set that saw the majority of the colour changes. This gives some credibility to the notion that there is a reaction occurring between this particular anionic emulsifier and the fragrance.

It should also be of note that formulation 16.01, was recorded as having a loss of odour in elevated temperatures after weeks one and two. However, by week four the emulsion had split at all storage conditions and therefore the odour was not tested in any storage condition after week two as the test was already classed as a fail.

Table 4-10 Formulation 16.01 Appearance and Odour results

Formulation No.	16.01						
Tests	0 days	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16
Appearance 40°C		50% SPLIT	50% SPLIT	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance 45°C		50% SPLIT	50% SPLIT	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance 20°C	Thick, white cream	AS INITIAL	AS INITIAL	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance Fridge		AS INITIAL	AS INITIAL	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Odour 40°C		LOSS OF TOP NOTE (2)	LOSS OF TOP NOTE (2)	NT	NT	NT	NT
Odour 45°C		LOSS OF TOP NOTE (2)	LOSS OF TOP NOTE (2)	NT	NT	NT	NT
Odour 20°C	Mango	AS INITIAL	AS INITIAL	NT	NT	NT	NT
Odour Fridge		AS INITIAL	AS INITIAL	NT	NT	NT	NT
			NT =	Not tested			

These results will be looked at in more detail in the Discussion section to assess the accuracy of the Accelerated Stability Model when applied to this accelerated and real-time data.

4.3 Organoleptic Discussion

4.3.1 Appearance

This section will firstly address the results which offered little or no useful data before focussing in more detail on those findings of more significance.

Of the 65 formulations made, 24 formulations were unstable with regard to appearance due to structural breakdown. Unfortunately 16 of these formulations were so unstable that no meaningful data was able to be collected because they broke down too quickly. However, from the remaining 49 formulations that completed the testing process some interesting discussion points and conclusions were drawn.

Firstly, in the 41 cases where the elevated and long-term stability had no change in appearance, the results would at first appear to strengthen the case for the Accelerated Stability Model as it correctly predicted the stability of the formulations

in the long term. However, the results actually showed only the stability of these formulations was sufficient to last for the duration of the test period of this research. These formulations, which were inherently thermodynamically unstable, were kinetically stabilised well enough to mean that the tests performed were not long enough either in accelerated or real-time conditions to allow a change in appearance to be observed. This was not a reflection that the Accelerated Stability Model was accurate for these cases, rather that the tests were not performed over a long enough period to see any change in appearance for these formulations. Hence no conclusions could be drawn from these 41 results on the accuracy of the Accelerated Stability Model.

Perhaps the most interesting data arose from the eight formulations that had a measurable amount of instability. As mentioned in the Results section, six of these eight formulations showed instability only at elevated temperature storage conditions, with no change to appearance over the whole of the long-term ambient storage time. Figure 5-1 for example shows formulation 2.01 appearance results discrepancy between long-term and accelerated data.

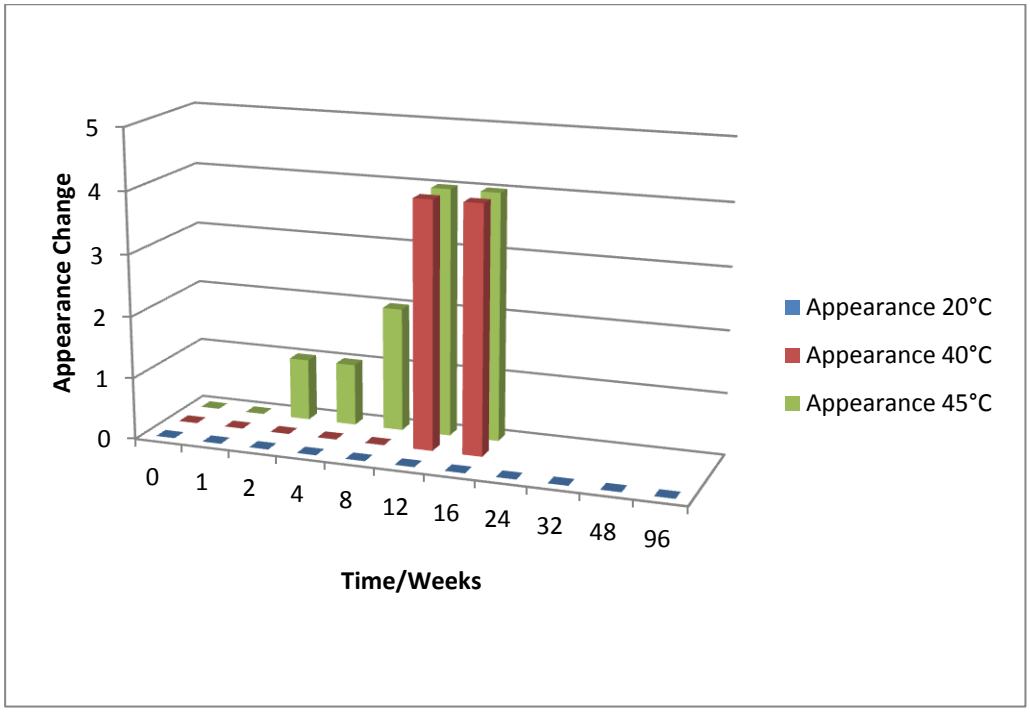


Figure 4-1 Formulation No. 2.01 Appearance Results

Overall, this meant that for 75% of the formulations showing a measurable instability, the Accelerated Stability Model gave a false result with regard to appearance. On an industry scale, this represented an enormous waste of resources to reformulate and retest formulations that failed Accelerated Stability

Tests but may have been adequate for market. To try to quantify the level of inaccuracy of the model on a wider scale, the mean value for the eight formulations' appearance result was calculated. The results are shown in the table 5-11 and figure 5-2 below:

Table 4-11 Average of eight Appearance changing formulations with Prediction Error calculation

weeks	0	1	2	4	8	12	16	24	32	48	96
Average Appearance 40°C	0	1.125	1.5	1.5	2.75	4.375	4.5				
Average Appearance 45°C	0	1.625	1.75	2.875	3.75	4.375	4.5				
Average Appearance 20°C	0	0	0	0.625	0.625	1	1.25	1.25	1.25	1.25	1.25
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				0.5	0.875	0.75	0.25	1.625	1.5	3.125	3.25
Average ASM Prediction Error											1.484375

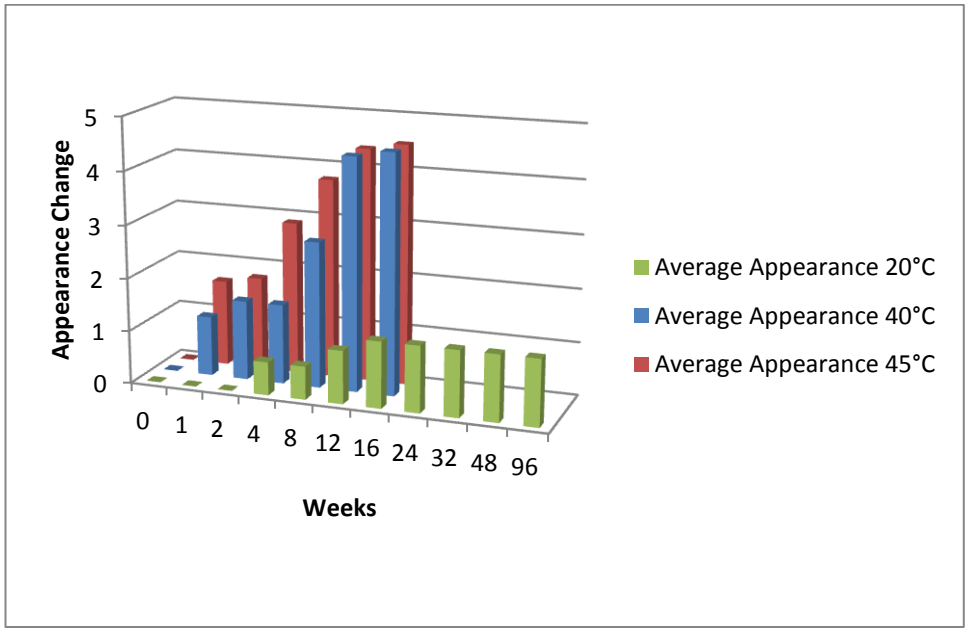


Figure 4-2 Average Appearance Change Over Time of Eight Appearance Unstable Formulations

This table and graph showed a Predictive Error Range of 3.00 against a target of 1.25, and an average predictive error across all the results of 1.48 against a target of 1. This suggested that the Accelerated Stability Model was neither an accurate nor precise predictor of appearance changes over time for this data set.

Further analysis of these results was done by plotting the Accelerated Stability Model Prediction Error against the real-time time points in weeks.

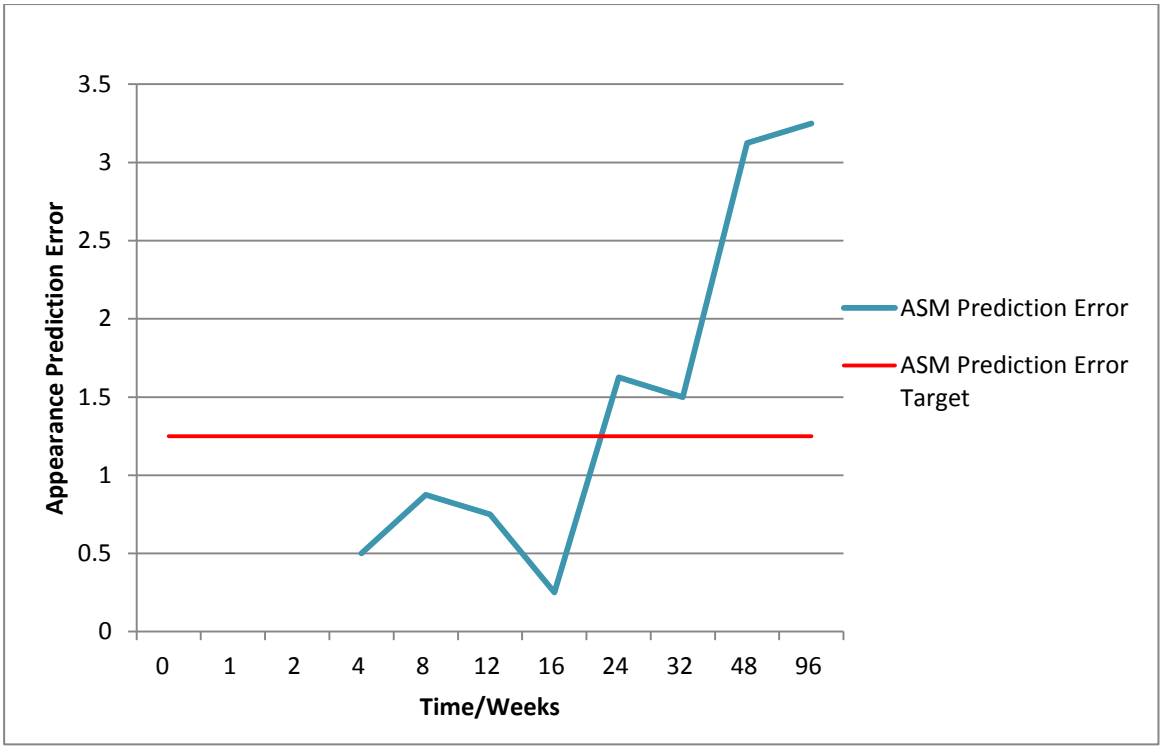


Figure 4-3 Average Appearance Change Prediction Error Over Time

As can be seen, the general trend of the graph was that the further into the future the Accelerated Stability Model was trying to predict appearance changes, the larger the prediction error became. This showed the Accelerated Stability Model became less accurate the further into the future it was predicting. It was also possible to see that, in the case of these eight formulations that showed a measurable change in appearance, the Accelerated Stability Model accurate prediction threshold was 16 weeks of real time testing. This was well short of the 96 weeks that the Cosmetics industry uses the Accelerated Stability Tests to model.

That being said, there was one formulation of the eight that seemed to have a very good correlation to the Accelerated Stability Model. Formulation 16.01 results are shown in Table 4-12:

Table 4-12 Formulation 16.01 Appearance results with Prediction Error included

Formulation No	16.01										
weeks	0	1	2	4	8	12	16	24	32	48	96
Appearance 40°C	0	5	5	5	5	5	5	[illegible]	[illegible]	[illegible]	[illegible]
Appearance 45°C	0	5	5	5	5	5	5	[illegible]	[illegible]	[illegible]	[illegible]
Appearance 20°C	0	0	0	5	5	5	5	5	5	5	5
	The colours indicate values that should be similar if the Accelerated Stability Model is accurate										
ASM Prediction Error				0	0	0	0	0	0	0	0
Average ASM Prediction Error											0

This formulation had a very abrupt appearance change, going from no change (0) to severe change (5) at all time points in one testing cycle. This change occurred at exactly the time points that the Accelerated Stability Model predicted, with an average prediction error of zero and a prediction error range of zero, which showed the Accelerated Stability Model was precise and accurate for this formulation. Whether this was a result of the abrupt nature of the appearance change, or that this particular formulation reaction adhered to the Accelerated Stability Model would have required further investigation.

It was also worth noting that no formulation passed the elevated stability tests and went on to fail the long-term ambient test condition, i.e Q_{10} was always greater than 2. This meant that every formulation that failed the long-term ambient testing was highlighted by the Accelerated Stability Model. For the purpose of industry, this result was positive as no formulation would have passed the Accelerated Stability Testing and then failed on market.

4.3.2 Colour Change

Of the 49 samples that underwent the accelerated and real time-testing, 18 showed a change in colour in the elevated temperature conditions that was significant enough to class the test as a fail. The other 32 samples had no change in colour for either elevated storage temperatures or long-term ambient storage. Although this might have appeared to support the predictive capacity of the Accelerated Stability Model, since the prediction of positive long-term stability result was accurate, this is not necessarily the case. All these results showed was that no change occurred in these formulations over the period of time that they were observed. It did not verify that the Accelerated Stability Model was accurate at predicting colour changes in cosmetic products, but rather that these products were stable for colour over the period of time and temperatures they were observed in this study.

Similarly to the appearance results, of the 18 formulations that showed a colour change, 14 did not reflect any colour change on long-term ambient stability. For example, formulation 1.01 results are given in Table 4-13 and Figure 4-4.

Table 4-13 Formulation 1.01 Colour Results

Formulation No	1.01										
weeks	0	1	2	4	8	12	16	24	32	48	96
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 40°C	0	2	2	2	3	3	4				
Colour 45°C	0	2	2	2	3	3	4				

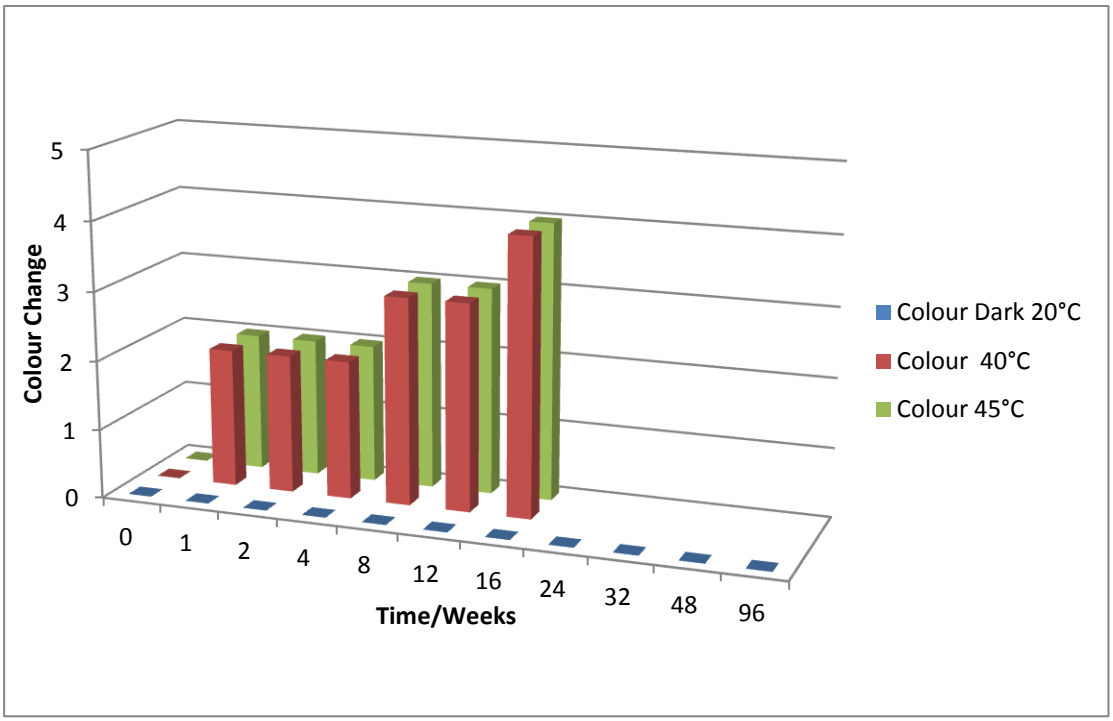


Figure 4-4 Formulation No. 1.01 Colour Change Results

Thus 78% of the formulations that displayed a colour change in elevated temperature storage conditions did not display a colour change in long-term ambient storage conditions. Therefore 78% of these formulations gave a false fail on Accelerated Stability testing. If these numbers were reflected on an industry scale, this would have represented an enormous waste of resources to reformulate and retest formulations that may have been suitable for market.

Taking the mean colour change across all 18 of these colour unstable formulations gave the results shown in Table 4-14 and Figure 4-5.

Table 4-14 Average of 18 colour changing results with Prediction Error calculation

weeks	0	1	2	4	8	12	16	24	32	48	96
Average Colour Change 40°C	0.00	0.76	1.12	1.82	2.06	2.50	2.75				
Average Colour Change 45°C	0.00	1.24	1.47	2.18	2.29	2.88	3.25				
Average Colour Change 20°C	0.00	0.12	0.12	0.12	0.18	0.41	0.65	0.69	0.75	0.88	0.88
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				0.65	0.94	1.06	1.18	1.49	1.31	1.63	2.38
Average ASM Prediction Error											1.33

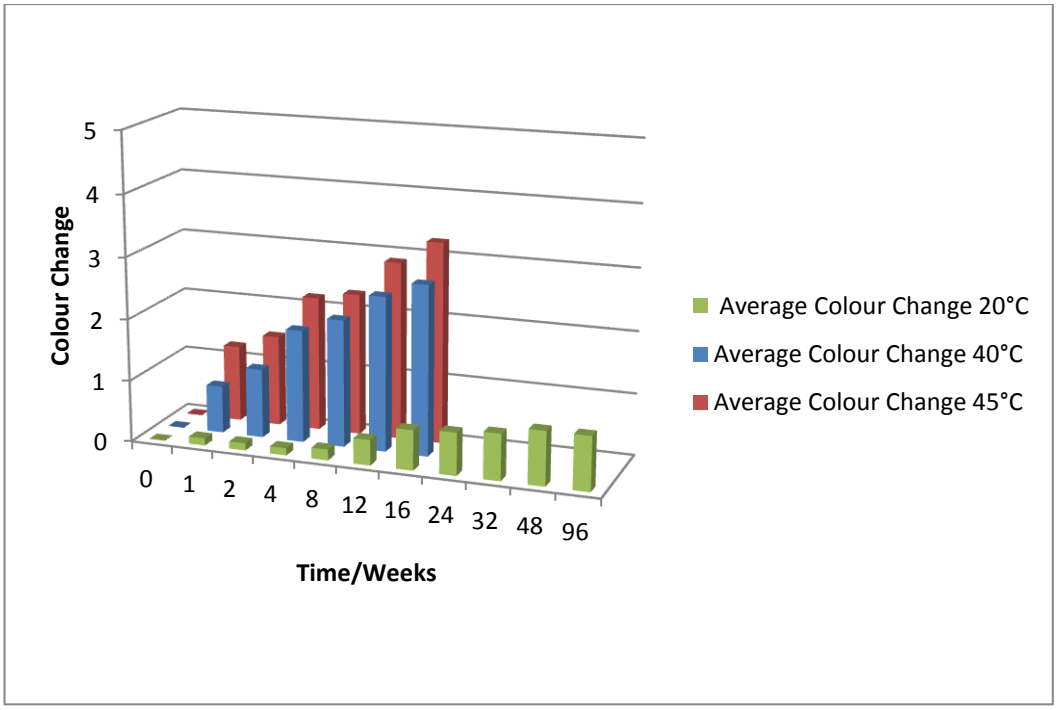


Figure 4-5 Average Change of the 18 Colour Unstable Formulations

As can be seen, the Average Predictive Error across these 18 formulations is 1.33 against a benchmark of 1 and the Predictive Error Range of 1.73 against a benchmark of 1.25. This further suggested that the Accelerated Stability Model was, on average, neither an accurate or precise tool to predict the colour changes within this set of formulations.

Further analysis of these results was done by plotting the Accelerated Stability Model prediction error against the time in weeks of the real-time test in Figure 5-6.

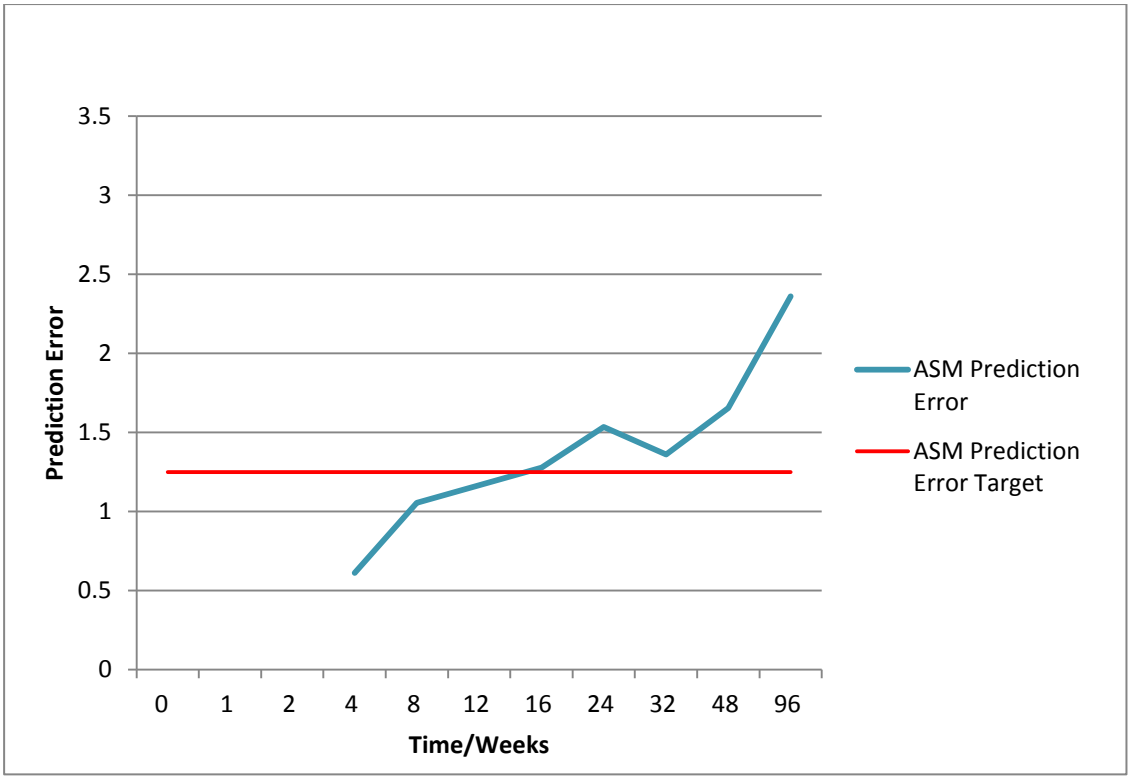


Figure 4-6 Average Colour Change Prediction Error Over Time

As can be seen, the general trend of the graph was that the further into the future the Accelerated Stability Model was trying to predict appearance changes, the larger the prediction error became. This showed the Accelerated Stability Model became less accurate the further into the future it was predicting. It was also possible to see that, in the case of these 18 formulations that showed a measurable change in colour, the Accelerated Stability Model Accurate Prediction threshold was 16 weeks of real-time testing. This was well short of the 96 weeks that the Cosmetics industry uses the Accelerated Stability Tests to model.

It was again worth noting that no formulation that passed the elevated stability tests went on to fail the long term ambient test condition, i.e. Q_{10}>2 for colour change for these formulations. This meant that every formulation that failed the long-term ambient testing was highlighted by the Accelerated Stability Model. For the purpose of industry, whilst potentially wasteful of resources, this result may have been seen as a positive one as it ensured that no formulation would have passed the Accelerated Stability Testing and then gone on to fail in the market.

4.3.3 Odour

Of the 49 formulations that went onto full testing only 2 showed a change in odour at elevated temperatures, and no formulation had a change in odour in long-term stability testing. Therefore 47 samples had no change in odour for either elevated storage temperatures or long-term ambient storage. Again, whilst this would have appeared to strengthen the predictive capacity of the Accelerated Stability Model, since the prediction of positive long-term stability result was accurate, this may not have been the case. These results showed only that no odour change occurred in these formulations over the period of time that they were observed. It did not verify that the Accelerated Stability Model was accurate at predicting odour changes in cosmetic products, but rather that this particular fragrance, which was used across all the formulations made, was stable in 96% of emulsion formulations over the period of time and temperatures they were observed for.

Only two formulations had a detectable amount of odour change at elevated temperatures, and both changed significantly enough to be classed as a '3 – significant change' by the end of the test, and hence would have failed the Accelerated Stability Model. In industry, these results would have meant that these two formulations would have been reformulated and retested. The long-term stability showed that both these results were false fails as neither showed any change in long-term ambient conditions. Hence the reformulation and retesting represented a wasted resource as the original formulations would not have failed on market.

The average odour change results of these two formulations are given in Table 4-15 and Figure 4-7:

Table 4-15 Average results of two odour changing formulations

weeks	0	1	2	4	8	12	16	24	32	48	96
Avaerage Odour 40°C	0.00	1.00	1.00	1.00	1.00	2.50	3.00				
Average Odour 45°C	0.00	1.00	1.00	1.00	1.50	3.00	3.00				
Average Odour 20°C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				1.00	1.00	1.00	1.00	1.00	1.00	2.50	3.00
Average ASM Prediction Error											1.44

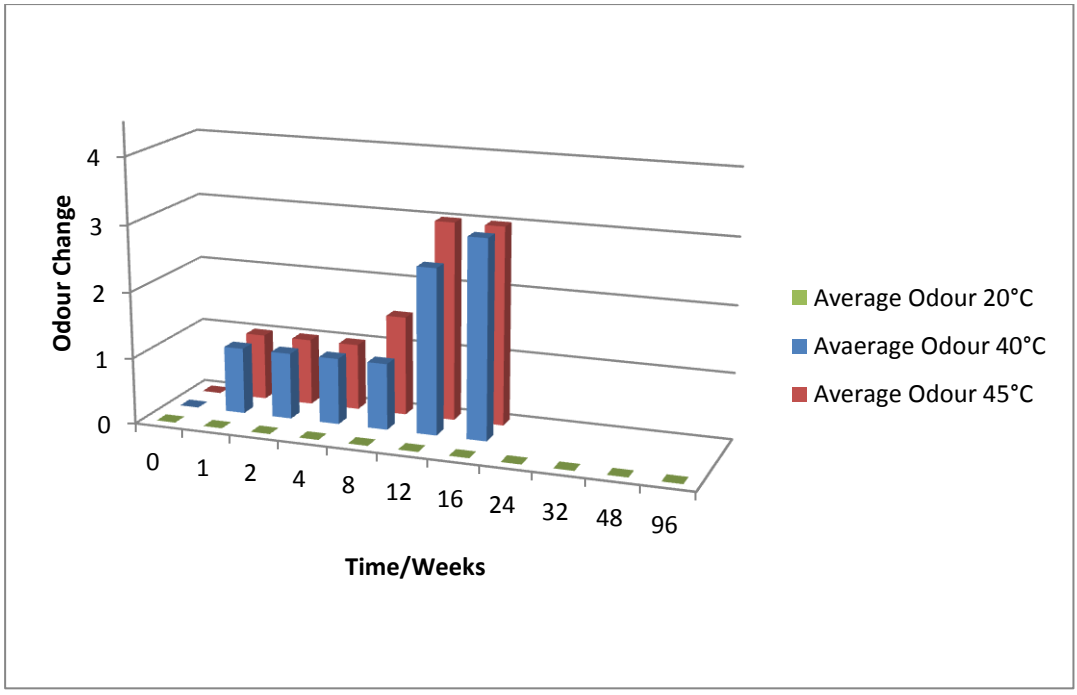


Figure 4-7 Average Change of the 2 Odour Unstable Formulations

As can be seen the Average Predictive Error across these formulations of 1.44 against a benchmark of 1 and the Prediction Error Range of 2.00 against a benchmark of 1.25. This suggested that the Accelerated Stability Model on average was neither accurate nor precise at predicting the odour changes within this set of formulations.

Further analysis of these results was done by plotting the Accelerated Stability Model prediction error against the time in weeks of the real time test in Figure 4-8.

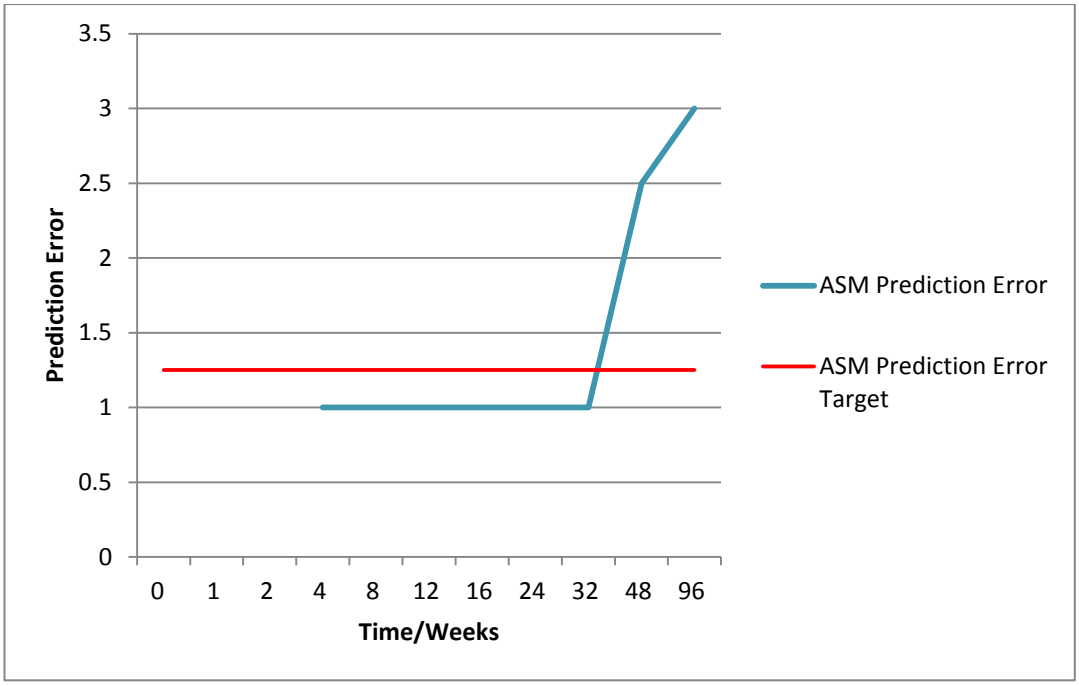


Figure 4-8 Prediction Error change over time for Odour

As can be seen, the general trend of the graph was that the further into the future the Accelerated Stability Model was trying to predict odour, the larger the prediction error became, suggesting the Accelerated Stability Model became less accurate the further into the future it was predicting. It was also possible to see that, in the case of the two formulations that showed a measurable change in odour, the Accelerated Stability Model accurate prediction threshold was 32 weeks of real time testing - well short of the 96 weeks that the Cosmetics industry uses the Accelerated Stability Tests to model.

It was also worth noting that no formulation passed the elevated stability tests and went on to fail the long term ambient test condition, i.e. Q_{10}>2 for odour change in these formulations. Again this meant that every formulation that failed the long term ambient testing was highlighted by the Accelerated Stability Model. For the purpose of industry, this result was a positive one as no formulation would have passed the Accelerated Stability Testing and then gone on to fail in the market.

It is perhaps not unexpected that fragrance changes at higher temperatures. By nature, fragrance is combination of volatile compounds that release from the surface of the product to be detected in the nose. Increasing temperature will change the rate at which the fragrance compounds are released from the product surface. For example, small fragrance compounds that give light or citrus fragrances are more volatile than the larger compounds that give wood or spice notes. Therefore

increasing temperature will encourage the more volatile compounds to release from the product surface, but the larger compounds may not release at the same accelerated rate – leading to fragrance change or drift (Blakeway et al. 1987) and (Steingass et al. 2017).

4.4 Organoleptic Conclusions

Given the testing parameters, there was no organoleptic parameter that was modelled well by the Accelerated Stability Model. This was shown by all three parameters having an Average Prediction Error above one and a Prediction Error Range above 1.25, which showed the model was neither accurate or precise for this data. It was also seen that no parameter had an accurate prediction threshold longer than 32 weeks as summarised in Table 4-16:

Table 4-16 Summary of Colour, Odour and Appearance results

	Appearance		Colour		Odour	
	Target	Result	Target	Result	Target	Result
No. of measurable unstable formulations	8		18		2	
Average Prediction Error (accuracy)	<1	1.48	<1	1.33	<1	1.44
Prediction Error Range (precision)	<1.25	3	<1.25	1.73	<1.25	2
Accurate Prediction Threshold (weeks)	>96	16	>96	16	>96	32

This showed that when a change was taking place within a formulation the Accelerated Stability Model was not accurate beyond 16 weeks predictions.

There were no cases across any of these parameters where a change occurred at long-term ambient conditions that had not been seen at elevated temperatures first. This meant that if these formulations were in fact being proposed for market, the ones that failed long-term stability would have been screened out by the Accelerated Stability Model. This was a positive observation for the use of the Accelerated Stability Model as it helped to justify its use in industry. However, it was also noted that in all of these parameters a significant proportion of the formulations that showed a change in elevated temperature storage conditions showed no change in long-term ambient conditions. For example, for the samples that showed a change in appearance in accelerated conditions, 75% did not show any change in real-time

testing. This figure is 76% for colour change and 100% for odour change. This represented a significant gap in the knowledge of formulators who would have assumed from the Accelerated Stability data that these formulations were unstable when in fact they were just unstable at elevated temperatures. This knowledge gap not only demonstrates a lack in our understanding of cosmetic and emulsion science but also potentially creates a large amount of wasted resource in reformulation and retesting.

4.5 Organoleptic Evaluation

There were three areas identified as methodology improvements or introduction of experimental error.

Firstly, the measurement of the organoleptic parameter was not directly measured but rather the change in an organoleptic parameter was placed on an arbitrary 0-5 scale, and hence was very subjective to the individual doing the testing. In this study, to try to minimise this subjective error, the results were checked by an experienced organoleptic stability technician from industry. The samples were provided and labelled only with the formulation name and unique reference number, along with the standard so that the formulations were unknown during testing. However, it is possible that the decision whether a change is slight, noticeable or significant could have been different from week to week and therefore could have created a subjective error in the data. This technique was chosen because it is the way the formulations' organoleptic parameters are measured in industry, and this study was a measure of those systems.

Another output of the 0-5 scale was that the scale was finite, where the parameter itself was on an infinite scale. For example, once a change in odour was classed as severe it could not go any higher than a change ranking of 5 on the arbitrary scale, when, in fact, the odour itself may still have been changing long after the change ranking of 5 was given. This questions the validity of using the 0-5 scale to give a true parameter change picture over time. However, it is worth noting that the use of this scale in industry is simply to detect if a significant change had occurred to stop the launch of a product, and not to profile the parameter in its fullest. Once a parameter had failed the test, the industry would have no interest in how it behaved over a longer period of time.

To overcome these limitations, each identified organoleptic parameter could have been measured analytically by calibrated instrumentation. For example, odour could have been quantified for both content and strength by using gas chromatography to analyse head space of each sample, this would have given a more accurate reflection of odour changes and may have detected changes that a nose could not have detected. Similarly, a colorimeter or UV/Vis spectrometer would have been able to give a more precise change in colour than the arbitrary 0-5 scale. These parameters may be a good starting point for future study in this area.

Secondly, the number of formulations that gave useful results to analyse was low for all these parameters. The worst case for this was the odour parameter, which only rendered two formulations with useful results to analyse. The difficulty here was in creating formulations that showed instability within the testing time parameters. The majority of formulations were either stable with regard to organoleptic parameters at all time points and temperatures (41 of 65) or too unstable and not making it to the first testing time point (16 of 65). This reduction in formulations that yielded useful results meant that the data sets from which conclusions may have been drawn from were correspondingly reduced and may not have been representative.

A possible area of future study may be to expand upon the formulations from this study to identify formulation areas that showed the ideal amount of instability and to place more formulations from those areas on test. It may also have been useful to use more than one fragrance across the formulations as in this study the same fragrance was stability tested 65 times. It should be unsurprising, therefore, that it gave the same result 96% of the time.

Lastly, the use of the fridge sample as the standard to which the test samples were compared could have given a misleading result. The reason for using this method was so that the test was directly comparing samples that came from the same batch, thus ensuring that the samples received exactly the same treatment during manufacturing. This assumed that keeping an emulsion in the fridge at 4ºC would have slowed any reactions and changes that the emulsion may otherwise have undergone. However, it was seen from many of the unstable formulations, including formulation 16.01 given in Table 4-17, that the 4ºC fridge sample separates along with the other time points:

Table 4-17 Formulation 16.01 Appearance Results

Formulation No.	16.01						
Tests	0 days	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16
Appearance 40°C		50% SPLIT	50% SPLIT	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance 45°C		50% SPLIT	50% SPLIT	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance 20°C	Thick, white cream	AS INITIAL	AS INITIAL	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)
Appearance Fridge		AS INITIAL	AS INITIAL	SPLIT (5)	SPLIT (5)	SPLIT (5)	SPLIT (5)

The alternative was to remake the formulation every time a test time point was due, so that the aged samples were compared to fresh samples. This, however, would have caused its own problems as the two samples would have been subjected to slightly different manufacturing conditions (raw material lot numbers, ambient manufacturing temperature, manufacturing vessel etc) and could not therefore have been guaranteed to be directly comparable.

A possible solution to this is similar to the 0-5 scale observations above: if direct measurement of the organoleptic parameters had been made by calibrated analytical equipment then there would have been no need for subjective comparison. Calibration of the analytical equipment would have ensured validity of the results, which would also have been more precise and informative of the parameters' changes.

Chapter 5 Chapter 5 – pH

The pH of a formulation is important to its safety, its ability to perform its function and in some cases its rheological properties. As such, although it is not likely to be directly perceived by the consumer, its consequence on the formulation may well be. For prospective products, any changes in the pH need to be accurately predicted so costly complaints or recalls for safety concerns or poor quality are avoided. This chapter will outline the method used to test pH as well as detail the results and conclusions of the predictive capacity of the Accelerated Stability Model.

5.1 pH Method

5.1.1 Equipment

- Mettler Toledo FE20 FiveEasy Benchtop pH Meter
- Capital Analytical pH buffer 4 and 7 solutions.
- Therma Handheld Lab Thermometer TA-288

Pictures of this equipment can be found in Appendix 5.

5.1.2 Sample Preparation

- Checked that the sample temperature was 25ºC ± 1ºC.
- Ensured that the pH meter wass calibrated and correctly serviced.
- Ensured that there was enough of the sample to cover the tip of the probe fully.

5.1.3 Sample Testing

- Ensured pH meter was in good working condition and had been calibrated using the standard buffer solutions within 24hrs before use.

- Ensured that the probe was clean and rinsed with de-ionised water to remove the buffer it has been stored in.
- Holding the probe by the shaft, the probe was inserted into the sample; gently moved around to ensure that the probe tip is fully covered.
- 'Read' button pressed to take measurement.
- Reading taken after the display has automatically settled.
- Shaft of probe gentle wiped and tip rinsed with DI water before returning probe to storage buffer solution.
- All readings recorded in the 'Coptis' database.

5.1.4 Pass/Fail Criteria

The pass/fail criteria for pH for a cosmetic product in industry is dependent on the effect that the pH has on the formulation's perceived quality to the consumer, the formulation's efficacy or the formulation's safety. For example, some thickening agents are very dependent on pH to give a certain rheology to a formulation. If the pH is unstable for that formulation over time the product's rheology may change during its time on market, diminishing the perceived quality of that product. Hence the pass/fail criteria for the pH stability of that formulation may be much tighter than for a product where pH is not so critical. Similarly, many active ingredients are more active at specific pH values, ascorbic acid (vitamin C), for example, it is more active at lower pH levels. Hence in order to gain the benefits of the active ingredients, the pH must not drift outside of the active material's optimum range, so the pass/fail criteria for those formulations may need to be stricter than in the absence of a pH sensitive active ingredient.

pH is also directly linked to safety, not only because application of an extreme pH to the skin may cause irritation, but also because some preservative systems used to stop microbiological contamination are only effective at an acidic pH. For example, sodium benzoate (a common preservative) is not very active in salt form, but dissociates to benzoic acid, an effective antimicrobial agent, in conditions below pH 5.5 (Rahn and Conn 1944), (Chipley 2005). Hence it would be very important for a product preserved with sodium benzoate not to be allowed a drift upward in pH to a point where the preservative becomes inactive. A drift downwards however would be acceptable, as long as it did not become an extremely acidic pH.

For the purpose of this study, and given the logarithmic nature of the pH scale, a divergence of more than 0.5 pH point from the initial result was considered to be a fail. That allowed a specification window of pH ±0.5 of the initial result, so that the ambient and elevated temperature storage conditions needed to fall within this range to be considered a pass.

To assess the Accelerated Stability Model's accuracy for pH two new parameters have been developed, designated the Average Prediction Error and Prediction Error Range. These were calculated by comparison of the values given by the Accelerated Stability Model and the real-time values that they predicted (Table 4-1 Results table and accuracy parameters)

The difference in these values was then calculated to give the prediction error at each time point. The difference from the largest and smallest prediction error value is the prediction error range, and gives an indication of the Accelerated Stability Model's precision. The average of the prediction errors can be calculated to give the average prediction error, and gives an indication of the Accelerated Stability Model's accuracy. This gives two indicators of how well the Accelerated Stability Model predicts the long term stability of a product, with a lower value showing a better predictive capacity of parameter changes. These values can be given for individual formulations' or a group of formulations' averaged results, to give a broader quantification of the Accelerated Stability Method's accuracy and precision.

For visualising the accuracy of the Accelerated Stability Model, a plot of Accelerated Stability Model's Prediction Error against the time in weeks was constructed. This plot showed at what time-point the predictive data became inaccurate when compared to real-time data. The aforementioned time point was designated the Accurate Prediction Threshold. For justification of use to assure cosmetic products' long term stability, the cosmetics industry needs the Accurate Prediction Threshold to be equal to, or greater than, 96 weeks.

For the purposes of this study, given the pass/fail criteria of ±0.5, an average prediction error of less than 0.25 would be considered an accurate prediction, with a prediction error range of less than 0.5 considered a precise prediction of stability.

5.2 pH Results

To view all the results in full, refer to Appendix 2 – pH Results.

Of the 65 formulations evaluated for this study 24 (36.9%) of them were unstable with regard to separation of the emulsion back into discreet phases, as detailed in Chapter 5 – Organoleptic Parameters. In 21 of these formulations, the product has changed so fundamentally that the pH results became unreliable and were not suitable to be collected for the whole study. These 21 cases where the testing has incomplete data sets for pH were therefore removed from the results that were considered for analysis of pH behaviour.

There were 44 formulations that maintained sufficient stability through all testing stages to obtain full data sets. The pH of these was recorded at every time point and storage temperature as shown in Table 5-1 Formulation 60.02 pH results below. Results falling within the pass/fail criteria were denoted in green, and failed results in red. For example formulation 60.02:

Table 5-1 Formulation 60.02 pH results

weeks	0	1	2	4	8	12	16	24	32	48	96
Storage condition	TRIAL CODE : 16900/AP.60.02										
pH 40°C	5.28	5.21	5.78	5.76	4.56	4.48	4.02				
pH 45°C	5.28	5.22	5.70	5.40	4.58	4.51	4.07				
pH 20°C	5.28	5.34	5.58	5.42	4.59	4.46	4.39	4.32	4.25	4.18	4.11
pH Fridge	5.28	5.36	5.50	5.44	4.46	4.41	4.37				

Initial (week 0) pH results range from 5.37, for formulation 12.01, to 7.71, for formulation 2.01.

The mean of the 44 tested formulations is given in Table 5-2 Averaged pH results, below:

Table 5-2 Averaged pH results

Weeks	0	1	2	4	8	12	16	24	32	48	96
Average pH 40°C	6.39	6.35	6.36	6.32	6.26	6.24	6.07				
Average pH 45°C	6.39	6.33	6.33	6.28	6.20	6.17	6.01				
Average pH 20°C	6.39	6.33	6.36	6.34	6.21	6.18	6.18	6.12	6.12	6.11	6.12
Average pH 4°C	6.39	6.40	6.41	6.38	6.28	6.24	6.25				

As can be seen, on average, all test points are within the ±0.5 of the initial sample, therefore are considered to be a test pass and that on average these formulations are stable with respect to pH.

Plotting these mean results against time shows a general drift downward of pH over time, but always within the pass/fail criteria:

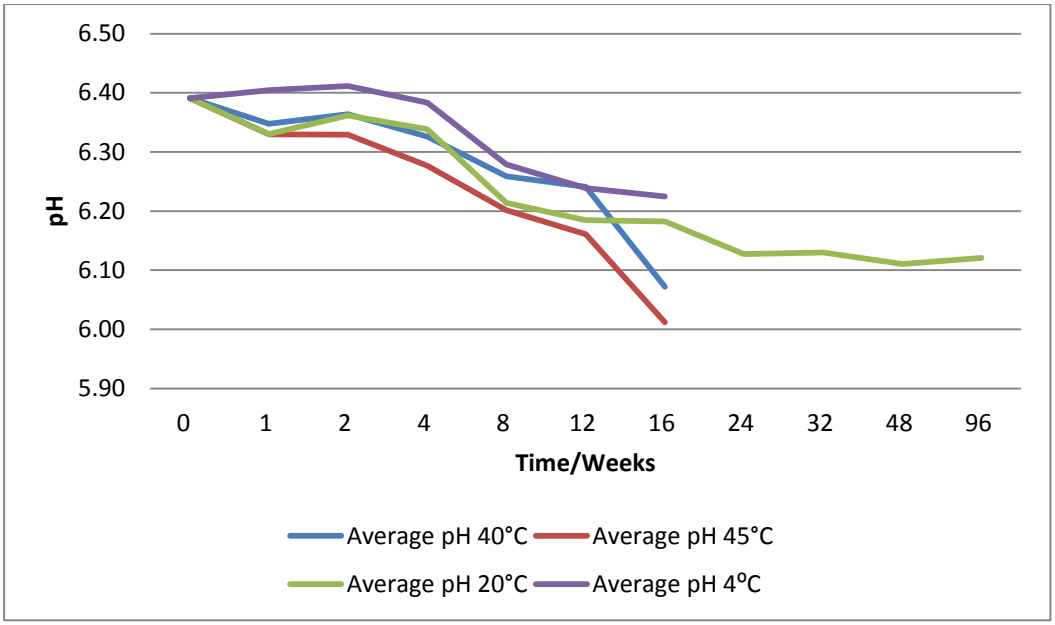


Figure 5-1 Averaged pH Results Over Time

This shows that there was a general change in the pH values over time and they did not, on average, remain static over the course of the testing.

Of the 44 formulations considered, 15 formulations remained within specification of the pass/fail criteria for the duration of the test. These would be considered a full pass in industry, for example the results of formulation 30.02 are shown below in table 5-4:

Table 5-3 Formulation 30.02 pH results

Weeks	0	1	2	4	8	12	16	24	32	48	96
BRAND : RESEARCH FORMULATIONS	TRIAL CODE : 16900/AP.30.02										
pH 40°C	6.33	6.30	6.30	6.33	6.44	6.40	6.44				
pH 45°C	6.33	6.31	6.31	6.30	6.43	6.41	6.41				
pH 20°C	6.33	6.33	6.33	6.11	6.34	6.31	6.30	6.41	6.34	6.35	6.30
pH Fridge	6.33	6.30	6.30	6.00	6.01	6.00	6.11				

These 15 formulations are stable over the time that they were observed for during this study with very little significant change. Their averaged results are given in table 5-5 below:

Table 5-4 Averaged pH results of 15 formulation with all 'pass' results

Weeks	0	1	2	4	8	12	16	24	32	48	96
Average pH 40°C	6.51	6.49	6.47	6.43	6.47	6.44	6.43				
Average pH 45°C	6.51	6.49	6.49	6.43	6.44	6.43	6.40				
Average pH 20°C	6.51	6.52	6.50	6.47	6.44	6.46	6.50	6.49	6.53	6.54	6.51
Average pH 4°C	6.51	6.58	6.57	6.52	6.49	6.46	6.41				

Table 5-4 Averaged pH results of 15 formulation with all 'pass' results, revealed that the largest change for these 15 formulations occurred on the 16th week of 45ºC

testing, with a change of 0.11 pH point from the initial. These pH results would appear to confirm the case for the Accelerated Stability Model's predictive capability in that the model predicted the real-time results would remain within the pass/fail criteria, and that was confirmed by real-time results. However, what these 15 results show is the rate of reaction (K) for a pH changing reaction is near zero for all temperature storage conditions, i.e. the activation energy for a pH changing reaction has not been met so there is no reaction proceeding at any tested temperature condition. Therefore the Accelerated Stability Model is not accurately modelling the change in rate of reaction as there is no reaction rate to model. These results do not describe anything other than that the activation energy required for a reaction to occur has not been met at any temperature points. Hence K has not changed linearly with time, as the Accelerated Stability Model suggests, rather it was not changing at all from zero. This skewed the results in favour of a 'good' predictive model for pH.

Excluding the 15 non-reacting formulations, left 29 formulations that had at least one result outside the pass/fail criteria, and a more accurate representation of the Accelerated Stability Models predictive accuracy of change in pH was achieved. Tabulating the average of these formulations gives:

Table 5-5 Average pH results of 29 formulations with at least one result outside of pass/fail criteria

Weeks	0	1	2	4	8	12	16	24	32	48	96
Average pH 40°C	6.35	6.30	6.33	6.30	6.17	6.16	5.91				
Average pH 45°C	6.35	6.27	6.27	6.21	6.11	6.05	5.84				
Average pH 20°C	6.35	6.26	6.31	6.29	6.13	6.07	6.05	5.97	5.94	5.90	5.92
Average pH 4°C	6.35	6.34	6.35	6.34	6.20	6.15	6.19				

As can be seen, for these 29 formulations, there is one temperature storage condition time point, 16 weeks at 45ºC, that on average fails the pass/fail criteria for pH change against the initial result. However, that result, which should model the reactions seen at 96 weeks of ambient storage, falls very closely to the true 96 week pH result (just 0.08 pH point away). More analysis of these results will be given in the discussion section.

Taking the 29 formulations that demonstrated a change large enough to produce a failed stability test individually, the results show that 27 failed in the elevated storage conditions, which in industry would have led to reformulation and retesting. Of these 27, 11 formulations remained within the pass/fail criterion of ±0.5 during long-term ambient testing, suggesting that the accelerated data in fact gave a false fail result

i.e. the value of Q_{10}>2. This would represent waste of resource in industry, reformulating and retesting a formulation that was perhaps adequate for market.

Perhaps more significant though are the two formulations that achieved a pass in the higher temperature storage conditions and then went on to fail the ambient long-term testing. These are formulations 2.02 and 55.01:

Table 5-6 Formulation 2.02 pH results

weeks	0	1	2	4	8	12	16	24	32	48	96
Formulation No. 02.02											
pH 40°C	7.61	7.60	7.35	7.60	7.43	7.14	7.11				
pH 45°C	7.61	7.35	7.33	7.58	7.26	7.16	7.13				
pH 20°C	7.61	7.43	7.40	7.55	7.40	7.12	7.22	7.20	7.07	7.00	7.02
pH Fridge	7.61	7.60	7.56	7.64	7.46	7.20	7.19				

Table 5-7 Formulation 55.01 pH results

weeks	0	1	2	4	8	12	16	24	32	48	96
Formulation No. 55.01											
pH 40°C	5.66	5.60	5.66	5.61	5.66	5.61	5.84				
pH 45°C	5.66	5.66	5.61	5.63	5.61	5.44	5.45				
pH 20°C	5.66	5.77	5.72	5.60	5.77	5.70	6.21	6.00	6.11	6.02	6.22
pH Fridge	5.66	5.70	5.71	5.70	5.73	5.71	6.56				

In industry, these would have passed the Accelerated Stability Testing and then gone on to fall outside of their specification in market conditions, the so called false pass which shows Q_{10}<2. It would depend on the attributes of the formulation whether this unpredicted change in pH would have a detrimental effect on the product's performance or safety.

5.3 pH Discussion

Firstly, taking the 44 data sets as a whole which are given in Table 5-9, the average change in pH remained within the pass/fail criteria of ±0.5:

Table 5-8 Average pH results of 44 formulations with one result outside of pass/fail criteria

Weeks	0	1	2	4	8	12	16	24	32	48	96
Average pH 40°C	6.39	6.35	6.36	6.32	6.26	6.24	6.07				
Average pH 45°C	6.39	6.33	6.33	6.28	6.20	6.17	6.01				
Average pH 20°C	6.39	6.33	6.36	6.34	6.21	6.18	6.18	6.12	6.12	6.11	6.12
Average pH 4°C	6.39	6.40	6.41	6.38	6.28	6.24	6.25				

There is a general downward drift in pH at all temperature storage conditions as shown by Figure 5-1. This data was taken further in Table 5-10 to look at the Prediction Error Range and the Average Prediction Error:

Table 5-9 Average pH results with Prediction Error calculation

Weeks	0	1	2	4	8	12	16	24	32	48	96
Average pH 40°C	6.39	6.35	6.36	6.32	6.26	6.24	6.07				
Average pH 45°C	6.39	6.33	6.33	6.28	6.20	6.17	6.01				
Average pH 20°C	6.39	6.33	6.36	6.34	6.21	6.18	6.18	6.12	6.12	6.11	6.12
Average pH 4°C	6.39	6.40	6.41	6.38	6.28	6.24	6.25				
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				0.01	0.15	0.14	0.14	0.16	0.13	0.13	0.11
Average ASM Prediction Error											0.12

The Prediction Error Range across all of the data was 0.15 against a target of less than 0.5 and the Average Prediction Error 0.12 against a target of 0.25. This showed that across all the data collected the Accelerated Stability Model was, on average, an accurate and precise predictive tool for pH changes.

This was verified by plotting the Accelerated Stability Model Prediction Error against time to view the Accurate Prediction Threshold shown in Figure 5-2:

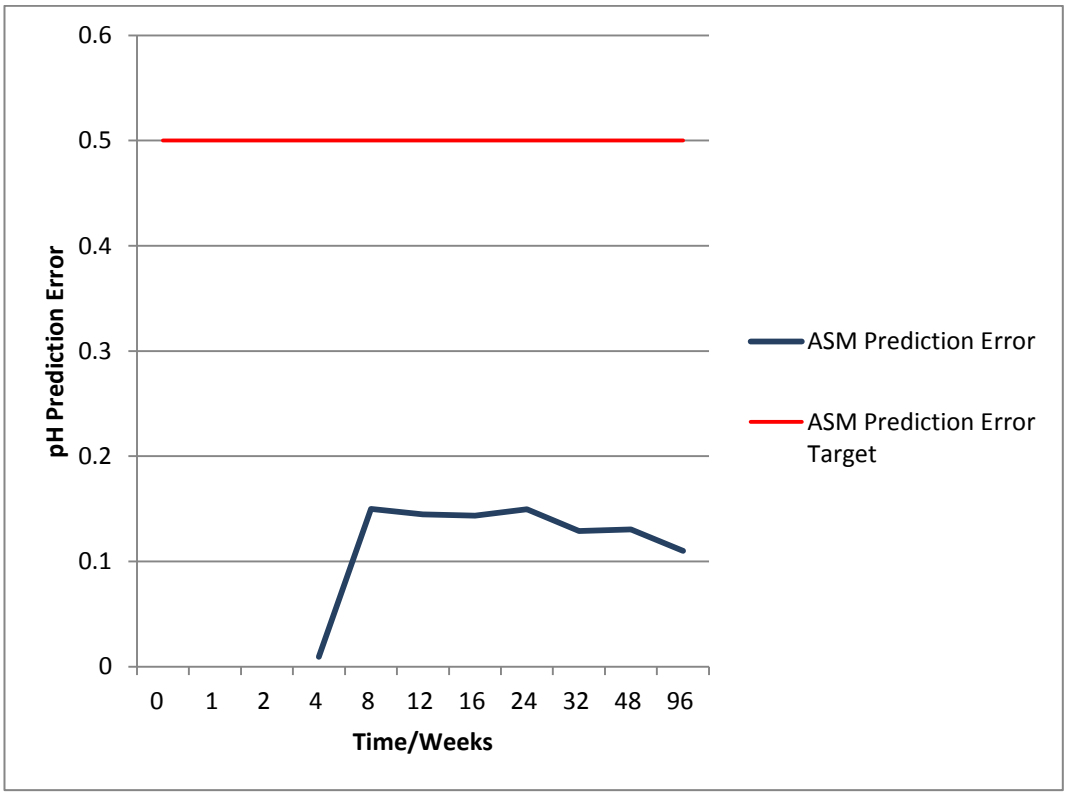


Figure 5-2 Total Average pH Change Prediction Error Over Time

This suggested that the Accurate Prediction Threshold for pH was beyond the 96 weeks that the cosmetic industry requires it to be.

As described in the Results section, there were 15 formulations that were removed from the analysis as they represented no change in pH and therefore no reaction

rate for the Accelerated Stability Model to predict. The 29 remaining formulation average results were given in Table 5-11.

Table 5-10 Average pH results of 29 formulations with at least one result outside of pass/fail criteria with Prediction Error calculation

Weeks	0	1	2	4	8	12	16	24	32	48	96
Average pH 40°C	6.35	6.30	6.33	6.30	6.17	6.16	5.91				
Average pH 45°C	6.35	6.27	6.27	6.21	6.11	6.05	5.84				
Average pH 20°C	6.35	6.26	6.31	6.29	6.13	6.07	6.05	5.97	5.94	5.90	5.92
Average pH 4°C	6.35	6.34	6.35	6.34	6.20	6.15	6.16				
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				0.01	0.21	0.19	0.24	0.25	0.23	0.27	0.08
Average ASM Prediction Error											0.19

As expected the removal of the 15 non-reacting formulations created a larger Average Prediction Error for the remaining 29 formulations. However, the Prediction Error Range across this data was still well within target at 0.24 against a target of less than 0.5, and the Average Prediction Error was also within target at 0.19 against a target of 0.25. This suggested that even when a reaction is taking place the Accelerated Stability Model was an accurate and precise predictor of the pH behaviour over time for these formulations. The Prediction Error was plotted against Time in Figure 5-3 to show the accurate prediction threshold.

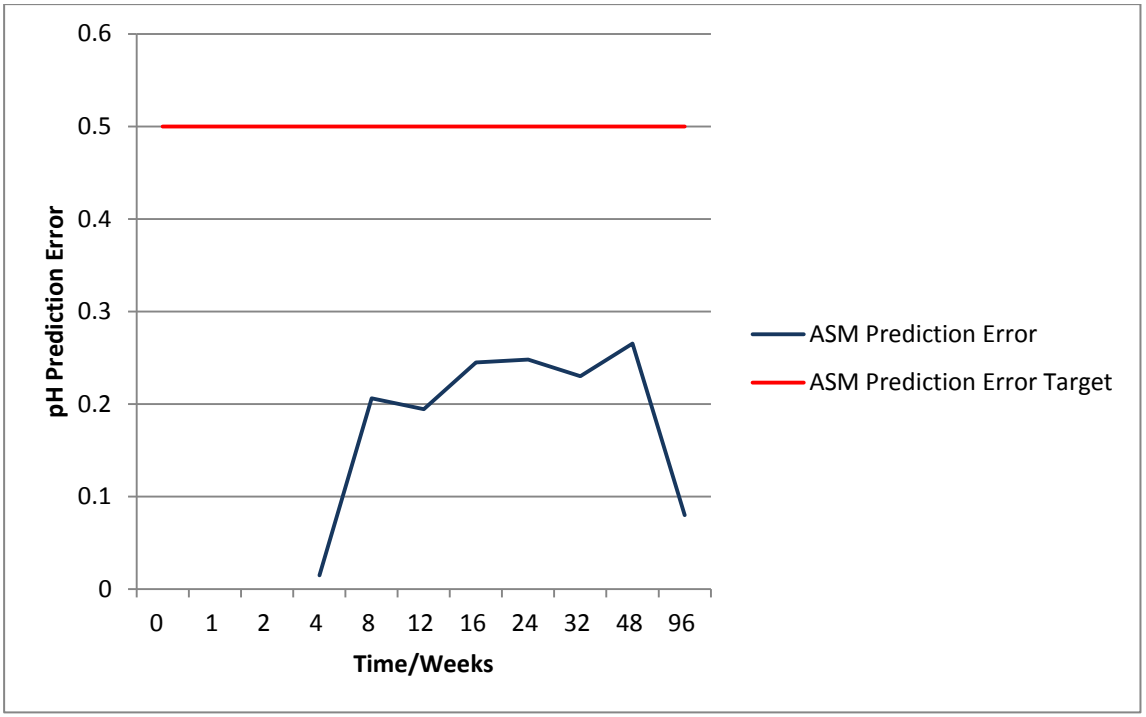


Figure 5-3 Plot of Average Prediction Error of pH Changing Formulations over Time

Figure 5-3 suggested that the accurate prediction threshold for pH was beyond the 96 weeks that the cosmetic industry requires it to be and even suggested that the prediction was better for 96 weeks than for 48 weeks.

If the average pH changes were modelled well by the Accelerated Stability Model, there were two formulation results that were worthy of note, 2.02 and 55.01. These formulations seemed to suggest that they would have passed Accelerated Stability Testing but then failed the long term ambient storage, i.e. would have failed on market. More analysis of these two formulations' results is given below.

Firstly, formulation 2.02 results were given in Table 5-12:

Table 5-11 Formulation 2.02 pH results with Prediction Error calculation

	TRIAL CODE : 16900/AP.02.02										
Weeks	0	1	2	4	8	12	16	24	32	48	96
pH 40°C	7.61	7.60	7.35	7.60	7.43	7.14	7.11				
pH 45°C	7.61	7.35	7.33	7.58	7.26	7.16	7.13				
pH Dark 20°C	7.61	7.43	7.40	7.55	7.40	7.12	7.22	7.20	7.07	7.00	7.02
pH Fridge	7.61	7.60	7.56	7.64	7.46	7.20	7.19				
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				0.05	0.05	0.21	0.38	0.38	0.36	0.14	0.09
Average ASM Prediction Error											0.21

For formulation 2.02, the Prediction Error Range was 0.33, still within the maximum target of 0.5 and the Average Prediction Error was 0.21, still within the target of 0.25, although both were higher than the average results. The overall trend for this formulation was a downward drift of pH, and all storage temperatures reflected this trend. Although the elevated storage conditions did show the decrease in pH, their results were very slightly higher in the results that should have reflected the 32, 48 and 96 weeks of ambient storage. In nominal terms, these differences were still within the predictive error target of 0.5 at 0.36, 0.14 and 0.09 respectively, so in prediction terms the Accelerated Stability Modelling was still considered acceptable. However, because the predictions remained slightly higher than true results, the ambient storage condition slipped outside of the pass/fail criteria, where the elevated temperature storage conditions were within the pass/fail criteria. The formulation's specific attributes would have determined whether this slightly lower pH would have been a problem for the product's efficacy or safety.

Secondly the results for formulation 55.01 are given in Table 5-13:

Table 5-12 Formulation 55.01 pH results with Prediction Error calculation

	TRIAL CODE : 16900/AP.55.01										
Weeks	0	1	2	4	8	12	16	24	32	48	96
pH 40°C	5.66	5.60	5.66	5.61	5.66	5.61	5.84	[illegible]	[illegible]	[illegible]	[illegible]
pH 45°C	5.66	5.66	5.61	5.63	5.61	5.44	5.45	[illegible]	[illegible]	[illegible]	[illegible]
pH Dark 20°C	5.66	5.77	5.72	5.60	5.77	5.70	6.21	6.12	6.11	6.14	6.22
pH Fridge	5.66	5.70	5.71	5.70	5.73	5.71	6.56	[illegible]	[illegible]	[illegible]	[illegible]
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				0.00	0.11	0.09	0.60	0.49	0.45	0.53	0.77
Average ASM Prediction Error											0.38

For formulation 55.01, the Prediction Error Range was 0.77, which was outside the target of 0.50, which showed the Accelerated Stability Model was not precise for this formulation. This was also reflected in the Average Prediction Error which was 0.38, much higher than the maximum target of 0.25, which showed that the Accelerated Stability Model was not accurate for this formulation. The overall trend for this formulation's pH was an upward drift but not all storage temperatures reflected this, as the 45ºC sample decreased overall and the 40ºC remained static until the last test time point as shown in Figure 5-4.

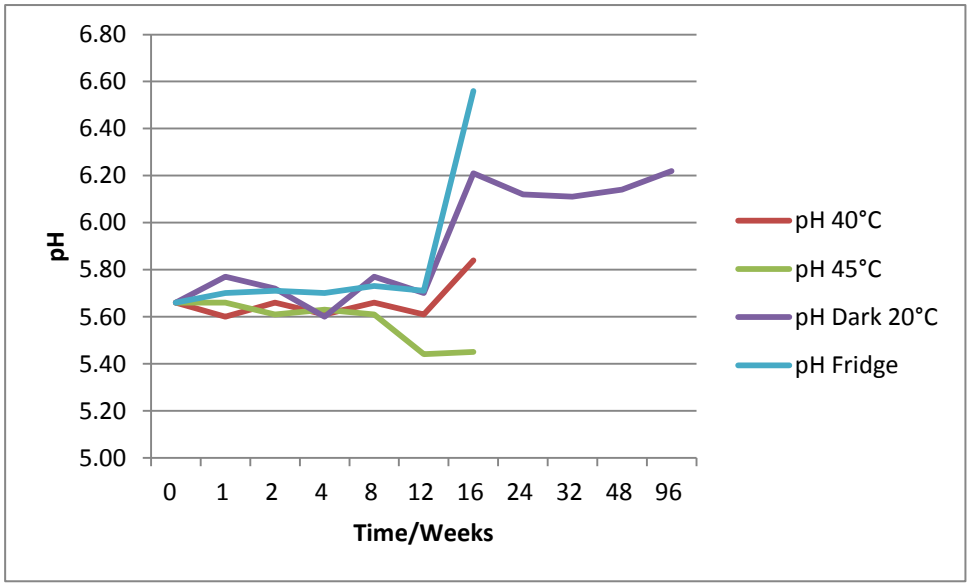


Figure 5-4 Plot of Formulation 55.01 pH Results Over Time

This data appeared to show that the fridge sample (4ºC) was the only temperature that displayed similar behaviour to the 20ºC sample. This is unusual, considering it was supposed to be the sample standard kept at lower temperature for slower reaction rates.

In this specific case the Accelerated Stability Model's accurate prediction threshold was 16 weeks as shown in Figure 5-5:

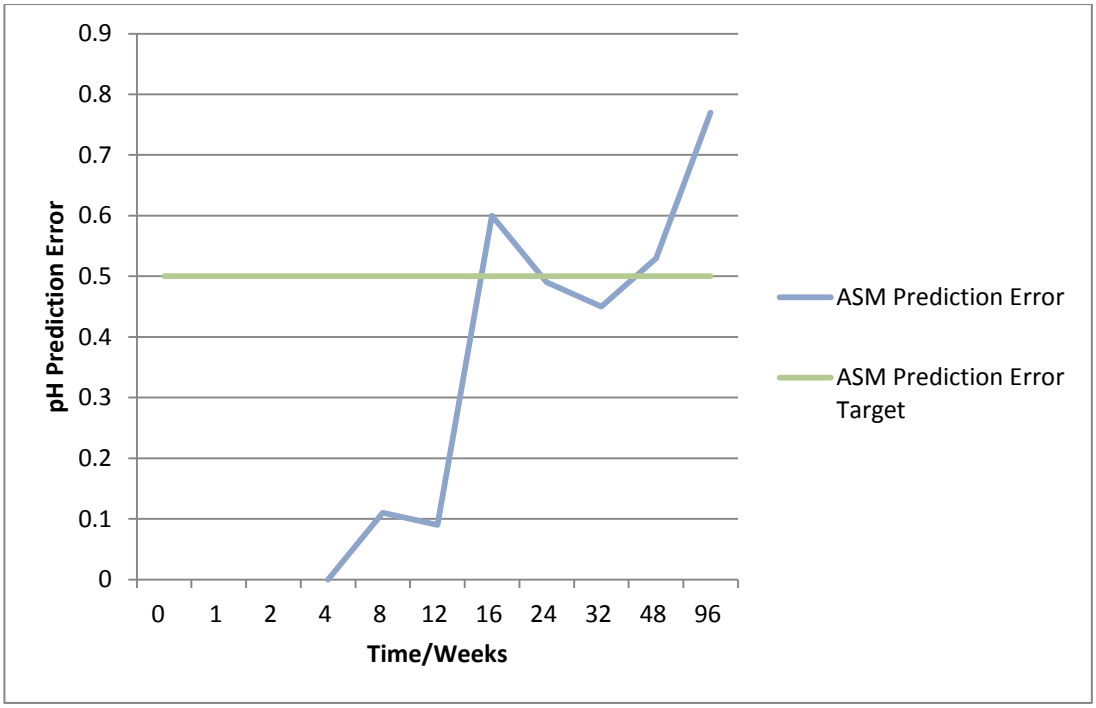


Figure 5-5 Formulation 55.01 Accurate Prediction Threshold

This suggested that for this specific case the Accelerated Stability Model was a poor predicting model of pH behaviour. Perhaps more importantly, in this case, the elevated temperatures results predicted no change in the real-time testing but the long-term ambient storage conditions did show a change, a false-pass, reflecting that Q_{10}<2. This was a significant result because if seen in industry, this formulation would have reached the market place without knowledge of the change in pH taking place. In the worst case, this could have led to a market recall of the product at huge expense to the brand owner.

This was a significant difference from previous parameter results where the Accurate Prediction Threshold result was less than 96 weeks. In those cases the elevated temperatures have showed a change that was not reflected in long-term storage conditions, resulting on reformulation and retesting. Whilst this represented a waste of resources it could be considered prudent to be overcautious with brand reputation, customer safety and financially.

It could be argued that, although the elevated temperature storage conditions did not accurately reflect the behaviour of this formulation's pH over time, the readings that were taken during the accelerated stability testing showed a 'fail' result at 16 weeks at 4ºC and 25ºC. It was possible that analysis of these unexpected results may have led to a re-evaluation of the suitability of this formulation for market.

However, adhering strictly to the Accelerated Stability Model would have allowed this formulation to proceed to the next development stage of safety assessment.

5.4 pH Conclusion

The results suggested that the Accelerated Stability Model was an accurate and precise predictor of pH changes over time. This was shown by both sets of data having stayed well within the prediction targets for Average Prediction Error, Prediction Error Range and the Accurate Prediction Threshold as summarised in Table 5-14.

Table 5-13 Summary of pH prediction results

	Target	All Formulations	pH Changing Formulations Only
Average Prediction Error	0.25	0.12	0.19
Prediction Error Range	0.5	0.15	0.24
Accurate Prediction Threshold (weeks)	>96 weeks	>96 weeks	>96 weeks

A possible explanation for this is that changes in pH were caused by reactions on a molecular scale with the association or disassociation of H^+ ions. This type of reaction is closely related to how the Arrhenius equation was derived, indeed the original data set used by Arrhenius was assessing the effect of temperature on the rate of association and disassociation of electrolytes in solution (Arrhenius 1889). Although Arrhenius would have been using simple solutions and not emulsions, the continuous phase of an emulsion is all that a pH probe can detect, and that environment is aqueous with ions present. Therefore, it was evident that this type of reaction was well modelled by the Accelerated Stability Model, given that it was based around the Arrhenius equation.

Although these average results suggested strong support for the Accelerated Stability Model with regard to pH, there were some individual cases where the results in elevated temperatures (40ºC and 45ºC) and the results in long-term ambient conditions were different. In 27 of the 44 formulations, 61%, the formulation failed at least one elevated storage condition test point but went on to pass the long-term ambient storage tests, the so-called false-fail. In industry, this 61% of formulations (unless the results were accounted for by a qualified person) would have been reformulated and retested unnecessarily. This would have represented a

large potential waste in resource and highlighted an area where there was a deficit in the knowledge of cosmetic formulation. It should be said however that for these 61% of formulations the Accelerated Stability Model was over-cautious, i.e. that stopping the advancement of formulations that did not need to be stopped (with resultant potential waste in resource) did prevent allowing a formulation on to market that could have become a liability.

However, in two formulations, 55.01 and 2.02 the results showed that the formulation passed elevated temperature storage testing, but went on to fail the long-term ambient condition testing. As discussed above, of these two formulations 2.02 results all remained within the targets for Prediction Error Range, Average Prediction Error and indeed for Accuracy Threshold, it was just that the pH had a downward drift to the limits of the pass/fail criteria and the long-term results just drifted out of the specification. It would have been down to the emulsion's specific attributes whether this drifting out of specification was detrimental to the product. The other formulation, 55.01 was more noteworthy because the results showed that the Accelerated Stability Model is an inaccurate and imprecise prediction tool for this formulation. With Prediction Error Range of 0.77 against a target of 0.5, an Average Prediction Error of 0.38 against a target of 0.25 and a Accuracy Threshold of just 16 weeks against a target of 96 weeks. Indeed these results were so poor that the 45ºC sample suggested a slight pH decrease, while the long-term ambient storage showed a significant increase to out of specification.

These results were important because they represented the capacity of the Accelerated Stability Model to understate the changes seen at ambient conditions – the so-called false-pass, showing $Q_{10}<2$. In industry these two formulations would have progressed to the next stage of development. This could have led to a formulation getting to market that would have changed beyond the Accelerated Stability Model prediction, possibly becoming a liability and causing a costly recall of the product from market. If these numbers were a reflection of industry-wide results, 1 in 49 (just over 2%) of emulsions on market could have displayed similar poor prediction by the Accelerated Stability Model, and undergone changes not seen at elevated temperatures, which represented a huge liability and risk for the cosmetics industry.

Overall these results showed that the Accelerated Stability Model was a good predictive tool for changes in pH of the formulations tested. However, there were some individual cases where not only did it demonstrate changes in elevated

temperatures that did not happen in long-term ambient conditions, but there were also cases where changes were seen in long-term storage which were not shown in elevated temperature conditions. The only way to verify that the short-term elevated storage condition results were valid, would be by comparison to long-term storage data.

5.5 pH Evaluation

There were three areas that were identified as methodology improvements or areas for future study.

Firstly, the nature of the formulation make-up was very similar across all the formulations. The only difference across the 65 samples made was that six different emulsifiers were used. The other seven chemicals remained the same, albeit with the oil phase ratio and emulsifier concentration changing from formulation to formulation. Although the emulsifier would have had an effect on pH, the make-up of the formulations meant that the same 7/8 chemical mixes were being tested 45 times. This could have been a possible explanation as to why the average results were so close together and were modelled so well by the Accelerated Stability Model.

A possible solution or area for further study to address this could have been to use more emulsifiers and a wider range of formulation ingredients to create a bigger data set from which to draw conclusions. It could also be noted that it is common to use non-corrosive acids such as citric or lactic acid to adjust pH in industry to the desirable level. This is another possible avenue of future research: by keeping all the other formulation parameters constant and adjusting the pH with a pH adjuster, would this improve or worsen the accuracy of the Accelerated Stability Model for a given emulsifier system?

Secondly, as an extension of the above point that these formulations were all very similar in their chemical make-up, it makes it very unusual that two formulation 55.01 and 2.02 would display such different behaviour to the rest of the data set. It cannot be discounted that these formulation had an error either in processing or in measurement.

A possible improvement to the experimental design could have been to make multiple batches of the same formulation and perform the stability protocols on all them to ensure that the results obtained are repeatable.

Lastly, given that the formulations did not have an active material or a pH sensitive thickening agent, and did have a preservative that was effective up to pH of 8, the pass fail criteria of ±0.5 was arbitrarily chosen as a significant change. Whilst, this did reflect a common specification range for pH in industry, it had no technical significance to the formulations made. Therefore a change of 0.5 may have been acceptable for these formulations. For example, in the case of the poorly modelled formulation 55.01, the maximum pH seen of 6.22 and the minimum of 5.44, had no detrimental effect on that formulation's colour, odour, appearance or viscosity results. Therefore, the fact that the Accelerated Stability Model did not predict behaviour very well was of little importance, given that all the changes seen had little detrimental effect on the formulation itself. Equally, in some cases the pH specification may have to be smaller than ±0.5 due to an activity or texture that is only achieved in a small pH window. If this was the case with this set of data and the specification required ±0.25 then the Accurate prediction threshold stops being greater than 96 weeks and instead becomes 48 weeks as shown in Figure 5-6 Accurate Prediction Threshold for the mean of all formulations if the specification for pH was ±0.25:

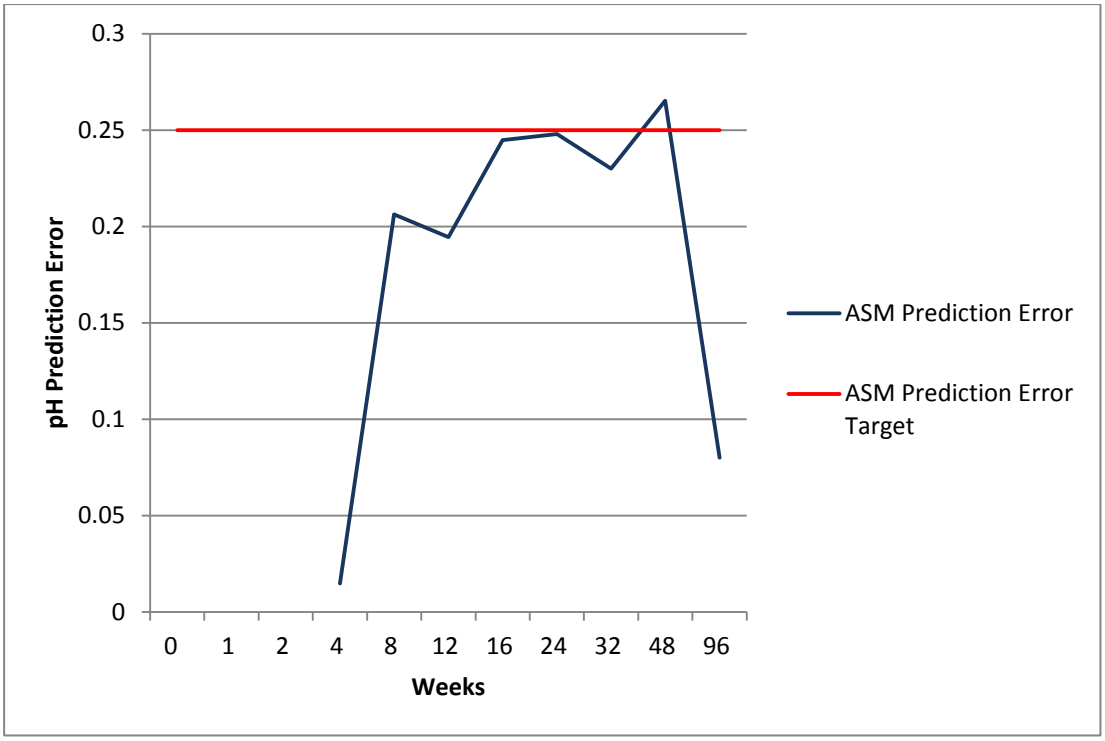


Figure 5-6 Accurate Prediction Threshold for the mean of all formulations if the specification for pH was ±0.25

A possible improvement to the methodology would have been to introduce some pH dependant materials into the formulation mixtures. For example, if a more pH sensitive preservative had been introduced, it would have meant that the pH of the formulations would have had to remain at the active level for that preservative, thus giving defined and relevant pass/fail criterion. To check the activity was still available, a full preservative efficacy test could have been performed at each test-point and the results compared from accelerated data and real-time data. This could be repeated for any active ingredient: for example vitamin C, which could have been monitored by vitamin C recovery; or a pH-sensitive thickening agent which would have been reflected in the viscosity results over time. This would have created a more relevant set of data to the industry.

Chapter 6 Chapter 6 – Viscosity

The viscosity of a formulation measures the material's resistance to flow. It can be directly perceived by a consumer when spreading the formulation onto skin or applying pressure to the formulation surface (formulation 'pick-up'). It is important to the product's perceived quality and significant changes can cause poor consumer experiences. As such, although small changes are not likely to be directly perceived by the consumer, they can give early indications of significant changes at a later date. For prospective products, any changes in the viscosity need to be accurately predicted so costly complaints or recalls for poor quality are avoided. This chapter will outline the method used to test viscosity as well as detail the results and conclusions of the predictive capacity of the Accelerated Stability Model.

6.1 Viscosity Method

6.1.1 Equipment

- Brookfield 'Low Viscosity' (LV) and 'Regular Viscosity' (RV) rotational dial viscometer, ensure the last calibration certificate is still valid at time of use.
- Height adjustable platform.
- Therma Handheld Lab Thermometer TA-288

Images of this equipment can be found in Appendix 5.

6.1.2 Sample Preparation

- The sample temperature was checked to be 25ºC +/- 1ºC.

6.1.3 Sample Testing

- Checked that the level bubble on the viscometer is central.
- Ensured that the correct spindle is attached to the viscometer and that the speed and spindle settings were correct as defined in the specification.

- Placed the sample on the viscosity platform for LV and RV and immersed the spindle to the correct depth as marked by the indentation on the spindle. Swirled it to remove any trapped air from underside of the spindle.
- Turned on the viscometer and allowed to run for one minute then reading taken.
- The Brookfield Viscometer gave a reading one to 100 scale called deflection points, and is most accurate between 30-70 deflection points. For first reading of a sample, i.e. all initial results, spindle size and rotation speed were adjusted to ensure reading of between 30-70 deflection points. For all subsequent readings of the aforementioned sample the same spindle and speed was used for comparison purposes.
- Viscosity calculated by multiplying the result with the appropriate factor on the Brookfield spindle and speed factors sheet (given in Table 6-1). Record this result and the deflection scale result, in the appropriate field in the 'Coptis' database.

Table 6-1 Brookfield Viscometer multiplication factors (Dial Reading Viscometer with Electric Drive Instruction Manual, Brookfield Engineering Laboratories inc., 2005)

LV Series Viscometer

Spindle Number							
1 & 61		2 & 62		3 & 63		4 & 64	
0.3	200	0.3	1K	0.3	4K	0.3	20K
0.6	100	0.6	500	0.6	2K	0.6	10K
1.5	40	1.5	200	1.5	800	1.5	4K
3	20	3	100	3	400	3	2K
6	10	6	50	6	200	6	1K
12	5	12	25	12	100	12	500
30	2	30	10	30	40	30	200
60	1	60	5	60	20	60	100

RV Series Viscometer

Spindle Number													
* 1		2		3		4		5		6		7	
0.5	200	0.5	800	0.5	2K	0.5	4K	0.5	8K	0.5	20K	0.5	80K
1	100	1	400	1	1K	1	2K	1	4K	1	10K	1	140K
2	50	2	200	2	500	2	1K	2	2K	2	5K	2	20K
2.5	40	2.5	160	2.5	400	2.5	800	2.5	1.6K	2.5	4K	2.5	16K
4	25	4	100	4	250	4	500	4	1K	4	2.5K	4	10K
5	20	5	80	5	200	5	400	5	800	5	2K	5	8K
10	10	10	40	10	100	10	200	10	400	10	1K	10	4K
20	5	20	20	20	50	20	100	20	200	20	500	20	2K
50	2	50	8	50	20	50	40	50	80	50	200	50	800
100	1	100	4	100	10	100	20	100	40	100	100	100	400

* Optional = Spindle = Spindle Speed = Factor K = 1000

6.1.4 Pass/Fail Criteria

The pass/fail criteria for viscosity for a cosmetic product in industry is dependent on the effect that the viscosity has on the formulation's perceived quality to the consumer or on the formulation's safety. We know from Stokes Law (Myers 1999) that viscosity is inversely proportional to the rate of creaming and sedimentation. Therefore, a decrease in viscosity is a key early indicator that the formulation may become susceptible to creaming and sedimentation. A change in emulsion structure, such as sedimentation and creaming, leads to safety concerns over build-up in concentrations of materials in the non-homogenous product. If the viscosity is unstable for a formulation over time, the product's rheological changes during its time on market, decreasing the perceived quality of that product. For example, if a

product is being dispensed from a pump, the formulation must stay fluid enough to go through the pumping mechanism to be dispensed. If the viscosity increases too much, the product may not be able to be dispensed, causing a decrease in the perceived quality of the product on market and perhaps a series of brand damaging complaints. Hence the pass/fail criteria for the viscosity stability of a given formulation will depend on how critical the viscosity is to the product's performance, quality or safety.

The Brookfield Viscometer measures resistance to flow by rotating a spindle of known surface area in the liquid being measured. The resistance is measured by a calibrated rotational spring that uncoils proportionally to the resistance on the spindle. The opening of this spring is placed on a scale of 1 to 100 which is the reading that the rotational plate viscometer gives. This number is then multiplied by a factor that depends on the rotational speed and the surface area of the spindle given in Table 6-1 Brookfield Viscometer multiplication factors (Dial Reading Viscometer with Electric Drive Instruction Manual, Brookfield Engineering Laboratories inc., 2005). This results in a situation where each spindle and speed have a defined range of apparent viscosities it can read. For example if the multiplication factor is 200, the highest reading that can be achieved is 200 x 100 = 20,000cps. Therefore, it is important to consider viscosity reading in context of the size of the scale being used and not just the viscosity reading itself. Hence, the deflection point reading is recorded alongside the apparent viscosity reading. These types of viscosity measurements are called single-point measurements and give an apparent viscosity based on the settings used. For the rest of this chapter, viscosity refers to the apparent viscosity given by a single point test.

It is rare to find a liquid that will give the same viscosity reading regardless of spindle chosen (shear stress) or rotational speed used (shear rate) (Morrison 2001). Liquids that display this behaviour are called Newtonian fluids. Far more common behaviour is Non-Newtonian, which change their apparent viscosity with a change in shear stress (spindle) or shear rate (speed of rotation). The reaction of viscosity to changes in shear rate and stress describes the rheological behaviour of the liquid and are discussed in more depth in the discussion section. Hence viscosity readings are intrinsically linked to the parameters of the viscometer being used and therefore when testing the same sample multiple times for comparison purposes the spindle and speed of rotation need to be kept constant.

For quality control purposes, it is common to use ±10 deflection point reading output at a prescribed spindle and speed setting to set the specification for a given formulation, rather than use a percentage change in the absolute viscosity values. For example, taking a product's viscosity using T bar C @ 5rpm (multiplication factor of 2000) may give a reading of 50 deflection points which is an apparent viscosity of 100,000cps. Instead of a specification of ±10% the viscosity reading (90,000 – 110,000 cps), a specification is set ±10 deflection points around the initial result (40-60 deflection points) which is an absolute viscosity range of 80,000 – 120,000cps. This creates a range specific to the scale that is available to the viscometer at those settings, and not just percentage points around a value i.e. the formulation has to stay within 10% of the scale that has been used.

For the purpose of this study, given there were no final packaging concerns as these were research formulations with the focus on the modelling of any changes, a change of 15 viscometer deflection points around the initial result was considered a significant change and regarded as a failed result for viscosity stability as this would be a noticeable change to texture. To make this clear throughout the chapter, all results will be expressed in both the absolute viscosity value and the defection point reading.

In order to compare viscosity changes across many different starting viscosities which may lead to different scales being used, the data was normalised. This was achieved by each result noting deflection point changes in viscosity from initial as well as absolute viscosity. This allowed analysis of the Accelerated Stability Model's accuracy on average across all the formulations.

To assess the Accelerated Stability Models accuracy for viscosity, the two new parameters have been developed, designated the Average Predictive Error and Prediction Error Range. These were calculated by comparison of the values given by the Accelerated Stability Model and the real-time values that they predicted as demonstrated in Table 4-1 Results table and accuracy parameters.

The difference in these values was then calculated to give the prediction error at each time point. The difference from the largest and smallest prediction error value is the prediction error range, and gives an indication of the Accelerated Stability Model's precision. The average of the prediction errors can be calculated to give the average prediction error, and gives an indication of the Accelerated Stability Model's accuracy. This gives two indicators of how well the Accelerated Stability Model predicts the long term stability of a product, with a lower value showing a better

predictive capacity of parameter changes. These values can be given for individual formulations' or a group of formulations' averaged results, to give a broader quantification of the Accelerated Stability Method's accuracy and precision.

A plot of the Accelerated Stability Model's Prediction Error against the time in weeks that it is predicting also renders a graph that shows when the Accelerated Stability Model becomes inaccurate. This shows at what time point the predictive data become inaccurate when compared to real time data, designated the Accurate Prediction Threshold. For justification of use to assure cosmetic product's long term stability, the cosmetics industry needs the Accurate Prediction Threshold to be equal to, or greater than, 96 weeks.

For the purposes of this study, given that the common specification range for viscosity is ±10 deflection points and the pass/fail criteria is ±15 deflection points, an Average Prediction Error of less than 10 deflection points would be considered an accurate prediction, with a Maximum Prediction Range of less than 15 deflection points considered a precise prediction of stability.

6.2 Viscosity Results

As discussed in previous chapters of the 65 formulations made for this study, 16 were not stable enough to reach the week 1 testing point in all conditions. These 16 formulations have no data on their viscosity behaviour over time because their structure changed fundamentally before any viscosity reading had been recorded thus these 16 have been removed from the results for viscosity.

There were a further eight formulations that displayed a change in appearance during the testing that would have resulted in a 'fail' result. Five of the eight changed appearance so fundamentally that a viscosity reading was not possible for at least one test point. This change did not necessarily happen in all temperature storage conditions and time-points, so the viscosity data sets for these formulations were incomplete. As viscosity can be an indicator of internal structure and of stability, through Stoke's Law (Myers 1999), these results were retained for further analysis in conjunction with the appearance results. However, as they were incomplete, they were excluded from the calculations to give the Average Prediction Error or Prediction Error Range.

Subsequently, there were 44 complete sets of viscosity results from the 65 initial formulations created for this study. The viscosity was taken at every time point and storage temperature. Results remaining within the pass/fail criteria of ±15 deflection points are denoted in green, with any fail results in red. As an example of this layout, formulation 26.01 results are noted in table 6-3.

Table 6-2 Formulation 26.01 viscosity results

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.26.01						TB@20rpm			x	200												
visc 45°C	43	8600	43	8600	40	8000	41	8200	43	8600	73	14600	72	14400								
visc 40°C	43	8600	40	8000	40	8000	40	8000	41	8200	40	8000	43	8600								
visc 20°C	43	8600	41	8200	43	8600	44	8800	44	8800	44	8800	45	9000	40	8000	41	8200	44	8800	43	8600
visc Fridge	43	8600	44	8800	44	8800	42	8400	41	8200	40	8000	30	6000								

To view all the results in full, refer to Appendix 3 – Viscosity Results.

A wide range of viscosities was displayed by the data, from very low viscosity of formulation 50.02 (showing an initial viscosity of 460cps and the lowest reading of 400cps during testing) given in Table 6-4, to the very viscous formulation 10.02 (having an initial viscosity reading of 240,000cps and a peak viscosity of 328,000cps during testing) given in table 6-5.

Table 6-3 Formulation 50.02 Viscosity Results

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.50.02						LV2@30rpm				10												
visc 45°C	46	460	48	480	44	440	40	400	60	600	40	400	40	400								
visc 40°C	46	460	40	400	47	470	48	480	42	420	40	400	58	580								
visc 20°C	46	460	41	410	47	470	40	400	44	440	42	420	40	400	42	420	40	400	40	400	36	360
visc Fridge	46	460	44	440	43	430	44	440	44	440	44	440	42	420								

Table 6-4 Formulation 10.02 Viscosity Results

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.10.02						TD@5rpm			x	4000												
visc 45°C	60	240000	74	296000	76	304000	76	304000	75	300000	74	296000	75	300000								
visc 40°C	60	240000	69	276000	66	264000	66	264000	60	240000	61	244000	62.5	250000								
visc 20°C	60	240000	79	316000	79	316000	79	316000	77	308000	75	300000	69.5	278000	67.5	270000	60	240000	60	240000	64	254000
visc Fridge	60	240000	80	320000	81	324000	81	324000	80	320000	82	328000	81	324000								

The averaged result of just the deflection point readings of these 44 sets of data was given in Table 6-6 below:

Table 6-5 Total Average Deflection Point Results

Week	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	43	44	45	45	48	46	60				
Visc 40°C	43	43	45	44	45	46	57				

Visc 20°C	43	48	49	47	52	50	62	61	61	62	64
Visc Fridge	43	49	48	48	53	51	62				

As can be seen, when all the results are averaged out, there was a general trend to a viscosity increase over time, although all test points remain within the pass/fail criteria of ±15 deflection points up to and including week 12. After this the only result that stayed within the pass/fail criteria was the 16 week 40ºC sample, with all other test results being above the upper fail limit, including all the real-time 20ºC test points. This can be shown more clearly when these results are plotted on a graph of Average Viscosity against Time (Figure 6-1):

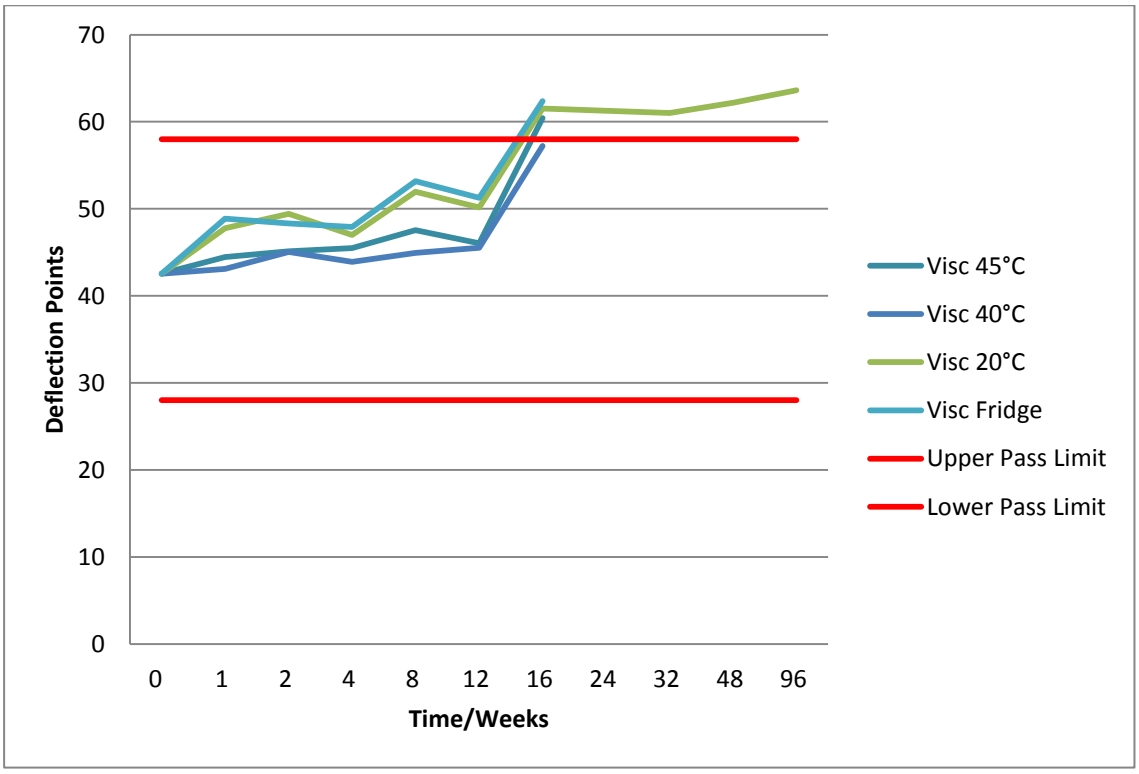


Figure 6-1 Graph of average Deflection Point results over Time

Thus the average results would be considered a test fail in industry and would therefore require a suitably qualified person to justify the advancement of these formulations to the next stage of development.

This general result of instability is supported by the finding that 35 of the 44 complete data sets had at least one result more than 15 deflection points away from the initial result in its data set, and would therefore be considered a stability test fail as demonstrated by Table 6-7.

Table 6-6 Table to show which formulations had a complete data sets for Viscosity

Emulsifier type				Anionic				Cationic		Non-ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
Percentage				1	2.5	1	3	2	4	2	4	2	4	0.75	1.5
Secondary variables		Mechanical Work (rpm)	Time (secs)												
phase ratio (W:O)															
80	20	3000	30	1.01	2.01	19.01	20.01								
		6000	30	1.02	2.02	19.02	20.02								
75	25	3000	30	3.01	4.01	17.01	18.01	23.01	24.01						
		6000	30	3.02	4.02	17.02	18.02	23.02	24.02						
70	30	3000	30	5.01	6.01	15.01	16.01		26.01	45.01	46.01	55.01	56.01	75.01	76.01
		6000	30	5.02	6.02	15.02	16.02		26.02	45.02	46.02	55.02	56.02	75.02	76.02
65	35	3000	30	7.01	8.01	13.03	14.03								
		6000	30	7.02	8.02	13.04	14.04								
60	40	3000	30	9.01	10.01	11.03	12.01				50.01		60.01		80.01
		6000	30	9.02	10.02	11.04	12.04		30.02		50.02		60.02		80.02

formulations too unstable to test/incomplete data set
formulations that fail Viscosity Stability
formulations that pass Viscosity Stability

There were nine formulations that remained within the pass/fail criteria for the duration of the test and would be considered a full pass in industry, for example formulation 4.01 results given in Table 6-8.

Table 6-7 Formulation 4.01 Viscosity Results

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.04.01						TC@5rpm			x	2000												
visc 45°C	26	52000	30	60000	31	62000	27	54000	30	60000	32	64000	31	62000								
visc 40°C	26	52000	27	54000	26	52000	25	50000	26	52000	25	50000	20	40000								
visc 20°C	26	52000	30	60000	31	62000	35	70000	35.5	71000	30	60000	31	62000	33	66000	31	62000	31	62000	31	62000
visc Fridge	26	52000	28	56000	27	54000	31	62000	31	62000	31	62000	30	60000								

These formulations were considered stable for the duration of time they were observed for, with no significant change. Their averaged results were given in Table 6-9:

Table 6-8 Average Deflection Point Result for Viscosity Stable Formulations

Week	0	1	2	4	8	12	16	24	32	48	96
visc 45°C	42.0	43.4	39.4	40.8	41.9	38.1	37.9				
visc 40°C	42.0	40.1	39.8	39.1	37.3	37.4	38.2				
visc 20°C	42.0	43.3	41.6	39.4	41.3	40.2	39.4	40.9	40.8	41.7	42.1
visc Fridge	42.0	44.1	43.2	43.9	42.8	42.3	41.6				

Table 6-9 revealed that the largest change, on average for these nine formulations, occurred in the 8th week of 40ºC testing, with an change of 4.7 deflection points from the initial. These results would appear to strengthen the case for the Accelerated Stability Model's predictive capability, however, what these nine results showed was a rate of reaction (K) for a viscosity change that is near zero for all temperature storage conditions, i.e. there was no reaction occurring at the given temperatures. If there was no reaction occurring that affects viscosity at any temperature points, then the Accelerated Stability Model was not predicting anything other than that the activation energy required for a reaction to occur has not been met at any temperature point. Hence K has not changed linearly with time, as the Accelerated Stability Model suggested, rather it was not changing at all from zero. This skewed the results in favour of a 'good' predictive model for viscosity. In the same way that formulations that were too unstable to yield useful data sets were removed from the analysis, formulations that show no change should also be disregarded when analysing the predictive capacity of the Accelerated Stability Model.

If we disregarded the nine non-reacting formulations, leaving 35 formulations that had at least one result outside the pass/fail criteria, we should achieve a more accurate representation of the Accelerated Stability Models predictive accuracy of change in viscosity. Table 6-10 gave the average deflection point result of these 35 formulations.

Table 6-9 Table of Averaged Deflection Point Results of Viscosity Unstable Formulations

Weeks	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	42.77	44.21	46.57	46.70	48.87	47.63	60.61				
Visc 40°C	42.77	43.24	46.37	45.16	46.89	47.60	63.54				
Visc 20°C	42.77	48.54	51.47	49.29	55.06	53.07	67.56	66.49	66.20	67.44	70.04
Visc Fridge	42.77	49.64	49.64	48.94	55.84	53.57	67.73				

As can be seen form Table 6-10, there was no viscosity result beyond 12 weeks at any temperature condition that had remained within the pass/fail criteria. This showed that there has been, on average, a significant change in these formulations' viscosities over the duration of the observation. The ability of the Accelerated Stability Model to predict these changes will be analysed in the discussion section below.

It was also worthy of note that of these 35 formulations, 17 failed in both accelerated and real-time testing, showing that these formulations would have been correctly stopped from progressing beyond stability testing. There were 15 formulations that failed accelerated stability testing but remained within the pass/fail criteria during real-time testing. This suggests that the Accelerated Stability Model gave false-fail results for these 15 formulations i.e. Q_{10}>2 for these 15 formulations with respect to viscosity. This would represent a waste of resource in industry, re-formulating and re-testing a formulation that was perhaps adequate for market.

Perhaps more interesting are the three for formulations that gave pass results on accelerated stability but went on to fail real-time testing – a false-pass, which shows that Q_{10}<2 for these 3 formulations. These are formulations 1.01, 1.02 and 24.01, and their results are shown in Table 6-11:

Table 6-10 Viscosity Results for Formulations 24.01, 1.02 and 1.01

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cp
	TRIAL CODE : 16900/AP.01.01					TC@5rpm				2000												
visc 45°C	33	66000	39	78000	30	60000	27	54000	26	52000	20	40000	20	40000								
visc 40°C	33	66000	44	88000	40	80000	31	62000	30	60000	23	46000	25	50000								
visc 20°C	33	66000	70	140000	77	154000	56	112000	55	110000	50	100000	54	108000	50	100000	51	102000	50	100000	51	102
visc Fridge	33	66000	70	140000	77	154000	56	112000	55	110000	50	100000	54	108000								
	TRIAL CODE : 16900/AP.01.02					TC@5rpm				2000												
visc 45°C	27.5	55000	32	64000	30	60000	27	54000	28	56000	20	40000	29	58000								
visc 40°C	27.5	55000	41	82000	40	80000	26	52000	29	58000	23	46000	20	40000								
visc 20°C	27.5	55000	56	112000	55	110000	46	92000	45	90000	50	100000	51	102000	55	110000	51	102000	56	112000	55	110
visc Fridge	27.5	55000	55	110000	55	110000	50	100000	50	100000	51	102000	53	106000								
	TRIAL CODE : 16900/AP.24.01					TB@10rpm				400												
visc 45°C	29	11600	32	12600	36	14200	36	14200	39	15400	38	15200	38	15000								
visc 40°C	29	11600	30	12000	32	12600	31	12200	30	12000	31	12200	44	17600								
visc 20°C	29	11600	31	12200	35	14000	34	13400	37	14600	39	15400	66	26400	72.5	29000	62.5	25000	50	20000	65	260
visc Fridge	29	11600	33	13200	33	13200	35	14000	36	14200	35	14000	60	24000								

If only the accelerated storage data was taken into account from these formulations, they would all have passed the Accelerated Stability Testing and then gone on to fall outside of their specification in market conditions. It would depend on the attributes of the formulation and packaging whether this unpredicted change in viscosity would have a detrimental effect on the products' performance or safety.

As discussed earlier, through Stokes Law, viscosity change can be an early indicator of a structure change. This is well demonstrated by formulation 50.01 when the viscosity and appearance data is viewed together in Table 6-12 and Table 6-13.

Table 6-11 Viscosity Results for Formulation 50.01

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.50.01						LV2@6rpm				50												
visc 45°C	62	3100	63	3150	66	3300	31	1550														
visc 40°C	62	3100	66	3300	60	3000	61	3050	40	2000												
visc 20°C	62	3100	61	3050	66	3300	63	3150	63	3150	61	3050	77	3850	72	3600	73	3650	73	3650	70	3500
visc Fridge	62	3100	66	3300	63	3150	63	3150	61	3050	60	3000	77	3850								

Table 6-12 Appearance Results for Formulation 50.01

Formulation No 50.01

weeks	0	1	2	4	8	12	16	24	32	48	96
Appearance 45°C	0	0	0	3	5	5	5				
Appearance 40°C	0	0	0	0	3	5	5				
Appearance Dark 20°C	0	0	0	0	0	0	0	0	0	0	0

As can be seen form Table 6-12 and Table 6-13, formulation 50.01 appearance had a severe change for separation at eight weeks for 45ºC and 12 weeks at 40ºC. These changes are indicated in the viscosity results by a severe decrease in viscosity at four weeks in the 45ºC and eight weeks at 40ºC. In this case, the viscosity results gave an indication that the emulsion structure was about to fail. It was worth noting that in fact the emulsion did not fail on real-time testing, indicating that this result was in fact a false fail.

6.3 Viscosity Discussion

Turning first to the 44 results that had a complete data set, the averaged results are given in table 6-14:

Table 6-13 Averaged Viscosity Results with Prediction Analysis

Week	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	42.55	44.46	45.11	45.49	47.56	46.02	60.41				
Visc 40°C	42.55	43.11	45.03	43.91	44.92	45.52	57.20				
Visc 20°C	42.55	47.76	49.44	46.99	51.97	50.15	61.51	61.25	61.02	62.18	63.63
Visc Fridge	42.55	48.89	48.32	47.92	53.17	51.26	62.39				
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				3.88	6.93	5.04	17.60	15.76	16.10	16.66	3.22
Average ASM Prediction Error									10.65		

The Average Prediction Error was 10.65 against a maximum target of 10, which showed that the Accelerated Stability Model was just outside the threshold for a precise prediction. The Prediction Error Range was 14.38 against a maximum target of 15, which showed the Accelerated Stability Model was just inside the threshold form an accurate prediction of viscosity changes.

Plotting the Prediction Error against time in Figure 6-2 showed that the Accurate Prediction Threshold was just 12 weeks, well short of the 96-week result that the Accelerated Stability Model needed to justify its use in the Cosmetics Industry:

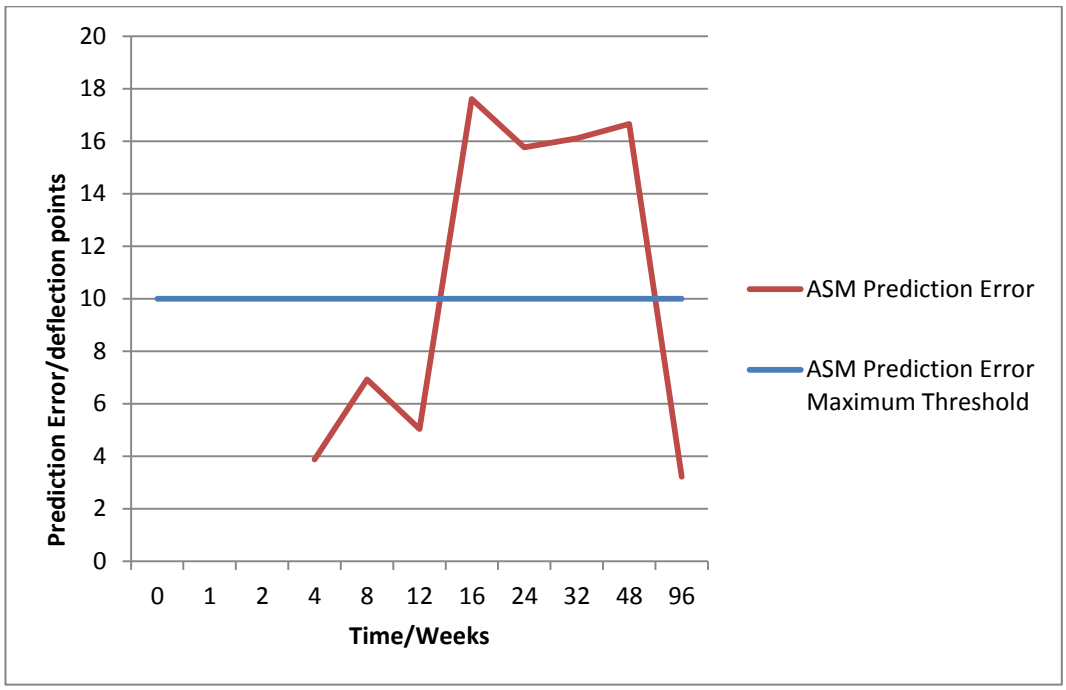


Figure 6-2 Plot of Average Prediction Error against Time for all Formulations

However, despite this data showing that the Accelerated Stability Model was on the threshold of being an accurate and precise predictive tool overall, this data was actually skewed towards a 'good' prediction tool due to the inclusion of nine results that did not change in either accelerated or long-term storage conditions as detailed in the Results Section. There were 35 of the 65 formulations made that were stable enough to have complete data sets but still had at least one result that fell outside of the pass/fail criteria. Taking only this data forwards allowed for the analysis of how accurately the Accelerated Stability Model predicted these changes, which will be the focus of this section.

The averaged results of these 35 formulations are given in Table 6-15:

Table 6-14 Average Deflection Point results for Viscosity unstable formulations

Weeks	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	42.77	44.21	46.57	46.70	48.87	47.63	60.61				
Visc 40°C	42.77	43.24	46.37	45.16	46.89	47.60	63.44				
Visc 20°C	42.77	48.54	51.47	49.29	55.06	53.07	67.56	66.49	66.20	67.44	70.04
Visc Fridge	42.77	49.64	49.64	48.94	55.84	53.57	67.73				

There was no result beyond 12 weeks that remained within specification, as shown in figure 6-3, when these results were plotted on a graph of deflection points over time:

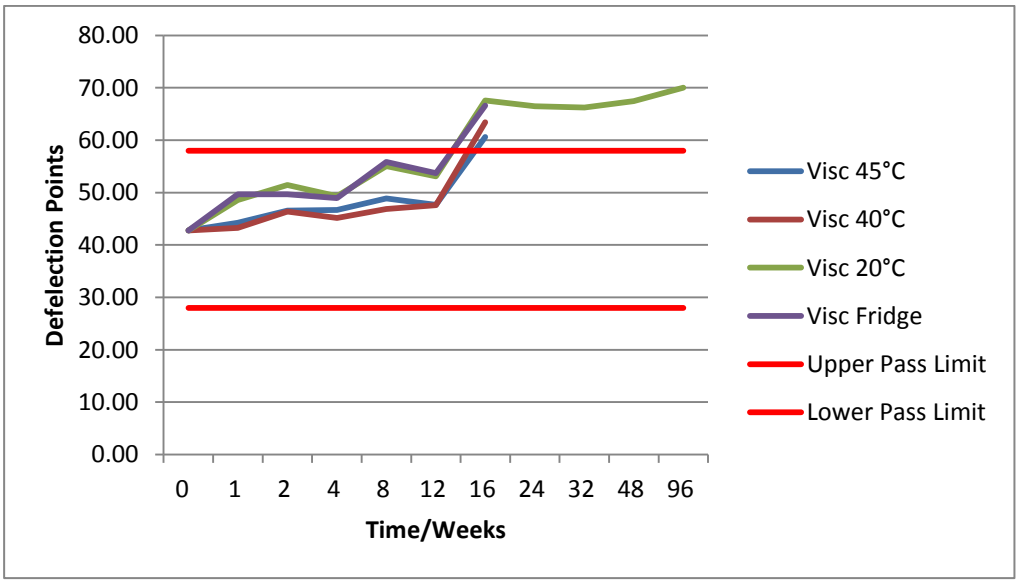


Figure 6-3 Plot of Average Viscosity Results over Time for viscosity changing formulations

Although this did reveal, as suspected, that the average viscosity for these formulations was unstable over time, it did not uncover any data on how well the Accelerated Stability Model predicted this behaviour. The data was taken on further in Table 6-16 to calculate Average Prediction Error and Prediction Error Range.

Table 6-15 Prediction Analysis of Viscosity Changing Formulations

Weeks	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	42.77	44.21	46.57	46.70	48.87	47.63	60.61				
Visc 40°C	42.77	43.24	46.37	45.16	46.89	47.60	63.44				
Visc 20°C	42.77	48.54	51.47	49.29	55.06	53.07	67.56	66.49	66.20	67.44	70.04
Visc Fridge	42.77	49.64	49.64	48.94	55.84	53.57	67.73				
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				6.04	8.69	6.50	22.40	19.79	19.31	19.84	9.43
Average ASM Prediction Error									14.00		

The Prediction Error Range across this data was 16.36, well above the threshold for a precise prediction of 15, the Average Prediction Error was 14 against the accuracy threshold target of 10. This showed that across this data the Accelerated Stability Model was, on average, neither an accurate or precise predictive tool for viscosity change.

This was verified further by plotting the Accelerated Stability Model Prediction Error against Time in Figure 6-4, to obtain the Accurate Prediction Threshold:

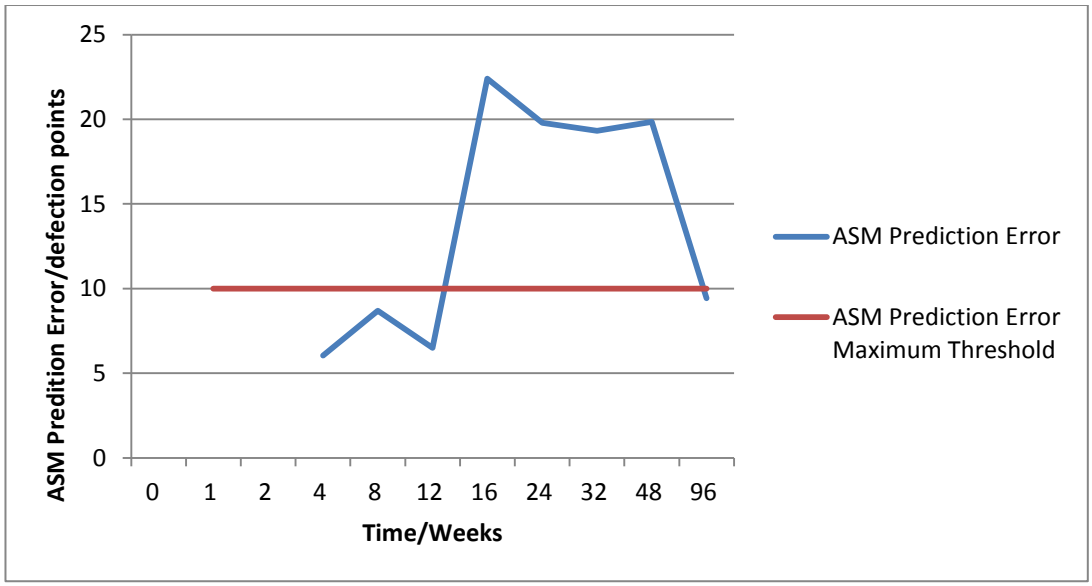


Figure 6-4 Plot of Average Prediction Error over Time for viscosity changing formulations

This graph showed that the Accurate Prediction Threshold for viscosity occurred at 12 weeks testing and at its worst had an average error of 20 deflection points, double the maximum limit for a good prediction. The Accurate Prediction Threshold of 12 weeks was well short of the 96-weeks required by the cosmetics industry to enable reliance on the Accelerated Stability Model.

It was noted that the 96-week result for Prediction Error was within the Maximum Threshold for an accurate prediction at 9.43. This result was especially unexpected given the previous four results for Prediction Error at 16, 24, 32 and 48 weeks were all around 20 (22.40, 19.79, 19.31, 19.84). The reason for this low Prediction error at 96-weeks is that there was, on average, a large increase in viscosity at the 45ºC temperature point from 12 to 16-week time points. This increase was not limited to the 45ºC storage temperature; all the temperature storage conditions saw a viscosity increase of between 13-16 deflection points at the 16-week test point from the 12-week result. As all these formulations had a similar ingredient make up, it

may have been that a common mechanism caused a viscosity increase 12 weeks after product manufacture. This mechanism's activation energy must have been reached below 4ºC, as all the temperature points displayed this increase in viscosity. After this increase, the 20ºC average viscosity plateaued for the remainder of the real-time test period, only increasing from 67.56 deflection points at the 16-week test to 70.04 deflection points at the 96-week test. As the 16-week 45ºC result was a prediction of the real-time 96-week result, the plateau in viscosity at 20ºC meant that the 96-week prediction was much closer to the Accelerated Stability result than the predictions for 24, 32 and 48-weeks which were predicted by results before the 16 week test. A typical example of a formulation that displayed this behaviour was 24.02 given in Table 6-17:

Table 6-16 Viscosity Results of Formulation 24.02

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.24.02						TB@20rpm				200												
visc 45°C	39	7800	44	8800	44	8800	44	8800	42	8400	44	8800	93	18600								
visc 40°C	39	7800	38	7600	43	8600	40	8000	41	8200	40	8000	84	16800								
visc 20°C	39	7800	39	7800	41	8200	44	8800	43	8600	44	8800	82	16400	80	16000	81	16200	82	16400	83	16600
visc Fridge	39	7800	40	8000	41	8200	41	8200	40	8000	41	8200	60	12000								

It could be seen that the 16-week increase in viscosity was not modelled by the Accelerated Stability model; if it had been, the 40ºC and 45ºC results would have shown similar increases at four weeks testing point. Whatever the mechanism causing this increase in viscosity was, its rate was not dependent on the temperature, i.e. its rate was zero order with respect to temperature, and therefore was a poor fit to the Accelerated Stability Model as it relies on first order rate kinetics. Therefore, for this set of data, it can be said that the Accelerated Stability model was a poor model of viscosity behaviour.

Whilst the Accelerated Stability Model was poor for this data, in industry, a formulator presented with the averaged results above would at least have seen, albeit at the very last test week, that the viscosity jumped after the week 12 results. This may have allowed them to assess the formulation's new increased viscosity's suitability for market before allowing the formulation to proceed onto the next developmental stage. This was more by fortune than scientific principle - if the increase in viscosity had occurred at 16-20 weeks instead of 12-16, the accelerated data would not have shown an increase and the formulation may have reached market place without the knowledge that the viscosity increases. In the worst case,

this could have led to a market recall of the product at huge expense to the brand owner.

In addition to the general poor predictive capacity of the Accelerated Stability Model for viscosity there were a few sets of results that could be significant and were worth some further analysis.

Firstly, the three formulations passed accelerated stability conditions but went on to fail real-time testing showing Q_{10}<2 for these formulations– the so-called false pass result. Table 6-18 gave formulation 1.01 and 1.02's results together as they were the same formulation with only manufacturing method differences and they displayed very similar behaviour:

Table 6-17 Viscosity Results for formulations 1.02 and 1.01

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
	TRIAL CODE : 16900/AP.01.01					TC@5rpm				2000												
visc 45°C	33	66000	39	78000	30	60000	27	54000	26	52000	20	40000	20	40000								
visc 40°C	33	66000	44	88000	40	80000	31	62000	30	60000	23	46000	25	50000								
visc 20°C	33	66000	70	140000	77	154000	56	112000	55	110000	50	100000	54	108000	50	100000	51	102000	50	100000	51	10200
visc Fridge	33	66000	70	140000	77	154000	56	112000	55	110000	50	100000	54	108000								
	TRIAL CODE : 16900/AP.01.02					TC@5rpm				2000												
visc 45°C	27.5	55000	32	64000	30	60000	27	54000	28	56000	20	40000	29	58000								
visc 40°C	27.5	55000	41	82000	40	80000	26	52000	29	58000	23	46000	20	40000								
visc 20°C	27.5	55000	56	112000	55	110000	46	92000	45	90000	50	100000	51	102000	55	110000	51	102000	56	112000	55	11000
visc Fridge	27.5	55000	55	110000	55	110000	50	100000	50	100000	51	102000	53	106000								

Table 6-18 data appeared to show that the ambient and fridge temperature storage conditions result in a building of viscosity to outside the pass/fail criteria after 1 week and the new viscosity was then fairly consistent for the remainder of the test. However, the elevated storage condition samples never received this initial viscosity increase and stayed within the pass/fail criteria for the duration of the 16-week test. Averaging these results and applying the Prediction Error Analysis was shown in Table 6-19:

Table 6-18 Average Viscosity Results for Formulations 1.01 and 1.02 with Prediction Error Analysis

Weeks	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	30.25	35.50	30.00	27.00	27.00	20.00	24.50				
Visc 40°C	30.25	42.50	40.00	28.50	29.50	23.00	22.50				
Visc 20°C	30.25	63.00	66.00	51.00	50.00	50.00	52.50	52.50	51.00	53.00	53.00
Visc Fridge	30.25	62.50	66.00	53.00	52.50	50.50	53.50				
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				8.50	10.00	20.00	24.00	25.50	21.50	30.00	28.50
Average ASM Prediction Error									21.00		

This showed the Average Prediction Error of 21 against a target of below 10, a poor result for accuracy, and a Prediction Error Range of 21.5 against a maximum target of 15, a poor result for precision. Plotting the Prediction Error against time in Figure 6-5 gave the Predictive Error Threshold:

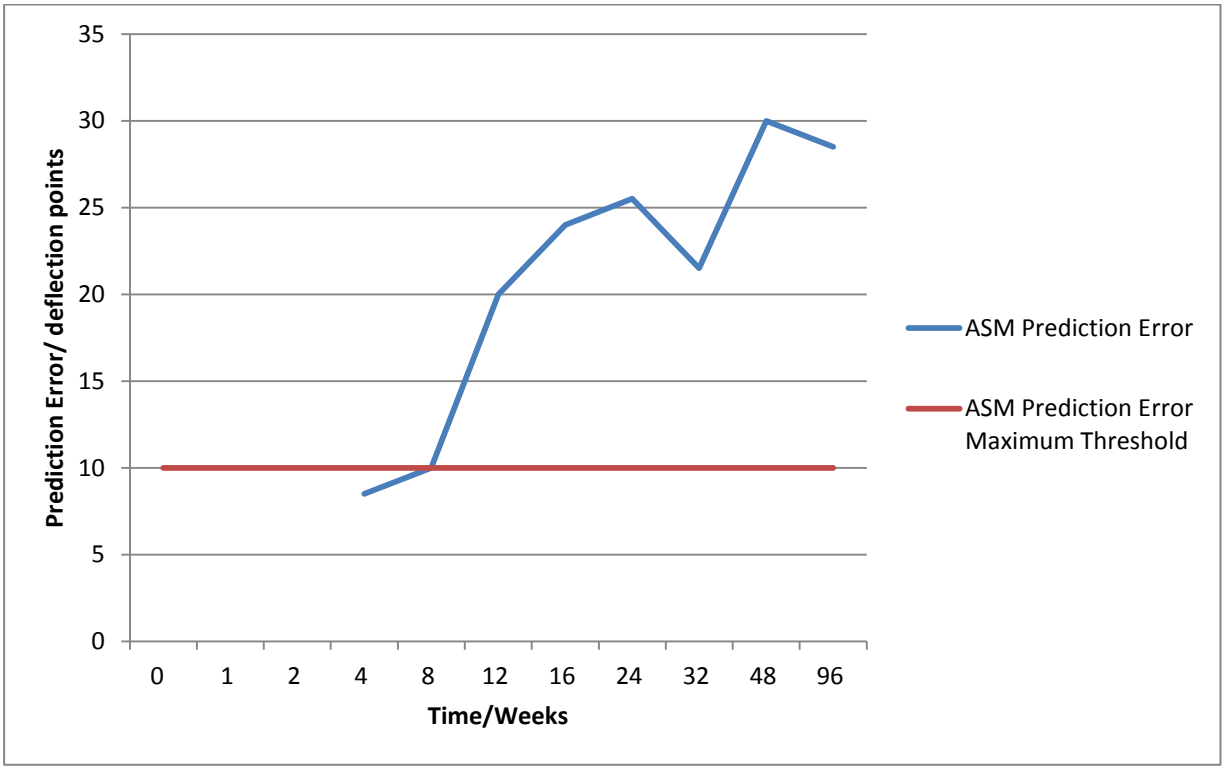


Figure 6-5 Plot of Average Prediction Error against Time for Formulations 1.01 and 1.02

Figure 6-5 showed that these two formulations had an Accurate Prediction Threshold of just eight weeks. Beyond this time the Accelerated Stability Model was neither an accurate or precise tool to predict viscosity behaviour for these formulations. More importantly the results stayed within specification under accelerated conditions and fell outside of specification in real-time testing – a false-pass. In industry, it could be argued that the fact the viscosity changes to outside of specification after 1 week at ambient temperatures would have given the formulator enough information to analyse the data and possibly stop this formulation progressing if this increase was inappropriate. The same cannot be said for the results of formulation 24.01 shown in Table 6-20.

Table 6-19 Viscosity Results for Formulation 24.01

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
	TRIAL CODE : 16900/AP.24.01					TB@10rpm				400												
visc 45°C	29	11600	32	12600	36	14200	36	14200	39	15400	38	15200	38	15000								
visc 40°C	29	11600	30	12000	32	12600	31	12200	30	12000	31	12200	44	17600								
visc 20°C	29	11600	31	12200	35	14000	34	13400	37	14600	39	15400	66	26400	72.5	29000	62.5	25000	50	20000	65	26000
visc Fridge	29	11600	33	13200	33	13200	35	14000	36	14200	35	14000	60	24000								

As can be seen from these results in Table 6-20, all the accelerated results for formulation 24.01 stayed within specification for the duration of their testing and, unlike 1.01 and 1.02, the ambient and fridge sample also stayed within specification until week 16 of testing. Further predictive analysis of these results was given in Table 6-21:

Table 6-20 Viscosity Results with Prediction Error Analysis for Formulation 24.01

Weeks	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	29	32	36	36	39	38	38				
Visc 40°C	29	30	32	31	30	31	44				
Visc 20°C	29	31	35	34	37	39	66	73	63	50	65
Visc Fridge	29	33	33	35	36	35	60				
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				3.50	5.00	3.00	35.50	37.00	32.50	19.50	27.50
Average ASM Prediction Error									20.44		

This data had the highest Prediction Error Range of any of the data sets so far with 33.50 against a maximum accuracy target of 15 and an Average Prediction Error of 20.44 against a maximum precision target of 10. It could also be seen that the Accurate Prediction Threshold was just 12 weeks as shown in Figure 6-6:

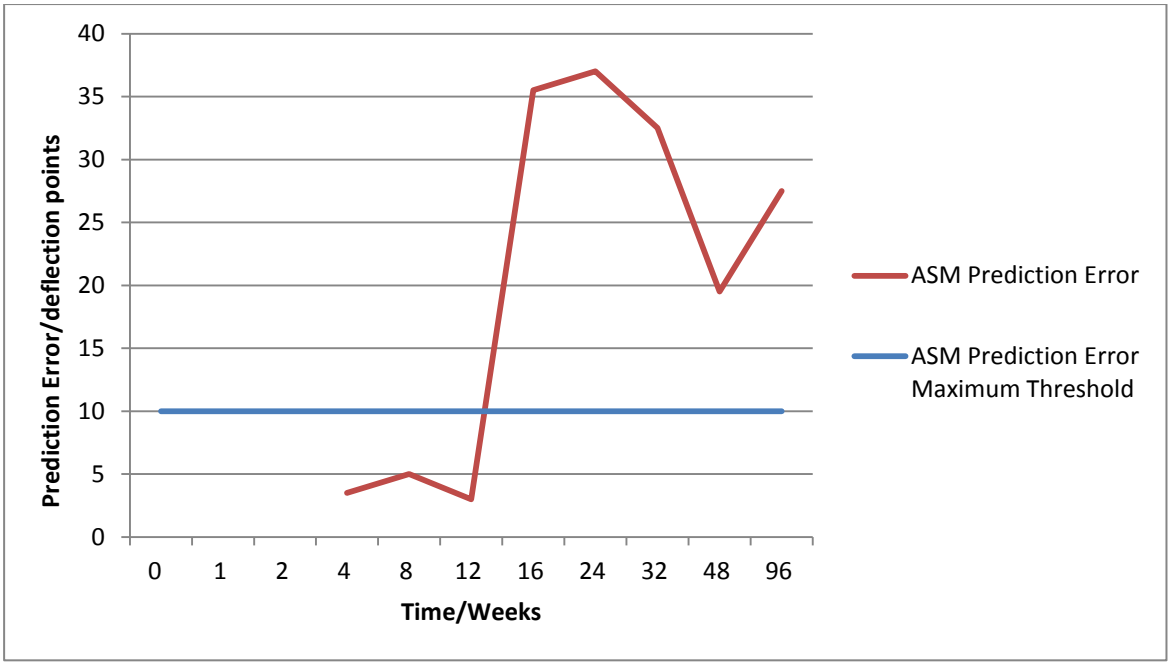


Figure 6-6 Plot of Prediction Error against Time for Formulation 24.01

However, perhaps more significant was that, given these results show Q10<2 for these formulations, in industry the accelerated data would not have stopped a formulation from advancing to the next stage of development, when in fact it may have been inappropriate to do so with an unpredicted, significant increase in viscosity occurring at ambient temperature. Although there was an increase at ambient temperature at 16 weeks, it was debatable whether this would have stopped the development, given the late stage of testing. In the worst case, this could have led to a market recall of the product at huge expense to the brand owner.

These three formulations, 1.01, 1.02 and 24.01, were a significant difference from previous parameter results where the Accurate Prediction Threshold result was less than 96 weeks. In those cases the elevated temperatures showed a change that was not reflected in long-term storage conditions, resulting in reformulation and retesting – a false-fail. Whilst this represented a waste of resources it could be considered prudent to be overcautious with brand reputation, customer safety and financially. Here however, the elevated temperature results did not show a change that happened in real-time – i.e. Q_{10} is less than 2 - a false pass. This represented a potential financial and reputational liability to the brand and consumer if the formulation had ever reached the market place.

Secondly, whilst the above results represented poor modelling of viscosity change, there were some results that seemed to show that the viscosity was affected

differently by storage at different storage conditions. For example formulation 9.01 are shown in Table 6-22 below:

Table 6-21 Viscosity Results for Formulation 9.01

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.09.01						TD@5rpm		factor		4000												
visc 45°C	46	184000	26	104000	28	112000	21	84000	20	80000	21	84000	20	80000								
visc 40°C	46	184000	28	112000	26	104000	20	80000	20	80000	22	88000	20	80000								
visc 20°C	46	184000	45	180000	62	248000	60	240000	64	256000	60	240000	62	248000	63	252000	52	208000	50	200000	63	252000
visc Fridge	46	184000	50	200000	50	200000	50	200000	55	220000	67	266000	61	244000								

This data showed the accelerated results decreasing whilst the ambient and fridge samples were increasing throughout the test:

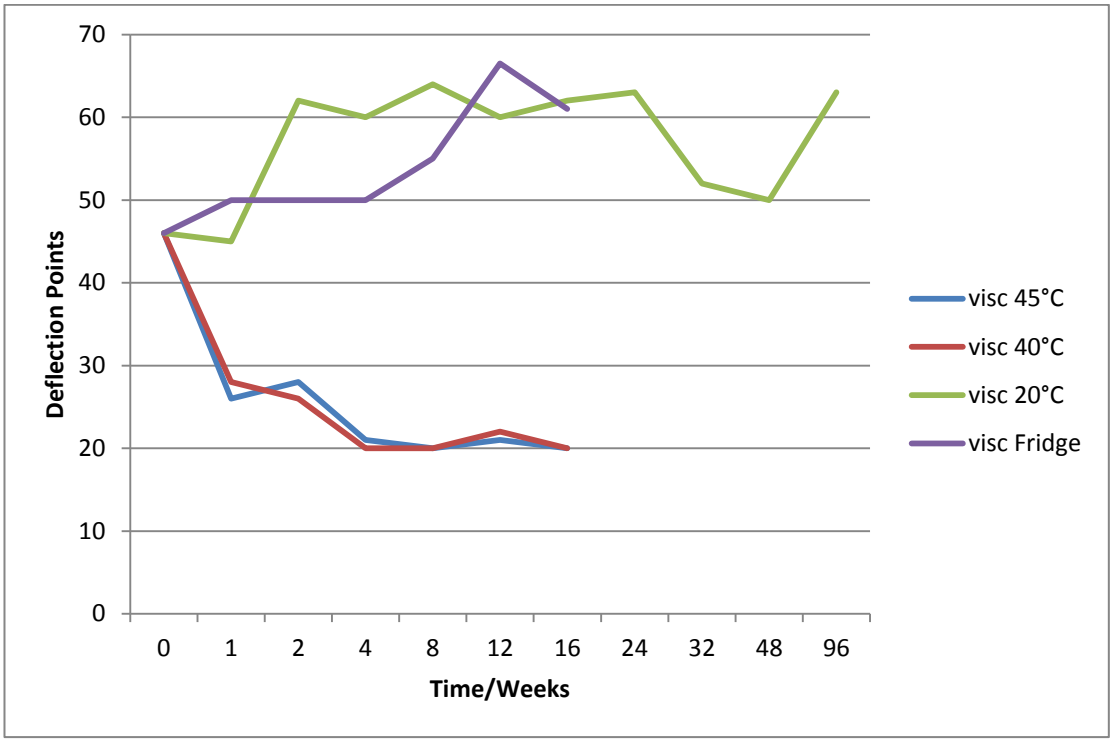


Figure 6-7 Plot of Viscosity over Time for Formulation 9.01

Table 6-22 and Figure 6-7 obviously indicated poor modelling by the Accelerated Stability Model, but also suggested that there was a mechanism occurring at elevated temperatures that was decreasing the viscosity. The data seemed to suggest that this mechanism had an activation energy between 20ºC and 40ºC, which resulted in the diverging results seen in Figure 6-7.

There was an even more interesting set of results given in Table 6-23 which gave formulation 56.02's results and extended the above point:

Table 6-22 Viscosity Results for Formulation 56.02

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.56.02						TB@20rpm			x	200												
visc 45°C	37	7400	20	4000	21	4200	20	4000	22	4400	20	4000	23	4600								
visc 40°C	37	7400	50	10000	50	10000	50	10000	50	10000	50	10000	93	18500								
visc 20°C	37	7400	40	8000	41	8200	44	8800	43	8600	40	8000	98	19500	95	19000	95	19000	97	19400	98	19600
visc Fridge	37	7400	50	10000	41	8200	39	7800	38	7600	33	6600	100	20000								

In Table 6-23 the fridge, 20ºC and 40ºC samples all increased viscosity slightly before the characteristic 16-week jump increased, while the 45ºC sample decreased viscosity and did not show the rapid increase from 12-16 weeks as illustrated in Figure 6-8.

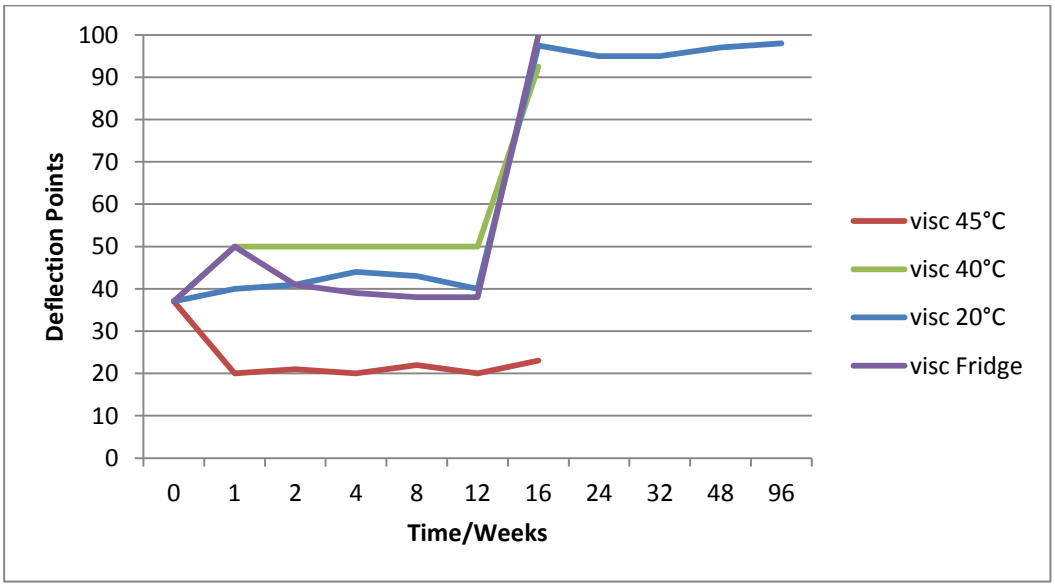


Figure 6-8 Viscosity Results over Time for Formulation 56.02

Similarly to formulation 9.01, there was a mechanism that was decreasing the viscosity in elevated temperatures and not in lower temperature storage conditions. In this case, however, the activation energy seemed to be reached between 40ºC and 45ºC. This resulted in divergence of viscosity behaviour between the storage temperature conditions, and a very poor prediction of viscosity behaviour by the Accelerated Stability Model.

6.4 Viscosity Conclusion

The results suggested that, in general the Accelerated Stability Model was neither an accurate nor precise predictive tool for viscosity behaviour over time for the 35

that showed a significant viscosity change in at least one test point as summarised in Table 6-24.

Table 6-23 Summary of Prediction Analysis for Viscosity

	Target	Viscosity Changing Formulations
Average Prediction Error	10	14.00
Prediction Error Range	15	16.36
Accurate Prediction Threshold	>96 weeks	12 weeks

A possible explanation of this was that viscosity changes had not been caused by molecular interactions, but rather the interactions and characteristics of the internal phase droplets. This type of interaction was not what the Arrhenius equation was derived from (Arrhenius 1889), and hence it should be no surprise that this type of parameter was poorly modelled by the Arrhenius equation.

Although the viscosity behaviour was poorly predicted by the Accelerated Stability Model, it could be argued that as long as the model achieved correct the pass/fail results correct for accelerated and real-time results the model would have worked by preventing an unpredicted change happening in real time. The logic being that the model may sometimes stop a formulation from proceeding that may have been acceptable for market with a false-fail result (accelerated pass, real-time fail) but a false-pass result (accelerated pass, real-time fail) would expose the brand and possibly the public to formulation liability.

In the 35 data sets there were 17 results that failed both accelerated and real-time testing, which showed that the model worked adequately in these cases. There were a further 15 (43%) that failed accelerated stability testing but did not fail real-time testing, the so-called false-fail showing $Q_{10}>2$ for these formulations. In industry, this 43% of formulations, unless the results were accounted for by a qualified person, would have been reformulated and retested unnecessarily. This represented a large potential waste in resource and highlighted an area where there was a deficit in the knowledge of cosmetic formulation. Although as detailed above, this was over-cautious by the Accelerated Stability Model and prevented any risk to the brand owner or public. However, in these viscosity results there were three cases out of the 44 complete data sets that could be considered false-passes, showing that $Q_{10}<2$ for these formulations. This was 6.8% of the sample size and if reflected in the wider industry represents a huge number of formulations that may have behaved

differently than expected by a qualified person when assessing the safety or quality of the product. It would have depended upon the products' specific attributes as to whether this unpredicted behaviour would have represented an efficacy, safety or quality problem.

The viscosity data also highlighted the problem of activation energy within the Accelerated Stability Model. If there was a reaction taking place that had an activation energy achieved between the various storage conditions, the Accelerated Stability Model could no longer have predicted behaviour, as it relies on linear reaction rate change with temperature, or first order rate Kinetic with respect to temperature. In this data, this manifested itself in a viscosity reducing mechanism progressing at elevated temperatures and not at ambient and fridge conditions. This phenomenon may not be limited to just viscosity in the wider industry, but within these formulations the viscosity results were the only parameters that showed this behaviour.

6.5 Viscosity Evaluation

There were three areas that were identified as methodology improvements or areas for future study.

Firstly, it had been decided that the method used to measure viscosity should be the same as the method used to measure viscosity in industry. That is, at the initial reading the spindle used and the speed of rotation was set to give a reading with the viscometer's most accurate range of 30-70 deflection points. This was referred to as a single point test as the viscosity was taken from a single shear rate (rotational speed) and shear stress (spindle surface area). This spindle and speed setting was recorded with the result and was used again in subsequent readings for that formulation so that results could be directly related and compared to the initial result. However, whilst this did enable direct comparison, perhaps a more useful measurement would have been a multi-point viscosity test to obtain a viscosity profile of each formulation at each test point. A multi-point test would have measured each sample's viscosity at a range of rotational speeds or shear rates. With this data a viscosity curve could have been obtained at a given shear stress (spindle). For further information, the spindle could then have been changed and the process of taking measurements at different rotational speeds repeated. This data would have enabled analysis of the flow characteristics of the formulation that a

single point test did not. For example, if viscosity drops with increasing shear rate, the flow is described as pseudoplastic or shear thinning:

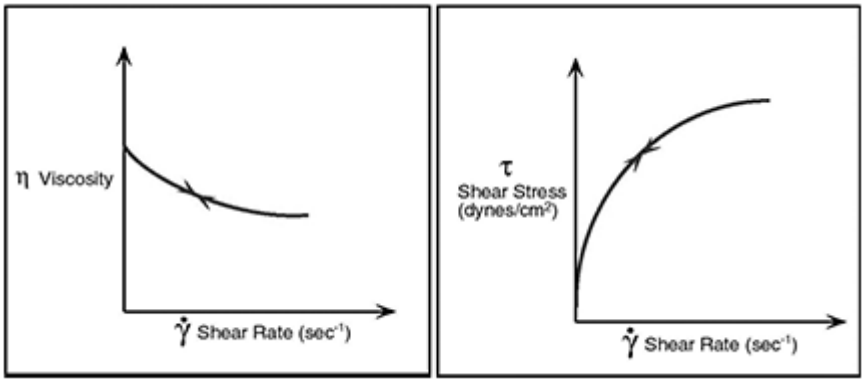


Figure 6-9 Relationship between Viscosity and Shear Rate and Shear Stress to show Pseudoplastic Behaviour

This gave an indication of how a formulation may feel under the high shear conditions of being spread on the skin or hair. It might be the case that formulations changed their viscosity profile over time or at elevated storage conditions and thus also the formulation's feel when being applied to the skin. Any such change would not have been picked up by a single point test but would be if full viscosity profiles were performed on each sample at each test point.

A possible area of further study would be to take the formulations that had complete data sets in this study and repeat the storage with complete viscosity profiles taken on each test point. It would be interesting to see if any of the formulations that showed no change during the single point testing of this study, would show a change in viscosity profile during the accelerated or real time testing and whether the Accelerated Stability Model predicted this profile change.

Secondly, and perhaps an extension to the problems with single point testing above, there were instances during this study where a viscosity increase meant the spindle and speed setting gave a reading off the 0-100 deflection point scale. This gave a problem as, in order to relate and average many different formulation results, the deflection point readings rather than absolute viscosity were used as a comparison tool. In order to record the increased viscosity reading, not just a deflection point of 100, the decision was made to keep shear rate the same and change the spindle (shear stress) to get a higher deflection point multiple which obtained an absolute viscosity reading. This absolute reading was then divided by the original deflection point multiple to give a deflection point reading above 100. For example formulation 55.01 results were given in Table 6-25.

Table 6-24 Viscosity Results for Formulation 55.02

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
TRIAL CODE : 16900/AP.55.02						TB@20rpm			x	200				TC@20	x	500						
visc 45°C	33	6600	33	6600	39	7800	40	8000	41	8200	43	8600	135	27000								
visc 40°C	33	6600	30	6000	30	6000	31	6200	30	6000	32	6400	125	25000								
visc 20°C	33	6600	31	6200	33	6600	33	6600	32	6400	30	6000	30	6000	31	6200	30	6000	30	6000	30	6000
visc Fridge	33	6600	33	6600	33	6600	39	7800	38	7600	31	6200	48	9500								

In this case, the 45ºC and 40ºC readings at 16-weeks were giving a reading of 'off the scale' for the original spindle and speed of TbarB @ 10rpm, which was a multiplier of 200. So the spindle was changed to TbarC, smaller surface area, while the rotational speed was kept the same, which gave a new multiplier of 500. A deflection point reading of 54 and 50 gave an absolute viscosity value of 27,000 cps and 25,000 cps. This figure was then divided by the original setting's multiplier of 200 to give an approximate deflection point value of the original spindle and speed setting. In this case the approximate deflection point values were 135 and 125 respectively, which of course were not possible deflection point readings but did enable these results to be compared to the other results in this data set. Where this technique was employed, the results are highlighted in orange and the alternative spindle and speed settings noted next to the original.

Whilst this assumption was reasonable, the magnitude of the approximation was unknown as the viscosity profiles of the formulations had not been taken. Had the profile been recorded, it would have been possible to show how much going from one shear stress viscosity curve to another would have changed the viscosity reading. Therefore, similarly to the single-point viscosity reading problem mentioned above, a solution to this problem would be to do full viscosity profiles at each time point. As a minimum, 2-3 different viscometer settings should be used to make sure that at least one of these settings remains within the deflection point range. This is, of course, difficult because at the beginning of the trial it is impossible to know how much the viscosity is going to increase or decrease.

Thirdly, the nature of the formulation make-up was very similar across all the formulations. The only difference across the 65 samples made was that six different emulsifiers were used. The other seven chemicals remained the same, albeit with the oil phase ratio and emulsifier concentration changing from formulation to formulation. This may have led to characteristic behaviour of these formulations being repeated again and again. For example, as was highlighted in the discussion section, in this data set there was a repeated behaviour of viscosity increase at all temperature conditions between 12 and 16 weeks testing. This increase occurred in

at least nine of the 35 complete data sets and affected the averaged data from which the conclusions were drawn (formulations 8.01, 24.01, 24.02, 26.02, 46.02, 55.01, 55.02, 56.01 and 56.02). It was noted that all of these formulations, although have different emulsifiers, have a similar size oil phase ratios as shown in Table 6-25 Table highlighting formulations that showed large jump in viscosity between 12-16 weeks.

Table 6-25 Table highlighting formulations that showed large jump in viscosity between 12-16 weeks

Emusion type				Oil in water emulsions											
Emulsfier type				Anionic				Cationic		Non-Ionic				Polymer	
Emulsifier				Sodium Steroly Glutamate (emulgin SG) (HLB = 23)		Glyceryl stearate SE (Cutina GMS SE) (HLB=18)		Behentrimonium Methosulfate and Cetyl Alcohol and Butylene Glycol (Incroquat Behenyl TMS-50)		PEG-100 Stearate and Glyceryl Stearate (Emugade 165) (HLB = 19)		Cetearyl Glucoside + Cetearyl alcohol (Tego Care CG90) (HLB = 11)		Sodium Polya (Cosmedia	
percentage				1	2.5	1	3	2	4	2	4	2	4	1	
Secondary variables		Mechanicl Work	Time (secs)												
80	20	3500	30	1.01	2.01	19.01	20.01								
		7500	30	1.02	2.02	19.02	20.02								
75	25	3500	30	3.01	4.01	17.01	18.01	23.01	24.01						
		7500	30	3.02	4.02	17.02	18.02	23.02	24.02						
70	30	3500	30	5.01	6.01	15.01	16.01		26.01	45.01	46.01	55.01	56.01	75.01	
		7500	30	5.02	6.02	15.02	16.02		26.02	45.02	46.02	55.02	56.02	75.02	
65	35	3500	30	7.01	8.01	13.03	14.03								
		7500	30	7.02	8.02	13.04	14.04								
60	40	3500	30	9.01	10.01	11.03	12.03				50.01		60.01		
		7500	30	9.02	10.02	11.04	12.04				50.02		60.02		

So, this viscosity behaviour may be inherent to the oil phase used in this study at specific phase ratios. This behaviour is not unusual as emulsions can change the lipid wax structure over time which builds viscosity, especially if there is no shear stress applied over time (Haj-shafiei et al. 2013).

A possible solution or area for further study to address this could be to use more emulsifiers and a wider range of formulation ingredients to create a bigger data set from which to draw conclusions. It should also be noted that it is common in industry to use gelling agent such as polymers and gums in order to adjust the product viscosity to the desirable level. This is another possible avenue of future research: by keeping all the other formulation parameters constant and adjusting the viscosity with various gelling agents, to investigate whether this improved or worsened the accuracy of the Accelerated Stability Model for a given gelling system.

Chapter 7 Digital Microscopy and Zeta Potential

Optical digital microscopy is the digital processing of an image collected using an optical microscope. It has the ability to give a lot of information about the internal structure of an emulsion. Most relevant for this study was to obtain information on the size of oil droplets created for each emulsion, given as droplet area, and then monitor the change in droplet size over time at different temperature storage points. This would give data on how fast droplets are coalescing and therefore emulsion stability. As well as this, a change in the range of droplet size can also give an indication of rate of disproportionation; large droplets getting larger and small droplets getting smaller as the internal phase migrates through the continuous phase driven by internal phase pressures.

Optical microscopy also allows the observation of the emulsion to investigate any unintended emulsion behaviour or unexpected structural changes.

7.1 Digital Microscopy - Method

7.1.1 Microscopy Equipment

- Keyence VHX 9000-F Series Digital Microscope with 250-2500x lens.
- Microscope slide and cover slips.

Images of this equipment can be found in Appendix 5.

7.1.2 Microscopy Sample Preparation

- The sample temperature was checked to be 25ºC +/- 1ºC
- Sample applied to microscope slide and cover with cover slip.
- Pressed cover slip to thin the sample until transparent.

7.1.3 Microscopy Sample Testing Method

- Digital Microscope screen turned in at the back and front.
- Checked correct lens is attached.
- Turned microscope on at the back – ensured plate was at the highest position and black tile was facing up.

- Pop up dialogue box asked if you want to initialise the XY stage –clicked Yes.
- Once initialised, turned the black tile over to white side.
- Placeed sample slide on the observing stage, set magnification to 500x on the lens.
- Clicked ‘Easy mode’ and selected focus – auto focus – execute. Microscope automatically focussed lens onto sample.
- A pop-up box will appeared asking if you would like to focus on something specific. Moved the green square to appropriate site and click OK then exit.
- If there were too many droplets in the field of vision to get a good focus and droplet separation, magnification was reset to 1000x.
- Clicked ‘Measure’ on vertical side bar and selected Auto Area Measure .
- Selected ‘Brightness’ and clicked measure.
- Four images were displayed, selected the one that has most completed circles (sensitivity was adjusted using slide bar). Selected ‘next’.
- Clicked ‘invert image’ (this gave the internal droplet areas).
- Clicked ‘eliminate grains’
- Pop up box – selected ‘remove large grains’ – used graph sizing distribution.
- Selected remove small grains – removed grains less than 2.5µm.
- Clicked delete tool and removed any areas that are not single droplets.
- Clicked ‘next’ – a table of results, histogram and list of extracted areas was created.
- Clicked ‘measurement item setting’ – added ‘circularity’ to table. Pressed ‘OK’.
- Opened table of results – checked at least 300 items have been measured.
    - If not, found a different area of the sample to measure and repeat procedure of focussing the lens onto the sample.
    - If so, clicked ‘save as CSV’ and saved image as required.

7.1.4 Microscopy Pass/Fail Criteria

The pass/fail criteria for a cosmetic product are dependent on the change affecting safety or the ability of the consumer to notice a difference. In the case of microscopy, neither of these criteria are affected as the size of internal droplets does not affect safety nor is it detectable by a consumer. An increase in droplet size is

only detectable to a consumer when it manifests itself into a change of appearance or viscosity, which has already been discussed in Chapters 5 and 7 respectively. Hence, in industry, microscopy is not routinely performed during stability testing protocols. It is sometimes used as a quality assurance tool during manufacturing scale-up to prove that the manufacture of a new formulation on the industrial scale has achieved the same droplet size as was seen on lab-scale batches. In this way, any testing data received for the lab-scale batch, be it stability, efficacy or organoleptic results, can be said to be relevant for the industrial scale manufactured bulk as well.

Therefore, microscopy does not have specific pass/fail criteria, as no microscopy result on its own would result in a failed stability test. Instead, it should be viewed in combination with the appearance and viscosity results, to see if a change in droplet size is an early indication of future changes in macro parameters. Any such changes, however, should still be predicted by the Accelerated Stability Model, and therefore results of the real-time testing can be compared directly to the accelerated test results as an assessment of the model's accuracy.

Many parameters can be measured by digital microscopy, but for the purposes of this research the parameters that were recorded were area (μm^2) as a measure of coalescence, and maximum and minimum diameter (μm) to give an indication of any disproportionation occurring. These parameters are given in Table 7-1:

Table 7-1 Microscopy measurement parameters

	Area	Unit	Perimeter	Unit	Max diameter	Unit	Min diameter	Unit	Circularity
Average Droplet		μm²		μm		μm		μm	

For the purposes of this study, where a change in droplet size is the crucial parameter, the value for droplet area is the most significant measurement and this parameter was the one taken forward to analysis. As all emulsions start from a different initial droplet size, direct comparison of results from one emulsion to the next could have given misleading results. To address this issue, results for droplet area were also expressed as percentage change from initial result.

To assess the Accelerated Stability Models predictive capacity, two new parameters have been developed, designated the Average Prediction Error and Prediction Error Range. These were calculated by comparison of the values given by the Accelerated Stability Model and the real-time values that they predicted as shown in Table 7-2:

Table 7-2 Comparable results of Accelerated Stability Model and Prediction Error

Weeks	0	1	2	4	8	12	16	24	32	48	96
Visc 45°C	initial		C	E			H				
Visc 40°C	initial	A	B	D	F	G					
Visc 20°C	initial			A1	B1	C1	D1	E1	F1	G1	H1
Visc Fridge	initial										
The colours indicate values that should be similar if the Accelerated Stability Model is accurate											
ASM Prediction Error				A-A1	B-B1	c-C1	D-D1	E-E1	F-F1	G-G1	H-H1
Average ASM Prediction Error									Average Prediction Error		

The difference in these values was then calculated to give the prediction error at each time point. The difference from the largest and smallest prediction error value is the prediction error range, and gives an indication of the Accelerated Stability Model's precision. The average of the prediction errors can be calculated to give the average prediction error, and gives an indication of the Accelerated Stability Model's accuracy. This gives two indicators of how well the Accelerated Stability Model predicts the long term stability of a product, with a lower value showing a better predictive capacity of parameter changes. These values can be given for individual formulations' or a group of formulations' averaged results, to give a broader quantification of the Accelerated Stability Method's accuracy and precision.

A plot of Accelerated Stability Model's Prediction Error against the time in weeks predicted also rendered a graph which showing when the Accelerated Stability Model became inaccurate. This showed at what time-point the predictive data became inaccurate when compared to real-time data, designated the Accurate Prediction Threshold. For justification of use to assure cosmetic product's long-term stability, the cosmetics industry requires the Accurate Prediction Threshold to be equal to, or greater than, 96 weeks.

For the purposes of this study an Average Prediction Error of less than 25% would be considered an accurate prediction, and a Prediction Error Range of less than 40% was considered a precise prediction.

7.1.5 Microscopy Results

To view all the results in full, refer to Appendix 4 – Microscopy Results.

As discussed in previous chapters, of the 65 formulations made for this study 16 were not stable enough to reach the week 1 testing point in all conditions. These

formulations yielded no microscopy data as their structure changed fundamentally before any microscopy images were taken, thus these 16 were removed from the results for microscopy.

Each reading was accompanied by the ‘true image’ of the droplets and a ‘measurement image’ of the shapes measured. As an example of this, formulation 60.02’s true image is shown below in Figure 7-1:

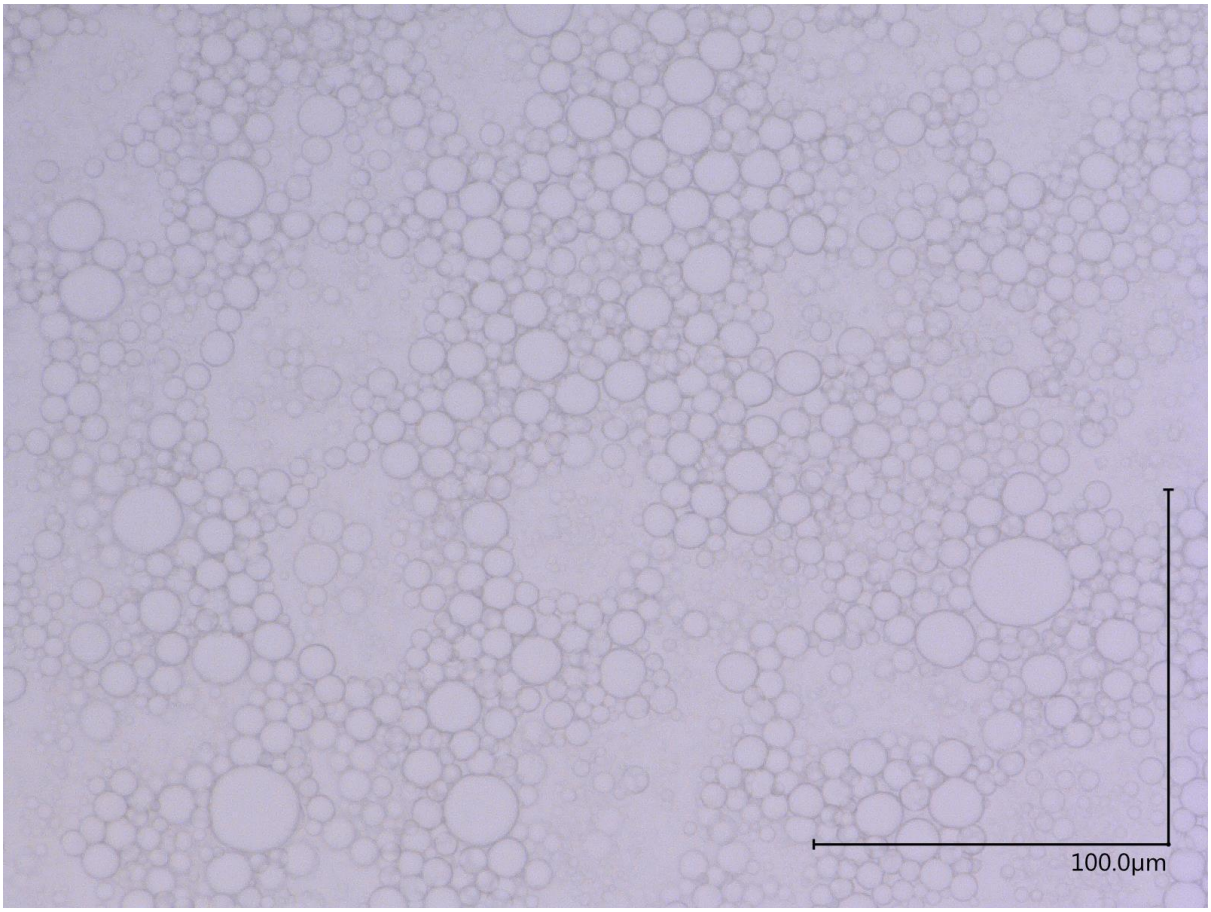


Figure 7-1 True image of formulation 60.02 initial result

Edge identifying software was then used to locate droplets and measure droplet area as shown in Figure 7-2.

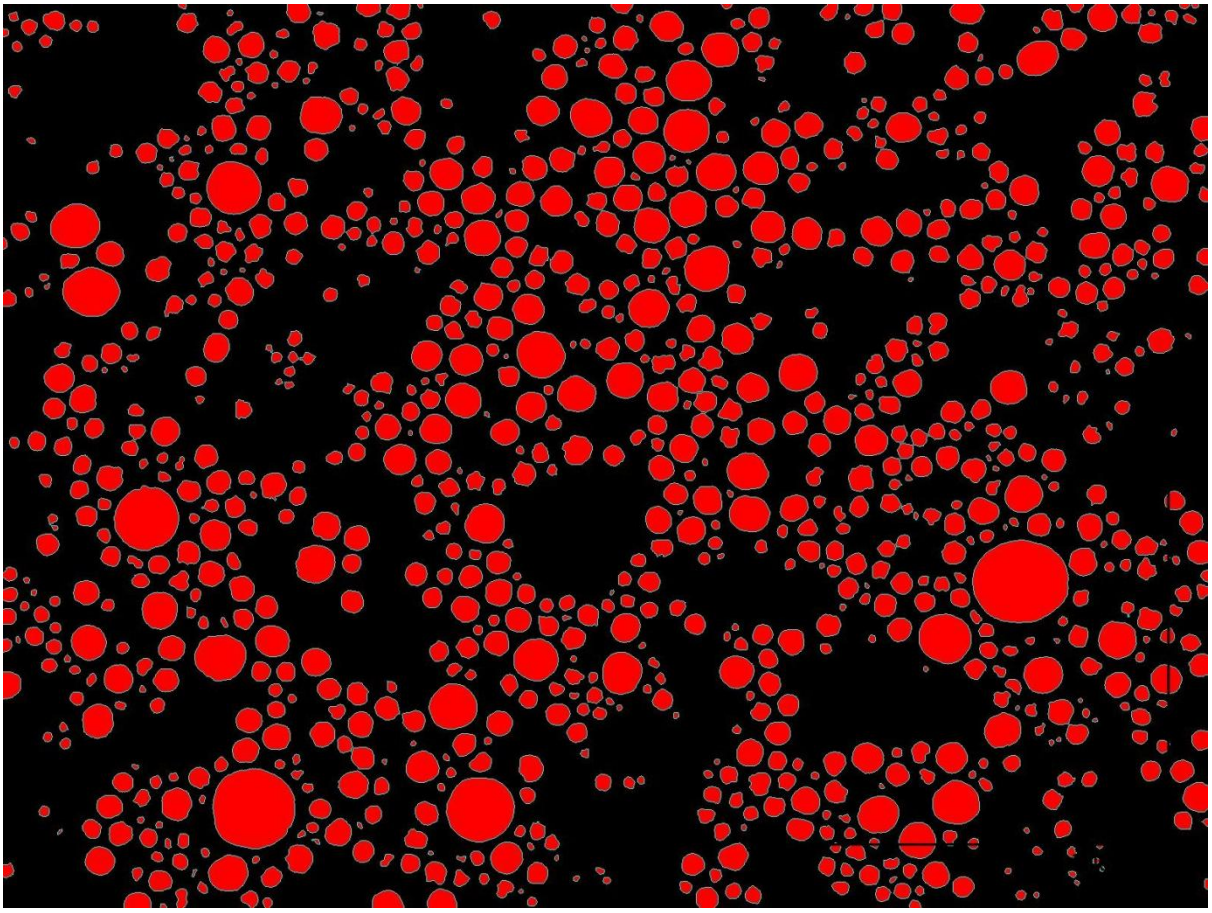


Figure 7-2 Area Measurement image of AP.60.02 Day 0 sample

The data of each image is produced in table form as demonstrated in Table 7-3 below.

Table 7-3 Table of Microscopy results of AP.60.02 Day 0 sample

	Area	Unit	Perimeter	Unit	Max diam	Unit	Min diame	Unit	Circularity
Average	24.5	µm²	16.2	µm	5.3	µm	4.5	µm	0.9
Standard Deviation	32.8	µm²	8.8	µm	2.7	µm	2.6	µm	0.1
Max	505.9	µm²	84.7	µm	27.3	µm	23.4	µm	1.3
Min	1.5	µm²	3.9	µm	1.3	µm	0.8	µm	0.7
Total	24603.7	µm²	16297.2	µm	5326.6	µm	4483	µm	927.3
Count	1004	pcs							
Area ratio	28.4	%							
Total region area	86682.4	µm²							

To view all the results for mean droplet size, refer to Appendix 4 – Microscopy Results.

Taking only the initial result first, below is a table of the initial mean droplet size results of each formulation in Table 7-4, along with the type of emulsifier and amount of energy input into the mixing stage (mechanical work):

Table 7-4 Initial mean droplet size results with formulation parameters

Emulsifier type				Anionic				Cationic		Non-ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
Percentage				1	2.5	1	3	2	4	2	4	2	4	0.75	1.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Initial mean droplet area (µm²)											
phase ratio (W:O)															
80	20	3000	30	57.6	37.2	n/a	10.5								
		6000	30	16.3	24.6	n/a	n/a								
75	25	3000	30	88.5	34.5	n/a	n/a	79.8	60.7						
		6000	30	15.5	6.1	n/a	n/a	26.3	14.8						
70	30	3000	30	38.7	26.5	n/a	106.4		75.6	184.0	135.5	12.9	18.4	157.9	68.4
		6000	30	6.9	89.0	n/a	n/a		9.7	14.9	11.2	6.2	6.9	30.7	24.2
65	35	3000	30	29.3	56.3	n/a	n/a								
		6000	30	7.0	5.8	n/a	n/a								
60	40	3000	30	90.2	49.6	n/a	153.3				111.5		185.6		103.6
		6000	30	10.7	7.1	n/a	19.4		13.5		43.6		24.5		64.3

As Table 7-4 showed, the highest initial reading was for formulation 60.01 which had an initial mean droplet size of 185.6µm² and true image is given in Figure 7-3.

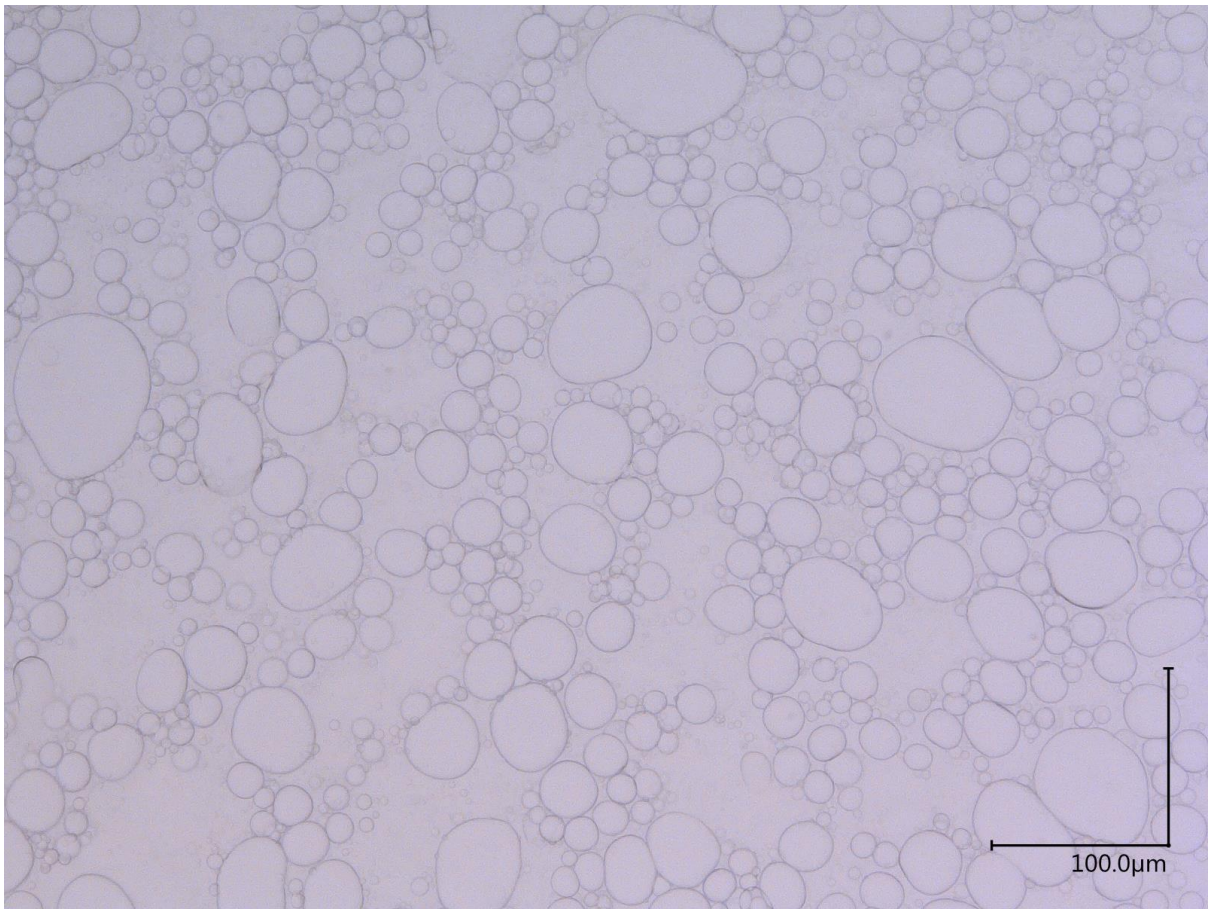


Figure 7-3 True image of formulation 60.01 initial result

The lowest was formulation 8.02 which had an initial mean droplet size of 5.8 µm², the true image of which is given in Figure 7-4.

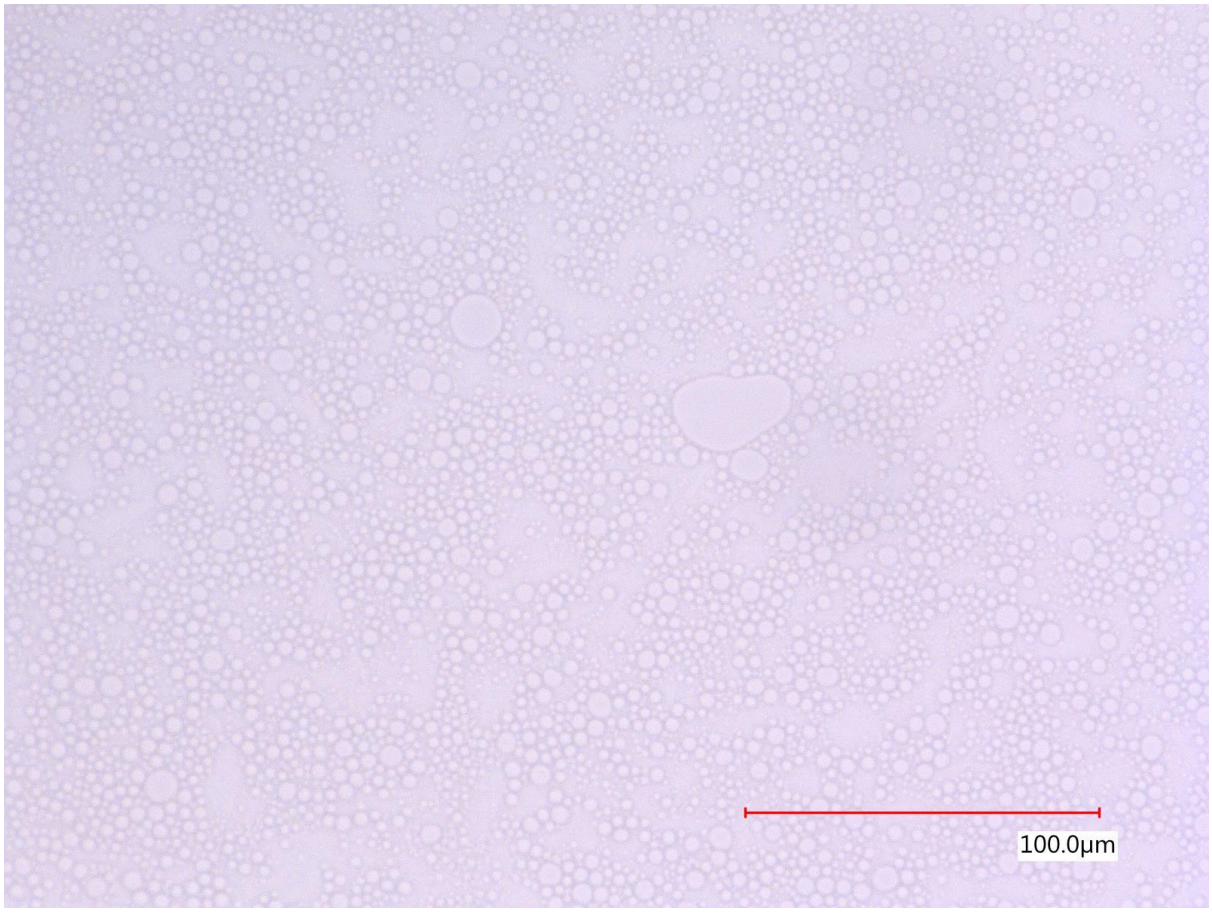


Figure 7-4 True image of formulation 8.02 initial result

The data also shows that, as expected, mechanical work had a negative correlation with droplet size, as every formulation except 6.01 to 6.02 saw a decrease in droplet size when mechanical work was increased. The degree of this decrease, however, had a wide range from the smallest; 34% reduction of 2.01 (37.2 µm²) to 2.02 (24.6 µm²); to the largest 92% reduction of 46.01 (135.5µm²) to 46.02 (11.2 µm²).

However, other expected behaviour was not seen. For example, with an increase in concentration of emulsifier, it was expected that the mean droplet size would decrease or remain the same. Remaining the same would show that there was enough emulsifier at both higher and lower concentration levels to stabilise all the new surface area created by the energy input. Decreasing would have shown that at the lower emulsifier concentration level, there was not enough emulsifier to stabilise the new surface area created by the mechanical work, leading to coalescence of the droplets and hence larger mean droplet size. On the other hand, at higher emulsifier concentration level there would have been more available emulsifier to stabilise the new surface area, retarding coalescence of droplets, resulting in lower overall mean

droplet size. Unexpectedly, of the 18 result pairs where this comparison was possible five showed an increase in droplet size as shown in Table 7-5.

Table 7-5 Mean initial droplet size with pairs of results highlighted

Emulsifier type				Anionic				Cationic		Non-ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
Percentage				1	2.5	1	3	2	4	2	4	2	4	0.75	1.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Initial mean droplet area (µm²)											
phase ratio (W:O)															
80	20	3000	30	57.6	37.2	n/a	10.5								
		6000	30	16.3	24.6	n/a	n/a								
75	25	3000	30	88.5	34.5	n/a	n/a	79.8	60.7						
		6000	30	15.5	6.1	n/a	n/a	26.3	14.8						
70	30	3000	30	38.7	26.5	n/a	106.4		75.6	184.0	135.5	12.9	18.4	157.9	68.4
		6000	30	6.9	89.0	n/a	n/a		9.7	14.9	11.2	6.2	6.9	30.7	24.2
65	35	3000	30	29.3	56.3	n/a	n/a								
		6000	30	7.0	5.8	n/a	n/a								
60	40	3000	30	90.2	49.6	n/a	153.3				111.5		185.6		103.6
		6000	30	10.7	7.1	n/a	19.4		13.5		43.6		24.5		64.3
		Formulation pairs that show an increase in droplet size with increased emulsifier concentration													
		Formulation pairs that show an decrease in droplet size with increased emulsifier concentration													
		Formulations that have no emulsifier concentration pairs to compare to													

These increases in mean droplet size are unexpected and cannot be explained easily given the tight formulation and method parameters of these formulations.

Similarly, the droplet size should have shown a correlation with increasing oil phase, given all other parameters were being kept the same. The expected behaviour was that the droplet size should either stay the same or become larger with increasing oil (internal) phase with a constant concentration of emulsifier. Remaining the same would shows that there was a sufficiently high concentration of emulsifier at all phase ratios to stabilise the droplets as they form. Increasing droplet size would have shown that there was not sufficient emulsifier to stabilise the increased surface area that more oil phase creates, and the system could only react by decreasing surface area until the emulsifier can cover all the interface, i.e. larger average droplet size. However, as can be seen for the results highlighted below, there was no such behaviour demonstrated in these results. Indeed the four longest data sets - the Anionic emulsifier series, showed a decrease in droplet size with increasing oil phase concentration at some point in the data as shown in Table 7-6.

Table 7-6 Mean initial droplet size of the Anionic 1 data series with related formulations highlighted

Emulsifier type				Anionic	
Emulsifier				1	
Percentage				1	2.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Initial mean droplet area (µm²)	
phase ratio (W:O)					
80	20	3000	30	57.6	37.2
		6000	30	16.3	24.6
75	25	3000	30	88.5	34.5
		6000	30	15.5	6.1
70	30	3000	30	38.7	26.5
		6000	30	6.9	89.0
65	35	3000	30	29.3	56.3
		6000	30	7.0	5.8
60	40	3000	30	90.2	49.6
		6000	30	10.7	7.1

	Colour denote data series that are related as described above

Given the tightly controlled formulation variances and method of manufacture used to create these formulations, this decrease in droplet size was difficult to explain, and suggests that there was an error either in the formulation's creation or in the method of measurement of the droplet size.

Looking beyond the initial results of all the formulations, again there were additional results that are difficult to explain. The expected behaviour was that the droplet size would increase as droplets coalesce or disproportionate to bigger droplets, or they would stay the same, as the emulsion was stable so that no coalescence or disproportionation occurred. As described in Chapter 1 – Introduction, the creation of new surface area within an emulsion by a decrease in droplet size is not spontaneous and requires energy through mechanical work. However, there were many cases within the data that showed a decrease in droplet size over time, and with no apparent pattern, for example formulation 9.01 results are given in Table 7-7.

Table 7-7 Microscopy data for formulation 9.01

weeks	0	1	2	4	8	12	16	24
TRIAL CODE : 16900/AP.09.01				units=	µm²			
microscopy 45°C	90.2	75.3	65.4	81.6	51.6	42.6	29.8	
microscopy 40°C	90.2	42.2	38.8	45.6	48.2	68.3	40.4	
microscopy 25°C	90.2	53.6	72.5	61.0	68.7	55.0	10.4	35.6
microscopy 4°C	90.2	68.4	66.5	55.7	45.6	50.2	78.6	

Of the 49 formulations that had microscopy data, 34 had a result that showed a decrease in droplet size of more than 10% from initial.

Furthermore, this decrease in droplet size was not the only unusual result in formulation 9.01 data set. The data also showed increases and decreases in the droplet size reading at each temperature over the observed time. This is more clearly seen when the data is put in graph of average droplet size (µm) from the initial result over time, shown in Figure 7-5:

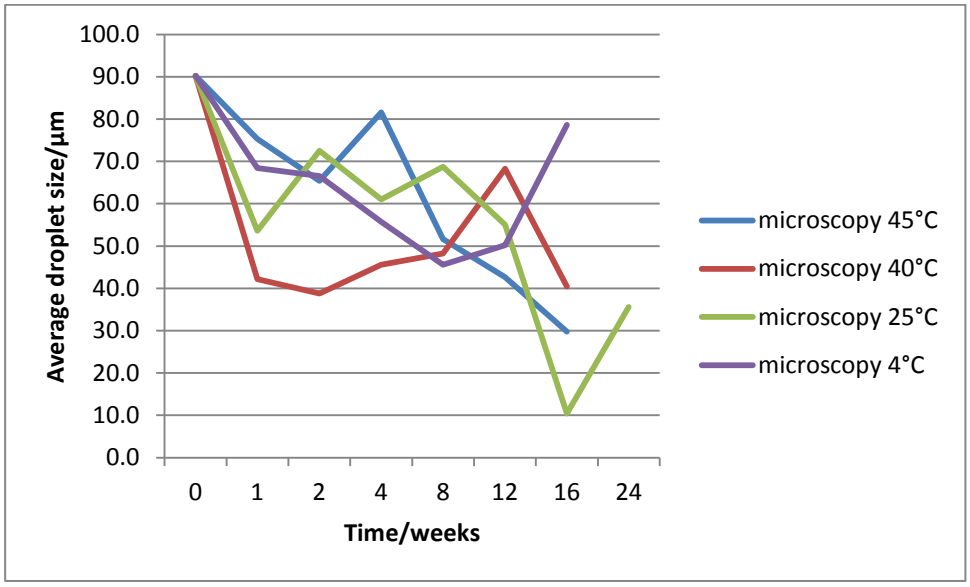


Figure 7-5 Formulation 9.01 microscopy results

There is no explanation for the mean droplet size to change in this manner without some mechanical energy input, which the samples did not receive.

This behaviour was not limited to formulation 9.01, the average of all the results when put as % change from initial result renders the graph in Figure 7-6.

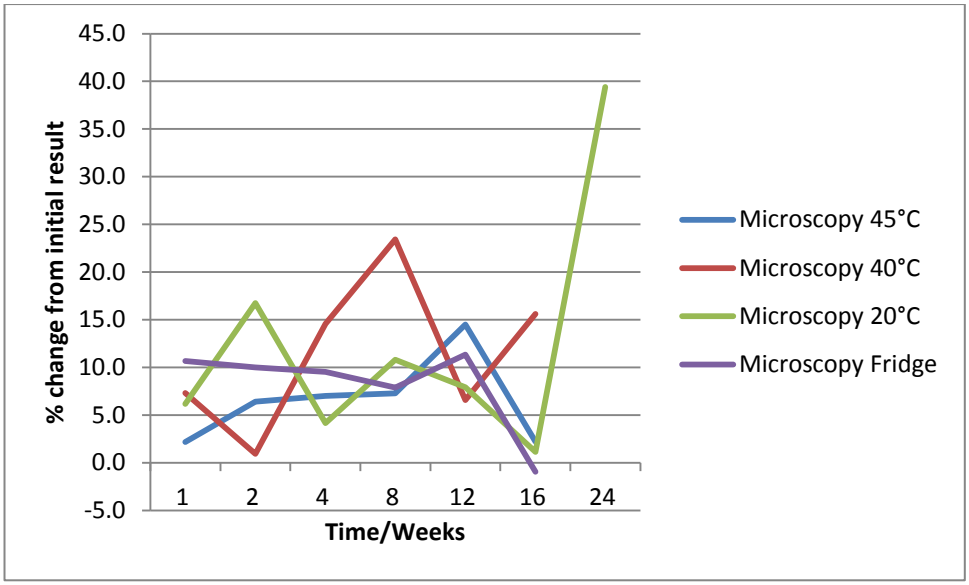


Figure 7-6 average % change from initial result for all formulations

As can be seen a seemingly random set of peaks and troughs in the results are present in each temperature data set.

This data pointed to a systematic error in the method used to obtain the results, and the testing was abandoned after 24 weeks testing as no useful data on the accuracy of the Accelerated Stability Model was being obtained.

7.1.6 Microscopy Discussion

With this experiment having been abandoned due to poor results there was no opportunity to evaluate the Accelerated Stability Model with this data. There were, however, some interesting images obtained that were worthy of note with respect to emulsion structure.

Firstly, formulation 2.02 16 week image from the fridge sample, Figure 7-7, appeared to show flocculation of the oil droplets:

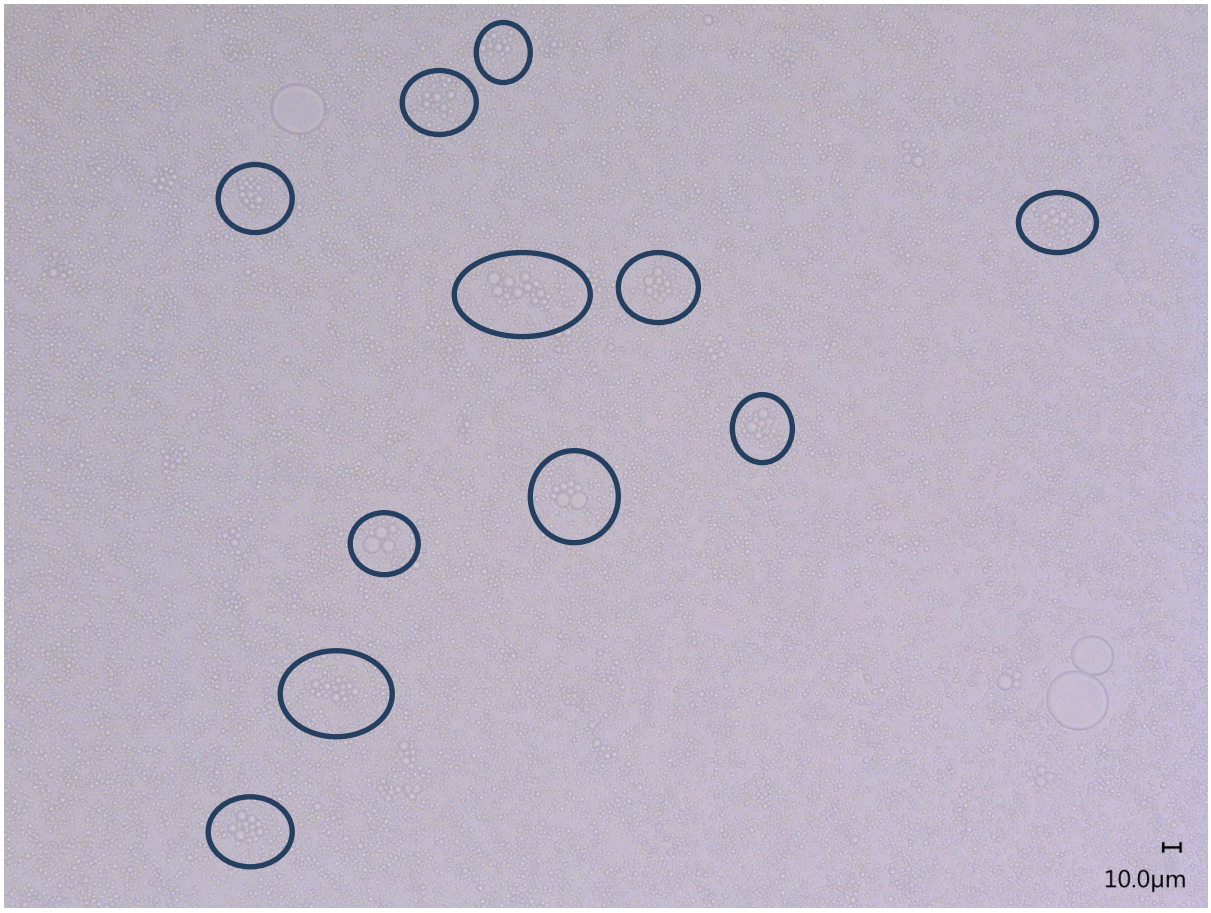


Figure 7-7 True image of formulation 2.02 16 weeks fridge sample showing flocculation

This image would enable a development chemist to see flocculation occurring in a sample before it starts to destabilise the system. It should be noted, however, that no other microscopy image in formulation 2.02 set showed this behaviour, for example the real-time 24 week image given in Figure 7-8.

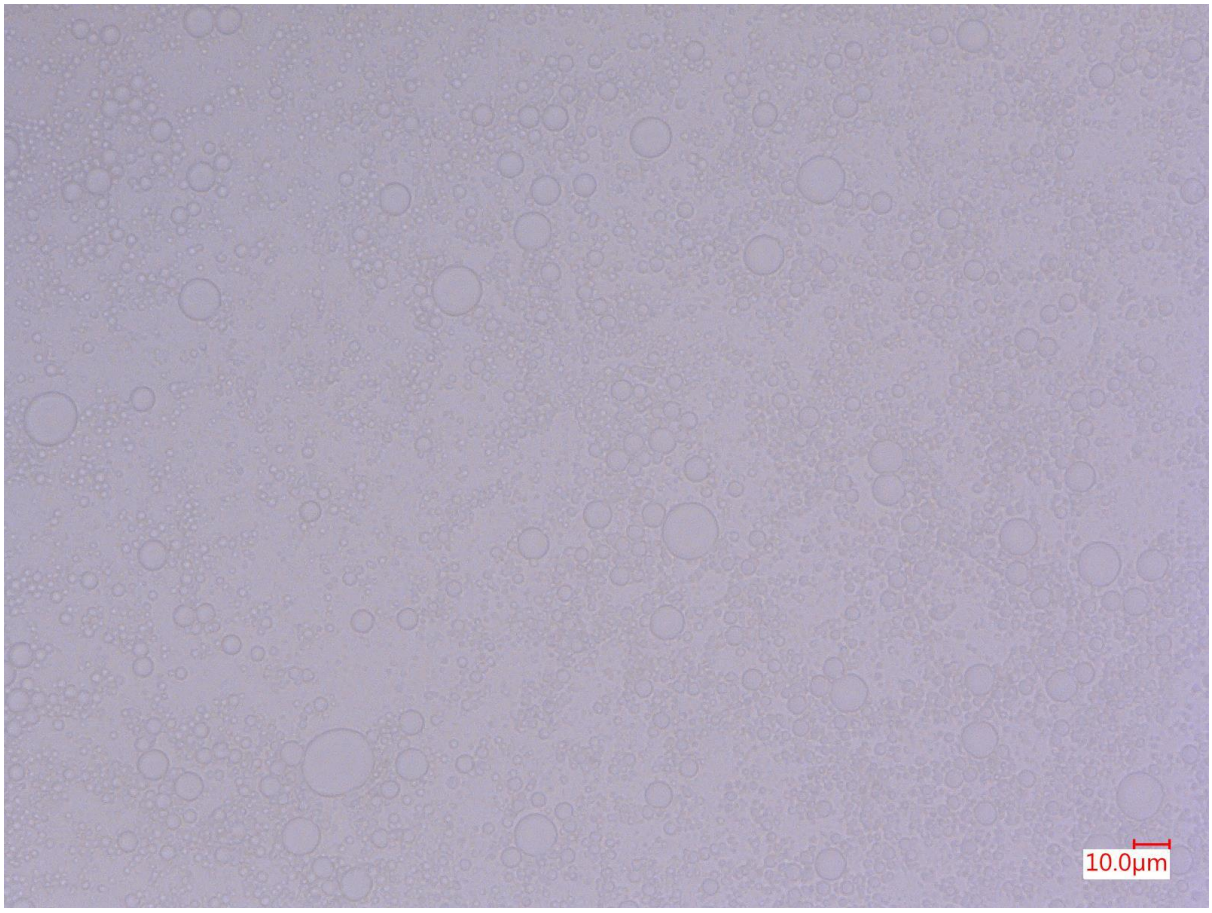


Figure 7-8 Formulation 2.02 24 week real time image showing no flocculation

Although an interesting image, it did not appear to be predictive of behaviour that may occur in real-time testing.

Secondly, formulation 50.01 and 50.02 showed a more complex structure than the intended oil-in-water emulsion:

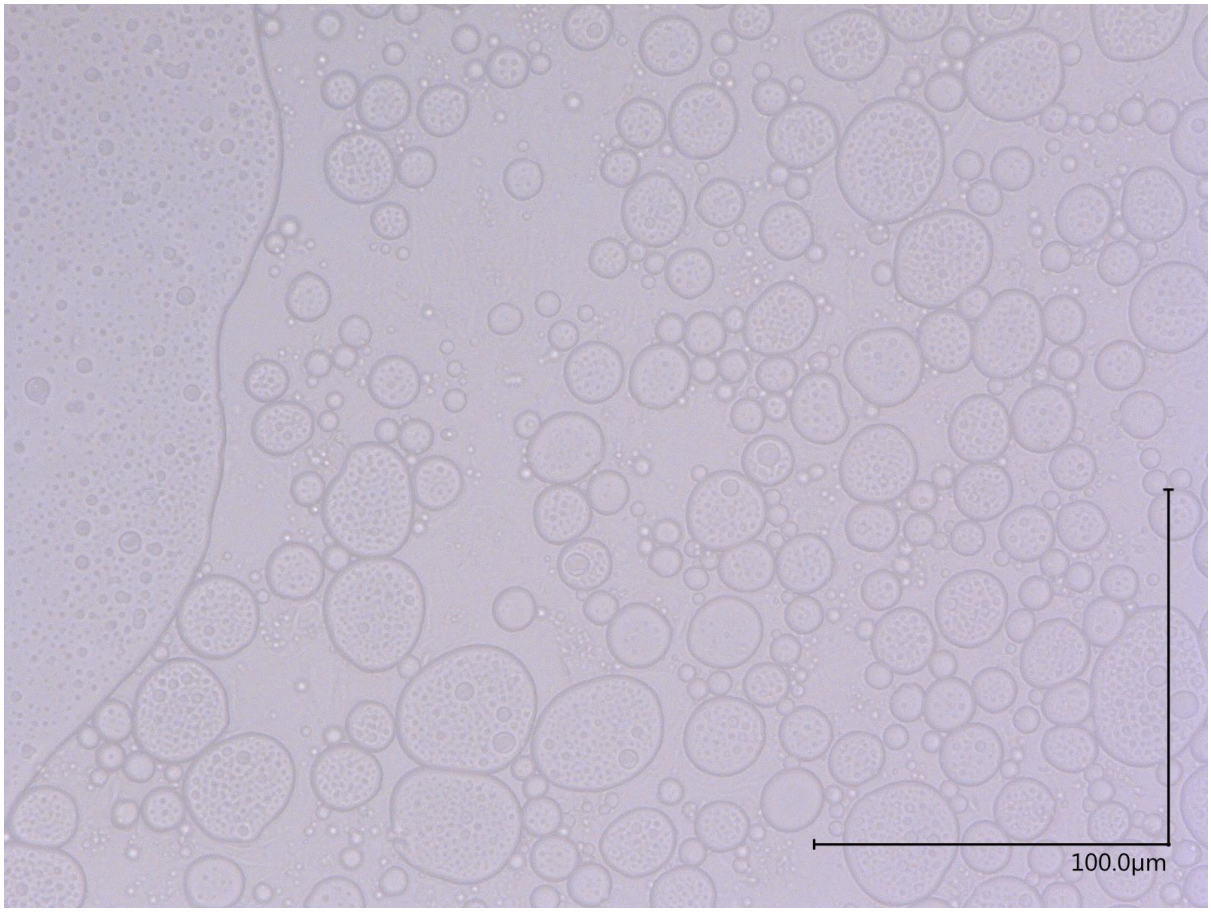


Figure 7-9 formulation 50.01 showing a complex water-in-oil-in-water emulsion structure

Figure 7-9 showed some of the water phase had been incorporated into the oil droplets as a secondary internal phase of formulation 50.01. The emulsifier used in this formulation was a non-ionic emulsifier blend of cetearyl glucoside and cetearyl alcohol with a HLB value of 11. This was the lowest HLB value of all the emulsifiers used and formulations 50.01 and 50.02 were the highest concentrations of this emulsifier system used. Therefore, there may have been an excess of emulsifier available in the system and with a low HLB value allowing the emulsifier to stabilise water-in-oil emulsions as well as oil-in-water emulsions, a more complex structure was stabilised. In formulation 50.02, which was the same formulation as 50.01 but with more mechanical work done, the same structure was seen, Figure 7-10, but to a much lesser extent because the droplet size of the primary emulsion was much smaller, leaving less space for a secondary emulsion within the droplets and more emulsifier was needed to stabilise the increased surface area of smaller droplets:

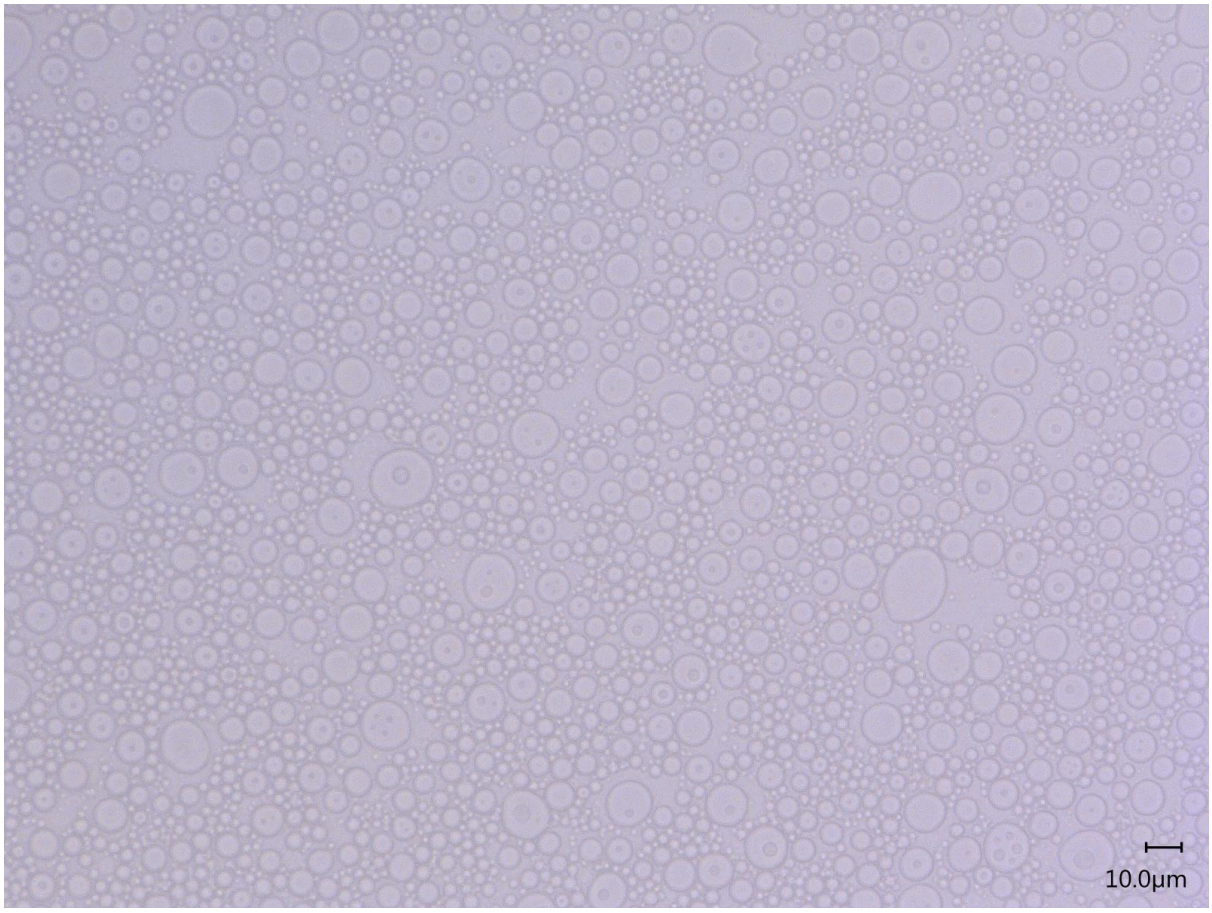


Figure 7-10 formulation 50.02 showing a complex emulsion structure

Although these results were interesting and would not have been seen with any other type of analysis, they did not answer any of the research questions of this study. Therefore, whilst these results may be the start point for some further study into emulsion structures, they will not be discussed further in this research.

7.1.7 Microscopy Conclusion

The data obtained from digital microscopy was of poor quality to have taken on to assess the accuracy of the Accelerated Stability Model for droplet size. However, the images obtained did enable the observation of the emulsion droplets which may be useful for understanding emulsion structures and behaviour. Therefore, microscopy remains a useful tool for emulsion development and perhaps as a quality tool for industrialisation of newly developed products. However, this technique is not recommended for inclusion into the Accelerated Stability Model.

7.1.8 Microscopy Evaluation

There were some crucial problems with the method employed to obtain emulsion droplet size. These included, but were not limited to:

- In order to obtain a useful image from the microscope, the emulsion needed to be just one droplet diameter thick. Therefore the microscope slides were pushed together with enough force to thin the sample out as much as possible. This process was not controlled and was adjusted during measurement to obtain an appropriate image to analyse. This process could have forced more mobile, smaller droplets out of the measurement sample, or crushed larger droplets with too much force, leading to a unreliable result.
- When the droplets were in close proximity or touching, the edge-finding software found it difficult to distinguish between droplets and the internal and external of droplets. This led to inclusion of shapes that were not genuine droplets, or exclusion of droplets that were in fact genuine.
- The range of droplet sizes meant that the number of droplets actually measured differed from one measurement to the next, with more smaller droplets able to be analysed than large droplets. For example, the largest droplet measurement, from formulation 60.01 (image above) analysed 748 droplets, whereas the smallest, from formulation 8.02 (image above) analysed 3791 droplets. This could have led to a skewing of results to a smaller average droplet size than true.
- The nature of microscopy only analyses a tiny proportion of a sample. The area was chosen at random but there is a possibility that, either by action to prepare the sample, or through non-homogenous sample, the area measured may not have represented the nature of the entire sample.

A solution to the above and an area of possible further study would be to use a different method of analysis for droplet size. A possible alternative method would be laser diffraction spectroscopy, which uses the diffraction patterns of a laser beam shone through a sample to analyse the geometric properties of the sample being measured (Stojanović et al. 2012). It is accurate down to the nano-meter range and would be appropriate to measure oil droplets in water. This method would measure many more droplets than the few hundred that microscopy analysis allowed. Laser diffraction would require the sample to be diluted in water until translucent enough for a laser to pass through, which would need to be done with a minimum of shear force to the sample to prevent the droplet size being affected.

7.2 Zeta Potential

The zeta potential of particles is determined by measuring their velocity while they are moving due to electrophoresis. Particles that have a zeta potential will migrate towards an electrode if a field is applied. The speed at which they move is proportional to the field strength and their zeta potential. As the field strength applied is known, measurement of the speed of movement, using laser Doppler electrophoresis, can be used to calculate the zeta potential. Specialised equipment from Malvern was used to make these measurements at Sunderland University – the methods used were those suggested by the Sunderland University measurement of zeta potential in emulsions, which were based on the Malvern instruction manual and online training courses.

7.2.1 Zeta Potential equipment

- Malvern Zetasizer Nano ZS90 with folded capillary cell.
- 100ml beaker
- 5% glycerine solution in water
- Magnetic hot plate and magnetic stirrer

Images of this equipment can be seen in Appendix 5.

7.2.2 Zeta Potential sample preparation

- The sample temperature was checked to be 25ºC +/- 1ºC
- 1g of sample emulsion added to 99g of 5% glycerine in water in the 100ml beaker.
- Sample stirred slowly with magnetic stirrer until sample is uniform.
- Sample added to the folded capillary cell.

7.2.3 Zeta Potential method

- Malvern Zetasizer turned on
- Selected ‘measure’ from the task bar.

- Selected ‘Open SOP’
- In the browser window that opens selected ‘Particle surface Zeta Potential measurement’
- From the browser that opens selected the ‘folded capillary cell’.
- When prompted for a background reading, ensured the cuvette chamber is empty and clicked ‘start’.
- Once background reading is completed, loaded the folded capillary cell into the cuvette chamber and clicked ‘Start’.
- The instrument read the zeta potential 30 times, and the average of these readings given as the final result.
- Saved the file under formulation number and date.

7.2.4 Zeta Potential Pass/Fail Criteria

Zeta potential was used as a measurement of the electrostatic repulsive forces between internal phase droplets. The higher the magnitude of the result the more the droplets repelled each other and hence became a barrier to coalescence. The value of ±30mv is often given as a threshold (Stubenrauch 2006) value, where a reading above would lead to long-term stability and below would give unstable systems. This value was used for this study and viewed in comparison to the long-term appearance stability results already collected.

7.2.5 Zeta Potential Results

The zeta potential was recorded once for each emulsion as it is not dependent on particle size, but rather the packing of the emulsifier at the droplet surface and electrolyte concentration of the continuous phase, which are not affected by droplet size. As discussed in previous chapters, of the 65 formulations made for this study 16 were not stable enough to have any measurements taken. These formulations therefore yielded no zeta potential data as their structure changed fundamentally before the reading could be taken, thus these 16 were removed from the results. The remaining results are given in Table 7-8.

Table 7-8 Zeta Potential results for all formulations

Emulsifier type				Anionic				Cationic		Non-ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
Percentage				1	2.5	1	3	2	4	2	4	2	4	0.75	1.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Formulation numbers											
phase ratio (W:O)															
80	20	3000	30	1.01	2.01	19.01	20.01								
		6000	30	1.02	2.02	19.02	20.02								
75	25	3000	30	3.01	4.01	17.01	18.01	23.01	24.01						
		6000	30	3.02	4.02	17.02	18.02	23.02	24.02						
70	30	3000	30	5.01	6.01	15.01	16.01		26.01	45.01	46.01	55.01	56.01	75.01	76.01
		6000	30	5.02	6.02	15.02	16.02		26.02	45.02	46.02	55.02	56.02	75.02	76.02
65	35	3000	30	7.01	8.01	13.03	14.03								
		6000	30	7.02	8.02	13.04	14.04								
60	40	3000	30	9.01	10.01	11.03	12.01				50.01		60.01		80.01
		6000	30	9.02	10.02	11.04	12.04		30.02		50.02		60.02		80.02

Emulsifier type				Anionic				Cationic		Non-ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
Percentage				1	2.5	1	3	2	4	2	4	2	4	0.75	1.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Zeta Potential (mv)											
phase ratio (W:O)															
80	20	3000	30	-45.2	-50.6	n/a	-41.2								
		6000	30	-10.1	-5.7	n/a	n/a								
75	25	3000	30	-70.9	-70.1	n/a	n/a	12.2	16.5						
		6000	30	-62.2	-62.4	n/a	n/a	12.7	15.9						
70	30	3000	30	-75.5	-44.4	n/a	-42.9		16.2	-14.3	-15.5	-17.2	-15.1	-23.9	-19.9
		6000	30	-57.6	-16.3	n/a	n/a		17.6	-14.4	-12.2	-14.0	-16.0	-24.1	-20.1
65	35	3000	30	-85.3	-75.6	n/a	n/a								
		6000	30	-76.3	-69.7	n/a	n/a								
60	40	3000	30	-48.9	-42.7	n/a	-47.3				-12.3		-13.3		-18.5
		6000	30	-42.2	-40.8	n/a	-43.1		11.6		-12.9		-12.6		-19.1

As Table 7-8 showed, there were 21 of the 49 formulations to be tested that had a result above the threshold value of ±30mv, with the highest value of -85.3mv for formulation 7.01. The rest were below the threshold value of ±30mv, with the lowest value of -5.7mv for formulation 2.02.

7.2.6 Zeta Potential Discussion

As expected, the zeta potential for the anionic emulsifiers were higher in magnitude than for the non-ionic and polymeric emulsifiers, due to the ionisation of the anionic polar head in solution, as opposed to the weaker dipole present on the non-ionic surfactant polar head. This full charge on the anionic surfactant head gives a higher surface charge to each oil droplet and hence there is more charge for the diffuse layer to overcome. However, along with the charged polar head, the anionic emulsifiers also give counter-ions into the aqueous continuous phase that non-ionic emulsifiers do not. In the case of the two anionic emulsifiers used in this study, sodium steroyl glutamate released sodium ions into solution, and Glyceryl Stearate

SE released potassium ions into the solution. These formed part of the electric double layer around the oil droplets and had a profound effect on zeta potential. An increase in counter ion concentration can lead to the slipping plane extending further into bulk solution, off-setting more of the surface charge and causing a reduction in zeta potential. It was expected therefore, that for the anionic emulsifier the zeta potential would decrease when the emulsifier concentration was increased for a similar formulation, given that oil phase size and mechanical work were kept the same. Indeed this decrease was seen in 8 of the 10 pairs of results. The other two formulation pairs showed a very small increase in zeta potential, which could indicate that the increase in counter ions had little effect on the distance of the slipping plane from the droplet surface for these formulations, hence the zeta potential remained similar as shown in Table 7-9.

Table 7-9 Effect of increasing anionic emulsifier 1 concentration on zeta potential

Emulsifier type				Anionic	
Emulsifier				1	
Percentage				1	2.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Zeta Potential (mv)	
phase ratio (W:O)					
80	20	3000	30	-45.2	-50.6
		6000	30	-10.1	-5.7
75	25	3000	30	-70.9	-70.1
		6000	30	-62.2	-62.4
70	30	3000	30	-75.5	-44.4
		6000	30	-57.6	-16.3
65	35	3000	30	-85.3	-75.6
		6000	30	-76.3	-69.7
60	40	3000	30	-48.9	-42.7
		6000	30	-42.2	-40.8

Pairs of formulations showing a decrease in Zeta potential with increased emulsifier concentration

Pairs of formulations showing an increase in Zeta potential with increased emulsifier concentration

Viewing the zeta potential data in conjunction with the long-term appearance data collected for the real-time experiments allowed analysis of the zeta potential's capacity as a guide to long term stability. The zeta potential results have been

overlaid with green where the formulation showed no change over time and orange where the formulation showed a change over time in Table 7-10.

Table 7-10 Zeta potential with appearance changing formulations highlighted

Emulsifier type				Anionic				Cationic		Non-ionic				Polymeric	
Emulsifier				1		2		1		1		2		1	
Percentage				1	2.5	1	3	2	4	2	4	2	4	0.75	1.5
Secondary variables		Mechanical Work (rpm)	Time (secs)	Zeta Potential (mv)											
phase ratio (W:O)															
80	20	3000	30	-45.2	-50.6	n/a	-41.2								
		6000	30	-10.1	-5.7	n/a	n/a								
75	25	3000	30	-70.9	-70.1	n/a	n/a	12.2	16.5						
		6000	30	-62.2	-62.4	n/a	n/a	12.7	15.9						
70	30	3000	30	-75.5	-44.4	n/a	-42.9		16.2	-14.3	-15.5	-17.2	-15.1	-23.9	-19.9
		6000	30	-57.6	-16.3	n/a	n/a		17.6	-14.4	-12.2	-14.0	-16.0	-24.1	-20.1
65	35	3000	30	-85.3	-75.6	n/a	n/a								
		6000	30	-76.3	-69.7	n/a	n/a								
60	40	3000	30	-48.9	-42.7	n/a	-47.3				-12.3		-13.3		-18.5
		6000	30	-42.2	-40.8	n/a	-43.1		11.6		-12.9		-12.6		-19.1

As a guide to long-term stability these zeta potential results are inconclusive. On the one hand the lowest zeta potential recording (-5.7mv) did indeed go on to show instability on long-term testing. However, 2.02 seemed to be the only result that followed this logic, with the other seven appearance unstable formulations having zeta potential results that were comparable to other formulations that were stable in the long-term studies.

Zeta potential is solely a measure of the electrostatic repulsion forces between droplets, and the resulting barrier to flocculation and coalescence. However, if these unstable formulations had been subject to a different mechanism of instability, for example sedimentation, creaming or disproportionation, then zeta potential would not have reflected this behaviour. Hence, taken on their own, these zeta potential results showed only part of the susceptibility of a formulation to instability.

If the Zeta potential data is observed in conjunction with the digital microscopy data it can be used to explain some of the more unusual behaviour observed in the microscopy results. One such case is the flocculation behaviour of formulation 2.02 (Figure 7-7 True image of formulation 2.02 16 weeks fridge sample showing flocculation), which had the lowest zeta potential, -5.7 mV. Using the DVLO theory introduced in the Introduction (Figure 1-8 DVLO theory graph of energy potential against distance of separation) the low zeta potential reading explains why the particles in this system get caught in the secondary minimum where Van der Walls forces are stronger than the repulsive forces, but not strong enough to overcome them to get to closer distances – hence flocculate. This formulation went on to

become unstable over time, showing that flocculation can indeed be a pathway to full instability.

7.2.7 Zeta Potential Conclusion

If the pass/fail criteria of ±30mv had been adhered to, 28 formulations would have been stopped at this stage. However, as the long-term stability data showed, 25 of these formulations showed no change in appearance over the testing time. In industry this would have represented a large waste of resource to reformulate and retest formulations that may have been suitable for market. Perhaps more significantly, of the 21 formulations that had a result over ±30mv, five went on to show instability at elevated temperatures and two (12.04 and 16.01) went on to fail long-term ambient conditions as well. These results would have been false passes and may have exposed consumers and brand owners to formulations inappropriate for market.

Therefore, given that zeta potential is only a measure of barrier to coalescence and flocculation and not other mechanism of instability, it became evident that zeta potential on its own seemed no better than the Accelerated Stability Model at predicting the long term stability of these emulsions.

7.2.8 Zeta Potential Evaluation

There were two areas in the method that could have introduced an experimental error. These both centre around the dilution of the sample to the correct opacity for the Malvern Zetasizer instrument to obtain a reading. Firstly, the dilution of the sample was into 5% glycerine solution, which was used as there was 5% glycerine in the water phase of the formulations. However, as discussed, the concentration of electrolytes in the continuous phase has a direct effect on the zeta potential, thus by diluting the emulsion in more continuous phase the concentration of electrolytes was also being diluted. This may have affected the zeta potential measurement. Secondly, the dilution itself required some stirring and mild heating to create a uniform substance. Although care was taken to minimise corruption of the sample, this action put energy into the system and possibly changed some parameters of the oil droplets which again may have affected the zeta potential reading.

A possible solution and an area for future study, would be to use the newly developed ‘high concentration cell’ from Malvern which is claimed not to need dilution of a sample in order to obtain a measurement.

Another possible area for future study would be to combine the zeta potential readings with measurement of other parameters which are indicators of the other mechanisms of instability. For example, if an emulsion was subjected to a centrifuge which artificially increased the gravitational force on the sample, it could show whether the sample was susceptible to creaming and sedimentation. A measurement could also be taken to assess the disparity in droplet size of the internal phase - a large disparity indicating increased susceptibility to disproportionation, with the rate of disproportionation falling to zero with a mono-dispersion of droplets. Taking all three of these measurements together, they could offer better guide to the behaviour of emulsions over time than using the elevated temperature techniques adopted by the Accelerated Stability Model. A future study would need to look at all these parameters in combination with long-term data to assess the validity of using these measurements as a guide for stability.

Chapter 8 Experimental Summary, Conclusion and Recommendations to Industry

8.1 Discussion

The table 8-1 below shows the overall accuracy parameters, along with targets, for the Accelerated Stability Model for each parameter measured in this study: appearance, colour, odour, pH and viscosity:

Table 8-1 Summary of mean results for each measurement parameter

	Appearance		Colour		Odour		pH		Viscosity	
	Target	Result	Target	Result	Target	Result	Target	Result	Target	Result
No. of measurable unstable formulations	8		18		2		29		35	
Average Prediction Error (accuracy)	<1	1.48	<1	1.33	<1	1.44	<0.25	0.19	<10	14.00
Prediction Error Range (precision)	<1.25	3	<1.25	1.73	<1.25	2	<0.5	0.24	<15	16.36
Accurate Prediction Threshold (weeks)	>96	16	>96	16	>96	32	>96	>96	>96	12

This table shows that the only parameter that remained within the Accelerated Stability Model's targets for accuracy and precision was pH, with all the others falling outside of targets for the emulsions measured. Perhaps the most significant result was the finding that for appearance, colour and viscosity the Accurate Prediction Threshold, that is the point in time that the accelerated predictions became inaccurate, was 16 weeks or shorter. Therefore, on average for these parameters the Accelerated Stability Model did not predict what was going to happen by the end of the testing time, let alone what would happen years into the future. This result suggested that on average it is possible, within Accelerated Stability Model timeframe, to identify that the model was inaccurate before the end of the test itself. It may be a recommendation to industry that every stability test should comment on the comparison of the already obtained real-time data and the early elevated temperature results, to justify the use of the Accelerated Stability Model.

In industry, the individual parameters would not be looked at in isolation, but all together, to assess whether a product should progress to the next stage of development. For example, a pH change on its own may not be a problem for the progression of a formulation, but if it was combined with a change in colour or odour, this may prevent the project progressing, as the change may be noticeable by the consumer either from a quality or safety perspective. Hence the research formulations were viewed in the same way to see if the results for any parameter in isolation would have been viewed differently in the context of all parameter results. The main focus of this analysis was on the false-pass results (Q_{10}<2), that is, formulations that in one parameter gave a pass result at accelerated temperatures which went on to fail real-time testing.

There were five formulations that gave a false pass result: 1.01, 1.02 and 24.01 for viscosity, and 2.02 and 55.01 for pH. Of these five, four gave a 'fail' result in at least one other parameter during accelerated testing, which indicated that the formulation may have been prevented from progressing to full-scale manufacture as shown in table 8-2 below.

Table 8-2 Summary of results for formulation displaying a false pass result

	1.01		1.02		24.01	
	Accelerated	Real-Time	Accelerated	Real-Time	Accelerated	Real-Time
Colour	FAIL	PASS	FAIL	PASS	PASS	PASS
Odour	PASS	PASS	PASS	PASS	PASS	PASS
Appearance	PASS	PASS	PASS	PASS	PASS	PASS
pH	FAIL	FAIL	FAIL	FAIL	PASS	PASS
Viscosity	PASS	FAIL	PASS	FAIL	PASS	FAIL

	2.02		55.01	
	Accelerated	Real-Time	Accelerated	Real-Time
Colour	FAIL	PASS	PASS	PASS
Odour	PASS	PASS	PASS	PASS
Appearance	FAIL	PASS	PASS	PASS
pH	PASS	FAIL	PASS	FAIL
Viscosity	FAIL	FAIL	FAIL	FAIL

However, formulation 24.01 passed all accelerated test parameters, and yet went on to fail real-time viscosity testing. Given these results in industry, all the accelerated data would not have prevented a formulation from advancing to the next stage of development when in fact it may have been inappropriate for it to do so, with an unpredicted, significant increase in viscosity occurring at ambient temperature. Whether this increase would have presented an issue to the safety or quality of the product would have depended on the characteristics of the cosmetic product, including packaging and application area. However, in the worst case this could have led to a market recall at great financial and reputational expense to the brand.

This single result was very important because it showed that a false pass was possible even after all accelerated data was taken into account. It also showed that the assigning of Q_{10} = 2 to the Q rule, as the most conservative value, can be inappropriate for these parameters as Q_{10}<2 for this case. If these ratios were repeated in industry, it represented 1.5% of the market. Given the size of the cosmetics market, or even just the emulsion sector, this was an enormous potential liability to the cosmetics industry.

It was also interesting to compare the results of 24.01 to 24.02 which had the same formulation make up but with more mechanical work done at the emulsification stage. The full results of both formulations are given in Appendix 1 – results, but are summarised below in Table 8-3:

Table 8-3 Summary of formulation 24.01 and 24.02 stability results

	24.01		24.02	
	Accelerated	Real-Time	Accelerated	Real-Time
Colour	PASS	PASS	PASS	PASS
Odour	PASS	PASS	PASS	PASS
Appearance	PASS	PASS	PASS	PASS
pH	PASS	PASS	FAIL	FAIL
Viscosity	PASS	FAIL	FAIL	FAIL

It would appear that the addition of extra mechanical work at the emulsification stage created a lower viscosity system which increased in viscosity on both accelerated and real-time conditions to give a fail result on both tests. It also created a slight change in pH readings but in this parameter the two formulations behaved very similarly.

This showed the fragility of the applicability of the Accelerated Stability Model. The same formulation was made with slightly different method parameters, one of which would have failed accelerated stability and thus accurately reflected the real-time failure. The other would have passed accelerated stability, which would have been a false pass, and represented a liability to industry. These results also highlighted the importance of reproducing the same formulation parameters on the industrial scale as were used in the stability batch created in the laboratory. Any change in energy input could have a significant effect on the finished product's immediate parameters and future changes in those parameters over time.

8.2 Experimental Conclusion

This study addressed several objectives including:

1) Using empirical data from experimentation of multiple cosmetic products that undergo both accelerated and real-time testing, does the industry standard Accelerated Stability Model deliver a reasonably accurate prediction of real-time stability?
2) Does a mathematical evaluation of the Arrhenius equation and its applicability to cosmetic products support or oppose the use of Accelerated Stability Models in cosmetic products?
3) Are there more appropriate or accurate tests that could be performed on these formulations?
4) Is there any action the industry can take to make the testing protocols more accurate or relevant?

To answer these question in turn:

1) For the products tested in this study, there were four parameters out of the five tested that showed that the Accelerated Stability Model was an inappropriate model for prediction of long-term stability. Indeed, in three of these parameters the model was not accurate beyond the extent of the accelerated testing time, 16 weeks, let alone beyond into the required shelf-life of a cosmetic product. It should be noted that the one parameter which was well modelled in this study, pH, was affected by molecular interactions and hence was a much better fit to the Arrhenius equation.

2) The application of the Arrhenius equation to cosmetic products to create the current Accelerated Stability Model makes many assumptions in its procedures and treatment of results. As was shown, these assumptions and procedures - including the approximation of activation energy; and the temperatures at which the products are tested - were inappropriate, and there appeared to be no stage of the implementation of the Arrhenius model that was justifiable.
   The only possible exception to this assertion was the chemical reactions taking place on the molecular scale, and even these would need validation from real-time data to show valid extrapolations - reactions that took place on a macro scale appeared not to be applicable to the Arrhenius model.

3) This study attempted to use additional tests of zeta potential and digital optical microscopy to ascertain whether measurement of other parameters could be a better guide to long-term stability. In both cases, the results appeared no better than the Accelerated Stability Model at predicting long-term stability on their own. However, the measurement of multiple parameters should be encouraged as it is able to give early indication or explanation of emulsion behaviour. In this study, for example, one formulation displayed flocculation (2.02) under microscopy, which could be explained using DVLO theory and the low Zeta potential measurement for that formulation -5.7mV. Therefore the measurement of zeta potential in combination with other parameters such as centrifugation and particle size analysis should allow better monitoring of the destabilising mechanisms of coalescence, flocculation, creaming, sedimentation and disproportionation, more than simply placing the samples in a high temperature oven.
4) See Recommendations to Industry Section below.

8.3 Experimental Evaluation

There are many areas in this study that have been identified as experimental improvements or possible areas of future study. These have been highlighted in the evaluation sections of each chapter, but in addition to those there are some more general improvements possible which suggest further avenues of future study.

Firstly, due to the time limitation on this study, the real-time testing could only run for 96-weeks. This was acceptable for evaluation of the Accelerated Stability Model as

the 45ºC at 16 weeks should mimic the 96-week real-time result. However, the majority of cosmetic products have a ‘PAO’ symbol on pack as the declaration of shelf-life. The EU regulation stipulates that to use this symbol the product must be stable for at least 30 months – or 135 weeks. It would therefore be of interest to extend the real-time results to 135 weeks to see if there were any changes in the parameter measurements between 96 and 135 weeks. This could highlight further shortcomings of the Accelerated Stability Model and suggest that any formulation that has only completed accelerated stability should not use the ‘PAO’ symbol until real-time data has been collected.

Secondly, as the results showed, the choice of emulsifier has a profound effect on the initial parameters and subsequent stability of an emulsion. Although six different emulsifiers were used for this study, spanning the four different types available, there are many more emulsifiers available to a cosmetic formulator. It was also noted that the data set from anionic emulsifier 2 (gylceryl stearate se) contained the majority of the formulations that were too unstable to start testing. If this study was repeated, this emulsifier would either be replaced or its concentration adjusted. It could be an area of future study to widen the formulations tested to include other emulsifiers, to see whether this affects the accuracy of the Accelerated Stability Model. Similarly, the oil phase itself was kept constant throughout the study, although the amount of oil phase added was varied. It could be an area of future research to use different oil phase constituents, either with the emulsifiers used in this study or different emulsifiers, to see whether the Accelerated Stability Model was a better predictive tool of those formulations.

Thirdly, emulsions are not the only type of formulations used in the cosmetics industry. Other product formats include aqueous gels, hydro-alcoholic sprays, solid suspensions, detergent gels, oils and balms. It would be interesting to perform similar studies on these product formats to see if the Accelerated Stability Model was more or less accurate across these formats. It may be the case that, because emulsions are reliant on macro structures rather than molecular interactions, they are particularly poorly modelled by the Accelerated Stability Model and other product formats may be modelled more accurately. This future work would be particularly interesting for the perfume format. Given that, by their nature, fragrances are volatile compounds, it would be expected that they would be significantly affected by the higher temperatures of accelerated stability. As suggested in the chapter on odour, it would be advantageous to perform both subjective nose tests and the more

analytical electronic nose or gas chromatography tests to assess the effects of elevated temperatures on fragrance.

Lastly, as suggested in the evaluation section of the organoleptic chapter, the assessment of changes for colour, odour and appearance were subjectively placed on a scale of 0-5 for the change from the initial sample and this may have introduced a subjective error into the data. Firstly, the comparison was done against the fridge sample as the 'standard' which assumed that the fridge sample did not change. This was done so that test samples were compared to a sample that had the same treatment during manufacturing; the alternative was to remake the formulation at every test-point to compare with the test samples, but this would also have introduced potential experimental error. To overcome these limitations, each identified organoleptic parameter could be measured analytically by calibrated instrumentation. For example, odour could have been quantified for both content and strength by using gas chromatography to analyse head space of each sample, which would have given a more accurate reflection of odour changes and may have detected changes that may not have been detected by the nose alone. Similarly, a colorimeter or UV/Vis spectrometer would have been able to give a more precise change in colour than the arbitrary 0-5 scale. These parameters may be a good starting point for future study in this area.

8.4 Recommendations to Industry

There are four recommendations to industry from this study:

Firstly, as was shown in this study, the Accelerated Stability Model was an unreliable prediction of future long-term behaviour. However, there are thousands of these tests being performed daily in the many development laboratories across the world. There is no reason that the regulatory bodies, in the various regulatory regions, could not insist on the commitment of the brands to keep the ambient sample on test for the desired shelf-life. This would not necessarily mean that the product cannot launch after completion of accelerated stability testing, but the ambient sample would continue to be monitored for any unexpected change. It is common for the testing samples to start testing between six months and one year before the formulation is manufactured on the industrial scale. Therefore, the brand and the formulation chemist would always be six months ahead of any unexpected changes in ambient conditions on market.

Secondly, it is recommended that there be more education of the consumer about what 'PAO' and expiry dates mean. Although the brands are taking the financial risk of a recall if the product is unstable in the long-term, the consumer is unknowingly taking a risk by placing a product on their skin which may not have passed long-term testing. Education of the customer could take the form of a statement on pack as to the nature of the testing performed, or possibly a pack may not display the 'PAO' symbol until long-term stability testing had been completed. Until this time, the product's pack should display an expiry date which is justifiable either by real-time data or strong accelerated data.

Thirdly, there is currently no requirement to justify the use of the Accelerated Stability Model for any given test, although occasionally there may be a statement by a qualified person justifying an out-of-spec result. By the time the 16-week test results are available, there are already four accelerated results that can be compared to the real time data as demonstrated in Table 8-4.

Table 8-4 Representation of results that are comparable within the accelerated stability model

weeks	0	1	2	4	8	12	16	24	32	48	96
Average Appearance 40°C		A	B	D	F	G					
Average Appearance 45°C			C	E			H				
Average Appearance 25°C				A1	B1	C1	D1	E1	F1	G1	H1

There should be a pre-agreed specification in place for the pass/fail criteria, making it simple to compare the ambient 4, 8, 12, and 16 week tests with the earlier accelerated data. If these were already producing different results at these points in the test, it may be supposed that extrapolated results would be a poor prediction as well.

A compromise could be to adopt the above technique and then use the data to decide whether an ambient test should be kept on testing beyond the 16 weeks due to the Accelerated Stability Models. If the Accelerated Stability Model showed a poor predictive capacity for that formulation, then the ambient sample should be kept on test for the duration of the product's desired shelf life.

Finally, it is recommended that more research be done into the use of more modern analytical equipment. The Accelerated Stability Models were constructed in a paper of 1985 and a monograph in 1991. Analytical equipment has become more reliable and affordable in the intervening years and their use as predictive tools for product shelf-life should be fully investigated. One example would be from the Evaluation section of Chapter 7: the combination of zeta potential measurement, controlled

centrifugation and particle size analysis by laser diffraction should give a full picture of an emulsion’s resistance to the five instability mechanisms. This type of targeted and educated measurement may be much more reliable than the many assumptions underlying the current practice of placing samples in elevated temperature conditions and extrapolating real-time results.

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Chapter 10 Appendices

10.1 Appendix 1 – Organoleptic Results

weeks	0	1	2	4	8	12	16	24	32	48	96
FORMULATION No.	1.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	2	2	2	3	3	4				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	2	2	2	3	3	4				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	1.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	2	2	2	3	3	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	2	2	2	3	3	3				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	2.01										
Appearance 40°C	0	0	0	0	0	4	4				
Appearance 45°C	0	0	1	1	2	4	4				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	3	3	3	nt	nt				
Colour Dark 20°C	0	0	0	0	0	4	4	5	5	5	5
Colour 45°C	0	0	3	3	3	nt	nt				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	2.02										
Appearance 40°C	0	0	0	0	0	3	3				
Appearance 45°C	0	0	0	0	0	3	3				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	3	3	3	nt	nt				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	3	3	3	nt	nt				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	3.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	1	2	2	4	4				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	3	3	4	4				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	3.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	2	3	3	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	3	3	3	3				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	4.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				

Colour 40°C	0	2	2	2	3	2	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	3	3	3	3	3	3				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	4.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	3	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	3	3				
Odour 40°C	0	2	2	2	2	3	3				
Odour 45°C	0	2	2	2	3	3	3				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	5.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	2	3	3	3	4	4				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	3	3	3	3	4	4				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	5.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	1	2	2	3	3	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	2	3	3	3	3	3				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0

Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	6.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	2	2	3	3	3	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	3	3	3	3	3	3				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	6.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	3	3	3	3				
Colour Dark 20°C	0	0	0	0	0	0	0	1	2	3	3
Colour 45°C	0	0	0	3	3	4	4				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	7.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	2	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	3	3				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	7.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0

Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	3	3	3	3				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	3	3	3	3				
Odour 40°C	0	0	0	0	0	2	3				
Odour 45°C	0	0	0	0	0	3	3				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	8.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	2	2	3	3	3	4				
Colour Dark 20°C	0	0	0	0	0	0	3	3	3	3	3
Colour 45°C	0	3	3	3	3	3	4				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	8.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	9.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				

Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	9.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	10.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	10.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	2	2	3	3	4	4				
Colour Dark 20°C	0	0	0	0	0	0	0	2	2	3	3
Colour 45°C	0	3	3	3	3	4	4				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	12.01										
Appearance 40°C	0	2	2	2	3	3	4				
Appearance 45°C	0	3	3	3	3	3	4				

Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	12.04										
Appearance 40°C	0	2	5	5	5	5	5				
Appearance 45°C	0	5	5	5	5	5	5				
Appearance 20°C	0	0	0	0	0	3	5	5	5	5	5
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	nt	nt	nt	nt	nt	nt				
Colour Dark 20°C	0	0	0	0	0	nt	nt	nt	nt	nt	nt
Colour 45°C	0	nt	nt	nt	nt	nt	nt				
Odour 40°C	0	0	0	0	nt	nt	nt				
Odour 45°C	0	nt	nt	nt	nt	nt	nt				
Odour Dark 20°C	0	0	0	0	0	nt	nt	nt	nt	nt	nt
Odour Fridge	0	0	0	0	0	nt	nt				

FORMULATION No.	16.01										
Appearance 40°C	0	5	5	5	5	5	5				
Appearance 45°C	0	5	5	5	5	5	5				
Appearance 20°C	0	0	0	5	5	5	5	5	5	5	5
Appearance Fridge	0	0	0	5	5	5	5				
Colour 40°C	0	nt	nt	nt	nt	nt	nt				
Colour Dark 20°C	0	0	0	nt	nt	nt	nt	nt	nt	nt	nt
Colour 45°C	0	nt	nt	nt	nt	nt	nt				
Odour 40°C	0	2	2	nt	nt	nt	nt				
Odour 45°C	0	2	2	nt	nt	nt	nt				
Odour Dark 20°C	0	0	0	nt	nt	nt	nt	nt	nt	nt	nt
Odour Fridge	0	0	0	nt	nt	nt	nt				

FORMULATION No.	20.01										
Appearance 40°C	0	0	0	0	3	5	5				
Appearance 45°C	0	0	0	3	5	5	5				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	nt	nt				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	nt	nt				
Odour 40°C	0	0	0	0	0	0	0				

Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0				
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	23.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	1				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	3				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	23.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	1				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	24.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	24.02							
Appearance 40°C	0	0	0	0	0	0	0	

Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	26.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	26.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	30.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				

Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	45.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	45.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	46.01										
Appearance 40°C	0	0	0	0	3	5	5				
Appearance 45°C	0	0	0	3	5	5	5				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	46.02

Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	50.01										
Appearance 40°C	0	0	0	0	3	5	5				
Appearance 45°C	0	0	0	3	5	5	5				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	50.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	55.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				

Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	55.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	56.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	56.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	60.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	60.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	75.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	75.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				

Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	76.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	76.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	80.01										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0

Odour Fridge	0	0	0	0	0	0	0				

FORMULATION No.	80.02										
Appearance 40°C	0	0	0	0	0	0	0				
Appearance 45°C	0	0	0	0	0	0	0				
Appearance 20°C	0	0	0	0	0	0	0	0	0	0	0
Appearance Fridge	0	0	0	0	0	0	0				
Colour 40°C	0	0	0	0	0	0	0				
Colour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Colour 45°C	0	0	0	0	0	0	0				
Odour 40°C	0	0	0	0	0	0	0				
Odour 45°C	0	0	0	0	0	0	0				
Odour Dark 20°C	0	0	0	0	0	0	0	0	0	0	0
Odour Fridge	0	0	0	0	0	0	0				

10.2 Appendix 2 – pH Results

weeks	0	1	2	4	8	12	16	24	32	48	96
Formulation No.	9.01										
pH 40°C	5.61	5.59	6.21	6.20	6.22	6.21	6.20				
pH 45°C	5.61	5.50	6.05	6.11	6.10	6.19	6.11				
pH 20°C	5.61	5.63	6.27	6.21	5.99	5.90	5.99	5.78	5.51	5.44	5.41
pH Fridge	5.61	5.59	6.21	6.00	6.59	6.44	6.43				
Formulation No.	9.02										
pH 40°C	5.51	6.06	6.10	6.01	6.00	6.11	6.06				
pH 45°C	5.51	6.11	5.94	5.99	5.90	5.93	5.90				
pH 20°C	5.51	5.50	6.05	6.04	6.04	6.01	6.13	5.78	5.55	5.51	5.50
pH Fridge	5.51	5.57	5.99	5.78	5.91	5.90	5.44				
Formulation No.	7.01										
pH 40°C	7.21	7.16	7.16	6.78	6.77	6.70	6.71				
pH 45°C	7.21	7.15	7.09	6.99	6.90	6.61	6.60				
pH 20°C	7.21	7.00	7.05	6.84	6.81	6.73	6.81	6.79	6.75	6.77	6.80
pH Fridge	7.21	7.16	7.04	6.97	6.93	6.91	6.61				
Formulation No.	7.02										
pH 40°C	7.13	7.00	7.02	7.04	7.11	7.13	7.11				
pH 45°C	7.13	7.01	7.00	7.00	7.01	7.00	7.01				
pH 20°C	7.13	6.99	7.03	7.04	7.06	7.05	7.06	7.06	7.04	7.05	7.05
pH Fridge	7.13	7.07	7.05	7.01	7.11	7.12	7.13				
Formulation No.	5.01										
pH 40°C	7.28	6.84	6.99	6.75	6.71	6.71	6.70				

pH 45°C	7.28	6.86	6.81	6.68	6.64	6.63	6.61				
pH 20°C	7.28	7.20	7.22	6.87	6.97	6.81	6.89	6.81	6.75	6.66	6.65
pH Fridge	7.28	7.25	7.31	7.09	6.90	6.99	6.90				
Formulation No.	5.02										
pH 40°C	7.21	6.95	6.90	6.91	6.80	6.81	6.80				
pH 45°C	7.21	6.93	6.99	6.93	6.64	6.61	6.61				
pH 20°C	7.21	7.23	7.26	7.22	6.68	6.63	6.61	6.63	6.75	6.60	6.65
pH Fridge	7.21	7.21	7.22	7.20	6.05	6.11	6.66				
Formulation No.	3.01										
pH 40°C	7.30	6.97	6.79	6.84	6.64	6.40	6.44				
pH 45°C	7.30	7.11	6.70	6.83	6.63	6.50	6.59				
pH 20°C	7.30	7.15	7.01	7.01	6.65	6.68	6.69	6.06	6.15	6.10	6.05
pH Fridge	7.30	7.29	7.13	7.33	7.14	6.83	6.68				
Formulation No.	3.02										
pH 40°C	7.29	6.85	6.75	6.96	6.82	6.79	6.11				
pH 45°C	7.29	6.90	6.72	6.87	6.74	6.91	6.13				
pH 20°C	7.29	7.15	7.04	7.13	7.12	6.73	6.33	6.30	6.25	6.11	6.12
pH Fridge	7.29	7.53	7.18	7.26	7.00	6.76	6.91				
Formulation No.	1.01										
pH 40°C	7.57	7.20	7.21	6.76	6.71	6.70	6.97				
pH 45°C	7.57	6.97	6.99	6.67	6.69	6.74	6.98				
pH 20°C	7.57	7.18	7.12	6.99	6.87	6.79	6.89	6.85	6.88	6.81	6.88
pH Fridge	7.57	7.53	7.18	7.26	7.00	6.76	6.91				
Formulation No.	1.02										
pH 40°C	7.57	6.98	6.99	7.06	6.80	6.71	6.90				
pH 45°C	7.57	6.90	6.81	6.82	6.76	6.64	6.91				
pH 20°C	7.57	7.18	7.13	7.01	6.83	6.77	6.78	6.79	6.71	6.70	6.61
pH Fridge	7.57	7.27	7.10	7.16	6.98	7.06	6.79				
Formulation No.	10.02										
pH 40°C	7.46	7.40	7.40	7.41	7.40	7.41	7.40				
pH 45°C	7.46	7.39	7.39	7.30	7.33	7.30	7.35				
pH 20°C	7.46	7.51	7.51	7.55	7.50	7.51	7.45	7.40	7.45	7.44	7.44
pH Fridge	7.46	7.54	7.54	7.51	7.33	7.31	7.28				
Formulation No.	10.01										
pH 40°C	5.78	5.98	5.89	5.87	5.81	5.81	5.81				
pH 45°C	5.78	5.99	6.02	6.06	6.01	6.06	5.99				
pH 20°C	5.78	5.81	5.85	5.87	5.88	5.90	5.91	6.00	6.03	6.08	6.02
pH Fridge	5.78	5.84	5.85	5.87	5.81	5.83	5.93				
Formulation No.	8.01										
pH 40°C	7.30	7.24	7.24	7.14	7.11	7.13	6.94				
pH 45°C	7.30	7.24	7.48	7.16	7.13	7.14	7.03				
pH 20°C	7.30	7.32	7.32	7.14	7.15	7.11	7.15	7.14	7.15	7.11	7.11
pH Fridge	7.30	7.29	7.29	7.21	7.20	7.22	7.22				
Formulation No.	8.02										
pH 40°C	5.74	5.98	5.94	5.11	5.00	5.66	5.60				

pH 45°C	5.74	6.01	6.11	6.00	6.11	5.69	5.11				
pH 20°C	5.74	5.89	5.99	5.91	5.71	5.99	5.90	5.69	5.62	5.66	5.60
pH Fridge	5.74	5.87	5.78	5.77	5.71	5.77	5.73				
Formulation No.	6.01										
pH 40°C	7.47	7.37	7.20	7.05	7.13	7.01	7.00				
pH 45°C	7.47	7.26	7.15	7.05	7.09	7.03	7.02				
pH 20°C	7.47	7.41	7.22	7.05	7.01	7.00	7.00	7.00	7.01	7.01	7.02
pH Fridge	7.47	7.56	7.53	7.05	7.53	6.99	7.00				
Formulation No.	6.02										
pH 40°C	7.49	7.44	7.44	7.16	7.11	7.13	7.12				
pH 45°C	7.49	7.40	7.40	7.10	7.13	7.11	7.12				
pH 20°C	7.49	7.41	7.41	7.36	7.50	7.71	7.65	7.60	7.70	7.65	7.55
pH Fridge	7.49	7.42	7.42	7.56	7.59	7.53	7.65				
Formulation No.	4.01										
pH 40°C	7.59	7.35	7.31	7.35	7.54	7.31	7.30				
pH 45°C	7.59	7.36	7.38	7.21	7.16	7.11	7.10				
pH 20°C	7.59	7.52	7.50	7.55	7.30	7.33	7.36	7.31	7.77	7.77	7.74
pH Fridge	7.59	7.63	7.68	7.61	7.51	7.50	7.11				
Formulation No.	4.02										
pH 40°C	7.70	7.27	7.36	7.36	7.00	7.11	7.10				
pH 45°C	7.70	7.31	7.36	7.26	7.01	7.13	7.11				
pH 20°C	7.70	7.48	7.36	7.36	6.97	6.98	7.00	6.90	6.98	7.01	7.03
pH Fridge	7.70	7.58	7.66	7.66	6.98	6.97	6.99				
Formulation No.	2.01										
pH 40°C	7.71	7.61	7.33	7.60	7.33	7.19	7.11				
pH 45°C	7.71	7.53	7.25	7.69	7.25	7.20	7.20				
pH 20°C	7.71	7.51	7.32	7.58	7.38	7.14	7.13	7.11	7.11	7.11	7.02
pH Fridge	7.71	7.73	7.52	7.57	7.52	7.10	7.19				
Formulation No.	2.02										
pH 40°C	7.61	7.60	7.35	7.60	7.43	7.14	7.11				
pH 45°C	7.61	7.35	7.33	7.58	7.26	7.16	7.13				
pH 20°C	7.61	7.43	7.40	7.55	7.40	7.12	7.22	7.20	7.07	7.00	7.02
pH Fridge	7.61	7.60	7.56	7.64	7.46	7.20	7.19				
Formulation No.	12.01										
pH 40°C	5.37	6.23	6.10	6.13	6.11	6.11	6.06				
pH 45°C	5.37	6.44	5.94	5.99	5.98	5.99	5.90				
pH 20°C	5.37	5.95	6.07	6.06	6.05	6.04	6.11	6.11	5.53	5.51	5.50
pH Fridge	5.37	6.43	5.92	5.93	5.40	5.40	5.41				
Formulation No.	23.01										
pH 40°C	6.10	6.00	6.30	6.00	6.61	6.63	6.34				
pH 45°C	6.10	6.11	6.11	6.18	6.20	6.00	6.14				
pH 20°C	6.10	6.10	6.22	6.20	6.21	6.22	5.87	5.81	5.90	5.95	5.99
pH Fridge	6.10	6.33	6.30	6.31	6.33	6.30	6.20				
Formulation No.	23.02										
pH 40°C	6.18	6.11	6.13	6.11	6.06	6.11	5.54				

pH 45°C	6.18	6.14	6.13	6.10	6.11	6.00	5.47				
pH 20°C	6.18	6.20	6.13	6.13	6.14	6.11	5.81	5.71	5.80	5.88	5.90
pH Fridge	6.18	6.21	6.13	6.14	6.13	6.12	5.94				
Formulation No.	30.02										
pH 40°C	6.33	6.30	6.30	6.33	6.44	6.40	6.44				
pH 45°C	6.33	6.31	6.31	6.30	6.43	6.41	6.41				
pH 20°C	6.33	6.33	6.33	6.11	6.34	6.31	6.30	6.41	6.34	6.35	6.30
pH Fridge	6.33	6.30	6.30	6.00	6.01	6.00	6.11				
Formulation No.	26.01										
pH 40°C	6.13	6.11	6.33	6.31	6.31	6.10	4.63				
pH 45°C	6.13	6.10	6.30	6.32	6.31	6.00	4.65				
pH 20°C	6.13	6.13	6.14	6.11	6.10	6.08	5.07	5.06	5.00	5.09	5.11
pH Fridge	6.13	6.14	6.20	6.21	6.20	6.21	5.04				
Formulation No.	26.02										
pH 40°C	6.27	6.21	6.11	6.10	6.21	6.11	6.01				
pH 45°C	6.27	6.23	6.10	6.13	6.14	6.13	5.96				
pH 20°C	6.27	6.21	6.13	6.14	6.15	6.14	6.64	6.51	6.60	6.65	6.50
pH Fridge	6.27	6.20	6.22	6.20	6.22	6.20	5.89				
Formulation No.	24.01										
pH 40°C	6.36	6.31	6.20	6.11	6.06	6.11	5.64				
pH 45°C	6.36	6.30	6.19	6.18	6.11	6.10	5.41				
pH 20°C	6.36	6.21	6.22	6.20	6.22	6.20	6.05	6.00	6.01	6.00	6.05
pH Fridge	6.36	6.22	6.33	6.30	6.31	6.33	6.27				
Formulation No.	24.02										
pH 40°C	6.32	6.30	6.30	6.33	6.31	6.30	5.52				
pH 45°C	6.32	6.11	6.11	6.10	6.13	6.11	5.43				
pH 20°C	6.32	6.14	6.18	6.19	6.18	6.19	6.20	6.22	6.20	6.00	5.59
pH Fridge	6.32	6.20	6.22	6.20	6.21	6.20	6.60				
Formulation No.	45.01										
pH 40°C	5.52	5.50	5.19	5.18	5.19	5.18	5.16				
pH 45°C	5.52	5.51	5.91	4.41	4.99	4.90	5.13				
pH 20°C	5.52	5.11	4.90	4.91	4.33	4.30	5.11	5.10	5.11	5.02	5.11
pH Fridge	5.52	5.10	4.99	5.11	5.00	5.00	5.00				
Formulation No.	45.02										
pH 40°C	5.51	5.99	5.90	5.99	5.11	5.11	5.14				
pH 45°C	5.51	5.44	5.11	5.13	5.10	5.16	5.10				
pH 20°C	5.51	5.40	5.10	5.16	5.13	5.13	5.12	5.11	5.10	5.00	5.05
pH Fridge	5.51	5.41	5.13	5.12	5.14	5.13	5.11				
Formulation No.	50.02										
pH 40°C	5.86	5.81	5.49	5.90	5.91	5.90	5.33				
pH 45°C	5.86	5.49	5.83	5.81	5.11	5.14	5.30				
pH 20°C	5.86	5.40	5.81	5.88	5.14	5.14	5.14	5.11	5.10	5.00	5.05
pH Fridge	5.86	5.81	5.99	5.90	5.44	5.13	5.11				
Formulation No.	46.02										
pH 40°C	5.55	5.56	6.00	6.00	6.11	6.10	6.11				

pH 45°C	5.55	5.51	6.01	6.01	6.13	6.11	6.10				
pH 20°C	5.55	5.59	6.11	6.11	6.14	6.14	6.10	6.11	6.13	6.00	6.12
pH Fridge	5.55	5.09	6.14	6.13	6.33	6.30	6.90				
Formulation No.	55.01										
pH 40°C	5.66	5.60	5.66	5.61	5.66	5.61	5.84				
pH 45°C	5.66	5.66	5.61	5.63	5.61	5.44	5.45				
pH 20°C	5.66	5.77	5.72	5.60	5.77	5.70	6.21	6.00	6.11	6.02	6.22
pH Fridge	5.66	5.70	5.71	5.70	5.73	5.71	6.56				
Formulation No.	55.02										
pH 40°C	5.83	5.81	5.89	5.80	5.84	5.81	5.08				
pH 45°C	5.83	5.99	5.90	5.90	5.99	5.99	5.28				
pH 20°C	5.83	5.81	5.83	5.88	5.83	5.83	6.09	6.01	6.00	6.00	6.50
pH Fridge	5.83	5.40	5.44	5.41	5.41	5.81	6.47				
Formulation No.	60.01										
pH 40°C	5.26	5.11	5.31	5.44	4.98	4.99	4.90				
pH 45°C	5.26	5.22	5.33	5.21	4.72	4.70	4.77				
pH 20°C	5.26	5.21	5.30	5.21	4.98	4.91	4.91	4.91	4.88	4.85	4.90
pH Fridge	5.26	5.26	5.44	5.20	5.54	5.50	5.51				
Formulation No.	60.02										
pH 40°C	5.28	5.21	5.78	5.76	4.56	4.48	4.02				
pH 45°C	5.28	5.22	5.70	5.40	4.58	4.51	4.07				
pH 20°C	5.28	5.34	5.58	5.42	4.59	4.46	4.39	4.32	4.25	4.18	4.11
pH Fridge	5.28	5.36	5.50	5.44	4.46	4.41	4.37				
Formulation No.	56.01										
pH 40°C	6.02	6.00	6.00	6.06	6.01	6.00	5.07				
pH 45°C	6.02	6.01	6.01	6.00	6.00	5.99	5.05				
pH 20°C	6.02	6.02	6.03	6.00	6.00	6.00	5.30	5.11	5.10	5.00	5.02
pH Fridge	6.02	6.03	6.04	6.03	6.01	6.09	5.48				
Formulation No.	56.02										
pH 40°C	5.98	5.99	5.96	5.90	5.91	5.91	5.20				
pH 45°C	5.98	5.96	5.44	5.44	5.40	5.44	5.05				
pH 20°C	5.98	5.49	5.41	5.49	5.41	5.41	5.62	5.66	6.00	6.02	6.05
pH Fridge	5.98	5.99	5.90	5.91	5.93	5.99	6.11				
Formulation No.	75.01										
pH 40°C	5.84	5.81	5.81	5.81	5.86	5.81	5.81				
pH 45°C	5.84	5.80	5.82	5.88	5.66	5.60	5.60				
pH 20°C	5.84	5.81	5.90	5.99	5.55	5.51	5.51	5.66	6.00	6.23	6.51
pH Fridge	5.84	5.89	5.89	5.91	5.61	5.60	5.60				
Formulation No.	75.02										
pH 40°C	5.59	5.51	5.56	5.56	5.66	5.61	5.60				
pH 45°C	5.59	5.59	5.61	5.61	5.64	5.63	5.61				
pH 20°C	5.59	5.58	5.51	5.51	5.11	5.10	5.11	5.23	5.24	5.33	5.33
pH Fridge	5.59	5.57	5.66	5.66	5.13	5.11	5.10				
Formulation No.	76.01										
pH 40°C	5.81	5.81	5.81	5.81	5.83	5.81	5.88				

pH 45°C	5.81	5.80	5.80	5.88	5.81	5.80	5.81				
pH 20°C	5.81	5.91	5.91	5.91	5.90	5.91	5.91	5.91	5.83	5.87	5.86
pH Fridge	5.81	5.99	5.99	5.99	5.91	5.99	5.90				
Formulation No.	76.02										
pH 40°C	5.76	5.81	5.81	5.81	5.83	5.81	5.88				
pH 45°C	5.76	5.80	5.80	5.88	5.81	5.80	5.81				
pH 20°C	5.76	5.91	5.91	5.91	5.90	5.91	5.91	5.91	5.83	5.87	5.85
pH Fridge	5.76	5.99	5.99	5.99	5.91	5.99	5.90				
Formulation No.	80.01										
pH 40°C	5.51	5.56	5.66	5.51	5.56	5.61	5.60				
pH 45°C	5.51	5.61	5.64	5.59	5.61	5.63	5.61				
pH 20°C	5.51	5.51	5.52	5.58	5.51	5.58	5.68	5.59	5.61	5.63	5.61
pH Fridge	5.51	5.66	5.53	5.57	5.66	5.69	5.65				
Formulation No.	80.02										
pH 40°C	5.64	5.81	5.83	5.81	5.81	5.81	5.88				
pH 45°C	5.64	5.88	5.81	5.80	5.80	5.80	5.81				
pH 20°C	5.64	5.91	5.90	5.91	5.91	5.91	5.91	5.83	5.81	5.81	5.81
pH Fridge	5.64	5.99	5.91	5.99	5.99	5.99	5.90				
Average Results											
Weeks	0	1	2	4	8	12	16	24	32	48	96
Average pH 40°C	6.39	6.35	6.36	6.33	6.26	6.24	6.07				
Average pH 45°C	6.39	6.33	6.33	6.28	6.20	6.16	6.01				
Average pH 20°C	6.39	6.33	6.36	6.34	6.21	6.18	6.18	6.13	6.13	6.11	6.12
Average pH 4ºC	6.39	6.40	6.41	6.38	6.28	6.24	6.22				
ASM Prediction Error				0.01	0.15	0.14	0.14	0.15	0.13	0.13	0.11
Average ASM Prediction Error											0.12

10.3 Appendix 3 – Viscosity Results

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
Formula No.		9.01				TD@5rpm				4000												
visc 45°C	46	184000	26	104000	28	112000	21	84000	20	80000	21	84000	20	80000								
visc 40°C	46	184000	28	112000	26	104000	20	80000	20	80000	22	88000	20	80000								
visc 20°C	46	184000	45	180000	62	248000	60	240000	64	256000	60	240000	62	248000	63	252000	52	208000	50	200000	63	252000
visc Fridge	46	184000	50	200000	50	200000	50	200000	55	220000	67	266000	61	244000								
Formula No.		9.02				TD@10rpm				2000												
visc 45°C	47	94000	30	60000	29	58000	29	58000	28	56000	20	40000	21	42000								
visc 40°C	47	94000	36	72000	35	70000	31	62000	30	60000	30	60000	30	60000								
visc 20°C	47	94000	66	132000	50	100000	55	110000	50	100000	55	110000	51	102000	51	102000	55	110000	56	112000	60	120000
visc Fridge	47	94000	60	120000	50	100000	27	54000	29	58000	28	56000	28	56000								
Formula No.		7.01				TC@5rpm				2000												
visc 45°C	50	100000	59	118000	59	118000	59	118000	90	180000	93	186000	91	182000								
visc 40°C	50	100000	56	112000	58	116000	59	118000	60	120000	61	122000	60	120000								
visc 20°C	50	100000	80	160000	82	164000	84	168000	61	122000	66	132000	61	122000	61	122000	66	132000	61	122000	62	123000
visc Fridge	50	100000	97	194000	98	196000	99	198000	60	120000	69	138000	63	126000								
Formula No.		7.02				TD@5rpm				4000												
visc 45°C	50	200000	30	120000	40	160000	50	200000	51	204000	50	200000	51	204000								
visc 40°C	50	200000	39	156000	34	136000	30	120000	31	124000	40	160000	40.5	162000								
visc 20°C	50	200000	59	236000	52	208000	46	184000	45	180000	47	188000	40	160000	40	160000	39	156000	40	160000	41	164000
visc Fridge	50	200000	59	236000	50	200000	47	188000	49.5	198000	49.5	198000	46.5	186000								
Formula No.		5.01				TD@10rpm				2000												
visc 45°C	36	72000	26	52000	50	100000	50	100000	52	104000	50	100000	40	80000								
visc 40°C	36	72000	28	56000	54	108000	50	100000	52	104000	50	100000	40	80000								
visc 20°C	36	72000	34	68000	60	120000	60	120000	61	122000	60	120000	61	122000	60	120000	61	122000	70	140000	71	142000
visc Fridge	36	72000	38	76000	68	136000	78	156000	60	120000	62	124000	60	120000								
Formula No.		5.02				TC@5rpm				2000												
visc 45°C	69	138000	49	98000	49	98000	34	68000	34.5	69000	34	68000	34.5	69000								
visc 40°C	69	138000	64	128000	66	132000	31	62000	30	60000	31	62000	30	60000								
visc 20°C	69	138000	90	180000	91	182000	45	90000	45.5	91000	45	90000	45.5	91000	45	90000	45.5	91000	45	90000	46	92000
visc Fridge	69	138000	79	158000	66	132000	39.5	79000	39	78000	38.5	77000	35	70000								
Formula No.		3.01				TC@5rpm				2000												
visc 45°C	44	88000	40	80000	25	50000	24	48000	27	54000	24	48000	29	58000								
visc 40°C	44	88000	45	90000	30	60000	31	62000	29	58000	28	56000	26	52000								
visc 20°C	44	88000	35	70000	30	60000	33	66000	26	52000	26	52000	27	54000	26	52000	26	52000	24	48000	23	45000
visc Fridge	44	88000	45	90000	30	60000	26	52000	50	100000	45	90000	44	88000								
Formula No.		3.02				TC@5rpm				2000												
visc 45°C	33	66000	45	90000	27	54000	40	80000	41	82000	21	42000	20	40000								
visc 40°C	33	66000	36	72000	29	58000	28	56000	25	50000	25	50000	24	48000								
visc 20°C	33	66000	45	90000	35	70000	37	74000	34	68000	36	72000	30	60000	31	62000	31	62000	31	62000	30	60000
visc Fridge	33	66000	30	60000	31	62000	39	78000	35	70000	33	66000	31	62000								

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
Formula No.		1.01				TC@5rpm				2000												
visc 45°C	33	66000	39	78000	30	60000	27	54000	26	52000	20	40000	20	40000								
visc 40°C	33	66000	44	88000	40	80000	31	62000	30	60000	23	46000	25	50000								
visc 20°C	33	66000	70	140000	77	154000	56	112000	55	110000	50	100000	54	108000	50	100000	51	102000	50	100000	51	102000
visc Fridge	33	66000	70	140000	77	154000	56	112000	55	110000	50	100000	54	108000								
Formula No.		1.02				TC@5rpm				2000												
visc 45°C	28	55000	32	64000	30	60000	27	54000	28	56000	20	40000	29	58000								
visc 40°C	28	55000	41	82000	40	80000	26	52000	29	58000	23	46000	20	40000								
visc 20°C	28	55000	56	112000	55	110000	46	92000	45	90000	50	100000	51	102000	55	110000	51	102000	56	112000	55	110000
visc Fridge	28	55000	55	110000	55	110000	50	100000	50	100000	51	102000	53	106000								
Formula No.		10.02				TD@5rpm				4000												
visc 45°C	60	240000	74	296000	76	304000	76	304000	75	300000	74	296000	75	300000								
visc 40°C	60	240000	69	276000	66	264000	66	264000	60	240000	61	244000	62.5	250000								
visc 20°C	60	240000	79	316000	79	316000	79	316000	77	308000	75	300000	69.5	278000	67.5	270000	60	240000	60	240000	64	254000
visc Fridge	60	240000	80	320000	81	324000	81	324000	80	320000	82	328000	81	324000								
Formula No.		10.01				TC@10rpm				1000												
visc 45°C	49	49000	35	35000	35	35000	40	40000	41	41000	46	46000	49	49000								
visc 40°C	49	49000	35	35000	35	35000	40	40000	44	44000	40	40000	41	41000								
visc 20°C	49	49000	45	45000	35	35000	30	30000	31	31000	30	30000	33	33000	42	42000	47	47000	55	55000	55	55000
visc Fridge	49	49000	49	49000	42	42000	40	40000	42	42000	41	41000	40	40000								
Formula No.		8.01				TC@5rpm				2000												
visc 45°C	60	120000	73	145000	72.5	145000	56	112000	61	122000	66	132000	62	124000								
visc 40°C	60	120000	70	140000	70	140000	72	144000	70	140000	70	140000	82	164000								
visc 20°C	60	120000	67	134000	67	134000	51	102000	50	100000	55	110000	89	178000	89	178000	89	178000	89	178000	90	180000
visc Fridge	60	120000	55	110000	55	110000	52	104000	53	106000	50	100000	79	158000								
Formula No.		8.02				TC@5rpm				2000												
visc 45°C	65	130000	60	120000	50	100000	56	112000	60	120000	61	122000	61	122000								
visc 40°C	65	130000	55	110000	52	104000	50	100000	50	100000	51	102000	50	100000								
visc 20°C	65	130000	69	138000	56	112000	51	102000	56	112000	53	106000	51	102000	51	102000	52	104000	60	120000	62	124000
visc Fridge	65	130000	70	140000	66	132000	66	132000	60	120000	60	120000	60	120000								
Formula No.		6.01				TC@5rpm				2000												
visc 45°C	60	120000	80	160000	82	164000	86	172000	99	198000	82	164000	88	176000								
visc 40°C	60	120000	65	130000	57.5	115000	50	100000	52	104000	50	100000	51	102000								
visc 20°C	60	120000	84	167000	67	134000	58	116000	68	136000	56	112000	54	108000	52	104000	58	116000	60	120000	62	124000
visc Fridge	60	120000	50	100000	48	96000	46	92000	94	188000	83.5	167000	70	140000								
Formula No.		6.02				TC@5rpm				2000												
visc 45°C	18	35000	32	64800	32.4	64800	45	90000	40	80000	50	100000	60	120000								
visc 40°C	18	35000	24	48000	24	48000	50	100000	53	106000	50	100000	51.5	103000								
visc 20°C	18	35000	24	48800	24.4	48800	50	100000	74	148000	67	134000	70	140000	80	160000	85	170000	95	190000	97	194000
visc Fridge	18	35000	26	52800	26.4	52800	65	130000	60	120000	55	110000	57.5	115000								
Formula No.		4.01				TC@5rpm				2000												
visc 45°C	26	52000	30	60000	31	62000	27	54000	30	60000	32	64000	31	62000								
visc 40°C	26	52000	27	54000	26	52000	25	50000	26	52000	25	50000	20	40000								
visc 20°C	26	52000	30	60000	31	62000	35	70000	35.5	71000	30	60000	31	62000	33	66000	31	62000	31	62000	31	62000
visc Fridge	26	52000	28	56000	27	54000	31	62000	31	62000	31	62000	30	60000								
Formula No.		4.02				TB@5rpm				800				TC@5rpm		2000						
visc 45°C	31	24800	71	56800	71	56800	71	56800	75	60000	39	78000	40	80000								
visc 40°C	31	24800	60	48000	61	48800	61	48800	55	110000	50	100000	52	104000								
visc 20°C	31	24800	61	48800	66	52800	66	52800	95	190000	81	162000	93	186000	91	182000	91	182000	93	186000	88	176000
visc Fridge	31	24800	66	52800	70	56000	70	56000	95	190000	83	166000	66	132000								
Formula No.		2.01				LV3@30rpm				40												
visc 45°C	30	1200	34	1360	90	3600	91	3640	92	3680	91	3640	93	3720								
visc 40°C	30	1200	30	1200	60	2400	66	2640	60	2400	80	3200	91	3640								
visc 20°C	30	1200	31	1240	42	1680	41	1640	54	2160	50	2000	50	2000	51	2040	51	2040	51	2040	50	2000
visc Fridge	30	1200	30	1200	46.5	1860	50	2000	61	2440	65	2600	92	3680								
Formula No.		2.02				LV3@30rpm				40												
visc 45°C	27	1080	66	2640	65	2600	69	2760	69	2760	65	2600	94.5	3780								
visc 40°C	27	1080	35	1400	57	2280	60	2400	61	2440	65	2600	90	3600								
visc 20°C	27	1080	30	1200	55	2200	56	2240	55	2200	53	2120	66	2640	50	2000	51	2040	50	2000	50	2000
visc Fridge	27	1080	33	1320	55	2200	61	2440	60	2400	50	2000	51	2040								
Formula No.		12.01				TB@5rpm				800												
visc 45°C	45	36000	20	16000	21	16800	21	16800	20	16000	10.5	8400	20	16000								
visc 40°C	45	36000	40	32000	40	32000	47.5	38000	40	32000	41	32800	40	32000								
visc 20°C	45	36000	43	34400	33	26400	21	16800	15	12000	18.5	14800	20	16000	30	24000	46	36800	42	33600	42	33200
visc Fridge	45	36000	39	30800	33	26400	10.5	8400	10	8000	10	8000	21	16800								

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
Formula No.		12.04				TB@5rpm				800												
visc 45°C	50	39600																				
visc 40°C	50	39600																				
visc 20°C	50	39600	45	36000	42.5	34000	40	32000	10	8000	10	8000										
visc Fridge	50	39600	43	34400	45	36000	46	36800	10.5	8400	7.5	6000										
Formula No.		16.01				TB@10rpm				400												
visc 45°C	30	12000																				
visc 40°C	30	12000																				
visc 20°C	30	12000	35	14000	10	4000																
visc Fridge	30	12000	34	13600	12	4800																
Formula No.		20.01				LV3@6rpm				200												
visc 45°C	31	6200	32	6300	29	5800																
visc 40°C	31	6200	30	6000	31.5	6300	30	6000	31.5	6300												
visc 20°C	31	6200	31	6100	30.5	6100	31.5	6300	38.5	7700	35	7000	80	16000	81	16200	88	17600	84	16800	86	17200
visc Fridge	31	6200	33	6600	30.5	6100	29	5800	29.5	5900	27.5	5500	85	17000								
Formula No.		23.01				TB@20rpm				200												
visc 45°C	30	6000	44	8800	43	8600	44	8800	51	10200	38	7600	74	14800								
visc 40°C	30	6000	31	6200	40	8000	40	8000	41	8200	39	7800	70	14000								
visc 20°C	30	6000	30	6000	41	8200	43	8600	44	8800	33	6600	45	9000	41	8200	44	8800	40	8000	42	8400
visc Fridge	30	6000	39	7800	39	7800	44	8800	50	10000	39	7800	52	10400								
Formula No.		23.02				LV3@6rpm				200				LV3@3rpm		400						
visc 45°C	43	8600	60	12000	61	12200	59	11800	58	11600	63	12600	91	36400								
visc 40°C	43	8600	44	8800	61	12200	60	12000	61	12200	60	12000	67	26800								
visc 20°C	43	8600	40	8000	66	13200	63	12600	63	12600	58	11600	78	15600	77	15400	70	14000	80	16000	70	14000
visc Fridge	43	8600	49	9800	50	10000	50	10000	57	11400	59	11800	65	13000								
Formula No.		30.02				TB@5rpm				800												
visc 45°C	48	38400	48	38000	45	36000	40	32000	41	32800	41	32800	42	33600								
visc 40°C	48	38400	49	38800	48.5	38800	47.5	38000	46.5	37200	46.5	37200	42.5	34000								
visc 20°C	48	38400	49	38800	48.5	38800	40	32000	41	32800	40	32000	41	32800	40	32000	41	32800	40	32000	41	32800
visc Fridge	48	38400	48	38400	43	34400	41	32800	42	33600	42	33600	42	33600								
Formula No.		26.01				TB@20rpm				200												
visc 45°C	43	8600	43	8600	40	8000	41	8200	43	8600	83	16600	82	16400								
visc 40°C	43	8600	40	8000	40	8000	40	8000	41	8200	40	8000	43	8600								
visc 20°C	43	8600	41	8200	43	8600	44	8800	44	8800	44	8800	45	9000	40	8000	41	8200	44	8800	43	8600
visc Fridge	43	8600	44	8800	44	8800	42	8400	41	8200	40	8000	30	6000								
Formula No.		26.02				TB@20rpm				200				TbarC@20rpm		500						
visc 45°C	40	8000	50	10000	41	8200	61	12200	73	14600	74	14800	64	32000								
visc 40°C	40	8000	41	8200	59	11800	59	11800	63	12600	60	12000	56	28000								
visc 20°C	40	8000	40	8000	55	11000	58	11600	70	14000	77	15400	65	32500	61	30500	66	33000	60	30000	68	34000
visc Fridge	40	8000	49	9800	55	11000	60	12000	71	14200	70	14000	54	27000								
Formula No.		24.01				TB@10rpm				400												
visc 45°C	29	11600	32	12600	35.5	14200	35.5	14200	38.5	15400	38	15200	62.5	25000								
visc 40°C	29	11600	30	12000	31.5	12600	30.5	12200	30	12000	30.5	12200	72.5	29000								
visc 20°C	29	11600	31	12200	35	14000	33.5	13400	36.5	14600	38.5	15400	66	26400	72.5	29000	62.5	25000	50	20000	65	26000
visc Fridge	29	11600	33	13200	33	13200	35	14000	35.5	14200	35	14000	60	24000								
Formula No.		24.02				TB@20rpm				200				TC@20rpm		500						
visc 45°C	39	7800	44	8800	44	8800	44	8800	42	8400	44	8800	45	22500								
visc 40°C	39	7800	38	7600	43	8600	40	8000	41	8200	40	8000	84	16800								
visc 20°C	39	7800	39	7800	41	8200	44	8800	43	8600	44	8800	82	16400	80	16000	81	16200	82	16400	83	16600
visc Fridge	39	7800	40	8000	41	8200	41	8200	40	8000	41	8200	60	12000								
Formula No.		45.01				LV3@30rpm				40												
visc 45°C	36	1440	30	1200	30	1200	31	1240	41	1640	40	1600	53	2120								
visc 40°C	36	1440	35	1400	36	1440	30	1200	31	1240	30	1200	41	1640								
visc 20°C	36	1440	30	1200	33	1320	31	1240	33	1320	31	1240	44	1760	40	1600	41	1640	41	1640	40	1600
visc Fridge	36	1440	31	1240	31	1240	33	1320	30	1200	44	1760	50	2000								
Formula No.		45.02				LV2@30rpm				10												
visc 45°C	42	420	46	460	43	430	40	400	59	590	46	460	40	400								
visc 40°C	42	420	40	400	47	470	46	460	43	430	40	400	66	660								
visc 20°C	42	420	41	410	47	470	41	410	44	440	41	410	40	400	41	410	44	440	40	400	36	360
visc Fridge	42	420	44	440	43	430	44	440	46	460	44	440	41	410								
Formula No.		50.01				LV2@6rpm				50												
visc 45°C	62	3100	63	3150	66	3300	31	1550	25	1250												
visc 40°C	62	3100	66	3300	60	3000	61	3050	40	2000												
visc 20°C	62	3100	61	3050	66	3300	63	3150	63	3150	61	3050	78	3900	72	3600	73	3650	73	3650	70	3500
visc Fridge	62	3100	66	3300	63	3150	63	3150	61	3050	60	3000	79	3950								

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
Formula No.		50.02				LV2@30rpm				10												
visc 45°C	46	460	48	480	44	440	40	400	60	600	40	400	40	400								
visc 40°C	46	460	40	400	47	470	48	480	42	420	40	400	58	580								
visc 20°C	46	460	41	410	47	470	40	400	44	440	42	420	40	400	42	420	40	400	40	400	36	360
visc Fridge	46	460	44	440	43	430	44	440	44	440	44	440	42	420								
Formula No.		46.01				LV3@12rpm				100				LV3@6rpm		200						
visc 45°C	32	3200	34	3400	40	4000	66	13200														
visc 40°C	32	3200	30	3000	40	4000	80	8000	77	15400												
visc 20°C	32	3200	31	3100	41	4100	82	8200	86	8600	88	8800	96	19200	93	18600	93	18600	82	16400	90	18000
visc Fridge	32	3200	33	3300	41	4100	88	8800	92	9200	96	9600	87	17400								
Formula No.		46.02				LV2@12rpm				25												
visc 45°C	35	875	39	975	53	1325	41	1025	85	2125	60	1500	82	2050								
visc 40°C	35	875	32	800	48	1200	41	1025	47	1175	98	2450	64	1600								
visc 20°C	35	875	33	825	53	1325	45	1125	83	2075	86	2150	82	2050	80	2000	45	1125	45	1125	44	1100
visc Fridge	35	875	36	900	47	1175	47	1175	81	2025	80	2000	100	2500								
Formula No.		55.01				TB@20rpm				200				TC@20		500						
visc 45°C	35	7000	41	8200	39	7800	39	7800	33	6600	32	6400	43	21500								
visc 40°C	35	7000	36	7200	30	6000	35	7000	34	6800	30	6000	45	22500								
visc 20°C	35	7000	39	7800	31	6200	30	6000	31	6200	30	6000	49	24500	44	22000	41	20500	48	24000	44	22000
visc Fridge	35	7000	40	8000	31	6200	39	7800	37	7400	31	6200	57	28500								
Formula No.		55.02				TB@20rpm				200				TC@20		500						
visc 45°C	33	6600	33	6600	39	7800	40	8000	41	8200	43	8600	78	39000								
visc 40°C	33	6600	30	6000	30	6000	31	6200	30	6000	32	6400	50	25000								
visc 20°C	33	6600	31	6200	33	6600	33	6600	32	6400	30	6000	32	16000	31	6200	30	6000	30	6000	30	6000
visc Fridge	33	6600	33	6600	33	6600	39	7800	38	7600	31	6200	39	19500								
Formula No.		60.01				TC@5rpm				2000												
visc 45°C	75	150000	80	160000	89	178000	75	150000	54	108000	51	102000	50	100000								
visc 40°C	75	150000	81	162000	82	164000	80	160000	87	174000	79	158000	49	98000								
visc 20°C	75	150000	88	175000	72.5	145000	79	158000	63	126000	75	150000	70	140000	71	142000	72	144000	77	154000	76	152000
visc Fridge	75	150000	90	180000	82	164000	71	142000	80	160000	76	152000	75	150000								
Formula No.		60.02				TC@10rpm				2000												
visc 45°C	45	90000	40	80000	42.5	85000	42	84000	28.5	57000	25	50000	30	60000								
visc 40°C	45	90000	46	91000	45	90000	45.5	91000	29.5	59000	29.5	59000	33.5	67000								
visc 20°C	45	90000	45	90000	45.5	91000	46	92000	45	90000	34.5	69000	35	70000	38.5	77000	39	78000	40	80000	41	82000
visc Fridge	45	90000	44	87000	42.5	85000	43	86000	43	86000	40.5	81000	38.5	77000								
Formula No.		56.01				TB@20rpm				200				TC@20rpm		500						
visc 45°C	58	11600	63	12600	63	12600	64	12800	63	12600	63	12600	73	36500								
visc 40°C	58	11600	60	12000	61	12200	60	12000	63	12600	58	11600	49	24500								
visc 20°C	58	11600	61	12200	66	13200	66	13200	63	12600	59	11800	63	31500	61	30500	60	30000	64	32000	70	35000
visc Fridge	58	11600	66	13200	61	12200	63	12600	63	12600	50	10000	74	37000								
Formula No.		56.02				TB@20rpm				200				TC@20		500						
visc 45°C	37	7400	20	4000	21	4200	20	4000	22	4400	20	4000	30	6000								
visc 40°C	37	7400	50	10000	50	10000	50	10000	50	10000	50	10000	92.5	18500								
visc 20°C	37	7400	40	8000	41	8200	44	8800	43	8600	40	8000	97.5	19500	95	19000	95	19000	97	19400	98	19600
visc Fridge	37	7400	50	10000	41	8200	39	7800	38	7600	33	6600	56	28000								
Formula No.		75.01				TB@5rpm				800												
visc 45°C	47	37600	54	42800	53.5	42800	60	48000	40	32000	42	33600	42	33600								
visc 40°C	47	37600	49	38800	48.5	38800	47.5	38000	41	32800	40	32000	41	32800								
visc 20°C	47	37600	40	32000	45	36000	41	32800	42.5	34000	41	32800	40	32000	48.5	38800	48.5	38800	46	36400	48	38000
visc Fridge	47	37600	49	38800	48.5	38800	45.5	36400	42.5	34000	40	32000	41	32800								
Formula No.		75.02				LV3@6rpm				200												
visc 45°C	38	7600	22	4400	16	3200	16	3200	10	2000	8	1600	9	1800								
visc 40°C	38	7600	20	4000	15	3000	15	3000	10	2000	10	2000	10	2000								
visc 20°C	38	7600	17	3400	16	3200	16	3200	8	1600	7	1400	7	1400	10	2000	16	3200	16	3200	20	4000
visc Fridge	38	7600	20	4000	14	2800	14	2800	10	2000	9	1800	8	1600								
Formula No.		76.01				TC@5rpm				2000												
visc 45°C	57	114000	45	90000	45	90000	66	132000	45	90000	40	80000	46	92000								
visc 40°C	57	114000	51	102000	51	102000	56	112000	51	102000	50	100000	50	100000								
visc 20°C	57	114000	55	110000	55	110000	59	118000	55	110000	51	102000	51	102000	55	110000	59	118000	62	124000	65	130000
visc Fridge	57	114000	49	98000	49	98000	61	122000	49	98000	49	98000	40	80000								
Formula No.		76.02				TC@5rpm				2000												
visc 45°C	37	74000	34	68000	34	68000	31	62000	34	68000	36	72000	36	72000								
visc 40°C	37	74000	34	68000	36	72000	36	72000	34	68000	38	76000	38	76000								
visc 20°C	37	74000	37	74000	34	68000	37	74000	41	82000	43	86000	41	82000	41	82000	41	82000	43	86000	47	94000
visc Fridge	37	74000	49	98000	49	98000	46	92000	49	98000	49	98000	47	94000								

Week	0		1		2		4		8		12		16		24		32		48		96	
	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps	def	cps
Formula No.		80.01				TC@2.5rpm				4000												
visc 45°C	40	160000	37.5	150000	36.5	146000	39	156000	38	152000	38	152000	37	148000								
visc 40°C	40	160000	37	148000	37.5	150000	34	136000	36.5	146000	37.5	150000	37.5	150000								
visc 20°C	40	160000	42	168000	41.5	166000	39.5	158000	41.5	166000	40	160000	42	168000	42	168000	43	172000	45	178000	44	176000
visc Fridge	40	160000	41.5	166000	42	168000	44	176000	43.5	174000	40.5	162000	41.5	166000								
Formula No.		80.02				TC@2.5rpm				4000												
visc 45°C	36	144000	35	140000	34	136000	34	136000	33	132000	32	128000	32.5	130000								
visc 40°C	36	144000	34.5	138000	34	136000	35.5	142000	34.5	138000	34	136000	33	132000								
visc 20°C	36	144000	37	148000	36	144000	34.5	138000	36.5	146000	36.5	146000	39	156000	39.5	158000	39.5	158000	41	162000	41	162000
visc Fridge	36	144000	37.5	150000	39	156000	39	156000	38	152000	41	164000	40	160000								

10.4 Appendix 4 – Microscopy Results

Results for mean droplet size:

weeks	0	1	2	4	8	12	16	24
Formulation No.	9.01			units=	µm²			
microscopy 45°C	90.2	75.3	65.4	81.6	51.6	42.6	29.8	
microscopy 40°C	90.2	42.2	38.8	45.6	48.2	68.3	40.4	
microscopy 20°C	90.2	53.6	72.5	61.0	68.7	55.0	10.4	35.6
microscopy 4°C	90.2	68.4	66.5	55.7	45.6	50.2	78.6	
Formulation No.	9.02			units=	µm²			
microscopy 45°C	10.7	8.9	7.9	11.3	9.9	12.4	14.2	
microscopy 40°C	10.7	7.6	9.5	8.5	7.6	19.6	12.3	
microscopy 20°C	10.7	14.6	11.3	8.6	8.2	9.5	7.4	10.5
microscopy 4°C	10.7	15.9	18.8	7.9	12.3	8.9	8.3	
Formulation No.	7.01			units=	µm²			
microscopy 45°C	29.3	21.3	16.9	25.7	15.9	24.6	11.5	
microscopy 40°C	29.3	38.5	28.6	11.5	32.6	6.4	29.3	
microscopy 20°C	29.3	44.6	52.8	69.8	56.3	54.1	60.6	69.3
microscopy 4°C	29.3	34.6	35.6	33.1	41.6	35.9	38.2	
Formulation No.	7.02			units=	µm²			
microscopy 45°C	7	8.8	8	8.6	7.5	9.5	7.3	
microscopy 40°C	7	9.5	8.5	8.3	6.9	7.6	8.7	
microscopy 20°C	7	7.6	6.6	6.9	8.4	9.8	7.6	8.3
microscopy 4°C	7	8.6	7.5	7.9	9.6	8.2	5.6	
Formulation No.	5.01			units=	µm²			
microscopy 45°C	38.7	32.6	39.5	33.9	32.2	25.5	26.3	
microscopy 40°C	38.7	33.6	34.7	44	23.8	20.8	24.6	
microscopy 20°C	38.7	47.6	35.2	37.1	34.5	33.6	39.4	32.1
microscopy 4°C	38.7	35.6	30.8	25.8	24.8	38.4	37.1	
Formulation No.	5.02			units=	µm²			
microscopy 45°C	6.9	8.6	11.6	8.9	5.8	9	10.1	
microscopy 40°C	6.9	6.3	5.4	6	8.4	7.6	11.3	

microscopy 20°C	6.9	7	5.2	5.2	6.9	5.6	8.2	7.1
microscopy 4°C	6.9	8.4	6.1	7.9	9.6	7.9	6.4	
Formulation No.	3.01			units=	µm²			
microscopy 45°C	88.5	65.7	76.8	56.3	98.6	73.9	46.9	
microscopy 40°C	88.5	62.8	115.7	78.6	119.6	127.4	65.6	
microscopy 20°C	88.5	74.1	70.2	54.3	64.3	85.6	40.1	142.2
microscopy 4°C	88.5	76.2	64.3	98.3	65.3	118.5	58.5	
Formulation No.	3.02			units=	µm²			
microscopy 45°C	15.5	37.6	69.3	78.3	45.8	77	68.2	
microscopy 40°C	15.5	26.4	45.9	116.4	78.3	62.1	61.9	
microscopy 20°C	15.5	20.3	102.3	15.5	45.6	77.3	50.6	110.3
microscopy 4°C	15.5	36.6	54.9	87.4	99.1	116.8	47	
Formulation No.	1.01							
microscopy 45°C	57.6	43.2	37.1	69.4	35.6	55.7	37.2	
microscopy 40°C	57.6	33.6	55.9	89.8	39.4	68.3	49.4	
microscopy 20°C	57.6	39.6	69.3	77.6	32.6	47.5	43.8	38.4
microscopy 4°C	57.6	48.6	68.1	92.3	49.3	89.4	45.3	
Formulation No.	1.02							
microscopy 45°C	16.3	19.4	12.9	13.4	3	16.1	16.3	
microscopy 40°C	16.3	12.8	8.4	9.4	37.6	9.4	19.8	
microscopy 20°C	16.3	7.6	21.6	32.4	33.1	13.9	9.4	122.9
microscopy 4°C	16.3	5.6	9.6	18.8	6.3	10.1	4.5	
Formulation No.	10.02							
microscopy 45°C	7.1	8.9	6.3	18.3	36.5	25.7	19.4	
microscopy 40°C	7.1	11.8	9.4	29.3	51.5	14.6	16.4	
microscopy 20°C	7.1	8.2	7.1	13.5	7.4	14.9	12.4	8.8
microscopy 4°C	7.1	9	8.4	16.3	6.4	15.7	13.8	
Formulation No.	10.01							
microscopy 45°C	49.6	56.8	67.6	42	64.9	57.1	38.5	
microscopy 40°C	49.6	38.5	55.7	67.4	34.6	51.3	75.1	
microscopy 20°C	49.6	64.2	58.3	37.2	32.9	66.1	44.3	67.1
microscopy 4°C	49.6	34.6	33.2	43.7	35.3	34.4	42.6	
Formulation No.	8.01							
microscopy 45°C	56.3	74.1	61.9	109.1	76.4	98.3	60.6	
microscopy 40°C	56.3	50.3	59.3	68.4	49.6	74.3	123.1	
microscopy 20°C	56.3	42.6	45.7	60.8	52.7	68.6	88.8	74.3
microscopy 4°C	56.3	59.3	68.4	96.4	74.6	63.3	115.8	
Formulation No.	8.02							
microscopy 45°C	5.8	5.9	4.9	4.5	7.6	7.8	6.4	
microscopy 40°C	5.8	4.6	3.9	4.4	4.6	4.6	4.6	
microscopy 20°C	5.8	10.6	6.5	4.7	4.5	4.6	4.1	4.9
microscopy 4°C	5.8	9.4	12.6	5.8	6.8	5.1	7.4	
Formulation No.	6.01							
microscopy 45°C	26.5	16.3	25.3	32.3	16.4	18.2	4.9	

microscopy 40°C	26.5	14.3	29.2	11.3	12.2	19.3	7.6	
microscopy 20°C	26.5	18.3	17.5	31.6	35.6	34.2	23.8	15.6
microscopy 4°C	26.5	16.5	34.6	32.6	19.6	35.4	33.8	
Formulation No.	6.02							
microscopy 45°C	89	64.2	66.6	72.1	71.6	97	89	
microscopy 40°C	89	83.6	141.6	88	65.7	123.4	150.5	
microscopy 20°C	89	92.6	65.3	75.6	63.7	96.3	69	74.3
microscopy 4°C	89	98.3	63.2	68.4	84.3	67.4	70.4	
Formulation No.	4.01							
microscopy 45°C	34.5	47.6	45.9	5.4	39.6	6.2	42.3	
microscopy 40°C	34.5	45.9	35.5	43.1	30	9.7	36.7	
microscopy 20°C	34.5	42.3	60.5	50	70.5	51.6	69.5	34.5
microscopy 4°C	34.5	62.3	57.6	39.1	46.6	23.7	41.7	
Formulation No.	4.02							
microscopy 45°C	6.1	14.9	12.6	8.4	16.5	17.3	8.4	
microscopy 40°C	6.1	15.6	4.2	7.6	8.8	4.4	9.6	
microscopy 20°C	6.1	6.7	21	7.6	18.6	8.6	7.4	6.1
microscopy 4°C	6.1	8.9	9.4	10.3	6.4	4	4.6	
Formulation No.	2.01							
microscopy 45°C	37.2	24.8	18.9	36.4	3	16.1	17.6	
microscopy 40°C	37.2	35.4	6.6	16.2	37.6	12.6	18.4	
microscopy 20°C	37.2	18.4	21.6	38.4	43.6	13.9	42.3	122.9
microscopy 4°C	37.2	24.1	35.6	32.6	6.3	10.1	32.5	
Formulation No.	2.02							
microscopy 45°C	24.6	35.6	15.6	18.6	12.4	2	9.6	
microscopy 40°C	24.6	31.4	12.7	23.3	1.7	2.6	7.8	
microscopy 20°C	24.6	23.3	17	15.6	3.5	3.1	5.4	24.6
microscopy 4°C	24.6	36.1	16.3	8.6	3.5	5.4	6.5	
Formulation No.	12.01							
microscopy 45°C	153.3	94.3	4.4	10.6	16.3	11.8	12.8	
microscopy 40°C	153.3	98.4	21.5	11	75.3	95.6	116.4	
microscopy 20°C	153.3	11.2	89.4	7.3	77.7	12.6	11.5	8.9
microscopy 4°C	153.3	96.8	98.6	11	40.4	19.6	11.3	
Formulation No.	12.04							
microscopy 45°C	19.4							
microscopy 40°C	19.4							
microscopy 20°C	19.4	23.8	32.5	36.4	12.9	24.3		
microscopy 4°C	19.4	24.4	12.3	16.4	10.3	8.9		
Formulation No.	20.01							
microscopy 45°C	10.5	9.4	16.4					
microscopy 40°C	10.5	14.4	15.6	19.1	25			
microscopy 20°C	10.5	15.6	12.3	21.6	19.3	16.4	13.7	13.6
microscopy 4°C	10.5	19.4	13.6	17.7	20.6	24.3	11.3	
Formulation No.	23.01							

microscopy 45°C	79.8	102.6	93.3	107.6	86	116.3	76.1	
microscopy 40°C	79.8	78	94	88	83.2	89.6	80	
microscopy 20°C	79.8	72.4	16.5	71.1	118.9	76.1	92.4	98.6
microscopy 4°C	79.8	71.4	93.1	84.9	100.3	94.4	97.6	
Formulation No.	23.02							
microscopy 45°C	26.3	15.1	12.7	13.1	15.5	13.1	15.6	
microscopy 40°C	26.3	12.6	15.8	13	15.4	19	21.1	
microscopy 20°C	26.3	16.5	17.7	15.5	18.4	15.5	13.4	23.1
microscopy 4°C	26.3	18	18.9	18	18.7	17.6	15.5	
Formulation No.	26.01							
microscopy 45°C	75.6	62.5	54.6	58.9	61.7	68.9	66.8	
microscopy 40°C	75.6	76.2	77.6	53.9	65.4	53.9	60.3	
microscopy 20°C	75.6	79.6	77.9	54.2	64.2	60.4	57.3	52.6
microscopy 4°C	75.6	78.6	79.5	62.7	69	62.9	80.7	
Formulation No.	26.02							
microscopy 45°C	9.7	9.2	13.6	9.6	10.2	8.4	9.7	
microscopy 40°C	9.7	9.4	15.6	9.6	13.4	12.3	8.6	
microscopy 20°C	9.7	8.9	9.4	9.2	9.9	8.6	10.1	12.6
microscopy 4°C	9.7	8.8	9.1	12.5	12.4	9.6	10.8	
Formulation No.	24.01							
microscopy 45°C	60.7	20.4	58.7	10.4	50.1	54.3	54.5	
microscopy 40°C	60.7	26.4	55.5	16.9	51.3	52.4	44.8	
microscopy 20°C	60.7	20.9	12.4	10.7	46.1	12.4	49.5	56.3
microscopy 4°C	60.7	21.6	51.3	11.2	54.4	59.3	48.5	
Formulation No.	24.02							
microscopy 45°C	14.8	9.7	10.7	10.6	11	9.3	8.2	
microscopy 40°C	14.8	11.8	12.8	11.3	12.2	8.9	9.8	
microscopy 20°C	14.8	13.5	12.6	15.7	14.7	7.2	8.2	23.6
microscopy 4°C	14.8	12.6	11.8	13.7	12.7	12.6	13.3	
Formulation No.	45.01							
microscopy 45°C	184	138.8	162.8	168.3	104.3	148.6	169.4	
microscopy 40°C	184	136.7	165.4	167.4	107.6	127.9	116.4	
microscopy 20°C	184	127.6	158.3	123.9	133.4	168.7	125.7	156.3
microscopy 4°C	184	124.6	111.6	123.5	123.8	156.7	136.4	
Formulation No.	45.02							
microscopy 45°C	14.9	9.5	17.6	11.1	10.3	12.9	13.7	
microscopy 40°C	14.9	19.4	18.1	16.4	17.1	9.1	12.1	
microscopy 20°C	14.9	16.3	19.5	13.8	11.6	10.8	11.1	15.6
microscopy 4°C	14.9	12.6	16.8	13.2	8.6	19.6	7.9	
Formulation No.	50.01							
microscopy 45°C	111.5	111.6	106.3	78.9	95.6			
microscopy 40°C	111.5	88.4	76.8	78.8	94.5			
microscopy 20°C	111.5	96.3	74.2	107.3	88.7	98.4	89.6	94.6
microscopy 4°C	111.5	109.2	79.5	69.6	102.5	83.6	75.1	

Formulation No.	50.02							
microscopy 45°C	43.6	47.6	46.3	13.7	22.4	46.3	34.5	
microscopy 40°C	43.6	19.9	9.1	12.1	26.5	44.6	44.1	
microscopy 20°C	43.6	44.2	10.8	11.1	48.6	12.8	44.6	35.6
microscopy 4°C	43.6	38.8	47.2	7.9	49.6	16.5	22.6	
Formulation No.	46.01							
microscopy 45°C	135.5	98.4	67.4	75.6				
microscopy 40°C	135.5	84.6	41.6	109	91.9			
microscopy 20°C	135.5	87.1	58.9	96.9	76.4	84.6	91	136.9
microscopy 4°C	135.5	74.9	57.4	104.3	80	92.6	64.8	
Formulation No.	46.02							
microscopy 45°C	11.2	9.5	13.4	18.1	13.7	11.1	12.9	
microscopy 40°C	11.2	19.4	14	12.1	12.1	16.4	9.1	
microscopy 20°C	11.2	16.3	20.4	21.4	11.1	13.8	10.8	
microscopy 4°C	11.2	12.6	8.8	12.5	7.9	13.2	19.6	
Formulation No.	55.01							
microscopy 45°C	12.9	32.5	22.4	53.9	43.2	25.4	36.9	
microscopy 40°C	12.9	36.4	26.5	58.7	51.5	29.8	43.4	
microscopy 20°C	12.9	48.6	58.4	52	39.2	48.6	39.4	26.5
microscopy 4°C	12.9	49.6	59.6	49.5	45.9	49.2	38.9	
Formulation No.	55.02							
microscopy 45°C	6.2	9.5	14.6	10.1	8.2	11.1	9	
microscopy 40°C	6.2	19.4	13.5	9.3	9.7	16.4	9.3	
microscopy 20°C	6.2	16.3	12.5	8	8.3	13.8	7.9	8.6
microscopy 4°C	6.2	12.6	11.3	6.5	8.2	13.2	7.9	
Formulation No.	60.01							
microscopy 45°C	185.6	164.3	138.8	98.4	132.4	156.7	167.1	
microscopy 40°C	185.6	167.1	136.7	96.3	152.9	124.3	157.4	
microscopy 20°C	185.6	137.1	127.6	125.4	123.6	184.3	123.9	147.3
microscopy 4°C	185.6	114.6	124.6	106.3	137.7	176.3	103.9	
Formulation No.	60.02							
microscopy 45°C	24.5	9.5	17.6	14.6	20.3	18.1	13.7	
microscopy 40°C	24.5	19.4	18.1	13.5	23.2	12.1	12.1	
microscopy 20°C	24.5	16.3	19.5	12.5	22.5	21.4	11.1	19.4
microscopy 4°C	24.5	12.6	16.8	11.3	19.8	12.5	7.9	
Formulation No.	56.01							
microscopy 45°C	18.4	23.8	15.6	16.5	21.5	14.6	23.8	
microscopy 40°C	18.4	32.6	25.4	17.2	18.9	13.5	32.6	
microscopy 20°C	18.4	23.5	24.7	21.3	24.3	12.5	23.5	24.9
microscopy 4°C	18.4	22.1	12.8	18.3	18.1	11.3	22.1	
Formulation No.	56.02							
microscopy 45°C	6.9	8.2	9	9.3	9.6	10.1	6.8	
microscopy 40°C	6.9	9.7	9.3	8.1	9.1	9.3	6.3	
microscopy 20°C	6.9	8.3	7.9	6.6	9.6	8	6.8	7.4

microscopy 4°C	6.9	8.2	7.9	8.9	11.5	6.5	8.4	
Formulation No.	75.01							
microscopy 45°C	157.9	132.4	126.6	156.7	132.4	164.3	98.4	
microscopy 40°C	157.9	152.9	124.3	124.3	152.9	167.1	96.3	
microscopy 20°C	157.9	123.6	113.2	184.3	123.6	137.1	125.4	147.3
microscopy 4°C	157.9	137.7	114.9	176.3	137.7	114.6	106.3	
Formulation No.	75.02							
microscopy 45°C	30.7	27.6	46.3	13.7	22.4	46.3	34.5	
microscopy 40°C	30.7	19.9	10.1	12.1	28.6	44.6	44.1	
microscopy 20°C	30.7	34.2	11.8	11.1	28.6	12.8	44.6	45.6
microscopy 4°C	30.7	38.8	37.2	7.9	29.6	16.5	22.6	
Formulation No.	76.01							
microscopy 45°C	68.4	64.2	66.6	72.1	71.6	97	89	
microscopy 40°C	68.4	83.6	141.6	88	65.7	123.4	150.5	
microscopy 20°C	68.4	92.6	65.3	75.6	63.7	96.3	69	74.3
microscopy 4°C	68.4	98.3	63.2	68.4	84.3	67.4	70.4	
Formulation No.	76.02							
microscopy 45°C	24.2	23.4	15.3	16.5	21.5	14.6	23.8	
microscopy 40°C	24.2	32.6	15.4	17.2	18.9	13.5	32.6	
microscopy 20°C	24.2	33.5	14.7	11.3	24.3	12.5	23.5	24.9
microscopy 4°C	24.2	32.1	12.8	18.3	18.1	11.3	22.1	
Formulation No.	80.01							
microscopy 45°C	103.6	65.7	76.8	56.3	98.6	73.9	46.9	
microscopy 40°C	103.6	62.8	115.7	78.6	119.6	127.4	65.6	
microscopy 20°C	103.6	74.1	70.2	54.3	64.3	85.6	40.1	142.2
microscopy 4°C	103.6	76.2	64.3	98.3	65.3	118.5	58.5	
Formulation No.	80.02							
microscopy 45°C	64.3	57.4	46.3	33.7	22.4	46.3	34.5	
microscopy 40°C	64.3	23.6	29.1	25.3	25.5	24.6	44.1	
microscopy 20°C	64.3	24.2	20.8	31.1	48.6	18.8	44.6	65.7
microscopy 4°C	64.3	38.6	47.2	38	39.6	16.5	52.6	

10.5 Appendix 5 – Pictures of Equipment

Pictures of equipment used during study:

Mettler Toledo FE20 FiveEasy Benchtop pH Meter

Therma Handheld Lab Thermometer TA-288

Brookfield ‘Low Viscosity’ (LV) and ‘Regular Viscosity’ (RV) viscometer

Height adjustable platform.

Keyence VHX 9000-F Series Digital Microscope with 250-2500x lens

Malvern Zetasizer Nano ZS90.

Folded capillary cell for Zeta Potential measurment.