CHAPTER 3

STABILITY STUDY OF MOISTURISER CONTAINING *Baccaurea motleyana hook f.* FRUIT'S EXTRACT

3.1 Introduction

Since cosmetic products are created and designed to meet specific consumer needs, formulators need to identify raw materials with the desired functionalities and combine these materials in specific ratios to yield an acceptable finished product that performs as intended and remains stable during its shelf life. Product rationale or specifications must be decided before any formulation works begin. This way the formulator has parameters against which to benchmark the success of their product. Schueller and Romanowski (1999d) stated that in successful product development, the following parameters should be considered

(a) Evaluation of aesthetic factors including colour, appearance, viscosity, texture and dispensability.

(b) Establishment of performance targets to provide a measure of how well the product should perform such as cleansing and moisturizing effect.

(c) Consideration of packaging attributes such as the size and shape of the container, materials of construction etc.

(d) Marketing claims that may raise the regulatory concerns, necessitate use of special or regulated ingredients or require the product to produce specific results; all of which may impact on the formulation work.

(e) Consideration of financial concerns which may affect the choice of raw materials, packaging components or even mode of product manufacture.

In reality, some degree of compromise may be required to produce a product with good performance at an acceptable cost. Schueller and Romanowski (1999d) have outlined the following product development approach as follows:

(a) Gathering and evaluating information on raw materials to be used including their functionality and safety.

(b) Preparation of prototypes and evaluating them for stability and functionality.

(c) Refining prototypes after evaluating them until a satisfactory formulation is achieved. Safety considerations at this time should also be assessed.

(d) Performance and consumer testing and modification of the product, if necessary.

(e) Finalising the formulation and confirm its stability and production feasibility.

Many sources of information can be obtained from suppliers' formularies, reference texts, patent literature and technical trade journal. Reference texts have the advantage that they are very thorough and often combine a discussion of cosmetic theory with practical formula information. The disadvantage is that it may not contain the most up-to-date information. The cosmetic ingredients may be identified in several ways (Schueller and Romanowski, 1999e) as follows:

(a) By trade name – the supplier's brand name.

(b) By the official INCI (International Nomenclature Cosmetics Ingredients) name as published in the International Cosmetic Ingredient Dictionary (published by the Cosmetic, Toiletry and Fragrance Association, CTFA).

(c) By the chemical name as designated by the International Union of Pure and Applied Chemistry (IUPAC).

The INCI dictionary provides the ingredients' chemical structure, names of suppliers and Chemical Abstracts Service number. The Material Safety Data Sheet (MSDS) provided by the raw materials supplier contain information related to specific hazards and precautions associated with those chemicals. Merck Index and CRC Handbook offer information on physical properties, mode of manufacture and materials' other uses. With the latest technology, we could find so many skincare products ingredients' produced by manufacturers with so many claims of their functions and positive effects on the skin. Some work well for cleansing, others are good for lubricating, healing or moisturising.

3.1.1 Emulsions

Cosmetic formulators develop products that are designed to deliver a variety of benefits to consumers which include cleansing, moisturising, conditioning and colouring. To achieve these benefits, formulas include active ingredients that usually cannot stand alone as finished products because they are difficult to use, aesthetically unappealing or require dilution. For these reasons, active ingredients are typically incorporated into cosmetic formulation or in a delivery system such as emulsions and vesicular systems like liposomes (Schueller and Romanowski, 1999c). At some point, the formulator needs to combine or mix incompatible materials into one product. Many methods have been utilised to achieve this but none has been utilised more than emulsions.

Emulsions are mixtures of oily and aqueous materials which can be defined as " A two phase system consisting of two completely immiscible or partially miscible liquids, one being dispersed in the other in the form of very fine molecules." (Knowlton, 1996a). The principle components are water and oil. Water is a good carrier for many materials, innocuous and inexpensive. It also evaporates without leaving residue. On the other hand, oily materials can be good moisturising and conditioning agents. Water and other materials which are polar and dissolve to some extent in the water will make up the water phase. Oil and other non-polar materials which exhibit some solubility in oily materials will make up the oil phase. Since the oil and water phases do not mix and the oil phase sits on top of the water phase, we need a third component that is an emulsifier to able these two phases to mix and form an emulsion.

In *Emulsions and their use in cosmetics*, Knowlton (1996a) has mentioned about the many advantages in using emulsion systems and these can be listed as follows:

(a) aesthetics purpose since emulsion promote product elegance and allow oily materials to be applied to the skin aesthetically.

(b) product's compatibility since it allows incompatible ingredients (ex. oil & water) to be brought together in the same product.

(c) inclusion of active ingredients since emulsion allows the incorporation of active materials to the skin.

(d) cost since emulsion often contains significant amount of water and therefore provide a cost-effective product.

(e) formulation flexibility which allows easy modification of product's characteristics such as viscosity, feel and appearance.

3.1.1 (i) Type of Emulsion System

According to Knowlton (1996c), the type of emulsion orientation, be it oil-in-water (o/w) or water-in-oil (w/o), which can be determined by

(a) the amount of dispersed and continuous phase used

Usually the continuous phase will have larger amount than the dispersed phase. There are however an exception cases where the emulsifying power of the emulsifier "overpowers" the type of emulsion produced and give the reverse result.

(b) the type of emulsifier used

Low and high Hydrophilic-Lipophilic Balance (HLB) emulsifier number indicate w/o and o/w emulsion system respectively.

(c) the order in which the continuous and dispersed are mixed

It is generally the case where the phase which is being added to the other phase will become the dispersed phase when the emulsion is fully formed. There is however an exception when a phase inversion occurred. According to this rule, if the water phase is added to the oil phase, then the resultant would be w/o emulsion but if the amount of water is more, then this would be converted to o/w emulsion. This is called *phase inversion.*

3.1.1 (ii) Particle Size of Emulsion

The particles that make up the emulsion are polydisperse (meaning they have variable sizes) and their average size is often used for emulsion classification (Schueller and Romanowski, 1999c). If their average size diameter is less than 100 Amstrong (A), it is referred to as a micellar emulsion and a particle diameter of 100 A to 2000 A is known as microemulsion. Larger particles make up macroemulsion. This is the most common type found in cosmetic formulations (Schueller and Romanowski, 1999c). The smaller the particle size of the emulsion will create a more stable emulsion system.

Particle size of an emulsion depends on a number of emulsion basic properties but the most obvious one is the mechanical energy used to produce the emulsion. It is often preferable to use high shear mixing devices to reduce the emulsion particle size. This will create higher interfacial area at the dispersed phase molecule and allow more molecules of the emulsifier to be adsorbed at the interface, hence promoting to a more stable emulsion. The emulsion which is made by phase inversion method will often produce a smaller dispersed phase particle sizes than the equivalent emulsion made by more conventional means (Knowlton, 1996c).

Brooks (2000) has reported the work carried out by Lin in 1970s in researching the many potential variables in controlling the particle size emulsion. In his research, Lin has proposed the use of low-energy emulsification process in which to hold back the large quantity of continuous phase (water) in an o/w emulsion system. He made an emulsion concentrate and then diluted it with cold water. This lead to an emulsion with a smaller particle size than if made by conventional techniques. In his research, Lin found that this technique worked much better on low solids emulsions. Generally, the more uniform and smaller the particle size of the dispersed phase, the more viscous is the emulsion system produced (Brooks, 2000).

3.1.1 (iii) Emulsion Appearance

Even though the appearance of the emulsion is largely indicated by the size of the dispersed phase particles, the appearance is also affected by the refractive indices of both the dispersed and continuous phases. In discussing the physical properties of emulsions, Knowlton (1996c) has tabulated the relationship of the emulsion's appearance with its particle size, Table 3.1.

Table 3.1: Relationship of particle size to emulsion appearance

Emulsion Appearance	Approximate Particle Size
Milky-White	Greater than 1 μ
Blue-grey	1.0 – 0.1 μ
Translucent	0.1 – 0.05 μ
Transparent	Less than 0.05 μ

3.1.1 (iv) Emulsion Components

As mentioned by Schueller and Romanowski, (1999c), one of the essential characteristics of an emulsion is that, given enough time or energy, the emulsion will separate into its original phases since it is a mixture of incompatible ingredients. Cosmetic products are not meant to last forever. The true challenge for the cosmetic formulator is designing systems that will remain stable over the useful life of the product. By understanding the ingredients characteristics in the emulsion that include oil phase, water phase (also known aqueous phase) and the emulsifier, will make the formulator task easier in formulating a good and stable products.

(a) Oil Phase

This phase is composed of non-polar compounds that are generally incompatible with water; such as oils, waxes, fats and their derivatives including fatty acids, fatty alcohols, esters, hydrocarbons, glycerides and silicones. These materials provide numerous benefit to the skin that act as emollients to provide an appealing feel, soften and improve the skin's moisture-retaining properties because they leave behind a water-repellent film (Schueller and Romanowski, 1999d, Knowlton, 1996a, Knowlton, 1993).

(b) Aqueous/Water Phase

This phase is made up of water and all other hydrophilic materials including humectant such as glycerine and propylene glycol, water-soluble polymers (as thickening agent), preservatives, colorants, electrolytes and active ingredients such as extracts. The aqueous phase also reduces the greasy feel of the oil phase and the cost of the overall product formulated (Schueller and Romanowski, 1999d, Knowlton, 1996a, Knowlton 1993).

(c) Emulsifiers

Emulsifiers are compounds which make emulsions possible by stabilising the dispersion of the internal phase in the continuous phase. They are the surfactants that reduce the interfacial tension between the oil and water phases so that these phases could be mixed together (Brooks, 2000, Schueller and Romanowski, 1999c, Knowlton, 1996b, Cannell, 1993). Emulsifiers are molecules that have hydrophilic and lipophilic portion. They are classified as anionic, cationic, nonionic or amphoteric; depending on the nature of their water-soluble head group (Schueller and Romanowski, 1999c, Knowlton, 1996a). When placed in water, emulsifiers have a tendency to align themselves in a manner that reduces the interaction between their hydrophilic and lipophilic ends. If enough emulsifier is present, spherical structures called micelles are formed, Figure 3.1. These structures aggregates in which the lipophilic tails are oriented toward the centre of the micelle and the hydrophilic form at the outer surface.

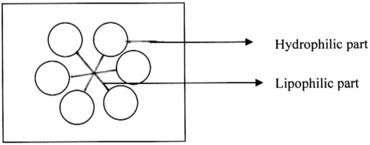


Figure 3.1: Emulsion micelles

When an emulsifier is added to a water-in-oil (w/o) emulsion system, the oil molecules stabilise because they are incorporated into the lipophilic interior part of the micelles whilst the external surface layer of the oil molecules are shielded with the hydrophilic part of the emulsifier, Figure 3.2. Since the hydrophilic part has the same charge, then it will repel each other. Thus, the oil particles are shielded from each other and their coagulation is inhibited, resulting in a stable emulsion. Although emulsifiers help to stabilise the interaction between the oil phase and the water phase, emulsions are still inherently unstable which eventually will separate (Schueller and Romanowski, 1999c, Vaughn, 1993). The speed at which this occurs or how complete the separation is depends on many factors and among them is the composition of the emulsion itself.

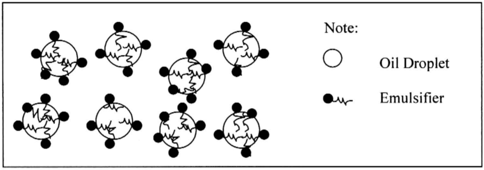


Figure 3.2: Emulsifier orientation in o/w emulsion system

Prior to any selection of emulsifiers to be used in the product, the type of emulsion system needs to be identified. This is very important because many aesthetic, functional and stability effects will depend on the emulsion type. According to Brooks (2000) and Knowlton (1996b), the water-in-oil (w/o) types permit direct contact of the oil phase to the skin, giving an immediate occlusive effect and slow released of emulsified water to the skin, giving little cooling sensation. Even though this type of product will give a rich and lubricious end-feel effect, but the emulsion is difficult to produce since the oil phase is continuous and only a rigid interfacial film and a crowded disperse phase serve to stabilise this emulsion, promoting a solid rather than fluid form of emulsions (Brooks, 2000, Leonard, 1996a). Brooks (2000) has reported that the o/w type of emulsion is preferable. Besides producing greater cooling effect on the skin by allowing water evaporation, the major advantages is that the emulsion produced is more stable. It has a non-oily application characteristics, light-bodied cosmetic effects, with the possibility of using extremely low oil concentrations (Brooks, 2000, Knowlton, 1996b). This type of emulsion is raw materials cost effective since higher percentage of the materials used is water. In this study, o/w type of emulsion is chosen.

3.1.1 (iv) (a) Emulsifiers and Method of Selection

Basically there are three groups of emulsifiers that is anionic, cationic and nonionic. For anionic emulsifier, the hydrophilic part carry a positive charge and a lipophilic part carry a negative charge. Example of anionic emulsifier is the monovalent soap such as sodium stearate (used in o/w emulsion system). Brooks (2000) has reported that most frequently encountered in practice are those products or emulsions system containing high concentrations of stearic acid and stearate emulsifiers. However, these products are succeptible to both gelation and shear thinning effects. He further stated that shelf life and freeze-thaw studies are useful for these products. Many ingredients which experience gelation due to their crystallisation at ambient temperatures would remain in solution at high temperatures. Brooks (2000) has suggested the following number of techniques to prevent gelation in anionic systems:

(a) by avoiding excessive concentrations of polyol higher fatty acid esters and fatty alcohols in the emulsion. Plasticising the dispersed phase with high concentration of mineral oil, triglyceride oil or even low freezing point esters such as isopropyl myristate with up to 10% is helpful.

(b) Incorporating small amounts of alkyl sulfates such as sodium lauryl sulfate into the formulation to raise the HLB value, with the amount from 0.1 to 0.5%.

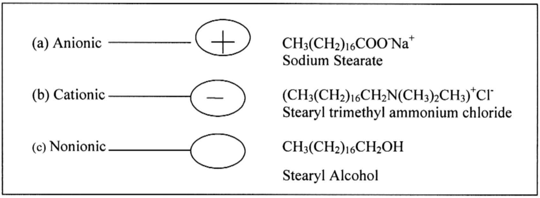
(c) Avoiding using large quantities of high freezing point liquid esters such as isopropyl palmitate or butyl stearate.

The use of cationic emulsifiers is mainly in the area of hair conditioners product where the cationic material function as a conditioning agent as well as stabilising the emulsion itself. Most common examples of cationic emulsifiers are quaternary ammonium compounds such as stearyl trimethyl ammonium chloride. Both anionic and cationic emulsifiers stabilise the emulsion by the application of an electrical charge to the oil dispersed phase molecule. However to ensure good emulsion stability, both these emulsifiers require a secondary co-emulsifier which is normally a non-ionic type (Knowlton, 1996a, Leonard, 1996b).

Non-ionic emulsifiers are by far the most commonly used type of emulsifiers by cosmetic scientists to stabilise emulsion systems. This type of emulsifier exhibit wide versatility and, unlike anionic and cationic emulsifiers, are stable to electrolytes (Knowlton, 1996b, Leonard, 1996b, Knowlton, 1993). They are compatible with other emulsifier types, making them suitable as co-emulsifiers in systems stabilised with anionic or cationic emulsifiers. This emulsifier stabilise the emulsion by interface strengthening of the dispersed phase molecules. Generally, non-ionic emulsifiers are unaffected by pH and salt concentration in the emulsion system (Schueller and Romanowski, 1999c, Knowlton, 1996b). All of the emulsifiers discussed above are illustrated in Figure 3.3 below.

3.1.1 (iv) (b) Hydrophilic-Lipophilic Balance (HLB) System

The most widely used method for choosing an emulsifier is known as Hydrophilic-Lipophilic Balance (HLB) system (Schueller and Romanowski, 1999c, Knowlton,



$CH_3(CH_2)_{16}COO^-Na^+$
Sodium Stearate

$(CH_3(CH_2)_{16}CH_2N(CH_3)_2CH_3)^+Cl^-$
Stearyl trimethyl ammonium chloride

$CH_3(CH_2)_{16}CH_2OH$
Stearyl Alcohol

Figure 3.3: Different types of emulsifiers

1996a, Leonard, 1996b, Knowlton,1993). According to Knowlton (1996a), this system is developed by W.C. Griffin in 1949 to quantify the balance of hydrophilic and lipophilic character within the same emulsifier molecule. This system uses a relative scale of 0 to 20 to rate emulsifiers, based on their affinity for oil and water. Compounds with low HLB value are more lipophilic, while higher HLB materials are hydrophilic. This means that if the material is 100% lipophilic, then the HLB value would be 0 and if it is 100% hydrophilic, its value would be 20. The type of emulsion that will be formed depends on the material's HLB value. Generally, materials with an HLB rating of 3 – 8 will result in a w/o emulsion, while those with a value of 10 – 20 will give an o/w emulsion.

In *Module 7 Surfactants*, Leonard (1996b) has summarised this HLB system by characterising the emulsifying properties of non-ionic surfactants. He gives examples of HLB ranges compared against their applications and their behaviour in aqueous solution, Table 3.2. It should be noted that HLB system can provide information about the type of emulsion a material can produce even though it does not give information about the concentrations required. These concentrations can only be found through experimentation. According to Leonard (1996b), this system does not select the best type of surfactant for a particular purpose but merely eliminates the great number of unsuitable surfactants for a given purpose.

Table 3.2: Hydrophilic-Lipophilic Balance Ranges

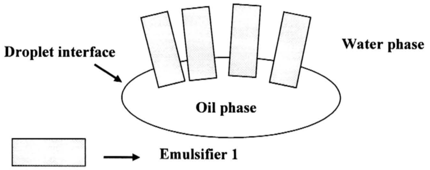
HLB Range	Behaviour in water	Examples of application	Types of emulsion formed (if any)
0 – 3	Insoluble	Dispersants Solids in oil	None
3 – 6	Dispersible	Co-emulsifiers W/O emulsifiers	Water-in-oil
6 – 8	Cloudily soluble	W/O emulsifiers Wetting agents	Water-in-oil
8 – 10	Turbidly soluble	O/W emulsifiers Wetting agents	Oil-in-water
10 – 13	Translucently soluble	O/W emulsifiers General detergents	Oil-in-water
13 – 15	Clearly soluble	O/W emulsifiers General detergents	Oil-in-water
> 15	Clearly soluble	Solubilisers	Solubilisation

3.1.1 (iv) (c) Emulsifiers Blends -Enhancing Emulsion Stability

A mixture of different types of emulsifiers could enhance the emulsion stability (Schueller and Romanowski, 1999b, Knowlton, 1996a). Non-ionic emulsifiers work to stabilise emulsions by mechanical strengthening mechanism. The more the emulsion interface is strengthened by the presence of non-ionic emulsifier in the system, the more stable will be the resultant emulsion. The mechanical strength of the interface will be determined by the quantity of emulsifier used, which in turn is determined by the packing density of the emulsifier at the interface. It is possible to achieve a higher packing density of emulsifier material at the emulsion interface by using a blend of emulsifiers rather than a single emulsifier, thereby producing an inherently more stable emulsion, Figure 3.4 (Knowlton, 1996a, Leonard, 1996b, Knowlton, 1993).

It is obvious from Figure 3.4(a) that no further emulsifier can reside at the emulsion interface due to the fact that there is no more room for further emulsifier molecules to

(a) A single emulsifier



(b) A blend of emulsifiers

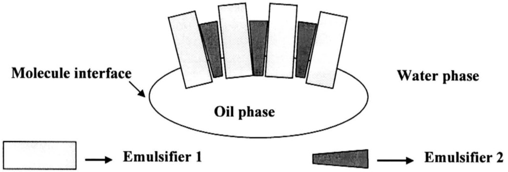


Figure 3.4: Packing of emulsifier at an emulsion interface

adsorb at the interface itself. On the other hand, by using a blend of emulsifiers we could see that the packing density of the emulsifiers molecules at the emulsion interface can be greater, Figure 3.4(b). More emulsifier molecules are able to adsorb at the emulsion interface, promoting more stable emulsion system.

3.1.1 (iv) (d) Micelle Formation and Effect on Emulsion Physiochemical Properties

In an o/w emulsion system, the emulsifier molecules will align themselves at the oil phase interface with the hydrophilic part is adsorbed at the water phase and the lipophilic part is at the oil phase. These emulsifier molecules will continue to align themselves at the oil interface until all the available space at the interface will be filled by the adsorbed emulsifier molecules. At this point, the interfacial tension at the oil phase interface will be at its lowest and cannot be reduced further by that particular emulsifier (Schueller and Romanowski, 1999b, Briggs, 1996b, Leonard, 1996a, Knowlton, 1996d). The remaining emulsifier molecules will then try to find another dispersed phase interface to adsorb. When all the oil phase interfaces have been filled with the emulsifier molecules, the remaining emulsifier molecules left at the water phase will try to adjust themselves in such a way to minimise the contact of their lipophilic tails with the water phase and this is acquired by forming an agglomerations in the form of spherical micelle with the hydrophilic part facing outwards in contact with the water phase, Figure 3.5. (Schueller and Romanowski, 1999b, Briggs, 1996b, Leonard, 1996a, Knowlton, 1996d).

The concentration at which the emulsifier molecules start agglomeration into micelles is known as **Critical Micelle Concentration (CMC)** (Leonard, 1996a, Knowlton, 1996d, Briggs, 1996b, Knowlton, 1993). It should be noted that micelles do not affect the interfacial tension between the dispersed phase and the continuous phase, Figure 3.6, but they have a marked effect on the bulk properties of the emulsion system (Briggs, 1996b). Different types of emulsifiers will have different CMCs because the molecules are different sizes and shapes which will pack differently at the dispersed phase interface. The emulsifier different physiochemical properties will also affect the way they are packed at the dispersed phase interface.

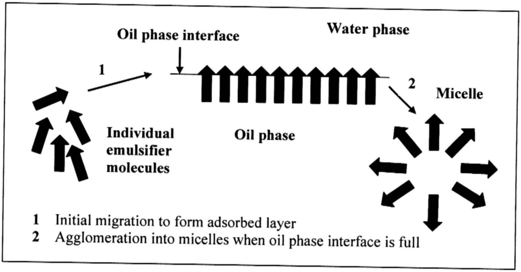


Figure 3.5: Behaviour of emulsifier molecules in o/w emulsion system

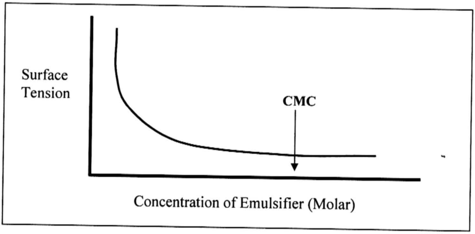


Figure 3.6: Relationship between surface tension with concentration of emulsifier

Micelles are not fixed structures. They are in a state of dynamic equilibrium, breaking down and reforming continually. At or above CMC, spherical type of micelles are formed (Leonard, 1996a). However, these spherical micelles can increase in size or change their shape with these following factors:

(a) Increase in emulsifier concentration in the emulsion system

Increasing emulsifier concentration will increase the amount of its molecule in the emulsion system. If the dispersed phase molecule interface has already been filled, then the remaining emulsifier molecules will be dispersed in the water phase. These molecules will align themselves to avoid their lipophilic part from the water phase and forming micelle. As higher emulsifier concentration is present in the water phase, more micelle formation is expected, causing an increase in the overall viscosity of the emulsion system (Brooks, 2000, Leonard, 1996a).

(b) Use of different types of emulsifiers in the emulsion system

Another important factor that can be considered is mixing different emulsifier types in the emulsion system. If ionic emulsifier is used, the micelle formed will have an electrostatic repulsion between the adjacent charged of the hydrophilic part molecules. This will limit the number of emulsifier molecules that can be packed into the micelles composed. Since non-ionic emulsifier does not carry any charge, then it does not have this problem. Under the same condition, the number of molecules packed in a purely non-ionic micelle is much higher compared to the ionic ones.

For an emulsion system that contains both ionic and non-ionic emulsifiers, the micelles formed could possibly produced a rod shape that contain non-ionic molecules slot in between the ionic molecules, Figure 3.7. The length of this micelle (Figure 3.7(b)) would increase if ionic molecules were added to the emulsion since they carry the same charge, thus would repulse each other. This will not happen if non-ionic molecules were added to the emulsion system. They would pack more closely to the other molecules as they are not affected by the electrostatic repulsion (Leonard, 1996a, Briggs, 1996b, Knowlton, 1993). All these changes can cause the micelles to grow in size and change their shapes, therefore would increase the viscosity of the emulsion system.

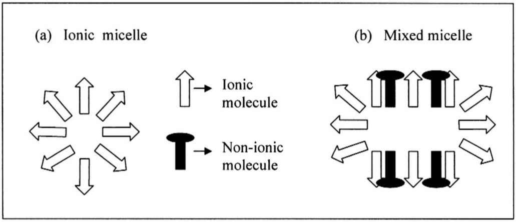


Figure 3.7: Micelle type and behaviour

(c) Addition of electrolytes to the emulsion system

Adding electrolytes to an ionic emulsion system increases the ionic strength of the water phase. The opposite charged molecules present in the water phase will be attracted to each other and this phenomenon will reduce the electrostatic repulsion between them. The molecules can then pack closer together and the micelles will grow in size and progress in shape thus increasing the viscosity of the emulsion system, Figure 3.8.

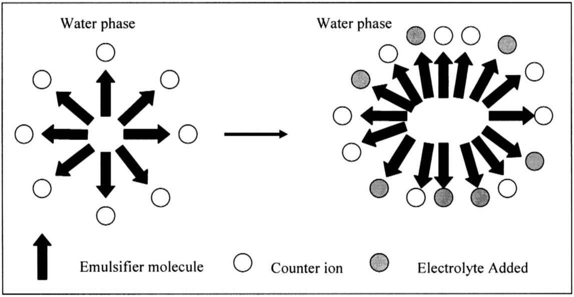


Figure 3.8: Electrolyte addition effect to micelle formation

However, if too much electrolytes are added to the emulsion, the ionic strength of the water phase will increase. This will not only reduce the electrostatic repulsion of the micelles formed but also breaking down the micelles and shorten their average life-span. The micelles networks are disrupted and the viscosity of the emulsion will dramatically drops (Leonard, 1996a).

(d) Variations in temperature and pressure of the emulsion system

Temperature and pressure can change the viscosity and structure of the emulsion (Vaughan, 1993). A change in either, will affect the solubility of the emulsifier molecule leading to a change in shapes and sizes of the micelles formed and alter the micelles' interaction (Leonard, 1996a). Increasing the temperature is of the most concern since it can cause a dramatic reduction in the viscosity, thus loss of structure in a product. According to Leonard (1996a), there is also a limitation to the size of the spherical micelles and this factor depends on the type of emulsifier. When the size limit is reached, either a new spherical micelle form or the shape changes. The spherical shape can elongate into a rod or spread into a disc. They can also agglomerate into different phases or even amalgamate and form orderly rows of orientated molecules when sufficient micelles are present in the emulsion system, Figure 3.9. The changes in micelles' sizes and/or shapes will result in physical changes to the viscosity and structure of the emulsions. As micelles interact to form or change phase, the structure of the emulsion system also alters. They can either produce a cubic, hexagonal or lamellar phases. A cubic or hexagonal phase would give a stiff gel structure whilst lamellar are usually more free flowing even though they are very viscous. This is due to the aligned layers of the molecules that can slip over one another relatively easily (Leonard, 1996a).

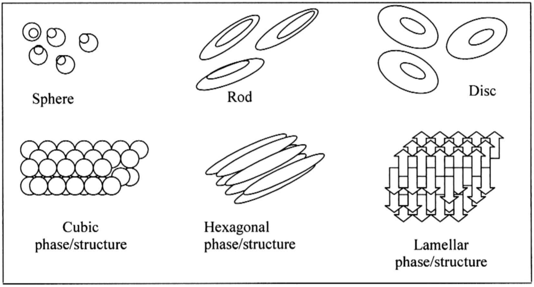


Figure 3.9: Micelles and their change in behaviour in emulsion system

3.1.1 (v) Physical Properties of Emulsions

As reported by Knowlton (1996c), properties of an emulsion depends on three factors that is the basic properties, macro-properties and micro-properties. These factors are interrelated to each other. The basic properties include emulsifier type and its quantity used in the emulsion system, energy input in developing the emulsion and the order of mixing the dispersed and the continuous phase. All these factors will affect the type of emulsion system produced, affecting the quality of the emulsion macro properties (its appearance, viscosity, density, pH and rheological properties) and the micro-properties (particle size, particle charge, dispersed phase viscosity and density of the emulsion) and finally affecting the emulsion stability.

Rheology is affected by the dispersed and continuous phases' viscosities and inter-dispersed phase particle interactions (Brooks, 2000, Knowlton, 1996c). Brooks (2000) and Knowlton (1996c) has reported that if the continuous phase is generally the major phase of the emulsion, then the properties of the emulsion rheology is normally similar to those continuous phase itself.

3.1.1 (vi) Intermolecular Forces in Emulsion System

The attractive forces which hold the molecules together in the emulsion system are known as van der Waals interactions (Briggs, 1996a, Vaughn, 1993). According to Briggs (1996a), the two types of van der Waals interaction which have the greatest impact on cosmetic product stability are London dispersion forces and hydrogen bonding.

(a) London Dispersion Forces

This type of force is known as universal attractive force where all molecules, whether polar or non-polar are attracted to each other. This force is relatively strong and will lead to particle coalescence. Example of this force is illustrated diagrammatically in Figure 3.10 below.

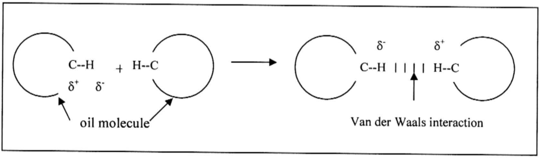


Figure 3.10: London dispersion forces acting on two oil molecules

(b) Hydrogen bonds

This force plays an important role in determining the properties of many interfacial systems (Briggs, 1996a). Hydrogen has a relatively low electronegativity. This means it does not hold onto its electron very tightly. When it is attached to an atom with high electronegativity such as oxygen (which holds onto electrons tightly), it allows its electron to be pulled away towards the oxygen atom. At this point, the hydrogen atom becomes slightly positive charged. The hydrogen atom with its positive charge is now attracted to any electronegative atom around it such as another oxygen atom. In this way, hydrogen forms a bridge or a hydrogen bond between the two electronegative atoms as shown in Figure 3.11 below.

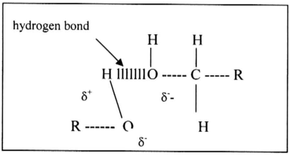


Figure 3.11: Hydrogen bond

An example of hydrogen bond is in water molecules, Figure 3.12. A considerable amount of energy is needed to break the hydrogen bonds between these molecules. That is the reason why water has such a high boiling point in spite of its apparently small molecule. The hydrogen bonds must be broken before the individual molecules can be vaporised (Briggs, 1996a).

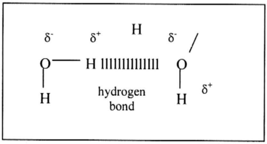


Figure 3.12: Hydrogen bonding between water molecules

3.1.1 (vii) Process Related to Emulsion Separation

According to Brooks (2000), Schueller and Romanowski (1999c) and Knowlton (1996d), the processes that cause the emulsion separation are due to the following phenomenon:

(a) Flocculation

This is a condition where the dispersed phase molecules clumps together or aggregates with each other, Figure 3.13. It is caused by inadequate surface charge on the molecules interface which reduces the repulsive force between the molecules (Schueller and Romanowski, 1999c, Brooks, 2000). However, the two molecules remain distinct. There is no change in the size of the molecules. Therefore, when the emulsion is agitated again, it will reverse to its original form. Flocculation is considered a less serious threat to emulsion stability. Transparent microemulsions can become translucent due to flocculation. According to Brooks (2000) and Knowlton (1996d), this problem may be at least partially rectified by increasing the emulsifier concentration and/or by choosing emulsifier such as anionic which are able to inhibit flocculation more effectively by generating a more complete electrical double layer phenomenon.

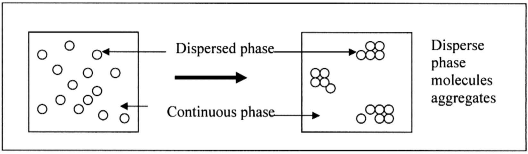


Figure 3.13: Process of flocculation

(b) Coalescence

This is the process whereby two or more dispersed phase molecules fuse together to form a single, larger particle. The resultant of this phenomenon is the reduction in the dispersed phase molecules surface area (Briggs, 1996a), Figure 3.14. Coalescence is an irreversible process and more of a problem for macroemulsions because it can cause a variation in rheology (Schueller and Romanowski, 1999c). Brooks (2000) and Schueller and Romanowski (1999c) reported that if enough dispersed phase molecules coalesce, they will result in a complete separation of the emulsion system. According to Brooks (2000), coalescence phenomenon may mostly be observed on samples stored at high temperature. He also suggested that this problem is due to insufficient emulsifier in the emulsion system.

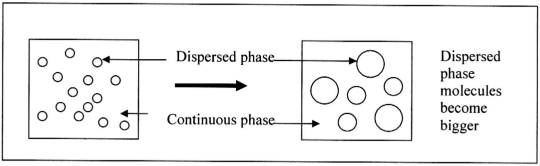


Figure 3.14: Process of coalescence

(c) Creaming

Brooks (2000) has reported that the most common instability phenomenon for fluid emulsion is creaming, Figure 3.15. It can be in the form of still-emulsified but fat-rich layer forms at the surface of the o/w emulsion or a thin transparent oily layer formed at the top of the still-emulsified o/w emulsion. The molecules in an emulsion have varying densities. This is a process in which the less dense particles tend to rise to the top. In an

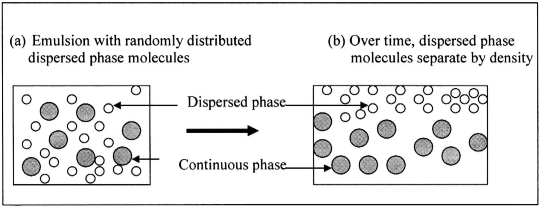


Figure 3.15: The process of creaming for o/w emulsion

o/w emulsion, the dispersed oil phase molecules are less dense than the water continuous phase, hence exhibit a tendency to rise (Knowlton, 1996d). Creaming represents a less serious stability problem because none of the particles actually combine. So, the emulsion can be reverse to its original state when it is shaken to redisperse the creaming layer (Schueller and Romanowski, 1999c, Brooks, 2000).

(d) Sedimentation/Downward Creaming

Like creaming, sedimentation also arises due to the difference between the dispersed and continuous phase of the emulsion whereby the water continuous phase which has greater density than the dispersed oil phase tend to fall at the bottom of the container, Figure 3.16.

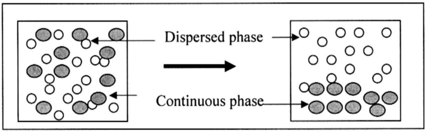


Figure 3.16: The process of sedimentation for o/w emulsion

Phase separation is often the most obvious sign of emulsion instability. It involves collision and coalescence of the dispersed phase molecules which result in visible appearance of separated oil and water phases. The rate of dispersed phase molecule separation can be described by Stokes Law (Klein, 2001, Knowlton, 1996d) given below:

$$V = \frac{2a^2(\rho_c - \rho_d)g}{9\eta}$$

where

V = Falling velocity of dispersed phase molecule (causing sedimentation/creaming)

a = Radius of dispersed phase molecule

g = Acceleration due to gravity

η = Viscosity of the continuous phase

ρ_d = Density of the dispersed phase

ρ_c = Density of the continuous phase

When dispersed phase molecules coalesce with each other, their radius become bigger. This will in turn increased the velocity of the dispersed phase molecule. The resultant from this action will cause further likeliness for the molecules to collide with one another. This will finally lead to higher coalescence of the dispersed phase and finally caused emulsion instability. From this law, it is also obvious that if the viscosity of the continuous phase is low, which means that the viscosity of the emulsion system is also low since the bulk of the continuous represent the emulsion (Schueller and Romanowski, 1999c), then the velocity of the dispersed phase will increase, contributing to emulsion instability. Knowlton (1996d) has suggested the following ways to minimise this problem:

(a) by reducing the particle size of the dispersed phase molecules, more interfacial area at this dispersed phase molecule is available, thus more number of the emulsifier molecules could be adsorbed at this interface.

(b) by strengthening the emulsion interface by using non-ionic emulsifiers so that if collision of the dispersed phase molecules do occur, they will not coalesce.

(c) by increasing the electrical charge on the interface of the dispersed phase molecules. This is only for o/w emulsion system.

(d) by increasing the viscosity of the continuous phase. One way is by adding a water phase thickeners such as carbomer or xanthan gum to the o/w emulsion or by adding high-melting point waxes such as hydrogenated castor oil or behenyl alcohol to the w/o emulsions systems (Klein, 2001, Brooks, 2000).

3.1.1 (viii) Parameters Contributing to Emulsion Instability

There are many factors that can expedite or lengthen the separation process of the emulsion. Among them are listed below.

(a) Incompatibility and/or insufficient amounts of ingredients used in the emulsion

Incompatibility and/or insufficient amounts of the primary ingredients used in the emulsion such as emulsifier, and/or thickening agent used in the product formulation will fasten the product's separation process. Brooks (2000) has reported that the viscosity of the emulsion system may be raised by increasing the dispersed phased concentration. He also mentioned that the higher the melting point of the dispersed phase in o/w emulsions, the more viscous the product is and an o/w system with a higher concentration of lipophilic emulsifier blends will produce higher product's viscosity. In general, the thinner the emulsion, the greater the difficulty in achieving a stable emulsion system (Brooks, 2000, Briggs, 1996a).

(b) Microbial contamination

The microorganisms that are relevant to cosmetic industries are bacteria, yeasts and molds. A contaminated product caused by either one of these microorganisms will change the product odour, colour and finally cause the product to separate. A suitable preservative system should be incorporated to reduce this effect. However, this study is not within the scope of this research.

(c) Storage condition of the emulsion product

The temperature at which an emulsion is stored normally has a marked effect on its stability. It is well known that elevated temperature storage condition accelerates the emulsion destabilisation process. Evaporation of the aqueous/water phase can reduce emulsion stability (Schueller and Romanowski, 1999c, Knowlton, 1996d). High temperature emulsion stability can be improved by using higher melting point waxes in the oil phase of the emulsion so when the emulsion is subjected under high temperature storage condition, it will not easily melt hence promoting stability of the emulsion. By choosing an emulsifier system with a suitable temperature-solubility curve will also help to reduce this problem (Brooks, 2000, Knowlton, 1996c). According to Knowlton (1996b and 1993) and Cannell (1993), to quantify the effect of temperature increase have on product stability, the following assumptions need to be made:

(i) similar changes that occur at elevated temperatures would also occur at moderate temperatures but over a longer period of time.

(ii) All physico-chemical changes which take place have first-order reaction kinetics which means that for approximately every 10°C rise in temperature, a double (two fold) reaction rate will take place for any product changes.

When the product was heated or stored at higher temperature, the dispersed phase molecules move faster. The increased in their movement velocity was under the influences of Brownian motion (Knowlton, 1996a), causing the dispersed phase molecules to move closer to each other. In *Emulsion instability and its minimisation,* Knowlton (1996a) has explained about the London-Van der waals forces effect when the moisturiser product was unstable. London-Van der waals forces are the forces of attraction which operate between two dispersed phase molecules when the molecules are closed to each other. He described this force in a simplified mathematical equation below:

$$F = -\frac{Aa}{12H}$$

where

F = London-Van der Waals force of attraction

A = Hamaker constant

a = radius of the dispersed phase molecules

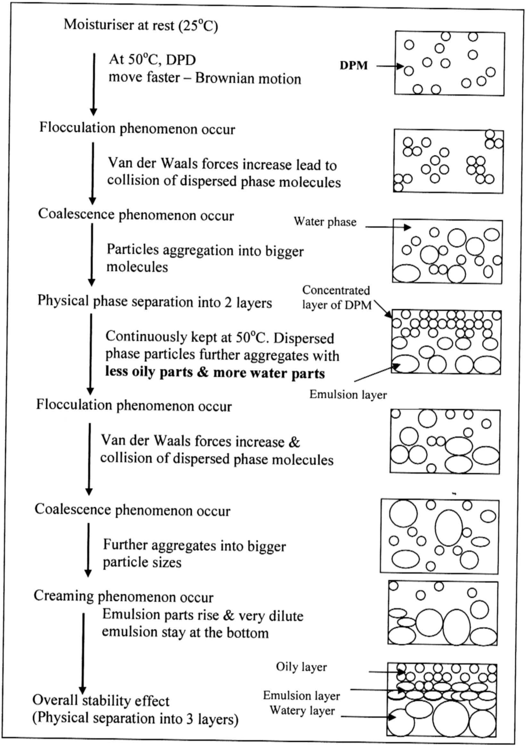
H = distance between two adjacent dispersed phase molecules

From the equation, it is obvious that when the dispersed phase move closer to each other (flocculation), the H magnitude is small. Hence, the London-Van der Waals force of attraction increased. As the product is continuously kept at 50°C, the dispersed phase molecules collide with each other. The collision of these dispersed phase molecules lead to coalescence phenomenon. The radius of the dispersed phase molecules (a value) will increased, contributing to the increase of London-Van der Waals force of attraction and finally, causing the emulsion to become unstable. For o/w emulsions system, the oily parts will move to the top of the bottle, producing a two-layer emulsion separation. At this stage, the emulsion at the bottom layer will contain less oily substance while the amount of water phase parts are not much different. The hydrogen-oxygen bonding in the water molecules are strong and make the water molecules hold onto each other.

Thus, a higher temperature is needed to break this bonding to cause water evaporation from the emulsion system (Briggs, 1996b). At this stage, the viscosity of the emulsion will be very much less.

Continuously subjecting the moisturiser product at higher temperature, will further caused the emulsion part to separate again. The remaining dispersed phase particles in the emulsion will again begin to flocculate. As they get closer to each other, Van der Waals attraction between the particles increased, inducing the particles to collide with one another. As the result, they coalesce and bigger particles are formed. At this point, the whole of the emulsion system will contain varying sizes and densities of dispersed phase molecules. The viscosity of the emulsion is also very much reduced. This condition will finally lead to the separation of the dispersed phase molecules according to their densities (Knowlton, 1996d) which is called *creaming*. The final resultant of this whole phenomenon will cause the moisturiser product to separate into three layers with the top layer comprise of only the oily substance, the middle layer is an emulsion and the bottom layer is a very dilute emulsion, as shown in Figure 3.17.

Knowlton (1996c) has suggessted about mechanically strengthening the interface of the dispersed phase to protect the emulsion interface from being ruptured by ice crystals when the product is stored at low temperature extremes. He has also recommended of avoiding the use of charged dispersed phase molecules as a means to stabilise the w/o emulsion system for the product to be stored under low temperature. If charged dispersed phase molecules are present, the effective electrolyte concentration in the water phase would rise steeply as the product begins to freeze, thus promoting emulsion instability.



Note: The particle means dispersed phase molecules (DPM) and the space in the box is the continuous phase.

Figure 3.17: Flowchart for possible physical separation of o/w moisturiser

(d) Over and/or insufficient mixing of the ingredients

A high speed mixing is needed during the mixture of oil phase and water phase ingredients to produce an emulsion product. However, over mixing a thickening agent ingredient such as a polymer, at high speed could break down its thickening properties, thus producing a thin emulsion.

3.1.1 (ix) Enhancement of Emulsion Stability

The following are a few techniques to improve the stability of an emulsion system.

(a) By increasing the viscosity of the continuous phase of the emulsion system

For o/w emulsion system, a thickening agent which is usually a polymeric gum is used whilst for w/o emulsion system, waxes such as bees wax and high molecular hydrogenated oils can be used (Knowlton, 1996d).

(b) By reducing the particle size of the dispersed phase molecules

This can be carried out by increasing the amount of mechanical energy used when producing the emulsion product. A high speed stirrer and/or a homogeniser are normally incorporated in the procedure to produce the emulsion product with smaller particle sizes.

(c) By stabilising the pH of the emulsion system

The lower the pH of the emulsion, the more unstable is the emulsion. Some ingredients such as fruits acids (AHAs) when incorporated at high percentage into the product's formulation could reduce the pH of the product tremendously. Thus causing the product to become unstable and easy to physically separated. To use high percentage of this type of ingredients but without jeopardising the pH value of the product formulated, a buffering system could be applied to the product. The principle behind the buffer system is that it contains both a weak acid and a weak base so that it has the ability to absorb small additions of either a strong acid or a strong base, causing very little changes in the pH of that respective solution system. When a small amount of a strong acid is added, its hydrogen ions is neutralised by the weak base in the buffer system.

(d) By applying ionic charge to reduce the dispersed phase molecule-molecule collisions

The principle behind this is to apply an ionic charge to the surface of the dispersed phase molecule. This method is only for o/w emulsion system. Therefore, when two molecules approach each other, the electrostatic charges cause them to repel each other, Figure 3.18. The most commonly used method to apply a charge to the cosmetic emulsions is by surface adsorption. Usually the sodium or triethanolamine salts of stearic acid is used (Knowlton, 1996a, Knowlton, 1993). The sodium salts of stearic acids (sodium stearate) or example, is produced *in situ* by adding stearic acid to the oil phase and let it react with sodium hydroxide as shown in the equation below:

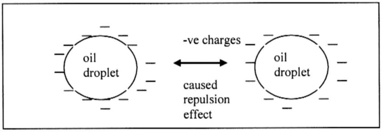


Figure 3.18: Repulsion of dispersed phase molecule by electrostatic charges

$$\underset{\text{Stearic acid}}{C_{17}H_{35}COOH} + \underset{\text{Sodium hydroxide}}{NaOH} \longrightarrow \underset{\text{Sodium stearate}}{C_{17}H_{35}COO^{-}Na^{+}} + \underset{\text{Water}}{H_2O}$$

When emulsion is formed, sodium stearate migrates to the interface between the dispersed (oil) phase molecules and the continuous (water) phase. The resultant of this phenomenon, all the negative charges will be at the interface of the dispersed phase molecules as shown in Figure 3.19.

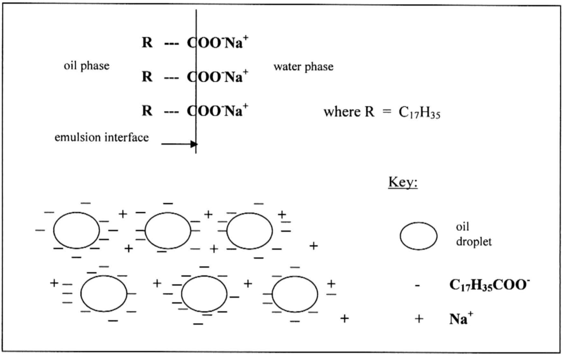


Figure 3.19: Ionisation by adsorption at emulsion interface

When more sodium hydroxide was added to the product, more sodium stearate was formed. As the result, more negative charges group from sodium stearate ($C_{17}H_{35}COO^-$) will be adsorbed at the surface of the dispersed phase molecules and the positively charge sodium ions (Na^+) are dispersed in the continuous phase. The negative charges carried by each oil molecules will repel each other. This phenomenon will reduce the likelihood of these oil molecules to collide with each other, hence the product will be more stable.

(e) Strengthening of the dispersed phase molecules and the continuous phase interface

This can be accomplished by incorporating the non-ionic surfactants in the product formulated. Non-ionic surfactants are stable to electrolytes (Knowlton, 1996b, Leonard, 1996b, Knowlton, 1993) and they are compatible with other emulsifier types, making them suitable as co-emulsifiers in systems stabilised with anionic or cationic emulsifiers. Generally, this emulsifier is unaffected by pH and salt concentration in the emulsion system (Schueller and Romanowski, 1999c, Knowlton, 1996b).

3.1.1 (x) Effect of Shear Rate to Viscosity of Emulsion

Emulsions are Non-Newtonian fluid which when sheared at high shear rate will decrease in viscosity. This type of fluids is called *pseudoplastic.* Schramm (2000) argued that many liquid products that seem homogenous are in fact composed of molecules (or particles) of irregular shape or molecules of one liquid dispersed in another liquid. He said that all these molecules when at rest will maintain their irregular internal order and are characterized by their internal resistance to flow, that is their high viscosity. With increasing shear rates, these molecules will be turned in the direction of the flow which cause these particles to disentangle, stretch and orient themselves parallel to the driving force, Figure 3.20. These molecular alignments allow the molecules to slip each other more easily, hence allow these molecules to flow faster. The result of this phenomenon, the viscosity reading measured shows a lower result when compared to the viscosity of the products measured at rest condition.

Schramm (2000) stated that most liquid materials experience a reversible shear thinning effect which means that these materials will regain their original high viscosity when the shearing action is slowed or even terminated, often with time lag. These liquids' molecules will return to their origin state of non-orientation and non-deformed molecules due to Brownian motion. Brownian motion will keep all molecules at random. At very low shear rates, pseudoplastic liquids behave similarly to Newtonian liquid where the liquid's viscosity is independent of the shear rate, often called the *zero shear viscosity* (Schramm, 2000). When the shear rate increases to such an extent that it is able to induce molecular orientation to exceed the randomizing effect of the Brownian motion, the viscosity drops drastically. At extremely high shear rates, the fluid's viscosity will approach asymptotically a finite constant level which cannot cause further shear thinning effect to the liquid. This is the condition where the optimum of the perfect orientation of the liquid has been reached.

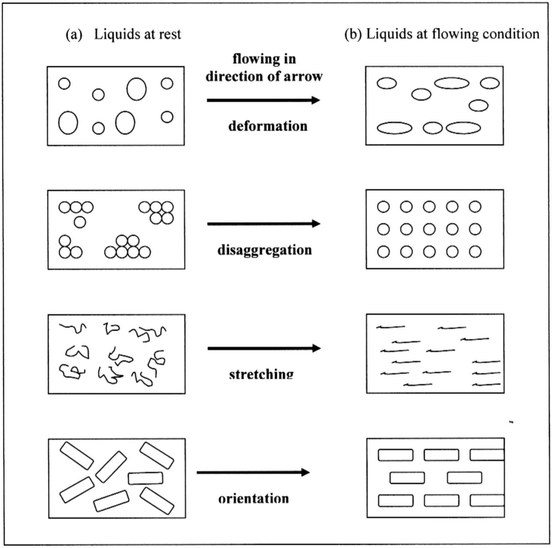


Figure 3.20: Example of molecular orientation when at rest and during flowing condition

Schramm (2000) has stated that another possible reason for shear thinning behaviour of a fluid is due to reduction of intermolecular interactions causing the flow resistance. When discussing about the plasticity and thixotropy behaviour of the pseudoplastic fluids, he says that most dispersions when at rest can build up an intermolecular or inter-particles network binding forces such as polar forces, Van der Waals forces and hydrogen and/or ionic bonds. According to him, these forces restrict positional charge of volume elements and give the substance a solid character with a infinitely high viscosity. If the forces acting from outside is smaller than those forming the network, then the solid character of the fluid will be deformed *elastically*. Only when the outside forces are strong enough to overcome the network forces, will cause the network to collapse. The volume elements will now change its position irreversibly. The solid turns into a flowing liquid. Schramm (2000) has also reported that many dispersions show a time-related molecules interaction which lead to bonds creating a three dimensional network structure. In comparison to the forces within molecules, these bonds which are often hydrogen or ionic bonds are relatively weak and rupture easily when the dispersion is subjected to shear over an extended period of time, Figure 3.21. When the network structure is disrupted, the viscosity drops until it reaches the lowest possible level for a given constant shear rate. Therefore, the possible reasons contributing to viscosity decreases with the increasing shear rates are as the result of combined breakdown of the molecules network structure and the changes in molecular orientation.

3.1.2 Moisturiser

In order to maintain skin elasticity and firmness, it is essential to hydrate the skin. Using a moisturiser with an effective active ingredients will not only help to moisturise the skin and prevent water lost from the skin but also will help to plump up the skin, minimising the appearance of fine lines and wrinkles (FDA, 1999). Moisturiser chosen for the study is an o/w emulsion type, in the form of cream. A few ingredients with different functions are selected to develop the product. Care have been taken to use only those ingredients that have been approved on their safety aspect in cosmetic product. The method and equipment required to prepare the cosmetic products depend on the types and quantity of products to produce. Generally, mixers with variable speed motors and fitted with propellers or paddle-types blades are sufficient for the ingredients mixing process. Products such as creams may require homogeniser that provide high shear to produce emulsions. Heating and cooling small batches of many cosmetic products could be done using hot plates.

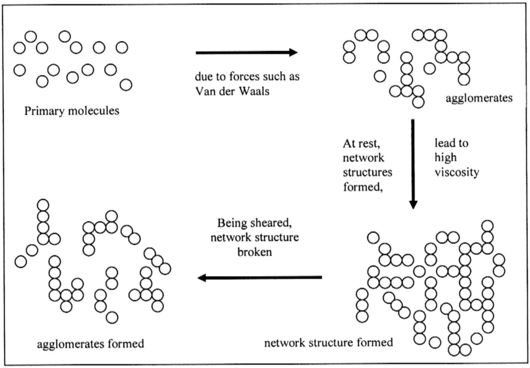


Figure 3.21: Molecules interaction in pseudoplastic (thixotropic) dispersions

3.1.2 (i) Assessment of Moisturiser Product

According to Knowlton (1996e), the selection of product's attributes for assessing the quality of any given product must be relevant to the product's aesthetics, functionality and safety. He has recommended a checklist for emulsion product attributes which is essential for product's stability assessment as listed in Table 3.3 below. He also stated that light stability test is normally required for products kept in transparent packaging. The evaluation is carried out either by visual observation or by spectrophotometric analysis. A skincare product should not be too viscous as it may not appear friendly to the user. On the other hand, it should have a rheological properties. Even though it is not flowable but it should be easy to flow upon slight application of pressure on the product. The types of packaging used will also depends on the product's viscosity. For example, a less viscous and flowable product may need a pump type of bottle. This type of product could not be packed in a tube form. For a more viscous product and unflowable, a squeeze type of tube or a jar could be used.

Table 3.3: Emulsion Product Attributes

No	Product attributes	Test Method
1	Appearance	Visual
2	Colour	Visual and Colour Spectrophotometer
3	Odour	Olfactive
4	pH	pH meter
5	Viscosity	Brookfield Viscometer & Haake Rheometer
6	Rheology	Haake Rheometer
7	Specific gravity	Densitometer
8	Stability (phase separation)	Visual observation
9	Microbial Assessment : Total Plate Count Mold & Yeast Count	Food and Drug Administration (FDA) Bacteriological Analytical Manual, 8th Edition, 1995

The pH of the product should not be too acidic or too alkaline since it can be too abrasive to the skin. It should be compatible with the skin's pH which is in the range of 4.5 to 5.5 for women and 4.3 to 5.5 for men (Courage & Khazaka, 1997).

Colour is an important factor for a cosmetic product. It should be appealing to the consumer. It can indicate whether a product is spoilt, oxidised or contaminated. By visual observation, colour is a matter of perception and subjective interpretation, thus different people could express different colour interpretation on the same issue. A variety of conditions could also affect on how a colour looks. This includes light-source, size and background differences (Minolta, 1998). Colours covering a large area will appear brighter than a small area. The background differences will create a contrast effect. For instance, an apple will appear duller when placed in front of a bright background than when it was placed in front of a dark background.

Due to these difficulties, various methods have been devised to interpret colour more easily and accurately. Most widely known method is the one developed by an international organization concerned with light and colour, that is the Commission Internationale de I'Eclairage (CIE). This method is based on assessing the colour quantitatively by the use of CIE colour values. Also referred to as CIELAB, the L*a*b* colour space which is devised in 1976 is to provide a more uniform colour differences in relation to visual differences. The L*, a* and b* parameters are believed to correlate with three separate stimuli with which colours are perceived by human visual cortex (Jokiman et.al., 2002). The L* parameter measure the change in the sample appearance along a grey scale which ranges from 0 for theoretical black to 100 for white. The increase in L* parameter correlates with increasing in lightness or brightness of the product's sample. The a* and b* parameters indicate the colour's direction. +a* indicates the red direction and the –a* indicates the green direction whereas +b* indicate yellow direction and -b* shows the blue direction. Increase in the value of a* and b* parameter correlates with increasing in redness and yellowness of the sample respectively.

Microbes spread through a variety of transmission substrates, including water, dust particles, insects and human beings. Usually, low levels of microbial contamination are inevitable and do not pose a significant threat to the products. However, uncontrol microbial contamination can cause product's spoilage and other problems. Therefore, it is essential to provide some built-in resistance to microbial growth since cosmetic products are likely to be exposed to some contamination. To inhibit the growth of microbes and prevent the growth of any further contamination the product might encounter, preservatives are added. *Baccaurea motleyana hook f.* fruit's extract and the moisturiser products formulated are added with preservatives to minimise the risk of product and extract contamination by microbes. In this research, the selected moisturiser product's attributes studied are pH, viscosity, odour, colour, appearance and and signs of physical product's separation.

3.1.2 (ii) Stability of Moisturiser Product

The stability of products is related to their shelf-life. It is a factor which determines whether a product is suitable for use or not over a specific period of time. The fundamental theory behind product stability testing is to subject the product to exaggerated environmental conditions, such that any changes that occur in either the characteristics of the product or its compatibility with its packaging do so more rapidly than would be expected in "normal" conditions of storage. Knowlton (1996b) has mentioned about the effect of poor product stability which among them are

(a) changes in appearance of the products, making it cosmetically unacceptable.

(b) changes in physical characteristics of the product, rendering it unpleasant (changes of odour), inconvenient (become thick gel or sticky leading to poor spreading ability) or unsuitable to use

(c) poor stability of the active ingredients causing the product to become ineffective.

Product stability is a vital consideration for the designers and manufacturers of cosmetic products. Even though, there is no legal requirements for a product to be stable (partly due to the difficulty in quantifying the point at which a product is judged to be unstable) in the market, but the cosmetic product manufacturer should be aware that the Consumer Products (Safety) requires that any cosmetic product should be clearly labelled with an expiry date (Knowlton, 1996b).

3.1.2 (ii) (a) Stability Testing Conditions

Several methods can be used to measure changes in product stability. However, the prime requirement is that each method should be sensitive enough to detect meaningful product changes and rapid enough to accommodate the repetitiveness of assessment which is necessary. In the cosmetics industry, there are a number of "standard" storage conditions that are frequently used in product stability testing. Knowlton (1996b), Cannell (1993) and Briggs (1996a) have stated that these conditions have been established using combinations of environmental extremes which are known to have good correlation with the ability to predict product stability fairly accurately.

For the purpose of this research, the conditions selected for product's stability testing are as follows:

(i) Accelerated test at higher temperature (50°C)

The increase of temperature can help us to determine the long term stability profile of a product in a shorter period of time. This is due to any physico-chemical changes that take place in a product, do so more rapidly at higher temperatures than if the product had been stored at room temperature. For countries experience cold climate, a temperature at 40°C would represent a significantly higher temperature condition than the product would be exposed to in the market place. But for a tropical country, a temperature at 40°C might represent a normal climatic condition. So in this case, Knowlton (1996b) has proposed to assess stability for a product in a tropical country at 50°C storage condition. Knowlton postulated that a product exhibiting good stability after 1 month at 50°C has a probable chance of exhibiting good stability at room temperature for about 8 months. In this study, an accelerated test at 50°C is selected. The products are stored in the oven at 50°C for a period of 5 weeks. This storage condition will be useful to assist in making an early prediction of the cosmetic product stability during the development phase.

(ii) Freeze-Thaw Stability Testing

Knowlton (1996b) and Cannell (1993) has recommended for freeze-thaw test to be carried out on all products in the form of solution, emulsion and suspension which are to be sold in climates with temperatures dropping to 0°C. The product is placed into a freezer and allowed to freeze for at least 24 hours and then re-equilibrated to room temperature (thaw) for another 24 hours before any product attributes are measured. This operation is known as one (1) freeze-thaw cycle. The successful completion of about 5 freeze-thaw cycles without serious product degradation such as product's separation into two layers or crystallisation, normally indicates that the product is stable (Knowlton, 1996b).

Miner (1993) has reported about the study made by Sherman in 1971 regarding the possibility of freeze-thaw effect on the o/w emulsion system. At temperature below freezing, the formation of ice crystals in o/w emulsion may cause oil particles to elongate and flatten. The lipophilic portions of the emulsifier molecules will also loose their mobility while the hydrophilic portions are simultaneously "dehydrated" due to freezing out of water. Any other changes that usually take place at RT condition will normally be retarded at this freezing condition (Knowlton, 1996e). However, as the sample is thawed, water is released and travels rapidly through the emulsion. If the emulsion can go back to its initial state before coalescence occurs, then the emulsion survives the freeze-thaw test. According to him, if the rate of re-dissolution of the ingredients is slow, then the product's instability may occur. Brooks (2000), In *Cosmetic Emulsions – Cream and Liquids* has also reported that the shelf life and freeze-thaw studies are useful for those product's formulation containing high concentrations of stearic acid and stearate emulsifiers since these chemicals are most susceptible to both gelation and shear thinning effects, thus could experience crystallization at ambient temperature. Expose a product to alternate freezing and thawing can be of value to indicate the tendency of the liquid products to cloud or crystallise, and the physical stability of creams and other liquid emulsions (Cannell, 1993). This test is carried out only for those products which have passed the accelerated stability test at 50°C.

3.1.2 (ii) (b) Stability Testing Schedule

In cosmetics industry, there is a widely accepted testing schedule for assessing the changes in product attributes over time. The most commonly quoted testing schedule for assessing product attributes as stated by Knowlton (1996b), Cannell (1993) and Miner (1993) is

(a) at initial assessment

(b) 1,2,3,6,9 and 12 months assessment (at all selected storage conditions)

However, it is not necessary to carry out product attributes measurements at all these schedules. The minimum requirement for product's testing is at initial assessment, at 1 month, 3 months and 1 year. More frequent assessments, particularly in the early part of the testing schedule are advisable if the formulation or product type is novel (Knowlton, 1996b, Cannell, 1993). Cannell (1993) has recommended to allow flexibility in the examination schedule to allow modifications be made in the light of information that becomes available as the test progresses.

3.1.3 Objective of the Study

The aim of the research is to study the effect of using *Baccaurea motleyana hook f.* fruit's extract on the stability of moisturiser product developed. Therefore the following two studies are conducted:

(a) Development of moisturiser base product

(b) Development of moisturiser product containing *Baccaurea motleyana hook f.* fruit's extract

3.2 Materials and Method

3.2.1 Raw Materials in Emulsion Preparation

The ingredients used in moisturiser product formulation are listed in Table 3.4 below.

Table 3.4: List of ingredients used in moisturiser product formulation

No	Ingredients	INCI/Technical Name	Function	Origin
1	Eumulium 22	Tribehenin PEG-20 Esters	This is an o/w non-ionic type of emulsifier. The long carbon chain (C22) will provide a long lasting effect and good spreading on the skin. The HLB value is 10.5.	Gattefosse s.a., France.
2	Eumulium Delta	Cetyl Alcohol (and) Glyceryl Stearate (and) PEG-75 Stearate (and) Ceteth-20 (and) Steareth-20.	This is an o/w non-ionic type of emulsifier. It is a good stabiliser and has HLB value of 9.	Gattefosse s.a., France.

Table 3.4: List of ingredients used in moisturiser product formulation, *continued*

No	Ingredients	INCI/Technical Name	Function	Origin
3	M.O.D	Octyldodecyl myristate	It is the ester of octyldodecanol and mysristic acid. It is a good skin conditioning agent. Also function as an emollient. It help to improve the feel and spreadability on the skin.	Gattefosse s.a., France.
4	Stearic acid	n-octadecanoic acid	It is a fatty acid that function as a consistency agent. Is an anionic emulsifier since it has hydrophilic and lipophilic group in its molecule (Schueller and Romanowski, 1999c). Reaction with sodium hydroxide, forms sodium stearate (soap), an anionic o/w type of emulsifier. It can also increase the viscosity of the product.	Henkel KGaA, Germany
5	Sodium Hydroxide (10% solution)		It is used as pH adjuster. But can react with stearic acid to form sodium stearate which is an anionic o/w type of emulsifier. It can increase the viscosity of the product.	Anwil s.a., Poland
6	Euxyl K400	Benzyl Alcohol (and) methylchloroiso-thiazolinone (and) methylisothiazolinone	It is a mixture of methyl dibromo glutaronitrile and 2-phenoxyethanol and function as a preservative. It has a broad spectrum effect against bacteria, yeast and mold.	Schulke & Mayr GmbH, Germany
7	Hydra-tonique RS11641		It is a fragrance.	Technico Flor, France.
8	Deionised Water		Function as a solvent.	
9	Citric acid		Used in the preparation of citrate buffer solution.	Sigma-Aldrich, US
10	tri-Sodium citrate		Used in the preparation of citrate buffer solution.	Sigma-Aldrich, US

3.2.2 Method

(a) Emulsion Stability Measurement

The initial base moisturiser product developed with the best criteria as analysed subjectively and with the least ingredients is chosen for further moisturiser base product development and stability study. The moisturiser was kept at RT for 48 hours to age. After 48 hours, these products were filled into universal bottles and their pH, appearance, colour and viscosity were analysed. The instrument used in the analysis study were listed in Table 3.5 below.

Table 3.5: Instrument used to analyse the product

No.	Analysis	Instrument
1	Appearance	Visual Observation
2	pH	Mettler Toledo 320 pH Meter
3	Colour	Visual Observation and Minolta Colour Spectrophotometer CN-3500d
4	Viscosity	Haake Rheometer, Model RS75
5	Physical Separation	Visual Observation

These products were then placed in the oven for accelerated stability testing study at 50°C for the period of 5 weeks. The products' samples were prepared triplicates and analysed after 48 hours (considered at Week 0). For the accelerated test, the products were kept in the calibrated oven (Protech Brand, Malaysia). Each week the products would be taken out from the oven and its properties such as appearance, pH, viscosity, colour, physical separation, if any, were analysed. All the analysis carried out were at RT except the viscosity of the product kept at 50°C where they were analysed at 50°C.

The freeze-thaw test is carried out if the products were stable at 50°C. The respective products' samples would be stored in the freezer for 24 hours. Once this period is completed, these products' samples would be taken out and thawed at RT (26-29°C) for another 24 hours. The total of these 48 hours would be considered as one freeze-thaw cycle. This testing condition would be repeated until 5 cycles are completed. If the products do not show any significant changes after completion of 5 cycles, then it would be regarded as stable.

(b) Rheology Measurement

The moisturiser product viscosity was measured using Haake Rheometer, Model RS75. The type of sensor system used was coaxial cylinders which comprise of measuring cup Z20 (D48 mm) and spindle Z20 DIN 53019. The measurement was done using a CR Steady State Flow Curve programme whereby the shear rate was fixed at 0.1288 s^{-1}, 0.2 s^{-1}, 0.5 s^{-1}, 1.0 s^{-1} and 1.2 s^{-1}. The temperature was set at 50°C for the stability test samples stored at 50°C and at 25°C for the samples stored at RT and freeze-thaw test. The deviation of temperature during the measurement was set at 0.5°C and the maximum waiting time was fixed at 20 seconds. For the viscosity measurement at 50°C, the moisturiser products' samples were let to stabilise in the measuring cup for 3 minutes before any measurement was taken.

(c) Colour Measurement

The product's colour was by visual observation and measured using Minolta Colour Spectrophotometer CN-3500d. The principal measurement of this instrument is using CIE colour values which is explained in detail under section 3.1.2 (i), 3rd paragraph.

(d) Panel Sensory Evaluation

An initial work was conducted where a few formulations were developed and the pH of the formulated products were measured. The other parameters such as the product's physical appearance in terms of its colour, appearance, feel and performance of the product when applied to the skin such as its spreadability, absorption rate, moisturising effect, oiliness and tackiness were subjectively evaluated. The following definition was used to describe the product's physical appearances:

(a) Appearance of product – the overall appearance of the product which includes the product's texture, smoothness, feel and consistency (uniformity). Rating scale used is very bad, bad, acceptable, good and very good.

(b) Colour of product - the product's colour in its container. Rating scale used is brownish, off white, white and milk white.

(c) Feel of product – the feel of the product by using the thumb and forefinger. Rating scale used is medium lotion, heavy lotion, very soft cream and soft cream. For freeze-thaw test study, the following rating is used; very hard, hard, slightly hard, soft and very soft.

(d) Texture of product – the feature and feels of the product. For freeze-thaw study, the rating used is very rough, rough, slightly rough, fine and very fine.

(e) Spreadability of product – easiness to spread or apply the product onto the skin. Rating scale used is very bad, bad, acceptable, good and very good.

(f) Absorption into the skin –the rate of moisturiser product being absorbed into the skin within 3 minutes. Rating scale used is very slow, slow, moderate, quick and very quick.

(g) Moisturising effect – degree to which the product moisturise the skin. Rating scale used is very little, little, moderate, much and very much.

(h) Tackiness of the skin – degree to which the product stick to the skin. Rating scale used is much, moderate, slight, very slight and not at all.

(i) Oiliness of the skin – degree to which the product feels oily or slippery on the skin. Rating scale used is much, moderate, slight, very slight and not at all.

3.2.2 (i) Emulsion Preparation Without Active Ingredients

In product development initial study (Table 3.6), the amount of each moisturiser product prepared was 100 grams/batch. Both Phase 1 and Phase 2 ingredients were weighed in a beaker separately. These ingredients were heated separately using a hot plate in a water-bath set-up until both temperature in the water bath reached 75°C. In each beaker, the ingredients were stirred using a magnetic stirrer. Once both phases have reached 75°C, Phase 2 solution was poured to Phase 1 solution slowly. The mixture was stirred continuously for about 5 minutes at this temperature. It was then slowly cooled down to about 35°C at RT (25-27°C) while continuously being stirred. When the emulsion has reached to about 35°C, it has become slightly thicken and Phase 3 ingredients were then added to the mixture. The mixture was continuously stirred for about 15 minutes and left for 48 hours at room temperature to age. This is to allow for all the possible reactions in the emulsion to take place and stabilised the whole emulsion system.

Table 3.6: Moisturiser formulation – initial study

Ingredients	Formulation Code				
	M1 (wt/wt%)	M2 (wt/wt%)	M3 (wt/wt%)	M4 (wt/wt%)	M5 (wt/wt%)
Phase 1					
Eumulium 22	6			3	3
Eumulium Delta		6	4	3	3
Octyldodecyl myristate	15	15	15	15	15
Stearic Acid				3	
Phase 2					
Deionised water	78.6	78.6	80.6	75.6	78.6
Phase 3					
Euxyl K400	0.1	0.1	0.1	0.1	0.1
Hydratonique RS11641	0.3	0.3	0.3	0.3	0.3
TOTAL	100	100	100	100	100

The objective of the study is to obtain the stable moisturiser base product formulation with the desired product's pH lies within the range of 5.5 to 6.5. The addition of *Baccaurea motleyana hook f.* fruit's extract to the products formulated will reduce the pH of the products formulated. Thus to increase the pH of the moisturiser product's developed, sodium hydroxide (10% solution) was used. Addition of sodium hydroxide cause the reaction with stearic acid used in the product's developed, forming sodium stearate, which is an anionic emulsifier. Sodium stearate can also thicken the moisturiser products formulated. Therefore, this would certainly help to stabilise the whole emulsion system. As higher wt/wt% of sodium hydroxide is added to the products developed, more sodium stearate is expected to form. In this study, nine moisturiser product base formulations were developed with various wt/wt% of sodium hydroxide (10% solution), Table 3.7.

Table 3.7: Moisturiser base formulation

Ingredients	Formulation Code								
	B0 (wt/wt%)	B1 (wt/wt%)	B2 (wt/wt%)	B3 (wt/wt%)	B4 (wt/wt%)	B5 (wt/wt%)	B6 (wt/wt%)	B7 (wt/wt%)	B8 (wt/wt%)
Phase 1									
Eumulium 22	3	3	3	3	3	3	3	3	3
Eumulium Delta	3	3	3	3	3	3	3	3	3
Octyldodecyl myristate	15	15	15	15	15	15	15	15	15
Stearic Acid	3	3	3	3	3	3	3	3	3
Phase 2									
Deionised water	75.4	74.9	74.85	74.8	74.75	74.65	74.55	74.45	74.35
Phase 3									
Euxyl K400	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Hydratonique RS11641	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
NaOH (10% solution)	0	0.5	0.55	0.6	0.65	0.75	0.85	0.95	1.05
TOTAL	100	100	100	100	100	100	100	100	100

3.2.2 (ii) Emulsion Preparation With Active Ingredients

The selected moisturiser base formulation was added with *Baccaurea motleyana hook f.* fruit's extract with the wt/wt% used in the range of 10 wt/wt% to 20 wt/wt%, Table 3.8. A stability study at 50°C and at freeze-thaw condition were then performed to find the stable product containing with the highest wt/wt% of the extract used in the product's formulation.

An attempt was made to improve the stability performance of the moisturiser product formulated at higher wt/wt% of the extract incorporated in the product's formulation. In this study, a citrate buffer is used to stabilise the pH of the moisturiser product so that when higher wt/wt%of the extract is added to the product's formulation, it will not reduce the pH of the product. The citrate buffer solution is prepared by adding 0.1M tri-

Table 3.8: Moisturiser formulation with *Baccaurea motleyana hook f.* fruit's extract

Ingredients	Formulation Code				
	MR1	MR2	MR3	MR4	MR5
Phase 1	(wt/wt%)	(wt/wt%)	(wt/wt%)	(wt/wt%)	(wt/wt%)
Eumulium 22	3	3	3	3	3
Eumulium Delta	3	3	3	3	3
Octyldodecyl myristate	15	15	15	15	15
Stearic Acid	3	3	3	3	3
Phase 2					
Deionised Water	64.35	61.35	59.35	57.35	54.35
Phase 3					
Euxyl K400	0.1	0.1	0.1	0.1	0.1
Hydratonique RS11641	0.5	0.5	0.5	0.5	0.5
NaOH (10% soltn.)	1.05	1.05	1.05	1.05	1.05
Baccaurea motleyana hook f. fruit's extract	10	13	15	17	20
Total	100	100	100	100	100

sodium citrate solution to 0.1M citric acid solution in the ratio of 3 : 1. In this study, the citrate buffer solutions was to replace all the deionised water portion used in the product's formulation. Four moisturiser products were developed with *Baccaurea motleyana hook f.* fruit's extract used in the products' formulations was increased from 17 wt/wt% to 25 wt/wt%, Table 3.9. A stability study at 50°C and freeze-thaw condition were conducted to identify the stable product containing with the highest wt/wt% of the extract.

In stability study, each moisturiser product formulations prepared was 500 grams/batch. The method of preparation is similar to the smaller batch which was explained earlier. Upon cooling of the product to 35°C, the product has thicken slightly and since more sample is prepared this time, the magnetic stirrer could not handle it. An overhead stirrer is needed to continuously mixed the emulsion formed. The speed used is 345 rotation per minute (rpm). Phase 3 ingredients were added to the emulsion at this state and the stirring was continued for about 15 minutes.

Table 3.9: Moisturiser with citrate buffer system

Ingredient	Formulation Code			
	BR1 (wt/wt%)	BR2 (wt/wt%)	BR3 (wt/wt%)	BR4 (wt/wt%)
Phase 1				
Eumulium 22	3	3	3	3
Eumulium Delta	3	3	3	3
Octyldodecyl myristate	15	15	15	15
Stearic Acid	3	3	3	3
Phase 2				
Citrate Buffer Solution	57.35	54.35	51.35	49.35
Phase 3				
Euxyl K400	0.1	0.1	0.1	0.1
Hydratonique RS11641	0.5	0.5	0.5	0.5
NaOH (10% soltn.)	1.05	1.05	1.05	1.05
Baccaurea motleyana hook f. fruit's extract	17	20	23	25
Total	100	100	100	100

It should be noted that a reverse process has been carried out to prepare this o/w emulsion product. This means that instead of adding Phase 1 ingredients into Phase 2, a reverse ingredients addition was implemented. Since we are not using homogeniser in the product's preparation, then a reverse addition of Phase 2 to Phase 1 is preferable to produce a smaller particle size of an o/w emulsion (Brooks, 2000, Knowlton, 1996c), creating more stable emulsion product. When Phase 2 solution was added to Phase 1 solution, what was formed initially was w/o emulsion but as higher concentration of Phase 2 solution was added, the w/o emulsion begin to invert to o/w emulsion. An efficient mixing of the emulsion at this stage is required though, since the emulsion gets more viscous near this inversion point (Brooks, 2000). The emulsion was also continuously being stirred upon cooling it to about 35°C. This is to maintain a fine particle size acquired throughout the whole process of producing the emulsion.

According to Brooks (2000), emulsion may coarsen during cooling process and fast cooling without efficient mixing may cause lumps to form in the emulsion.

3.3 Result and Discussions

3.3.1 Development of Moisturiser Product Base Formulation

3.3.1 (i) Initial Moisturiser Base Development Study

During the initial study, five moisturiser product formulations were prepared and evaluated subjectively, just to see their physical characteristics during their application on the skin. The result obtained for this study is summarised in Table 3.10. The pH values and properties for all the moisturiser products analysed are not much different from each other, except for the viscosity, feel and absorption rate. Compared to other product's formulations, product M4 gives a better result in term of its feel or thickness. The product is soft cream while the other is either a softer cream or in a lotion form. A more viscous properties is needed to stabilise the product since at higher temperature stability testing condition, a less viscous product could be easily separated.

Table 3.10: Initial moisturiser base development study

Variables	Formulation Code				
	M1	M2	M3	M4	M5
pH	3.06	3.04	3.10	2.93	3.06
Observation of Product's Properties					
Appearance	Good	Good	Acceptable	Good	Acceptable
Feel	Very soft cream	Very soft cream	Heavy lotion	Soft cream	Medium lotion
Absorption Rate	Very quick	Very quick	Moderate	Very quick	Moderate
Colour	Milk white	Milk white	Milk white	Milk white	Milk white
Spreadability	Very good	Very good	Acceptable	Very good	Acceptable
Moisturising Effect	Much	Much	Much	Much	Much
Tackiness	Not at all	Not at all	Very slight	Not at all	Very slight
Oiliness	Not at all	Not at all	Very slight	Not at all	Very slight

3.3.1 (ii) Moisturiser Base Product Development Study

The overall result obtained when the products were analysed at RT (Week 0) was shown in Table 3.11. Even though the product's L* value increases slightly as higher wt/wt% of sodium hydroxide was added to the product but by visual observation, all the products are milk white in colour. The pH and viscosity of the moisturiser products also increased as higher wt/wt% of sodium hydroxide was added to the product. The increased in the product's viscosity was due to the formation of sodium stearate which was earlier discussed under section 3.1.1 (ix) (a).

Table 3.11: Moisturiser base formulation – physical properties at RT

Formulation Code	B1	B2	B3	B4	B5	B6	B7	B8
wt/wt% NaOH	0.5	0.55	0.6	0.65	0.75	0.85	0.95	1.05
pH	4.29	4.48	4.65	4.75	5.10	5.42	5.79	6.09
Viscosity (cps) at 0.1288 s^{-1}	47361	51183	55170	58276	64701	69913	74898	79997
Colour (Visual Observation)	milk white	milk white	milk white	milk white	milk white	milk white	milk white	milk white
Colour Spectrophotometer:								
L* value	93.08	93.11	93.16	93.21	93.29	93.37	93.43	94.03
a* value	-0.71	-0.73	-0.74	-0.76	-0.77	-0.79	-0.80	-0.85
b* value	0.41	0.42	0.43	0.45	0.49	0.52	0.55	0.39

3.3.1 (iii) Stability Study at 50°C

(a) pH and physical separation profile

The pH values and the physical separation of the products formulated were shown in Table 3.12. The shaded column means that the product is unstable and has physically separated.

Table 3.12: Moisturiser base formulation - pH and physical separation profile for stability test at 50°C

Formula Code	B1	B2	B3	B4	B5	B6	B7	B8
wt/wt% NaOH	0.5	0.55	0.6	0.65	0.75	0.85	0.95	1.05
Time, Week\pH								
0	4.29	4.48	4.65	4.75	5.10	5.42	5.79	6.09
1	4.27	4.45	4.63	4.73	4.95	5.27	5.55	5.86
2	4.24	4.38	4.57	4.71	4.83	5.19	5.35	5.74
3	4.02	4.35	4.54	4.61	4.77	5.11	5.27	5.62
4	3.99	3.99	4.32	4.40	4.56	4.99	5.24	5.52
5	3.89	3.92	3.95	3.99	4.41	4.83	5.25	5.53

Note:

Unshaded column - The product is stable.
Darker shade column - The product has separated into two layers.
Lighter shade column - The product has separated into three layers.

(i) Physical Separation

The result shows that the product formulated with less than 0.95wt/wt% of sodium hydroxide (10% solution) in the formulation was unstable and has separated either into 2 or 3 layers. It can be seen that as higher wt/wt% of sodium hydroxide was added to the product's formulation, the product has become more stable. From 8 products formulated, 4 products have finally separated into 3 layers (B1, B2, B3 and B4), 2 products have separated into 2 layers (B5 and B6) and the other 2 formulations are stable (B7 and B8) until 5 weeks period of stability testing. The moisturiser product developed is an o/w type. This means water phase ingredients is the continuous phase and oil phase ingredients is a dispersed phase. Since the amount of the dispersed phase is small, then the assumption of the physical separation in the o/w emulsion system is caused by the dispersed phase molecules which has become unstable when stored at 50°C. The possible explanation to the products' instability at this temperature was earlier explained under Section 3.1.1 (viii) (c) and thee process took place was summarised in Figure 3.17.

(a) Correlation of Sodium Hydroxide Used with Moisturiser Product's Stability

The product's stability can be improved by adding the ionic charge to the surface of the dispersed phase molecules and strengthening the interface between the dispersed phase molecules and the continuous phase, as earlier discussed under Section 3.1.1 (ix)(d). The moisturiser product developed contains stearic acid. When sodium hydroxide was added to the product, it reacted with stearic acid to form sodium stearate. Sodium stearate molecules migrate to the interface between the dispersed phase molecules and the continuous (water) phase causing all its negative charges group be adsorbed at the surface of the dispersed phase molecules and the positively charge sodium ions (Na^+) be dispersed in the continuous phase. The higher wt/wt% of sodium hydroxide added to the product, more sodium stearate molecules were formed. The negative charges carried by each dispersed phase molecules will repel each other, therefore will reduce the likelihood of them to collide with one another, hence producing a more stable product.

(b) Strengthening the dispersed phase molecules and the continuous phase interface

In this study, two types of emulsifiers were used to stabilise the o/w emulsion system that is Eumulium Delta, Eumulium 22, which both are non-ionic types and sodium stearate, which was formed *in situ* during the formation of the emulsion, is an anionic type. The amount of Eumulium Delta and Eumulium 22 used in all the product's formulated were fixed, that is 3 wt/wt% each, thus the effect of the product's stability caused by these two emulsifiers are assumed to be the same for all the products' formulated. The only difference in the products' formulations are the amount of sodium stearate formed, consequently will affect the reactions at the oil and water phase interface. Therefore, the discussions will focus on the effect of sodium stearate formed in the moisturiser products' formulated.

The possible reaction causing an increase in the product's stability as higher wt/wt% of sodium hydroxide was added to the formulation is illustrated diagrammatically in Figure 3.22 below. Assuming all the NaOH solution added to the formulation reacts 100% with stearic acid to form sodium stearate, thus as more NaOH used in the product's formulated, more sodium stearate is formed. Knowlton (1996a) says the emulsifiers will align themselves at the dispersed phase molecule interface. The mechanical strength of the dispersed phase molecules will be determined by the quantity of emulsifiers present at the interface, which in turn is determined by the emulsifiers' packing density at the molecules' interfaces. When sodium stearate is formed in the emulsion, it is adsorbed at the dispersed phase molecules' interfaces, providing a a "physical bridge" between the oil phase and the water phase, thus reduce the interfacial tension between the two phases (Knowlton, 1996a). This will mechanically strengthens the emulsion interface. Thus, when dispersed phase molecule-molecule collisions take place, the chances of coalescence to occur are significantly reduced. This will increase the stability of the emulsion system. Further adding of NaOH solution to the product formulated will form more sodium stearate. The sodium stearate molecules will again move to the oil molecule interface and align themselves until come to a state where all the available space at the oil molecule interface will be fully filled by those molecules. At this stage, the interfacial tension of the oil interfacial will be at its lowest and cannot be further reduced by other sodium stearate molecules (Leonard, 1996a, Briggs,1996b). The higher packing density of the emulsifiers at the emulsion interface will produce an inherently more stable emulsion (Knowlton, 1996b).

(ii) pH Profile

There was a reduction trend in the pH value observed within 5 weeks period of stability testing for all the moisturiser products formulated, Table 3.12. Towards the end of 5 weeks stability testing period at 50°C, the pH value obtained for all the products were much lower than when measured at Week 0. At the beginning of Week 0, the pH

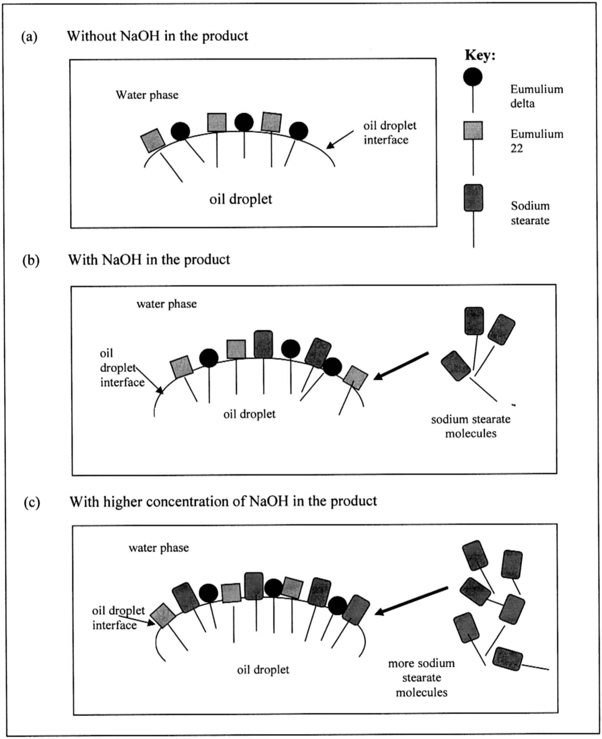


Figure 3.22: Possible reaction contributing to increase in product's viscosity

value measured give a reading which is in the range of about 4.3 – 6.1. However, towards the end of Week 5 stability testing period, the pH value obtained is in the range of 3.89 to 5.53. In relation to the product's stability, the result clearly showed that the lower the pH value of the moisturiser product, the more unstable is the product, which have separated either into 2 or 3 layers. In this study, moisturiser product with the initial pH value of at least 5.79 (Week 0) is stable throughout the 5 weeks period of stability testing. Even though there is a slight decrease in the pH value for both B7 and B8, but by week 4 of stability testing period, the pH value for both of the products are almost stable.

There is also a possibility of inaccuracy in the pH value measured for the unstable product. The measurement of the product's pH was taken at the centre of the product's container. When taking the pH measurement of the product which is unstable and has separated either into 2 or 3 layers, the pH probe will first touch the top layer of the product in the sample's container which in this case, is made up of oily layer. The height of the oily layer formed depends on how bad the product's separation has occurred. As the product becomes more unstable, the height of the oily layer increase. Thus, when measuring the pH of this product, the pH value obtained are mostly due to the oily substances which have separated from the emulsions and being at the top layer, this oily substances will form a thin layer adhere on the head of the pH meter's probe. Therefore, for the unstable product that has badly separated into obvious separation layer, the pH measurement taken will be likely due to the pH of the oily substance.

The oily layer is found to be acidic with the pH value is in the range of 3.88 to 4.0. That is the reason the pH value obtained for the products which have badly separated either into 2 or 3 layers were not much different from each other and if more of the oily layer was formed, the pH value acquired could be in the range of 3.8 to 4.1. If the moisturiser product has separated into 2 layers with a thin oily layer formed, then the pH value obtained will be close to the pH of the emulsion layer. Due to this fact, it is not clear at which point the pH value of the product started to separate. What is apparent is the fact that as the pH of product is higher, the product become more stable. In this study, the pH of the stable moisturiser base product formulation is about 5.79 when measured at RT, Week 0.

(b) Viscosity profile

The moisturiser products become more viscous with higher addition of wt/wt% of sodium hydroxide in the product formulation, Figure 3.23. At 0.5wt/wt% of NaOH measured at Week 0 (RT), the apparent viscosity is 47361 cps (0.1288 s^{-1} shear rate) and this viscosity has increased to 79997 cps (Week 0, RT) when 1.05wt/wt% of NaOH was added to the formulation, measured at similar shear rate. The probable reaction leading to the increase in the product's viscosity with respect to the addition of NaOH in the product's formulation is explained below.

Once all the dispersed phase molecules interfaces have been fully filled with sodium stearate, the remaining excess of sodium stearate molecules in the water phase will try to adjust themselves in such a way to minimise the contact of their lipophilic tails with the water phase by forming an agglomerations in the form of spherical micelle. Since micelles are not fixed structures, they can reform continually by increasing in size or change in shape depending on the concentration of sodium stearate molecules present in the emulsion system. When the maximum size limit of the micelles is reached, either new spherical micelles formed or the shape changes (Leonard, 1996a). The spherical shape can either elongate in a rod or spread into a disc. When sufficient micelles are

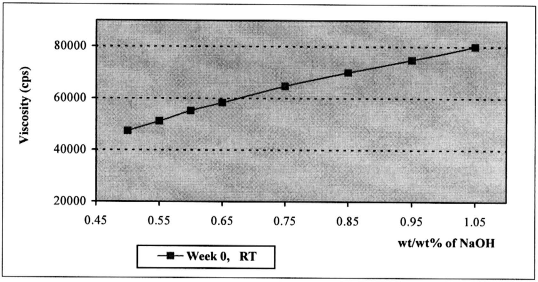


Figure 3.23: Viscosity profile with increase of NaOH addition to moisturiser base product at RT condition ($0.1288\ s^{-1}$, Week 0)

present, they can agglomerate into phases/structures such as a cubic, hexagonal or lamellar structure, or even agglomerate into phases and form orderly rows of orientated molecules. This phenomenon will result in physical changes to the viscosity and structure of the emulsion system. If the micelle is composed of ionic emulsifier molecules alone, there will be electrostatic repulsion between the adjacent like-charged hydrophilic part. Therefore this will limits the number of molecules that can pack into a micelle structure (Leonard, 1996a). Non-ionic emulsifier molecules do not have this limitation since they do not carry any charges. So, the number of molecules in a purely non-ionic micelle is much higher under the same conditions.

In this study, the product contains two types of emulsifiers that is anionic (sodium stearate) and non-ionic (Eumulium Delta and Eumulium 22). So, the micelles formed will be a combination of these emulsifiers, that is in the form of mixed micelles. The non-ionic emulsifier molecules are able to slot themselves in between the anionic molecules, ending with a rod shape micelle. As the micelles grow in size and change shape, the viscosity of the emulsion also increases (Leonard, 1996a). This is the reason that as higher wt/wt% of NaOH solution was added to the formulations, the product's viscosity increased. Since the product formulated is easily spread when being sheared, there is also a possibility that the micelle structure formed is lamellar. Leonard (1996a) has stated that lamellar structure are usually more free flowing, even though they look viscous because the aligned layers of the emulsifier molecules can slip over on one another relative easily when compared to a cubic or hexagonal phase which usually give a stiff gel structure.

(i) Moisturiser Product's Viscosity Profile with Increasing Shear Rate

The moisturiser product's viscosity profile showed a decreased in viscosity value with increasing shear rate, Figure 3.24. The product's behaviour that lead to this profile is as discussed previously under Section 3.1.1 (x).

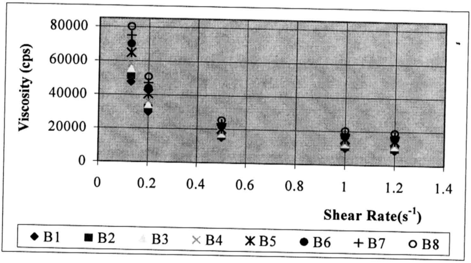


Figure 3.24: Viscosity profile for moisturiser base products stored at RT condition with increasing in shear rate value

(ii) Moisturiser Product's Viscosity Profile Stored at 50°C Stability Condition

The viscosity of the moisturiser products formulated are in the range of 35,500 cps to 52,900 cps when measured using Haake Rheometer RS75 (50°C, 0.1288 s^{-1} shear rate, CR programme) for the products' samples are kept at Week 0, RT condition. When the moisturiser products are subjected to 50°C stability testing condition their viscosity have decreased, Figure 3.25. At high temperature, most fluids become less viscous due to the products' physical changes. The phenomenon of products' physical changes at high temperature have been discussed earlier under Section 3.1.1 (viii)(c). Basically, at 50°C the dispersed phase molecules move faster causing them to aggregate and form bigger sizes molecules. During this aggregation and coalescence stages, the distribution of the dispersed phase molecules in the emulsion were uneven where some parts in the emulsion system could be more concentrated than the other, leading to creaming process and finally cause a physical separation of the product, either into 2 or 3 distinct

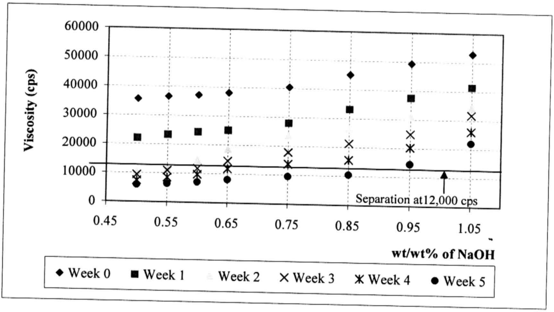


Figure 3.25: Viscosity profile for moisturiser base products with increased in wt/wt% of NaOH, stored at 50°C condition, ($0.1288s^{-1}$)

layers. Obviously from Figure 3.26, the longer the product is kept at 50°C condition, the less viscous and unstable is the product. In this study, the viscosity of the products that have separated into 2 distinct layers is about 12,000 cps. These products if continuously stored at 50°C will further degrade and separate into 3 layers. The viscosity values for those products which have separated into 3 layers are in the region of 7,000 cps to 9,300 cps. However, it should be noted that there might be an inaccuracy during the viscosity measurement for the separated products since their products' molecules are not evenly distributed producing a different types of physical separation profiles (2 or 3 layers). For example, if the product has separated into 2 layers with the top layer containing a minor layer of the oil phase and a major layer of the emulsion phase, then the viscosity measured will give a higher viscosity value as compared to those products which have separated into 2 equal layers and those products which have separated into 3 layers.

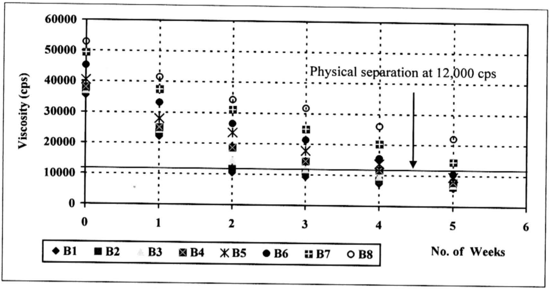


Figure 3.26: Viscosity profile for moisturiser base products stored at 50°C condition within 5 weeks of stability testing period (0.1288 s^{-1})

Eight formulations have been developed but only two (B7 and B8) were found to be stable until 5 weeks period of stability testing. From these two formulations, B8 with 1.05wt/wt% of NaOH in its formulation still look intact by end of 5 weeks of stability testing period. Starting with about 53,000 cps at Week 0, measured at 50°C (0.1288 s^{-1} shear rate), this product has reduced to almost half of its viscosity value (22,084 cps) after 5 weeks stored at 50°C. Therefore, formulation B8 is selected as base product formulation for stability study containing *Baccaurea motleyana hook f.* fruit's extract.

(c) Colour Profile

The colour of the moisturiser products formulated are only measured for those stable products. By visual observation, the colour for all of the moisturiser products developed are milk white and no obvious changes of the colour was seen when they are stored for 5 weeks at 50°C. This shows that the colour of the products formulated were stable throughout the 5 weeks period of stability testing. The colour for the products developed are shown in Figure 3.27.

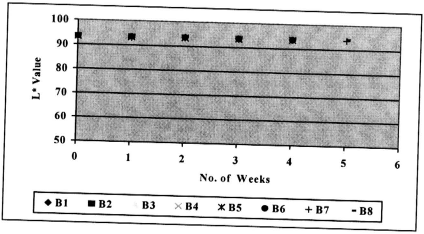


Figure 3.27: Colour profile (L* value) for moisturiser base products within 5 weeks of stability testing period at 50°C

3.3.1 (iv) Freeze-Thaw Stability Study

The freeze-thaw stability study results are shown in Table 3.13 below.

Table 3.13: Freeze-thaw stability testing for moisturiser base product

Formulation Code	B7		B8	
wt/wt% NaOH	0.95		1.05	
	Week 0	After 5 freeze-thaw cycles	Week 0	After 5 freeze-thaw cycles
pH Value	5.79	5.73	6.09	6.01
Physical Separation	NO	NO	NO	NO
Viscosity measured at 0.1288 s^{-1} (cps), 25°C	74898	76513	79997	80815
Colour (Observation)	Milk white	Milk white	Milk white	Milk white
Colour Spectrophotometer:				
L(White +/Black -)	93.43	93.39	94.03	94.00
a(Red +/Green -)	-0.80	-0.86	-0.85	-0.90
b(yellow +/blue -)	0.55	0.51	0.39	0.36
Physical Assessment				
Texture	Fine	Slightly Rough	Fine	Slightly Rough
Feel	Soft	Slightly Hard	Soft	Slightly Hard

The product's properties analysed after completion of 5 freeze-thaw cycles such as its pH, colour and viscosity value are quite similar to those values measured at Week 0, RT condition. However, after completion of 5 freeze-thaw cycles, it was observed that both B7 and B8 products' texture were slightly rough and the products are not as shiny as when compared to the appearance observed at Week 0 (RT condition). The products are also slightly hard, most probably due to the freezing effect. As earlier mentioned under Section 3.1.2 (ii) (a)(ii), stearic acid and stearate emulsifiers could cause crystallization to the emulsion system. Hence, could change the physical appearance and properties of the product formulated when stored under freezing condition. In this study, sodium stearate is formed *in situ*. Assuming 100% of NaOH (10% solution) has fully reacted with stearic acid used in the product formulation, this means the amount of stearic acid used to form sodium stearate is 21.15% (0.6346 gm of stearic acid in 100 gm product) for formulation B7 (containing 0.95 wt/wt% NaOH) and 23.38% (0.7014 gm of stearic acid in 100 gm product) for B8 (containing 1.05 wt/wt% NaOH). The detail of the calculation could be referred in Appendix B. Sodium stearate is a co-emulsifier in the product, function to further strengthen the dispersed phase molecules interfaces, preventing coalescence of dispersed phase molecules. This matter has been discussed earlier under Section 3.1.1 (iv) (a).

A low freezing point esters such as isopropyl myristate has been added to the products' formulations. Brooks (2000) has reported that this chemical when used at high percentage (up to 10%) could prevent the formation of gelation or crystallisation effect in the emulsion system that contains sodium stearate. In this study, the products' developed contain 15wt/wt% of isopropyl myristate. This is probably the reason that only slight changes of the products' appearances, textures and feels were observed after completion of freeze-thaw study. The products have also conform to their original condition upon slight stirring. Since there was no product's physical separation observed, these products are considered stable.

3.3.2 Development of Moisturiser Product Containing *Baccaurea motleyana hook f.* Fruit's Extract

Five moisturiser products' were developed with the amount of *Baccaurea motleyana hook f.* fruit's extract added to the formulations was in the range of 10 wt/wt% to 20 wt/wt%. The results are presented in Table 3.14. As higher wt/wt% of *Baccaurea motleyana hook f.* fruit's extract was added to the product, the pH of the product reduced. Without the extract, the pH of the moisturiser base product is 6.09 and the value was significantly reduced within the range of 4.62 to 4.10 when 10 wt/wt% to 20 wt/wt% of the extract was added to the product's formulation. Since *Baccaurea motleyana hook f.* fruit's extract is very acidic with its pH value in the region of 2.78 to

3.0, the higher amount of extract added into the product's formulation has reduced the product's pH.

The colour of the product without the extract is milk white. As more extract was added to the product, the colour of the product has become slightly darker and the overall effect has caused the colour of the product to become off white. This effect is caused by the colour of the extract which is mild yellowish (visual observation). By using Minolta Colour Spectrophotometer, this colour changes is indicated by the L*, a* and b* value which shows the reduction in the L* value, increase in a* value and decrease in b* value as more extract was added to the products.

Table 3.14: Moisturiser containing *Baccaurea motleyana hook f.* fruit's extract at Week 0 (RT condition)

Formula Code	B8	MR1	MR2	MR3	MR4	MR5
wt/wt% *Baccaurea motleyana hook f.* Extract	0	10	13	15	17	20
Result:						
pH	6.09	4.62	4.49	4.38	4.24	4.12
Viscosity (RT, 0,1288 s^{-1})	79997	67961	64306	59380	53510	48504
Colour (Visual Observation)	milk white	white	white	white	off white	off white
Colour Spectrophotometer						
L*	94.03	93.12	92.96	92.80	92.63	91.84
a*	-0.85	-0.55	-0.61	-0.69	-0.78	-0.36
b*	0.39	3.53	3.81	3.77	3.74	4.10

The viscosity of moisturiser product stored at RT condition, measured at 25°C and 0.1288 s^{-1} shear rate is about 80,000 cps. The product's viscosity has decreased when the extract was added to the product, Figure 3.28. The possible mechanism leading to the reduction in the product's viscosity might be due to the breakdown of micelles network formation in the o/w emulsion system.

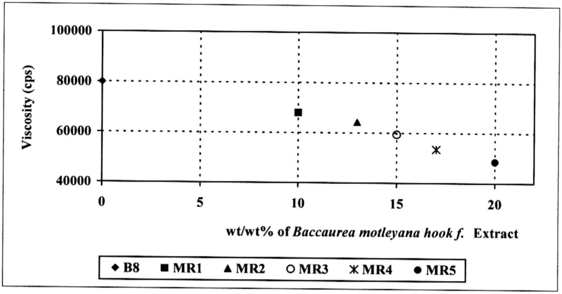
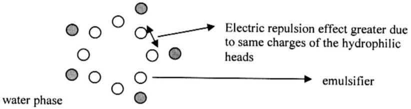


Figure 3.28: Viscosity profile of moisturiser containing *Baccaurea motleyana hook f.* fruit's extract (0.1288 s^{-1}, 25°C)

Micelles are formed when excess emulsifiers' molecules are present in the emulsion system. The shape of the micelles formed, be it spherical, cubic, rod etcetera, are dependent on the concentration of excess emulsifiers' molecules available in the emulsion system. Higher wt/wt% of *Baccaurea motleyana hook f.* fruit's extract in the emulsion system means higher concentration of electrolytes or ions present in that emulsion system. This will increase the ionic strength of the water phase in the emulsion system, simultaneously reducing electric repulsion effect between the adjacent hydrophilic heads of the emulsifier molecules. As the result, these emulsifiers' molecules could pack more closely together, Figure 3.29. Leonard (1996a) has reported that this phenomenon will at first enables the micelles to grow and progress in shape, ending with viscosity increase in the o/w emulsion system, Figure 3.29(a). However, he has also stated that if too much of electrolytes were added to the o/w emulsion system, the average life-span of the micelles formed would be shortened. The presence of higher concentration of electrolyte or ionic charges in the o/w emulsion system could

(a) Less concentration of electrolyte or ionic charges in the o/w emulsion system leading to product thickening effect.



(b) High concentration of electrolyte or ionic charges in the o/w emulsion system leading to reduction in product's viscosity.

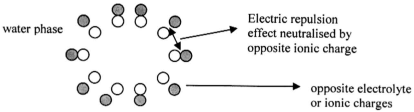


Figure 3.29: Possible reduction of electric repulsion effect of the emulsifier molecules in the o/w emulsion system

cause the hydrophilic head of the emulsifier molecules to be attracted to the opposite ionic charges present in the o/w emulsion system. Since micelles are constantly breaking down and reforming (Leonard, 1996a), this reaction could delay the micelles formation in the emulsion system. Therefore, if the life-span of the micelles formed is shorter than their reforming time, the micelles network formation are disrupted, causing the viscosity of the whole emulsion system to be dramatically decreased. This is probably the reason that as higher concentration of *Baccaurea motleyana hook f.* fruit's extract is added to the product, the viscosity of the moisturiser product is reduced.

3.3.2 (i) Stability Study at 50°C

(a) pH and Physical Separation Profile at 50°C Stability Testing

Out of 5 products developed, 2 have separated, Table 3.15. The results showed the product containing 17wt/wt% and 20wt/wt% of *Baccaurea motleyana hook f.* fruit's extract have separated into 2 layers with the top layer consists of oily substance and the bottom layer is an emulsion at Week 5 and Week 3 respectively. The possible mechanism leading to this product's separation might be due to the charge shielding effect. When particles are dispersed in a liquid such as water, they tend to acquire a charge, usually by ionisation process that is when the dispersed particles either lose an ion or pick up an ion from the surrounding medium (in both cases, often an hydrogen ion, H^+) (Briggs, 1996a). *Baccaurea motleyana hook f.* fruit's extract contains organic acids which is a weak acid. The ionisation of weak acids in water in terms of Bronsted-Lowry equilbrium can be described as follows:

Table 3.15: Moisturiser containing *Baccaurea motleyana hook f.* fruit's extract – pH and physical separation profile at 50°C

Formulation Code	B8	MR1	MR2	MR3	MR4	MR5
wt/wt % *Baccaurea motleyana hook f.* fruit's extract	0	10	13	15	17	20
Week 0	6.09	4.62	4.49	4.38	4.24	4.10
Week 1	5.86	4.62	4.36	4.30	4.19	4.07
Week 2	5.74	4.47	4.35	4.26	4.18	4.06
Week 3	5.62	4.44	4.32	4.24	4.16	4.02
Week 4	5.52	4.42	4.26	4.23	4.10	3.95
Week 5	5.43	4.34	4.27	4.22	4.01	3.93

Note: Shaded column indicated a two-layer product separation.

$$HA + H_20 \rightleftharpoons H_30^+ + A^-$$

Or simplified as

$$HA \rightleftharpoons H^+ + A^-$$

Baccaurea motleyana hook f. fruit's extract contains two types of organic acids, tartaric acid and malic acid. So, the possible ionisation mechanisms for these two weak acids in water can be described as follows:

(a) DL-Malic acid: $CH_2CHOH((COOH)_2) \rightleftharpoons \underset{\text{positive charged ion}}{2H^+} + \underset{\text{negative charged ion}}{CH_2CHOH(COO^-)_2}$

(b) L(+)-Tartaric acid: $2(CHOH.COOH) \rightleftharpoons \underset{\text{positive charged ion}}{2H^+} + \underset{\text{negative charged ion}}{2CHOHCOO^-}$

The addition of *Baccaurea motleyana hook f.* fruit's extract to the moisturiser product cause more of the negative and positive charge ions from these two acids, to be dispersed in the continuous phase. Knowlton (1996a) argued that some of the positive charged ions dispersed in the continuous phase will be attracted towards the negative charged ions at the surface of the dispersed phase molecules, causing the positive charged ions inadjacent to the negative charged ions as illustrated in Figure 3.30.

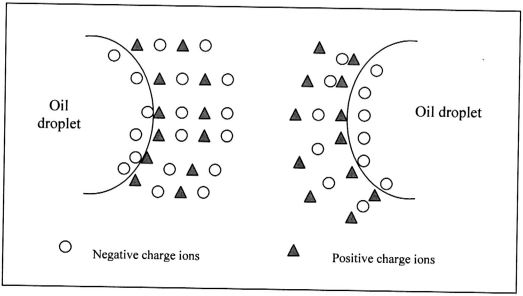


Figure 3.30: Electrical charges configuration at the surface of a dispersed phase molecule in an o/w emulsion

This condition is referred as *Helmholtz double-layer* or *electrical double-layer.* He said that when electrical double layer is formed, the repulsive effects of the negative charges are neutralised to some extent by the positive charges. As the result, the dispersed phase molecules will have the tendency to move closer to each other since the repulsive effects of the negative ions were reduced. This phenomenon is called the *charge shielding effect* and is a contributing factor to emulsion instability.

As higher amount of *Baccaurea motleyana hook f.* fruit's extract was added to the moisturiser product, higher concentration of hydrogen ions (H^+) were dispersed in the continuous phase. These hydrogen ions would migrate towards the surface of the negative charged dispersed phase molecules and increase the magnitude of the charge shielding, consequently reducing the repulsion effect between the dispersed phase molecules, leading to flocculation, coalescence and creaming phenomenon. Finally contributing to product's physical separation and these reactions are accelerated at higher temperature condition.

(ii) pH Profile

There is an obvious pH reduction seen when the product was continuously stored at 50°C condition throughout the 5 weeks period of stability testing, Table 3.15. Even though the initial pH (Week 0) of the product containing *Baccaurea motleyana hook f.* fruit's extract is much lower (pH 4.1-4.62) than the unstable moisturiser base products (pH 4.29-5.42), but the moisturiser product containing this extract showed a more consistent profile compared to the moisturiser base product stability study. This is perhaps due to the stable base moisturiser product formulation chosen in *Baccaurea motleyana hook f.* fruit's extract study. So, even when the extract is incorporated in the product formulation, lowering the pH of its o/w emulsion system, the product is still quite stable at that lower pH values. In other words, once the base formulation used is stable, the effect of product's instability caused by *Baccaurea motleyana hook f.* fruit's extract in the product formulation is much reduced.

(b) Viscosity Profile at 50°C Stability Condition

The viscosity of the products formulated with *Baccaurea motleyana hook f.* fruit's extract, measured at Week 0 (50°C, 0.1288s^{-1}) is in the region of 32,063 cps to 43,004 cps. The higher the amount of extract added to the product's formulation, the less viscous is the product, Figure 3.31. The similar viscosity profile trend like in the moisturiser base product formulation stability study was observed when the products formulated with *Baccaurea motleyana hook f.* fruit's extract were stored at 50°C for 5 weeks of stability testing period. Thus, the similar phenomenon that leads to the product's thinning and physical separation can be referred to the previous discussion under section 3.3.1 (iii) (b) (ii). The physical separation of the product also occurred

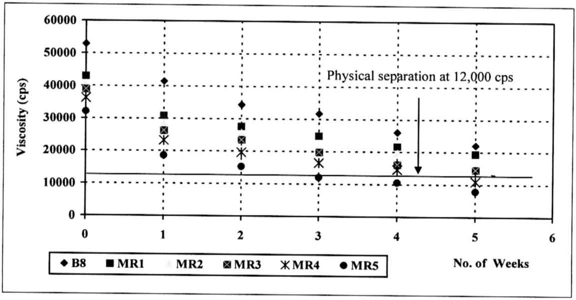


Figure 3.31: Viscosity profile of moisturiser product containing *Baccaurea motleyana hook f.* fruit's extract (50°C, 0.1288 s^{-1})

when the viscosity reaches about 12,000 cps. In this study, the stable moisturiser product with the highest percentage of *Baccaurea motleyana hook f.* fruit's extract is 15wt/wt%.

(c) Colour Profile at 50°C Stability Testing Condition

Not much changes on the colour profile seen for the stable products' formulated. The products developed either with or without *Baccaurea motleyana hook f.* fruit's extract is quite stable throughout the 5 weeks of stability testing at 50°C, Figure 3.32. By observation, the moisturiser base, B8 is milk white, MR1, MR2 and MR3 is white and MR4 and MR5 is off white in colour.

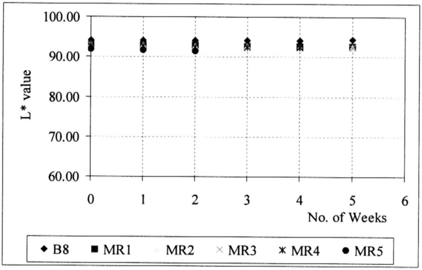


Figure 3.32: Colour profile (L* value) for moisturiser containing *Baccaurea motleyana hook f.* fruit's extract (50°C)

3.3.2 (ii) Freeze-Thaw Stability Study

The result is presented in Table 3.16. Most of the product's properties analysed after completion of 5 freeze-thaw cycles such as its pH, colour and viscosity value are quite similar to those values measured at RT condition. The similar products' physical assessment results like in the moisturiser base study was seen with the products

Table 3.16: Freeze-thaw stability profile for moisturiser containing *Baccaurea motleyana hook f.* fruit's extract

Formulation Code	MR1		MR2		MR3	
wt/wt% Extract	10		13		15	
Testing Condition	Week 0	After 5 freeze-thaw cycles	Week 0	After 5 freeze-thaw cycles	Week 0	After 5 freeze-thaw cycles
pH	4.62	4.58	4.49	4.46	4.38	4.35
Physical separation	NO	NO	NO	NO	NO	NO
Viscosity at 0.1288 s^{-1} (cps)	67961	68573	64306	65873	59380	60813
Colour (Observation)	white	white	white	white	white	white
Colour Spectrophotometer						
L* value	93.12	93.10	92.96	92.93	92.80	92.78
a* value	-0.55	-0.57	-0.61	-0.63	-0.69	-0.72
b* value	3.53	3.50	3.81	3.78	3.77	3.74
Physical Assessment						
Texture	Fine	Slightly Rough	Fine	Slightly Rough	Fine	Slightly Rough
Feel	Soft	Slightly Hard	Soft	Slightly Hard	Soft	Slightly Hard

Note: wt/wt% Extract refers to wt/wt% *Baccaurea motleyana hook f.* fruit's extract.

formulated with *Baccaurea motleyana hook f.* fruit's extract. After 5 freeze-thaw cycles have been completed, the products' texture was slightly rough and not as shiny as when they were observed at Week 0, stored at RT condition. The products are also slightly hard. In spite of this, the products are still able to conform to their initial condition in terms of their appearances and textures when they are slightly stirred. This behaviour was due to the freezing effect which was previously discussed under section 3.3.1 (iv). Since there is no product's physical separation seen, all the products studied are considered stable.

3.3.3 Enhancement of Stability Performance for Moisturiser Product Containing *Baccaurea motleyana hook f.* Fruit's Extract

In this study, the stability of the o/w emulsion system was improved by using citrate buffer system which comprised of 0.1M citric acid and 0.1M tri-sodium citrate in a ratio of 1:3. The pH of the citrate buffer is 5.36. In water, citric acid would dissociates into hydrogen ion molecules and citrate ion molecules whilst tri-sodium citrate would completely dissociated into citrate ion molecules and sodium ion molecules as in the following equation:

Citric acid: $C_3H_4(OH)(CO_2H)_3 \rightleftharpoons 3H^+ + C_6H_5O^-_7$

hydrogen ion citrate ion

Tri-Sodium citrate: $C_6H_5Na_3O_7 \longrightarrow Na^+ + C_6H_5O^-_7$

sodium ion citrate ion

The results is presented in Table 3.17.

Table 3.17: Moisturiser containing *Baccaurea motleyana hook f.* fruit's extract using citrate buffered system at Week 0 (RT condition)

Formulation Code	BR1	BR2	BR3	BR4
wt/wt% *Baccaurea motleyana hook f.* fruit's extract	17	20	23	25
Result:				
pH	5.29	5.20	5.12	5.05
Viscosity (25°C, 0.1288 s^{-1})	64412	59127	52987	48372
Colour (Visual Observation)	white	off white	off white	off white
Colour Spectrophotometer:				
L* value	92.79	91.98	90.83	90.65
a* value	-0.63	-0.21	-0.35	-0.47
b* value	3.86	4.17	4.73	4.70

The pH of the product containing *Baccaurea motleyana hook f.* fruit's extract with citrate buffer is in the range of pH 5.05 to 5.29. Even though higher percentage of *Baccaurea motleyana hook f.* fruit's extract is added to the product's formulation which in this case is up to 25wt/wt%, the pH acquired are much higher than when the same amount of the extract is added to the product formulated without citrate buffer which is pH 4.12 (20wt/wt% of extract) and pH 4.24 (17wt/wt% of extract). *Baccaurea motleyana hook f.* fruit's extract contains malic acid and tartaric acid. In water, these acids would dissociates into hydrogen ion molecules and their conjugate base molecules respectively.

Malic acid: $CH_2CHOH((COOH)_2) \rightleftharpoons 2H^+ + CH_2CHOH(COO^-)_2$

Tartaric acid: $2(CHOH.COOH) \rightleftharpoons 2H^+ + 2CHOHCOO^-$

These hydrogen ions molecules from *Baccaurea motleyana hook f.* fruit's extract can react with citrate ion molecules from citrate buffer solutions and formed citric acid. This

$$\underset{\substack{\text{hydrogen ion}\\\text{from malic and tartaric acid}}}{H^+} + \underset{\text{citrate ion}}{C_6H_5O^-_7} \longrightarrow \underset{\text{citric acid}}{C_3H_4(OH)(CO_2H)_3}$$

reaction will occur until all the citrate ion molecules available in the emulsion system have reacted with hydrogen ion molecules. The measurement of product's pH means the measurement of hydrogen ions concentration in the product. Since most of the hydrogen ions were bounded to citrate ion molecules, there would be only small amount of hydrogen ion molecules available in the emulsion system, finally giving a higher pH reading even though higher concentration of *Baccaurea motleyana hook f.* fruit's extract was added to the product's formulation, Table 3.18. The higher the pH of the product, the more stable is the product.

The viscosity of the product formulated with citrate buffer is in the range of 48,372 cps to 64,412 cps (25°C, $0.1288s^{-1}$). With similar amount of extract added to the product, the viscosity value attained is much higher than when the product is formulated without using citrate buffer. The viscosity of the moisturiser containing 17wt/wt% and 20wt/wt% of *Baccaurea motleyana hook f.* fruit's extract (without citrate buffer), measured at 25°C ($0.1288s^{-1}$) is 53,510 cps and 48,504 cps respectively whilst with similar amount of extract used, measured at similar condition but with citrate buffer, the product's viscosity obtained is much higher that is 64,412 cps (17wt/wt% of extract) and 59,127 cps (20wt/wt% of extract). The increase in the product's viscosity is probably due to the formation of sodium stearate through the reaction between sodium ions from dissociation of tri-sodium citrate molecules and excess of stearic acid in the product.

Stearic acid used in the product reacted with NaOH to form sodium stearate, thickening and also help stabilising the moisturiser products formulated. However, there is a limitation in using NaOH solution in the product since it is a strong base. High concentration of NaOH solution added to the product could increase the products pH to alkaline. Moisturiser base product, B8, contains 1.05wt/wt% of NaOH solutions and 3wt/wt% of stearic acid. As calculated, 4.491% of NaOH (10% solution) is needed to react with 3% of stearic acid to form sodium stearate (Appendix B). Since only 1.05wt/wt% of NaOH was used in the product, there is still about 2.3% excess of unreacted stearic acid available in the product. Therefore, there is a possibility that sodium ions from dissociation of tri-sodium citrate molecules, reacted with excess of stearic acid in the product and formed sodium stearate, further thickening the product. This might be the reason that the buffered moisturiser products, for example BR1 and BR2, have a viscosity higher than those product formulated without using the buffer, MR4 and MR5, even with similar wt/wt% of *Baccaurea motleyana hook f.* fruit's extract was used in the product.

By visual observation, the product formulated with 17wt/wt% of the extract is white whilst those formulated with higher wt/wt% of the extract is off white. As more extract is added to the product's formulation, the colour of the product become slightly darker, Table 3.17.

3.3.3 (i) Stability Study at 50°C

(a) pH and physical separation

A similar pH reduction trend was observed when the products were subjected to 50°C stability testing condition, like those moisturiser products containing the extract but formulated without citrate buffer, Table 3.18. From four products developed, two have physically separated, but this time with higher wt/wt% of *Baccaurea motleyana hook f.* fruit's extract used in the product. The products containing citrate buffer and formulated with 23 wt/wt% and 25 wt/wt% of *Baccaurea motleyana hook f.* fruit's extract have separated into two layers at Week 4 and Week 3 respectively under 50°C stability testing condition, Table 3.19. The products containing 17 wt/wt% and 20 wt/wt% of *Baccaurea motleyana hook f.* fruit's extract formulated with citrate buffer is stable throughout the 5 weeks of stability testing period at 50°C, whilst the products contains similar wt/wt% of the extract but formulated without citrate buffer (MR4 and MR5)

Table 3.18: Buffered moisturiser product – pH and physical separation profile at 50°C

Formulation Code	BR1	BR2	BR3	BR4
wt/wt % *Baccaurea motleyana hook f.* fruit's extract	17	20	23	25
pH Value:				
Week 0	5.29	5.20	5.12	5.05
Week 1	5.19	5.09	4.96	4.91
Week 2	5.10	4.98	4.87	4.82
Week 3	4.99	4.89	4.79	4.73
Week 4	4.94	4.84	4.60	4.59
Week 5	4.92	4.80	4.49	4.40

Note: Shaded column showed a two-layer physical separated product.

have separated into two layers at Week 5 and Week 3 respectively when stored under similar stability testing condition.

As earlier discussed under section 3.3.2 (i), the possibility of the physical separation of the product is due to the charge shielding effect caused by the presence of high concentration of charged ions in the continuous phase. When citrate buffer is added to the product's formulation, these charged ions were bounded to citrate ions, finally reducing the *charge shielding* effect and producing a more stable product. This is probably the reason that the product containing 17wt/wt% and 20wt/wt% of *Baccaurea motleyana hook f.* fruit's extract using citrate buffer is more stable than those products formulated without it, but containing similar wt/wt% of *Baccaurea motleyana hook f.* fruit's extract. Therefore, a much higher wt/wt% of the extract was needed to cause physical separation of the product. The behaviour of these products, leading to their physical separation are explained earlier under Section 3.1.1 (viii).

(b) Viscosity Profile

The viscosity of the buffered moisturiser products formulated with *Baccaurea motleyana hook f.* fruit's extract is in the range of 32,241 cps to 40,845 cps when measured at Week 0 (50°C, $0.1288s^{-1}$). A similar viscosity trend like those moisturiser products formulated with and without *Baccaurea motleyana hook f.* fruit's extract was observed when the product was stored at 50°C throughout the 5 weeks period of stability testing, Figure 3.33. The viscosity of the products that have separated into 2 layers are about 12,000 cps. The reason of reduction in the product's viscosity during the 5 weeks stability study period have been explained earlier in Section 3.3.1(iii)(b) (ii).

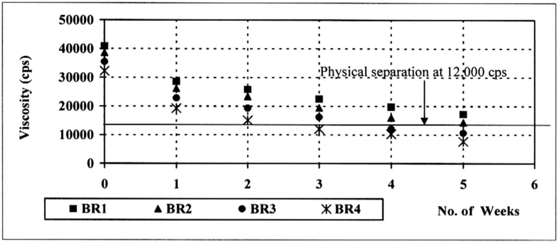


Figure 3.33: Viscosity profile of buffered moisturiser containing *Baccaurea motleyana hook f.* fruit's extract (0.1288 s^{-1}, 50°C)

(c) Colour Profile

Throughout the 5 weeks of stability testing period at 50°C, the colour for all the products' measured are quite stable. There are no significant differences in the results obtained, Figure 3.34. By observation, the product containing 17wt/wt% of *Baccaurea motleyana hook f.* fruit's extract is white whereas those products containing higher than this are off white.

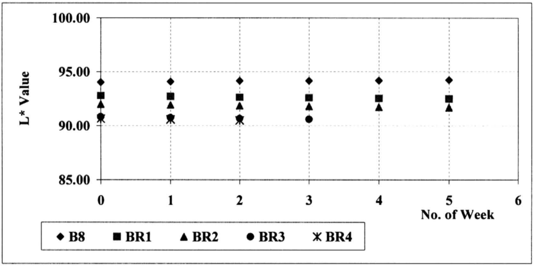


Figure 3.34: Colour profile (L* value) for buffered moisturiser (50°C)

3.3.3 (ii) Freeze-Thaw Stability Profile

The results are as presented in Table 3.19. In general all of the product's properties measured after completion of 5 freeze-thaw cycles such as its pH, colour and viscosity value, except the products' physical assessment, are quite close to those values measured at RT condition. The buffered moisturiser products' texture and feel are not as good as when the products were kept at RT condition, measured at Week 0. The products' texture is slightly rough and its feel is slightly hard, probably due to the freezing effect which was earlier discussed under section 3.3.1 (ii) (d). However, these characteristics are easily reversible to its origin condition upon slight stirring. On the other hand, there are also no product's physical separation seen. Therefore all the products are considered stable.

Table 3.19: Freeze-thaw stability study for moisturiser product containing *Baccaurea motleyana hook f.* fruit's extract

Formulation Code	BR1		BR2	
wt/wt% *Baccaurea motleyana hook f.* fruit's extract	17		20	
Testing Condition	Week 0	After completion of 5 cycles	Week 0	After completion of 5 cycles
pH	5.29	5.25	5.20	5.16
Physical separation	NO	NO	NO	NO
Viscosity at 0.1288s^{-1} (cps)	64412	65103	59127	59845
Colour (Visual Observation)	white	white	Off white	Off white
Colour Spectrophotometer:				
L* value	92.79	92.81	91.98	91.95
a* value	-0.63	-0.69	-0.21	-0.26
b* value	3.86	3.90	4.17	4..21
Physical Assessment				
Texture	Fine	Slightly Rough	Fine	Slightly Rough
Feel	Soft	Slightly Hard	Soft	Slightly Hard

3.4 Conclusion

M4 with 3wt/wt% stearic acid was selected for its good spreading characteristics – it was a soft cream, neither oily nor tacky. Adding 0.95% and 1.05% NaOH solution to the M4 respectively (coded B7 and B8) gave stable emulsions even in storage at 50°C. The good stability was also due to their higher viscosities produced of 14,000 cps to 22,000 cps. B8 was selected for further evaluation with the content *of Baccaurea motleyana hook f.* extract varied from 10wt/wt% to 20wt/wt% (coded MR1 to MR5). Incorporation of more extract lowered the pH from 6.09 to 4.12 – 4.62, probably due to the increased charge shield formed around the oil droplets in the oil/water emulsion. The viscosity and colour were also affected by the amount of extract in the formulation. MR3 with 15wt/wt% extract, pH 4.22 and viscosity of over 12,000 cps was found to a stable system.

Furthermore, using the citrate buffer to maintain the skin pH at 5.6 helped stabilise the system so that the extract could be increased to 17wt/wt% and 20wt/wt% (BR1 and BR2). The stabilisation was due to excess H^+ from malic and tartaric acids (rambai extract) binding with the citrate ion to produce citric acid. A higher viscosity resulted, leading to the increased stability compared to the unbuffered system with the same amount of extract. A further increase in the viscosity is possible from the sodium ions (produced from dissociation of trisodium citrate in the buffering system) reacting with stearic acid to produce sodium stearate.