FORMULATION, *IN VITRO* AND *IN VIVO* EVALUATION OF COSMETIC NANO-CREAM FROM VIRGIN COCONUT OIL, KOJIC ACID DIPALMITATE AND EMULIUM KAPPA

SARMAD SABAH NASSER AL-IDRESI

UNIVERSITI SAINS MALAYSIA
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by

SARMAD SABAH NASSER AL-EDRESI

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In the name of Allah who is gracious and merciful

This thesis is dedicated to my wife, daughter,

parents, brother and sisters

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
°C	Degree centigrade
cm	Centimeter
cm^2	Square centimeter
conc.	Concentration
c.p.	Centipoises
DF	Degree of freedom
D-optimal	Design-optimal
DOE	Design of Experiment
EIP	Emulsion Inversion Point
EK	Emulium kappa
Eq.	Equation
FDI	Cream formulated with deionized water
FDW	Cream formulated with distilled water
Fig	Figure
FNW	Cream formulated with nano-water
FRW	Cream formulated with reverse osmotic water
g	Gram
G'	Storage modulus
G"	Loss modulus
HLB	Hydrophile-lipophile balance
HPLC	High Performance Liquid Chromatography
hr	Hour

Hz	Hertz
ICH	International Conference of Harmonization
i.d.	Internal diameter
KDP	Kojic acid dipalmitate
Kp	Permeation coefficient
L	Liter
$\mu g.mL^{-1}$	Microgram per milliliter
µS	Microsiemens
min	Minute
mins	Minutes
mL	Milliliter
mm	Millimeter
MP	Methyl paraben
mV	Millivolt
N	Number of replications
nm	Nanometer
o/w	Oil in water
P	Probability
Pa	Pascal
PG	Propylene glycol
PIT	Phase Inversion Temperature
PP	Propyl paraben
R^2	Correlation coefficient
RE	Residual Error

rpm	Rotation per Minute time
RSD	Residual Standard eviation
RSM	Response Surface Methodology
s	Second
S.D.	Standard deviation
SED	Statistical Experimental Design
SEM	Scanning Electron Microscope
Sig.	Significant
Sp.	Spreadability
SS	Sum of squares
SC	Stratum corneum
t.	Time
T.	Temperature
THF	Tetrahydrofuran
USP	United State Pharmacopoeia
V	Volt
VCO	Virgin Coconut Oil
v/v	Volume per volume
w/o	Water in oil
w/w	Weight per weight
X_1	First component in mixture design (EK/PG)
X'_1	First pseudo-component in mixture design (EK/PG)
X_2	Second component in mixture design (VCO)
X'_2	Second pseudo-component in mixture design (VCO)

X_3	Third componnt in mixture design (Deionized water)
X'_3	Third pseudo-component in the mixture design (Deionized water)
Y_1	Response of apparent viscosity
Y_2	Response of yield value
Y_3	Response of spreadability

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Formulasi, Penilaian *In vitro* dan Penilaian *In vivo* terhadap Kosmetik Krim-Nano daripada Minyak Kelapa Tulen, Asid Kojik Dipalmitat dan Emulium Kappa

ABSTRAK

Minyak kelapa tulen (*Virgin Coconut Oil*-VCO) dalam air distabilkan oleh Emulium Kappa® yang digunakan sebagai agen pengemulsi untuk menyediakan krim kosmetik. Asid kojic dipalmitate [*Kojic Acid Dipalmitate* (KDP)] yang dilarutkan dalam VCO adalah suatu bahan pemutihan. Ciri terakhir formulasi krim bergantung pada nisbah fasa minyak, agen pengemulsi/emulsi bersama dan air yang dianggapkan sebagai pembolehubah utama. Rajah fasa ternari bersama grafik kontur digunakan untuk menilai kesan-kesan perubahan pada pembolehubah. Sistem itu direka bentuk dengan menggunakan model Scheffé. Kesan-kesan komponen pada kelikatan, nilai alah, dan keboleh sebaran (*spreadability*) yang jelas (kriteria utama) ditaksirkan melalui penggunaan perisian *Design-Expert*®. Penetapan kriteria formulasi terakhir dibuat berdasarkan ciri-ciri dua krim pemutih yang terdapat dalam pasaran. Kemudian, kestabilan formulasi terakhir ditaksirkan melalui penggunaan dua ujian kestabilan tercepatkan, iaitu ujian beku-nyahbeku selama dua minggu dan ujian tercepatkan klasik (*classical accelerated test*) selama enam bulan. Krim nano disediakan melalui penggunaan kaedah *Emulsion Inversion Point* (saiz partikel < 300nm). Pertumbuhan Ostwald (Ostwald Ripening) adalah faktor yang mengurangkan kestabilan (*destabilizing*) bagi emulsi nano yang boleh dikurangkan dengan cara menambahkan

minyak yang tidak boleh larut, iaitu skualena, kepada fasa minyak. VCO:skualena dengan nisbah 10:0, 9.8:0.2, 9.6:0.4, 9.4:0.6, 9.2:0.8, 9:1 dan 8:2 adalah dinilaikan. Kestabilan emulsi nano dinilaikan melalui ciri-ciri elektroforesis titisan-titisan emulsi. Akhir sekali, KDP yang dimuatkan ke dalam krim nano dan krim biasa diuji untuk menentukan keupayaannya menembusi kulit tiruan dengan menggunakan sel pembauran Franz, sementara tingkah laku penyimpanan KDP dalam liang-liang bulu tikus Wistar dikaji dengan menggunakan teknik penanggalan pita (*tape stripping technique*). Hasilnya menunjukkan bahawa *Mixture Design*, dengan dibantu oleh *Design-Expert®*, boleh digunakan untuk menambah baikkan formulasi krim yang terdiri daripada KDP, VCO dan EK. Tambahan pula, ujian *freeze-thaw* boleh dianggapkan sebagai ujian alternatif yang berkesan berbanding ujian-ujian klasik. Krim yang mengandungi air tanpa ion (*de-ionized water* - FDI) telah terbukti sebagai krim yang paling stabil. Lagipun, pembahagian antara titisan-titisan secara berterusan adalah disebabkan oleh skualene dalam penurunan pertumbuhan Ostwald (*Ostwald ripening*). Nilai potensi zeta menjadi semakin bertambah apabila kadar peratusan skualena dipertingkatkan. Akhir sekali, tidak terdapat sebarang variasi yang signifikan pun antara krim nano dan krim biasa, dari segi pelepasan drug, pada nilai $p > 0.05$, sementara KDP yang dimuatkan ke dalam krim nano menjadi terperangkap di dalam liang-liang bulu tikus selama > 7 hari dan KDP yang dimuatkan ke dalam krim biasa menjadi terperangkap selama ≤ 3 hari, iaitu mengalami kadar pengurangan (*depletion rate*) yang lebih pantas.

Formulation, *In vitro* and *In vivo* Evaluation of Cosmetic Nano-cream from Virgin Coconut Oil, Kojic Acid Dipalmitate and Emulium Kappa

ABSTRACT

Virgin Coconut Oil (VCO)-in-water stabilized by Emulium Kappa® (EK) as an emulsifier was used to prepare a cosmetic cream. Kojic acid dipalmitate (KDP) dissolved in VCO was the whitening ingredient. The final characteristic of the cream formulation depends on the ratio of the oil phase, emulsifier/coemulsifier and water which are considered as the main variables. Ternary phase diagram with contour graphics was used to assess the effects of variable changes. The system was designed by using Scheffé model. The effects of the components on the apparent viscosity, yield value, and spreadability (the main criteria) were assessed by using Design-Expert® software. The criteria of the final formulation were determined based on the properties of two commercially available whitening creams. Then, the stability of the final formulation was assessed by using two accelerated stability tests, namely the freeze-thaw test for two weeks and classical accelerated tests for six months. Nano-cream was prepared by using Emulsion Inversion Point method (particle size < 300nm). Ostwald ripening is the main destabilizing factor for nano-emulsion that can be reduced by the addition of non-soluble oil, namely squalene to the oil phase. VCO:squalene in the ratio of 10:0, 9.8:0.2, 9.6:0.4, 9.4:0.6, 9.2:0.8, 9:1, and 8:2 were evaluated. The stability of nano-emulsions was evaluated by the electrophoretic properties of the emulsion droplets. Finally, KDP loaded into normal and nano-cream were tested for their capabilities to

permeate through artificial skin using Franz diffusion cell, while the storage behavior of KDP in the hair follicles of Wistar rat was investigated using tape stripping technique. The results indicated that the Mixture Design, with the aid of Design-Expert® could be used successfully and efficiently to optimize cream formulation composed of KDP, VCO, and EK. Furthermore, freeze-thaw test could be considered as an efficient alternative test to the classical methods. Cream that contains deionized water (FDI) was proven to be the most stable. Moreover, continuous partitioning between the droplets due to squalene resulted in the decline of Ostwald ripening. The zeta-potential value increased as the percentage of squalene increased. Finally, no significant variation between normal and nano-cream, in terms of drug release, was found at p-value > 0.05, while KDP loaded into nano-cream was trapped in the hair follicles for > 7 days and KDP loaded into normal cream was trapped for ≤ 3 days i.e. was subjected to a faster depletion rate.

CHAPTER 1: INTRODUCTION

1.1 Emulsions

A thermodynamically unstable dispersion of two immiscible liquid, such as water and oil, is called an emulsion (Imbert *et al.*, 2002, Goodwin, 2004). The presentation of one of these two liquid is in the form of finely distributed spherical droplets in the second liquid i.e. the continuous phase. The emulsion is referred to as an oil-in-water (o/w) emulsion if the oil is dispersed in water (Frelichowska *et al.*, 2009), while the reverse produces water-in-oil (w/o) emulsion. Table 1.1 lists a number of immiscible phase components (Mollet and Grubenmann, 2001b, Dickinson, 2009).

These disperse systems, in terms of free energy, are greater by the amount of surface energy if compared with that of macroscopically extended systems. Thus, coalescence can form as a result of droplet collisions in pure emulsions which can cause the separation of the emulsion into different phases, which will be in a state of lower energy (Mollet and Grubenmann, 2001b, Goodwin, 2004). Furthermore, coalescence has greater practical significance than does sedimentation, in terms of stability issues, since droplets may exist collectively together for a long time without actually coalescing. Moreover, a third component is required, namely an emulsifier, in order to produce a stable emulsion i.e. a long lived technical emulsion (Rieger, 1996). A protective layer formed from the accumulation of the emulsifier at the interface must prevent the droplet from coalescing and this layer must be tough and elastic to enhance the stability of the emulsion. Sometimes, mixtures of emulsifiers or emulsifier/co-emulsifiers are used to optimize emulsion properties (Block, 1996, Mollet and Grubenmann, 2001b).

Table 1.1 Some immiscible phases components (Rieger, 1996)

Phase	Examples
Polar ingredients	Polyols
	Butylene glycol
	Glycerin
	Polyethylene glycol
	Propylene glycol
	Water
Non polar ingredients	Esters
	Fats
	Lanolin
	Synthetic e.g. isopropyl myristate and isopropyl palmitate
	Vegetable oil
	Ethers
	Perfluropolyether
	Polyoxypropylene
	Fatty acids
	Fatty alcohols
	Hydrocarbons
	Butane, propane
	Microcrystalline waxes
	Mineral oils
	Petrolatum
	Squalene
	Miscellaneous
	Halohydrocarbons e.g. perflurocarbons
	Waxes, plant and animal
	Silicone fluids

1.1.1 Emulsifiers

Rieger (1996) defined emulsifiers as "Amphiphilic compounds which are (i) soluble in at least one phase of the system, (ii) forming monolayers oriented at phase interfaces, (iii) exhibits equilibrium concentrations at phase interfaces higher than those in the bulk solution and forms micelles at specific concentrations and (iv) exhibits one or more of

the following characteristics: detergency, foaming, wetting, emulsifying, solubilizing and dispersing".

1.1.1.1 Types of common emulsifiers

There are 4 types of common emulsifiers.

i) o/w emulsifiers (low molecular weight of hydrophilic nature):

a) Anionic: soaps e.g. (Na, K, NH_4 and morpholinium salts of fatty acids), sodium lauryl sulfate, sodium mersolate,
b) Cationic: they carry positive charges e.g. laurylpyridinium chloride, lauryltrimethylammonium.
c) Nonionic e.g. polyoxyethylene fatty alcohol ethers.

ii) w/o emulsifiers (low molecular weight of lipophilic nature) such as magnesium stearate, magnesium oleate, aluminum stearate and calcium stearate.

iii) Less pronounced properties emulsifiers (low molecular weight) such as fatty acid esters of polyols and polyoxyethylene.

iv) High molecular weight emulsifiers: e.g. albumin, casein and gelatin.

(Rieger, 1996, Mollet and Grubenmann, 2001b).

1.1.2 Emulsion stabilizers

Emulsion creaming, droplet flocculation or coalescence can be inhibited after the emulsification process for the achievement of emulsion stabilization. This can be done by increasing the viscosity of continuous phase, equalizing phase densities and by adsorbing stabilizing substances at the oil-water interface (Rieger, 1996, Dickinson, 2009).

1.1.3 The Hydrophile-Lipophile Balance (HLB) system

Hydrophile-Lipophile Balance concept was introduced by Griffin in 1949 and developed in the 1950s. This concept has a mean of characterizing surfactant (Orafidiya and Oladimeji, 2002, Wu *et al.*, 2004, Guo *et al.*, 2006). HLB system provided the formulators with relevant information regarding emulsion formulation (Mollet and Grubenmann, 2001b, Ishii and Nii, 2005). A scale of surfactant lipophilicity (0-20) was provided by this HLB system that simplified the selection and blending of emulsifiers (Constantinides and Scalart, 1997). W/o emulsions can be obtained by using a surfactant with a low HLB (≤ 6), while o/w emulsions stabilization requires a higher HLB (≥ 8) (Table 1.2). Algebraic manipulation is necessary when a blend of surfactant, with a known HLB, is used for a particular emulsification (Rieger, 1996, Guo *et al.*, 2006). Thus, the HLB values of a mixture of emulsifiers, $HLB_{Mixture}$, are calculated by the following equation:

$$HLB_{Mixture} = HLB_1.g_1 + HLB_2.g_2 + \ldots\ldots \quad \text{Eq. (1.1)}$$

g_1, g_2, ... are the mass fractions of the components.

Table 1.2 HLB values and its applications (Mollet and Grubenmann, 2001b)

	HLB value	Application
Lipophilic	0-3	Defoamers
↓	3-8	w/o emulsions
	7-9	Wetting agents
	8-18	o/w emulsions
	11-15	Detergents
hydrophilic	15-18	Solubilizers

1.2 Cosmetics

Substances or preparations which are intended for external use or in the buccal cavity for care, cleaning and to modify the appearance or odor are called cosmetics (Umbach, 1991c, De Groot, 1997, Mitsui *et al.*, 1997c, Kütting and Drexler, 2003). Cosmetics are used for curing, reducing and preventing physical damage and this is the difference from medicament in that every cosmetic treatment should start with cleansing that should remove germs, unwanted dirt and improve physiological and physical well-being (Umbach, 1991b). Sometimes the distinction between cosmetics and medicament is difficult to interpret in terms of the above definitions e.g. sebum flow can be affected by skincare preparations (Mitsui *et al.*, 1997c, Mollet and Grubenmann, 2001a).

1.2.1 Cosmetic preparations

The physiology of the skin can be affected by cosmetics (Umbach, 1991a). This effect can be desirable or harmful, thus this factor must be considered carefully (Mitsui *et al.*, 1997d). Emulsions, whether o/w or w/o, are particularly important in cosmetic

formulation. Due to the large number of raw materials, many combinations of emulsifiers can make up optimized products for particular applications such as (i) cosmetics for the skin, (ii) nail care, (iii) cosmetics for the hair and (iv) shaving aids (Rieger, 1996).

1.2.1.1 Emulsions in cosmetics

1.2.1.1(a) Types of emulsion

The development of a stable emulsion that is safe for the skin and meets modern requirements, which means higher stability and lower emulsifier concentration, is one of the hardest tasks to be accomplished in the field of cosmetics. Furthermore, it is very difficult to combine the stability and the modern requirements, thus the emulsion that tends to be unstable at higher temperature ranges may have good skin compatibility or soreness that might be caused from very stable emulsions (de Groot, 1998). The most popular emulsions are o/w because these emulsions have initial cooling effects due to evaporation of water, thus giving a good feeling to the skin (Miller *et al.*, 1999, Sinko and Martin, 2006d). Moreover, o/w emulsions do not make the skin look very shiny and are less likely to block pores. These properties will increase their acceptance by the consumers (Swarbrick and Boylan, 1996). On the other hand, w/o emulsion forms a thin film on the skin surface and this will control the dehydration of the cornified layer which makes this type of emulsion correspond closely to the physiological conditions of the skin since this is incorporated by the skin's own fatty exudates (Mitsui *et al.*, 1997a). Finally, multiple emulsions, such as o/w/o or w/o/w, are those in which a dispersed

phase is contained within another dispersed phase and they contain at least two types of surfactants (Mollet and Grubenmann, 2001a).

1.2.1.1(b) Hydrocolloids

Hydrocolloids are gel-forming thickeners used to keep the emulsifier film firm, and can also act as protective colloids and consistency regulators (Mollet and Grubenmann, 2001a). Hydrocolloids are of two types, either organic or inorganic. Hydrocolloids are gathering around the droplets and reinforcing their stabilizing layer (Sinko and Martin, 2006d).

1.2.1.2 Basic composition of cosmetic emulsion

The following ingredients are part of a cosmetic emulsion. (i) *the oil phase*, to which belongs the emulsifier system, consistency regulator, oil soluble preservatives and oil-soluble antioxidant as well as the actual oil components (Mitsui *et al.*, 1997d), (ii*) the aqueous phase*, which makes up to 85-95% of the emulsion, containing water soluble preservatives and any humectants and thickeners and (iii) *the remaining ingredients* such as active substances, perfume oils and colorants (Mollet and Grubenmann, 2001a, Sinko and Martin, 2006d).

1.2.1.3 Solutions

The defatting effect of washing can be counteracted by skin oils and fats which are used as solvents for active substances. These solutions are also intended to soften, smooth and protect the skin. They can also serve as lubricant for massaging (Mollet and Grubenmann, 2001a).

1.2.1.4 Gels

Gels are solid or semisolid systems of at least two components. As condensed mass enclosed by a liquid forms gels (Gallegos and Franco, 1999, Sinko and Martin, 2006d). Gels contain large amounts of glycerin (up to 20%). They are fat-free base used for plant extract and water. In addition, gels contain various gel formers such as gelatin and agar-agar.

1.2.2 Preservation of cosmetics

Contamination of cosmetics can be prevented by adding preservatives which suppress the proliferation of microorganisms and kill them in time (Soni *et al.*, 2001, Atemnkeng *et al.*, 2007). However, it is necessary to use as small amount of preservatives as possible to decrease their adverse effects on human beings (Mitsui *et al.*, 1997b). Routinely, parabens are first used as antimicrobial preservatives in the mid 1920s, with methyl and propyl-paraben being the most commonly used (Soni *et al.*, 2005). Parabens are widely used in cosmetics and pharmaceutical products because they have no taste, no

odor, low toxicity, broad spectrum of action, neutral pH, do not cause the discoloration of the product and are cheap (Soni *et al.*, 2005, Polati *et al.*, 2007).

1.3 Rheology

Rheological and mechanical properties reflect the response of pharmaceutical materials to an externally applied stress (Korhonen *et al.*, 2000, Jiménez Soriano *et al.*, 2001). Rheology is the study of this response, while Rheometry or Viscometry is the application of measurement techniques and instrumentation. By definition, rheology is the study of the flow and deformation of matter (Barry, 1971, Khunawattanakul *et al.*, 2008). When pharmaceutical materials are subjected to the externally applied stress, they undergo flow or deformation e.g. creams, ointments, foams and compacted powders. The parameters which describe the viscoelastic properties of a system are of particular significance. There are two types of pharmaceutical system deformation: (i) flow (irreversible deformation) and (ii) elasticity (spontaneously reversible deformation) (Radebaugh, 1988, Tamburic *et al.*, 1996, Korhonen *et al.*, 2001).

1.3.1 The elements of rheology

Three parameters namely stress, strain in solids or its liquid equivalent (shear rate) and time can describe the elementary rheological properties of most materials.

i. **Stress:** is defined as the internal force acting on the area of the cube of the material. Since this force acts to balance out the applied force and keeps the

material cube in equilibrium, it is defined as an internal force. There are two types of stress, normal and shear stress. Pascal (Pa) is a unit of shear stress.

ii. **Strain:** is the relative deformation of a solid body in response to a stress. Elongation or compression strain and shear strain are the two types of strain. Shear strain is unitless.

iii. **Time:** is the third parameter. Rheological properties of material can be affected by time. Reciprocal time is the unit for shear rate. The response to stress depends on both the length of time the material is subjected to the stress and the magnitude of stress.

1.3.2 Elasticity and viscosity

The interaction between stress and either strain or shear rate yields a very important relationship. Elasticity is the proportionality constant between stress and strain and the unit of measure is Pascal (Pa) since strain is unitless. Elasticity can also be referred to as storage moduli (G'). On the other hand, a measure of a liquid resistance to flow is known as viscosity which represents the relationship between shear stress and shear rate. Viscous modulus is also known as loss modulus (G") and has Pascal second unit (Pa.s). Viscosity, η, is calculated using the following equation:

$$\eta = \frac{\sigma}{\gamma} \qquad \text{Eq. (1.2)}$$

Where σ is shear stress and γ is shear rate.

A combination of viscous and elastic properties is termed viscoelasticity (Radebaugh, 1988). The tangent between G" and G' defines the phase angle as:

$$\tan \delta = \frac{G''}{G'} \qquad \text{Eq. (1.3)}$$

1.3.3 Fluid flow behavior

The basic shear diagram of shear rate versus shear stress is shown in Fig 1.1. Fluids for which viscosity remains consistent regardless of shear rate and shear stress are known as Newtonian fluids (Rao, 1999, Muller-Goymann, 2004). While fluids for which the viscosity depends on shear stress or shear rate are said to show non-Newtonian behavior and are classified as dilatants, peudoplastic and plastic as shown in Fig 1.1 (Radebaugh, 1988, Liang *et al.*, 2008).

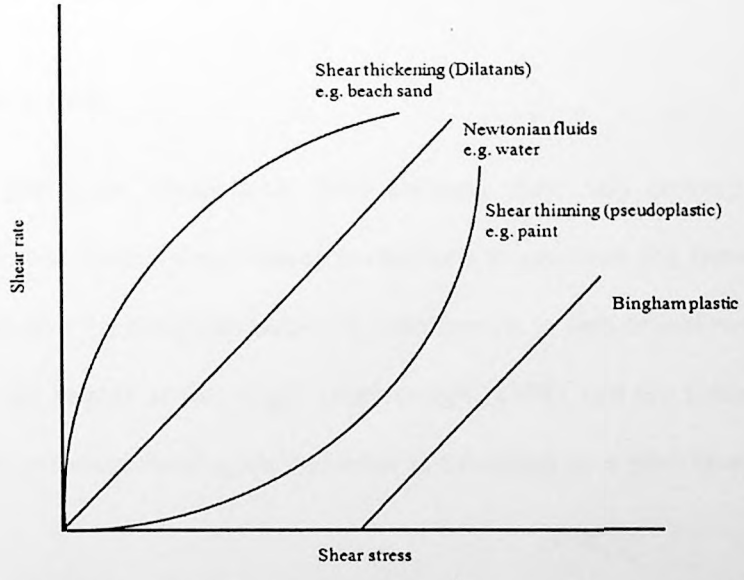

Figure 1.1 Basic shear diagram of shear rate versus shear stress for flow behavior classification

1.3.3.1 Newtonian behavior

In Newtonian behavior, the plot begins at the origin and there is a direct proportional relationship between shear rate and shear stress. Compounds of low molecular weight represent typical Newtonian fluids e.g. sugar solution (Rao, 1999, Muller-Goymann, 2004). Usually, there are very few true Newtonian fluids i.e. very few liquids exhibit a constant viscosity at all shear rates (Radebaugh, 1988).

1.3.3.2 Non-Newtonian behavior

A wide range of behavior is displayed by non-Newtonian fluid. In this type of fluid, viscosity is not directly proportional to the shear rate (Radebaugh, 1988, Liang *et al.*, 2008).

1.3.3.2(a) Plastic flow

They termed plastic or viscoelastic flow because they only deform elastically and reversibility. Initial stress (yield value) is required to generate the flow. There are two types of plastic flow (i) Bingham behavior corresponds to that of a Newtonian liquid but the plot does not begins at the origin (Radebaugh, 1988) and (ii) Casson's curve. The simplest non-Newtonian rheological behavior is exhibited by a pure Bingham liquid (Fig 1.1).

1.3.3.2(b) Pseudoplastic flow

Fig 1.1 presents pseudoplastic behavior of many emulsions, dispersions and polymeric solutions (Muller-Goymann, 2004). Commonly, pseudoplastic flow are called shear-thinning (Rao, 1999). The curve begins at the origin of the shear stress-shear rate plot but it convexes upwards with shear-thinning fluids (Liang *et al.*, 2008). Shear stress increase gives a more proportional increase in shear rate. The viscosity decreased as the shear stress increased and no yield value is required to start the flow. Most non-Newtonian fluids exhibit shear thinning behavior.

1.3.3.2(c) Dilatant flow

Dilatant flow is also called shear-thickening behavior. The curve begins at the origin of the shear stress-shear rate plot but it concaves downwards. Shear stress increase gives a less proportional increase in shear rate. The viscosity increased as the shear stress increased. Dilatancy implies an increase in the volume of the sample during the test (Rao, 1999, Liang *et al.*, 2008).

1.3.3.3 Time-dependent behavior

Emulsions are said to exhibit thixotropic flow behavior when they exhibit time-dependent shear thinning behavior (Rao, 1999, Jiménez Soriano *et al.*, 2001, Liang *et al.*, 2008). Thus, a reversible time-dependent decrease in viscosity at constant shear rate is defined as thixotropy (Barry, 1971). The breakdown and the re-forming of gel-

solution-gel structure is the mechanism of thixotropy (Radebaugh, 1988). Gelatin, mayonnaise and many emulsion systems are examples of thixotropic materials. Thixotropy can be quantified by using several proposed methods. One of these proposed methods is the area of hysteresis loop (Barry, 1971, Jiménez Soriano *et al.*, 2001).

Anti-thixotropy or negative thixotropy is the time-dependent increase in viscosity at constant shear rate. Dispersion that contains 1-10% (v/v) solid shows negative thixotropy that is in contrast with dilatants systems which contain more than 50% (v/v) solid. A phenomenon in which a solution forms a gel when gently shaken or sheared is known as rheopexy which corresponds to negative thixotropy (Radebaugh, 1988).

1.3.4 Yield value

Yield value is a threshold value of stress after which the materials start to flow. Yield value only exists in Plastic flow while it is zero in Newtonian, Pseudoplastic and Dilatant flow. Yield stress value is a concept that is useful in pharmaceutical process design, modeling and sensory assessment (Rao, 1999).

1.3.5 Apparent viscosity

Apparent viscosity is a viscosity at any given shear rate that can be obtained from basic shear (shear stress-shear rate) diagram (Rao, 1999). Viscosity is not constant for non-Newtonian fluids because viscosities are changing depending on shear stress applied. Apparent viscosity can also be calculated from equation 1.2 with some modifications:

$$\eta_j = \frac{\sigma}{\gamma_j} \qquad \text{Eq. (1.4)}$$

Where η_j is the viscosity at a given shear rate γ_j.

1.4 Optimization in pharmaceutical formulation

1.4.1 Pharmaceutical experimental design

The Design of Experiment (DOE) or Statistical Experimental Design (SED) is a concept for the planning of informatics experiments which can be used in many formulations (Martinello *et al.*, 2006). In pharmaceutical technology, DOE is recommended greatly (Huisman *et al.*, 1984). DOE requires prior knowledge of the procedure used so that a robust and valid statistical model, for the examined factors, can be achieved (Srinivasan *et al.*, 2000) with a minimum number of experiments i.e. minimum time, resources and effort (Loukas, 1998, Petrovic *et al.*, 2006, Rajin *et al.*, 2007). For stable and effective dosage forms development, careful selections of integral components are essential which can be achieved through pre-formulation studies (Mura *et al.*, 2005). In the cosmetic cream formulations, DOE plays a vital role in product development, because it is not easy to predict the optimum values of the formulation properties, such as spreadability and viscosity (Contreras and Sanchez, 2002).

1.4.1.1 Experimental design protocol

Screening is usually carried out at the first stage in order to reduce the number of factors and to determine the important outcomes under which both squared and interaction terms in the model are of interest; while optimization is mostly carried out after screening, thus the best setting for the important variables are determined. These two elements (i.e. screening and optimization) are considered as the main elements of the DOE concept (Martinello *et al.*, 2006, Rispoli and Shah, 2007, Sayyad *et al.*, 2007, Zivanovic *et al.*, 2008). Due to the involvement of multivariable process parameter, the optimization process is considered as a tedious process (Sayyad *et al.*, 2007). Furthermore, optimization process involves three major steps (i) performing the statistically design experiments, (ii) estimating the coefficient in mathematical model and (iii) predicting the response and checking the adequacy of the model (Srinivasan *et al.*, 2000).

1.4.1.2 Techniques of experimental design

There are several techniques of DOE used for formulation development, such as Cross Technique, Factorial Design and Mixture Design (Rajin *et al.*, 2007). Factorial Design is the most popular experimental design used to study the systems having independent factors (Huisman *et al.*, 1984, Loukas, 1998, Rajin *et al.*, 2007) and to determine the relationship between two or more components (Contreras and Sanchez, 2002). In the mixture components, the ratios of the components are dependent on one another, where

the sum has to be equal to 1 or 100% (Huisman *et al.*, 1984, Mura *et al.*, 2005, Rajin *et al.*, 2007, Rispoli and Shah, 2007). In addition, mathematical model has to be used too.

1.4.1.3 Mixture Design

Mixture Design has been used to explore how much change, in a mixture composition, will affect the properties of the mixture (Huisman *et al.*, 1984, Mura *et al.*, 2005, Martinello *et al.*, 2006) and it has been adopted to optimize the composition of the systems to describe the response as a function of the mixture composition by means of a mathematical model (Huisman *et al.*, 1984, Patel *et al.*, 2007, Rajin *et al.*, 2007, Rispoli and Shah, 2007, Zhu *et al.*, 2008). For a three-component system, Mixture Design can be represented by an equilateral triangle of two dimension space (Loukas, 1998, Patel *et al.*, 2007, Zhu *et al.*, 2008). The relationship between the formulation variables was investigated effectively by Statistical Mixture Design (Rajin *et al.*, 2007).

1.4.1.3(a) Response surface methodology

It is important to obtain knowledge about potential physical and chemical interaction between mixture components so as to rapidly accelerate drug development (Mura *et al.*, 2005) and this can be achieved by using Response Surface Methodology (RSM) (Martinello *et al.*, 2006, Sayyad *et al.*, 2007) in order to visualize and select optimal condition immediately (Martinello *et al.*, 2006, Rispoli and Shah, 2007, Sayyad *et al.*, 2007, Zivanovic *et al.*, 2008). In the 1950s, Response Surface Methodology (RSM) was

developed by Box and Wilson (Khoo and Chen, 2001). Response surface contour can be depicted only after the discovery of acceptable statistical model function (Huisman *et al.*, 1984). Response surface plots clearly show the influence of two factors on recovery value in the investigated area and are presented in three dimensional spaces (Zivanovic *et al.*, 2008). The examination of these three dimension graphs may help to determine a region with acceptable values of responses (Huisman *et al.*, 1984) because RSM combines experimental design and statistical technique for model optimization and building (Khoo and Chen, 2001). Moreover, linear or quadratic effect of experimental variables and the response can be mapped onto the surface contour plot (Khoo and Chen, 2001, Sayyad *et al.*, 2007).

1.4.1.3(b) Computer software

In pharmaceutical industry, computer software can be used with DOE (Martinello *et al.*, 2006, Ismail *et al.*, 2008). An example of such software is Design-Expert® which has been much described by many authors (Srinivasan *et al.*, 2000, Petrovic *et al.*, 2006, Rajin *et al.*, 2007). In reality, these software can analyze multi-responses simultaneously, efficiently and very accurately (Khoo and Chen, 2001). Therefore, Design-Expert® was used in this study. Design-Expert® can screen for vital factors and locate ideal process settings to discover optimal product formulations.

1.4.1.3(c) Ternary phase diagram

It is important to point out that most of the three components of Mixture Design employ ternary phase diagram in the pre-formulation study. This ternary phase diagram was described by many researchers (Wu *et al.*, 2001, Minardi *et al.*, 2002, Shafiq *et al.*, 2007, Shah *et al.*, 2007, Dixit and Nagarsenker, 2008, Zhu *et al.*, 2008). Phase diagram can capture the relationship between a mixture phase behavior and its composition. The construction of ternary phase diagram is usually time consuming, particularly, to delineate a phase boundary. Pseudo-ternary phase diagram is useful for the identification of the region of interest e.g. o/w emulsion region (Shafiq *et al.*, 2007).

1.5 Stability study of cream formulation

Cream is a liquid that is dispersed through external liquid phase in the form of small droplets either o/w or w/o containing one or more drug substances dissolved or dispersed in suitable base. Cream is complex and the thermodynamically unstable semisolids dosage forms a system, due to physicochemical interaction, hydrodynamic interaction, micelles formation and liquid crystalline formation (Block, 1996, Peramal *et al.*, 1997, Jiménez Soriano *et al.*, 2001, USP, 2005, Buhse *et al.*, 2005, Masmoudi *et al.*, 2005, Florence and Attwood, 2006, Salvador *et al.*, 2007). Creams should be stabilized by emulsifiers (surfactants) with a range of oil (Miller *et al.*, 1999, Korhonen *et al.*, 2001). The mechanism of surfactant stabilization is by forming monolayer at the droplet surface which reduces the interfacial tension that reduces the possibility of collision (Wu *et al.*, 2004). Recently, cosmetics creams have been restricted to o/w emulsions because they

are easily water washable; they do not stain clothes and they are more cosmetically and aesthetically acceptable (USP, 2005, Sinko and Martin, 2006d).

1.5.1 Cream instabilities

Cream system instability may involve (i) creaming or sedimentation: as a result of disparities in densities (Manoj *et al.*, 2000, Mollet and Grubenmann, 2001b, Masmoudi *et al.*, 2005, Florence and Attwood, 2006, Sinko and Martin, 2006d), (ii) flocculation and coagulation of the dispersed liquid droplets (Miller *et al.*, 1999, Masmoudi *et al.*, 2005, Florence and Attwood, 2006), (iii) coalescence leading to the breaking of the emulsion: the emulsion is only disrupted and fused when the droplets coalesce (Block, 1996, Mollet and Grubenmann, 2001b, Masmoudi *et al.*, 2005) and (vi) phase inversion (Sinko and Martin, 2006d). Some types of cream instabilities are shown in Fig 1.2.

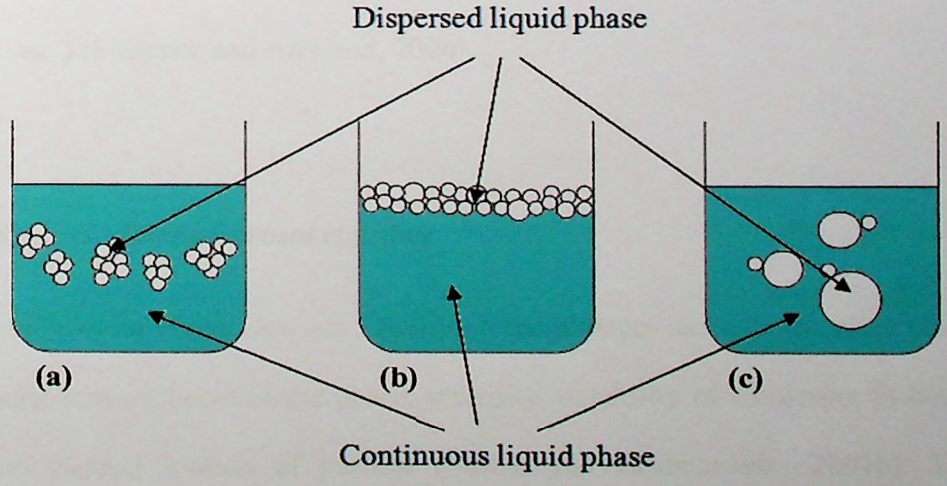

Figure 1.2 Emulsion instabilities. (a) Coagulation; (b) Creaming; (c) Coalescence

1.5.2 Methods of stability evaluations

Cream stability, initially, can be evaluated by testing the samples from laboratory batches. They are usually based on the instability acceleration technique which may necessitate complex analytical methodologies (Mollet and Grubenmann, 2001b, COLIPA, 2004, Stiller *et al.*, 2004, USP, 2005, Masmoudi *et al.*, 2005, Florence and Attwood, 2006, Sinko and Martin, 2006d, Salvador *et al.*, 2007) Many methods are applied to evaluate the destabilization process but none is actually recognized (Masmoudi *et al.*, 2005). The manufacturer should develop proper stability data for his product and to consider many external conditions that can affect potency, purity and quality (COLIPA, 2004, USP, 2005). The stability of creams must be considered in terms of physical and chemical stability which are of practical consequences (Block, 1996, Di Mambro and Fonseca, 2007). It must be done to assure the (i) stability and physical integrity under appropriate condition of storage, transportation and use (ii) chemical stability, (iii) microbiological stability and (vi) the functions and aesthetics. The stability of emulsion may be affected by additives (e.g. preservatives, coloring agents, etc.) (Florence and Attwood, 2006).

1.5.3 Factors affecting cream stability

The instability of cream depends heavily on temperature since temperature changes the interfacial tension between the phase, viscosity, solubility of emulsifier in both phases and the thermal motion of particle (Mollet and Grubenmann, 2001b). Therefore, accelerated stability tests like heating/cooling cycle and freeze-thaw cycle have been

employed for stability testing of cream (Block, 1996). Freeze-thawed stability testing is the only predictive accelerated testing methodology being applied because the destabilizing process functions only during the freezing and thawing and not during the storage under frozen condition (Block, 1996). Due to the short development cycle of cosmetic product, this type of accelerated test was developed to enable the prediction of stability. However, it does not provide kinetic data necessary to estimate shelf life (Block, 1996, COLIPA, 2004). Combined analytical techniques, including HPLC methodology, are used to obtain chemical stability data over time (Guaratini *et al.*, 2006). This is because rapid chemical and physical decomposition/changes in the formulation may happen due to thermal variation and can usually be detected by quantification of some parameters such as viscosity, solubility, particle size, pH and conductivity over time. Conductivity is the most sensitive technique applied for the physical changes (Bjerregaard *et al.*, 1999, Stiller *et al.*, 2004, Masmoudi *et al.*, 2005, Guaratini *et al.*, 2006). The duration of the stability test depends on the product storage period: usually 6 months stability study has to be carried out for products that stored ≥ 6 months, but the storage period of stability study of < 6 months is determined case-by-case (Block, 1996, USP, 2005), for example, the development cycle of cosmetic is relatively short and it is important that new products are marketed as quickly as possible (COLIPA, 2004, Masmoudi *et al.*, 2005). The viscoelastic properties of emulsion are amenable to rapid, quantitative, measurement and can serve as the basis for quality control or stability indicating methodology (Block, 1996, Marquardt and Sucker, 1998, Florence and Attwood, 2006, Guaratini *et al.*, 2006).

1.5.4 Shelf life prediction

Shelf life is the suitability of a drug that could be determined by an acceptance criterion throughout its re-test period, i.e. the combination of chemical, physical, biological and microbiological tests. The expiration date is the date placed on a drug product container label to design the time after which it must not be used and prior to which (if stored under defined condition) a batch of the product is expected to remain within the approved shelf life specification. The acceptance criteria is usually a 5% change in assay from its initial value (ICH, 2003).

The prediction of shelf lives on only chemical parameter is not comprehensive especially for cosmetic formulation where physical stability is of utmost importance and other parameters should be analyzed also (Guaratini *et al.*, 2006).

1.6 Nano-emulsions

1.6.1 Definition

Nano-emulsions are oil-in-water (o/w) or water-in-oil (w/o) transparent or translucent colloidal dispersions, diameter of droplets usually in the 20-500nm size range (Santos-Magalhães *et al.*, 2000, Porras *et al.*, 2004, Usń *et al.*, 2004, Al-Edresi and Baie, 2009) , formed by the dispersion of one liquid phase into the second liquid phase to form a droplet (Fernandez *et al.*, 2004, Solans *et al.*, 2005, Maestro *et al.*, 2006, Anton *et al.*, 2007). The interest on studies of this type of emulsion began in the early 19^{th} century, but it exploded recently due to cosmetic and pharmaceutical applications of novel

systems generating nano-particles (Anton *et al.*, 2007, Kamiya *et al.*, 2008). Nano-emulsions also are known as miniemulsions (Fernandez *et al.*, 2004, Tadros *et al.*, 2004, Solans *et al.*, 2005, Liu *et al.*, 2006, Maestro *et al.*, 2006, Anton *et al.*, 2007), fine disperse emulsions (Liu *et al.*, 2006), submicron emulsions (Fernandez *et al.*, 2004, Solans *et al.*, 2005, Liu *et al.*, 2006, Maestro *et al.*, 2006), ultrafine emulsions (Fernandez *et al.*, 2004, Solans *et al.*, 2005, Porras *et al.*, 2008), translucent emulsions (Fernandez *et al.*, 2004, Ee *et al.*, 2008), emulsoides (Maestro *et al.*, 2006) and unstable microemulsions (Maestro *et al.*, 2006).

1.6.2 Stability of nano-emulsions

Nano-emulsions have a long term kinetic stability due to their very small droplet sizes, (Tadros *et al.*, 2004, Solans *et al.*, 2005, Liu *et al.*, 2006, Maestro *et al.*, 2006) which result in a large reduction in the gravitational force. Thus, Brownian motion suffices to overcome gravity (Betz *et al.*, 2005, Solans *et al.*, 2005, Maestro *et al.*, 2006) and prevent sedimentation and creaming during storage.

1.6.3 Benefits of nano-emulsions

Nano-emulsion is an attractive system for many industrial applications (Wang *et al.*, 2007, Gutiérrez *et al.*, 2008) due to their purity, simplicity (Sonneville-Aubrun *et al.*, 2004), the ability to sterilize them through filtration and the increased bioavailability of drugs solubilized in them (Wang *et al.*, 2007, Kotyla *et al.*, 2008). These properties