PH DRIVEN ENCAPSULATION OF CURCUMIN IN SELF-ASSEMBLED NIOSOME CURCUMINOID: PHYSICAL CHARACTERIZATIONS, ENCAPSULATION EFFICIENCY & STABILITY STUDY

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CPP	Critical Packing Parameter
DCP	Dicetylphosphate
DLS	Dynamic Light Scattering
EE%	Drug Entrapment Efficiency
HLB	Hydrophilic-Lipophilic Balance
MLV	Multilamellar Vesicles
PBS	Phosphate-Buffered Saline
STR	Stearylamine
SUV	Small Unilamellar Vesicles
TEM	Transmission Electron Microscopy
TFH	Thin-Film Hydration
ULV	Unilamellar Vesicles
WHO	World Health Organization

DORONGAN PH KE ATAS PENGKAPSULAN KURKUMIN DALAM NIOSOM KURKUMINOID: PENCIRIAN FIZIKAL, KECEKAPAN PEMERANGKAPAN & KAJIAN STABILITY

ABSTRAK

Kurkumin adalah antioksidan dengan pelbagai ciri-ciri pemyembuhan tetapi mempunyai kelarutan yang rendah dan kurang bioavailabiliti disebabkan oleh pengaruh pH yang tinggi. Niosom terdiri daripada surfaktan bukan ionik yang bertindak sebagai vesikel pengangkutan untuk mengatasi batasan kurkumin sebagai ubat berpotensi. Tujuan kajian ini adalah untuk mengkaji pengaruh pH yang berbeza ke atas sifat fiziokimia kurkumin niosom. Niosom terdiri daripada nisbah 1:1 daripada Span 60: Tween 60 digunakan sebagai ejen membentuk vesikel dan disediakan dengan menggunakan kaedah penghidratan filem nipis. Pengaruh rumusan kepada saiz vesikel, kecekapan pemerangkapan (EE) dan kestabilan kurkumin niosom telah disiasat. Mikroskop transmisi elektron (TEM) mendedahkan bahawa niosom pada pH 3 dan 7 mempunyai bentuk sfera dan taburan yang seragam, manakala pH 9 menghasilkan niosom yang tidak teratur. Hasil kajian menunjukkan bahawa penubuhan kurkumin dalam niosom pada pH 3 menghasilkan vesikel dengan saiz yang lebih besar (394.07 ± 17.35 nm sehingga 556.37 ± 28.44 nm) berbanding formulasi pH lain sebelum penyemperitan. Walau bagaimanapun, selepas penyemperitan saiz telah dikurangkan dengan ketara (150.30 ± 2.29 nm sehingga 156.30 ± 2.17 nm). Selain itu, EE daripada kurkumin niosom pada pH 3 adalah paling tinggi (75.23%) berbanding dengan niosom pada pH lain. Tambahan pula, potensi zeta pada pH 3 adalah amat rendah berbanding pH 7 dan 9. Selepas disimpan selama 1 bulan, didapati bahawa keadaan beku (-4^{o}C) mempunyai kestabilan tertinggi dengan penurunan EE yang paling rendah. Saiz niosom kurkumin meningkat dan mula menggumpal apabila suhu dan tempoh simpanan bertambah, menunjukkan kestabilan yang lebih rendah pada suhu yang tinggi.

PH DRIVEN ENCAPSULATION OF CURCUMIN IN SELF-ASSEMBLED NIOSOME CURCUMINOID: PHYSICAL CHARACTERIZATIONS, ENCAPSULATION EFFICIENCY & STABILITY STUDY

ABSTRACT

Curcumin is an antioxidant with various healing properties but possesses limitation due to its poor solubility and reduced bioavailability as pH influences greatly on curcumin. Niosomes are composed of non-ionic surfactants and act as transport vesicle for drug delivery to overcome the limitations of curcumin as a potential drug. The aim of this study was to investigate the influence of different pH on the physiochemical properties of curcumin-loaded niosomes. The niosomes were composed of 1:1 mole ratio of Span 60: Tween 60 as vesicle-forming agents and prepared using thin film hydration method. The influence of formulation on vesicle size, entrapment efficiency (EE) and stability of curcumin-loaded niosomes was investigated. Transmission electron microscopy (TEM) revealed that niosomes at pH 3 and 7 had a spherical shape and uniform distribution, while pH 9 produced irregularly shaped niosomes. The results showed that the incorporation of curcumin into niosome at pH 3 yielded vesicles with larger sizes (394.07 ± 17.35 nm to 556.37 ± 28.44 nm) compared to other pH formulation before extrusion. However, after extrusion the size has been significantly reduced to smaller sizes (150.30 ± 2.29 nm to 156.30 ± 2.17 nm). Furthermore, the EE of curcumin niosomes at pH 3 was highest (75.23%) than that of niosomes at pH 7 and 9. The zeta potential at pH 3 was also remarkably lower than pH 7 and 9, yet decreases gradually over time. After 28 days storage, it was found that freezer storage (-4^{o}C) had the highest stability with less reduction in EE. The size of all the formulation increased and started to aggregate when the temperature and storage period increases indicating lower stability at a higher temperature.

CHAPTER ONE

INTRODUCTION

This chapter introduces the overview of this research and how the effect of pH is significant for the encapsulation of curcumin niosome. In general, this chapter outlines the research background of curcuminoid niosomes, the problem statement and objectives of this study along with the scope of research. Next, a brief review of the study is provided through the organization of thesis.

1.1 Research Background

The development of nanoscale technologies has widely spread over the years due to the vast advancement in research. The increasing nanoscale technologies have been applied for medicinal purposes in terms of not only disease diagnosis but also to extent of treatment and prevention. The recent development of nanoscale technologies in the medicinal sector includes nanoparticle delivery which involves encapsulation of drugs for various disease prevention. The physical properties of nanoparticles having large surface area results in the ability to bind, adsorb and carry compounds such as drugs, probes and proteins. However, the challenges may arise when it comes loading of sufficient amount of drug into the particles (De Jong and Borm, 2008).

Nanoparticles are widely available due to the physical properties that it have the ability to replicate or alter biological processes. Nanoparticles are often colloids within the size range of 10–1000 nm (Kreuter, 1994) but other definitions include nanoparticles with size less than 100 nm as studies demonstrated that smaller nanoparticles have number of advantages than larger ones because it is best suited for

intravenous delivery and can be targeted on more smaller parts of body (Singh and Lillard, 2009).

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-hepadiene-3, 5-dion] is a major component derived from the naturally occurring yellow pigment called turmeric has received much attention due to its excellent medicinal properties for the past few decades (Tavano *et al.*, 2014). It is scientifically proven to show medical benefits such as anti-inflammatory (Lantz *et al.*, 2005), antimicrobial, anticancer (Aggarwal *et al.*, 2003), antioxidant, anti-amyloid (Yang, Giselle P. Lim, *et al.*, 2005), anti-Alzheimer, anti-cystic fibrosis (Egan *et al.*, 2004) and wound healing properties (Gopinath *et al.*, 2004). Various studies of curcumin on animals (Shankar *et al.*, 1980; Qureshi *et al.*, 1992) and human (Cheng *et al.*, 2001) proved that curcumin is completely safe even at high dosage.

However, curcumin has major drawbacks such as low aqueous solubility under acidic condition and subjected to biodegradation under basic conditions (Wang *et al.*, 1997). The hydrophobic property of curcumin making it less feasible to dissolve in aqueous media. It is therefore subjected to poor solubility and lack of bioavailability within the body since poor intrinsic activity, poor absorption and high rate of metabolism can prevent its effective medicinal properties (Anand *et al.*, 2007). The recent development of the drug delivery has proven that the solubility and the bioavailability of curcumin can be improved through encapsulation. Encapsulation of curcumin can promote dispersion in aqueous media and protects from chemical degradation (Sari *et al.*, 2015). There are various types of encapsulation that is still being employed in industries such as surfactant micelles (Mandal *et al.*, 2017), nanospheres (Pandit *et al.*, 2018), nanoparticles (Wang *et al.*, 1997), nanoemulsions (Arbain *et al.*, 2018), liposomes (Yao *et al.*, 2016) and niosomes. One of the vesicular

nanocarriers that have come to recent attention is a non-ionic surfactant based vesicle called niosomes.

Niosomes are composed of hydrated non-ionic surfactant which is capable of encapsulating both hydrophilic and lipophilic substances. It has proven to have the ability to encapsulate various drugs, genes and proteins. Surfactants can be classified into three categories mainly, ionic (cationic or anionic), non-ionic and zwitterionic (amphoteric) based on the head charge. Surfactant selection is mainly based on hydrophilic-lipophilic balance (HLB) ranging 0-20. The HLB is a dimensionless parameter that commonly used to distinguish surfactants and plays an important role in controlling drug entrapment efficiency (EE). A lower HLB value (<9) indicates lipophilic surfactant (oil-loving group predominates) while a high HLB value (>11) refers to hydrophobic surfactant (water-loving group predominates). In this present study, Tween 60 and Span 60 will be used with HLB values of 14.9 and 4.7 respectively (Moghassemi *et al.*, 2014).

Over the past few years, niosomes have been an imposing alternative to other drug carriers such as liposome due to its greater stability, low cost and compatibility. Besides that, studies showed that drug entrapment efficiency in the liposome is much lower compared to niosome due to a high concentration of cholesterol in the liposome (Kazi *et al.*, 2010). The structure of the niosome being both hydrophilic and lipophilic enhances various drug delivery. Niosomes are often prepared by various methods of targeting for higher entrapment efficiency and stability. One of the methods that will be discussed in this study will be on thin layer hydration (TFH) method.

1.2 Problem Statement

Curcumin is found to be practically insoluble in acidic and neutral pH but soluble in basic condition. Previous studies have been done solely on pure curcumin at different pH since curcumin are highly pH dependent (Ravindran *et al.*, 2007). Studies demonstrated that curcumin is poorly absorbed by the gastrointestinal tract and/or subjected to presystemic transformation since it can be affected by the change in pH along the digestion process (Ammon *et al.*, 1991). It was also found that more than 90% of curcumin decomposed rapidly in neutral-basic pH buffer (Oetari *et al.*, 1996; Wang *et al.*, 1997). This is because proton removed from the phenolic group of curcumin resulting in the destruction of this structure. However, it is stable below pH 7.0 but with decreasing pH values, the dissociation equilibrium shifts towards neutral resulting in very low aqueous solubility (Tønnesen and Karlsen, 1985; Wang *et al.*, 1997). In order to sustain the downside of curcumin degradation and improves its stability, encapsulating curcumin able to provide enhanced drug delivery system.

A number of studies have been done on the effect of pH on pure curcumin, but there is minimal literature showed on the effect of pH on encapsulated curcuminoid. Zheng *et al.* (2017) demonstrated that curcumin encapsulated in chitosan nanoparticle appeared to show good resistance towards degradation at neutral pH of 7. Mokhtar *et al.* (2008) found that at acidic pH of 5.5, high entrapment of flurbiprofen was obtained with a maximum encapsulation efficiency of 94.6%. The amount of flurbiprofen encapsulated increased 1.5 times as pH decreased from basic (pH 8.0) to acidic (pH 5.5) but decreased significantly at pH>6.8. The lowest entrapment of flurbiprofen occurred at pH 7.4 and 8 with no significant difference between them. Enhanced drug delivery system which includes curcumin encapsulation able to provide more

promising results not only in terms of its bioavailability but also stability at different pH and temperature.

It has been discovered that niosomes from a combination of non-ionic surfactants such as Tween 60 and Span 60 have the highest entrapment efficiency compared to other mixtures of non-ionic surfactants such as Span/Tween 80 and Span/Tween 40 (Taymouri and Varshosaz, 2016). This is because mixtures of Span 60 and Tween 60 gives optimum hydrophobic and high hydrophilic properties providing excellent entrapment efficiencies. Similarly, it has been also reported that entrapment efficiencies for niosomes prepared using the mixtures of Span 60 and Tween 60 were higher as compared to those prepared using solely single surfactants either of Span 60 or Tween 60 (Junyaprasert *et al.*, 2012). Although the combination of Span/Tween 60 in the formation of curcumin niosome as model drug have been reported by Pakir (2016), the effect of pH has not been reported to date. Hence, in this current study, the effect of pH will be studied on the formation of niosome and encapsulation of curcumin by using Tween 60 and Span 60 as the selection of non-ionic surfactants.

1.3 Research Objectives

1. To study the effect of pH on the encapsulation of curcumin in self-assembled niosome curcuminoid.
2. To synthesize curcumin niosome and characterize it in terms of encapsulation efficiency, size and stability.

1.4 Research Scope

In this study, curcumin niosomes were synthesized based on the formulation and prepared accordingly. After synthesis, the samples were characterized using

transmission electron microscope (TEM), dark field microscope, UV-Vis spectrophotometer and zetasizer. The characterizations were done to observe and study the curcumin niosomes in terms of encapsulation efficiency, size, structure and charge. Lastly, stability studies were performed by storing the sample under different temperature conditions for 28 days to observe the changes in encapsulation efficiency, size, structure and charge.

1.5 Organization of Thesis

This thesis consists of five main chapters and each chapter contributes to the sequence of this study. The following are the contents of each chapter in this study:

Chapter 1 introduces the overview of this research and the significance of pH on the encapsulation of curcumin niosome, problem statement, research objectives, research scope and the organization of thesis.

Chapter 2 discusses the literature review of this study. An insight into niosomes in general and curcumin as the model drug, a discussion on types of surfactant, Hydrophilic-Lipophilic Balance (HLB), Critical Packing Parameter (CPP), the effect of additives, and processing variables are elaborated. Also, explanations on size reduction techniques and the influence of operating parameters are included as well.

Chapter 3 covers the experiment materials required for this study and the details of methodology from the start of this research project. It discusses the description of equipment and materials used, characterization steps, stability studies, experimental procedures and description of parameters affecting the encapsulation of curcumin.

Chapter 4 refers to the experimental results and discussions of the data obtained. Further elaboration on the characterization results, stability studies and effect of pH are provided in this chapter.

Chapter 5 concludes all the findings achieved in this research study. Recommendations or future studies on this research topic are included as well.

CHAPTER TWO

LITERATURE REVIEW

Chapter two briefly presents the previous discoveries and reviews available from credible scientific records and references that are related to this research topic. In general, this chapter outlines the overview and elaboration of curcumin as a model drug, niosomes, niosomal formulation used in encapsulating curcumin and the effect of processing variables particularly on the effect of pH on curcumin encapsulation. Then, a review of types of surfactant is presented to signify the importance of use in this research.

2.1 Curcumin as drug

Curcumin is the major component of a naturally occurring yellow-orange pigment called the turmeric has received a lot of attention in the medicinal field due to its excellent properties. The dried ground rhizome of the perennial herb *Curcuma longa* or turmeric has been widely used in Asia as traditional medicine as well as a household remedy against various diseases, including hepatic disorders, cough, sinusitis, rheumatism, and biliary since the second millennium BC (Kumar *et al.*, 2010). *Curcuma spp*. contain turmerin (an antioxidant protein), essential oils (such as turmerones, atlantones and zingiberene) and curcuminoids including curcumin. Curcumin is a phenolic compound which is generally characterized by low molecular weight polyphenol (MW=368.37 g/mol) with a melting point of 183°C. Commercial grade curcumin contains the curcuminoids desmethoxycurcumin (MW=338 g/mol; typically 10–20%) and bisdesmethoxycurcumin (MW=308 g/mol; typically less than

5 %) (Sharma *et al.*, 2005). General structures of curcumin in rodents and human are represented in Figure 2.1.

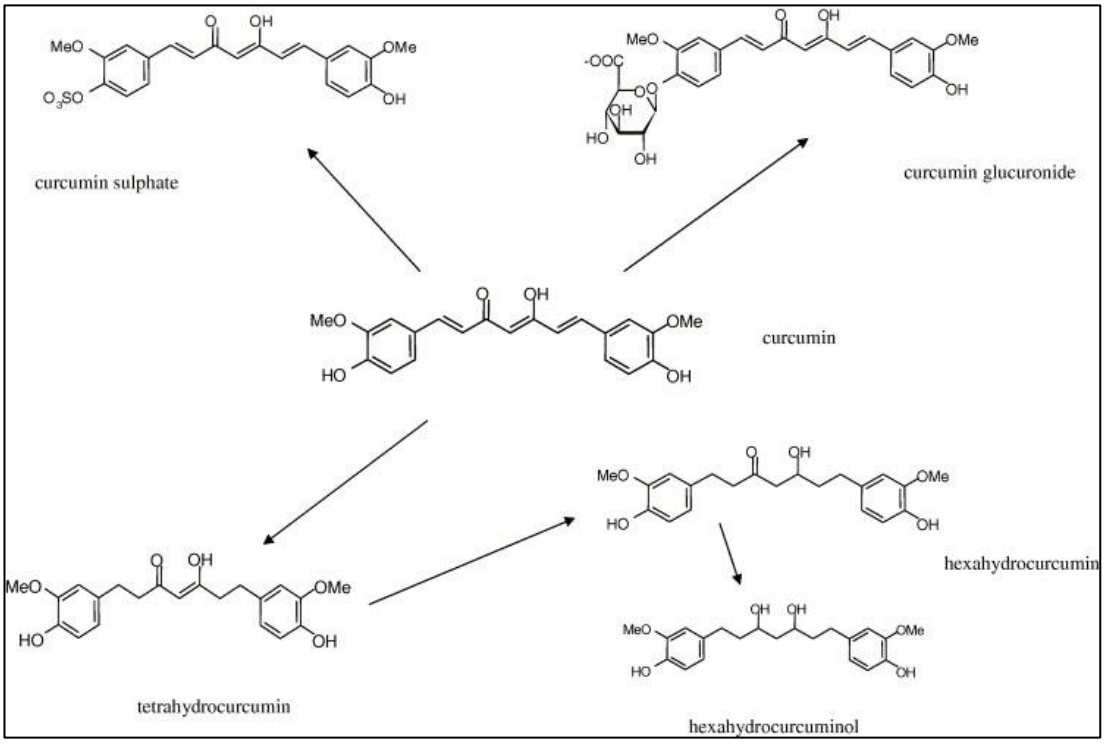

Figure 2.1: Chemical structures of major metabolites of curcumin in rodents and humans (Sharma et al., 2005)

Extensive literature is available showing the properties of curcumin as a model drug and capable of treating numerous diseases. Curcumin possesses various properties such as anti-inflammatory (Ullah *et al.*, 2017), antimicrobial, anticancer (Khan *et al.*, 2018), antioxidant, anti-mutagenic, anti-amyloid (Yang *et al.*, 2005), anti-Alzheimer (Cheng *et al.*, 2015), anti-cystic fibrosis (Egan *et al.*, 2004), hypoglycaemic and wound healing properties (Gopinath *et al.*, 2004) without any toxic effect that have been reported using different delivery vesicles.

Pharmacological activities have been studied in humans and animals have proven that curcumin is safe and well tolerated even at very high doses. In phase I clinical trial of curcumin, a single daily oral dose is given to patients with high-risk or

pre-malignant lesions ranging from 500 to 8000 mg/day for 3 months showed no toxicity and urinary excretion of curcumin was undetectable. The serum concentration of curcumin usually peaked at 1 to 2 hours after oral intake of curcumin and gradually declined within 12 hours. The World Health Organization (WHO) have outlined that the acceptable daily intake of curcumin as a supplementary additive as 0–1 mg/kg body weight (Cheng *et al.*, 2001).

However, poor aqueous solubility and lack of bioavailability are the major constraints that hold its medicinal properties as an effective therapeutic drug. It is found to be soluble in acetone, ethanol, methanol, chloroform and glacial acetic acid, and particularly insoluble in water. It is found that pH influences greatly on the stability of curcumin. Acidic pH (between 1 to 6) shows curcumin are stable with very minimal degradation. This results in the compatibility of curcumin in the stomach and small intestine favouring lower pH (Belkacemi *et al.*, 2011). Nevertheless, another study demonstrated that curcumin is poorly absorbed by the gastrointestinal tract and/or subjected to presystemic transformation since the kinetics and change in pH occurs along the digestion process (Ammon *et al.*, 1991). On the other hand, at neutral and basic pH curcumin is unstable and undergoes degradation up to 90% within 30 mins forming trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexanal as major degradation product while ferulic acid, feruloylmethane and vanillin as the minor product (Wang *et al.*, 1997).

2.2 Niosome as drug delivery vesicle

Nanocarriers are commonly used in drug delivery system for therapy and diagnosis in the field of medicine. Nanoscale vesicle carriers have gained great attention lately due to its high entrapping efficiencies, providing targeted (cellular or tissue) delivery of drugs, improved bioavailability, and able to sustain release of drugs.

One of the prominent vesicular drug carriers is niosome that have come to attention recently as a promising drug carrier.

Niosomes are non-ionic surfactants having surfactant bilayer membrane similar to liposomes. However, non-ionic surfactants are often used as active compounds in drug delivery systems instead of phospholipids as in liposomes due to their compatibility, stability and toxicity are quite significant compared to the ionic or amphoteric surfactants. The structure of niosome itself being both hydrophilic and lipophilic able to be used as the delivery device for various drugs. This can be achieved by entrapping hydrophilic components in the vesicular aqueous core or adsorbed on the bilayer surfaces while the lipophilic substances are encapsulated within the lipophilic bilayers (Moghassemi and Hadjizadeh, 2014). Figure 2.2 shows the graphical representation of niosome molecule being able to entrap both lipophilic and hydrophilic molecules.

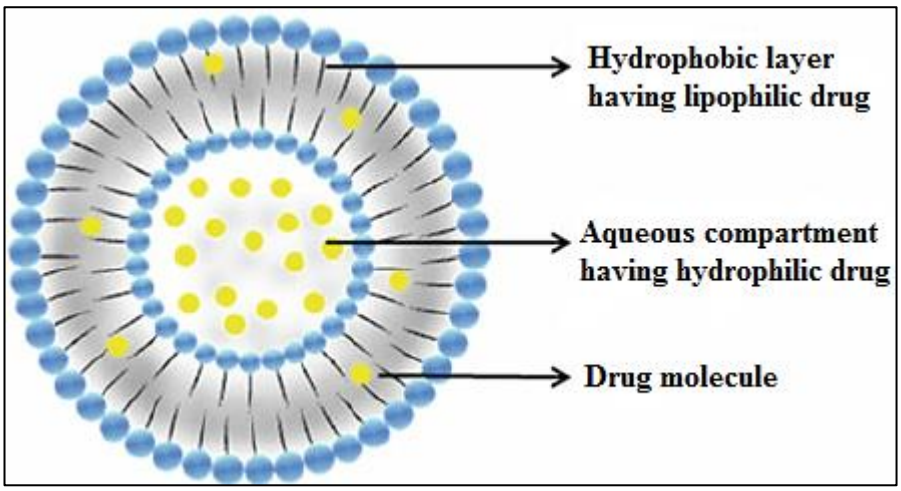

Figure 2.2: Structure of niosome (Rageeb and Usman, 2017)

Niosomes are widely used via various routes such as transcutaneous route (Bal *et al.*, 2010), intravenous injection (Dufes *et al.*, 2004), ocular delivery (Rentel *et al.*, 1999) , pulmonary delivery (Marianecci *et al.*, 2010) ,dermal delivery and transdermal delivery (Alsarra *et al.*, 2005; Schreier and Bouwstra, 1994; Shatalebi *et al.*, 2010).

The unique features of niosomes exhibits and stands out as the best delivery vesicle compared to other drug carriers available. The advantages and disadvantages of niosome are listed as below:

Table 2.1: Advantages and disadvantages of niosomes (Rageeb and Usman, 2017)

Advantages	**Disadvantages**
• Act as a storage for controlled release of the drug.	• Physical instability over the difference in formulation and condition.
• Osmotically active and stable over a long period of storage.	• Undergoes aggregation over a long period of storage.
• Low toxicity and high compatibility due to the nature of surfactant being biodegradable and non-immunogenic.	• May undergoes fusion with other neighbouring particles.
• Can be administrated through various routes (oral, transdermal, ocular, pulmonary and etc.)	• Leakage of the entrapped drug due to instability.
• A wide variety of drugs (hydrophilic or hydrophobic) with different solubility can be entrapped.	• Hydrolysis of encapsulated drugs which limits the lifespan of the suspension.
• Flexible and easily modified due to the functional groups on the hydrophilic heads.	

Niosomes can be unilamellar or multilamellar depending on the method used to prepare them. Unilamellar vesicles can be subdivided into two size ranges which are small unilamellar (SUV) and large unilamellar vesicles (LUV). Multi-lamellar vesicles (MLV) are vesicles with more than one bilayer is present. However, for targeted delivery of drugs often require nanoscale (<100nm) carriers which is much

more preferable in the medicinal field. Besides that, smaller niosomes will have more surface energy and tend to aggregate to lower surface energy (Moazeni *et al.*, 2010). Thus, smaller particles are much stable than bigger ones. There are various drug deliveries consisting of natural materials that can be used as a transport medium for drug delivery. A clearer representation of the types of possible vesicle formations is listed in Table 2.2.

Table 2.2: Types of vesicles (Ohshima, 2017)

Type of vesicle	**Abbreviation**	**Diameter**
Small unilamellar vesicle	SUV	10-100 nm
Large unilamellar vesicle	LUV	>100 nm
Giant unilamellar vesicle	GUV	>1 μm
Oligolamellar vesicle	OLV	0.1-1 μm
Multilamellar large vesicle	MLV	>0.5 μm
Multivesicular vesicles	MVV	>1 μm

There are several types of niosomes that can be classified based on the size of chains. Polyhedral niosomes are first obtained from surfactant classes as non-uniform vesicles whereby having 4-12 straight edges with similar length. Polyhedral niosomes are found to be stable for 36 days and able to entrap and release slowly water-soluble markers carboxyfluorescein and nucleotides. *In vitro* studies demonstrated that polyhedral niosomes studied using luteinizing hormone-releasing hormone (LHRH) as a model peptide was more stable in 5% rat skeletal muscle homogenate than in rat plasma. It is found that polyhedral niosomes undergo transformation into spherical structures, while spherical niosomes remain intact even at a higher temperature (Arunothayanun *et al.*, 1999).

Discomes are another class of niosome which are generally larger about 11-60 µm. Discomes are found to be stable for up to 6 months when stored at 4^{o}C and such large particle can be advantageous for drug delivery in the field of ophthalmology (Ucbegbu *et al.*, 1992). It is reported that discomes are capable of encapsulating Naltrexone Hydrochloride (NTX) which is a promising therapy for corneal disorders associated with diabetes mellitus (diabetic keratopathy). Discomes offers favourable properties for enhanced corneal uptake of the hydrophilic drug (NTX) and as well protects from photo-oxidation which the major instability problem encountered by NTX (Ucbegbu *et al.*, 1992). However,discomes are generally unstable at 37^{o}C and such preparation condition must be avoided.

Proniosomes are the dry form of niosomes which are obtained from water-soluble transport carrier coated with a thin layer of dry non-ionic surfactant. The carriers are completely safe, free from toxic, have good water solubility for ease of hydration. Advantages of proniosomes include higher stability such as from aggregation, fusion and leakage of the drug over time (Nelson *et al.*, 2009). Proniosomes offer a versatile vesicle delivery concept with potential for delivering drugs via transdermal route. It is reported that proniosome estradiol selected as a model lipophilic drug has very high entrapment efficiency and their ability to rearrange on dilution to form a stable niosome suspension (Fang *et al.*, 2001).

The physical nature of niosome vesicles plays an important role in determining the amount of drug loading and its ability to deliver sufficient amount of drug on targetted parts of the body. The physical state of the vesicle has a direct correlation to one another, therefore, it is necessary to evaluate the parameters to get an optimum result. Figure 2.3 shows the some of the important factors affecting the physical nature of niosome.

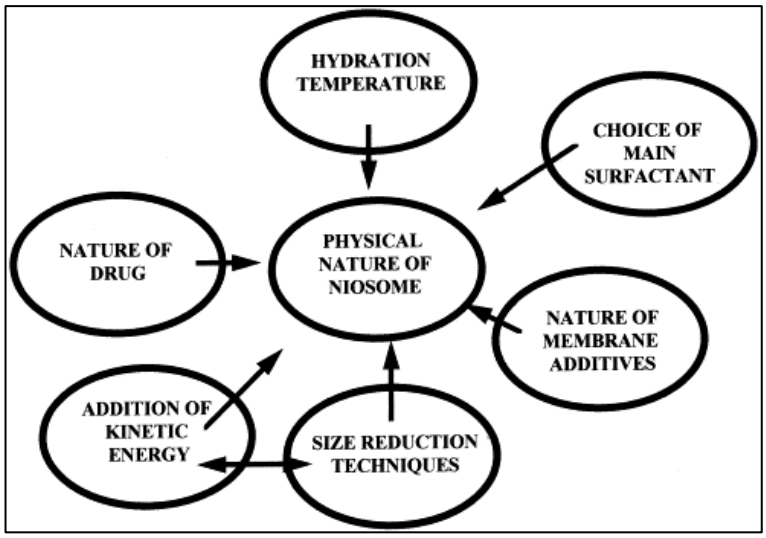

Figure 2.3: Factors affecting the physical nature of niosome (Uchegbu and Vyas, 1998)

2.2.1 Niosomal formulation

The different niosomal formulation will give different values in terms of stability, entrapment efficiency, size, charge and morphology. Several studies showed that niosomes prepared with a molar ratio of 1:1 molar ratio of Span 60 and Tween 60 have highest entrapment efficiency with respect other combination of Spans and Tweens (Junyaprasert *et al.*, 2012; Varshosaz *et al.*, 2014; Taymouri and Varshosaz, 2016). However, this statement conflicts with finding as stated that the least entrapment efficiency was found to be niosomes prepared with 1:1 molar ratio of Span 60 and Tween 60. It is found that higher composition of Tween 60 results in larger particle size and thus in the direct relationship with higher entrapment efficiency (Basiri *et al.*, 2017). In this study, a combination of Span 60 and Tween 60 has been studied based on its properties mentioned previously. In terms of size, niosomes prepared with 1: 1 molar ratio of Span 60 and Tween 60 will be considerably smaller and stable due to the rigidity of the bilayers composed of stearyl alkyl chain (C18) in Span/Tween 60 niosomes in comparison to palmityl alkyl chain (C16) of Span/Tween 40 (Varshosaz *et al.*, 2014).

There are various methods of niosome preparation and one of the simplest and common methods to produce is by thin film hydration method (TFH). This method is widely used whereby surfactants and additives are dissolved in organic solvents such as methanol, chloroform or ethanol in a round-bottomed flask. It is then subjected to near vacuum pressure using a rotary evaporator to remove the organic solvents leaving a thin film on the surface of the flask. An aqueous solution such as distilled water or PBS (phosphate buffer saline) [containing drug] is added to hydrate the thin film above the transition temperature (T_C) of the surfactant. This will eventually form multilamellar vesicles (MLV) and can be further homogenized into single unilamellar (SUV) by either sonication and/or extrusion.

2.2.2 Nature of surfactants

Surfactants can be classified into anionic, cationic, nonionic and amphoteric. The most common types of nonionic amphiphiles used for vesicle formation are alkyl ethers, alkyl esters, alkyl amides and esters of fatty acids. While naturally occurring phospholipids such as liposome having double alkyl chain, niosomes often form a single chain (Uchegbu and Florence, 1995).

Sorbitan fatty acid ester is a class of fatty acid esters often used in cosmetics to solubilize essential oils in water-based products. Esters of plain (non-PEGylated) sorbitan with fatty acids is usually referred to as Span. Spans are insoluble but dispersible in water. The gel transition temperature T_C increases accordingly with the length of the acyl chain. With sorbitan monostearate (Span) surfactants, a hydrophilic-lipophilic balance (HLB) number of between 4 and 8 was found to be compatible with vesicle formation (Yoshioka *et al.*, 1994; Uchegbu and Florence, 1995). Sorbitan esters can form vesicles in the presence or absence of molecules which operate cooperatively to form bilayer membrane such as cholesterol.

Yoshioka *et al.* (1994)have demonstrated on the preparation and properties of niosomes using series of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan trioleate (Span 85). It is reported that molecular weight of a nonionic surfactant was also found to directly influence the drug entrapment efficiency. It is observed that from the drug entrapment efficiency increases with molecular weight for Span 20, Span 40 and Span 60. However, Span 65 and span 80 had lower entrapment since both have an unsaturated alkyl chain which results in increased permeability and decreased entrapment (Yoshioka *et al.*, 1994). On the other hand, Span 60 having higher T_C have the highest entrapment and forms less leaky niosome at ambient temperature (~25ºC). The phase transition temperature T_C of non-ionic surfactants also affects the drug entrapment efficiency (Ohshima, 2017; Biswal *et al.*, 2008). Table 2.3 shows common Span series commonly employed as the choice of surfactant and their respective properties.

Table 2.3: Common Span series surfactant properties

Surfactant	**HLB**	**T_C (°C)**	**Chain length (number of carbons)**
Span 20 (Sorbitan monolaurate)	8.6	16	C12
Span 40 (Sorbitan monopalmitate)	6.7	42	C16
Span 60 (Sorbitan monostearate)	4.7	53	C18
Span 80 (Sorbitan monooeleate)	4.3	12	C18

Similarly, Tweens are polyoxyethylene fatty acid esters with HLBs from 10-17 usually used for O/W emulsion. Tweens are composed of four hydrophilic polyoxyethylene chains that can form mono-, di-, or triester with fatty acids. The HLB values of Tweens are determined based on the number polyoxyethylene and fatty acid chains. Unlike Spans, Tweens are soluble and dispersible in water and usually added

together to form both oil/water and water/oil emulsions which are more stable than single emulsifier alone (Ma and Hadzija, 2013). Table 2.4 represents common Tween series with their HLB values and chain length.

Table 2.4: Common Tween series surfactants

Surfactant	**HLB**	**Chain length (number of carbons)**
Tween 20 (Polyoxyethylene sorbitan monolaurate)	16.7	11
Tween 40 (Polyoxyethylene sorbitan monopalmitate)	15.6	15
Tween 60 (Polyoxyethylene sorbitan monostearate)	14.9	17
Tween 80 (Polyoxyethylene sorbitan monooleate)	15.0	17

Ruckmani and Sankar (2010) have reported that, though Tween 80 has a longer alkyl chain than Tween 20 and Tween 40, it showed lower entrapment due to the presence of unsaturated alkyl chain. On the other hand, it was found that Tween 60 has the longest saturated chain and higher drug entrapment efficiency compared to other Tweens.

2.2.2.1 Hydrophilic-Lipophilic Balance (HLB)

In this system, each surfactant is assigned with a numerical value which identified as Hydrophilic-Lipophilic Balance (HLB) which determines the balance of the size and strength of the hydrophilic (water-loving or polar) and the lipophilic (oil-loving or non-polar) groups of the surfactants even though two surfactants may have the same HLB and yet exhibit different solubility characteristics. Therefore, the HLB factor directly related to the solubility of that compound. A surfactant that has

lipophilic characteristics is assigned a low HLB number (<9.0), whereas hydrophilic surfactant is assigned with a high HLB number (>11.0). Those in the range of 9-11 are intermediate categories. When two or more surfactants are blended, the resulting HLB value differs based on the blending composition (Uniqema, 2004).

Shahiwala and Misra (2002) reported that surfactant with an HLB value in the range 14–17 is not suitable to produce niosomes while surfactant with an HLB value of 8.6 gives the highest entrapment efficiency of niosome. Entrapment efficiency decreases as the HLB value decreases from 8.6 to 1.7. For surfactants with HLB greater than 6, cholesterol must be added in order to form a bilayer vesicle and for lower HLB values, cholesterol enhances the stability of vesicles (Biswal *et al.*, 2008; Lawrence *et al.*, 1996; Shahiwala and Misra, 2002). The guideline for the selection of surfactant based on HLB value with respective usage is presented in Table 2.5.

Table 2.5: Range of HLB with respective usage

HLB	**Usage**
4-6	Water/Oil emulsifiers
7-9	Wetting agents
8-18	Oil/Water emulsifiers
13-15	Detergents
10-18	Solubilizers

2.2.2.2 Critical packing parameter (CPP)

The geometry of the vesicle results in different structures which can be predicted with critical packing parameter. This dimensionless parameter allows predicting the shape of the amphiphilic aggregates and can be defined using following equation 2.1 as below:

$$\text{CPP (Critical packing parameter)} = \frac{v}{lc \times a_0} \qquad (2.1)$$

Where v is the volume of the hydrocarbon portion, a_o is the effective area of the head group and l_C is the length of the hydrophobic tail.

CPP is helpful in predicting the structure of niosome vesicles in following way; spherical micelles formed if CPP <1/2, bilayer micelles are formed if ½ <CPP <1 and inverted micelles are formed if CPP >1 (Bagheri *et al.*, 2014).

2.2.3 Stability-enhancing additives

Cholesterol is an additive that is commonly used in niosomal drug delivery. The position of cholesterol in the bilayer of a vesicle and its hydrogen bond with a hydrophilic head of a surfactant. In addition to surfactant properties, cholesterol content tends to affect the important vesicular properties such as entrapment efficiency, storage time, drug release and stability. In number of cases, cholesterol is required in the formulation to prevent vesicle aggregation by stabilizing the system against the formation of aggregates by repulsive steric or electrostatic effects (Moghassemi and Hadjizadeh, 2014).

Cholesterol is primarily known to terminate the gel to liquid phase transition of niosome systems (Cable, 1989), resulting in less leaky niosomes (Rogerson *et al.*, 1987). This will eventually form niosomes which are more stable without any cholesterol additives. Conversely, cholesterol can also act in another way which can increase the chain order of liquidated bilayer. Normally, cholesterol is added at 1:1 molar ratio with the surfactants in most of the formulations (Abdelbary and El-gendy, 2008). Higher concentration of cholesterol provides greater stability to the surfactant bilayer by promoting the gel-liquid transition temperature (T_C) of the vesicle (Uchegbu and Vyas, 1998). This has been seen in the niosomal formulation using combination Span 60 and Tween 60 with higher cholesterol concentration resulting in greater

stability and entrapment efficiency (Varshosaz *et al.*, 2014). However, even after the addition of cholesterol, the intrinsic phase transition behaviour of vesicle-forming surfactants still impacts the properties of the dispersion such as the membrane permeability, encapsulation efficiency, bilayer rigidity, toxicity and etc. (Abdelbary and El-gendy, 2008).

Charged additives are often added to stabilize the niosome by adding a charged molecule to the bilayer and also prevents the aggregation of niosomes. dicetylphosphate (DCP) and phosphatidic acid are negatively charged additives, while stearylamine (STR) and cetylpyridinium chloride are positively charged additives, which are both commonly used for preventing aggregation of niosomes (Uchegbu and Vyas, 1998). It is reported that addition of the membrane additives has the most stable formulation due to the high zeta potential (Junyaprasert *et al.*, 2008).

However, it is reported that the addition of DCP has no significant changes in paclitaxel (PCT) encapsulated niosomes. Conversely, DCP has alters the entrapment efficiency dependent on the alkyl side chain of the surfactants. Since DCP are negatively charged molecules, they may tend to possibly interact electrostatically with positively charged drugs and change the entrapment efficiency (Abdelkader *et al.*, 2011). The drawbacks of DCP is that increasing amount of DCP increases the size and the membrane thickness of the niosome (Sezgin-Bayindir and Yuksel, 2012). Therefore, the addition of the charged molecules should not exceed the limit and normally added 2.5–5 mol% which will prevent the formation of niosomes (Hu and Rhodes, 1999).

2.3 Processing variables

2.3.1 Temperature of hydration

The temperature of hydration medium generally phosphate buffer solution effects on the self-assembly of surfactant into niosomes and results in different shape, size and the yield of niosomes. The hydration temperature normally kept above the gel transition temperature of the surfactant; i.e. Span 60 having gel transition temperature (T_C) of 53ºC must be hydrated with a temperature slightly above than that (Kazi *et al.*, 2010). Additionally, other contributing factors such as the volume of hydration medium and duration of hydration must be accounted as well (Biswal *et al.*, 2008).

2.3.2 Types of solvent

Solvent utilized have also directly impact on the vesicle size and permeability of the active compound (drug). The type of solvent is based on the ability of the drug to dissolve completely. Solvent such as alcohol has been reported to have an adverse effect on mainly on the vesicle size and entrapment. Vesicles formed from different alcohols have different size and have the following order: Ethanol > Propanol > Butanol > Isopropanol. It is reported that ethanol gives the highest niosome size due to the high aqueous solubility of non-ionic surfactants while lowest size encountered with isopropanol due to branched-chain present in it. Besides that, the solvent selection also affects the rate of hydration which results in the formation of niosomes. When isopropanol and butanol are used, niosomes are formed more spontaneously due to faster phase separation of isopropyl alcohol and butanol due to their low aqueous solubility (Yadav *et al.*, 2010). However, the absorption band of curcumin in most polar solvents such as methanol is red-shifted and broad with λ_{max} at ~420 nm. Using methanol as solvent have also shown a varying effect on curcumin's stability towards

degradation in alkaline solutions such as resulting in broader adsorption peak (Lee *et al.*, 2013).

2.3.3 Separation of unentrapped drug

Separation of unentrapped drug solution can be achieved through various techniques such as dialysis, gel filtration and centrifugation. Various researchers have used centrifugation to purify niosomes as it offers less time consumption and high removal of unentrapped drugs. The process involves removal of large or free drugs using centrifugal force and obtain a large fraction of pellets with a uniform distribution of size and shape. However, the drawback of centrifugation is that repeated washing of pellets may be required by using buffer solution in order to remove completely the unentrapped drug. However, the choice of the method including the duration and speed of centrifugation must be optimized in order to achieve high removal of unentrapped drug (Uchegbu *et al.*, 2013).

2.3.4 Size reduction techniques

There are several size reduction techniques applicable for post-preparation of niosomes such as sonication, extrusion, or high-pressure homogenization. The reason size reduction is necessary for the post preparation of niosome is that particle size and particle size distribution have an important role in their biodistribution. It is always desirable to have a narrow particle size distribution for drug carriers. The size of niosomes has a major impact on their *in-vitro* and *in-vivo* performance (Homaei, 2016). The most common method is either probe sonication or bath sonication which involves sound energy to agitate particles which adversely effects on the entrapment efficiency of the active agents. The sonication time must be optimized in order to form vesicles with a uniform unilamellar structure. It is reported that zidovudine niosomes able to

achieve mean diameter of 801nm after probe sonication from 2-3.5µm vesicle size earlier (Ruckmani and Sankar, 2010).

Although probe sonication more effective than bath sonication, probe sonication exposed to high energy input resulting in an increase in temperature of the solution and possible shedding of particles. This may require additional steps such as centrifugation in order to remove the large particles from the dispersion. The size reduction technique can also be incorporated by having both sonication and extrusion to obtain nano-sized niosome particles (Uchegbu *et al.*, 1995). This is because extrusion able to provide a promising size distribution of particles based on the size of the pores on the membrane. This has been discovered using extrusion of liposomes with and without cholesterol able to produce the same mean diameters of vesicles of 112 ± 6 nm using 100 nm polycarbonate membrane extruder. Although it is reported that sonication results in much smaller size particles of 28 ± 2 nm without cholesterol and 64 ± 3 nm with cholesterol but this can be controlled by the duration of sonication (Lapinski *et al.*, 2007).