# FORMULATION AND PHYSICOCHEMICAL CHARACTERIZATION OF DIETHYLAMINOETHYL DEXTRAN COATED LIPOSOME AS A DRUG CARRIER AND ITS EFFECT ON THE RHEOLOGICAL PROPERTIES OF CARBOPOL GEL

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**THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

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## ABSTRACT

In search of improved topical delivery products, efforts are aimed to develop new drug carriers or modify existing drug carriers mainly to exhibit adequate penetration properties as well as to assure localization of the drug within the skin. Liposome has played a significant role in therapeutics as an effective drug carrier. However, physicochemical instability of liposomes reduces its possibilities and potentials to be used in drug delivery systems. The purpose of this work was to study the potential of diethylaminoethyl dextran (DEAE-DX) coated liposomes as drug carriers for topical applications. Thin film hydration method was employed to prepare three types of liposomes composed of lecithin, 1,2-dipalmitoyl-sn-glycero-3-phospocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phospocholine (DSPC), respectively. As stability is a general problem with liposomes, DEAE-DX was included in the liposome formulation to promote steric stabilization by coating the surface of the lecithin, DPPC and DSPC liposomes. It was found that liposomes stabilized by DEAE-DX were superior to the corresponding non-coated liposomes. The critical vesicular concentration (CVC) was determined in order to investigate the minimum concentration of lipid required to form vesicles via surface tension analysis. Also, the surface behavior of the lipids was investigated through surface tension analysis before and after the addition of DEAE-DX. All of the liposomes were evaluated based on their morphology, particle size and zeta potential. A thirty-five-day stability study showed that DEAE-DX coated liposomes were stable at room temperature. Encapsulation efficiencies of these liposomes were studied via the loading of curcumin as the hydrophobic drug and diphenhydramine hydrochloride (DPH) as the hydrophilic drug into the liposomes. An *in vitro* release experiment demonstrated a slower drug release profile for DEAE-DX coated liposomes compared to non-coated liposomes. Although liposomes are exhibited as potential carriers for the topical formulation, the practical application of these

formulations onto the skin is less due to its liquid nature. In this study, liposome formulation was incorporated into carbopol gel in order to ease the mode of application. The rheological results indicated that presence of DEAE-DX coated liposomes in the gel has modified viscoelastic and flow characteristics of the gel. Furthermore, it was observed that the presence of DPH in the gel has lowered the storage modulus, *G'* of the gel whereas curcumin has no significant effect on the rheological properties of carbopol gel.

## ABSTRAK

Dalam pencarian produk penghantaran topikal yang bermutu, usaha telah diperuntukan bertujuan untuk menghasilkan pembawa ubat atau mengubah suai pembawa ubat yang sedia ada supaya dapat mempamerkan sifat penembusan yang mencukupi dan memastikan penyetempatan ubat dalam kulit. Liposom telah memainkan peranan penting dalam bidang terapeutik sebagai pembawa ubat yang berkesan. Walau bagaimanapun, ketidakstabilan fizikokimia liposom mengurangkan potensi liposom untuk digunakan dalam sistem penghantaran ubat. Tujuan penyelidikan ini adalah untuk mengkaji potensi liposom disaluti dietilaminoetil dektran (DEAE-DX) sebagai pembawa ubat untuk aplikasi topikal. Kaedah penghidratan filem nipis telah digunakan untuk menyediakan tiga jenis liposom yang terdiri daripada lesitin, 1,2-dipalmitoyl-sn-glycero-3-phospocholine (DPPC) dan 1,2-distearoyl-sn-glycero-3-phospocholine (DSPC). Oleh kerana ketidakstabilan merupakan masalah umum liposom, DEAE-DX telah digunakan untuk memberi kesan penstabilan sterik dengan menghasilkan lapisan yang meliputi permukaaan liposom lesitin, DPPC dan DSPC. Keputusan kajian menunjukkan bahawa permukaan liposom yang diubah suai dengan penyalutan DEAE-DX adalah lebih stabil berbanding liposom yang tidah diubah suai. Kajian telah dijalankan untuk memperoleh kepekatan kritikal pembentukan liposome yang bertujuan untuk menyiasat kepekatan minimum lipid yang diperlukan untuk membentuk vesikel melalui analisi tengangan permukaan. Selain itu, sifat permukaan lipid sebelum dan selepas penambahan DEAE-DX juga disiasat melalui analisi tegangan permukaan. Sifat morfologi, saiz zarah dan keupayaan zeta bagi semua liposom juga telah dikenalpasti. Kajian saiz dan keupayaan zeta selama 35 hari pada suhu bilik menunjukkan bahawa kestabilan liposom telah dipertingkatkan melalui penyalutan permukaan liposom dengan DEAE-DX. Keberkesanan pengkapsulan liposome telah dikaji dengan memasukkan kurkumin sebagai ubat hidrofobik dan difenhidramin hidroklorida (DPH) sebagai ubat

hidrofilik. Kajian *in vitro* pelepasan menunjukkan bahawa pelepasan ubat daripada liposom bersalut DEAE-DX adalah lebih perlahan berbanding liposom yang tidak disaluti DEAE-DX. Walaupun liposom mempamerkan sifat sebagai pembawa ubat yang berpotensi untuk formulasi topikal, aplikasi praktikal rumusan ini pada kulit adalah terhad kerana sifat cecairnya. Dalam kajian ini, liposom telah dimasukkan ke dalam matriks gel karbopol untuk memudahkan mod aplikasi. Keputusan reologi menunjukkan bahawa kehadiran DEAE-DX dalam gel liposom telah mengubah kekenyalan, kelikatan serta ciri aliran gel. Tambahan pula, pengurangan dalam nilai modulus penyimpanan, *G'* telah diperhatikan dengan kehadiran DPH dalam gel liposom. Manakala, kurkumin tidak menunjukkan kesan ketara ke atas sifat reologi gel liposom.

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| *CE* | Cohesive energy |
| CUR | Curcumin |
| CVC | Critical vesiculation concentration |
| DEAE-DX | Diethylaminoethyl-Dextran |
| DLS | Dynamic light scattering |
| DPH | Diphenhydramine hydrochloride |
| DPPC | Dipalmitoylphophatidylcholine |
| DPPC-DEAE-DX | DEAE-DX coated DPPC liposomes |
| DSC | Differential scanning calorimetry |
| DSPC | Disteroylphosphatidylcholine |
| DSPC-DEAE-DX | DEAE-DX coated DSPC liposomes |
| EE | Encapsulation efficiency |
| *G'* | Elastic modulus |
| *G"* | Viscous modulus |
| LEC | Lecithin |
| LEC-DEAE-DX | DEAE-DX coated LEC liposomes |
| LG-DPPC-DEAE-DX | DEAE-DX coated DPPC liposomes in gel |
| LG-DPPC-DEAE-DX-CUR | CUR loaded DEAE-DX coated DPPC liposomes in gel |
| LG-DPPC-DEAE-DX-DPH | DPH loaded DEAE-DX coated DPPC liposomes in gel |
| LG-DSPC-DEAE-DX | DEAE-DX coated DSPC liposomes in gel |
| LG-DSPC-DEAE-DX-CUR | CUR loaded DEAE-DX coated DSPC liposomes in gel |
| LG-DSPC-DEAE-DX-DPH | DPH loaded DEAE-DX coated DSPC liposomes in gel |

| | |
|---|---|
| LG-LEC-DEAE-DX | DEAE-DX coated LEC liposomes in gel |
| LG-LEC-DEAE-DX-CUR | CUR loaded DEAE-DX coated LEC liposomes in gel |
| LG-LEC-DEAE-DX-DPH | DPH loaded DEAE-DX coated LEC liposomes in gel |
| LVR | Linear viscoelastic region |
| PEG | Polyethylene glycol |
| *PLI* | Power Law Index |
| QELS | Quasi-elastic light scattering |
| $T_c$ | Liquid crystalline phase transition temperature |
| TEA | Triethanolamine |
| TEM | Transmission electron microscope |
| $T_m$ | Melting temperature |
| UV-Vis | Ultraviolet-visible |
| $\Gamma$ | Surface tension |
| $\gamma_b$ | Break point |
| $\gamma_c$ | Critical strain |
| $\eta$ | Shear viscosity |
| $\sigma_p$ | Yield stress |

# CHAPTER 1: INTRODUCTION

## 1.1 Preface

Liposomes are artificially constructed spherical vesicles of a few nanometers to micrometers in diameter composed of phospholipids or amphipathic lipids enclosing an aqueous solution. The word liposome is derived from two Greek words of "lipos" and "soma" with the meaning of fat for the former and "body" for the latter (Dua et al., 2012). It is made up of material similar to cell membrane; therefore liposomes represent an advanced vehicle for administration of substances such as nutrients and pharmaceuticals drugs into the cell, out of the cell and between different parts of a cell (Meland et al., 2014). Because of their amphiphilic structure; liposome can entrap hydrophilic drug in their aqueous compartment and lipophilic drug within the lipid membrane (De & Venkatesh, 2012) (Figure 1.1).

Hydrophilic components of the lipid bilayer are directed towards aqueous phases whereas hydrophobic components for both the lipid layers are directed towards one another to form the internal layer of a membrane. Also, it has the ability to protect and sustain the release of the encapsulated drug. As liposomes are highly biocompatible, they were initially conceived of as a delivery system for intravenous delivery (Egbaria & Weiner, 1990).

Throughout the years, liposomes have made substantial advancement for medical application, particularly as carrier of anticancer agents for chemotherapeutics owing to its excellent properties such as biodegradability, non-toxic and flexibility. Furthermore, liposomal formulations provide an innovative system for vaccination, gene therapy and radiopharmaceuticals. Liposomes also have attracted much attention among the colloidal drug carrier systems proposed for site-specific drug delivery (Dass et al., 1997; Harasym et al., 1998; Kim, 1993; Lasic, 1998; Storm & Crommelin, 1998).

Apparently, liposome also can be functionalized through topical administration route. In the dermatological field, liposomes were initially preferred as a drug carrier due to its moisturizing and restoring properties. Later, various potentials of liposomes such as the ability to encapsulate various types of drug as well as administration of these drugs to the epidermal cells and deeper cell layers were investigated (Patel et al., 2012).

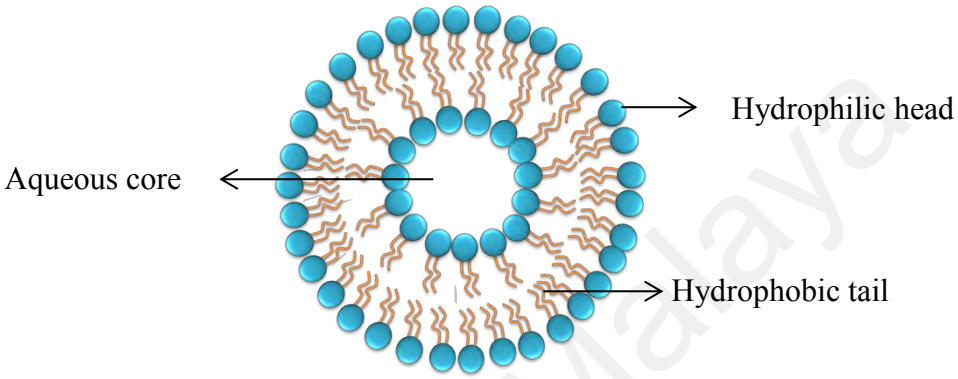



**Figure 1.1**: Illustration of liposome.

The first report on the development of liposome for topical application was presented at FIP 1979 congress (Mezei & Gulasekharan, 1979). This study indicated that drug deposition in the skin was increased whereas the drug absorption into central blood supply was reduced upon the topical application of liposomal encapsulated drug. Meizi and Gulasekharan compared the topical application of liposomal triamcinolone acetonide and the control ointment for 5 days. Amazingly, drug deposition in the epidermis and dermis using liposomal system was found to be higher compared to the control ointment. They also reported that urinary excretion of the drug was diminished (Mezei & Gulasekharan, 1980). It is evident that liposomes are beneficial for the development of dermatological products due to various proven benefits. In 1988, Peverly Lipogel was the first topical applied product commercialized in Switzerland using liposome technology (Barel et al., 2014). Over the years, many liposomal

products are being developed and marketed which includes Celadrin®, Optisome™, Lipo C™ Liposome and Lipo-Gest™ (Argan & Harikumar, 2012).

The formulation of an appropriate liposomal system as a drug carrier depends on the composition of the liposomes. Nature of the lipids composing liposomes affects the membrane fluidity, charge density, steric hindrance and permeability of the vesicles (Prathyusha et al., 2013). In general, naturally-derived phospholipids such as egg phosphatidylcholine (Egg-PC) and soy phosphatidylcholine (Soy PC) with varying fatty acyl chain are used to prepare the lipidic vesicles. Phospholipids from natural sources are more favored compared to synthetic phospholipid due to the low cost of production (Madrigal-Carballo et al., 2010). Soy lecithin is one type of phospholipid from natural sources which are extensively used in the preparation of lipidic vesicle for various applications (Alvarez et al., 2007). Soy lecithin composes a mixture of phosphatidylcholine, -ethanolamine and -inositol as the main components (Gibis et al., 2014). However, the behavior of these naturally derived lipids which exists as a mixture is hardly defined hence less preferred for medical application especially for intravenous administration of drug. Thus, synthetic or semi-synthetic lipids which comprise of only one lipid species such as 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) are more preferable for medical applications (Uchegbu et al., 2013).

Another issue often related to liposomes is its stability. Unmodified liposomes, regardless composing of synthetic lipid or naturally derived lipid results in aggregation of the vesicles. Firstly, they tend to attach to each other which results in aggregation of the vesicles. Subsequently, the changes in particle size distributions will result in leakage of encapsulated material (Park et al., 2014). On the other hand, it has been reported that liposomes could only deliver drugs to the upper layer of stratum corneum

and therefore accumulates in skin appendages for topical applications (Zhou et al., 2014). The challenge continues when liposomes are introduced into the blood stream as they exhibit short circulation half-life (Chung et al., 2014; Mady, et al., 2009).

As stability is a general problem with lipid vesicle, surface modification of liposome with bio adhesive and polymeric membrane is necessary to overcome the limitations of liposomes. Polymer coating of liposomes is considered as a robust technique to enhance the stability of liposomes (Panneerselvam et al., 2014). Several authors have developed liposomes coated with chitosan (Park et al., 2014), pectin (Zhou et al., 2014) and polyvinyl alcohol (Mu & Zhong, 2006) in order to increase their stability towards drug release. Polyethylene glycol (PEG) is another polymer which is commonly used to present the stabilizing effects (Mu & Zhong, 2006). However, PEG has to be modified or functionalized with active ingredients in order to produce sterically stabilized liposomes which eventually involve a higher production cost. Thus, in order to overcome these limitations, we have incorporated a polycationic polymer Diethylaminoethyl-Dextran (DEAE-DX) in the formulation in order to promote the stability of liposomes via deposition of positively charged dextran on liposomal surface. For the first time, the effect of DEAE-DX on the stability of liposomes was studied.

Diethylaminoethyl-dextran (DEAE-DX) was used in this study because of its excellent properties such as biodegradability and biocompatibility (Abioye et al., 2014). Positively charged DEAE-DX similar to chitosan also has the possibilities to interact with and enhance skin permeation as there is a stronger interaction with skin. We found that DEAE-DX shows the stabilizing effects by simply mixing the liposome suspension with DEAE-DX solution. As such DEAE-DX coating may represent a more convenient technique compared to PEGylation. Also, DEAE-DX has few important applications in therapeutics. It has been reported that DEAE-DX shows inhibiting effects towards tumor growth (Thorling et al., 1971). Furthermore, it is able to form conjugates with

various drug molecules to enhance its therapeutic activities (Abioye et al., 2014). Besides that, DEAE-DX was designed as a potential candidate as adjuvants for lymphatic targeted drug delivery system (Feng et al., 2010).

We have studied the effect of DEAE-DX coating on liposomes composed of lecithin, DPPC and DSPC, respectively for topical drug delivery system. The variations in physicochemical characteristic of these liposomes were evaluated. The surface tension study was included in order to determine the CVC of LEC, DPPC and DSPC solution at a temperature of 30.0 ºC. The effect of DEAE-DX on the surface tension and CVC was also reported herein in order to elucidate the interaction of DEAE-DX polymer with lipid in the bulk solution. In this study, curcumin as the hydrophobic drug and diphenhydramine hydrochloride as the hydrophilic drug were loaded into the liposomes.

Curcumin is a yellow lipid-soluble natural pigment derived from the rhizome of *Curcuma longa*. It is a hydrophobic polyphenol compound which has numerous medicinal benefits such as anti-inflammatory, anti-carcinogenic, anti-microbial and antioxidant properties which give rise to a promising clinical application (Hasan et al., 2014). Furthermore, curcumin was reported to be useful in the treatment of allergic diseases as it exhibits anti-allergic property when examined in animal via models of allergy (Lee et al., 2008). All these properties of curcumin make it very promising in the therapeutics. However, the delivery of curcumin is limited due to its high hydrophobicity which restricts its transdermal use (Chen et al., 2012).

Previous work has studied the conjugation of curcumin with carriers consist of macromolecules, nano-formulation, cyclodextrin, liposomes or hydrogels solely to enhance its water solubility. It was found that the circulation time and bioavailability of curcumin was increased with the use of these carriers. Without these carriers, clinical

application of curcumin is limited due to its poor water solubility and low systemic bioavailability (Dhule, 2012). Thus in this study, curcumin was loaded into liposome which is a potential vehicle in order to enhance the delivery of curcumin. Moreover, curcumin loaded liposome was coated with DEAE-DX which influences the stability of the liposome.

Diphenhydramine hydrochloride (DPH) is an antihistamine used to treat severe allergic symptoms such as itchiness, common cold, insect bites and bee stings. DPH has potential effect for the treatment of allergic skin disorders as it distributes into the skin efficiently and sustains higher concentrations than in serum. Unfortunately, high dosage administration of DPH has side effects such as sedation and drying of mouth which limits its usage (Shahi et al., 2013). It also has been reported that high dosage administration of DPH may increase sensitivity to sun lights which eventually leads to skin rash and sunburn (Yamada et al., 1998). The present study hypothesized that liposome encapsulated DPH will attain a prolonged delivery thereby reducing the potential dose-related side effects.

The formulated liposomes were chosen to serve as the topical drug delivery system due to their potential as a safe, stable and effective hydrophobic and hydrophilic carrier for sustained drug delivery which can also reduce dosing frequency of drugs. However, the major limitation of using liposomes topically is the liquid nature of preparation (Wasankar et al., 2012). Considering that, incorporation of liposomes into the gel matrix where original structure of the vesicles is preserved could be an attractive mode of topical application. Liposomal products whereby liposomes are incorporated in the gel are also commercially available. One of the evident products is FUNGISOME$^{TM}$ gel which is uniquely designed liposomal formulation of Amphotericin B (Jadhav et al., 2010).

Gelling agents that are commonly used for the preparation of topical gel is carbopol. Carbopol gels are approved for pharmaceuticals use in several different administration routes. They also exhibit good rheological properties resulting in a stable gel system. Moreover, carbopol gels are anionic with good buffering capacity, which may contribute to the maintenance of the desired pH. It has also been confirmed that liposomes are fairly compatible with carbopol (Gupta et al., 2012; Pavelić et al., 2001). Due to the fore mentioned advantages of carbopol gels, liposomes were incorporated into carbopol gel. The effect of formulated liposomes on the rheological properties of carbopol gel was investigated. The drug release profile of liposomal gel was studied. In overall, this study investigated the stabilization of liposome via DEAE-DX coating and presents liquid–state liposomes as a potential drug carrier for topical application by incorporating into gel matrix where original structure of liposome is preserved as illustrated in Figure 1.2.

## 1.2 Main Objective

To improve the stability of liposome via incorporation of DEAE-DX into liposomal formulation.

### 1.2.1 Objectives

- To study the physical properties of uncoated and DEAE-DX coated liposome.
- To investigate the rheological properties of liposomal gel.
- To study the *in vitro* release of the liposome.

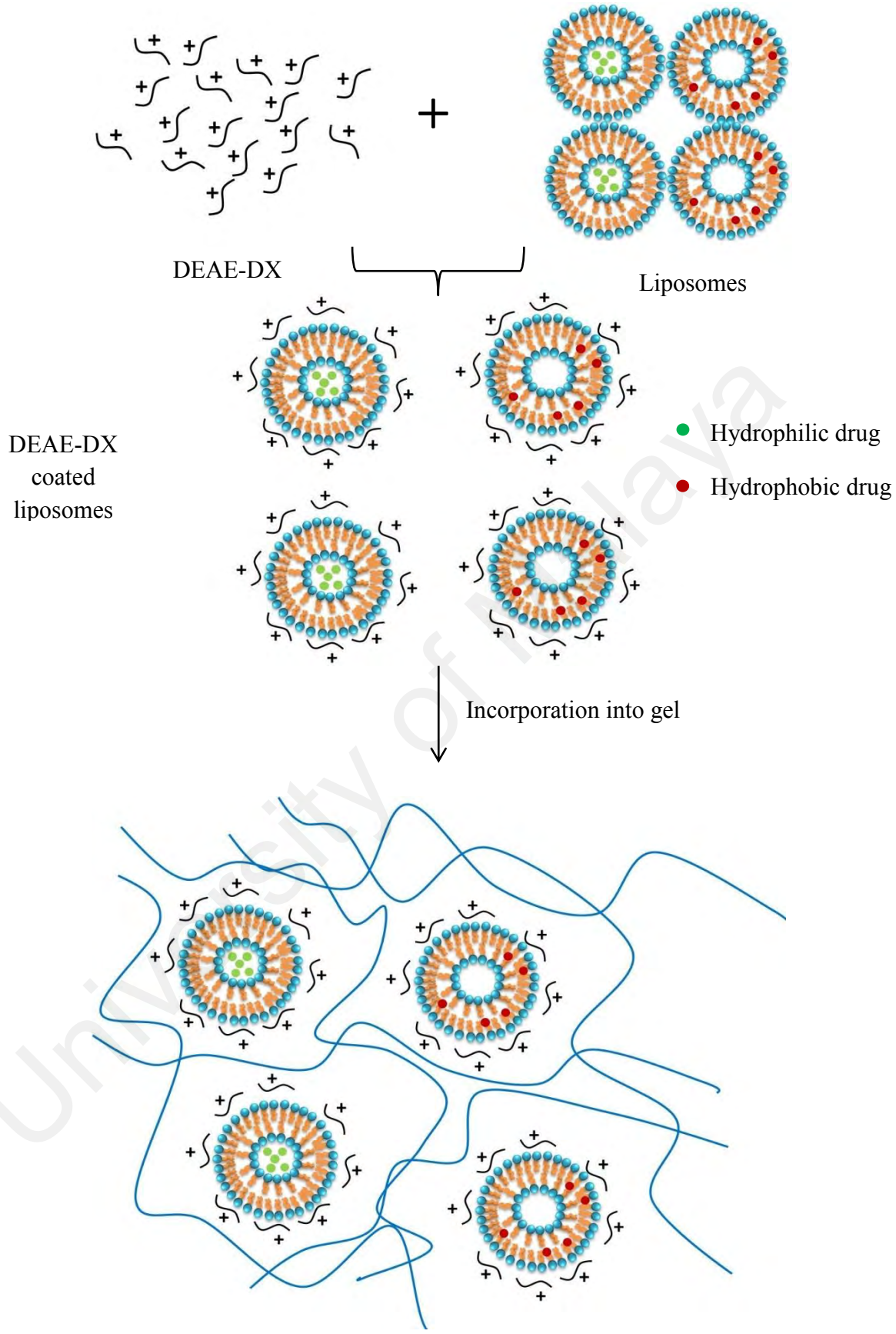



**Figure 1.2**: A schematic illustration of stabilization of liposomes via DEAE-DX coating and incorporation of liposomes into gel matrix

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction to liposomes

Formulation of spherical structures was discovered by British haemotologist by the name Dr. Alec D. Bangham and R.W. Horne. Bangham first observed liposomes when he and his colleague R.W Horne tested a new electron microscope at the Babraham Institute, in Cambridge, UK (Shurtleff & Aoyagi, 2016; Bangham & Horne, 1964). They observed that hydration of dry phospholipids film with negative staining agent spontaneously formed small structures with closed membranes that resembled the bilayer of living cells and established the basis of model membrane system (Bangham, 1968). Within a few years, a variety of enclosed phospholipid bilayer structure consisting of single bilayers, initially termed 'bangosomes' and then the spherical structure was named as a liposome or artificial vesicle (Allen & Cullis, 2013). Their publication in 1964 has brought to the development of liposome studies (Bangham & Horne, 1964). Since that time, the utility of liposomes has been recognized across a wide variety of fields, from medicine to cosmetics.

The molecular structure of phospholipid with amphiphilic nature of a surfactant defines the way in which they form phospholipid liposomes. Surfactants are usually organic compounds that have the adsorbing ability on the surface or interfacial of air/liquid, liquid/liquid and solid/liquid. This is due to their amphiphilic nature, meaning they contain both hydrophobic tail groups and hydrophilic head groups. In the presence of water, the head is attracted to water and line up to form a surface layer facing the water, while the tail group, which composes long hydrocarbon chain, is repelled by water and line up to form a surface away from the water. Disruption of phospholipid membrane causes the membrane to reassemble into tiny spheres either as a bilayer or monolayer which are referred as liposomes (Dua et al., 2012).

Generally, liposomes can be prepared using natural and/or synthetic phospholipids (phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylinositol) (Figure 2.1). Phosphatidylcholine and phosphatidylethanolamine compose the two major structural components of most biological membranes (Cooper, 2000). Phospholipids consist of two hydrocarbon fatty acid chain linked to phosphate containing the polar head group.

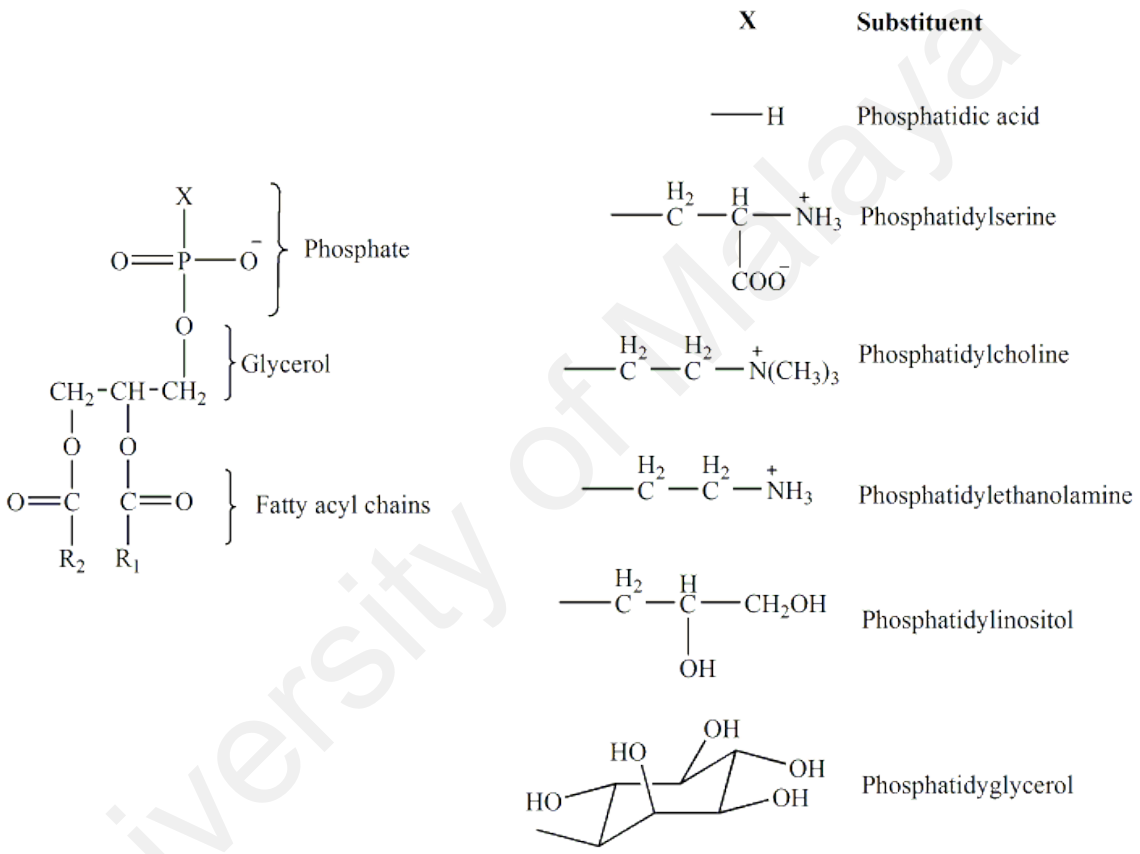



**Figure 2.1:** Types of phospholipids.

Phospholipid consists of fatty acids which may differ in their chain length and saturation of the chain. The most common lipid used is phosphatidylcholine (also known as lecithin) which is composed of a choline head group and glycerophosphoric acid, with a variety of fatty acids (Sipai et al., 2012). Besides phospholipids, liposome may also consist of other constituents such as cholesterol, hydrophilic polymer

conjugated lipids and water. The rigid hydrocarbon ring structure of cholesterol improves the membrane fluidity of the phospholipids (Lee et al., 2005).

According to Allen and Cullins (2013), one of the early pioneers, Gregory Gregoriadis, established the concept that liposomes could entrap drugs and be used as drug delivery systems. The ability of liposome to encapsulate both hydrophilic and hydrophobic drug makes it a potential non-toxic drug carrier (Heurtaul et al., 2003; Lidgate et al., 1988; Popovska et al., 2013). They can accommodate water soluble drugs, in the central aqueous core, water-insoluble drugs in the lipid membrane and peptide and small proteins at lipid-aqueous interface (Sipai et al., 2012). Thus, liposomes exhibit three potential compartments that able to accommodate any desirable drug.

The preparation method of liposome is dependent on the following conditions: 1) the physiochemical characteristics of the liposomal ingredients; 2) the nature of dispersion medium; 3) concentration of the entrapped substance and its potential toxicity; 4) additional processes involved during application/delivery of the vesicles; 5) optimum size, polydispersity and shelf-life of the vesicles for the intended application and 6) batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products (Kaur et al., 2012; Sirisha et al., 2012).

Attractive biological properties of liposome whereby the size and surface behavior of liposomes can be easily altered by introducing new ingredients in the formulation or by varying the preparation methods have enabled liposome to become a pharmaceutical carrier for numerous practical applications (Torchilin, 2005). Evolution of liposome-based technology from the first generation “conventional vesicles” to stealth liposomes, targeted liposomes and stimuli-sensitive liposomes (Bharali et al., 2009; Immordino et al., 2006) has resulted in the appearance of unique technology

involving liposomes. One of the stealth liposomes that have been marketed is DOXIL® for the treatment of Kaposi's sarcoma (Immordino et al., 2006). These liposomes show increased pharmacokinetics effects by having a longer circulation time and a lower rate of elimination. Besides long circulating liposome, active targeting also can be achieved via coupling of liposome with site-specific ligands (Allen & Cullis, 2013). As for topical application, liposomes increase the permeability of skin for various entrapped drugs. The delivery of drugs onto the skin is recognized as an effective means of therapy. Besides that, they diminish the side effects of the drug as a low dose of the drug is sufficient for a sustained drug release. Also, exposure of sensitive tissues to toxic drugs is reduced (Sipai et al., 2012).

## 2.2 Characterizations of liposome

### 2.2.1 Critical vesiculation concentration (CVC)

Transition of the physical properties with respect to the concentration of lipid solution will occur as the concentration of monomers exceeds a certain level. This implies that monomers are assembled into vesicles. The concentration at which this transition occurred is known as critical vesiculation concentration (CVC). CVC is a quantitative parameter which is also referred as the minimum concentration required for lipid monomers to self-assembly into a bilayer structure of liposome. Thus through physical property measurement on a series of lipid solution with various concentration, the CVC value is obtained. Physical properties such as surface tension, turbidity, molar conductivity, calorimetry analysis and osmotic pressure are suitable for the estimation of CVC.

The transition of surface tension with respect to concentration of lipid solution was employed for the determination of CVC in this research. Inflection point from the plot of surface tension as a function of lipid concentrations estimated the CVC (Figure 2.2). When lipids are dissolved in water, they orientate at the surface so that the

hydrophobic regions are pointed away from the aqueous environment. The reason for the reduction in the surface tension when surfactant molecules adsorb at the water surface is that the surfactant molecules replace some of the water molecules at the surface and the forces of attraction between surfactant and water molecules are less than those between two water molecules, hence the contraction force is reduced (Attwood & Florence, 2012). As a consequent, the surface tension is reduced.

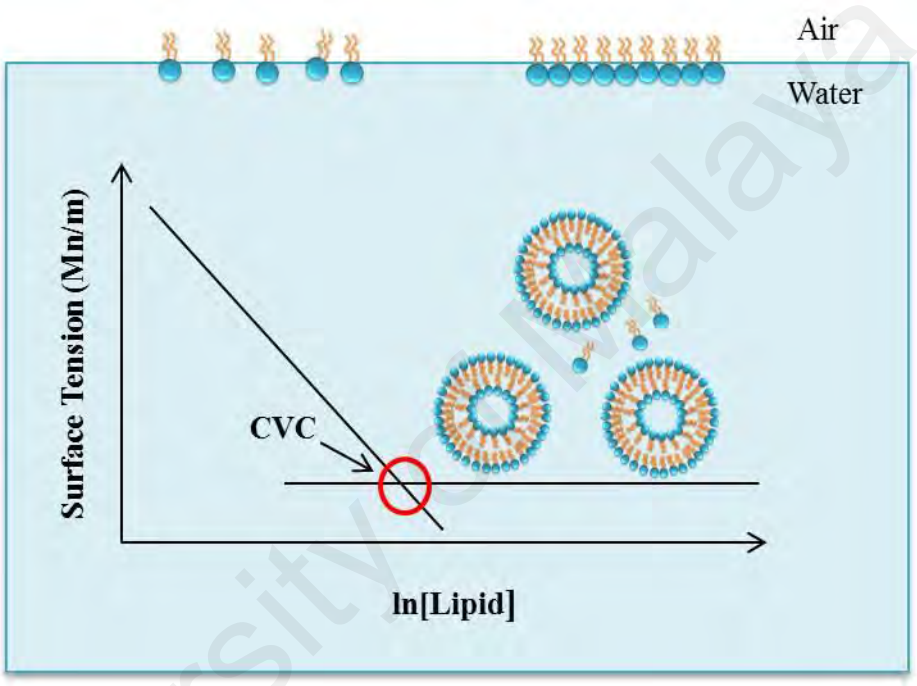



**Figure 2.2**: Inflection point from the plot of physical property as a function of lipid concentrations estimated the CVC.

The balance between hydrophilic and hydrophobic properties of a lipid affects the surface activity of the lipid. As for example, an increase in the length of hydrocarbon chain of lipid increases their surface activity for a homologous series of lipid such as DPPC and DSPC. Thus, the surface tension is reduced. Besides that, the longer the hydrocarbon chain, the stronger the hydrophobicity hence the lower the concentration of fatty acid required for the formation of the liposome (Zhang & Marchant, 1996). It has been reported that long-chain fatty acid oleic acid self-assemble approximately at a concentration 20 μM whereas short chain species such as decanoic

acid and octanoic acid self-assembles at a concentration of 40 μM and 250 μM respectively (Budin et al., 2014). Factors such as temperature, ionic strength and pH of a medium also may influence the CVC value. For instance, increasing the temperature or ionic strength of a system decreases the CVC value. Besides that, previous work by Tan & Misran (2013) shows that surface modification of oleic acid liposome by low-molecular-weight chitosan exhibited lower CVC value compared to the unmodified oleic acid liposomes. It was found that presence of chitosan had disturbed the packing of oleic acid molecules at the air/water interface, therefore resulting in the increase of surface area occupied by the oleic acid at the air/water interface. Subsequently, the chitosan bound to oleic acid are removed from the air/water interface to the bulk medium. Hence, oleic acid-chitosan liposomes formations were more favorable.

It is important to study the CVC of liposome in order to avoid preparation of either too high or too low concentration of liposome solution for physicochemical studies. And, previous studies also mentioned that rate of flocculation is slower in the solution with low vesicular concentration (Ninham et al., 1983). Besides that, an implication of vesicle stability is obtained whereby the smaller the CVC, the more stable the vesicle.

### 2.2.2 Morphology

Transmission electron microscopy is a simple and relative method to observe the morphology of the liposome. The electron microscope is a type of microscope that uses a beam of electrons to create an image of the specimen. It is capable of much higher magnifications and has a greater resolving power than a light microscope, allowing it to see much smaller objects in finer detail. There are few methods involving TEM in the morphological evaluation of the liposomes. One of the methods is cryo-electron microscope method whereby molecular motion in the bilayer is frozen by rapid cooling of the copper grid with liposome dispersion to -160 $^{o}$C in liquid ethane without using

staining agents. This method is preferable for observation of undisturbed liposome (Almgren et al., 2000). However, the images obtained are low in contrast.

On the other hand, freeze-fracture electron microscopy is an optimal technique for examining the ultrastructure of rapidly frozen biological samples by TEM. As for freeze-fracture transmission electron microscope, liposome suspension is rapidly frozen and fractured into two symmetry portions followed by deposition of platinum and carbon on the exposed faces prior to being examined. This method is suitable for dispersion with high concentration. In addition, this method reveals the interior morphology of the liposome (Coldren et al., 2003). However, the preparation of the samples which involves cryofixation, fracturation and shading with evaporated platinum or gold is a tedious and lengthy process (Frederik et al., 1996; Robenek & Severs, 2008).

According to Ruozi et al. (2011), negative staining is the simplest and convenient procedure. According to this procedure, liposomes display little contrast compared to their surrounding in producing the image as biological materials for instance lipids are nearly transparent to the electron beam. Image of the sample without staining agent will appear white in the image as the electrons are transmitted and hit the phosphor screen and produce fluorescence. Thus, staining agents composed of heavy metals such as lead and uranium are used to scatter imaging electrons and therefore produce contrast between different structures. Staining the samples with heavy metals results in addition of electron density to it which causes more interactions between the electrons in the primary beam and those of the sample. Hence, there is a contrast in the resultant image. The area with a staining agent will appear as light grey to black in the image depending on the proportion of electron diffracted.

However, there are some drawbacks of negative staining as staining and drying process may introduce a certain level of artifacts such as liposome fusion and flattening of spherical liposomes (Forte & Nordhausen, 1986). The phenomenon is due to the high sensitivity of liposomes to the changes in the environment, particularly concentration and composition. Furthermore, other limitation includes the structural collapse of samples such as emulsion and liposome as a result of exposure to a vacuum pressure of $10^{-6}$ mbar. TEM technique will be a very useful tool to observe the morphology provided the liposome produced is very stable and strongly elastic in nature before being subjected to a low-pressure environment.

### 2.2.3 Dynamic light scattering (DLS)

Dynamic light scattering (DLS), otherwise known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS) is a technique extensively used to determine size distribution profile of small particles in a suspension or polymers in a solution. This technique is used widely in the diverse area of science and technology which includes studies on colloids, polymer, polyelectrolyte solution and glass forming liquids (Berne & Pecora, 2013).

In the scope of DLS, the time-dependant fluctuations of light scattered from particles experiencing Brownian motion is measured (Crooke, 2007). The Brownian movements of the particles are due to the collision between suspended particles and solvent molecules (Bettelheim et al., 2015). When light hits the particles suspended in a solution, it scatters light in all direction (Rayleigh scattering) (Ganguly, 2013). This phenomenon is due to the difference in index of refraction of the particle and the suspending solvent. This also means that the polarizability of the particle differs from the solvent. Thus, arriving electric field is oscillating and is able to displace the cloud of electrons and thereby cause atoms to oscillate (Syvitski, 2007). The advantage of using this technique is the ability to measure innate samples. Besides that, it also measures

large size range of particles which comprise of low nanometer to low micrometer range. Other advantages include the minimal sample volume, concentration and preparation requirements. Despite all these strengths, DLS technique also has some limitation as it may report the size of aggregates and impurities that are present although in small quantities along with the desired particle size (Berger et al., 2001; Kölchens et al., 1997).

Particle size measurement is one of the factors which govern the topical delivery of active ingredients into the skin. It has been highlighted previously by Verma et al. (2012) that particles generally larger than 600 nm are unable to deliver their contents into deeper layer of skin. On the other hand, particles with size smaller than 300 nm are able to deliver its content into deeper layer of skin. Most research papers presented the liposomal size of less than 300 nm for topical application. For an example, Tsai et al. (2015) reported average particle size of all liposome formulated for topical application ranging from 123.7 nm to 177.7 nm. Moreover, Park et al. (2013) found the stable formulation of chitosan coated liposome to be 279.9 nm. Also, the average particle size for multilayered liposome obtained by Gibis et al. (2014) is approximately 200 nm. Particle size is mainly dependent on the composition of the liposome. It was found that liposome formulated with high level of cholesterol gives a larger particle size (Tsai et al., 2015). In addition, some polymers such as chitosan also resulted in larger particle size upon coating on negatively charged liposome due to the formation of layer on the surface via adhesion of chitosan. On the contrary, high level of Tween 80 reduces the particle size of liposome due to the destabilization of lipid bilayer (Tsai et al., 2015).

### 2.2.4 Zeta potential

Solids, liquids, and gases are three fundamental states of matter. If one of these states is finely dispersed in another then it is said to form a ‘colloidal system’. Most colloidal dispersions in aqueous media carry an electric charge (Remington et al., 1975). The

nature of the particle and its surrounding medium affects the surface charge (Boisseau & Lahmani, 2009). The zeta potential of a particle is the overall charge that a particle acquires in a particular medium (Pathak, 2011). It is a physical property which is exhibited by any particle in suspension. It has long been recognized that the magnitude of zeta potential is a very good index of the interaction between colloidal particles (Ghosh, 2006).

There are two parts of liquid layer surrounding the particles; an inner region (Stern layer) where the ions are strongly bound and an outer region (diffuse layer) where the ions are less firmly associated. There is a notional boundary within the diffuse layer in which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity), ions within the boundary move along. Those ions beyond the boundary stay with the bulk dispersant. The potential at this boundary (surface of hydrodynamic shear) is the zeta potential. In other words, zeta potential is the potential difference between dispersion medium and stationary layer of fluid attached to dispersed particles (Blum et al., 2011; Luque et al., 2012; Ma & Kim, 2011).

Measurements of zeta potential are commonly used to predict the stability of colloidal systems (Lindon et al., 2016; Shah et al., 2014; Starov & Griffiths, 2012). If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency to form aggregates (Nimesh & Chandra, 2011). However, if the particles have low zeta potential values then there will be no force to prevent the particles flocculating. Particles suspension with zeta potentials > +30 mV or < −30 mV are normally considered stable (Hunter et al., 2013; Kanade & Bhattacharya, 2016; Verwey et al., 1999).

Previous work has reported the change in magnitude of zeta potential of liposomes upon coating with polymeric membrane. For example, coating of 0.1 % (m/v)

of chitosan for liposome composed of soy lecithin resulted in the change of zeta potential from -9.4 mV to +26.5 mV (Park et al., 2013). And upon increasing the concentration of chitosan to 0.3 % (m/v) and 0.5 % (m/v), the zeta potential was found to increase to +34.5 mV and +39.2 mV respectively. Besides that, another finding by Gibis et al. (2014) shows the layer-by-layer electrostatic deposition of chitosan followed by pectin in order to formulate multilayered liposomes. The zeta potential of the liposome changed from -20 mV to +70 mV for chitosan coating and to approximately -20 mV for pectin coating. Often, such change in magnitude of zeta potential indicates the successful coating of polymer on the surface of the liposome. Therefore, the multilayered liposome was found stable from the 30 days stability study.

### 2.3 Limitation of liposome

Liposomes have great potential in drug delivery; however liposome-based drug could not be marketed in great numbers. Sterilization method is also one of the challenges faced for wide usage of the liposome (Toh & Chiu, 2013). Phospholipids are thermos labile sensitive to the use of heat, radiation and /or chemical sterilizing agents as they easily cause degradation of liposome components and may leave toxic components under certain condition. Filtration of liposomes through sterile membranes is a method available for sterilization of liposomes. However, filtration does not remove contaminants such as viruses (Sipai et al., 2012). Besides that, this method is not suitable for vesicles larger than 0.2 μm.

The major problem that limits the manufacture and development of liposomes has been the stability issues (Gaonkar et al., 2014; Ohshima & Makino, 2014). Both physical and chemical stability have been an issue hence final liposome formulation has short shelf-lives (Arias, 2016). Chemical instability is caused by hydrolysis of ester bond and/or oxidation of unsaturated acyl chains of lipids (Chow, 1992). The pH, buffer

concentration, ionic strength and temperature also may influence the hydrolysis kinetics of phospholipid (Grit & Crommelin, 1993).

On the other hand, physical stability is due to the aggregation of vesicle that eventually forms larger particles that leads to drug leakage (Gad, 2008; Ma, 1990; Torchilin & Weissig, 2003). This phenomenon of drug leakage and change in liposome size affects the in vivo performance of the drug formulation. Consequently, the therapeutic index of the drug is reduced since the large liposomes are rapidly cleared by phagocytic cells of the mononuclear phagocyte system (MPS), which is also referred to as the reticuloendothelial system (RES). Larger liposomes are eliminated from circulation faster compared to smaller liposomes. Liposome accumulation is higher in the liver and spleen because of the rich blood supply and also the presence of a greater amount of macrophages.Furthermore, partitioning of hydrophobic drug from the bilayer into the solvent upon long term storage also affects the stability of the dispersion. Other limitation of liposomes includes its high production cost which involves incorporation of costly materials into the formulation of liposome for stabilization purpose (Kaur et al., 2013). Also, expensive equipment needed in order to increase the manufacturing of liposomes (Mozafari, 2005).

## 2.4 Polymer coating of liposomes

As stability is a general problem with lipid vesicle, surface modification of liposome with bioadhesive and polymeric membrane is necessary to overcome the limitations of liposomes. Many attempts have been made to enhance the physical stability of liposomes. Coating of liposome with polymers is a promising strategy whereby liposome becomes mucoadhesive thus they have a longer residence time in the respective biological environment (Klemetsrud et al., 2013). Liposomes can be coated by electrostatic deposition of an oppositely charged polymer.

The adsorption of the polymer molecule at the active sites of the liposomal surface leads to a number of possible polymer conformations which can be described in terms of trains, loops and tails (Klemetsrud et al., 2013). Besides that, liposomes are usually surface modified by hydrophilic polymers to improve their applicability, for instance, the resultant liposomes exhibit good stability, reduce nonspecific adsorption and provide reactive sites for covalent binding of targeting ligands.

As for topical application, coating with positively charged polymers can improve the skin permeation because of the increased interaction between liposome and skin. Improvement on skin permeability by coated liposome also helps to overcome the limitation of liposome whereby conventional liposome could only deliver drugs to the upper layer of the stratum corneum and accumulate in the skin appendages. An important characteristic of polymer is that they create a protective impermeable layer over the liposome surface. During the past years, natural polymers such as amylopectin, pullulan, hydroxylmethyl cellulose, chitosan, pectin and many more, have been utilized as coating materials in order to overcome the limitation of the liposome.

Chitosan, a biodegradable material has emerged as a promising coating material for liposomal drug delivery system. Chitosan is extensively used in pharmaceutical preparation due to its availability, good compatibility, anti-microbial property and absorption-enhancing agent. Chitosan is also inexpensive as it is derived from chitin which is a major structural polysaccharide found in invertebrate animals and lower plants. Due to its natural abundance and bioadhesives, chitosan has received much attention for liposomal coating in novel bioadhesive drug delivery system. Besides that, the potential use of chitosan polymer which is positively charged is much promising as chitosan could improve skin permeation. Several studies highlighted that liposome coated with chitosan could carry drugs across the skin barrier hence enhancing the drug delivery via improved skin permeation. Prolonged and sustained released may be

achieved via the combination of chitosan and liposomal characteristics. The chitosan-coated liposomes were formed via ionic interaction between positively charged chitosan and negatively charged diacetyl phosphate on the surface of the liposomes (Guo et al., 2003). Studies have shown that coating of chitosan decreases the lipid oxidation rate. At an elevated temperature, hydrophilic heads of lecithin undergo dehydration which alters their optimum curvature. At this condition, lecithin liposome may coalescence into larger particle. However, upon coating with chitosan, this type of coalescence could be prevented as the vesicles are not closely in contact with each other. The electrostatic repulsion between coated liposomes is larger than the uncoated ones due to the presence of positive charge on the surface of chitosan-coated liposomes. Therefore strong electrostatic repulsive forces are generated between the coated particles. Thus, pro-oxidant metals are repelled from the vesicle surface, and the lipid oxidation rate decreases (Gibis et al., 2013).

Another polymer preferably employed in the surface coating of the liposome is pectin. Pectins are well known for its safe use as gelling and thickening agent and stabilizers in food industries. In addition to their capability to form gels, pectin also is a great potential coating material for liposomes as the production cost is low. Pectin is negatively charged at neutral pH due to the presence of carboxylic acid groups in the galacturonic residues of the pectin chain. This feature enables the surface coating of pectin. Citrus pectin is a naturally occurring polysaccharide which consists mainly of D-galacturonic acid units with varying degrees of methyl esterified carboxyl groups. Pectin can be categorized into two main groups which are characterized by degree of esterification (DE); high methoxy pectin (HM-pectin) has DE above 50%, and low methoxy pectin (LM-pectin) having a DE value below 50%. LM-pectin can be further amidated to modify its properties (Klemetsrud et al., 2013). The previous study mentioned that pectin also can be a good polymer candidate for bonding onto liposome

and pectin coated liposome has shown to improve the drug delivery in the gastrointestinal tract (Klemetsrud et al., 2013). Besides that, pectin coated liposome may be used as bioadhesive liposomes in dental applications. Studies have showed that pectin coated liposomes seems to retain better for a longer time intervals on dental enamel than uncoated negatively charged liposomes. It was hypothesized that pectin may help to prolong the adhesion of liposomes on the tooth surfaces (Nguyen, 2011).

These polysaccharide polymers have received wide attention due to its carbohydrate moieties in the structure which have the ability to interact specifically with various receptors in target cells (Duo & Yeo, 2012). However, several challenges are associated with polysaccharide due to its high degree variability in molecular weight and structure which gives inconsistent properties that may influence its biological properties. Hence, standardization procedures are needed to be established for purification purposes (Duo & Yeo, 2012).

On the other hand, studies on liposomal system stabilized by synthetic polymers such as polyethylene glycol (PEG), polyvinylalcohol and polyacrylic acid also have been conducted. Surface engineering of liposome with polyethylene glycol (PEG) had shown a remarkable progress and breakthrough in the development of stealth technologies. Polyethylene glycol (PEG) is another polymer which is widely used to present the stabilizing effects (Ning et al., 2011). PEG represents an outstanding polymer for surface modification of liposome as they are amphiphilic and they become non-ionic after being attached to hydrophobic molecules. Amphiphilicity behavior of PEG enables them to possess high solubility in both aqueous and organic media. As PEG is soluble in organic solvent, it is possible to solubilize lipids and PEG in organic solvent hence facilitates the formation of stealth liposomes. PEG is also characterized by its excellent biocompatibility, lack of toxicity, low immunogenicity and antigenicity and good excretion kinetics. In addition, PEG can be synthesized with a wide range of

molecular weight (MW, 400 Da to 50 kDa) and with a low polydispersity index (PDI, below 1.1). They also can be synthesized into different end group functionalities such as PEG-lipid conjugates (Nag & Awasthi, 2013). The resultant PEGylated liposomes are arguably the best-engineered long-circulating nanocarriers. For instance, PEGylated liposomes exhibit half-life circulation of 15–24 hours in rodents and as high as 45 hours in humans.

Although synthetic PEGs conjugated to phospholipids have successfully reached clinical applications but it was not easy to functionalize PEG-coated liposomes with active ligands. PEG is a linear polymer and only has two reactive sites per molecule for surface grafting chemistry. In addition, modification and functionalization of PEG with active ingredients in order to produce sterically stabilized liposomes involves a higher production cost (Mady et al., 2009).

**Table 2.1**: Polymers used for stabilization of liposome.

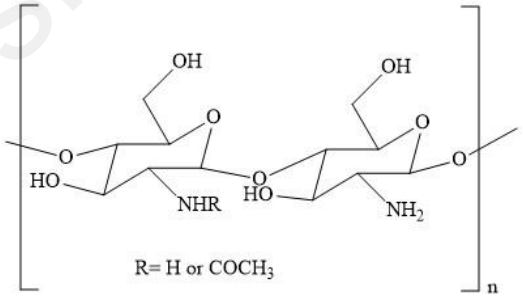
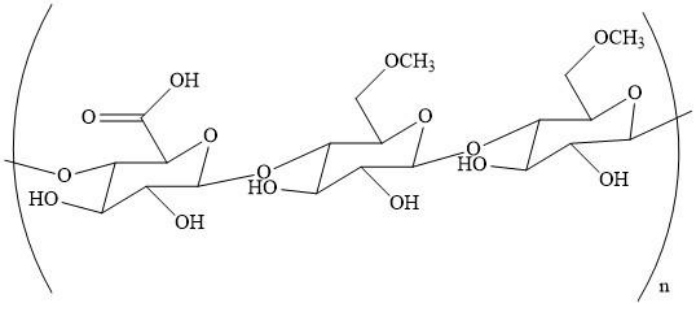
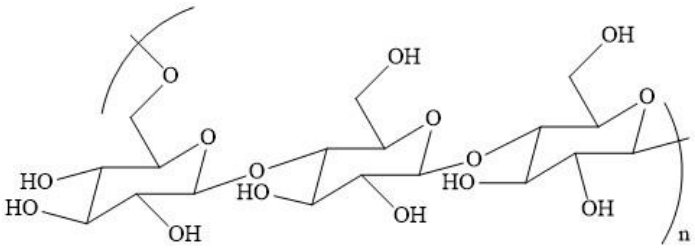
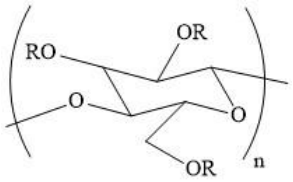
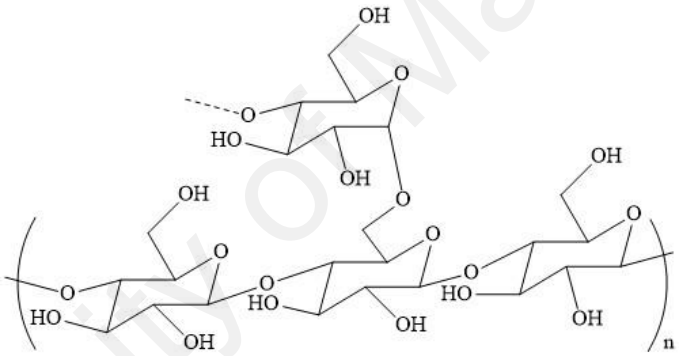
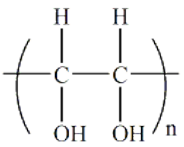
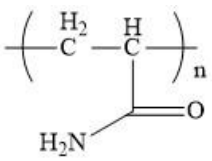
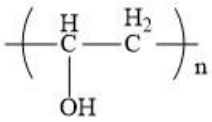
| Polymer | Structure | Reference |
|---|---|---|
| Chitosan |   | (Liu et al., 2009; Mady et al., 2009) |
| Pectin |   | (Nguyen et al., 2011). |

**Table 2.1**: (continued)

| | | |
|---|---|---|
| Pullulan |  | (Kang et al., 1997; Sehgal & Rogers, 1995) |
| Hydroxylmethyl cellulose |  | (Takeuchi et al., 2001). |
| Amylopectin |  | (Miyazaki et al., 1992) |
| PEG |  | (Abuchowski et al., 1977; Beugin et al., 1998; Woodle and Lasic 1992). |
| Polyacrylamide |  | (Rescia et al., 2011) |
| Polyvinylalcohol |  | (Rescia et al., 2011) |

## 2.5 Diethylaminoethyl-Dextran

Diethylaminoethyl-Dextran (DEAE-DX) is a polycationic derivative of dextran. It is produced via the reactions of diethylaminoethyl chloride and dextran in an aqueous medium in the presence of alkali metal hydroxide to etherify the OH of dextran (Usher & Patel, 1985). The degree of substitution is approximately one DEAE-substituent per three glucose units which gives an average molecular weight of 1 million. The structure of DEAE-DX is depicted in Figure 2.3.

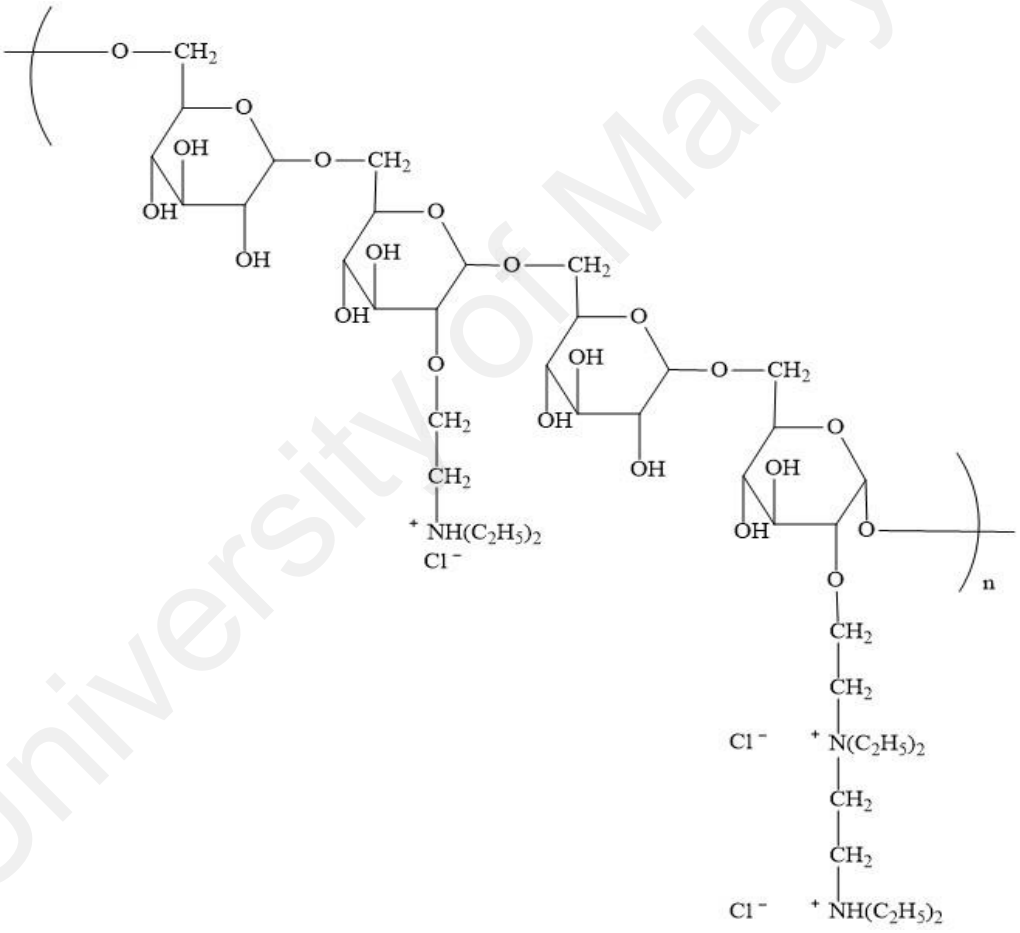



**Figure 2.3:** Structure of DEAE-DX.

DEAE-DX is widely used in many fields such as molecular biology and health-care sector due to the factor that they are highly purified and produced in microbiological quality. DEAE-DX as a polycationic is highly versatile and has been

reported to exhibit a wide range of chemical and biological properties. Some applications of DEAE-DX are well documented in Table 2.2. Other application of DEAE-DX is its uses in drug delivery. DEAE-Dextran-insulin complex has been studied as an oral delivery system (Manosroi et al., 1990). Besides that, the retention capacity calcium alginate bead was compared using DEAE-DX by a formation of polyelectrolyte membrane at the bead surface. Moreover, it was also reported that DEAE-DX is advantageous as it can be used at any pH.

**Table 2.2:** Applications of DEAE-DX.

| Applications | References |
|---|---|
| DEAE-Dextran in vaccine production | (Westrook et al., 1994), (Joo et al., 1988) |
| DEAE-Dextran enhances protein and nucleic acid uptake | (Fox et al., 1977), (Rigby et al., 1969), (Schenborn et al., 2000), (Mack et al., 1998) |
| Gene therapy | (Kaplan et al., 1998) |
| Enhancer of viral infectivity | (Fiala et al., 1969), (Park et al., 1998) |
| Stabilization of proteins | (Gibson et al., 1992), (Gavalas et al., 2000) |

Commercially available DEAE-DX that exists as dry powder shows excellent temperature stability. On the other hand, in solution form, DEAE-DX is stable in a pH range of 4-10 at an ambient temperature. Also, DEAE-DX can be stored in an airtight container more than 3 years at room temperature. Besides that, controlled stability study at a temperature of 8, 25, and 40 °C for 3 years also shows that the quality and efficacy of DEAE-DX is maintained. The stability of DEAE-DX itself serves as the main reason for it to be widely exploited for various applications (Gibson et al., 1992)

## 2.6 Diphenhydramine Hydrochloride (DPH)

Diphenhydramine hydrochloride belongs to a class of drug which is known as antihistamines (Figure 2.4). Antihistamine prevents the effects of histamine released by the immune system when the body is exposed to allergens. Histamines are chemicals

that cause itching, sneezing, runny nose and watery eyes. Thus, antihistamine plays a role in preventing histamines from attaching to the body cells which eventually stops the cascading effect of allergies. DPH as topical formulations is commonly used to relief the effect of itching that caused by allergic reaction. However, administration of high concentration of antihistamine such as DPH for the treatment of allergies leads to complications such as nervous system, cardiovascular system, respiratory system and gastrointestinal system.

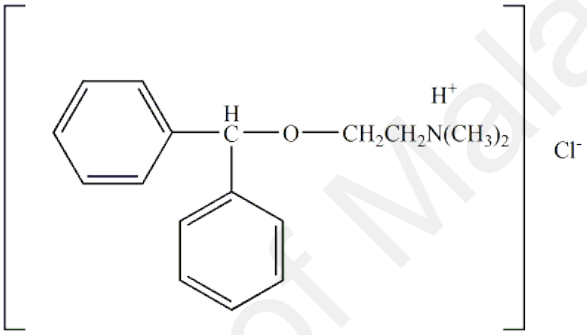



**Figure 2.4:** Structure of DPH.

For above reasons, previous studies have suggested the encapsulation of DPH into liposomes. Iwanaga et al. (2000) reported the action of liposome on the anti-histaminic effect of DPH in rats to highlight the usefulness of liposome as an intranasal formulation for topical application. The findings show that DPH was released slowly from liposomes and to the nasal cavity and this phenomenon prolonged the effect of DPH. Therefore, the adverse effect of this drug can be minimized.

Besides that, Shahi et al. (2013) also have reported research work on the administration of diphenhydramine hydrochloride into topical liposomal formulation. According to the results obtained, in vitro release of DPH from liposomal formulation via guinee pig skin exhibited sustained release action for 8 hours as compared to drug

solution. This finding also revealed that the incorporation of DPH into topical liposomal drug delivery system helps to reduce adverse effects of DPH.

Other than liposomes, other drug carriers for topical delivery of DPH through the skin using microemulsion, sodium alginate emulgel, hydroxyethylcellulose gel and carbopol cream were characterized for its release behavior by Sanna et al. (2010). It was reported that microemulsion gives the highest diffusion rate of DPH. Then, *in vivo* performance of these carriers showed the absence of skin irritation and also reduction in the response induced by histamine.

## 2.7 Curcumin

Curcumin, polyphenolic compound of turmeric plant is well known for its pharmacological properties (Table 2.3). Most importantly, curcumin has not been associated with any significant side effects even at high doses (Anand, 2007). Also, curcumin is sold at affordable prices. Many studies have been taken place to reveal its potential for medical application. However, despite being the subject of numerous studies, curcumin exhibits poor water solubility and low bioavailability due to the hydrophobic nature of the molecule (Figure 2.5). These properties of curcumin have been highlighted as the main problem in delivering curcumin for medical application. Poor absorption, fast systemic clearance and rapid metabolism of curcumin results lower the retention time of curcumin in the plasma.

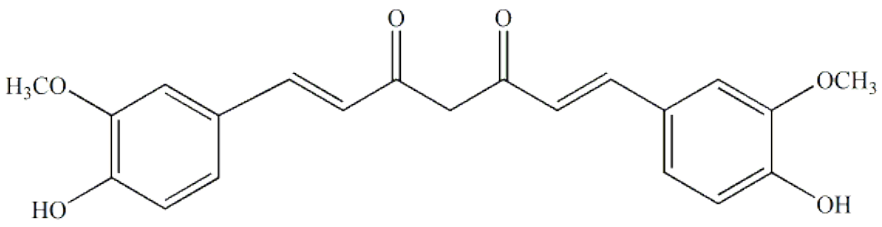



**Figure 2.5:** Structure of curcumin

Great number of efforts has been taken in order to improve the bioavailability of curcumin. For an example, delivery of curcumin via liposomal formulations delivery has attracted the attention of researchers. Liposomes are considered as potential drug carriers due to their capacity to encapsulate hydrophobic compound. Finding show that, oral administration of liposome-encapsulated curcumin in rat showed high bioavailability of curcumin. Another study showed that bioavailability of curcumin loaded flexible liposome is 2.35 fold higher that of curcumin suspension. Moreover, the bioavailability of curcumin in silica-coated liposome was found 3.31 fold higher than the uncoated liposomes (Prasad et al., 2014).

Besides liposomes, encapsulation of curcumin into other nanocarriers such as polymeric nanoparticles, micelles, microemulsions, nanogels and solid-lipid nanoparticles also helps to protect and improve the half-life of the curcumin (Chen et al., 2015). In a recent study, Dr. Heng stated that topical curcumin gel inhibits phosphorylase kinase and reduces inflammation due to improved penetration of curcumin into the skin. Hence, curcumin gel appears to treat burns and scalds effectively (Heng, 2017; Pensoft Publishers, 2017).

**Table 2.3:** List of pharmacological activities of curcumin

| Pharmacological Activities | References |
|---|---|
| Antiaging or antiwrinkle | (Saraf et al., 2011) |
| Antioxidant | (Lee et al., 2008) |
| Anti-irritant | (Saraf et al., 2011) |
| Anti-inflammatory | (Lee et al., 2008), (Mullaicharam & Maheswaran, 2012) |
| Anti-microbial | (Saraf et al., 2011), (Patel et al., 2009), |
| Anti-cancer | (Patel et al., 2009), (Lee et al., 2008) |
| Anti-allergy | (Lee et al., 2008) |

## 2.8 Topical delivery system

The topical delivery system is a localized drug delivery system and has been an attractive way to prevent or treat a wide variety of diseases, especially diseases related to skin (Jain et al., 2011). Moreover, topical route of administration is extensively employed to deliver cosmeceutical active ingredients into the skin in order to maintain, improve and nourish the youthful appearance. Skin is the largest and most accessible organ of the body and it plays an important role as a formidable barrier for the administration of most therapeutic compounds (Benson & Watkinson, 2012).

Topical products are mostly developed intentionally to produce an effect at a definite part at the site of the application via the penetration of the drug into the underlying layers of skin or mucous membrane (Mohamed, 2004). Drugs and active ingredients could be delivered through a variety of topical formulations. Examples of topical formulations are creams, lotions, ointments, gels, solutions, suspensions, pastes, sprays and transdermal patches (Ballington, 1998). One of the advantages of drug delivered via topical route is that the drug could be delivered directly to the site of action for an extended period of time. Besides that, the drug concentration is higher at the localized area as the mean resident time of drug is longer. Also, the ease of application of topical formulation provides a convenient and pain-free self-administration for patients (Paudel et al., 2010). For an example, instead of daily injection, diabetes patients could apply an anti-diabetic transdermal patch which able to reduce the painfulness. However, drug delivery system using topical route also offers challenges as barrier property of skin limits the absorption of a wide range of substance (Paudel et al., 2010).

In the past, liposomes as a drug delivery system have been broadly employed to improve the potential of drug delivery via several routes of administration. It has been reported that delivery of the drug via liposomal system was found to be superior compared to conventional dosage forms especially for the intravenous and topical route of delivery (Egbaria & Weiner, 1990). A liposomal formulation which is composed of

lipid mixtures similar to those found in stratum corneum would be a potential delivery system for the topical route of administration. Also, liposomal formulations provide sufficient quantity of drug into deeper tissue and at the same time enhance systemic absorption of the drug. It has been reported that topical formulation of the liposomal system significantly enhances the accumulation of drug at the site of administration as a result of the high compatibility of liposomes with biological membranes (Egbaria & Weiner, 1990).

Studies on the treatment of various skin diseases using liposomal formulation have been reported by many researchers. For instance, treatment of acne via encapsulation of drug such as benzoyl peroxide (Fluhr et al., 1999), salicyclic acid (Bhalerao & Harsyahl, 2003), lauric acid (Yang et al., 2009) or tea oil (Xu et al., 2006) in liposome exhibited better results compared to conventional system by releasing higher deposition of drug on the target site. Other study involving liposomal formulation includes the treatment psoriasis. Methotrexate which is a commonly used drug in treating psoriasis was found penetrate adequately into the skin after being loaded into liposomal formulation (Katare et al., 2010)

## 2.9 Incorporation of liposome in semisolid dosage form

Despite all the advantages of liposomes, a major limitation of using liposomes topically is the liquid nature appearance (Gad, 2008). As a result, liposomes may leak when applied topically (Dragicevic & Maibach, 2015). Thus this can be overcome by incorporation of the liposome in an adequate vehicle where the original structure of the liposome is preserved whereas their rheology and mucoadhesive properties are altered (Antimisiaris et al., 2008). This can be achieved via the incorporation of liposomes into semisolid forms such as cream or gel (Thassu et al., 2007). The utility of semisolid bases for incorporation of liposome also helps to prolong the time of drug to be

delivered at the application site. This is an important characteristic of a pharmaceutical formulation as prolonged release of drug helps reduce the dosing frequency of the drug.

Creams are present in the form of semi-solid in which the dispersion consist of an emulsion, aqueous microcrystalline of long-chain fatty acids or alcohol that are cosmetically and aesthetically acceptable. On the other hand, the gel can be categorized as either water-based or organic solvent-based (Gupta & Garg, 2002). However, aqueous-based gel is a common approach for topical liposomal formulation. It has been reported that pharmaceutical formulations that are prepared in gel forms have extended skin contact time. Gels posses some degrees of flexibility and at the same time, they can withstand their own weight without collapsing. Thus, gels are categorized as soft solid masses which encompass three-dimensional macroscopic networks that can entrap large volume of solvent to form an infinite rigid network structure (Kaur, 2013).

Polymeric gels are commonly used as a drug carrier in pharmaceutical and cosmeceutical industries as they exhibit better physical property and stability as compared to cream and ointment. Polymeric gels are a cross-linked network of polymer chains which are formed via covalent cross-link or physical entanglement of the chain. Polymeric gels can be prepared using either synthetic polymers such as silicon and poly (N-isopolyacrylamide) or natural polymers such as polysaccharides (Peppas & Huang, 2002). Incorporation of liposomes in semi-solid forms also helps to minimize the problem caused by skin penetration enhancers (Mezei & Gulasekharam, 1980).

Pharmaceutical or cosmeceutical semisolid formulation uses skin penetration enhancers such as sulphoxides, azones, terpenes and pyrrolidones to increase the delivery efficacy of drug through the percutaneous route. Skin penetration enhancers induce structural changes in stratum corneum by disrupting the tightly packed lipid layer (Barry, 1991; Williams & Barry, 2012). Therefore, it causes undesired side effects

such as irritation, allergy or inflammation. Furthermore, skin penetration enhancers also cause the penetration of other small lipophilic compounds such as fragrance and preservatives along with the active ingredients through the skin (Dayan, 2005). Thus, encapsulation of drugs in liposomes and further incorporation of drug loaded liposomes into semisolid forms helps to overcome these problems. For example, development of an antifungal agent, Tolnaftate, loaded topical liposomal gel for the treatment of fungal diseases investigated by Meghana et al. (2014) shows that the developed gel formulation attributed to a higher permeation which leads to faster cure rate compared to the available marketed formulation. Besides that, Makky (2014), highlighted liposomal tazarotene gel for the treatment of psoriasis has resulted in higher drug retention and minimal skin irritancy compared to commercial tazarotene gel.

## 2.10 Liposomes in carbopol gel

In this study, the liposomes were incorporated into the carbopol gel (Figure 2.6). Carbopol has been used diversely for pharmaceutical and cosmeceutical application. Carbopol polymers are polymers of acrylic acid crosslinked with divinyl glycol (Puoci, 2014) and its IUPAC name is poly (acrylic acid).

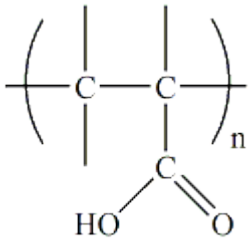



**Figure 2.6:** Molecular structure of carbopol

Carbopol is favored in the formulation of drug delivery system as these polymers readily absorb water, get hydrated and swell. As carbopol is dispersed in water, the solution will exhibit a low value of pH. For example, 1.0 % carbopol dispersion recorded a pH range of 2.5 - 3.0 whereas 0.5 % carbopol dispersion gives a pH range of

2.7-3.5. Neutralizing agents such as sodium hydroxide, potassium hydroxide, or triethanolamine is added to increase the pH of the polymer dispersion. Increasing the pH until the pH 5 until causes the viscosity of the solution to increase rapidly. At pH 5, a plateau viscosity is achieved. The system remains at this viscosity through pH of 10 (Sheng, 2010).

Upon addition of a neutralizing agent, ionization of the carboxylic groups on the polymer backbone results in electrostatic repulsion of the chain segment. Uncoiling of the polymer chains occur which adds to the swelling of the polymer. Carbopol polymers are very well suited for topical dosage forms as they are safe and effective (Guo, 2003). Products consist of carbopol polymers such as anti-inflammatory, anti-allergic and analgesic gel, with a wide range of viscoelasticity and flow behavior have been successfully formulated and commercialized. Carbopol polymers have been used in a variety of commercial formulations containing active pharmaceutical ingredients such as lidocaine, benzoyl peroxide, nystatin, fluorouracil and many more.

Topical formulations containing carbopol exhibit minimal irritancy and non-sensitizing properties after being applied on the skin. Also, carbopol polymers have a wide range of buffering capacity that enables the preparation of gel with desired pH (Merclin et al., 2004). Besides the excellent properties of carbopol gel, it has also been demonstrated that the derivatives of acrylic acid are compatible with liposomes (Reza et al., 2003). Considering all the above mentioned, liposome vesicle embedded into carbopol gel matrix could be a potential candidate as drug delivery vehicle for topical application.

## 2.11 Rheology of gel and its topical application

Rheology is a study of the deformation of matter resulting from the application of force (Goodwin & Hughes, 2008). Rheology is a commonly used technique to characterize the flow and viscoelastic behavior polymeric gel. These behaviors are important as it predicts on the deformability, spreadability and stability of topical gel formulation (Brummer, 2006; Brummer & Godersky, 1999). The viscoelastic behavior of the gel can be studied from the amplitude and frequency test. Few important parameters such as the linear viscoelastic region (LVR) and critical strain ($\gamma_c$) reflects the flexibility of the gel whereas, elastic modulus (*G'*) and cohesive energy (CE) shows the elasticity and cohesiveness of the gel. The high values of *G'* and $\gamma_c$ show high rigidity due to the presence of strong internal gel network. A high $\gamma_c$ value indicates that the gel is more resistant to deformation.

Flow property such as gel viscosity influences the spreadability of the gel. The gel which is highly viscous may have poor spreadability and this phenomenon reduces the homogeneity of liposome's dispersion in the skin (Garg et al., 2002; Ueda et al., 2009). Thus, the treatment consistency at the targeted sites is affected. This consequently affects the diffusion rate of gel into the skin. Moreover, incorrect dosage of drug may have been transferred due to the poor consistency of gel (Glavas-Dodov et al., 2003; Ivens et al., 2001). On the other hand, a gel that exhibits low viscosity may not be stable as the gel is not strong enough to trap the dispersed liposomes. Thus, a suitable concentration of carbopol is required for the preparation of liposomal gel with desired viscosity. Besides that, skin feel upon application also could be evaluated from the rheological data. For topical application, the sensation on the skin upon gel application can be studied from the shear thinning of the gel. The gel which exhibits shear thinning behavior allows even spreading onto the skin for optimum adsorption of the loaded carrier or drug (Brummer, 2006; Jelvehgari & Rashidi, 2007).

# CHAPTER 3: METHODOLOGY

## 3.1 Materials

1,2-dipalmitoyl-sn-glycero-3-phospocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phospocholine (DSPC), lecithin (LEC) and diethylaminoethyl-dextran (DEAE-DX) (average molecular weight of 500 000, degree of substitution corresponds to one DEAE-substituent per three glucose units) were purchased from Sigma-Aldrich (St. Louis, USA). Diphenhydramine hydrochloride (DPH) was purchased from Cayman Chemical (Michigan, USA). Chloroform of Emprove grade, curcumin and triethanolamine (TEA) were purchased from Merck. Carbopol 934 was a general gift from Corel Pharma Chem (Ahmedabad, India). All samples were prepared using deionized water with a resistivity of 18.2 Ω cm.

## 3.2 Methods

### 3.2.1 Surface tension analysis

LEC, DPPC and DPPC 0.5 % (w/v) were dissolved in chloroform respectively. The solutions were dried under reduced pressure by using rotary evaporator (Model Buchi Rotavapor R114, Switzerland) which results in the formation of thin layer lipid film on the wall of the flask. The thin film was hydrated and gently shaken at temperature of 55 ºC using warm deionized water. The surface tension of LEC, DPPC and DSPC solutions with varying concentration after an appropriate dilution was determined respectively by Du Noüy ring method using Force Tensiometer (Model Sigma 702, Finland) at 30.0 ºC. The critical vesicular concentration (CVC) was determined from the intersection point of a graph of surface tension as a function of natural logarithm concentration of lipid solution. Similar procedures were applied to measure the surface tension of mixture composing LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX respectively, while keeping the DEAE-DX concentration constant and varying the lipid

concentration. On the other hand, the effect of increasing DEAE-DX concentration from 0.02 % (w/v) to 0.08 % (w/v) on the surface behavior of the lipid was also investigated.

### 3.2.2 Preparation of LEC liposome

LEC liposomes were prepared at a concentration of 0.4 % (w/v). LEC was dissolved in chloroform and the solution was dried under reduced pressure by using rotary evaporator (Buchi, Switzerland) which results in the formation of a thin layer of lipid film on the wall of the flask (Figure 3.1). For curcumin encapsulated liposome, concentration of curcumin ranging from 0.01 % (w/v) to 0.09 % (w/v) was dissolved in chloroform together with soy lecithin 0.4 % (w/v).

(a) (b)

**Figure 3.1:** Formation of thin film for (a) DPH encapsulated liposome (b) Curcumin encapsulated liposome.

Then, warm deionized water of 5 ml was added to the flask. As for DPH encapsulated liposomes, the dry films LEC liposomes were hydrated with 5 ml of DPH solution with a concentration ranging from 0.03 % (w/v) to 0.19 % (w/v) respectively. The solutions were subjected to sonication using bath type sonicator to obtain uniformly sized particles. The similar procedure was applied for preparation of 0.4 % (w/v) DPPC and DSPC liposome solution respectively.

### 3.2.3 Preparation of LEC-DEAE-DX liposome

Liposomes coated with various concentration of DEAE-DX were prepared by slowly adding LEC liposomes of 0.4 % (w/v) into equal volume of DEAE-DX solution (0.01, 0.02, 0.03, 0.04 and 0.05 % w/v) under magnetic stirring. The LEC-DEAE-DX mixture solution was stirred for approximately 1 hour at room temperature. The resulting mixture solution contains 0.2 % (w/v) of LEC and 0.005, 0.01, 0.015, 0.02 or 0.025 % w/v DEAE-DX respectively. Similarly, LEC-DEAE-DX liposomes encapsulated curcumin or DPH were prepared by adding the liposome solution into equal volume of DEAE-DX solution. The final concentration of curcumin ranges from 0.005 % (w/v) to 0.045 % (w/v) and DPH ranges from 0.015 % (w/v) to 0.095 % (w/v). The similar procedure was applied for preparation of DPPC-DEAE-DX and DSPC-DEAE-DX.

### 3.2.4 Transmission electron microscopy (TEM)

The morphology of uncoated and DEAE-DX coated liposomes after a storage period of 5 days were obtained using a 200 kV TEM (Model JEOL JEM-2100F, Japan). A drop liposomal suspension was placed onto a carbon-coated copper grid, and the excess solution was drawn off with filter paper. It was then negatively stained using phosphotungstic acid solution 2 % (w/v) and air-dried at room temperature. The grid was then ready to be examined under TEM.

### 3.2.5 Particle size and zeta potential measurement

The average hydrodynamic particle size and zeta potential of the liposome and DEAE-DX coated liposome solutions were measured using Malvern NanoSeries Zetasizer (Malvern Instrument, UK) at a constant temperature of 25 ºC. The stability of the uncoated and DEAE-DX coated liposomes were monitored over a period of 35 days at room temperature. The size and zeta potential measurements were measured in triplicates and the average value was reported.

### 3.2.6. Differential scanning calorimetry (DSC)

Thermal behaviors of uncoated and DEAE-DX coated liposomes after freeze drying were characterized by using Pyris 6 DSC (PerkinElmer, USA). Approximately 5 mg of sample was weighed into an aluminum sample pan. An empty pan was used as a reference. The heating run was performed from 25 ºC to 100 ºC with a heating rate of 6 ºC /min.

### 3.2.7. Encapsulation efficiency of DEAE-DX coated liposome

The percentage of curcumin and DPH incorporated in liposomes was determined by centrifuge method (Model Eppendorf 5804R Centrifuge). The drug loaded liposome was centrifuged at 10 000 rpm for 60 minutes in order to separate entrapped drug in the liposome from the free drug. The absorbance of free drug in the clear supernatant with appropriate dilution was determined by ultraviolet-visible (UV-Vis) spectrometer (Cary 50 UV-Vis spectrometer, Agilent Technologies, USA) at a wavelength of 425 nm for curcumin and 258 nm for DPH respectively. The concentration of curcumin was determined from the calibration curve (1 µg/ml – 6 µg/ml)(Figure 3.2) whereas the concentration of DPH was determined from the calibration curve (0.01 mg/ml – 0.1 mg/ml)(Figure 3.3). The encapsulation efficiency of liposomes was calculated as in equation 1.

$$EE\ (\%) = \frac{T-S}{T} \times 100\ \% \qquad \text{(Equation 1)}$$

Where EE is the Encapsulation efficiency, T is the theoretical concentration of drug added and S is the concentration of drug detected in the supernatant.

**(a) Preparation of calibration curve of curcumin**

A stock solution of 10 µg/ml of curcumin solution was prepared by dissolving 500 µg of curcumin in 25 ml ethanol and then made up to 50 ml in a volumetric flask. After that, a series of curcumin solution with the concentration of 6 µg/ml, 5 µg/ml, 4 µg/ml,

3 µg/ml, 2 µg/ml and 1 µg/ml were prepared by diluting the curcumin stock solution. The prepared curcumin solutions were measured spectrophotometrically by using Varian UV-visible spectrophotometer model Cary 50 (Agilent Technologies, US) at the wavelength of 425 nm and the standard concentration curve of curcumin was plotted (Figure 3.2)

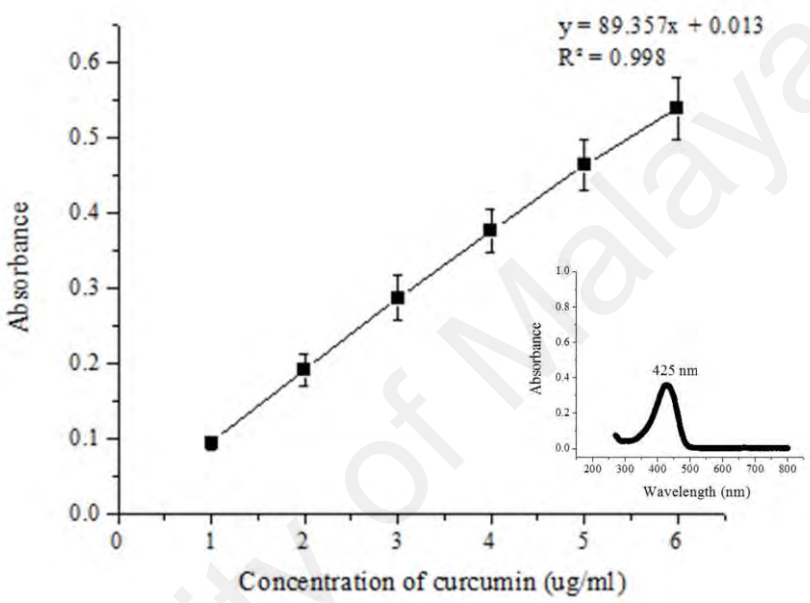



**Figure 3.2:** Calibration curve of curcumin in ethanol and UV-Vis absorption spectrum (inset).

**(b) Preparation of calibration curve of DPH**

A stock solution of 0.1 mg/ml of DPH solution was prepared by dissolving 5 mg of DPH in 25 ml deionized water and then made up to 50 ml in a volumetric flask. After that, a series of DPH solution with the concentration of 0.01 mg/ml, 0.02 µg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml and 0.1 mg/ml were prepared by diluting the DPH stock solution. The prepared DPH solutions were measured spectrophotometrically by using Varian UV-visible spectrophotometer model Cary 50 (Agilent Technologies, US) at the wavelength of 258 nm and the standard concentration curve of DPH was plotted (Figure 3.3)

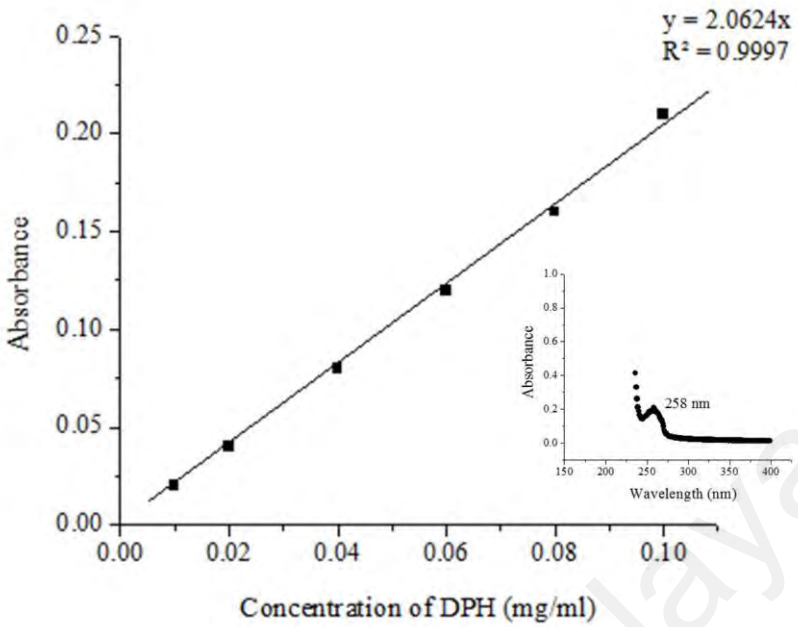



**Figure 3.3:** Calibration curve of DPH in deionized water and UV-Vis absorption spectrum (inset).

### 3.2.8 Preparation of gel

Gel was prepared using carbopol 934 (0.1, 0.15, 0.25, 0.5 and 1.0 % w/v). The appropriate quantity of carbopol 934 powder was dispersed into distilled water by stirring for 24 hours at room temperature. Then, the mixture was neutralized by dropwise addition of 5 % w/v triethanolamine (TEA). Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 7.4. The obtained gels were kept at room temperature for 24 hours before the rheological studies. The gel with desired rigidity and elasticity was selected for the preparation of liposomal gel.

### 3.2.9 Preparation of liposomal gel

Liposomal gel formulations were prepared by dispersing the prepared liposomes (non-coated or DEAE-DX coated liposomes) into the selected gel described previously under constant stirring with a glass rod. The similar procedure was applied for the preparation of DPH or curcumin loaded liposomal gel. The liposomal gels prepared consist of 0.2 %

of liposomes in the gel. The liposomal gels were stored at room temperature for 24 hours before rheological study.

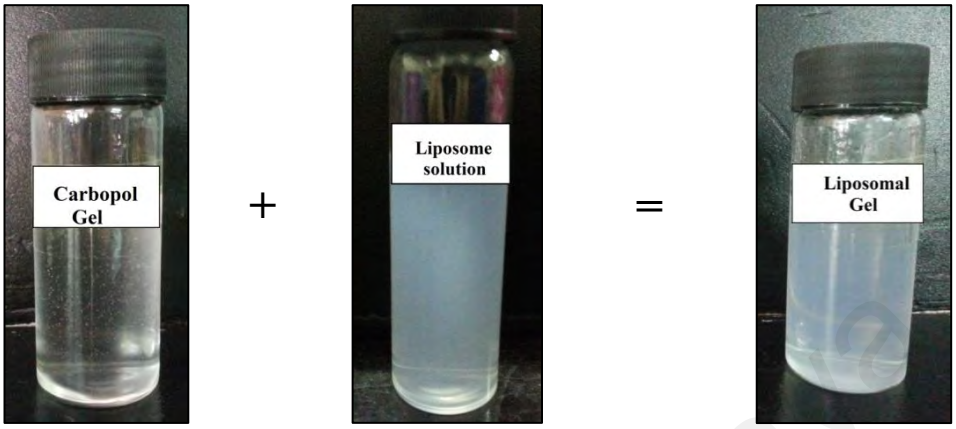



**Figure 3.4:** Preparation of liposomal gel.

### 3.2.10 Morphological study of liposomal gels

The distribution of liposomes in the selected gel system was observed using TEM (Model LIBRA 120, Germany). The liposomal gel was placed onto a carbon-coated copper grid. It was then negatively stained using phosphotungstic acid solution 2 % (w/v) and air-dried at room temperature. The grid was then ready to be examined under TEM.

### 3.2.11 Rheological measurement

Rheological properties of the gels were measured using a stress/rate controlled Bohlin Gemini CVO-R Rheometer (Malvern, UK) with a temperature controller. All of the measurements were performed at 25.0 ± 0.1º C with a 4º/40 mm cone and plate geometry, and a 0.100 mm gap. Firstly, dynamic oscillation measurement which consists of amplitude sweep test and frequency sweep test was carried out. In the amplitude sweep test, the gels were subjected to sinusoidal deformation at a constant frequency and resulting response stress was measured. As for frequency sweep test, the gel was subjected to a constant strain and the response stress was measured as a

function of frequency. Secondly, flow measurement was conducted in order to obtain the viscosity curve of the gels. All measurements were carried out in triplicates and the average values were reported.

(a) Dynamic oscillation measurement

An amplitude sweep was performed first with the frequency fixed at 0.5 and the applied strain ranging from 0.0001 to 1 unit. Then, a frequency sweep was performed in a controlled strain mode (a low deformation strain was chosen from the linear viscoelastic region (LVR) of the amplitude sweep) by varying the frequency from 0.2 to 20 Hz. The critical strain ($\gamma_c$), break point ($\gamma_b$), elastic modulus (*G'*), viscous modulus (*G"*) and cohesive energy (CE) of the gel were determined from their dynamic rheological behaviors in order to study the viscoelastic behavior of the gels (Figure 3.6). The cohesive energy (CE) of the gel is calculated as shown in the equation 2 (Sohm & Tadros, 1989).

$$CE = \frac{1}{2} G' \gamma_c^2 \qquad \text{(Equation 2)}$$

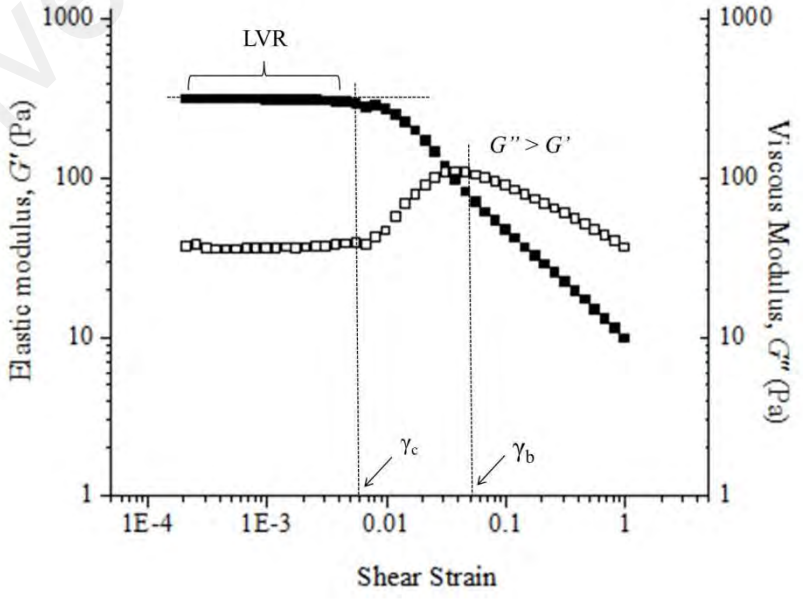



**Figure 3.5:** The variation of *G'* ( ■ ) and *G"* ( □ ) as a function of applied strain.

(b) Flow measurement

The flow behavior of the gels was measured at a controlled mode varying the shear rate from 0.0001 to 200 $s^{-1}$. The flowability of the gels was indicated from the yield stress ($\sigma_p$) value determined from the shear stress versus shear rate curve. Also, the shear viscosity profiles were fitted with Power-Law model in order to determine the degree of shear thinning as shown in the equation 3:

$$\eta = k\dot{\gamma}^{n-1} \quad \text{(Equation 3)}$$

Where $\eta$ is the shear viscosity of the gel, $k$ is the consistency index, $\dot{\gamma}$ is the shear rate and $n$ is the Power Law Index (PLI). The range of PLI varies from '0' for very shear thinning materials to '1' for Newtonian materials. The numerically equal value of the consistency 'k' is the viscosity at 1.

(a)

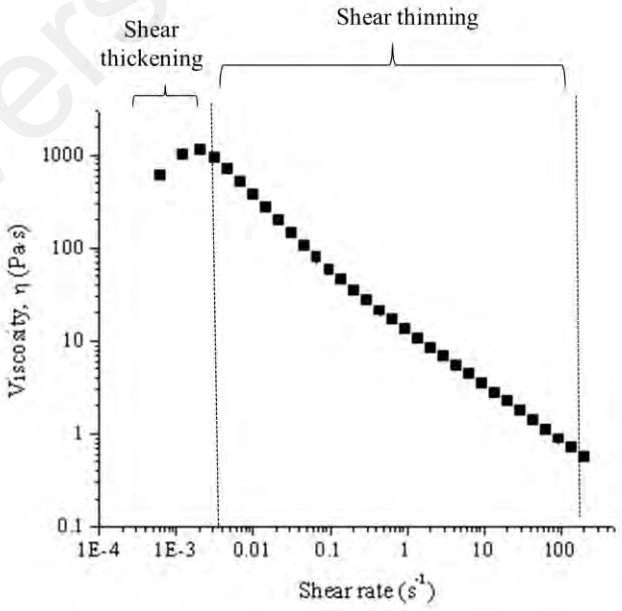



**Figure 3.6:** (a) A typical viscosity curve showing shear thickening at low shear rate and shear thinning at high shear rate and (b) Yield stress ($\sigma_p$) determination from shear stress versus shear rate curve.

(b)

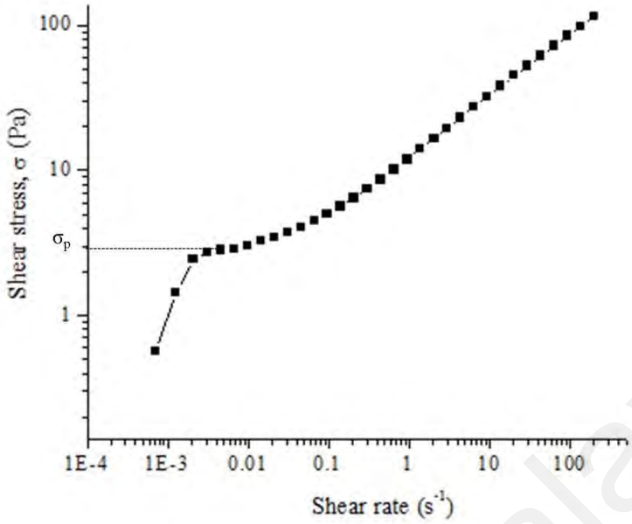



**Figure 3.6:** (continued)

### 3.2.12 *In Vitro* drug release

In vitro drug release of uncoated, DEAE-DX coated liposomes and liposomal gel was evaluated by using Automated Franz Diffusion Cell System (Microette Autosampling System, Hanson Research Co., USA) with 0.636 $cm^2$ of effective diffusion area. In order to maintain the sink condition, 0.5 % Tween 80 with 20 % ethanol v/v in 10 mM PBS solutions (pH 7.4) was used as receptor medium for detection of curcumin (Chen et al., 2012) whereas 10 mM PBS solutions (pH 7.4) for detection of DPH.

The receptor medium was continuously stirred at a constant speed of 400 rpm and the temperature was equilibrated at 37 ± 1 ºC. In this experiment, regenerated cellulose membranes of 5000 Da molecular weight cut-off was sandwiched between the donor and receptor compartments. The membranes were pretreated by soaking in the receiving medium overnight before being mounted to Franz Diffusion Cells. Each sample of about 1 ml was introduced into the donor compartments. Receptor phase sample was withdrawn at predetermined intervals throughout 24 h experimental period

and was refilled with fresh receiving medium to maintain a constant volume. The absorbance of drug released at each interval was obtained using UV-spectrophotometer. The amount of drug release was determined from the calibration curves (Figure 3.7-Figure 3.8).

**(a) Preparation of calibration curve of curcumin**

A stock solution of 15 µg/ml of curcumin solution was prepared by dissolving 750 µg of curcumin in 25 ml of 0.5 % Tween 80 with 20 % ethanol v/v in 10 mM PBS solutions (pH 7.4) and then made up to 50 ml in a volumetric flask. After that, a series of curcumin solution with the concentration of 0.5 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml and 1 µg/ml were prepared by diluting the curcumin stock solution. The prepared curcumin solutions were measured spectrophotometrically by using Varian UV-visible spectrophotometer model Cary 50 (Agilent Technologies, US) at the wavelength of 425 nm and the calibration curve of curcumin was plotted (Figure 3.7)

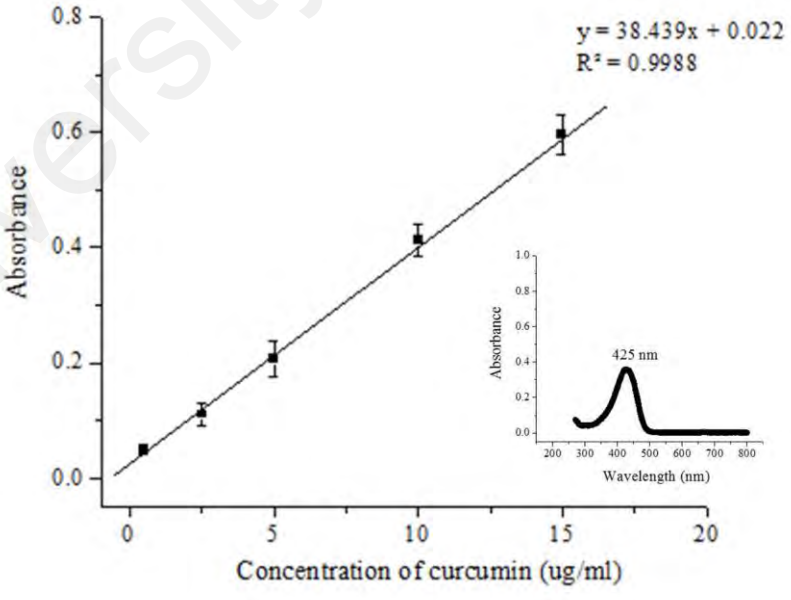



**Figure 3.7:** Calibration curve of curcumin in Tween 80/ethanol/PBS and UV-Vis absorption spectrum (inset).

**(b) Preparation of calibration curve of DPH**

A stock solution of 0.1 mg/ml of DPH solution was prepared by dissolving 5 mg of DPH in 25 ml of 10 mM PBS solutions (pH 7.4) and then made up to 50 ml in a volumetric flask. After that, a series of DPH solution with the concentration of 0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml and 0.1 mg/ml were prepared by diluting the DPH stock solution. The prepared DPH solutions were measured spectrophotometrically by using Varian UV-visible spectrophotometer model Cary 50 (Agilent Technologies, US) at the wavelength of 258 nm and the calibration curve of DPH was plotted (Figure 3.8)

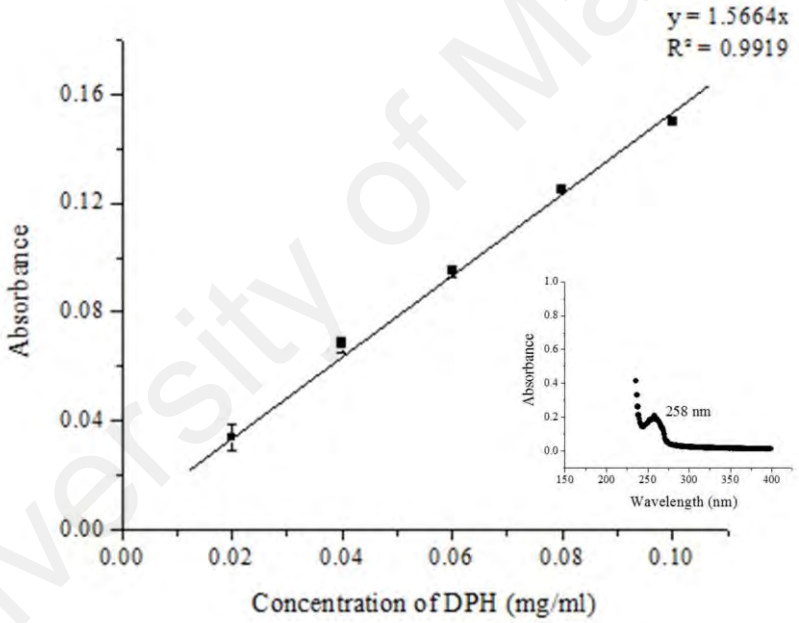



**Figure 3.8:** Calibration curve of DPH in PBS and UV-Vis absorption spectrum (inset).

### 3.2.13 Kinetic model evaluation

The *in vitro* release of DPH and curcumin from liposomal dispersion and liposomal gel was curve fitted to Zero-order, First-order, Higuchi and Korsmeyer Peppas models by DDSolver software to understand their release kinetics. DDSolver is a menu-driven adds-in program for Microsoft Excel that features built in mathematical models in its

library (Figure 3.9). Thus, the DDSolver program is used as a tool to facilitate the modeling, speed up the calculation, reduce user errors and also provide a convenient way of reporting data (Zhang et al., 2010).

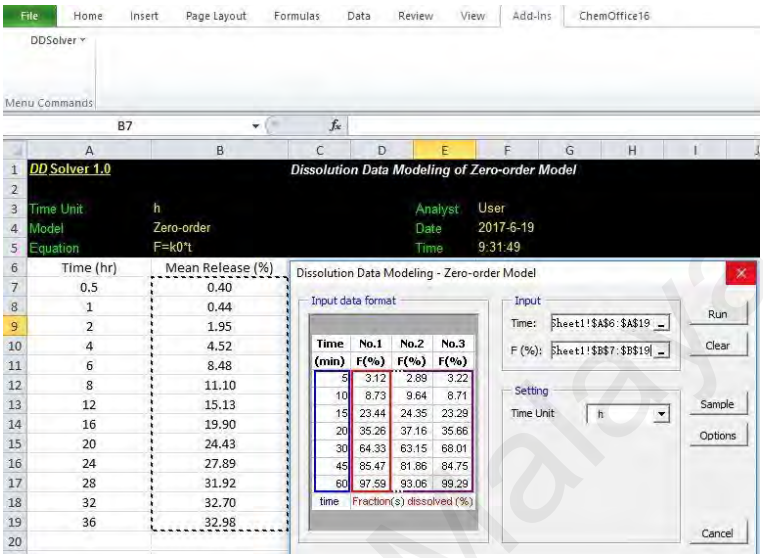



**Figure 3.9**: Interface of the DDSolver program.

Zero-order, First-order, Higuchi and Korsmeyer Peppas models that are implemented in the program were used to compare and study the drug release data obtained in this research. Table 1 summarizes the mathematical models whereby F is the percentage of drug released at time t. The release data obtained was fitted to the following models by quick generation of plots with fitted curves for each individual dataset.

**Table 3.1**: Model available in DDSolver for curve fitting drug release data.

| Model | Equation |
|---|---|
| Zero-order | $F = k_0 t$ |
| First-order | $F = 100(1 - e^{-k_1 t})$ |
| Higuchi | $F = k_H t^{0.5}$ |
| Korsmeyer-Peppas | $F = k_{KP} t^n$ |

# CHAPTER 4: RESULTS & DISCUSSION

## 4.1 Surface tension

A graph of surface tension (γ) versus ln [lipid] was plotted for the determination of critical vesicular concentration (CVC) of LEC, DPPC and DSPC solutions respectively (Figure 4.1). The result shows that surface tension of all liposomal solutions decreased with increasing the concentration of lipid until reaching a concentration whereby the surface tension deviate to a constant value. CVC is an important quantitative parameter determined via the inflection point from the γ profile. The value of CVC reflects the minimum concentration of lipid required in forming liposomes which also implies the tendency of lipid to self-assemble.

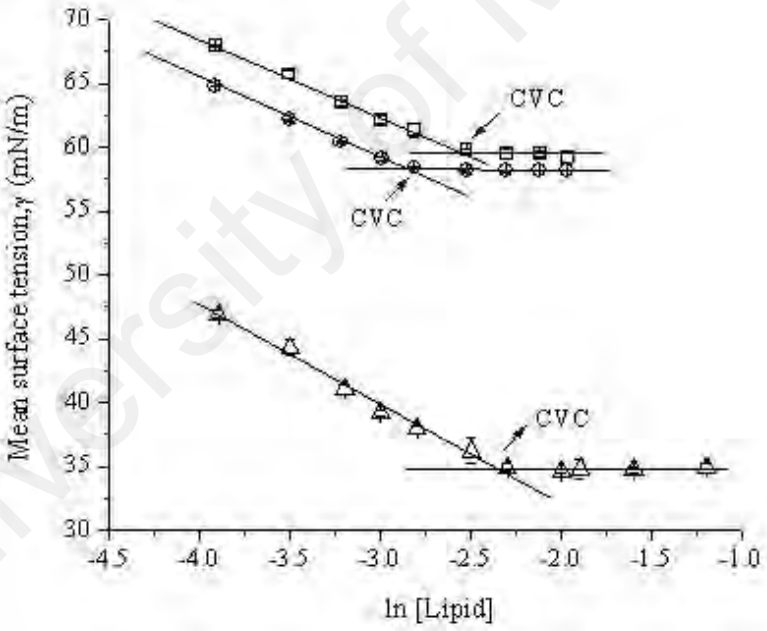



**Figure 4.1:** Surface tension profile LEC ( △ ), DPPC ( □ ) and DSPC ( ○ ) liposome solutions.

When lipids dissolved in water, they adsorb and orientate at the water surface as such that the hydrophobic regions are removed from the aqueous environment. The adsorption of lipid which replaces the water molecules at the surface causes the reduction of surface tension which arises from the reduction in contraction force at the

air/water surface due to a lower intermolecular force between lipid and water molecules compared to those between two water molecules (Attwood & Florence, 2012).

The CVC of LEC, DPPC and DSPC was obtained at 0.1 % (w/v), 0.08 % (w/v) and 0.06 % (w/v), respectively. DSPC which has longer alkyl chain poses stronger hydrophobic force thus lower the concentration of DSPC required to form liposomes (Zhang & Marchant, 1996). This study implies that LEC, DPPC and DSPC at a concentration above 0.1 (w/v), 0.08 % (w/v) and 0.06% (w/v), respectively tend to self-assemble into vesicles. Besides that, it is important to study the CVC of the liposome to avoid preparation of either too high or too low concentration of liposome solution for physicochemical studies (Ninham et al., 1983). The changes in the γ behavior of LEC, DPPC and DSPC after the addition of DEAE-DX were also studied (Table 4.1).

**Table 4.1:** Surface behavior of LEC, DPPC and DSPC after addition of DEAE-DX.

| **Concentration of DEAE-DX (w/v %)** | **LEC** | | **DPPC** | | **DSPC** | |
|---|---|---|---|---|---|---|
| | Surface Tension (mN/m) | CVC (w/v %) | Surface Tension (mN/m) | CVC (w/v %) | Surface Tension (mN/m) | CVC (w/v %) |
| 0 | 34.9 | 0.1 | 59.7 | 0.08 | 58.2 | 0.06 |
| 0.02 | 42.9 | 0.1 | 63.7 | 0.05 | 63.4 | 0.04 |
| 0.04 | 50.3 | 0.09 | 64.3 | 0.04 | 64.9 | 0.03 |
| 0.08 | 51.8 | 0.05 | 64.8 | 0.04 | 65.0 | 0.03 |

It was observed that upon addition of DEAE-DX, γ of LEC, DPPC and DSPC have shown an increase. The changes in the γ profile were mainly attributed to the presence of DEAE-DX. When the DEAE-DX concentration was further increased from 0.02 % (w/v) to 0.08 % (w/v), the surface tension increases slightly and the CVC value was found to be reduced for all the liposome solution. The obtained results suggested that LEC, DPPC and DSPC are bound to DEAE-DX respectively and this promotes the removal of lipid from the surface to the bulk phase. Therefore, in the presence of

DEAE-DX, less amount of lipid is required for the formation of vesicles. This phenomenon clearly explains the reason for the slight decrease of CVC upon addition of DEAE-DX.

Another plausible reason is that without the addition of DEAE-DX, the lipid monomers were more closely packed at the air /water interface. It can be deduced that in the presence of DEAE-DX, the arrangement of lipid monomers have been disrupted. Thus, leading to an increase in the area occupied at air/water interface which eventually causes the increase in the surface tension of the DEAE-DX and liposomal mixture. The results obtained from the DSC analysis in section 4.4 also supports the fact whereby arrangement of lipid monomers are disrupted by the presence of DEAE-DX and thus the $T_m$ obtained for DEAE-DX coated liposome is slightly lower than then non-coated liposomes.

### 4.2 Particle size of DEAE-DX coated liposome

The effects of adding DEAE-DX ranging from 0.005 % (w/v) to 0.025 % (w/v) on the hydrodynamic diameter of LEC, DPPC and DSPC liposomes were investigated (Figure 4.2). Concentrations of the lipids were fixed at 0.2 % (w/v) which is above their respective CVC value. The particle size of uncoated LEC liposomes was found to be (85 ± 1) nm with polydispersity index 0.3, particle size of DPPC liposomes was found to be (99 ± 2) nm with polydispersity index 0.4, while particle size of DSPC liposomes was (72 ± 1) nm with polydispersity index 0.2.

In overall, DSPC liposomes have smaller particle size compared to LEC and DPPC liposomes. It is expected that DSPC with additional of two carbon in the alkyl chain promotes a higher flexibility in the molecule. Thus, leading to the formation of more fluidic lipid bilayer which corresponds to a lesser bending rigidity (Park et al., 2011). Convolution of this dynamics encourages the formation of liposomes with higher curvature and hence smaller size. Coating of the LEC liposomes with 0.005 % (w/v)

DEAE-DX resulted in an increase of the hydrodynamic diameter of the liposome to (105 ± 2) nm with a polydispersity index of 0.4. Further increase in the concentration of DEAE-DX to 0.02 % (w/v) attributed to a significant increase in the liposomal size from (105 ± 2) nm to (195 ± 6) nm with a polydispersity index of 0.4. This might be due to the ionic interaction between negatively charged LEC liposome and the positively charged polymer.

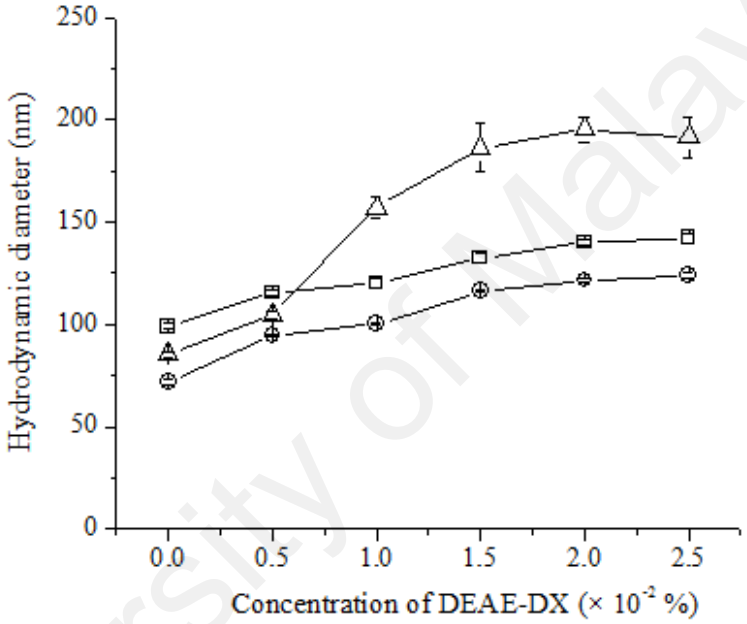



**Figure 4.2:** Variation in the size of DEAE-DX coated LEC ( △ ), DPPC ( □ ) and DSPC ( ○ ) liposomes with varying concentration of DEAE-DX.

On the other hand, the addition of DEAE-DX up to 0.020 % (w/v) resulted in an increase of the hydrodynamic diameter of the DPPC and DSPC liposome to (140.1 ± 0.6) nm and (122 ± 1) nm with polydispersity index 0.4 respectively. The increase in the size of liposome indicated the interaction between DEAE-DX and liposomes. However, upon addition of 0.025 % (w/v) of DEAE-DX, there was no significant change in the liposomal size compared to liposome coated with 0.02 % (w/v) DEAE-DX. This indicated that surface of liposome was saturated with DEAE-DX.

### 4.3 Zeta potential of DEAE-DX coated liposome

Zeta potential is a parameter that has been used to investigate the potential stability of the colloidal system. Particles in a suspension with larger magnitude of zeta potential have a greater tendency of repulsion between particles. Thus, more stable is the suspension (Mady et al., 2009). The variation in the magnitude of the zeta potential of LEC, DPPC and DSPC liposomes after coating with DEAE-DX is shown in Figure 4.3.

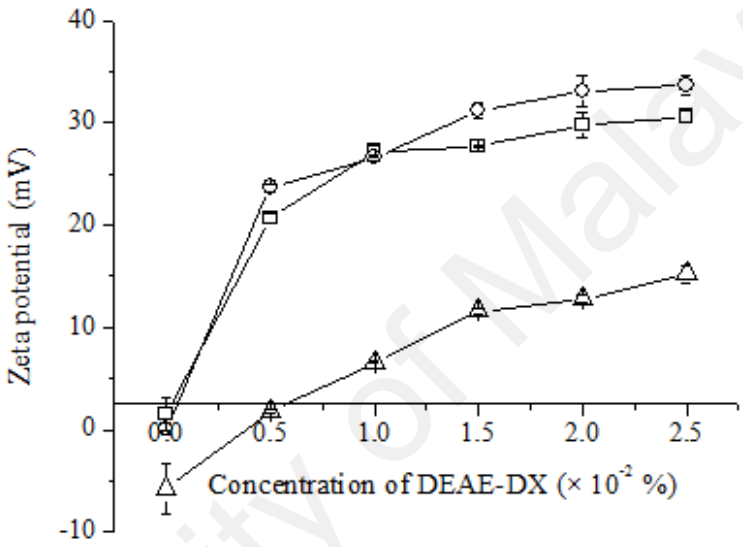



**Figure 4.3:** Variation in zeta potentials of DEAE-DX coated LEC (△ ), DPPC ( □ ) and DSPC ( ○ ) liposomes with varying DEAE-DX concentration.

Coating of the LEC liposomes with DEAE-DX resulted in a significant shift of zeta potential values from negative to positive. The uncoated liposomes had a negative zeta potential of -5.8mV; whereas 0.005, 0.01, 0.015, 0.020 and 0.025 % (w/v) DEAE-DX coated liposome had positive zeta potentials of +1.82, +6.58, +11.6 and +15.3 mV, respectively. The shift from negative to positive zeta potentials indicated the successful coating of the positively charged DEAE-DX on the surface of negatively charged lecithin liposome. On the other hand, DPPC and DSPC which are pure lipids have a relatively lower magnitude of zeta potential compared to LEC. However, addition 0.005

% (w/v) DEAE-DX to DPPC and DSPC liposomes resulted in a significant increase of zeta potential value to +20.7 mV and +23.7 mV, respectively.

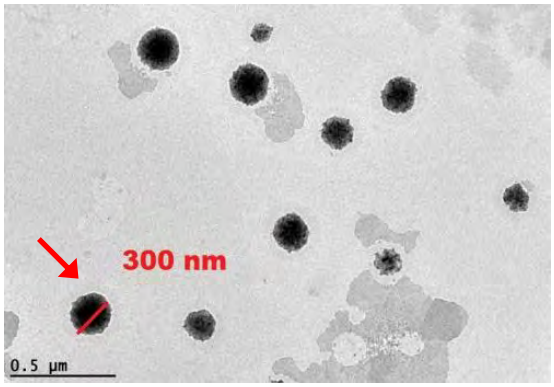
The tremendous increase in the magnitude of zeta potential after addition of DEAE-DX explains the successful coating of positively charged DEAE-DX on the surface of the lipids. It was also observed that as the concentration of DEAE-DX increases to 0.02 % (w/v), the magnitude of the zeta potential of DPPC and DSPC liposomes also increases to +30.6 mV and +33.7 mV, respectively. Further increase of DEAE-DX concentration to 0.025 % (w/v) did not significantly modify the magnitude of zeta potential for both types of liposomes significantly. In comparison, DSPC liposomes have a slightly higher magnitude of zeta potential compared to DPPC liposomes. This result indicated that DSPC liposomes which are of smaller particle size have greater mobility and they present greater steric repulsion than DPPC liposomes.

### 4.4 Evaluation of stability of uncoated and DEAE-DX coated liposome

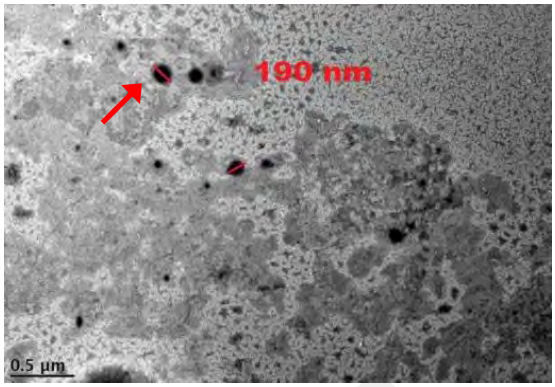
The entire sample was kept for a period of 5 days before analysis. Surface morphological studies on the shape of LEC, DPPC and DSPC liposome indicated that the particles were almost spherical. The uncoated LEC, DPPC and DSPC liposome [Figure 4.4 (a), (c) and (e)] were larger in size compared to the DEAE-DX coated liposome [Figure (b), (d) and (f)] under the same preparation condition (Figure 4.4).

The increase in size indicated the aggregation of the particles within a short period of time. The morphological behavior of the liposomes was in agreement with the result obtained from the stability of particle size measurement (Figure 4.5) in which the uncoated liposome exhibited an increase in size compared to the coated liposome after 35 days of evaluation. This phenomenon is due to the low repulsion force between the uncoated liposomes which has accelerated the aggregation process. On the other hand, DEAE-DX coated liposomes will tend to repel each other, thereby discouraging aggregation and providing a more stable colloidal dispersion.

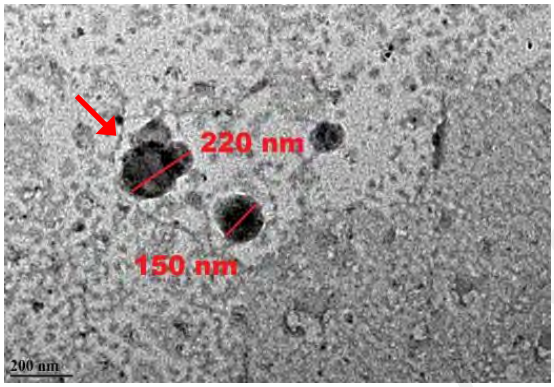
(a)



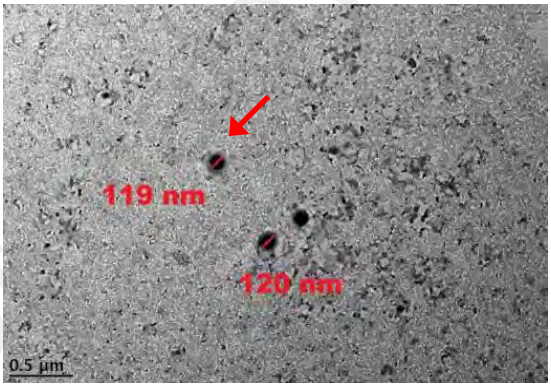

(b)



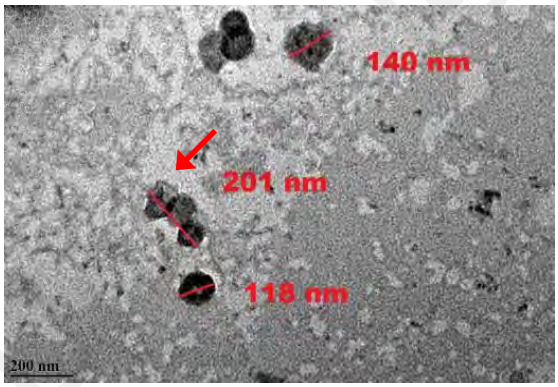

(c)




(d)




(e)




(f)

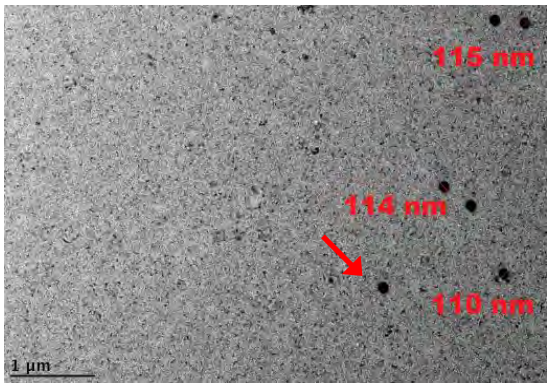
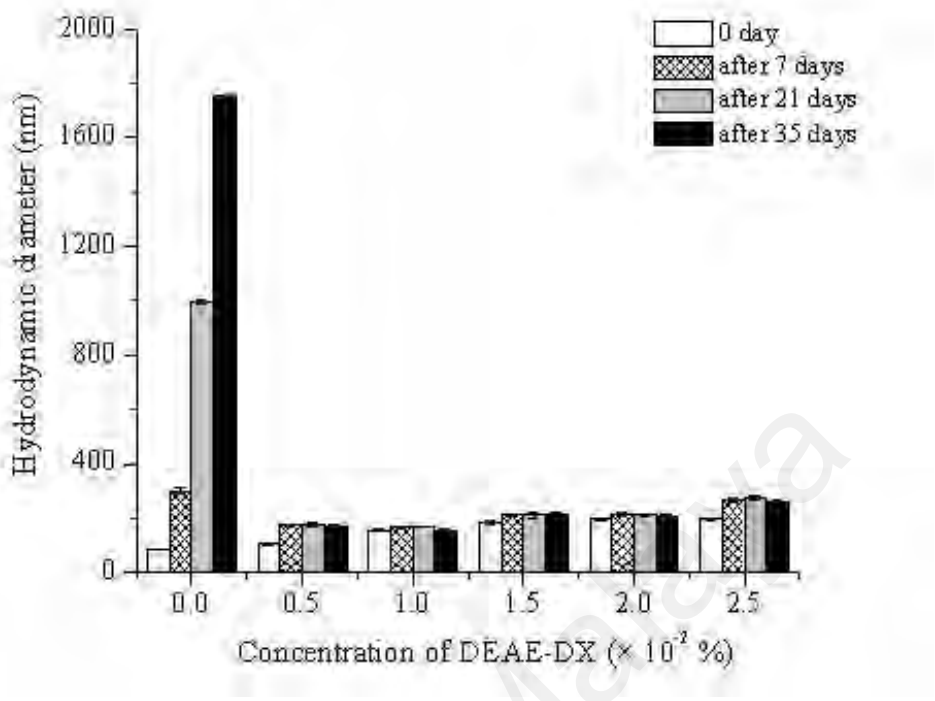



**Figure 4.4:** TEM micrographs of liposomes (a) uncoated LEC liposomes, (b) 0.02 % (w/v) DEAE-DX coated LEC liposomes, (c) uncoated DPPC liposomes, (d) 0.02 % (w/v) DEAE-DX coated DPPC liposomes, (e) uncoated DSPC liposomes, (f) 0.01 % (w/v) DEAE-DX coated DSPC liposomes.

The change in particle size of uncoated and DEAE-DX coated liposomes were analyzed in order to investigate the colloidal stability of these liposomes which were kept at room temperature over a period of 5 weeks (Figure 4.5). All the uncoated liposomes have shown a drastic increase in particle size within the storage period. The obtained result explains the aggregation of the particles into a cluster at a significant rate thus exhibiting larger size along with time. After 35 days of evaluation, the particle size of uncoated LEC liposome continued to increase from (85 ± 1) nm to (1752 ± 1) nm, uncoated DPPC liposome increased from (99 ± 2) nm to (721 ± 4) nm and uncoated DSPC liposome increased from (72 ± 1) nm to (451 ± 1) nm. The drastic increase in size may be explained by the aggregation of particles which was indicated by low zeta potential measurement obtained for uncoated liposomes.

This phenomenon clearly shows that uncoated LEC, DPPC and DSPC liposomes were not stable. However, it was observed that the extent of particle size increment in DSPC liposome is slightly lower compared to LEC, DPPC liposomes over a period of 35 days. This could be due to the nature of DSPC liposome which has smaller size thus moving rapidly in the solution and hence lower tendency to form aggregates compared to DPPC liposome. The particle size of coated liposome displayed very little change throughout 35 days of evaluation. DEAE-DX coated liposome was observed to be more stable as the variation in particle size was not obvious as compared to the uncoated liposomes. On coating the liposomes with DEAE-DX, strong mutual repulsion between the adjacent bilayers arise, which improves their stability.

(a)




(b)

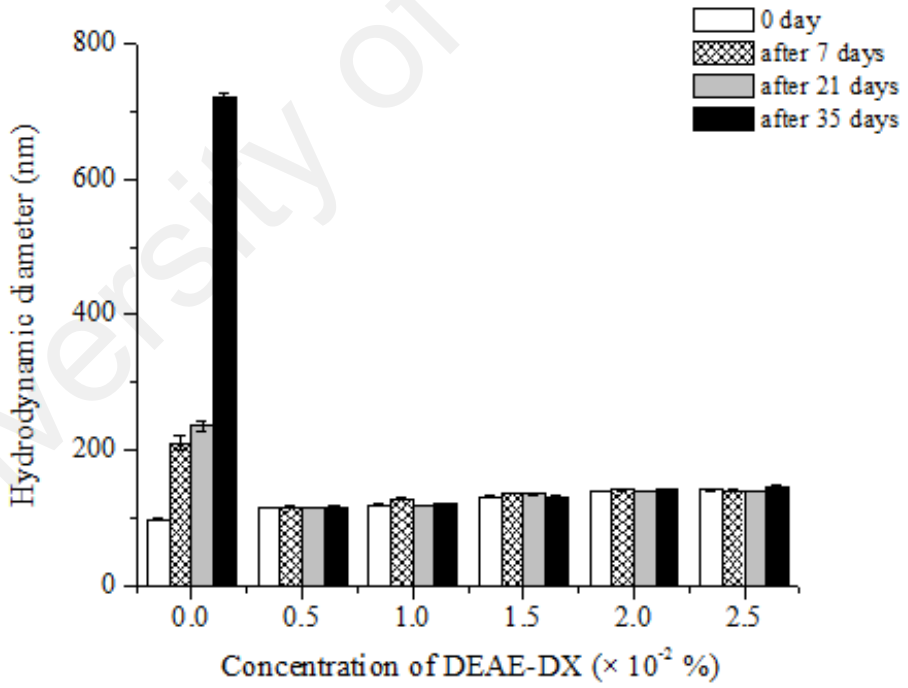



**Figure 4.5:** The influence of DEAE-DX concentration on the size of (a) LEC, (b) DPPC and (c) DSPC liposomes over a period of 35 days.

(c)

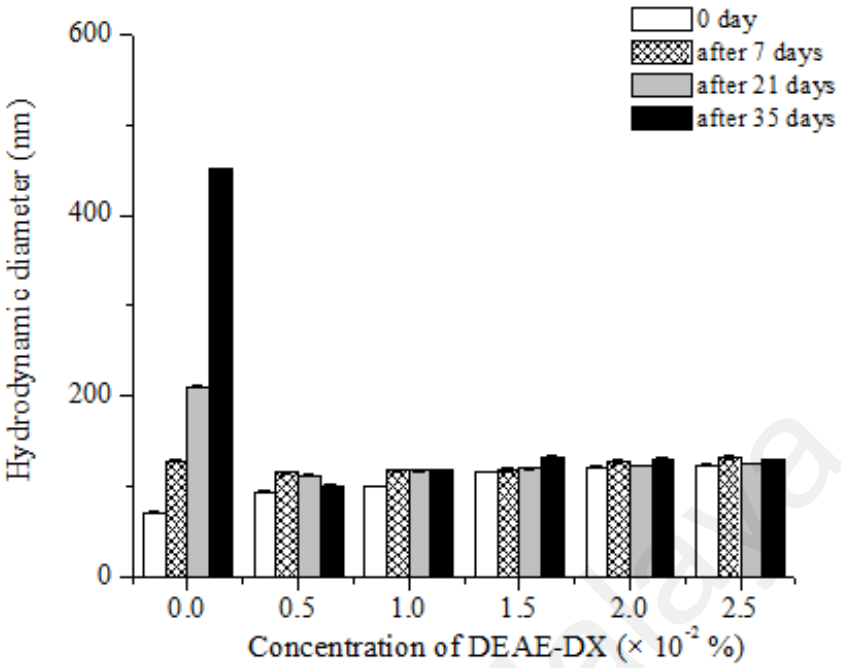



**Figure 4.5:** (continued)

Also, the coated liposomes had a polydispersity index of less than 0.4 whereas the uncoated liposome had a polydispersity index of 0.9 after 35 days of evaluation. The high polydispersity index of uncoated liposome shows that the sample may contain large particle or aggregates that could be slowly sedimenting (Figure 4.6). This phenomenon proves that DEAE-DX coating could enhance the colloidal stability of LEC, DPPC and DSPC liposomes.

(a) (b)

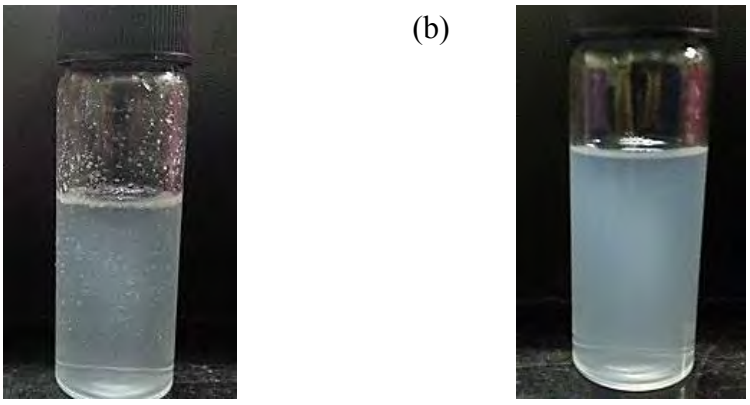


**Figure 4.6:** (a) Non-coated liposome solution and (b) DEAE-DX coated liposome solution after 35 days of storage at room temperature.

Figure 4.7 demonstrated the changes of the zeta potential of uncoated and DEAE-DX coated liposomes which were monitored over 35 days. The larger magnitude of zeta potential indicated the increase in stability of the colloidal system. As the zeta potential increases, the greater the repulsion between particles and this phenomenon contributes to a more stable colloidal dispersion (Park et al., 2014). It has been stated elsewhere that particles in a suspension with large negative or positive zeta potentials will have low tendency to form aggregates. Herein, a relatively lower magnitude of zeta potential was observed for uncoated LEC, DPPC and DSPC liposomes and its value fluctuated over the storage period.

(a)

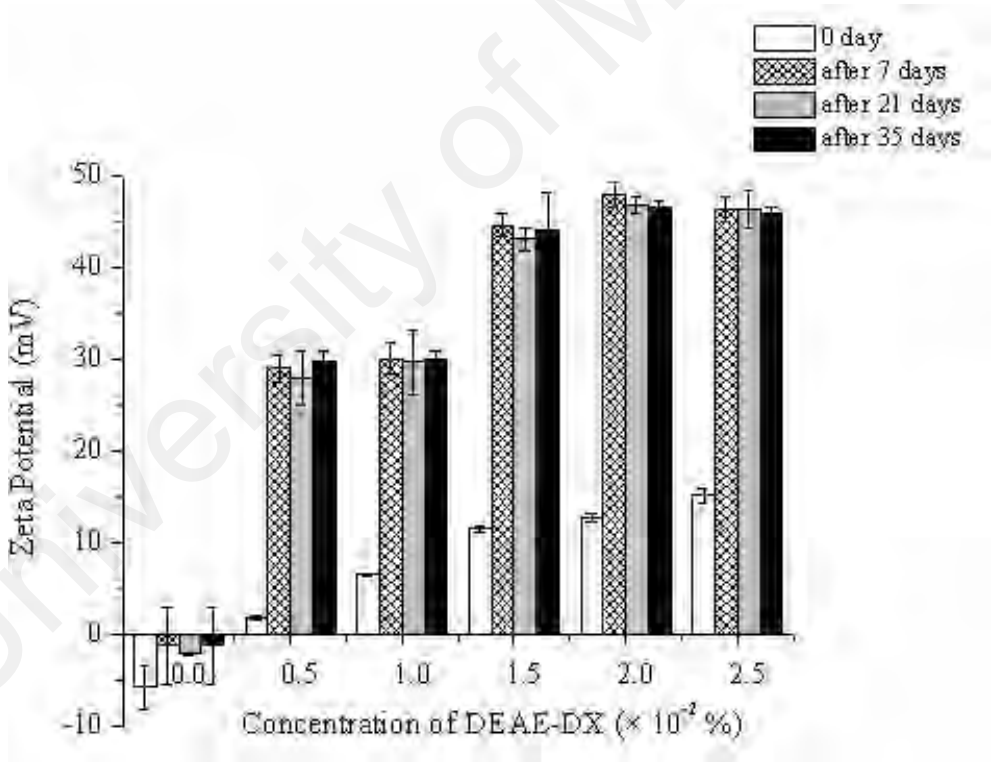
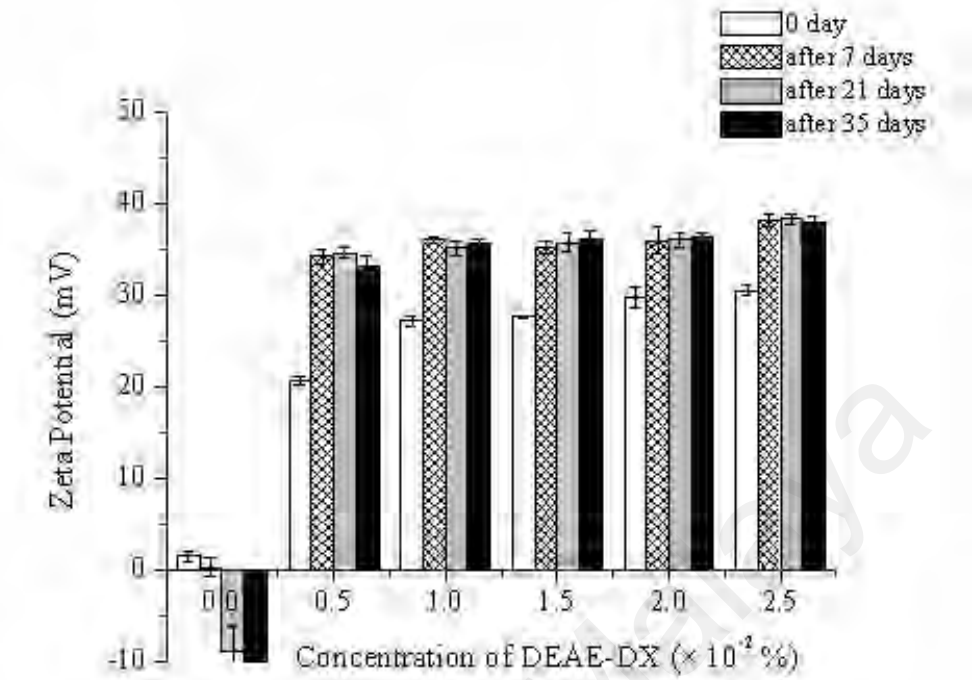



**Figure 4.7:** The influence of DEAE-DX concentration on zeta potential of (a) LEC, (b) DPPC and (c) DSPC liposomes over a period of 35 days.

(b)




(c)

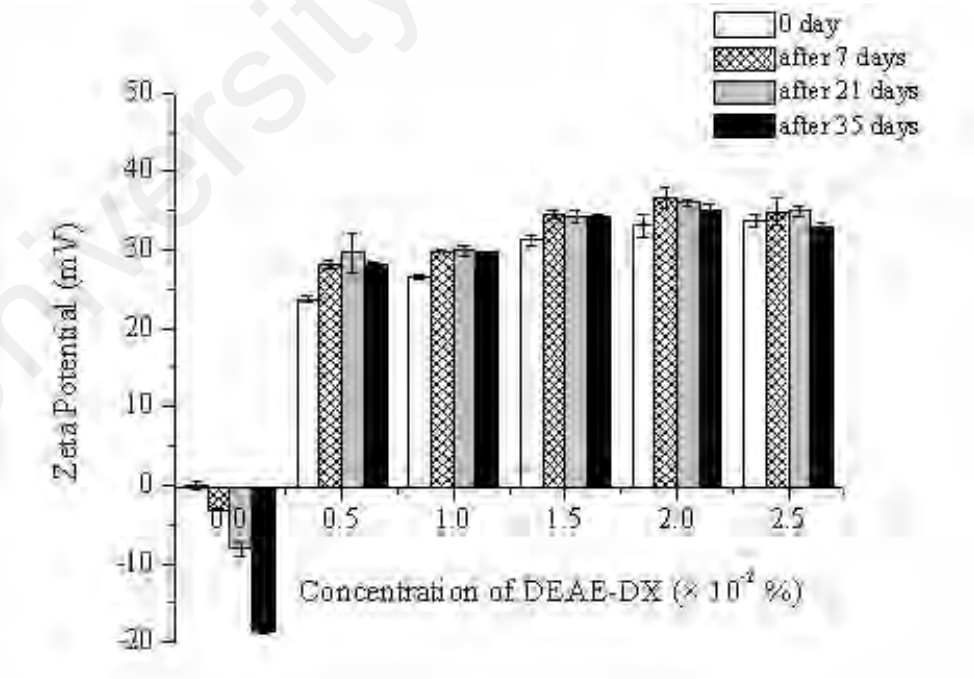



**Figure 4.7:** (continued)

On the other hand, the zeta potential of DEAE-DX coated LEC, DPPC and DSPC liposomes was observed to increase after 7 days of storage and was stabilized after 35 days of evaluation. This phenomenon is due to the adsorption of polymer on the liposome from solutions (Reboiras et al., 2001). Hence, the coiling and uncoiling of the DEAE-DX polymer on the surface of liposome during the first 7 days may explain the increase in zeta potential. After 7 days, the adsorption of the polymer onto the surface of liposome achieved equilibrium and therefore a constant zeta potential was observed from day 7 to day 35.

Besides that, the coated LEC, DPPC and DSPC liposomes had a larger magnitude of zeta potential compared to the uncoated liposomes. Thus, these particles have greater repulsion between particles which contributes to a lower tendency of aggregation. Therefore, the increase of magnitude in zeta potential value by DEAE-DX coating on liposomal surface attributed to the improvement in the stability of the liposomes. This phenomenon supports the particle size results over 35 days in which the coated liposomes exhibited little variation in particle size.

In overall, 0.02 % (w/v) DEAE-DX coated LEC liposome, 0.02 % (w/v) DEAE-DX coated DPPC liposome and 0.01 % (w/v) DEAE-DX coated DSPC liposome were observed to be the most stable dispersion as they show very little changes in particle size and zeta potential over a period of 35 days. Thus, this formulation was chosen to study the encapsulation efficiency and drug release.

### 4.5 Thermal behavior

Thermal analysis using differential scanning calorimetry (DSC) was carried out to show the interaction of DEAE-DX with lipid. All of the coated liposomes show a lower transition temperature ($T_m$) compared uncoated liposomes (Figure 4.8). This might be due to the presence of DEAE-DX which disrupt the packing of the phospholipids. This

results also indicated the interaction between lipid and DEAE-DX, therefore shows successful coating of DEAE-DX on the surface of liposomes.

The $T_m$ obtained for dehydrated LEC, DPPC and DSPC liposomes was 56.5 $^oC$, 65.6 $^oC$ and 78.5 $^oC$, respectively. In the absence of water, the spacing between the phospholipid headgroup decreases, thereby giving rise to increased van der Waals interaction between lipid hydrocarbon chain (Ohtake et al., 2006). Thus the obtained $T_m$ reported here is higher than $T_m$ of hydrated LEC, DPPC and DSPC found in the literature.

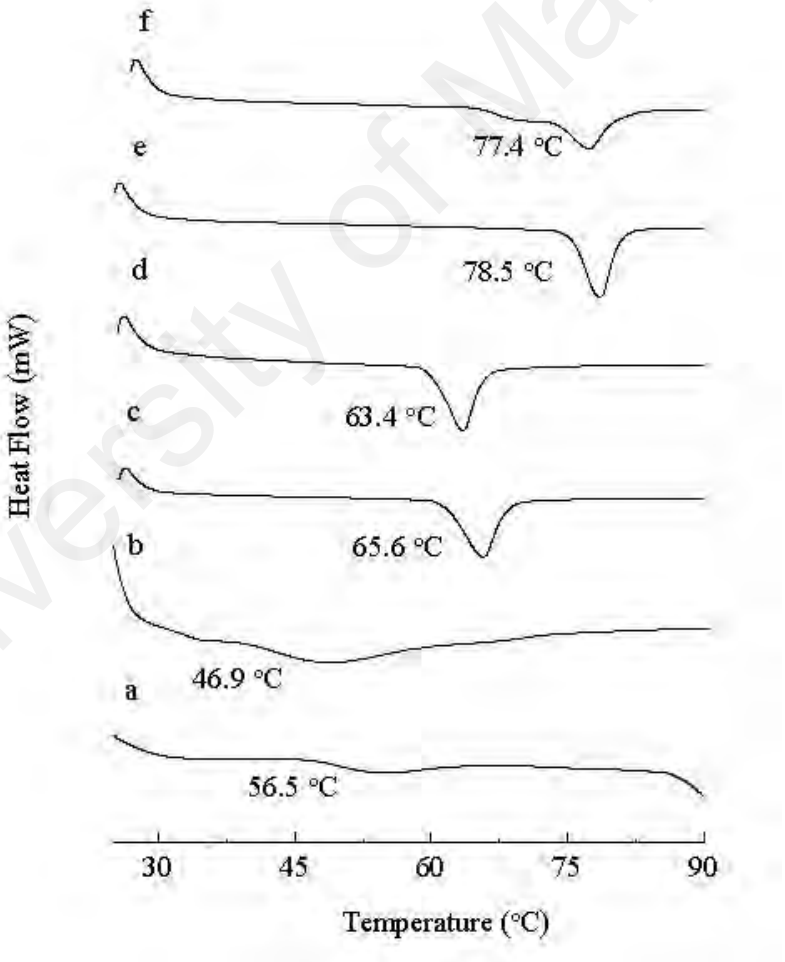



**Figure 4.8:** Thermal behaviors of (a) LEC liposomes, (b) LEC-DEAE-DX liposomes, (c) DPPC liposomes, (d) DPPC-DEAE-DX liposomes, (e) DSPC liposomes and (f) DSPC-DEAE-DX liposomes.

## 4.6 Encapsulation efficiency

### 4.6.1 Curcumin

The encapsulation efficiency of curcumin in the liposome was studied by varying the concentration of curcumin and maintaining the composition of liposomes at 0.2 % (w/v) and 0.02 % (w/v) DEAE-DX respectively for LEC and DPPC liposome whereas for DSPC liposomes, 0.01% (w/v) DEAE-DX was applied (Figure 4.9).

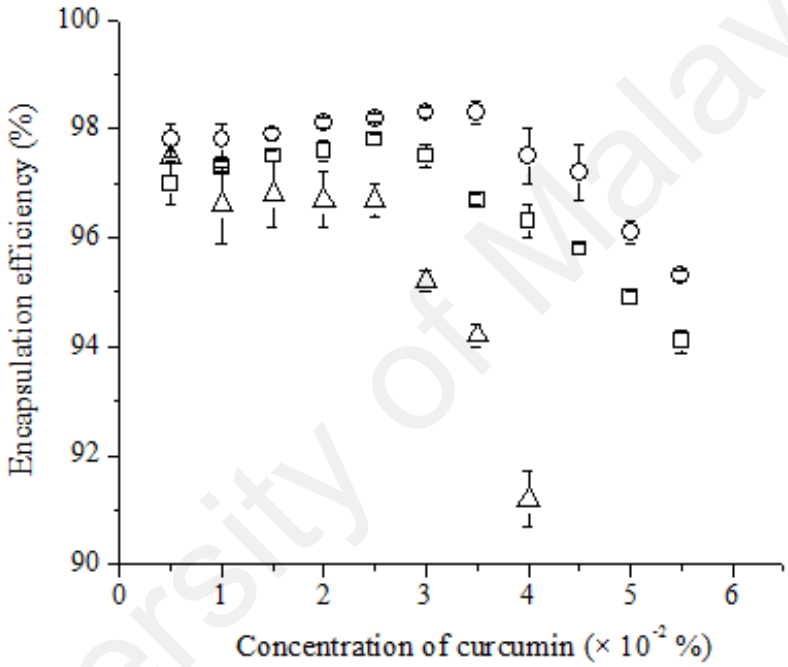



**Figure 4.9:** Encapsulation efficiency of LEC-DEAE-DX ( △ ), DPPC-DEAE-DX ( □ ) and DSPC-DEAE-DX ( ○ ) liposomes with varying concentration of curcumin.

The optimum encapsulation efficiency of LEC-DEAE-DX liposome was found at 0.025 % (w/v). Once the bilayer membrane of the LEC-DEAE-DX liposomes is saturated with curcumin, further increase of curcumin to 0.03 % (w/v) and above resulted in a decrease of encapsulation efficiency. This indicated the achievement of optimum encapsulation of LEC-DEAE-DX at 0.025% (w/v) with the efficiency of 96.7 %. A similar trend was observed for DPPC-DEAE-DX with optimum encapsulation of 0.025 % (w/v) with 97.8 % efficiency whereas DSPC-DEAE-DX with optimum

encapsulation of 0.035 % (w/v) with 98.3 % efficiency. The high encapsulation efficiency is because of the hydrophobicity of curcumin, therefore the amount of drug in external aqueous phase will be limited by its solubility. We expected that DSPC-DEAE-DX which has smaller particle size will have the lowest entrapment for curcumin due to its smaller trapping volume.

However, the results show that DSPC-DEAE-DX able to entrap more curcumin than LEC-DEAE-DX and DPPC-DEAE-DX. The plausible reason for this observation is based on the greater hydrophobicity nature of DSPC lipid which contributes to a rigid bilayer packing. As a consequence, curcumin has a lower tendency to leak and a higher tendency to reside in the bilayer which results in higher encapsulation efficiency.

### 4.6.2 DPH

The optimum concentration of DPH that can be incorporated into the liposomes composed of LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX was investigated respectively. It was observed that at very low concentration of DPH, the encapsulation efficiency is low, while increase in the concentration of DPH leads to higher encapsulation efficiency (Figure 4.10). The reason that affects the encapsulation efficiency is due to the high solubility (1000 mg/ml) of DPH in the bulk medium. Therefore, at low concentration of DPH, the probability of DPH to be entrapped in liposomes is lower. Nevertheless, the probability of DPH to be entrapped in liposome during bilayer convolution is expected to be higher as the concentration of DPH increases. However, once the liposome is saturated with DPH, a further increase in DPH causes a reduction of encapsulation efficiency.

DPPC-DEAE-DX liposomes showed optimum encapsulation at 0.065% (w/v) DPH with the efficiency of 37.2 % whereas LEC-DEAE-DX and DSPC-DEAE-DX liposomes showed optimum encapsulation at 0.055% (w/v) DPH with the efficiency of

29.9 % and 36.1 %, respectively. The plausible reason for higher entrapment amount of DPH in DPPC-DEAE-DX liposomes probably due to its greater trapping volume compared to DSPC-DEAE-DX liposomes (Ahmad, 2015). This result agrees with the particle size measurement in which hydrodynamic diameter of DPPC liposome is larger compared to DSPC liposomes.

On the other hand, LEC-DEAE-DX which has the largest particle size supposed to have greater trapping capacity. Therefore, it was expected to entrap more DPH compared to DPPC-DEAE-DX and DSPC-DEAE-DX. Nevertheless, LEC-DEAE-DX was observed to have lowest encapsulation efficiency. This could be due to the leakage of DPH from LEC-DEAE-DX which arises from the instability of the liposome as compared to DPPC-DEAE-DX and DSPC-DEAE-DX.

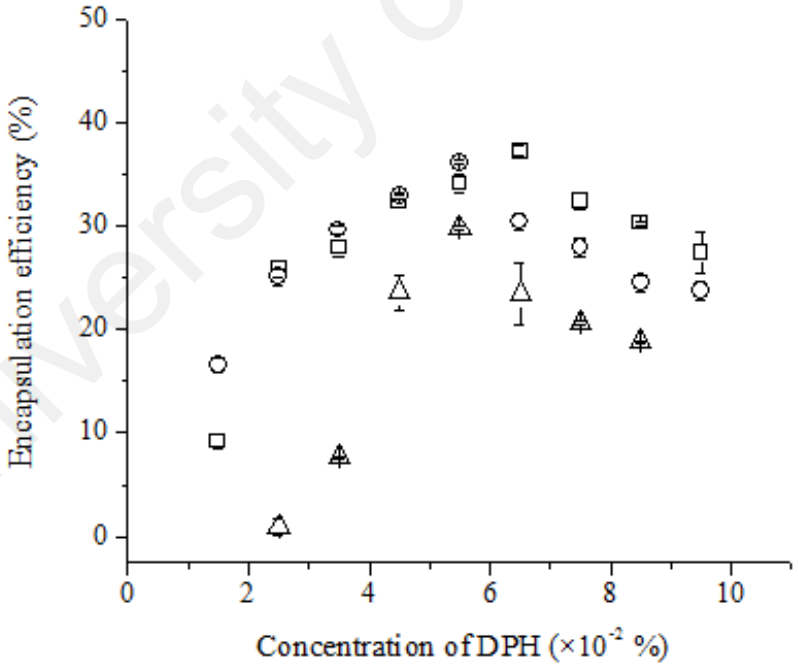



**Figure 4.10:** Encapsulation efficiency of LEC-DEAE-DX ( △ ), DPPC-DEAE-DX ( □ ) and DSPC-DEAE-DX ( ○ ) liposomes with varying concentration of DPH.

## 4.7 Rheological analysis

### 4.7.1 Dynamic behavior of pure carbopol gel

Liposomes are dispersed in water, hence their viscosity is low. The liquid-like appearance of the liposomal solution may not be suitable for the delivery of drug via topical administration. Therefore, it is essential to increase the viscosity of liposome by formulating the liposomes into a gel matrix. Carbopol, a hydrophilic polymer is considered most suitable for topical application due to its minimal irritancy and non-sensitizing properties. Also, previous studies have shown that liposomes are compatible with derivatives of acrylic acid which is a type of synthetic polymers (Ortan et al., 2011). However, the concentration of the polymer forming the gel matrix can affect the stability of the incorporated liposomes.

Carbopol which imparts the desired semisolid consistency was chosen so as to ease the process of administration via topical application. Therefore, in order to understand rheological characteristics of liposomal gels and selection of optimum concentration of carbopol with desired rheological characteristic, various concentrations of carbopol (0.1, 0.15, 0.25, 0.5, 1.0 % w/v) were prepared and analyzed for its rheological characteristics. The rheological behavior of amplitude sweep test was first examined by subjecting the gels to an oscillating strain at a constant frequency.

The amplitude sweep test was conducted in order to obtain parameters such as the linear viscoelastic region (LVR), critical strain $\gamma_c$, elastic modulus *G'*, and cohesive energy CE values. The flexibility of 0.1, 0.15, 0.25, 0.5 and 1 % w/v carbopol gel was studied based on the LVR and $\gamma_c$. On the other hand, the elasticity of the gel and cohesiveness of the gel network were evaluated from the values of *G'* and CE respectively. The *G'* of the gel was determined from the LVR obtained from the amplitude sweep profile whereas $\gamma_c$, represents the end of LVR. Also, the inter-particle interactions are represented by the value of cohesive energy, CE values.

Figure 4.11 display amplitude sweep test that was performed by increasing amplitude at a constant temperature of 25.0 ºC and a constant frequency of 0.5 Hz. At a lower strain range, elastic modulus, *G'* and loss modulus, *G"* responses nearly parallel and exhibiting a LVR until *G'* is declined and being dominated by *G"* which indicated the deformation of solid like gel while the gel starts to flow. Results show that *G',* $\gamma_c$ and CE value increases as the concentration of carbopol increases from 0.1 % to 1% w/v (Table 4.2). This phenomenon shows that increasing carbopol concentration probably leads to the formation of a stronger internal gel network due to the increase of the physical entanglement between the polymer chains (A-sasutjarit et al., 2005). Also, high-value *G'* indicated that the formulated gel is stiffer and longer LVR showed that the gel poses high structural integrity.

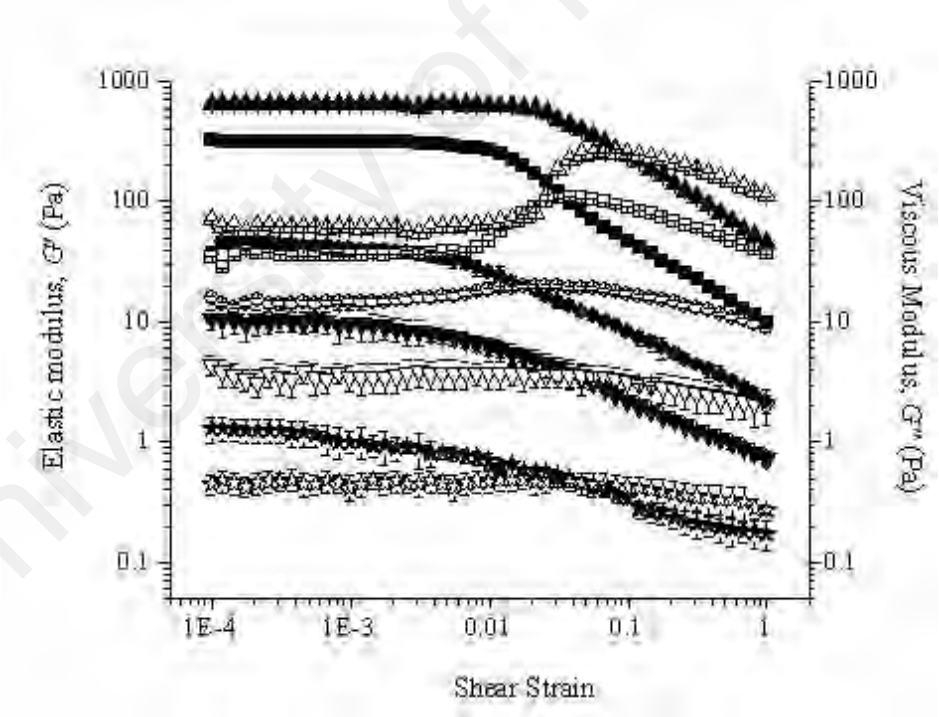



**Figure 4.11:** The variation of *G'* (closed symbol) and *G''* (open symbol) as a function of applied strain for pure carbopol gel at concentration of ( ★ ) 0.1 %, ( ▼ ) 0.15 %, ( ● ) 0.25 %, ( ■ ) 0.5 %, and ( ▲ ) 1.0 % obtained at 25 ºC.

**Table 4.2:** The critical strain ($\gamma_c$), elastic modulus (*G'*) and cohesive energy (CE) for carbopol concentration ranging from 0.1 to 1.0 (w/v %) determined from the respective dynamic rheological behaviors.

| Concentration of carbopol (w/v %) | Critical strain, $\gamma_c$ | Elastic modulus, *G'* (Pa) | Cohesive energy, CE (Pa) |
|---|---|---|---|
| 0.1 | 0.0007±0.0002 | 1.3±0.3 | 0.0000003±0.0000002 |
| 0.15 | 0.0010±0.0003 | 10.5±2.0 | 0.000053±0.000002 |
| 0.25 | 0.0015±0.0002 | 42.4±5.5 | 0.00005±0.00002 |
| 0.5 | 0.0082±0.0001 | 317±2 | 0.011±0.001 |
| 1.0 | 0.0214±0.0003 | 642±1 | 0.147±0.003 |

Rheological properties of the carbopol gel were further characterized using a frequency sweep test at a strain below the critical strain, $\gamma_c$ after the linear viscoelastic region of carbopol gels has been defined by amplitude test (Figure 4.12).

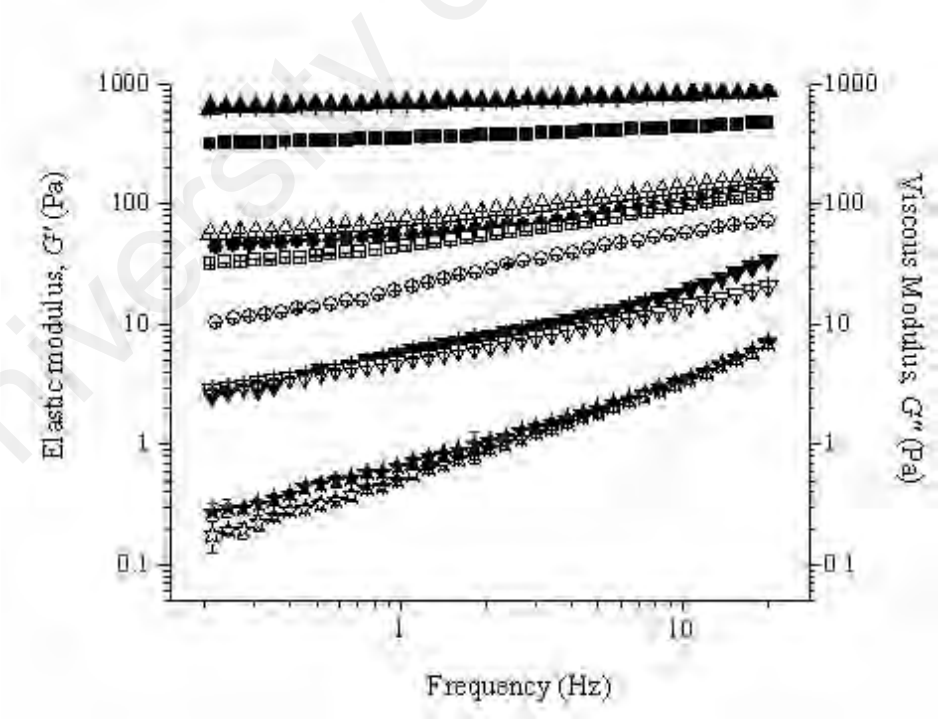



**Figure 4.12:** The variation of *G'* (solid symbol) and *G''* (open symbol) as a function of frequency for pure carbopol gel at concentration of ( ★ ) 0.1 %, ( ▼ ) 0.15 %, ( ● ) 0.25 %, ( ■ ) 0.5 %, and ( ▲ ) 1.0 % obtained at 25 ºC.

The frequency sweep test of 0.1 % and 0.15 % carbopol gel displayed a narrow gap between its *G'* and *G"* which indicated that forces within the gel is weaker and hence has low capacity to store energy. However upon increasing the concentration of carbopol above 0.25 %, the gap between *G'* and *G"* was found to be greater over the studied frequency range. This implied that the applied energy can be stored more effectively in the elastic component of the gel with higher carbopol concentration (Ikeda & Foegeding, 2003). Thus, indicating solid-like behavior for carbopol.

### 4.7.2 Flow behavior of pure carbopol gel

Besides viscoelastic properties, the flow behavior of carbopol gel with various concentrations was also evaluated by measuring its complex viscosity as a function of frequency which was obtained from the frequency sweep (Figure 4.13). As expected, the higher the concentration of carbopol results in higher value of complex viscosity and thus stronger the internal gel network structure.

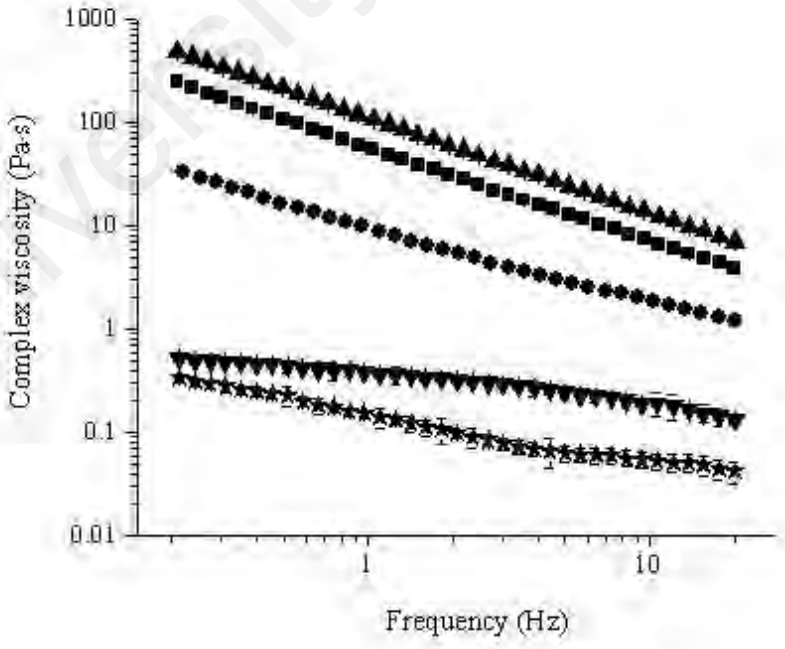



**Figure 4.13:** The complex viscosity as a function of frequency for pure carbopol gel at concentration of ( ★ ) 0.1 %, ( ▼ ) 0.15 %, ( ● ) 0.25 %, ( ■ ) 0.5 %, and ( ▲ ) 1.0 % obtained at 25 ºC.

Complex viscosity was found to decrease upon increasing of frequency which indicated a shear thinning behavior. Shear thinning behavior is an example of non-Newtonian flow because the viscosity is a not constant at a constant temperature and composition as stated by Newton law of viscous flow (Troy, 2004). The shear thinning behavior arises from the deformation of the polymer chains and alignment of the chain in the flow direction (Tan & Misran, 2014).

Rheological studies such as elasticity and flow behavior are important as to evaluate the feasibility of these pure gels to be included in the preparation of liposomal gels. Gel with a high *G'* and CE can limit the dispersion and reduce the homogeneity of the liposomes within the gel network of carbopol (Rodríguez-Hernández & Tecante, 1999). Also, a very high complex viscosity will result in poor spreadability of the gel and hence unable to disperse liposomes evenly on the skin which may delay the diffusion rate of the encapsulated drug onto the skin. On the other hand, gel with low *G'*, CE and complex viscosity have weak internal gel network structure which may not be strong enough to hold and stabilize the dispersed liposomes. For these reasons, a suitable carbopol concentration of 0.25 % (w/v) which exhibits not too high nor too low *G'*, CE and complex viscosity was chosen in order to prepare liposomal gel system. Also, 0.25 % (w/v) carbopol gel forms the desired consistency of gel which is neither too rigid nor flowy (Figure 4.14).

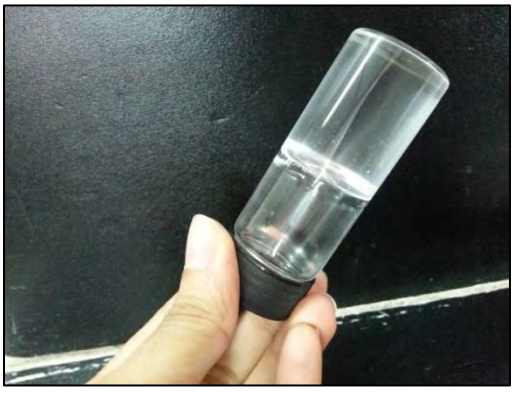


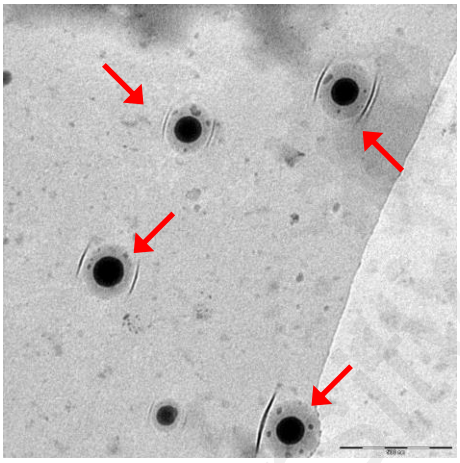
**Figure 4.14:** 0.25 % (w/v) carbopol gel.

## 4.8 Liposomal gel

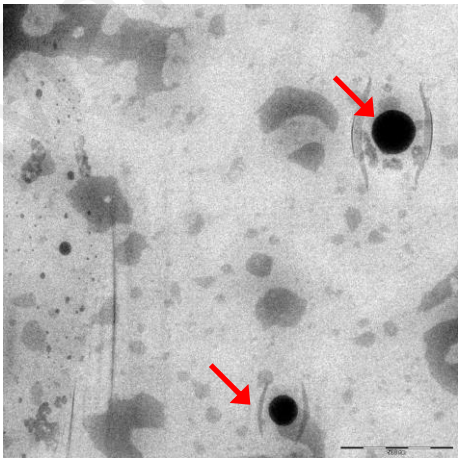
### 4.8.1 Morphology of liposomal gel

All of the liposomes were incorporated into carbopol gel of concentration 0.25 % (w/v). The morphology of LEC-DEAE-DX-liposome-in-gel (LG-LEC-DEAE-DX), DPPC-DEAE-DX-liposome-in-gel (LG-DPPC-DEAE-DX) and DSPC-DEAE-DX-liposome-in-gel (LG-DSPC-DEAE-DX) were evaluated using a transmission electron microscopy as shown in Figure 4.15. The entrapped liposomes in the gel matrix showed spherical morphology.

(a)



(b)



(c)

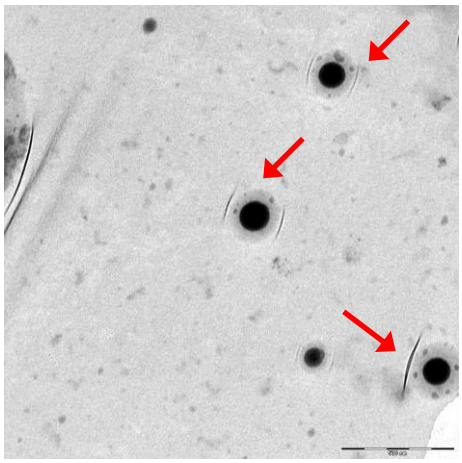


**Figure 4.15:** TEM micrographs of liposomal gel (a) LG-LEC-DEAE-DX, (b) LG-DPPC-DEAE-DX, (c) LG-DSPC-DEAE-DX. The liposomes were indicated with arrows at the scale bar of 500 nm.

Previous work by Tan & Misran (2014) showed that liposome dispersed in gel matrix appeared as spherical morphology. In addition, the observed liposomes did not undergo any change in their particle size. Dragicevic-Curic et al. (2009) also mentioned that incorporation of lecithin liposomes into carbomer hydrogels of different polymer concentration did not lead to a remarkable change in the particle size. The polymer chains do not disrupt the structure of liposome, but the liposomes are accommodated into the voids of the gel network (Chieng & Chen, 2010).

On the other hand, Bochot et al. (1998) reported that incorporation of the thermosensitive liposome in a poloxamer gel may modify the particle size as poloxamer adsorp indifferently to negatively or positively charged or neutral liposomes. Herein, the obtained results show that all of the liposomes were found to be entrapped in the gel. Also, all the particles exhibited a particle size of less 200 nm. This shows that incorporation of liposomes in the gel matrix did not alter the size of liposomes to a larger extent.

### 4.8.2 Rheological properties of liposomal gels

In this work, viscoelastic and flow behavior of gel after incorporation of DEAE-DX coated liposomes and non-coated liposomes were compared. Six different types of liposomal gels were prepared, namely LEC-in-liposome-gel ( LG-LEC), LEC-DEAE-DX-liposome-in-gel (LG-LEC-DEAE-DX), DPPC-liposome-in-gel (LG-DPPC), DPPC-DEAE-DX-liposome-in-gel (LG-DPPC-DEAE-DX), DSPC-liposome-in-gel (LG-DSPC) and DSPC-DEAE-DX-liposome-in-gel (LG-DSPC-DEAE-DX). Each liposomal gel consists of 0.2 % (w/v) of liposomes which is above the CVC values of the respective lipids.

#### 4.8.2.1 Dynamic behavior of liposomal gel

The amplitude profile of all the gels upon incorporation of liposome and DEAE-DX coated liposomes are shown in Figure 4.16-4.18. A significant increase of *G'* was observed upon incorporation of LEC, DPPC and DSPC liposomes, respectively into the gel. The *G'* value of the gel increased from 42.4 Pa to 82.7 Pa, 86.5 Pa and 87.7 Pa after incorporation of lecithin, DPPC and DSPC liposomes, respectively into 0.25% carbopol gel (Table 4.3). Results show that upon addition of the liposome in gel, the elasticity of carbopol gel increases due to natural rigidity within the liposome (Mourtas et al., 2008). On the other hand, LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX exhibited *G'* values of 105 Pa, 96.3 Pa and 123 Pa respectively. It was noticeable that upon addition of coated liposomes, the elasticity of the gel was found to be even greater. Under applied shear, coated liposomes could effectively store the applied stress in its elastic component compare to the non-coated liposomes and hence increased the *G'* of liposomal gel.

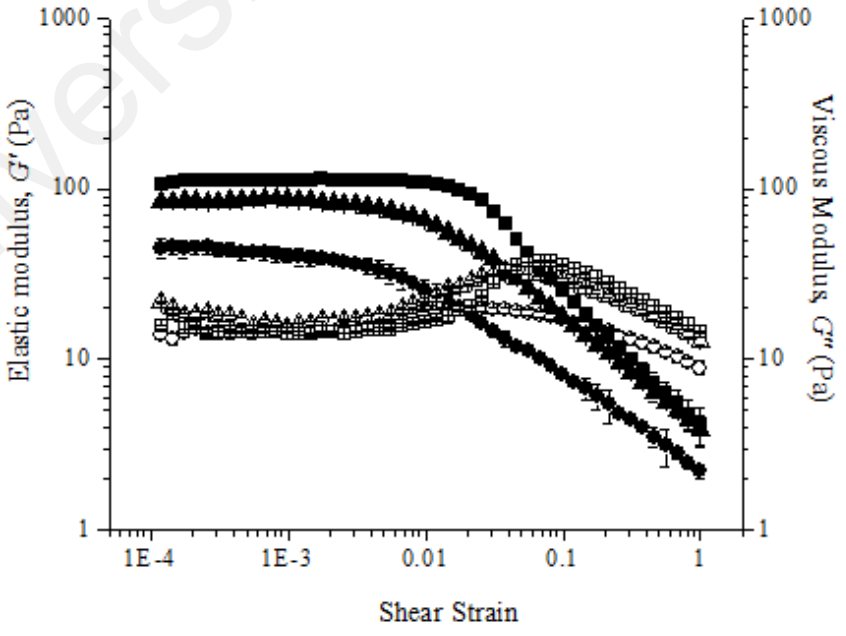



**Figure 4.16:** The variation of *G'* (solid symbol) and *G''* (open symbol) as a function of applied strain for carbopol gel ( ● ), LG-LEC ( ▲ ) and LG-LEC-DEAE-DX ( ■ ).

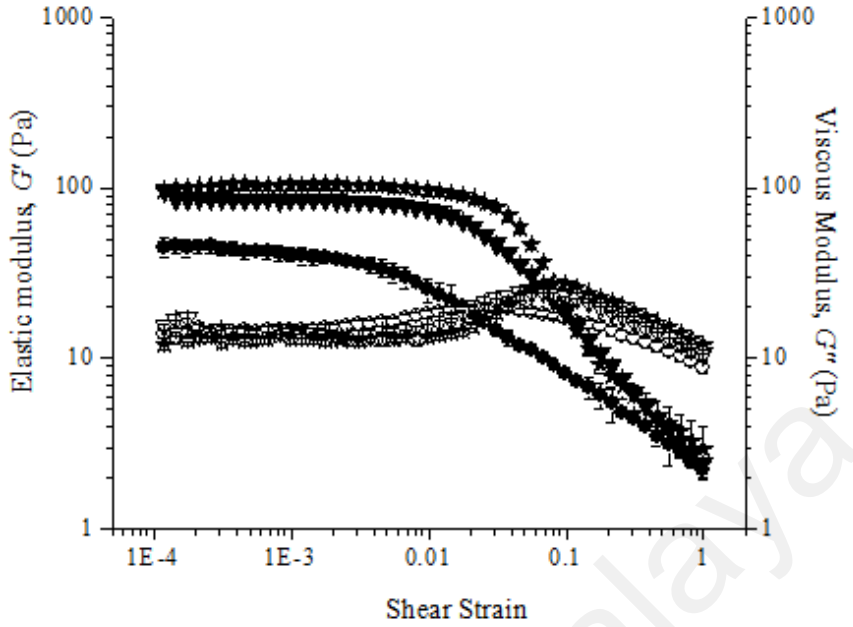



**Figure 4.17:** The variation of *G'* (solid symbol) and *G''* (open symbol) as a function of applied strain for carbopol gel ( ● ), LG-DPPC ( ▼ ) and LG-DPPC-DEAE-DX ( ★ ).

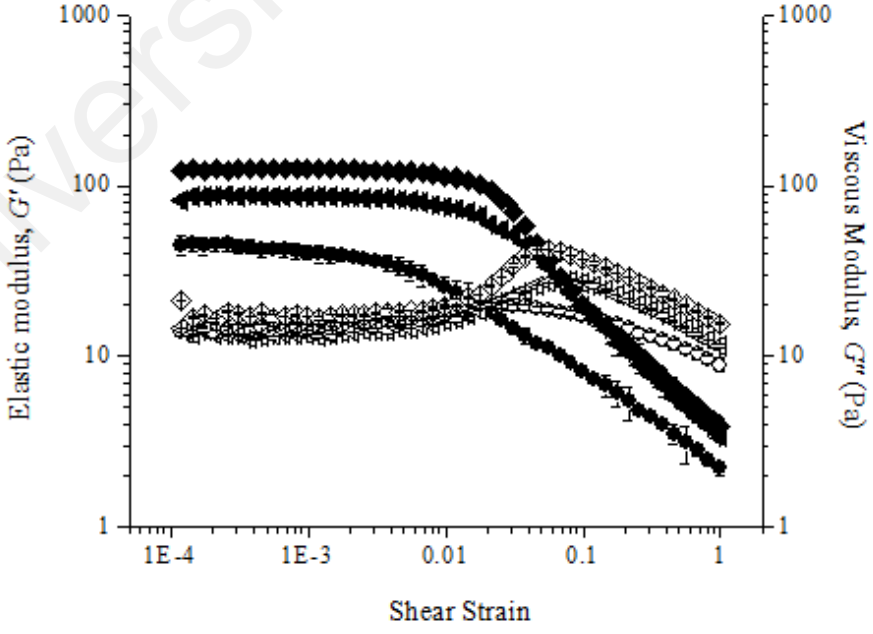



**Figure 4.18:** The variation of *G'* (solid symbol) and *G''* (open symbol) as a function of applied strain for carbopol gel ( ● ), LG-DSPC ( ◄ ) and LG-DSPC-DEAE-DX ( ◆ ).

**Table 4.3:** The critical strain ($\gamma_c$), elastic modulus (*G'*) and cohesive energy (CE) of liposomal gels which were determined from their dynamic rheological behaviors.

| Concentration of carbopol (w/v %) | Critical strain, $\gamma_c$ | Elastic modulus, *G'* (Pa) | Cohesive energy, CE (Pa) |
|---|---|---|---|
| 0.25 % Carbopol gel | 0.0015±0.0002 | 42.4±5.5 | 0.00005±0.0002 |
| LG-LEC | 0.0026±0.0004 | 82.7±0.1 | 0.0003±0.0005 |
| LG-LEC-DEAE-DX | 0.0068±0.0001 | 105±2 | 0.0024±0.0001 |
| LG-DPPC | 0.0046±0.0003 | 86.5±0.1 | 0.0009±0.0002 |
| LG-DPPC-DEAE-DX | 0.0120±0.0001 | 96.3±0.1 | 0.0069±0.0002 |
| LG-DSPC | 0.0056±0.0003 | 87.7±3 | 0.0014±0.0003 |
| LG-DSPC-DEAE-DX | 0.0082±0.0003 | 123±1 | 0.0041±0.0001 |

This result is further supported by the higher value of $\gamma_c$ obtained for the liposomal gel which the formulated liposomal gel become more resistant to deformation. After exceeding the $\gamma_c$, *G'* for all the gel was found to decrease with increased applied strain until it reached the break point ($\gamma_b$). This is a characteristic point where the gel began to flow (*G'' > G'*). In comparison, $\gamma_b$ for all the liposomal gel is higher than pure carbopol gel. This means liposomal gel have lower tendency to flow compared to pure carbopol gel. Besides that, the changes in CE of gel from 0.00005 Pa to 0.0003, 0.0009 and 0.0014 for LG-LEC, LG-DPPC and LG-DSPC respectively explains that the elastic strength of gel network structure in the liposomal gel is stronger than pure carbopol gel. The plausible reason for a formation of stronger gel network is due to the hydrophobic interaction between polymer chains and liposome bilayer (Grijalvo et al., 2016).

Besides that, all the gels with DEAE-DX coated liposomes have longer LVR and show higher $\gamma_c$ as well as CE values compared to gels containing non-coated

liposomes. Thus, it can be deduced that liposomal gel containing coated liposomes will be more resistant to breakdown by stress for instance during transportation or handling. The increase in the elastic behavior of liposomal gel after incorporation of coated liposomes may be due to the interaction between negatively charged carbopol gel and positively charged DEAE-DX at the surface of liposomes. In a previous study for the development of controlled release formulation, the combination of chitosan and carbopol polymers enhanced the gel strength significantly. Polyelectrolyte complexes are formed via the electrostatic interaction of chitosan between the protonated amine ($NH_3^+$) group of chitosan and the carboxylate ($COO^-$) group of carbopol. As a result, the performance of these polymers in a controlled release matrix was improved (López, 2016).

Herein, the enhancement of gel elasticity after incorporation of DEAE-DX coated liposome would indicate a special interaction between DEAE-DX and carbopol that could promote the internal forces holding the polymers to become stronger. Thus, attributing to the adhesiveness of the resultant gel due to the oppositely charged polymers. However, it is important to ensure that formulated gel is not too rigid. Strong cohesive energy retards the flow of liposomal gel and limits the spreadability of the gel which will affect the homogeneity and consistency of gel upon application. Although the obtained results show that CE of the liposomal gel has increased in the presence of DEAE-DX, however the increase in $\gamma_c$ supports the formation of more elastic and flexible network structure for LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX. Thus, the flowability and spreadability of the liposomal gel are retained.

After analyzing the linear viscoelastic region for all the liposomal gel, frequency sweep was performed in order to attain more information on the forces or interaction in the gel system (Franck, 2004). The frequency sweep in which all liposomal gel

exhibited solid like behavior with *G'* greater than *G"* further supports the fact whereby energy was stored more effectively in the liposomal gel (Figure 4.19-4.21).

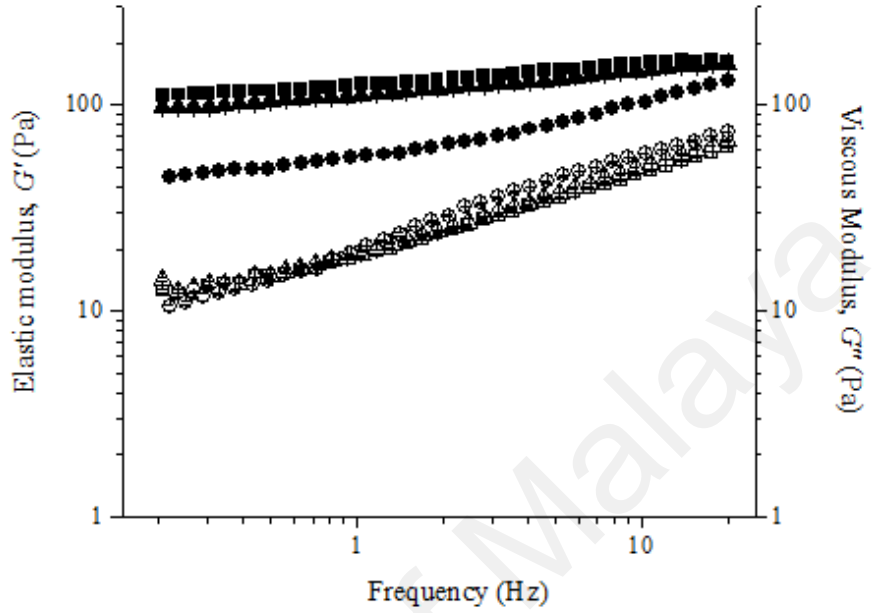



**Figure 4.19:** The variation of *G'* (solid symbol) and *G''* (open symbol) as a function of frequency for carbopol gel ( ● ), LG-LEC ( ▲ ) and LG-LEC-DEAE-DX ( ■ ).

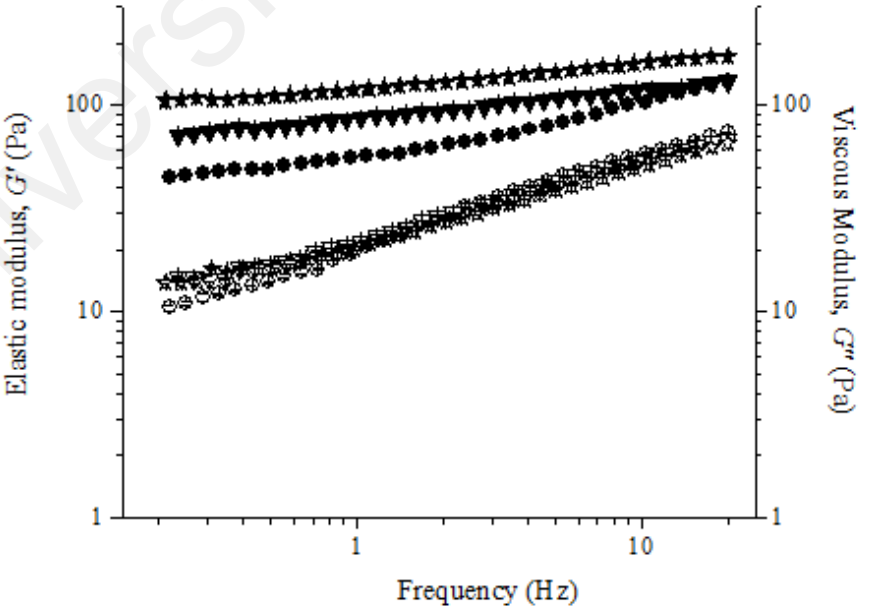



**Figure 4.20:** The variation of *G'* (solid symbol) and *G''* (open symbol) as a function of frequency for carbopol gel ( ● ), LG-DPPC ( ▼ ) and LG-DPPC-DEAE-DX ( ★ ).

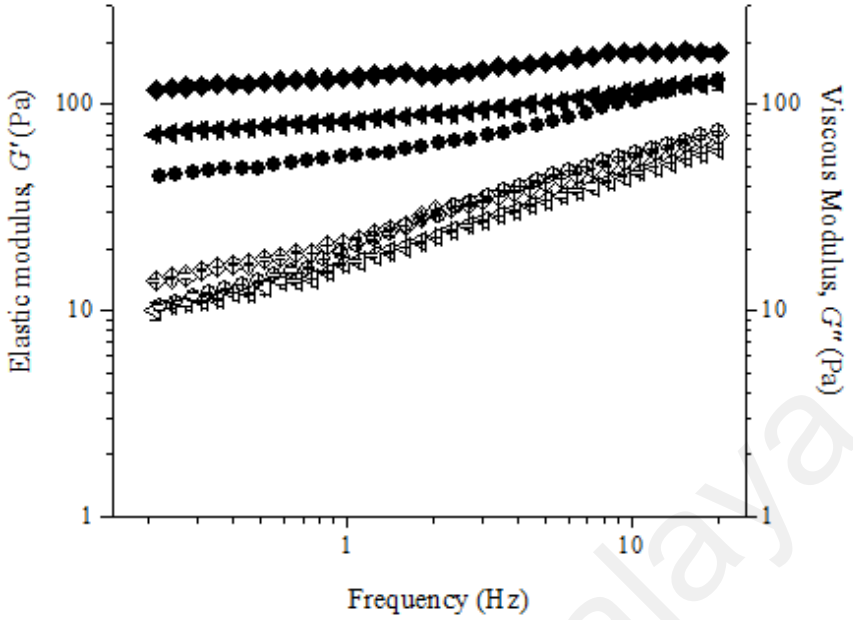



**Figure 4.21:** The variation of *G'* (solid symbol) and *G''* (open symbol) as a function of frequency for carbopol gel ( ● ), LG-DSPC ( ◄ ) and LG-DSPC-DEAE-DX ( ◆ ).

The slope of *G'* of the liposomal gel was found to decrease and show lower dependency towards frequency as compared to the pure carbopol gel. The decrease in slope implied that the liposomal gel is more solid-like behavior than pure carbopol gel. In this case, sedimentation is unlikely to occur.

Figure 4.22 showed the tan δ profile solid-like behavior of liposomal gel. The tan δ is defined as *G''*/ *G'* which indicates solid-like behavior of liposomal gel or the overall viscoelasticity. The results show that gel with the presence of liposome was highly elastic and its tan δ profile is lower than tan δ profile obtained for pure carbopol gel. On the other hand, it was also observed that gel which contains the coated liposomes exhibit greater elasticity compared to gel which contains non-coated liposomes. The tan δ of the LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX was lower than the LG-LEC, LG-DPPC and LG-DSPC which showed gel containing coated liposomes were more solid-like.

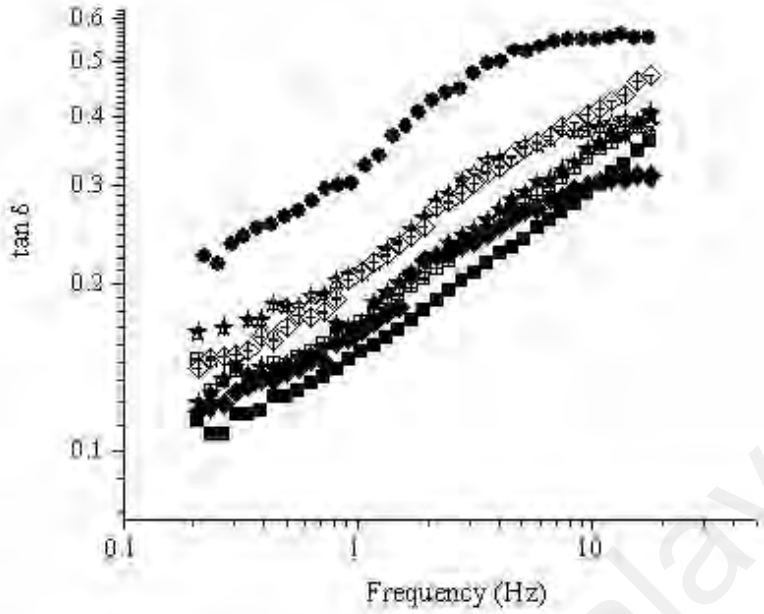


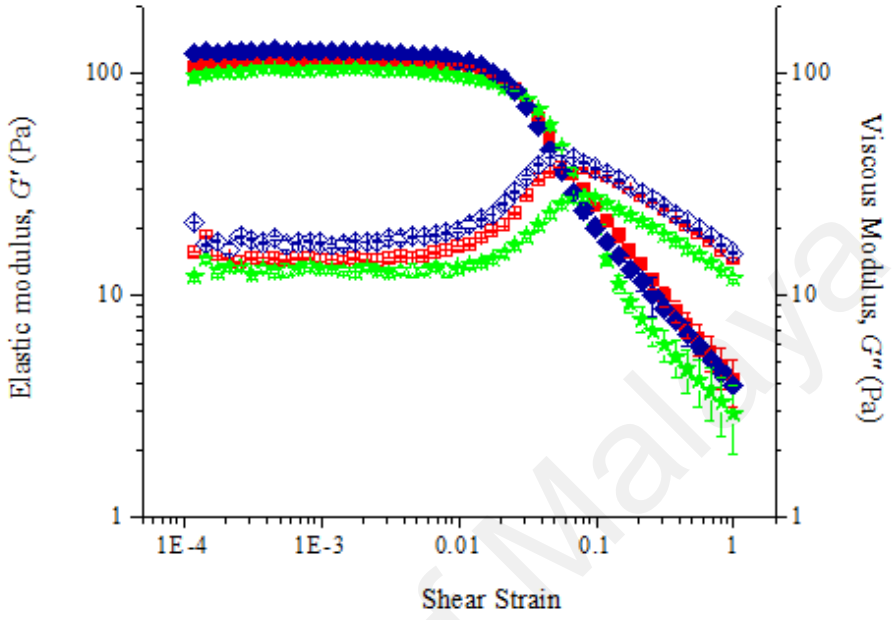

**Figure 4.22:** The tan δ of carbopol gel ( ● ), LG-LEC ( □ ) and LG-LEC-DEAE-DX ( ■ ), LG-DPPC ( ☆ ) and LG-DPPC-DEAE-DX ( ★ ), LG-DSPC ( ◇ ) and LG-DSPC-DEAE-DX ( ◆ ).

The rheological behavior of three different types of liposomal gel namely LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX, LG-DEAE-DX-DSPC was also compared (Figure 4.23). From the information shown in Table 4.3, *G'* of LG-DSPC-DEAE-DX was the highest which indicates its high elasticity. This result was mainly due to the higher phase transition of DSPC compared to LEC and DPPC liposome which is more rigid at the experiment temperature of (25.0 ± 0.1) ºC.

However, the $\gamma_c$ of the liposomal gel is highest in LG-DPPC-DEAE-DX followed by LG-LEC-DEAE-DX and LG-DSPC-DEAE-DX. Thus, LG-DPPC-DEAE-DX exhibited the longest LVR among the liposomal gels. On the other hand, LG-DSPC-DEAE-DX which is more rigid limits the mobility of polymer chains and thus led to formation of less flexible (low $\gamma_c$) liposomal gel compared to LG-DPPC-DEAE-DX. Besides that, the CE value which implies the elastic strength of gel network

structure was found highest for LG-DPPC-DEAE-DX followed by LG-DSPC-DEAE-DX and LG-LEC-DEAE-DX.

(a)




(b)

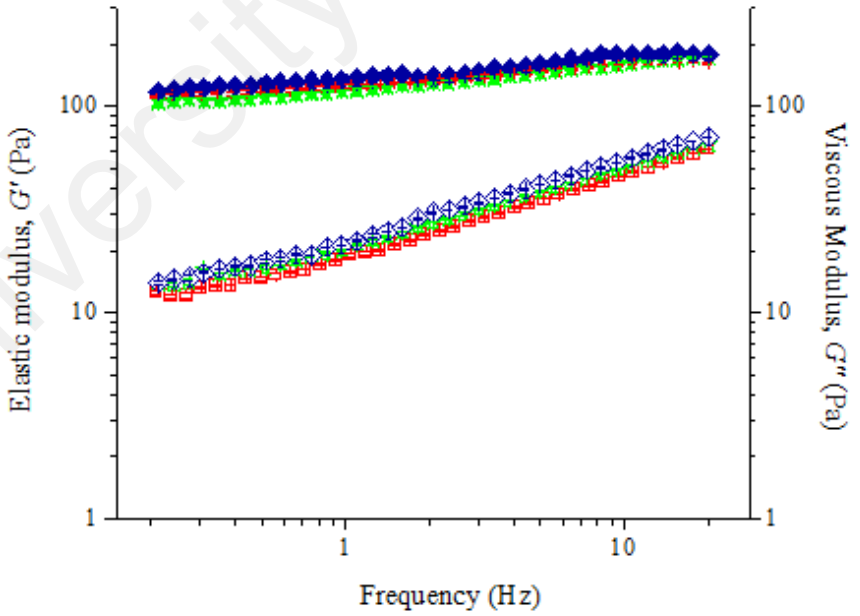



**Figure 4.23:** The comparison of *G'* (solid symbol) and *G''* (open symbol) of LG-LEC-DEAE-DX ( ■ ), LG-DPPC-DEAE-DX ( ★ ), and LG-DSPC-DEAE-DX ( ◆ ) as a function of (a) applied strain and (b) frequency.

#### 4.8.2.2 Flow behavior of liposomal gels

Generally, polymeric gel systems are non-Newtonian and exhibit shear thinning behavior. Similar to carbopol gel, the liposomal gel also exhibited shear thinning behavior. The spreadability and flowability of the liposomal gel were studied by measuring its shear viscosity as a function of shear rate and shear stress as to compare the degree of shear thinning of the liposomal gel (Garg et al., 2002). The shear viscosity profile of all the liposomal gel was found to be slightly higher than carbopol gel due to the rigidity of the liposomes itself (Figure 4.24-4.26).

(a)

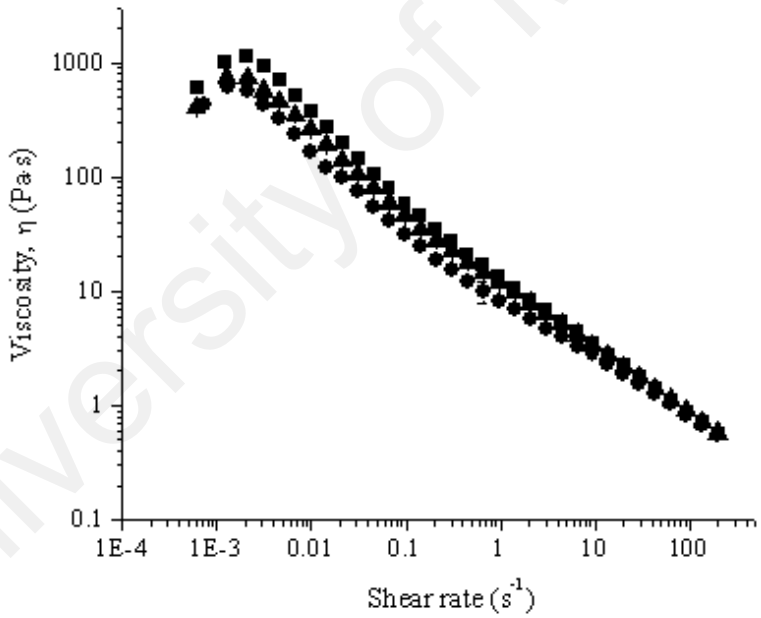



**Figure 4.24:** The flow behavior of carbopol gel ( ● ), LG-LEC ( ▲ ) and LG-LEC-DEAE-DX ( ■ ) where (a) is the shear viscosity profile with increasing the shear rate and (b) is the shear stress versus shear rate profile.

(b)

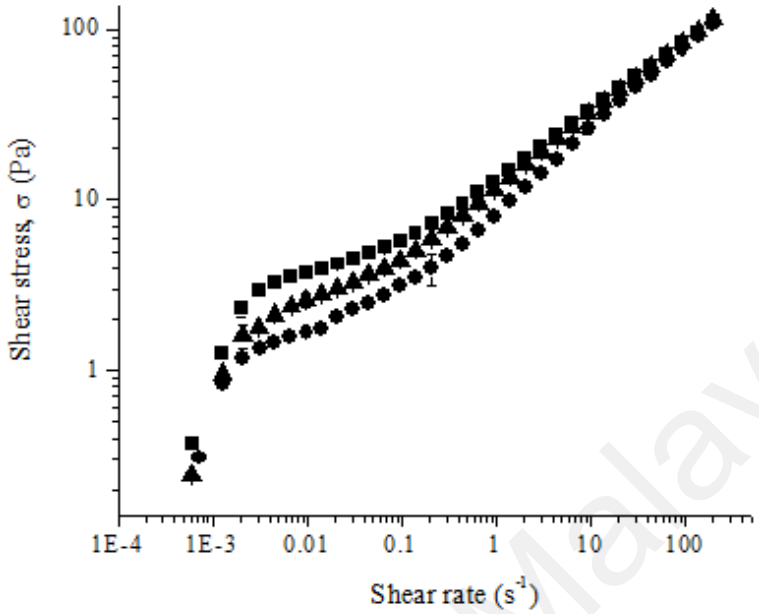



**Figure 4.24:** (continued)

(a)

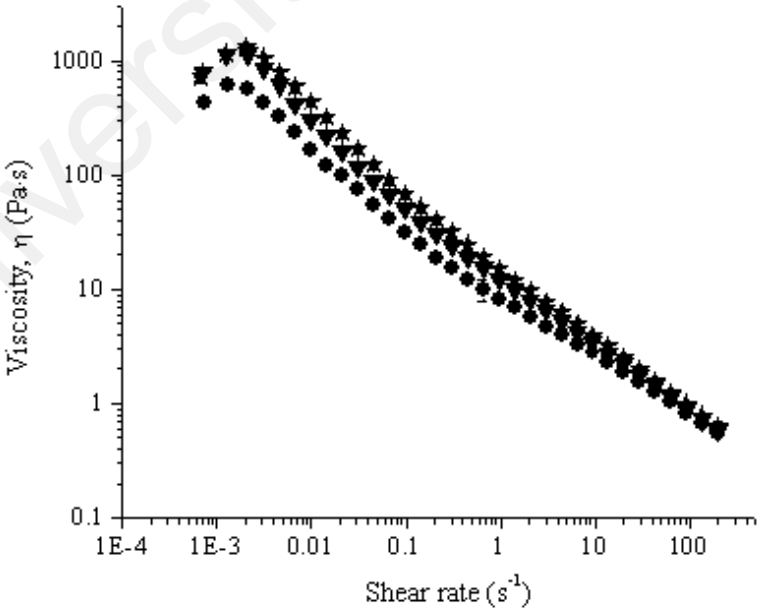



**Figure 4.25**: The flow behavior of carbopol gel ( ● ), LG-DPPC ( ▼ ) and LG-DPPC-DEAE-DX ( ★ ) where (a) is the shear viscosity profile with increasing the shear rate and (b) is the shear stress versus shear rate profile.

(b)

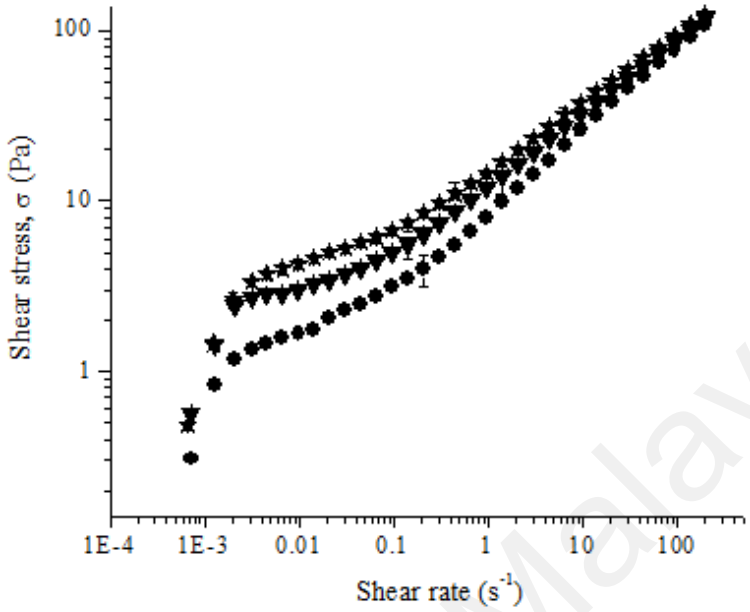



**Figure 4.25**: (continued)

(a)

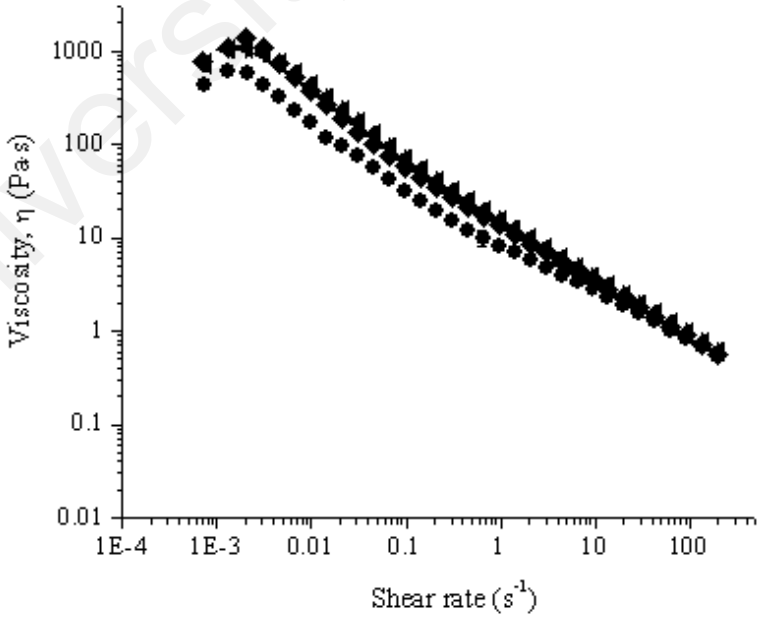



**Figure 4.26**: The flow behavior of carbopol gel ( ● ), LG-DSPC ( ◄ ) and LG-DSPC-DEAE-DX ( ◆ ) where (a) is the shear viscosity profile with increasing the shear rate and (b) is the shear stress versus shear rate profile.

(b)

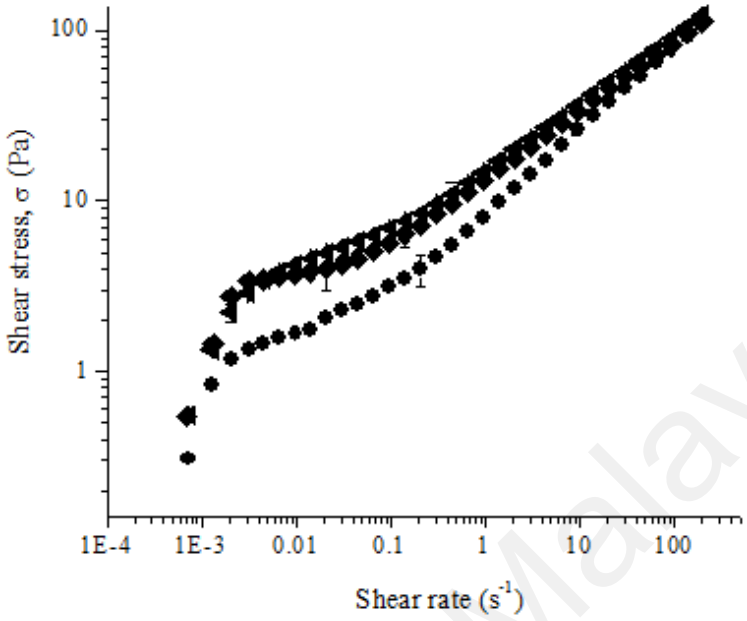



**Figure 4.26**: (continued)

Also, the yield stress ($\sigma_p$) of the liposomal gel was found greater than pure carbopol gel. Yield stress ($\sigma_p$) is the stress applied in order to make the structure fluid flow (Table 4.4). The value of yield stress is significant as it imparts the degree of deformation of a structured fluid. Furthermore, higher viscosity at low shear rate is desirable for topical gel during storage period (Yang et al., 2008).

The obtained result which shows that liposomal gel have higher shear viscosity and yield stress ($\sigma_p$) might be attributed to the presence of liposomes which has increased the rigidity of the formulation as discussed in the previous section whereby all the liposomal gel exhibited greater storage modulus (*G'*). Besides that, the presence of liposome may have added an extra bridging effect which linked the adjacent polymer chains in the gel which causes the shear viscosity and $\sigma_p$ to increase. As a result, the gel is more resistant to flow.

**Table 4.4:** Yield stress ($\sigma_p$) of liposomal gels which was determined from the steady rheological behaviors.

| Name | Yield stress,$\sigma_p$ (Pa) |
|---|---|
| 0.25 % Carbopol gel | 1.35 |
| LG-LEC | 1.78 |
| LG-LEC-DEAE-DX | 2.96 |
| LG-DPPC | 2.72 |
| LG-DPPC-DEAE-DX | 3.36 |
| LG-DSPC | 2.89 |
| LG-DSPC-DEAE-DX | 3.40 |

Shear thickening was observed at low shear rate region (Goodwin & Hughes, 2008). This result is probably due to the stiff inner structure of the gels (Benchabane and Bekkaour, 2008). However, shear thinning behavior was observed as the shear stress was increased and exceeded the yield stress, $\sigma_p$. The shear viscosity decreased with increasing shear rate. In practice, this means that the flow encounters less resistance at the higher shear rate. This type of behavior is typically observed for toothpaste and tomato paste. Shear viscosity profile of were fitted with Power-Law model in order to compare the degree of shear thinning behavior of pure carbopol gel, uncoated liposomal gel and DEAE-DX coated liposomal gel. The PLI value indicates the deviation of a fluid from Newtonian behavior (PLI for Newtonian fluid is equal to 1).

The PLI of the formulated liposomal gel exhibited lower values than pure carbopol gel (Table 4.5). This result indicated that the formulated liposomal gel performs better spreading ability as compared to pure carbopol gel (Garg et al., 2002).

Thus a thinner layer of semisolid dosage could be applied to the skin and which results in more rapid absorption of drug (Brummer, 2006).

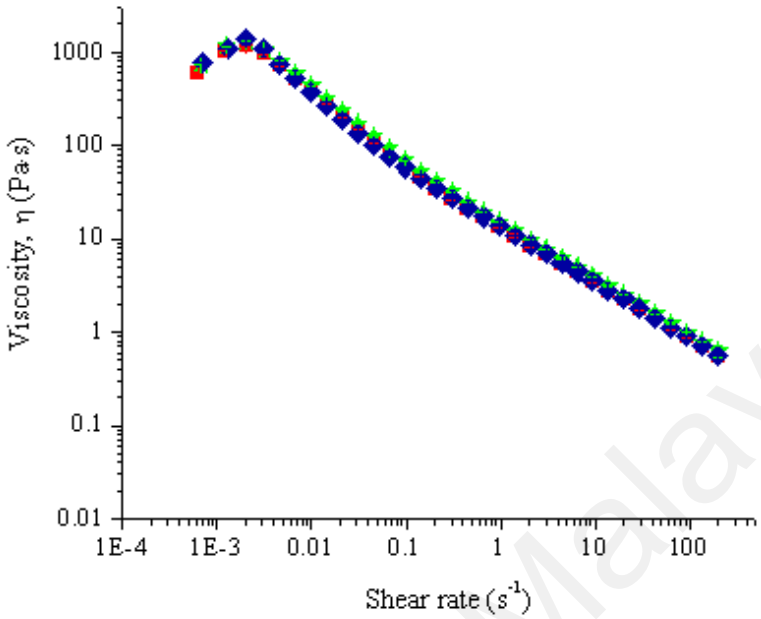
**Table 4.5:** Power Law Index (PLI) of liposomal gels which was determined from the steady rheological behaviors.

| Name | Power Law Index, PLI |
|---|---|
| 0.25 % Carbopol gel | 0.416 |
| LG-LEC | 0.401 |
| LG-LEC-DEAE-DX | 0.351 |
| LG-DPPC | 0.364 |
| LG-DPPC-DEAE-DX | 0.359 |
| LG-DSPC | 0.356 |
| LG-DSPC-DEAE-DX | 0.351 |

Flow behavior results also indicated that liposomal gels containing DEAE-DX coated liposomes exhibited minimal difference in their extent of shear thining compared to a liposomal gel containing non-coated liposomes. However, there is an increase in yield stress of liposomal gel upon incorporation of DEAE-DX coated liposomes compared to non-coated liposomes. This result supports the findings obtained from the amplitude and frequency test whereby addition of DEAE-DX enhances the rigidity of the gel; therefore the resultant gel is more resisting to flow.

There was no obvious difference observed in the flow behavior among the three different types of liposomal gel (Figure 4.27). However, LG-DSPC-DEAE-DX exhibited slightly higher value of yield stress followed by LG-DPPC-DEAE-DX and LG-LEC-DEAE-DX. As expected, LG-DSPC-DEAE-DX is the most elastic gel based on the amplitude sweep test due to its rigidity.

(a)




(b)

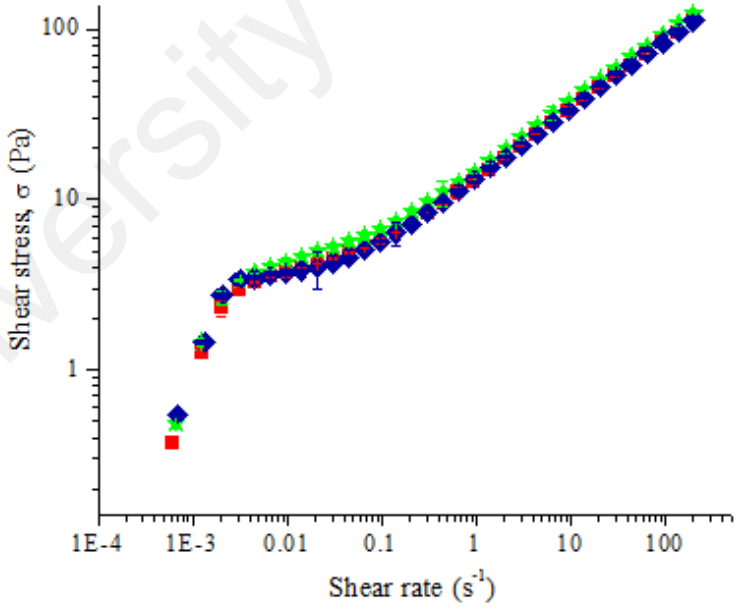



**Figure 4.27:** The comparison of flow behavior LG-LEC-DEAE-DX ( ■ ), LG-DPPC-DEAE-DX ( ★ ) and LG-DSPC-DEAE-DX ( ◆ ) where (a) is the shear viscosity profile of the mixed gels with increasing the shear rate and (b) is the shear stress versus shear rate profile.

### 4.8.3 Rheological properties of drug loaded liposomal gel

As discussed earlier, two different types of drug namely DPH and curcumin have been incorporated into the liposome system (Figure 4.28). The effect of drug loaded liposome on rheological behavior of carbopol gel was investigated. As mentioned in the previous section, carbopol gel after incorporation of DEAE-DX coated liposomes has shown improvement in the flexibility and CE of the gel. And, all of the liposomal gel revealed a domination of elastic over viscous behavior, as the magnitude of *G'* was higher than *G"*.

Thus, it is also important to study whether incorporation of drugs will alter the rheological properties of the liposomal gel that eventually influences the deformability, spreadability, and stability of the gel. The viscoelasticity of the liposomal gel has to be maintained as such where incorporation of the drug does not modify the internal gel structure to a larger extent. Also, another factor to be considered is the flow property of the gel, as it could affect the homogeneity and spreadability of liposome dispersion.

(a) (b)


**Figure 4.28:** (a) Curcumin loaded liposomal gel and (b) DPH loaded liposomal gel

#### 4.8.3.1 Dynamic behavior of DPH loaded liposomal gel

Based on the rheological profile displayed on Figure 4.29, all of the DPH loaded liposomal gel shows a lower *G'* compared to liposomal gel. This means that liposomal gels have lost its elasticity in the presence of DPH. This result was further supported by the critical strain $\gamma_c$ and CE values which indicated the decrease in the cohesiveness and the internal forces of the gel structure (Table 4.6).

(a)

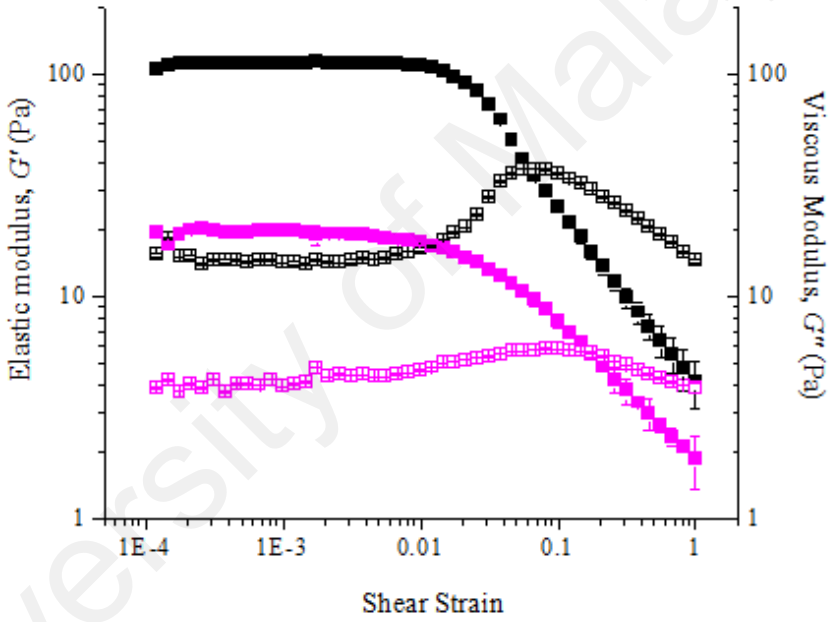
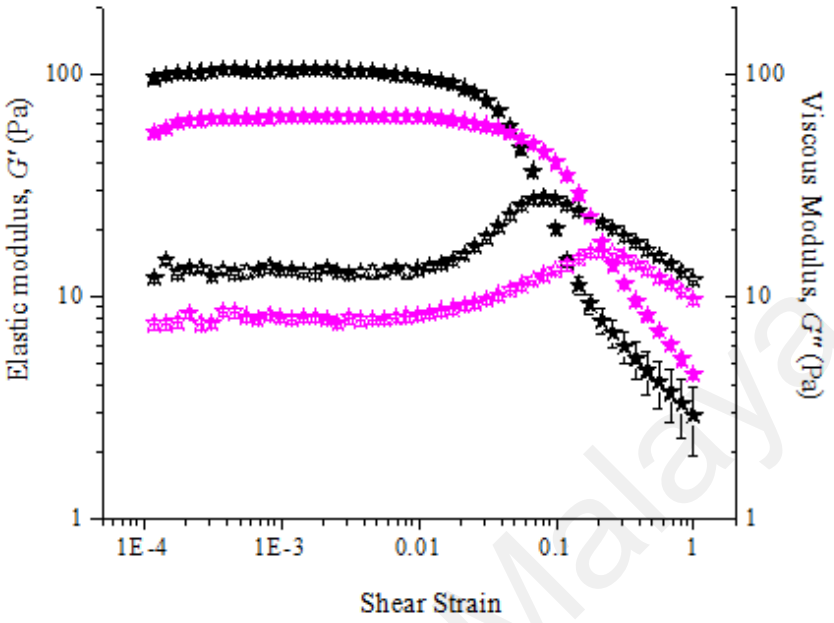



**Figure 4.29**: The variation of *G'* (solid symbol) and *G"* (open symbol) as a function of applied strain of (a) LG-LEC-DEAE-DX ( ■ ) and LG-LEC-DEAE-DX-DPH ( ■ ), (b) LG-DPPC-DEAE-DX ( ★ ) and LG-DPPC-DEAE-DX-DPH ( ★ ), (c) LG-DSPC-DEAE-DX ( ◆ ) and LG-DSPC-DEAE-DX-DPH ( ◆ ).

(b)




(c)

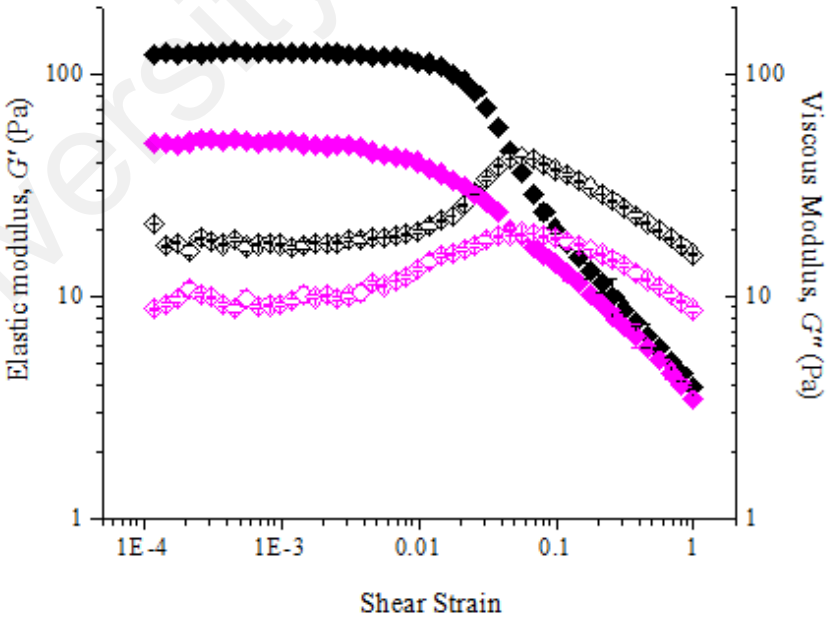



**Figure 4.29**: (continued)

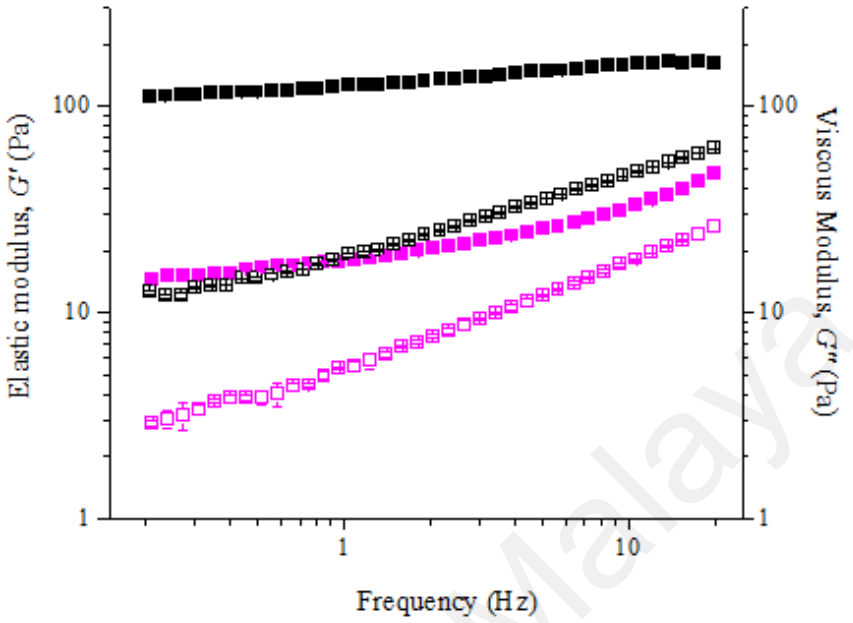
**Table 4.6:** The critical strain ($\gamma_c$), breakpoint ($\gamma_b$), elastic modulus (*G'*) and cohesive energy (CE) of liposomal gels and DPH encapsulated liposomal gels which were determined from their dynamic rheological behaviors.

| Name | Critical strain, $\gamma_c$ | Elastic modulus, *G'* (Pa) | CE (Pa) |
|---|---|---|---|
| LG-LEC-DEAE-DX | 0.0068±0.0001 | 105±2 | 0.0024±0.0001 |
| LG-LEC-DEAE-DX-DPH | 0.0046±0.0001 | 19.7±0.2 | 0.0002±0.0001 |
| LG-DPPC-DEAE-DX | 0.0120±0.0001 | 96.3±0.1 | 0.0069±0.0002 |
| LG-DPPC-DEAE-DX-DPH | 0.0120±0.0001 | 62.5±0.2 | 0.0045±0.0003 |
| LG-DSPC-DEAE-DX | 0.0082±0.0003 | 123±1 | 0.0041±0.0003 |
| LG-DSPC-DEAE-DX-DPH | 0.0026±0.0001 | 48.8±0.1 | 0.0002±0.0004 |

The contribution of DPH to the elastic component of the liposomal gel is also shown in frequency sweep (Figure 4.30). According to the frequency sweep, all liposomal gel including those encapsulated DPH exhibited viscoelastic behavior with *G'* larger than *G"* over the selected frequency range. However, *G'* profile of DPH containing liposomal gel was shifted to a lower value compared to liposomal gel without DPH.

Also, the tan δ of the liposomal gel is lower than the DPH-liposomal gel (Figure 4.31). This result further supports the findings where the presence of DPH in the liposomal gel reduces the solid-like properties and rigidity of the gel. The plausible reason is due to the formation of the less extended structure of gel due to the presence of DPH which shields the electrostatic forces between carbopol polymers (Figure 4.32).

(a)




(b)

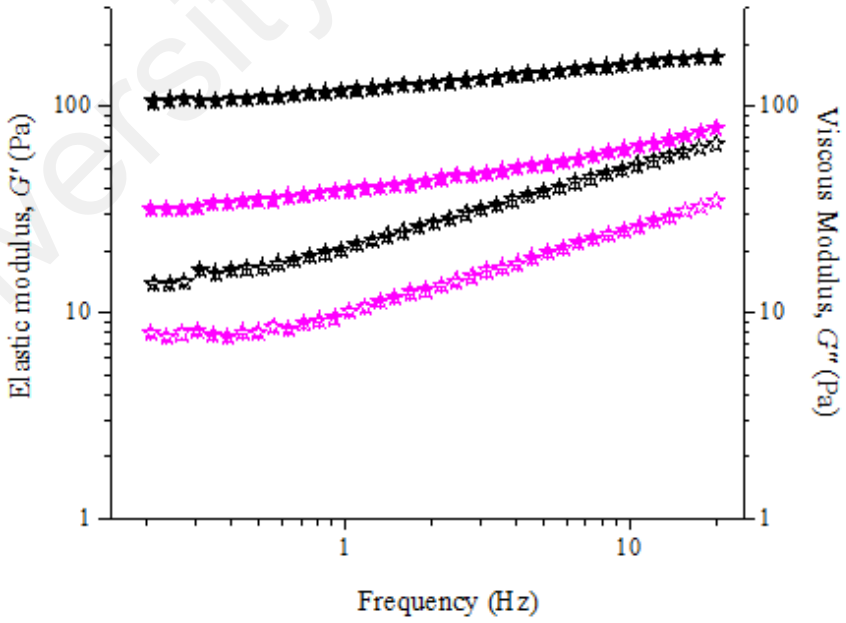



**Figure 4.30** The variation of *G'* (solid symbol) and *G"* (open symbol) as a function of frequency of (a) LG-LEC-DEAE-DX ( ■ ) and LG-LEC-DEAE-DX-DPH ( ■ ), (b) LG-DPPC-DEAE-DX ( ★ ) and LG-DPPC-DEAE-DX-DPH ( ★ ), (c) LG-DSPC-DEAE-DX ( ◆ ) and LG-DSPC-DEAE-DX-DPH ( ◆ ).

(c)

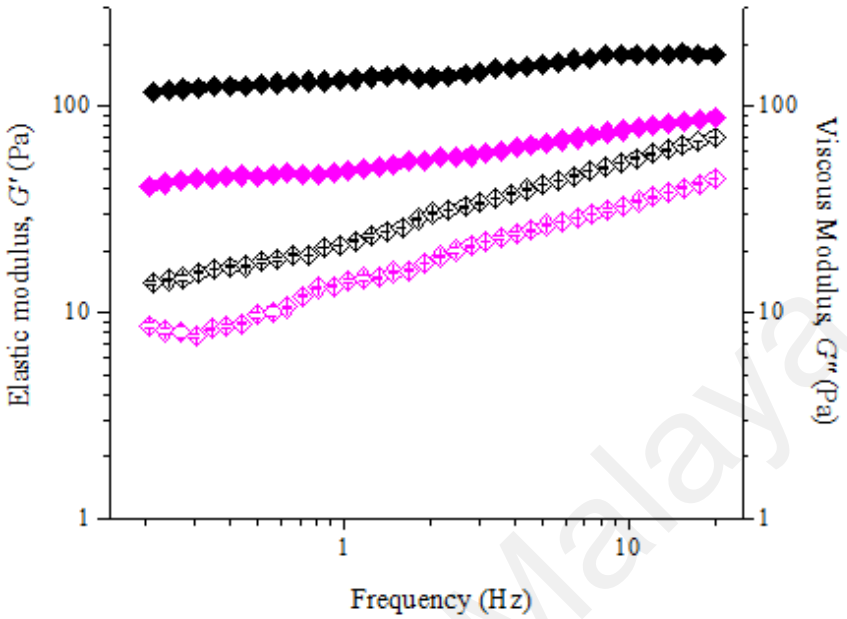



**Figure 4.30**: (continued)

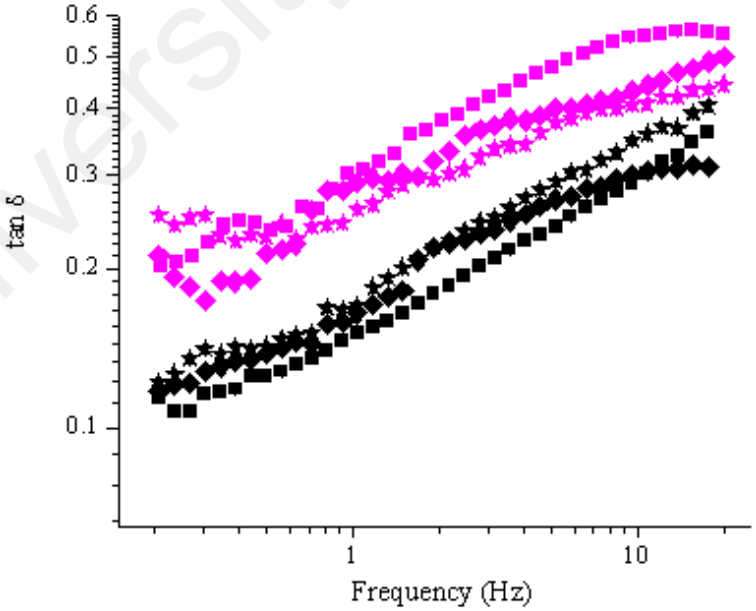



**Figure 4.31:** The tan δ of LG-LEC-DEAE-DX ( ■ ) and LG-LEC-DEAE-DX-DPH ( ■ ), LG-DPPC-DEAE-DX ( ★ ) and LG-DPPC-DEAE-DX-DPH ( ★ ), LG-DSPC-DEAE-DX ( ◆ ) and LG-DSPC-DEAE-DX-DPH ( ◆ ).

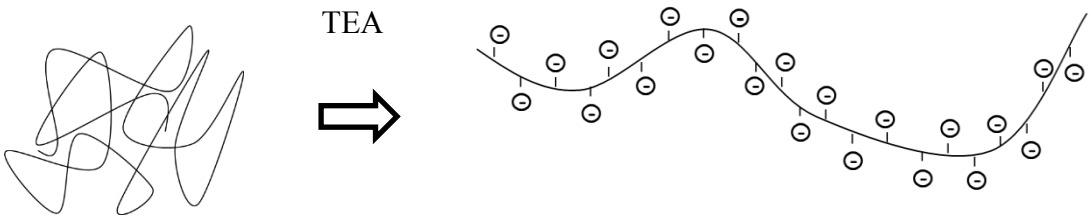



**Figure 4.32**: Illustration of polymer uncoiling.

Formation of carbopol based gel involves the swelling of the polymer after the addition of neutralizing agent, triethanolamine (TEA). TEA removes proton from the side chains of carbopol and subsequently carboxylate ions are formed (Rivers & Umney, 2007). Hence, negative charges are generated along the backbone of the polymer (Figure 4.32). Thus, electrostatic repulsion between carboxylate groups ($-COO^-$) results in uncoiling and formation of expanded gel structure. This will eventually impart rigidity to the gel structure. However, in the presence of DPH, electrostatic repulsive forces between the carboxylate groups ($-COO^-$) were screened and hence the rigidity of the gel structure decreases (Figure 4.33).

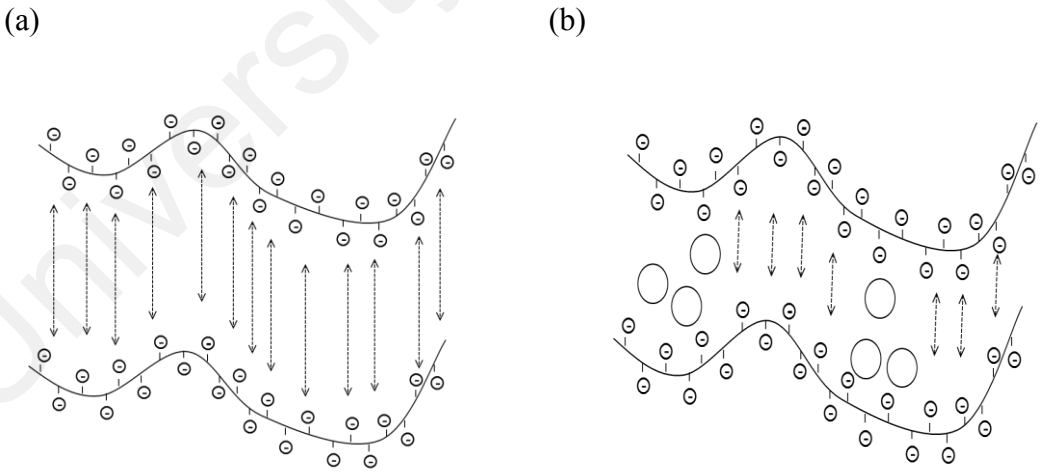



**Figure 4.33**: Illustration of gel (a) in the absence of DPH and (b) in the presence of DPH. The length of the dotted line is a measure of the magnitude of electrostatic repulsion.

Next step was to increase the concentration of the gelling agent, carbopol while maintaining the concentration of liposomes and DPH. The analysis was repeated using

carbopol of 0.5 % (w/v) and 1.0 % (w/v) (Figure 4.34-4.36). We hypothesized that increasing the concentration of gelling agents could help to enhance the elasticity of the gel after incorporation of DPH. The interaction between polymer chains would be greater and it may counteract the shielding of the carboxylate group by drug molecules hence forming more expanded gel structure.

On the other hand, the frequency sweep further supports the discussed point where there is a minimal difference of *G'* observed between the DPH loaded liposomal gel and liposomal gel prepared using of 0.5 % and 1.0% carbopol gel over the selected frequency range. Unlike liposomal gel prepared using 0.25 % CP whereby a significant decrease in *G'* was observed for liposomal gel after incorporation of DPH over the selected frequency range.

(a)

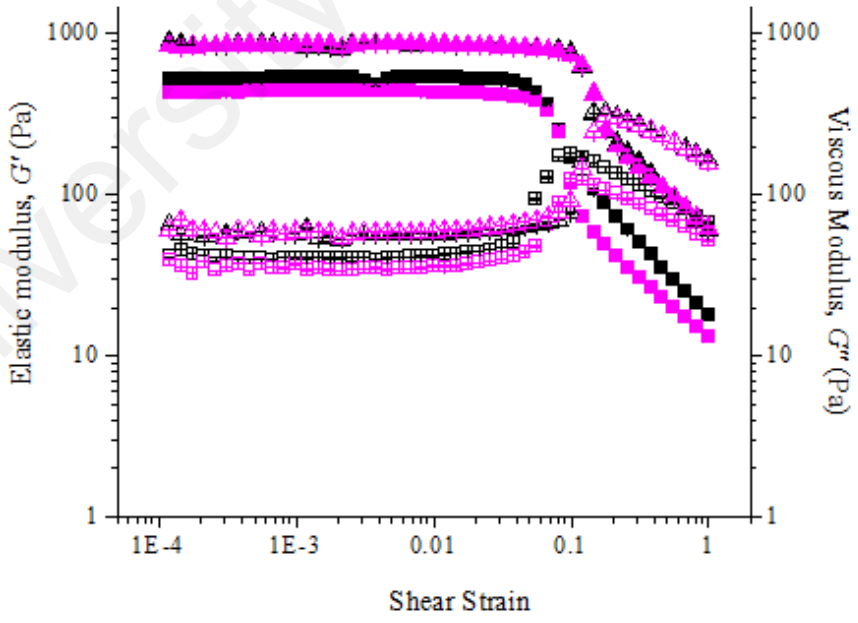



**Figure 4.34**: The variation of *G'* (solid symbol) and *G"* (open symbol) of LG-LEC-DEAE-DX ( ■ ) and LG-LEC-DEAE-DX-DPH ( ■ ) prepared using 0.5 % carbopol and LG-LEC-DEAE-DX ( ▲), LG-LEC-DEAE-DX-DPH ( ▲ ) prepared using 1.0 % carbopol (a) as a function of applied strain and (b) as a function of frequency.

(b)

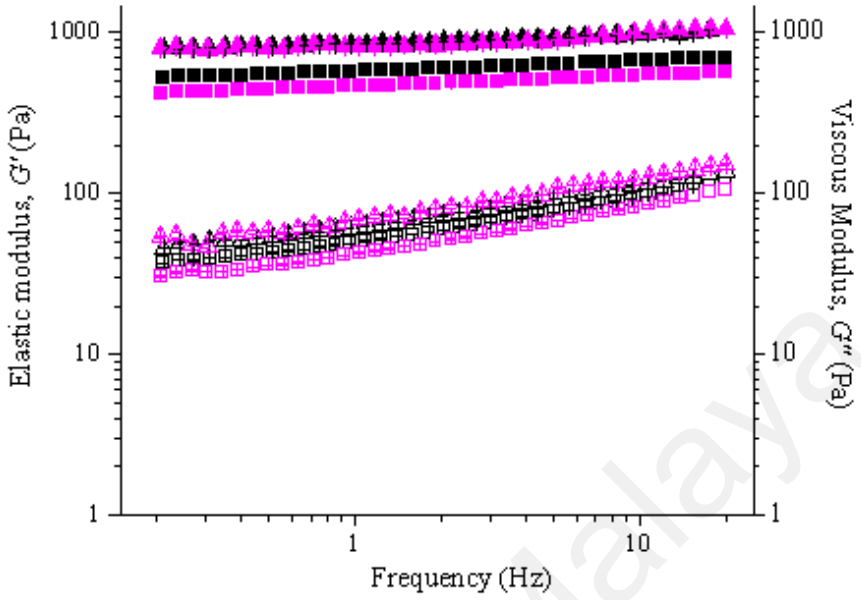



**Figure 4.34:** (continued)

(a)

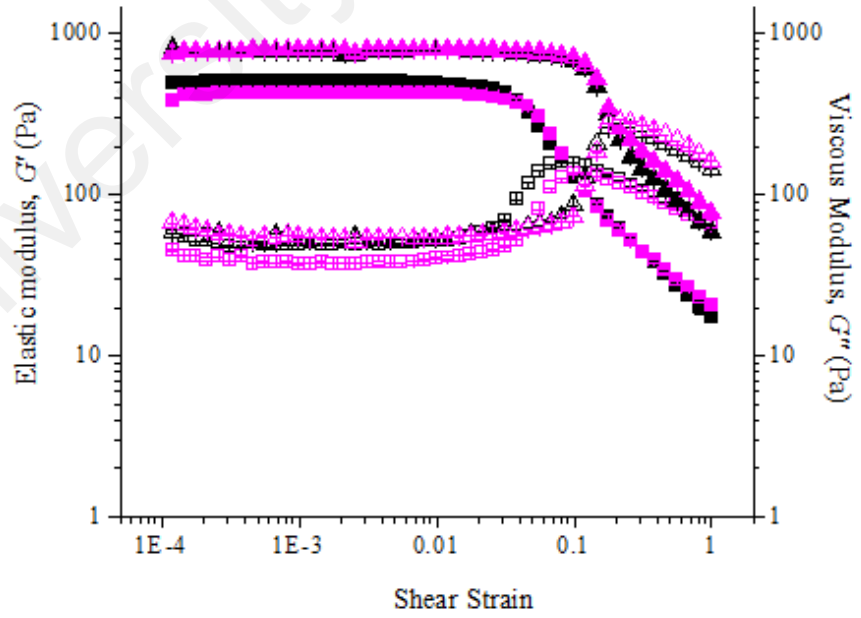



**Figure 4.35:** The variation of *G'* (solid symbol) and *G"* (open symbol) of LG-DPPC-DEAE-DX ( ■ ) and LG-DPPC-DEAE-DX-DPH ( ■ ) prepared using 0.5 % carbopol and LG-DPPC-DEAE-DX ( ▲ ), LG-DPPC-DEAE-DX-DPH ( ▲ ) prepared using 1.0 % carbopol (a) as a function of applied strain (b) as a function of frequency.

(b)

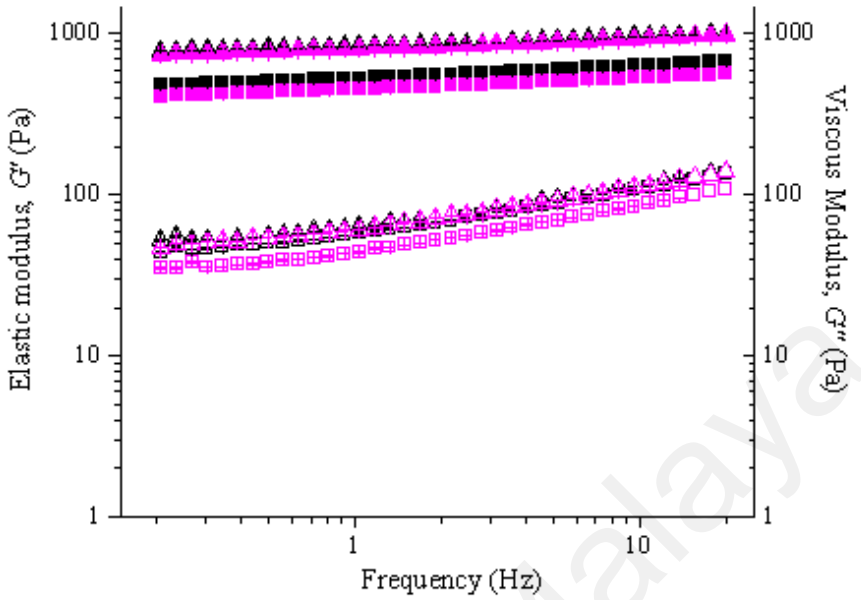



**Figure 4.35:** (continued)

(a)

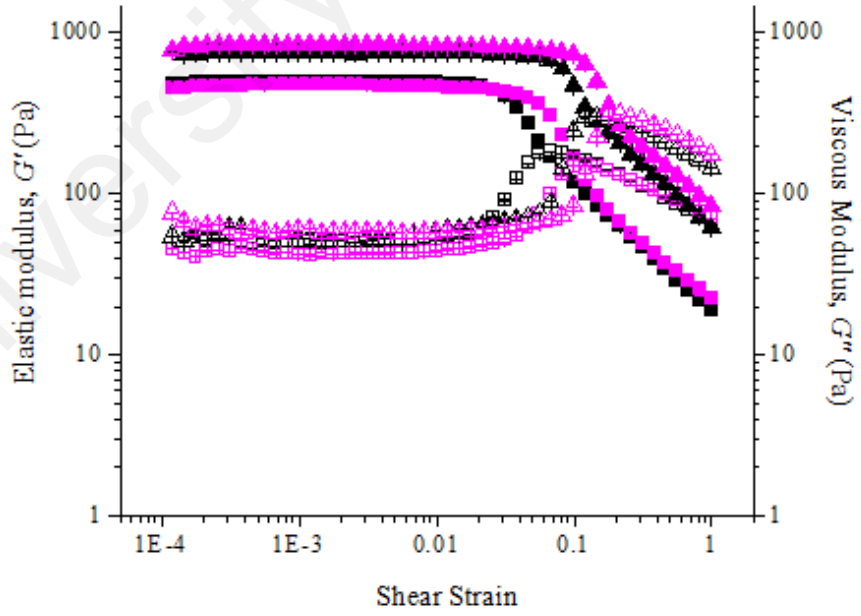



**Figure 4.36**: The variation of *G'* (solid symbol) and *G"* (open symbol) of LG-DSPC-DEAE-DX ( ■ ) and LG-DSPC-DEAE-DX-DPH ( ■ ) prepared using 0.5 % carbopol and LG-DSPC-DEAE-DX ( ▲ ), LG-DSPC-DEAE-DX-DPH ( ▲ ) prepared using 1.0 % carbopol (a) as a function of applied strain and (b) as a function of frequency.

(b)

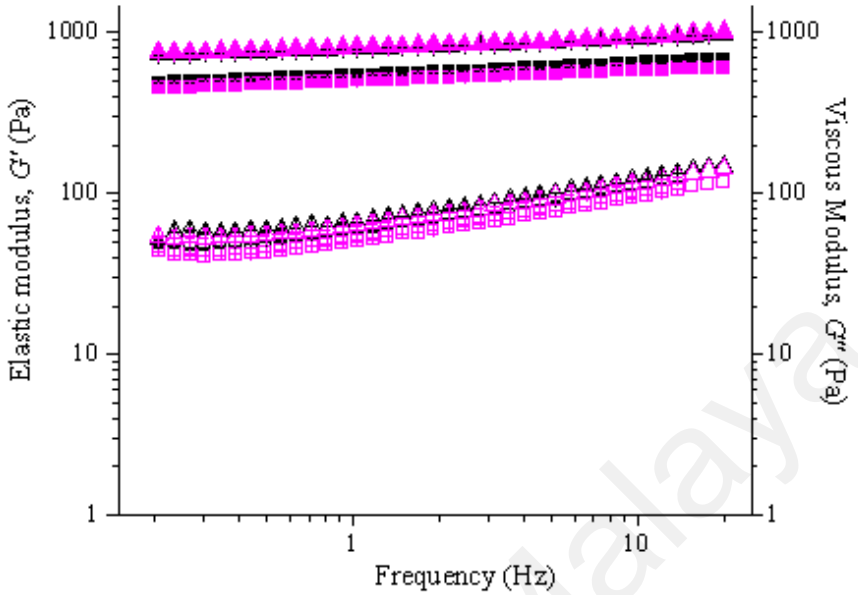



**Figure 4.36: (**continued)

As the concentration of carbopol increased, the *G'*, critical strain and breakpoint of all the liposomal gel increased (Table 4.7). Similarly, the critical strain and breakpoint of DPH loaded liposomal gel prepared using 0.5 % and 1.0 % carbopol exhibited higher value than those prepared using 0.25 % carbopol. In fact, there is not much difference in *G'* of DPH loaded liposomal gel compared to *G'* of a non-loaded liposomal gel prepared using 0.5 % and 1.0 % carbopol

**Table 4.7:** The critical strain ($\gamma_c$), breakpoint ($\gamma_b$), elastic modulus (*G'*) and cohesive energy (CE) for liposomal gels and DPH encapsulated liposomal gels prepared using 0.5 % and 1.0 % carbopol which were determined from their dynamic rheological behaviors.

| Name | Critical strain, $\gamma_c$ | Breakpoint, $\gamma_b$ | Elastic modulus, *G'* (Pa) |
|---|---|---|---|
| | **0.5 % Carbopol** | | |
| LG-LEC-DEAE-DX | 0.0314±0.0002 | 0.0994±0.0006 | 540±5 |
| LG-LEC-DEAE-DX-DPH | 0.0381±0.0003 | 0.0995±0.0003 | 436±3 |
| LG-DPPC-DEAE-DX | 0.0214±0.0001 | 0.082±0.001 | 484±6 |
| LG-DPPC-DEAE-DX-DPH | 0.0259±0.0001 | 0.0994±0.0001 | 387±5 |
| LG-DSPC-DEAE-DX | 0.0177±0.0001 | 0.0677±0.0001 | 471±6 |
| LG-DSPC-DEAE-DX-DPH | 0.0259±0.0001 | 0.12±0.04 | 411±4 |

**Table 4.7**: (continued)

| Name | Critical strain, $\gamma_c$ | Breakpoint, $\gamma_b$ | Elastic modulus, *G'* (Pa) |
|---|---|---|---|
| | **1.0 % Carbopol** | | |
| LG-LEC-DEAE-DX | 0.0677±0.0001 | 0.146±0.001 | 899±4 |
| LG-LEC-DEAE-DX-DPH | 0.0677±0.0001 | 0.177±0.001 | 872±5 |
| LG-DPPC-DEAE-DX | 0.0677±0.0001 | 0.177±0.001 | 814±3 |
| LG-DPPC-DEAE-DX-DPH | 0.0994±0.0001 | 0.214±0.001 | 751±2 |
| LG-DSPC-DEAE-DX | 0.059±0.001 | 0.146±0.001 | 775±7 |
| LG-DSPC-DEAE-DX-DPH | 0.082±0.001 | 0.177±0.001 | 693±9 |

All of the liposomal gel prepared by using 0.5 % and 1.0% of carbopol gel exhibited tan δ values lower than 0.2 as shown in Figure 4.37. The obtained results support the hypothesis whereby the elasticity of DPH loaded liposomal gel could be enhanced by increasing the concentration of carbopol gel. However, too high concentration of gelling agent will lead to the formation of a very rigid gel which eventually affects the homogeneity of dispersed liposome. Thus, 0.5 % carbopol was chosen for the preparation of DPH loaded liposomal gel instead of 1.0 % carbopol.

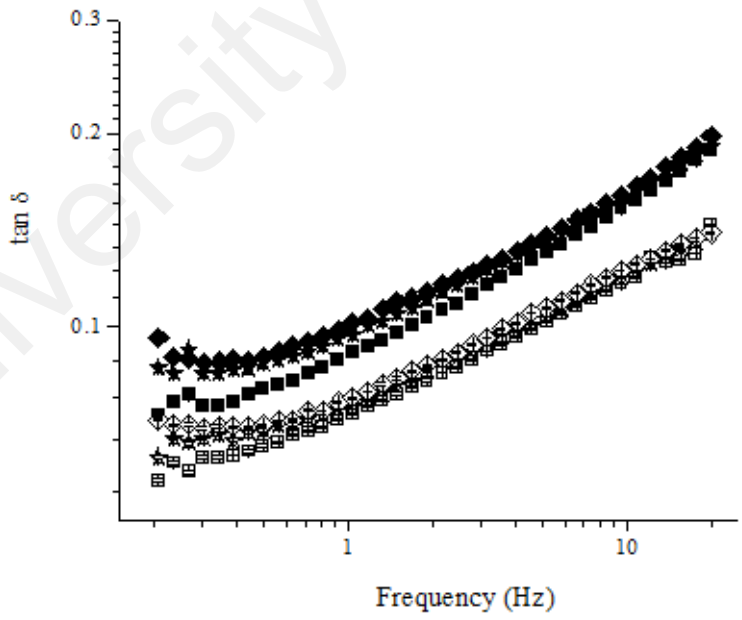



**Figure 4.37:** The tan δ of LG-LEC-DEAE-DX-DPH ( ■ ), LG-DPPC-DEAE-DX-DPH ( ★ ) and LG-DSPC-DEAE-DX ( ◆ ) prepared at 0.5 % CP and LG-LEC-DEAE-DX-DPH ( □ ), LG-DPPC-DEAE-DX-DPH ( ☆ ) and LG-DSPC-DEAE-DX-DPH ( ◇ ) prepared at 1.0 % CP.

#### 4.8.3.2 Flow behavior of DPH loaded liposomal gel

The results obtained in previous section revealed that DPH loaded liposome in 0.25 % carbopol gel has lost its rigidity. This result was further supported by shear viscosity and shear stress profile. The effect becomes pronounced when a shear rate is applied as it causes the polymer chains to come closer without any impedance due to the fact that the repulsive electrostatic force between carboxyl groups is masked by DPH. We also observed the effect of adding DPH to pure 0.25 % and 0.50 % carbopol gel respectively. As expected 0.25 % carbopol gel containg DPH is flowy compared to 0.5 % carbopol gel (Figure 4.38).

(a) (b)

**Figure 4.38:** (a) 0.25 % carbopol gel containing DPH and (b) 0.50 % carbopol gel containing DPH.

As a result, increasing carbopol concentration from 0.25 % to 0.5 % for DPH loaded liposomal gel has improved the rigidity of the gel. Figure 4.39 to Figure 4.41 compares the flow behavior of liposomal gel with or without DPH that were prepared using carbopol concentration of 0.25 %. Also, these profiles were compared to DPH loaded liposomal gels containing 0.5 % carbopol. Based on results obtained, 0.25 % carbopol gel containing DPH loaded liposomes exhibited lower viscosity compared to

the 0.25 % carbopol gel containing non-loaded liposomes as a result of reduced rigidity of the gel structure as discussed in the previous section. Besides that, the yield stress ($\sigma_p$) value also shown significant decrease. The yield stress ($\sigma_p$) value was decreased from 2.96 to 0.95 for LG-LEC-DEAE-DX, 3.36 to 1.16 for LG-DPPC-DEAE-DX and 3.40 to 1.26 for LG-DSPC-DEAE-DX after incorporation of DPH (Table 4.8). This implies that the presence of DPH in liposomal gel prepared using 0.25 % carbopol has lower resistance to flow. Besides that, the PLI value of DPH encapsulated liposomal gel has increased indicates that the gel is less shear thinning and has poor spreading ability compared to the plain liposomal gel.

**Table 4.8:** Power Law Index (PLI) and yield stress ($\sigma_p$) of liposomal gel and DPH encapsulated liposomal gels prepared using 0.25 % carbopol which were determined from the steady rheological behaviors.

| Name | Power Law Index, PLI | Yield stress,$\sigma_p$ (Pa) |
|---|---|---|
| LG-LEC-DEAE-DX | 0.351 | 2.96 |
| LG-LEC-DEAE-DX-DPH | 0.418 | 0.95 |
| LG-DPPC-DEAE-DX | 0.355 | 3.36 |
| LG-DPPC-DEAE-DX-DPH | 0.397 | 1.16 |
| LG-DSPC-DEAE-DX | 0.351 | 3.40 |
| LG-DSPC-DEAE-DX-DPH | 0.404 | 1.26 |

On the other hand, DPH loaded liposomal gel prepared at 0.5 % carbopol exhibited higher viscosity as well as yield stress ($\sigma_p$) value (Table 4.9). This result was predictable as liposomal gel prepared at 0.5 % carbopol had shown greater elasticity from the result obtained in the previous section. Also, the PLI value of the DPH loaded liposomal gel containing 0.5 % carbopol was found lower compared to those containing

0.25 % carbopol. The obtained value implies that the formulated gel has better spreading ability which leads to rapid absorption of drug after being applied to the skin.

**Table 4.9:** Power Law Index (PLI) and yield stress ($\sigma_p$) of DPH encapsulated liposomal gel prepared using 0.5 % carbopol which were determined from the steady rheological behaviors.

| Name | Power Law Index, PLI | Yield stress,$\sigma_p$ (Pa) |
|---|---|---|
| LG-LEC-DEAE-DX-DPH | 0.337 | 9.24 |
| LG-DPPC-DEAE-DX-DPH | 0.302 | 15.7 |
| LG-DSPC-DEAE-DX-DPH | 0.294 | 18.1 |

(a)

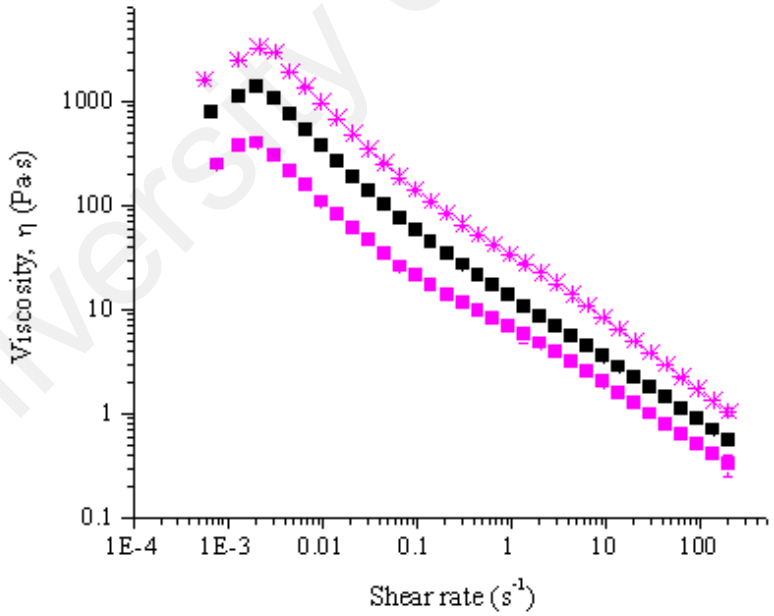



**Figure 4.39:** The flow behavior of LG-LEC-DEAE-DX ( ■ ) and LG-LEC-DEAE-DX-DPH ( ■ ) prepared using 0.25 % carbopol and LG-LEC-DEAE-DX-DPH prepared using 0.5 % carbopol ( ✳ ) where (a) is the shear viscosity profile with increasing shear rate and (b) is the shear stress versus shear rate profile.

(b)

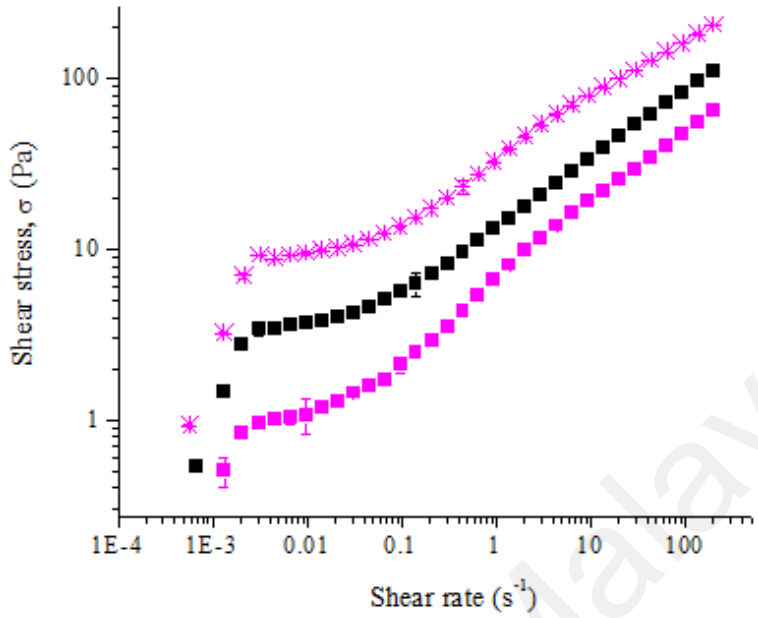



**Figure 4.39: (**continued)

(a)

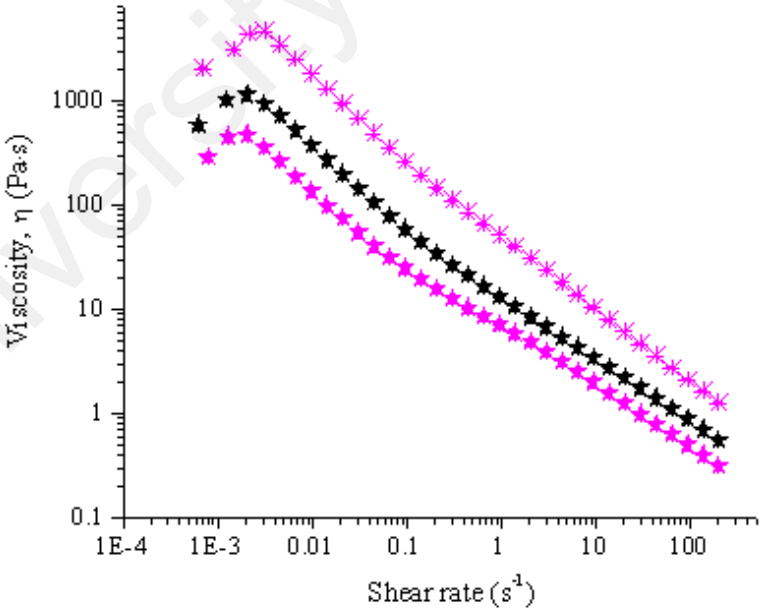



**Figure 4.40**: The flow behavior of LG-DPPC-DEAE-DX ( ★ ) and LG-DPPC-DEAE-DX-DPH ( ★ ) prepared using 0.25 % carbopol and LG-DPPC-DEAE-DX-DPH prepared using 0.5 % carbopol ( ✳ ) where (a) is the shear viscosity profile with increasing shear rate and (b) is shear stress versus shear rate profile.

(b)

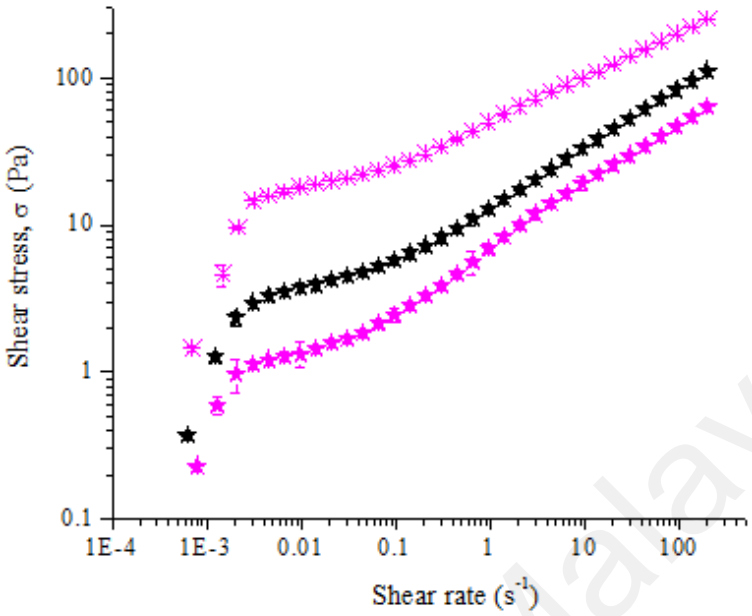



**Figure 4.40:** (continued)

(a)

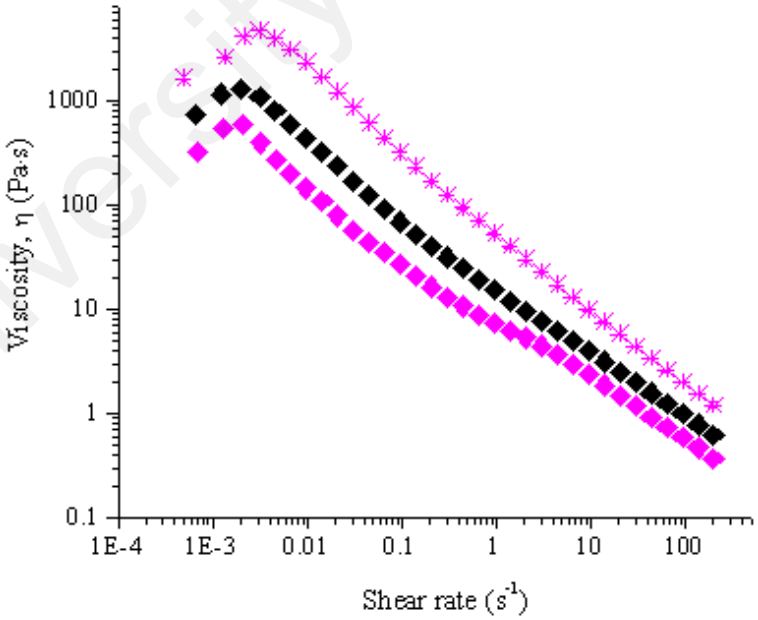



**Figure 4.41:** The flow behavior of LG-DSPC-DEAE-DX ( ◆ ) and LG-DSPC-DEAE-DX-DPH ( ◆ ) prepared using 0.25 % carbopol and LG-DSPC-DEAE-DX-DPH prepared using 0.5 % carbopol ( ✳ ) where (a) is the shear viscosity profile with increasing shear rate and (b) is the shear stress versus shear rate profile.

(b)

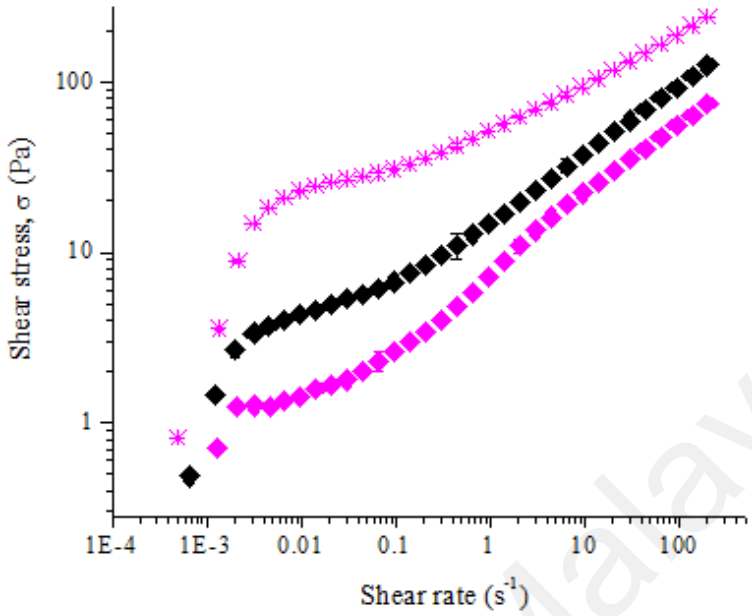


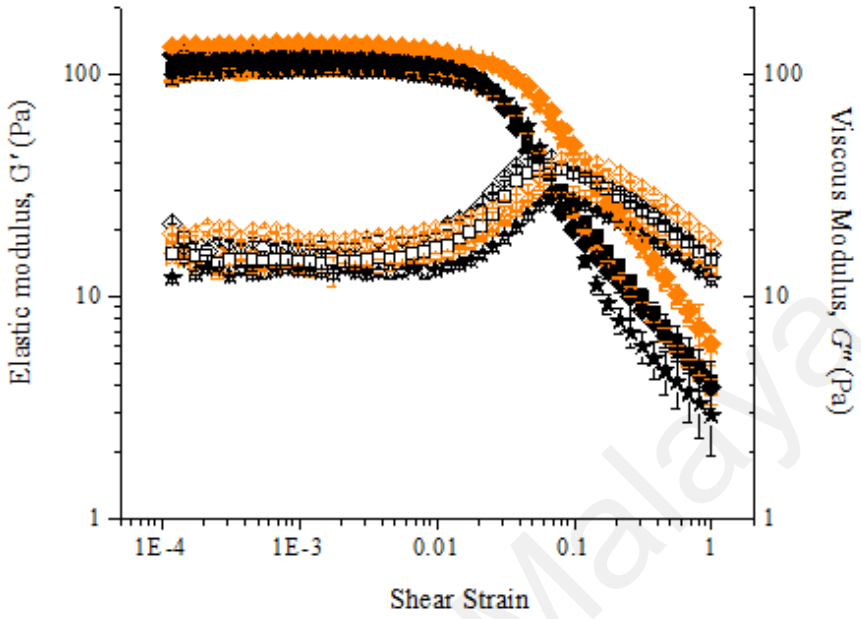

**Figure 4.41:** (continued)

In a conclusion, formulation of 0.5 % carbopol in gel has helped to improve the viscoelastic and flow behavior of DPH loaded liposomal gel by providing more possibilities for the polymers to repel and form an extended structure due to the presence of higher concentration of polymer despite some region are being masked by DPH.

### 4.8.3.3 Dynamic behavior of curcumin loaded liposomal gel

Liposome encapsulated curcumin was incorporated into carbopol gel prepared at concentration of 0.25 %. The amplitude sweep profiles of curcumin encapsulated liposomal gels showed that *G'* was predominant over *G"* (Figure 4.42a). The liposomal gel had no significant change in *G'* after incorporation of curcumin encapsulated liposomes. Similar to liposomal gel, frequency sweep profile of curcumin liposomal gel also exhibited viscoelastic behavior with *G'* larger than *G"* over the selected frequency range (Figure 4.42b).

(a)




(b)

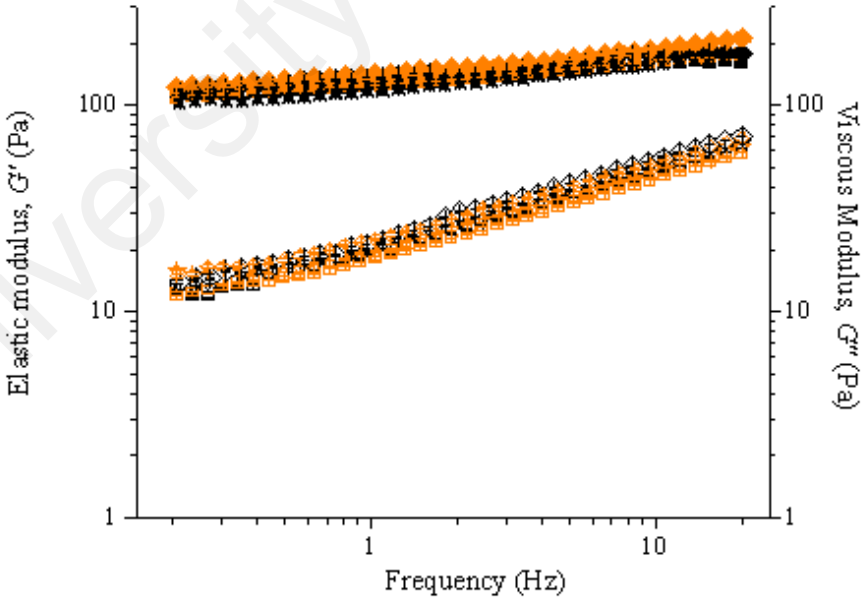



**Figure 4.42:** The variation of *G'* (solid symbol) and *G"* (open symbol) of LG-LEC-DEAE-DX ( ■ ), LG-LEC-DEAE-DX-CUR ( ■ ), LG-DPPC-DEAE-DX ( ★ ), LG-DPPC-DEAE-DX-CUR ( ★ ), LG-DSPC-DEAE-DX ( ◆ ), LG-DSPC-DEAE-DX-CUR ( ◆ ) where (a) as a function of applied strain and (b) as a function of frequency.

However, the $\gamma_c$, and $\gamma_b$ of curcumin liposomal gel exhibited slightly higher compared to liposomal gel (Table 4.10). This result indicated that curcumin encapsulated liposomal gel has slightly greater elastic network and exhibited greater resistivity against deformation. This was further supported by the higher CE values obtained for curcumin liposomal gel.

**Table 4.10**: The critical strain ($\gamma_c$), breakpoint ($\gamma_b$), elastic modulus (*G'*) and cohesive energy (CE) of the liposomal gels and curcumin encapsulated liposomal gels which were determined from their dynamic rheological behaviors.

| Name | Critical strain, $\gamma_c$ | Breakpoint, $\gamma_b$ | Elastic modulus, *G'* (Pa) | CE (Pa) |
|---|---|---|---|---|
| LG-LEC-DEAE-DX | 0.0068±0.0001 | 0.0068±0.001 | 105±2 | 0.0024±0.0002 |
| LG-LEC-DEAE-DX-CUR | 0.0120±0.0003 | 0.082±0.001 | 102±1 | 0.0073±0.0004 |
| LG-DPPC-DEAE-DX | 0.0120±0.0001 | 0.099±0.002 | 96.3±0.1 | 0.0069±0.0003 |
| LG-DPPC-DEAE-DX-CUR | 0.0150±0.0001 | 0.146±0.005 | 132±3 | 0.0149±0.0001 |
| LG-DSPC-DEAE-DX | 0.0082±0.0003 | 0.056±0.002 | 123±1 | 0.0041±0.0001 |
| LG-DSPC-DEAE-DX-CUR | 0.0180±0.0001 | 0.146±0.004 | 130±5 | 0.0211±0.0002 |

The plausible reason for the increase in critical strain and breakpoint for curcumin liposomal gel may be due to the increase in rigidity of liposome as a result of the entrapped curcumin. Curcumin being a hydrophobic drug interacts with the hydrophobic region of the liposome, thus producing a more compact and rigid arrangement of bilayers. This will eventually add on the rigidity of the liposomes. As such, a slight increase in elasticity of liposome gel was observed upon incorporation of curcumin.

Although, there is no significant decrease in tan δ between curcumin encapsulated liposomal gel and plain liposomal gel, but the slight shift of curcumin liposomal gel to a lower value of tan δ suggested the incorporation of curcumin improved the elasticity of liposomal gel (Figure 4.43). In overall, we suggest that incorporation of curcumin loaded liposomes in 0.25 % carbopol slightly increased the elasticity of the gel unlike incorporation of DPH loaded liposomes in 0.25 %.

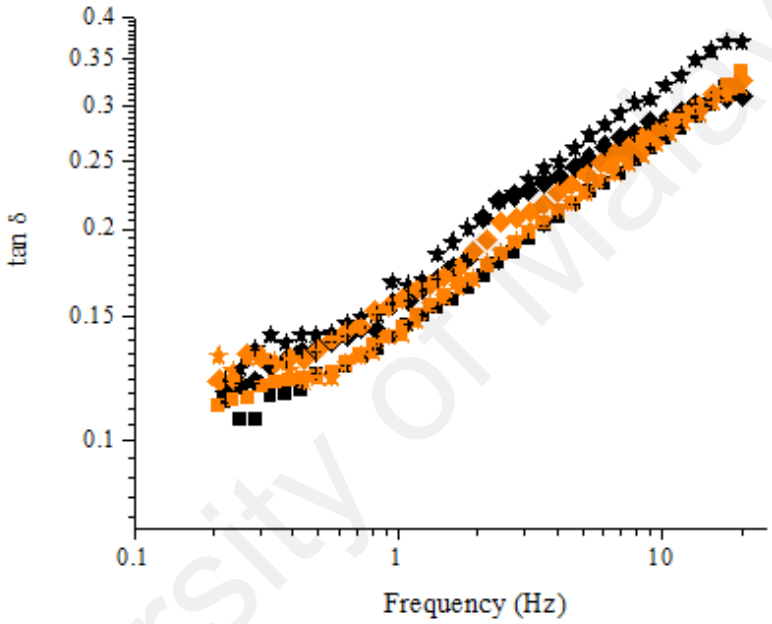



**Figure 4.43:** The tan δ of LG-LEC-DEAE-DX ( ■ ) and LG-LEC-DEAE-DX-CUR ( ■ ), LG-DPPC-DEAE-DX ( ★ ) and LG-DPPC-DEAE-DX-CUR ( ★ ), LG-DSPC-DEAE-DX ( ◆ ) and LG-DSPC-DEAE-DX-CUR ( ◆ ).

### 4.8.3.4 Flow behavior of curcumin loaded liposomal gel

The flow behavior of curcumin loaded liposomal gel was shown in Figure 4.44. There is no drastic change in the viscosity of the liposomal gel after incorporation of curcumin. However, the yield stress value has increased from 2.96 to 3.81 for LG-LEC-DEAE-DX, 3.36 to 4.58 for LG-DPPC-DEAE-DX and 3.40 to 5.17 for LG-DSPC-DEAE-DX after incorporation of curcumin (Table 4.11).

**Table 4.11:** Power Law Index (PLI) and yield stress ($\sigma_p$) of the liposomal gel and curcumin encapsulated liposomal gels prepared using 0.25 % carbopol which was determined from the steady rheological behaviors.

| Name | Power Law Index, PLI | Yield stress,$\sigma_p$ (Pa) |
|---|---|---|
| LG-LEC-DEAE-DX | 0.351 | 2.96 |
| LG-LEC-DEAE-DX-CUR | 0.355 | 3.81 |
| LG-DPPC-DEAE-DX | 0.355 | 3.36 |
| LG-DPPC-DEAE-DX-CUR | 0.340 | 4.58 |
| LG-DSPC-DEAE-DX | 0.351 | 3.40 |
| LG-DSPC-DEAE-DX-CUR | 0.324 | 5.17 |

(a)

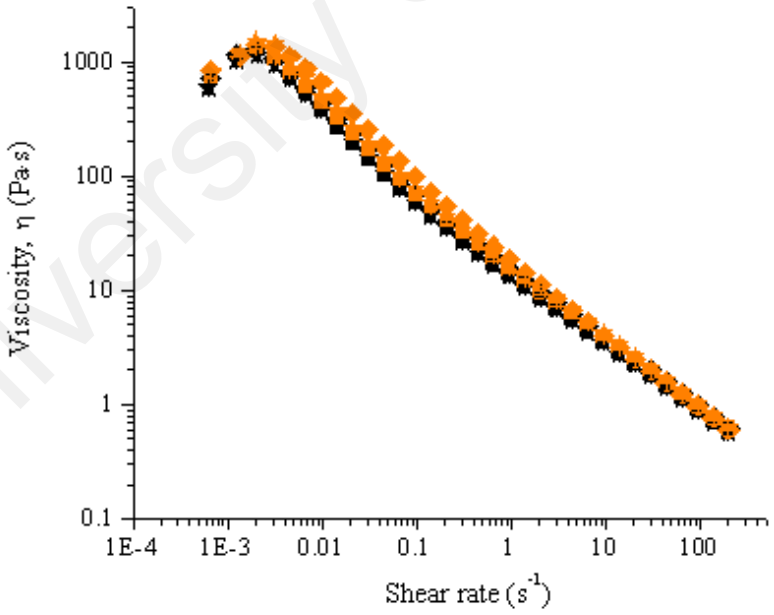



**Figure 4.44**: The flow behavior of LG-LEC-DEAE-DX ( ■ ), LG-LEC-DEAE-DX-CUR ( ■ ), LG-DPPC-DEAE-DX ( ★ ), LG-DPPC-DEAE-DX-CUR ( ★ ), LG-DSPC-DEAE-DX ( ◆ ), LG-DSPC-DEAE-DX-CUR ( ◆ ) where (a) is the shear viscosity profile with increasing shear rate and (b) is the shear stress versus shear rate profile.

(b)

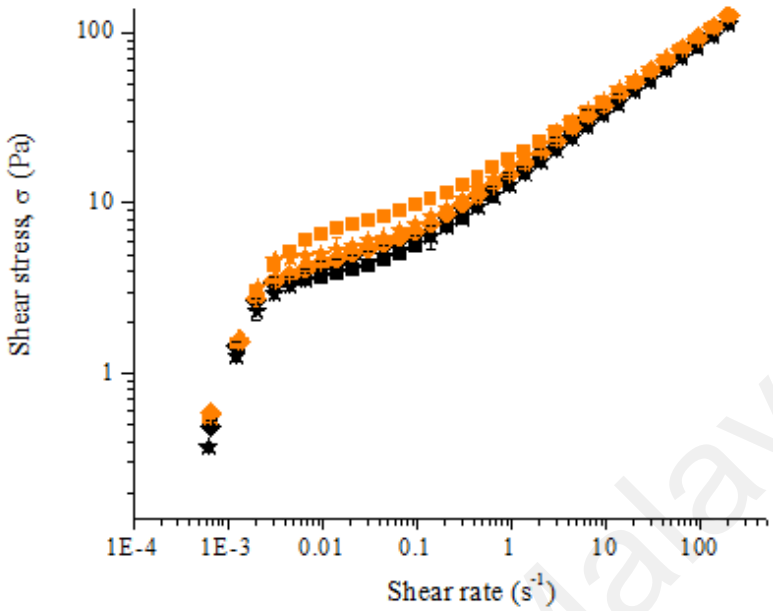



**Figure 4.44**: (continued)

As mentioned above, curcumin encapsulated liposomal gels seems to have stronger elastic network and exhibits greater resistivity against deformation based on the obtained $\gamma_c$, $\gamma_b$ and CE value from the amplitude sweep test. Thus, slightly higher shear stress is needed to cause the curcumin encapsulated liposomal gel to yield and flow. On the other hand, the PLI values did not change significantly after incorporation of curcumin into liposomal gel (Table 4.11). This indicated that curcumin liposomal gel is also as shear thinning as the plain liposomal gel hence has good spreading ability which may contribute to a rapid absorption of curcumin after being applied on the skin.

## 4.9 *In vitro* release

Liposomal solutions with optimum formulation properties based on the results obtained from surface tension, particle size and encapsulation efficiency analysis were subjected to *in vitro* release investigation. Besides the drug release property of liposomal solution, the release of drug after incorporation of the liposome into gel matrix was also investigated. The crucial study of liposomal drug delivery system is the rate at which the drug is released from the liposome as a carrier. *In vitro* release tests are generally performed in order to distinguish whether the system portrays either slow or fast release. Also, release studies reveal details on the release mechanisms and the kinetics. Without exception, *in vitro* release analysis is an essential tool employed to study the behavior of various drug delivery system which enables the development of drug release products (D'Souza, 2004).

### 4.9.1 DPH release

#### 4.9.1.1 Liposomal dispersion system

DPPC-DEAE-DX with 0.065 % (w/v) of DPH, LEC-DEAE-DX and DSPC-DEAE-DX with 0.055 % (w/v) of DPH respectively were selected in this study owing to it's optimized formulation property based on the results obtained from encapsulation efficiency analysis. In this section, we have studied the influence of LEC, DPPC and DSPC liposome respectively on the release of DPH by comparing with the release of plain DPH from a solution. Moreover, the effect of DEAE-DX coating on the release behavior of all the liposome formulation was also studied.

The cumulative amount of DPH releases from all samples was plotted against time as shown in Figure 4.46. For the first 2 hours, there was not much difference in the rate of release for the liposomal system compared to free drug solution. This could be due to the presence of free DPH molecules in the aqueous phase which diffuses rapidly

through the membrane. However, the release rate of liposomal system begun to slow down compared to the free drug solution after 2 hours. This phenomenon explains the retention of DPH in the liposome thus demonstrating a slower rate of diffusion through the membrane. Free drug solution diffuses directly from the solution to the diffusate chamber. On the other hand, diffusion of DPH in liposomal dispersion involves two-step whereby DPH firstly diffuses from the liposome to the aqueous solution followed by diffusion from the aqueous solution to the diffusate chamber as illustrated in Figure 4.45.

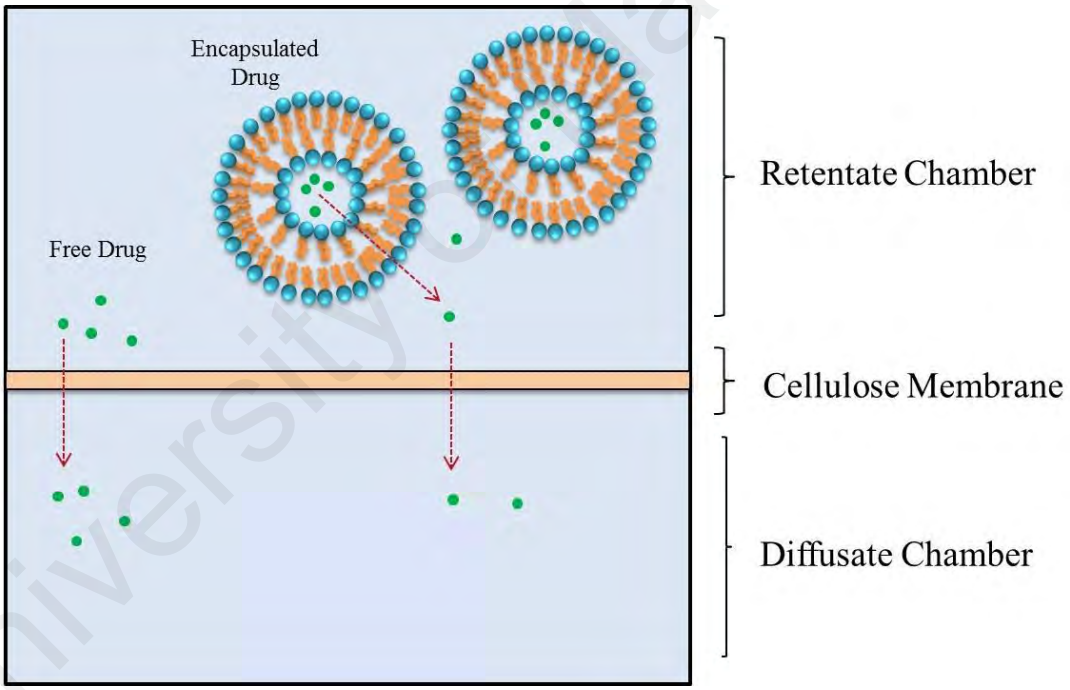



**Figure 4.45:** Illustration of drug release process.

In overall, this study showed that the cumulative drug release of the liposomal system was found lower compared to free drug solution containing same drug concentration for 24 hours release profile (Figure 4.46). Thus revealing DPH molecules were successfully incorporated to liposomes.

Besides that, the effect of DEAE-DX coated liposome on the DPH release was also studied. For the first 8 hours, coated and uncoated LEC liposome released almost the same amount of DPH. However, for the next 16 hours, DEAE-DX coated LEC liposomes exhibited slower release compared to the uncoated liposomes. The same phenomenon is observed for DPPC and DSPC liposome in which the DEAE-DX coated liposome has a slower release for the subsequent 16 hours compared to uncoated liposome. This information shows that in the presence of DEAE-DX, the liposome may be protected against drug leakage. As a consequence, the drug is retained in the liposome for a longer period of time.

(a)

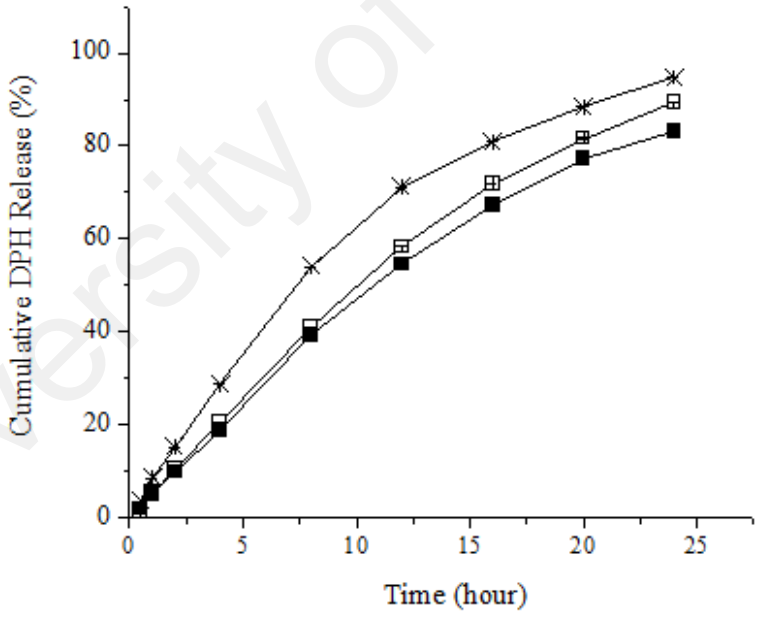
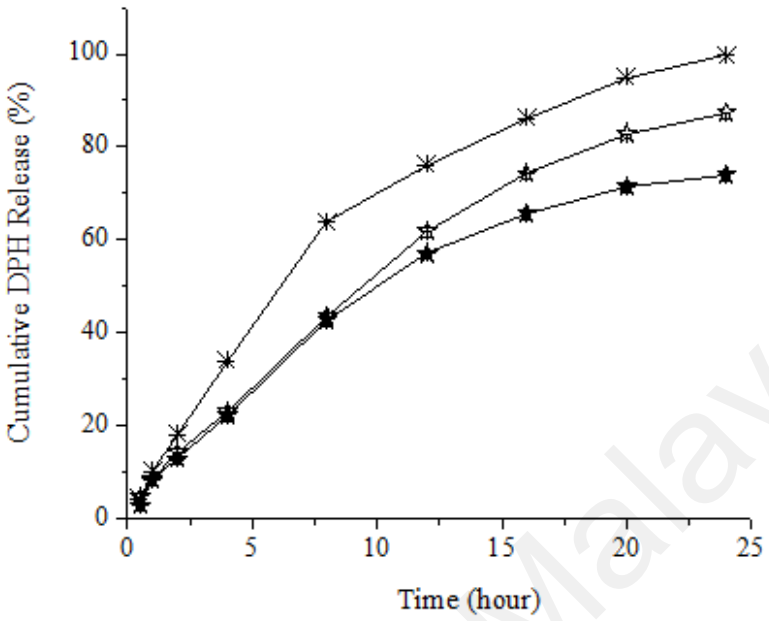



**Figure 4.46:** In vitro release of (a) 0.055 % (w/v) DPH solution ( ✳ ), LEC ( □ ) and LEC-DEAE-DX ( ■ ), (b) 0.065 % (w/v) DPH solution ( ✳ ), DPPC ( ☆ ) and DPPC-DEAE-DX ( ★ ), (c) 0.055 % (w/v) DPH solution ( ✳ ), DSPC ( ◇ ) and DSPC-DEAE-DX ( ◆ ), (d) Comparison of LG-LEC-DEAE-DX ( ■ ), LG-DPPC-DEAE-DX ( ★ ) and LG-DSPC-DEAE-DX ( ◆ ) over a period of 24 hours.

(b)




(c)

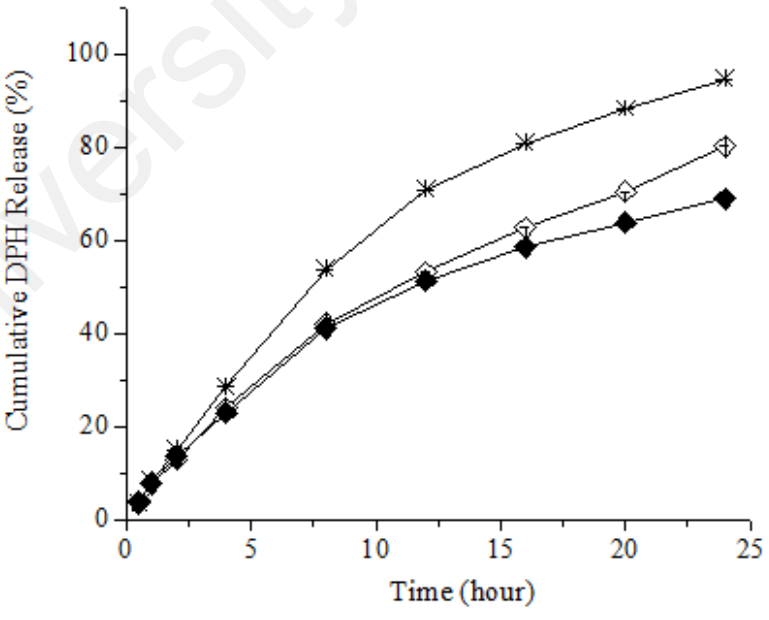



**Figure 4.46:** (continued)

(d)

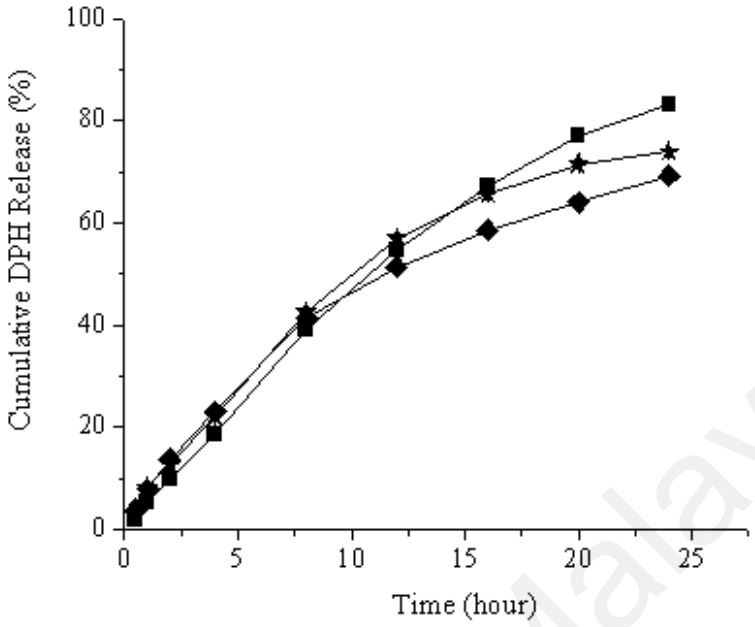



**Figure 4.46:** (continued)

This study reveals that the formulated carrier system is a slow release carrier as compared to its plain drug solution which has reached maximum release of almost 100 % at the 24$^{th}$ hour. Also, this study revealed that coating of liposome with DEAE-DX could prolong the release of DPH. Such release pattern is advantageous as sustained release of DPH from the liposomal system could help to reduce the adverse effects of DPH.

We also compared the in vitro release of DPH from three different formulations which consist of LEC-DEAE-DX, DPPC-DEAE-DX or DSPC-DEAE-DX liposomes (Figure 4.46d). After 24 hours of release analysis, LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX show the cumulative release of 83.1 %, 74.0 % and 69.2 %, respectively. Results show that DSPC-DEAE-DX had the slowest release thus may serve as the best system for sustained release of DPH. DSPC with a longer alkyl chain and higher phase transition temperature as compared to LEC and DPPC exhibits slowest

release due to stronger van der Waals interaction between the lipid chains (Begum et al., 2012). This result is in agreement with the results obtained from the DSC analysis in Section 4.4. Thus, higher energy is required to disrupt the binding packing of DSPC bilayers hence slowing down the release of the DPH.

Besides that, obtained result also supports the fact whereby DPH is entrapped in the core of liposomes instead of being attached to the liposomal surface. DPH is a hydrophilic drug; hence there is a possibility for it to be located either in the core region of the liposome or at the surface of the liposome by attaching to the hydrophilic chain of DEAE-DX. However, the slowest release of DPH from DSPC liposomes shows that DPH diffusion is affected by the long alkyl chain length of phospholipid bilayer and stronger bilayer interaction which delayed the process of DPH diffusion through the bilayer. Hence, we suggest that DPH is located in the core region of liposomes as its diffusion mainly affected by the disruption of the bilayer in the liposome.

#### 4.9.1.2 Liposomal gel system

As the release behavior of liposome solution was obtained, it is also important to investigate and compare the release behavior of the liposome after incorporation into carbopol gel. Similar to *in vitro* release of liposomal dispersion, LG-DPPC-DEAE-DX with 0.065 % (w/v) DPH whereas LG-LEC-DEAE-DX and LG-DSPC-DEAE-DX liposomes with 0.055 % (w/v) of DPH were subjected for *in vitro* drug release analysis. The carbopol concentration used for the preparation of gel is 0.5 % (w/v) as it displayed the desired gel consistency according to the results obtained from the rheological analysis. The release of DPH from the plain gel was also compared with the release of DPH from the liposomal gel in order to demonstrate the retarding effect of the liposome in delivering drugs.

Figure 4.47(a-c) shows the release of DPH from all samples plotted against time. All of the liposomal gel exhibited slower release of DPH compared to the plain gel. In overall, 0.055 % DPH gel and 0.065 % DPH gel shows release at 82.7% and 88.2 %, respectively whereas LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX show release at 69.9 %, 66.0 % and 59.7 %, respectively for a period of 48 hours. This result was as expected as the presence of liposomes in the gel matrix acts as a barrier and hold DPH for longer period compared to the DPH from plain gel that diffuses directly from the gel matrix to diffusate chamber. This result also reveals the successful incorporation of DPH loaded liposomes into the gel matrix without being distorted by the polymers network in the gel. Besides that, LG-DSPC-DEAE-DX exhibited the slowest release of DPH as expected due to its rigid structure (Figure 4.47d).

(a)

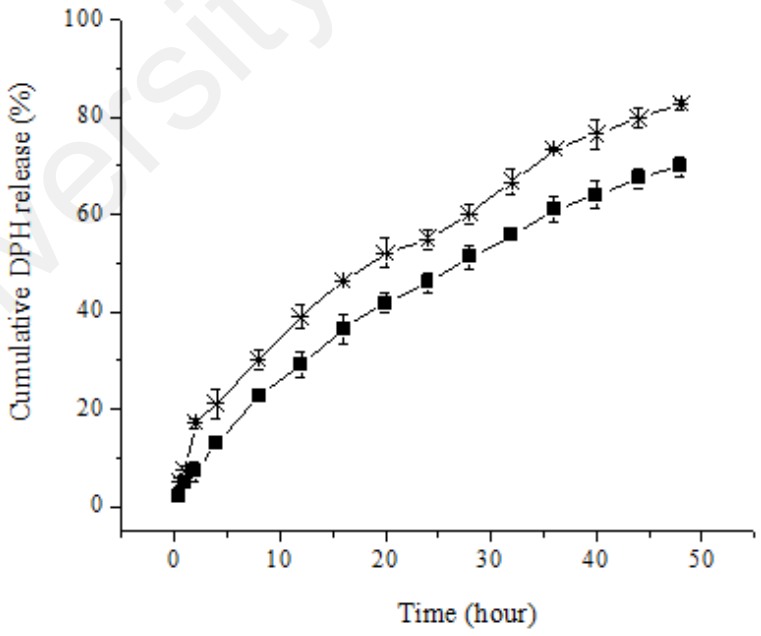
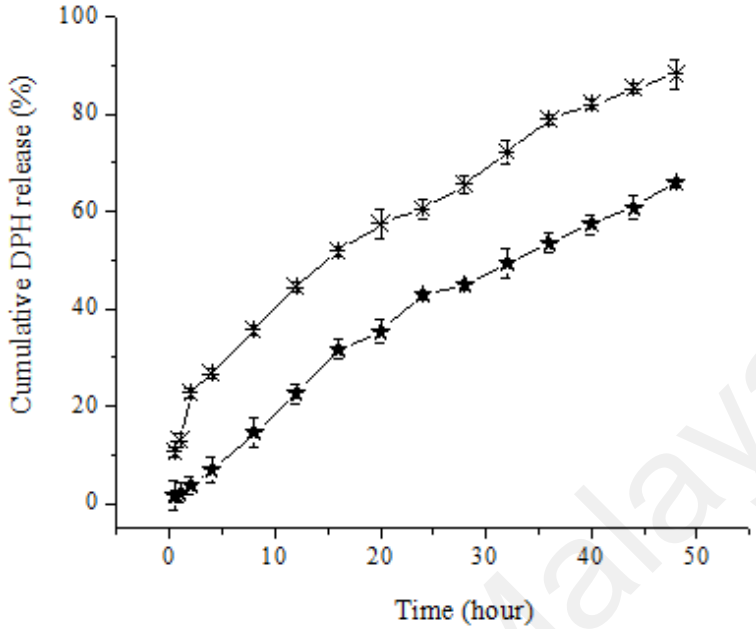



**Figure 4.47:** In vitro release of (a) 0.055 % (w/v) DPH gel ( ✳ ) and LG-LEC-DEAE-DX( ■ ), (b) 0.065 % (w/v) DPH gel ( ✳ ) and LG-DPPC-DEAE-DX ( ★ ), (c) 0.055 % (w/v) DPH gel ( ✳ ) and LG-DSPC-DEAE-DX ( ◆ ), (d) Comparison of LG-LEC-DEAE-DX ( ■ ), LG-DPPC-DEAE-DX ( ★) and LG-DSPC-DEAE-DX ( ◆ ) over a period of 48 hours.

(b)




(c)

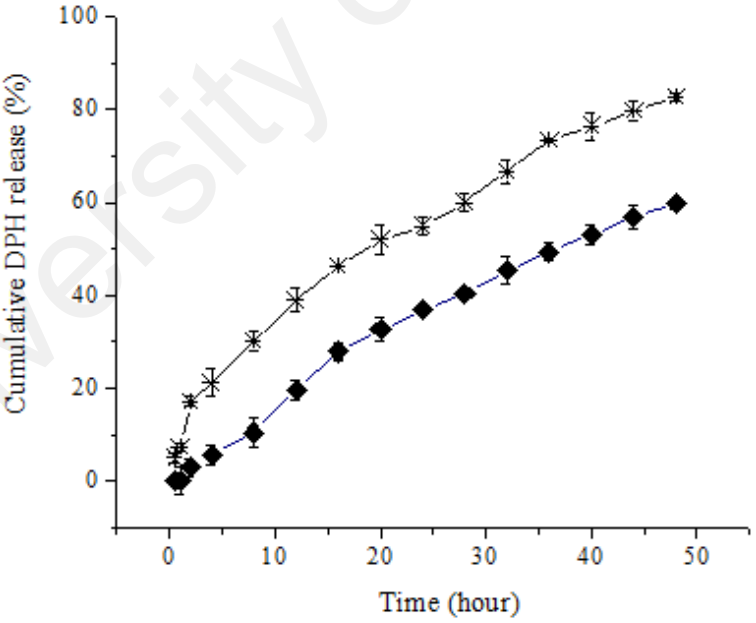



**Figure 4.47:** (continued)

(d)

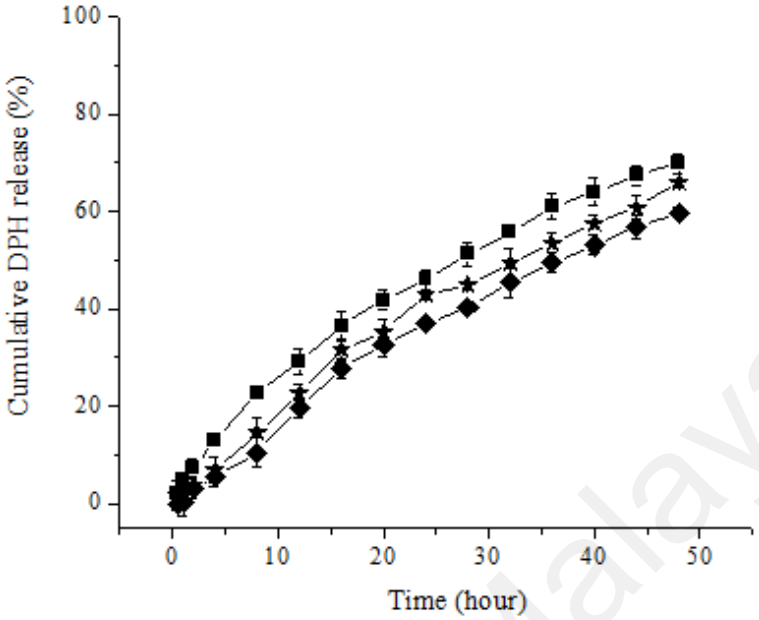



**Figure 4.47:** (continued)

#### 4.9.1.3 Comparison of DPH release between liposomal solution and liposomal gel

The release rate of the drugs from liposomal dispersion and liposomal gel was also compared respectively. Based on the results obtained, the release rate of LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX after formulated into the gel revealed the retention of the drug by the gel. Based on Figure 4.48, the cumulative DPH releases for LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX at 24$^{th}$ hour are 83.0 %, 74.0 % and 69.2 % respectively. However, at the 24$^{th}$ hour the release of LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX only reaches 46.0 %, 42.8 % and 37.1 %, respectively. This shows that the release rate of DPH has decreased about two times after incorporation of liposomes into gel. The DPH release from the liposomal gel was investigated for 48 hours since only less than 50 % of DPH was released from the liposomal gel for the first 24 hours of analysis. And it was found that up to 48 hours of

analysis, the release of DPH for LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX reached 69.9 %, 66.0 % and 59.7 %, respectively (Figure 4.47d).

Conventional drugs such as DPH are associated with some adverse effects such as dry mouth and throat, increased heart rate, pupil dilation and urinary retention (Rezaeifar, 2016). The *in vitro* release result demonstrated that the advantages of liposomal gel in improving the therapeutic efficacy by prolonging the release of the drug. Also, it may be possible to omit the side effects of these drugs by using liposomal gel delivery method with a low dosage of DPH which enable better therapeutic performance. Therefore, development of DPH encapsulated liposomes followed by incorporation of these liposomes into carbopol gel certainly has become a feasible way to design an effective transdermal delivery system.

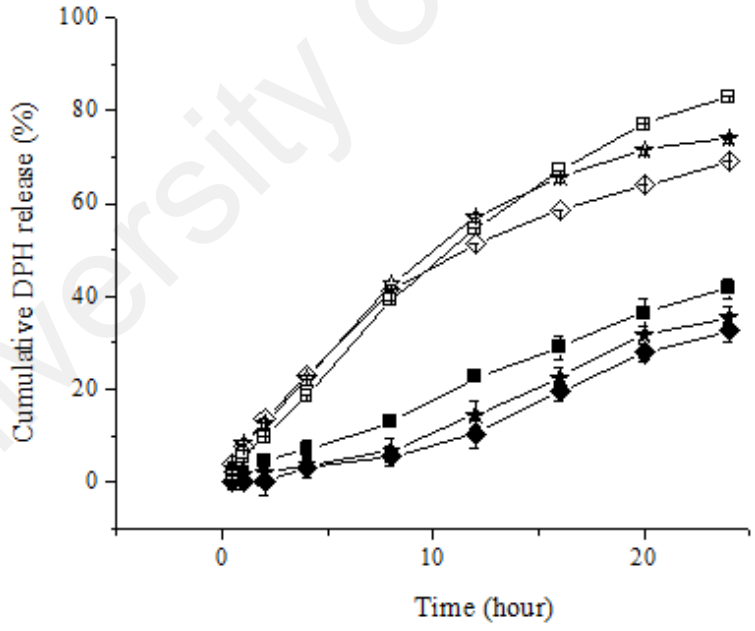



**Figure 4.48:** In vitro release of DPH from LEC-DEAE-DX ( □ ), DPPC-DEAE-DX ( ☆ ) and DSPC-DEAE-DX ( ◇ ) and LG-LEC-DEAE-DX ( ■ ), LG-DPPC-DEAE-DX ( ★ ) and LG-DSPC-DEAE-DX ( ◆ ) over a period of 24 hours.

### 4.9.2 Curcumin release

#### 4.9.2.1 Liposomal dispersion system

Curcumin is practically insoluble in water and soluble in methanol, ethanol, dimethylsulfoxide and acetone (Jefferson, 2015). Curcumin is considered highly hydrophobic based on the predicted log P value ranging from 2.56 to 3.29 (Grynkiewicz & Slifirski, 2012). Since free curcumin is insoluble in water, free curcumin released in aqueous buffer could not be quantified. Thus, a suitable sink condition which consists of Tween 80 and ethanol in PBS was employed to quantify the free curcumin released from the liposomal system. However, the challenge continues as high concentration of curcumin in liposomal system could not be detected when it was released into the receptor medium. Thus, we have loaded a low concentration of curcumin (0.005 % w/v) into LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX solely to study their release properties. The cumulative release from all samples was plotted against time as shown in Figure 4.49.

The release behavior of non-coated liposomes and DEAE-DX coated liposomes was compared to study the effect of DEAE-DX coating on the release of curcumin. The obtained result shows that coated liposome exhibited a slower release than the non-coated liposomes. This might be due to the retarding effect of DEAE-DX that surrounds the liposomal surface which reduces the permeability of liposome thus lowering the amount of curcumin released to the receptor medium. Besides that, the *in vitro* release studies for LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX were compared (Figure 4.49d). After 36 hours of release analysis, LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX showed a cumulative release of 33.9 %, 26.9 % and 17.6 % respectively. Results show that DSPC-DEAE-DX had the slowest release. The result may be due to the fact that increase in alkyl chain length of lipids increases the phase transition of lipid chains due to the strong van der Waals interaction between lipid

chains. Consequently, higher energy is required to disrupt the ordered packing which in turn affects the release of curcumin from the liposome. Furthermore, larger lipidic area of longer chain lipids may enhance the hydrophobic drug binding with lipid bilayer resulting slower release (Begum et al., 2012; Chen et al., 2012). It can be deduced that the nature of the lipid affects the drug release properties.

(a)

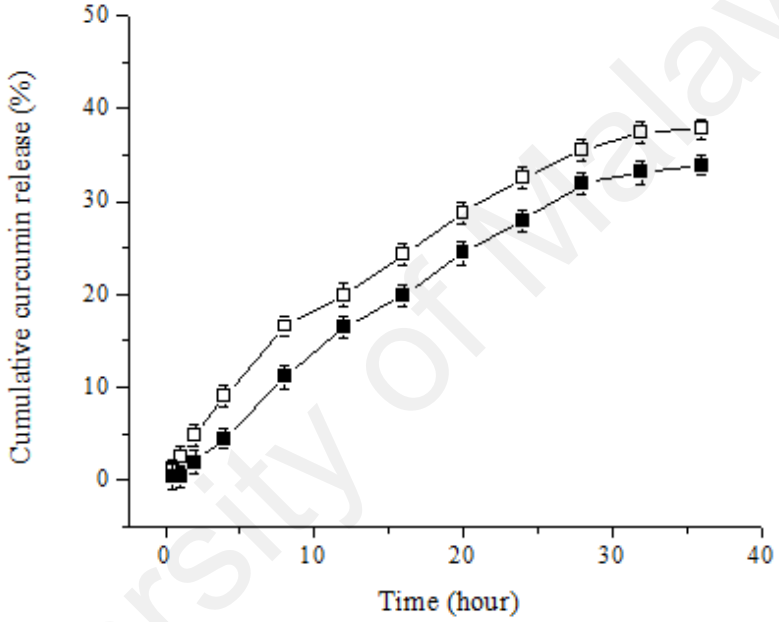
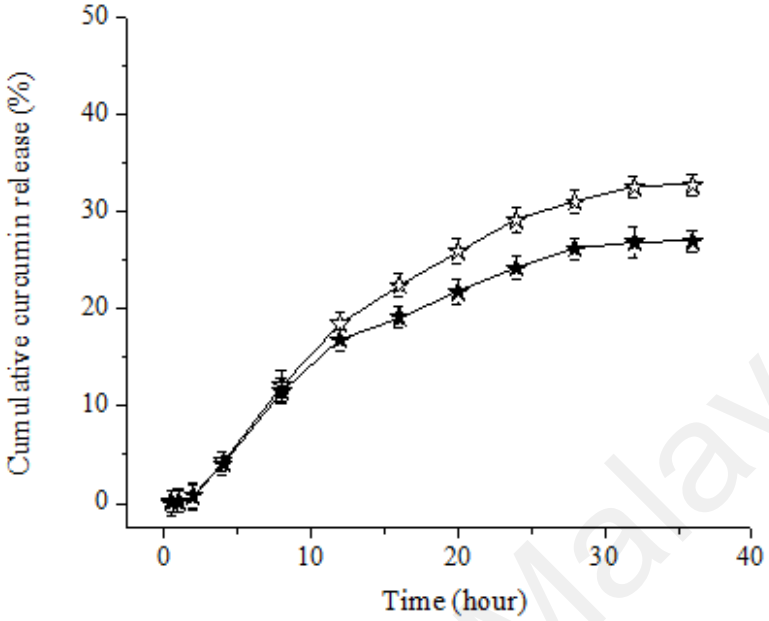



**Figure 4.49:** In vitro release of curcumin from (a) LEC ( □ ) and LEC-DEAE-DX ( ■ ), (b) DPPC ( ☆ ) and DPPC-DEAE-DX ( ★ ), DSPC ( ◇ ) and DSPC-DEAE-DX ( ◆ ), (d) Comparison of LEC-DEAE-DX ( ■ ), DPPC-DEAE-DX ( ★ ) and DSPC-DEAE-DX ( ◆ ) over a period of 24 hours.

(b)




(c)

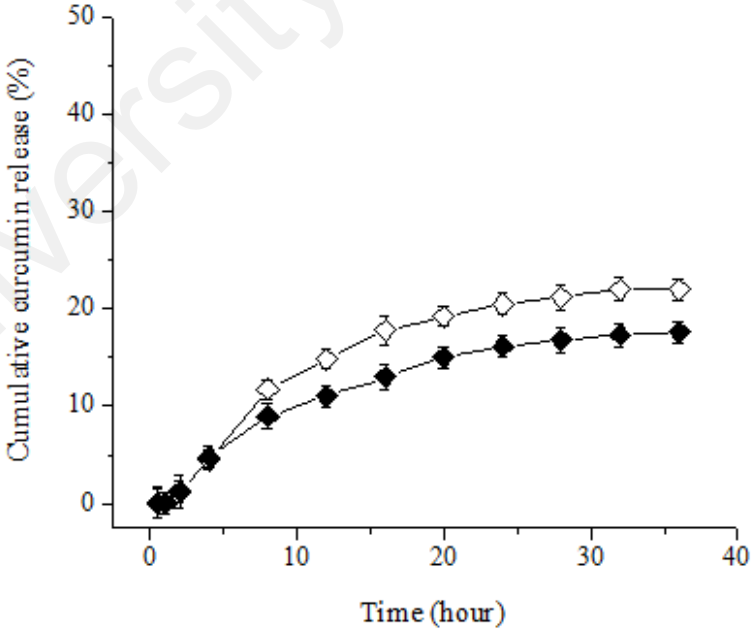



**Figure 4.49:** (continued)

(d)

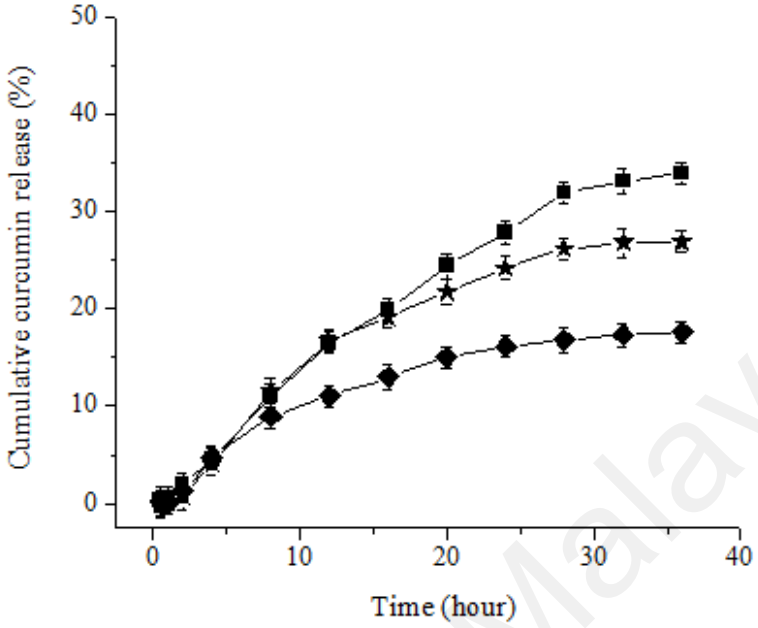



**Figure 4.49:** (continued)

#### 4.9.2.2 Liposomal gel system

The *in vitro* release of curcumin from LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX was also investigated respectively. Similar to *in vitro* release of liposomal solution, curcumin with concentration of 0.005% (w/v) was loaded into the liposomal gels as well as the plain gel in order to compare the difference in the release pattern. The carbopol concentration used for the preparation of gel is 0.25 % (w/v) because it gives the desired gel consistency as shown in the rheological analysis in the previous section.

As observed in Figure 4.50, all of the liposomal gel exhibited slower release of curcumin compared to the plain curcumin gel. The plain curcumin gel showed cumulative release of 60.6 % of at the 48$^{th}$ hour. On the other hand, the amount of curcumin released has reduced about 25.0 %, 33.6 % and 44.0 % for LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX, respectively. This result also

supports the fact whereby the presence of liposome has slower down the release of curcumin. Thus, longer period required for curcumin to be released to the diffusate chamber.

The release of curcumin from LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX was also compared and the results obtained are 35.6 %, 27.0 % and 16.6 %, respectively. Similar to the previous results, the slowest release of curcumin was observed in the formulation of LG-DSPC-DEAE-DX for a period of 48 hours (Figure 4.50d). DSPC liposome bearing longer alkyl chain compared to DPPC and lecithin forms more rigid liposomes. Thus, curcumin has a higher affinity towards the bilayers hence longer time is required to diffuse from liposomes to matrix solution.

(a)

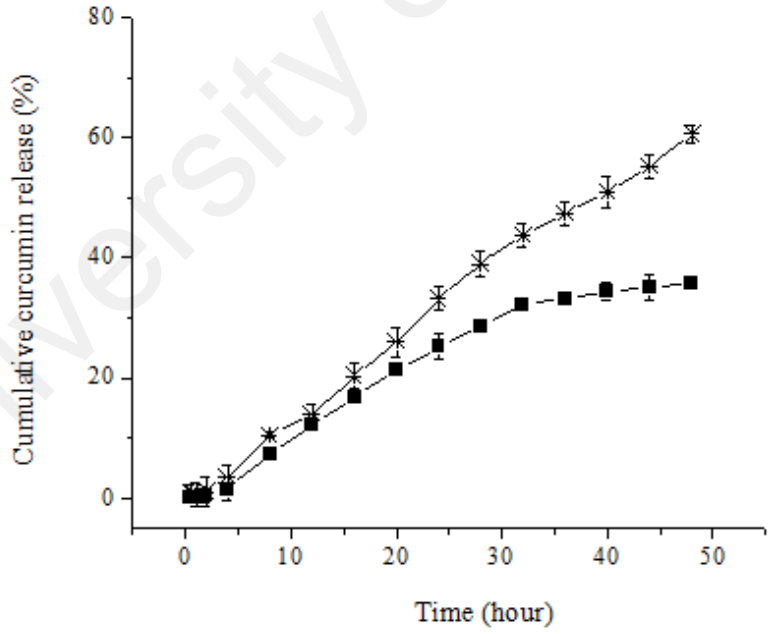
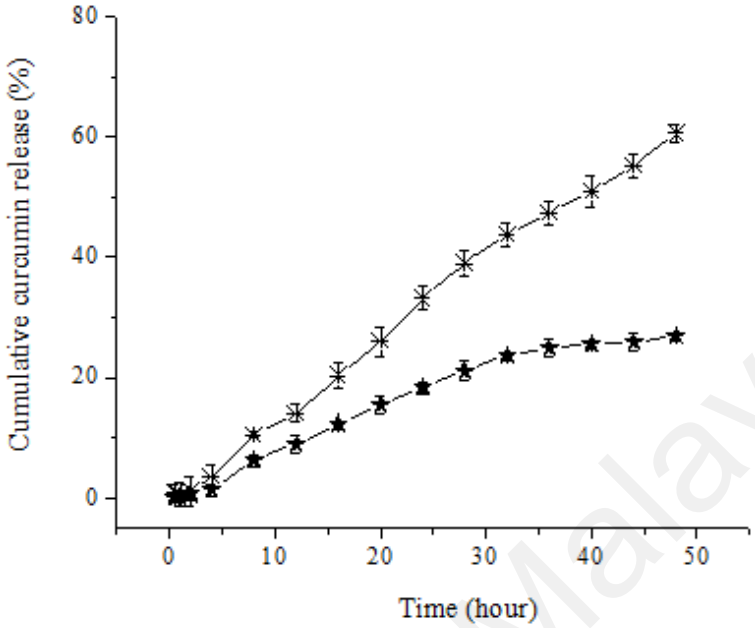



**Figure 4.50:** In vitro release of curcumin from (a) plain gel ( ✳ ) and LG-LEC-DEAE-DX ( ■ ), (b) plain gel ( ✳ ) and LG-DPPC-DEAE-DX ( ★ ), (c) plain gel ( ✳ ) and LG-DSPC-DEAE-DX ( ◆ ), (d) Comparison of LG-LEC-DEAE-DX ( ■ ), LG-DPPC-DEAE-DX ( ★ ) and LG-DSPC-DEAE-DX ( ◆ ) over a period of 48 hours.

(b)




(c)

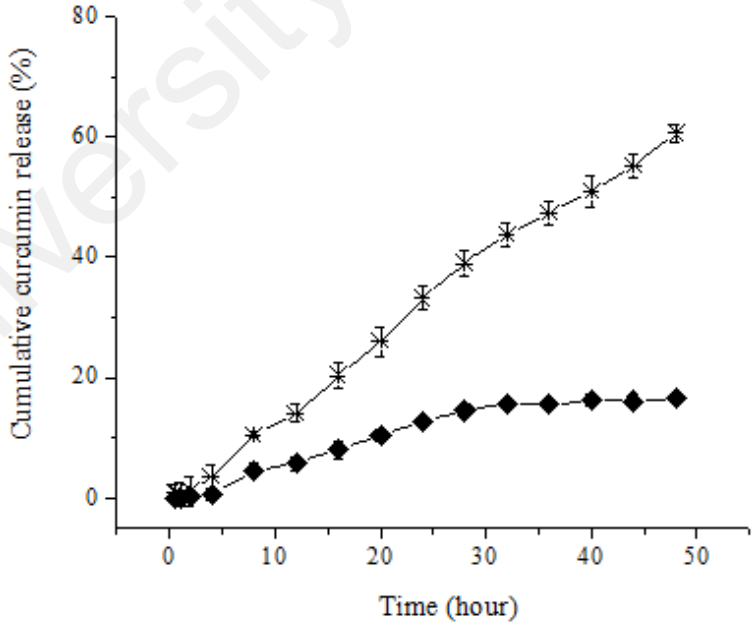



**Figure 4.50:** (continued)

(d)

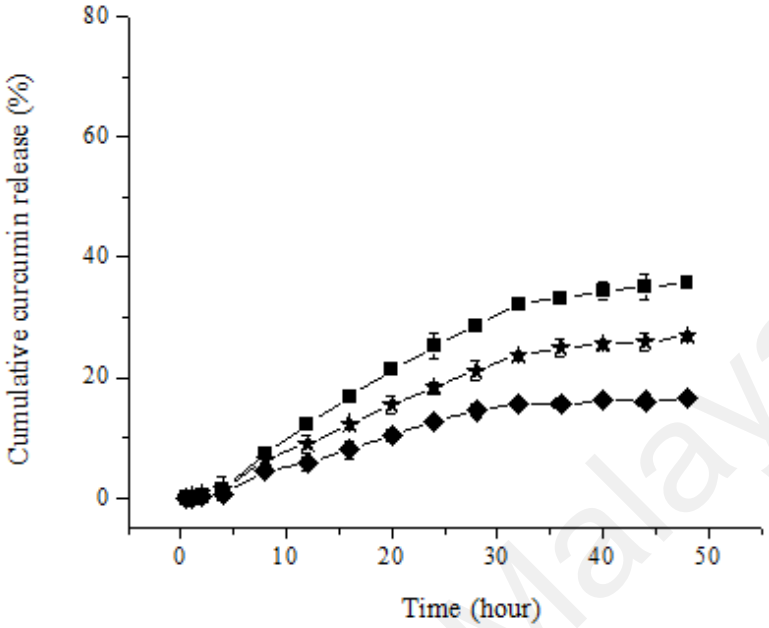



**Figure 4.50:** (continued)

#### 4.9.2.3 Comparison of curcumin release between liposomal solution and liposomal gel

Figure 4.51 compares the curcumin release between liposomal dispersion and liposomal gel for 36 hours of analysis. The release rate of curcumin from LG-LEC-DEAE-DX, LG-DPPC-DEAE-DX and LG-DSPC-DEAE-DX also exhibited slower release compared to LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX liposomal solution. As expected, presence of gelling agent delays the release of curcumin. Development of curcumin encapsulated liposomes followed by incorporation of these liposomes into carbopol gel could provide possibilities to be exploited as a sustained delivery system.

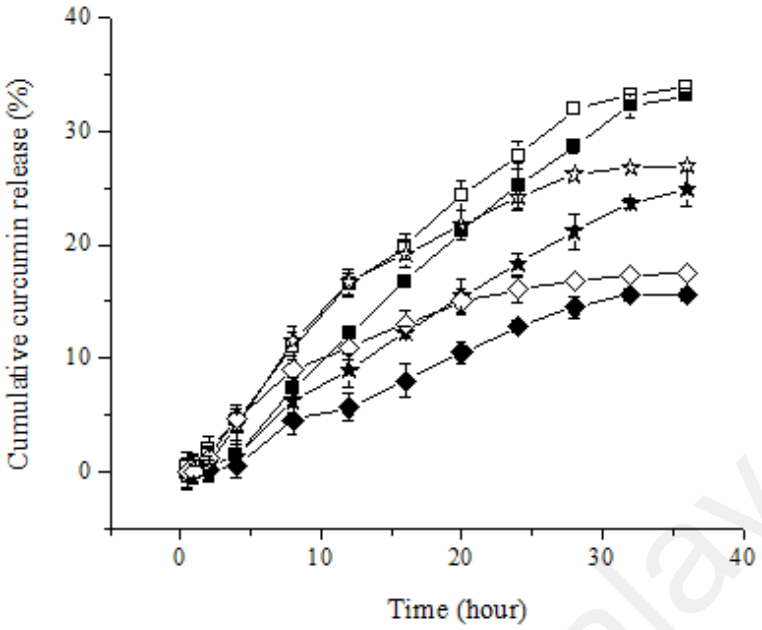



**Figure 4.51:** In vitro release of curcumin from LEC-DEAE-DX ( □ ), DPPC-DEAE-DX ( ☆ ) and DSPC-DEAE-DX ( ◇), LG-LEC-DEAE-DX ( ■ ), LG-DPPC-DEAE-DX ( ★ ) and LG-DSPC-DEAE-DX ( ◆ ) over a period of 36 hours.

### 4.9.3 Kinetic model evaluations

Kinetic modeling predicts the mechanism of drug release and also describes the rate at which drug is released from the drug delivery system. There are several ways in which drug release can be described. For instance, immediate release drug products provide rapid dissolution of the drug after administration whereas delayed release drug products do not immediately disintegrate or release the drug into the body (Shaikh et al., 2015). On the other hand, extended drug release products are designed to release their medication over a prolonged period of time. Also, extended drug release products allow the reduction in dosage frequency as the drug is released gradually at a predetermined rate which could give the desired therapeutic effect. The extended release can be achieved using either a controlled-release dosage forms or sustained-release dosage forms (Perrie, 2010).

Application of mathematical models to study drug release process of pharmaceutical dosage forms helps to elucidate the mechanism of the drug release. Herein, the *in vitro* release of drug from liposomal dispersion and liposomal gel was curve fitted to Zero-order, First-order, Higuchi and Korsmeyer-Peppas models by using DDSolver software to understand their release kinetics (Table 4.12-4.13).

Correlation coefficient values were high in all cases. However, a model with the highest or the values of correlation coefficient nearest to 1 was considered to be the best model. Table 4.12 shows the kinetic modeling of DPH release from liposome dispersion and liposomal gel. The correlation coefficient of the first order and Korsmeyer-Peppas was found nearest to the value of 1 for each of the formulation (Figure 4.52-Figure 4.53). First order model involves the process that is directly proportional to the drug concentration involved in the process. It shows that the release of DPH is a concentration-dependent process. First order kinetic also often used to describe the release of water soluble drugs in porous material in pharmaceutical dosage forms. Besides that, the rate constant which was determined from the slope of the kinetic models was compared between the liposomal dispersion and liposomal gel. The rate constant values obtained for liposomal gel was found lower compared to the ones obtained for liposomal dispersion explained slower release of DPH from liposomal gel compared to liposomal dispersion.

DPH release also complies with Korsmeyer-Peppas model as the $R^2$ value was found nearer to the value of 1. The value of diffusion exponents, n was found more than 0.43 and less than 0.89 which indicates the release mechanism is according to a fickian release. Accordingly, $0.43 < n < 0.89$ indicated diffusion controlled release and swelling-controlled release which is also known as anomalous transport (Mady, 2014; Wu et al., 2011).

**Table 4.12:** Kinetic model evaluation of DPH release for liposomal dispersions and liposomal gels.

| Sample | Zero Order | | First Order | | Higuchi | | Korsmeyer-Peppas | |
|---|---|---|---|---|---|---|---|---|
| | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ |
| LEC-DEAE-DX | 3.887 | 0.967 | 0.067 | 0.992 | 15.751 | 0.922 | 7.395 | 0.991 |
| LG-LEC-DEAE-DX | 1.674 | 0.923 | 0.026 | 0.994 | 9.678 | 0.973 | 5.772 | 0.998 |
| DPPC-DEAE-DX | 3.391 | 0.871 | 0.056 | 0.982 | 14.064 | 0.970 | 10.521 | 0.986 |
| LG-DPPC-DEAE-DX | 1.496 | 0.967 | 0.022 | 0.997 | 8.548 | 0.935 | 3.439 | 0.993 |
| DSPC-DEAE-DX | 3.699 | 0.893 | 0.065 | 0.993 | 15.265 | 0.953 | 10.337 | 0.979 |
| LG-DSPC-DEAE-DX | 1.363 | 0.977 | 0.019 | 0.996 | 7.748 | 0.917 | 2.607 | 0.992 |

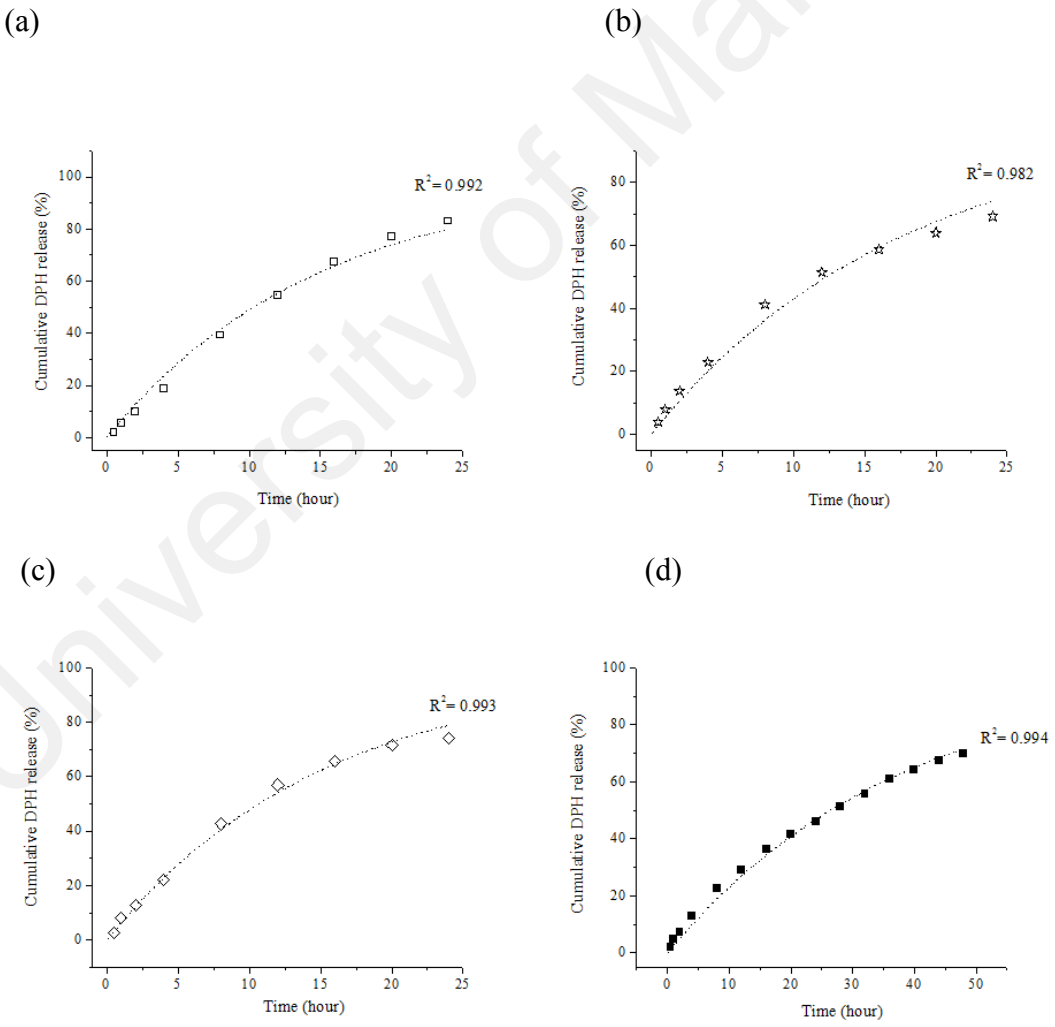



**Figure 4.52:** First order curve fitting for DPH release of (a) LEC-DEAE-DX ( □ ), (b) DPPC-DEAE-DX ( ☆ ), (c) DSPC-DEAE-DX ( ◇ ), (d) LG-LEC-DEAE-DX ( ■ ), (e) LG-DPPC-DEAE-DX ( ★ ) and (f) LG-DSPC-DEAE-DX ( ◆ ).

(e) (f)

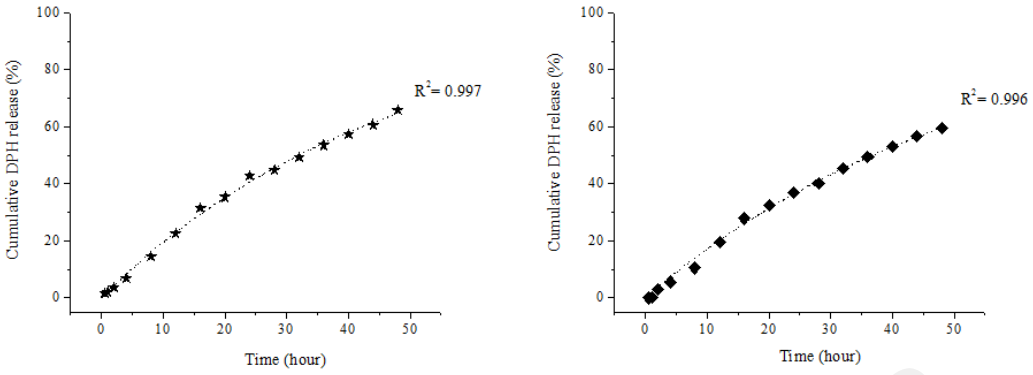



**Figure 4.52:** (continued)

(a) (b)

(c) (d)

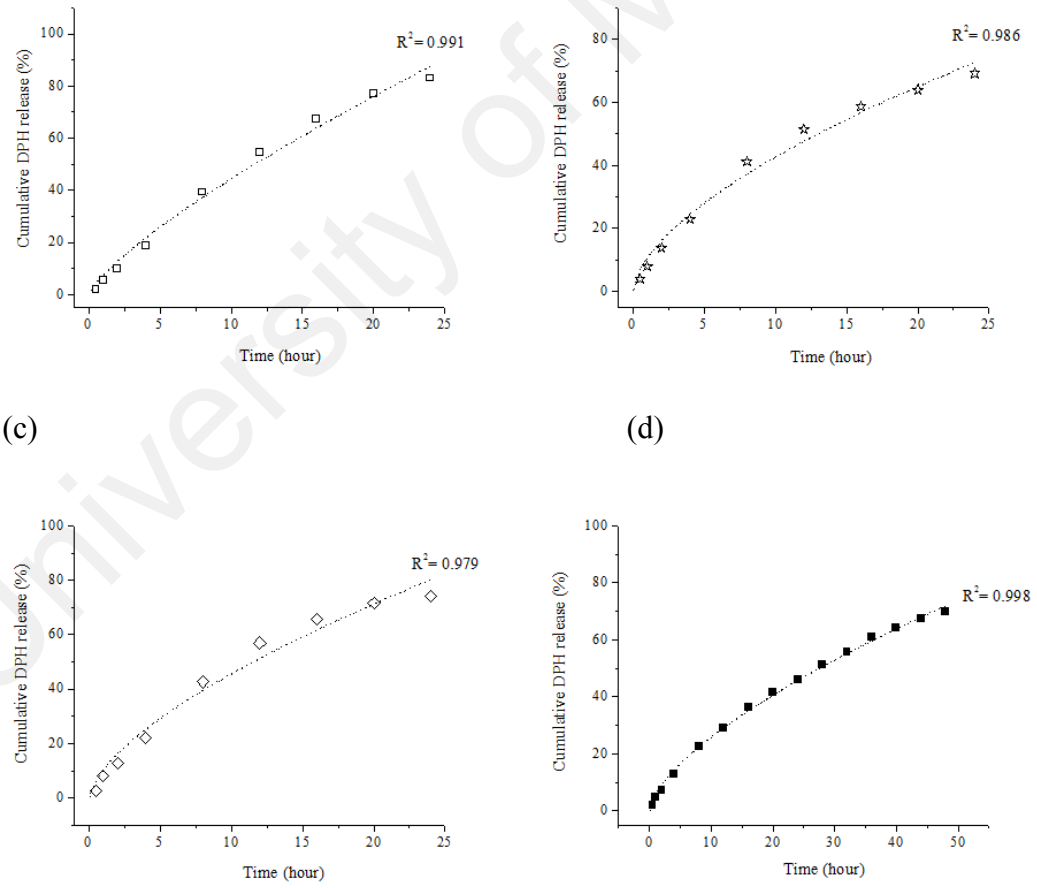



**Figure 4.53:** Korsmeyer-Peppas curve fitting for DPH release of (a) LEC-DEAE-DX ( □ ), (b) DPPC-DEAE-DX ( ☆ ), (c) DSPC-DEAE-DX ( ◇ ), (d) LG-LEC-DEAE-DX ( ■ ), (e) LG-DPPC-DEAE-DX ( ★ ) and (f) LG-DSPC-DEAE-DX ( ◆ ).

(e) (f)

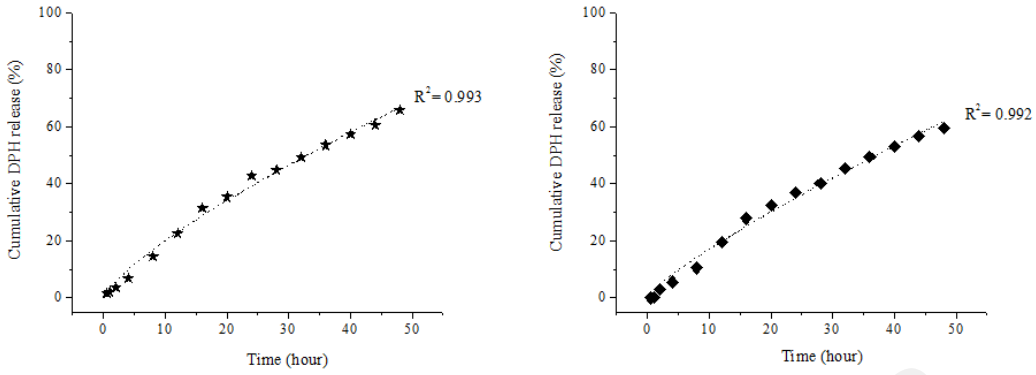



**Figure 4.53:** (continued)

On the other hand, Table 4.13 shows that curcumin release was only best fitted with Korsmeyer-Peppas model (Figure 4.54). The value of diffusion exponents, n was found more than 0.43 and less than 0.89 which indicates a fickian release. Accordingly, *0.43< n < 0.89* indicated diffusion controlled release and swelling-controlled release which also known as anomalous transport (O. Mady, 2014; Wu et al., 2011). Similarly, the rate constant values obtained for liposomal gel containing curcumin was lower compared to the ones obtained for liposomal dispersion which explained slower release of curcumin from liposomal gel compared to liposomal dispersion.

**Table 4.13:** Kinetic model evaluation of curcumin release for liposomal dispersions and liposomal gels.

| Sample | Zero Order | | First Order | | Higuchi | | Korsemeyer Peppas | |
|---|---|---|---|---|---|---|---|---|
| | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ |
| LEC-DEAE-DX | 1.086 | 0.965 | 0.013 | 0.986 | 5.374 | 0.919 | 2.184 | 0.986 |
| LG-LEC-DEAE-DX | 0.881 | 0.951 | 0.011 | 0.975 | 5.013 | 0.901 | 1.807 | 0.970 |
| DPPC-DEAE-DX | 0.918 | 0.900 | 0.011 | 0.935 | 4.599 | 0.924 | 2.592 | 0.960 |
| LG-DPPC-DEAE-DX | 0.655 | 0.957 | 0.008 | 0.975 | 3.730 | 0.908 | 1.352 | 0.977 |
| DSPC-DEAE-DX | 0.608 | 0.849 | 0.007 | 0.880 | 3.079 | 0.944 | 2.208 | 0.959 |
| LG-DSPC-DEAE-DX | 0.420 | 0.924 | 0.005 | 0.940 | 2.406 | 0.902 | 1.024 | 0.956 |

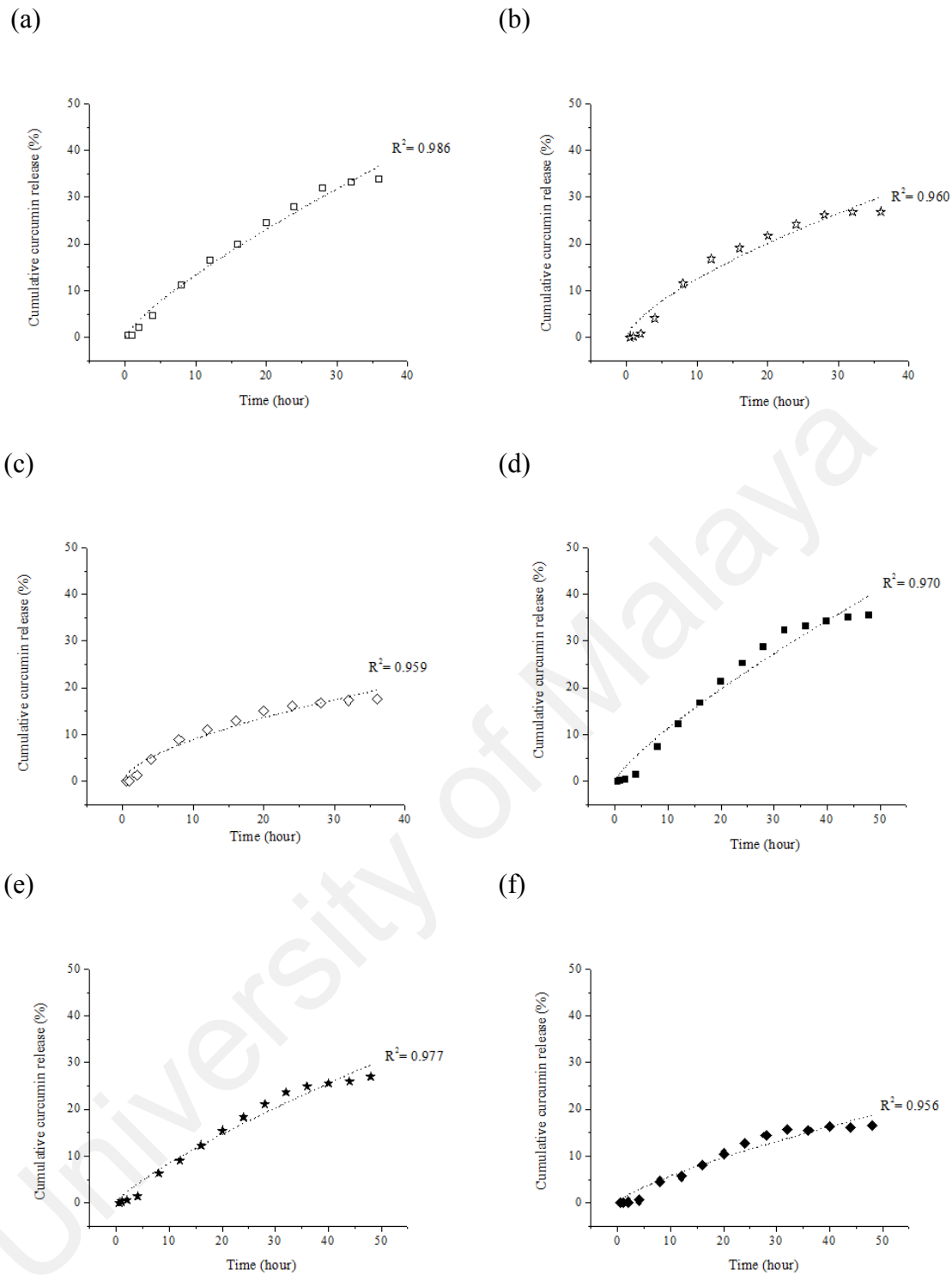



**Figure 4.53:** Korsmeyer-Peppas curve fitting for curcumin release of (a) LEC-DEAE-DX ( □ ), (b) DPPC-DEAE-DX ( ☆ ), (c) DSPC-DEAE-DX ( ◇ ), (d) LG-LEC-DEAE-DX ( ■ ), (e) LG-DPPC-DEAE-DX ( ★ ) and (f) LG-DSPC-DEAE-DX ( ◆ ).

# CHAPTER 5: CONCLUSION

The potential of Diethylaminoethyl-dextran (DEAE-DX) coated liposomes as drug carriers was investigated in this research. All of the liposomes were prepared using thin film hydration method at a concentration of 0.2 % (w/v) which is above the critical vesicular concentration (CVC) of the respective lipids. The CVC of lecithin (LEC), dipalmitoylphosphatidylcholine (DPPC) and distearoylphophatidylcholine (DSPC) were found at 0.1 % (w/v), 0.08 % (w/v) and 0.06 % (w/v), respectively which indicated that formation of liposome consisting of long alkyl chain lipid is more favorable. It was also found that addition of DEAE-DX influenced the arrangement of lipid at air/water interface. In the presence of DEAE-DX, surface tension has increased due to the larger area occupied at air/water interface.

As instability of liposomes still limits their application, polymer coating method was employed using DEAE-DX in order to provide steric repulsion between particles. There was a huge difference in the morphological evaluation between non-coated and DEAE-DX liposomes. TEM micrographs show that DEAE-DX coated liposomes are able to retain the spaces between the particles, unlike the non-coated liposomes that show particles coagulation. The DEAE-DX coating effect on liposome is further intensified by the results obtained from particle size and zeta potential.

Particle size and zeta potential increases upon addition of DEAE-DX show a successful coating of DEAE-DX and indicated a favorable interaction between the DEAE-DX and the liposome. Thirty-five days stability study shows that particle size and zeta potential of DEAE-DX coated liposome were stable at room temperature. In overall, 0.02 % (w/v) DEAE-DX coated LEC and DPPC liposomes respectively, 0.01 % (w/v) DEAE-DX coated DSPC liposomes were found to be the most stable formulation. At this composition, LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX had

particle size of (195.3 ± 6) nm, (140.1 ± 0.6) nm and (121.6 ± 1.0) nm, respectively. On the other hand, LEC-DEAE-DX, DPPC-DEAE-DX and DSPC-DEAE-DX had a zeta potential of +11.6 mV, +30.6 mV and +33.7 mV, respectively.

Thermal behaviors of liposomes before and after addition of DEAE-DX were revealed using Differential scanning calorimetry (DSC). Overall, in the presence of DEAE-DX, the packing of the phospholipids is interrupted. This explains the decrease in $T_m$ for physical DEAE-DX coated liposomes and indicates the interaction between lipid and DEAE-DX. Therefore successful coating of DEAE-DX on the surface of liposomes is proved. In comparison, DSPC-DEAE-DX liposomes exhibited the higher $T_m$ value than LEC-DEAE-DX and DPPC-DEAE-DX liposomes.

The most stable formulations which were chosen according to particle size and zeta potential analysis were loaded with a hydrophilic drug namely diphenhydramine hydrochloride (DPH) and also curcumin as a hydrophobic drug. Results show that encapsulation efficiency of the formulated liposomes was found more than 95.0 % for curcumin. On the other hand, all the liposomes had encapsulation efficiency less than 40 % for DPH. The nature of the drug certainly has affected the encapsulation efficiency of the liposomal system.

Also, the amount of drug encapsulated is mainly affected by the type of lipid forming the liposomes. The amount of curcumin encapsulated in DSPC-DEAE-DX is 0.035 % whereas LEC-DEAE-DX and DPPC-DEAE-DX had an optimum encapsulation of 0.025 %, the amount of DPH encapsulated in LEC-DEAE-DX and DSPC-DEAE-DX liposomes are 0.055 % whereas DPPC-DEAE-DX had an optimum encapsulation of 0.065 %. DPPC-DEAE-DX liposomes with greater trapping volume have higher tendency to accommodate more DPH in the core region of liposomes

whereas DSPC-DEAE-DX liposome with longer alkyl chain could entrap more curcumin.

To serve the purpose of the application, liposome formulation was incorporated into carbopol gel. We found that incorporation of the liposome into carbopol gel enhanced the rigidity of the gel. Furthermore, the presence of DEAE-DX coated liposome contributed to the rigidity of the gel as well. However, the presence of DPH in the gel system has affected the viscoelasticity of the gel. DPH loaded liposomal gel was found to be flowy. On the other hand, curcumin loaded liposomal gel maintain its viscoelasticity and did not exhibit significance change in the flow behavior.

An *in vitro* release experiment demonstrated that coated liposome have a slower release compared to the non-coated liposome. This result proves that DEAE-DX could be a potential candidate for designing a controlled release system. As for the gel system, liposomal gel exhibited a slower release compared to the gel containing only drug. Retardation of the liposome in releasing the drug definitely fulfills the attempts of producing sustained release of drug by reducing its side effects. Also, development of liposomal gel helps tackling problems such as poor solubility and low bioavailability that are often associated with hydrophobic drugs.

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