INCORPORATION OF CURCUMIN IN LIPID BASED DELIVERY SYSTEMS AND ASSESSMENT OF ITS BIOACCESSIBILITY

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ABSTRACT OF THE THESIS

INCORPORATION OF CURCUMIN IN LIPID BASED DELIVERY SYSTEMS AND ASSESSMENT OF ITS BIOACCESSIBILITY

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Dr. Qingrong Huang

Curcumin, the major curcuminoid compound from turmeric (*Curcuma longa*) is a well-studied nutraceutical with many health promoting biological properties. The benefits of curcumin greatly depend on its solubilization (bioaccessibility) and subsequent absorption through cell lining so that it can reach systemic/general circulation. Unfortunately, curcumin has low water solubility and undergoes rapid metabolism on oral delivery.

Lipids have shown to affect the absorption of poorly soluble nutraceuticals such as curcumin by enhancing solubilization in the intestinal milieu through alterations to the composition and character of the colloidal environment – e.g. vesicles, mixed micelles and micelles when delivered orally. Thus, the purpose of this study was to formulate three lipid-based delivery systems with curcuminoids – gel like emulsions, nanoemulsions and organogels, and investigate their impact on *in vitro* solubilization or bioaccessibility.

Curcumin was dissolved in MCT oil with the help of Span 20 to be used as the lipid phase. Gel-like emulsions, nanoemulsions and organogels were then formulated using the curcumin-MCT oil lipid phase using different types of sugar esters as organogelators and emulsifiers. The organogels and gel-like emulsions are viscoelastic in nature with a solid dominant behavior which was supported by the rheological data. Optical and fluorescence microscopic imaging helped understand the morphology of curcumin in the lipid based delivery systems as well as the systems themselves. Digital scanning calorimetry was used to study the phase transitions that occur in the systems. The bioaccessibility of the different lipid based delivery systems was evaluated using *in vitro* lipolysis experiments (dynamic pH stat lipid digestion model). Results suggest that lipid based delivery systems have more bioaccessibility when compared to that of unformulated curcuminoids. The bioaccessibility of curcumin increased at least 6.4 folds when it was incorporated in a lipid based delivery system as compared to unformulated curcuminoids. Out of the three systems tested, gel-like emulsions had the highest stability and thus can be good candidate for incorporation and delivery of curcumin.

Acknowledgement

A Master's thesis is a structure that is built over the course of a student's most productive years when one is transitioning from the phase of basic learning to advance learning. This structure serves as groundwork for most part of one's career in terms of development of lifelong work ethics and lessons like patience, determination, focus, risk-taking and ultimately success or failure. I have been fortunate enough to be at one of the world's most renowned institutes in my field, and lay the foundation for my entire life's work here.

But this foundation could not be built without the strength of the pillars supporting it. Here I acknowledge those pillars on which me and my work stand today.

To my research advisor Dr. Qingrong Huang, who has been my guiding light and without whose phenomenal support from the moment he took me in as a student to the completion of this project, I would not have found motivation to be in the field of nanotechnology.

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List of Abbreviations

bFGF:	Basic fibroblast growth factor
VEGF:	Vascular endothelial growth factor
EAT cells:	Ehrlich-Lettre Ascites cells, mouse breast adenocarcinoma cells
NIH3TC cells:	Mouse embryonic fibroblast cells
KDR gene:	Kinase insert domain receptor gene
HUVEC:	Human umbilical vein endothelial cells
HIV:	Human immunodeficiency virus
BEC:	Buccal epithelial cells
GI:	Gastrointestinal
O/W:	Oil in water
W/O:	Water in oil
HLB:	Hydrophobic lipophilic balance
M8:	Mannitol dioctanoate
S8:	Sorbitol dioctanoate
FFA:	Free fatty acid

MCT:	Medium chain triglyceride
Span 20:	Sorbitan monolaurate
NEOBEE 1053:	Medium chain triglycerides composed of 4% C_8 and 96% C_{10} triglycerides
HPLC:	High performance liquid chromatography
RPM:	Rotations per minute
S270:	Sucrose stearate, HLB 2
S570:	Sucrose stearate, HLB 5
P90:	Sucrose monoplamitate, HLB 18
DSC:	Digital scanning calorimetry
G':	Storage modulus
G":	Loss modulus
PCS:	Photon correlation spectroscopy
PDI:	Polydispersity index
NaTDC:	Sodium taurodeoxycholate
LCT:	Long chain triglycerides

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CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

1.1 CURCUMIN – THE ORIGIN

Natural products have been a major source for new drug discovery. Many new drugs developed recently are either natural products or natural product-derived (Butler 2008). These include plants, animals and microbe based products that have been under the spotlight because of the pharmacological properties demonstrated by these products in various studies (Harvey 2008). One such plant derived product is curcumin. Curcumin has been a well studied compound for its pharmacological properties; however, it suffers from shortfalls such as poor absorption, rapid metabolism and excretion which will be discussed in detail here.

Turmeric, rhizome of the perennial plant *Curcuma longa* belonging to the *Zingiberaceae* (ginger) family, is not only a popular spice in South East Asian cuisine, but has been used for medicinal purposes since ancient times. Turmeric, often available in powdered form, consists of bioactive compounds such as the water soluble peptide turmerin, essential oils such as turmerones, atlantones, and zingiberen and about 3-5% hydrophobic curcuminoids (Sharma, Gescher et al. 2005). Curcuminoids, the crystalline polyphenolic compounds, are responsible for the yellow color of turmeric and many of its biological activities. 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione or diferuloylmethane, called curcumin by its common name, is one of the major curcuminoids found in turmeric.

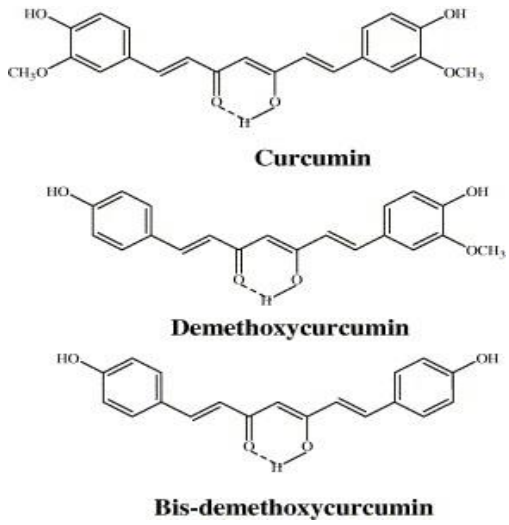


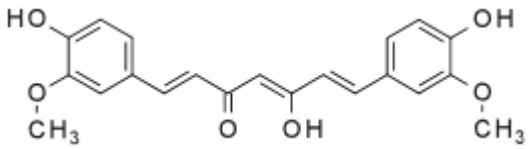
Fig.1. Chemical Structures of Curcuminoids [Reprinted (Jayaprakasha, Jaganmohan Rao et al. 2006), with permission from Elsevier].

1.1.1 CHEMICAL PROPERTIES OF CURCUMIN

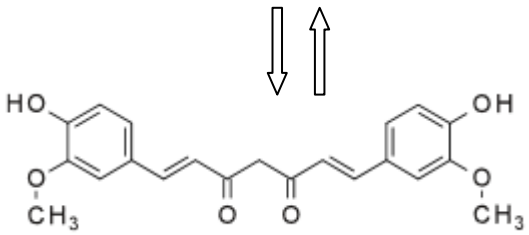
Curcumin found commercially, comes as a mixture of as much as 77% curcumin along with about 17% demethoxycurcumin and 3% bisdemethodycurcumin (Goel, Kunnumakkara et al. 2008). The latter two are other two major curcuminoids found in turmeric.

Curcumin is low molecular weight compound with molecular weight of 368.37g/mol, melting point of 183°C and chemical formula $C_{21}H_{20}O_6$. It is chemically a bis-α,β-unsaturated β-diketone exhibiting keto-enol tautomerism as shown in Figure 2 (Sharma, Gescher et al. 2005), with the bis-keto form being predominant in acidic and neutral solutions as well as cell membranes and enol form in alkaline solutions (Wang, Pan et al. 1997). Curcumin is poorly soluble in water; however, it dissolves in organic solvents such as acetone, dimethylsulphoxide and ethanol. Maximum absorption of curcumin

occurs at 415- 420nm spectrophotometrically, and the molar absorptivity of curcumin dissolved in ethanol varies from 4.95×10^4 - 6.73×10^4 L cm^{-1} M^{-1}.



Enol Form



Keto Form

Fig.2. Keto-enol tautomerism of Curcumin at different conditions.

1.1.2 PHARMACOLOGICAL PROPERTIES OF CURCUMIN

Throughout the past few decades, curcumin has been studied for a great variety of beneficial biological and pharmacological properties; yielding positive results for many of the claimed properties and inconclusive or ineffective results for many others.

Curcumin has proven to be an effective anti-cancer, angiogenesis- modulating, anti-oxidant and anti-fungal agent. Anti-mutagenic, anti-virus, immunomodulatory and enhanced wound healing have been cited as some other benefits of curcumin (Maheshwari, Singh et al. 2006).

Cancer is characterized by neoplasia or unregulated cell growth along with other symptoms caused by dysregulation of cell signaling pathways at multiple steps. Multi-action curcumin molecule modulates an array of targets involved in the cell signaling

pathways such as the activation of transcription factors, receptors, kinases, cytokine, enzymes, and growth factors which result in either neoplasia, inflammation, unregulated apoptosis or a combination of these (Anand, Sundaram et al. 2008).

Angiogenesis, the growth of new vascular capillaries from preexisting ones, is an important physiological process involved in healing and reproduction. Unregulated angiogenesis leads to tumor growth, edema, rheumatoid arthritis and other illnesses. bFGF, VEGF and other growth factors are the active mediators of angiogenesis. Curcumin has been shown to inhibit bFGF-mediated corneal neovascularization (Arbiser, Klauber et al. 1998), the expression of VEGF, angiopoietin1 and angiopoietin 2 genes in EAT cells, VEGF and angiopoietin 1 genes in NIH3T3 cells, and KDR gene in HUVEC (Gururaj, Belakavadi et al. 2002).

Oxidative stress plays a major role in development of diseases like Alzheimer's, Parkinson's, and cancer. Curcumin has been an effective inhibitor of iron-catalyzed lipid peroxidation, possibly by chelating iron (Reddy and Lokesh 1992; Sreejayan and Rao 1994). Curcumin has also been shown to exhibit anti-oxidant property by inhibiting an array of reactive oxygen species such as superoxide anion radicals, hydroxyl radicals (Reddy and Lokesh 1994) and nitrogen dioxide radicals (Unnikrishnan and Rao 1995).

Immunocompromised patients suffering from diseases like Human Immunodeficiency Virus (HIV), cancer, diabetes mellitus or neonates are very susceptible to nosocomial infections. Fungal infection is one of the very common nosocomial infections contracted by these patients (Beck-Sague and Jarvis 1993; Shyh-Ming Tsao 2000). Curcumin in combination with amphotericin B or fluconazole acts synergistically as an effective antifungal agent (Shyh-Ming Tsao 2000). Curcumin has been show to be more effective

antifungal agent against *P.basiliensis* than the commercial antifungal agent fluconazole, also inhibiting the adhesion of *Candida* species isolated from HIV infected patients to the human buccal epithelial cells (BEC) (Martins, da Silva et al. 2009). It has been tested to be effective against some fluconazole resistant strains of *Candida* species (Khan, Shreaz et al. 2012).

Thus, multi-targeting curcumin deserves special attention pertaining to its delivery and efficacy of delivery systems among all other phytochemicals under research.

1.2 BIOAVAILABILITY AND PHARMACOKINETICS OF CURCUMIN

Bioavailability of a bioactive compound is defined as the fraction of the compound ingested by a living organism, that ends up in systemic circulation and is available at the site of action (Versantvoort and Rompelberg 2004; Versantvoort, Oomen et al. 2005; Acosta 2009). In case of lipophilic compounds such as curcumin, the bioavailability (F) can be presented as the product of different physiochemical and physiological factors as shown in Equation 1 (Versantvoort and Rompelberg 2004):

$$F = F_B * F_A * F_H \quad \ldots (1)$$

Here, F_B is the fraction of compound that is released from the delivery system matrix into the digestive juices of the GI tract (Versantvoort and Rompelberg 2004; Versantvoort, Oomen et al. 2005). The compound is then solubilized in vesicles, micelles or mixed micelles formed with the help of the bile juices, phospholipids and lipid digestion

products, if any present from diet or delivery matrix; thus becoming bioaccesible or ready to be absorbed in the next step to bioavailability.

F_A is the fraction of F_B (bioaccesible compound) that is transported across the layer of intestinal epithelium (Versantvoort and Rompelberg 2004) .This absorption step depends upon the solubilization of the bioactive in the carrier structures like vesicles, micelles and mixed micelles formed, that help in the transport process passively or actively. Whether the transport will be active or passive again depends on the characteristics of the compound and also its function in the body. Highly hydrophobic compounds have low permeability and hence are actively transported(Acosta 2009).

F_H is the fraction of F_A (transported bioaccesible compound) that reaches systemic circulation without being metabolized during the transport (Versantvoort and Rompelberg 2004), is available at the site of action and is responsible for the beneficial effects of bioactives.

Bioavailability and pharmacokinetics are inter-related. A good understanding of the pharmacokinetics of the active compound, or in laymen terms ‘what the body does to the active compound’, is important to design efficient delivery systems that increase the bioavailability of the active compound.

Numerous studies have been conducted to study the pharmacokinetics of curcumin in rats as well humans. The first pharmacokinetics study was carried out on Sprague-Dawley rats, where an oral administration of 1g/kg of curcumin resulted in excretion of about 75% in feces and negligible amount in the urine. Plasma level measurements

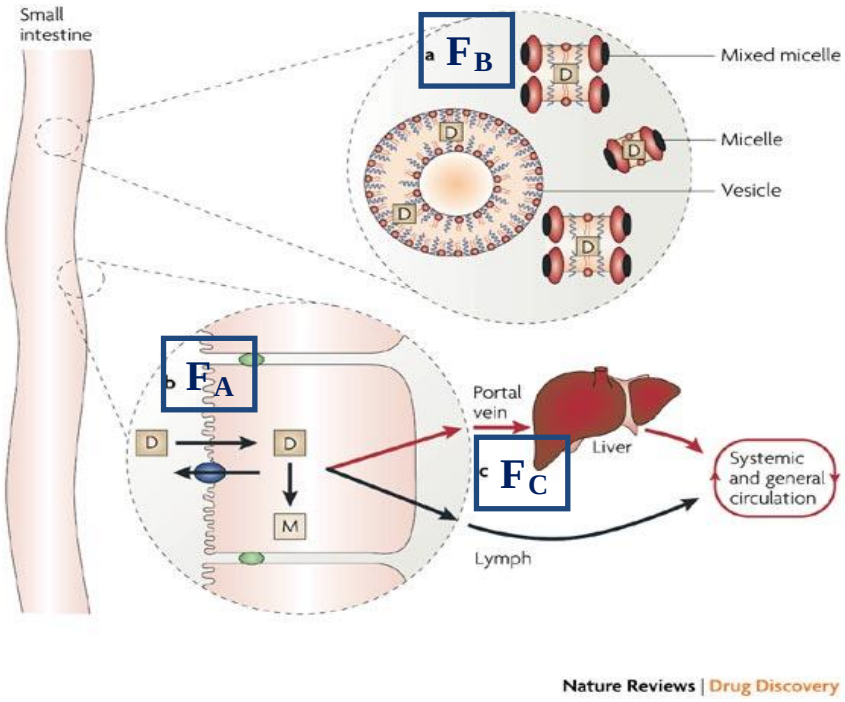


Fig.3. Absorption of lipophilic bioactives: Bioaccessibility – F_B , Absorption - F_A and Metabolism – F_H [Reprinted by permission from Macmillan Publishers Ltd: Nature Review Drug Discovery (Porter, Trevaskis et al. 2007)].

and billiary excretion suggested poor absorption of curcumin from gut while intravenous injection resulted in active transportation of curcumin into bile owing to strong concentration gradients, but a majority of it was metabolized rapidly (Wahlstrom and Blennow 1978). In another study, deuterium and tritium labeled curcumin was administered orally as well as intraperitoneally, where majority of curcumin was excreted in the feces. In the same study when the curcumin was administered intravenously and intraperitoneally in cannulated rats, it was excreted in the bile and major biliary metabolites were found to be glucuronides of tetrahydrocurcumin and hexahydrocurcumin, while dihydroferulic acid along with traces of ferulic acid were identified as minor biliary metabolites, suggesting poor absorption of curcumin in the gut

(Holder, Plummer et al. 1978). The pharmacokinetic fate of curcumin in humans is in agreement to that in rats as observed in several studies (Ireson, Jones et al. 2002; Garcea, Jones et al. 2004; Garcea, Berry et al. 2005), suggesting poor absorption and rapid metabolism upon oral administration. It has been observed through various studies that curcumin metabolizes via reduction as well as conjugation. When administered orally it undergoes conjugation resulting in formation of curcumin glucuronide and sulfates, while on intravenous or intraperitoneal administration curcumin reduces to form tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin (Pan, Huang et al. 1999; Asai and Miyazawa 2000; Ireson, Orr et al. 2001; Garcea, Berry et al. 2005; Hoehle, Pfeiffer et al. 2006).

It is also unclear whether curcumin metabolites are as active as the pure compound itself. Some studies indicate that curcumin metabolites such as curcumin glucuronides and tetrahydrocurcumin are less active than curcumin (Ireson, Orr et al. 2001; Sandur, Pandey et al. 2007), while some studies suggest otherwise (Kim, Araki et al. 1998; Okada, Wangpoengtrakul et al. 2001; Naito, Wu et al. 2002; Murugan and Pari 2006; Pari and Murugan 2007; Pfeiffer, Hoehle et al. 2007).

Thus, due to its low solubility, poor absorption, rapid metabolism and excretion, curcumin is a poorly bioavailable compound.

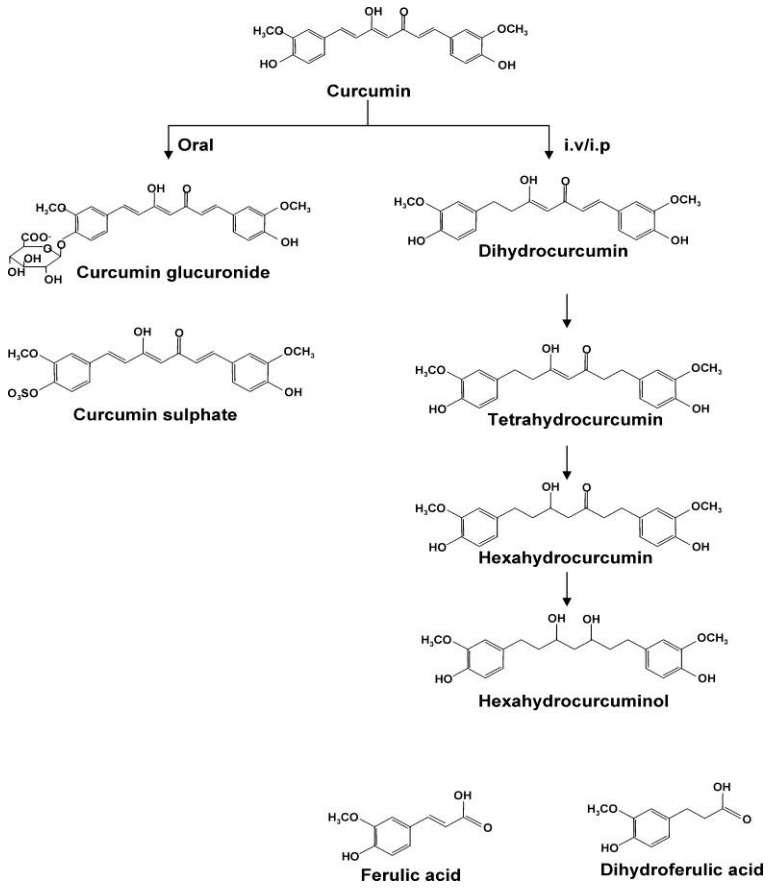


Fig.4. Curcumin and its metabolites

1.3 SAFETY OF CURCUMIN

Although curcumin is a diet derived bioactive, it will be unwise to assume that doses exceeding that in the normal diet would be harmless. Studies conducted on animals have shown that curcumin is well tolerated at high doses (Wahlstrom and Blennow 1978; Sharma, Ireson et al. 2001). Anecdotal incidences can still be found, suggesting adverse effect of curcumin in some subjects (Burgos-Moron, Calderon-Montano et al. 2010). At least three Phase I clinical trials have established that curcumin is safe at doses as high as 12g/day when ingested without the use of enhanced delivery systems (Shoba, Joy et al. 1998; Cheng, Hsu et al. 2001; Lao, Demierre et al. 2006).

1.4 CURRENT APPROACHES TO DELIVERY OF CURCUMIN

Several approaches have been explored for efficient curcumin delivery and thereby increase its bioavailability. Adjuvants (Shoba, Joy et al. 1998), nanoparticles (Shaikh, Ankola et al. 2009), phospholipid complexes(Gupta and Dixit 2011) , polysaccharide complexes (Tønnesen, Másson et al. 2002), nano emulsions and emulsions (Wang, Jiang et al. 2008; Ahmed, Li et al. 2012) are just a few examples.

Shoba *et al*. (Shoba, Joy et al. 1998) studied curcumin combined with an adjuvant piperine, which is a known inhibitor of hepatic and intestinal glucuronidation in rat as well as human subjects. Curcumin administered alone in dose of 2g/kg in rats resulted in moderate serum concentration after a period of 4 hours, but when piperine (20mg/kg) was administered simultaneously the serum concentrations of curcumin increased and so did the bioavailability, by 154%. The same combination of curcumin and piperine was studied in humans, and the bioavailability of curcumin increased by 2000%. Shaikh *et al*. (Shaikh, Ankola et al. 2009) prepared polymer nanoparticles encapsulated with curcumin and studied the stability and *in vitro* behavior. The particles were spheres with particles size of 264nm and polydispersity index of 0.31. An entrapment of 76.9% with a loading of 15% was obtained. An increase of 9- fold was observed in the bioavailability of curcumin. The release was due to diffusion phenomenon and followed by Higuchi's release pattern which helps to understand the release kinetics of drugs encapsulated in polymer matrix in to the surrounding fluid, when the drug loading exceeds its solubility in the polymer matrix. A recent study carried out by Ahmed *et al.* (Ahmed, Li et al. 2012) compared the bioaccessibility of curcumin from nano emulsions and conventional

emulsions using different lipids such as short chain triglycerides, medium chain triglycerides and long chain triglycerides. Greater bioaccessibility was observed for medium chain triglycerides as compared to short chain and long chain triglycerides. Also, slightly higher bioaccessibility was reported for conventional emulsions as compared to nano emulsions. Similar study carried out in our lab (Wang, Jiang et al. 2008) to study the effect of curcumin encapsulated in O/W nanoemulsions made using high pressure homogenization suggested enhanced anti-inflammatory action of curcumin in mouse ear inflammation model. Another study from our lab (Yu and Huang 2010) studied curcumin encapsulated in micelles made from hydrophobically modified starch and reported increased activity of encapsulated curcumin as compared to free curcumin.

Thus, even though the therapeutic use of curcumin suffers from many shortfalls like low absorption, rapid metabolism and excretion, it is possible to remedy the short comings through various delivery systems.

1.5 LIPID BASED SYSTEMS FOR ORAL DELIVERY

It has been well known that lipids enhances the absorption of poorly water soluble compounds or lipophilic bioactives and increases their oral bioavailability (Charman, Porter et al. 1997; Humberstone and Charman 1997; Fleisher, Li et al. 1999). This has led to the popularity of use of lipid based delivery systems to increase the oral bioavailability of poorly soluble drugs as well as nutraceuticals.

The mechanisms involved have been well explained in many reviews (Porter and Charman 2001; Porter, Trevaskis et al. 2007; Dahan and Hoffman 2008), a brief summary of which is as follows:

- **Increase solubilization:** The presence of lipids in the GI tract stimulates the contraction of gall bladder. This leads to the release of biliary and pancreatic secretions such as bile salts (BS), phospholipids (PL), cholesterol (Ch), pancreatic lipase and other enzymes. BS, PL and Ch form intestinal mixed micelles which increase the solubilization of lipophilic bioactives. In addition, the digestion of lipids leads to the formation of free fatty acids and monoglycerides, these along with biliary compounds and emulsifiers or co-solvents used in formulation, aid in the formation of mixed micelles, micelles, liposomes and vesicles which further prevent the precipitation of lipophilic bioactives and increase their solubilization. The solubilized bioactives are easily absorbed through the intestinal cell wall. Thus this mechanism directly affects the bioavailability of lipophilic bioactives by increasing its bioaccessibility.
- **Alter the gastric residence time:** The presence of lipids in the GI tract increases the transit time by delaying gastric emptying. This avails more time for solubilization for lipophilic bioactives.
- **Stimulate the lymphatic transport:** Orally administered bioactives usually enter the systemic circulation via portal blood; however, lipids have shown to enhance the extent of intestinal lymphatic transport of lipophilic bioactives.

Lipids have also been shown to alter the physical barrier system of GI tract and hence increase the absorption of solubilized lipophilic bioactives.

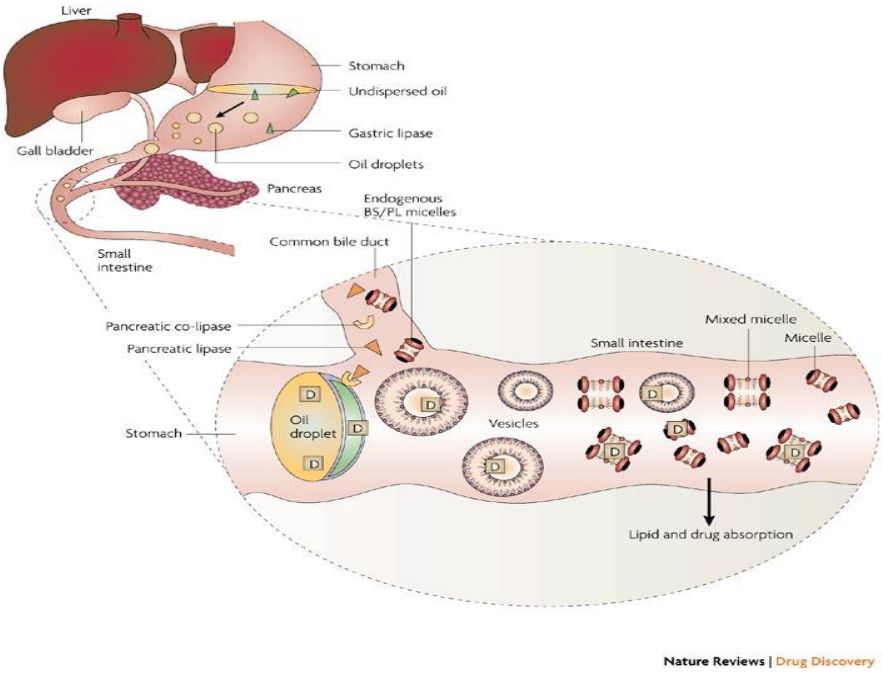


Fig.5. Effect of lipids and lipid digestion on solubilization of bioactive [Reprinted by permission from Macmillan Publishers Ltd: Nature Review Drug Discovery (Porter, Trevaskis et al. 2007)].

Emulsions, nanoemulsions, liposomes, organogels and self emulsifying drug delivery systems are some examples of lipid based delivery systems. The lipid based delivery systems for this study are limited to gel-like emulsions and nanoemulsions.

1.5.1 EMULSIONS

Emulsion is a mixture of two immiscible liquids, with one liquid dispersed as small spherical droplets in the other. Depending on the various characteristics, it is divided into sub categories such as microemulsions, nanoemulsions, high internal phase emulsions, gel-like emulsion, etc.

Two types of emulsion systems have been explored in this study:

- Gel-like emulsions
- Nanoemulsions

1.5. 1.1 GEL-LIKE EMULSIONS

For the purpose of this study, emulsions exhibiting gel-like viscoelastic behavior will be addressed as gel-like emulsion. Usually, when dispersed phase volume fraction exceeds the value of 0.73 (Princen 1979), highly concentrated emulsions or viscoelastic gel emulsions are formed. The emulsions prepared in this study do not have dispersed phase volume fraction greater than 0.73; however, they do exhibit viscoelastic behavior as observed from the rheological measurements carried out on the systems. Similar behavior has been observed of emulsions made from sugar esters; however, those systems are W/O emulsions, while the ones we made are O/W type. Increased viscosity of the continuous phase and close packing of dispersed phase could be possible reasons for the gel-like behavior of these systems. These systems will be discussed in detail in Chapter 2.

1.5.1.2 NANO EMULSIONS

Nanoemulsions are kinetically stable emulsions with droplet size less than 200nm diameter (Solans, Izquierdo et al. 2005). They could be either oil in water (O/W) type or water in oil (W/O) type. They are a well studied class of lipid based delivery systems for drug and bioactive delivery (Gutiérrez, González et al. 2008). Upon oral delivery, O/W nanoemulsions are digested quickly and efficiently owing to the smaller particle size and larger surface area (Borel, Armand et al. 1994). Hence, nanoemulsions are a good lipid

based delivery system candidate. Nanoemulsions have been employed for encapsulation of curcumin in past (Wang, Jiang et al. 2008; Ahmed, Li et al. 2012), but with different formulations.

1.5.2 ORGANOGELS

Gels have been described as materials that are "easier to recognize than define", many definitions have been given over the years. However, gels have been accepted as semi-solid materials comprising of low concentrations (<15%) of gelator molecules that self-assemble to form a network that entraps the solvent, preventing flow due to surface tension (Vintiloiu and Leroux 2008).

Organogels can be distinguished from hydrogels in that, the solvent is organic in nature. For this study, we have used an edible medium chain triglyceride since the delivery system is supposed to be administered via oral route. Molecular organogels, the focus of this study, are thermo-reversible and visco-elastic in nature, and are composed of low molecular weight gelator molecules that self assemble into three dimensional networks and immobilize the organic solvent. The aggregation of the gelator molecules is driven by weak interactions such as dipole-dipole, van der Waals, and hydrogen bond interactions. (Friggeri, Gronwald et al. 2002). Organogels have been rarely investigated for oral delivery. Out of the couple of studies conducted (Murdan, Andrysek et al. 2005; Yu and Huang 2012), one of the studies has been conducted in our lab (Yu and Huang 2012).

1.6 SUGAR BASED ESTERS

Sugar based esters are compounds made by esterification of sugars or sugar based compounds with fatty acid or acyl chains. Two types of sugar based esters have been included in this study: sucrose esters (as emulsifiers) and sugar alcohol esters (as organogelators).

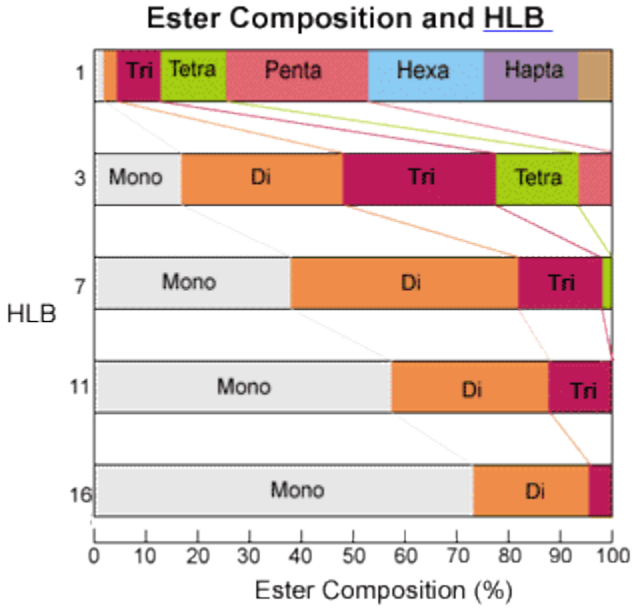


Fig.6. The effect of ester substitution on HLB of sucrose esters [Courtesy of Mitsubishi-Kagaku Food Corporation, Japan]

(http://www.mfc.co.jp/english/whatsse.htm**).**

Sucrose esters are non ionic emulsifiers. The hydrophilic part is the sugar moiety while the hydrophobic part consists of fatty acid chain. Sucrose by nature is polyhydric, with three primary hydroxyls –C6, C1', C6'. These three hydroxyls are most reactive and easily substituted with fatty acids, forming mono-, di- and tri-esters and hence are most commonly available commercially. The fatty acids affect the properties of the sucrose

ester. The degree of substitution also affects the HLB value. Low HLB value is obtained by high ester substitution, while high HLB value can be obtained by low ester substitution (Whitehurst 2008).

Figure 6 demonstrates the effect of the degree of substitution on the HLB value of the sucrose esters used as emulsifiers.

Sugar alcohols are hydrogenated carbohydrate, where the ketone or aldehyde group has been replaced with hydroxyl group. Just like sucrose, sugar alcohols are polyhydric in nature, with multiple hydroxyl groups present. Hence, just like sucrose they are good candidates for conversion into amphiphilic molecules. Hence the concept of forming amphiphilic molecules by reaction of acyl chains with sugar alcohols has been explored in many studies (J. A. Van, J. G et al. 1977; Chopineau, McCafferty et al. 1988; Jadhav, Vemula et al. 2010).

The sugar alcohol esters used in this study are a kind gift from D George John's lab at CUNY, NY and have been used as organogelators. The organogelators in focus are Mannitol Dioctanoate (M8) and Sorbitol Dioctanoate (S8) synthesized by enzyme mediated trans-esterification of sugars with fatty acid donors. The numeral suffix of '8' denotes that the amphiphile molecule has two C_8 tails, while the letters 'M' and 'S' denote the head group composed of Mannitol and Sorbitol respectively. The ability of these amphiphiles to exhibit self assembly is because of their ability to interact with other amphiphile molecules. The specific (e.g. hydrogen bonding) and non specific (e.g. surface tension, van Der Waals forces) forces between gelator-gelator and gelator-solvent molecules have been reported to be responsible for organogel formation using sugar alcohol esters. The interaction among gelator molecules leads to self assembly into

crystalline network, while the solvent-gelator interactions are responsible for solvent entrapment into the matrix of gelator network (Jadhav, Vemula et al. 2010).

1.7 *IN VITRO* DIGESTION MODEL

Successful incorporation of bioactives into delivery systems is an important step in the development of an efficient delivery system. At the same time, it is vitally important to assess the ability of the delivery system to carry out the function it was designed for i.e. deliver the bioactive. In the case of a lipid based delivery system, it is the ability of the system to solubilize the bioactive that needs to be validated. *In vitro* digestion model is one of the methods, which can help assess the bioaccessibility of the bioactive incorporated in lipid based delivery systems. The advantage of *in vitro* methods over *in vivo* methods is that, they consume fewer resources and are easier to carry out. *In vitro* methods may not match the accuracy of *in vivo* methods, but they provide a good alternative when it comes to ease of utilization (Hur, Lim et al. 2011).

The *in vitro* digestion model utilized for this study is the pH stat model as shown in Figure 7. It is a dynamic method wherein the GI tract environment is simulated using digestion buffers and enzymes. The delivery system along with the digestion buffer is placed in the reaction vessel. Enzyme solution is added to the reaction vessel, which starts the lipid digestion. FFAs are released, resulting in a pH drop. These FFAs are hydrolyzed using NaOH solution. The quantity of NaOH added and time of addition is monitored throughout the span of the experiment.

A good understanding of the lipid digestion process is vital, when using the above method. Lipid digestion primarily occurs in the small intestine. Lipid digestion is a

complex process that involves emulsification, hydrolysis, micellization and finally, absorption through enterocytes. The release of phospholipids and bile in the GI tract, aids in emulsification of the lipids. The emulsification step is important, as it provides the necessary surface conditions for the pancreatic lipase and co-lipase to act upon (Aloulou, Rodriguez et al. 2006). The triglycerides are hydrolyzed by the lipolytic enzymes, releasing free fatty acids and monoglycerides (Porter, Trevaskis et al. 2007). These digestion products may further enhance the solubilization of the bioactive by forming micelles, mixed micelles and vesicles along with the bile salts and phospholipids, which carry the bioactive (Porter, Pouton et al. 2008). These systems are then absorbed through the enterocytes and the bioactive reaches the systemic circulation.

The whole lipid digestion product from the reaction vessel is ultra-centrifuged after about 2 hours, which is usually the time it takes to digest lipids in lumen. The bioaccessibility of the bioactive is assessed by analysis of the aqueous phase present in the whole lipid digestion product that was separated by ultra-centrifugation. Depending on the type of lipid and the extent of digestion of the delivery system, the digestion product will separate into about three phases. The top layer consists of undigested oil, the middle aqueous phase consists of the micellar and vesicular systems with solubilized bioactive while the bottom layer is a pellet composed of precipitated unsolubilized bioactive and calcium salts of fatty acids (Porter and Charman 2001). The pH stat model not only allows the determination of bioaccessibility, but it also helps study the rate and extent of digestion of delivery systems. This information can be useful in designing delivery systems with desired release properties.

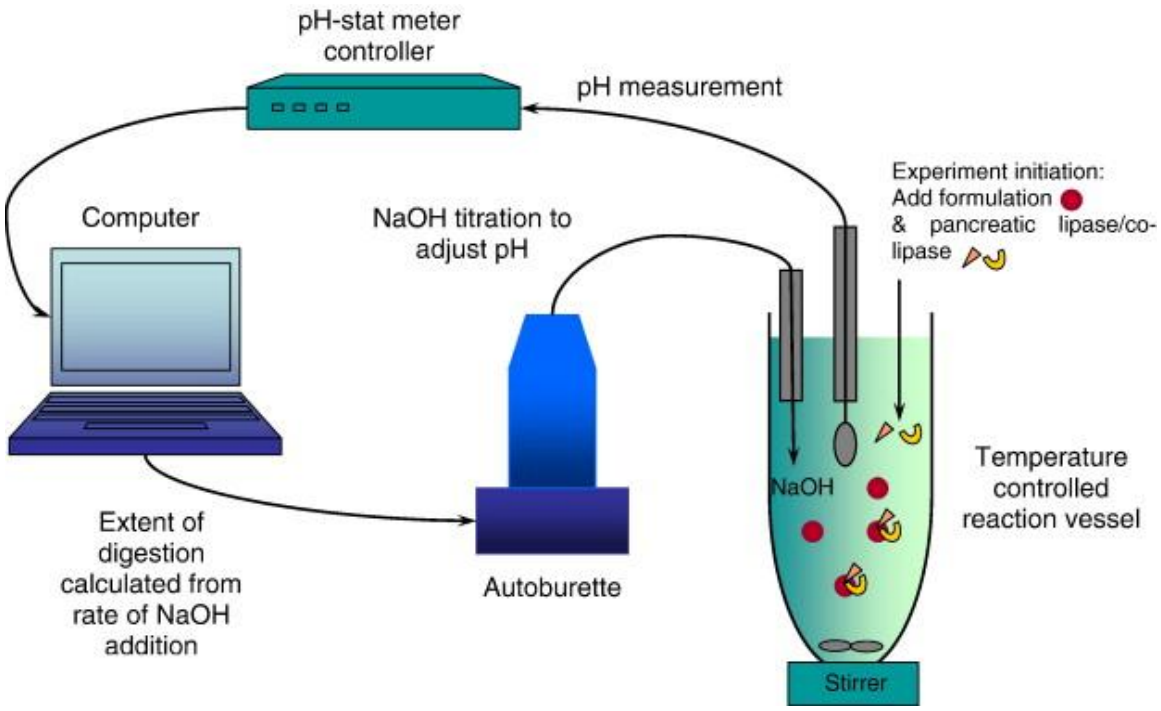


Fig.7. Lipid Digestion Model – The pH-stat method [Reprinted by permission from Macmillan Publishers Ltd: Nature Review Drug Discovery (Porter, Trevaskis et al. 2007)].

The pH-stat model quantifies the extent of lipid digestion indirectly, via measuring the release of free fatty acids. This in turn is dependent on the degree of ionization of fatty acids and availability of titrable fatty acids (Sek, Porter et al. 2001; Sek, Porter et al. 2002). Thus, this is the major limitation of the pH-stat model for lipid lipolysis. However, the various classes of lipolysis products such as fatty acids, monoglycerides and diglycerides lack chromophoric groups, thus otherwise detection and quantification of these products is difficult (Sek, Porter et al. 2001).

CHAPTER 2

LIPID BASED DELIVERY SYSTEMS FOR CURCUMIN: PREPARATION AND CHARACTERIZATION

2.1 LIPID PHASE WITH CURCUMIN

The solubility of curcumin is low in oils (Cui, Yu et al. 2009; Lin, Lin et al. 2009). Although it can be dissolved in heated oils, curcumin soon precipitates, usually within a short period of time of few hours. Solubility and bioaccessibility of curcumin in different oils has been studied in our lab(Yu and Huang 2012) and it was observed that use of Span 20 (10%) in oil increases the loading of curcumin. It was also observed that dissolving curcumin in MCT oil increases its bioaccessibility. Hence for my experiments, I have used MCT oil along with Span 20 (10%) to dissolve curcumin and use it as the lipid phase for all the subsequent experiments.

2.1.1 MATERIALS

Commercial grade curcumin (mixture of curcumin 82%, demethoxycurcumin 15%, and bisdemethodycurcumin 3%) was obtained from Sabinsa Corporation (East Windsor, NJ). Medium Chain Triglyceride, a mixture of caprylic ($C_{8)}$ and capric (C_{10}) triglycerides (NEOBEE 1053) was a kind gift provided by Stepan Company (North Field, Illinois). Span 20 (Sorbitan monolaurate) was purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade water was obtained from Alfa Aesar (Lawrence, KS). Glacial acetic acid was obtained from J.T. Baker (Phillipsburg, NJ). HPLC grade acetonitrile was purchased from Pharmaco – AAPER (Brookfield, CT).

2.1.2 PREPARATION OF LIPID PHASE WITH CURCUMIN

MCT oil was heated to 60°C with continuous stirring. Span 20 was added and dissolved. 5% curcumin was then added to the mixture under continuous stirring and heated to ~140°C until the curcumin was completely dissolved. The mixture was then left to cool to bring down the temperature to ambient. The mixture was then centrifuged at 20,000 RPM for 15 min. Some curcumin precipitated, while the supernatant MCT oil + Span 20 mixture with dissolved curcumin was stored for further use. This MCT oil + Span 20 mixture with dissolved curcumin will henceforth be referred to as the lipid phase for simplicity sake.

2.1.3 CHARACTERIZATION

To determine the final loading of curcumin in the lipid phase, the lipid phase was diluted by 1000 times with 1:2 (v/v) Distilled Water: Acetonitrile mixture. A 25D UV-VIS absorption detector (Dionex) equipped UltiMate 3000 HPLC system was used for the analysis. A C18 3.9_150mm Nova-Pak column was used. Methodology earlier developed in our lab (Yu and Huang 2012) was employed, which is as follows:

The mobile phase solvents were: (A) Water with 2% acetic acid, and (B) Acetonitrile; elution conditions were: 0-2min, 65% A and 35% B, 2-17 min of linear gradient from 35% B to 55% B, 17-22min held at 55% B, 22-23 min B linearly went back to 35% . Flow rate of 1ml/min was maintained, the detection wavelength was set to 420nm and 50µl of sample was injected. Duplicates were analyzed.

Also, the stability of the lipid phase was observed visually for 5 days.

2.1.4 RESULTS AND DISCUSSION

The area under the curve was calculated using the standard curve of curcumin. The value obtained was multiplied by the dilution factor to obtain the loading of curcumin. The loading of curcumin in lipid phase was calculated to be ~34mg/g, which agrees with previous work done in our lab (Yu and Huang 2012). On visual observation, the lipid phase was quite stable even after 5 days of storage at room temperature with no curcumin fall out. The Span 20 is thought to reduce the interfacial tension leading to increased solubility of curcumin in MCT oil.

2.2 SELECTION OF SUCROSE ESTER COMBINATION FOR GEL-LIKE EMULSIONS AND NANO EMULSIONS

Oils have required HLB numbers that identify the HLB necessary to give good o/w emulsification. More stable emulsions are produced when two or more emulsifiers are mixed to obtain a combined HLB value matching the required HLB value of oil used, when compared to using a single emulsifier. Hence, two combinations of emulsifiers made from S270 (sucrose stearate, HLB 2), S570 (sucrose stearate, HLB 5) and P90 (sucrose monoplamitate, HLB 18) were tested for both, gel emulsions and nano emulsions:

1) S270 (HLB 2) + P90 (HLB ~18) - called **Com. 2** henceforth

2) S570 (HLB 5) + P90 (HLB ~18) - called **Com. 5** henceforth

The required HLB value of MCT oil is in the range of 9-13. The required HLB value of 12 was selected for all experiments in this study. Equation 2 was used to calculate the proportion of the emulsifiers for the combination:

$$HLB = \frac{[(Qty._{Emulsifier\ 1})(HLB_{Emulsifier\ 1}) + (Qty._{Emulsifier\ 2})(HLB_{Emulsifier\ 2})]}{[Qty._{Emulsifier\ 1} + Qty._{Emulsifier\ 2}]} \quad \ldots(2)$$

Thus, the proportion (w/w) as shown in Table 1 was calculated for Com. 2 and Com. 5 which gives a total HLB of 12 for the mixture:

Combination	**S270/S570 (%)**	**P90 (%)**
Com. 2 (S270)	37.5	62.5
Com. 5 (S570)	46.2	53.8

Table 1.Proportion (w/w) of Emulsifiers to achieve final HLB number of 12, this is the required HLB number of the oil used.

2.3 GEL-LIKE EMULSIONS

Gel-like emulsions are emulsions that exhibit gel-like viscoelastic behavior. Once formed, they do not flow under gravity like conventional emulsions. No study utilizing gel-like emulsions as a delivery matrix to incorporate curcumin was found during

literature survey. As per our knowledge, this is the first attempt to utilize gel-like emulsions for curcumin delivery.

Two different emulsifier combinations Com.2 and Com.5 were used for preparation of the gel-like emulsions. The morphology and other characteristics were observed using optical and polarized light microscopy, digital scanning calorimetry, and rheology.

Gel-like emulsions were very stable for couple of months with no visible curcumin fallout.

2.3.1 MATERIALS AND METHODS

2.3.1.1 MATERIALS

Sucrose esters S270 and S570 were a kind gift from Mitsubishi-Kagaku Food Corporation (Japan). Sucrose ester Habo Monoester P90 was a kind gift from Compass Foods (Singapore). Lipid phase previously prepared was used. Milli-Q water (18 MΩ) was used throughout the experiments.

2.3.1.2 GEL EMULSION PREPARATION AND OPTIMIZATION

Creation of a ternary phase diagram was tried initially, however, since the sucrose esters are in powder form, it was very difficult to dissolve them in the respective phase (based on their HLB values). Oil concentration of 40% or more yielded unstable emulsions. Thus O/W region of the phase diagram was selected as the area of interest as shown in Figure 8, and emulsions with lipid phase up to 30% were prepared.

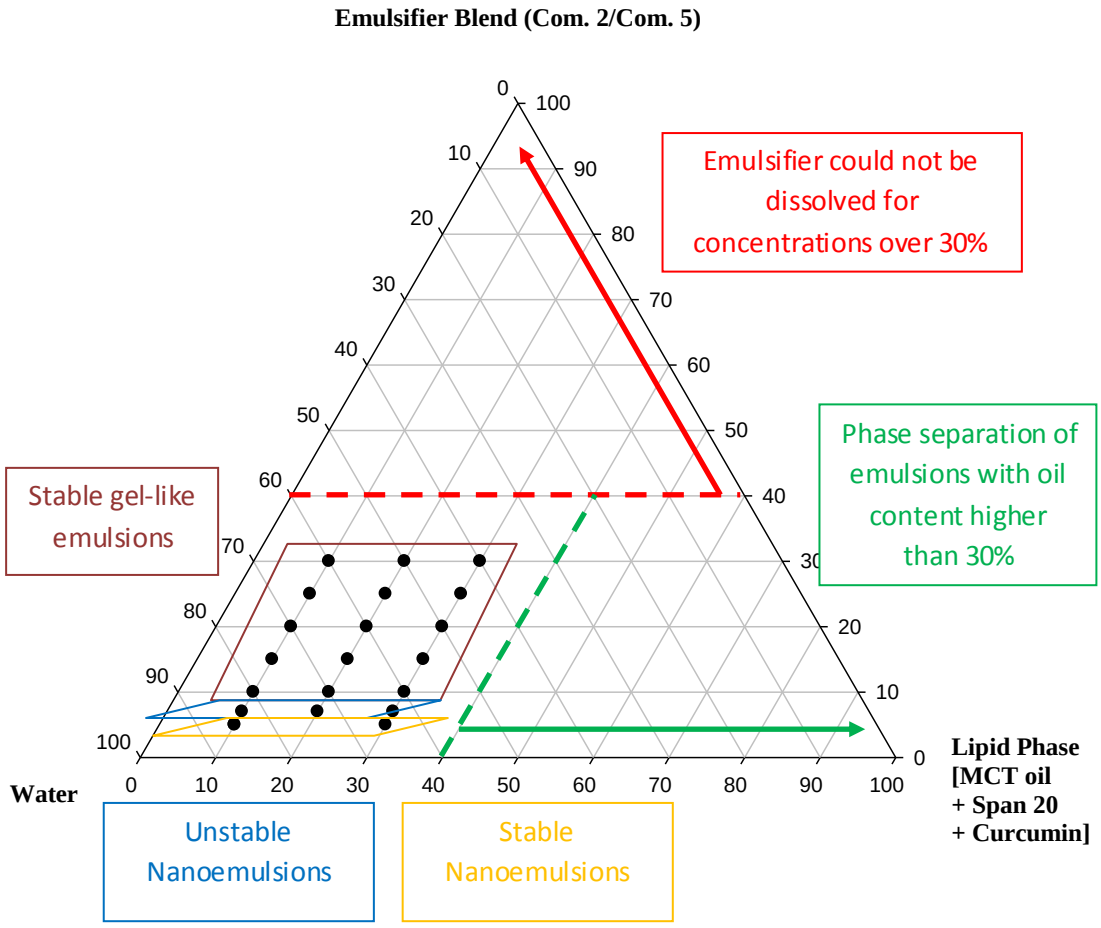


Fig.8. Ternary Phase Diagram of Emulsions. (●) represents emulsions that were prepared.

Emulsions were prepared using the lipid phase as the dispersed phase, while water was used as the continuous phase. Both, the lipid phase and water were measured and emulsifier mixture was added and stirred for 10 minutes to facilitate dispersion of sucrose esters emulsifiers. It was then heated and stirred for 15 minutes to guarantee complete dissolution. The coarse emulsion was then removed from heat and immediately homogenized at high speed of 16000 RPM for 1 minute using Ultra Turrax (T 25 basic,

IKA Works) high speed homogenizer. The emulsion was then cooled and stored at room temperature. The phase separation was observed visually.

2.3.1.3 CHARACTERIZATION

Optical and fluorescence imaging was carried out using Olympus BX41 Epifluorescence microscope equipped with a black and white CCD camera (C8484, Hamamatsu). For fluorescence imaging, the laser was adjusted to green fluorescence mode; images were obtained with an exposure time of 350ms. A small sample was mounted on the slide and covered with a glass cover slip.

The thermo-chemical properties of the gel emulsions were characterized using differential scanning calorimetry (DSC) analysis. Approximately 3-4mg of samples were weighed in 40µl aluminum pans and sealed with lids. An empty aluminum pan with lid was used a control. The temperature ramp speed was fixed at 10°C/min for all samples. The heat flow in the range between 27 and 230°C was recorded. The temperature was controlled using liquid nitrogen.

The rheological measurements on gel emulsions were performed using ARES strain controlled rheometer (Rheometric Scientific). Cone plate geometry (25mm diameter, 1rad cone angle) was used for all measurements. The linear viscoelastic region was determined by dynamic strain sweep test at a frequency of 1Hz. This was followed by frequency sweep measurements in the linear viscoelastic region.

2.3.2 RESULTS AND DISCUSSION

The stability of gel-like emulsions was observed visually. The gel-like emulsions were stable for a period of observation of two months. No visible curcumin fallout was noted during this period. It was observed that gel emulsions with emulsifier concentration less than 25% phase separated when sharp shear was applied while taking out the samples for characterization using a spatula suggesting that gel-like emulsions with emulsifier concentration less than 25% are prone to phase separation on application of sharp shear, due to disruption. All stable gel-like emulsions with emulsifier concentrations 25% and 30% were not prone to shear induced phase separation described above.

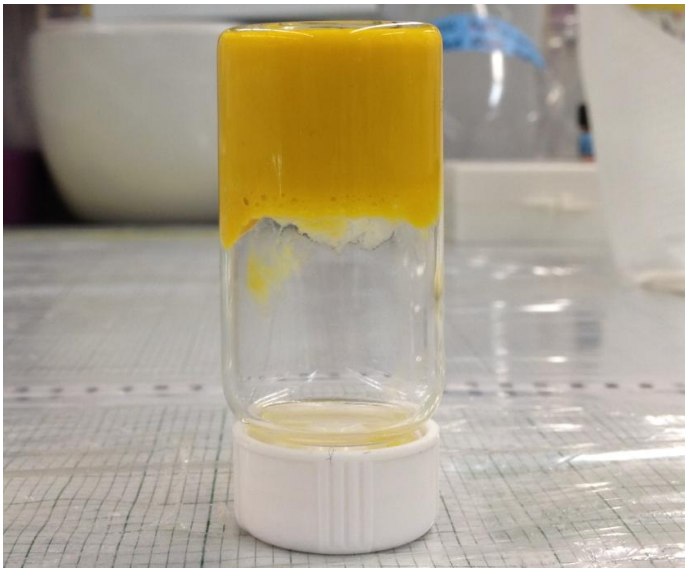


Fig.9. Gel-like emulsions do not flow under gravity. (30/20, Com. 2 shown in figure).

Since the particle size of droplets could not be determined by dynamic light scattering experiments, optical micrographs were used to understand the morphology of the gel-like

emulsions. The micrographs in Figure 10 are of the formulation containing 30% lipid phase and 20% emulsifier (Combinations Com. 2 and Com. 5). It was observed that the emulsion droplet size was in the range of micrometers, suggesting large particle size.

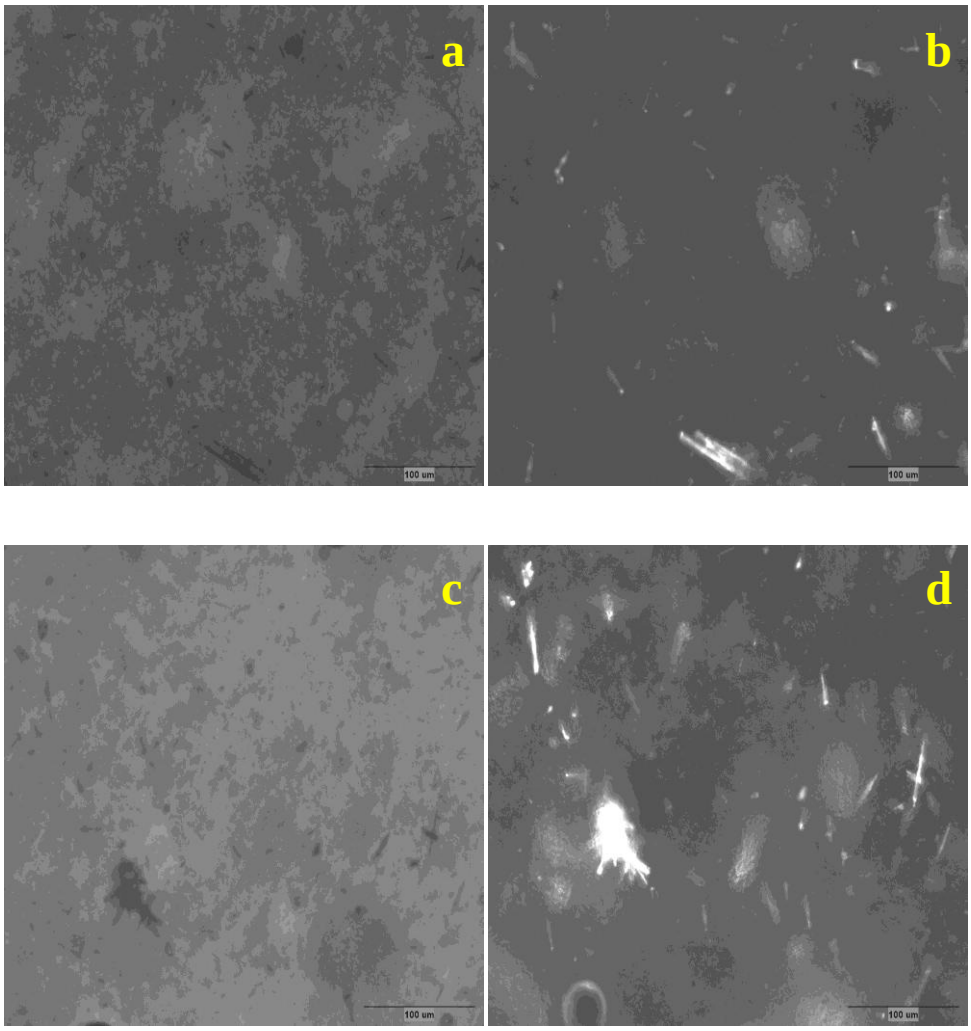
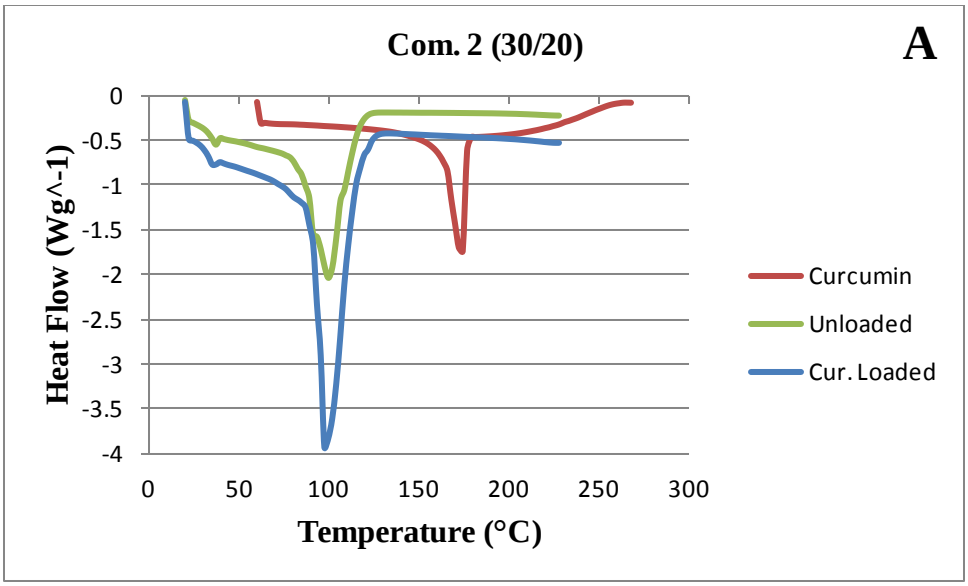


Fig.10. Micrographs of gel-like emulsions. (a) & (c) Optical micrographs of Com. 2 (30/20) and Com.3 (30/20) gel-like emulsions respectively. (b) & (d) Fluorescence micrographs of Com. 2 (30/20) and Com.3 (30/20) gel-like emulsions respectively. Scale shown is 100µm.

Crystals can also be observed in the optical micrographs of both Com. 2 and Com. 5 formulations which are suspected to be precipitated curcumin. The Com. 2 gel-like emulsion appeared more uniform and monodispersed as compared to that of Com. 5. Curcumin is naturally fluorescent in the visible green spectrum. Thus to determine whether the crystals present were precipitated curcumin, fluorescence micrographs were obtained. The fluorescence micrographs confirmed the presence of precipitated curcumin in both Com.2 and Com.5 gel-like emulsions observed as the bright areas in from Figure 10 (b) and (d).

The precipitation of the curcumin could be the result of temperature stress and shear during gel-like emulsion preparation. Sugar esters form aggregate structures in aqueous phase (Sadtler, Guely et al. 2004; Aramaki, Hoshida et al. 2012). It could be possible that excess emulsifier in the gel-like emulsions are forming aggregates structures in the continuous aqueous phase and consequently aiding the precipitation of curcumin in the system.

Digital Scanning Calorimetry (DSC) experiments were carried out to study the thermal properties of the gel-like emulsions. The results as shown in Figure 11 could not validate the presence of curcumin crystals. It should be noted that the sample size used for DSC experiments was very small, of the order of 3-4mg, and thus the sample size tested may not contain any curcumin crystals at all or may only contain a very small quantity which is below the detection.



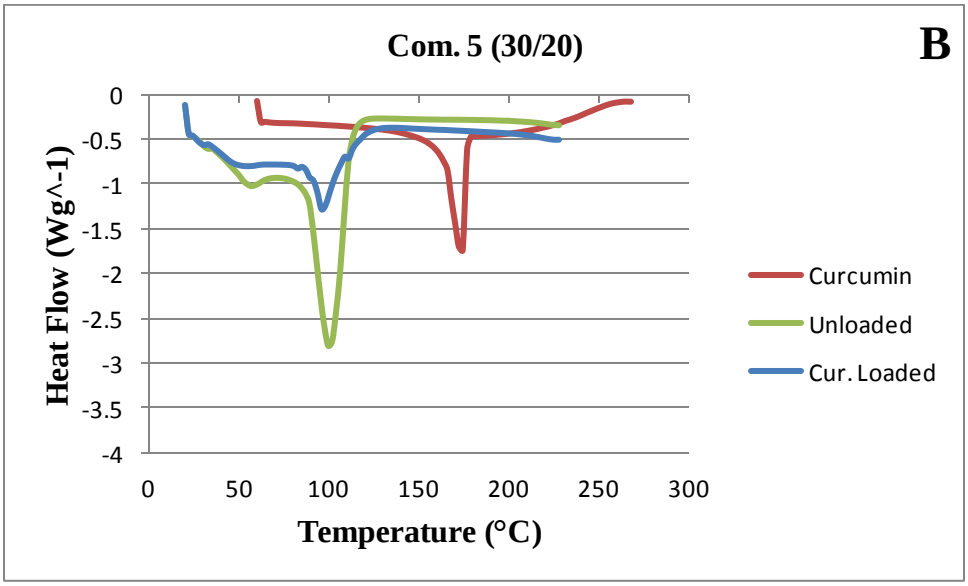


Fig.11. DSC results of gel-like emulsions (30/20: lipid phase/emulsifier). (A) Composite graph of blank and curcumin loaded gel-like emulsions using emulsifier combination Com.2 and curcumin (B) Composite graph of blank and curcumin loaded gel-like emulsions using emulsifier combination Com.5 and curcumin.

As mentioned previously, sugar esters have been known to form aggregate structures in aqueous solution (Stubenrauch 2001; Sadtler, Guely et al. 2004). It is possible that the excess of sugar ester in the continuous phase of gel-like emulsions used in this study form such aggregates. The peaks in Figure 11 (A) and (B) at around 50°C could be due to the phase transition of the emulsifier aggregate structures owing to the temperature elevation, however, this needs to be validated. The peak observed at 100°C is due to water evaporating from the system in both gel-like emulsions tested.

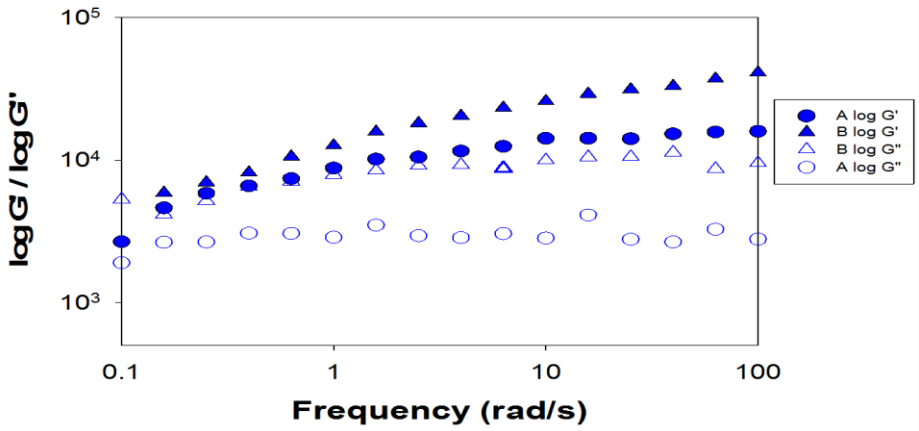


Fig.12. Frequency sweep data for gel-like emulsions (30/20: lipid phase/emulsifier) at 0.1% strain. (A) Gel-like emulsion with emulsifier combination Com.2. (B) Gel-like emulsion with emulsifier combination Com.5.

Rheological measurements were also carried out on the gel-like emulsions to determine their behavior. For all the gel-like emulsions tested, the G' values were higher than the G" values suggesting a viscoelastic solid behavior.

As observed from the micrographs, the gel-like emulsions seem closely packed. Also, if the excessive emulsifier in the aqueous phase is aggregating as discussed before, this

could also result in increased viscosity of the continuous phase which stabilizes the dispersed phase and prevents aggregation and phase separation. This would explain the high stability of the gel-like emulsions to phase separation in to oil and water.

Com. 2 emulsifier combination contains higher amount of water soluble emulsifier P90 when compared to Com.5 emulsifier combination. If the excess emulsifiers form aggregate structures in the continuous phase and increases the viscosity of the continuous phase, the gel-like emulsions with higher amount of water soluble emulsifier (Com.2), should have higher gel strength than gel-like emulsions with lower amount of water soluble emulsifier (Com. 5), which is the case as observed from Figure 12. However, this needs to be validated further.

2.4 NANOEMULSIONS

Nanoemulsions are emulsions with particle size of dispersed phase less than 200nm. They are kinetically stable systems, but they do phase separate over time, depending on various factors, such as formulation, method of preparation, stresses, etc.

Nanoemulsions have been previously used to incorporate curcumin (Wang, Jiang et al. 2008; Ahmed, Li et al. 2012) successfully and they have also been tested for efficacy in these studies. However, sucrose esters have not been used as emulsifiers for curcumin incorporation so far.

Two different emulsifier combinations Com.2 and Com.5 were used for preparation of the nanoemulsions with curcumin. Particle size and stability were observed after preparation.

The nanoemulsions were not very stable; the most stable ones phase separated after a period of 48-72 hours.

2.4.1 MATERIALS AND METHODS

2.4.1.1 MATERIALS

Sucrose esters S270 (sucrose stearate, HLB 2) and S570 (sucrose stearate, HLB 5) were a kind gift from Mitsubishi-Kagaku Food Corporation (Japan). Sucrose ester Habo Monoester P90 (sucrose monoplamitate, HLB ~18) was a kind gift from Compass Foods (Singapore). Lipid phase previously prepared was used. Milli-Q water (18 MΩ) was used throughout the experiments.

2.4.1.2 NANOEMULSION PREPARATION AND OPTIMIZATION

Emulsions were prepared using the lipid phase as the dispersed phase, while water was used as the continuous phase. Both, the lipid phase and water were heated up to 70-80°C to facilitate dissolving of sucrose esters emulsifiers. Low HLB value emulsifier was added to the lipid phase while high HLB value emulsifier was added to water and stirred for 10 minutes to guarantee complete dissolution. The dispersed phase (combination of low HLB emulsifier + lipid phase) was added under constant stirring to the continuous phase (combination of high HLB emulsifier + water) and further stirred for 15 minutes; the temperature of 70-80°C was maintained. The emulsion was then removed from heat and immediately homogenized at high speed of 16000 RPM for 1 minute using Ultra Turrax T 25 basic(IKA Works, Willimington, USA) high speed homogenizer. This coarse emulsion was then subjected to high pressure homogenization at 100MPa using

EmuliFlex-C3 (AVESTIN Inc., Ottawa, Canada) for up to 12 passes. Samples were collected at 3rd, 6th, 9th and 12th pass for characterization.

Two combinations of sucrose esters (Com.2 and Com. 5) were used to prepare two different sets of nanoemulsions.

The formulations (w/w) shown in Table 2 were made for both the sets; water was added to make up the proportion to total of 100%.

Lipid Phase (%)	Emulsifier Blend (%)
10	5
20	5
30	5

Table 2. Nanoemulsion formulations prepared.

2.4.1.3 CHARACTERIZATION

Particle size of the nano emulsions was measured using a photon correlation spectroscopy (PCS)-based BIC 90 plus particle size analyzer. The particle size analyzer was equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corporation, New York, NY). The light source of the particle size analyzer was a solid-state laser operating at 658 nm with 30 mW power. Signal detection was by a high-sensitivity avalanche photodiode detector. All measurements were made at a fixed scattering angle of 90° and temperature of 25.0 ± 0.1 °C. The sample was diluted to avoid multiple scattering.

The stability of the emulsions was observed visually.

2.4.2 RESULTS AND DISCUSSION

Emulsifier concentration as low as 10% would form gel-like emulsions for both Com.2 and Com.5 emulsifier combinations during the study. Fluid emulsions which can be processed in high pressure homogenizer could not be formed at emulsifier concentration as low as 10%.

Com. 2	**3 Pass**	
(lipid phase/emulsifier)	**Z –Avg. (nm)**	**PDI**
10/5	190 ±0.2	0.324±0.009
20/5	44.3±1.8	0.265±0.009
30/5	15.8±0.5	0.245±0.002

(A)

Com. 5	**3 Pass**	
(lipid phase/emulsifier)	**Z –Avg. (nm)**	**PDI**
10/5	139.8±2.8	0.298±0.008
20/5	44.6±1.1	0.285±0.014
30/5	20.8±25.3	0.224±0.012

(B)

Table 3. Particle size distribution of nanoemulsions with (A) Com.2 and (B) Com.5 emulsifier combinations on 3rd pass at 100MPa in HPH system.

Thus, an emulsifier concentration of 5% and 7% with dispersed phase content varying from 10%-30% for emulsions preparation was experimented with. The emulsions made thusly, were passed through high speed homogenization at 100MPa. Samples were collected at 3rd, 6th, 9th and 12th passes; these were then tested for particle size distribution and stability. The particle size analysis is reported as the average diameter of the particle (Z – Average) and the polydispersity index (PDI) in Table 3 (A) and (B).

It was observed that 7% emulsifier concentration did not give stable emulsions. The emulsions phase separated within a couple of hours.

Some emulsions made with 5% emulsifier concentration yielded stable emulsions. The emulsions made at 3rd pass were the most stable ones, while the rest of 5% emulsifier proportion emulsions phase separated in a couple of hours.

Fig.13. Nano emulsions at 3rd Pass are the most stable (10/5, Com. 2).

It could be observed from Table 3 that as the lipid phase proportion increases, the particle size decreases.

2.5 ORGANOGELS

Organogels are gels formed by three dimensional structures of organogelators which entrap an organic solvent.

They have been investigated as possible delivery matrix for curcumin in our lab using different organogelators(Yu and Huang 2012) than the ones used in this study.

Two different organogels were used for the preparation of the organogels with curcumin. The morphology and characteristics were studied using optical and fluorescence microscopy, digital scanning calorimetry and rheology.

2.5.1 MATERIALS AND METHODS

2.5.1.1 MATERIALS

Sugar ester gelators M8 (Mannitol Dioctanoate) and S8 (Sorbitol Dioctanoate) were kindly provided by Dr. George John's lab at CUNY (New York). Lipid phase prepared as stated previously was used.

2.5.1.2 ORGANOGEL PREPARATION

The sugar ester gelator was added at 10% to the lipid phase in a glass vial with screw cap. It was heated till all of the gelator dissolved under constant stirring. The mixture was cooled to room temperature. To confirm gelation, the vial was inverted to check for flow.

2.5.1.3 CHARACTERIZATION

Optical and fluorescence imaging was carried out using Olympus BX41 Epifluorescence microscope equipped with a black and white CCD camera (C8484, Hamamatsu). For

fluorescence imaging, the laser was adjusted to green fluorescence mode; images were obtained with an exposure time of 350ms. A small sample was mounted on the slide and covered with a glass cover slip.

The thermo-chemical properties of the organogels were characterized using differential scanning calorimetry (DSC) analysis. Approximately 3-4mg of samples were weighed in 40µl aluminum pans and sealed with lids. An empty aluminum pan with lid was used a control. The temperature ramp speed was fixed at 10°C/min for all samples. The heat flow in the range between 27 - 230°C was recorded. The temperature was controlled using liquid nitrogen.

The rheological measurements on organogels were performed using ARES strain controlled rheometer (Rheometric Scientific). Cone plate geometry (25mm diameter, 1rad cone angle) was used for all measurements. The linear viscoelastic region was determined by dynamic strain sweep test. This was followed by frequency sweep measurements in the linear viscoelastic region.

2.5.3 RESULTS AND DISCUSSION

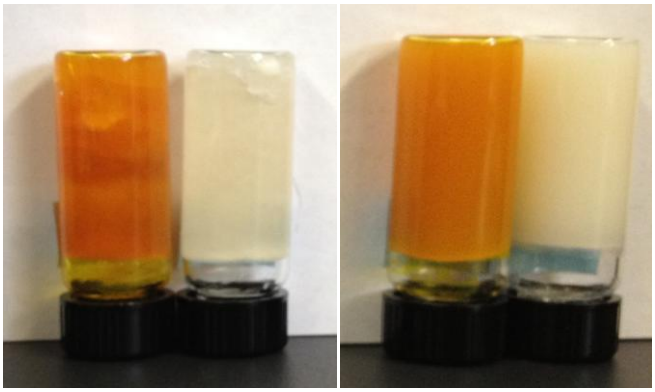


Fig.14. Loaded and Unloaded organogels. M8 organogel (left) and S8 organogel (right).

The two organogelators used in the study have been extensively studied by Dr. George John's group and according to them the M8 gelator forms ribbon like gelator network while the S8 gelator forms plate like structures. Similar results have been observed in organogels formed in this study as can be seen in the micrographs in Figure 15 (A) & (C).

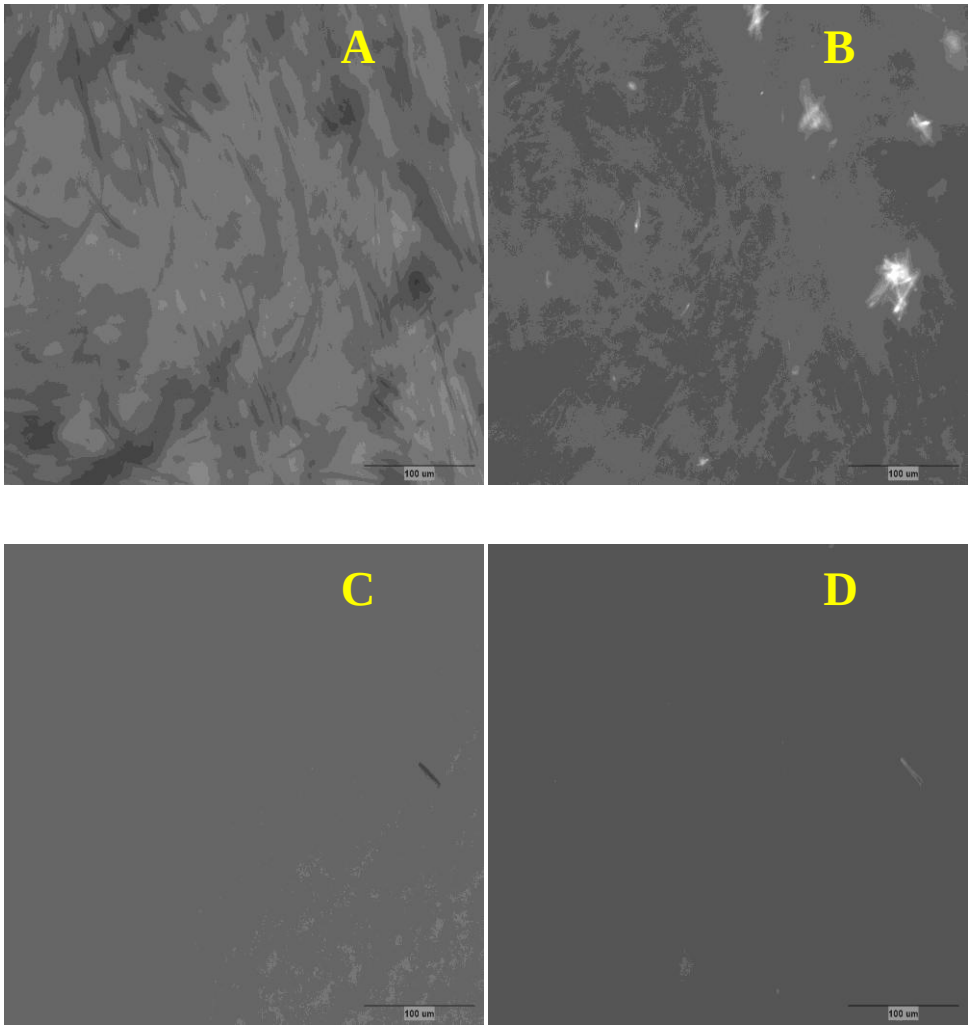


Fig.15. Micrographs of Organogels. (A) & (C) Optical micrographs of M8 and S8 gel respectively. (B) & (D) Fluorescence micrographs of M8 and S8 gel respectively. Scale shown is 100µm.

Also, the precipitated curcumin crystals are clearly visible as the bright region in the fluorescence micrographs of the organogels in Figure 15 (B) & (D). The precipitation of curcumin could be due to the temperature stress during organogel formation.

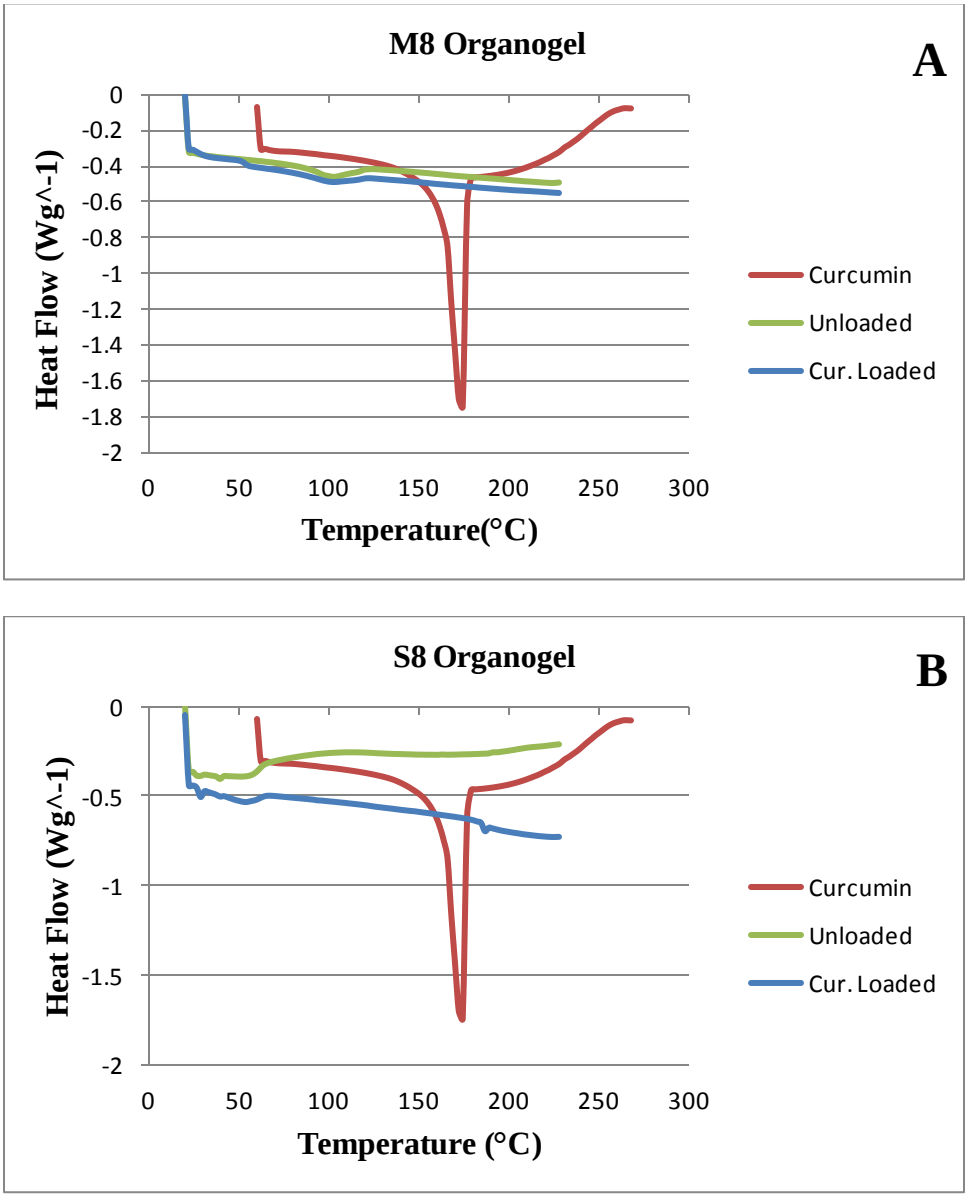


Fig.16. DSC results of organogels. (A) Composite graph of blank and curcumin loaded M8 organogels and curcumin (B) Composite graph of blank and curcumin loaded S8 organogels and curcumin.

The Digital Scanning Calorimetry (DSC) experiments were conducted to study the thermal properties of the organogels. Again, the DSC measurements could not validate the presence of crystals observed in fluorescence micrographs. Non- homogenously distributed curcumin crystals and presence of very small crystalline curcumin could be responsible for undetected presence of curcumin crystals in DSC measurements.

Peaks can be observed at around 100°C for M8 organogels (Figure 16 A), and between 50-60°C for S8 organogels (Figure 16 B). These could be the result of the melting and disruption of the three dimensional structure of organogelators in both the organogels. This can be further tested by observing samples under microscope with temperature ramp application.

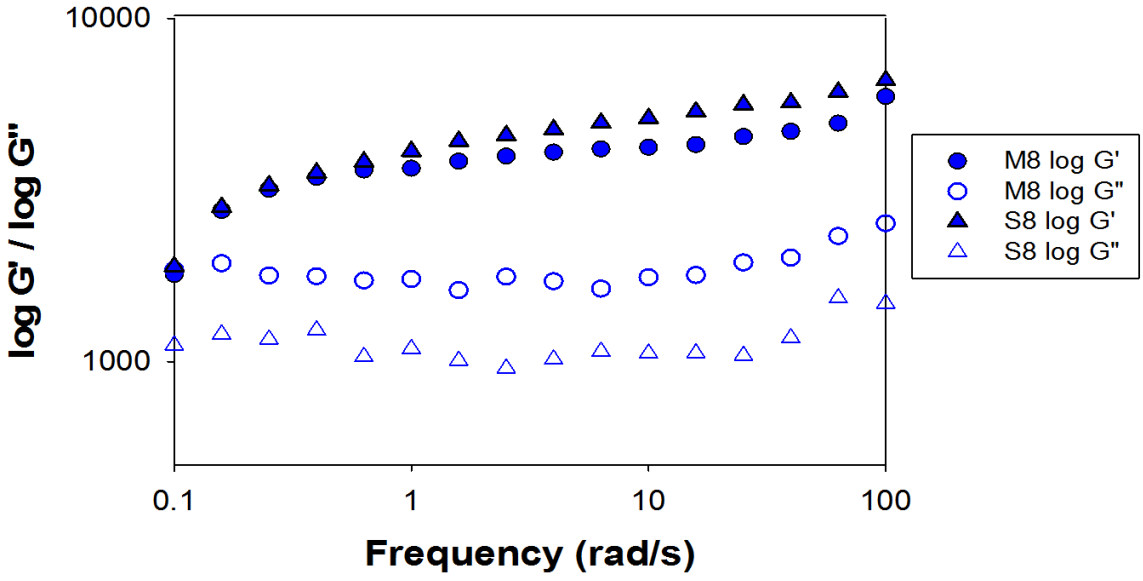


Fig.17. Frequency sweep data for organogels at 0.1% strain.

Rheological measurements for organogels also revealed viscoelastic solid like behavior for both M8 and S8 organogel. When compared to gel-like emulsions, organogels are

weaker (Comparing Figure 12 and Figure 17), which is relevant to shelf life and also the release of curcumin on oral delivery. Weaker gels will disintegrate faster on mastication as well as other stresses during its path to the lumen. Unloaded organogels were observed to be stronger than loaded organogels. This could be due to the presence of curcumin crystals in the loaded system disrupting or weakening the three dimensional network formed by the organogelators.

2.6 CONCLUSION

Three different lipid based delivery systems were successfully prepared for curcumin incorporation. The most stable nanoemulsions, gel-like emulsions and organogels were later tested for bioaccessibility using *in vitro* digestion model.

However, the morphology and characteristics of gel-like emulsions are very intriguing and need further investigation. Also, precipitation of curcumin in the system is an important factor for the stability of all three lipid based delivery systems. The nucleation and growth of curcumin crystals over time eventually leads to disruption and phase separation in all three lipid based delivery systems. Thus it is important to understand the speed with which the curcumin crystallizes in different systems. This will help in correct assessment of the stability and shelf-life of the systems.

CHAPTER 3

LIPID BASED DELIVERY SYSTEMS: CHARACTERIZATION USING *IN VITRO* DIGESTION MODEL

3.1 *IN VITRO* DIGESTION MODEL

The *in vitro* digestion model utilized in this study is the pH-stat model. The delivery system to be tested is added to the reaction vessel along with the digestion buffer, under constant agitation to simulate the environment *in vivo*. The fed or unfed state of the lumen affects the bioaccessibility of lipid based delivery systems as the concentration of bile salts and phospholipids are different in fed condition and unfed condition. Different recipes of buffer solutions are used to mimic either the fed or unfed condition of the lumen. The scope of my study is limited to testing digestion of the delivery systems in fed condition of lumen. A solution of gastric enzymes is added which hydrolyze the triglycerides into fatty acids and monoglycerides. The pH-stat titration apparatus monitors the progress of the triglyceride lipolysis.

3.2 MATERIALS AND METHODS

3.2.1 MATERIALS

Calcium chloride dihydrate, sodium taurodeoxycholate (Na TDC) and pancreatin lipase were purchased from Sigma (St. Louis, MO). Tris maleate was obtained from Research Organics (Cleveland, OH) and sodium chloride and 0.25N NaOH were bought from BDH - VWR (West Chester, PA). Phosphotydylcholine was purchased from Lipoid (Newark, NJ).

3.2.2 *IN VITRO* DIGESTION

Pancreatin solution was prepared in advance for this experiment and stored in ice. 1g of porcine pancreatin was mixed in 5ml of fed-state buffer under constant stirring to activate the enzymes. This mixture was then centrifuged and stored. A fresh pancreatin solution was made every day to ensure optimum enzyme activity.

For the *in vitro* lipolysis experiment, the lipid based delivery system with curcumin was added to 9ml of the fed-state buffer (it mimics the fed state conditions of the lumen) and pH was adjusted to 7.5. 1ml of pancreatin solution was added to start the lipolysis. A pH of 7.5 ± 0.2 was maintained by addition of 0.25N NaOH throughout the experiment, temperature of the reaction vessel was maintained at 37 ± 1°C. As a control experiment, dispersion of curcumin in water was also subjected to digestion. The experiments lasted 2 hours and were carried out in duplicates.

The lipolysis fed-state buffer was prepared using the recipe in Table 4:

Ingredient	**Quantity (mM)**
Tris maleate	50
NaCl	150
$CaCl_2$ $2H_2O$	5
NaTDC	20
Phosphatidylcholine	5

Table 4. Recipe of fed-state lipolysis buffer.

3.2.3 POST DIGESTION CHARACTERIZATOIN

After the *in vitro* digestion experiment, the whole digestion product was ultra centrifuged at 50,000 RPM for 40 min in a Beckman Coulter ultracentrifuge (Type 60 Ti rotor). The aqueous phase was collected through syringes and filtered using 0.22 µm syringe filter. The particle size of the micelles was measured using this filtrate in a Brookhaven Particle Size Analyzer. The remaining filtrate was stored for bioaccessibility analysis.

3.3 RESULTS AND DISCUSSION

Fed state buffer was used for all the experiments, as it was demonstrated in a previous study in our lab that the extent of lipolysis of lipid phase by itself was higher in fed state as compared to unfed state (Yu and Huang 2012). However, in the same study it has been observed that the extent of lipolysis is lesser in fed state as compared to unfed state for organogels used in that study.

In vitro lipolysis was carried out for the most stable of nanoemulsions, gel-like emulsions and organogels.

3.3.1 IN VITRO DIGESTION TITRATION PROFILES

The percentage of FFAs produced during triglycerides digestion in the dynamic *in vitro* digestion experiments gives the extent of lipolysis.

The extent of lipid digestion was calculated using Equation 3:

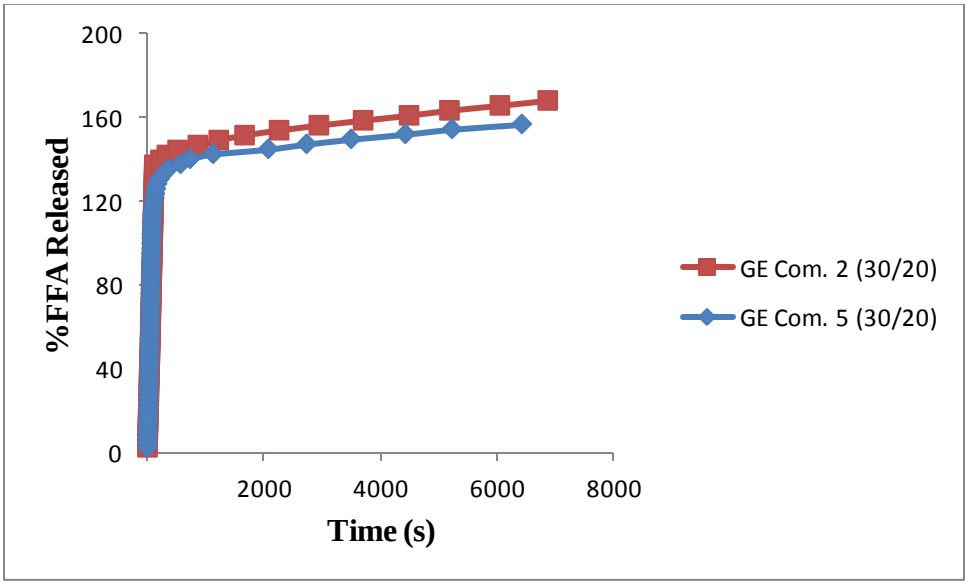
$$\%Extent\ of\ Lipolysis\ = \frac{Vol._{NaOH} \times Conc._{NaOH}}{No.of\ FFA\ released \times Moles_{Triglycerides}} \times 100 \quad \ldots(3)$$

Here, the $Vol._{NaOH}$ is the total amount of NaOH consumed in the lipolysis, Concentration of NaOH used for the experiment was 0.25 M, No. of FFA released was assumed to be 2 from a mole of triglyceride hydrolyzed. Mole of triglyceride for the amount of MCT oil in the formulation was calculated using molecular weight of MCT oil obtained using Equation 4:

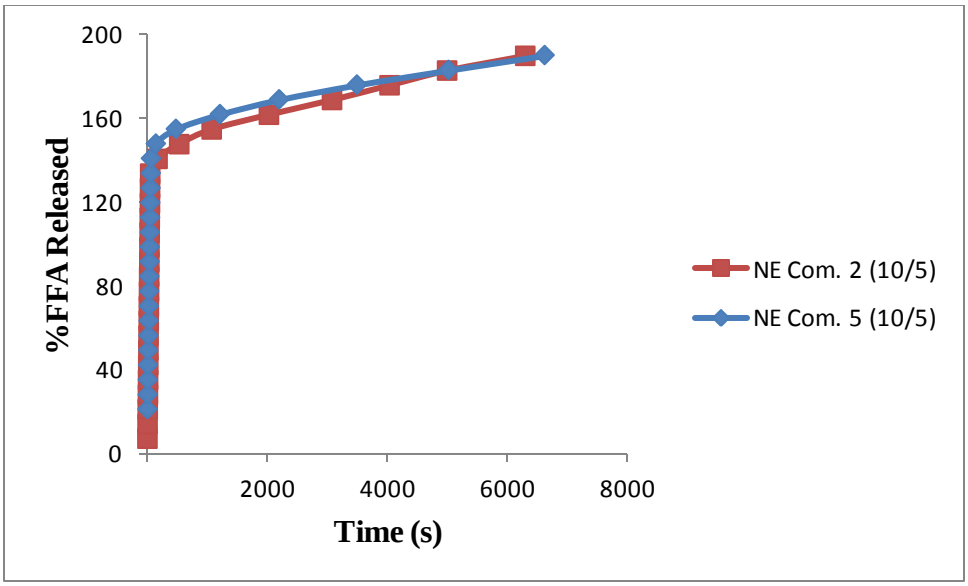
$$Molecular\ Weight_{Triglyceride} = \frac{3 \times 1000 \times Molecular\ Weight_{KOH}}{Saponification\ Value} \quad \ldots(4)$$

In the above equation, Saponification Value for MCT oil of 334 was used.

The titration profiles were generated for all the systems tested by plotting the %FFA released as a function of time.



(A)



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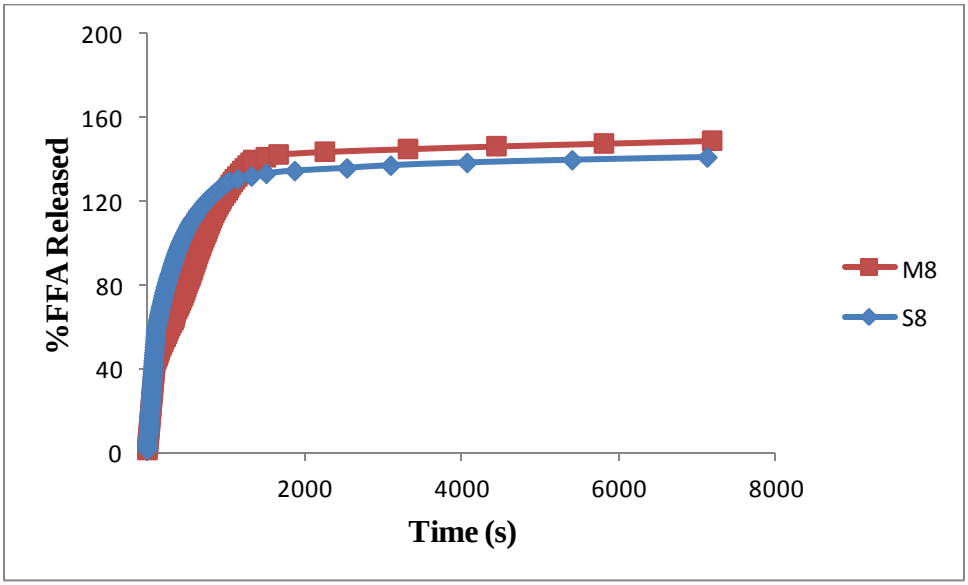


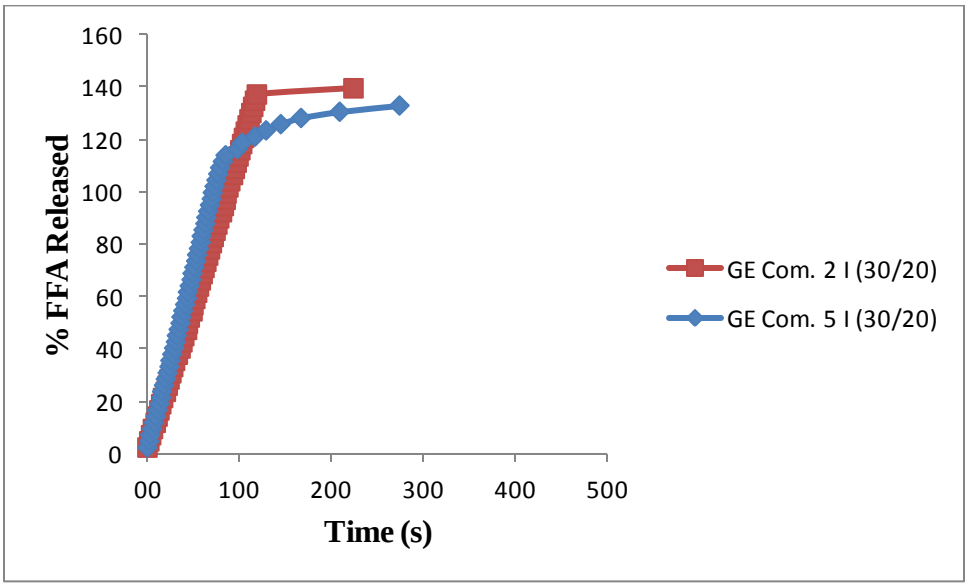
(C)

Fig.18. Titration profiles of lipid based delivery systems. (A) Titration profiles of gel like emulsions (30/20: lipid phase/emulsifier) (B) Titration profiles of nanoemulsions (10/5: lipid phase /emulsifier) (C) Titration profiles of organogels.

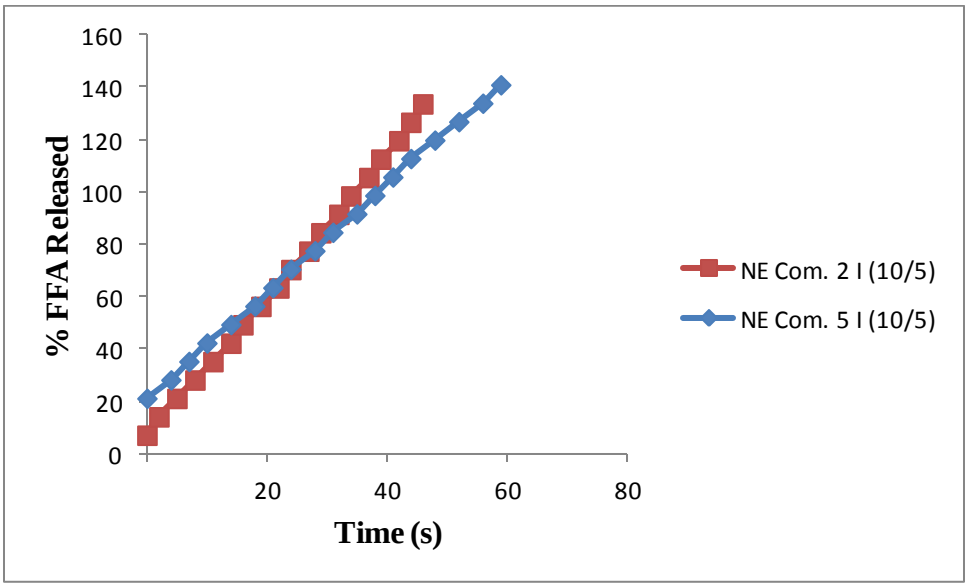
It can be observed from Figure 18, that the release of FFAs is very fast in the initially and then it declines rather abruptly. Rapid release profile of FFAs has been observed when MCT oil is digested *in vitro* in other studies as well (Sek, Porter et al. 2001; Sek, Porter et al. 2002; Ahmed, Li et al. 2012). Also, the extent of digestion for all systems is greater than a 100%, however this will be discussed later. When triglycerides are hydrolyzed, the released FFAs tend to accumulate at the oil water interface and inhibit lipase activity if they are not immediately solubilized to form micelles and mixed micelles or precipitated in presence of calcium ions to form calcium soaps. The migration of released FFAs away from the oil water interface and into the aqueous phase depends to an extent on the dispersibility of the FFAs in aqueous phase. The medium chain FFAs released from digestion of MCT oil are more dispersible in aqueous phase when compared to the long chain FFAs released from LCT oils. They easily migrate away from the oil water interface, and hence allow lipase to access the remaining oil for further hydrolysis and subsequent rapid digestion of MCT oils (Sek, Porter et al. 2001; Sek, Porter et al. 2002). The oil used for all the systems in the current study is MCT oil, and rapid digestion was expected.

As seen in Figure. 19, a majority of the FFAs are released within first 500 seconds for gel like emulsions, first 80 seconds for nanoemulsions and it takes up to as long as 2000 seconds for organogels. The digestion of nanoemulsions is the fastest, followed by gel like emulsions and then organogels. When lipids enter the lumen, they are first emulsified with the help of phospholipids present in the gastric juice, and then the lipase acts upon the emulsified oil droplets and hydrolyses the triglycerides. Lipids in nanoemulsions are already emulsified; also the droplet size is small which offers a greater surface area for

the lipase to act upon. Hence, the digestion is rapid. Gel like emulsions also contain already emulsified lipids, and hence they are rapidly digested as well. Organogels on the other hand contain lipids entrapped in a gelator network.



(A)



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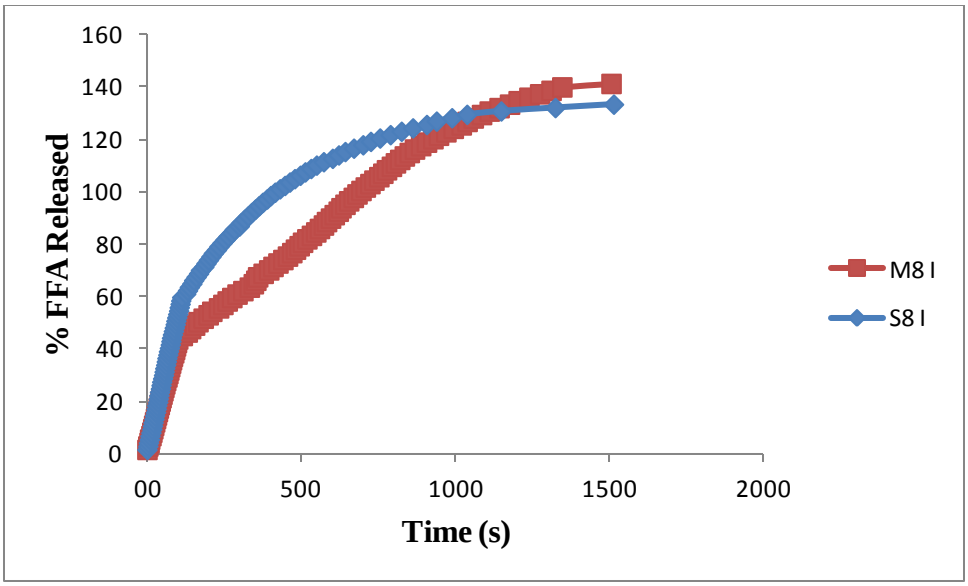

(C)

Fig.19. Titration profiles of lipid based delivery systems for initial period of rapid digestion. (A) Titration profiles of gel like emulsions (30/20: lipid phase/emulsifier) for initial 500 seconds (B) Titration profiles of nanoemulsions (10/5: lipid phase /emulsifier) for initial 80 seconds (C) Titration profiles of organogels for initial 2000 seconds.

Hence, the lipid first needs to be emulsified, so that enough lipid surfaces are available for the lipase to act upon resulting in longer time taken for digestion.

Also, it should be kept in mind that the lipid content of all the systems is different, which also affects the titration profile (Ahmed, Li et al. 2012).

3.3.2 EFFECT OF EMULSIFIERS AND GELATORS ON EXTENT OF LIPOLYSIS

It can be observed from Table 5 that the extent of lipolysis for all the formulations is more than 100%. Ideally, during lipolysis, lipase hydrolyses the triglyceride and releases a diglyceride and an FFA. Then the diglyceride is hydrolyzed into a 2-monoglyceride and an FFA. Thus, when one mole of triglyceride is digested, two moles of FFAs and one mole of 2-monoglycerides are released as shown in Figure 20.

Com. 2 Gel-like emulsion	**%Extent of Lipolysis**
(30/20) (lipid phase/ emulsifier)	171.74 ± 1.01
Com. 5 Gel-like emulsion	**%Extent of Lipolysis**
(30/20) (lipid phase/ emulsifier)	157.37± 1.80

(A)

Com. 2 Nanoemulsion	**%Extent of Lipolysis**
(10/5) (lipid phase/ emulsifier)	196.90±2.35
Com. 2 Nanoemulsion	**%Extent of Lipolysis**
(10/5) (lipid phase/ emulsifier)	190.24±1.40

(B)

Organogels	**%Extent of Lipolysis**
M8	152.19 ± 0.50
S8	143.68 ± 1.20

(C)

Table 5. %Extent of lipolysis of the lipid based delivery systems.

The 2-monoglyceride may isomerize to 1-monoglyceride which may further hydrolyze into third FFA and glycerol, but this process is limited *in vivo* (Mattson and Volpenhein 1964). When the triglycerides are completely digested, 100% extent of lipolysis is achieved.

MCT oil is almost completely hydrolyzed within a period of almost 30 minutes (Sek, Porter et al. 2002). In the systems studied here, greater than 100% of extent of lipolysis could be due to several reasons. One of the reasons could be that the lipase is hydrolyzing the emulsifiers used for the lipid based delivery systems, explained later. The other reason could be the production of assembled digestion products that are a result of breakdown of phospholipid and bile salt components. This could lead to a drop in pH that is independent of the drop in pH due to FFAs released from lipid digestion only (Ahmed, Li et al. 2012).

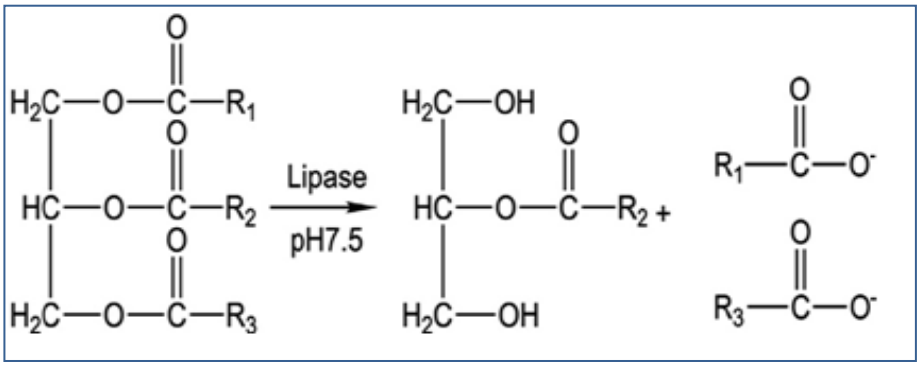

Fig.20. Hydrolysis of triglyceride by lipase enzyme (Yu and Huang 2012)

Lipase has an affinity for ester bonds (Beck 1955). All the emulsifiers and organogelators used in this study are fatty acid esters of sugar or sugar alcohols. These

gelators and emulsifiers have multiple ester bonds, which the lipase can attack and hydrolyze releasing additional FFAs other than the ones released by lipolysis of the oil.

Emulsifier	Molecular Weight (g/moles)	No. of FFAs released (assumed)	Theoretical Volume of NaOH (µl)	Experimental Volume of NaOH (µl)
S270	608.76	1	656.8	700
S570	608.76	1	656.8	700
P90	508.72	1	786.2	800
M8	434.56	2	1840.88	1750
S8	434.56	2	1840.88	1800

Table 6. Comparison of theoretical volume of NaOH consumed on 100% lipolysis of emulsifier and experimental volume of NaOH consumed on lipolysis of 100mg sample weight of emulsifier.

A simple experiment was conducted to verify whether the emulsifiers were being hydrolyzed. 100mg of all emulsifier and organogelators were lipolysed using same fed state buffer and pancreatin lipase. The results showed the release of FFAs as the pH decreased progressively after addition of lipase enzyme. The FFAs were neutralized using NaOH. Thus, this experiment proved that some of the excessive extent of lipolysis of the systems is due to the presence of the emulsifiers and gelators used in the study and that they undergo hydrolysis and release FFAs.

Emulsifier/ MCT oil	Molecular Weight (g/moles)	No. of FFAs released (assumed)	Theoretical Volume of NaOH (µl)	Experimental Volume of NaOH (µl)
MCT Oil	503.083	2	2104.4	-
S 570	608.76	1	305.38	-
P90	508.72	1	425.56	-
Total for Sample	-	-	2835.34	3300

Table 7. Breakdown of theoretical and experimental volume of NaOH consumed upon 100% digestion of emulsifier/MCT oil for gel like emulsions Com.5 (30/20: lipid phase/emulsifier) for a total sample weight of 505mg.

Table 7 shows the comparison of the theoretical volume of NaOH consumed on 100% lipolysis of the emulsifiers and MCT oil used for gel-like emulsion (30/20: lipid phase/emulsifier) Com. 5. The weight of individual components was calculated from the proportions used in the formulations for a total sample weight of 505mg of gel like emulsions used for lipolysis in one of the experiments. The theoretical volume of NaOH consumed was calculated by using the Equations 3 by assuming 100% of lipolysis. It was assumed that lipolysis of one mole of MCT oil released 2 mole of FFAs, while that of the emulsifiers released 2 FFAs.

Lipid Based Delivery System	Sample Weight (mg)	Theoretical Volume of NaOH on 100% lipolysis (MCT oil + Emulsifiers) (µl)	Experimental Volume of NaOH on 100% lipolysis (MCT oil + Emulsifiers) (µl)
Gel like emulsions Com. 2 (30/20)	505	2858.02	3550
Gel like emulsions Com. 5 (30/20)	503	2835.34	3300
Nanoemulsions Com. 2 (10/5)	511	899.51	1350
Nanoemulsions Com. 5 (10/5)	510	894.88	1350
M8	302	4345.32	5650
S8	308	4432.77	5450

Table 8. Comparison of theoretical volume of NaOH consumed on 100% lipolysis of (MCT oil + Emulsifier) in the sample to the experimental volume of NaOH consumed on lipolysis.

Also, it was assumed that S270 and S57 are pure sucrose stearate monoester emulsifiers and P90 is pure sucrose palmitate emulsifier with no impurities and their hydrolysis releases one mole of FFA for one mole of emulsifier digested. The experimental volume of NaOH consumed during the lipolysis of the emulsifiers as shown in Table 6. shows that the theoretical value is very close to the experimental value, and hence the assumptions are reasonable.

Table 7 shows that an excess of 464.6 µl of NaOH has been consumed in the actual experiment, after the lipolysis of 100% of oil and emulsifiers present in the sample. Similar results can be seen for other lipid based delivery systems tested as shown in Table 8. Thus, it can be said that assembled digestion products that are a result of breakdown of phospholipid and bile salt components present in the fed state buffer along with the FFAs released on lipolysis of emulsifiers used in the system are responsible for the total extent of lipolysis of lipid based delivery systems being greater than 100% .

3.3.3 POST DIGESTION CHARACTERISTICS

The aqueous phase of the post digestion lipolysis products were further subjected to particle size analysis. The aqueous phase of the post digestion lipolysis products contains the systems like mixed micelles, micelles and vesicles which are responsible for the solubilization of the bioactive.

Table 9 shows that the particle size are very large than the typical micelle size, suggesting either aggregation of the micelles and mixed micelles or formation of other structures such as vesicles and liposomes.

Com. 2 Gel-like emulsions	**Z – Average (nm)**	**PDI**
(30/20)	187.4±4.0	0.164± 0.08
Com. 5 Gel-like emulsions	**Z – Average (nm)**	**PDI**
(30/20)	173.0±1	0.386±0.01

(A)

Com. 2 Nanoemulsion	**Z – Average (nm)**	**PDI**
(10/5)	102.3±12	0.244±0.01
Com. 2 Nanoemulsions	**Z – Average (nm)**	**PDI**
(10/5)	157.0±4.0	0.278±0.04

(B)

Organogels	**Z – Average (nm)**	**PDI**
M8	124.1±0.6	0.318±0.06
S8	144.5±17	0.317±0.19

(C)

Table 9. Particle size distribution in aqueous phase of post digestion lipolysis products.

3.4 CONCLUSION

The lipid based delivery systems were subjected to *in vitro* lipolysis using the fed state buffer to mimic the fed state in the lumen. The extent of lipolysis for all systems

exceeded 100%. Further investigation revealed that there is additional decrease in pH due to the release of FFAs from other sources besides the triglycerides. The presence of excess of FFAs is partly due to the hydrolysis of sugar and sugar alcohol esters that have been used as emulsifiers and gelators. In addition, there are other products in the systems that undergo hydrolysis, possibly the phospholipids and proteins present in the buffer, resulting in decrease in pH.

CHAPTER 4

LIPID BASED DELIVERY SYSTEMS: ASSESSMENT OF BIOACCESSIBILITY AFTER *IN VITRO* DIGESTION

4.1 BIOACCESSIBILITY

Bioaccessibility is described as the fraction of ingested bioactive that is released from delivery matrix and is solubilized in the digestive juices of GI tract and becomes available for absorption. Hence, bioaccessibility is the first step to bioavailability. It is comparatively convenient and cheaper to assess the bioaccessibility of bioactives than bioavailability, and it can be used as a screening tool before further assessment of delivery systems for bioavailability of the bioactive they carry.

In this chapter, the bioaccessibility has been assessed for the three lipid based delivery systems for curcumin incorporation that were developed in the span of this study.

4.2 MATERIALS AND METHODS

4.2.1 MATERIALS

HPLC grade water was obtained from Alfa Aesar (Lawrence, KS). Glacial acetic acid was obtained from J.T. Baker (Phillipsburg, NJ). HPLC grade acetonitrile was purchased from Pharmaco – AAPER (Brookfield, CT).

4.2.2 HPLC ANALYSIS

The same method used previously to determine the curcumin dissolved in lipid phase was used for the analysis of post digestion aqueous phase. However, the sample preparation

was slightly different. Here, the filtrate obtained post digestion was acidified using HCl. It was then mixed with two volumes acetonitrile and filtered into HPLC analysis vials with screw caps.

Again, a 25D UV-VIS absorption detector (Dionex) equipped UltiMate 3000 HPLC system was used for the analysis. A C18 3.9_150mm Nova-Pak column was used. Methodology earlier developed in our lab (Yu and Huang 2012) was employed, which is as follows –

The mobile phase solvents were: (A) Water with 2% acetic acid, and (B) Acetonitrile; elution conditions were: 0-2min, 65% A and 35% B, 2-17 min of linear gradient from 35% B to 55% B, 17-22min held at 55% B, 22-23 min B linearly went back to 35% . Flow rate of 1ml/min was maintained, the detection wavelength was set to 420nm and 50µl of sample was injected. Duplicates were analyzed.

4.3 RESULTS AND DISCUSSION

After the digestion of the lipid based delivery systems, the aqueous phase was separated using ultra-centrifugation. The bottom pellet was discarded, since it is only the aqueous phase that contains the systems like mixed micelles and micelles that are responsible for the transport of the bioactive across the epithelium. The unsolubilized curcumin settles at the bottom after ultra-centrifugation, as it is not soluble in water. This results in only the solubilized curcumin to be successfully quantified for the bioaccessibility assessment.

Fig.21. Post Digestion Solution after Ultra-Centrifugation.

The aqueous phase was analyzed using HPLC assay method previously developed in our lab (Yu and Huang 2012).

The bioaccessibility of the systems was determined using the following equation:

$$\%Bioaccessibility = \frac{Mass\ of\ solubilized\ curcumin}{Mass\ of\ curcumin\ in\ lipid\ phase} \times 100 \quad \ldots(5)$$

Here, the mass of solubilized curcumin was the product of the concentration of solubilized curcumin in the aqueous phase of the post digestion products of lipolysis and volume of the aqueous phase. The mass of curcumin was further calculated using the mass of the curcumin dissolved in oil, mass of oil in the system which was obtained from the volume of oil used and the density of oil.

The mass of the solubilized curcumin is again calculated using the AUC and the equation obtained from the standard curve of curcumin (Appendix III).

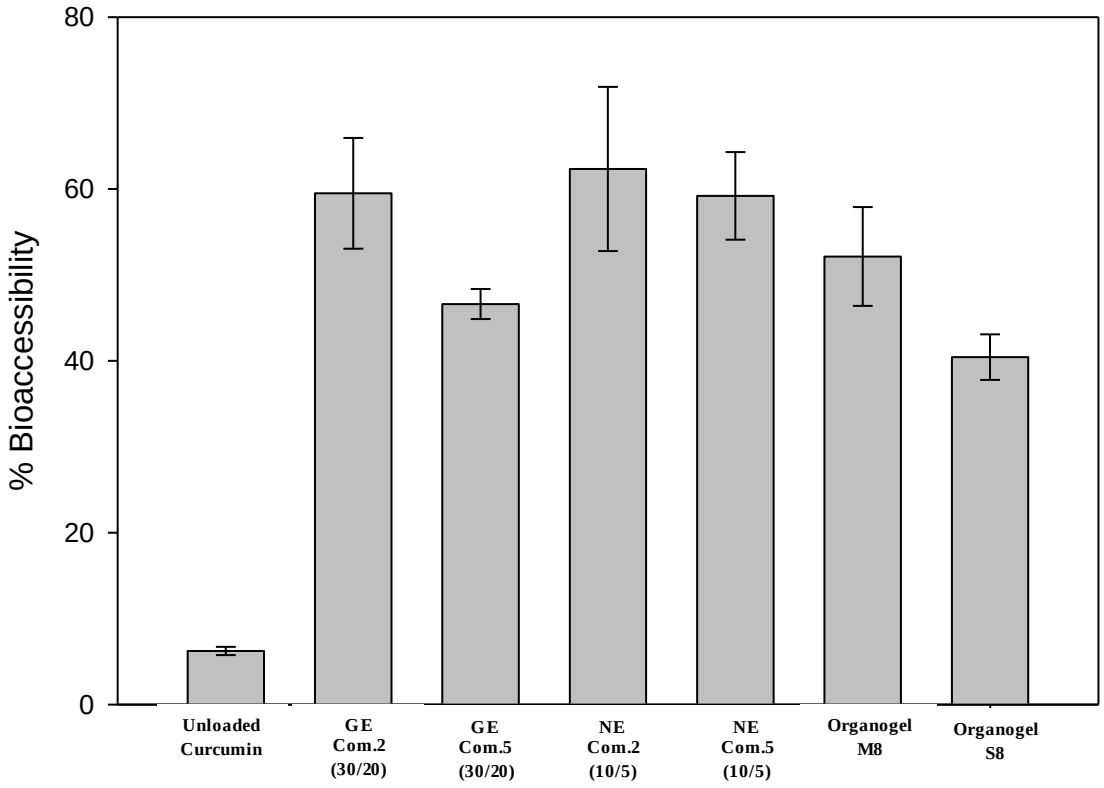

Fig.22. % Bioaccessibility of different lipid based delivery systems (n=2). [GE – Gel-like emulsions and NE – Nanoemulsions].

From Figure 22, it can be seen that the bioaccessibility of all the lipid based delivery systems is significantly higher than that of the dispersion of curcumin in water. It also suggests that the bioaccessibility of systems containing emulsifier combination Com. 2 is higher than that of the ones containing Com. 5 for gel emulsion. The higher content of hydrophilic emulsifier in the emulsifier combination Com.2 could be responsible for higher bioaccessibility by enhancing solubilization of curcumin into mixed micelles.

This is an interesting observation, however further study is required to understand the underlying phenomenon responsible for this discrepancy. At the same time, there is no significant difference in the bioaccessibility of the two nanoemulsions tested. The oil droplets in nanoemulsions are more easily accessible to lipase because nanoemulsions are fluid and not viscous like gel-like emulsions. A large surface of oil droplets is readily available for lipase action in nanoemulsions. This could be the reason why there is a difference in the bioaccessibility of nanoemulsions and gel-like emulsions made using different emulsifier combination.

	%Bioaccessibility	**%Extent of Lipolysis**
Curcumin Dispersion in Water	6.22±0.48	---

(A)

Com. 2 Gel-like emulsions	**%Bioaccessibility**	**%Extent of Lipolysis**
(30/20) (lipid phase/ emulsifier)	59.48±6.45	171.74 ± 1.01
Com. 5 Gel-like emulsions	**%Bioaccessibility**	**%Extent of Lipolysis**
(30/20) (lipid phase/ emulsifier)	46.60±1.75	157.37± 1.80

(B)

(Continued)

Com. 2 Nanoemulsion	%Bioaccessibility	%Extent of Lipolysis
(10/5) (lipid phase/ emulsifier)	62.32±9.56	196.90±2.35
Com. 5 Nanoemulsions	**%Bioaccessibility**	**%Extent of Lipolysis**
(10/5) (lipid phase/ emulsifier)	59.19±5.105	190.24±1.40

(C)

Organogels	%Bioaccessibility	%Extent of Lipolysis
M8	52.13±5.75	152.19 ± 0.50
S8	40.43±2.65	143.68 ± 1.20

(D)

Table 10. %Bioaccessibility of lipid based delivery systems.

There is a significant difference between the bioaccessibility of M8 organogel and S8 organogel. S8 organogel is weaker and readily disintegrates in the reaction vessel under continuous stirring. This makes the oil readily susceptible emulsification followed by lipase attack for digestion. At the same time, rapid disintegration could also lead to rapid release of curcumin from oil resulting in lower bioaccessibility.

There is also a positive correlation between the % extent of lipolysis of the systems and the % bioaccessibility of the systems. For all the lipid based delivery systems tested, the % bioaccessibility was noticed to increase with an increase in % extent of lipolysis as can be seen in Table 10. As the extent of lipolysis increases, the amount of micelles, mixed

micelles and vesicles formed in the aqueous phase increases, which in turn solubilize more curcumin resulting in an increase in the bioaccessibility of curcumin.

Overall, there is almost at least a 6.4 fold increase in the bioaccessibility of curcumin upon incorporation into a lipid based delivery system. Due to higher stability as compared to nanoemulsions or organogels, gel-like emulsions are better candidates for curcumin incorporation and delivery via oral route.

4.4 CONCLUSION

The bioaccessibility of the different lipid based delivery systems was assessed. It was observed that the bioaccessibility of the bioactive compound curcumin increases significantly when incorporated into lipid based delivery systems such as nanoemulsions, gel-like emulsions and organogels in the fed state. The bioaccessibility of curcumin increases with increase in the extent of lipolysis. Also, the type of emulsifiers used also result in difference in the bioaccessibility of the bioactive. But this needs to be validated by further studies.

The bioaccessibility of this study was limited to the fed state, the assessment of the bioaccessibility of these systems is required using the fasted state condition *in vitro*.

CHAPTER 5

OVERALL CONCLUSIONS

The bioavailability and bioaccessibility of many lipophilic bioactive compounds is limited by poor solubility, poor absorption, rapid metabolism, excretion, etc. This limits the use of such bioactives, despite excellent pharmacological efficacy. And curcumin is one such compound. A multi-target molecule, curcumin is effective anti-cancer, angiogenesis modulating, anti-fungal, anti-mutagenic, anti-virus agent.

An attempt was made in this study to overcome one of the shortfalls of curcumin molecule i.e. poor solubilization and subsequent absorption. Three types of lipid based delivery systems were evaluated for increasing bioaccessibility of curcumin. All three types of lipid based delivery systems tested, namely nanoemulsions, gel-like emulsions and organogels proved effective in increasing the bioaccessibility of curcumin when compared to the dispersion of the compound in water.

However, there are other areas to be explored and questions to be answered. Some of these issues are related to the morphology of the gel-like systems, the mechanism and kinetics of curcumin precipitation in the systems.

In conclusion, the knowledge gained in the process of this thesis can be used in manufacture of effective delivery systems for lipophilic bioactives such as curcumin. It is also a stepping stone towards a better understanding of the delivery systems and their effect on bioaccessibility of bioactives.

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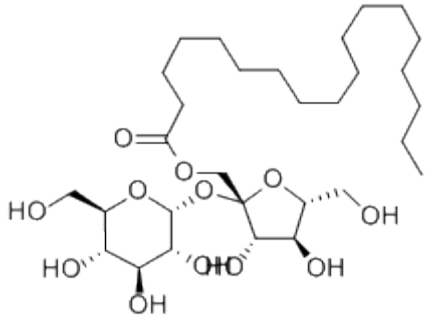
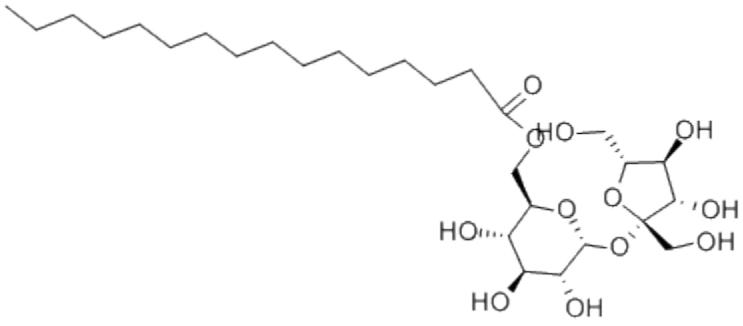
APPENDIX

Appendix I Chemical structures of sucrose stearate and sucrose monoplamitate.

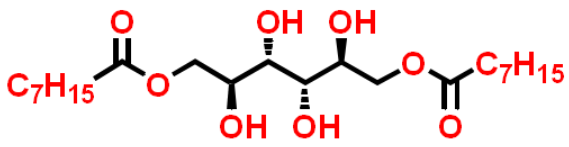


Chemical Structure of Sucrose stearate

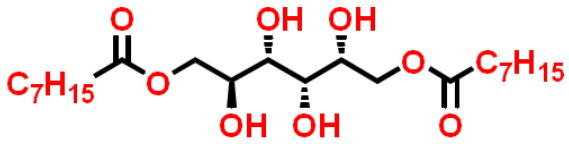


Chemical Structure of Sucrose palmitate

Appendix II Chemical Structure of Mannitol dioctanoate and Sorbitol dioctanoate



Chemical Structure of Mannitol dioctanoate (M8)



Chemical Structure of Sorbitol dioctanoate (S8)

Appendix III Standard curve of total curcumin, dissolved in DMSO, diluted using 1:1 acetonitrile: water to correlate UV-VIS absorption to concentration of curcumin.