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MECHANISTIC STUDIES TO ELUCIDATE THE ROLE OF LIPID VEHICLES
ON SOLUBILITY, FORMULATION AND BIOAVAILABILITY OF POORLY
SOLUBLE COMPOUNDS

BY

INAYET DUMANLI

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

IN

APPLIED PHARMACEUTICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2002

DOCTOR OF PHILOSOPHY DISSERTATION OF
INAYET DUMANLI

APPROVED:

Dissertation Committee:

Major Professor

The block contains three handwritten signatures, each written over a horizontal line. The top signature is the most prominent and appears to be 'William J. ...'. The middle signature is 'John H. ...'. The bottom signature is 'Harold W. ...'.

DEAN OF GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

2002

ABSTRACT

Lipids have been utilized to increase the bioavailability of poorly soluble drugs which resulted in with advent of High Throughput Screening (HTS). Although lipids have been used to improve bioavailability of a few drugs for almost twenty years, they are still not well characterized. There are limited publications about the effects of physicochemical properties of the lipid vehicle, such as class of lipid (i.e. glyceride, propylene glycol ester), fatty acid chain length, MW and polarity on lipid solubility, formulation and bioavailability of poorly soluble drugs. Knowledge of such relationship can improve quick screening of these vehicles. The goals of this study are to identify physicochemical properties of the lipids, to investigate the relationship of these properties with solubility of the model drugs, namely nifedipine and griseofulvin, to design lipid-drug formulations with Cremophor EL, to evaluate *in vitro* dissolution of selected formulations and to compare the bioavailability of nifedipine from the formulations tested. It has been shown that the lipids used improved the solubility of nifedipine and griseofulvin compared to the solubility of drugs in water. Calculated solubility parameter was not sufficient to predict the solubility of the drugs in the lipids. Calculated and measured properties of lipids analyzed with stepwise regression analyses showed that MW, dielectric constant, surface tension and fatty acid chain length are the common factors that govern the lipid solubility. However, the estimates of each factor were different for each drug indicating that the nature of the drug played an important role in lipid solubility. Incorporation of Cremophor EL, a nonionic surfactant into lipid-based nifedipine formulation enhanced the solubility and dissolution of nifedipine. The solubility of

nifedipine showed a linear correlation with increasing surfactant concentration. While solution rate was dependent on the type of lipid used, the nature of the lipid had no affect on the dissolution of nifedipine in presence of the surfactant. Dissolution of nifedipine from the lipids showed that as the fatty acid chain length increases, the dissolution rate increases. This is due to lower solubility of nifedipine in the lipids that have longer chain length. The effect of lipids on dissolution rate and extent of nifedipine showed that even though physicochemical properties of lipids (HLB, interfacial tension, viscosity, density) and solubility of nifedipine in lipids play a role, only partitioning of the drug from lipid to the dissolution medium and the particle size of the formulation in dissolution medium provided a good correlation with dissolution extent of nifedipine. The bioavailability of nifedipine obtained with different lipid formulations in beagle dogs showed that the type lipid and surfactant used in the formulation play important roles. Dissolution is a good predictor for *in vivo* performance of nifedipine lipid formulation when it was formulated with non-digestible lipids (mostly mono-glycerides). Although, the solubility of nifedipine in lipids, the particle size of the formulation in dissolution medium and partitioning of the drug from the formulation to dissolution medium seem to affect the *in vivo* performance of the drug, dissolution performance of the formulation and digestibility of the lipid used in the formulation are the major factors in bioavailability of nifedipine from the lipid-based formulations.

ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. M. Serpil Kislalioglu for acting as my major professor. I found in her a very generous person willing to share her knowledge with deep dedication. She not only guided me for my studies but also listened to me as a friend and supported me in my career.

This dissertation research was carried out in Hoffmann-La Roche Laboratories, Nutley, NJ 07110. I would like to extend my appreciation to Dr. A. Waseem Malick (Vice President, Pharmaceutical and Analytical R & D) and Dr. Navnit Shah (Distinguished Research Leader) of Hoffmann-La Roche, Nutley, NJ 07110, for providing me a graduate fellowship from January 1999 to May 2002. Their dedication and commitment to pharmaceutical research via joint university-industry program is deeply acknowledged. Dr. Shah shared his expertise in drug development by co-advising me in my research. His novel ideas and dedication to science were a constant source of inspiration to me.

My gratitude is also extended to Dr. Wantanee Phuapradit (co-advisor on my research), Dr. Martin Infeld, Dr. Hashim Ahmed, Dr. Gordon Beck, Dr. Harpreet Sandhu, Dr. Raman Iver, Mrs. Maria Bachynsky, Dr. Hitesh Chokshi, Dr. Tarik Roshdy, Dr. Rodolpho Pinal, Dr. Fang-Chung Chen, Mr. Ashish Chatterjee, Mr. Mohommad Rashed, Mr. Antonio Albano, Mr. Maurice Munroe, Mr. Michael Lanyi, Mrs. Oksana Bohoslawec, Dr. Navita Mallaieu, Dr. Aruna Railkar, Mr. Zahid Qureshi,

Mr. Giuseppe Ciccone, Mr. Nick Lalli, Ms. Vicky Pacholec, Ms. Kathy Lang, Mr. Donald Wright, Mr. Leonard Williams, Mr. Steve Lumiscio and Mr. Ken Zhu (Hoffmann-La Roche Inc.) for considering me as a part of their team with their support and willingness to help by giving extra time besides their duties.

I want to thank to Dr. Jyh-Hong Wang for his help in statistical analysis and Drs. Thomas Needham, Norman Campbell and Stanley Barnett for serving as my committee members. Also my gratitude is extended to Ms. Kathleen Hayes (Senior Administrative Assistant of Applied Pharmaceutical Sciences) who always had a smile and was willingness to help me.

Finally, my deepest feelings and regards go to my parents, my family and friends for their endless support and full trust that enabled me to go through all the challenges in an environment of love.

PREFACE

This work has been prepared in accordance with the manuscript format option for dissertation preparation, as outlined in Section 11 of the Graduate Student Manual of the University of Rhode Island. Contained within is the body of the work divided into three sections.

Section I includes one manuscript which introduces the subjects of dissertation to the reader and specific objectives of my research.

Section II includes three manuscripts, containing the findings of the research. These manuscripts are written in the format required by the journals to which they will be submitted.

Section III contains appendices and bibliography, arranged in alphabetic order by the author's last name.

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Abstract: The advent of High Throughput Screening (HTS) and Combinatorial Chemistry in drug discovery has resulted in compounds which are more lipophilic, having $\log P > 4$. As a consequence, the application of lipid delivery systems has more recently received greater attention in the pharmaceutical industry. This paper presents a review of lipid vehicles used in the oral drug delivery of poorly soluble drugs. Lipids that are available in the US market are defined and classified and different types of lipid formulations have been cited from the literature. Formulation considerations and the effect of lipids on lipolysis as well as systemic and lymphatic drug absorption are discussed. In addition, the characterization techniques of formulation, stability, *in vivo* / *in vitro* correlation and absorption mechanisms for lipid-based formulations are summarized.

Keywords: Lipid vehicles, lipid-based formulations, formulation considerations, lipolysis, lymphatic absorption, characterization techniques

Glossary:

DG: Diglyceride

DSC: Differential scanning calorimeter

GER: Gastric emptying rate

GML: Glyceryl mono-laurate

GMO: Glyceryl mono-oleate

GMS: Glyceryl mono-stearate

HLB: Hydrophile-lipophile balance

IT: Interfacial Tension

L: Liquid

LCT: Long chain triglyceride

LFDS: Low frequency dielectric spectroscopy

MBLA: N- α -methylbenzylinoamide

MCM: Medium chain monoglyceride

MCT; medium chain triglyceride

MG: Monoglyceride

MP: Melting point

N/A: Not available

PEG: Polyethylene glycol

PGG: Polyglycolized glycerides

S: Solid

SEDDS: Self-emulsifying drug delivery system

SMEDS: Self-microemulsifying drug delivery system

SEOF: Self-emulsifying oil formulation

SLN: Solid lipid nanoparticles

SS: Semisolid

TG: Triglyceride

TBUs: Tributyrin units

1. INTRODUCTION

During the development of oral pharmaceutical dosage forms of new drug candidates, the formulation scientists are faced with many challenges. One challenge which is very often encountered is poor bioavailability. With the advent of High Throughput Screening (HTS) and Combinatorial Chemistry in drug discovery, modern drug molecules are more lipophilic which often results in their being poorly bioavailable. The use of lipid vehicles has generated considerable attention in recent years for improving the bioavailability of poorly soluble drugs as one of the approaches to improve their oral bioavailability. The well-known effect of food in improving the bioavailability of many poorly soluble drugs such as griseofulvin, carbamazepine and danazol is evidence that lipids can be beneficial to drug absorption (Charman et al., 1992, 1993, 1997; Humberstone and Charman, 1997). Lipids can improve the bioavailability of drugs through several mechanisms such as by enhancing solubility/dissolution (Pozzi et al., 1991; Sheen et al., 1991; Charman et al., 1992; Shah et al., 1994; Matuszewska et al., 1996; August et al., 1997), by providing uniform gastric emptying and uniform absorption rate (Yamahira et al, 1979a, b, 1980) and by increasing lymphatic absorption (Cheema et al., 1987; Charman and Stella, 1992; August et al., 1993; Porter and Charman, 1997).

Although lipids have been used to improve bioavailability for almost twenty years, they are still not well characterized. In addition, mechanistic studies that explain the effect of physicochemical properties of lipid vehicles on formulation and drug absorption, the use of appropriate techniques to characterize lipid-based systems and

in vivo/in vitro correlations have not been adequately addressed and well understood.

In this paper, information regarding these issues is collected and discussed in the following order: (I) definition and classification of lipids, (II) type of lipid formulations, (III) formulation considerations of lipid based systems, (IV) effects of lipids during the absorption of poorly soluble drugs and (V) characterization techniques of lipid based systems.

2. DEFINITION AND CLASSIFICATION OF LIPIDS

Lundberg and Weiner define lipids as a “chemically heterogeneous group of substances, having in common the property of insolubility in water but solubility in non-polar solvents” and the science that deals with lipids is referred to as ‘Lipidology’ (Lundberg, 1984; Weiner, 1993). However, many lipids have some limited water solubility and are commonly used as vehicles in pharmaceutical formulations. Lipids can be classified based on chemical characteristics such as carbon chain length of fatty acid and ester type rather than on their solubility. Surfactants also fall into this category. Sometimes, there is no distinct difference between a lipid and a surfactant. Some lipids have surface active properties, for example medium chain monoglycerides can lower interfacial tension and easily dispersed in aqueous media.

Types of lipids include natural and synthetic fatty acids, oils, fats, waxes, phospholipids and some surfactants. Fatty acids can be defined as organic acids with straight hydrocarbon chain and can be classified based on the length of the hydrocarbon chain. Fatty acids with hydrocarbon length C_2 - C_6 are considered to be short-chain, with C_8 - C_{12} medium-chain and with C_{14} and higher long-chain fatty acids.

Oils are unsaturated fatty acid esters of glycerol whereas fats are saturated fatty acid esters of glycerol. Waxes are the esters of fatty acids with alcohols other than glycerol. Surfactants that are in the lipid category are commonly fatty acid esters of PEGs. Lipids also include some lipid fractions and synthetic accommodations of glycerides with PEG esters. The fractionated esters are seldom pure and tend to exist as mixtures of various combinations. Commonly used lipid vehicles in oral drug delivery have at least one fatty acid esterified with a polyol such as glycerol/s, propylene glycol or PEG. Tables 1 A-F present lipid vehicles commonly used in the US pharmaceutical industry in oral drug delivery products. Phospholipids are excluded from the scope of this paper since they are mainly used in parenteral formulations.

3. TYPE OF LIPID FORMULATIONS

In addition to simple solutions of drug molecules in lipids, solid dispersions, micelles, liquid crystals, emulsions including self-emulsifying systems and microemulsions and solid lipid nanoparticles (SLN) are also commonly used formulation approaches for lipid based systems.

Because of their unacceptable taste, many lipid formulations are administered in soft or hard gelatin capsules as unit dosage forms.

Lipid formulations were classified by Pouton (1999 and 2000) as Types I, II, IIIA and IIIB. This classification is based on composition such as amount (%) of the glyceride, surfactants and hydrophilic co-solvents and physical attributes including particle size

of the dispersion, impact of aqueous dilution and digestibility (Table 2). This classification can be useful in comparing different studies and can serve as a guide to estimate physicochemical properties of the lipid formulation of interest. The incorporation of a drug in the vehicle can significantly change the physicochemical properties of the system in terms of particle size and dispersibility following aqueous dilution and may result in a shift of the system from one type to another. Therefore, such classification cannot be a significant predictor of the physicochemical properties of the end product and is especially so for surface active and high dose drugs. The classification of lipid systems as solutions, suspensions, solid dispersions, micelles, emulsions including self-emulsifying systems (SEDDS) and microemulsions and solid lipid nanoparticles appears to be universally acceptable. Examples of these formulations that are cited in the literature are summarized in Tables 3- 9. As can be seen from these tables, SEDDS are the most commonly used formulations and drugs with molecular weights around 300 are the most commonly used model drugs. By presenting these tables, we wanted give a guideline for the readers. Additional information on selected examples of lipid solutions, suspensions, emulsions and solid dispersions can be found in a review by Humberstone and Charman (1997), SEDDS by Pouton (1997) and Gershanik and Benita (2000), and solid lipid nanoparticles by Muller et al. (2000).

Despite the numerous studies shown in these tables, only a relatively few commercial formulations of lipid based systems are available on the market. These are cyclosporine (Sandimmune™ and Neoral™, Novartis), saquinavir (Fortovase™,

Roche), ritonavir (Norvir™, Abbott) and fat-soluble vitamins. Possible reasons for the rarity of commercially available lipid-based products are the physical complexity of lipids, reliability of supply (quality), lack of knowledge of mechanisms of drug absorption and fate of the lipid in the gastrointestinal tract. An additional factor may be the limited solubility of pharmaceutical compounds in lipid solvents. Overall, the lack of mechanistic understanding of the role of lipids in drug absorption is a key reason for their underutilization for formulation development.

4. FORMULATION CONSIDERATIONS OF LIPID BASED SYSTEMS

4.1. Selection of lipid vehicles

For a lipid to be used in a drug delivery system it must be safe and ,therefore, the first requirement for these excipients is to be classified as GRAS (generally recognized as safe) materials. The majority of lipids used in marketed products do fulfill this requirement. In lipid formulation development, potential toxicity of any surfactants need should be considered carefully. This evaluation includes not only the type of the surfactant but also the concentration/dose (Swenson et al., 1994).

Drug solubility in the lipid is an important factor in the selection of the lipid as a vehicle. Determination of solubility of drugs in lipids is a major challenge since solubility of drugs in lipid based system is a poorly understood concept. There is no published systematic study to show a relationship between solubility in lipid and physicochemical properties of the drug such as molecular weight, logP and melting point. It has been shown that in the case of an investigational anti-HIV agent solubility

increases as ester bond concentration of lipid increases (Anderson and Marra, 1999). Determining the solubility parameter of a lipid vehicle and thereby quantifying its cohesive energy can offer a solution for screening of lipid candidates. Calculated solubility parameters can be used for this purpose. The closer the solubility parameter of a drug to that of a lipid vehicle, the more soluble would be that drug in such a vehicle (Dumanli et al., 2000).

The formulation scientist should also take into account the fact that the solubility of a drug may change due to the changes in the vehicle during the products shelf life. For example, partitioning of water from the gelatin capsule shell into the lipid can have a significant effect on the solubility of the drug. Therefore, the hygroscopicity of the lipid used in such formulation should be carefully evaluated (Fig.1). Furthermore, the dissolved drug in a solid lipid may precipitate out during processing when the formulation is cooled down to room temperature. The solubility of a drug in solid lipids should be significantly higher than final concentration in the formulation. Solubility should be determined under extreme conditions that the product would be subjected to during processing, manufacture and its shelf life. It should also be kept in mind that the release of a drug from the vehicle is the inverse function of its solubility in the lipid solvent. Therefore, chemical potential (concentration/solubility) and partitioning of the drug from lipid to immediate aqueous environment should be considered in addition to drug solubility and concentration (Armstrong and James, 1980).

To optimize both the lipid solubility and release of the drug, co-solvent and surfactant/s may be added to the formulation. Cremophor EL a commonly used surfactant has increased the solubility of lipophilic drugs, griseofulvin and nifedipine, Table 10. The ratio of co-solvents should be optimized to avoid potential precipitation of the drug following aqueous dilution. Lipid vehicle, surfactant, co-solvent and drug combinations can be evaluated upon aqueous dilution using phase diagrams (Shah et al., 1994; Constantinides, 1995 and Kim et al. 2000).

4.2. Effect of HLB

As mentioned above, there is no distinct difference between a surfactant and a lipid that is surface active. In order to achieve good dispersibility and absorption, it may be necessary to use both surfactant/s and lipid vehicle/s in formulations such as SEDDS, emulsions and microemulsions. HLB plays an important role in the selection of lipid vehicles in these types of lipid formulations. Even though most of the lipids have no surface active properties, some of them like medium chain mono-glycerides and PEG esters exhibit surfactant properties. These can have HLB values up to 14, (Table 1F). HLB of a lipid vehicle/surfactant can affect the solubility of a drug. For example, as the HLB increases, the solubility of griseofulvin increases (Fig. 2) (Dumanli et al., 2000). The effect of HLB on drug release rate from a lipid based formulation is shown in Fig.3. HLB value of a lipid vehicle also significantly influences the self-emulsification and dissolution properties of a SEDDS formulation (Shah et al., 1994, Kim et al., 2000 and Nazzal et al., 2002).

4.3. Drug Loading

The factors determining the loading capacity of a drug in solid lipid nanoparticles include as (I) drug solubility in molten lipid, (II) miscibility of molten drug in a lipid melt, (III) chemical and physical structure of solid lipid matrix and (IV) polymorphic state of lipid material (Muller et al. 2000).

It is not advisable to use a saturated solution in solid lipids because some of the drug may precipitate out at ambient temperature causing structural variations within the system. Resulting crystallization may lower the bioavailability.

In lipid formulations with high drug concentration, determination of the saturation point of the drug becomes a critical factor in the formulation design. If the drug precipitates out in gastrointestinal tract, its bioavailability may be reduced. However, the use of appropriate lipid vehicle at the optimum level can overcome this problem as it has been shown in the case of lipid formulation of DMP 323, a poorly soluble HIV protease inhibitor. A formulation containing Gelucire 44/14 (a mixture of glycerides and PEG esters) increased DMP 323 aqueous solubility and dissolution rate, and enhanced the bioavailability at high doses (August et al., 1997).

Drug loading can also affect physiochemical properties of the formulation. For example, increasing concentration of L-365,260, a poorly soluble benzodiazepine derivative, caused changes in droplet size of emulsions prepared with Labrafil M 2125CS (Craig et al., 1993).

A poorly soluble drug substance dissolved in MCM can significantly affect rheological properties and particle size of the formulation as a function of its concentration (Fig. 4). Change in slope of zero shear viscosity vs. concentration shows that the lipid solvent cannot accommodate too many solute molecules so that the drug reaches the saturation point where further addition of the drug may cause entanglement of the molecule (Dumanli and Kislalioglu, 1999).

Concentration and surface active properties of a drug in a lipid vehicle can modify the phase transition of a lipid. However, such an effect is dependent on the properties of the drug. For example, griseofulvin facilitates liquid crystal formation in glyceryl mono-oleate (GMO) at saturated concentration whereas nifedipine does not have the same influence. At the physiological temperature, GMO forms a cubic phase via reverse micellar transformation which is concentration specific. Above a certain concentration, lipophilic drugs such as ibuprofen and propranolol transformed the cubic phase into an inverted hexagonal phase where the system becomes less viscous resulting in modification of the release properties (Chang and Bodmeier, 1997). As the water content of cubic phase is increased, the system may become more viscous (Engstrom, 1990) and may sustain the release of the drug. On the other hand this phenomenon may enhance the stability. Enhanced stability of cefazolin and cefuroxime in glyceryl mono-oleate cubic phase gels showed its potential as a chemical stability enhancer, protecting the antibiotics from β -hydrolysis and oxidation as a result of reduced motility of water, in the gel (Sadhale and Shah, 1998).

4.4. Manufacturing and Stability Considerations

Most of the lipid-based formulations especially the ones that contain semi-solid/solid lipids involve the application of heat during their preparation. Duration of heat application, temperature and cooling rate can have a significant effect on the quality and stability of the final product. Scale-up processes of these formulations are also challenging because duration of heat application and cooling rate can change with increased scale. Therefore, optimal process should be always considered during development to assure scale-up certainty and robustness. DSC and viscosity measurements can be useful tools to monitor the effect of temperature, holding time and cooling rate. Many lipids may exhibit polymorphic transitions upon storage or during processing. It has been shown that cooling rate can affect endotherms of Gelucires upon aging (Sutananta et al., 1994).

Most of liquid and semi-solid lipids contain unsaturated bond that photo- and auto-oxidize. Peroxide formation with oxidation can damage the drug and induce toxicity. Lipid peroxides may also form due to autoxidation, which increases with unsaturation level. Hydrolysis of the lipid may be accelerated due to the pH of the solution or from processing energy such as ultrasonic radiation (Weiner, 2002). Including an anti-oxidant (i.e. α -tocopherol, propyl gallate, ascorbate or BHT) can be necessary in lipid-based formulation. Hygroscopicity of the vehicles (Fig. 1) is also important for stability of gelatin capsules. Water migration into capsule fill can cause cracking of the capsule shell.

5. EFFECTS OF LIPIDS DURING ABSORPTION OF POORLY SOLUBLE DRUGS

5.1 Lipolysis and systemic absorption

Understanding of lipid digestion process is important in design of lipid-based formulations. Digestion process involves 3 basic steps (Fig. 5): (I) dispersion of lipid into gastric media and emulsification by gastric motility, (II) enzymatic hydrolysis of tri-glycerides into mono-glyceride and fatty acids, (III) the formation of mixed micelles with hydrolysis products and bile salts and ultimately absorption through the intestinal wall (Carey et al., 1983). The lipid digestion process has been exclusively described for tri-glycerides. However, there is little information on the fate in Gastro-intestinal (GI) lumen of lipids such as propylene glycol esters of fatty acids, polyglycerol and polyethylene glycol esters.

Micelle formation of lipolytic products with bile salts is the crucial step that enhances drug solubilization and absorption. The total solubility of certain drugs is proportional to taurocholate concentration, a major bile salt in the small intestine. A linear relationship was shown between solubilization and logP of steroids, cyclosporine A, griseofulvin (Mithani et al., 1996). A detailed discussion of the lipid digestion process and its impact on drug absorption from the gastro-intestinal tract can be found in other reviews (Eldem and Speiser, 1989; Embleton and Pouton, 1997; Humberstone and Charman, 1997; MacGregor et al., 1997).

The nature of lipids used in the formulation can affect the lipolysis process. The *in vitro* lipolysis process can be used to elucidate the effect of lipids on lipolysis. The details of the process are described in section 6.2.2.

The nature of oil as an influencing factor on absorption of SEDDS has been discussed (Craig et al. 2000; Gershanik and Benita 2000). The fatty acid chain length directly influences lipolysis. The long-chain fatty acids are more inhibitory than the medium-chain ones (Bernback et al., 1987; Hutchison, 1994; Zangenberg et al., 1998; Olbrich and Muller, 1999; Lacy et al., 2000). The results in Fig.6 show that the shorter chain length allows more rapid lipolysis among tributyrine, MCT (Miglyol 812) and LCT (soybean oil) (Hutchison, 1994). Lipase enzyme is active only at interface. The greater the interface the higher the lipase activity is. Therefore, the extent of lipolysis is very low for triacetin, this is because it is miscible with aqueous medium so that no oil/water interface can be formed. The comparison study of *in vitro* lipolysis between medium chain and long chain triglycerides quantified by titrimetric, highperformance thin-layer chromatography (HPTLC) and ultracentrifugational techniques (Sek et al., 2002) showed that the rate and extent of digestion of the medium chain triglycerides was greater than long chain lipids and independent of bile salt concentration.

The surfactants have also important effect on lipolysis. Hydrophilic surfactants such as Brij 96 (polyoxyethylene 10 oleoyl ether), Tween 80 (polysorbate 80) and Cremophor RH40 (polyoxyl 40 hydrogenated castor oil) that have HLB value more than 10 inhibits lipolysis while lipophilic ones such as Span 20 (sorbitan mono-laureate) and

Crill 4 (sorbitan mono-oleate) that have HLB less than 10 do not. This inhibitory effect with hydrophilic surfactants can be minimized by adding lipophilic co-surfactants like medium chain mono-/di-glycerides to the formulation of interest. In a recent US Patent (Lacy et al., 2000), it was found that transesterification products of polyoxyethylene glycol with glycerol esters of capric and caprylic acids, do not inhibit the *in vivo* lipolysis of digestible oils. Labrasol (glyceryl caprylate/caprate and PEG caprylate/caprate) and Softigen 767 (PEG-6 caprylic/capric glyceride) with HLB values of 14 were given as examples hydrophilic surfactants that do not have inhibitory effect on lipolysis.

A rapidly digested lipid would promote maximum drug absorption (Hutchison, 1994). For example, when MCT and LCT have been compared for the absorption of vitamin E, MCT enhanced intestinal absorption of vitamin E (Gallo-Torres et al., 1978). However, this effect can not be generalized. CyclosporineA absorption, in contrast to vitamin E, was higher in LCT than in MCT (Behrens et al., 1996; Renner et al. 1988a, b). This difference was interpreted as the result of formation of mixed micelles which occurs during the digestion.

Fluidity and volume of the lipid can affect the gastric retention as it was shown in the absorption of SL-512, an anti-inflammatory agent, (Yamahira et al., 1978) and Phenytoin (Shinkuma et al., 1985). Medium chain tri-glyceride, medium chain mono-glyceride, corn oil and N-methylbenzylolinoleamide (MBLA) has been investigated as model lipid vehicles. The solubility of SL-512 was found to be 0.5, 4.2, 0.3 and 2.2%

(w/v) in each vehicle, respectively. Even though, MCM provided the highest solubility of SL-512, it has not provided the highest serum level and gastric emptying time. It was concluded that serum level and gastric emptying time of SL-512 was correlated with fluidity of the lipids.

Gastric emptying was also affected by the chain length of fatty acids (Hunt and Knox, 1968). Fatty acids with 12-18 carbon atoms slowed down gastric emptying more than those with up to 10 carbon atoms. C₁₄ (myristate) was the most effective one.

The effect of chain length of fatty acid of the lipid vehicle on formulation design and the bioavailability of Halofantrine (Hf) has been studied (Khoo et al., 1998). The solubility of Hf was enhanced by increasing the proportion of Captex (MCT) for the medium-chain glyceride formulations based on Captex/Capmul (MCM) of combinations. However, this approach led to less efficient emulsification. A 2:1 (w/w) ratio of Captex to Capmul provided a good balance between drug loading and efficient emulsification. Hf was less soluble in long-chain glycerides compared to the medium chain ones. Better emulsification was obtained with the formulations that contained medium-chain glycerides. The authors observed a trend towards higher bioavailability of the drug with the long-chain tri-glyceride formulations compared with medium chain glyceride formulations in the beagle dogs. Such anomalous findings apparently based on differences in intra-luminal processing of the medium- and long-chain glycerides where the bile salts and the formation of mixed micelles can influence absorption.

Lipid digestion products pass across an unstirred water layer (UWL) in the small intestine. Within the UWL an acidic microclimate aids micellar dissociation so that monomers pass passively across the brush-border membrane. For long-chain fatty acids passage across the unstirred water layer is rate limiting, whereas passage of short- and medium-chain fatty acids is limited by the brush-border membrane (Thomson et al., 1993).

5.2. Lymphatic absorption

Once the lipolysis products, fatty acids and mono-glycerides, are taken up by enterocytes, a re-synthesis of the fatty acids and monoglycerides takes place in the endoplasmic reticulum. Together with cholesterol, phospholipid and proteins, lipoproteins are synthesized. Lipoproteins are characterized as high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. Among all these, chylomicrons are the most abundant in the intestine cells. They contain the largest amount of tri-glycerides and they are the largest in size. Since they cannot be taken up by the blood capillaries because of their size, they enter the lymph capillaries (Tso, 1985; Nijs, 1987; Porter and Charman, 1997). Absorption of a drug through lymphatic system escapes first-pass effect. In order to incorporate a drug in the tri-glyceride core of the chylomicrons, the drug must be lipophilic. Probucol, lipophilic vitamins and halofantrine are the most commonly used model drugs to investigate lymphatic absorption. A lipid used in lymphatic delivery is important to determine the extent of lymphatic drug transport. The type of lipid, their degree of saturation, chain length and physical state play an

important role in lymphatic transport of drugs. LCT lipids are more favorable for the lymphatic absorption than MCT. Fatty acids containing 14 or more carbon atoms are considered to be taken up in the lymph, and those containing 8-12 carbon atoms enter the systemic circulation through the portal vein (Armstrong and James, 1980).

The effect of different oils on the absorption of probucol has been studied in rat (Palin et al., 1984). The plasma concentration of probucol was determined following its administration in arachis oil (LCT), Miglyol 812 (MCT) and liquid paraffin. The total absorption of the drug from lymphatic and portal system was significantly greater for arachis oil formulation than with the other two aforementioned vehicles. The literature also cites the reverse effect: The lymphatic and portal transport of retinol (vitamin A) in unanesthetized rats (Hollander, 1980) demonstrated that addition of short-chain fatty acids to the infusate did not change the appearance rate of vitamin A in the bile or lymph. Lymphatic uptake rate of vitamin A peaked following the addition of medium chain fatty acids to the infusate. Addition of long-chain unsaturated fatty acids, oleic, linoleic and arachidonic to the infusate inhibited the lymphatic appearance of retinol as the chain length and degree of unsaturation increased. The absorption rate of retinol in the bile was increased significantly when long-chain polyunsaturated fatty acids, linoleic and arachidonic acids, were added to the infusate. This indicated that polyunsaturated fatty acids also shifted the exit of vitamin A from the lymphatic to the portal circulation. The preferential transport of the polyunsaturated fatty acids into the portal circulation may also shift the transport of vitamin A into the portal circulation by a co-transport mechanism.

In another study with rats, three retinoids, namely isotretinoin, etretinate and temarotene, have been used to investigate the effects of solubility and lipophilicity on lymphatic uptake after oral administration in each of three oily vehicles, cottonseed oil, Miglyol 812 and linoleic acid (Nankervis et al., 1996). Lipid solubility increased with increasing partition coefficient of the retinoid. However, the rank order of increasing lymphatic uptake from each of three oils shows an inverse relationship with solubility of the retinoid in each oil. The reason was explained as follows; the retinoid is likely to escape from the dosing vehicle before passing across the diffusional barrier of the unstirred water layer in the gut. It is related to the solubility of the retinoid in the oil. The easier the diffusion of a drug into the intestine, the more thermodynamically favorable it will be for the retinoid to partition out of the oil.

The animal model used is important in studies related to lymphatic absorption. Rat is a commonly used animal model to investigate lymphatic absorption of drugs. The physiological state of the animal is also important. The effect of type of lipid and formulation on lymphatic transport of Halofantrine (Hf.) has been studied in the conscious and the triple-cannulated anesthetized rat model (Porter et al. 1996a,b). It was found that the lymphatic transport of Hf. in conscious rat was independent of the type of lipid (triglyceride or fatty acid) and the type of formulation (micellar or regular solution). On the other hand, the lymphatic transport of Hf in anesthetized rat was dependent on the type of formulation (micellar, emulsion and solution). It was proposed that the lipid vehicle effects in anesthetized rat model reflected the lack of

gastric processing by preduodenal lipase and the shear action of the stomach that presents in the conscious rat model.

The degree of unsaturation of lipid was also reported as a factor on the absorption of drug molecules (Craig et al. 2000). The effect of unsaturation on intestinal lymphatic drug absorption has been investigated. The lipoprotein fractions were monitored in mesenteric lymph following intraduodenal administration of arachis oil and fatty acids such as oleic, linoleic and linolenic to rats. It was shown that the greater the degree of unsaturation of the fatty acid, the more rapid the onset of chylomicron synthesis as indicated by more lymphatic absorption of the fatty acid (Renner et al., 1986; Cheema et al., 1987). However, absorption of halofantrine was largely independent on triglyceride unsaturation in a conscious rats (Holm et al., 2001).

Lymphatic uptake was also affected by isomers of lipid used. The effect of elaidic (9-cis) and oleic acids (9-trans) on lymphatic uptake studied showed that oleic acid exhibited higher lymphatic recovery rate compared to elaidic acid (Bernard et al., 1987).

6. CHARACTERIZATION OF LIPID BASED FORMULATIONS

Lipids enhance bioavailability for poorly soluble drugs. However, they are challenging to work with because of their physical complexity. It is imperative to use a technique that does not alter any physico-chemical properties of a lipid-based system. Methods used for characterization of SEDDS and SLN were summarized by Gershanik and

Benita (2000) and Muller et al. (2000), respectively. In this review, more detailed techniques are cited from the literature as well as from our experience for lipid based delivery systems.

6.1. Formulation and Stability Evaluations:

Thermal and particle size analysis are the common methods that can be used to evaluate formulation factors such as HLB, concentration of the components and processing conditions and stability.

6.1.1. Thermal Analysis:

Differential Scanning Calorimeter (DSC) is the most commonly used thermal analysis technique for characterization of lipid based systems. Some of the lipid vehicles are solid or semi-solid, therefore formulations containing such vehicles are processed at temperatures higher than room temperature. Therefore, it is important to characterize the system with thermal analysis before the formulation process.

DSC analysis will be useful to determine possible interaction between drug and lipid vehicle especially in solid dispersions. It can be helpful to construct a phase diagram of the drug of interest versus temperature. Solid dispersions of UC-781, an antiviral agent, with Gelucire 44/14 and PEG 6000 have been characterized using DSC.

Melting points peaks were used to construct the phase diagram for solid dispersions. Based on these diagrams, no eutectic mixtures were observed. Phase diagrams of solid dispersions and those of physical mixtures were similar suggesting that there was no

significant chemical change or interaction between the drug and PEG 6000 or Gelucire 44/14. It was also showed that based on DSC data, the drug can be dissolved up to a concentration of 25% (w/w) in the liquid phase in PEG 6000 and Gelucire 44/14 (Damian et al., 2000).

DSC can be a useful tool in determination of stability of lipid based systems. The thermal behavior of stored different types Gelucires has been studied using DSC to examine the relationship between preparation condition and stability (Sutananta et al., 1994). Based on DSC results, it was concluded that the thermal traces observed during storage are associated with the segregation or recombination of the Gelucire components into different microscopic regions within the sample, rather than polymorphic changes. They also conducted tensile strength measurements indicated that the strength of Gelucires changed during storage. Modulated DSC has been utilized in softgel capsules to screen the variables such as temperature and humidity influencing the hardness of gelatin capsules (Nazzal and Wang, 2001). The gelling mechanism that occurred in solid lipid nanoparticles that contains Compritol was investigated using DSC (Freitas and Muller, 1999). The results showed that the crystallization occur during gelation. However, if the lipid based system is liquid, this kind of stability problem may not be detected by DSC (unpublished data). Therefore, it can be said that DSC analysis is more useful for solid based lipid systems.

6.1.2. Particle size analysis:

Most lipids are either in emulsion/microemulsion form or they can form an emulsion upon mixing with gastric fluid (self-emulsifying systems). Droplet or particle size will influence performance of the ultimate dosage form. Therefore, particle size analysis is an important analytical tool in characterization of lipid based delivery system. There are number of techniques which are available to measure the particle size. These include optical microscopy, scattering methods such as light scattering and photon correlation spectroscopy and electron microscopy. Scattering methods are mostly commonly used since they cover a wide range particle size (from 2nm to 2 mm).

Particle size measurements provide a quick assessment in comparison of emulsion systems. For example, the efficiency of self-emulsification by measuring the rate of emulsification has been studied by monitoring the relative intensity of light scattered by dispersion and particle size distribution of resulted emulsion using light microscopy and a Coulter Nano-Sizer (Pouton, 1985a). The mixture of Miglyol 812 (medium chain tri-glyceride) or Miglyol 840 (propylene glycol ester of caprylic/capric acid) with the surfactant Tween 85 (polysorbate 85) provided more efficient self-emulsification system than the mixture liquid paraffin or oleic acid with the surfactant Tween 85. It was concluded that the Coulter Nano-Sizer provided a quick, non-invasive technique for comparing the mean particle sizes of resultant emulsions but was inappropriate for sizing all self-emulsifying systems. It was shown that the determination of particle size with light scattering is more important than comparison of emulsification rate. Light scattering has been used to measure the emulsification rate to explain the mechanism of the spontaneity of self-emulsifiable oils. The

mechanism was explained by liquid crystal formation and it was concluded that spontaneity was dependent on the nature of the material used in the system (Groves and Mustafa, 1974). The mechanism of action for self-emulsified systems consisting of Tagat TO (ethoxylated glycerol tri-oleate) and Miglyol 812 (medium chain triglyceride) has been also studied using Low Angle Laser Light Diffraction for the emulsions with droplet distribution above 1 μm (Wakerly et al., 1986). Quasi-elastic light scattering was used for investigations of submicron dispersions. Ternary phase studies showed a specific region of lamellar liquid crystal dispersed in the isotropic phase of solubilized water.

The particle size measurements can be useful to characterize the factors such as HLB of surfactant, type and concentration of co-surfactants affecting the efficacy of self-emulsifying oral delivery system. Such study has been conducted using self-emulsifying formulations containing surfactants (Spans and Tweens) with different HLB values (4.3 to 16.7), co-surfactants (mono-glycerides) with varying fatty acid chain length (C_8 to C_{18}) and vegetable oils (peanut, soybean and safflower oil) with different fatty acid composition (C_8 to C_{18}). The system was characterized using dissolution test and particle size measurements (Bachynsky et al., 1997). It was concluded that a surfactant with an HLB in the range of 11-15 at 5% concentration and a co-emulsifier consisting of a monoglyceride of caprylic/capric acids at 17% concentration were most effective in a formulation having good self-emulsifying properties in terms of particle size and satisfactory dissolution characteristics of an aratinoid model drug used.

One should be aware of that using laser diffraction requires dilution of the sample that may alter the properties of self-emulsifying systems. Therefore, when the particle size of the sample is above 1000 nm, which is the upper limit of dynamic light scattering, electron microscopy may be used.

6.1.3. Other methods

X-Ray diffraction can be used to monitor any polymorphic changes and to detect possible lipid-vehicle interactions along with FT-IR (Tandon et al., 2001). Low frequency dielectric spectroscopy (LFDS) has been used to elucidate the mechanism of self-emulsification. It was shown that self-emulsification with Imwitor 742 or Labrafil M2125 CS and Tween 80 occurs via liquid crystal formation depending on surfactant/oil ratio. It was also shown that LFDS is a useful technique to examine the individual components in order to investigate the effects of drug inclusion. Surface tension and particle size measurements were also conducted in order to determine the effect of drug concentration (Craig et al., 1993a and b, 1995). Refractive index, conductance and density measurements were conducted to compare the water-in-oil microemulsions containing long- versus medium chain glycerides (Constantinides and Scalart, 1997). Rheological investigations and NMR can be used to investigate the effect of drug loading in MCM (Dumanli et al., 1999). In this study it has been shown that beyond a certain concentration, the solution reaches a saturation level causing a change in the slope of concentration vs. zero-shear viscosity and particle size (Fig 4). The effect of drug loading has been further investigated with NMR showing that as the

concentration of the drug in MCM increased, the line-widths of drug broadened while line-widths of MCM remained same.

Zeta potential measurements can help to evaluate physical stability of dispersed lipid based systems and charged lipid-based formulations (Washington, 1996; Gershanik and Benita, 2000). Freitas and Muller (1998) studied the gelation mechanisms seen in aqueous dispersions of solid lipid nanoparticles consisting of 10% Compritol and 1.2% Pluronic F68 by measuring zeta potential of the system. The possible mechanisms of gel formation are explained as the structural changes of the lipid phase leading to zeta potential reduction and particle growth. Recently, positively charged self-emulsifying formulations were developed by Gershanik et al. (1996 and 1998) to enhance the interaction with mucosal surface and increase cellular uptake. Zeta potential measurements were conducted to characterize such systems, to investigate the effect of progesterone incorporation on the charge of the formulation and to show that the enhanced electrostatic interactions of positively charged droplets with rat intestinal mucosal surface are responsible for the uptake of cyclosporine A.

6.2. In vitro/in vivo correlation (IVIVC)

6.2.1 *Dissolution studies*

Dissolution testing for poorly soluble drugs requires different media than those normally used for water-soluble compounds. The incorporation of surfactant into dissolution media was found useful (Serajuddin, 1988; Roman, 1999; Dressman and Reppas, 2000). The dissolution of UC-781, an inhibitor of HIV-1 replication and a poorly soluble drug, has been carried out in aqueous polysorbate 80 solutions (Damian

et al., 2000). The dissolution of physical mixtures and solid dispersions of the drug compared with PEG 6000 and Gelucire 44/14 showed that the drug release from Gelucire 44/14 was higher than from physical mixture and PEG 6000. It has been also shown that the micelle is sensitive to impurities and electrolytes influencing size, loading capacity, solubility and dissolution rate (Wasan, 2001). The type of surfactant used in dissolution media is important since it may interact with the capsule shells as in the case of sodium lauryl sulfate (SLS), an anionic surfactant that may interact with cationic charges of gelatin at gastric pH (Pillay and Fassihi, 1999). These interactions may retard the disintegration of the shell. Therefore, two-phase dissolution test method has been proposed for the poorly soluble compounds by Grundy et al. (1997a, b). The test system was used as modification of the USP XXII Apparatus 2. The important feature of this system is containing lower phase that has dissolution medium of 750 mL SIF and upper phase that has dissolution medium of 250 mL n-octanol. Improved *in vivo-in vitro* correlation was demonstrated with two phase dissolution test by employing Nifedipine gastrointestinal therapeutic system (GITS). The test was used for dissolution studies of lipid-filled capsules employing Nifedipine as a model drug (Pillay and Fassihi, 1999).

Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration by RRSBW distribution, known as the Weibull distribution, showed that prediction of plasma profile was possible in seven out of eleven classes (Nicolaidis et al., 2001). In this study, it has been also shown that the plasma profile of a lipophilic drug can be predicted with appropriate *in vitro* dissolution data.

provided that the absolute bioavailability of the drug is known and the drug has dissolution limited absorption.

6.2.2. *In vitro* lipolysis

A physiologically representative medium should be used to establish *in vitro* lipolysis. For this purpose, the media can be simulated intestinal fluid containing 50 mM Tris-maleate buffer (pH=6.5), 5mM CaCl₂·2H₂O, 150 mM NaCl, 8 mM sodium taurocholate and 1.5 mM lecithin. Lipase solution should be added according to required enzyme activity (20 TBUs per mL of lipase-colipase is recommended). Lipolysis starts when the enzyme solution is added to tri-glyceride emulsion. The reaction can be followed by monitoring fatty acid generation via continued titration with pH-stat. (Hutchison, 1994; Carriere et al., 1997; MacGregor et al., 1997; Zangenberg et al., 2001). Attention should be given to the surfactant that may be present in tri-glyceride emulsion because most of the surfactants used in lipid formulation inhibit lipolysis. There are not many published studies that correlate *in vitro* lipolysis and *in vivo* results. Only preliminary results were given for progesterone with good *in vivo* correlation (MacGregor et al., 1997) whereas no IVIVC was established for cyclosporine. Olive oil as LCT and Miglyol 812 as MCT have been compared for cyclosporine intestinal absorption both *in vitro* and *in vivo* (Reymond et al., 1988a, b). An *in vitro* lipid digestion method established was used to compare LCT and MCT in terms of the drug partitioning into aqueous phase. It was demonstrated that lipid digestion promotes the partition of the drug in the aqueous phase for MCT, whereas for olive oil presence of lipolysis products in the aqueous

phase after digestion decreases the distribution of Cyclosporine into this phase. However, their *in vivo* results showed that drug permeation is higher with LCT. Digested vehicles promoted the absorption compared to the non-digested ones. It was concluded that *in vitro* phase quantification could not simulate *in vivo* absorption events.

6.3. Absorption mechanisms

Increased absorption mechanisms with lipids involve increasing drug solubility/dissolution, changing gastric and intestinal transit time, stimulation of bile flow and increased intestinal permeation (Muranishi, S., 1985; Aungst et al., 1993, 1996). Reduced particle/droplet size can be another reason that lipid-based formulation can provide increased absorption. These mechanisms can be elucidated with measuring dissolution test and particle size of the formulation, using cell monolayers and imaging techniques. Zeta potential measurements can also be useful to study the mechanism of charged lipid based systems (Gershanik et al, 1998). Particle size measurements can be used to show the mechanism of increased bioavailability of certain drugs. For example, enhanced the bioavailability of poorly soluble Cyclosporine A has been studied in o/w microemulsions consists of capric/caprylic triglyceride as an oil(Captex 355), polyoxyethylated castor oil (Cremophor EL™) as a surfactant, Transcutol™ as co-surfactant and saline (Gao et al., 1998). It was concluded that the enhanced bioavailability of Cyclosporine A loaded in this microemulsion system was due to the reduced droplet size of microemulsion systems.

Physicochemical interactions between the self-emulsifying system and intestinal mucosa can also affect drug absorption. The interaction of a self-emulsifying lipid drug delivery system with the everted rat intestinal mucosa has been demonstrated as a function of droplet size and surface charge (Gershanik et al., 1998). A positively and a negatively charged self-emulsifying system was prepared with ethyl-oleate and oleylamine using Cyclosporine A as a lipophilic model drug. Transmission electron microscopy has been used to measure the particle size of the self-emulsifying systems. They found that the enhanced electrostatic interactions of positively charged droplets with the mucosal surface are mostly responsible for the preferential uptake of Cyclosporine A from the positively charged droplets as compared to negatively charged droplets. Positively charged self-emulsifying oil formulation containing Tween 80, benzyl alcohol, ethyl oleate and oleylamine also elicited the highest and most satisfactory absorption profile for progesterone (Gershanik and Benita, 1996).

6.3.1. Imaging techniques

Imaging techniques such as scintigraphy, ultrasound and magnetic resonance imaging can be used to get detailed visualization of transit absorption of lipids in the body. The effect of oleic acid on human ileal brake was determined by measuring the transit of radio-labeled tablets by gamma scintigraphy in volunteers (Dobson et al., 1999). It was shown that oleic acid activated the ileal brake that slowed the transit of tablets through small intestine. Readers can refer to a review by Wilson et al. (1997) for more details about imaging techniques.

6.3.2. The use of cell monolayers

The Caco-2 cell, derived from a human colorectal carcinoma, has been used extensively to study the permeation of drug compounds. It is especially useful to predict the intestinal absorption mechanisms. Mechanisms of lipid uptake and metabolism have been studied in the nutrition area using Caco-2 cells as a model. This information can be applied to study lipid based drug delivery. It has been reported that many of the biochemical and metabolic features of lipid processing *in vivo* have been shown to exist in Caco-2 cells (O'driscoll, 1998). These features include fatty acid uptake, esterification and triglyceride formation, cholesterol absorption, esterification and synthesis, synthesis and secretion of lipoproteins, expression of P-glycoproteins (P-GP) and CYP3A-like cytochrome P450 activity. However, it is important to be aware of the fact that there are also some differences such as no fatty acid binding proteins (FABP) in Caco-2 cells.

Efficacy and toxicity screening of various enhancers has been studied using Caco-2 cell (Quan et al., 1998). The findings indicated that the effectiveness of absorption enhancers in the Caco-2 monolayer system was similar to an *in vivo* rat system. The Caco-2 cell model was also used to investigate the charge dependent interactions of positively charged SEOF with human intestinal epithelial cells (Gershanik et al., 2000). It was shown that the positively charged emulsions affected the barrier properties of the cell monolayer at high concentrations and reduce the cell viability. However, no detectable cytotoxic effect was observed with following dilution.

The effects of Capmul MCM on physiological properties of rabbit ileum and distal colon, including active ion transport, trans-epithelial resistance and passive permeability have been investigated *in vitro* (Yeh et al., 1994). It was found that Capmul MCM inhibited active ion transport.

7. CONCLUSIONS

Lipids can play a significant role in improving absorption of poorly soluble drugs. Their physicochemical and physiological aspects should be well defined. Physicochemical aspects including characteristics of lipids and its affect on drug solubility and stability should be studied. In physiological aspects, understanding the lipid digestion process will help to design a rational lipid drug delivery systems. Judicial selection of lipids through knowledge of a lipid's physicochemical and physiological properties can overcome absorption of poorly soluble drugs. This is rapidly evolving area and the future of lipid drug delivery system is quite promising. Certainly, further research on areas like lipid digestion and absorption is desirable. More mechanistic studies that will include different variety of lipids are needed. The type of model drugs used in research purpose should be extended to be more representative and rational. The drug/lipid relationship should be carefully investigated to design a cost-effective formulation with enhanced bioavailability together with optimum stability and manufacturability.

8. ACKNOWLEDGEMENT

This study is part of Ms. Dumanli's Ph.D. dissertation research supported by Hoffmann-La Roche. The research was conducted at Hoffmann-La Roche facilities in Nutley, NJ.

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Table 1: Examples of Commonly Used Lipids in Oral Drug Delivery

Table 1A: Examples of Fatty Acids Commonly Used in Oral Drug Delivery

Chemical Structure	Chemical Name	Examples of Trade Names	Some Physico-chemical Properties*				
			Form [#]	MP (°C)	HLB	Density ¹	IT ²
Fatty Acids COOH (CH ₂) _n CH ₃	Caprylic acid (C ₈)	Emery 657 (Henkel)	L	16.5	-	0.8995	2.52
	Capric acid (C ₁₀)	Emery 659 (Henkel)	L	31.5	-	N/A	N/A
	Lauric acid (C ₁₂)	Emery 652 (Henkel)	S	43-45	-	N/A	N/A
	Myristic Acid (C ₁₄)	Emery 655 (Henkel)	S	53-55	-	N/A	N/A
	Palmitic Acid (C ₁₆)	Emersol 143 (Henkel)	S	60-68	-	N/A	N/A
	Stearic Acid (C ₁₈)	Emersol 153 NF (Henkel)	S	64-70	-	N/A	N/A
	Oleic acid (C _{18:1})	Crossential O 94	L	8-10	-	-	-
		(Croda)	L	8-10		0.8842	10.44
		Emersol 6313 (Henkel)					
	Linoleic acid (C _{18:2})	Crossential L98 (Croda)	L	-5	-	-	-
		Emersol 315 (Henkel)				0.8942	11.00
	Linolenic acid (C _{18:3})	Crossential LN75 (Croda)	L	-11	-	0.9036	9.43

Table 1B: Examples of Commonly Used Glyceryl Mono- And Diesters

Chemical Structure	Chemical Name	Examples of Trade Names	Some Physico-chemical Properties*				
			Form [#]	MP (°C)	HLB	Density ¹	IT ²
50 Glyceryl mono- di-esters $\text{H}_2\text{C} - \text{OH}$ $\text{H} \text{C} - \text{OH}$ $\text{H}_2\text{C} - \text{O COR}$ Mono-glyceride $\text{H}_2\text{C} - \text{OH}$ $\text{H} \text{C} \text{O} - \text{COR}$ $\text{H}_2\text{C} \text{O} - \text{COR}$ Diglyceride	Glyceryl	CapmulMCMC8 Abitec)	SS	-	5-6	N/A	N/M
	monocaprylate	Imwitor 308 (Sasol)	S	30-34	6-7	1.0132	N/M
	Glyceryl mono/di-caprylate	Imwitor 988 (Sasol)	L	20-25	4 -6	0.9931	1.20
	Mono/di-glyceride of caprylic/capric acid	Capmul MCM (Abitec)	L	25-30	5-6	1.0010	N/M
		Imwitor742	L	25-30	5-6	-	-
	Glyceryl mono-laurate	Imwitor 312 (Sasol)	S	56-60	5-6	N/A	N/A
	Glyceryl palmitostearate	PecirolATO (Gattefosse)	S	53-57	2	N/A	N/A
	Glyceryl mono-stearate	Capmul GMS50 (Abitec)	S	57-62	3-4	N/A	N/A
		Imwitor 191 (Sasol)	S	66-71	4	N/A	N/A
	Glyceryl mono-oleate	Pecceol (Gattefosse)	L	-	3	0.9352	3.32
		Capmul GMO (Abitec)	L	-	3-4	0.9378	3.58
	Glyceryl	Maisine (Gattefosse)	L	-	4	0.9385	3.87
	Glyceryl behenate	Compritol 888ATO	S	69-74	2	N/A	N/A

Table 1C: Examples of Commonly Used Triglycerides

Chemical Structure	Chemical Name	Examples of Trade Names	Some Physico-chemical Properties*				
			Form [#]	MP (°C)	HLB	Density ¹	IT ²
Triglycerides H ₂ C O-COR	Caprylic /	Crodamol GTCC (Croda)	L	-	-	0.9325	15.20
	Capric triglyceride	Labrafac CC (Gattefosse)	L	-	1	0.9331	13.96
		Miglyol 810 (Sasol)	L	-	1.5	0.9404	12.06
		Captex 300 (Abitec)	L	-	1.5	0.9414	14.76
		NeobeeM5 (Stepan)		-	-	0.9354	15.14
HC-O- COR	Glyceryl tricaprato	Captex1000 (Abitec)	S	-	1.5	N/A	N/A
H ₂ CO- COR	Glyceryl trimyristate	Dynasan 114 (Sasol)	S	55-58	1	N/A	N/A
	Glyceryl tripalmitate	Dynasan 116 (Sasol)	S	61-65	1	N/A	N/A
	Glyceryl tristearate	Dynasan 118 (Sasol)	S	70-73	1	N/A	N/A
	Glyceryl trioleate	Captex GTO (Abitec)	L	-	1	0.9056	16.87

Table 1D: Commonly Used Propylene Glycol Esters

Chemical Structure	Chemical Name	Examples of Trade Names	Some Physico-chemical Properties*				
			Form [#]	MP (°C)	HLB	Density ¹	IT ²
Propylene glycol esters $\begin{array}{c} \text{H}_3\text{C} \\ \\ \text{H} - \text{C} - \text{O} - \text{COR} \\ \\ \text{H}_2\text{C} - \text{O} - \text{COR} \end{array}$	Propylene glycol monocaprylate	Capmul PG-8 (Abitec)	L	-	2-3	0.9372	3.2
		Capryol 90 (Gattefosse)	L	-	6	0.9360	5.9
	Propylene glycol dicaprate	Captex 100 (Abitec)	L	-	2-3	N/A	N/A
	Propyleneglycol dicaprylate/ dicaprate	Captex 200 (Abitec)	L	-	3	0.9078	11.3
	Propylene glycol monolaurate	Lauroglycol 90	L	-	5	0.9136	6.7
		(Gattefosse)	L	-	4	N/A	N/A
		Capmul PG-12 (Abitec)					
	Propylene glycol monooleate	E-32238 (Cognis)	L	-	-	N/A	N/A
	Propylene glycol dioleate	Emery 2379-U (Cognis)	L	-	-	N/A	N/A

Table 1E: Commonly Used Polyglycerol Esters

	Chemical Structure	Chemical Name	Examples of Trade Names	Some Physico-chemical Properties*				
				Form [#]	MP (°C)	HLB	Density ¹	IT ²
Σ	Polyglycerol esters	Diglyceryl mono-oleate	Caprol 2GO (Abitec)	L	-		0.9733	2.66
	CH ₂ – O-COR	Triglyceryl mono-oleate	Capmul 3GO (Abitec)	L	-	6.5	0.9756	2.34
	CH – OH	Triglyceryl mono-stearate	Capmul 3GS (Abitec)	S	-	6.5	N/A	N/A
	H ₂ C-O	Hexaglyceryl di-oleate	Caprol 6G2O (Abitec)	L	-	8.5	-	-
	CH ₂	Hexaglyceryl di-stearate	Plurol Oleique (Gattefosse)	L	-	6	1.0211	1.59
	HC OCOR		Caprol 6G2S (Abitec)	S	-	8.5	-	-
	O-CH							
	H ₂ C	Decaglyceryl tetraoleate	Caprol 10G4O (Abitec)	L	-	6	N/A	N/A
	H C – OH	Decaglyceryl decaoleate	Caprol 10G10O (Abitec)	L	-	3.5	N/A	N/A

Table 1F: Commonly Used PEG Esters

Chemical Structure	Chemical Name	Examples of Trade Names	Some Physico-chemical Properties*			
			Form [#]	MP (°C)	HLB	Density ¹
Poly-ethyleneglycol esters COR O (CH ₂ CH ₂ O) _n H	PEG200 caprylate/caprata	E-32237 (Cognis)	L	-	-	N/A
	PEG200 monolaurate	Emerest 2620 (Cognis)	L	-	9	N/A
	PEG200 monooleate	Emerest 2624 (Cognis)	L	-	8	N/A
	PEG400 caprylate/caprata	Softisan 767 (Sasol)	L	-	14	1.0651
	PEG400 monolaurate	KesscoPEG400ML (Stepan)	L	-	13	0.0179
	PEG400 dilaurate	KesscoPEG400DL (Stepan)	L	-	10	0.9786
	PEG400 monooleate	Emerest 2646 (Cognis)	L	-	12	N/A
	PEG400 dioleate	Kessco PEG 400 (Stepan)	L	-	8.5	0.9690
	PEG1500 laurate/myristate	Gelucire 44/14 (Gattefosse)	SS	44	14	N/A
	PEG1500 palmitate/stearate	Gelucire 50/13 (Gattefosse)	SS	50	13	N/A

*Our calculations and experimental findings

[#] L: Liquid, S: Solid, SS: Semi-solid

¹Density measurements (g/cm³) were done using Paar Oscillating U-Tube method at 37°C.

²Interfacial tension (IT) (mN/m) against gastric fluid were conducted with Kruss K 12 Tensiometer using the ring method at 37°C, N/M: It could not be measured due to spontaneous emulsification N/A: Not available

Table 2: Properties of Type I, II, IIIA and IIIB Lipid Formulations Based on Pouton Classification (Redrawn with the permission of publisher of ref. Pouton, 1999)

	Increasing Hydrophilic Content
	Type I	Type II	Type IIIA	Type IIIB
Typical Composition				
Triglycerides or mixed glyceride	100%	40-80%	40-80%	< 20%
Surfactants	-	20-60% (HLB<12)	20-40% (HLB>11)	20-50% (HLB >11)
Hydrophilic cosolvents	-	-	0-40%	20-50%
Particle size of dispersion	Coarse	100-250 nm	100-250 nm	50-100 nm
Significance of aqueous dilution	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity	Significant phase changes & potential loss of solvent capacity
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial may be inhibited	Not required Not likely

Table 3. Lipid Systems: Solution and Suspension

Composition of lipid formulation used	Drug investigated	Purpose of the study	Outcome of the study	Reference
3% (v/v) Phenol red – 0.1N Potassium salts of saturated fatty acids (C ₂ –C ₁₈) – Water to make 1000 mL	Phenol red MW= 354, oil-soluble model compound	To seek a relationship between the chain length of fatty acids and the slowing of gastric emptying	Fatty acids from C ₂ to C ₁₀ do not change gastric emptying time, while fatty acids with C ₁₂ to C ₁₈ carbon atoms delayed in gastric emptying	Hunt and Knox 1968
– 0.5% (w/v) solution of drug in MCT, MCM and MBLA – 0.5% suspension of drug in corn oil	SL-512 MW =296.5, anti-inflammatory agent	To investigate the relationship between drug absorption and gastric emptying of lipid formulations	Higher viscosity and volume of lipids (≥ 100 µL/rat) delayed the absorption of the drug due to longer retention time of drug in the stomach	Yamahira et al. 1978

Table 3. Lipid Systems: Solution and Suspension (continued)

Composition of lipid formulation used	Drug investigated	Purpose of the study	Outcome of the study	Reference
1% (w/v) drug solution in: – MCT – MBLA	SL-512 MW =296.5, anti-inflammatory agent	To investigate the absorption characteristics of the drug in rats in relation to digestibility of lipids	Serum level of the drug was found to be much higher from MCT (digestible lipid) than from MBLA (non-digestible lipid)	Yamahira et al. 1979a
– 5% drug (as solution in ethanol) – qs. to 100% olive oil as LCT or Miglyol812 as MCT	Cyclosporine A MW =1202, immunosuppressive	To compare the effect of LCT and MCT on absorption in conscious dogs	Absorption of the drug was 10 times higher with LCT than with MCT. The difference was due to the formation of mixed micelles which occurs during digestion of LCT but not MCT	Behrens et al. 1996
Unit dose consists of: 100 mg/kg drug and 0.5 mL of one of following lipid vehicles – Arachis oil, – Miglyol 812 – Liquid paraffin	Probucol MW = 516.9, hypocholesterolemic	To investigate the effect of lipid vehicles on the gastrointestinal absorption of probucol in rats	Digestible oils (Arachis oil and Miglyol 812) provided higher plasma concentrations than Liquid paraffin. Arachis oil provided the highest plasma and lymph concentrations	Palin et al. 1983

Table 3. Lipid Systems: Solution and Suspension (continued)

Composition of lipid formulation used	Drug investigated	Purpose of the study	Outcome of the study	Reference
<ul style="list-style-type: none"> – Oleic acid – Linoleic acid – Linolenic acid – Arachis oil 	-	To determine the effect of unsaturation on lipoprotein fractions in the lymph	The use of fatty acids with greater unsaturation should be considered in lymphatic drug delivery because they increase the onset of chylomicron synthesis	Cheema et al. 1987
7.1% Drug in: <ul style="list-style-type: none"> – Glycerol monooleate 	Ubidecarenone MW= 863, used in angina pectoris	To improve the absorption of the drug	Bioavailability was same compared to the physical precipitate of the drug	Pozzi et al. 1991
Solution of 3 retinoids (3mg/mL) in each vehicle: <ul style="list-style-type: none"> – Cottonseed oil – Miglyol 812 – Linoleic acid 	Isotretinoin MW = 300, Etretinate MW = 354, Temarotene MW = 304, used in acne treatment	To investigate lymphatic transport of three retinoids in the rat in terms of effect of lipophilicity and lipid vehicle	Lipid solubility increased as log P increased but lymphatic uptake decreased as the solubility increased	Nankervis et al. 1996

Table 3. Lipid Systems: Solution and Suspension (continued)

Composition of lipid formulation used	Drug investigated	Purpose of the study	Outcome of the study	Reference
Unit dose contains: – 4% (w/v) Drug – 50µL mixture of oleic acid and glyceryl mono-oleate (2:1 w/w)	Halofantrine MW= 500.5, antimalarial	To investigate the lymphatic transport of Halofantrine	Lymphatic transport was evaluated using micellar, emulsion and solution formulations. The lipid solution provided the least intestinal lymph and plasma uptake of the drug in the triple-cannulated anesthetized rat model	Porter et al. 1996 a & b
7.5 % (w/v) drug in Peceol	Ontazolast MW =335, used for asthma	To compare the bioavailability of the drug as aqueous suspension, emulsion and SEDDS	All lipid formulations provided a 14-19-fold increase in bioavailability compared to an aqueous solution. Lipid solution provided the highest lymphatic absorption	Hauss et al. 1998
200 mg drug with MCM in unit dose	Saquinavir MW = 671, HIV protease inhibitor	To compare the powder and lipid formulation of the drug	Bioavailability of Saquinavir in lipid is 3 times higher than that of powder formulation	Perry & Noble 1998

Table 4. Lipid Systems: Micellar Solutions

Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
350nM retinol – 2.5mM fatty acid (Butyric acid (C ₄), Octanoic acid (C ₈), Oleic acid (C _{18:1}), Linoleic acid (C _{18:2}) or Arachidonic acid (C _{20:4}) – 5-15mM Sodium taurocholate	Retinol MW= 286.5, vitamin A	To seek the influence of pH, bile and fatty acids on retinol lymphatic and portal transport	Caprylic acid provided the highest lymphatic transport whereas linoleic and arachidonic acids provided the highest portal transport	Hollander, 1980
– 19.2mM Fatty acid (palmitic, oleic or linoleic) – 19.2 Monoolein – 20mM Taurocholate	-	To investigate the effect of saturated and unsaturated lipids on the composition of lymph tri-glycerides in rats	There were no differences in the size of chylomicrons formed with saturated and unsaturated lipids but their clearance rates varied	Renner et al., 1986
Unit dose contains: – 4% (w/v) Drug – 50μL mixture of oleic acid and glyceryl monooleate (2:1 w/w) – 4% (w/v) Tween 80 – Saline to make 2.88 mL	Halofantrine MW= 500.5, antimalaria l	To investigate lymphatic transport of Halofantrine	Micellar solution provided higher lymph and plasma concentration than an emulsion and a solution in the triple-cannulated anesthetized rat model	Porter et al., 1996b

Table 5. Lipid Systems: Emulsions and Microemulsions

	Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
	0.0005% (w/v) Vitamin E – 5.7% (v/v) Trioctanoin (MCT) or Triolein (LCT) – carbohydrate, saline and proteins	D- α -(5-Methyl-H) tocopherol MW = 430, vitamin E	To develop MCT formulation for Vitamin E and compare the bioavailability between MCT and LCT	MCT enhanced the intestinal absorption because it increased water solubility of vitamin E	Gallo-Torres et al. 1969 and 1978
	1% (w/v) micronized drug 1% (w/v) Polysorbate 60 40% (v/v) corn oil water to make 100%	Griseofulvin MW = 353, antifungal	To investigate absorption characteristics of the drug from o/w emulsions and suspensions	The bioavailability has been increased 2.5-fold with emulsions compared to aqueous suspension	Carrigan and Bates 1973
9	0.334 % (w/w) drug in glyceryl mono-oleate emulsion	Danazol MW = 337.5, steroid hormone	To compare the bioavailability of danazol from an emulsion and a tablet in human in fed and fasted state	Emulsion formulation increased the bioavailability of drug 4-fold compare to tablets in fasted subjects but the differences was not significant in the fed state.	Charman et al. 1993

Table 5. Lipid Systems: Emulsions and Microemulsions (continued)

	Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
	<ul style="list-style-type: none"> – Sandimmune- Emulsion form(25, 100 mg drug in corn oil, glycerol, LabrafilM2125CS and alcohol) – Neoral- Microemulsion form (15, 30, 60 mg drug in corn oil, polyoxyl 40 hydrogenated castor oil and alcohol) 	Cyclosporine A MW =1202, immunosuppressive	To compare the pharmacokinetics of a microemulsion and an emulsion in human	Microemulsion provided 3 times higher absorption than the emulsion and reduced the inter-subject variability	Mueller, et al., 1994
2	4% (w/v) Drug <ul style="list-style-type: none"> – 50µL mixture of oleic acid and glyceryl mono-oleate (2:1 w/w) – 0.2% (w/v) Tween 80 – Saline to make 2.88 mL 	Halofantrine MW= 500.5, antimalarial	To compare lymphatic transport of Halofantrine with emulsion, solution and micelle	Emulsion provided higher lymphatic transport than solution, lower than micellar solution	Porter et al. 1996b
	Microemulsion of the drug with various ratios of with Captex355, ChremophorEL, Transcutol and saline	Cyclosporine A MW =1202, immunosuppressive	To compare bioavailability of an o/w microemulsion with marketed products, Sandimmun and Neoral	The bioavailability of the drug in the microemulsion system was increased 3.3 and 1.25 fold compared to Sandimmun and Neoral, respectively	Gao et al., 1998

Table 6. Lipid Systems: SEDDS/SMEDDS

Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
A range of concentrations of benzoic acid were dissolved in: <ul style="list-style-type: none"> – 30% (w/w) Tween 85 – 70% (w/w) Miglyol 812 	Benzoic acid MW = 122, model compound	To investigate the inclusion of a model drug on the performance of SEDDS	Formulations containing 1.5-2% benzoic acid were the most efficient.	Pouton, 1985b
Ternary phase of: <ul style="list-style-type: none"> – Tagat TO, ethoxylated glycerol trioleate, – Miglyol 812 		To investigate the mechanism of action of SEDDS	Self-emulsification was a result of aqueous penetration into the liquid crystal aided by mechanical dispersion	Wakerly et al., 1986
Neobee M5 Tagat TO	WIN 54954 antipicornavirus	To develop a SEDD formulation and compare the bioavailability with PEG solution in beagle dogs	SEDDS improved the reproducibility of the plasma profile in terms of the maximum plasma concentration	Charman et al., 1992

Table 6. Lipid Systems: SEDDS/SMEDDS (continued)

Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
2 and 6% (w/v) Drug – 56, 60 and 62% (w/v) Labrafil M 2125 CS – 30% (w/v) Tween 80 – 3% (w/v) propylene glycol – 5% (w/v) water	L-365,260 MW = 398, benzodiazepine derivative	Self-emulsification of the system with and without drug was investigated using LFDS, surface tension measurements and particle size analysis	LFDS investigations show the effect of drug loading on individual components. Incorporation of drug changed the surface tension of the system and droplet size of the resulting emulsion	Craig et al. 1993
5% (w/w) Drug was formulated in various extent of PGG, MCM, PEG-25 glyceryl trioleate Polysorbate80 and peanut oil/neobee oil	Ro 15-0778 MW = 304, Used in acne treatment	To investigate the ability of PGG with varying fatty acid and PEG chain lengths to produce self-emulsification	SEDDS gave 3-fold greater AUC than solution, tablet and capsule of wet-milled spray dried powder in dogs	Shah et al., 1994

Table 6. Lipid Systems: SEDDS/SMEDDS (continued)

	Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
	10% Progesterone – 25% Tween 80 – 10-50% Benzyl alcohol – 2.5-3% Oleylamine – to 100% Ethyl oleate	Progesterone MW = 314, hormone	To develop and characterize a positively charged self-emulsifying oil formulation	Positively charged emulsion provided the highest AUC among a PEG 300 solution, a suspension, ethyl oleate and a negatively charged emulsion in rats. Further toxicity studies were recommended	Gershanik and Benita, 1996
8	7.5% (w/v) drug in mixture of Peccol and Gelucire 44/14 (either equal or 2:8)	Ontazolast MW = 335, used for asthma	To compare the bioavailability of drug with an aqueous suspension, an emulsion and SEDDS	All lipid formulations provided 14-19 fold increase in bioavailability compare to aqueous solution. The emulsion and solution provided better lymphatic absorption than SEDDS	Hauss et al. 1997
	– Imwitor 742 – Tween 80	L-683,453 Used in benign prostatic hyperplasia	To compare the bioavailability between aqueous suspension and SEDDS	SEDDS provided 13.7% increase in bioavailability compared to the suspension	Matuszewska et al. 1996

Table 6. Lipid Systems: SEDDS/SMEDDS (continued)

Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
5.66 % (w/w) drug in a series of self-emulsifying systems containing Myvacet9-45, Captex200, LabrafacCM-10, Lauroglycol and Labrasol	Ubidecarenone (CoenzymeQ ₁₀) MW= 863, used in angina pectoris	To develop and characterize SEDDS of Q ₁₀ using PGG as emulsifier and to evaluate bioavailability in dogs	A two-fold increase in the bioavailability was observed compare to tablet formulations	Kommuru, et al., 2001
100 mg/mL Cyclosporine A in mixture of: – 25% (w/w) Tween 80 – 30% ethanol – 3% (w/w) oleylamine – qs.100% (w/w) ethyl oleate	Cyclosporine A MW =1202, immunosuppressive	To investigate the interaction of positively charged self-emulsifying oil formulations with rat everted intestinal mucosa	Electrostatic interactions were responsible for uptake of the drug from the positively charged droplets	Gershanik et al., 1998

Table 7. Lipid Systems: Solid Dispersions/Solutions

Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
15-45% drug - 55-85% one of the following vehicles: Gelucire 44/14, PEG1000, PEG, 1450and PEG 8000	REV 5901 MW=355, 5-lipoxygenase inhibitor	To investigate the effect of vehicle amphiphilicity on dissolution and bioavailability in dogs	The dissolution and bioavailability were higher from the formulation with Gelucire 44/14 than that from PEGs.	Serajuddin, et al. 1988
18% drug - 82% gelucire44/14 and PEG 400 mixture (6:1)	REV 5901 MW=355, 5-lipoxygenase inhibitor	To compare the bioavailability from tablets and solid dispersions in human	Solid dispersion with lipid increased the bioavailability of the drug 5-fold compared to tablets because of improved dissolution rate	Sheen et al. 1991
5 % drug - 30% Gelucire 44/14 - 65% Soya lccithin	Ubidecarenone MW= 863, used in angina pectoris	To compare the absorption physical mixture, lipid solution and solid dispersion	Solid dispersion provided the highest AUC in dogs compared to physical mixture and lipid solution	Pozzi et al., 1991

Table 7. Lipid Systems: Solid Dispersions/Solutions (continued)

Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
Gelucires 43/01, 50/02, 55/18, 50/13	Theophylline MW = 180, Diuretic, vasodilator	To investigate the effect of humidity during storage of Gelucires using dielectric, viscosity, thermal and dissolution studies	Gelucires containing more PEG esters showed more changes than those containing more glycerides. No fundamental explanation was given for mechanism of dissolution differences under high humidity for both	Sutananta et al. 1996
Unit dose contained 350 mg drug in Gelucire 44/14	DMP 323 HIV protease inhibitor	To improve the bioavailability of drug at high doses	Bioavailability was increased 50% using semi-solid Gelucire 44/14 formulation compared with the glycol vehicles	Aungst et al. 1997
3.75% (w/v) drug in mixture of Gelucire 44/14 and Labrasol	Nifedipine MW = 346, vasodilator	To develop a new method for dissolution studies of lipid-filled capsules for Nifedipine	Two-phase dissolution system has been developed and used. It has been suggested to use in lipid based formulation as an alternative to USP method	Pillay and Fassihi, 1999
Solid dispersion of drug was prepared at various concentration with Gelucire 44/14 and PEG 6000	UC-781 MW=335.5, antiviral thiocarboxanilide	To prepare and characterize solid dispersions of the drug with PEG 6000 and Gelucire 44/14 to improve dissolution properties	Enhanced dissolution rate was obtained with solid dispersions compared with physical mixture and DSC, X-ray, IR were used in characterization of the system	Damian et al., 2000

Table 8. Lipid Systems: Solid Lipid Nanoparticles

Composition of lipid formulation	Drug used	Purpose of the study	Outcome of the study	Reference
Mixtures of follows at different ratios Dynasan 116 Lecithin Tetronic 908 Pluronic F127	-	To investigate the gel formation of phospholipid-stabilized SLN	Gel formed because of crystallization of melt-homogenized tripalmitate and it can be prevented by the addition of co-emulsifier	Westesen & Siekmann, 1997
5% one of the lipid (cetylpalmitate, Dynasan 116 or Dynasan 118) 0.5% one of the surfactant solution (Cholic acid sodium salt, Lipoid E80, Poloxamer 407 or Tween 80	-	To investigate the effect of surfactant and chain length of fatty acid on enzymatic degradation of SLN	Longer the fatty acid chains in the glycerides provided the slower the degradation. Cholic acid sodium accelerated the degradation while poloxamer 407 slowed down	Olbrich & Muller, 1999
10% (m/m) Compritol 888 ATO 1.2% (m/m) Pluronic F68 Water to make 100%	-	To investigate long-term stability of SLN in terms of crystallinity of lipid phase	Crystallization occurred during the storage leading gelation	Freitas & Muller, 1999

Table 9.Lipid Systems: Cubic Phase

Composition of lipid formulation used	Drug used	Purpose of the study	Outcome of the study	Reference
– Glyceryl monooleate (GMO)		To form Cubic phase dispersion called Cubosome™ and compare with emulsion and liposome formulations	Lipophilic drugs incorporated better to Cubosome than to emulsion	Engstrom, 1990
– 10% (w/w) drug in Glyceryl monooleate (GMO)	Griseofulvin MW=352, antifungal Ibuprofen MW= 206, analgesic Propranolol MW= 259, β -blocker	To investigate the effect of cubic phase formed with GMO on dissolution profiles of drug binding of drugs to mono-glyceride matrix systems and the influence of the addition of drugs on the phase behavior	The solubility of drugs in mono-glyceride and surface tension of drugs affected the dissolution profiles and phase transitions. Above the certain concentrations, the drugs having good solubility in glyceryl monooleate transformed the cubic phase into an inverted hexagonal phase	Chang and Bodmeier, 1997
Cefazolin (50 and 200 μ g/g) and cefuroxime (200 μ g/g) in GMO	Cefazolin & Cefuroxime MW = 454 & 424, antibiotics	To evaluate the ability of the cubic phase gel to act as a chemical stability enhancer	The enhanced stability of both drugs in GMO cubic phase gel has been demonstrated	Sadhale & Shah, 1998

Table 10: Solubility of griseofulvin and nifedipine in lipid/Chremophor EL mixtures

Lipid vehicle	Lipid/Chremophor EL ratio (g/g)	Griseofulvin solubility (mg/g) (mean $\pm$ SEM)	Nifedipine solubility (mg/g)
Miglyol 810 Glyceryl tri caprylate (C ₈)	10/0	0.683 $\pm$ 0.008	3.359 $\pm$ 0.029
	9/1	0.867 $\pm$ 0.004	4.863 $\pm$ 0.043
	8/2	1.001 $\pm$ 0.007	10.183 $\pm$ 0.087
	7/3	1.578 $\pm$ 0.008	17.940 $\pm$ 0.123
	6/4	2.249 $\pm$ 0.005	23.702 $\pm$ 0.091
	5/5	4.235 $\pm$ 0.025	18.276 $\pm$ 0.083
Capmul MCM Glyceryl mono- caprylate (C ₈)	10/0	4.031 $\pm$ 0.005	10.273 $\pm$ 0.091
	9/1	6.542 $\pm$ 0.028	14.368 $\pm$ 0.037
	8/2	6.696 $\pm$ 0.059	16.738 $\pm$ 0.047
	7/3	6.069 $\pm$ 0.032	23.873 $\pm$ 0.089
	6/4	6.475 $\pm$ 0.048	23.720 $\pm$ 0.023
	5/5	5.915 $\pm$ 0.019	26.478 $\pm$ 0.124
Capmul GMO Glyceryl mono-oleate (C _{18:1})	10/0	3.292 $\pm$ 0.019	3.115 $\pm$ 0.111
	9/1	3.127 $\pm$ 0.008	8.878 $\pm$ 0.094
	8/2	3.776 $\pm$ 0.043	11.758 $\pm$ 0.087
	7/3	5.235 $\pm$ 0.034	16.456 $\pm$ 0.108
	6/4	8.457 $\pm$ 0.062	18.591 $\pm$ 0.056
	5/5	6.110 $\pm$ 0.059	24.825 $\pm$ 0.083
Caprol 2GO 2-glyceryl mono-oleate (C _{18:1})	10/0	2.275 $\pm$ 0.006	3.026 $\pm$ 0.037
	9/1	2.916 $\pm$ 0.012	7.055 $\pm$ 0.148
	8/2	3.551 $\pm$ 0.005	10.221 $\pm$ 0.097
	7/3	4.005 $\pm$ 0.025	14.657 $\pm$ 0.084
	6/4	4.278 $\pm$ 0.009	19.548 $\pm$ 0.174
	5/5	4.567 $\pm$ 0.025	24.143 $\pm$ 0.267

Table10: Solubility of griseofulvin and nifedipine in lipid/Chremophor EL mixtures (continued)

Lipid vehicle	Lipid/Chremophor EL ratio (g/g)	Griseofulvin solubility (mg/g) (mean± SEM)	Nifedipine solubility (mg/g)
Caprol3GO 3-glyceryl mono-oleate (C _{18:1})	10/0	1.386 ± 0.002	3.193 ± 0.099
	9/1	1.975 ± 0.015	6.217 ± 0.122
	8/2	2.494± 0.008	10.679 ± 0.165
	7/3	2.554± 0.005	12.353 ± 0.465
	6/4	3.133± 0.018	19.385 ± 0.345
	5/5	3.900± 0.030	24.304 ± 0.655
Capmul PG-8 Propylene glycol mono- caprylate (C ₈)	10/0	3.774 ± 0.018	16.983 ± 0.555
	9/1	4.497± 0.028	19.256 ± 0.426
	8/2	4.758± 0.020	23.304 ± 0.457
	7/3	5.342± 0.019	27.750 ± 0.589
	6/4	7.883± 0.033	31.226 ± 0.891
	5/5	6.516± 0.186	40.032 ± 0.924
Capmul PG-12 Propylene glycol mono- laurate (C ₁₂)	10/0	2.197 ± 0.006	8.118 ± 0.243
	9/1	2.376 ± 0.012	10.717 ± 0.094
	8/2	3.410 ± 0.014	13.287 ± 0.243
	7/3	4.250± 0.007	18.792 ± 0.145
	6/4	4.755 ± 0.026	23.359 ± 0.456
	5/5	6.067 ± 0.043	18.055 ± 0.754
Capmul PG-18 Propylene glycol mono- oleate (C _{18:1})	10/0	1.913 ± 0.007	5.068 ± 0.035
	9/1	1.658 ± 0.012	8.728 ± 0.216
	8/2	2.255 ± 0.020	10.990 ± 0.556
	7/3	2.280 ± 0.026	14.683 ± 0.423
	6/4	2.929 ± 0.013	21.050 ± 0.589
	5/5	4.142 ± 0.044	30.176 ± 0.812

Figure 2. Effect of HLB on release rate of Ro-0778 (ref. Shah et al, 1994)

A. Labrafil M2125; B. Labrafac Hydro; C. Labrafac CM6 BM290; D. Labrafil WL2609; E. Lanrafac CM8Bm 284; F. Labrafac CM 10 BM 287; G. Labrasol

Figure 3. Effect of cooling rate on viscosity of wax based vehicle at different temperatures (Shah et al., 1996)

Figure 6. Effect of acyl chain length on the rate of triglyceride lipolysis (Hutchison, 1994)

SECTION I

OBJECTIVES

- a. To evaluate solvent properties the lipids available in the US using two poorly soluble drugs, nifedipine and griseofulvin.
- b. To select most suitable lipids and prepare nifedipine lipid formulation to evaluate its dissolution properties.
- c. To assess the bioavailability of the nifedipine formulations in beagle dogs and to seek a correlation of *in vivo* data to that of *in vitro* test results.

SECTION II

MANUSCRIPT II

A PRACTICAL APPROACH TO DETERMINE CONTRIBUTION OF MEASURED AND CALCULATED PHYSICAL AND CHEMICAL PARAMETERS ON SOLVENT EFFECT OF LIPIDS

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Keywords: Lipids, poorly soluble drugs, properties of lipids, lipid solubility

1. INTRODUCTION

The use of lipid vehicles has generated considerable attention in recent years to improve the bioavailability of poorly soluble drugs. The well-known effect of food in improving the bioavailability of many poorly soluble drugs such as griseofulvin, carbamazepine and danazol is the evidence that lipids can be beneficial to drug absorption (Charman et al., 1992, 1993, 1997; Humberstone and Charman, 1997). Lipids can improve the bioavailability of drugs through several mechanisms. Enhancing the solubility/ dissolution of the drug is one of the commonly observed effects (Pozzi et al., 1991; Sheen et al., 1991; Charman et al., 1992; Shah et al., 1994; Matuszewska et al., 1996; Aungst et al., 1997).

Although lipids have the potential for enhancing drug absorption, only few commercial formulations of lipid-based formulations are available on the market. These are cyclosporine (Sandimmune™ and Neoral™, Novartis), saquinavir (Fortovase™, Roche), ritonavir (Norvir™, Abbott) and fat-soluble vitamins. One of the reasons for rarity of commercially available lipid-based products is the physical complexity of lipids.

Drug solubility in a lipid is the most important factor in the selection of a lipid as a vehicle. However, due to their physical complexity such as existing as mixtures, solubility of drugs in lipids is a poorly understood concept. Formulation scientists many times select an effective lipid that dissolves a given lipophilic drug by trial and error. The lipids that are screened for this purpose as routine are given in Table 1. In

general, the lipids that contain a fatty acid distribution and ester combinations provide better solubility than purer ones. For example, Capmul MCM that contains caprylic (C_8) and capric acid (C_{10}) provides better solubility than Capmul MCMC8 that contains only caprylic acid. Since liquid type lipid or lipid combinations are used in the oral formulations as routine, solubility studies are limited to oily vehicles.

There are a number of studies to show the effects of the physiochemical properties of the drug on lipid solubility. The melting point and partition coefficient of the drugs are the key factors affecting solubility in trioleates (Yamaoka et al, 1983; Patton et al, 1984; Mithani et al., 1995; Alvarez-Nunez and Yalkowsky, 2000 and Larsen et al., 2002). However, systematic studies showing a relationship between lipid solubility of a drug and physicochemical properties of lipid are limited. Only Anderson and Marra, (1999) reported chemical properties that contributed solvent property of synthetic and natural oils, tributyrin and tricaprylin. It was shown that as the ester concentration of the lipid increased, the solubility of an investigational anti-HIV agent in the lipids used increased. It has been also shown that oils having larger molecular volumes (ethyl oleate, Miglyol 812 and soybean oil) solubilized testosterone less than the oils with smaller molecular volumes (ethyl butyrate and ethyl caprylate) (Malcolmson et al., 1998 and Warisnoicharoen et al., 2000).

In general, solvent properties of lipids depend on the chemical, electrical and structural effects. The polarity, hydrophille-lipohille balance, ester concentration,

solubility parameters, surface tension and molecular volume can play a role in their solvent property.

Polarity of a solvent plays an important role in the solubility. Polar solvents are capable of solvating molecules through dipole interaction forces, particularly via hydrogen-bond formation, which is a major mechanism in the solubility of a compound. Polarity of solvents can be defined by dielectric constant (ϵ), which is an important property related to the solubility and hydrophilic-lipophilic balance (Gorman and Hall, 1963 and 1964, Carstensen, J.T., 1971, Cave et al, 1979 and Rabaron et al. 1993). It has been shown that the solubility of a solute decreased as the dielectric constant of solvent decreased (Carstensen et al., 1971, Trivedi et al., 1996). Therefore, dielectric constants of lipids can be evaluated to predict their solvent properties.

An understanding of cohesive energy between the drug and the lipid molecules may help to determine how a lipid will behave as a solvent. Cohesion is result of the London forces, polar interactions and specific ones like hydrogen bonding (Hansen and Alan, 1971, Barton, 1975). The most commonly used approach in quantifying the cohesion between a solvent and a solute is the solubility parameter, δ , which is defined as the square root of the cohesive energy density, expressed as the energy of vaporization.

$$\delta = (\text{CED})^{1/2} = (\Delta E_v/V_m)^{1/2} \quad (1)$$

Where CED is cohesive energy density ΔE_v is the energy of vaporization and V_m is the molar volume.

This parameter may be useful to predict the solvating ability of a lipid or the lipid mixture. When solubility parameters of lipid and the drug are similar, they are expected to become miscible (Scatchard, 1931, Small, 1953, Krevelen and Hoftyzer, 1972). Determining the solubility parameter of a lipid vehicle and thereby quantifying its cohesive energy may offer a solution for screening solvent properties of the lipid candidates.

There are numbers of indirect and direct methods available that can be used to determine solubility parameter. The practical methods include vapor pressure or boiling point determinations, solubility/miscibility measurement in liquids with known cohesive energy, solution calorimetry, and surface energy measurements.

Theoretically, and more often the group contribution method is used (Fedors, 1974, Samaha et al., 1990, Subrahmanyam et al., 1996, Hancock et al, 1997, Breitreutz, 1998, Ohta et al., 1999). According to this calculation, the solubility parameter:

$$\delta_F = [\sum \Delta e / \sum \Delta v]^{1/2} \quad (2)$$

Where Δe : The additive atomic group contributions for the energy of vaporization

Δv : The additive atomic group contributions for the molar volume

In this study, the group contribution method given above (2) was used to calculate the solubility parameter from knowledge of the structural formula of the selected lipids and drug compounds. This approach is especially useful in the initial stages of the

pharmaceutical research and development process, where the properties of a new chemical entity, which is very often in short supply, can be estimated in light of very little experimental data. Because of lipid's instability during heating, their boiling point often can not be obtained. Therefore, Fedor's method appears to be the most applicable one to calculate solubility parameters of lipids.

The hydrophilic-lipophilic balance (HLB) was developed by Griffin to characterize the polarity of nonionic surfactants. Most of the lipids used in oral drug delivery have surface-active properties. For example, Capmul MCM has HLB value of 5-6 like Spans. Capmul MCM has surface tension of around 26 dynes/cm². Like surfactants, the interaction between a lipophilic drug and the lipid can take place at the hydrophobic part of the lipid vehicle. HLB of surfactants like nonoxynols was correlated with their solubility parameters (Samaha and Naggar, 1988, Schott, 1984 and 1995, Poulain et al., 1997). Similarly, such correlation may exist in lipids. If so this property can be a very useful tool to select a suitable lipid to dissolve a given drug.

It has been also shown that surface free energy of solids was correlated well with solubility parameters (Samaha and Naggar, 1990). Therefore, surface tension, defined as the surface free energy change per unit area increase, can be also a useful tool to predict solvent property of lipids.

Saponification value is another important chemical property to predict the solvent power of lipids. It is defined as the weight of KOH used to saponify the ester and fatty acids. Therefore, it has been used to earlier obtain ester concentration and correlated well with solvent power of lipids (Anderson and Marra, 1999).

Most of the commonly used solvents like water, ethanol and methanol are small molecules. Lipids differ from these solvents in terms of molecular weight and purity. Lipids like triglycerides are much larger molecules. Therefore, they cannot accommodate large amount of solute. It has been also shown that the oils with larger molar volume like ethyl oleate, Miglyol 812 and soybean oil solubilized testosterone less than the oils with smaller molar volumes (ethyl butyrate and ethyl caprylate) (Malcolmson et al., 1998).

The aim of this study is to seek a relationship among the calculated and measured properties of lipids (MW, SAP, dielectric constant, HLB, solubility parameter and surface tension) on estimating the solubility of the selected poorly soluble drugs. The effect of hydrophilic head (glycerine vs. propylene glycol) and lipophilic tail (fatty acid chain length) of lipid has been also evaluated. Multiple linear regression (MLR) used to predict aqueous solubility of various drugs previously (Jorgensen and Duffy, 2002) can be utilized to predict the solubility of lipophilic drugs in lipids. MLR is the technique of predicting a response by a linear combination of several variables. In this statistical analysis, the variables that are highly correlated to each other should not be used. Correlation coefficient between the properties given above and the solubility of

the drugs in lipids and the contribution of each property can be found with MLR. The predictive ability of the model can be tested by regression coefficient which can be facilitated with a statistical package like JMP®. Nifedipine and griseofulvin have been selected as the model lipophilic drugs for this study. Nifedipine (Figure 1) is a pyridine carboxylate and griseofulvin (Figure 2) is benzofuran dione. Both drugs have similar MW around 350.

2. MATERIALS AND METHODS:

2.1. Materials

2.1.1. Model Drugs

Nifedipine (Figure 1) is a calcium antagonist, which is used to treat angina and hypertension. It is 3, 5-pyridinedicarboxylic acid, 1, - dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester. It has a molecular weight of 346.34. Nifedipine is practically insoluble in water (9.5 µg/ml at 25°C) and less soluble in ethanol. It is also light sensitive and converts to a nitrosophenylpyridine derivative when exposed to daylight and UV light. Therefore, all nifedipine experiments were carried out under yellow light.

Griseofulvin (Figure 2) is an antifungal agent (7-Chloro-2', 4, 6-trimethoxy-6'-methylspiro[benzofuran-2(3H), 1'-[2]cyclohexene]-3,4'-dione). It is practically insoluble in water (18 µg/ml at 25°C) and slightly soluble in ethanol. It has molecular weight of 352.77. It is a classical example used as a model to study solubility related problems.

2.1.2. Lipids

Table I. lists the lipids used in this study. Their fatty acids distribution is also presented in the same table. The lipids used were limited to the liquid. Selection of the liquid lipid has been made in such a way that the effect of hydrophilic head (glyceryl vs. propylene glycol), number of glyceryl groups and lipophilic tail (fatty acid chain lengths) can be investigated. There was no purification involved; they were used as obtained so that the findings can be helpful for the formulation scientists who use these materials as provided.

2.2. Methods

2.2.1. Experimental Solubility Determination

The solubility of the drugs in each lipid vehicle and water was determined by adding an excess amount of the drug into 10 g vehicle and equilibrating the mixture at $25^{\circ} \pm 0.1^{\circ}\text{C}$ for 72 hr. The mixture was centrifuged for 10 minutes and filtered through a $0.45\mu\text{m}$ filter. The amount of griseofulvin dissolved was determined by UV spectroscopy at a wavelength of 328 nm. The calibration curve used for calculations is given in the appendix, Figure1. Nifedipine dissolved was quantified by an HPLC method. A Hewlett Packard 1050 HPLC system with a UV detector ($\lambda = 236\text{ nm}$) was used. The stationary phase consisted of a micro Bondapak, C_{18} reverse phase column (3.9 x 300 mm, Waters Corp., Milford, MA). The mobile phase used was acetonitrile: methanol: water (2: 3: 3). The samples

were diluted with methanol before the run. The flow rate was 1.0 mL/min with 30 minutes of total run time per injection.

A standard solution for Nifedipine (0.1 mg/mL) and lipid vehicles was prepared in methanol and was run with HPLC. The data acquisition was made using the software Turbochrome 6.1.1.0.0 (Perkin Elmer Corp, Wellesley, MA). Nifedipine standard curve for the method is given in the Appendix, Figure 2. HPLC chromatographs of lipids were also obtained to make sure that they don't interfere with nifedipine peak. All studies were performed in triplicate and coefficient of variance (CV) for precision of the experiments was $\pm 2\%$.

2.2.2. Surface Tension

A Kruss K 12 Tensiometer (Kruss USA, Charlotte, NC) was used to determine surface tension. The plate method was selected. Lipids were equilibrated and surface tension was measured at 25°C with platinum plate cleaned with flame.

2.2.3. Density Measurements

The density of lipids was measured to use in molecular volume calculations.

The density of a lipid was determined using a Paar Oscillating U-Tube (Anton PAAR, Ashland, VA) at 25°C. The instrument was calibrated with water. The U-Tube was filled slowly with the lipid by making sure that there are no air bubbles. The display after equilibration was recorded as density (g/cm^3).

2.2.4. FT-IR studies

IR spectra for drugs, vehicle and solution were obtained using a Nicolet FT-IR with Attenuated Total Reflection (ATR) accessory (Nicolet Analytical, Madison, WI). The lipid and lipid solution of the drug was added to a sample holder. Solid samples were diluted in mineral oil. First, the spectra of lipids and the model drugs were taken (Appendix, Figures 3-15). The spectra of drugs solution in lipids were also recorded. Omnic software Version 6 (Nicolet Analytical, Madison, WI) was used to obtain spectra and for subtraction of lipid spectrum from solution spectrum.

2.2.5. HLB calculation

The HLB value of each lipid vehicle was estimated as follows:

$$HLB = 20 \left(1 - \frac{S}{A}\right) \quad (3)$$

Where S is the saponification number of the ester and A is the acid number of the fatty acid. The HLB of Capmul MCM (Glyceryl mono-caprylate), for which S=250 and A= 370, is $20 \left(1 - \frac{250}{370}\right) = 6.5$.

2.2.6. Calculation of Solubility Parameters

Solubility parameters (δ_f) of lipids and drugs were calculated using the group contribution method devised by Fedors (Eq. 2)

Using equation (2), calculation of solubility parameter of oleic acid $\{CH_3-(CH_2)_6-CH_2-CH=CH-CH_2-(CH_2)_6-COOH\}$ is as follows:

(Refer to Table II)

$$\delta_F = [\sum \Delta e / \sum \Delta v]^{1/2} = [26305/314.4]^{1/2} = 9.1469 \text{ (cal/cm}^3)^{1/2} \quad (2)$$

In this model, the contribution of hydrogen bonding is not included. Therefore, hydrogen bonding contribution was calculated as:

$$\delta_H = (5000m/V)^{1/2} \quad (4)$$

where m is the number of hydrogen donor and acceptors, and V is the molar volume (MW/density)

Total solubility parameter (δ_T) was calculated by adding hydrogen bonding contribution (δ_H) to the Fedor's solubility parameter (δ_F):

$$\delta_T = (\delta_F^2 + \delta_H^2)^{1/2} \quad (5)$$

Solubility parameters for griseofulvin and nifedipine were calculated by equation (5). Griseofulvin has δ_G of $14.77 \text{ (cal/cm}^3)^{1/2}$ and nifedipine has $\delta_N = 17.38 \text{ (cal/cm}^3)^{1/2}$. These findings differ from the calculations that appear in the literature as $\delta_G = 10.44$ and $\delta_N = 10.75$ (Hancock et al, 1997, Squillante et al., 1997) due to addition of hydrogen bonding contributions.

2.2.7. Refractive Index Measurements and Calculations of Dielectric Constant

The refractive index (N) of each lipid vehicle was measured at 25°C using Abbe Mark II Model 10480 Digital refractometer (Sodium light at 589 wavelengths) (Cambridge Instrument Inc., Buffalo, NY).

Dielectric constant (ϵ) of each vehicle was calculated using the equation (6).

$$N^2 = \epsilon \quad (6).$$

2.3. Statistical Analyses

In order to evaluate the contribution of all the factors together, a stepwise linear regression analysis has been selected for multiple linear regression analyses of factors that affect the solubility of drugs. It is an approach to select a subset of effects for a regression model and facilitates searching and selecting among many models. Stepwise regression is used when there is little theory to guide the selection of terms for a model and the modeler. It can be done with forward or backward elimination methods. Forward brings in the regressor that most improves the fit, given that term is significant at the level specified by probability to enter. Backward removes the regressor that affects the fit the least, given that term is not significant at the level specified in probability to leave. Backward elimination method has been used in this study. The correlation coefficient (R_{adj}^2) and coefficient for each factor was determined. The model used for this analysis is as follows:

$$S = \sum a_i c_i + a_0 \quad (7)$$

Where S is the solubility, a_i is the coefficient determined by regression analysis to maximize R^2 , c_i is the factor (one of the physicochemical properties of lipid: MW, dielectric constant, saponification number, surface tension and solubility parameter), i is the set of factor and a_0 is the intercept of the linear regression.

The effect of lipid's lipophilic tail (fatty acid chain length), hydrophilic head (glycerol or propylene glycol and number of glycerol) has been investigated using single factor ANOVA. The model used for this analysis:

$$S_{ij} = \mu + \Gamma_i + \epsilon_{ij} \quad (8)$$

Where S is the solubility of the drug, i is the level of each factor: The factor lipophilic tail had 3, glyceryl vs. propylene glycol had 2 and number of glycerol had 3 levels, j is repetition (solubility experiments were conducted in triplicates), μ is mean, Γ is the effect of treatment and ϵ is the random error.

JMP Statistical Software version 4.0.4 (SAS Institute Inc, Cary, NC) has been used for all analyses. Significance level of 0.05 has been used for the analyses. The models adequacy has been checked with residual analysis and lack of fit.

3. RESULTS AND DISCUSSION

The saturated solutions of griseofulvin and nifedipine in each lipid when viewed under a microscope were all crystal free with exception of griseofulvin in glyceryl mono-oleate (GMO) which probably dissolved slowly by forming liquid crystals, Figure 3. The FT-IR studies demonstrated no measurable chemical interaction between the drugs and lipid vehicles. Figure 4 is an example of the FT-IR spectrum for nifedipine in Capmul Pg-8. Possible interactions of a lipid and the drug may occur through the hydrogen bonding and the other polar and hydrophobic interactions. However, there is no changes in the band appearing at 3400 cm^{-1} corresponding to hydrogen bond stretching (O-H), at 1740 cm^{-1} corresponding to carboxylic acid and the bands between 1470 and 1200 cm^{-1} which correspond to C-H and O-H bending. Since there is no chemical interaction was observed, the interaction between the lipid and the drug may be the result of London forces or polar interactions.

The experimental solubility results for griseofulvin in the tested lipids ranged from 0.318 to 4.031 mg/g and the solubility of nifedipine from 0.322 to 16.983 mg/g (Table III). Glyceryl mono-caprylate (Capmul MCM) was the best solvent for griseofulvin increasing the solubility 224 times compared to water. For nifedipine, propylene glycol mono-caprylate (Capmul PG-8) was the best solvent and the solubility was increased 1790 times as compared to water solubility.

Lipids used were better solvents for nifedipine than for griseofulvin in increasing solubility because nifedipine has higher lipophilicity with a log P of 4 (Squillante et al., 1997) compared to griseofulvin with log P of 2 (Mithani et al, 1996 and Nelsen et al. 2001). The solubility values obtained were used to correlate them with solubility parameter, MW, polarity and surface tension of lipids that are given in Table IV.

The solubility parameter calculated for griseofulvin is $\delta_G = 14.77 \text{ (cal/cm}^3)^{1/2}$. Capmul MCM that has the closest solubility parameter to that of griseofulvin provided the highest solubility among all lipids used. However, the same correlation could not be observed with nifedipine. The calculated solubility parameter for nifedipine is $\delta_N = 17.38 \text{ (cal/cm}^3)^{1/2}$ and the lipid that has closest solubility parameter is glyceryl mono-caprylate/caprate (Capmul MCM; $\delta = 15.12 \text{ (cal/cm}^3)^{1/2}$) but the highest solubility is provided by propylene glycol mono-caprylate (Capmul PG-8). Overall, calculated solubility parameter appeared to be a poor predictor for the expected solvent effects of the lipids.

The comparison of solubility of the drugs and HLB of lipids showed that only the solubility of griseofulvin correlated well with HLB of the lipids. As the HLB of the lipid increased, the solubility of griseofulvin increased, Figure 5, with a correlation coefficient of 0.9014. However, the solubility of nifedipine did not show any correlation with HLB of the lipids.

Comparing the solubility of drugs against an individual property of the selected lipid did not show any common correlation. Therefore, stepwise analysis has been conducted to evaluate overall effects of all variables. The stepwise analysis has been selected because there are independent multiple variables. The factors included into analyses are MW, dielectric constant (DC), SAP, surface tension (ST) and solubility parameter (δ). HLB was excluded from the the model because it is calculated using SAP value. Regression coefficient and significance (p) were evaluated to determine the weight of contribution of each property of lipids.

Table V shows stepwise analyses steps for prediction of nifedipine solubility and Table VI for griseofulvin. As it can be seen from the tables, MW, DC, SAP and surface tension were significant factors for nifedipine ($p < 0.05$). Solubility parameter is not significant factor ($p > 0.05$) so that it has been removed from the model. On the other hand, SAP is not a significant factor for griseofulvin solubility while MW, DC, surface tension are significant factors ($p < 0.05$). From these analyses, the relationship to estimate the solubility of nifedipine (S_{nif} , mg/g) can be expressed as $(Radj)^2 = 0.8686$):

$$S_{nif} = -173 + (-0.054)MW + (80.95)DC + (0.056)SAP + (0.52)ST \quad (9)$$

Where MW is molecular weight, DC is dielectric constant, SAP is saponification number and ST is surface tension (dyne.cm⁻²).

The relationship to estimate the solubility of griseofulvin (S_{gris} , mg/g) can be expressed as: ($R^2 = 0.8187$)

$$S_{gris} = -24.61 + (-0.0082)MW + (14.14)DC + (-0.14)ST + (0.34)\delta \quad (10)$$

Where MW is molecular weight, DC is dielectric constant, ST is surface tension (dyne.cm⁻²) and δ is solubility parameter ((cal/cm³)^{1/2}).

The common properties of the lipids that contribute for the solubility of both drugs are MW, dielectric constant and surface tension. The contribution of MW is the highest for both drugs ($R^2 = 0.7721$ for nifedipine and $R^2 = 0.5701$ for griseofulvin). However, the estimates of each property are different between two drugs indicating a major role of the drug in lipid solubility. Saponification value representative of ester concentration was not a commonly shared contributor for both drugs as opposed to previously shown (Anderson and Marra, 1999). Therefore, it cannot be generalized. It can be a contributor for more lipophilic drugs like nifedipine as opposed to griseofulvin.

The effect of hydrophilic head (glycerine vs. propylene glycol, number of glyceryl groups) and lipophilic tail (fatty acid chain length) of lipid has been tested with ANOVA for both drugs in accordance with the model described in Section 2.3,

Equation 8. The results in Table VII showed that the effect of fatty acid chain length is significant for both drugs ($p < 0.05$). The solubility of drugs was increased as the alkyl chain decreased. Also, griseofulvin being more hydrophilic than nifedipine seems to be more sensitive to and affected from hydrophilic head changes.

4. CONCLUSIONS

In this study, it has been shown that the lipids used improved the solubility of nifedipine and griseofulvin compared to the solubility of drugs in water. Calculated solubility parameter was not sufficient to predict the solubility of drugs in the lipids. Calculated and measured properties of lipids analyzed with stepwise regression analyses showed that MW, dielectric constant, surface tension are the common factors that govern the lipid solubility. However, the estimates of each factor were different for each drug indicating that the drug played important role in lipid solubility. Overall results showed that MW, dielectric constant, surface tension and fatty acid chain length contribute the solvent action of lipids.

5. ACKNOWLEDGMENT

This study is part of Ms. Dumanli's Ph.D. dissertation research supported by Hoffmann-La Roche. The research was conducted at Hoffmann-La Roche facilities in Nutley, NJ. The main author wishes to thank to Dr. Jyh-Hone Wang of Industrial Engineering, University of Rhode Island for his help in statistical analyses and to Dr. Rodolfo Pinal of Hoffmann-La Roche for his valuable discussion on this topic.

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Table I: Lipid vehicles used in this study

Chemical Name	Trade Names	Fatty acid distribution*
Oleic acid (C _{18:1})	Emersol6313 (Henkel)	75 % C _{18:1} 6% C _{18:2} 6% C _{16:2} 5% C ₁₆ 3% C _{14:1} 3% C ₁₄ 2% C _{18:3}
Glyceryl tri-oleate	Captex GTO (Abitec)	92% C _{18:1} 4% C _{18:2} 3% C ₁₈
Caprylic (C ₈) / Capric (C ₁₀) triglyceride	Miglyol 810 (Sasol)	71% C ₈ 29% C ₁₀
	Captex 300 (Abitec)	67% C ₈ 33% C ₁₀
Mono-and di-glyceride of caprylic(C8)/capric(C10) acid	Capmul MCM (Abitec)	81% C ₈ 17.4% C ₁₀
Glyceryl mono-oleate	Capmul GMO (Abitec)	80% C _{18:1} 4% C ₁₆ 4% C ₁₈ 11% C _{18:2}
Diglycerylmonooleate	Caprol 2G0 (Abitec)	90% C _{18:1} 3% C ₁₈ 3% C _{18:2}
Triglyceryl monooleate	Capmul 3GO (Abitec)	90% C _{18:1} 3% C ₁₈ 3% C _{18:2}
Propylene glycol monocaprylate	Capmul PG-8 (Abitec)	99% C ₈ 1% C ₁₀
Propylene glycol mono-laureate	Capmul PG-12 (Abitec)	99% C ₁₂
Propylene glycol mono-oleate	Capmul PG-18 (Abitec)	93% C _{18:1} 3% C ₁₈ 3% C _{18:2}
Propyleneglycol dicaprylate (C8)/dicaprinate (C10)	Captex 200 (Abitec)	64% C ₈ 34% C ₁₀ 2% C ₆

* C₆: Caproic, C₈: Caprylic, C₁₀: Capric, C₁₂: Lauric, C₁₄: Myristic, C_{14:1}: Myristoleic, C₁₆: Palmitic, C_{16:1}: Palmitoleic, C₁₈: Stearic, C_{18:1}: Oleic, C_{18:2}: Linoleic and C_{18:3}: Linolenic acid

Table II: Group contributions to the energy of vaporization and the molar volume at 25°C for the functional groups of oleic acid

Group	Δe (cal/mol)	$\Delta v(\text{cm}^3/\text{mol})$
1 CH ₃	1125	33.5
14 CH ₂	16520	225.4
1 COOH	6600	28.5
2 -CH=	2060	27
Total	26305	314.4

Table III: Experimental solubility results for nifedipine and griseofulvin

Lipid vehicle	Griseofulvin solubility mg/g (mean $\pm$ SEM, n=3)	Nifedipine solubility mg/g (mean $\pm$ SEM, n=3)
Emersol 6313 (Henkel) Oleic Acid (C _{18:1})	0.951 $\pm$ 0.006	0.3220 $\pm$ 0.0269
Captex GTO (Abitec) Glycerltrioleate(C _{18:1})	0.318 $\pm$ 0.015	1.3028 $\pm$ 0.0103
Miglyol 810 (Huls) Glyceryl tri caprylate(C ₈)	0.683 $\pm$ 0.008	3.3595 $\pm$ 0.0292
Capmul MCM (Abitec) Glyceryl monocaprylate(C ₈)	4.031 $\pm$ 0.005	10.2733 $\pm$ 0.0913
Capmul GMO (Abitec) Glycerylmonooleate (C _{18:1})	3.292 $\pm$ 0.019	3.1149 $\pm$ 0.1112
Caprol2GO (Abitec) Diglycerylmonooleate(C _{18:1})	2.275 $\pm$ 0.018	3.0264 $\pm$ 0.03712
Caprol 3GO (Abitec) 3 glycerylmonooleate (C _{18:1})	1.386 $\pm$ 0.002	3.1930 $\pm$ 0.0996
Capmul PG-8 Propyleneglycol monocaprylate (C ₈)	3.774 $\pm$ 0.018	16.9831 $\pm$ 0.5556
Capmul PG-12 Propyleneglycol monolaurate (C ₁₂)	2.197 $\pm$ 0.006	8.1186 $\pm$ 0.2429
Capmul PG-18 Propyleneglycol monooleate (C _{18:1})	1.913 $\pm$ 0.007	5.0682 $\pm$ 0.0353
Captex 200(Abitec) propylene glycol dicaprylate(C ₈)	0.817 $\pm$ 0.004	4.1052 $\pm$ 0.0668
water	0.018 $\pm$ 0.001	0.0095 $\pm$ 0.0005

Table IV: Calculated and measured properties of lipids used

Lipid	SAP	δ (cal/cm ³) ^{1/2}	HLB	Density (g/cm ³)	MW	Molar volume (cm ³)	Refractive index	Dielectric constant	Surface tension (mN/m) at 25°C
Oleic acid	-	10.54	1	0.8886	282	307	1.4585	2.1272	32.40
Captex GTO	190	10.63	1	0.9123	863	946	1.4685	2.1565	32.78
Miglyol810	340	12.16	1.5	0.9437	495	525	1.4477	2.0958	29.58
CapmulMCM	250	15.12	6.5	1.0022	275	273	1.4508	2.1048	28.21
CapmulGMO	165	12.12	4	0.9451	490	518	1.4688	2.1574	31.36
Caprol2GO	141	12.26	5	0.9814	421	429	1.473	2.1697	29.77
Caprol3GO	143	14.29	6	0.9776	494	505	1.4728	2.1691	37.61
CapmulPG-8	300	13.19	5	0.9387	202	215	1.4363	2.0630	29.3
CapmulPG-12	225	12.26	4	0.9183	258	245	1.4444	2.0863	29.53
CapmulPG-18	162	11.44	3.5	0.9096	340	374	1.4592	2.1293	32.15
Captex 200	329	11.82	3	0.9173	328	375	1.4397	2.0727	30.17

SAP: Saponification, HLB: Hydrophille-lipophile balance, MW: Molecular weight, δ : Solubility parameter

Table V. Linear regression analysis with stepwise fit for solubility of nifedipine

Stepwise Regression Control

Alpha 0.05

Current Estimates						
SSE	DFE	MSE	RSquare	RSquare Adj	Cp	AIC
58.907758	19	3.100408	0.8914	0.8686	4.020048	31.55006
Parameter	Estimate	nDF	SS	"F Ratio"	"Prob>F"	
Intercept	-173.23706	1	0	0.000	1.0000	
MW	-0.0545075	1	117.7817	37.989	0.0000	
Dielectric constant	80.958237	1	14.02595	4.524	0.0467	
SAP	0.05630996	1	38.5523	12.435	0.0023	
Surface tension	0.5228523	1	23.83656	7.688	0.0121	
Solubility parameter	.	1	0.065537	0.020	0.8890	

Step History						
Step	Parameter	Action	"Sig Prob"	Seq SS	RSquare	Cp
1	MW	Entered	0.0000	418.9455	0.7721	17.824
2	Dielectric constant	Entered	0.0443	22.12014	0.8129	13.057
3	SAP	Entered	0.0457	18.78224	0.8475	9.3117
4	Surface tension	Entered	0.0121	23.83656	0.8914	4.02
5	Solubility parameter	Entered	0.8890	0.065537	0.8916	6
6	Solubility parameter	Removed	0.8890	0.065537	0.8914	4.02

Table VI. Linear regression analysis with stepwise fit for solubility of griseofulvin

Stepwise Regression Control

Alpha 0.05

Current Estimates						
SSE	DFE	MSE	RSquare	RSquare Adj	Cp	AIC
3.9353886	19	0.207126	0.8502	0.8187	4.2928	-33.3931
Parameter	Estimate	nDF	SS	"F Ratio"	"Prob>F"	
Intercept	-24.609009	1	0	0.000	1.0000	
MW	-0.0082946	1	7.90434	38.162	0.0000	
Dielectric constant	14.1378874	1	2.821204	13.621	0.0016	
SAP	.	1	0.062991	0.293	0.5951	
Surface tension	-0.1443072	1	2.321461	11.208	0.0034	
Solubility parameter	0.34747891	1	3.551889	17.148	0.0006	

Step History

Step	Parameter	Action	"Sig Prob"	Seq SS	RSquare	Cp
1	MW	Entered	0.0000	14.97806	0.5701	32.51
2	Solubility parameter	Entered	0.0065	3.423615	0.7004	18.596
3	Dielectric constant	Entered	0.0342	1.61624	0.7619	13.084
4	Surface tension	Entered	0.0034	2.321461	0.8502	4.2928
5	SAP	Removed	0.5951	0.062991	0.8502	4.2928

Table VII. Effect of hydrophilic head (glyceryl/propylene glycol and # of glyceryl group) and lipophilic tail (fatty acid chain length) on solubility of nifedipine and griseofulvin analyzed by single factor ANOVA

Functional group		Lipids	Nifedipine			Griseofulvin		
	i=Level		Solubility(mg/g)	F ratio	p	Solubility(mg/g)	F ratio	p
<u>Factor 1:</u> Glyceryl vs. propylene glycol	1	CapmulMCM	10.273	1.1993	0.1918	4.031	0.3994	0.0950
		CapmulGMO	3.115			3.292		
	2	CapmulPG8	16.983			3.774		
		CapmulPG18	5.068			1.913		
<u>Factor 2:</u> # of glyceryl group	1	CapmulGMO	3.115	0.2258	0.8044	3.292	2207.144	0.0001
	2	Caprol2GO	3.026			2.275		
	3	Caprol3GO	3.193			1.386		
<u>Factor 3:</u> Fatty acid chain length	1	CapmulPG8	16.983	220.30 08	0.0001	3.774	3951.706	0.0001
	2	CapmulPG12	8.118			2.197		
	3	CapmulPG18	5.068			1.913

Figure 1. Nifedipine chemical structure

GRISEOFULVIN

Figure 2. Chemical structure of griseofulvin

Figure 3: Liquid crystal formed with griseofulvin in glyceryl mono-oleate.

Figure 4: FT-IR profile of nifedipine in mineral oil and Capmul PG-8

Figure 5: Effect of HLB on solubility of griseofulvin

SECTION II

MANUSCRIPT III

ROLE OF LIPID PROPERTIES AND CREMOPHOR EL CONCENTRATION ON FORMULATION DEVELOPMENT AND DISSOLUTION PERFORMANCE OF LIPID-BASED NIFEDIPINE FORMULATIONS

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Keywords: lipid vehicles, nifedipine, *in vitro* characterization

1. INTRODUCTION

The use of lipid vehicles has generated considerable attention in recent years for improving the bioavailability of poorly soluble drugs as one of the approaches to improve their oral bioavailability. One of the advantages of lipids in delivery of poorly soluble drugs is to improve the bioavailability of drugs by enhancing solubility/dissolution (Pozzi et al., 1991; Sheen et al., 1991; Charman et al., 1992; Shah et al., 1994; Matuszewska et al., 1996; August et al., 1997).

Lipid-based drug development phases can be classified as (I) selection of lipid vehicle/s and design of dosage form, (II) dissolution, product performance and bioavailability testing. Selection of the best lipid vehicle is one of the important chores in lipid formulations.

A suitable solvent for the lipid formulation usually contains a lipid and a surfactant. Depending on the solubility/concentration of the drug incorporated, saturation can influence the absorption of the drug. A lipid that is suitable for a drug delivery system must be safe and, therefore, the first requirement for these excipients is to be classified as GRAS (generally recognized as safe) materials. The potential toxicity of the surfactant incorporated should be considered carefully especially in chronic use. This evaluation includes not only the type of the surfactant but also its concentration/dose (Swenson et al., 1994).

To optimize the lipid solubility and particularly the release of the drug, addition of a co-solvent and surfactant/s can be useful. Ratios of the co-solvent, lipid and surfactant should be optimized to avoid potential precipitation of the drug in the gastro-intestinal (GI) media. To understand the performance of the formulation in GI media, the lipid, surfactant, co-solvent and drug combinations can be evaluated upon aqueous dilution using the phase diagrams (Shah et al., 1994; Constantinides, 1995 and Kim et al., 2000). Since only few lipids are capable of maintaining the drug in the solution form in the GI fluid, the surfactant addition may be necessary to maintain a good dispersibility of the drug.

Based on these facts, the solubility of a drug in a lipid is an important factor in selection of the lipid as a vehicle. This issue has been discussed earlier by the same group (Manuscript 2). Although, MW, fatty acid chain length and dielectric constant were determined as the common contributors to the solvent property of a given lipid, specific drug – lipid interactions are still important factors.

On the other hand, release of a drug from the vehicle was found to be an inverse function of its solubility in the lipid system (Armstrong and James, 1980). Therefore, chemical potential (concentration/solubility) and partitioning of the drug from the lipid to the immediate aqueous environment should be considered in lipid-based formulation design.

HLB plays an important role in the selection of the lipid vehicles in such formulations. Even though most of the lipids have no surface-active properties, medium chain mono-glycerides exhibit surfactant properties. HLB provides reliable practical information about the water miscibility of the lipid and the surfactant. HLB of polysorbates varied the dissolution of aratinoid (Bachynsky et al., 1994). Shah et al. (1994) showed that HLB of the oil surfactant mixtures affected the release property of a freely oil soluble drug.

Dissolution testing for poorly soluble drugs requires modification of the routinely used media. Incorporation of a surfactant into the dissolution media improved dissolution of poorly soluble drugs such as REV5901, UC781 (Serajuddin, 1988; Roman, 1999; Dressman and Reppas, 2000 and Damian et al., 2000). The mechanism of surfactant facilitated dissolution of poorly soluble drugs has been reviewed by Crison et al. (1996) and summarized as micelle-facilitated dissolution. Polysorbate 80, sodium lauryl sulfate and polyoxyl-35 castor oil are the most commonly used surfactants added to the medium to improve dissolution of the poorly soluble drugs.

The surfactant concentration selected depends on the critical micellar concentration of the surfactant and micellar solubility of the drug. The type of surfactant used in the dissolution medium is also important because of the possible interactions with the capsule shells as it happens with sodium lauryl sulfate (SLS). This anionic surfactant may interact with cationic charges of gelatin at gastric pH (Pillay and Fassihi, 1999).

These interactions may retard the disintegration of the capsule shell and thus dissolution of the drug.

Larsen et al., (2002 and 2000) demonstrated that partitioning of testosterone from the vegetable oils into a pH 6 buffer was correlated with *in vitro* release rates. Viscosities of oils have not influenced the release of the drug. However, the viscosities studied were very close to each other.

In the lipid based formulations, the effect of the properties of lipids on dissolution rate and extent of the drugs has been hardly studied. Therefore, in this study, formulations of nifedipine, developed with different classes of lipids and a surfactant combination, were examined to investigate the effect of surfactant on the solubility and dissolution of the drug. The effects of physicochemical properties of lipids on dissolution of nifedipine were evaluated. Effect of particle size and partitioning behavior of nifedipine on dissolution properties was also examined.

2. MATERIALS AND METHODS

2.1. Materials

Lipids that are used for this study listed in Table I were selected based on their class and GRAS (Generally Recognized as Safe) status. The selected lipids included glycerides (glyceryl tri- caprylate/caprate, glyceryl mono-caprylate/caprate and glyceryl mono-oleate), polyglycerol (diglycerol mono-oleate and triglycerol mono-oleate) and propylene glycol esters (propylene glycol mono-caprylate, -laurate and -

oleate). By selecting these lipids, the effects of hydrophilic (glyceryl, polyglycerol and propylene glycol) and hydrophobic (fatty acid chain) groups of lipids on the dissolution can be determined. The fatty acid distributions of lipids are also given in the same table. The measured/calculated properties of the lipids (HLB, density, MW, polarity and viscosity) were summarized in Table II. The details of these measurements/calculations are already given in the Manuscript II. Because there is no purification involved in the formulations with lipids, they were used as obtained.

The model drug chosen for this study was nifedipine, a calcium antagonist that is used to treat angina and hypertension. It is 3, 5-pyridinedicarboxylic acid, 1, - dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester. It has a molecular weight of 346.34.

Nifedipine is practically insoluble in water (9.5 µg/mL at 25°C) and less soluble in ethanol. It is also light sensitive and converts to a nitrosophenylpyridine derivative when it is exposed to daylight and UV light. Therefore, all nifedipine related experiments were carried out under yellow light.

Cremophor EL (BASF Corp., Mount Olive, NJ), polyoxyl 35 castor oil, was used as the surfactant to improve the water miscibility of lipids. Cremophor EL (Polyoxyethyleneglycerol triricinoleate 35) is a liquid non-ionic surfactant with a critical micelle concentration (CMC) of 0.0015 % w/v (Figure1). It is produced by the chemical reaction of ethylene oxide with castor oil. The major components of Cremophor EL are tri-glycerides which contain about 87% of ricinoleic acid. The surfactant is presently used as a vehicle in the pharmaceutical preparations to increase

the solubility of lipophilic drugs, including cyclosporine A and paclitaxel (Sparreboom et al., 1996 and Meyer et al., 2001).

2.2. Methods

2.2.1. Solubility Determination

The mixtures of Cremophor EL and lipid vehicles shown in Table I were prepared at varying lipid/surfactant ratios of 10/0, 9/1, 8/2, 7/3, 6/4, 5/5 w/w, which corresponds to 0, 10, 20, 30, 40 and 50% (w/w) Cremophor EL concentration, respectively. Visual observation of these mixtures was recorded. All lipid-surfactant combinations produced clear solutions except Miglyol 810, which formed cloudy mixtures at all ratios.

The solubility of nifedipine was determined in each lipid-surfactant combination and in Simulated Gastric Fluid (SGF) by adding an excess amount of the drug into 10 g of solvent and equilibrating the mixture for 72 hr. at $25^{\circ} \pm 0.1^{\circ}\text{C}$ for lipid/surfactant mixtures and $37^{\circ} \pm 0.1^{\circ}\text{C}$ for SGF. The mixture was centrifuged for 10 minutes and filtered through a $0.45\mu\text{m}$ filter. The amount of nifedipine dissolved was determined by an HPLC method.

A Hewlett Packard 1050 HPLC system with a UV detector ($\lambda = 236\text{ nm}$) was used for nifedipine determination. The stationary phase consisted of a micro Bondapak, C_{18} reverse phase column (3.9 x 300 mm. Waters Corp., Milford, MA). The mobile phase used was acetonitrile: methanol: water (2: 3: 3) combinations. The samples were

diluted with methanol before the runs. The flow rate used was 1.0 mL/min with 30 minutes of total run time per injection. The data acquisition was made using the software Turbochrome 6.1.1.0.0 (Perkin Elmer Corp, Wellesley, MA). All studies were performed in triplicate.

2.2.2. Interfacial Tension

A Kruss K 12 Tensiometer (Kruss USA, Charlotte, NC) was used to determine the interfacial tension between the selected lipid and simulated gastric fluid (SGF), USP. The ring method was selected for the measurements. Both the lipids and SGF were used at 37°C.

All the lipids used except Capmul MCM in Table I were less dense than SGF, USP. First, surface tension of the lipids was measured in a vessel then the vessel was emptied and cleaned. The heavy phase (SGF) was placed in the vessel, and then the ring was dipped in the liquid. The lipids were slowly added into the vessel by making sure no mixing occurred at the interface. Interfacial tension was determined by pulling the ring away from interface, the “pull” method.

2.2.3 Formulation Development

Among all surfactant/lipid mixtures, the ones containing 0, 10, and 40% w/w Cremophor EL have been selected for formulation development of nifedipine. The drug (10 mg) was added to the lipid/surfactant combination (4.5 g) and mixed in a shaker at the room temperature until it was completely dissolved. The final solutions

that were examined under a polarizing microscope were crystal-free. The solutions obtained were filled into “000” size hard-gelatin capsules and sealed with a bending solution that contained 20% w/v gelatin, 0.8% w/v polysorbate80 and water to make final volume 100 mL. The capsules were kept at the room temperature and assayed for nifedipine. The assay conducted using the HPLC method showed that the formulations were stable at the room temperature for two months containing $100 \pm 2\%$ nifedipine.

2.2.4. Dissolution Test

The USP II apparatus with paddle at a speed of 50 rpm and a temperature of $37^{\circ} \pm 0.1^{\circ}\text{C}$ was used to obtain dissolution profiles for the nifedipine lipid capsules.

Simulated gastric fluid (pH =1.2, 900 mL) was used as the dissolution medium. Since the solubility of nifedipine is low, Cremophor EL was added to dissolution medium to increase the solubility of drug. Cremophor EL concentration of 3% (w/v) was selected to increase the solubility of nifedipine (Table III) and can differentiate dissolution of nifedipine from different formulations.

Four capsules that have total of 10-mg drug were placed in capsule cages and dipped into the dissolution medium. Samples (3 mL) were withdrawn using a syringe that was attached to a cannula with a 10 μ filter at 3, 5, 8, 10, 15, 20, 40 and 60 minutes. The withdrawn sample was replaced with 3 mL fresh dissolution medium. The amount of dissolved nifedipine was determined by the HPLC method as explained in Section 2.2.1.

The percentage of nifedipine dissolved at 60 minutes was taken as the dissolution extent of the formulation. The dissolution rates were calculated using the Kitazawa equation (Pillay and Fassihi, 1999):

$$C_t = C_0 \cdot e^{kt} \text{ or}$$

$$\ln(C_t) = C_0 - kt \quad \text{Equation 1}$$

where C_t is the remaining concentration of nifedipine at time t , C_0 is the initial concentration and k is the dissolution rate. Therefore, the slope of the plot $\ln(C_t)$ vs. time in the linear region was taken as the dissolution rate.

2.2.5. Partition Coefficient

Partitioning of nifedipine from a respective formulation to the dissolution medium was obtained by mixing an equal amount of formulation and SGF + 3% Cremophor EL for 1 hr at 25°C. The aqueous and oily phases of the mixture were separated by centrifuging the mixtures for 10 min at 2000 rpm. The oily phase separated was assayed for nifedipine.

2.2.6. Particle Size Measurement

Light scattering was used for the particle size measurements of the formulations that were obtained by dissolving them in SGF. The dissolution conditions were used for the particle size determination. Four capsules that have total of 10-mg drug were placed in capsule cages and dipped into the dissolution medium (900 mL), containing 3% w/v Cremophor EL. 5 mL sample was withdrawn at 10, 20, 40 and 60 minutes and

the particle sizes were measured using a Dynamic Light Scattering (Brookhaven Instrument Corp. Holtsville, NY). The measurement duration was one minute at the wavelength 488 nm and the detector angle 90°. Polydispersity index monitored was within 0.2% range.

2.2.7. Statistical Analyses

The effect of Cremophor EL concentration on the solubility of nifedipine in lipids has been analyzed using linear regression. The model used is expressed as:

$$S_N = S_L + aC_S \quad \text{Equation 2}$$

Where S_N is the solubility of nifedipine (mg/g) in the mixture, S_L is the intercept and represents the solubility of nifedipine (mg/g) in the lipid, C_S is the surfactant concentration (% w/w) and a is the slope and represents solubility rate. Correlation coefficient (R_{adj}^2) has been obtained. Goodness of fit test has been performed using Shapiro-Wilk hypothesis. For the Shapiro-Wilk test, a test statistic, W , is calculated and is used to test following hypotheses:

H_0 : The distribution is normal distribution

H_a : The distribution is different from normal distribution

If the probability of W is higher than 0.05, the conclusion is fail to reject H_0 .

Similarly, the effect of physicochemical properties of lipids, partitioning of nifedipine and particle size of nifedipine lipid-based formulations on dissolution has been analyzed using a simple linear regression analysis and correlation coefficient (R_{adj}^2) has been obtained. Goodness of fit test has been performed.

JMP Statistical Software version 4.0.4 (SAS Institute Inc, Cary, NC) has been used for all analyses.

3. RESULTS AND DISCUSSIONS

3.1. The Role of Cremophor EL and on the Solubility and Dissolution of Nifedipine in Lipids

The mixtures of lipid and Cremophor EL were clear except the Miglyol810/Cremophor EL mixtures. The solubility of nifedipine in these mixtures is given in Table IV. As it can be seen from the results, addition 10-50% (w/w) of Cremophor EL increases the solubility of the drug around 10 to 600 times. Surfactant related increase in solubility (%) results in Table IV showed that the highest percent increase was observed with Caprol 2GO (662%), which has the lowest nifedipine solubility and the lowest percent increase was observed with Capmul Pg-8 (12%), which provided the highest solubility for nifedipine. These results indicated that the effect of surfactant on the solubility of nifedipine is more dominant, if the solubility of the drug in a lipid is low. The increase in solubility is proportional to the amount surfactant added, Figure 2 and the surfactant concentration and solubility obeyed:

$$S_N = S_L + aC_S \quad \text{Equation 3}$$

All the statistical parameters, the slope (a), intercept S_L (solubility of nifedipine in lipid) and correlation coefficient (R_{adj}^2) values obtained using Equation2 are tabulated in Table V. Goodness of fit test showed that normal distribution is followed for all

lipids ($p > 0.05$) and the model is adequate. Data in Table V also showed that slopes are the same for the same homolog series of lipids. For example, polyglycerols like CapmulGMO (Glyceryl mono-oleate), Caprol2GO (Di-glyceryl mono-oleate) and Caprol3GO (Tri-glyceryl mono-oleate) have a slope of 0.41 and propylene glycol esters namely CapmulPG-8 (Propylene glycol mono-caprylate), CapmulPG-12 (Propylene glycol mono-laurate) and CapmulPG-18 (Propylene glycol mono-oleate) have the slope around 0.38. The increasing surfactant concentration affected polyglycerol esters more than propylene glycol esters because the effect of surfactant on the solubility of nifedipine is more dominant with lipids dissolves the drug at lower extent.

Dissolution profiles of the formulations that contained Cremophor EL showed that addition of 10% (w/w) surfactant in the formulation increased the rate of dissolution, Figure 3. An additional increase of surfactant in the formulation did not show further difference, regardless of the type of lipid. Figure 3 is an example to the dissolution performance of 10 -40% Cremophor EL containing nifedipine lipid formulation. Overall findings indicated that the presence of 10% Cremophor EL has hidden the variations involved in the dissolution caused by the lipids because micelle-facilitated dissolution takes over when there is sufficient surfactant presents in the formulation.

3.2. Effect of Lipids on Nifedipine Dissolution

The dissolution profiles of the formulations containing 10 mg nifedipine, 4.5 g of lipid and no surfactant are given in Figure 4. The highest and complete dissolution of

nifedipine was obtained with Capmul MCM. Although the highest solubility of nifedipine was obtained in Capmul PG-8, it showed the lowest dissolution rate and extent for nifedipine.

When the dissolution performance of the drug in a lipid series containing increasing fatty acid chain length compared, it was found that increasing chain length increased the dissolution rate and extent, Figure 5. The decrease in solubility of nifedipine with increasing fatty acid chain length may be the reason of the increment observed in the dissolution rate and extent. However, Table IV and Figure 4 demonstrated that solubility of nifedipine in Capmul MCM (glyceryl mono-caprylate/caprate) is similar to Capmul PG-8 solubility (Propylene glycol mono-caprylate) and yet Capmul MCM provided much higher dissolution extent and rate. This occurrence suggests the role of additional factors that may influence the dissolution of the drug from the lipids. These factors may be partitioning behavior of the drug, viscosity, polarity and density of the lipid.

The dissolution rate and the extent of nifedipine given in Table VI compared with the solubility of the drug in the lipids, physicochemical properties of lipids (HLB, density, viscosity and interfacial tension) given in Table II showed that the effect of each factor is significant on dissolution rate and extent ($p < 0.005$ with one way ANOVA). When the drug has high lipid solubility, lipid tends to keep the drug itself and does not let it release into aqueous medium. However, if the lipid is hydrophilic which can be

defined by HLB, interfacial tension, polarity, dissolution extent of the drug can be improved highly like it happens in Capmul MCM (96% dissolved in an hour).

Density and viscosity were the other important properties of lipids affected dissolution rate and the extent of nifedipine, Tables II and VI. Relatively low density of the lipid resulted in floating of the formulation on surface of the dissolution fluid and slowed the release of the drug. Also as the viscosity of the lipid increased, the mixing time of the formulation was extended hence the dissolution rate and the extent was lowered.

The dissolution performance of nifedipine in the lipids was further evaluated using two parameters; dissolution extent and rate of nifedipine from each lipid given in Table VI. These values used to investigate the contribution of each factor to dissolution parameters. The statistical evaluations were carried out with the linear regression analyses. Among individual tests, only meaningful relationship was found between dissolution extent and partition coefficient of the drug ($R_{adj}^2 = 0.991$).

Partition coefficient of nifedipine from formulations to the dissolution medium has been given in Figure 6. The drug partitioned in propylene glycol (PG) esters in the highest order. The increasing fatty acid chain from C8 to C18 decreased the partition coefficient from 9.6 to 7.8. Partitioning of drug from lipid to aqueous phase decreased as the solubility of drug increased in the lipid. Dissolution extent was significantly affected by partitioning, Figure 7. As the partition coefficient of drug from increased,

dissolution rate decreased producing a correlation coefficient of $R_{adj}^2 = 0.991$ between dissolution extent and partition coefficient of nifedipine.

Particle size of the lipid-formulation in the GI fluid can also be an important factor that affects the dissolution of nifedipine. The smallest particle size was obtained from Capmul MCM which provided as small particle as the Miglyol810/CremophorEL mixture did, Table VII. During one hour mixing, the particle size of formulation did not change by time. The linear regression analyses showed that the particle size of each lipid at 60 minutes was correlated well with the dissolution extent at 60 minutes ($R_{adj}^2 = 0.9758$ and $p = 0.1733$ for goodness of fit) and partition coefficient of nifedipine ($R_{adj}^2 = 0.9835$ and $p = 0.3146$ for goodness of fit). As the particle size increased, dissolution extent decreased and partition coefficient (Po/w) increased, Figure 8. These results also confirm the findings by Shah et al., (1994). As the particle size decreases, the surface area increases so that dissolution enhances.

4. CONCLUSION

Incorporation of Cremophor EL, a nonionic surfactant, into lipid-based nifedipine formulation enhanced the solubility and dissolution of nifedipine. The solubility of nifedipine showed a linear correlation with surfactant concentration. While solubility rate was dependent on the type of lipid used, the dissolution of nifedipine in presence of surfactant was the same regardless of lipid used.

Dissolution of nifedipine from lipids showed that as the fatty acid chain length increases, the dissolution rate increases due to lower solubility of nifedipine in the lipids that have higher chain length. The effect of lipids on dissolution rate and extent of nifedipine investigated showed that even though physicochemical properties of lipids (HLB, interfacial tension, viscosity, density) and solubility of nifedipine in lipids play role, only partitioning of the drug from lipid to aqueous medium and particle size of the formulation in dissolution medium were provided a good correlation with dissolution extent of nifedipine.

5. ACKNOWLEDGMENTS

This study is part of Ms. Dumanli's Ph.D. dissertation research supported by Hoffmann-La Roche. The research was conducted at Hoffmann-La Roche facilities in Nutley, NJ.

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Table I. Lipids used in this study

Classification and chemical structure	Chemical Name	Trade Names	Composition (% fatty acid distribution)
Triglycerides	Caprylic (C ₈) / Capric (C ₁₀) triglyceride	Miglyol 810	C ₈ : 71 C ₁₀ : 29
Glycerol mono-/ di-esters	Mono-and di-glyceride of caprylic /capric acid	Capmul MCM	C ₈ : 81 C ₁₀ : 18 C ₆ : 1
	Glycerol mono-oleate	Capmul GMO	C _{18:1} : 80 C _{18:2} : 10 C ₁₈ : 4 C ₁₆ : 4 C ₁₄ : 2
Polyglycerol esters	Diglycerol mono-oleate	Caprol 2G0	C _{18:1} : 90 C _{18:2} : 3 C ₁₈ : 3 C ₁₆ : 1
	Triglycerol mono-oleate	Capmul 3GO	C _{18:1} : 90 C _{18:2} : 3 C ₁₈ : 3 C ₁₆ : 1
Propylene glycol esters	Propylene glycol mono-caprylate	Capmul PG-8	C ₈ : 99 C ₁₀ : 1
	Propylene glycol mono-laureate	Capmul PG-12	C ₁₂ : 98 C ₁₄ : 2
	Propylene glycol mono-oleate	Capmul PG-18	C _{18:1} : 93 C _{18:2} : 3 C ₁₈ : 3 C ₁₆ : 1

* C₆: Caproic, C₈: Caprylic, C₁₀: Capric, C₁₂: Lauric, C₁₄: Myristic, C_{14:1}: Myristoleic, C₁₆: Palmitic, C_{16:1}: Palmitoleic, C₁₈: Stearic, C_{18:1}: Oleic, C_{18:2}: Linoleic and C_{18:3}: Linolenic acid

Table II. Calculated and measured properties of lipids used

Lipid	HLB	Density (g/cm ³)	MW	Viscosity (cP)	Dielectric constant	Interfacial tension (mN/m) at 37°C
Miglyol 810	1.5	0.9437	495	15	2.0958	12.00
Capmul MCM	6.5	1.0022	275	65	2.1048	Not measurable
Capmul GMO	4	0.9451	490	325	2.1574	3.16
Caprol 2GO	5	0.9814	421	760	2.1697	2.70
Caprol 3GO	6	0.9776	494	1090	2.1691	2.43
Capmul PG-8	5	0.9387	202	1	2.0630	3.23
Capmul PG-12	4	0.9183	258	15	2.0863	3.12
Capmul PG-18	3.5	0.9096	340	25	2.1293	3.15

Table III. Nifedipine solubility in Simulated Gastric Fluid at 37°C containing Cremophor EL

Cremophor EL (% w/v)	Solubility (mg/mL)
0	0.0054
0.5	0.0548
1	0.1032
2	0.2104
3	0.3148
5	0.5739
7	0.7885
10	1.1641
12	1.4551
15	1.7048

Table IV. Solubility of nifedipine in lipid/Chremophor EL mixtures

Lipid vehicle	Cremophor EL concentration %	Nifedipine solubility mg/g mean$\pm$ SEMn=3	% increase in solubility
Miglyol 810 Glyceryl tri caprylate (C ₈)	0	3.359 $\pm$ 0.029	0
	10	4.863 $\pm$ 0.043	43
	20	10.183 $\pm$ 0.087	197
	30	17.940 $\pm$ 0.123	418
	40	23.702 $\pm$ 0.091	577
	50	18.276 $\pm$ 0.083	415
Capmul MCM Glyceryl mono- caprylate (C ₈)	0	10.273 $\pm$ 0.091	0
	10	14.368 $\pm$ 0.037	39
	20	16.738 $\pm$ 0.047	61
	30	23.873 $\pm$ 0.089	129
	40	23.720 $\pm$ 0.023	126
	50	26.478 $\pm$ 0.124	151
CapmulGMO Glycerylmono- oleate (C _{18:1})	0	3.115 $\pm$ 0.111	0
	10	8.878 $\pm$ 0.094	182
	20	11.758 $\pm$ 0.087	269
	30	16.456 $\pm$ 0.108	411
	40	18.591 $\pm$ 0.056	472
	50	24.825 $\pm$ 0.083	657
Caprol 2GO 2-glyceryl mono- oleate (C _{18:1})	0	3.026 $\pm$ 0.037	0
	10	7.055 $\pm$ 0.148	131
	20	10.221 $\pm$ 0.097	231
	30	14.657 $\pm$ 0.084	370
	40	19.548 $\pm$ 0.174	521
	50	24.143 $\pm$ 0.267	662

Table IV: Solubility of nifedipine in lipid/Chremophor EL mixtures (continued)

Lipid vehicle	CremophorEL concentration %	Nifedipine solubility mg/g mean$\pm$ SEM n=3	% increase in solubility
Caprol3GO 3-glyceryl mono- oleate (C _{18:1})	0	3.193 $\pm$ 0.099	0
	10	6.217 $\pm$ 0.122	93
	20	10.679 $\pm$ 0.165	230
	30	12.353 $\pm$ 0.465	279
	40	19.385 $\pm$ 0.345	490
	50	24.304 $\pm$ 0.655	635
Capmul PG-8 Propylene glycol mono-caprylate (C ₈)	0	16.983 $\pm$ 0.555	0
	10	19.256 $\pm$ 0.426	12
	20	23.304 $\pm$ 0.457	34
	30	27.750 $\pm$ 0.589	58
	40	31.226 $\pm$ 0.891	76
	50	40.032 $\pm$ 0.924	123
Capmul PG-12 Propylene glycol mono-laurate (C ₁₂)	0	8.118 $\pm$ 0.243	0
	10	10.717 $\pm$ 0.094	30
	20	13.287 $\pm$ 0.243	60
	30	18.792 $\pm$ 0.145	122
	40	23.359 $\pm$ 0.456	173
	50	18.055 $\pm$ 0.754	110
Capmul PG-18 Propylene glycol mono-oleate (C _{18:1})	0	5.068 $\pm$ 0.035	0
	10	8.728 $\pm$ 0.216	70
	20	10.990 $\pm$ 0.556	112
	30	14.683 $\pm$ 0.423	178
	40	21.050 $\pm$ 0.589	293
	50	30.176 $\pm$ 0.812	455
Cremophor EL	0	58.23 $\pm$ 1.632	-

Table V. List of parameters for Equation 2 to investigate the effect of surfactant concentration on the solubility of nifedipine (S_N) in lipids

Lipid	R^2_{adj}	a (slope)	S_L	p for goodness of fit
Miglyol810 (Glyceryl tri-caprylate)	0.782	0.40	3.12	0.9637
CapmulMCM (Glyceryl mono-caprylate)	0.915	0.36	10.51	0.3470
CapmulGMO(Glyceryl mono-oleate)	0.981	0.41	3.76	0.7663
Caprol2GO (Di-glyceryl mono-oleate)	0.994	0.42	2.57	0.1856
Caprol3GO (Tri-glyceryl mono-oleate)	0.967	0.42	2.21	0.1153
CapmulPG-8 (Propylene glycol mono-caprylate)	0.987	0.37	16.30	0.2789
CapmulPG-12 (Propylene glycol mono-laurate)	0.964	0.38	7.146	0.6537
CapmulPG-18 (Propylene glycol mono-oleate)	0.955	0.38	4.52	0.8131

Table VI. Dissolution parameters of nifedipine in various lipids (n =3)

Lipid	Dissolution rate (k)* (Mean $\pm$ SD)	Dissolution extent (%) (Mean $\pm$ SD)
Miglyol810 (Glyceryl tri-caprylate)	0.012 $\pm$ 0.000	50.19 $\pm$ 1.44
Capmul MCM (Glyceryl mono-caprylate)	0.254 $\pm$ 0.009	96.05 $\pm$ 0.28
Capmul GMO (Glyceryl mono-oleate)	0.021 $\pm$ 0.002	79.64 $\pm$ 1.44
Caprol2GO (Di-glyceryl mono-oleate)	0.074 $\pm$ 0.002	76.58 $\pm$ 0.99
Caprol3GO (Tri-glyceryl mono-oleate)	0.081 $\pm$ 0.009	73.12 $\pm$ 0.43
Capmul PG-8 (Propylene glycol mono-caprylate)	0.006 $\pm$ 0.000	30.27 $\pm$ 3.54
Capmul PG-12 (Propylene glycol mono-laurate)	0.008 $\pm$ 0.002	31.85 $\pm$ 2.93
Capmul PG-18 (Propylene glycol mono-oleate)	0.015 $\pm$ 0.001	45.73 $\pm$ 2.50

*k: calculated using Equation 1

% dissolved nifedipine at 60 minutes

Table VII. Results of particle size of nifedipine in each lipid at 37°C

Lipid	Particle size (nm, mean $\pm$ SD, n=3)			
	10 min	20 min	40 min	60 min
Miglyol810	N/A*	N/A*	N/A*	N/A*
Capmul MCM	10 $\pm$ 0.5	12 $\pm$ 1.1	12 $\pm$ 1.5	11 $\pm$ 0.5
Capmul GMO	322 $\pm$ 11	328 $\pm$ 8	337 $\pm$ 12	333 $\pm$ 28
Caprol 2GO	473 $\pm$ 36	434 $\pm$ 28	433 $\pm$ 28	454 $\pm$ 45
Caprol 3GO	459 $\pm$ 24	419 $\pm$ 28	418 $\pm$ 32	417 $\pm$ 28
Capmul PG-8	800 $\pm$ 4.7	786 $\pm$ 24	765 $\pm$ 27	777 $\pm$ 32
Capmul PG-12	732 $\pm$ 26	726 $\pm$ 9	728 $\pm$ 26	724 $\pm$ 5
Capmul PG-18	727 $\pm$ 23	723 $\pm$ 21	730 $\pm$ 26	707 $\pm$ 8
Miglyol810/ Cremophor EL	11 $\pm$ 1.0	11 $\pm$ 2.1	10 $\pm$ 1.3	10 $\pm$ 1.2

*N/A: not applicable. The mixture is too coarse to measure

Figure 1. Critical micelle concentration (CMC) of Cremophor EL (n=3)

Figure 2: The effect of concentration of Cremophor EL on the solubility of nifedipine in lipids

- ♦ Glyceryl tri-caprylate
- Di-glyceryl mono-oleate
- + Propylene glycol mono-laurate
- Glyceryl mono-caprylate
- x Tri-glyceryl mono-oleate
- Propylene glycol mono-oleate
- Glyceryl mono-oleate
- Propylene glycol mono-caprylate

Figure 3: Effect of surfactant concentration on dissolution of nifedipine from Capmul PG-8

Figure 4. Effect of lipid on dissolution of nifedipine

Figure 5. Effect of fatty acid chain length on dissolution of nifedipine from propylene glycol esters

Figure 7. Effect of partitioning of lipids from nifedipine formulation to dissolution medium on dissolution extent of nifedipine

Figure 8: Effect of partitioning and dissolution extent on particle size of the nifedipine formulation in lipids

SECTION II

MANUSCRIPT IV

BIOAVAILABILITY OF NIFEDIPINE LIPID FORMULATIONS IN BEAGLE DOGS

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Keywords: lipid vehicles, nifedipine, *in vitro-in vivo* correlation

1. INTRODUCTION

The use of lipid formulations generated considerable interest in the last two decades because the modern drugs produced are highly lipophilic as the result of High Throughput Screening (HTS). The lipids may improve the bioavailability of drugs through several mechanisms such as by enhancing their solubility/ dissolution (Pozzi et al., 1991; Sheen et al., 1991; Charman et al., 1992; Shah et al., 1994; Matuszewska et al., 1996; August et al., 1997), by homogenously dispersing the drug into the gastric fluid and providing and uniform absorption rate (Yamahira et al, 1979a, b, 1980) and by increasing lymphatic absorption (Cheema et al., 1987; Charman and Stella, 1992).

Despite numerous studies with lipid-based system to increase the bioavailability of poorly soluble drugs, only a relatively few commercial formulations of lipid-based systems are available on the market. These are cyclosporine (Sandimmune™ and Neoral™, Novartis), saquinavir (Fortovase™, Roche), ritonavir (Norvir™, Abbott) and fat-soluble vitamins. Among the reasons for limitation of their use may be not to have sufficient knowledge of *in vitro* and *in vivo* performance of these type of formulations. Lipolysis that takes place in upper intestine is a physiological event that makes it more difficult to predict *in vivo* performance of lipid-based formulations.

Understanding of the lipid digestion process is important in design of the lipid-based formulations. The digestion process involves three basic steps: (I) dispersion of lipid solution into the gastric media and emulsification of the solution by the gastric movements, (II) enzymatic hydrolysis of di- and tri-glycerides into mono-glyceride

and fatty acids, (III) formation of the mixed micelles with hydrolysis products and bile salts and ultimately absorption of lipids through the intestinal wall (Carey et al., 1983). Only tri-glycerides are digested physiologically. The pharmaceutical grade di-glycerides are rare. When a mono-glyceride is used in a formulation, it is assumed that it skips the enzymatic hydrolysis step. Therefore, dissolution studies may be predictive of the bioavailability of such systems.

Micelle formation of lipolysis products with bile salts is the crucial step that enhances the solubility and absorption of poorly soluble drugs. The total solubility of compounds such as steroids, griseofulvin, cyclosporine A, danazol, pentazocaine, triamcinolene and diazepam was found to be proportional to taurocholate concentration, a major bile salt present in the small intestine. A linear relationship was shown between solubilization and partition coefficient of steroids, cyclosporine A, and griseofulvin (Mithani et al., 1996).

A detailed discussion of the lipid digestion process and its impact on drug absorption from the gastro-intestinal tract can be found in several reviews (Eldem and Speiser, 1989; Embleton and Pouton, 1997; 1997; MacGregor et al., 1997; Dumanli et al, 2002). However, there are not many published studies investigated the correlation between *in vitro* lipolysis and *in vivo* results. MacGregor et al. (1997) briefly mentioned the lipolysis of progesterone that was formulated with two SEDDS and compared with plasma profiles an oily suspension. The AUCs of the lipid formulations were 8-10 times more the suspension. The SEDDS formulations formed small

droplets, uniformly dispersed in the GI system and undergone lipolysis were demonstrated good *in vitro in vivo* correlation. Reymond et al. (1988a and b) described an *in vitro* study where lipid emulsion was formulated with controlled particle size and followed the bioavailability of drug from such emulsions in the male rats via urine excretion. They have concluded that *in vitro* lipolysis could not simulate *in vivo* performance of the formulations.

In order to correctly predict *in vivo* performance of lipid formulations, more than one evaluation technique such as dissolution, particle size determination and solubility of the drug in the lipids and GI fluids may be needed.

In this study, the bioavailability of nifedipine from different lipid formulations was determined in the beagle dogs. The types of lipids were determined from our previous study in which *in vitro* dissolution performance of a nifedipine formulation in CapmulPG-8 (propylene glycol mono-caprylate), CapmulPG-12 (propylene glycol mono-laurate), CapmulPG-18 (propylene glycol mono-oleate), CapmulMCM (glyceryl mono-caprylate/caprate), Miglyol810 (glyceryl tri-caprylate), Caprol2GO (diglyceryl mono-oleate), Caprol3GO (tri-glyceryl mono-oleate) and CapmulGMO (glyceryl mono-oleate) was investigated (Manuscript III). Among these lipids, we have selected CapmulMCM, which provided the highest dissolution rate, CapmulPG8, which provided the lowest dissolution rate and Miglyol810, which provided relatively moderate dissolution rate, for the further *in vivo* studies. The latest lipid is a tri-glyceride which undergoes to lipolysis, while CapmulMCM and CapmulPG-8 are

mono-glycerides and therefore are not subjected to the lipolysis process. To seek an *in vitro* and *in vivo* correlation, the solubility, partitioning and particle size of nifedipine in the given lipids were also determined.

2. MATERIALS AND METHODS

2.1. Materials

Nifedipine and butamben (an internal standard for HPLC analyses of nifedipine in plasma) were purchased from Sigma, Louis, MO, Capmul MCM (Mono-glyceryl caprylate/caprate) and Capmul PG-8 (Propylene glycol caprylate) were provided by Abitec, Columbus, OH. Miglyol810 (tri-glyceryl caprylate/caprate) and Cremophor EL (PEG-35 Castor oil,) were gifts from Sasol, NJ and BASF, Germany, respectively. All the lipids have GRAS (Generally Recognized as Safe) status. Methanol, acetonitrile, chloroform, acetone, phosphoric acid were purchased from Fisher Scientific., Springfield, NJ. All the chemicals were used as received.

2.2. Methods

2.2.1. Preparation of Formulations

Nifedipine (100 mg) was dissolved in each lipid vehicle (4.5 g) at 25°C in a shaker. All formulations were in clear liquid form and there were no changes observed when they were kept at 4°C for a month. The components of the formulations were given in Table 1. The formulations A-C contained 100 mg nifedipine and 45 g lipid in each batch. Formulation D contained 100 mg nifedipine, 4.5 g Cremophor EL and 40.5 g Miglyol 810. Each formulation (1.1250g containing 2.5 mg nifedipine) was filled into

a “000 size” hard-gel capsule sealed with a bending solution to prevent the leakage. The bending solution contained 20% (w/v) capsule shells, 0.8% (w/v) Tween80 and distilled water and was kept at 40°C during sealing.

2.2.2. Nifedipine Assay in the Formulations

Nifedipine content of the each formulation was determined by an HPLC method described in Section 2.2.3.1. The determination was made by weighing around 0.14 g of the formulation that contained 0.078 mg nifedipine, diluting with methanol and analyzing at 238 nm.

2.2.3. *In vitro* Tests

2.2.3.1. Solubility Determination

The solubility of nifedipine was determined in each lipid vehicle by adding an excess amount of the drug into 10 g of the vehicle mixture and equilibrating the mixture at $25^{\circ} \pm 0.1^{\circ}\text{C}$ for 72 hr. The mixture was centrifuged for 10 minutes and filtered through a 0.45 μm filter. The amount of nifedipine dissolved was determined by the HPLC method.

A Hewlett Packard 1050 HPLC system with a UV detector ($\lambda = 236\text{ nm}$) was used.

The stationary phase consisted of a micro Bondapak, C_{18} reverse phase column (3.9 x 300 mm, Waters Corp., Milford, MA). The mobile phase used was acetonitrile: methanol: water (2: 3: 3) mixture. The samples were diluted with methanol before the run and the flow rate was 1.0 mL/min with 30 minutes of total run time per injection.

The data acquisition was made using the software Turbochrome 6.1.1.0.0 (Perkin Elmer Corp, Wellesley, MA). All studies were performed in triplicates.

2.2.3.2. Dissolution Test

The USP II apparatus with paddle at 50 rpm and $37^{\circ} \pm 0.1^{\circ}\text{C}$ was used to obtain dissolution profiles for the nifedipine lipid capsules. Simulated gastric fluid (SGF) (pH1.2, 900 mL) was used as the dissolution medium. Since the solubility of nifedipine is low in SGF, Cremophor EL, a nonionic surfactant, was added to the dissolution medium to increase the solubility of drug. Cremophor EL concentration of 3% (w/v) was high enough to improve and maintain the solubility of nifedipine and to differentiate dissolution of nifedipine formulations used. Four capsules containing total 10-mg of drug were placed in capsule cages then dipped into dissolution vessel. Samples (3 mL) were withdrawn using a syringe attached to a cannula that has a 10 μ filter at the tip at 3, 5, 8, 10, 15, 20, 40 and 60 minutes. The sample withdrawn was replaced with 3 mL fresh dissolution medium. The amount of dissolved nifedipine was determined by the HPLC method as explained in Section 2.2.3.1.

The amount of dissolved nifedipine (%) at 60 minutes was taken as the dissolution extent of the formulation.

2.2.3.4. Partition Coefficient

The partition coefficient of the drug between the formulation and the Simulated Gastric Fluid (SGF) USP, was obtained by mixing equal amount of formulation and

SGF + 3% Cremophor EL for 1 hr at 25°C. The phases were separated by centrifuging the mixtures for 10 min at 2000 rpm. The oily phase was assayed for the amount of the drug analyzed by the HPLC method given in Section 2.2.3.1.

2.2.3. 5. Particle Size Measurements

The dissolution apparatus described in Section 2.2.3.2 was used for the particle size measurements. Four capsules that contained a total of 10-mg drug were placed in the capsule cages and dipped into the dissolution medium (900 mL SGF contained 3% w/v Cremophor EL). Sample (5 mL) was withdrawn at 10, 20, 40 and 60 minutes and the particle sizes were measured using a Dynamic Light Scattering (Brookhaven Instrument Corp. Holtsville, NY). The measurement duration was one minute at 488 nm and at 90° of detector angle. Polydispersity index monitored was within 0.2% range.

2.2.4 *In vivo* Absorption Study Design

2.2.4.1. Test Animals

The bioavailability of nifedipine lipid formulations was tested on six fasted beagle dogs using a single dose crossover design. The washout period between the experiments was one week. The dogs were supplied by The Marshall Farms, North Rose, NY. They were approximately 7-10 kg in weight and 13-16 months old and were acclimatized for at least three weeks prior to study.

The study group consisted of 3 males and 3 females. Each dog had an ear tattoo and was housed individually in a stainless steel cage that has an identification card showing compound number, study number, dose level and dog number and sex. Room temperature and humidity was maintained at approximately $72^{\circ} \pm 4^{\circ}\text{F}$ and $50\% \pm 19\%$, respectively. The animal room was on an approximate 12-hour light/dark cycle. Dogs were exercised outside the cage at least three times a week for at least 15 minutes.

2.2.4.2. Dosage forms, frequency and method of dosing

The dogs were fasted overnight prior to experiment. Each dog received four 2.5 mg nifedipine capsules (Details of the protocol were given in Table II). Each dog received approximately 500g Teklad Global Certified 21% protein Dog Diet 2021C 6 hours after dosing. Water, purified via reverse osmosis (RO), was available *ad libitum* by means of an automatic watering system. The RO water supply for each active animal room was monitored for bacterial contamination at least once a month by the Department of Laboratory Animal resources. In addition, chemical analysis of water was performed at approximately quarterly intervals by the Environmental Monitoring and Support Laboratory. No contaminants expected to interfere with the study were known to be present in the feed or water.

2.2.4.3. Blood sampling

Blood samples (1mL) were collected via jugular vein from each dog at 0, 1, 2, 3, 6, 10 and 24 hours and placed into glass tubes containing EDTA used as an anticoagulant. The tubes were kept at 4°C to prevent decomposition. The plasma was separated from

the whole blood with centrifugation at 4°C, transferred to screw- cap vials and was kept frozen in a -70°C freezer until assay time.

2.2.5. Assay of nifedipine in plasma

Nifedipine in all samples was assayed using a modified version of the HPLC method described by Mehta et al. (2002). In this method, a vortex mixer (Scientific Industries Inc., Bohemia, NY) was used to equilibrate the frozen samples at room temperature. Methanol (100 µl) containing 2 µg/mL butamben (as the internal standard) and acetonitrile (2 mL) were added to 0.5 mL plasma and vortex mixed for 30 minutes. After centrifugation at 4000 rpm for 15 minutes with a Hermle Centrifuge (Model Z382K, Denville Scientific, Metuchen, NJ), 2 mL of supernatant was transferred into a test tube containing 1 mL of distilled water. Acetone-chloroform mixture (4.5 mL, 1:1 v/v) was added to this mixture and was shaken for 1 hour. This solution was further centrifuged at 4000 rpm for 15 minutes to separate organic phase from aqueous phase. The aqueous phase was discarded. The organic phase transferred into an amber color test tube, and dried in a Turbovap LV ZW700 evaporator (Zymark Corp., Hopkington, MA) at 45°C for an hour under nitrogen. The residue was dissolved in 100 µl mobil phase, vortex-mixed for 30 seconds, transferred into Eppendorf tubes and centrifuged at 14000 rpm for 10 min. The aliquot (25 µl) obtained was injected to the HPLC system for nifedipine determination.

A Hewlett Packard 1050 HPLC system with a UV detector ($\lambda = 238$ nm) was used.

The stationary phase used was a reverse phase Zorbax RX-C18, 5-micron, 25cm x 4.6

mm column (Agilent Tech., Palo Alto, CA). The column temperature was kept at 55°C. The mobile phase consisted of 0.01 M disodium hydrogen phosphate buffer-methanol (45:55, v/v) mixture. The pH of the buffer was adjusted to pH=6.1 with 40% phosphoric acid. Run time used was 20 minutes and the flow rate was 0.8 mL/min. The data acquisition was made using the software Turbochrome 6.1.1.0.0 (Perkin Elmer Corp, Wellesley, MA).

The calibration graph was obtained by adding nifedipine solution in methanol to drug-free plasma. Nifedipine plasma solutions were prepared at concentration of 0, 10, 20, 40, 80, 120, 160, 200 and 240 ng/mL and stored in a -70°C freezer for pending analysis. The samples were processed as described above. The ratios of the peak area of nifedipine to that of butamben were used to construct a calibration graph, Appendix, Figure16. CV for precision of the experiment was within 2%.

2.3.6. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated from nifedipine plasma concentration. Maximum plasma concentration (C_{max}), time of occurrence of C_{max} (T_{max}) and area under the curve (AUC_{0-24 h}) was calculated using WinNonlin software by Pharsight Corporation (Mountain View, CA).

2.3.7 Statistical Analyses

Results for plasma concentrations were presented as mean ± SEM (standard error mean). Statistical comparisons of pharmacokinetic parameters were performed using t-

test for each pair. JMP Statistical Software version 4.0.4 (SAS Institute Inc, Cary, NC) has been used for analysis.

3. RESULTS AND DISCUSSION

The assay demonstrated that nifedipine lipid formulations contained 99-102 % of the original nifedipine loading. The plasma concentrations obtained after dosing each animal with respective formulations are presented in Table III. One of the female dogs (F4) vomited the Formulation A 10 minutes after the administration. Therefore, the results obtained from this dog excluded from further calculations.

Table IV shows the mean pharmacokinetic parameters (C_{max} , T_{max} , $AUC_{0-24 h}$) of the respective formulations and Figure 1, the plasma concentration profiles obtained during 10 hours following administration. As seen in Table IV and Figure 1, T_{max} values obtained from formulation A, B, C and D were not significantly different from each other ($p>0.05$) demonstrating that neither the lipids nor the surfactant added influences the absorption time of nifedipine. On the other hand, C_{max} and AUC_{0-24} values of formulations were significantly different from each other ($p<0.05$). The highest plasma profile was obtained with nifedipine formulations containing Miglyol810/Cremophor EL (9/1) and the lowest with the formulation containing CapmulPG-8. The highest C_{max} and AUC_{0-24} are the result of the micelle formation ability of Cremophor EL in GI medium and thus improved solubility of nifedipine. The bioavailability of nifedipine from formulation D is followed by $C>B>A$. The reason for higher bioavailability with Miglyol810 (glyceryl tri-caprylate) compared

with CapmulMCM (glyceryl mono-caprylate/caprate) can be explained by lipolysis. Miglyol810 is a tri-glyceride and broken down to mono-glyceride and fatty acids during lipolysis whereas CapmulMCM is a mono-glyceride and stays as it is during the digestion process. Although both form micelles with bile salts, digestion of Miglyol810 will generate higher amount of fatty acids. Therefore, the amount of micelles produced in the presence of bile salts with Miglyol810 will be higher than the micelles formed with CapmulMCM. Sek et al. (2002) also clearly demonstrated that the effect of bile salt on Miglyol812 was more pronounced than on CapmulMCM by an *in vitro* lipolysis experiment. A higher bile salt concentration can result in more micelles for solubility, partitioning and subsequent absorption of nifedipine. Therefore, in the presence of Miglyol810, higher plasma concentration of nifedipine was obtained.

The dissolution profiles of the same formulations demonstrated a different picture in simulated gastric fluid as shown in Figure 2. The Formulation B containing CapmulMCM and D containing CremophorEL provided the highest dissolution rate and extent while Formulation C containing Miglyol810 provided 50% release for nifedipine after 60 minutes. The lowest dissolution profile was obtained with Formulation A containing Capmul PG-8 which also provided the lowest bioavailability of nifedipine. The only significant difference observed between *in vitro* dissolution and *in vivo* plasma concentration profiles was with Formulations B and C. Formulation B containing CapmulMCM provided the higher dissolution profile than

Formulation C with Miglyol810. The opposite result observed the *in vivo* test was the result of lipolysis that was absent during the dissolution tests.

As the result of the findings, it can be said that the dissolution test by itself is not sufficient to predict the *in vivo* performance of formulations containing digestible lipids.

The differences of the effects of CapmulMCM and CapmulPG-8 can be explained by the contribution of the hydrophilic part to the lipid solubility and dissolution of nifedipine (Manuscript II and III). CapmulMCM is a glyceride ester of caprylic/capric acid and has a molecular weight of 275. CapmulPG-8 is a propylene glycol ester of caprylic acid and has a molecular weight of 202. Both lipids are surface active. The dissolution of samples containing either lipid is carried out in an environment contains 3% Cremophor EL. Under these conditions, CapmulMCM formed particles of approximately 12 nm and Capmul PG-8 around 700 nm, Table V. This may be one for the reasons of the differences observed in dissolution, Figure 2. Similarly, the formulation that was dispersed to larger particle size emulsion performed poorer bioavailability, Figure 1. The particle size effect may be true for the formulations that contain only mono-glycerides. However, it is not relevant for comparison of Formulations C and B. If the particle size is a factor, the lower bioavailability should be obtained for Formulation C containing Miglyol 810.

The effect of solubility of nifedipine in lipids on bioavailability also showed an inverse effect for the formulations without surfactant. The highest nifedipine solubility was obtained in CapmulPG-8 and this formulation provided the lowest bioavailability. Miglyol810 provided the lowest solubility for nifedipine, but the highest bioavailability among the lipids excluding the formulation with surfactant, Table V. This property affects the partitioning of nifedipine from the formulations to the dissolution medium. Partitioning values were also included in the same table. It provided the similar effect on bioavailability of nifedipine as dissolution does.

4. CONCLUSION

Bioavailability of nifedipine obtained with different lipid formulations in beagle dogs showed that the type lipid and surfactant used in the formulation plays an important role. Dissolution is a good predictor for the *in vivo* performance of nifedipine lipid formulation when it was formulated with non-digestible lipids (mostly mono-glycerides).

Although, the solubility of nifedipine in lipids, particle size of the formulation in dissolution medium and partitioning of the drug from the formulation to dissolution medium seem to affect the *in vivo* performance of the drug, dissolution rate and extent of the formulation and digestibility of the lipid used in the formulations played the major roles in bioavailability of nifedipine from lipid-based formulations.

5. ACKNOWLEDGMENT

The primary author wishes to thank Mrs. Oksana Bohoslawec, Department of Non-Clinical Drug Safety, Hoffmann-La Roche Inc., Nutley, NJ for her support in beagle dog experiments. Assistance in performing pharmacokinetic analysis by Dr. Navita Mallalieu, Department of Discovery Pharmacology, Hoffmann-La Roche Inc., Nutley, NJ is kindly acknowledged.

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Table I. Lipid formulations that were used in the *in vivo* studies

Formulation	A	B	C	D
Nifedipine	100 mg	100 mg	100 mg	100 mg
CapmulPG-8	45 g	-	-	-
Capmul MCM	-	45 g	-	-
Miglyol810	-	-	45 g	40.5 g
Cremophor EL	-	-	-	4.5 g

Table II. *In vivo* study experimental design (crossover study in fasted beagle dogs)

Formulation	Dose (mg/dog)	Number of capsules	Animal Numbers	
			Male	Female
A	10	4	3	3
One week wash-out period				
B	10	4	3	3
One week wash-out period				
C	10	4	3	3
One week wash-out period				
D	10	4	3	3

Table III. Nifedipine plasma concentrations (ng/mL)

Formulation A									
Time (hr)	M1	M2	M3	F4	F5	F6	Mean	SD	SEM
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	7.11	7.10	6.23	0.00	11.73	21.31	10.70	6.32	2.58
1	12.99	6.90	13.05	0.00	17.92	84.47	27.07	32.33	13.20
2	7.18	5.64	9.12	0.00	10.23	58.50	18.13	22.63	9.24
3	0.00	0.00	5.09	0.00	6.80	23.97	11.95	10.44	4.26
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Formulation B									
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	19.46	27.45	38.20	14.93	9.44	34.55	24.01	11.31	4.62
1	9.91	28.12	31.41	22.50	48.19	67.23	34.56	20.28	8.28
2	19.23	18.12	32.27	19.22	21.24	19.93	21.67	5.29	2.16
3	8.71	10.14	27.10	16.35	19.07	18.76	16.69	6.71	2.74
6	8.27	0.00	13.56	0.00	13.05	0.00	11.63	2.92	1.19
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table III: Nifedipine plasma concentrations (ng/mL) (continued)

Formulation C									
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	15.34	61.28	47.73	89.32	12.27	72.27	49.70	30.98	12.64
1	6.56	61.50	77.97	66.12	8.75	56.42	46.22	30.72	12.54
2	5.56	47.75	57.04	82.63	13.13	52.55	43.11	28.90	11.80
3	0.00	34.25	25.88	51.03	18.65	55.63	37.09	15.91	6.49
6	0.00	20.02	6.40	19.84	7.46	21.63	15.07	7.47	3.05
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Formulation D									
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	141.78	47.37	110.87	136.30	115.80	80.60	105.45	35.77	14.60
1	57.84	38.94	48.63	46.61	69.23	54.09	52.56	10.43	4.26
2	18.58	47.49	24.85	44.95	89.69	37.35	43.82	25.13	10.26
3	16.91	13.87	33.98	32.81	76.15	16.90	31.77	23.40	9.55
6	5.52	6.41	32.02	19.31	53.54	10.75	21.26	18.66	7.62
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table IV. Mean pharmacokinetic parameters of nifedipine lipid formulations with 6 beagle dogs

Formulation	Tmax $\pm$ sem¹ (hr)	Cmax $\pm$ sem² (ng/mL)	AUC₀₋₂₄ $\pm$ sem³ (ng.h/mL)	Digestibility
A (CapmulPG-8, propylene glycol mono-caprylate)	0.9 $\pm$ 0.1	23.66 $\pm$ 11.03	54.47 $\pm$ 18.80	Yes
B (Capmul MCM, glyceryl mono-caprylate/caprate)	0.83 $\pm$ 0.11	37.28 $\pm$ 7.39	154.64 $\pm$ 22.81	Skips
C (Miglyol810, glyceryl tri-caprylate)	0.75 $\pm$ 0.11	55.84 $\pm$ 12.83	252.31 $\pm$ 54.24	Skips
D (Miglyol810/Cremophor EL, 9/1)	0.58 $\pm$ 0.08	105.47 $\pm$ 14.59	362.44 $\pm$ 108.05	Inhibited

¹ p < 0.1806 Tmax values are not significantly different² p < 0.006 Cmax values are significantly different³ p < 0.0235 AUCs are significantly different

Table V. Summary of *in vitro* results for nifedipine lipid formulations

Formulation	Solubility (mg/g)	Dissolution (%) at 60 min	Partition coefficient (o/w)	Particle size (nm)
Miglyol 810	3.36 ± 0.03	50.19 ± 1.44	5.6 ± 0.45	Coarse
Capmul MCM	10.27 ± 0.09	96.05 ± 0.28	2 ± 0.18	11.6 ± 0.5
Capmul PG-8	16.98 ± 0.55	30.27 ± 3.54	9.6 ± 0.76	777 ± 32
Miglyol 810/ Cremophor EL	4.86 ± 0.04	97.23 ± 2.52	1.1 ± 0.02	10 ± 1.0

Figure 2: Dissolution of nifedipine from the formulations used for in vivo study

SECTION III

APPENDIX

Figure 1. Calibration curve for griseofulvin in acetone

Figure 2. Standart curve for HPLC analysis of nifedipine

Figure 3. FT-IR spectrum of nifedipine in mineral

Figure 4. FT-IR spectrum of griseofulvin in mineral

Figure 5. FT-IR spectrum of oleic acid

Figure 6. FT-IR spectrum of Captex GTO

Figure 2. Standart curve for HPLC analysis of nifedipine

Figure 3. FT-IR spectrum of nifedipine in mineral

Figure 4. FT-IR spectrum of griseofulvin in mineral

Figure 5. FT-IR spectrum of oleic acid

Figure 6. FT-IR spectrum of Captex GTO

Figure 7 FT-IR spectrum of Miglyol 810

Figure 8. FT-IR spectrum of Capmul MCM

Figure 9. FT-IR spectrum of Capmul GMO

Figure 10. FT-IR spectrum of Caprol2GO

Figure 11. FT-IR spectrum of Caprol3GO

Figure 12. FT-IR spectrum of Capmul PG-8

Figure 13. FT-IR spectrum of Capmul PG-12

Figure 14. FT-IR spectrum of Capmul PG-18

Figure 15. FT-IR spectrum of Captex200

Figure 16. Standard curve for nifedipine plasma calculation

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