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The Use of Liquid Self-Microemulsifying Drug Delivery Systems Based on Peanut Oil/Tween 80 in the Delivery of Griseofulvin

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Peanut oil and Tween 80 blends devoid of any cosurfactant were employed in the formulation of different batches of liquid self-microemulsifying drug delivery systems (LSMEDDS) and their suitability as vehicles for the delivery of a typical lipophilic drug—griseofulvin—was investigated. The LSMEDDS were evaluated using the following parameters: phase separation, globule size, viscosity, solubility of griseofulvin, and partition coefficient. The release profile of griseofulvin from the optimized LSMEDDS was evaluated in citrate/phosphate buffer solutions of pH 2.0, pH 6.5, and pH 7.4. The results obtained indicated that there was significantly higher ($\alpha \leq 0.05$) percentage cumulative amounts of griseofulvin released from the LSMEDDS in comparison with that released from peanut oil alone. The release of griseofulvin from the LSMEDDS into aqueous media of pH 6.5 and pH 7.4 showed enhanced and controlled dissolution of the drug from the formulation. Incorporation of griseofulvin into this proposed formulation is suggested as a strategy to overcome the irregular dissolution and absorption behaviors often associated with conventional griseofulvin tablets.

Keywords liquid self-microemulsifying drug delivery system; peanut oil; Tween 80; griseofulvin; release profile

INTRODUCTION

Peroral drug administration is the preferred route for chronic drug therapy. Numerous potent lipophilic drugs exhibit low oral bioavailability due to their poor aqueous solubility. The Biopharmaceutics Classification System (Amidon, Lennernas, Shah, & Crison, 1995) classifies drugs into four categories depending on their solubility and permeability characteristics. According to this scheme, griseofulvin belongs to class (or case) II drugs whose solubility is too low to be consistent with complete absorption. For this class of compounds, defined as "low solubility/high permeability class," dissolution in the lumen environment is the rate-controlling step in the absorption process (Amidon et al., 1995). Efforts are ongoing to enhance the oral bioavailability of lipophilic drugs to increase their clinical efficacy. The most popular approach is the incorporation of the active lipophilic component into inert lipid vehicles (Hong, Kim, Song, Park, & Kim, 2006), such as oils (Burcham, Maurin, Hausner, & Huang, 1997), surfactant dispersions (Aungst et al., 1994; Serajuddin, Sheen, Mufson, Bernstein, & Augustine, 1988), self-emulsifying formulation (Aungst, 1993; Charman et al., 1992; Craig, Lievens, Pitt, & Storey, 1993; Shah, Carvajal, Patel, Infeld, & Malick, 1994; Wakerly, Pouton, Meakin, & Morton, 1986), emulsions (Kararli et al., 1992; Myers and Stella, 1992; Palin, Philips, & Ning, 1986; Stella et al., 1978; Toguchi, Ogawa, Iga, Yashiki, & Shimamoto, 1990a), and liposome (Schwendener & Schott, 1996). These formulation approaches have their specific advantages and limitations. Potential advantages of the formulations resulting from these formulation approaches include enhanced oral bioavailability enabling reduction in dose, more consistent temporal profiles of drug absorption, selective targeting of drug(s) toward specific absorption window in gastrointestinal (GI) tract, and protection of drug(s) from the hostile environment in the gut (Kim, Cho, & Gao, 2001; Pouton, 1997). A major limitation of these systems is that it is difficult to predict the in vivo behavior of drug formulations for both nonaqueous and emulsified phases and this is ascribable to several physical and physiological factors. These factors include (1) whether the drug is formulated in an oil or emulsified form and in the latter form how it is being distributed between the two phases, (2) the absorption pathway of

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the drug, (3) the nature and particle size of the in vivo emulsion, (4) the role of surfactant/enhancers, (5) the metabolic pathway of the oil (triglyceride), and (6) the tendency of the formulation to slow gastric motility and to promote emptying of the gall bladder (Constantinides, 1995).

Liquid self-microemulsifying drug delivery systems (LSMEDDS) have been previously described in the literature as homogeneous mixtures of natural synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and cosolvents (Constantinides, 1995; Craig, 1993; Gao, Witt, Haskell, Zamora, & Shifflett, 2004; Taha, Samy, Kassem, & Khan, 2005). The principal characteristic of these systems is their ability to form fine oil-in-water (o/w) emulsions or microemulsions upon mild agitation following dilution by aqueous phases. This property makes LSMEDDS good candidates for the oral delivery of hydrophobic drugs especially those, which have adequate solubility in oil or oil/surfactant blends. Griseofulvin represents a classical example of drugs exhibiting poor aqueous solubility and complex technical formulation problems. Vegetable oils such as fractionated arachis and peanut oils are employed as vehicles for preparing oil solutions for injection (Reilly, 2000). The formation of microemulsions usually involves a combination of three to five components, namely: oil, water, surfactant, cosurfactant, and electrolyte. The novelty embodied in this study lies in the formulation of LSMEDDS using peanut oil and a nonionic surfactant (Tween 80) blends devoid of any cosolvent or cosurfactant. These cosurfactants are usually short-chain alcohols such as ethanol, polyethylene glycol or polyethylene glycol and they may help to dissolve large amounts of either the hydrophilic surfactant or the drug in the lipid phase and may sometimes play the role of the cosurfactant in the microemulsion systems. In order words, the cosurfactant increases the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film due to the void space among surfactant molecules (Constantinides et al., 1994). The objective of this study, therefore, was to design and formulate LSMEDDS incorporating griseofulvin for the purpose of enhancing the dissolution of the drug. The suitability of peanut oil/Tween 80 blends for this purpose was assessed by evaluating some of the physicochemical properties of the emulsion formed as well as the efficiency of incorporation of the drug in the LSMEDDS.

MATERIALS AND METHODS

Materials

Griseofulvin (AstraZeneca, Brussels, Belgium), Tween 80 (Merck, Darmstadt, Germany), sodium acetate, acetic acid (BDH, Midlands, England, UK), and pharmaceutical grade peanut oil (Croda, New York, NY, USA) were used as procured from their suppliers without further purification. Distilled water was obtained from an all-glass still. All other reagents were analytical grade and used as such.

Formulation of Various Liquid Self-Microemulsifying Drug Delivery Systems

LSMEDDS were formulated with different proportions of peanut oil and Tween 80 as shown in Table 1 to obtain blends containing from 5 to 40% Tween 80 while keeping the volume of water constant at 5 mL. An excess amount of griseofulvin powder (1 g) was added to the various oil/surfactant blends in test tubes and mixed by gentle stirring followed by mild agitation at 50 rpm for 2 min in a reciprocating mixer to ensure thorough homogenization while maintaining a temperature of 25 ± 1°C in a thermostated water bath. The mixtures wee finally kept for 12 h to attain equilibrium. The equilibrated samples were centrifuged at 600 × *g* for 10 min to remove the undissolved griseofulvin. The supernatant was taken and diluted for spectrophotometric quantification of griseofulvin at 291 nm.

Phase Separation Studies of the Various LSMEDDS

Using a micropipette, 0.05 mL volume of each LSMEDDS was added to a glass test tube containing 5 mL of distilled water at room temperature (25°C). The mixtures were again placed in the reciprocating agitator and homogenized for 2 min and allowed to stand maintained at the same temperature for a period of 12 h. The samples were assessed to determine possible separation of the phases by visual inspection. The above experiment was repeated with griseofulvin-loaded LSMEDDS.

Measurement of Globule Sizes of the LSMEDDS

The mean globule sizes of the various unloaded and griseofulvin-loaded LSMEDDS in various media (distilled water, 0.1 N HCl, and citrate/phosphate buffer, pH 7.4) were measured at room temperature using a microscope with a calibrated eyepiece (Koywa, Tokyo, Japan). The mean size of 100 globules measured on a single mount was used for each preparation.

Viscosities of the LSMEDDS

The viscosities of the various griseofulvin-loaded and unloaded LSMEDDS in different media were measured using a

TABLE 1
Quantities of Materials Used in Formulating the LSMEDDS

Batch	Peanut Oil (mL)	Tween 80 (mL)
A	12.375	0.0625
B	12.375	0.125
C	12.375	0.1875
D	12.375	0.250
E	12.375	0.3125
F	12.375	0.375
G	12.375	0.4375

torsion viscometer (Gallenkamp, Loughborough, England, UK) at room temperature. About 5 mL of the dispersion was employed for each determination and the mean of five determinations was taken as the viscosity of each preparation.

Determination of the Partition Coefficient of Griseofulvin Between the Oil and Buffer Solutions

The partition coefficient was determined at 37 ± 1°C. Each buffer solution (2 mL) containing griseofulvin (5%) was added to 2 mL of peanut oil. The mixture was agitated at 37 ± 1°C for 24 h using a thermostated water bath shaker. After standing for 1 h, 1 mL each of both the oil and buffer phases was collected and diluted with 9 mL of ethanol. The griseofulvin concentrations in both phases were determined spectrophotometrically at a predetermined wavelength of 291 nm using a digital UV–Vis double-beam spectrophotometer (SP8–100, Pye Unicam, Victoria, Melbourne, Australia) by extrapolation from a standard Beer's plot. The mean of three replicate runs was taken as the partition coefficient.

Solubility of Griseofulvin in the LSMEDDS

Griseofulvin (500 mg) was incorporated into the various LSMEDDS to achieve approximate concentrations of 25 mg/mL of the drug in each system. Each mixture was agitated for about 5 min using a stirrer and further vigorously agitated at 37 ± 1°C using a water bath shaker to equilibrate griseofulvin in the peanut oil and Tween 80 dispersion. Twelve hours was adjudged sufficient time to achieve equilibrium distribution of the drug in the two phases. The equilibrium mixture was centrifuged at $600 \times g$ for 10 min and 0.1 mL of the supernatant was taken and diluted with ethanol and the concentrations of griseofulvin determined spectrophotometrically at 291 nm.

Release Profile of Griseofulvin from the LSMEDDS

The USP 1999 Method II (paddle stirred system) was employed as follows: a volume of the LSMEDDS containing 200 mg of griseofulvin was transferred into a dialysis bag and the open end tied up to prevent leakage. The dialysis bag was immersed in 300 mL of each buffer solution maintained at 37 ± 0.5°C, held to the bottom of the vessel attaching to a stainless-steel gauze and stirred continuously at a rate of 50 rpm. The paddle clearance from the dialysis bag was about 2 cm. At predetermined time intervals, 1 mL portions of the dissolution medium were withdrawn, appropriately diluted and their absorbance determined in a spectrophotometer. The volume of the dissolution medium was kept constant by replacing it with 1 mL of fresh buffer solution after each withdrawal. The concentrations of the drug in the samples were determined with reference to the standard Beer's plot. The experiment was similarly repeated using an equivalent amount of griseofulvin powder dispersed in peanut oil alone. Four replicate release studies were carried out.

Statistical Data Analysis

The data from the different formulations were compared for statistical significance using the Student's *t* test with a level of significance (α) set at 0.05. All results were expressed as $M \pm SD$.

RESULTS AND DISCUSSION

Phase separation studies were initially carried out as a preliminary test for the efficiency of the self-microemulsification between the surfactant/oil mixture (Tween 80/peanut oil) and water. The principal characteristic of self-emulsifying systems is their ability to form fine o/w emulsions or microemulsions spontaneously upon mild agitation following dilution by aqueous phases. This is as a result of thermodynamic stability of self-emulsifying systems as opposed to the regular emulsions that are thermodynamically unstable. The formulated LSMEDDS showed no detectable phase separation during the 12-h period and were subjected to subsequent studies. Incorporation of griseofulvin did not affect the stability of the formulation throughout the course of the experiment. To confirm the long-term stability of both the griseofulvin-loaded and unloaded LSMEDDS formulations, they were stored at room temperature (25°C) on a bench for 4 weeks. Even after this long period of storage, no detectable changes in the integrity of the formulations could be observed. Figure 1 shows the variation of globule size of the LSMEDDS with concentration of the surfactant used in the formulation. Increasing concentration of the surfactant (Tween 80) decreased the globule size of the LSMEDDS until an optimum (minimum) value was attained at

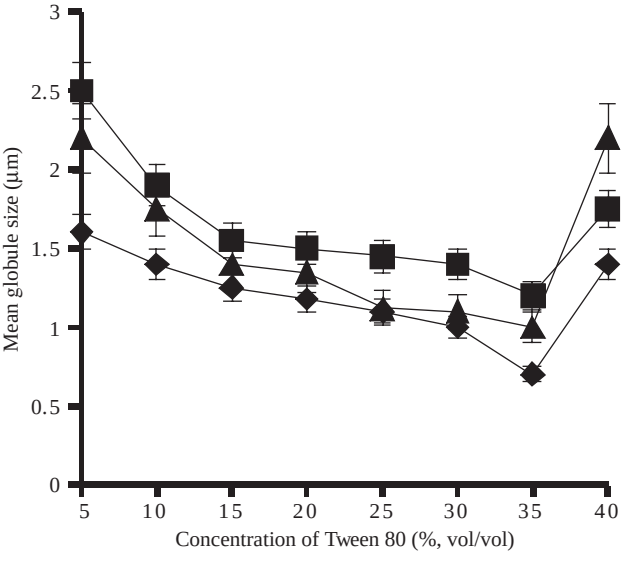

FIGURE 1. Variation of mean globule size of the unloaded LSMEDDS with concentration of Tween 80 in different media. —✦—, distilled water; —■—, 0.1 N HCl; —▲—, citrate/phosphate buffer. Data are expressed as $M \pm SD$ of 100 globules.

35% surfactant concentration. It is discernible from Figure 2 that the globule size at the optimal value of surfactant concentration showed a slight but insignificant increase ($\alpha \leq 0.05$) in the presence of griseofulvin. This may be due to some interference, of the drug, with self-emulsification process by altering the optimal oil/surfactant ratio and by interacting with the liquid crystalline or gel phase of the emulsion droplets (Gershanik & Benita, 2000). Emulsion droplet size is known to be a very important factor in the formulation of liquid self-emulsifying systems because of its influence on the rate and extent of drug release and absorption (Shah et al., 1994; Tarr & Yalkowsky, 1989). Some authors (Shah et al., 1994) have defined efficient self-emulsification as a system, which produces mean emulsion droplet diameter values of less than 5 μm. The optimal formulation, with or without the drug yielded emulsion with droplet size below 1 μm indicating that the oil/surfactant pair employed in this formulation has very efficient self-emulsification ability. Two factors have been identified to favor emulsion stability in the case of self-emulsifying systems: (1) relatively small volume of the dispersed oil phase and (2) narrow range of droplet size distribution (Shah et al., 1994). These two factors were found to be operative in our formulations; hence the high stability of the various LSMEDDS formulated in this study as noted from the phase separation studies. For a given combination of components, emulsions with small, uniform droplet size such as seen in our formulations, will take longer to break. Larger droplets are less stable than small droplets due to their larger area to volume ratio, and so will tend to grow at the expense of the smaller droplets (Shaw, 1980).

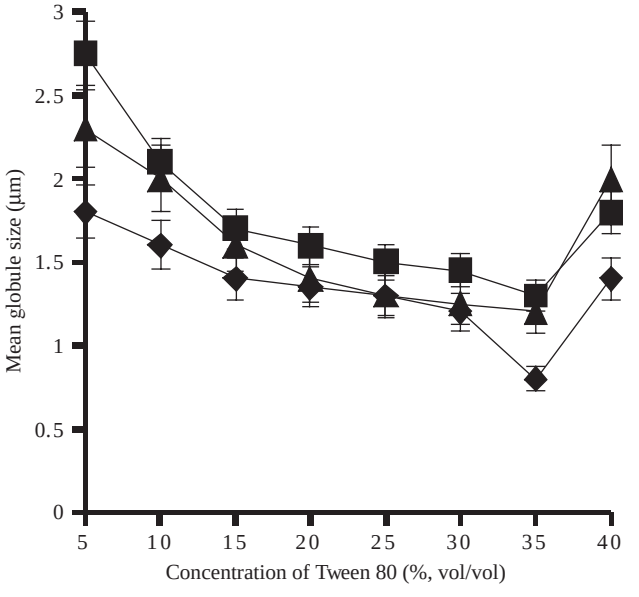

FIGURE 2. Variation of mean globule size of the loaded LSMEDDS with concentration of Tween 80 in different media. —✦—, distilled water; —■—, 0.1 N HCl; —▲—, citrate/phosphate buffer. Data are expressed as $M \pm SD$ of 100 globules.

The optimal surfactant concentration of 35% that yielded the optimal formulation is also ideal for a preparation that will traverse the GI tract. It has been reported that the usual surfactant concentration in liquid self-emulsifying formulation required to form and maintain an emulsion state in the GI tract ranged from 30 to 60% (wt/wt) (Gershanik & Benita, 2000). A large quantity of surfactant may irritate the GI tract. It is noteworthy from Figure 1 that distilled water provided the most suitable aqueous phase for enhanced self-emulsification performance in comparison with 0.1 N HCl and citrate/phosphate buffer, pH 7.4. In an earlier work (Wakerly et al., 1986), it was suggested that the ease of emulsification could be associated with the ease with which water penetrates into the various liquid crystalline or gel phases formed on the surface of the droplet. This ease of penetration of water into the gel phases of the droplet was observed visually in this study to be faster in distilled water than in 0.1 N HCl or citrate/phosphate buffer pH 7.4.

Partition coefficient values of 1.43, 1.26, and 1.21 were obtained in buffer solutions of pH 5.5, pH 6.5, and pH 7.4 respectively. This shows a slight decrease in partition coefficient values as the pH increased from 5.5 to 6.5. Conversely, there appears to be no significant variation ($\alpha = 0.05$) in the partition coefficient value as the pH increased from 6.5 to 7.4; an indication that at a near neutral pH, the partition coefficient of griseofulvin between the two phases is unaltered. Additionally, the intermediate values of partition coefficient obtained are an indication of easy partitioning of griseofulvin between lipid and aqueous phases (Gulati, Grover, Singh, & Singh, 1998). This is remarkable considering the fact that drug release from LSMEDDS depends on diffusion rate of the drug from the oil phase to the aqueous phase. This occurs as the droplets are transported along the GI tract in the presence of aqueous intestinal fluid. Griseofulvin is known to be poorly water soluble but in this formulation, there is high diffusion rate of the drug to the aqueous phase, which ensured its rapid dissolution. It may be reasonable to infer that in a medium of pH 5.5, griseofulvin incorporated in LSMEDDS may be released very rapidly.

The variation of mean viscosity of the LSMEDDS with concentration of Tween 80 in different media is depicted in Figure 3. It is apparent from Figure 3 that the viscosities of the LSMEDDS increased gradually with increasing concentration of the surfactant up to the optimal surfactant concentration of 35% (wt/wt) after which, the mean viscosity remained relatively constant until it began to decrease beyond a surfactant concentration of 70% (wt/wt). The presence of griseofulvin did not cause any remarkable change in the viscosity values recorded. A possible explanation is that the drug was completely solubilized in the oil/surfactant mixture prior to the final formation of the LSMEDDS. Viscosity is known to have some correlation (a inverse relationship at low shear rates such as were applicable in this study) with mean globule or particle size. A smaller particle size generally increases the low shear

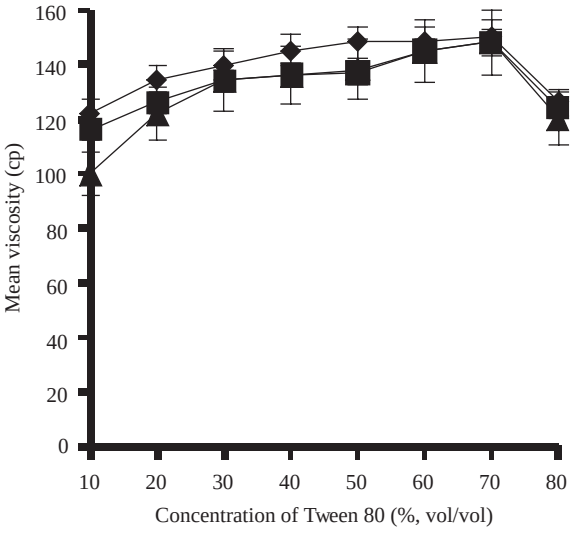

FIGURE 3. Variation of mean viscosity of the unloaded LSMEDDS with concentration of Tween 80 in different media. —✦—, distilled water; —■—, 0.1 N HCl; —▲—, citrate/phosphate buffer. Data are expressed as $M \pm SD$ ($n = 5$).

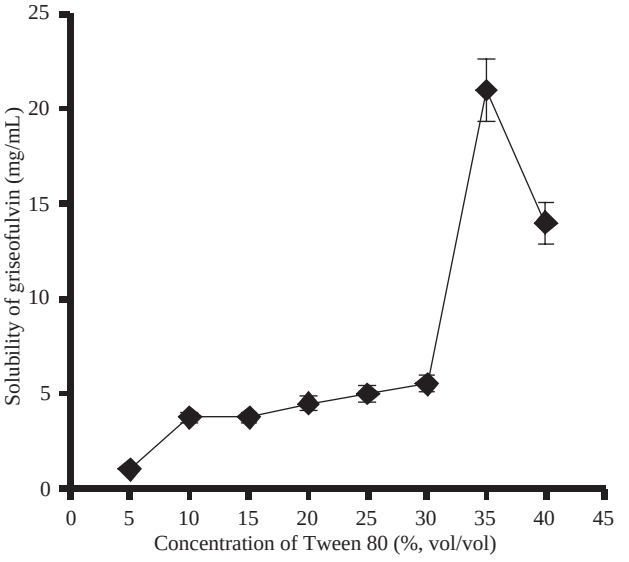

FIGURE 4. Solubility of griseofulvin in LSMEDDS containing varying concentrations of Tween 80 ($n = 5$).

viscosity due to colloidal interactions in emulsions. However, viscosity is often independent of particle size at higher shear rates as hydrodynamic forces dominate. A wide particle size distribution usually gives a lower viscosity than narrow ones due to better particle packing (Malvern, UK document, http://www.malvern.com/LabEng/industry/adhesives.htm.). An inverse relationship between the surfactant concentration and the mean globule size of the resulting LSMEDDS below the optimal concentration has earlier been noted in this study (see Figures 1 and 2). In addition, the highest mean viscosity was recorded in LSMEDDS formulated in distilled water as medium, further confirming distilled water as the most suitable aqueous phase for self-emulsification involving peanut oil and Tween 80 in comparison with the other aqueous phases. Generally, viscosity of LSMEDDS is known to give indication as to the emulsion globule size (Malvern, UK document, http://www.malvern.com/LabEng/industry/adhesives.htm.), which is observed to further influence the rate and extent of drug release and absorption.

The effect of concentration of the surfactant on the solubility of griseofulvin in the LSMEDDS is shown in Figure 4. The solubility of griseofulvin was highest in LSMEDDS composed of 35% (wt/wt) Tween 80. Beyond this optimal surfactant concentration, the solubility decreased sharply, confirming this concentration to be the optimum needed for efficient self-emulsification performance. Earlier studies (Pouton, 1995; Wakerly, Pouton, & Meakin, 1987) had revealed that the self-emulsification process is specific to (1) the nature of the oil/surfactant pair, (2) the surfactant concentration and oil/surfactant ratio, and (3) the temperature at which the process occurs. These important discoveries were further supported by the fact that only very specific combinations of pharmaceutical excipients led to efficient self-emulsifying systems (Chanana & Sheth, 1995; Kimura et al., 1994). It has also been argued that the liquid crystalline phase which forms between the oil/surfactant and water phases may be expected to be highly dependent on the proportions of oil, surfactant, and water present (Pouton, 1985). This may explain the observation from this study where addition of the surfactant (Tween 80) above 35% (wt/wt) almost always resulted in changes in the characteristics of the formulated LSMEDDS. The surface active agents are ampliphilic by nature, and they are therefore usually able to dissolve and even solubilize relatively high quantities of hydrophobic drugs. This ability to solubilize hydrophobic drugs has been found to be of prime importance for preventing precipitation within the GI lumen and for the prolonged existence of the drug molecules in soluble form, which is vital for effective absorption (Serajuddin et al., 1988; Shah et al., 1994).

Figure 5 shows the release profile in different media of griseofulvin from the LSMEDDS and the peanut oil at an agitation speed of 50 rpm. There was rapid initial release of griseofulvin within the first 1 h followed by a much slower release over the remaining release period. Up to 65% of griseofulvin was released from the LSMEDDS within 8 h at pH 6.5 and pH 7.4, whereas about 53% of the drug was released at an acidic pH of 2.0 within the same period. The dissolution experiment was carried out for up to 24 h. However, it was not possible to obtain 100% release of the drug even after this long period. It is probable that some amounts of the griseofulvin already solubilized in peanut oil/surfactant mixture must have been adsorbed onto and/or trapped in the dialysis membrane. The slightly higher amount

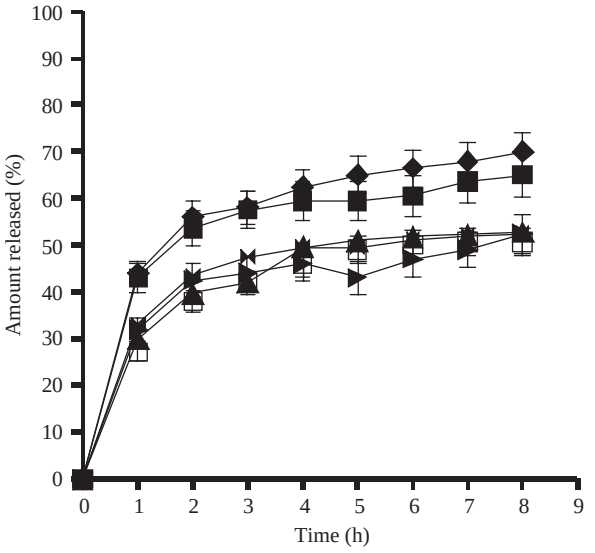

FIGURE 5. Release profile of griseofulvin from LSMEDDS and from the drug/oil mixture. —✦—, drug/LSMEDDS in buffer pH 6.5; —■—, drug/LSMEDDS in buffer pH 7.4; —▲—, drug/LSMEDDS in buffer pH 2.0; —►◄—, drug/oil in buffer pH 6.5; —►—, drug/oil in buffer pH 7.4; —□—, drug/oil in buffer pH 2.0 (n = 3).

of griseofulvin released at pH 6.5 than at pH 7.4 may be due to the slightly higher partition coefficient value of griseofulvin at the former pH in comparison with that at the latter pH. However, the difference in the amounts of griseofulvin released at both pH values was found not to be significant ($\alpha = 0.05$). This seems to closely mirror the results from partition coefficient studies. Release of the drug from the LSMEDDS was significantly higher ($\alpha = 0.05$) than from peanut oil alone. This is foreseen since the presence of surfactant in LSMEDDS has been shown to promote effective dispersion of hydrophobic drug in the lumen by the solubilization process, in addition to drug spreading in oil droplets (Toguchi, Ogawa, & Shimamoto, 1990b; Yoon & Burgess, 1996). It is equally discernible from Figure 5 that there was an extended release of griseofulvin from the LSMEDDS and from the oil as less than 65% of the drug was released within a 6-h period. This may be an advantage for a drug such as griseofulvin, which is used in the treatment of chronic fungal infections; the rapid initial release within the first 1h, as earlier noted, causing a rapid attainment of the minimum inhibitory concentration in vitro. This may have interesting implications in in vivo situations. Release of griseofulvin as noted above was also observed to be higher in media of high pH (6.5 and 7.4) in comparison with that at low pH of 2.0. This is also expected since griseofulvin, a weakly acidic drug, should show increased solubility in an alkaline environment. Release of griseofulvin from the LSMEDDS may occur essentially from the globules following their rupture or disintegration in the agitated medium. It is possible that some amount of drug may be released by passive diffusion from the oil globules, but the rupture of the globules in the infinitely diluted medium seems to play a predominant role in the release of the griseofulvin from the LSMEDDS formulation.

CONCLUSION

Peanut oil/Tween 80 admixtures have been formulated to show the formation of a microemulsified system under conditions of gentle agitation, similar to those which could be encountered in the GI tract for the delivery of a known poorly water soluble drug, griseofulvin. However, it should be acknowledged that the formulation proposed in this study may have some implications and even limitations for a segment of the world population that are prone to allergies resulting from peanut consumption. The emulsion droplet size was generally small, indicating that the formulation has efficient self-emulsification ability. The formulated LSMEDDS enhanced the dissolution and extended the release of griseofulvin. A separate investigation to correlate these results with in vivo bioavailability is currently being carried out.

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