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European Journal of Pharmaceutics and Biopharmaceutics 53 (2002) 343–352

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Formulation of an oral dosage form utilizing the properties of cubic liquid crystalline phases of glyceryl monooleate

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Received 25 September 2001; accepted in revised form 11 February 2002

Abstract

Glyceryl monooleate is a Food and Drug Administration-approved food additive which has the ability to form various liquid crystalline phases in the presence of various amounts of water. The unique properties of the cubic liquid crystalline phase that result upon the presence of excess body fluids at body temperature were utilized to formulate an oral dosage form containing furosemide as the model drug. The aim was to develop a formula, which has both bioadhesive and sustained release properties of the resultant cubic phase, so that increasing gastric residence time to improve bioavailability of the drug and at the same time obtaining a sustained action. The system was found to be affected by the limited solubility of furosemide in both the carrier system and the pH of surrounding medium. As a consequence, the addition of some solubility modifiers was investigated in order to obtain the desired properties of the expected liquid crystalline system.

Keywords: Glyceryl monooleate; Monoolein; GMO; Furosemide; Sustained release; Liquid crystal; Drug delivery; Formulation; Dissolution; Drug release

1. Introduction

Glyceryl monooleate (monoolein or GMO), a Food and Drug Administration-approved food additive, is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate, and has the ability to form different types of lyotropic liquid crystals in the presence of water [1]. The use of liquid crystalline phases of GMO as drug delivery systems has been widely investigated by many co-workers [1–7]. The unique properties of cubic liquid crystalline phases formed from GMO systems have been utilized for the preparation of controlled release systems [1–5]; and in topical and mucosal drug delivery systems due to their adhesive properties [4,9–12].

The model drug investigated in this research is furosemide; a diuretic agent that is used in the treatment of hypertension and edema associated with heart failure [13]. Several attempts were made in order to prepare sustained release formulations of furosemide with the aim of avoiding the short period of peak diuresis observed with conventional dosage forms in addition to reduction of associated side effects [14]. Nonetheless, sustained release formulations were faced with reduced bioavailability of the drug in comparison with immediate release dosage forms [15]. Ritschel et al. [16] had postulated that due to the site specificity and mechanism of absorption a peroral modified release dosage form having a longer gastric residence time could possibly increase the bioavailability of furosemide. This theory had been proved by Menon et al. [17] and Akiyama et al. [18] where different formulations designed to increase gastric residence time were associated with maximizing drug absorption. Moreover, a correlation was made between gastrointestinal transit and furosemide absorption [19].

In this study, the aforementioned properties of the cubic liquid crystalline phases of GMO were investigated to formulate an oral drug delivery system for furosemide. Furosemide (40 mg dose) was dispersed in GMO and filled into hard gelatin capsules, which when exposed to gastrointestinal fluids at body temperature, the mixture would swell forming the cubic liquid crystalline phase. Such a system was assumed to produce sustained release formulation in addition to being retained in the stomach through its bioadhesive nature. This is a first part of the study that deals

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0939-6411/02/$ - see front matter
PII: S0939-6411(02)00016-4

with the investigation of phyiscochemical properties of the cubic liquid crystalline phases arising from incorporation of furosemide in GMO and the exposure of the resultant mixture to gastrointestinal fluid. Furthermore, the effect of the pH of the surrounding medium and other additives that had modified the release rates were also studied.

2. Materials and methods

2.1. Materials

GMO is supplied from Eastman Fine chemicals, USA as a trade name GMOrphic-80. Furosemide (BP) is supplied from Chemische Fabrik, Italy. Polyethylene glycol 400 was manufactured by Riedel-de-Haën, Germany. Sodium chloride, disodium hydrogen orthophosphate dihydrate and potassium chloride were supplied from BDH Analar Chemicals, Poole, UK. Citric acid anhydrous and trisodium phosphate were obtained from Fluka AG, Switzerland. Sodium dihydrogen orthophosphate was manufactured by Merck, Germany. The water used was distilled water.

2.2. Equipment

The equipment used includes: Erweka dissolution tester model DT6R (Erweka, Frankfurt, Germany); Cary IE UV-spectrophotometer (by Varian Australia PTY Ltd., Australia); shaker water bath (Heto Lab Equipment SBD50 BIO, Denmark); Bausch & Lomb polarizing optical microscope (Bausch & Lomb, USA); Leitz Wetzlar GmbH polarizing microscope (Leitz, Germany); Kofler hot-stage (Optische Werk, Austria); differential scanning calorimeter (DSC 20), Mettler TC10A processor, TA 4000 system configuration with measuring cells DSC20 (Mettler, Switzerland); peristaltic pump P-3 (Pharmacia Fine Chemicals, Sweden); pH meter CD720 WPA (WPA Linton, Cambridge, UK) and water bath GFL (GFL, Germany).

2.3. Preparation of mixtures of GMO with other materials

GMO was allowed to melt at about 45 °C, in a hot water bath. Other materials which include furosemide, polyethylene glycol 400 (PEG 400) and trisodium phosphate (TSP) were added with continuous mixing until completely dispersed. The mixture was stored at 5 °C in a dark place.

2.4. Preparation of samples for phase behavior

Accurately weighed quantities of GMO were placed in glass vials, and melted at about 50 °C. The tested aqueous media including water, simulated intestinal fluids without enzymes (SIF) (United States Pharmacopeia (USP) 24), and simulated gastric fluids without enzymes (SGF) (USP 24) were warmed to about 40 °C, then added to the melted GMO with mixing. Samples were well closed and stored at 37 °C in a dark place for 12 h in order to reach equilibrium conditions before testing.

2.5. Identification of phase behavior

Samples of GMO and GMO mixtures with other aqueous solutions (water, SGF and SIF) were examined for their liquid crystalline phases by observing their viscosities and optical properties. Phase diagrams were constructed at different temperatures upon heating at constant rate (4 °C/min) on a hot stage connected to polarizing microscope.

2.6. Preparation of samples for studying the effect of additives on the formation of cubic phase

Mixtures of different percentages of additives were mixed with melted GMO in glass vials at 50 °C until completely dissolved or dispersed. The final weight of each sample was equivalent to 3 g. Two grams of distilled water (a quantity sufficient to form the cubic phase) preheated to 40 °C were added gradually and mixed. Samples were tightly closed and left for 12 h at 37 °C to reach equilibrium.

2.7. Observation of melting behavior

The melting behavior of GMO, furosemide and their mixtures was investigated using a hot-stage microscope. A constant heating rate was applied at 4 °C/min, and the sample was placed on a glass slide and examined during heating at 40×-magnification power.

2.8. Thermal analysis (DSC)

Differential scanning calorimetry (DSC) thermograms of samples including GMO, furosemide each alone and mixtures containing 18% (w/w) furosemide in GMO and 50% (w/w) furosemide in GMO (5–15 mg) were recorded using standard aluminum crucibles, that were pierced before testing. Calibration was performed with flat pressed indium (high purity, melting point 158.4 °C, heat of fusion is 28.45 J/g at a rate of 10 K/min). The heating rate for the tested samples was 10 °C/min.

2.9. Equilibrium solubility studies

Equilibrium solubility for furosemide was carried out using aqueous solutions with different pH values. An excess of each drug powder (500–4000 mg) was introduced into 75 ml of media in a 100-ml Erlenmeyer flask with a glass stopper. The flasks were fixed on the sample holder in a thermostatically maintained water bath (37.0 ± 0.5 °C) protected from light and mechanically shaken at a fixed rate of 200 strokes/min. The solutions were shaken for 48 h before sampling. Aliquots (10 ml) of the solution were drawn and filtered through 0.45-μm membrane filter and suitably diluted. Samples were redrawn after 72 and 96 h to ensure that the solutions had reached the equilibrium solubilities. Drug concentrations were determined by measuring the UV absorbance at 275 nm using a Cary IE UV spectrophotometer. Solubility measurements were done in triplicate.

2.10. Dissolution tests

Dissolution tests were carried out in triplicates using USP apparatus II (paddle) containing 900 ml of the dissolution media maintained at 37 ± 0.5 °C and stirred constantly at 100 rpm speed, unless otherwise stated. Samples accurately weighed containing the equivalent of 40 mg furosemide were filled into hard gelatin transparent capsule size (0) and used for dissolution testing within 24 h of preparation. The dissolution water bath and all samples containing furosemide were protected from light to avoid photodegradation of furosemide. At suitable intervals, 10-ml aliquots were withdrawn and filtered through a 0.45-μm membrane filter. Replacement of the withdrawal samples was done with the same dissolution medium maintained at 37 °C. The absorbance of furosemide was determined using UV spectrophotometer at 275 nm. Suitable dilutions were made using the same dissolution media where necessary.

For dissolution media with low pH values, i.e. in pH 3.0 citrate-phosphate buffer and SGF, the aforementioned dissolution method was modified in order to prevent saturation of furosemide solution inside the dissolution vessel. The dissolution vessel containing 900 ml of dissolution medium was connected to 3 l of the same media in an Erlenmeyer flask at 37 °C via silicon tubing. A peristaltic pump was used to ensure a constant flow rate (7.0 ml/min) in and out of the dissolution vessel. Samples were withdrawn from both solutions and treated as mentioned above.

2.11. Partitioning of furosemide between aqueous solutions and GMO in the cubic liquid crystalline form

A known concentration of furosemide (about 14 μg/ml, or 0.04 M) was prepared in the selected test solution. The aqueous solutions studied include distilled water, SGF (without enzymes) (pH 1.2), SIF (without enzymes) (pH 7.5), pH 3.0 citrate-phosphate buffer, pH 5.8 phosphate buffer, pH 10.0 phosphate buffer, 0.2 g NaCl/100 ml ($\mu = 0.034$), 0.4 g NaCl/100 ml ($\mu = 0.068$), 0.8 g NaCl/100 ml ($\mu = 0.137$), 1.6 g NaCl/100 ml ($\mu = 0.274$) and 3.2 g NaCl/100 ml ($\mu = 0.548$). One hundred milliliters of each solution were placed in a 100-ml stoppered conical flask in a shaker water bath maintained at 37.0 ± 0.5 °C. The water bath was protected from light to avoid photodegradation of furosemide. The initial absorbance of each solution was determined spectrophotometrically at 275 nm after filtration through a 0.45-μm membrane filter. One gram of GMO was added to each flask with continuous shaking at 200 strokes/min. At each selected time interval, 5-ml aliquots were withdrawn, filtered through a 0.45-μm membrane filter and measured spectrophotometrically at 275 nm. Runs were done in triplicate.

2.12. Assessment of mucoadhesion properties

An experiment was designed to test the mucoadhesion of the system to the biological membrane. A female rabbit stomach was selected for the experiment as representative of the biological membranes to which the material is supposed to adhere. The selected stomach membranes were taken immediately after death and stored in saline solution at ambient room temperature and used within 8 h of death. The tested pieces were GMO, pectin, Carbomer 934P and mixtures containing 2% w/w furosemide in GMO and 5% w/w furosemide in GMO, in addition to a formula containing furosemide/TSP/PEG 400/GMO in the ratio 5:5:10:80. The bioadhesive characteristics of the test pieces were assessed under a stream of water, SGF and pH 3.0 citrate-phosphate buffer. About 800 mg of the tested piece was allowed to stand over the surface of the selected tissue to be examined. About 1 ml of normal saline was added over the piece for wetting and remained in a horizontal position for 15 min. The sample adhered to the stomach surface was then placed vertically on a polystyrene support and a stream of fluid was allowed to flow over it in a vertical direction and the rate of flow was calculated each time. The flow rate was increased gradually until the piece had fallen down where the time and flow rates were recorded. Each test was repeated at least twice.

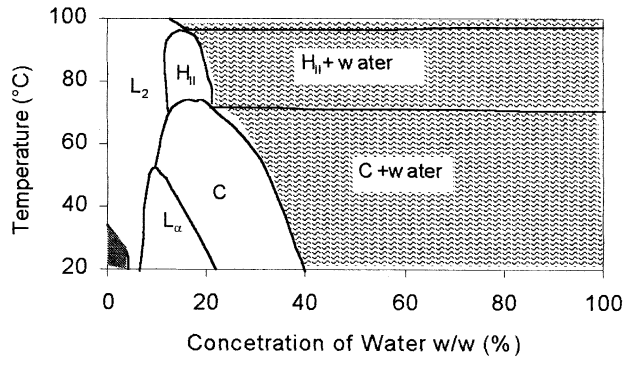
3. Results and discussion

3.1. Identification of phase diagrams of GMO

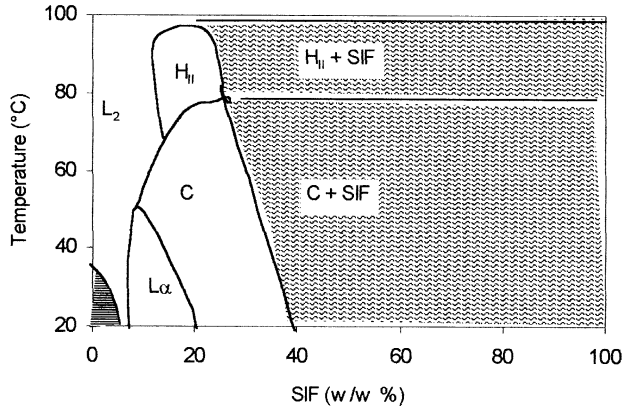
Phase diagrams of GMO were investigated in different aqueous media that included distilled water, simulated gastric fluid (SGF) without enzymes and simulated intestinal fluid (SIF) without enzymes. The last two media were selected so as to resemble gastrointestinal fluids in which the GMO is supposed to encounter.

The reversed micellar phase (L_2) was identified as being clear liquid and isotropic. The cubic phase was identified as being very viscous gel and isotropic when examined under polarizing microscope. Other phases were less viscous than the cubic liquid crystalline phase and look radiant when viewed between crossed polarizers. The lamellar phase (L_α) had a pattern of 'oily streaks' and Maltese crosses; while angular or fan-like textures was observed for the reversed hexagonal phase. These observations were in agreement with those found in the literature [8].

Fig. 1a shows the phase diagram for the GMO/water system. The diagram was similar to that of pure GMO as reported by Engstrom [1] in that L_2, lamellar and cubic phases appeared in the same relative position. The most noticeable difference was the appearance of the reversed hexagonal phase at temperatures below 80 °C instead of 85 °C as reported with pure GMO. Similar results were obtained by Geraghty et al. [8] where the reversed hexagonal phase had started to appear above 57 °C for Myverol 18-99, which is another commercial product of GMO. The phase diagram for GMO in SIF was similar to that of distilled water as shown in Fig. 1b. In SGF, the reversed



(a)



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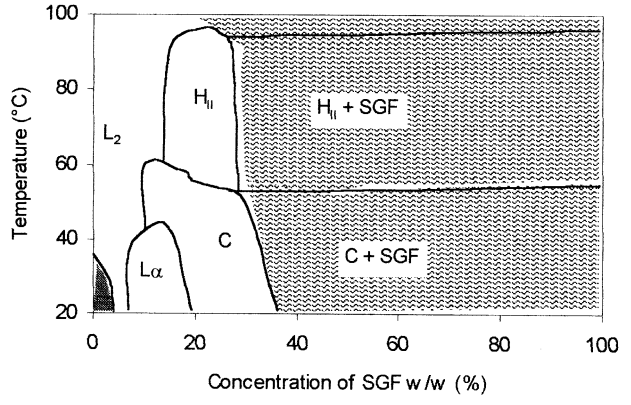

(c)

Fig. 1. Phase diagrams of (a) GMO/distilled water system, (b) GMO/SIF system, (c) GMO/SGF system. SIF, simulated intestinal fluid; SGF, simulated gastric fluid; L_2, reversed micellar phase; L_α, lamellar phase; C, cubic phase; H_{II}, reversed hexagonal phase.

hexagonal phase had started to appear at lower temperatures in comparison to that in SIF and water as shown in Fig. 1c. A similar finding had been recorded by Aota-Nakano et al. [20] where lowering the pH induced a phase transition from the cubic to the reversed hexagonal phase (H_{II}). These results were discussed in terms of the spontaneous curvature of the monolayer membrane and critical packing parameter of the membrane. These results could be utilized to prove that the cubic phase of GMO would exist at body temperature in the presence of gastrointestinal fluid.

3.2. Solubility profile for furosemide

The pH equilibrium solubility profile of furosemide was similar to that obtained by Doherty and York [21]. Since furosemide is a weakly acidic drug, its solubility is dependent on the pH of the dissolution medium. The equilibrium solubilities for furosemide in various dissolution media at 37 °C were found to be 2.5 mg/100 ml in simulated gastric fluid (SGF) without enzymes, 2.0 mg/100 ml in pH 3.0 citrate-phosphate buffer, 42.9 mg/100 ml in pH 5.8 phosphate buffer, 635.8 mg/100 ml in pH 7.5 phosphate buffer and 5.7 mg/100 ml in distilled water.

3.3. Investigations of mixtures containing furosemide and GMO

3.3.1. Effect of furosemide on the phase behavior of GMO

For mixtures containing 1–20% w/w furosemide in GMO with excess water at 37 °C, furosemide crystals were dispersed in the system as revealed by microscopic examination, which means that it was not dissolved. However, the presence of furosemide crystals did not affect the formation of cubic phase which was viscous with isotropic background under the polarizing microscope.

A complete phase diagram of GMO was investigated with the incorporation of 5% w/w of furosemide in GMO with distilled water (Fig. 2). At low temperatures, the presence of furosemide did not seem to affect the phase behavior of liquid crystalline phases of GMO in distilled water, but at high temperatures, the reversed hexagonal phase (H_{II}) was not formed. Instead, the cubic phase had existed. This might be due to the increase of solubility of furosemide in GMO at higher temperatures and so affecting the critical packing parameter at such temperatures.

3.3.2. Melting behavior of GMO mixtures containing different ratios of furosemide

Using a hot-stage microscope, mixtures containing 5% w/w and 10% w/w of furosemide in GMO were found to

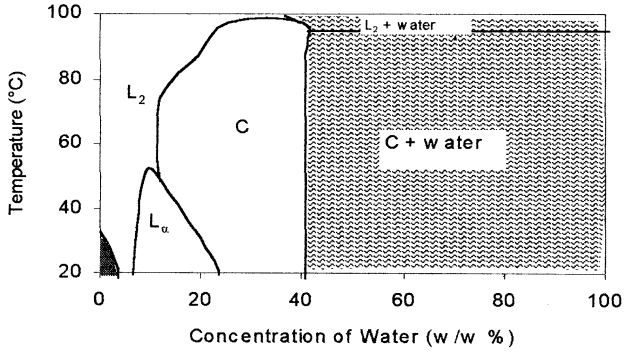

Fig. 2. Phase diagram of GMO containing 5% furosemide in water.

dissolve at about 170 and 190 °C, respectively, indicating that furosemide dissolved in GMO at high temperatures. At the same time, no decomposition was detected at the melting range of furosemide. For 20% w/w mixture of furosemide in GMO, melting was observed at 206–208 °C, followed by decomposition of the sample, indicating that complete dissolution at this range of temperature in GMO was not achieved.

The differential scanning calorimetry (DSC) thermogram for furosemide alone was similar to that reported in the literature [22] (Fig. 3a). For mixtures containing furosemide and GMO (Fig. 3), it seemed that the endothermic event attributed to the melting of GMO was not affected by the presence of furosemide.

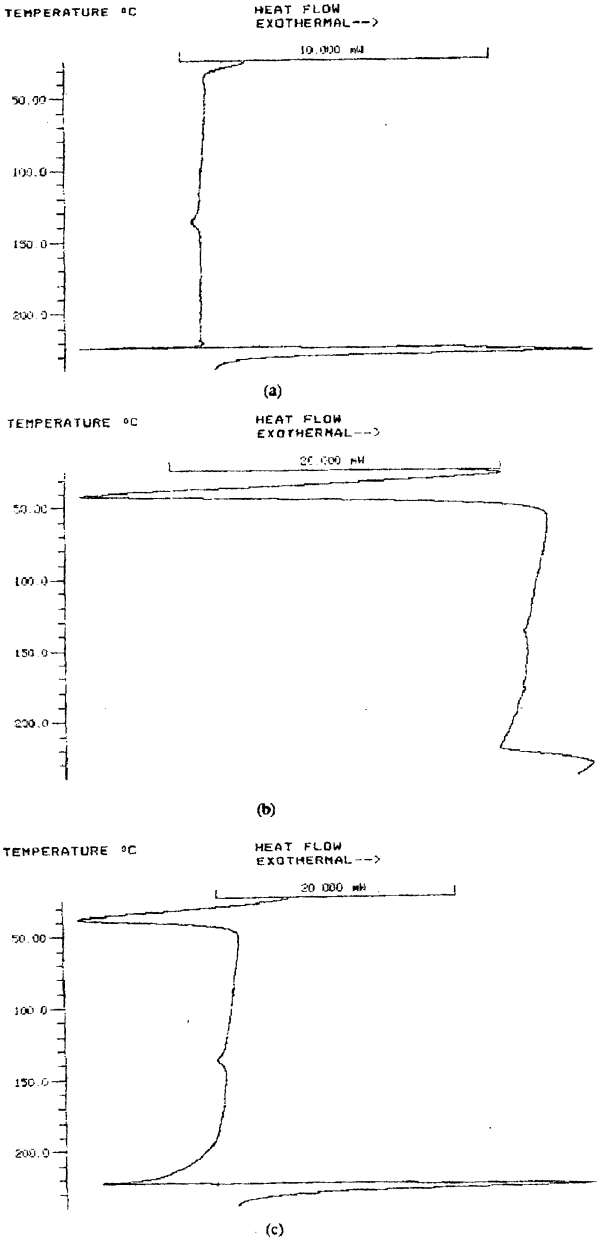

Fig. 3. DSC thermograms for furosemide alone (a), 18% furosemide in GMO (b) and 50% furosemide in GMO (c).

3.3.3. Partitioning of furosemide to the cubic liquid crystalline phases

In order to study the influence of pH of the aqueous media on the partition behavior of furosemide to the GMO cubic phase, a simple experiment was designed where the decrease in the concentration of furosemide in aqueous media containing a specific concentration of furosemide and a piece of cubic phase was measured until equilibrium was reached. The results were used to determine the lipid bilayer/water partition coefficient, denoted $K_{bl/w}$. The equation used for partition coefficient is defined [23]:

$$K_{bl/w} = \frac{[X]_{bl}}{[X]_w} \quad (1)$$

where X refers to furosemide. The concentration of X in the aqueous phase, $[X]_w$, was readily determined from direct measurement of UV absorbance of the aqueous solution after reaching equilibrium. The concentration of X in the lipid bilayer, $[X]_{bl}$ was impossible to determine by direct means, so assumptions that were proposed by Engstrom et al. [23] were used. The first assumption was that all GMO made up a bilayer in the cubic phase (due to its very low water solubility), and the second was that the concentration of X in the aqueous domain of the cubic phase was equal to $[X]_w$. $[X]_{bl}$ was obtained by calculating the amount of furosemide absorbed to the cubic phase of GMO from both the initial concentration of furosemide in the aqueous phase and its concentration after reaching the equilibrium. The effect of pH of the aqueous media on the partition coefficient is shown in Fig. 4. It can be clearly seen that the apparent partition coefficient was dependent on pH, with the most dramatic change occurring in the pH range 3–6, suggesting the partition coefficient to be strongly affected by the degree of ionization. These findings agreed with the results obtained by Engstrom et al. [23] where a pH-dependent partitioning of different drugs in GMO cubic phases was obtained. In order to exclude the effect of ionic strength on $K_{bl/w}$, the same experiment was conducted using sodium chloride in different concentrations with different ionic

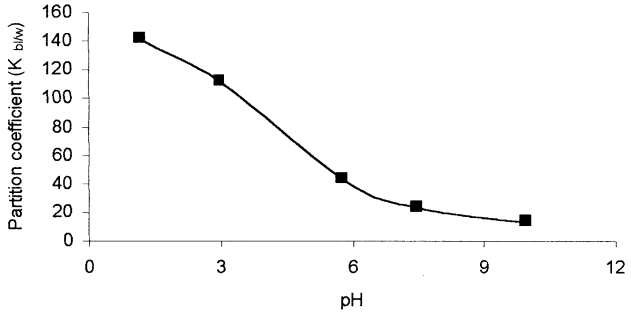

Fig. 4. The apparent lipid bilayer/water partition coefficient of furosemide versus the corresponding pH value (at 37 °C).

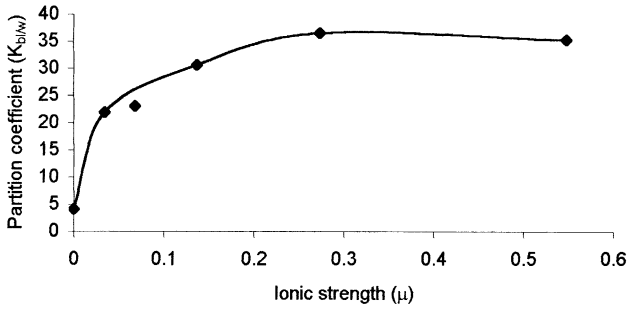

Fig. 5. The apparent lipid bilayer/water partition coefficient of furosemide versus the corresponding ionic strength (at 37 °C).

strengths comparable to those of the buffer system (Fig. 5). The increase in ionic strength was found to increase the $K_{bl/w}$ until a plateau was reached. However, the values of $K_{bl/w}$ in NaCl solutions were much less than those obtained with buffer systems, so the effect of ionic strength was considered to be relatively negligible. In all cases the cubic phase was formed, which was viscous and isotropic as revealed by cross-polarizers. Similar results were obtained by Chang and Bodmeier [24], where a pH-dependent drug absorption was observed with cationic drug, propranolol HCl. More drug was absorbed at a higher pH, which was concomitant with the complexation with free fatty acids being present in the monoglyceride [24]. However, in the case of furosemide, complexation between the free fatty acids and furosemide was unlikely to occur especially at low pH, where both were present in the unionized form.

3.3.4. Dissolution behavior of mixtures of furosemide and GMO

Release behavior of mixtures containing furosemide and GMO was investigated using SGF (without enzymes) as the dissolution medium at different drug loadings while keeping the dose of furosemide as 40 mg per capsule (Fig. 6). The results had shown that dissolution rates were very slow for the intended use. Increase in drug loading capacity from 5 to 20% furosemide in GMO had resulted in a slight decrease in the dissolution rate, but this decrease was considered insignificant when compared to dissolution rate of immediate release capsule (Fig. 6). Maximum release obtained after 24 h was about 20%. The drug release from cubic phase under these conditions is too slow to be used as a sustained release dosage form of the drug. This is most probably due to the low solubility of furosemide in acidic medium and in the aqueous phase of the cubic liquid crystalline system, in addition to the fact that at low pH, furosemide favors partitioning into the lipid bilayer of the cubic liquid crystal. Chang and Bodomeier [24] explained incomplete release of water-soluble drugs from matrices of GMO to be due to drug binding to the matrix systems. The solubility of the drugs was not a determining factor for the incomplete drug release with the monoglyceride matrices. Nonetheless, the incomplete or slow release of furosemide from cubic phase can be rather explained by both the solubility and the partitioning into the lipid bilayer of the resultant matrix.

Since the solubility of furosemide was found to change by changing the pH of the aqueous medium, the effect of changing the pH of the dissolution medium was studied (Fig. 7). It was found that the dissolution rate was affected by the pH of the surrounding medium. As the drug solubility inside the aqueous medium increased, the release rate increased. In addition, the release behavior was following the Higuchi diffusion model for the conditions of the experiment, since the percentage of the drug dissolved was linear with the square root of time.

3.4. Effect of additives on the liquid crystalline phases of furosemide/GMO mixtures

Based on the previous results, it could be seen that the release rate of furosemide from the cubic liquid crystalline phase was very slow, especially in acidic medium that resembled the pH of the stomach where the drug was supposed to be retained. Even at higher pH value (pH

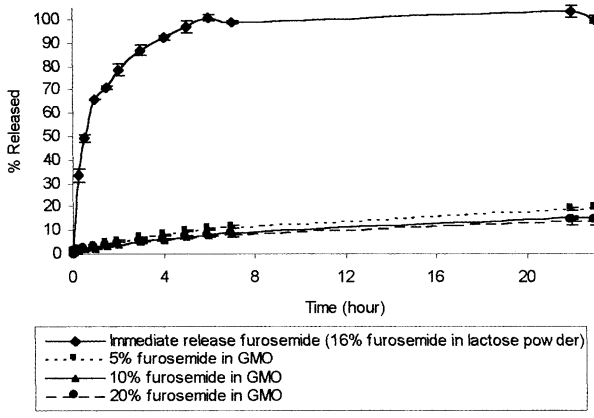

Fig. 6. Release rates of furosemide from GMO containing different drug loadings in comparison with an immediate release furosemide capsule using SGF at 37 °C.

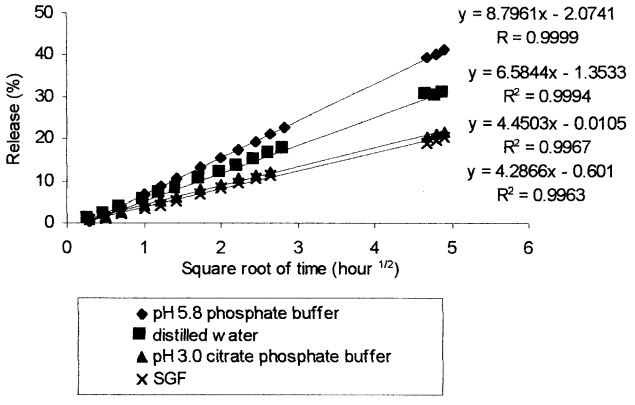

Fig. 7. Effect of changing the pH of the dissolution medium on the release rate of furosemide from mixture containing 5% furosemide in GMO as a function of square root of time.

5.8), which is the pH recommended by USP for the dissolution test of furosemide tablets, less than 50% was released in 24 h. This finding contradicted the objective of this study, which was based on targeting the drug to the stomach where it would be released in a reasonable rate that leads to enhancement of bioavailability. These results may lead to incomplete bioavailability of the drug. So attempts were focused on increasing the release rate especially in acidic medium.

Different additives; like Tween 80, PEG 400, PEG 1000, trisodium citrate and trisodium phosphate; were tested with the aim of increasing the solubility inside the cubic liquid crystalline phase, so as to gain better release behavior. Among several additives tested, two additives, PEG 400 and TSP, were selected based on maximum enhancement of dissolution rate of furosemide from cubic liquid crystalline phase. PEG 400 was chosen due to its high solubilization power for improvement of dissolution rate of furosemide in solid dispersions [25]. TSP was selected as being buffering agent in order to increase the pH inside the cubic liquid crystalline phase and thus increasing the solubility of furosemide. The buffering action of dissolving substances was used to improve the dissolution rate of furosemide by including basic compounds or buffering systems in the formulation [26].

3.4.1. Effect of additives on the formation of cubic phase

The effect of the selected additives on the formation of cubic liquid crystalline phase was tested. In the case of PEG 400, the addition of 1–30% w/w to GMO did not alter the formation of cubic liquid crystalline phase, but as the concentration of PEG 400 increased; the viscosity of the cubic liquid crystalline phase was decreasing. Alfons and Engestrom [27] had found that PEG swells the bicontinuous cubic phase of the GMO/water system and form what is called the sponge phase at constant water content (30%).

For TSP, addition of 1–5% w/w of TSP to GMO did not affect the formation of cubic phase. But above this concentration the reversed hexagonal phase (H_{II}) had started to appear. From 10–30% w/w TSP in GMO, two liquid crystalline phases were existing in addition to the excess water. These were the reversed hexagonal phase (H_{II}) and the micellar phase (L_2) which were existing as separate liquid phases one is viscous and anisotropic, and the other as an isotropic oily liquid. A similar behavior was observed by Engestrom et al. [23], where at high pH values above 9, the hexagonal structure was detected in addition to the cubic liquid crystalline phase. This was explained to be due to lipid decomposition resulting from base catalyzed hydrolysis of the ester bonds, which resulted in increasing the amount of free fatty acids and affecting the critical packing parameter.

In order to investigate the effect of addition of both PEG 400 and TSP, a partial ternary phase diagram was plotted (Fig. 8) by varying the concentrations of GMO, TSP and PEG 400. The concentration of furosemide and water were kept constant (5% w/w and 40% w/w, respectively). The phase diagram shows that increasing the amount of PEG 400 alone had no effect on the formation of cubic phase. However, addition of large amount of PEG 400 (above 20%) had resulted in decrease in the viscosity of the cubic phase. On the other hand, TSP at low concentration (below 7%) had shown no effect on the formation of cubic phase. But increasing its concentrations had first resulted in formation of reversed hexagonal phase (H_{II}), then formation of three liquid layers which were identified as reversed micellar phase (L_2), reversed hexagonal phase (H_{II}) and aqueous solution.

3.4.2. Effect of additives on the release behavior of furosemide from liquid crystals of GMO

The effect of increasing the concentration of TSP from 0 to 10% while keeping the concentration of PEG 400 constant at 10% is shown in Fig. 9a. The release rate of furosemide was increasing by increasing the concentration of TSP up to 5%. However, addition of 10% TSP had shown a dissolution profile similar to that of using 2.5% TSP. This was most probably due to the change of the liquid crystalline phase to the reversed hexagonal (H_{II}) rather than the cubic phase above 5% TSP, which was revealed using polar-

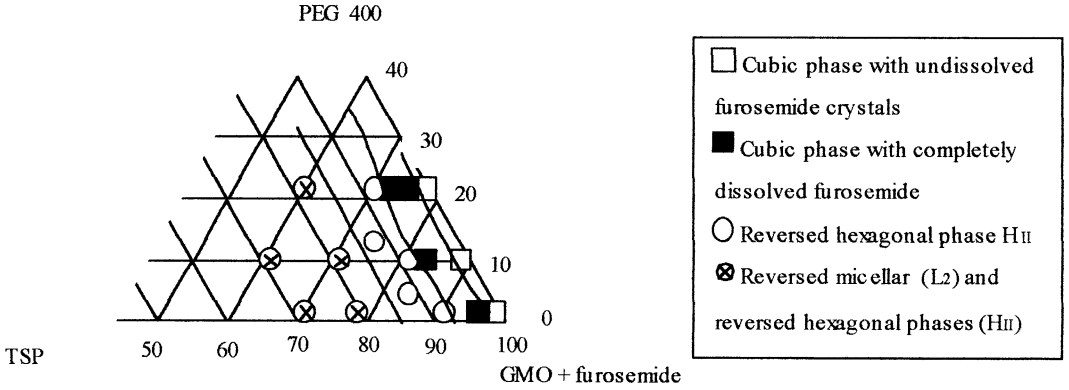

Fig. 8. Partial ternary phase diagram for GMO, polyethylene glycol 400 (PEG 400) and trisodium phosphate (TSP) with presence of 5% w/w furosemide in 40% distilled water.

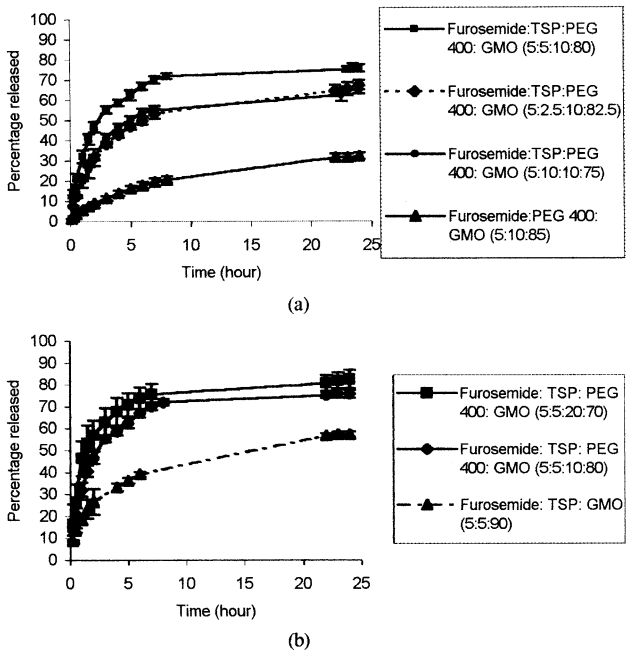

Fig. 9. Effect of addition of (a) TSP and (b) PEG 400 on the release rates of furosemide from cubic phase using pH 3.0 citrate-phosphate buffer, apparatus II.

izing microscope. The reversed hexagonal phase was previously reported to have the most favorable sustained release properties, compared with those from the cubic form [10]. The explanation was that the diffusion pathway is more obstructed in the reversed hexagonal form than in the cubic one. The closed water channels of the reversed hexagonal phase slowed down the diffusion of dissolved drug through the matrix. On the other hand, due to its lipophilic properties, furosemide might be entrapped in the lipid bilayer of the reversed hexagonal phase which contributed to the slower release rate from such phase.

Fig. 9b shows the effect of addition of PEG 400 to the formula containing 5% w/w TSP. It seems that addition of 10% of PEG 400 had significantly increased the dissolution rate, while addition of 20% PEG 400 had shown only slight improvement in the dissolution rate of furosemide in comparison to 10% PEG 400. The effect of PEG 400 on the release rate of furosemide from the cubic liquid crystalline phase could be either due to its solubilizing effect on furosemide and/or its hydrophilic properties, which facilitated diffusion through the water channels. Since the viscosity of the cubic liquid crystalline phase decreased at concentration of 20% PEG 400, this concentration was no longer considered, especially with relatively high standard errors of the data obtained from the dissolution profile.

The effect of varying the dissolution medium was studied for formula containing 5% of TSP and 10% PEG 400. The dissolution media selected were SGF without enzymes (pH ~1.2), pH 3.0 citrate-phosphate buffer and pH 5.8 phosphate buffer, using USP apparatus I instead of apparatus II so as not to disturb the resultant liquid crystalline phase

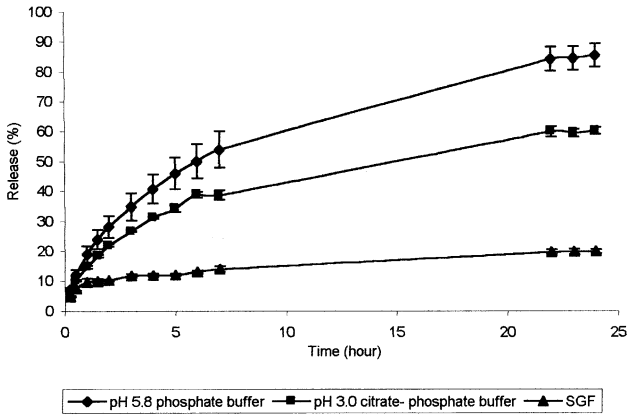

Fig. 10. Effect of changing the pH of the dissolution medium on the release rate of furosemide from formula containing furosemide/TSP/PEG 400/GMO (5:5:10:80) using apparatus I; 100 rpm.

during dissolution. The results are shown in Fig. 10. At low pH (1.2), the dissolution rate of furosemide was very slow, and the release rate had increased with increasing the pH to 3.0. Fastest release was obtained in the case of pH 5.8. Incomplete release at low pH values could be due to the neutralization effect of the acidic medium on TSP, in addition to the poor solubility of furosemide in acidic medium.

In order to simulate in vivo situation, the dissolution profile for the selected formula was studied by changing the pH of the dissolution medium with time. The results are shown in Fig. 11, in comparison with results obtained under the same conditions but without change with time (Fig. 10). It is shown that the release rate increased with increasing the pH of the dissolution medium. However, when using multi-stage dissolution medium, increasing the pH of the dissolution medium showed slower release rate in comparison with the same medium alone. This could be due to the neutralization of TSP by the acidic medium, and thus decreasing the pH inside the matrix. So, the main determin-

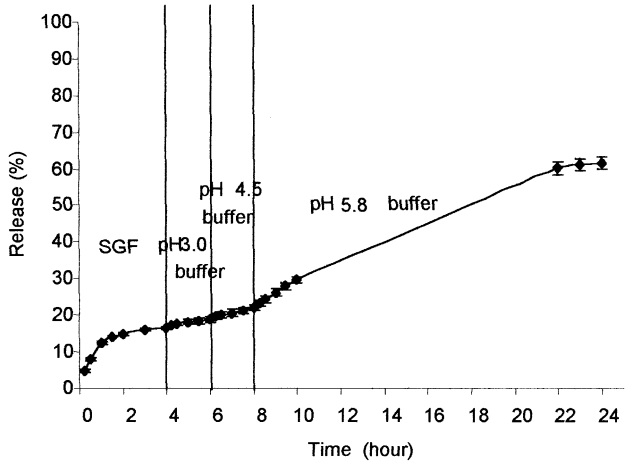

Fig. 11. Effect of using multi-stage dissolution media on the release rate of furosemide from formula containing furosemide/TSP/PEG 400/GMO (5:5:10:80) using apparatus I; 100 rpm.

ing factor in this case was not the pH of the dissolution medium, rather the pH inside the liquid crystalline phase.

3.4.3. Change of phase behavior of mixtures of furosemide, GMO and additives through dissolution conditions

The phase behavior of the resultant liquid crystalline phases of GMO was studied during multi-stage dissolution. It was observed that the cubic phase was formed within half an hour and remained throughout the dissolution test (i.e. 24 h) at all pH changes. This indicated that the cubic liquid crystalline phase is stable within the test conditions. However, needle-like crystals were observed which indicated the recrystallization of furosemide at acidic conditions. This behavior might be due to first solubilization of furosemide at high pH inside the cubic liquid crystalline phase due to presence of TSP. But, the pH value will start to decrease by the diffusion of the acidic dissolution medium inside the cubic phase which would result in recrystallization of furosemide. These needle-like crystals were recorded by Matsuda and Tatsumi [22] and identified as being the same polymorph of furosemide but different crystal habit. However, no attempt was made to isolate these crystals and further characterize them. These crystals were not found after 22 h in pH 5.8 buffer media, indicating their complete solubilization with increasing the pH value of the surrounding medium.

3.5. Assessment of mucoadhesive properties

Preliminary trials were made to study the mucoadhesive properties of the previous trials. The results of these experiments had shown that GMO alone has comparative mucoadhesive properties to that of carbomer, while the presence of furosemide as a dispersion in GMO had affected such properties and resulted in failure of adhesion to the rabbit stomach. However, for a formula containing furosemide/TSP/PEG 400/GMO in the ratio 5:5:10:80, the mucoadhesive properties were similar to those of GMO alone. Such results had indicated that the presence of large amount of drug crystals on the surface of the resultant cubic liquid crystalline phase had prevented the interaction with mucous membrane. Nonetheless, the mucoadhesion of such systems needs further much more studies.

4. Conclusion

An oral drug delivery system was developed with the aim of targeting the active compound (furosemide) to its absorption site (the stomach) and at the same time utilizing the sustained properties of liquid crystalline phase of GMO. Such a system might be more advantageous than conventional dosage forms of the model drug by increasing the bioavailability of the furosemide, which is characterized by window absorption, and at the same time improves patient compliance by its sustained effect. Due to its low solubility in acidic medium of the stomach and its high lipid partitioning to the cubic phase lipid bilayer, a simple direct mix between GMO and furosemide was not able to fulfill the desired requirements. So, additives that increased the solubility of furosemide inside the cubic phase were utilized in order to achieve the desired dissolution rate and mucoadhesive properties. A formula containing furosemide/TSP/PEG 400/GMO in the ratio 5:5:10:80, respectively, was found to have optimum properties concerning release characteristics and mucoadhesion. However, future work needs to be concentrated on the evaluation of in vivo mucoadhesive studies on the selected formulation.

References

[1] S. Engstrom, Drug delivery from cubic and other lipid-water phases, Lipid Technol. 2 (1990) 42–45.
[2] S. Engstrom, L. Engstrom, Phase behavior of the lidocaine-monoolein-water system, Int. J. Pharm. 79 (1992) 113–122.
[3] S. Engstrom, L. Lindahl, R. Wallin, J. Engblom, A study of polar lipid drug carrier systems undergoing a thermoreversible lamellar-to-cubic phase transition, Int. J. Pharm. 86 (1992) 137–145.
[4] S. Engstrom, H. Ljusberg-Wahren, A. Gustafsson, Bioadhesive properties of the monoolein water system, Pharm. Tech. Eur. 2 (1995) 14–17.
[5] M. Longer, P. Tyle, J. Mauger, A cubic-phase oral drug delivery system for controlled release of AG337, Drug Dev. Ind. Pharm. 22 (7) (1996) 603–608.
[6] D. Wyatt, D. Dorschel, A cubic phase delivery system composed of glyceryl monooleate and water for sustained release of water soluble drugs, Pharm. Tech. 10 (1992) 116–130.
[7] J. Lee, I.W. Kellaway, In vitro peptide release from liquid crystalline buccal delivery systems, Int. J. Pharm. 195 (2000) 29–33.
[8] P.B. Geraghty, D. Attwood, J.H. Collet, H. Sharma, Y. Dandiker, An investigation into the bioadhesive nature of Myverol 18-99, 15th Pharmaceutical Technology Conference, Christ Church College and the Town Hall, Oxford, UK, March 19–21, 1996, pp. 493–504.
[9] C.M. Chang, R. Bodmeier, Monoglyceride based liquid crystalline topical drug delivery systems, Pharm. Res. 11 (1994) S185.
[10] T. Norling, P. Lading, S. Engstrom, K. Larsson, N. Krog, S.S. Nissen, Formulation of a drug delivery system based on a mixture of monoglycerides and triglycerides for use in the treatment of periodontal disease, J. Clin. Periodontol. 19 (1992) 687–692.
[11] L.S. Nielsen, L. Schubert, J. Hansen, Bioadhesive drug delivery systems 1. Characterization of mucoadhesive properties of systems based on glyceryl mono-oleate and glyceryl monolinoleate, Eur. J. Pharm. Sci. 6 (1998) 231–239.
[12] P.B. Geraghty, D. Attwood, J.H. Collet, H. Sharma, Y. Dandiker, An investigation of the parameters influencing the bioadhesive properties of Myverol 18-99/water gels, Biomaterials 18 (1997) 63–67.
[13] W.P. Leary, A.C. Asmal, Slow release furosemide in the treatment of mild to moderate hypertension, Curr. Ther. Res. Clin. Exp. 28 (1980) 549–553.
[14] H. El-Shattawy, A. Kassem, M. El-Razzaz, Controlled release furosemide microcapsules: preformulation studies, Drug Dev. Ind. Pharm. 17 (1991) 2529–2537.
[15] F.S. Farshi, Y. Capan, S. Kes, A.A. Hincal, Formulation and comparative bioavailability of conventional and sustained release furosemide tablets, STP Pharm. Sci. 5 (1995) 361–366.
[16] W.A. Ritschel, A. Menon, A. Sakr, Biopharmaceutic evaluation of furosemide as a potential candidate for a modified release peroral dosage form, Methods Find. Exp. Clin. Pharmacol. 13 (1991) 629–936.
[17] A. Menon, W. Ritschel, A. Sakr, Development and evaluation of a

monolithic floating dosage form for furosemide, J. Pharm. Sci. 83 (1994) 239–245.
[18] Y. Akiyama, N. Nagahara, E. Nara, M. Kitano, S. Iwasa, I. Yamamoto, J. Azuma, Y. Ogawa, Evaluation of oral mucoadhesive microspheres in man on the basis of the pharmacokinetics of furosemide and riboflavin, compounds with limited gastrointestinal absorption sites, J. Pharm. Pharmacol. 50 (1998) 159–166.
[19] G. Santus, C. Lazzarini, G. Bottoni, E.P. Sandefer, R.C. Page, W.J. Doll, U.Y. Ryo, G.A. Digens, An in vitro–in vivo investigation of oral bioadhesive controlled release furosemide formulations, Eur. J. Pharm. Biopharm. 44 (1997) 39–52.
[20] Y. Aota-Nakano, J.S. Li, M. Yamazaki, Effects of electrostatic interaction on the phase stability and structures of cubic phases of monoolein/oleic acid mixture membranes, Biochim. Biophys. Acta 1461 (1999) 96–102.
[21] C. Doherty, P. York, The in-vitro pH-dissolution dependence and in-vivo bioavailability of frusemide-PVP solid dispersions, J. Pharm. Pharmacol. 41 (1989) 73–78.
[22] Y. Matsuda, E. Tatsumi, Physicochemical characterization of furosemide modifications, Int. J. Pharm. 60 (1990) 11–26.
[23] S. Engstrom, T.P. Norden, H. Nyquist, Cubic phases for studies of drug partition into lipid bilayers, Eur. J. Pharm. Sci. 8 (1999) 243–254.
[24] C. Chang, R. Bodmeier, Binding of drugs to monoglyceride-based drug delivery systems, Int. J. Pharm. 147 (1997) 135–142.
[25] N. Ozdemir, S. Ordu, Dissolution rate of furosemide from polyethylene glycol solid dispersions, Farmaco 52 (1997) 625–629.
[26] A.F. Marias, J.G. Van Der Watt, Relationship between the pH of the diffusion layer and the dissolution rate of furosemide, Drug Dev. Ind. Pharm. 17 (1991) 1715–1720.
[27] K. Alfons, S. Engstrom, Drug compatibility with the sponge phases formed in monoolein, water and propylene glycol or polyethylene glycol, J. Pharm. Sci. 87 (1998) 1527–1530.