Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 294 (2005) 161–171
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Cubic liquid crystalline glyceryl monooleate matrices for oral delivery of enzyme

Manish H. Shah, Anant Paradkar*

Department of Pharmaceutics, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandawane, Pune 411038, Maharashtra State, India

Received 21 November 2004; received in revised form 20 January 2005; accepted 22 January 2005

Abstract

In situ cubic phase transforming system of glyceryl monooleate (GMO) has been prepared which offers protection to the metaloenzyme, seratiopeptidase (STP), in gastric environment and provides delayed and controlled release with no initial burst after oral administration. Effect of magnesium trisilicate (MTS) on floating, proteolytic activity and drug release was studied. Gelucire® 43/01 was incorporated in the system to provide prolonged lag time. The drug-loaded matrices required 100 mg of MTS to overcome floatability of GMO matrix. Plain GMO matrices showed 85.3% loss of proteolytic activity in acidic medium, whereas matrices containing MTS showed retention of activity (111.6%). The hydrophobic nature of MTS induced formation of cubic phase at faster rate and the existence of cubic phase was confirmed by polarizing light microscopy. Furthermore, MTS provided alkaline microenvironment, which prevented acid-catalyzed hydrolysis and protein unfolding. The magnesium ions restored the activity of STP. The release of STP was decreased with increasing amount of MTS in the matrix. Gelucire did not affect proteolytic activity. The water uptake of matrices with gelucire was decelerated due to formation of hexagonal phase. However, the rate of STP release from these matrices was very slow due to incorporation of gelucire into lipid bilayers, which provided resistance to movement of STP. Thus, microenvironment-controlled in situ cubic phase transforming GMO matrices provided protection to STP and controlled release.

Keywords: In situ cubic phase transformation; Glyceryl monooleate; Proteolytic activity; Serratiopeptidase; Gelucire® 43/01

1. Introduction

Systems containing high concentration of amphiphilic surfactant, which exhibit three-dimensional arrangement of surfactant molecules capable of being transformed into each other in a definite sequence un-

* Corresponding author. Tel.: +91 20 25437237; fax: +91 20 25439383.
E-mail addresses: shawari1@yahoo.com (M.H. Shah), arparadkar@rediffmail.com (A. Paradkar).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.01.019

der certain circumstances, are termed as lyotropic liquid crystals (Makai et al., 2003). Different lyotropic liquid crystalline phases include lamellar, cubic and hexagonal phase. Cubic phase contains water channels surrounded by saddle-like curved bilayer of the amphiphile extended in three dimensions. The structure so formed separates two continuous networks of water channels (Shah et al., 2001).

Glyceryl monooleate (GMO) is a synthetic compound, which is an unsaturated monoglyceride belonging to the class of water-insoluble amphiphilic lipids (Engstrom et al., 1995). It forms different lyotropic liquid crystals depending on the water content and temperature. As water content and temperature increases, system forms cubic phase via reversed micellar and lamellar phases (Nessem, 2001; Sallam et al., 2002). Cubic liquid crystalline phase is of much interest due to the fact that it is highly viscous, thermodynamically stable and coexists in equilibrium with excess of water. They are isotropic and have good temperature stability as well as relative insensitivity to salts and solvents (Longer et al., 1996). These cubic liquid crystalline phases are mechanically robust and resistant to physical degradation. The interfacial area of cubic phase is about 400 m^2/g and the pore size of fully swollen cubic phases is about 5 nm (Engstrom et al., 1995; Wyatt and Dorschel, 1992). A typical globular protein has same size as the dimensions of water channels in the bicontinuous cubic phases. Protein entrapment in the cubic phase depends on the type of protein, its interaction with the lipid bilayer and dimensions of the water channels. It is difficult to incorporate macromolecular enzymes in the cubic phase, since this can modify the structure of protein and cubic phase. However, the enzyme-like glucose oxidase (M.W. 160 kDa) has been successfully entrapped in cubic phase. These characteristics of cubic phase thus make it potentially useful excipient for peptide drug delivery (Nylander et al., 1996).

Various researchers have been working on the cubic phase as carrier for drug delivery system. Wyatt and Dorschel (1992) demonstrated that the cubic-phase matrix provided sustained release of different drugs with varying solubilities in water and molecular weight. Sadhale and Shah (1998, 1999) showed that liquid crystalline cubic phase protected peptide-like insulin from agitation-induced aggregation and the peptide was biologically active in the cubic gel. They also showed that cubic phase enhanced chemical stability of drugs like cefazolin and cefuroxime.

In these drug delivery systems, cubic phases were prepared in advance and used as controlled release reservoir for drug substance. However, cubic phase is difficult to prepare and process due to the property of the raw materials and rheological properties of the liquid crystalline phases themselves. Lipids that form cubic phases are typically waxy solids at room temperature. Therefore, cubic phase is prepared by equilibrating lipid with water at high temperature over many hours since the transport of water is very slow through solid lipid. Processes that require longer hold-up time at high temperature to prepare cubic phases are not economically practicable (Makai et al., 2003; Nessem, 2001; Sadhale and Shah, 1999).

Liquid crystalline phases can be produced using precursor, which in situ can transform into cubic phase. Engstrom et al. (1992) used lamellar phase precursor, which in situ transformed into cubic phase and sustained the release of variety of drugs. Kumar et al. (2004) demonstrated application of GMO matrix in floating drug delivery system, which in situ formed cubic phase. In situ transformation into cubic phase proceeds through a low-viscosity lamellar phase. The lamellar phase is less efficient in controlling drug release and protection by immobilization of the drug-like peptides.

Present study is an attempt to design a GMO matrix system, which acts as a cubic phase precursor where its floating in stomach and fast initial release has been reduced by addition of magnesium trisilicate (MTS). MTS made the matrices heavier and also provided alkaline microenvironment in the swollen cubic phase, which was required for stabilization of model acid-labile enzyme, serratiopeptidase (STP; M.W. 52 kDa), when subjected to gastric pH (about 1.2). Hydrophobic lipid Gelucire® 43/01, a commercially available glyceride base, was incorporated to achieve desired prolonged drug release.

The prepared formulations were evaluated for proteolytic activity, water uptake and in vitro drug release. In vivo non-floatability of the formulation was studied in healthy human volunteers by gamma-scintigraphy. The different phases of lyotropic liquid crystals of GMO were characterized using polarizing light microscopy.

2. Materials and methods

2.1. Materials

Glyceryl monooleate (Rylo™ MG Pharma 19) was a generous gift from Danisco Cultor (Denmark). Gelucire® 43/01 and STP were gifts from Gattefosse S.A. (France) and Advanced Biochemicals Ltd. (India), respectively. MTS was purchased from Loba chemicals (India). Licaps® capsules (size 0, hard gelatin capsule specially designed for lipid formulations) were obtained as gift sample from Capsugel (India). All other chemicals used were of analytical grade.

2.2. Preparation of GMO matrices

2.2.1. Plain GMO matrices

GMO (300 mg) was taken in a beaker and heated at 50 °C on a water bath until it was fluid. To the fluid GMO, STP (20 mg) was added with stirring. The molten mass was poured in fabricated stainless steel cylindrical moulds (inner diameter of 8.5 mm, height of 10 mm) and frozen at −15 °C for 5 min. The matrices were kept in desiccator at room temperature over silica gel for 12–24 h before being subjected to any further evaluation.

2.2.2. GMO matrices containing MTS and gelucire

MTS containing batches were prepared by melting GMO (300 mg) in a beaker at 50 °C, and then added MTS (100, 110, 120, 130, 140, and 150 mg) and STP (20 mg) with stirring. Similarly, gelucire-containing matrices were prepared by melting GMO (300 mg) with gelucire (10, 20, and 30 mg), and then, MTS (100 mg) and STP (20 mg) were added with stirring. The matrices were prepared by the procedure described above. The effect of additives on floatability and water uptake was studied with and without STP. The matrices were kept in desiccator at room temperature over silica gel for 12–24 h before being subjected to any further evaluation.

2.3. Evaluation of GMO matrices

2.3.1. Optimization of MTS concentration

The matrices were tested for buoyancy lag time and matrix integrity using USP 24 type II dissolution test apparatus (Electrolab TDT-08L, India). Matrices were added in phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C and stirred constantly at 100 rpm. Time required to float was reported. Matrix floatability and integrity were inspected visually. In this study, the amount of MTS and gelucire in the matrix was varied in between 25–100 and 10–30 mg, respectively.

2.3.2. Gamma scintigraphy

Six healthy male subjects of age in between 23 and 30 years and having weight in the range of 55–70 kg participated in the study as volunteers. The purpose of the study was fully explained to each volunteer. ^{99m}Tc 0.1 mCi was uniformly mixed with the molten GMO containing MTS (100 mg) or combination of MTS (100 mg) and gelucire (30 mg). The molten mass was filled into Licap® capsules using a preheated glass Pasteur pipette and allowed to solidify at −15 °C for 5 min. After overnight fasting, volunteers were asked to swallow one capsule each with 200 ml of water in the morning. Images were recorded at different time intervals up to 4–5 h using millennium MPR Gamma camera (low-energy high-resolution collimeter integrated to ENTEGRA work station). An external marker was used to allow correct alignment of subjects during successive imaging. The subjects were served with light breakfast after 2 h.

2.3.3. Polarizing light microscopy

The matrices were placed in a USP 24 type II dissolution apparatus (Electrolab TDT-08L, India) containing 900 ml of phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C and allowed to hydrate under stirring at 100 rpm. The hydrated samples were removed at intervals of 1 and 8 h and examined under polarizing light microscopy (Nikon, Kanagawa, Japan) using λ1/4 compensator in order to study the existence of birefringence under crossed polars employing magnification of 200×. Photomicrographs of these samples were taken at room temperature. The lamellar, cubic and hexagonal phases were identified according to classification established by Rosevear (1954).

2.3.4. Proteolytic activity

This test was carried out in triplicate using USP 24 type II dissolution test apparatus (Electrolab TDT-08L, India). The matrices were placed separately in the vessels containing 900 ml of 0.1N hydrochloric acid

(pH 1.2) or phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C and stirred constantly at 100 rpm. After 2 h, the matrices were removed from the vessels and crushed in a test-tube containing 100 ml of Tris–buffer (pH 7.0). These samples were then assayed for proteolytic activity.

The proteolytic activity was determined as per the method reported in Food Chemical Codex (2003). The assay was based on a 30 min proteolytic hydrolysis of casein at 37 °C and pH 7.0. Unhydrolyzed casein was removed by filtration and the solubilized casein was determined spectrophotometrically at wavelength of 275 nm. In this method, the protease activity is expressed as PC units of preparation derived from *Bacillus subtilis* var. and *Bacillus licheniformis* var. One bacterial protease unit (PC) is defined as quantity of enzyme that produces 1.5 μg/ml equivalent of L-tyrosine per minute under the condition of the assay.

Activity of enzyme was calculated by Eq. (1).

$$\frac{\mathrm{PC}}{\mathrm{g}} = \left(\frac{A_\mathrm{U}}{A_\mathrm{S}}\right)\left(\frac{22}{30W}\right) \quad (1)$$

where A_U is value obtained by subtracting blank reading from test reading, A_S absorption of standard solution, 22 the final volume in ml of the reaction mixture, 30 the time of the reaction in minutes and W the weight of the original sample taken in 'g'.

2.3.5. Water uptake studies

Water uptake studies were carried out by equilibrium weight gain method (Kumar et al., 2004). The study was performed using USP 24 type I dissolution test apparatus (Electrolab TDT-06P, India). The GMO matrices containing different additives were accurately weighted and placed in a dissolution basket. The baskets were immersed in dissolution vessel containing 900 ml of phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C and rotated at 100 rpm. At regular intervals, the basket-matrix systems were removed from the dissolution vessels, blotted with tissue paper to remove excess water and re-weighted. Increase in the weight was reported and percent water uptake was calculated.

2.3.6. Drug release studies

Drug released from the matrices was studied by using USP 24 type II dissolution test apparatus (Electrolab TDT-08L, India). The dissolution test for each batch was performed in triplicate. The matrices were placed in 900 ml of phosphate buffer (pH 7.4) maintained at temperature 37 ± 0.5 °C and stirred constantly at 100 rpm. Aliquots (5 ml) were withdrawn at pre-determined time intervals and replenished with fresh dissolution medium maintained at 37 ± 0.5 °C. The aliquots were assayed spectrophotometrically at 220 nm.

3. Results and discussion

3.1. Optimization of MTS concentration

The amount of MTS required in the formulation was decided on the basis of its ability to impart non-floating characteristic to the matrix. It was observed that matrices without drug containing 25 mg of MTS floated within 5.5 min, but did not float when 50 mg of MTS was incorporated. MTS has high density and it

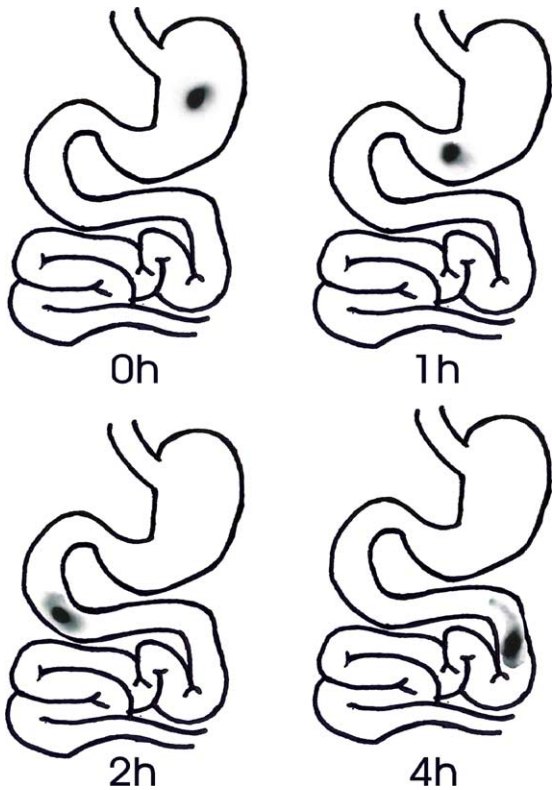

Fig. 1. Gamma scintigraphy of GMO matrix containing magnesium trisilicate.

is hydrophobic in nature (Kearney, 2000). Presence of drug in matrix caused significant difference in amount of MTS required. The drug-loaded matrices required 100 mg of MTS to make it non-floating. Density of matrix determines its floating ability, and it is dependent on matrix swelling and increase in density due to MTS. To counteract reduction in density due to high water uptake, higher amount of MTS was required to make the matrix non-floating.

The gamma scintigraphy images (Fig. 1) obtained at different time intervals after administration of the matrices confirmed that matrix did not float in vivo due to high density and passes to the intestine within 2 h.

Matrix did not show any significant erosion when amount of MTS was increased up to 150 mg, but above this it caused erosion of matrix. It could be due to interference in the liquid crystalline structure because of high solid content. Addition of gelucire did not affect floating and erosion.

3.2. Polarizing light microscopy

The liquid crystalline structures of different matrices after hydration are presented in the Table 1. The plain matrices hydrated for 1 and 8 h showed the existence of the lamellar structures (Fig. 2a). The incorporated water-soluble drug had transformed the cubic phase into lamellar phase. The matrices hydrated for 1 h containing different amount of MTS revealed the existence of optically isotropic cubic phase, which was characterized by dark background under polarized light (Fig. 2b). Hydrophobic nature of MTS increased free water in the aqueous channels, which favored the conversion of lamellar phase to cubic phase. However, these matrices after hydration for 8 h showed the existence of optically birefringent homogeneous hexagonal structure (Fig. 2c). Similar structural transformation was noted in matrices containing gelucire. After initial hydration of 1 h, the matrices with 10 and 20 mg of gelucire indicated the existence of cubic phase. On the contrary, the matrices with 30 mg of gelucire were transformed into hexagonal phase but the percentage of hexagonal phase in the hydrated matrices was very less. After hydration of 8 h, all the three matrices with gelucire were in hexagonal phase. This effect of hydrophobic gelucire is in confirmation with our previous observation where increase in content of hydrophobic drug diazepam favored formation of hexagonal phase (Kiran et al. 2004). Similarly, Caboi et al. (2001) showed that the incorporation of hydrophobic additive induced the formation of hexagonal phase with increase in either concentration or time after preparation. Chang and Bodmeier (1997) reported that the solubilization of hydrophobic additive in the lipophilic domain increased the apparent hydrophobic chain volume of the lipid, and therefore, affected topology and shape of the aggregate.

Table 1
Polarizing light microscopic characteristics of liquid crystalline phases as a function of concentration of additives

Amount of MTS	Amount of Gelucire 43/01	Structure of liquid crystalline phases	
		1 h	8 h
Plain matrix	–	Lamellar	Lamellar
100	–	Cubic	Hexagonal
110	–	Cubic	Hexagonal
120	–	Cubic	Hexagonal
130	–	Cubic	Hexagonal
140	–	Cubic	Hexagonal
150	–	Cubic	Hexagonal
100	10	Cubic	Hexagonal
100	20	Cubic	Hexagonal
100	30	Cubic/Hexagonal	Hexagonal

3.3. Proteolytic activity

The proteolytic activity of different matrices was evaluated separately before and after treating them for 2 h in acidic (0.1N HCl, pH 1.2) and basic media (phosphate buffer, pH 7.4) and results are presented in Fig. 3. The plain GMO matrix showed about 85.3% loss of proteolytic activity in acidic medium. This result seems to be different from previous studies. Ericsson et al. (1991) have reported that cubic phase protected the oligopeptides form enzymatic degradation. Similarly, Sadhale and Shah (1998, 1999) have investigated protection of proteins and peptides by cubic phase. They showed that cubic phase caused physical and chemical stabilization of peptide molecule due to reduced mobilization of drug as well as water. The work done by Ericsson and Sadhale had utilized preformed cubic phase. In present work, the system in situ attempts to transform into cubic phase, as it coexists in equilibrium with excess of water. Initial rate of water uptake was higher due to formation of lamellar phase. Further-

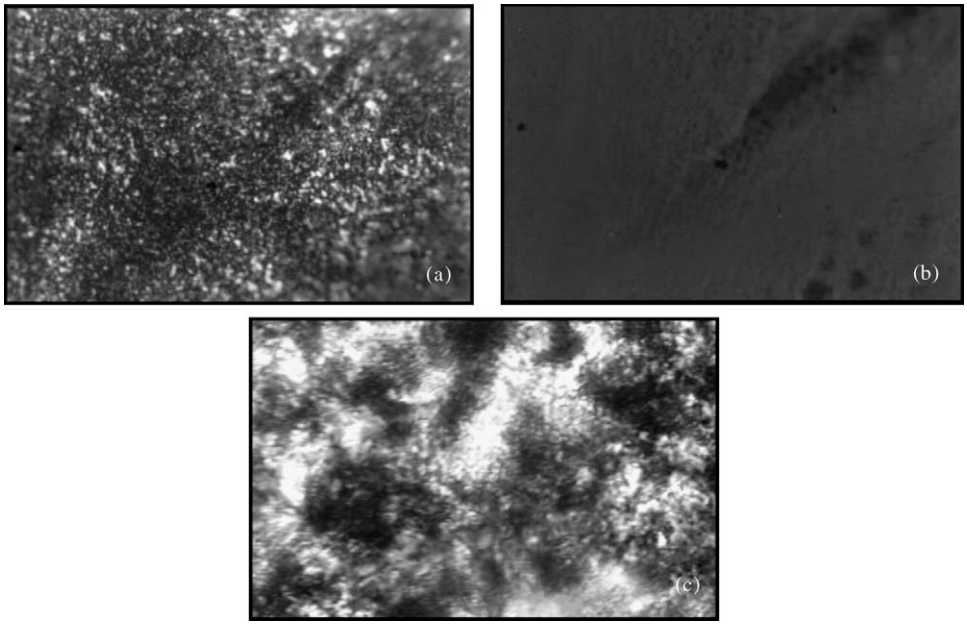

Fig. 2. Polarizing light microphotographs of GMO matrices showing different phases of liquid crystalline systems: (a) lamellar phase; (b) cubic phase; (c) hexagonal phase.

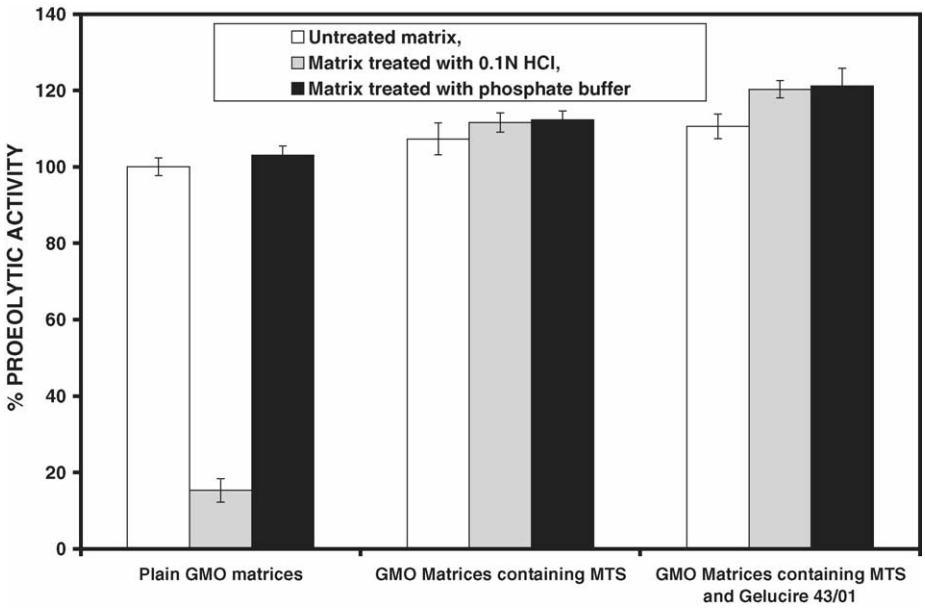

Fig. 3. Activity of different matrix containing serratiopeptidase.

more, formation of these low-viscosity lamellar phases allowed burst release following Fickian diffusion kinetics (Kumar et al., 2004), which caused peptide degradation in acidic medium. The formation of lamellar phase in plain matrix was confirmed by polarizing light microscopy.

In the acidic medium, the proteolytic activity of matrices containing MTS was 111.6%. This showed that inclusion of MTS offered protection to STP. The water uptake study showed that MTS reduced water uptake and its hydrophobic nature induced the formation of highly ordered stiff cubic phase at faster rate, which was rate-limiting step in further water absorption. The formation of cubic phase after hydration of 1 h in MTS-containing matrices was verified by polarizing light microscopy. MTS provided alkaline microenvironment and prevented acid-catalyzed enzyme hydrolysis as well as protein unfolding, which caused aggregation (Fu et al., 2000). As reported by Kim and Kim (1993), magnesium ions restored the activity of inactivated metaloenzyme STP and exhibited increase in initial activity. Inclusion of gelucire does not affect the proteolytic activity of matrices. The matrices with different additives were found to retain proteolytic activity in basic medium.

3.4. Water uptake studies

Water uptake profiles of GMO matrices with and without drug containing different amount of MTS are shown in Fig. 4. Water uptake was significantly higher in drug-loaded matrices as compared to matrices without drug. Increased water uptake in presence of drug was in confirmation with previous report (Kumar et al., 2004). Water uptake decreased linearly with increase in the amount of MTS in the matrix (Fig. 5). This effect was encountered due to hydrophobic nature of MTS. The rate of water uptake in the matrices with and without drug was found to be significantly high in the initial stages, which was attributed to formation of lamellar phase, further water uptake led to formation of viscous cubic phase which restricted additional water uptake.

Similarly, addition of gelucire, hydrophobic mixture of triglycerides, has also caused significant decrease in water uptake (see Fig. 6). Water uptake decreased with increase in amount of gelucire. Incorporation of hydrophobic material transformed the cubic phase into the hexagonal phase. The formation of hexagonal phase resisted further water uptake, which was in accordance with the report by Farkus et al. (2000).

3.5. Drug release studies

The drug release profiles (Figs. 7 and 8) of GMO matrices containing different amount of MTS and combination of MTS with gelucire were characterized by an initial lag of about 60–120 min. It was interesting to note that less than 10% of total drug released during the lag. Thereafter, the rate of drug release was increased. This was probably due to hydrophilic channels available during the release of STP increased with increasing initial water uptake.

It was observed that as the amount of MTS increased, the release of STP was decreased. Release rate was in well correlation with the water uptake. MTS decreased the water uptake and drug release as its amount increased. Due to the hydrophobic nature, it reduced volume of water in the aqueous channels, thus limited release of drug by diffusion.

Since the proportion of incorporated MTS was high ($\geq$100 mg), it favored the formation of cubic phase at faster rate, giving initial lag time in drug release. The formation of cubic phase after hydration of 1 h was confirmed by polarizing light microscopy. Furthermore, polarizing light microscopy data after hydration of 8 h indicated that the hexagonal phase was formed in the matrices containing MTS. This showed that the system was transforming from cubic to hexagonal phase. During this transformation, drug release was also increased. In hexagonal phase, the hexagonally packed cylindrical aggregates can move freely along their length, which provided faster drug release as compared to three-dimensional arrangement of cubic arrays. In later stage, the drug release was controlled by dynamic swelling behaviour of hexagonal phase, and such release profile corresponded to time independent zero-order kinetics (Farkus et al., 2000).

Similar structural transformation from cubic to hexagonal phase was also noticed in the gelucire-containing matrices (Fig. 6). The rate of transformation of cubic to hexagonal phase was higher in matrices containing 30 mg of gelucire. However, the rate of drug release from the GMO matrix containing gelucire was very slow. Gelucire comprises of mix-

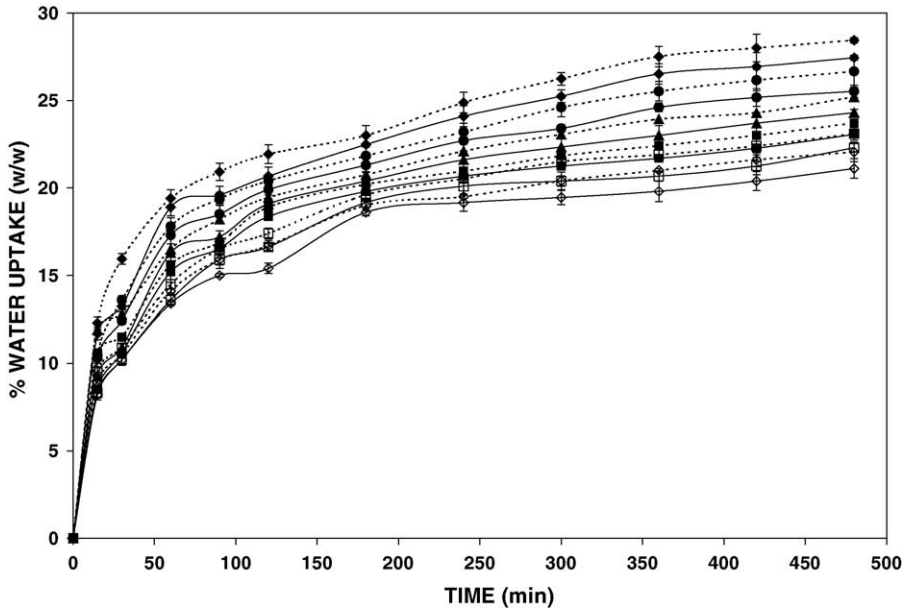

Fig. 4. Effect of amount of MTS on water uptake of GMO matrices with (dotted lines) and without drug (bold lines): 100 mg (♦); 110 mg (●); 120 mg (▲); 130 mg (■); 140 mg (□); 150 mg (◊).

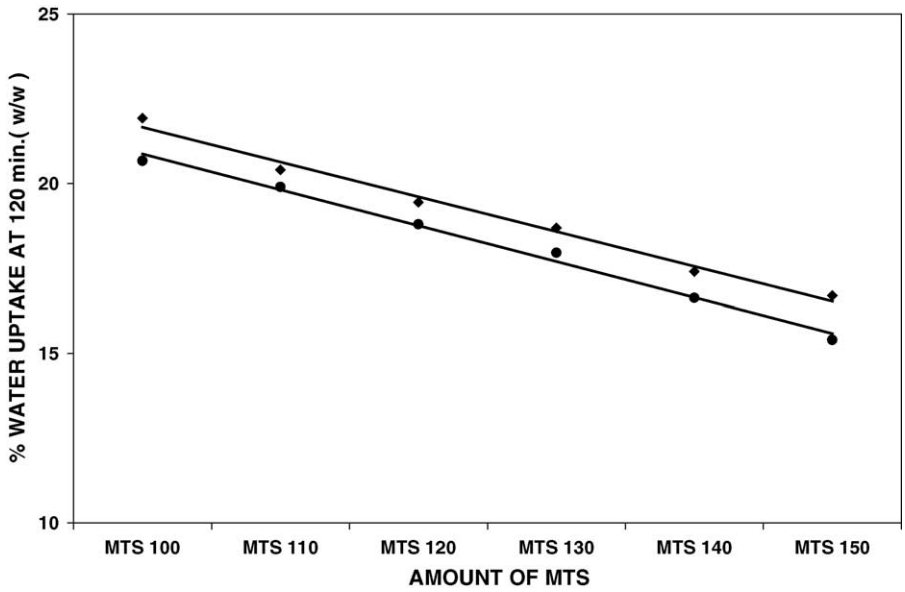

Fig. 5. Effect of amount of MTS on water uptake by GMO matrix: (♦) with drug and (●) without drug.

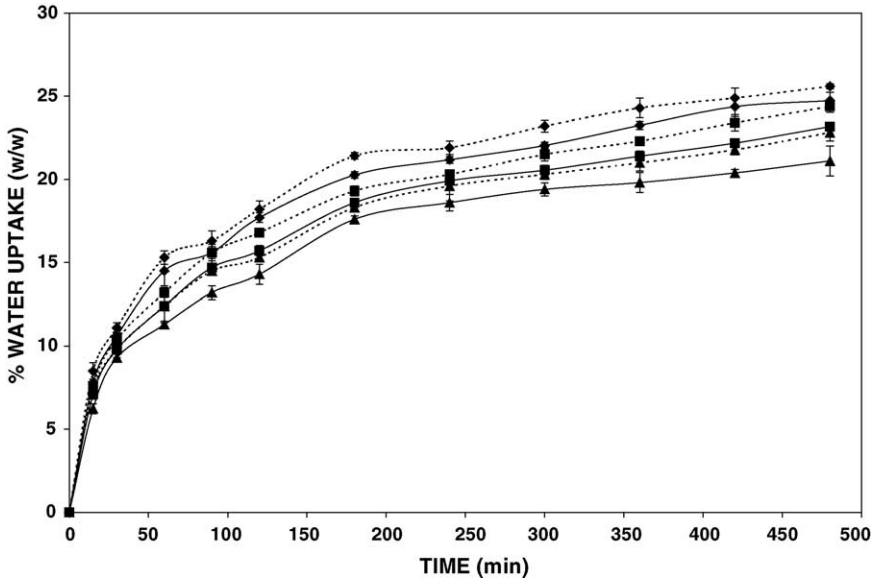

Fig. 6. Effect of amount of gelucire on water uptake of GMO matrices with (dotted lines) and without drug (bold lines): 10 mg (♦); 20 mg (■); 30 mg (▲).

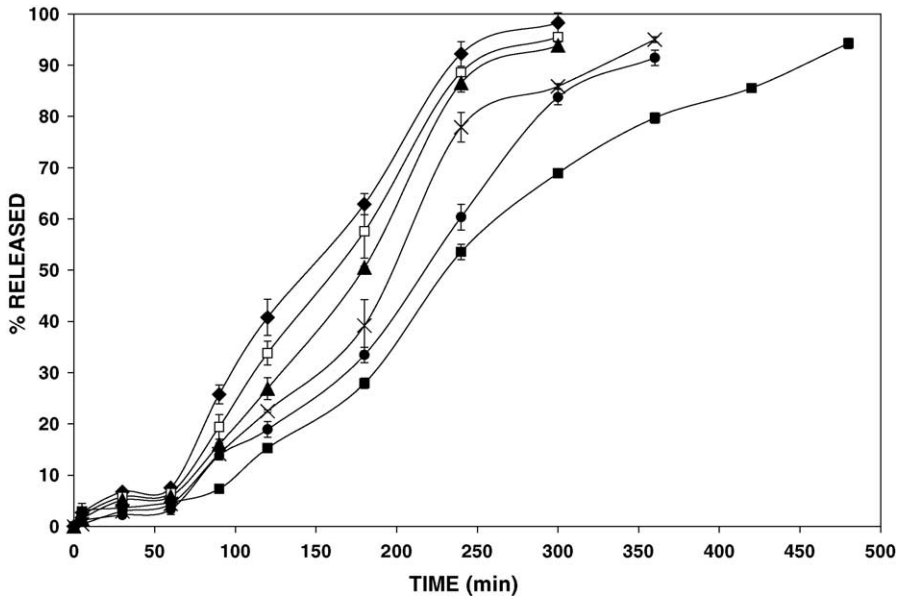

Fig. 7. Effect of amount of MTS on release of serratiopeptidase: 100 mg (♦); 110 mg (□); 120 mg (▲); 130 mg (×); 140 mg (●); 150 mg (■).

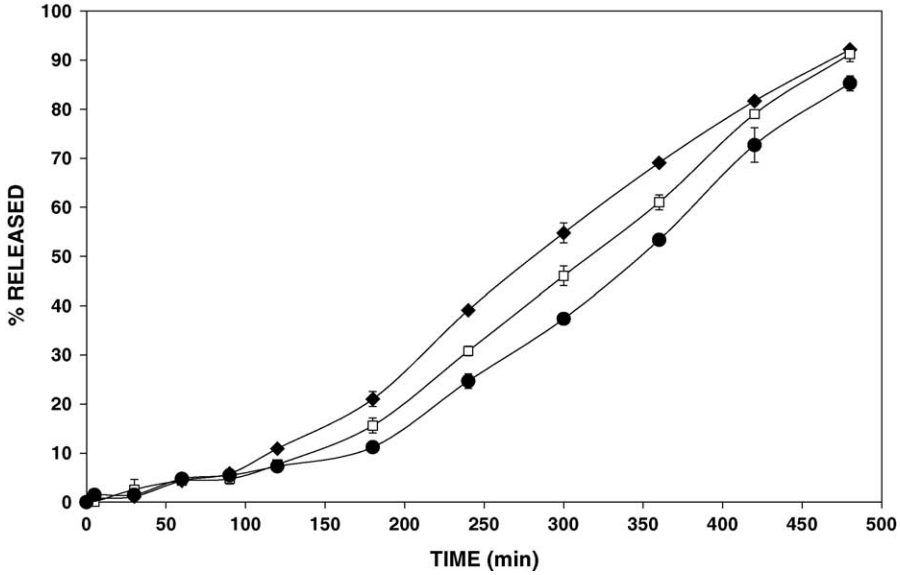

Fig. 8. Effect of amount of gelucire on release of serratiopeptidase: 10 mg (♦); 20 mg (□); 30 mg (●).

ture of saturated triglycerides of different fatty acids, which includes C_8 (3%), C_{10} (2%), C_{12} (29%), C_{14} (%), C_{16} (17%), and C_{18} (36%). It is extremely hydrophobic (HLB = 1) due to absence of PEG esters, and therefore, caused reduced volume of water in aqueous channels and thereby decreased drug release by potentiating effect of MTS. Incorporation of triglycerides in the bilayers provided resistance to the movement of STP molecule. Sutananta et al. (1995) also reported sustained release matrices of gelucire where only 17% theophylene was released over a period of 20 h.

Thus, microenvironment-controlled in situ cubic phase transforming GMO matrices provided protection to acid-labile metaloenzyme STP and prolonged its release.

4. Conclusion

The in situ cubic phase transforming system can be used as a carrier for protein molecules by altering the microenvironment and water uptake by the matrix. Incorporation of MTS was found to improve stability of STP and control its release from GMO matrix. Gelucire can be used in the system to provide delayed lag time.

Acknowledgements

Authors MHS and AP are thankful to University Grants Commission for providing financial assistance in terms of Junior Research Fellowship and Major Research Project, respectively. Authors are also thankful to Danisco Cultor, Denmark, and Gataffose, France for the gift samples of GMO and Gelucire® 43/01, respectively. Authors are thankful to Capsugel India for providing the gift sample of Licaps® and Mr. V.L. Rathi and Ms. Shilpa Risbud of Advanced Biochemicals Ltd. for gift sample of STP and analytical support. Authors are indebted to Dr. Shrikant Solav, Spect-Lab, Pune, for providing the facilities of gamma-scintigraphy and to Dr. B. K. Das and Dr. Rajshekhar, Agharkar Research Institute, Pune, for providing the facilities of polarizing light microscopy.

References

Caboi, F., Amico, G.S., Pitzalis, P., Monduzzi, M., Nylender, T., Larsson, K., 2001. Addition of hydrophilic and lipophilic compound of biological relevance to the monoolein/water system. I Phase behavior. Chem. Phys. Lipids. 109, 47–62.

Chang, M.C., Bodmeier, R., 1997. Effect of dissolution media and additives on the drug release from cubic phase delivery system. J. Control. Release 46, 215–222.

Engstrom, E., Lindahl, L., Wallin, R., Engblom, J., 1992. A study of polar lipid drug carrier system undergoing a thermoreversible lamellar to cubic phase transformation. Int. J. Pharm. 86, 137–145.

Engstrom, H., Wahren, A., Gustafosan, 1995. Bloodhesive properties of the monoolein–water system. Pharm. Technol. 2, 14–17.

Ericsson, B., Eriksson, P.O., Loefroth, J.E., Engstroem, S., Ferring, A.B., Malmoe, S., 1991. Cubic phase as delivery system for peptide drugs. ACS Symp. Ser. 469, 251–265.

Farkus, E., Zelko, R., Nemeth, Z., Palinkas, J., Morton, S., Raczs, I., 2000. The effect of liquid crystalline structure on chlorehexidine diacetate release. Int. J. Pharm. 193, 239–245.

Food Chemical Codex, 2003. pp. 495–496.

Fu, K., Klibanov, A.M., Langer, R., 2000. Protein stability in controlled-release system. Nature Biotechnol. 18, 24–25.

Kearney, A.S., 2000. Magnesium trisilicate. In: Kibbe, A.H. (Ed.), Handbook of Pharmaceutical Excipients. Pharmaceutical Press, London, pp. 309–310.

Kim, N., Kim, S.I., 1993. Metal ion relevance for the stability and activity of a 52-kDa Serratia proteinase. Biotechnol. Biochem. 57, 29–33.

Kumar, K.M., Shah, M.H., Ketkar, A., Mahadik, K.R., Paradkar, A., 2004. Effect of drug solubility and different excipients on floating behaviour and release from glyceryl mono-oleate matrices. Int. J. Pharm. 272, 151–160.

Longer, M., Tyle, P., Mauger, J.W., 1996. A cubic-phase oral drug delivery system for controlled release of AG 337. Drug Dev. Ind. Pharm. 22, 603–608.

Makai, M., Csanyi, E., Palinkas, J., Eros, I., 2003. Structure and drug release of lamellar liquid crystals containing glycerol. Int. J. Pharm. 256, 95–107.

Nessem, D.I., 2001. Formulation and evaluations of itraconazole via liquid crystal for topical delivery system. Int. J. Pharm Biomed. Anal. 26, 387–399.

Nylander, S.T., Mattisson, C., Razumas, V., Miezis, Y., Jakansson, B., 1996. A study of entrapped enzyme stability and substrate diffusion in a monoglycerides-based cubic liquid crystalline phase. Colloids Surf. 114, 311–320.

Rosevear, F.B., 1954. The microscopy of the liquid crystalline neat and middle phases of soaps and synthetic detergents. J. Am. Oil Chem. Soc. 31, 628–639.

Sadhale, Y., Shah, J.C., 1998. Glyceryl monooleate cubic phase gel as chemical stability enhancer of cefazolin and cefuroxime. Pharm. Dev. Technol. 3, 549–556.

Sadhale, Y., Shah, J.C., 1999. Biological activity of insulin in GMO gels and the effect of agitation. Int. J. Pharm. 191, 65–74.

Sallam, A.S., Khalil, E., Ibrahim, H., Freij, I., 2002. Formulation of an oral dosage form utilizing the properties of cubic liquid crystalline phases of glyceryl monooleate. Eur. J. Pharm. Biopharm. 23, 343–352.

Shah, J.C., Sadhale, Y., Chilukuri, D.M., 2001. Cubic phase gels as drug delivery systems. Adv. Drug Deliv. Rev. 47, 229–250.

Sutananta, W., Craig, D.Q.M., Newton, J.M., 1995. An evaluation of the mechanism of drug release from glycerides bases. J. Pharm. Pharmacol. 47, 182–187.

Wyatt, D.M., Dorschel, D., 1992. A cubic-phase delivery system composed of glyceryl monooleate and water for sustain release of water-soluble drugs. Pharm. Technol. 16, 116–130.