Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 323 (2006) 18–26
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Spray dried glyceryl monooleate–magnesium trisilicate dry powder as cubic phase precursor

Manish H. Shah, Shailesh V. Biradar, Anant R. Paradkar *

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

Received 30 December 2005; received in revised form 7 May 2006; accepted 23 May 2006
Available online 26 May 2006

Abstract

Glyceryl monooleate (GMO) is a polar amphiphilic lipid, which forms different sequential lyotropic liquid crystals upon hydration. GMO has been utilized for various delivery systems and routes of administrations. Owing to sticky and waxy nature of GMO, preparation of oral solid dosage form utilizing GMO is still a challenge for pharmaceutical researchers. Therefore, the objective of the present work was to fabricate dry powder precursors using GMO, which upon hydration in situ forms cubic phase and can be wisely used for fabrication of oral solid dosage forms. In addition to this, dry powder precursor was evaluated for drug loading, in vitro release behavior and in vivo performance of model drug diclofenac sodium (DiNa). The dry powder precursor was obtained by spray-drying GMO with DiNa using magnesium trisilicate (MTS) as adsorbent. The percent drug entrapment of various batches of powder precursor was in the range of 84–93% indicating high content uniformity. SEM and image analysis showed that as the amount of MTS in powder precursor was increased, the particle size decreased. Furthermore, the viscosity of powder precursor was function of amount of MTS. The rate of water uptake of powder precursor was higher due to uniform layer of GMO on the MTS surface, which led to faster transformation of lamellar phase into cubic phase. The polarizing light microscopy confirmed that cubic phase was formed upon hydration of powder precursor. The drug released from powder precursor was initially governed by the cubic phase formed and in later stage it depends upon dynamic swelling behavior of hexagonally packed cylindrical aggregates. The drug loaded powder precursor was found to have more effective and prolonged anti-inflammatory and analgesic activity as compared to pure drug. Thus the dry powder precursor of cubic phase was prepared in which drug release was entirely governed by the mesophases formed.

Keywords: Glyceryl monooleate; Cubic phase precursor; Spray-drying; Anti-inflammatory activity; Analgesic activity

1. Introduction

Glyceryl monooleate (GMO), an amphiphilic lipid self-associate to form sequential liquid crystalline mesophases, viz., lamellar, cubic and hexagonal when placed in an aqueous media (Shah et al., 2001; Kumar et al., 2004). Being non-toxic, biodegradable and biocompatible it has found its utility in various delivery systems and routes of administrations (Ganem-Quintanar et al., 2000). Delivery system based on partially hydrated lamellar phase (Farkus et al., 2000; Makai et al., 2003), hydrated cubic gel (Sallam et al., 2002), cubic phase dispersions (Siekmann et al., 2002; Spicer et al., 2002) and matrix (Kumar et al., 2004; Shah and Paradkar, 2005) have been explored by many researchers.

Cubic phase coexists in equilibrium with the excess water and being highly viscous has gained much attention for sustained release. The sustained release may be due to slow drug diffusion or increased residence time in its solublized form. Further, its isotropic nature, relative insensitivity to salts and solvents, robustness and resistance to physical degradation make it most preferred candidate for sustained drug delivery.

However, design and development of cubic phase based palatable solid dosage form of GMO has limitations due to its intrinsic properties like stickiness and stiffness. Preparation of dry powder precursors, which can be quickly transformed into cubic phase *in situ,* will promote industrial application of the system.

* Corresponding author. Tel.: +91 20 25437237; fax: +91 20 25439383.
E-mail address: arparadkar@rediffmail.com (A.R. Paradkar).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.05.040

Recently, Spicer et al. (2002) developed spray dried dry powder cubosomes by encapsulating monoolein using ternary (starch–monoolein–water) and quaternary (dextran–monoolein –ethanol–water) systems. The water based ternary system hydrates monoolein and generate highly viscous cubic phase. This require additional dispersion step to minimize sticking of powder to drying chamber. The quaternary system was processed without high shear dispersion; however, the final product has fairly high solvent content (13% water and 3% ethanol) exceeding the permissible ICH and regulatory guideline limits for residual solvents. Kim et al. (2000) has developed freeze-dried powder that can form a dispersed cubic phase in water. Cost effective production of this dosage form in a stipulated time limits its commercial application. Szoka et al. (1998) has prepared dry powder formulation of polynucleotidase complexes for inhalation using freeze-drying. However, the vesicles formed were spherical lamellar liquid crystal shells that have lower bilayer area per volume and were more shear sensitive.

In this study dry powder precursors were produced by spray-drying. The drug and magnesium trisilicate (MTS; as a carrier) were dispersed whereas GMO was dissolved in isopropyl alcohol. During spray-drying GMO coats MTS and drug surfaces. The resulting powder has advantage of increased surface area and residual solvent content within standard ICH and regulatory limits. The dry powder precursors obtained by this modified method can directly be used for capsule or tablet preparations; the most popular dosage forms. Further, spray-drying technique, offers flexibility to alter and control powder properties as particle size distribution, bulk density, flowability, solid-state properties and moisture content (Broadhead et al., 1992; Cornigan, 1995), making it suitable method for preparation of pharmaceutical powder. Diclofenac sodium (DiNa) was selected as model drug and the powder precursors were evaluated for percent yield, drug content, image analysis, surface topography, residual solvent content, phase behavior, physical interactions, rheology, *in vitro* drug release, *in vivo* anti-inflammatory and analgesic activity.

2. Materials and methods

2.1. Materials

Glyceryl mono-oleate (Rylo^{TM} MG Pharma19) was generous gift from Danisco Cultor, (Copenhagen, Denmark). Licaps® capsules (size 0, hard gelatin capsule specially designed for lipid formulations) were obtained as gift sample from Capsugel (India). Magnesium trisilicate was purchased from Loba Chemicals, Mumbai, India. MTS has average particle size of 5.7 μm (d 0.5) determined by Laser Diffractometer, Mastersizer 2000 with distilled water as dispersant (Mastersizer Ver. 2, Malvern Instruments, Malvern, UK). Diclofenac sodium was gift from Bluecross Laboratories Ltd., Nasik (India). Subsyde®-CR capsules (Raptakos Brett and Co. Ltd., Roha, India.) containing controlled release beads of DiNa were purchased from local pharmacy shop. All other chemicals used were of analytical grade.

Table 1
Composition of powder precursor

Batch code	Composition of powder precursor (parts by weight)		
	GMO	MTS	DiNa
A	1	0.5	0.5
B	1	1	0.5
C	1	1.5	0.5
D	1	2	0.5
E	1	2.5	0.5

2.2. Preparation of powder precursor

GMO was dissolved in sufficient amount of isopropyl alcohol and then dispersed MTS and DiNa in it (proportions are shown in Table 1). Total solid content of dispersion of all batches was 5.0%. Spray-drying was carried out using laboratory scale spray dryer (Jay Instruments and Systems Pvt. Ltd., Mumbai, India) consisting of a cylindrical chamber with two cyclone collector at the air exit. Dispersions to be spray dried was kept under stirring on magnetic stirrer and fed into a twin-fluid nozzle at the top of the spray dryer body with a liquid orifice size of 0.1 cm using peristaltic pump. Aspiration air at a pressure of 300 mm WC (mm of water column) was pumped through 0.25 cm annular air orifice. The inlet temperature of drying air was 95 °C (outlet temperature 85 °C). The dispersion consisting of GMO, MTS and DiNa was pumped through the liquid side of the twin-fluid atomizer at a rate 10-ml/min with atomization air pressure of 1,96,133 Pa (2 kg/cm^2). Samples of powder precursor 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 powder precursor

2.3.1. Percent yield

The weight of powder precursor obtained after spray-drying was considered as observed yield and percent yield was calculated by using following formula:

$$\text{percent yield} = \left(\frac{\text{observed yield}}{\text{theoretical yield}}\right) \times 100 \quad (1)$$

2.3.2. Drug content

Powder precursor equivalent to 60 mg of DiNa was weighed accurately and dissolved in suitable quantity of methanol. This solution was filtered through 0.45 μm filter and absorbance was determined at 276 nm by UV spectrophotometer (V-530, JASCO, Japan) after appropriate dilution. The DiNa content was calculated using the absorbance obtained by repeating the same procedure for 60 mg of pure DiNa.

2.3.3. Image analysis

For image analysis, the images were captured using a stereomicroscope (Carl Zeiss, Germany) attached with a digital camera (Watec, Wat-202, Japan). The captured images were analyzed using Biovis Image Plus software (Expert Tech Vision,

India). Around 200 particles were analyzed with 20X magnification for average diameters.

2.3.4. Scanning electron microscopy (SEM)

SEM was used to observe the morphological characteristics of powder precursor. Samples were mounted on a double faced adhesive tape and sputtered with thin gold–palladium layer by sputter coater unit (VG Microtech, UK) and surface topography was analyzed with a Cambridge Stereoscan S120 scanning electron microscope (Cambridge, UK) operated at an acceleration voltage of 10 kV.

2.3.5. Residual solvent content

The amount of isopropyl alcohol remained in the powder precursor following spray-drying was determined using thermogravimetric analysis (TGA; TGA-50, Shimadzu Corporation, Japan). The flow rate of nitrogen employed for flushing was 30 ml/min in the temperature range 25–90 °C. The heating rate was 5 °C/min and the weight of sample was about 10 mg.

2.3.6. Diffused reflectance infrared Fourier transform spectra (DRIFTS)

DRIFTS of pure DiNa, MTS, GMO and powder precursor were obtained after appropriate background subtraction using a FTIR spectrometer (FTIR-8400, Shimadzu Corporation, Japan) equipped with a diffuse reflectance accessory (DRS-8000, Shimadzu Corporation, Japan) and a data station. About 2–3 mg of sample was mixed with dry potassium bromide and the sample was scanned from 4000 to 400 cm^{-1}.

2.3.7. Rheological behavior

The rheological examination was carried out using Brookfield LV-DV III programmable rheometer equipped with spindle CP40 (Brookfield Engineering Laboratories Inc., Middleboro). A cone and plate sensor having a diameter of 2.4 cm was used and the cone angle was 0.8°. The thickness of sample in the middle of sensor was 0.0127 mm. Samples of powder precursor (300 mg) were hydrated for 1 h in a petriplate with 5 ml of phosphate buffer (pH 6.8) maintained at 37 ± 0.5 °C. The hydrated sample was loaded on rheometer plate at temperature 25 ± 0.3 °C and the initial linear viscoelastic region of the samples was determined and 100 rpm was chosen as a suitable shear rate for all systems investigated. The data obtained was further analyzed by regression analysis.

2.3.8. Polarizing light microscopy

The samples of powder precursor (300 mg) were hydrated for 1 and 8 h with 5 ml of phosphate buffer (pH 6.8) maintained at 37 ± 0.5 °C. The hydrated samples were examined under polarizing light microscope (Nikon, Kanagawa, Japan) using λ 1/4 compensator in order to study the texture of anisotropic phases. The phase boundaries were examined at a magnification of 200X. Photomicrographs of these samples were taken at room temperature after hydration of 1 and 8 h. The different mesophases were identified according to classification established by Rosevear (1954).

2.3.9. Water uptake studies

The water uptake of the powder precursor was examined gravimetrically (Farkus et al., 2000). The powder precursor (100 mg) was weighed into the donor chamber of the diffusion cell sealed by pre-hydrated semipermeable membrane. The donor chamber was weighed at analytical accuracy and then mounted on the receiver compartment of cell. The receiver compartment was filled with phosphate buffer (pH 6.8) and assembled cell was maintained at 37 ± 0.5 °C. At pre-determined time intervals the cell was disassembled and the donor chamber with powder precursor sample was blotted with tissue paper to remove excess of water and re-weighed. The donor cell with powder sample was remounted on to the cell.

2.3.10. Drug release studies

Drug release study of the powder precursor was carried out using USP 24 type II dissolution test apparatus (Electrolab TDT-08L, India). The dissolution test for each batch was performed in triplicate. The powder precursor was filled into the Leaps® capsules. Capsules were placed in 900 ml of phosphate buffer (pH 6.8) 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 276 nm.

2.3.11. In vivo anti-inflammatory activity in rats

Anti-inflammatory activity of powder precursor (batch E) was determined by the carrageenan-induced rat paw edema test as described by Winter et al. (1962). Wistar rats of either sex (150–200 g) were divided into different groups containing six animals each. Animals were fasted for 12 h before experiment and only water was allowed. While the first group was the control and received the vehicle (water 1 ml per rat), the second group received DiNa (5 mg kg^{-1} body mass). Third group received the Subsyde®-CR (containing DiNa equivalent to 5 mg kg^{-1} body mass). The remaining group received the powder precursor (batch E, containing DiNa equivalent to 5 mg kg^{-1} body mass) orally. All the suspensions for oral dose were prepared in water rather than in buffer as only first one was permitted by Ethics committee and administered in a constant volume of 1 ml per rat. One hour after the administration of the powder precursor, Subsyde®-CR and pure DiNa, 0.1 ml of 1% (m/v) suspension of carrageenan was injected into the left hind paw intraplantar of control and test animals. The initial paw volume was measured immediately using a plethysmometer (UGO Basile, Varese, Italy), and thereafter the paw volume was measured every 1 h up to 24 h. The research protocol of animal experimentation was approved by the 'Institutional animal Ethics Committee' of Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune, India.

2.3.12. In vivo analgesic activity in rats

The method of Randall and Selitto (1957) was followed to measure pain in the inflamed paw. Animals were fasted for 12 h before experiment and only water was allowed. Assessment of pain consisted of measurement of threshold stimulus for reac-

tion (escape or paw withdrawal) using a weight applied to the pads of the hindpaw of Wistar rats of either sex (150–200 g). The threshold for pain sensation was measured before (basal) and up to 8 h after intraplantar injection of 1% carrageenan (0.2 ml) using Analgesy-meter (UGO Basile, Varese, Italy). Pure DiNa, Subsyde®-CR, powder precursor (batch E) and water (vehicle) were administered orally 15 min priors to carrageenan injection. The research protocol of the animal experimentation was approved by the 'Institutional animal Ethics Committee' of Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune, India.

3. Results and discussions

3.1. Percent yield and drug entrapment

The percent yields of different batches of powder precursors prepared by spray-drying are shown in Table 2. It was observed that as the amount of MTS in spray-drying dispersion was increased, the percent yield increased. In case of batches (batch A) with lower MTS content, sticking was observed on side wall of drying chamber. This sticking was attributed to incomplete adsorption of GMO on inadequate amount of MTS particles, resulting in lower yield. With increase in MTS content, yield was improved significantly, which may be attributed to uniform coating of GMO on MTS particles. The product thus obtained was found to be comparatively non-sticky one. Interestingly high density of MTS reduced the escaping tendency of the powder and increases the percent yield. Percent drug entrapment of various batches was in the range of 84–93% (Table 2). The percent drug entrapment in powder precursors with lower amount of MTS (A, B and C) was almost similar whereas the batches with high amount of MTS (D and E) presented considerable improvement in drug entrapment. With bathes A, B and C with lower MTS content, significant sticking was observed leading to loss of drug during processing while this loss was reduced with increase in MTS content.

3.2. Scanning electron microscopy (SEM) and image analysis

The photomicrographs were obtained at 1–5 KX magnification in order to gather information about the powder characteristics (particle size and shape) and surface (texture). The SEM microphotographs of starting materials MTS and DiNa are shown in Fig. 1. The microphotograph of MTS showed that it has irregularly shaped clusters of microparticles. Microphotograph of pure DiNa had presented arrangement of large crystals with fine particles or microparticles covering their surface, which may be generated due to micronization or any other size reduction process. On the other hand the different batches of powder precursor showed that GMO had coated the entire dispersion of DiNa and MTS and provided smooth texture to powder precursor in which the crystals of DiNa and MTS cannot be identified. Representative SEM photograph of different batches (A, B, C, D and E) of powder precursor are shown in Fig. 2. The batches with lower amount of MTS (A and B) showed significantly bulkier sized aggregates. In the batches with higher concentration of MTS (C, D and E) the aggregation of powder precursor was significantly reduced and the sample appeared irregular

Table 2
Effect of amount MTS on the different characteristics of powder precursor

Batch code	Percent yield (%)[a]	Percent drug entrapment (%)[a]	Particle size (μm)[b]	Structure of liquid crystalline mesophases	
				1 h	8 h
A	60.50 ± 3.2	84.1 ± 1.2	498.28 ± 40.2	Cubic	Hexagonal
B	63.67 ± 2.8	85.0 ± 1.4	310.12 ± 60.1	Cubic	Hexagonal
C	68.90 ± 2.2	86.6 ± 1.6	226.15 ± 55.2	Cubic	Hexagonal
D	71.26 ± 2.5	90.1 ± 1.8	106.80 ± 54.6	Cubic	Hexagonal
E	82.70 ± 1.2	93.3 ± 1.1	80.33 ± 30.3	Cubic	Hexagonal

[a] Results reported as mean ± S.D., $n = 3$.
[b] Mean ± S.D., $n = 200$.

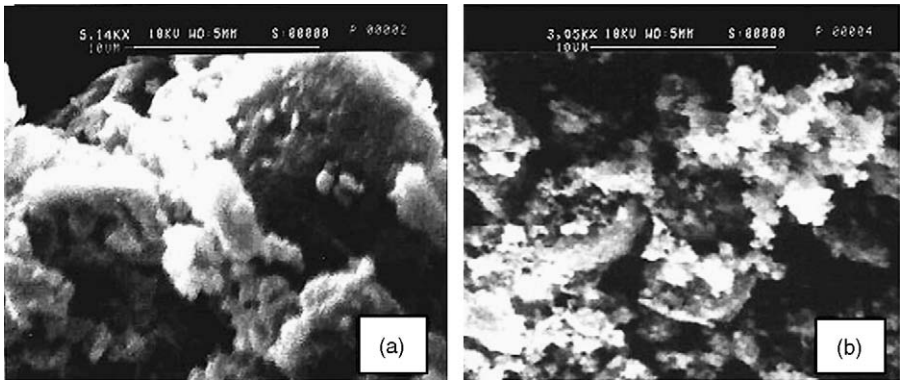

Fig. 1. Scanning electron microphotographs of pure DiNa (a) and MTS (b).

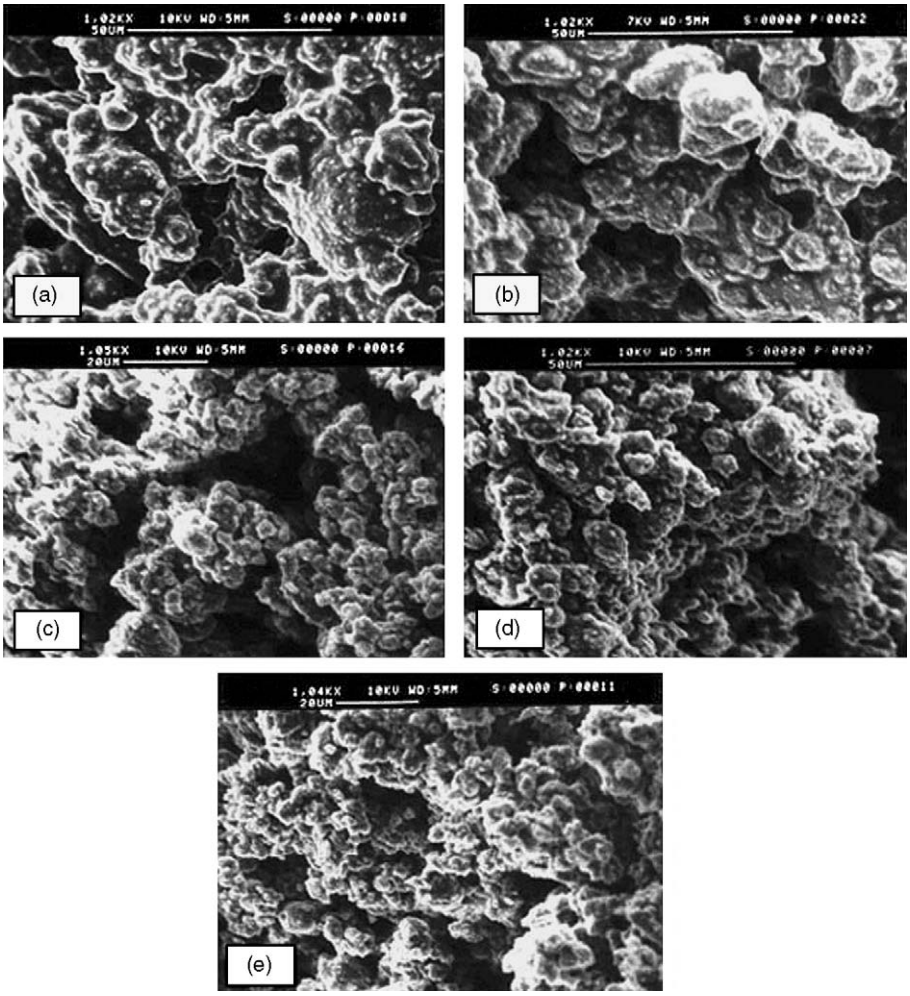

Fig. 2. Scanning electron microphotographs of different batches of powder precursor: (a) batch A; (b) batch B; (c) batch C; (d) batch D; (e) batch E.

shaped agglomerates with small particles having smooth surface. The small semispherical powder particles had been packed together closely due to sticky nature of GMO leading to aggregation after spray-drying. Similar results were shown by image analysis data (Table 2). As the amount of MTS in powder precursor was increased, the particle size decreased. The batch A with lowest amount of MTS has particle size 498.28 ± 40.2 μm whereas batch E with highest amount of MTS has particle size 80.33 ± 30.3 μm.

3.3. Residual solvent content

Residual solvent present was determined using TGA. The TGA curves represent the weight loss of the sample as a function of temperature. TGA curves of different batches of powder precursor are shown in Fig. 3. TGA curve for initial samples of batch A represents 1.53% (w/w) loss of the total solvent of the powder precursor in between 41 and 59 °C and the total weight loss of the sample observed was 2.34% (w/w) (Fig. 3a). Similarly, for batch B there was 1.013% (w/w) loss of the total solvent of the powder precursor in between 40 and 52 °C and had 2.88% (w/w) (Fig. 3b) of total weight loss. In case of these batches, higher amount of solvent was entrapped in GMO owing

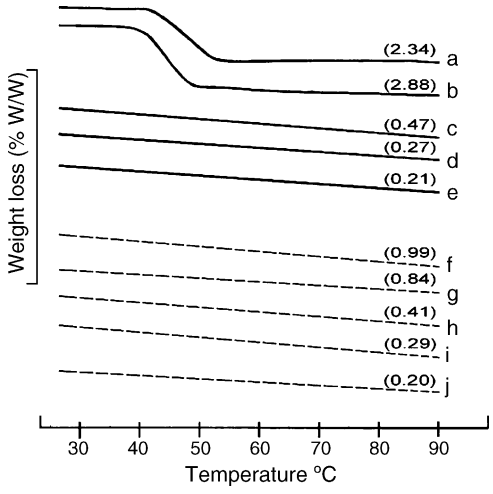

Fig. 3. Thermogravimetric curves of different batches of powder precursor immediately after spray-drying (bold line) and after 1 month (dotted lines). (a and f) TGA curve for batch A samples at initial stage and after 1 month, respectively; (b and g) TGA curve for batch B samples at initial stage and after 1 month, respectively; (c and h) TGA curve for batch C samples at initial stage and after 1 month, respectively; (d and i) TGA curve for batch D samples at initial stage and after 1month, respectively; (e and j) TGA curve for batch E samples at initial stage and after 1 month, respectively.

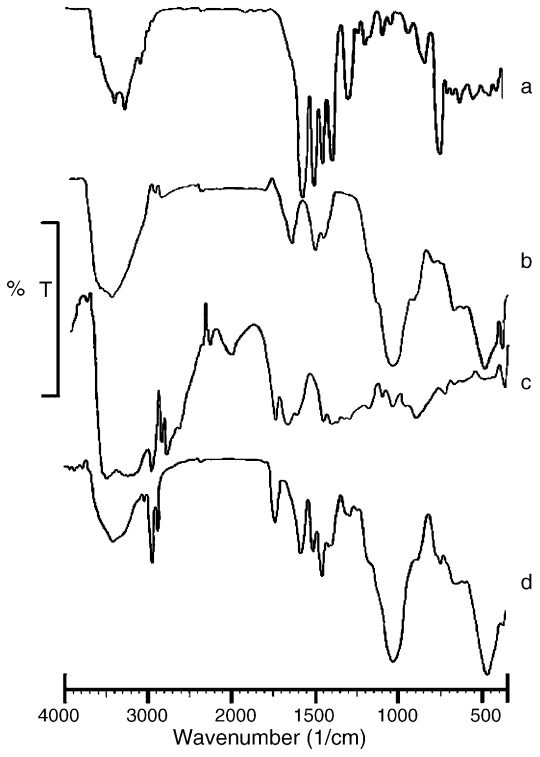

Fig. 4. DRIFT spectra corresponding to DiNA (a); MTS (b); GMO (c); powder precursor of batch A (d).

to its incomplete adsorption on MTS particles, which was evaporated at lower temperature resulting from melting of GMO. After 1 month of spray-drying these batches showed 0.99% (w/w) (Fig. 3f) and 0.84% (w/w) (Fig. 3g) total weight loss for batch A and batch B, respectively.

The batches C, D and E presented the total weight loss 0.47% (w/w) (Fig. 3c), 0.27% (w/w) (Fig. 3d) and 0.21% (w/w) (Fig. 3e), respectively. In these batches the amount of MTS was higher; therefore more surface area was made available for adsorption of GMO and yielded particles with lower particle size rather than lumped and aggregated as in case of batches A and B. This was also conformed by image analysis (Table 2). Therefore high surface area and thin layer of GMO may be responsible for lower entrapment of solvent. The residual solvent content of these batches remained unchanged after storage of 1 month after spray-drying (Fig. 3h–j). According to ICH guidelines (1997) isopropyl alcohol is a class III solvent and permissible limit is 0.5%, thus the spray-drying method used for these batches was found to be suitable.

3.4. DRIFT spectroscopy

Interaction between the drug and carrier often leads to identifiable changes in the IR spectra. To study possible interactions between DiNa, GMO and MTS, IR spectra were obtained (Fig. 4). The characteristic peaks of DiNa (Fig. 4a) occurred at 3382.9, 3259.5, 3076.2, 1569.9 and 752.2 cm^{-1}. The absorption of the amino group was located in the bands at 3382.9, 3259.5 and 3076.2 cm^{-1}. The wave number observed at 1569.9 and 752.2 cm^{-1} was due to strong carboxylate anion stretching (C=O) and C–Cl stretching vibration, respectively. Whereas IR spectra of MTS (Fig. 4b) showed characteristic peak of strong silanol linkage (Si–O) at 1033.8 cm^{-1}. The spectra of GMO (Fig. 4c) revealed OH-stretching vibration at 3544.9 cm^{-1}. To find out interactions, the presence or absence of peak associated with specific structural characteristic of the molecule in IR spectra of powder precursor was studied. No physical interaction was observed in the spectra of precursor as spectral bands presenting all functional group of the DiNa, GMO and MTS were seen unchanged. Therefore, it was apparent that no interaction had been occurred. The representative spectrum of powder precursor (batch A) is given in Fig. 4d.

3.5. Rheological behavior

The effect of amount of MTS on the viscosity of hydrated powder precursor is shown in Fig. 5. Viscosity powder precursor was found to be the function of amount of MTS as defined by the following equation:

$$\text{viscosity} = 2.41(\text{amount of MTS in the powder precursor}) \quad (p = 0.002) \tag{2}$$

It was noted that as the amount of MTS in the powder precursor increased, viscosity was increased linearly ($r^2 = 0.9631$). The powder precursor with high amount of MTS (batch E) had highest viscosity. With high content of MTS, GMO was completely coated on MTS. As a result, when such system was hydrated; it induced formation of cubic phase, having high viscosity.

3.6. Water uptake studies

Effect of amount of MTS on water uptake of powder precursor is shown in Fig. 6. It was observed that water uptake was decreased with increasing amount of MTS. This result was in agreement with our previous report (Shah and Paradkar, 2005). Batch A had shown maximum water uptake as impact of hydrophobicity of MTS on transformation of lamellar phase into cubic phase was comparatively low. While batch E had shown

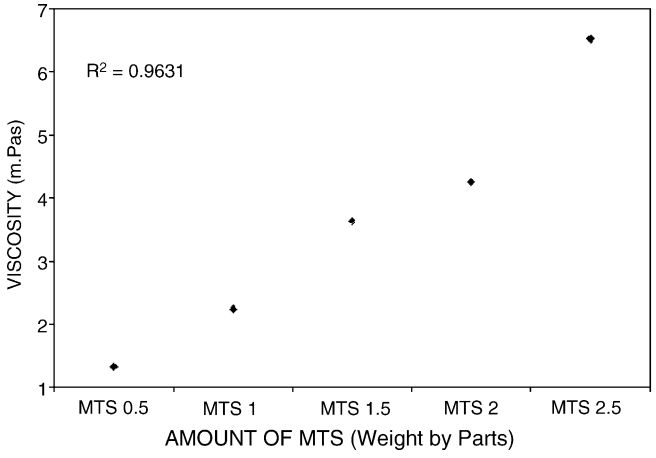

Fig. 5. Effect of amount of MTS on viscosity of powder precursor (mean ± S.D., $n = 6$).

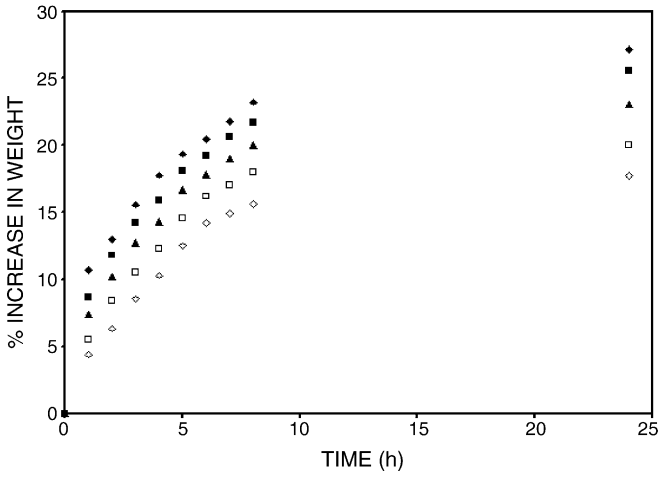

Fig. 6. The water uptake by powder precursor as a function of time: batch A (♦); batch B (■); batch C (▲); batch D (□); batch E (◊) (average values, $n = 3$).

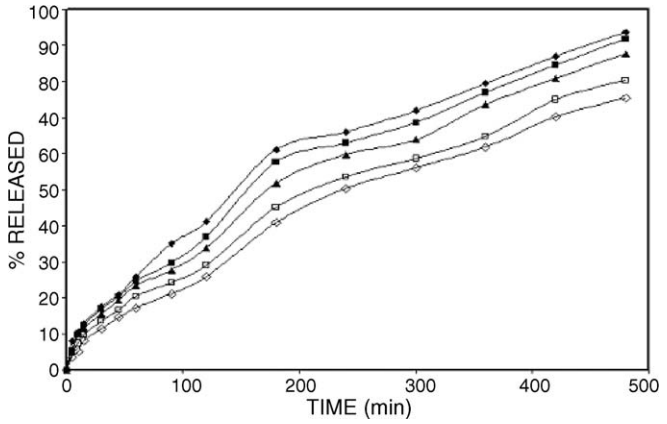

Fig. 7. Diclofenac sodium release form different powder precursors: batch A (♦); batch B (■); batch C (▲); batch D (□); batch E (◊) (average ± S.D., $n = 3$).

relatively faster transformation to cubic phase due to pronounced effect of hydrophobicity of MTS and hence had shown lower water uptake. When compared to the water uptake of powder precursor without drug (data not shown), it can be concluded that presence of drug in powder precursor did not significantly affect the water uptake. This may be due to high amount of MTS in powder precursor batches. Hydrophobic nature of MTS counteracted the hydrophilic nature of drug (Kumar et al., 2004). Furthermore, the amount water uptake was comparatively lower than the earlier reports (Kumar et al., 2004; Shah and Paradkar, 2005). Since this study was performed using diffusion cell, it had much slower rate of swelling as less surface area was exposed to the buffer media and restriction of media access to the powder precursor by membrane. There was no further increase in water uptake after 10 h owing to transformation of cubic phase into hexagonal phase in presence of hydrophobic additive (Shah and Paradkar, 2005) and was assured by polarizing light microscopy (Table 2).

3.7. Drug release study

The drug release profile of powder precursor with different amount of MTS is shown in Fig. 7. The release profile had not shown burst release at the initial stages, which has been previously reported for matrix system due to formation of lamellar phase. However, Shah and Paradkar (2005) recently found that the matrix incorporated with MTS had shown initial lag of 60–120 min and less than 10% of total drug was released during the lag. In the present work the system was prepared using spray-drying technique. It had generated the small agglomerates with high surface area, which led to comparatively faster drug release in the initial stages. The water uptake study had showed that there was formation of cubic phase in early stage conformed by polarizing light microscopy (Table 2; Fig. 8a). After 8 h, observation of systems under polarizing light microscope revealed that the system was transformed into hexagonal phase (Table 2; Fig. 8b). This was in conformation with our previous work (Shah and Paradkar, 2005), which showed that hydrophobic nature MTS transformed cubic phase into hexagonal phase. The drug released in later was governed by the dynamic swelling behaviour of hexagonally packed cylindrical aggregates.

SEM and image analysis studies indicated that as the amount of MTS in powder precursor was increased, the particle size was decreased from 498.28 ± 40.2 μm (batch A) to 80.33 ± 30.3 μm (batch E). However, batches with high amount of MTS (D and E) released the drug at very slow rate though they had smaller particle size. The possible explanation for this might be given by coating of GMO on the powder surface. As amount of MTS increased, high product recovery was obtained, so it could be appropriate to state that with increasing amount of MTS entire dispersion of drug and MTS was coated with GMO. Similarly, as amount of MTS increased viscosity was also increased and batch E had highest viscosity. This could be correlated to the amount of

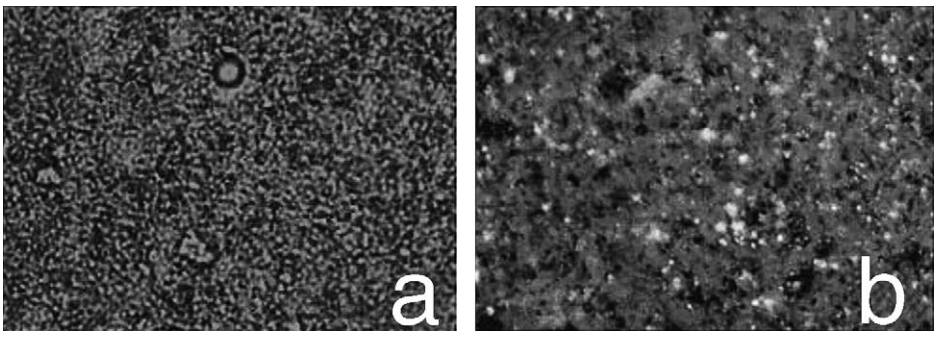

Fig. 8. Polarizing light microphotographs of powder precursor showing liquid crystalline mesophases: (a) cubic phase; (b) hexagonal phase.

Table 3
Effect of different preparations on percent inhibition of rat paw edema in rats

Time points (h)	DiNa (%)	Subsyde®-CR (%)	Powder precursor (%)
1	17.5	19.7	21.9
2	31.2	34.8	40.3
3	43.4	45.3	49.3
4	27.1	39.7	48.7
5	24.9	34.8	43.5
6	19.8	33.2	41.8
7	19.5	32.9	41.4
8	19.5	32.6	42.9
24	38.3	41.5	45.1

Average values, $n = 6–8$.

GMO in powder precursor. Further increase in amount of MTS (>2.5 parts by weight) led to instability and drug release was no longer controlled.

Therefore, it could be concluded that, though the particle size of the powder precursor reduced with increasing amount of MTS, the slow release was associated with the coating of GMO on the surface of drug. Furthermore, as there was no interaction found in IR studies, the release retarding effect was purely governed by the mesophases formed in the powder precursor system.

3.8. Anti-inflammatory activity

Carrageenan-induced rat paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility (Vinegar et al., 1969). Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 h (Williams and Morley, 1973; Williams and Peck, 1977) and the swollen paw maintained about the same degree of edema for several hour. DiNa shows its activity in second phase.

The powder precursor (batch E) was subjected to preliminary testing of anti-inflammatory activity in comparison with the pure DiNa and Subsyde®-CR to evaluate activity with pure drug and also with marketed controlled release formulation. The reduction in the inflammation (i.e. reduction in the left hind paw edema volume of the animals) after administration of carrageenan was recorded and percent inhibition was calculated and presented in Table 3. Pure DiNa, powder precursor and Subsyde®-CR caused reduction in rat paw edema and maximum inhibition of the carrageenan-induced rat paw oedema was occurred at the end of 3 h. Pure DiNa showed 43.4% ($p < 0.01$) percent inhibition at 3 h, thereafter the percent inhibition gradually decreased to 38.3% ($p < 0.05$) after 24 h. The powder precursor and Subsyde®-CR presented improvement in inhibitory activity as compared to pure drug. Subsyde®-CR showed increase in percent inhibition at 3 h (45.3%; $p < 0.01$) and the percent activity decreases to 41.5% ($p < 0.01$) after 24 h. The powder precursor causes remarkable increase in percent inhibition after 3 h (49.3%; $p < 0.01$) and inhibition was remained nearly same after 24 h (45.1%; $p < 0.01$). Powder precursor and Subsyde®-CR inhibited prostaglandins (PGs) for long time due to release of drug in controlled manner and therefore maintained the activity for prolonged period of time. PGs has been well established as mediators of several components of inflammatory responses and due to vasodilator effect, it increases microvasodilator permeability and causes oedems by potentiating the microvascular effect of other mediators such as bradykinin, substance P and histamin (Ferreira, 1972; Williams and Morley, 1973; Williams and Peck, 1977). The decrease in percent inhibition after 3 h may be due to other autocoids released due to carrageenan in the first phase, which caused increase in inflammation.

3.9. In vivo analgesic activity in rats

The data obtained after subjecting animals to Randall and Selitto test is shown in Table 4. Weight threshold required for the animal response was reduced due to intraplantar administration of carrageenan. DiNa had significantly increased the weight threshold to 10.89 ± 1.51 ($p < 0.01$) at 3 h as compared to water treated animals (3.86 ± 1.44) but at the 8 h the weight threshold decreased to normal (basal) level (4.82 ± 0.49; $p > 0.05$). The Subsyde®-CR (11.19 ± 1.96; $p < 0.01$) and powder precursor (11.46 ± 1.29; $p < 0.01$) showed significant increase in weight threshold at 3 h and maintained with slight

Table 4
Effect of different preparations on pain in inflamed tissue (Randall and Selitto test) in rats

Time points (h)	Response latencies ($g \times 20$)			
	Water	DiNa	Subsyde®-CR	Powder precursor
Basal	5.17 ± 1.25	5.04 ± 2.36	6.00 ± 0.73	6.48 ± 2.20
1	4.95 ± 1.27	5.58 ± 1.88	6.07 ± 0.81	6.66 ± 0.53
2	4.81 ± 1.55	5.42 ± 1.08	6.01 ± 1.97	6.71 ± 1.03
3	3.86 ± 1.44	10.89 ± 1.51	11.19 ± 1.96	12.49 ± 0.94
4	3.40 ± 1.35	5.10 ± 0.83	10.48 ± 1.64	11.46 ± 1.29
5	3.67 ± 0.99	4.67 ± 0.61	11.96 ± 1.48	11.00 ± 1.27
6	3.32 ± 1.15	5.00 ± 0.83	10.46 ± 1.55	10.69 ± 1.37
7	3.60 ± 1.04	4.36 ± 0.61	10.05 ± 1.33	9.03 ± 0.75
8	3.77 ± 1.16	4.82 ± 0.49	9.90 ± 0.49	9.98 ± 0.95

Results reported as mean ± S.E.M, $n = 6–8$.

decrease up to 8 h (9.90 ± 0.45; $p < 0.01$ for Subsyde®-CR and 9.98 ± 0.95; $p < 0.01$ for powder precursor). Powder precursor and Subsyde®-CR inhibited PGs, which induces pain in inflammatory sites, for long time and therefore increased and maintained weight threshold. Slight declination in the weight threshold after at 3 h may be attributed to other mediators such as bradykinin, substance P and histamin released due to carrageenan injection, which produced further inflammation and was not inhibited by DiNa.

4. Conclusion

A dry powder precursor of cubic phase had been fabricated. The SEM studies and image analysis demonstrated that the particle size of powder precursor was dependent on amount of MTS present. The residual solvent content was in compliance with limit set by ICH guideline. Powder precursor formed cubic phase at faster rate and drug release was entirely governed by the mesophases formed. The DiNa loaded powder precursor had presented more effective and prolonged anti-inflammatory and analgesic activity as compared to pure drug owing to controlled release of drug. The powder precursor thus prepared can be utilized for preparation of oral solid dosage forms such as tablet and capsule.

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 gift sample of Rylo™ MG Pharma19. Authors are grateful to Capsugel India for providing the gift sample of Licaps®. Authors are also thankful to Drs. B.K. Das and Rajshekhar, Agharkar Research Institute, Pune, for providing the facilities of polarizing light microscopy and Drs. S.S. Apte and Y. Madhusudhanrao of Kakatia University, Warangal, for providing the facility for rheological studies.

References

Broadhead, J., Rouan, E.S.K., Rhodes, C.T., 1992. The spray-drying of pharmaceuticals. Drug Dev. Ind. Pharm. 18, 1169–1206.

Cornigan, O.I., 1995. Thermal analysis of spray dried products. Thermochim. Acta 248, 245–248.

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.

Ferreira, S.H., 1972. Prostaglandins. Aspirin-like drugs and analgesia. Nature New Biol. 240, 301–307.

Ganem-Quintanar, A., Quintanar-Guerrero, D., Buri, P., 2000. Monoolein: a review of the pharmaceutical applications. Drug Dev. Ind. Pharm. 26, 809–820.

International Conferences on Harmonization, Impurities, Guidelines for Residual Solvents, 1997. Q3C. Federal Register. 62, p. 67377.

Kim, J.S., Kim, H.K., Chung, H., Sohn, Y.Y., Kwon, I.C., Jeong, S.Y., 2000. Drug formulations that form a dispersed cubic phase when mixed with water. Proc. Int. Symp. Control. Rel. Bioact. Mater. 27, 1118–1119.

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

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.

Randall, L.D., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissues. Arch. Int. Pharmacodyn. Ther. 113, 223–249.

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.

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, M.H., Paradkar, A., 2005. Cubic liquid crystalline glyceryl monooleate matrices for oral delivery of enzyme. Int. J. Pharm. 294, 161–171.

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

Siekmann, B., Bunjes, H., Koch, M.H.J., Westesen, K., 2002. Preparation and structural investigation of colloidal dispersions prepared from cubic monoglyceride-water phase. Int. J. Pharm. 244, 33–43.

Spicer, P.T., Small, W.B., Lynch, M.L., Burns, J.L., 2002. Dry powder precursors of cubic liquid crystalline nanoparticles (cubosomes). J. Nanoparticle Res. 4, 297–311.

Szoka, F.C., Rolland, A., Wang, J., 1998. Dry powder formulations of polynucleotide complexes for inhalation delivery to the respiratory tract. US Patent 5,811,406, 22 September.

Vinegar, R., Schreiber, W., Hugo, R., 1969. Biphasic development of carrageenin oedema in rats. J. Pharmacol. Exp. Ther. 166 (1), 96–103.

Williams, T.J., Morley, J., 1973. Prostaglandin as potentiators of increased vascular permeability in inflammation. Nature 246, 215–217.

Williams, T.J., Peck, M.J., 1977. Roll of prostaglandin-mediated vasodilatation in inflammation. Nature 270, 530–532.

Winter, C.A., Risley, E.A., Nuss, C.W., 1962. Carrageenin-induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. Proc Soc. Exp. Biol. Med. 111, 544–547.