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Research Article

Exploring Microstructural Changes in Structural Analogues of Ibuprofen-Hosted *In Situ* Gelling System and Its Influence on Pharmaceutical Performance

Sharvil S. Patil,[1,2] Edakkal Venugopal,[2] Suresh Bhat,[2] Kakasaheb R. Mahadik,[1,4] and Anant R. Paradkar[3,4]

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ABSTRACT. The present work explores inner structuration of *in situ* gelling system consisting of glyceryl monooleate (GMO) and oleic acid (OA). The system under study involves investigation of microstructural changes which are believed to govern the pharmaceutical performance of final formulation. The changes which are often termed mesophasic transformation were analysed by small angle X-ray scattering (SAXS), differential scanning calorimetry (DSC), rheology and plane polarised light (PPL) microscopy. The current work revealed transformation of blank system from W/O emulsion to reverse hexagonal structure upon addition of structural analogues of ibuprofen. Such transformations are believed to occur due to increased hydrophobic volume within system as probed by SAXS analysis. The findings of SAXS studies were well supported by DSC, rheology and PPL microscopy. The study established inverse relationship between log *P* value of structural analogues of ibuprofen and the degree of binding of water molecules to surfactant chains. Such relationship had pronounced effect on sol–gel transformation process. The prepared *in situ* gelling system showed sustained drug release which followed Higuchi model.

KEY WORDS: flurbiprofen; hexagonal phase; ibuprofen; ketoprofen; liquid crystal; sustained drug release.

INTRODUCTION

Lyotropic liquid crystals (LLC) are of point of attraction for many researchers due to their wide range of applications in the area of drug delivery, material science, food technology, *etc.* They have ability to entrap various guest molecules with different polarity including biomolecules. Additionally, they show structural resemblance to human membranes having large surface area (1–3). Formation of different lyotropic liquid crystalline mesophases by amphiphilic or polar lipids upon contact with water has been reported in literature (4). Structural properties of lipids, water content and temperature of the system govern the type of liquid crystal (LC) phase or mesophase formed within system. Most commonly used techniques for mesophase identification include small angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR) and optical microscopy (5). Different LC phases like lamellar, hexagonal and transparent cubic phases have been reported by researchers in lipid/water systems including isotropic solutions containing micelles or vesicles (6–9). The geometrical packing properties of lipid in particular environment govern the formation of a particular phase (10).

One of the metabolites obtained after lipolysis of triglycerides is glycerol monooleate (GMO), a polar lipid which is commonly used as a food emulsifier (11). It forms wide variety of LC phases when mixed with water. GMO/water binary systems have been well explored which depict formation of reverse isotropic micellar solution (L_2), lamellar (La), inverted type hexagonal (H_{II}) and cubic (V_2) liquid crystalline phases (12–16). These LC phases have different physical properties, and hence, they are well explored as drug delivery systems. Moreover, these phases exhibit potential applications in food, pharmaceutical and cosmetic industry (17–24).

Considering the wide range of applications associated with GMO-based systems, *in situ* gelling system comprising of GMO, oleic acid and polyethylene glycol 400 (PEG 400) was prepared and characterised. It was expected that such system will transform into gel upon hydration. Further, it is believed that such sol to gel transformation may involve formation of different LC phases (mesophases) depending on the type of additive and water content. This process is often termed as mesophasic transformation (25).

Thus, the objective of the current work is to investigate and explore the microstructural properties of *in situ* gelling system involving sol to gel transformation and further, to probe the effect of additive on the process of mesophase transformation in terms of release characteristics (pharmaceutical performance) of the prepared formulations. The additives used in the present study are structural analogues of ibuprofen.

[1] Poona College of Pharmacy, Department of Pharmaceutics, Bharati Vidyapeeth Deemed University, Erandwane, Pune, Maharashtra 411 038, India.
[2] Polymer Science and Engineering Division, National Chemical Laboratory, Pune, 411 008, India.
[3] Centre for Pharmaceutical Engineering Science, University of Bradford, Bradford, West Yorkshire BD7 1DP, UK.
[4] To whom correspondence should be addressed. (e-mail: krmahadik@rediffmail.com; a.paradkar1@bradford.ac.uk)

MATERIALS AND METHODS

Materials

Ketoprofen, flurbiprofen and ibuprofen were generous gifts by Glenmark Pharmaceuticals, FDC Limited, Mumbai and Torrent Pharmaceuticals, Gujarat, respectively. GMO (Rylo MG 19 Pharma) was a gift by Danisco culture, Denmark. Oleic acid (OA) and PEG were purchased from Loba Chemie Pvt. Ltd, Mumbai and Merck Limited, Mumbai, India, respectively.

Methods

Preparation of Blank In Situ Gelling System (BGOP)

Initially, various ratios of GMO and OA were screened for preparation of *in situ* gelling system. PEG 400 was used as a channel forming agent. GMO/OA/PEG in 3:1:1 *v/v* ratio was found to be optimum for preparation of *in situ* gelling system. To describe in brief, GMO was melted at 40°C. Molten GMO and oleic acid (3:1 *v/v*) were taken in a vial (15 mL) and mixed together on a vortex mixer (Heidolph, India). Finally, PEG 400 was introduced into the system. GOP system thus obtained was mixed (vortex mixer) to obtain a homogeneous mixture.

Preparation of Drug-Loaded In Situ Gelling System

Ketoprofen (0.13 M) was dissolved in BGOP separately to obtain ketoprofen-loaded BGOP (KGOP). Similarly, ibuprofen (0.13 M)-loaded BGOP (IGOP) and flurbiprofen (0.13 M)-loaded BGOP (FGOP) were prepared. The prepared systems were subjected to further characterisation.

Hydration of Prepared In Situ Gelling Systems

The prepared *in situ* gelling systems (BGOP, IGOP, KGOP and FGOP) were hydrated separately at hydration levels between 5% and 35% *v/v* with deionised water and analysed after 24 h.

Characterisation

Determination of In Situ Gelling Ability

In situ gelling ability of prepared systems was estimated by diluting them with water. To describe in brief, fixed amount (100 µl) of *in situ* gelling system (BGOP, IGOP, KGOP and FGOP) was added to 100 mL distilled water separately and analysed for *in situ* gelling behaviour.

Estimation of Drug Content

Ketoprofen, ibuprofen and flurbiprofen from preweighed KGOP, IGOP and FGOP respectively, were determined by dissolving the systems in 50 mL methanol separately. The solutions thus obtained from KGOP, IGOP and FGOP were analysed spectrophotometrically (UV Spectrophotometer, Jasco V-530, Japan) at 259, 264 and 246 nm, respectively.

Rheological Investigation

Intermediate hydrated samples of BGOP, KGOP, IGOP and FGOP were subjected to rheological measurements. Rheological analysis was performed using a controlled stress rheometer (Viscotech Rheometer, Rheologica Instruments AB, Lund, Sweden). RheoLogic Basic software version 5.0 was used in order to analyse the data. Rheometer with cone (1.0°) and plate (25-mm diameter) geometry was used. Rheological measurements were carried out at 25±0.5°C (26). Intermediate hydrated samples of BGOP, KGOP, IGOP and FGOP were analysed for viscometry and creep recovery.

Viscometry. Varying shear stress in the range of 0.1–50 Pa was applied on intermediate hydrated samples (5–35% *v/v*) of BGOP, KGOP, IGOP and FGOP.

Creep recovery. The samples gelled upon controlled hydration were subjected to a constant stress of 1 Pa (as obtained from linear viscoelastic region) for 100 s followed by sample recovery for 200 s. The creep compliance, J (defined as ratio between measured strain and applied stress), was recorded against time (25).

Polarised Light Microscopy

The mesophase type in the hydrated samples was analysed using polarised light microscopy (27). Hydrated *in situ* gelling system was transferred to a glass tube (internal diameter 0.5 cm) and observed for birefringence (if any) under a microscope at 25±0.5°C with a $\lambda/4$ plate oriented at 45° to the polarizer axes under ×40 magnification (Nikon Eclipse E600, Nikon Instech Co., Japan).

SAXS Investigation

SAXS experiments were performed on a Bruker NANOSTAR with rotating Cu anode and pinhole geometry. A current of 100 mA was used for anode operation along with a potential difference of 45 kV. A quartz capillary of 2-mm diameter and 10-µm wall thickness was used for sample analysis. Background subtraction from an empty capillary was done after accounting for the sample absorption. Temperature was controlled during the whole analysis using Bruker Peltier heating cooling unit. HISTAR gas-filled multiwire detector was used for data collection. The scattering from intermediate hydrated samples of BGOP, KGOP, IGOP and FGOP was measured for long enough until the scattered intensity gave at least three million counts on the detector.

Differential Scanning Calorimetry (DSC)

Mettler Toledo 821e instrument equipped with an intracooler (Mettler Toledo, Switzerland) was used for analysing the prepared samples. Indium and zinc standards were used for calibration of instrument. A 10±3 mg of hydrated (15% *v/v*) BGOP, KGOP, IGOP and FGOP sample was placed in aluminium crucibles separately. The crucibles were cooled to −25°C at a rate of 10°C/min once sealed hermetically. Each sample was maintained at −25°C for 10 min and

subjected to heating from −25°C to 5°C at the scanning rate of 3°C/min.

In Vitro Drug Diffusion Studies

KGOP, IGOP and FGOP were evaluated for *in vitro* drug diffusion studies using dialysis bag technique. One millilitre of KGOP (equivalent to 30 mg ketoprofen) was placed in pretreated cellulose dialysis tubing (7 cm in length) after tying one of its ends with thread. Dialyzing medium (distilled water, 5 mL) was also added into the bag, and the other end of tubing was also secured with thread. The bag was placed in dissolution vessel of USP 24 type II dissolution test apparatus containing 900 mL dialyzing medium (distilled water) maintained at 37±0.5°C and stirred continuously at 100 rpm. Similar method was used for analysing placebo formulation (BGOP) so as to check interference, if any. Aliquots were collected at predetermined time intervals. Sink condition was maintained throughout the analysis. Spectophotometric analysis of aliquots was performed at 259 nm for determining ketoprofen content. Similar procedure was used for *in vitro* drug diffusion study of IGOP and FGOP. The aliquots were analysed spectrophotometrically at 264 and 246 nm, respectively. PCP Disso v 3i software was used for analysing the data.

RESULTS AND DISCUSSION

In the preliminary work, different ratios of GMO and oleic acid were screened for preparing *in situ* gelling system. Stiff gel was formed by the prepared systems upon hydration which showed negligible amount of drug release even after 7–8 h. Therefore, a channel forming hydrophilic additive, PEG 400, was introduced into the system which modulated the appearance of gel upon hydration and also the drug release characteristics of the systems. It was observed that BGOP transformed into gel at 30% *v/v* of water, whereas samples containing structural analogues of ibuprofen required 35% *v/v* of water for transformation into gel. To investigate this further, microstructural changes associated with *in situ* gelling process were monitored by plane polarised light microscopy, SAXS, DSC and rheology.

Plane Polarised Light Microscopy

The samples of intermediate hydration regimes were in the form of W/O emulsion initially which transformed into gel upon successive hydration. The gel thus formed showed birefringence with fan-like structures suggesting conversion of W/O emulsion to H_{II} phase (Fig. 1) (27). SAXS analysis of samples was performed in order to confirm the results of PPL microscopy.

Small Angle X-ray Scattering Investigation

There are reports stating utility of SAXS for investigation of microstructure of colloidal systems (28–31). The extent to which alterations in the inner structuration of drug-loaded *in situ* gelling system are induced when compared to BGOP was investigated by using SAXS. Samples from intermediate hydration regimes were selected for the same purpose. Figure 2 depicts the SAXS patterns of prepared samples. The samples from intermediate hydration regimes before transformation into gel showed broad peak which became sharp upon complete transformation of the sample into gel after hydration. The sharp peak observed for the gel indicated formation of reverse hexagonal phase (32). The results supported our readings of plane polarised light microscopy and confirmed a mesophasic transformation that has taken place in the prepared samples.

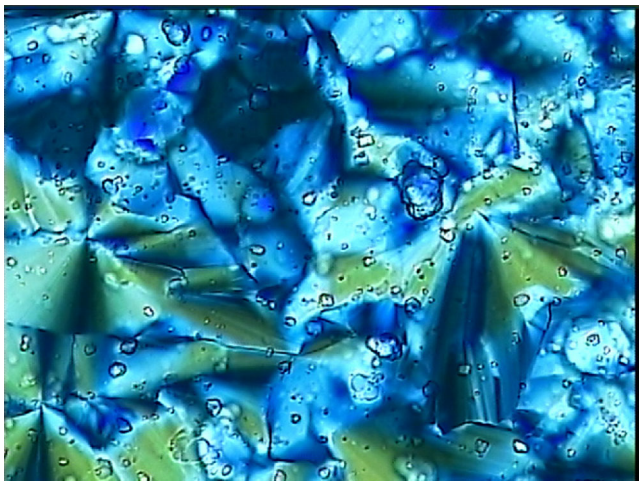

Fig. 1. Polarised optical microscope image of reverse hexagonal phase

The formation of reverse hexagonal phase upon addition of oleic acid to GMO has been well reported (33,34). Presence of oleic acid in the system increases apparent hydrophobic volume of the lipid which ultimately increases the packing parameter. Log *P* value gives an idea about the polarity of molecule, the higher log *P* value more will be the nonpolar nature of molecule. In the present work, structural analogues used are ketoprofen, ibuprofen and flurbiprofen having log *P* values 0.97, 3.6 and 4.24, respectively (35,36). Thus, addition of hydrophobic structural analogues of ibuprofen might have caused further increase in hydrophobic volume of lipid leading to transformation of W/O emulsion to reverse hexagonal phase. This assumption was further supported by the amount of water required for such sol–gel transformation, *i.e.* 35% *v/v* water for samples containing structural analogues of ibuprofen and 30% *v/v* of water for BGOP. The dehydration of surfactant chains induced by the hydrophobic structural analogues of ibuprofen might have contributed to requirement of higher water for sol–gel transformation. These findings of SAXS were further supported by DSC and rheology.

Differential Scanning Calorimetry

The thermodynamic property of water associated with *in situ* gelling process may be altered by microstructural changes that take place during transformation of the system into gel. These changes can be readily analysed by using subzero temperature differential scanning calorimetry. Nature of water associated with *in situ* gelling process can be obtained from the position of endothermic peaks observed in DSC thermogram. Depending on the peak position, water can be bound (which is associated to hydrophilic groups and melts below −10°C), interphasal water (defined as water confined within the interface of dispersed system, which melts at about −10°C) or free water which melts at ~0°C (37,38). Figure 3 shows

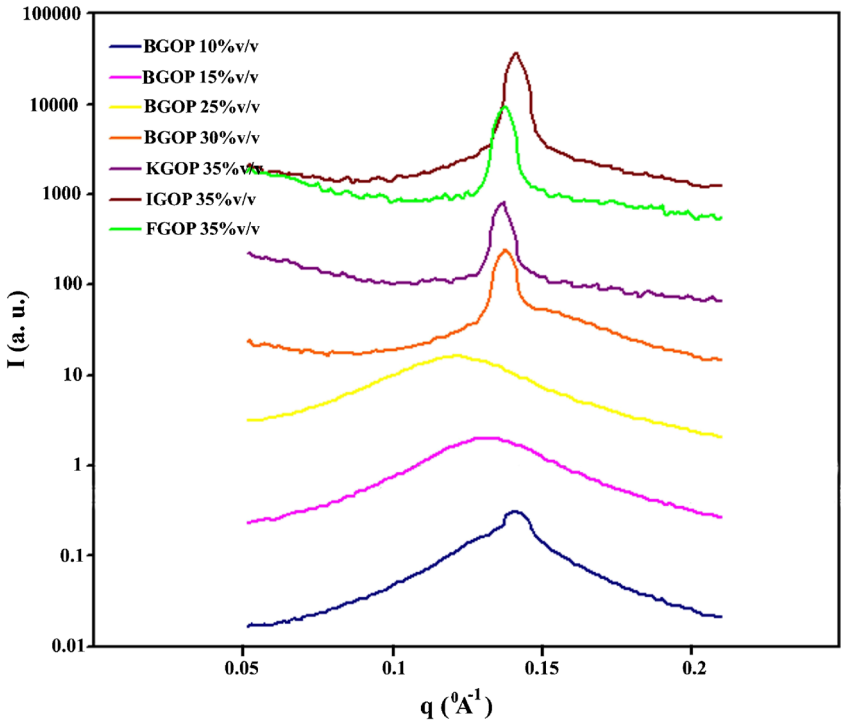

Fig. 2. Representative SAXS patterns of prepared samples at different hydration levels

DSC thermogram of intermediate hydrated sample (15% *v/v*) of BGOP, KGOP, IGOP and FGOP. All the endothermic peaks were in range of −4°C to −2°C indicating water present in the system was interphasal water. However, BGOP and KGOP showed extra endothermic peak at about 0°C corresponding to free water. The endothermic events observed in DSC study have been listed in Table I.

The fusion temperatures and enthalpies of interphasal water within selected intermediate hydration were used to determine the degree of binding strength of water with the surfactant and drug molecules. BGOP and KGOP showed endothermic peak at −2.37°C and −2.73°C with enthalpies −4.54 and −3.56 Jg^{-1}, respectively. Thus, there was a significant rise in enthalpy (around 21%) when ketoprofen was introduced in the system indicating that the thermodynamic properties of water associated with GMO and PEG chains at the interface were significantly altered which could be attributed to dehydration induced by hydrophobic ketoprofen.

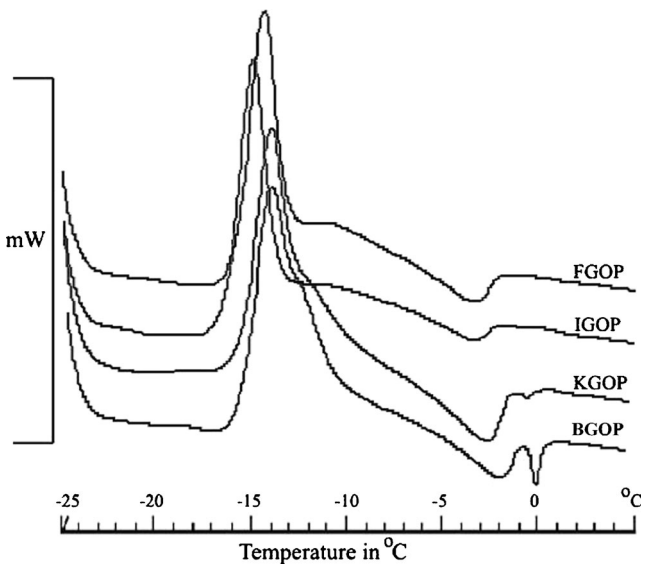

Fig. 3. DSC thermogram of intermediate hydrated sample (15% *v/v*) of BGOP, IGOP, KGOP and FGOP

The comparison of thermodynamic events of IGOP and FGOP with BGOP indicated 58.37% and 59.47% rise in enthalpy, respectively. Thus, the order of decrease in enthalpy can be represented as FGOP > IGOP > KGOP. Flurbiprofen having highest log *P* amongst other drugs used in the study might have disrupted the structure of water associated with GMO and PEG chains to a larger extent due to its stronger hydrophobic nature as compared to KGOP and IGOP. Moreover, in addition to interphasal water, BGOP and KGOP showed endothermic peak at about 0°C indicating presence of free water in the selected intermediate regime. The existence of free water suggests complete hydration of GMO and PEG chains owing to smallest log *P* value of ketoprofen amongst the structural analogues of ibuprofen. However, IGOP and FGOP did not show peak for free water suggesting that the systems had the ability to incorporate more water into themselves before their transformation into gel. Thus to summarise, thermodynamic properties of intermediate hydrations of BGOP, KGOP, IGOP and FGOP were investigated using DSC. Hydrophobic nature of structural analogues of ibuprofen delayed gel formation process when the systems were subjected to controlled hydration. Microstructure of the system was further investigated by shear rheology.

Rheological Investigation

In the present work, we have analysed rheological properties of intermediate hydrated regimes using viscometry and

Table I. Thermal Behaviour of Systems at 15% *v/v* Dilution with Water

		Endothermic peak temperature (°C)		Enthalpy (J g^{-1})	
Sr. no.	Sample	Interphasal water	Free water	Interphasal water	Free water
1	BGOP	−2.37	0	−4.54	−1.38
2	KGOP	−2.73	0.016	−3.56	−0.13
3	IGOP	−3.2	–	−1.89	–
4	FGOP	−3.16	–	−1.84	–

creep recovery studies. The linearity shown by the plot of shearing stress *versus* shear rate confirmed that all the samples screened using viscometry studies possessed Newtonian flow (not shown). Figure 4 shows plot of viscosity *versus* hydration (% *v/v*) for intermediate regimes of BGOP, KGOP, IGOP and FGOP.

The reports state that progressive hydration of surfactant headgroups enhances their mobility which leads to progressive loosening-up of the headgroup packing as the process is entropy driven (39). Such increment in mobility of surfactants might have caused loosening of the boundaries by the adjacent fluid lamellae thus interfering with each other ultimately raising the viscosity of the system (40). Moreover, such increase in viscosity confirmed sol to gel transformation within the system with controlled hydration.

The rheological screening of the samples showed highest viscosity for BGOP when compared to drug-loaded samples. The order in which reduction in viscosity was observed can be represented by BGOP > KGOP > IGOP > FGOP. The highest viscosity associated with BGOP may be attributed to unhindered hydration of surfactant head groups as compared to drug-loaded samples. The addition of hydrophobic structural analogues of ibuprofen reduced viscosity of the samples. Amongst KGOP, IGOP and FGOP, lowest viscosity associated with FGOP was attributed to stronger hydrophobic nature of flurbiprofen owing to its highest log *P* value which affected the degree of binding of water molecules to GMO and PEG chains, whereas ketoprofen being less nonpolar showed highest viscosity suggesting its lower hindrance towards hydration of GMO and PEG chains.

Creep recovery studies were used to elucidate the elastic component present within samples upon their transformation into gel (41). Percent creep recovery was calculated by using the formula given below:

$$\delta J = \left\{ \frac{J(100s) - J(300s)}{J(100s)} \right\} \times 100$$

The percent creep value is directly proportional to elastic component present within system. Figure 5 shows creep recovery curves for all the prepared samples. Table II shows percent creep recovery data for the prepared gels. It was observed that addition of ibuprofen analogues to BGOP prolonged sol–gel transformation. BGOP transformed into gel upon addition of 30% *v/v* water, whereas samples containing structural analogues of ibuprofen required 35% *v/v* water for transformation into gel. Hydrophobic structural analogues of ibuprofen might have hindered the process of hydrogen bonding of water molecules to surfactant chains thus delaying

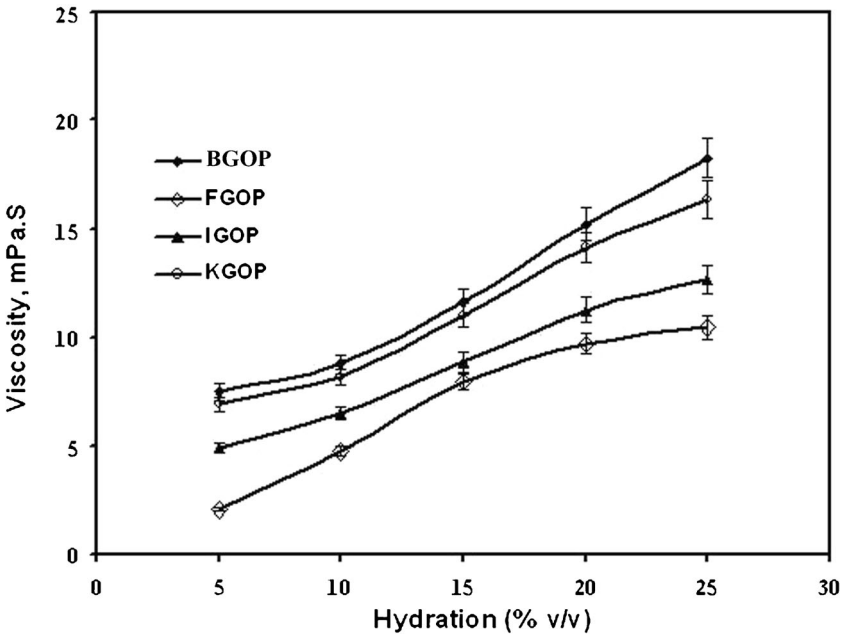

Fig. 4. Plot of viscosity *versus* hydration (% *v/v*) for intermediate regimes of BGOP, KGOP, IGOP and FGOP (*n*=3)

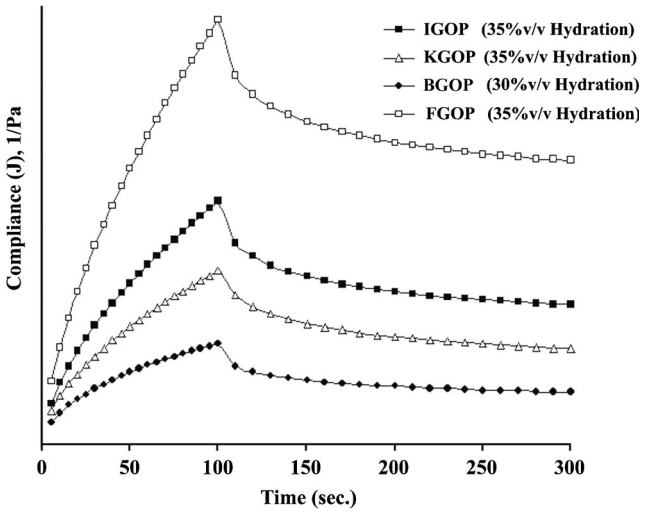

Fig. 5. Representative graph of creep recovery test for the prepared *in situ* gels of BGOP, KGOP, IGOP and FGOP

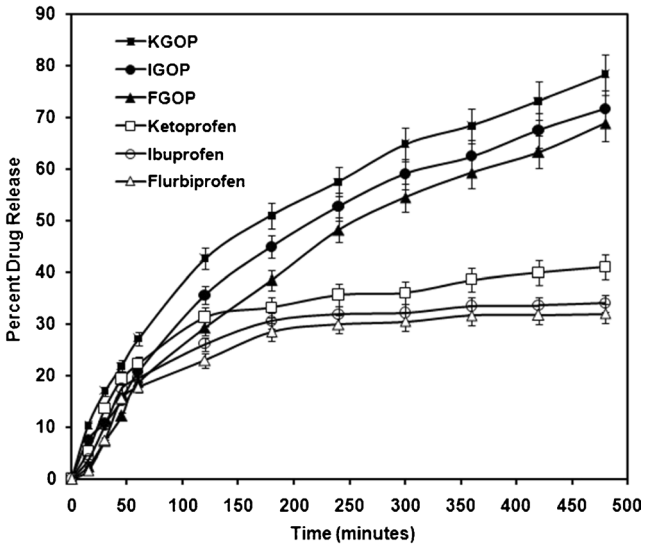

Fig. 6. *In vitro* drug release profiles of IGOP, KGOP, FGOP, ibuprofen, ketoprofen and flurbiprofen alone (n=3)

gel formation process. The creep of KGOP was lowest (17.01 ±2.93%), whereas that of FGOP was highest (53.59±3.56%) amongst the drug-loaded samples. The reason for such difference may be attributed to the difference in log *P* values of structural analogues of ibuprofen since log *P* value depicts nonpolar nature of the molecule. Thus, sample containing ketoprofen showed lower creep value, whereas sample containing flurbiprofen had high creep value. This may be attributed to the hindrance in the hydrogen bonding process between water molecules and surfactant chains which governs their flexibility. Higher log *P* value in case of flurbiprofen showed larger hindrance to hydrogen bonding process between water and surfactant chains thus keeping their flexibility intact showing highest creep value. Thus, the results of rheological studies were in agreement with DSC studies.

In Vitro Drug Release Study

The drug release from lyotropic liquid crystals has been shown to be governed by the type of mesophase formed upon hydration of the system (42). The present work highlights effect of additive (drug) on inner structuration of *in situ* gelling system which in turn governs the characteristics of the gel formed ultimately influencing drug release kinetics. Thus, *in vitro* drug release study can be used as tool to assess performance of the prepared gel formulations. Drug release study of samples containing ketoprofen, ibuprofen and flurbiprofen was performed in distilled water where the solubility for these drugs was limiting parameter (Fig. 6). All the samples containing structural analogues of ibuprofen showed sustained drug release following Higuchi model. Thus, the drug release was linear function of square root of time which may be due to encapsulation of drug within the hexagonal structure of gel matrix (25). KGOP showed highest percent drug release, whereas FGOP had lowest value for percent drug release amongst all the prepared samples. An inverse relationship between log *P* of drug and percent drug release was observed. Moreover, the samples containing structural analogues of ibuprofen showed improvement in drug release when compared to ketoprofen, ibuprofen and flurbiprofen alone which may be attributed to the presence of hydrophilic PEG molecules and the structure of reverse hexagonal phase wherein the aqueous compartments consists of closed extended micellar columnar structures, and the lipophilic drugs are located within the lipid domain.

Table II. Percent Creep Recovery for All the Samples After Transformation into Gel

Sample	Hydration (% *v/v*)	Percent creep recovery
BGOP	30	18.19±1.907
KGOP	35	17.01±2.93
IGOP	35	42.26±3.03
FGOP	35	53.59±3.56

Mean±SD, n=3

CONCLUSION

In the current work, microstructural properties of *in situ* gelling system involving mesophasic transformations have been investigated. The study indicated that addition of hydrophobic structural analogues of ibuprofen to plain *in situ* gelling system increased critical packing parameter and transformed W/O emulsion to reverse hexagonal phase. The study established inverse relationship between log *P* value of structural analogues of ibuprofen and the degree of binding of water molecules to surfactant chains. Such relationship had a pronounced effect on sol–gel transformation process. The hexagonal phase formed by the systems containing structural analogues of ibuprofen showed sustained drug release kinetics following Higuchi model.

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Conflict of Interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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