molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

Probing Influence of Mesophasic Transformation on Performance of Self-Emulsifying System: Effect of Ion

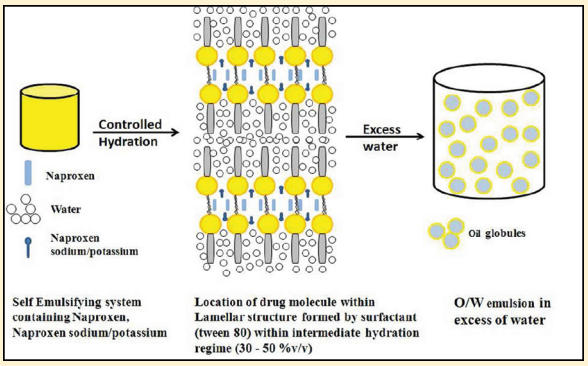
Sharvil S. Patil,[†,‡] Edakkal Venugopal,[‡] Suresh Bhat,[‡] Kakasaheb R. Mahadik,*[,†] and Anant R. Paradkar*[,§]

[†]Department of Pharmaceutics, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune-411 038, Maharashtra, India

[§]Centre for Pharmaceutical Engineering Science, University of Bradford, Bradford BD7 1DP, U.K.

[‡]Polymer Science and Engineering Division, National Chemical Laboratory, Pune-411 008, India

ABSTRACT: Self-emulsifying systems are mixtures of oils and surfactants, ideally isotropic, sometimes including cosolvents, which emulsify under conditions of gentle agitation, similar to those which would be encountered in the gastrointestinal tract. The process of self-emulsification has remained the center of attraction for most researchers. Controlled hydration of self-emulsifying systems shows formation of an intermediate gel phase which upon rupture forms an emulsion. Current work was undertaken to understand and explore the microstructural properties of intermediate gel phase which are believed to influence the performance (droplet size) of the final formulation. The effect of additives on microstructural properties of intermediate gel phase has also been investigated. Microstructural elucidation of hydrated samples of intermediate regimes was done by using techniques such as small angle X-ray scattering, differential scanning calorimetry and rheology. Samples from intermediate regimes showed formation of local lamellar structure which swelled with hydration. In the present work, the effect of addition of salt form of naproxen (sodium and potassium) and naproxen (base) on microstructural properties of intermediate regimes was investigated. Systems containing naproxen salts formed larger droplets whereas naproxen base formed smaller ones. Microstructural properties of intermediate lamellar structures were well correlated with performance of the final formulation. The current studies indicate that by controlling the properties of intermediate regimes optimized formulations with desired performance can be tailor-made.



KEYWORDS: *self-emulsifying system, naproxen, microstructure, lamellar structure, liquid crystal, emulsion*

INTRODUCTION

Among the several active pharmaceutical ingredients discovered in the current era, 40% suffer from a drawback of poor water solubility.[1] To overcome this barrier one of the recently used alternatives includes lipid based formulations with special emphasis on self-emulsifying drug delivery systems (SEDDS).[2] SEDDS are mixtures of oils and surfactants, ideally isotropic, and sometimes containing cosolvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation.[3] Thus, for lipophilic drugs that exhibit dissolution rate limited absorption, these systems may offer an improvement in the rate and extent of absorption (i.e., bioavailability). Moreover, these systems present potential advantages which include possible reduction in dose, more consistent temporal profiles of drug absorption, selective targeting of drug toward specific absorption window in GI tract, and protection of drug from the hostile environment in GI lumen.[4] The process of self-emulsification leading to formation of an emulsion has remained a point of attraction for most researchers.

Many researchers have made attempts to describe the mechanism governing the process of self-emulsification.[5,6] It has been reported that self-emulsification occurs when the entropy change that favors dispersion is greater than the energy required to increase the surface area of the dispersion.[7] The inner structuration of the microemulsion has been widely studied, and the most common structures formed during microemulsion formation are water in oil (W/O), bicontinuous structures or LC (liquid crystal) phases and finally oil in water (O/W) emulsion. Considering the fact that SES also generally leads to formation of microemulsion, one of the mechanisms claimed by researchers to describe the process of self-emulsification involves formation of a liquid crystal (LC) phase at the interface which ruptures upon successful penetration by water. Thus it is obvious that the rate of emulsification will be governed by the rate and extent of water penetration into the intermediate gel phase (involving LC phase) formed during the self-emulsification process.[8−12] In other words, "LC phase formation and its rupture" can be

Received: October 24, 2011
Revised: December 27, 2011
Accepted: January 4, 2012
Published: January 4, 2012

regarded as the droplet size (and in turn performance of the system) controlling step identical to the "rate" controlling step in progress of a specific reaction. Our group has studied viscoelastic properties of the intermediate LC phase formed during the process of self-emulsification.[13] It was observed that intermediate hydration of SES systems which have a higher viscous component present smaller droplets and vice versa. The objective of the current work is to understand and explore the microstructural properties of hydrated intermediate regimes (involving intermediate gel phase) of SES consisting of nonionic surfactants and further to investigate the effect of additive on the microstructural properties of hydrated intermediate regimes affecting the performance of the final formulation.

In the present work the inner structuration of SES composed of pharmaceutically permitted ingredients such as Captex 355 (oil), Tween 80 (surfactant) and Capmul MCM (CMCM, cosurfactant) has been studied in detail. Moreover the influence of addition of different salt forms of the same drug on intermediate hydrated regimes and its effect on performance of the final emulsion formed have been investigated. Thus systems containing naproxen (base form), naproxen sodium and naproxen potassium (3% w/v) were prepared, and the effect of ion on mesophasic transformation was studied. The microsctructural evaluation of intermediate hydrated regimes of prepared SES was performed using tools such as plane polarized light microscopy, small angle X-ray scattering (SAXS), rheology and differential scanning calorimetry (DSC). The formed emulsions were analyzed for droplet size and *in vitro* drug release study. The current work has been planned to find out possible correlation between droplet size, SAXS, DSC and rheology since such work will help us to understand the influence of mesophasic transformations on the pharmaceutical performance of the system.

EXPERIMENTAL SECTION

Materials. Captex 355 (caprylic/capric triglyceride) and Capmul MCM (CMCM, glyceryl mono- and dicaprate) were generous gift samples from Abitec Corporation, USA. Tween 80 (polyoxyethylene 20 sorbitan monooloeate) was purchased from Merck Chemicals, Mumbai, India. Naproxen and naproxen sodium were obtained as a generous gift from Lupin Research Park, Pune. Naproxen potassium was synthesized by dissolving equimolar amounts of naproxen and potassium hydroxide in ethanol under gentle heating (40−45 °C). The clear solution thus obtained was filtered through a Whatman filter paper (no. 41), and ethanol was evaporated to obtain a white powder. Formation of salt was confirmed by solubility, FTIR and DSC studies (data not shown).

Methods. *Formulation of Plain SES (PSES).* PSES was formulated after screening different ratios of Captex 355 and Tween 80 in the range of 0−100% w/w. The optimized formula for PSES consisted of 40% w/w Captex 355 and 60% w/w of surfactant (consisting of 40% w/w Tween 80 and 20% w/w CMCM).

Formulation of Drug Loaded SES. Naproxen sodium, naproxen potassium and naproxen (3% w/v, 0.12−0.13 M) were dissolved in PSES separately to obtain NSSES, NKSES and NSES respectively. The prepared SES was subjected to further characterization.

Hydration of SES. The prepared SES were hydrated at hydration levels in the range of 10−70% v/v with deionized water and stored for 24 h before analysis.

Characterization. *Emulsifying Properties.* A fixed amount (40 μL) of SES was added to 100 mL of distilled water at 25 ± 0.5 °C, and performance of spontaneous emulsification was observed visually and analyzed according to droplet formation pattern.[13] This test was performed in triplicate.

Drug Content. Naproxen and naproxen salts (sodium and potassium) from preweighed SEDDS were extracted by dissolving in 25 mL of methanol and water respectively. Drug content in the extract was analyzed spectrophotometrically at 271 nm in triplicate.

Droplet Size Measurement. Droplet size of all the prepared SES was measured by BIC 90 Plus particle size analyzer (Brookhaven Instruments Corporation, USA). For measurement, SES was prediluted by addition of 0.5 mL of SES to 100 mL of distilled water under slow agitation at room temperature (25 ± 0.5 °C). Dynamic light scattering from the sample was used to determine hydrodynamic radius. NSES, NSSES and NKSES were analyzed using a similar procedure. Analysis was done in triplicate.

Rheological Studies. Rheological measurements of hydrated SES were performed using a controlled stress rheometer (Viscotech Rheometer, Rheologica Instruments AB, Lund, Sweden). Data analysis was done with Stress RheoLogic Basic software, version 5.0. A cone and plate geometry of 25 mm diameter and cone of 1.0° was employed. Viscometry test was performed for intermediate hydrated samples (30−50% v/v) of PSES, NSES, NSSES and NKSES with shear stress varying in the range of 0.1−100 Pa at 25 ± 0.5 °C.

Polarized Light Microscopy. Plane polarized light microscopy of hydrated SES was performed for identifying the type of mesophase formed in the prepared sample.[14] Hydrated SES was transferred to a specially fabricated glass tube (internal diameter 0.5 cm) and then viewed for the presence or absence of birefringence under a polarizing microscope at 25 ± 0.5 °C with λ 1/4 compensator under 40× magnification (Nikkon Eclipse E 600, Nikon Instech Co., Japan).

Small Angle X-ray Scattering (SAXS) Studies. SAXS experiments were done on a Bruker Nanostar with rotating type anode and pinhole geometry. The instrument uses a copper Kα radiation of wavelength 1.54 Å with a current of 100 mA and a potential difference of 45 kV having a sample to detector distance of 105 cm. The samples were taken in a quartz capillary of 2 mm thickness and 10 μm wall thickness. Empty capillary and air were taken as background with glassy carbon as reference. A Peltier heating cooling unit associated with the SAXS instrument was used for temperature control in the system. The data was collected on a HISTAR gas detector and was azimuthally averaged to get a 1D curve. The scattering from intermediate hydrated samples of PSES, NSES, NSSES and NKSES was measured for sufficient amount of time for at least 3 million counts. The data was analyzed using SASfit software developed by the Paul Sherrer Institute, Switzerland, for fitting of the Teubner−Strey (T−S) equation,[15]

$$I(q) = \frac{1}{a + bq^2 + cq^4} \quad (1)$$

where a, b and c are fitting parameters. The solid curves in Figure 1 are least-squares fits of the above equation through the data with a, b, and c as adjustable constants. The fitting constants can be used to determine the periodic domain spacing (d) and the correlation length (ξ) by

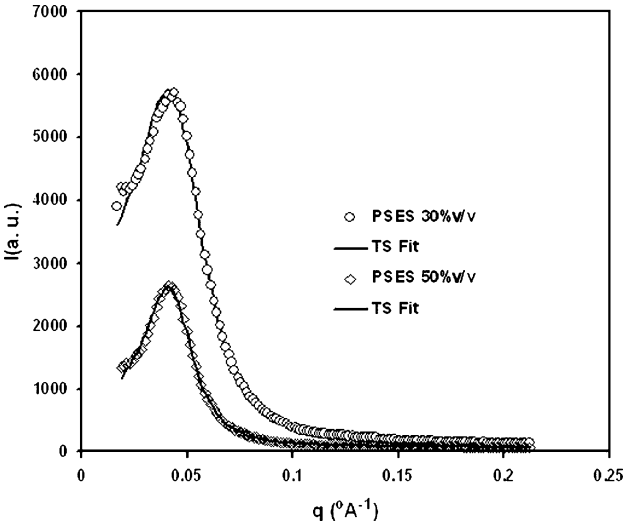


Figure 1. Representative plot of Teubner–Strey fit for PSES sample.

$$d = 2\pi\left[\frac{1}{2}\left(\frac{a}{c}\right)^{1/2} - \frac{1}{4}\left(\frac{b}{c}\right)\right]^{-1/2} \quad (2)$$

$$\xi = \left[\frac{1}{2}\left(\frac{a}{c}\right)^{1/2} + \frac{1}{4}\left(\frac{b}{c}\right)\right]^{-1/2} \quad (3)$$

Differential Scanning Calorimetry (DSC). Calorimetric measurements were performed with a Mettler Toledo 821e instrument equipped with an intracooler (Mettler Toledo, Switzerland). 10 ± 3 mg of 45% v/v hydrated PSES, NSES, NSSES and NKSES samples were placed in closed aluminum crucibles and cooled to −30 °C at the rate of 10 °C/min. They were maintained at −30 °C for 10 min and subjected to heating from −30 to 10 °C at the scanning rate of 3 °C/min. To ensure accuracy of caloric data the instrument was calibrated with indium/zinc.

In Vitro Drug Diffusion Studies. *In vitro* diffusion studies were carried out for NSES and naproxen salts (NSSES and NKSES) using the dialysis technique in triplicate. One end of pretreated cellulose dialysis tubing (7 cm in length) was tied with thread, and 1 mL of self-emulsifying formulation (equivalent to 30 mg of naproxen) was filled in it along with 5 mL of dialyzing medium (distilled water). The other end of the tubing was also secured with thread and was allowed to rotate freely in the dissolution vessel of a USP 24 type II dissolution test apparatus containing 900 mL of dialyzing medium (distilled water) maintained at 37 ± 0.5 °C with stirring at 100 rpm. Placebo formulation (PSES) was also tested likewise simultaneously under identical conditions so as to check interference, if any. Aliquots were collected periodically and replaced with fresh dialyzing medium. Aliquots, after filtration through Whatman filter paper (no. 41), were analyzed spectrophotometrically at 271 nm for naproxen content. A similar procedure was followed for *in vitro* drug diffusion study of NSSES and NKSES. The data was analyzed using PCP Disso v 3.0 software.

RESULTS AND DISCUSSION

Medium chain triglycerides (MCT) are most commonly used as oil in self-emulsifying systems.[4] Captex 355, being MCT, hence was selected as the model oil in this study. Tween 80 was selected as a surfactant for preparing SES. Various ratios of Captex 355:Tween 80 showed formation of a stiff gel when diluted with water. The gel was so stiff that it did not disperse in the water even after shaking. These observations indicated the necessity of cosurfactant in the formulation, and hence CMCM was introduced into the formulation. The ratio of oil:surfactant:cosurfactant was optimized on the basis of tendency of spontaneous emulsification and appearance of formed emulsion. "Good" emulsification was noted when droplets were formed spontaneously in water resulting in a transparent emulsion while it was noted as "moderate" for spontaneous emulsification with a milky appearance. Thus optimized model SES used in the present study consisted of 40% w/w of Captex 355 and 60% w/w of surfactant mixture consisting of 40% w/w of Tween 80 and 20% w/w of CMCM.

Droplet Size Analysis. The prepared emulsions were analyzed for droplet size distribution (Figure 2). The droplet

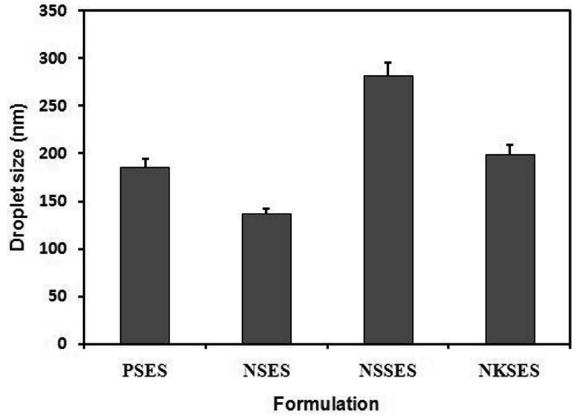


Figure 2. Droplet size analysis of various SES formulations (n = 3).

size of PSES (185.3 ± 5.1 nm) was found to be significantly altered after incorporation of drug since $P < 0.01$ as per one way ANOVA followed by Dunnett multiple comparison test. Droplet size of NSES (135.3 ± 4.6 nm) was found to be reduced whereas that of NSSES (284.7 ± 6.6 nm, $P < 0.01$) and NKSES (198.4 ± 5.2 nm, $P < 0.05$) was increased when compared to PSES. It was thought that such a significant change in droplet size of the prepared emulsion may be due to alteration of inner structuration of PSES by the guest molecule (drug). To investigate this further, all the prepared systems were subjected to controlled hydration, and the intermediate hydrated regime (30–50% v/v) showed formation of gel phase, leading to a drastic increase in viscosity, which then transformed into an emulsion upon subsequent increment in water. Thus microstructural properties of the intermediate gel phase were studied by plane polarized microscopy, SAXS, rheology and DSC to understand its influence on droplet size of final formulation. The hydrated intermediate regimes were screened for birefringence using a plane polarized light microscope, but all the samples showed optical isotropy i.e. absence of birefringence.[14]

Small Angle X-ray Scattering. The microstructure of the colloidal systems can be investigated by using SAXS.[16–18] Thus samples in the hydration range 30–50% v/v were screened by SAXS to study the extent to which the fundamental microstructural features of the intermediate gel phase of drug loaded samples (NSES, NSSES and NKSES) may differ from

dx.doi.org/10.1021/mp200541r | *Mol. Pharmaceutics* 2012, 9, 318–324

those of hydrated PSES systems. The SAXS diffraction curves obtained for all the hydrations were fitted with the Teubner–Strey equation to obtain correlation length (persistence length) ξ and periodic domain spacing $d = (2\pi/k)$, which is a measure of the quasi-periodic polar–nonpolar repeat distance.[15,19,20] Table 1 lists the values for ξ, d and the product $k\xi$ (k obtained from d), which is well above 2. The plot of $1/d$ versus hydration (% v/v) was found to be linear (Figure 3).

Table 1. Droplet Size, Periodicity (d), Persistence Length (ξ) and the Product $k\xi$ Calculated from SAXS Measurements

sample	droplet size of emulsion (nm), mean ± SD	hydration (% v/v)	d (Å)	ξ (Å)	$k\xi$
PSES	185.3 ± 5.1	30	137	49.51	2.27
		35	137.46	63.63	2.9
		40	140.61	70.56	3.15
		45	143	72.79	3.2
		50	144.58	72.79	3.16
NSSES	284.7 ± 6.6**[a]	30	111.89	58.76	3.30
		35	112.08	65.50	3.67
		40	113.98	71.51	3.94
		45	114.75	75.052	4.11
		50	115.23	75.051	4.10
NSES	135.3 ± 4.6**	30	143.65	56.41	2.47
		35	149.18	59.47	2.50
		40	152.12	60.89	2.51
		45	160.20	65.48	2.57
		50	165.86	65.14	2.47
NKSES	198.4 ± 5.2*	30	117.96	47.77	2.54
		35	114.19	60.82	3.35
		40	113.92	68.59	3.78
		45	114.61	70.20	3.85
		50	114.94	71.34	3.90

[a]**P < 0.01, *P < 0.05 for Dunnett multiple comparison test.

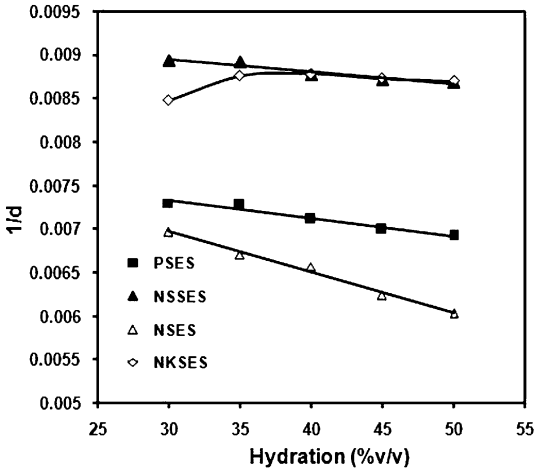


Figure 3. Plot of $1/d$ versus the volume fraction of water, ϕ_w, of intermediate hydrations of PSES, NSES, NKSES and NSSES.

Figure 4 shows the plot of correlation length or persistence length (ξ) versus hydration (% v/v) for all the prepared systems. The increased hydration showed increase in persistence length in the case of all the samples which remained constant after 40% v/v hydration. It can be observed that the persistence length was highest for NSSES whereas it was lowest for NSES. The order of decrease in persistence length observed can be represented as NSSES > NKSES > PSES > NSES, indicating decrease in stiffness of the surfactant/cosurfactant film in a similar order. Similarly decreasing order of d value (interdomain periodicity) can be represented by NSES > PSES > NSSES = NKSES (Table 1).

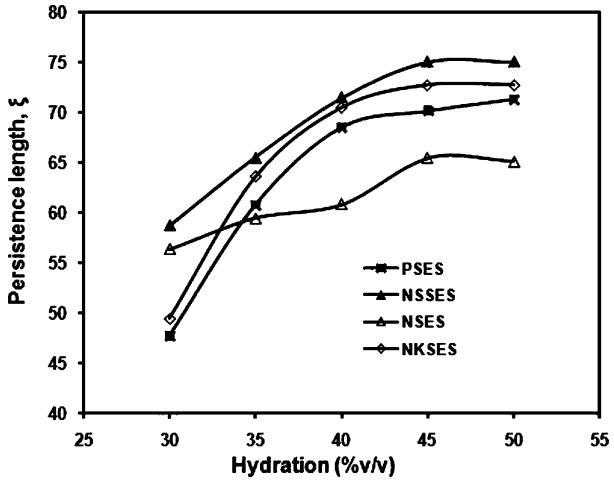


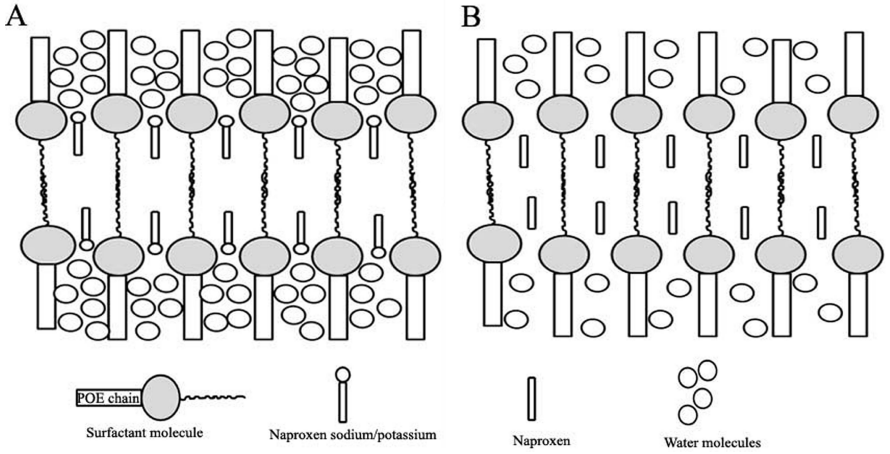
Figure 4. Plot of correlation length or persistence length (ξ) versus hydration (% v/v) of intermediate hydrations of PSES, NSES, NKSES and NSSES.

The plot of $1/d$ versus hydration (% v/v) often indicates dimensionality of swelling of intermediate microstructures. The linearity shown by the plot indicates one-dimensional swelling of the intermediate microstructures. Moreover, it supports the existence of "local" lamellar structures. Such structures are small stacks of layers randomly oriented in space so as to result in overall optical isotropy, but with local uniaxial behavior. Thus in such a case scattered fragments of lamellae are insufficient to cause solution birefringence.[21,22] Bicontinuous structures may also show one-dimensional swelling, but it has been well recorded that for bicontinuous microstructures the product $k\xi$ falls in the narrow range between 1.5 and 2.0. All the samples from intermediate regime screened in current work show values of the product $k\xi$ well above 2.0, so it is unlikely that any bicontinuous structures are formed.[22]

Scheme 1 is a schematic representation of lamellar structure with probable location of the drug molecule depending on its polarity. Hydrophilic drug (naproxen sodium and potassium) will be located near the headgroups of the surfactant (at the interface) whereas hydrophobic drug will be positioned in the core of the lamellae. NSSES and NKSES showed lower d value when compared to PSES. According to the Hofmeister series the ions can be classified as salting out ions and salting in ions. Salting out ions, also called kosmotropic ions or water structure makers, are usually small and have relatively small polarizability, have high electric fields at short distances and lose their water of hydration with great difficulty. Salting in ions, also called chaotropic ions or water structure breakers, have the opposite characteristics. Naproxen sodium solute is kosmotropic while the behavior of potassium ions is chaotropic.[23−26] The lower interdomain periodicity in the case of salt forms may be attributed to hydration of the hydrophilic polyoxyethelene (POE) chains of Tween 80 and their protrusion into the polar domain (water). In the case of NSES the water repellent nature of naproxen might have prevented hydration of POE chains of the surfactant thus showing higher d values. The stiffness of the

dx.doi.org/10.1021/mp200541r | Mol. Pharmaceutics 2012, 9, 318−324

Scheme 1. Schematic Representation of Location of Drug Molecule Depending on Its Nature within Lamellar Structure



surfactant/cosurfactant film can be better explained by using the persistence length, as the shorter the persistence length, the softer the film. Highest persistence length associated with NSSES may be attributed to the salting out effect of sodium ions leading to hydrogen bond formation between oxygen atoms of POE groups and water. In the case of potassium ions the chaotropic effect might have disrupted the hydrogen bonds between oxygen atoms of POE groups and water thus lowering the stiffness of the interface when compared to NSSES.[27] Owing to the hydrophobic nature of naproxen present in NSES, such disruption of hydrogen bonds associated with POE groups was highest and thus NSES showed lowest persistence length indicating formation of softer film. Systems with softer film might have presented lower resistance toward bending of surfactant film thus forming smaller droplets whereas the reverse was the case for systems with stiffer surfactant film which might have shown high resistance toward bending and hence formed larger droplets during the process of self-emulsification. To support our assumption we also performed differential scanning calorimetric study on intermediate hydrated sample (45% v/v) of PSES, NSES, NKSES and NSES.

Differential Scanning Calorimetry. Microstructural changes that are taking place during the process of self-emulsification alter the thermodynamic properties of water, which can be studied by using subzero temperature differential scanning calorimetry. Depending on the position of endothermic peaks in the DSC thermogram, water can be bound (which is associated with 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.[28−31] Figure 5 shows DSC thermograms of intermediate hydrated samples (45% v/v) of PSES, NSSES, NKSES and NSES. All the endothermic peaks were in the range of −4 to −6 °C, indicating the water present in the system was interphasal water. However, NSES showed an extra endothermic peak at about 0 °C corresponding to free water. The endothermic events observed in the DSC study have been listed in Table 2. Additionally, all the prepared samples showed exothermic peak at about −10 to −20 °C which may be attributed to the crystallization of surfactant chains at lower temperatures.

The degree of binding strength of water with the surfactant and drug molecules was evaluated by comparing the fusion temperatures and enthalpies of water within selected intermediate hydration. PSES and NSSES showed endothermic

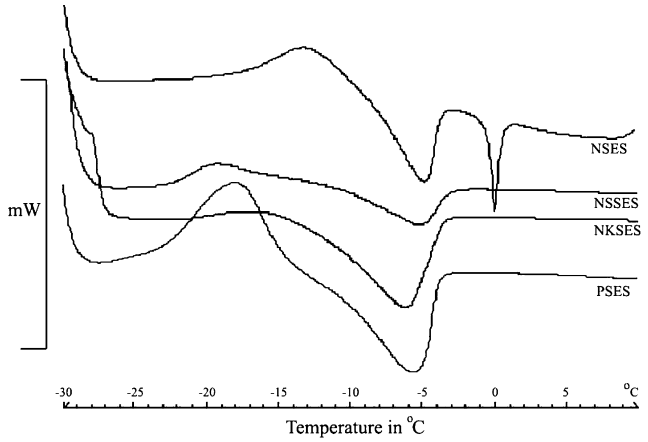


Figure 5. DSC thermogram of intermediate hydrated sample (45% v/v) of PSES, NSSES, NKSES and NSES.

Table 2. Thermal Behavior of Systems at 45% v/v Dilution with Water

		endothermic peak temp (°C)		enthalpy (J g^{-1})	
no.	sample	interphasal water	free water	interphasal water	free water
1	PSES	−5.67		−12.47	
2	NSES	−4.84	0	−7.1	−2.26
3	NSSES	−5.2		−2.27	
4	NKSES	−6.38		−12.22	

peaks at −5.67 °C and −5.2 °C with enthalpies −12.47 J g^{-1} and −2.27 J g^{-1} respectively. Thus there was significant rise in the enthalpy (around 82%) when naproxen sodium was introduced in the system. This indicates stronger binding of water molecules to the POE chains of surfactant and sodium ions at the interface. The endothermic peak observed for water in the case of NKSES was at −6.38 °C, indicating that it was toward peaks reported for the bound water. The comparison of endothermic events of NKSES to PSES suggested that there was about 2% increase in enthalpy, indicating that potassium ions did not alter degree of binding of water molecules to POE chains of surfactant. However, NSES showed two endothermic peaks at −4.84 and 0 °C corresponding to interphasal water and free water respectively. The presence of an endothermic peak for free water indicated stronger water repellant behavior

dx.doi.org/10.1021/mp200541r | Mol. Pharmaceutics 2012, 9, 318−324

of naproxen. The increase in enthalpy for water present in NSES was 43%, which indicates that there was binding of water to the surfactant tails even in the presence of naproxen. Thus to summarize, thermodynamic properties of intermediate hydration (i.e., hydration before transformation of the system into emulsion) of PSES, NSSES, NKSES and NSES were investigated using DSC. The presence of a free water peak in the case of NSES indicated its faster transformation toward emulsion with hydration whereas NSSES and NKSES did not show an endothermic peak for free water, which suggests that still the system had the ability to accommodate the water added in the system. It has been well documented that rheology can be used to investigate the microstructure of the systems. In the present work we have analyzed rheological properties of intermediate hydrated regimes using viscometry studies.

Rheological Studies. All the samples screened using viscometry studies possessed Newtonian flow since the plot of shearing stress versus shear rate showed linearity. Figure 6 shows a plot of viscosity versus hydration (% v/v) for intermediate regimes of PSES, NSSES, NKSES and NSES.

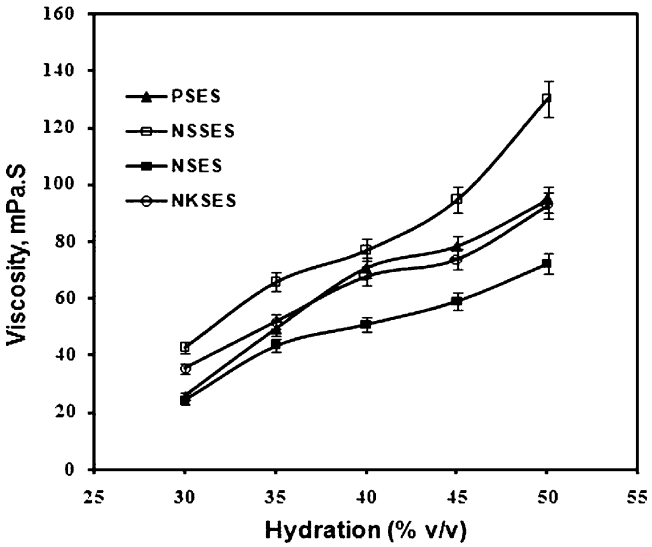


Figure 6. Plot of viscosity versus hydration (% v/v) for intermediate regimes of PSES, NSSES, NKSES and NSES (n = 3).

All the samples showed increase in the viscosity with hydration supporting the swelling of intermediate microsctructures as probed by SAXS and DSC. NMR studies of lipids have shown that progressive hydration of surfactant headgroups enhances their mobility. This process is entropy driven and leads to progressive loosening up of the headgroup packing.[32] Thus increase in the viscosity with hydration in the case of all the studied samples may be attributed to loosening of the boundaries by the adjacent fluid lamellae thus interfering with each other. Such interference might have caused laminar flow to change to turbulent flow increasing the energy dissipation ultimately raising the viscosity of the system.[33] The viscosity of NSSES was highest whereas that of NSES was lowest within different samples screened by rheology. The viscosity of PSES and NKSES was comparable. The increase in viscosity in the case of NSSES may be attributed to the salting out effect of sodium ions as discussed previously. The findings of rheological studies were found to be in agreement with the results of DSC studies indicating no alterations in degree of binding of water molecules to POE groups of surfactant, and thus viscosity of NKSES was similar to that of PSES. The lowest viscosity associated with NSES may be attributed to incomplete swelling of the microstructures due to hindrance created by hydrophobic naproxen towards hydration of POE groups of surfactant.

***In Vitro* Drug Release Study.** There are reports suggesting that the type of mesophase formed upon hydration of lyotropic liquid crystals governs drug release.[34] The present work highlights effect of additive (drug) on inner structuration of SES, which in turn governs the droplet size of the final formulation. Drug release studies for naproxen sodium, naproxen potassium, and naproxen, NSSES, NKSES and NSES were performed in distilled water where the solubility was the limiting parameter. Drug release profiles observed for both naproxen sodium and NSSES were similar, indicating that there was no effect on drug release profile even in the presence of surfactant (Figure 7). However there was improvement in the solubility of naproxen when formulated as SES in comparison to naproxen powder. Formulation of naproxen in SES presented naproxen in smaller droplets, which enormously increased the surface area for dissolution and at the same time the surfactants promoted wetting of the drug molecule leading to increased dissolution rate.[35]

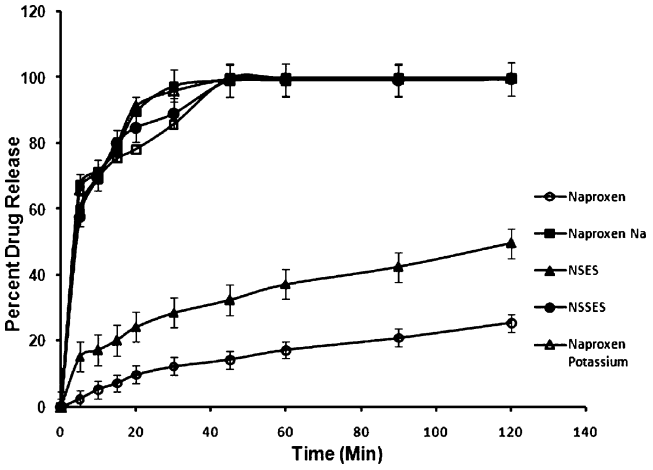


Figure 7. *In vitro* drug release profiles of naproxen, naproxen sodium, naproxen potassium and SES formulations of the same (n = 3).

■ CONCLUSION

The current work explores microstructural properties of intermediate gel phase of SES, and its influence on droplet size of final formulation. Moreover, the effect of a guest molecule (i.e., drug) on the microstructure of an intermediate gel phase has also been addressed. SAXS studies on selected intermediate regime confirmed formation of local lamellar structure. DSC and rheology studies suggested swelling of formed lamellar structure with progressive hydration. In the present study the effect of ion (sodium and potassium) associated with the model drug (naproxen) on microstructure of intermediate regime and ultimately performance (droplet size) of final formulation has been investigated. Systems containing salt forms of naproxen presented larger droplets when compared with systems containing naproxen (base). Kosmotropic and chaotropic effects associated with sodium and potassium ions, respectively, influenced the microstructure of the intermediate lamellar structure as observed from SAXS,

DSC and rheology which ultimately governed the droplet size of the final formulation. Thus to conclude, the nature of additive strongly influences the properties of the intermediate local lamellar structures formed during the self-emulsification process which ultimately affect the performance of the system. The current studies indicate that tailor-made formulations can be prepared if one can control the properties of the intermediate regimes.

AUTHOR INFORMATION

Corresponding Author

*Centre for Pharmaceutical Engineering Science, University of Bradford, Bradford, West Yorkshire, BD7 1DP, U.K. Tel: +44 1274 233 900. Fax: +44 1274 234679. E-mail: a.paradkar1@bradford.ac.uk. Poona College of Pharmacy, Bharati Vidyapeeth University, Pune, Maharashtra, India. Tel: +91 20 25437237. Fax: +91 20 25439383. E-mail: krmahadik@rediffmail.com.

ACKNOWLEDGMENTS

The authors thank Dr. Guruswamy Kumaraswamy, Scientist, Polymer Chemistry, National Chemical Laboratory, Pune for providing facility of Small Angle X ray Scattering and for extending his cooperation in SAXS data analysis and discussion. Sharvil Patil thank Dr. S. H. Bhosale, Head, Department of Pharmaceutical Chemistry, Poona College of Pharmacy for providing guidance in synthesis of naproxen potassium.

REFERENCES

(1) Radtke, M. Pure drug nanoparticles for formulation of poorly soluble drugs. *New Drugs* **2001**, *3*, 62−68.

(2) Gursoy, R.; Benita, S. Self-emulsifying drug delivery systems for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother.* **2004**, *58* (3), 173−182.

(3) Charman, S.; Charman, W.; Rogge, M.; Wilson, T.; Pouton, C. Self-emulsifying drug delivery systems: formulation and biopharmaceutical evaluation of an investigational lipophilic compound. *Pharm. Res.* **1992**, *9*, 87−93.

(4) Pouton, C. Formulation of self emulsifying drug delivery system. *Adv. Drug Delivery Rev.* **1997**, *25*, 47−58.

(5) Pouton, C. Lipid formulations for oral administration of drugs: nonemulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur. J. Pharm. Sci.* **2000**, *11*, S93−S98.

(6) Pouton, C.; Porter, C. Formulation of lipid-based delivery systems for oral administration: Materials, methods and strategies. *Adv. Drug Delivery Rev.* **2008**, *60*, 625−637.

(7) Reiss, H. Entropy induced dispersion of bulk liquids. *J. Colloid Interface Sci.* **1975**, *53*, 61−70.

(8) Groves, M.; De Galindez, D. The self-emulsifying action of mixed surfactants in oil. *Acta Pharm. Suec.* **1976**, *13*, 361−372.

(9) Wakerly, M.; Pouton, C.; Meakin, B. Evaluation of the self-emulsifying performance of a non-ionic surfactant-vegetable oil mixture. *J. Pharm. Pharmacol.* **1987**, *39*, 6−10.

(10) Craig, D.; Barker, S.; Banning, D.; Booth, S. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. *Int. J. Pharm.* **1995**, *114*, 103−110.

(11) Rang, M.; Miller, C. Spontaneous emulsification of oils containing hydrocarbon, non-ionic surfactant, and oleyl alcohol. *J. Colloid Interface Sci.* **1999**, *209*, 179−92.

(12) Lopez-Montilla, J.; Herrera-Morales, P.; Pandey, S.; Shah, D. Spontaneous Emulsification: Mechanisms, Physicochemical Aspects, Modeling, and Applications. *J. Dispersion Sci. Technol.* **2002**, *23* (1−3), 219−268.

(13) Biradar, S.; Dhumal, R.; Paradkar, A. Rheological Investigation of Self-emulsification Process. *J. Pharm Pharm. Sci.* **2009**, *12* (1), 17−31.

(14) Rosevear, F. The microscopy of the liquid crystalline neat and middle phases of soaps and synthetic detergents. *J Am. Oil Chem. Soc.* **1954**, *31*, 628−639.

(15) Teubner, M.; Strey, R. Origin of the scattering peak in microemulsions. *J. Chem. Phys.* **1987**, *87* (5), 3195−3200.

(16) Salonen, A.; Muller, F.; Glatter, O. Dispersions of Internally Liquid Crystalline Systems Stabilized by Charged Disklike Particles as Pickering Emulsions: Basic Properties and Time-Resolved Behavior. *Langmuir* **2008**, *24* (10), 5306−5314.

(17) Glatter, O.; Orthaber, D.; Stradner, A.; Scherf, G.; Fanun, M.; Garti, N.; Clément, V.; Leser, M. Sugar-Ester nonionic microemulsion: structural characterization. *J. Colloid Interface Sci.* **2001**, *241*, 215−225.

(18) Glatter, O.; Strey, R.; Schubert, K.; Kaler, E. Small-Angle Scattering Applied to Microemulsions. *Ber. Bunsen-Ges. Phys. Chem.* **1996**, *100*, 323−335.

(19) Chen, S.; Chang, S. Structural Evolution of Bicontlnuous Microemulsions. *J. Phys. Chem.* **1991**, *95*, 7427−7432.

(20) Bauer, C.; Bauduin, P.; Diat, O.; Zemb, T. Liquid interface functionalized by an ion extractant: The case of winsor III microemulsions. *Langmuir* **2011**, *27* (5), 1653−1661.

(21) Cabos, C.; Delord, P. Marignan, Local lamellar structure in dense microemulsions. *J. Phys. Rev. B* **1988**, *37*, 9796−9799.

(22) Regev, O.; Ezrahi, S.; Aserin, A.; Garti, N.; Wachtel, E.; Kaler, E.; Khan, A.; Talmon, Y. A study of the microstructures of a four component non-ionic microemulsion by cryo TEM, NMR, SAXS and SANS. *Langmuir* **1996**, *12*, 668−674.

(23) Collins, K.; Washabaugh, M. The Hofmeister effect and the behaviour of water at interfaces. *Q. Rev. Biophys.* **1985**, *18* (4), 323−422.

(24) Cacace, M.; Landau, E.; Ramsden, J. The Hofmeister series: salt and solvent effects on interfacial phenomena. *Q. Rev. Biophys.* **1997**, *30*, 241−277.

(25) Dong, R.; Hao, J. Complex fluids of Polyoxyethylene Monoalkyl Ether nonionic surfactants. *Chem. Rev.* **2010**, *110*, 4978−5022.

(26) Efrat, R.; Shalev, D.; Hoffman, R.; Aserin, A.; Garti, N. Effect of Sodium Diclofenac Loads on Mesophase Components and Structure. *Langmuir* **2008**, *24*, 7590−7595.

(27) Ray, A.; Némethy, G. Effects of ionic protein denaturants on micelle formation by nonionic detergents. *J. Am. Chem. Soc.* **1971**, *93* (25), 6787−6793.

(28) Senatra, D.; Lendinara, L.; Giri, M. W/O microemulsions as model systems for the study of water confined in microenvironments: Low resolution ^{1}H magnetic resonance relaxation analysis. *Prog. Colloid Polym. Sci.* **1991**, *84*, 122−128.

(29) Ezrahi, S.; Aserin, A.; Fanun, M.; Garti, N. Subzero temperature behaviour of water in microemulsions. In *Thermal behaviour of dispersed systems: surfactant science series*; Garti, N., Ed.; Marcel Dekker, Inc.: New York, 2001; Vol. *93*, pp 59−120.

(30) Kogan, A.; Shalev, D.; Raviv, U.; Aserin, A.; Garti, N. Formation and Characterization of Ordered bicontinuous microemulsions. *J. Phys. Chem. B* **2009**, *113*, 10669−10678.

(31) Schulz, P.; Puig, J.; Barreiro, G.; Torres, L. Thermal transitions in surfactant-based lyotropic liquid crystals. *Thermochim. Acta* **1994**, *231*, 239−256.

(32) Ulrich, A.; Watts, A. Molecular response of the lipid headgroup to bilayer hydration monitored by ^{2}H-NMR. *Biophys. J.* **1994**, *66*, 1441−1449.

(33) Mezzenga, R.; Meyer, C.; Servais, C.; Romoscanu, A.; Sagalowicz, L.; Hayward, R.. Shear rheology of Lyotropic liquid crystals: a case study. *Langmuir* **2005**, *21* (8), 3322−3333.

(34) Negrini, R.; Mezzenga, R. pH-Responsive Lyotropic Liquid Crystals for Controlled Drug Delivery. *Langmuir* **2011**, *27*, 5296−5303.

(35) Patil, P.; Joshi, P.; Paradkar, A. Effect of formulation variables on preparation and evaluation of gelled self emulsifying drug delivery system of Ketoprofen. *AAPS PharmSci.* **2004**, *5* (3), 1−8.