Soluplus Based Polymeric Micelles and Mixed Micelles of Lornoxicam: Design, Characterization and *In vivo* Efficacy Studies in Rats

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ABSTRACT

Aim: To investigate potential of polymer Soluplus (SP), to form micellar/mixed micellar systems and its ability to improve solubility and consequently therapeutic efficacy of model BCS Class-II drug Lornoxicam (LNX). **Methods:** Soluplus based micellar systems were prepared using thin film hydration method. CMC (critical micelle concentration) was determined using two methods, namely, Iodine UV spectroscopy and Pyrene fluorescence spectroscopy. Micellar systems were characterized for their particle size, drug loading, CMC and SANS study to understand the dynamics of micellar systems. The LNX loaded micellar systems were evaluated *in vivo*, to evaluate their effectiveness in improving the anti-inflammatory activity of LNX in rat-paw edema model and also to observe possible reduction in ulcerogenic potential of LNX. **Results:** SP as well as its mixed micellar systems could effectively form stable micelles with mean diameter in range of 60-70 nm and promising LNX loading efficiency. It was evident from CMC studies (using Iodine UV spectroscopy and Pyrene fluorescence spectroscopy methods) and with rubinghs regular solution theory that Soluplus could effectively form stable mixed micelles with other micelle forming agents like Solutol HS 15 (ST) and Phospholipon 90 H (PL 90H). SANS spectra confirms the presence of micellar systems and applying sphere model of SASFIT software, it was found that micelles exhibited spherical shape. *In vivo* anti-inflammatory study indicated that LNX loaded micellar systems resulted in superior therapeutic efficacy than LNX suspension, even at a reduced dose ($p < 0.05$). The ulcerogenic potential of LNX was also significantly reduced when LNX was incorporated into the micellar system. **Conclusion:** Soluplus possessed ability to form stable micellar systems which demonstrated promising potential to improve solubilization of a BCS class II drug, LNX and improved its therapeutic efficacy with reduction in adverse effects when tested *in vivo* in rats.

Key words: Polymeric micelles, Lornoxicam, Soluplus, Iodine, CMC, Mixed micelles.

INTRODUCTION

Nanoparticulate systems possess a very diverse classification and have become an important stream of research in pharmaceutical sciences. With advances in technology and understanding of diseases and disorders at cellular and molecular level, applications of nanoparticles in drug delivery are increasing. Of various nanocarriers being reported in literature, micellar systems are important drug delivery systems offering numerous advantages. Importantly, these are formed by self assembly of surfactants/polymers above their critical micelle concentration (CMC), thus enabling their fabrication by simple procedure. Micelles can effectively entrap lipophilic molecules in to their hydrophobic core and are therefore suitable for improving solubility of various BCS class II and IV molecules. Owing to their small size, usually 10-100 nm, they can be employed for intravenous delivery of actives and are able to target tumor site due to enhanced permeation and retention effect (EPR). Some polymers employed for yielding micelles are also amenable to surface modifications to render them target specific.

Submission Date : 07-03-2015
Revised Date : 11-08-2016
Accepted Date : 16-02-2016

DOI: 10.5530/ijper.50.2.8

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Micelles are also capable of improving solubility and thus the oral bioavailability of BCS class II and IV molecules. However, in spite of a plethora of advantages possessed by micelles, there have been a few imperative limitations, as demonstrated by the fact that only a few micellar products are in clinical trials and even fewer being available commercially.[1,2] Surfactants such as Cremophor EL, at high concentrations are reported to possess severe neurotoxicity and hematological adverse reactions. Tween 80 possess higher CMC, thus they no longer remain as stable as micelles on undergoing significant dilution in different anatomical locations. There is continued search to identify more micelle forming agents, which can form stable micelles with adequate drug loading capability and minimum/no toxicity.[3,4] Literature reports concept of mixed micellar systems wherein two or more micelle forming agents are combined to obtain advantages of each agent and minimize their individual drawbacks.

Soluplus (SP) is a polymeric agent that has been explored for its ability to form solid solution with many lipophilic drugs such as danazol, fenofibrate, itraconazole on being hot melt extruded thus improving their solubility.[5] However, a little is known about its ability to yield stable micelles. Its distinctive chemical structure as shown in Figure 1 enables the polymer molecules to self assemble and form stable amphiphilic aggregates i.e micelles. Studies were designed to evaluate SP in combination with other surfactants for forming mixed micellar systems.

The model drug selected for the study was Lornoxicam (LNX), a potent NSAID, from BCS Class II. It has limitation of poor aqueous solubility and causes severe gastrointestinal (GI) adverse effect such as bleeding and ulcers on chronic administration.[6,7] It was hypothesized that loading of LNX in SP based mixed micellar systems would improve its solubility and consequently its oral absorption that would aid in rapid pain relief. It was also expected that improved solubility and quick GI absorption should reduce GI adverse events related to LNX. To the best knowledge of authors, it is for the first time that micellar and mixed micellar systems comprised of SP have been reported. The study also investigates ability of micellar systems to improve oral efficacy of LNX and reduce their GI side effects which is of immense importance to improve performance of drugs with properties similar to LNX.

MATERIALS AND METHODS

MATERIALS

Soluplus® (SP) and Solutol HS 15 (ST) were received as kind gift samples from BASF. Phospholipon 90H (PL 90H) was received as a gift sample from Lipoid, GmBH, Germany. Methanol and chloroform were purchased from Fisher Scientific (Fair Lawn, NJ, USA). LNX was obtained as gift sample form Sun Pharma Pvt. Ltd., Mumbai. Pyrene and iodine was purchased from Sigma Aldrich.

Determination of CMC of Soluplus polymeric micelles and mixed micelles

CMC of different micellar/mixed micellar solutions were determined using two methods: Iodine UV spectroscopy and Pyrene fluorescence spectroscopy. Iodine UV spectroscopy method as reported by Gasiford *et al* 1997 was used to determine CMC of micelles.[8] Briefly, a stock solution of 0.5% KI/I2 was prepared. For determination of CMC a series of aqueous micellar/mixed micellar solutions in varying concentration were prepared. 1 ml of standard preparation KI/I2 was added to each of these micellar/mixed micellar solutions. The solutions were incubated for 2 hrs at room temperature in dark. The UV absorbance of varying polymer concentrations at 366 nm was measured using UV–Visible spectrophotometer.

In second method, a stock solution of pyrene (6×10^{-3} M) in acetone was used. 1 ml of the solution was transferred to 10 ml amber vials and acetone was evaporated at 65°C using water bath. To each of these vials, aqueous micellar/mixed micellar solutions in varying concentration were added. The concentration of pyrene in final solution was adjusted to 6×10^{-7} M. Micelles were allowed to equilibrate with pyrene for 24 hrs in dark and fluorescence intensity was measured at emission wavelength of 373 nm using Perkin fluorescence spectrometer.[9]

Preparation and characterization of LNX loaded micellar systems

The micelle forming agents were dissolved in chloroform in a round bottom flask and evaporated to form a thin film which on hydration with filtered distilled water with intensive shaking formed micelles/mixed micelles. SP alone or in combination with either ST or PL90H was employed to form micelles or mixed micelles. The micellar dispersions were sonicated for few minutes and then filtered through 0.45 μ filter membrane to remove any aggregates.

LNX loaded micellar systems were prepared using same above procedure, except that LNX was added along with micelle forming agents in chloroform and the same procedure was repeated.

Mean particle size analysis

The mean particle size (MPS) and polydispersity index (PI) of LNX polymeric micelles/mixed

micelles were determined using Malvern Zetasizer Nanoseries, UK.

LNX solubilization efficiency

UV spectrophotometric method was used to determine the amount of LNX solubilized in the micelles/mixed micelles. LNX micelle/mixed micellar solution was diluted suitably with methanol to extract all LNX from micelles and analyzed for its content employing standard plot developed on UV-visible spectrophotometer (JASCO V-530, Japan) at 376 nm.

Small angle neutron scattering (SANS) study

SANS experiments were carried out using the SANS diffractrometer at the Dhruva Reactor, Bhabha Atomic Research Centre, Trombay, India. The diffractrometer makes use of a beryllium oxide-filtered beam of mean wavelength (λ) 5.2Å. The angular distribution of the scattered neutrons was recorded using a one dimensional position-sensitive detector (PSD). The accessible wave vector transfer [$Q = (4\pi \sin \theta)/\lambda$, where 2θ is the scattering angle] range of the diffractrometer is 0.017-0.35 Å-1. The PSD allows simultaneous recording of data over the full Q range. The samples were held in a quartz sample holder of 0.5 cm thickness. In all measurements, the temperature was kept fixed at 45°C. The measured SANS data were corrected and normalized to a cross sectional unit, using standard procedures. SANS studies were carried out for micellar and mixed micellar solution of SP. All the solutions were prepared in D_2O at 25ºC.

In vivo studies

In vivo studies were performed to evaluate whether encapsulation of LNX in micelles can improve its oral absorption and ultimately improve its anti-inflammatory activity and also, to evaluate if the ulcerogenic potential of LNX can be reduced. Wistar rats (200-250 grams) were used for the *in vivo* studies. Animal care and handling throughout the experimental procedure were performed in accordance to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The experimental protocol was approved by institutional animal ethics committee.

Evaluation of Anti-inflammatory activity

Anti-inflammatory activity was determined using carrageenan induced rat paw edema model.[10] Animals were fasted overnight and divided into six groups as mentioned below:

The overnight fasted animals were divided into six groups (n=6) as follows

Group 1: Control (No treatment)

Group 2: LNX suspension equivalent to 0.4 mg/kg LNX

Group 3: LNX loaded mixed micelles (SP &PL 90H) at dose 0.27 mg/kg LNX

Group 4: LNX loaded mixed micelles (SP & ST HS 15) at dose 0.27 mg/kg LNX

Group 5: LNX loaded SP polymeric micelles at dose 0.27 mg/kg

Group 6: Blank SP polymeric micelles

Rats of all six groups were challenged by a subcutaneous injection of 0.1 ml of a 1% w/v carrageenan solution, into the plantar site of the left hind paw. All formulations were administered orally after induction of paw edema. The paw volume in ml was measured using plethysmometer at 0, 0.5, 1, 2, 4 and 6 h after carrageenan administration and the mean difference of paw volume (paw edema) at each time interval was calculated using following formula.

Paw edema/increase in paw volume of animal during each time interval = the difference in paw volume between the left and right hind paw of the same animal during that time interval.

Evaluation for reduction in ulcerogenecity

The ulcerogenic potential of LNX suspension and LNX micelles when administered orally was evaluated by a reported method.[10,11] Wistar rats were used for the study and were divided randomly into four groups (n=4) as follows:

Group 1: Control (No treatment)

Group 2: LNX suspension equivalent to 0.4 mg/kg LNX

Group 3: LNX loaded SP micelles equivalent to 0.4 mg/kg LNX

Group 4: LNX loaded SP/PL 90H mixed micelles equivalent to 0.4 mg/kg LNX

All the groups received regular diet daily for 14 days. On the 14th day, rats were sacrificed and their abdomen was opened. The stomach was incised along the greater curvature and gently washed with water. Hemorrhagic lesions, produced in the glandular portion were observed under a dissection microscope (X20 magnification) and evaluated by the following score:

0.0 Normal (no injury, bleeding and latent injury).

0.5 Latent injury or widespread bleeding.

1.0 Slight injury (2 to 3 dotted lines).

2.0 Severe injury (continuous lined injury or 5-6 dotted injuries).

3.0 Very severe injury (several continuous lined injuries).

4.0 Widespread lined injury or widened injury.

Statistical analysis

Data for all measurements were considered as mean ± standard error of mean (SEM) of three separate experiments. One-way ANOVA followed by Bonferroni test was used to evaluate the results of *in vivo* studies. Results were considered significant if $p<0.05$ at 95% confidence limit.

RESULTS

Preparation of LNX loaded micellar systems

Soluplus was evaluated alone and in combination with other surfactants to form micelles and was loaded with LNX. Micelles were observed to be formed as indicated by particle size analysis studies conducted on Malvern, zetasizer, Nanoseries, UK.

Determination of CMC of micellar systems

For determining CMC of SP, a series of solutions of varying concentration 0.00001% to 0.05 % of SP were prepared and CMC was determined on basis of change in slope of plot of log conc. vs absorbance/intensity. Figure 2a and 2b is graphical representation of CMC of SP determined by Iodine UV spectroscopy method and Pyrene Fluorescence spectroscopy method respectively. CMC of SP was found be 7.934×10^{-5} mM and 7.079×10^{-5} mM by the two methods respectively. Similarly, mixed micelles composed of SP in combination with either PL90H or ST HS 15 at different molar ratios were evaluated for CMC determination. The results of same are reported in Table 1 and 2. The experimental CMC values of PL90H /SP and STHS 15/SP mixed micellar systems were found to be lower than theoretical values, derived by applying Rubingh's regular solution theory (RST).

Determination of particle size and polydispersity index

The particle size of blank SP micelle was found to be in the range of 65 nm to 70 nm with unimodal size distribution (Figure 3). The mean particle size of different micellar formulations composed of SP alone, SP-PL90H and SP-ST loaded with LNX was also found to be unimodally distributed in same range as the blank ones as depicted in Table 3.

LNX solubilization efficiency of micellar/mixed micellar systems

Various concentrations of SP were employed to study the entrapment efficiency of LNX in SP micellar solution. The loading capacity for LNX increased with increasing concentration of SP polymer from 0.01% to 2% as shown in Table 4. 2% solution of SP could successfully load 2.783 ± 0.119 mg of LNX with narrow particle size distribution. LNX loaded SP micellar solutions were optically clear yellow solutions. SP was further combined individually with surfactants ST

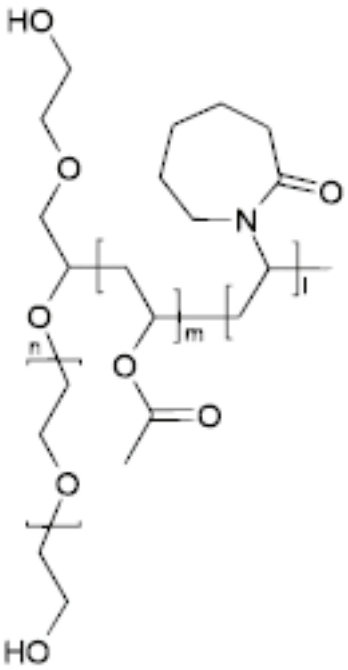

Figure 1: Structure of Soluplus

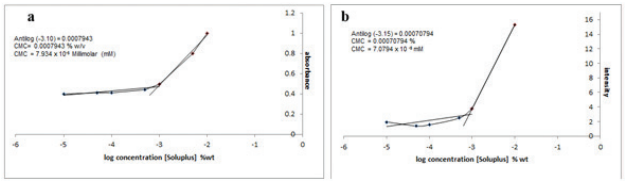

Figure 2a: Plot of UV intensity of I_2 vs. concentrations of Soluplus micelles in deionized water

Figure 2b: Flourescence intensity of Pyrene vs. concentrations of Soluplus micelles in deionized water

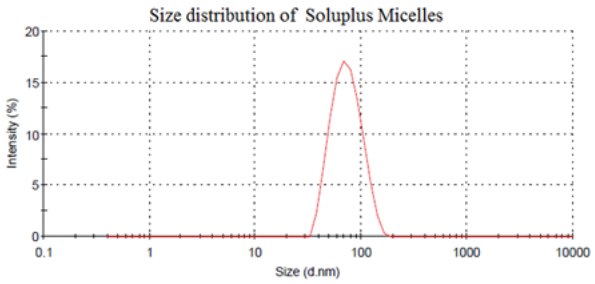

Figure 3: Particle size distribution by intensity of blank SP micelles as obtained on Malvern zetasizer nanoseries

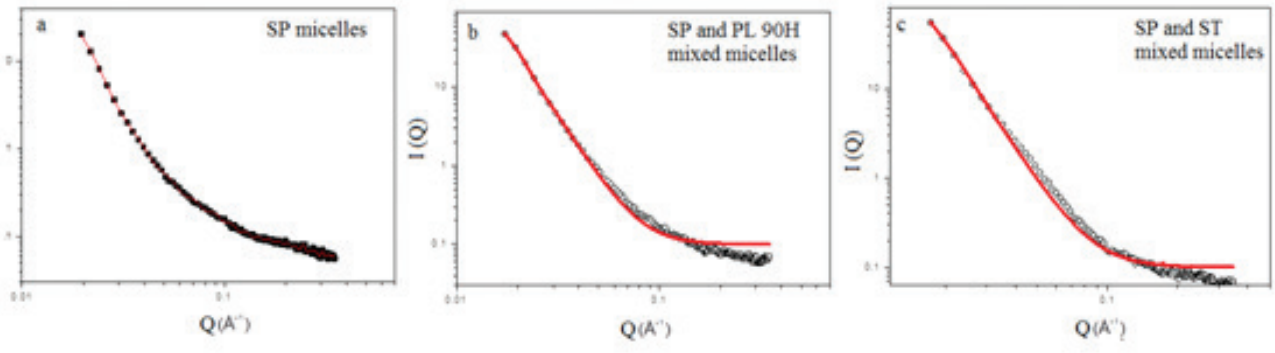

Figure 4: SANS plot of a)SP, b)SP and PL 90H mixed micelles and c)SP and ST mixed micelles

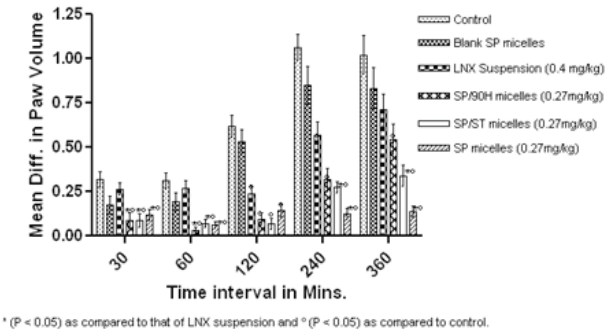

Figure 5: Anti-inflammatory activity of LNX *(paw edema ± SEM)* during each time interval for each group (n=6)* (P<0.05) as compared to that of LNX suspension and °(P<0.05) as compared to control. (One-way ANOVA followed by Bonferroni test).

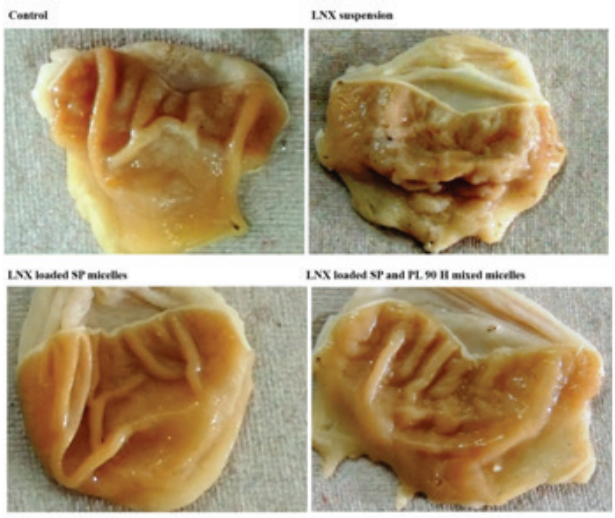

Figure 6: Photographic images of the incised stomach tissues of rat

and PL 90H in 1:1 molar ratios and their drug loading efficiency was determined. Though the LNX loading efficiency was found to increase in following order, SP micelles <SP-PL90H micelles< SP-ST micellar systems (Table 5), there was no significant difference observed in LNX loading between individual SP micelles and SP mixed micelles.

SANS (small-angle neutron scattering) study

The small-angle scattering intensity, I (Q) as a function of scattering vector Q (=4π sinθ/λ, where 2θ is the scattering angle and λ is the wavelength of the incident radiation) for a micellar solution can be expressed as I(Q) = n P (Q) S (Q) where n is the number density of the particles, P(Q) is the intra particle structure factor and depends on the shape and size of the particles, S(Q) is the inter particle structure factor and is decided by the spatial distribution of the particles.

For dilute non-interacting particles, S(Q) is unity. In the present study, the particles are assumed to be spheres for which the term P(Q) can be obtained as P(Q) = K(Q)2

$$K(Q,R,\Delta\eta) = \frac{4}{3}R^3\Delta\eta 3\frac{\sin QR - QR\cos QR}{(QR)^3}$$

$\Delta\eta$ being the scattering length density difference between particle and solvent, R is the radius of the particle. To improve the quality of fit, a polydispersity in particle size has been introduced, assuming log-normal distribution of particle size. Figure 4 shows SANS spectra of SP, SP/PL 90H and SP/ST HS 15 mixed micellar solution respectively.

The pattern and evolution of the SANS spectra indicates presence of micelles. Effective core radius of SP micelles was obtained from SANS analysis of 2% SP solution using sphere model of SASFIT software for sans evaluation. The effective core radius of micelles/mixed micelles along with polydispersity index is listed in Table 6.

In vivo studies

Evaluation of Anti-inflammatory activity

Figure 5 depicts the graphical representation of anti-inflammatory activity of all LNX containing formula-

Table 1: CMC and Interaction parameter (β) values of SP and PL 90H mixed micelles

	Formulation (SP and PL 90H) Molar ratio						
Concentration in mM	**1:0**	**1:1**	**1:2**	**1:4**	**2:1**	**4:1**	**0:1**
Experimental CMC mM	7.93×10^{-5}	7.94×10^{-5}	2.55×10^{-5}	5.01×10^{-5}	5.01×10^{-5}	7.94×10^{-5}	0.01
Calculated CMC mM	7.6×10^{-5}	1.39×10^{-4}	2.07×10^{-4}	3.41×10^{-4}	1.05×10^{-4}	8.73×10^{-4}	0.01
β	NA	-6.48	-13.80	-11.04	-8.40	-11.78	NA

mM – millimolar, SP- Soluplus PL 90H- Phopholippon 90 H, NA- not applicable.

Table 2: CMC and Interaction parameter (β) values of SP and ST HS 15 mixed micelles

	Formulation (SP and ST) Molar ratio						
Concentration in mM	**1:0**	**1:1**	**1:2**	**1:4**	**2:1**	**4:1**	**0:1**
Experimental CMC mM	7.93×10^{-5}	1.26×10^{-4}	1.58×10^{-4}	2.01×10^{-4}	1.00×10^{-4}	5.01×10^{-5}	0.01
Calculated CMC mM	7.6×10^{-5}	1.59×10^{-4}	2.38×10^{-4}	3.96×10^{-4}	1.2×10^{-4}	9.91×10^{-5}	0.01
β	NA	-12.60	-5.74	-3.58	-7.94	-11.56	NA

mM – millimolar, SP- Soluplus ST- Solutol HS 15, NA- not applicable.

Table 3: Particle size and polydispersity of LNX loaded SP micellar/ mixed micellar solution

Formulation	Mean Particle size in nm ± SEM	P.I ± SEM
SP micelles	67.65 ± 2.129	0.077 ± 0.006
SP and PL 90H mixed micelles	70.13 ± 2.239	0.076 ± 0.040
SP and ST mixed micelles	63.82 ± 2.371	0.080 ± 0.007

Table 4: Drug content of LNX loaded SP micellar micellar solution

SP conc. % w/v	Initial added drug (mg)	Mean Drug content (solubilized drug) in mg± SEM
0.01	4	0.123 ± 0.009
0.1	4	0.397 ± 0.049
1	4	1.313 ± 0.202
2	4	2.783 ± 0.119

Table 5: Entrapment efficiency of LNX loaded SP micellar/ mixed micellar solution

Formulation	Theoretical added drug (mg)	Mean experimental drug content ± SEM(mg)	% Mean drug loading efficiency ± SEM
SP micelles	4	3.03 ±0.060	69.58 ± 2.973
SP-PL 90H mixed micelles	4	3.29 ± 0.132	75.83 ± 1.502
SP-ST mixed micelles	4	3.19 ± 0.009	82.50 ± 3.307

Table 6: Micellar parameters obtained from SANS Analysis using Sphere model structure.

Micelles	Core Radius (Rc)	Polydispersity (s)	Shape
SP	30.0	0.175	Spherical
SP/PL 90H	29.5	0.198	Spherical
SP/ST HS 15	31.7	0.287	Spherical

tions. It is evident from Figure 5 that all LNX loaded micelles/mixed micelles showed significantly higher anti-inflammatory activity ($P<0.05$) as compared to control at all the time points. LNX loaded micelles/mixed micelles with even lower dose of LNX, showed significantly higher anti-inflammatory activity ($P<0.05$) as compared to LNX suspension. It was found that there was no significant difference in efficacy between different LNX loaded micelles/mixed micelles (except during 4 and 6 hrs where SP micelles showed higher anti-inflammatory activity as compared to SP mixed micelles) and all micellar systems were found almost equally promising in improving therapeutic efficacy of LNX. Blank SP micelles had no anti-inflammatory potential as can be seen in Figure 5.

Evaluation for reduction in ulcerogenecity

Figure 6 are the photographic images of the incised stomach tissues of rats from control group, LNX suspension and LNX SP micelles groups respectively. The control group showed no ulceration which was expected. The ulcer score obtained for LNX suspension was 3.13 ± 0.315 (mean ulcer score ± SEM). High ulceration index of LNX suspension was due to prostaglandins inhibition property of LNX along with high local concentration of LNX crystals in contact with gastric mucosa. Micellar systems loaded with LNX again demonstrated promising results wherein they did not cause as severe ulceration as LNX suspension. LNX loaded micelles showed very low ulceration index. The ulcer score obtained for LNX SP micelles and LNX SP/PL 90 H micelles were 0.70 ± 0.144 and 00.44 ± 0.214 which was significantly ($p<0.05$) lower than LNX suspension.

DISCUSSION

Micellar systems are one of the most promising delivery systems for BCS Class II drugs such as LNX, which have good permeability but compromised or limited water solubility responsible for their poor oral absorption. Over the years, a variety of agents have been screened and found useful to formulate micellar solutions of various drugs, although most of them suffer through some limitations. The problems generally encountered are poor drug loading capacity, safety at concentrations used to load single dose of actives, high CMC values responsible for breakdown upon dilution and so on. Thus, the search for discovering newer and better micelle forming agents is always on. Mixed micellar systems composed of two or more micelle forming agents also to some extent are known to overcome above mentioned drawbacks. In an attempt to prepare stable micellar systems loaded with adequate load of LNX, studies were planned to evaluate a novel polymer that has been launched commercially only recently by BASF Pvt. Ltd., namely Soluplus (SP). Chemically known as polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer, the unique chemical structure of Soluplus had promising ability to self-assemble and form micelles with outer hydrophilic shell and inner hydrophobic core. The polymer has already demonstrated great potential to formulate hot melt extrudates and to improve solubilization of various drugs absorption of various poorly soluble drugs.[12,13] However, its capability to form drug loaded micelles has not been reported so far. In addition, it was also planned to evaluate ability of SP to form stable drug loaded mixed micelles with ST and PL 90H.

Particle size analysis revealed that blank as well as LNX loaded micelles composed of SP alone or in combination with other biocompatible and well tolerated surfactants, ST and PL 90 H, resulted in almost same particle size of approximately 70 nm with unimodal distribution as expected for a micellar system.

CMC value of SP was found to be (7.6×10^{-5} millimolar i.e 7.6×10^{-8} Molar) which was in accordance with reported CMC value of SP as per BASF.[14] As hypothesized, micelles formed by SP possessed low CMC that would offer them greater resistance to deformation upon unlimited dilutions that can happen under *in vivo* conditions. Being pegylated polymer, SP micelles also could be of utmost importance for parenteral delivery of actives due to their stealth property.

Fabrication of mixed micellar systems of SP was mainly driven to understand feasilibility of SP to form mixed micelles and also with aim of incorporatinghigher amount of LNX in to the micelles. ST is reported to improve drug loading in various nanocarriers and PL90H is reported to improve oral absorption of various actives.[15-17]

The theoretical (calculated) CMC of mixed micelles were determined using by using the Rubinghs regular solution theory.[18-20]

Mixed CMC ($C_{12)}$ of binary mixture of surfactant 1 and 2 can be calculated using Equation (1)according to Rubingh's regular solution theory.

$$\frac{1}{C_{12}} - \frac{\alpha_1}{C_1} + \frac{(1-\alpha_1)}{C_2} \quad (1)$$

where (C_{12}) is theoretical mixed CMC for a binary mixture of surfactant 1 and 2, α_1 is the mole fraction of surfactant 1 in the total mixed solution and C_1 and C_2 are the CMCs of the individual surfactants 1 and 2, respectively.

The magnitude of interaction between two surfactant species in a binary mixture can be determined by calculating interaction parameter β using RST by Equation (2).

$$\beta_{12} = \frac{\ln(\alpha_1 C^* | x_1 C_1)}{(1-x_1)^2} \quad (2)$$

Where α_1 is the mole fraction of surfactant 1 in the mixed micellar solution, C* is CMC of mixed micelles, C_1 is the CMC of the surfactant 1 and x_1 is the micellar mole fraction of surfactant 1.

The micelle mole fraction x_1 can be calculated by solving iteratively the following equation (3),

$$\frac{x_1^2 \ln(\alpha_1 C^* | x_1 C_1)}{(1-x_1^2)^2 \ln\left((1-\alpha_1) C^* | (1-x_1) C_1\right)} = 1 \quad (3)$$

The β value demonstrates the extent of interaction between the two surfactant molecules and measures deviation from ideal behavior. If the value β is negative, then the two surfactant molecules exhibit attractive forces and there is synergism in mixed micelles, whereas a positive value indicates antagonism and if β=0 then mixed micelle formation is ideal. Larger the absolute value of β (positive or negative) stronger is the interaction (repulsion or attraction) between the surfactants.

Based on the results as presented in Table 1 and 2, a negative β value was obtained for both mixed micellar systems of soluplus which indicates that the mixed micelles exhibited synergism and attractive interaction in their mixed state. The values for micelle mole fraction $x_1$shows that in mixed micelles the molar fraction of SP (x_1) is always higher than molar fraction in solution (α_1), indicating that the contribution of polymer SP is significantly higher in mixed micelle than compared to 90H or ST HS 15. This was expected because SP has very low CMC and will preferentially partition into micelles.[21] Hence, by applying Rubingh regular solution theory it was concluded that (SP + PL90 H) and (SP + ST HS 15) mixed micelles formation was thermodynamically favored and exhibited synergism in their mixed micellar state.

Entrapment efficiency studies demonstrated results as expected wherein SP on combination with PL 90 H and ST HS 15 resulted in increased entrapment of LNX. ST HS 15 and PL90 H have been widely used as promising surfactants owing to their potential to improve solubilization of actives by virtue of their micellization capability. The same was expected to result in improved entrapment efficiency in present scenario. SANS spectra along with Particle size analysis confirms presence of micelles with spherical structure. It was found that the particle size and SANS spectra of micellar/mixed micellar systems were quite similar. The molecular weights of ST and PL 90 H are small compared to high mol. wt SP block polymer; hence the volume fraction occupied by ST/Pl 90 H in 1:1 molar micellar mixture with block polymer SP is quite small. This could be the reason why there are no significant changes in SANS spectra as well as particle size between SP micelles and mixed micelles. The sphere model was found to be best fitted with sans spectra, hence it was concluded that the micelles exhibited spherical type of geometry.

The objective of carrying out *in vivo* studies was to evaluate if the anti-inflammatory potential of LNX was improved on being loaded in micellar systems and secondly, to examine any possible reduction in its major adverse effect, namely gastro-intestinal irritation.The study included comparison of activity when low dose of LNX was administered. The result presented evidence that micelles/mixed micelles had significantly ($P<0.05$) higher anti-inflammatory activity with only 0.27 mg/kg dose (i.e. at lower dose) of LNX when compared with control. Anti-inflammatory effect of all micellar systems were significantly higher ($P<0.05$) as compared with LNX suspension at 0.4 mg/kg (Figure 5). This is beneficial for anti-inflammatory drugs and will help in reducing its dose and consequently dose related adverse events. It is clear from Figure 5 that all LNX loaded micelles/mixed micelles showed quick onset of action (30 minutes) as compared to that of LNX suspension (2 h). It may be attributed to the fact that micellar nano carriers facilitate quick absorption which leads to rapid onset of action. It was also noteworthy that the LNX loaded micellar carriers could maintain the significantly high anti-inflammatory effect for a longer duration. Enhanced activity and prolong duration is anticipated to help reduce the frequency of dose. This study clearly indicated that loading of LNX in micellar system could successfully increase its therapeutic efficacy and can prolong its action even at lower therapeutic dose of LNX. This is an indirect indication of improved bioavailability.

It has been well known that most of the NSAIDs are prone to cause gastrointestinal ulcers on long term administration. LNX has been reported to produce gastrointestinal ulcers and erosion on chronic oral administration in rats.[7] In the present study, LNX suspension showed significantly higher ulcerative effect as compared to that of control as well as micellar systems loaded with LNX ($P<0.05$) and the results were according to our aforementioned hypothesis. The possible explanation for this could be that with micellar systems LNX

was expected to be immediately dispersed and dissolve in GI fluids. Also administration of micellar solution prevents the direct contact of free drug with the gastric mucosa thus lowering damage to GI mucosa and subsequent ulceration. The slightly lower score observed with SP/ PL 90 H mixed micelles may be attributed by gastro protective effect of phospholipids on GI tract.[22]

CONCLUSION

SP based micellar systems presented all the desirable qualities, viz., nanometric size (between 10 to 100 nm), self assembling at low concentration and good drug loading capacity, as is expected from micelles. Loading LNX into SP based micelles maintained its therapeutic efficacy when tested *in vivo* in rats, even at a lower dose while providing rapid onset and longer duration of action, with reduction in ulceration, which is of great advantage. Thus, SP, a novel amphipihilc polymer demonstrated promising potential to form micellar systems with adequate drug load and colloidal stability, for improving therapeutic effectiveness of loaded molecules.

ACKNOWLEDGEMENTS

The authors are thankful to Sun Pharma and BASF, India for providing gift samples of Lornoxicam and Soluplus respectively. Ronak S. Bhuptani is thankful to All India Council for Technical Education (AICTE) for providing her financial assistance. Ankitkumar S. Jain is thankful to Amrut Mody Research Fund (AMRF), Mumbai and University Grants Commission (UGC), New Delhi for funding his research activities. Dinesh T. Makhija is also thankful to AMRF, Mumbai for funding his research activities. Authors are very thankful to Dr. A.V. Karnik and Mr. Milind Thigle from Chemistry Department, University of Mumbai, for giving permission to conduct flourescence studies in their department.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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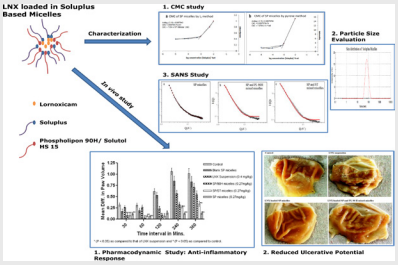
PICTORIAL ABSTRACT



ABBREVIATIONS USED

SP: Soluplus; **LNX:** Lornoxicam; **PL 90H:** Phospholipon 90H; **ST HS 15:** Solutol HS 15; **CMC:** Critical micelle concentration; **NSAID:** Non steroidal antiinflammatory drug; **BCS:** Biopharmaceutical Classification System; **SANS:** Small angle neutron scattering; **PI:** Polydispersity index; **GI:** Gastrointestinal; **EPR:** Enhanced permeation and retention; **MPS:** Mean particle size; **RST:** Rubingh's regular solution theory.

SUMMARY

- Soluplus, a polymeric agent has ability to form micellar and mixed micellar systems that could improve solubilization of Lornoxicam, a BCS Class II drug.
- Micellar and mixed micellar systems were found promising nanocarriers when evaluated for their particle size, CMC and drug loading capacity.
- SANS studies were performed and are reported for the first time, to the best knowledge of authors, for soluplus based micellar systems, that confirmed its nanomicellar structure and geometry.
- Soluplus based micellar and mixed micellar systems improved therapeutic efficacy of Lornoxicam when tested *in vivo* in rats for inflammation relief and reduction in ulcerogenicity of Lornoxicam.

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