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Study of Polymeric Mixed Micelle System of Sulphasalazine for Improvement of Oral Bioavailability

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ABSTRACT

Sulfasalazine (SSZ) has been recommended for rheumatoid arthritis, ulcerative colitis, and Crohn's disease. However, low aqueous solubility and reduced bioavailability obstruct its clinical application. The aim of this study was to formulate a mixed micelles (MM) system composed of two biocompatible copolymers Soluplus and Pluronic F127 to improve the solubility and oral bioavailability of insoluble drug SSZ. SSZ-MM was prepared by an ethanol solvent evaporation method and optimized using 32 factorial designs with respect to quantity of polymers. The average size, zeta potential and entrapment efficiency of the optimized formulation were found to be 59.12 nm, -16.4 mV and 62.04% respectively. The SSZ-MM showed sustained release up to 24 h in in-vitro release study. Ex-vivo endocytic uptake studies revealed involvement of endocytic pathways in the uptake of mixed micelles from the intestine. The in-vivo oral bioavailability study in Wistar rats showed 2.19 folds higher AUC of SSZ-MM than free SSZ, indicating the mixed micelles of Soluplus and Pluronic F127 is a feasible drug delivery system to promote insoluble drug oral absorption in the gastrointestinal tract.

Keywords: Sulfasalazine, Bioavailability, Absorption, Factorial design, Micelles, Pharmacokinetics.

INTRODUCTION

The oral route of drug administration is the most important route for administering drugs for systemic effects. The concept of BCS to provides a better understanding of the relation between drug release from the product and the absorption process [1,2]. The bioavailability will be affected by the *in-vivo* performance of the dosage form if the dissolution, drug release is rate limiting. The BCS takes into account three major factors dissolution, solubility and intestinal permeability which govern the rate and extent of drug absorption from a solid dosage form [2,3]. Poor bioavailability due to poor solubility of drugs is predominant amongst the potential problems encountered in oral drug delivery, especially with the drug candidates belonging to BCS class II and IV. Formulation and development of an efficacious delivery system for BCS class II and IV drug is challenging task. Some techniques employed to increase bioavailability are a lipid-based delivery system, polymer-based nanocarriers, liquesolid technology, crystal engineering (nanocrystals and co-crystals), self-emulsifying solid dispersion [1].

Nanocarriers are important components in novel drug formulation they increase bioavailability, protect and stabilize sensitive agents and minimize side effects. Polymeric mixed micelles (MM) have evoked special interest as nanosized drug delivery system for the poorly water-soluble drug because they provide increased stability of hydrophobic drug and also due to their *in-vivo* advantages versus the free drug. The hydrophobic drug can be entrapped in the core of micelles and hydrophobic shell mask the core from the biological environment. They are made up of two or more amphiphilic polymers that can self-assemble into nano-structure with size ranging between 20-200 nm. This thermodynamically driven process occurs above a co-polymer determined concentration commonly known as critical micellar concentration (CMC) [4].

The aim of this work is to develop novel mixed micelles to enhance the oral bioavailability of sulfasalazine (SSZ). Sulfasalazine is BCS class II drug used in the treatment of mild to moderate ulcerative colitis, and as adjunctive therapy in severe ulcerative colitis; and also in rheumatoid arthritis. Sulfasalazine is an anti-inflammatory agent having chemical name 5-([P-Pyridylsulfomoyl)Phenyl]azo)salicylic acid. Sulfasalazine is also proven to have potential anti-rheumatic activity as a disease-modifying drug [5-7]. Being a BCS class II compound SSZ shows low aqueous solubility and hence limits its absorption through the gastrointestinal tract and eventually reduces the oral bioavailability. The oral bioavailability of SSZ is 15%, which is due to low aqueous solubility and PgP efflux. MM are known to improve the bioavailability of drugs due to increased solubility, inhibition of PgP efflux and lymphatic uptake [8-12]. The present study describes the formulation and optimization of mixed micelles of sulphasalazine using a suitable polymer combination. The formulation is optimized and evaluated for *in-vitro, ex-vivo* and *in-vivo* performance.

MATERIALS AND METHODS

Materials

Sulfasalazine was received as a kind gift from IPCA laboratories [Mumbai, Maharashtra, India]. Soluplus was received as a gift sample from INTAS Pharmaceuticals LTD. [Ahmedabad, Gujrat, India]. Pluronic F 127 was purchased from Sigma Aldrich [Mumbai, Maharashtra, India] and all other chemicals were procured from the local sources.

Methods

Drug excipient compatibility study

FTIR spectra of Soluplus, Pluronic F127, SSZ and mixture of these stored at 40°C for two weeks were recorded on FTIR spectrophotometer (JASCO FTIR-8400, Japan) in the range of 4000–400 cm^{-1}.

Determination of CMC of polymers

Iodine UV-visible spectroscopy method was used for the determination of CMC of individual as well as a binary mixture of polymers [10-16]. Briefly, a stock solution of (0.3% w/v) KI/I2 was prepared. A series of aqueous micellar/mixed micellar solutions (0.000001% to 0.1% w/v) in varying concentration were prepared. 1 ml of a prepared standard solution of KI/I2 was added to each of these micellar/mixed micellar solutions. The solution was incubated for overnight at room temperature in dark. The UV-absorbance of varying polymer concentration at 225 nm was measured using a UV-visible spectrophotometer. CMC value was determined by plotting absorption intensity of iodine against the amount of polymer.

Preparation of SSZ-MM

Ethanol solvent evaporation method was used for the preparation of SSZ-MM. SSZ(100 mg) was blended in a binary mixture of Soluplus and Pluronic F127 and dissolved in 20 ml of ethanol and then sonicated for 20 min [1,2]. This solution was vacuum-dried in a rotary evaporator under a vacuum of -0.1 M Pa at a temperature of 55°C to remove ethanol. The formed film was rehydrated in 10 ml of deionized water and shaken for 30 min, non-incorporated SSZ was removed from the solution by filtration with 0.45 µm membrane and the SSZ-MM was obtained.

Optimization of SSZ-MM Using 32 factorial design

A full-factorial experimental design was used and data analysis using design expert fitted to significant quadratic polynomial models with high correlation coefficient. In order to optimize the preparation of formulations, the Soluplus (X1) and Pluronic F127 (X2) were chosen as independent variables and at three levels. According to a 32 full factorial experimental table (Table 1).

Table 1: A 32 full factorial experimental design layout.

Formulation code	Coded level	
	Soluplus (Factor 1) mg	**Pluronic F 127 (Factor 2) mg**
F-1	400	300
F-2	400	200
F-3	400	100
F-4	300	300
F-5	300	200
F-6	300	100
F-7	200	300
F-8	200	200
F-9	200	100

Characterization of micelles

Particle size analysis

MM formulations were diluted with ultrapure water to produce a suitable concentration for particle size analysis. The particle size analysis of the prepared SSZ-MM dispersion were performed using zetasizer ZS 90 (Malvern Instruments, Worcestershire, UK), utilizing laser diffraction with beam length 2.40 mm, range lens of 300 RF mm, and at 14.4% obscuration. The mean diameter of each batch is recorded in Table 2.

Table 2: Values of particle size, entrapment efficiency and zeta potential of SSZ-MM (R1-R9) as per full factorial design.

Formulation code	Particle size (nm)	% EE	Zeta potential (mV)
F-1	60.06	70.02	-19.00
F-2	50.41	61.96	-18.70
F-3	50.05	62.09	-16.40
F-4	58.40	85.74	-19.00
F-5	46.31	59.82	-20.50
F-6	47.23	63.41	-18.20
F-7	62.73	52.08	-18.00
F-8	61.97	37.53	-19.02
F-9	67.00	25.12	-18.50

Zeta potential (ZP) measurements

ZP was determined by measuring the electrophoretic mobility using Malvern Zetasizer Nano ZS 90 (Malvern Instruments, UK). Samples were prepared using double distilled water. The field strength applied was 20 V cm−1. The conversion into the zeta potential was performed by the software using the Helmholtz–Smoluchowski equation:

$$\varsigma = EM \times 4\pi\eta/\varepsilon \text{----------Eq. 1}$$

Where, ςis zeta potential, EM is electrophoretic mobility, η is viscosity of the dispersion medium, and ε is dielectric constant. Prior to the measurement, all samples were diluted using distilled water.

Determination of entrapment efficiency

SSZ-MM solution was diluted with methanol and bath sonicated for 20 minutes to disrupt the micellar structure [1]. Absorbance was measured using UV-visible spectrophotometer at 360 nm. EE was calculated according to the following equation:

% EE = (The amount of entrapped drug in MM)/(The amount of entrpped drug in MM and free drug in dispersion) × 100 ------- Eq. 2

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed on a Mettler-Toledo DSC 821e instrument, and an empty standard aluminium pan was used as reference. DSC scan were recorded at a heating rate of 10° C/Min in a temperature range 30-300°C. DSC measurements were carried out on pure sulfasalazine and MM loaded with sulfasalazine

In-vitro drug release study

Comparative drug release of A-SSZ (SSZ (100 mg)) dispersed in distilled water (10 ml) and sonicated for half hour) and SSZ-MM was carried out by dialysis bag method. 5 ml of both the samples were placed in a dialysis bag which was immersed into a beaker containing 300 ml phosphate-buffered saline (PBS, pH 7.4) with continuous magnetic stirring at 100 rpm/min. At predetermined time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 12 and 24 h) 5 ml of sample was withdrawn from the dissolution media while the sink conditions were maintained. The samples were analyzed for SSZ content using UV-visible spectroscopy at 360 nm [13,17].

HPLC procedure for determination of SSZ

Preparation of stock solutions of sulfasalazine

Stock solution 1 mg/ml was prepared by dissolving sulfasalazine in methanol. Working stock solution for sulfasalazine was prepared by diluting appropriately stock solution to get the final desirable concentration range.

Preparation of spiked plasma samples

The linearity range was chosen as 2-10 µg /ml spiked plasma was prepared by taking 0.5 ml plasma, to which 0.5 ml solution of sulfasalazine. The contents of the tubes were vortexed for 5 min. Later 1 ml Methanol was added to it vortex mixing was done for 10min. It was kept for partitioning for 30 minutes vortexing it intermittently at 5 minutes interval. After which the organic layer was collected and one more successive washing to plasma was given with methanol. Then all the collected organic solvent was evaporated dryness and later reconstitution with Mobile phase was done 50 µl aliquots of each concentration [15].

***Ex-vivo* endocytic uptake study**

Endocytic uptake study of the prepared MM across rat intestine was evaluated using everted rat intestine model [14]. One end of the isolated intestine everted using glass rod was clamped and secured with a silk suture, while from the other open end 1 ml of Phosphate Buffer pH 6.8 was filled using a syringe. The proximal end was then carefully secured using silk suture and the resultant sac was incubated in a dispersion of SSZ-MM (effective concentration equivalent to 1 mg/ml). Everted gut sacs incubated with specific endocytic inhibitors like chlorpromazine (CZ) (concentration of 10 µg/ml) at 37°C for 30 min. After 30 min of incubation, the phosphate buffer from the intestinal sacs was carefully collected in a test tube and subjected to HPLC analysis.

***In-vivo* pharmacokinetic study**

For *in-vivo* pharmacokinetic studies, Male Wistar rats weighing 200–230 g were used. The protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of Pune University, Pune, India. The animals were divided into three groups (n = 6). Group I is in control. Group II and III were administered 30 mg/kg Body weight of sulfasalazine either as a solution of the free drug (group II) or as sulfasalazine-MM (group III) orally using an oral feeding cannula. The blood samples (0.5 ml) were withdrawn from retro-orbital and, collected into heparinized microcentrifuge tubes (containing heparin equivalent to 50 µL per ml blood) at different time intervals. Plasma was separated by centrifuging the blood samples at 6000 rpm for 10 min. After centrifugation, the plasma obtained was stored at −20°C until analysis [18,19].

RESULTS AND DISCUSSION

Determination of CMC of polymers

The CMC of individual polymer i.e., Pluronic F127 and Soluplus was found to be 4.8 mM and 7 × 10^{-4} mM, respectively. Combination of Soluplus and Pluronic F127 in different combinations such as 1:1, 1:4 and 4:1 had CMC values 1.125*10-2, 5.63 × 10^{-2} and 5 × 10^{-2}. (Figure 1) Combination of soluplus and pluronic in ratio 1:1 resulted in significant reduction in CMC value. Hence the polymers were used in a ratio (1:1) for further studies. The CMC of mixed micelles observed to be lower than CMC of individual polymer and gives a certainty of MM formation. According to the literature, higher hydrophobic chain length and its interaction with micellar structure decrease the CMC and help to retain the structural integrity and stability of MM upon dilution [20].

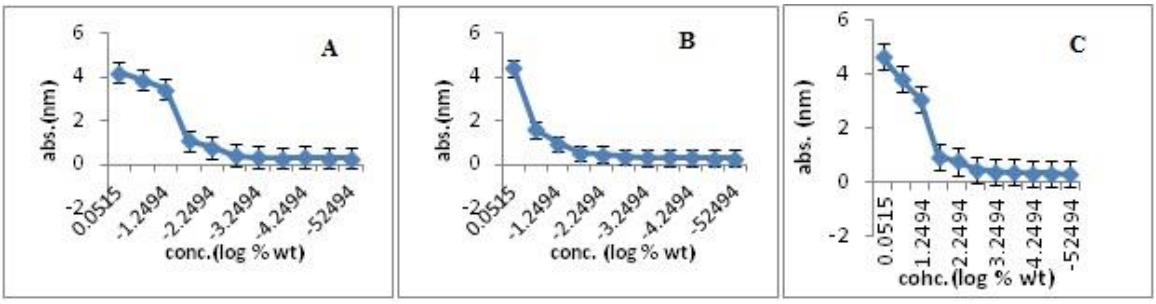

Figure 1: Critical micelle concentration of binary mixture of soluplus: pluronic F127 ratios. A) 1:1 B) 1:4 C) 4:1

Experimental design and statistical analysis

Micelle formation may not occur if the hydrophobic content in the system is too high while a rise in the hydrophilic region may lead to destabilization of the system [13]. Hence, selection of the optimum amount of the polymers was a necessity for stable micelle formation. Based on preliminary studies and CMC calculations concentration of Soluplus and concentration of Pluronic F127 were selected as independent variables and particle size, zeta potential and entrapment efficiency as a response parameter (Table 2). A full factorial design was selected as it helps to study the effect of response parameters by changing both variables simultaneously with a minimum number of experimental runs. The factorial design has been reported to highlight the relationship

between the variables through minimum experimental error. SSZ-MM average particle size was found in the range of 40-70 nm indicates the dependence of particle size on the amount of both polymers and it has been mathematically expressed in equation 3.

PS = 47.53-2.82A+5.20B+3.57AB+4.68A2-18.19B2 -------Eq. 3

The regression coefficient R2 = 0.9408 indicated an excellent correlation between the polymer amount and the micellar size, all the factors selected for study were found statistically significant.

It was seen that micellar size was directly proportional to the amount of pluronic and inversely proportional to the amount of soluplus. As pluronic is a hydrophobic polymer, hydrophobic segment formed inner core and is able to realize high drug loading, hence as the concentration of pluronic increases particle size increases [21-25]. The simultaneous increase in the amount of both the polymers (A & B) shows a positive effect on micelle size and can be graphically correlated using a response surface plot represented in Figure 2.

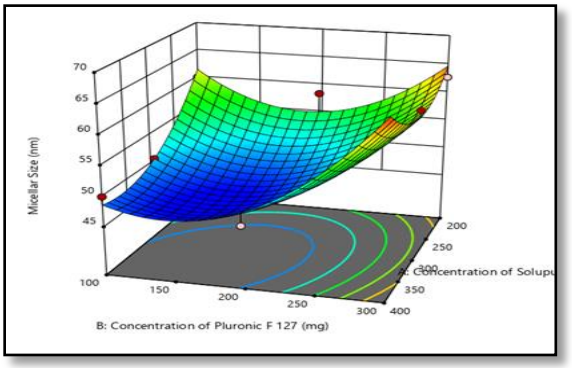

Figure 2: Three-dimensional response surface plots for particle size.

Hydrophobic segment of the polymer reduces the interfacial free energy in micelle formation and therefore introducing the hydrophobic segment might have eased micelle formation. Furthermore, a combination of hydrophobic linkages in pluronic with a higher percentage of hydrophilic PEO linkages in soluplus prevented the aggregation of particles and ensured micelle size integrity in a nanometer range [19].

EE of SSZ-MM was found to be in the range of 25-85% indicating that response was strongly dependent on the amount of polymers. The effect can be explained with the help of equation 4.

% EE = +65.23-9.54A-13.22B-4.76AB+6.64A2-18.19B2 -------Eq. 4

Regression coefficient exhibits an excellent correlation between the polymer amount and EE indicating the high significance of the model. The coefficient of variable A (-9.54) and B (-13.22) was found to be negative depicting the inverse correlation between both the polymers and EE. Interaction of both shows negative value, concentration of Pluronic F127 shows more effect on entrapment than Soluplus. As Pluronic is a lipophilic polymer, thus from equation 4 and response surface (Figure 3) the shell

forming property of Soluplus due to hydrophilic linkages and the hydrophobic linkage in pluronic lead to incorporation of a large amount of drug in the micellar core. The decrease in entrapment of SSZ at high polymer concentrations can be attributed to the cumulative increase in the hydrophilic linkages on both the polymers. An increase in the quantity of Pluronic can also be related to the negative influence of on the entrapment of SSZ as it would eventually lead to distortion of micelle integrity [8,23,26].

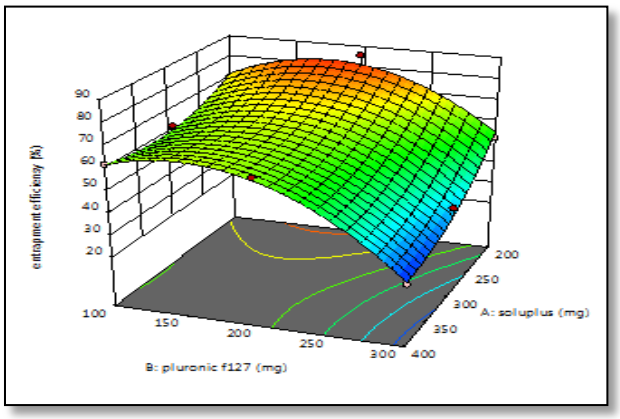

Figure 3: Three-dimensional response surface plots for % entrapment efficiency.

Zeta potential measurement was carried out to investigate the charge present on the micelles and to predict the physical stability of MM (Figure 4). However, due to the presence of non-ionic polymers low value of zeta potential were obtained (16-21 mV (eq.5)). These can contribute to the stabilization of micelles; improve the electrostatic stabilization which thereby reduces the interfacial tension of the formulation.

Zeta potential = -20.05+0.4833A-0.2367B-0.7750B+1.22A2+0.9633B2 -------Eq. 5

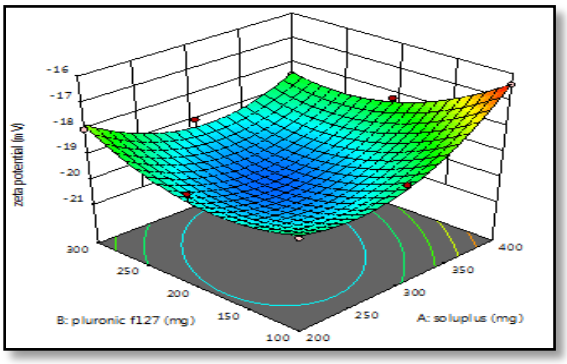

Figure 4: Three-dimensional response surface plots for Zeta potential.

After analyzing the polynomial equations depicting the dependent and independent variables, a further optimization and validation process by means of the design expert software was undertaken with desirable characteristics to search the optimal

formula solution of SSZ-MM which depended on the prescriptive criteria of maximum EE and minimum particle size. The predicted values of particle size, EE and zeta potential were 48.11 nm, 63.56% and -16.61mV respectively. Desirability of this solution was 0.819 in order to confirm the predicted model, a new batch of SSZ-MM according to the optimal formulation factors levels was prepared. The optimized formulation had an average particle size of 59.12 nm, entrapment efficiency of 62.04% and -16.4 ±1 Mv.

Drug excipient compatibility study

From FTIR study, the characteristic peak of drug such as of the aromatic O-H stretch (3654 cm^{-1}), N-H stretch (3413 cm^{-1}), C-H stretch (3031-2819 cm^{-1}), N-H bend (1677 cm^{-1}), Carbonyl group band (1757-167 cm^{-1}), C-O stretch (1668 cm^{-1}) disappeared and were replaced by the peak of Polymer where remaining peaks also either shifted or were replaced in the IR spectrum of the formulation shown in Figure 5 hence shows the drug compatibility with the polymers.

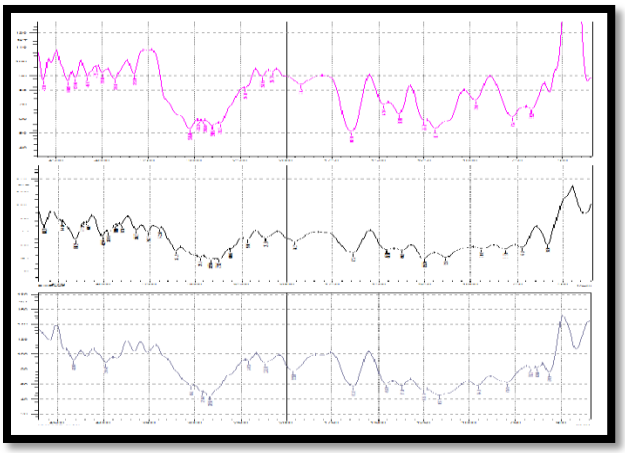

Figure 5: FT-IR Spectra of (a) Drug+Soluplus (b) Drug+Pluronic F127(c) Drug+Soluplus+Pluronic

DSC

DSC analysis was carried out to study the endothermic behavior and intermolecular interaction between the drug and polymer in the micellar system. The single sharp endothermic peak of SSZ was obtained at 256°C (reported 255º-260°C). The SSZ MM had melting endotherm at 178°C, SSZ-MM (Figure 6).

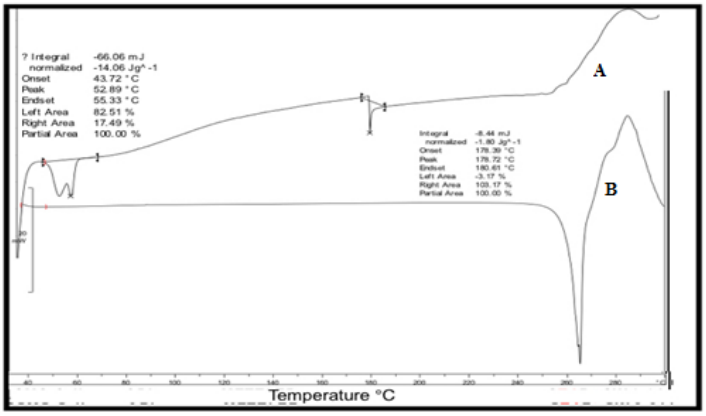

Figure 6: Differential scanning colorimetry thermograms. A) SSZ-MM B) SSZ.

The shifting and disappearance of sharp melting endotherm corresponding to SSZ indicate the transformation of crystalline nature of drug into amorphous form thus suggesting entrapment of drug into the hydrophobic core.

***In-vitro* drug release**

The drug release studies performed by dialysis bag technique over three replicates using PBS (pH 7.4) as the dissolution medium. The dissolution profile of SSZ-MM (Figure 7) fitted Korsemeyer Peppas kinetic model (R^2= 0.9882) with 42.21% & 78.07% drug release for A-SSZ & SSZ-MM respectively, at the end of 24 h.

In-vitro drug release from A-SSZ and SSZ-MM over a period of 24 h, could be correlated with the amount of drug and polymer in the micellar formulation. Entrapment of SSZ in the hydrophobic core sequestered it from the surrounding solvent and hydrophilic shell surrounding the core aided in sustaining the drug release up to 24 h [19,21]. It is supposed that a drug is released through several mechanism like diffusion through microchannels in polymer matrix and release by polymer degradation and solubilization. MM formed from soluplus and Pluronic F127 would exhibit significantly improved kinetic stability under various physiological conditions and facilitate the drug release.

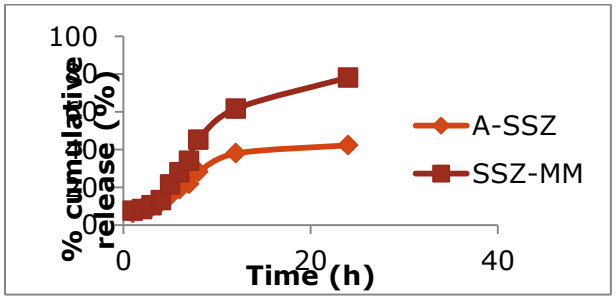

Figure 7: *In-vitro* release profile of SSZ-MM and A-SSZ (n = 6, mean ± S.D.)

***Ex-vivo* study**

There is abundant energy needed for the uptake of nanocarriers from the intestine. There are two receptor-mediated processes known as clathrin and caveolae-mediated endocytosis and an actin-dependent [14]. To confirm the involvement of these receptor-mediated processes in the uptake of MM from the intestine, studies were carried out in the presence of inhibitors of the clathrin and caveolae receptors. Chlorpromazine is an amphiphilic cationic drug of clathrin in endosomes and further reduces expression of the clathrin on the surface of the enterocytes.

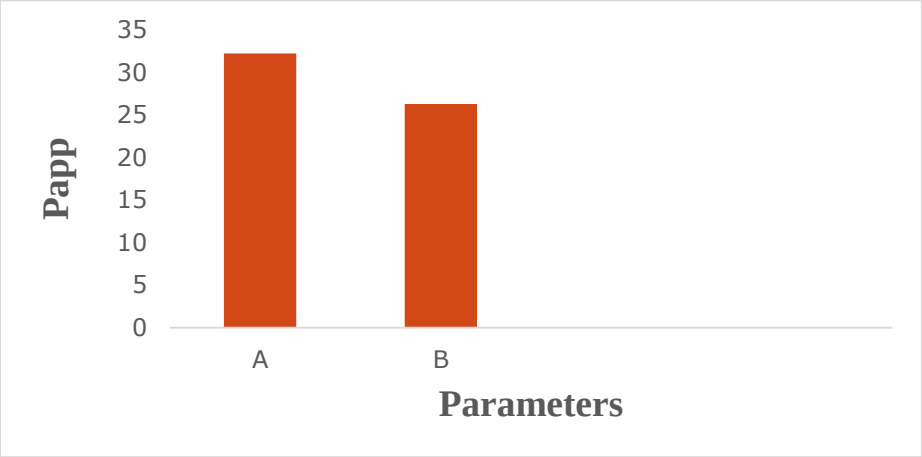

Figure 8: *Ex vivo* apparent permeability of SSZ-MM at A-37°C, B-in the presence of CPZ at 37°C (n=3).

Figure 8 represents the apparent permeability of SSZ-MM at 37°C and in the presence of CZ at 37°C, in the presence of CZ at 37°C measured in 30 min. The reduction in the apparent permeability of SSZ-MM in the presence of CZ at 37°C by 18.44% as compared with SSZ-MM at 37°C concludes involvement of receptor-mediated process in MM uptake of the intestine.

In-vivo pharmacokinetic study

The concentration-time curve after a single oral dose of A- SSZ and SSZ-MM formulations in rats is shown in Figure 9. The pharmacokinetic parameters after oral administration (Table 3) shows the concentration-time curve of SSZ-MM and A-SSZ. It was observed that for all the time points, the mean plasma concentration of SSZ-MM was greater than A-SSZ and it confirmed the superiority of SSZ-M. Around, 2.19 fold rises in the AUC_{0-24} of SSZ-MM was observed in comparison to A-SSZ after oral administration.

SSZ incorporated into micellar nanoformulation aid in bypassing extensive first-pass metabolism due to hydrophobic drug core association. Also, the micellar systems have an ability to reducing the metabolism and elimination of the drug from the biological system due to its nano size. The nonionic nature of polymers decreased the electrostatic repulsion between the drug molecules and formed a hydrophilic shell around the core which drastically increased the solubility and stability of the formulation.

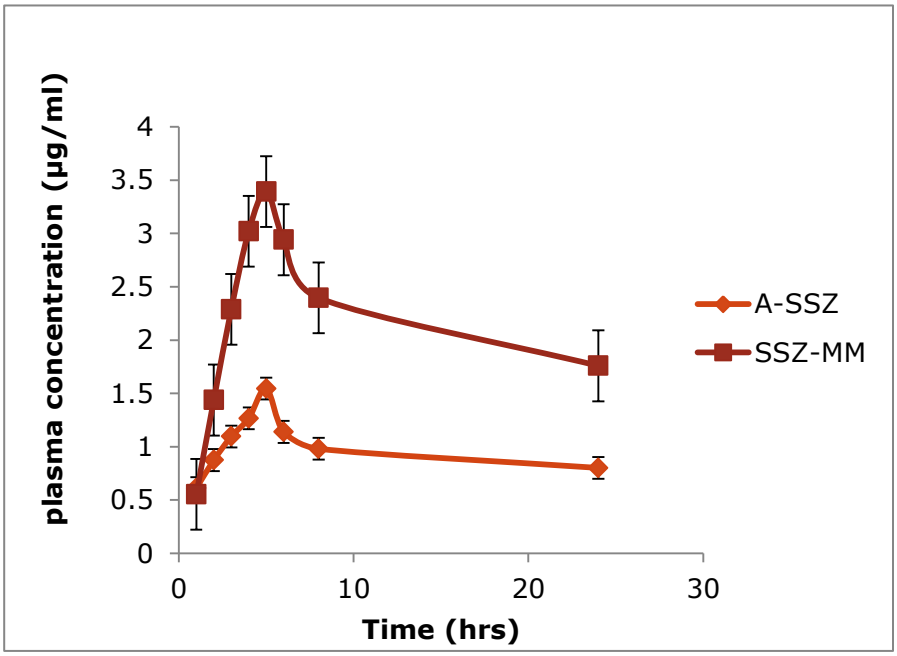

Figure 9: *In-vivo* plasma concentrations profile of A-SSZ and SSZ-MM in rats (n=3, ± S.D.)

Table 3: *In-vivo* pharmacokinetic parameters of A-SSZ & SSZ- MM in rats.

Parameters	**Sulfasalazine suspension (A-SSZ)**	**Sulfasalazine-MM (SSZ-MM)**
C_{max}	1.545 µg/ml	3.391 µg/ml
T_{max}	5 hour	5 hour
$T_{1/2}$	2.5 hour	2.4 hour
AUC	22.3416	50.7663

CONCLUSION

The potential for proficient oral delivery of poorly water-soluble drugs such as SSZ using MM was demonstrated. A combination of Soluplus and Pluronic F127 provided mixed micelles with small particle size and good entrapment which provided sustained drug release and was demonstrated in *in-vivo* studies in rats. Mixed micelles established itself to be a stable formulation and offered a potential approach for enhancing the bioavailability of BCS class II drugs.

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REFERENCES

1. Sachan, N., et al. Biopharmaceutical classification system: A strategic tool for oral drug delivery technology. *Asian Journal of Pharmaceutics*. **2009**. 3(2):76.
2. Chavda, H., et al. *Biopharmaceutics classification system. Systematic Reviews in Pharmacy*. **2010**. 1(1):62.
3. Tsume, Y., Dissolution effect of gastric and intestinal pH fora BCS class II drug, Pioglitazone: New *in-vitro* dissolution system to predict *in-vivo* dissolution. *Journal of Bioequivalence & Bioavailability*. **2013**. 05(06).
4. Cagel, M., et al. Polymeric mixed micelles as nanomedicines: Achievements and perspectives. *European Journal of Pharmaceutics and Biopharmaceutics.* **2017.** 113: 211-228.
5. Mols, R., et al. Sulfasalazine transport in *in-vitro*, *ex-vivo* and *in-vivo* absorption models: contribution of efflux carriers and their modulation by co-administration of synthetic nature-identical fruit extracts. *Journal of Pharmacy and Pharmacology*. **2005.** 57(12): 1565-1573.
6. Chungi, V., et al. A simple and rapid liquid chromatographic method for the determination of major metabolites of sulfasalazine in biological fluids. *Journal of Pharmaceutical Sciences*. **1989.** 78(3): 235-238.
7. Box, S., Sulphasalazine in the treatment of rheumatoid arthritis. *Rheumatology*. **1997.** 36(3): 382-386.
8. Bernabeu, E., et al. Novel Soluplus ® —TPGS mixed micelles for encapsulation of paclitaxel with enhanced *In-vitro* cytotoxicity on breast and ovarian cancer cell lines. *Colloids and Surfaces B: Biointerfaces*, **2016.** 140: 403-411.
9. Hou, J., et al. Preparation and evaluation of icariside II-loaded binary mixed micelles using Solutol HS15 and Pluronic F127 as carriers. *Drug Delivery*. **2016.** 23(9): 3248-3256.
10. Bhuptani, R., Soluplus based polymeric micelles and mixed micelles of Lornoxicam: Design, characterization and *in-vivo* efficacy studies in rats. *Indian Journal of Pharmaceutical Education and Research*. **2016.** 50(10.5530): 277-286.
11. Muynedo, L., Micelles of TPGS modified apigenin phospholipid complex for oral administration: Preparation, *in-vitro* and *in-vivo* evaluation. *Journal of Biomedical Nanotechnology*. **2013**. 9(10.1166): 2034-2047.
12. Yu, J., et al. Enhancement of oral bioavailability of the poorly water-soluble drug silybin by sodium cholate/phospholipid-mixed micelles. *Acta Pharmacologica Sinica.* **2010.** 31(6): 759-764.
13. Kanade, R., et al. Self-assembling raloxifene loaded mixed micelles: Formulation optimization, *in-vitro* cytotoxicity and *in-vivo* pharmacokinetics. *AAPS PharmSciTech.* **2017**.
14. Bhalekar, M., et al. Fabrication and efficacy evaluation of chloroquine nanoparticles in CFA-induced arthritic rats using TNF-α ELISA. *European Journal of Pharmaceutical Sciences*. **2016.** 84: 1-8.
15. Hierrezuelo, J., et al. Micellar properties of a mixed surfactant system constituted by n-octyl-β-d-thioglucopyranoside and sodium dodecyl sulphate. Colloids and Surfaces A*: Physicochemical and Engineering Aspects.* **2005.** 264(1-3): 29-36.
16. Jindal, N., and Mehta, S., Nevirapine loaded poloxamer 407/pluronic p123 mixed micelles: Optimization of formulation and *in-vitro* evaluation. *Colloids and Surfaces B: Biointerfaces*. **2015.** 129: 100-106.

17. Joshi, G., et al. Bioavailability enhancement, Caco-2 cells uptake and intestinal transport of orally administered lopinavir-loaded PLGA nanoparticles. *Drug Delivery*. **2016**. 23(9): 3492-3504.
18. Zhang, Z., et al. Improved solubility and oral bioavailability of apigenin via Soluplus/Pluronic F127 binary mixed micelles system. *Drug Development and Industrial Pharmacy*. **2017**. 43(8): 1276-1282.
19. Maximiliano, C., et al. Polymeric mixed micelles as nanomedines: Achievments and Perspectives. *European Journal of Pharmaceutics and Biopharmaceutics*. **2016.** 1-80.
20. Mols, R., et al. Sulfasalazine transport in *in-vitro*, *ex-vivo* and *in-vivo* absorption models: contribution of efflux carriers and their modulation by co-administration of synthetic nature-identical fruit extracts. *Journal of Pharmacy and Pharmacology*. **2005**. 57(12): 1565-1573.
21. Zhou, Q., et al. Thermosensitive pluronic F127-b-poly(ε-caprolactone) mixed micelles. *Journal of Controlled Release.* **2011**. 152: e125-e127.
22. Mingkwan, T., Pluronic® P123/TPGS and pluronic® F127/TPGS mixed micelles for the entrapment of itraconazole. *Chiang Mai J Sci*. **2015.** 42(1550-7033): 946-956.
23. Chauhan, R., Quantitative method development and validation of sulfasalazine in tablet dosage form by UV-spectroscopy. *International Journal of Pharm Sci Rev Res*. **2012.** 15(0976 – 044X): 120-122.
24. Mehanny, M., et al. Bisdemethoxycurcumin loaded polymeric mixed micelles as potential anti-cancer remedy: Preparation, optimization and cytotoxic evaluation in a HepG-2 cell model. *Journal of Molecular Liquids*. **2016.** 214:162-170.
25. Kulthe, S., et al. Polymeric micelles: Authoritative aspects for drug delivery. *Designed Monomers and Polymers*. **2012**. 15(5): 465-521.
26. Cui, X., et al. Mechanism of the mixed surfactant micelle formation. *The Journal of Physical Chemistry B.* **2010**. 114(23): 7808-7816.