INTESTINAL PERMEABILITY STUDIES OF SULPIRIDE INCORPORATED INTO SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEM (SMEDDS)

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ABSTRACT

The objective of the present study was to determine the intestinal absorption of sulpiride incorporated into SMEDDS by means of single-pass intestinal perfusion method (SPIP) in rat and to compare the effective permeability coefficient obtained with that of drug solution and micellar solution. The prepared SMEDDS and micelles formulations were investigated for droplets size. SPIP experiment was performed using the three formulations in three of the secluded regions of the small intestine (duodenum, jejunum, and ileum). The amount of the drug in the samples was estimated by HPLC and the effective permeability coefficients in rats were calculated. The human intestinal permeability was predicted based on rat effective permeability coefficient value. The dilution stability of the formulations was also determined. The average droplet size of SMEDDS and micelles was 9.27 nm and 7.20 nm respectively. The effective permeability coefficient of sulpiride was appreciably lower in the ileum weighed against jejunum and duodenum when administered as a solution ($p<0.05$). The estimated human absorption of sulpiride for the SMEDDS dilutions was superior to that from solution ($p<0.05$) and similar to micellar solution. The micellar dilutions were unstable whereas the SMEDDS dilutions were stable. Based on the above results, SMEDDS can be a potential candidate for improving the peroral absorption of the sulpiride.

Keywords: Sulpiride, single-pass intestinal perfusion technique, SMEDDS, micellar solution, effective permeability coefficient.

INTRODUCTION

Drug molecules which are poorly soluble and moderately and/or poorly permeable are classified under Biopharmaceutic Classification System (BCS); Class IV (Amidon *et al.,* 1995). Drug belonging to this class present a major obstacle for development of dosage form viable for oral administration and in most cases these drugs are administered parenterally by addition of solubility enhancers into the formulation (Sachan *et al.,* 2009). Even though most of the BCS class IV drugs show an outstanding *in vitro* pharmacological affect at minute concentrations they donot enter the later stages of drug development as they have low solubility and therefore low and variable absorption when administered orally.

In this decade a lot of research has been focused on developing, lipid drug delivery systems in order to enhance the oral bioavailability of drugs that are poorly soluble and in particular the self-microemulsifying drug delivery systems (SMEDDS). Generally these systems are isotropic mixtures of oils, surfactants and co-solvents/co-surfactants (Gursoy and Benita, 2004). Once administered into the gastrointestinal system, they are diluted with gastro intestinal fluid and the gastric motility provides the agitation for the formation of a fine oil-in-water (o/w) micro emulsions (SMEDDS) (Shah *et al.,* 1994). The difference between a SEDDS and SMEDDS is that the former when diluted results in a droplet size between 100 and 300 nm and the later results in a droplet size of less than 50 nm (Gursoy and Benita, 2004). For drugs which are lipophilic in nature this system would enhance the dissolution rate and therefore might improve the oral bioavailability (Cooney *et al*., 1998; Porter and Charman, 2001; Shen and Zhong, 2006; Singh *et al.,* 2009).

Sulpiride is a drug with proven antipsychotic and antidepressant activity. It is reported to be a selective dopamine D2 antagonist. Erratic absorption of this drug is been reported when given orally and the T_{max} reported was 2 to 6 hours after oral administration. One of the causes for this low oral bioavailability (30%) was poor solubility in water **(**Parikh *et al.,* 2009**).** This drug undergoes very minimal metabolism, as shown by the amount excreted in the urine unchanged. About 70% to 90% of i.v dose and 15% to 25% of oral dose is excreted unchanged in the urine. However, most of the drug has been recovered in the feces. The plasma elimination half-life of the drug has been reported to be 6 to 8 hours (Haung *et al.,* 2001). This drug has been reported to be a BCS class IV molecule (Rinaki *et al.,* 2003). Baluom *et al.* (2001) reported the drug to be a substrate for P-

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glycoprotein (P-gp). Henceforth, the poor oral absorption of sulpiride can be attributed to low solubility/dissolution rate, low permeability and P-gp efflux. According to the data published by our group, SMEDDS formulation was able to significantly increase the oral bioavailability of sulpiride in rabbits (Chitneni *et al.,* 2010). We speculated that enhancement of dissolution rate, permeability and P-gp inhibition as the factors for enhanced oral bioavailability of sulpiride. However, enhancement of dissolution rate was only reported in that paper by experimentation. Further research was done to know whether enhancement in the permeability also contributed to the overall enhancement in the oral bioavailability.

Among the various techniques available for determination of drug intestinal permeability, in situ single-pass rat intestinal perfusion method is widely used. A strong relationship between the jejunum permeability in rats and percent drug absorbed in human has been reported in the literature (Fagerholm *et al.,* 1996; Zakeri-Milani *et al.,* 2007).

The objective of the present study was to determine the permeability of sulpiride prepared as SMEDDS in comparison with pure drug solution and the micellar solution in three intestinal segments, duodenum, jejunum and ileum using single-pass intestinal perfusion technique in rats. Colon section was not considered as minimal absorption takes place from there (Liu *et al.,* 2009).

MATERIALS AND METHODS

Materials

Sulpiride was procured from Shanghai PI Chemicals Ltd (China). Acetonitrile and methanol of HPLC grade were purchased from J.T.Baker (Phillipsburg, NJ, USA). Sodium chloride, potassium chloride, sodium bicarbonate, magnesium chloride, sodium dihydrogen phosphate, glucose, Tween 80 and propylene glycol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Oleic acid was purchased from Fischer chemicals (USA). All the other chemicals used were of analytical grade.

Krebs-Ringer perfusion solution preparation

The perfusion solution was prepared by dissolving 7.8 gm sodium chloride, 0.35 gm potassium chloride, 1.37 gm sodium bicarbonate, 0.02 gm magnesium chloride, 0.22 gm sodium dihydrogen phosphate and 1.48 gm glucose in 1000 mL of distilled water (Song *et al.,* 2006).

Preparation of drug solution, SMEDDS and micellar solution

The formulation details are shown in table 1. The drug solution was prepared by dissolving required amount of sulpiride in Krebs-Ringer's buffer solution to yield 400 μg/mL. A mixture of the surfactants, co-surfactant and oils was prepared as per the formula. Sulpiride was then dissolved in the above mixture by stirring. Before the perfusion experiment, appropriate amount of Krebs-Ringer's buffer solution was added to adjust the formulation to 400 μg/mL concentration. The micellar solution containing equivalent surfactant and co-surfactant concentration was prepared in the similar way.

Table 1: Formulation compositions of sulpiride SMEDDS and micelle

Ingredients	Formulations	
	SMEDDS	Micelles
Sulpiride (mg)	20	20
Oleic acid (mg)	47.60	-
Tween 80 (mg)	634.90	634.90
Propylene glycol (mg)	317.50	317.50

Droplet diameter analysis

Dynamic light scattering (DLS) or photon correlation spectroscopy (PCS) is a method which has been used and quoted to be a suitable technique for determining the droplet diameter of microemulsions and SMEDDS (Craig *et al.,* 1995; Constantinides and Scalart, 1997; Khoo *et al.,* 1998; Porter *et al.,* 2004). Photon correlation spectrometer using laser light scattering (Zeta sizer 1000 HS, Malvern Instruments Ltd., Worcestershire, UK) was employed for droplet diameter determination. PCS is a technique of measuring particles using the principle of Brownian motion. The samples were put into a cuvette in a thermostatic chamber. Light scattering was monitored at 90° angle and a wavelength of 633 nm at 25°C. The experiments were run in triplicate.

Animals

Male Sprague-Dawley rats weighing between 180 to 230 gm were used for the study. The animals were placed in polypropylene cages at 25 ± 1°C and 60 ± 5% RH with free access to food and water. All the studies that involved the use of animals were performed in accordance with the Animal Ethical Guidelines for Investigations in Laboratory Animals and were approved by the Ethical Committee for Animal Experimentation of Universiti Sains Malaysia.

Study of drug solution stability in Krebs-Ringer perfusion solution

This study was conducted to find out if the drug was stable in Krebs-Ringer solution and Krebs-Ringer solution that passed through the intestinal segments. After a 12 hr overnight fast, the rats were anesthetized by intraperitonial injection of pentobarbital sodium (Nembutal injection, Ceva, France) at 60 mg/kg body weight. Upon verification of the loss of pain reflux, a midline abdominal incision of 3-4 cm was made using an electrical cautery (RB Model 708, RB Medical Engineering Ltd., Herefordshire, UK) and intestinal segments, namely, duodenum (8 cm), jejunum (10-15 cm)

and ileum (10-15 cm) were isolated and cannulated at both ends with plastic tubing. The plastic tubing at one end was connected to a perfusor (Perfusor secura FT, Braun Inc., Melsungen, Germany) to which a 50 mL syringe containing Krebs-Ringer solution was attached. The other end of tubing was used to collect the perfusate. The segment of interest was first rinsed with normal saline to clear the segment. The Krebs-Ringer buffer solution was then passed through the segment at a rate of 0.2 mL/min. The blank perfusate was collected at the outlet and used to prepare sulpiride (400 μg/mL) for stability study. All the prepared solutions were incubated at 37 °C for 3 hr in an oven. The drug content before and after incubation was estimated using validated HPLC method.

Binding studies

This study was conducted to ensure that the drug loss from perfusion was not due to binding of drug with the intestine wall. The intestinal homogenate was prepared after excising the required part of the intestine. The excised intestinal segment was washed with ice cold buffer and then inverted. Mucosa was collected from the inverted intestinal segment by scrapping the intestine using a blunt spatula. The intestinal mucosa was weighed and homogenized using a homogenizer (MSE homogenizer, MSE Scientific Instruments, England, UK) with ice cold Krebs-Ringer buffer to make a solution containing 20% w/v homogenate. The homogenate was centrifuged at 4000 rpm for 10 min (Labofuge 200, Heraeus Sepatech GmbH, Oesterode, Germany). The supernatant was collected and utilized to prepare drug solution as described earlier. The solutions were then incubated at 37 °C for 3 hr in an oven (Song *et al.,* 2006). The drug content in the intestinal homogenate before and after incubation was estimated using validated HPLC method.

Permeability study using single-pass intestinal perfusion technique

In this experiment, 36 male Sprague-Dawley rats were divided equally into nine groups. The experiment was carried out on three intestinal segments, duodenum, jejunum and ileum using drug solution, SMEDDS and micelle solution for each intestinal segment. The intestinal segment was isolated as follows: duodenum segment of 8 cm was isolated starting from the pylorus; jejunum segment of 10-15 cm was isolated 25 cm from the pylorus; and ileum segment of 10-15 cm was isolated 20 cm upwards from caecum (Song *et al.,* 2006).

The permeability study using single-pass intestinal perfusion technique was conducted using gravimetric method for estimation of net water flux (Sinko *et al.,* 1995; Svensson *et al.,* 1999; Song *et al.,* 2006). Sutton *et al.* (2001) reported that the gravimetric method was as accurate as the "nonabsorbed" marker method. The rats were treated as described under section Study of drug solution stability in Krebs-Ringer perfusion solution. The surgical area was wetted with normal saline, which was then covered with wet cotton to avoid loss of fluid. To initiate the experiment, a pump was connected to a syringe containing sulpiride (400 μg/mL). The perfusate samples were collected in pre-weighed Eppendorf® tubes and the weight recorded. The time was set to zero once the Krebs-Ringer buffer solution was completely pushed out of the intestinal segment. Each perfusion experiment lasted for 120 min and the perfusate was collected at time intervals of 5 min. At the end of the experiment, the length of the perfused intestinal segment was measured without any stretching. The animals were euthanized with a cardiac injection of saturated potassium chloride. The collected samples were stored at -20°C until further analysis using validated HPLC method.

HPLC method used for analysis of samples

A 500 μL aliquot of the rat intestinal perfusate solutions containing sulpiride was transferred into an Eppendorf® tube, followed by the addition of 50 μL of metoclopramide internal standard solution (60 μg/ml), 25 μl of 60% perchloric acid and 50 μL of acetonitrile. The mixture was vortexed (REAX 2000, Heidolph, Schwabach, Germany) for 2 min and then the supernatant was transferred to a new Eppendorf® tube. 500 μL of chloroform was then added and vortexed for 2 min. The mixture was then centrifuged at 10,000 rpm for 15 min (Labofuge 200, Heraeus Sepatech GmbH, Oesterode, Germany). A 100 μL of the aqueous supernatant was injected into the column. The calibration curve exhibited an excellent linearity over the concentration range of 50 to 4000 ng/mL. The LOD obtained was 30 ng/mL while the LOQ was 50 ng/mL. The recovery values were above 95 % for sulpiride and internal standard. The accuracy and precision values were within the USFDA limits.

Estimation of steady state intestinal effective permeability

The effective permeability was estimated using parallel tube model (Laurent *et al.,* 2001; Song *et al.,* 2006; Zakeri-Milani *et al.,* 2007) at steady state. Steady state was thought to be reached when the concentration of the drug in the perfusate was constant. The steady state intestinal effective permeability coefficient value for each segment was calculated using the equation:

$$P_{eff} = [Q_{in} \cdot \ln (C_{in} / C_{out}')] / A$$

Where Qin is the rate of perfusion (0.2 ml/min), A is the surface area within the intestinal segment that is assumed to be the area of a cylinder ($2\pi rL$) with the length (L) (measured at the end of the experiment) and radius (r) of 0.18 cm (Komiya *et al.,* 1980; Kararli, 1995), C_{in} and C_{out} are the inlet and fluid-transport-corrected outlet solution concentrations, respectively. The C_{out} was calculated by using the formula given below (Issa *et al*., 2003).

C_{out} (corrected) = $C_{out} \cdot (Q_{out}/Q_{in})$

Extrapolation of rat jejunal permeability to human absorption

The data from the rat jejunal permeability studies were extrapolated using the equation given below. The fraction absorbed (fa) is

$$fa = 1\text{-}e^{-\{2 \,.\, P_{eff,man} \,.\, t/r \,.\, 2.8\}}$$

Where, effective permeability coefficient in man

$$P_{eff,man} = 3.6.\ P_{eff,rat} + 0.03.\ 10^{-4}$$

t = 3 hr, the residence time in the human jejunum; r = 1.75 cm, the radius of the intestinal segment in human (Fagerholm *et al.,* 1996).

Evaluation of changes in droplet diameter of SMEDDS and micellar solutions

One mL of SMEDDS/micellar solution was diluted with 10 mL of 0.1 M HCl, phosphate buffer pH 6.8 and distilled water. After dilution, the droplet diameter of the dispersion was measured using Malvern zetasizer. The same sample was then placed in a water bath maintained at 37 ^{o}C for 24 hr. The droplet diameter of the sample was again measured. Monitoring the droplet size changes over a period of 24 hr was adequate as the drug hardly stays for more than 24 hr in the gastrointestinal tract. The surface morphology and droplet size of fresh and 24 hr incubated formulations was also examined using transmission electron microscopy (TEM; Leo 912 AB Eftem, Oberkochen, Germany). A drop of the diluted SMEDDS or micellar solution was directly deposited on the holey film grid and the morphology and droplet size of formulations was observed.

STATISTICAL ANALYSIS

The results are presented as mean ± standard deviation. The effective permeability coefficients obtained across various intestinal segments using different formulations were treated statistically using two-way analysis of variance. When there was a statistically significant difference, a post-hoc Tukey-HSD (Honestly Significant Difference) test was performed. To evaluate the stability of the drug in the intestine, binding of the drug to the intestinal segment and to evaluate the changes in droplet diameter of micellar solution, a paired sample t-test was used. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Stability of drug in Krebs-Ringer buffer solutions

There was no statistically significant changes, in drug concentration in the perfused Krebs-Ringer buffer solutions for sulpiride (duodenum (p= 0.187), jejunum (p = 0.159) and ileum (p = 0.073)). Drug was also stable in unperfused Krebs-Ringer buffer solutions.

Binding studies

There was no statistically significant changes, in the drug concentrations in all the intestinal segment homogenates (duodenum (p = 0.169), jejunum (p = 0.129) and ileum (p = 0.158)).

Droplet diameter measurement

The droplet diameter values of the formulations studied are presented in table 2. The droplet size of the SMEDDS and micellar solution were comparable. The small polydispersity values for all the formulations indicated, uniformity in the dispersed phase droplet size.

Table 2: Droplet diameter results of SMEDDS and micellar formulations. Mean ± S.D., N=3.

Formulation	Droplet diameter (nm)	Polydispersity
SMEDDS	9.27 ± 1.00	0.08 ± 0.01
Micelles	7.20 ± 1.01	0.07 ± 0.01

Permeability study using single-pass intestinal perfusion technique

The steady state was reached after 45 min as shown in fig. 1. The effective permeability coefficient values obtained, for different intestinal segments are shown in table 3. From the results presented in table 3, it can be observed that the effective permeability coefficient value for sulpiride solution decreased from the duodenum, to jejunum and ileum segment. The permeability coefficient values between duodenum and ileum were significantly different ($p < 0.05$), unlike duodenum and jejunum as well as between jejunum and ileum.

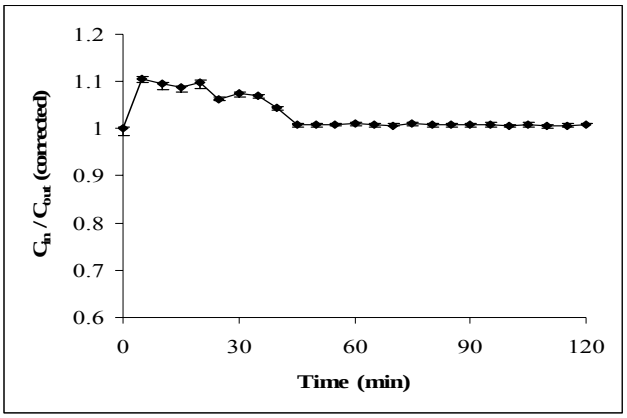


Fig. 1: Representative plot of the sulpiride concentration ratio of the outlet and inlet concentrations after correction with net water flux (C_{in}/ C_{out}) against time. Mean ± S.D., N=4.

When sulpiride was administered in SMEDDS and micellar solutions, the effective permeability coefficient values across all the intestinal segments were significantly increased statistically ($p<0.05$), which were approximately 3 times for duodenum, 4.5 times for jejunum and 5 times for ileum when compared with the drug solution. Nevertheless, there was no statistically

Table 3: Effective permeability coefficients (*P*eff) of different rat intestinal segments using different formulations of sulpiride. Mean ± S.D., N=4.

Formulation	Intestinal Segment P_{eff} X 10^{-6} (cm/sec)			
	Duodenum	Jejunum	Ileum	
Drug solution	3.10 ± 0.88	2.95 ± 0.95	0.92 ± 0.14	$p < 0.05$
SMEDDS	9.06 ± 0.53	9.12 ± 1.47	5.86 ± 0.62	
Micellar solution	8.69 ± 0.72	8.39 ± 0.79	5.64 ± 0.74	

significant difference ($p>0.05$) in the effective permeability coefficient values between SMEDDS and micellar solution across all the intestinal segments.

The permeability coefficient values of SMEDDS and micellar solutions increased from duodenum to ileum. The increase was significant between ileum and jejunum as well as between ileum and duodenum ($p < 0.05$). However, the permeability coefficient values of jejunum and duodenum were closely similar and not significantly different statistically ($p > 0.05$).

The rat jejunal effective permeability coefficient values obtained with different formulations were extrapolated to calculate the human oral absorption as the rat effective jejunum permeability data can be correlated to the human absorption (Fagerholm *et al.,* 1996; Laurent *et al.,* 2001; Zakeri-Milani *et al.,* 2007). The estimated % absorption values for sulpiride for different formulations are presented in table 4. There was a significant improvement in the estimated oral absorption of sulpiride in human when administered as SMEDDS or micellar solution, when compared with drug solution. There was an increase of approximately 2.2 times in percent drug absorbed. The mean % human oral absorption value estimated for sulpiride solution was 31.78 %.

Table 4: Estimated % human oral absorption from the rat jejunum effective permeability coefficient values for different sulpiride formulations. Mean ± S.D., N=4.

Formulation	Estimated human absorption (%)	
Drug solution	31.78 ± 6.10	$p < 0.05$
SMEDDS	70.70 ± 5.24	
Micellar solution	68.85 ± 4.13	

Changes in droplet diameter of SMEDDS and micellar solutions

The changes in droplet diameter of SMEDDS and micellar solutions containing sulpiride are presented in table 5. When observed under TEM, (fig. 2) the droplets appeared to be dark with bright surroundings and were found to be spherical in shape for the two formulations. The initial droplet size for both the formulations was less than 10 nm (fig 2a and 2c). It could be observed that the droplet diameter of micellar solutions increased significantly in 0.1 N HCl after incubation for 24 hr (fig 2b). Similar, results were observed for droplet size of micellar solution in other media also after 24 hr incubation (table 5). It can be noted that there was a slight increase in the values of mean droplet diameter for the SMEDDS formulation, in 0.1 M HCl, phosphate buffer pH 6.8, and distilled water, after 24 hr incubation. However, the increase in droplet size in all the media after 24 hr incubation was statistically insignificant ($p > 0.05$).

DISCUSSION

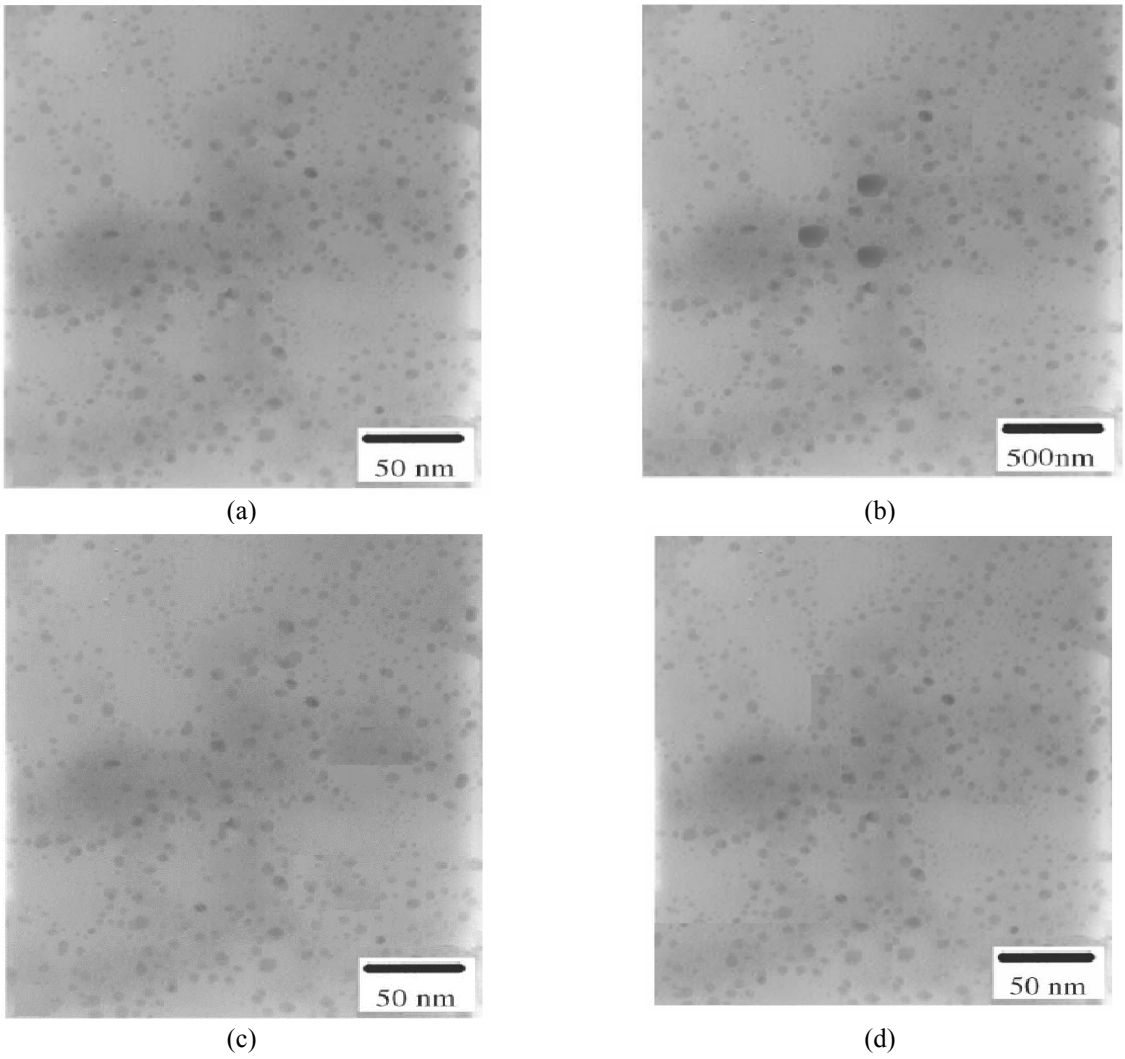
Drug was sufficiently stable throughout the duration of experiment (120 min) in all the solutions studied. As the aqueous solubility of sulpiride was 800 µg/mL (Kohri *et al.,* 1996), a lower concentration of 400 µg/mL was used in this study. The decrease in the permeability coefficient in the ileum segment may be attributed to P-gp efflux of sulpiride, as there is a moderate expression of P-gp expression in the duodenum and jejunum and maximal expression is found in the ileum (Yumoto *et al.,* 1999; Cao *et al.,* 2005). Similar, results were reported by Watanabe *et al.* (2004) for sulpiride in their study. The mean rat jejunum effective permeability coefficient value obtained, for sulpiride in the present study was 2.05 x 10^{-6} cm/sec, relatively lower than the value reported by Fotaki *et al.* (2005), which was 5 x 10^{-6} cm/sec. The difference in the permeability might be due to the highly variable absorption of sulpiride in rats, which was in the range of 20 to 50% (Zhao *et al.,* 2003; Watanabe *et al.,* 2004). The rat duodenal and ileum permeability coefficient values were 1.5 times more and 2 times less than that of the jejunum permeability. This result was in line with the findings of Watanabe *et al.* (2004) and Fotaki *et al.* (2005).

The presence of surfactant, Tween 80 in the SMEDDS and micellar formulations may have induced membrane perturbation and P-gp inhibition that enhanced the permeability of sulpiride (Zhang *et al.,* 2003; Shono *et al.,* 2004; Wu *et al.,* 2006). Tween 80 when administered to the rats was reported to cause intestinal membrane perturbation (Lo, 2003). Tween 80 consisted of a polyoxyethylene and hydrocarbon chain, which inculcate both lipophilic and hydrophilic properties to the surfactant, allowing it to partition between lipid and protein domains in the intestinal membrane and disrupting

its integrity, and may have increased the permeability of sulpiride (Swenson *et al.,* 1992). The multi-drug transporter, P-gp is phosphorylated by protein kinase C and phosphorylation modulates its transport function (Yang *et al.,* 1996). Tween 80 was reported to modulate the P-gp efflux by inhibition of protein kinase C activity and thereby reducing the phosphorylation of P-gp (Tang *et al.,* 2007). Similar, results were reported for nobiletin that no statistically significant difference ($p > 0.05$) in the permeability coefficient values between SMEDDS and

Table 5: Stability in terms of droplet diameter of sulpiride containing SMEDDS and micellar solutions upon dilution. Mean ± S.D., N=3.

Formulation	Medium	Droplet diameter (nm)			Polydispersity	
		Initial	After 24 hr		Initial	After 24 hr
SMEDDS	0.1 M HCl	9.28 ± 0.09	9.60 ± 0.27	$p > 0.05$	0.06 ± 0.02	0.07 ± 0.01
	Phosphate buffer pH 6.8	9.34 ± 0.10	9.39 ± 0.10		0.06 ± 0.01	0.07 ± 0.00
	Distilled water	9.29 ± 0.09	9.36 ± 0.05		0.06 ± 0.01	0.05 ± 0.01
Micellar solution	0.1 M HCl	7.42 ± 0.20	194.72 ± 13.96	$p < 0.05$	0.07 ± 0.02	0.44 ± 0.11
	Phosphate buffer pH 6.8	7.31 ± 0.20	190.49 ± 10.17		0.07 ± 0.01	0.49 ± 0.11
	Distilled water	7.08 ± 0.45	203.33 ± 11.62		0.08 ± 0.02	0.56 ± 0.12



(a) (b) (c) (d)

Fig. 2: TEM micrographs of (a) Fresh micellar solution (b) Micellar solution after 24 hr of dilution (c) Fresh SMEDDS dilution (d) SMEDDS after 24 hr of dilution.

micelles was obtained across all the intestinal segments (Yao *et al.,* 2008).

From the results, SMEDDS dilutions were found to be stable whereas micellar dilutions were unstable. The reason behind the instability of micellar solution may be the dissociation of the micelle into monomers and the precipitation of the entrapped drug (Rangel-Yagui *et al.,* 2005). The results obtained were consistent with the results obtained for acyclovir loaded SMEDDS which contained 90% of surfactant co-surfactant mixture of Tween 60/glycerin (2: 1) and 9% of sunflower oil (Patel and Sawant, 2007) and nobiletin loaded SMEDDS with 90% of surfactants (Cremophor® EL and Tween 80 (1:1))/co-surfactant (PEG-400) at 3.5:1 and 10% of oil (cradamol and tea oil (3:1)) (Yao *et al.,* 2008). Similarly, Quan *et al.* (2007) reported that there was no phase separation or coalescence after centrifugation at 4000 rpm for 15 min of a microemulsion obtained from dilution of puerarin loaded SMEDDS with water at a ratio of 1:250. The SMEDDS was composed of 13.5% of drug puerarin, 17.5% of oleic acid, 34.5 % Tween 80 and 34.5 % of propylene glycol. The higher percentage of propylene glycol present in the formulation did not result in loss of integrity of the microemulsion on dilution.

Introduction of SMEDDS pre-concentrate into the aqueous medium under gentle agitation results in the formation of fine oil-in-water microemulsion. Since the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous (Craig *et al.,* 1995; Kim *et al.,* 2000). Surfactants form a layer around the emulsion droplets and reduce the interfacial energy as well as provide a mechanical barrier to coalescence (Gursoy and Benita, 2004; Khoo *et al.,* 1998*)* and hence provide a stable emulsion.

CONCLUSION

In conclusion, the permeability coefficient was higher with the SMEDDS and micellar solutions containing sulpiride across all the intestinal segments compared to the drug solution. The estimated absorption of sulpiride in human for the SMEDDS dilutions was higher than that for drug solution ($p<0.05$) and similar with that for the micellar solution ($p>0.05$). Moreover, the sulpiride dilutions were more stable than the micellar dilutions. Therefore, SMEDDS would be an ideal drug delivery system for bioavailability enhancement of sulpiride.

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