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Preparation and characterization of reverse micelle based organogels of piroxicam

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Received September 13, 2002, accepted August 18, 2003

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Pharmazie 59: 191–193 (2004)

The solubilization of piroxicam to increase transdermal permeation rate was attempted by incorporating the drug in reverse micelle systems consisting of lecithin/isopropyl myristate/water [RMS-1] and sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/isooctane/water [RMS-2]. The change in polarity of water present in the water pool formed by reverse micelles resulted in a solubilization of piroxicam. These systems were used for the formation of reverse micellar organogels RMO-1 and RMO-2 by means of either varying hydration ratio (W_o) or by addition of a macromolecule, e.g. gelatin, into the system or by taking both the parameters in consideration. These systems were evaluated for physical properties, toxicology, *in vitro* and *in vivo* transdermal permeation. Significant ($p < 0.01$) inhibition of carrageenan induced rat paw oedema was observed for products RMO-1 and RMO-2 and a marketed transdermal product after 3 h.

1. Introduction

The reverse micelle composed of surfactants with hydrocarbon tails projecting towards the non-polar environment in the presence of a polar solvent can serve as a vector for the transdermal permeation of poorly soluble or less skin permeable drugs. The properties of water localized in reverse micelles differ from the bulk water. The system is cheap, non-toxic, more effective and amenable to release medication. The main disadvantage of the reverse micelle system is the use of organic solvents in the dispersed system, which may be nonbiocompatible with body fluids. The surfactants which have mostly been studied in this respect are sodium bis(2-ethylhexyl) sulfosuccinate (AOT), Tweens®, Spans®, poly(ethylene oxide), alkyl trimethyl ammonium halides e.g. CTAB and phospholipids e.g. phosphotidyl choline (Robinson et al. 1984). Among the organic solvents used as dispersion media are n-octane, iso-octane, heptane, cyclohexane, benzene and chloroform.

The oral administration of piroxicam is associated with adverse effects like nausea, vomiting, vertigo and peptic ulcer sometimes leading to gastric bleeding. It is absorbed from the gastrointestinal tract but is subjected to first pass metabolism. The manifestation associated with the gastric distress could be avoided by application in transdermal therapeutic systems.

For the present study, reverse micelle systems consisting of lecithin/isopropyl myristate/water (RMS-1) and AOT/iso-octane/water (RMS-2) were used for improving solubility and transdermal permeability of piroxicam. Lecithin was used in these systems because it is a naturally occuring surfactant with amphiphilic character. Further, the lecithin/isopropyl myristate/water system is bio-compatible, while the AOT/iso-octane/water system was taken for comparison. These systems were used for the formation of organogel. The other systems like Tween/isopropyl myristate/water, Span 60/isopropyl palmitate and Tween/squalene/water were also tried but they exhibited temperature dependent solubility characteristics.

2. Investigations, results and discussion

The critical micelle concentration (CMC) of lecithin and AOT was determined using an Abbe refractrometer. A sharp change in the refractive index of solution was observed at CMC. The CMC values of lecithin in isopropyl myristate and AOT in isooctane were found to be 1.6% w/v and 0.24% w/v respectively.

Table 1: Polarity probe studies; variation in λ_{max} of the RMS-1 and RMS-2 as a function of molarity ratio of water to surfactant (W_0)

Value of W_0	Changes in λ_{max} (nm) in	
	RMS-2	RMS-1
1	254.0	–
2	251.0	257.0
3	248.5	–
4	246.5	254.0
5	246.0	–
6	–	251.5
7	–	–
8	–	249.0
9	–	–
10	–	247.0
11	–	–
12	–	246.0

Table 2: Drug content in reverse micellar systems and organogels

Product	Drug content	
	mg	% of labelled amount
RMS-1	4.264	85.28
RMS-2	4.415	88.30
RMO-1	4.422	88.44
RMO-2	4.418	88.36

The reverse micellar gel based transdermal drug delivery systems of piroxicam were made using two reverse micelle systems RMS-1 and RMS-2. These systems were utilized for the formation of organogels RMO-1 and RMO-2 by means of either varying hydration ratio (W_0) or by addition of gelatin into the system or by taking both the parameters in consideration. W_0 is the molar ratio of water to surfactant i.e. $[H_2O]/[S]$ where [S] is the molarity of surfactant. The control products RMO-1C and RMO-2C were formulated similarly omitting the addition of drug.

The formation of reverse micelles was confirmed by investigation of the polarity of the water pool present in reverse micelles using aqueous nitrate polarity probes. Polarity probe studies showed that the nitrate ion had an n-π^* band around 246 nm in bulk water where non-bonding n electrons are less tightly bound as compared to π or σ electrons. The solvent sensitivity of this band arises from the differences in the hydrogen bonding ability of the ground and excited states of the oxygen electrons of nitrate. The variation in λ_{max} of n-π^* band of the nitrate ions as a function of hydration ratio (W_0) was investigated (Table 1). At low W_0 values, the n-π^* band appeared at higher wavelengths i.e. around 257 nm for RMS-1 and at 254 nm in case of RMS-2 indicating a lower strength of interaction between the pool water and nitrate ion. At higher W_0 values, the strength of the nitrate ion-water pool interaction increases and hence blue shift of the band to 246 nm occurs which is its normal value in bulk water (Leser et al. 1986).

The drug content in the reverse micellar systems and organogels was estimated spectroscopically at 520 nm (Suryanarayana et al. 1988) on a Shimadzu 1601 UV Spectrophotometer employing a dialysis membrane (MWCO-12400) in isopropyl myristate for RMS-1 and RMO-1 and in iso-octane for RMS-2 and RMO-2 (Table 2).

The *in vitro* permeation of piroxicam from the organogel formulations across the abdominal skin of rat using a Keshary-Chien diffusion cell (Keshary et al. 1984) maintained at a constant temperature of 37 ± 2 °C showed a linear relationship with time (Fig.). The lag time, which was about 45 min, could be attributed to solvation and permeation of drug (free/micelllar) across the skin into the receptor compartment. The cumulative percent release from different products after 8 h was 40.1% (RMO-1), 25.1% (RMO-2), 14.5% (RMO-1C) and 13.1% (RMO-2C).

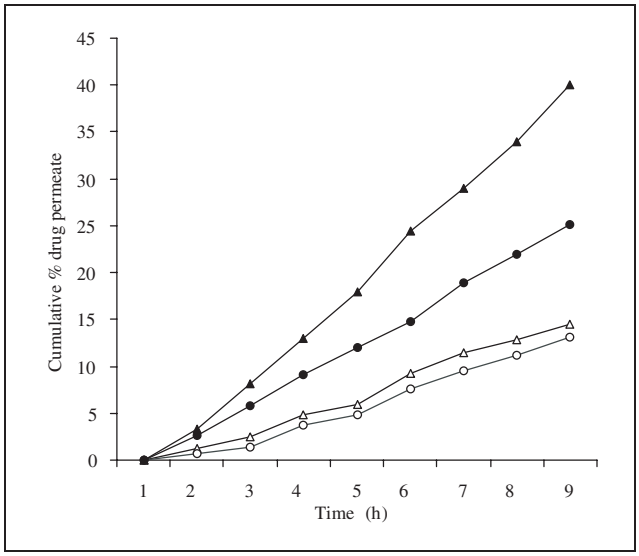

Fig: *In vitro* permeation profile of piroxicam
—△— RMO-1C, —▲— RMO-1, —○— RMO-2C, —●— RMO-2

Draize rabbit patch test (Mc Fadden et al. 2001] was performed for testing skin irritancy. The preparations produced no sensitization during three days observation period.

The anti-inflammatory effect was measured as inhibition in carageenan induced rat paw oedema (Bascatter et al. 1996) using a plethysmometer and compared with the marketed product containing an equivalent amount of drug. The mean percentage of paw oedema inhibition (measured in 5 rats) showed that RMO-1 was most active while no significant difference existed between the marketed product and RMO-2. The better activity of RMO-1 can be attributed to the presence of the naturally occurring surface active agent, lecithin.

$$\text{Percentage inhibition} = \frac{V_c - V_t}{V_c} \times 100 \qquad (1)$$

where V_c is oedema volume for control (maximum), V_t is oedema volume at 3 h. The percentage inhibition of oedema volume after transdermal application was in order RMO-1 > RMO-2 > marketed product (Table 3).

3. Experimental

3.1. Material

Piroxicam was obtained as gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad and Sigma Inc. U.S.A provided a gift sample of soya lecithin (MW 750). Sodium bis(2-ethylhexyl) sulfosuccinate (AOT, MW 444.5) and

Table 3: Data for in vivo evaluation of prepared products in albino rats

Time (h)	Change in hind paw volume (ml) on application of different products			
	Control	RMO-1	RMO-2	Marketed Gel
1.0	0.99 ± 0.02	0.92 ± 0.014*	0.95 ± 0.008 ns	0.97 ± 0.02 ns
2.0	1.75 ± 0.01	1.56 ± 0.006**	1.70 ± 0.011 ns	1.72 ± 0.018 ns
3.0	2.28 ± 0.008	0.95 ± 0.007**	1.50 ± 0.012**	1.67 ± 0.009**
4.0	1.95 ± 0.012	0.92 ± 0.002**	1.01 ± 0.01**	1.70 ± 0.006**
5.0	1.85 ± 0.009	0.90 ± 0.012**	0.91 ± 0.009**	1.80 ± 0.013*

* $P < 0.05$, ** $P < 0.01$, ns- not significant, One way ANOVA test (Dunneett's t Test), Readings are average of five animals ± standard deviation.

gelatin were procured from Ranbaxy Laboratories, Mumbai while other solvents and reagents used were procured from Central Drug House, Mumbai. Dialysis membrane (MWCO 12,400) was procured from Sigma Inc., USA. All solvents and chemicals used were of laboratory reagent grade.

3.2. Critical Micelle Concentration (CMC)

Solutions of lecithin in isopropyl myristate were prepared in the range 0.4% to 2.4% and that of AOT in iso-octane in the range 0.06 to 0.36% w/v. The CMC was determined using an Abbe refractrometer. A sharp change in the refractive index of solution was observed at CMC.

3.3. Preparation of reverse micelles

3.3.1. System 1: lecithin/ isopropyl myristate/water (RMS-1)

Step I: A 200 mM solution (25 ml) of lecithin was prepared by dissolving 3.75 g of lecithin in 25 ml of isopropyl myristate with continuous stirring at room temperature for 2 h using a magnetic stirrer. Double distilled water (0.18 ml) was added by means of a microlitre syringe ($W_0 = 2$). The dispersion of water could be achieved by subsequent vigorous stirring of the solution for a few minutes. Complete mixing was finally accomplished by slow stirring for 1–2 h.
Step 2: Accurately weighed piroxicam (20 mg) was added to the system under constant stirring. The stirring was continued for 15 min so as to help the micellar solubilisation of the drug.

3.3.2. System II: AOT/iso-octane/water (RMS-2)

Step 1: A 200 mM solution (25 ml) of AOT was prepared by dissolving 2.225 g AOT in 25 ml of iso-octane with constant stirring at room temperature using a magnetic stirrer for 2–3 h. Double distilled water (0.18 ml) was added with the help of a microlitre syringe to make $W_0 = 2$. The dispersion of water could be achieved by subsequent vigorous stirring of the solution for a few minutes. Complete mixing was finally accomplished by slow stirring for 1–2 h. Step 2 remained same as described for system I.

3.4. Characterization of reverse micellar systems

3.4.1. Polarity probe studies

Water was gradually injected in varied quantities, so as to produce hydration ratio (W_0) 1–12 in case of RMS-1 and 1 to 5 for RMS-2. The nitrate ions from potassium nitrate or sodium nitrate were used as probe. The concentration of potassium nitrate was used in such a way that $[H_2O : NO_3]$ was equal to 50 : 1 and each system was scanned for λ_{max}.

3.5. Conversion of RMS into organogel

3.5.1. Organogel from RMS-1

For preparation of organogel RMO-1 the previously prepared RMS-1 containing drug was used. From this the gel was prepared by stepwise addition of 0.09 ml double distilled water by means of a microlitre syringe to make W_0 equal to 3 at room temperature under constant stirring. Gel formation took place within 30 s after the addition of the critical amount of water.

3.5.2. Organogel from RMS-2

Similarly the organogel RMO-2 was prepared using RMS-2 containing drug. A concentrated gelatin solution (0.1 ml, 9.8% w/v) was added by means of a microlitre syringe to make $W_0 = 3$ at 40–50 °C under constant stirring. To this 0.5 ml of water-soluble crosslinking agent, glutaraldehyde was added so that the crosslinking readily takes place in the water pool. The stirring was continued with cooling to 30 °C until it turns very viscous and homogeneous. This cloudy viscous mixture was allowed to stand at room temperature, till it becomes a clear gel.
The control products RMO-1C and RMO-2C were also formulated similarly omitting the addition of the drug (step 2) in reverse micellar system formulation.

3.6. Drug content determination

The dialysis tube was washed with running water for 3–4 h in order to remove glycerin. The sulphate ions were removed by treating the tube with a 0.3% w/v solution of sodium sulfite at 80 °C for 1 min then washed with hot water at 60 °C for 2 min and acidified with 0.2% v/v solution of sulphuric acid. The tube was finally washed several times with hot demineralised water. An accurately weighed quantity (approx. 1g) of preparation was filled in the treated dialysis tube. After closing both ends of the tube, it was put into a beaker containing 50 ml of organic solvent i.e. isopropyl myristate for RMS-1 and RMO-1 and iso-octane for RMS-2 and RMO-2. The whole assembly was kept for 6 h for equilibrium dialysis in case of reverse micelle preparations and for 24 h in case of organogels. The tube was taken out and the contents were dissolved in methanol. After appropriate dilutions the drug content was determined spectrophotometrically at 520 nm.

3.7. In vitro permeation studies

The skin of albino rats from their abdominal region was carefully excised and hairs were removed by means of a razor cautiously ascertaining that hair roots are not cut out and thus barrier functions persist. The dermal side of the skin was cautiously cleaned of any adhering subcutaneous tissues and/or blood vessels with the help of tweezers and it was preserved in a normal saline solution. The excised skin (3 cm^2) was mounted on the receptor compartment of the diffusion cell. Such an amount of the product (aprox. 1 g), which is equivalent to 5 mg of the drug, was applied. The stratum corneum side faced the donor compartment while the dermal side was facing and touching the eluting bulk of the receptor compartment. From the receptor compartment containing 50 ml of saline phosphate buffer pH 7.2 samples (5 ml each) were withdrawn every hour and replaced with 5 ml of fresh PBS solution in order to maintain the sink condition. The withdrawn samples were diluted appropriately with a mixture of PBS (pH 7.2) and methanol (1 : 1). The samples were analyzed spectrophotometrically for drug content.

3.8. Toxicological evaluation

The hairs on both side of back of two rabbits were shaved and the skin was washed with antiseptic solution. One side of the back of each rabbit was taken as control, the other side for application of transdermal preparations RMO-1 and RMO-2. The observations were continued for three days for appearance of any sensitization reaction.

3.9. In vivo evaluation of the preparation

Five groups of albino rats weighing 250 ± 12 g were taken. The abdominal portion of each was cleaned and shaved. The marketed transdermal product (treatment 1), RMO-1 (treatment 2) and RMO-2 (treatment 3) containing drug equivalent to 250 μg and control formulations RMO-1C and RMO-2C were applied separately to different groups of rats. Carrageenan suspension (1%, 0.5 ml) were injected after one hour in right hind paw of the albino rats. A plethismometer was used to measure the paw volume in treated and control groups.

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