ENHANCED TRANSDERMAL DELIVERY OF KETOPROFEN FROM BIOADHESIVE GELS

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ABSTRACT

The aim of this study was to evaluate and compare the *in vitro* and *in vivo* transdermal potential of bioadhesive gels of ketoprofen by using gelling polymers like sodium carboxymethylcellulose, xanthan gum, poloxamer 407 and carbopol 934P as bioadhesive polymer with and without penetration enhancer (oleic acid). The effect of oleic acid as a penetration enhancer was examined when it was added to the bioadhesive formulations. Gels were evaluated for bioadhesive force and viscosity. To study the *in vitro* potential of these formulations, permeation studies were performed with Franz diffusion cell using excised rat abdominal skin. Carrageenan induced rat paw edema model was used to investigate their *in vivo* performance. The commercial formulation of ketoprofen was used as a reference formulation. The *in vitro* permeation studies indicate that ketoprofen bioadhesive gel of poloxamer 407 with penetration enhancer was superior to gels of sodium carboxymethylcellulose and xanthan gum with penetration enhancer (oleic acid). The permeation rate of ketoprofen from poloxamer 407 based bioadhesive gel with 15% v/w penetration enhancer was higher (rat abdominal skin flux = 0.421 ± 0.032 mg/cm^2/h) than the permeation rate of sodium carboxymethylcellulose and xanthan gum based bioadhesive gel with 15% v/w penetration enhancer. In the paw edema test poloxamer 407 based bioadhesive gel with 15% v/w penetration enhancer showed the best permeation and effectiveness. The *in vitro* and *in vivo* studies showed that bioadhesive gels of ketoprofen could be used for effective therapy.

Keywords: Ketoprofen; bioadhesive gel; permeation; rat skin; *in vivo* study.

INTRODUCTION

Ketoprofen (KP) is a potent non-steroidal anti-inflammatory drug that inhibits prostaglandin synthetase cyclooxygenase. KP is widely used for acute and long-term management of rheumatoid arthritis. However, the oral administration of this drug is usually accompanied by severe gastric and duodenal irritation, and because of its short half-life of 1.1-4 h, it requires frequent oral dosage (Lanza *et al*., 1998; El-kattan *et al*., 2000).

Percutaneous absorption of KP would be a possible alternative offering distinct advantages, such as elimination of gastric and duodenal irritation and delivering the drug directly to the inflamed site and thereby producing prolonged therapeutic local concentrations.

Percutaneous drug delivery has some advantages of providing the controlled delivery of drugs. In case of their application such as ointments, creams, it is difficult to expect their effects, because wetting, movement and contacting easily remove them. There is a need to develop the new formulations that have suitable bioadhesion. The percutaneous administration of bioadhesive gels has good accessibility and can be applied, localized and removed easily. Because of its excellent accessibility, self-placement of a dosage form is possible (Shin and Cho, 2006).

But skin is a natural barrier for transdermal administration and only a few drugs can penetrate the skin easily and in sufficient levels to be effective (Barry 2001; Moser *et al*., 2001).

Therefore, in recent years, penetration enhancers such as hydrogenated soybean phospholipids, ethanol, alcohols with long carbon chains (C_8 to C_{14}), n-octanol and cyclic monoterpenes, nonionic surfactants, propylene glycol and isopropyl myristate have been used in many studies to increase the percutaneous absorption of drugs (Nishihata *et al*., 1988; Parsaee *et al*., 2002; Ho *et al*., 1994; Iwasa *et al*., 1991; Santoyo *et al*., 1995). Membranes from rats, mice, pigs, guinea pigs, snakes, rabbits, and humans as well as synthetic membranes have been used for these drug diffusion studies (Nair and Panchagnula, 2004; Tokudome and Sugibayashi, 2003).

Recently, bioadhesive gels have been developed for piroxicam, triamcinolone acetate, benzocaine, griseofulvin and methotrexate (Santoyo *et al*., 1995; Shin *et al*., 1999; Shin *et al*., 2000; Shin *et al*., 2003; Vlachou *et al*., 1992; Lu and Jun 1998). These gels were studied for the bioadhesive character and diffusion parameters.

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Table 1: Composition of the bioadhesive gel formulations

Ingredients	Batch code											
	S	$S0_5$	$S0_{10}$	$S0_{15}$	X	$X0_5$	$X0_{10}$	$X0_{15}$	P	$P0_5$	$P0_{10}$	$P0_{15}$
Ketoprofen (g)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Na CMC (g)	1	1	1	1	-	-	-	-	-	-	-	-
Xanthan gum (g)	-	-	-	-	1	1	1	1	-	-	-	
Poloxamer 407 (g)	-	-	-	-	-	-	-	-	22	22	22	22
Carbopol 934P (g)	1	1	1	1	1	1	1	1	1	1	1	1
Ethanol (ml)	30	30	30	30	30	30	30	30	30	30	30	30
Oleic acid (ml)	0	5	10	15	0	5	10	15	0	5	10	15
Distilled water (g)*	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

*Final quantity was adjusted with distilled water to 100 g for constant 2.5%w/w of ketoprofen containing gel.

Oleic acid (OA) is considered to have good permeation enhancing property by acting as a lipid disrupting agent that increases the fluidity of stratum corneum lipid by increasing the formation of water channels (Naik *et al*., 1995; Fang *et al*., 2003; Francoeur *et al*., 1990).

Carbopols are excellent bioadhesive polymers but they have very low pH in the range of 2.5-3.0 (1% aqueous solution) (Ahuja *et al*., 1997). If used alone, they may cause irritation following topical application due to there low pH. Its irritant properties can be reduced by combining it with other non-irritant bioadhesive polymers.

Therefore, it was proposed to develop a topical bioadhesive gel systems of KP with and without penetration enhancer (OA). Polymers used are sodium carboxymethylcellulose (NaCMC), xanthan gum (XG), poloxamers 407 (PL407) and carbopol 934P (CB934P).

Bioadhesion properties, viscosity studies and *in vitro* permeation of these selected gels were studied through the excised full thickness rat abdominal skin. The prepared gels were compared with the conventional marketed gel formulation. Furthermore, pharmacodynamic studies of gels were evaluated for anti-inflammatory activity on a carrageenan-induced rat paw edema model. This study aimed to enhance topical penetration of KP from different bioadhesive gels and to compare with the commercial formulation available.

EXPERIMENTAL

Materials

Ketoprofen (KP) was obtained as gift sample from Themis Labs Pvt. Ltd., Mumbai, India. Sodium carboxymethylcellulose (NaCMC) and oleic acid (OA) were kindly supplied by s.d fine-chem. Ltd, (Mumbai). Xanthan gum (XG) and carbopol 934P (CB934P) were supplied from Loba Chemie Pvt. Ltd., (Mumbai). Poloxamer 407 (PL407) was supplied from BASF India Ltd., (Mumbai). All other chemicals used were of analytical grade.

Preparation of ketoprofen gel with NaCMC and XG

The required amount of gelling polymer (NaCMC or XG) and CB934P were weighed (table 1). Weighed polymers were added slowly in the beaker containing distilled water (40 ml) with continuous stirring at 400-600 rpm. The mixture was stirred continuously for 1 h until it forms a clear gel. Accurately weighed KP was dissolved in 30 ml of ethanol and the ethanolic solution of drug was added slowly with stirring (400-600 rpm) in the previously prepared polymer gel. Penetration enhancer OA was added with stirring. The final quantity was made up to 100 g with distilled water. The prepared gel was kept for 24 h for complete polymer desolvation.

Preparation of ketoprofen gel with PL407

PL407 possesses reverse thermal gelling property and therefore the gel containing PL407 was prepared by cold method (Schmolka 1972). The required amount of PL407 and CB934P were weighed (table 1). Weighed PL407 was added slowly in the 5^{o}C cold distilled water (20 ml) with stirring (400-600 rpm) and CB934P was separately dissolved in normal distilled water (5 ml) and stirred. Then CB934P solution was added to PL407 solution with stirring. Accurately weighed KP was dissolved in 30 ml of ethanol and the ethanolic solution of drug was added slowly in the previously prepared polymer gel with stirring at 400-600 rpm. Penetration enhancer OA was added with stirring. The final quantity was made up to 100 g with distilled water. The prepared gel was kept for 24 h at room temperature for complete polymer desolvation.

Bioadhesive testing

The method developed by Kim *et al*., 1999 and Choi *et al*., 2003 was slightly modified for studying the bioadhesive character of the prepared gels. The modified

apparatus used in the study is shown in fig. 1. The apparatus used for study comprised of two arm balance, one side of which contains two glass plates and other side contains a container. One of the two glass plates were attached permanently with the base of the stage and other one attached with the arm of the balance by a thick strong thread. The membrane used for bioadhesive testing was fresh rat intestinal membrane. Fresh rat intestine was glued to the upper side of the lower plate and another was glued to the lower side of the upper plate by using cyanoacrylate adhesive. The weighed gel (0.5 g) was placed on the rat intestine glued to the upper side of the lower plate. Then upper plate was placed over the lower plate and 50 g preload force (or contact pressure) was applied for 5 min (preload time). After removal of the preload force, the water kept in a bottle at some height was siphoned in the container (fig. 1) at the rate of 10 ml per min till the plates were detached from each other. The rate of dropping of water was controlled with on-off switch same as in infusion bottle. The weight of water required for detachment of glass plates was considered as the bioadhesion force of the applied gel.

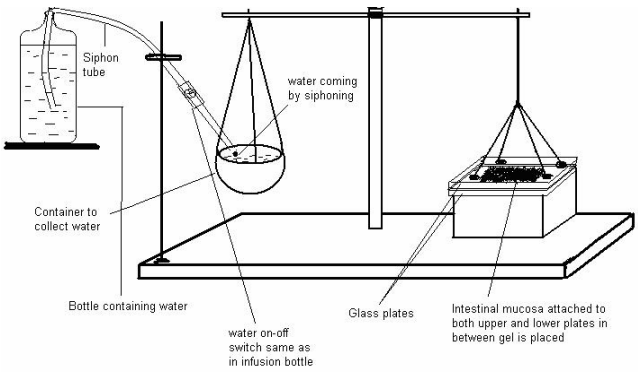


Fig. 1: Schematic diagram of fabricated modified bioadhesive force testing apparatus.

Viscosity studies

The viscosities of different KP bioadhesive gels were determined using a cone and plate geometry viscometer (Brookfield digital viscometer-III Rheometer V 3.3 HB) (spindle: 51 cps) at 10 rpm and 25 ± 0.1°C.

In vitro permeation studies

The percutaneous permeation studies were done by using modified Franz diffusion cell (Diameter of 1.5 cm with a diffusional area of 1.76 cm^2) and membranes used were shaved, full thickness, excised rat abdominal skin (Santoyo *et al*., 1995; Schmook *et al*., 2001).

Collection and preparation of rat abdominal skin samples: The animal study protocol was reviewed and approved by the Animal Ethics Committee at the Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, India. Male wistar rats weighing 160-180 g were used to excise full thickness skin. Rats were anaesthetized by ether and then hair of abdominal skin was removed by using electric clipper. Special care was taken while removing hairs, so as not to destroy the stratum corneum. A piece of full thickness skin sample (2.5 x 2.5 cm) was then excised from the shaved abdominal region. The dermal side of the skin was cleaned off any adhering subcutaneous tissues and/or blood vessels and the skins free of any adhering subcutaneous tissues and/or blood vessels were used for the permeation study.

In vitro permeation studies of gels: Prepared skin samples (rat skin) was mounted on the receptor compartment of the permeation cell with the stratum corneum facing upward and the dermis side facing downward. The donor compartment was kept on the receptor compartment and secured tightly with the help of clamps. The receptor compartment was then filled with 30 ml of pH 7.4 phosphate buffer. The temperature of media was maintained at 37 ± 0.5°C with the help of temperature controlled water jacket. Weighed bioadhesive gel or marketed gel (2 g equivalent to 50 mg of KP) was then placed in the donor compartment. The receptor compartment containing pH 7.4 phosphate buffer was stirred at 200 ± 5 rpm to maintain the hydrodynamics of the receptor fluid. Sampling (0.5 ml) was carried out at the different intervals up to 8 h. The volume of release media was maintained by adding equal volume of the fresh media after every sampling. The concentration of KP in the sample was measured spectrophotometrically at 258 nm.

Anti-inflammatory effect test

The anti-inflammatory activity of KP bioadhesive gels were evaluated with rat paw edema test. The bioadhesive gel or marketed gel (1 g) was applied to the shaved abdominal skin of male Wistar rats weighing 180-200 g. Just before administration of bioadhesive gels, 1% carrageenan-saline solution (0.1 ml) was injected into each hind paw of rats. The thickness of paw edema induced by carrageenan was measured by using a standard screw gauge during 8 h after application of KP bioadhesive gel.

STATISTICAL ANALYSIS

Results are given as mean ± standard deviation (S.D). The cumulative amount (mg/cm^2) of KP permeation through rat skin was plotted as a function of time (h). The slope of the linear portion of the plot is presented as the flux ($mg/cm^2/h$). The results were analyzed statistically using Student's *t*-test or one way analysis of variance (ANOVA). Significance was determined at $P < 0.05$.

RESULT AND DISCUSSION

Physicochemical characteristics of ketoprofen gel

The results of bioadhesive force and viscosity of KP bioadhesive gels were given in table 2.

The concentration of the different polymers NaCMC, XG and PL407 was selected to obtain the bioadhesive force between 80-100 gf and viscosity between 700-1000 cps (table 2). Increasing the concentration (5% v/w to 15% v/w) of penetration enhancers (OA) did not show any significant difference ($P<0.05$) in the bioadhesive force but significantly decreased ($P<0.05$) the viscosity of the bioadhesive gels.

Table 2: Bioadhesive force and viscosity of different bioadhesive gels.

Batch code	Bioadhesive force (gf) (mean ± *S.D**)	Viscosity (cps) (mean ± *S.D***)
S	96 ± 3.0	793.4 ± 1.52
$S0_5$	97± 5.2	785.6 ± 0.89
$S0_{10}$	95± 7.4	779.5 ± 2.01
$S0_{15}$	92 ± 1.2	760.1 ± 2.2
X	85 ± 4.3	789.5 ± 1.05
$X0_5$	86 ± 3.0	771.6 ± 1.87
$X0_{10}$	84 ± 4.0	765.3 ± 1.54
$X0_{15}$	80 ± 2.1	760.5 ± 2.54
P	98 ± 7.6	1174.6 ± 0.25
$P0_5$	101 ± 4.8	1092.1± 2.36
$P0_{10}$	99 ± 6.9	1080.5 ± 2.45
$P0_{15}$	100 ± 1.5	1054.1± 1.25
Marketed gel	88 ± 4.4	759.1± 0.25

* *n=5; S.D*: standard deviation for five determinations
** *n=3; S.D*: standard deviation for five determinations

Permeation studies using rat abdominal skin

As expected the flux of KP from OA containing bioadhesive gels were found significantly higher ($P<0.05$) than the flux of KP bioadhesive gel without OA (table 3). Increasing the concentration (5% v/w to 15% v/w) of penetration enhancers (OA) showed significant difference ($P<0.05$) in the flux of KP (Shin and Cho, 2006). The highest flux and enhancement factor for KP from the bioadhesive gels containing OA were found to be 0.421 ± 0.032 mg/cm^2/h (in the batch PO_{15}) and 29.71 (in the batch XO_{15}) respectively.

The highest cumulative amount permeated of KP at 8 h from bioadhesive gels of NaCMC, XG and PL407 with OA was found to be 2.220 ± 0.067 mg/cm^2, 3.649 ± 0.072 mg/cm^2 and 4.119 ± 0.196 mg/cm^2, respectively. The maximum amount permeated from bioadhesive gels of PL407 can be attributed to the surfactant effect of the PL407 (Santoyo *et al*., 1995).

Table 3: Permeation parameters of ketoprofen from different bioadhesive gels through rat abdominal skin (mean ± *S.D, n=3*)

Batch code	Rat abdominal skin	
	Flux (mg/cm^2/h)	Enhancement factor
S	0.022 ± 0.009	1.00
$S0_5$	0.124 ± 0.021	5.63
$S0_{10}$	0.163 ± 0.031	7.40
$S0_{15}$	0.220 ± 0.029	10.00
X	0.014 ± 0.008	1.00
$X0_5$	0.201 ± 0.022	14.35
$X0_{10}$	0.316 ± 0.034	22.57
$X0_{15}$	0.416 ± 0.030	29.71
P	0.084 ± 0.009	1.00
$P0_5$	0.141 ± 0.021	1.67
$P0_{10}$	0.257 ± 0.012	3.05
$P0_{15}$	0.421 ± 0.032	5.01
Marketed gel	0.159 ± 0.014	-

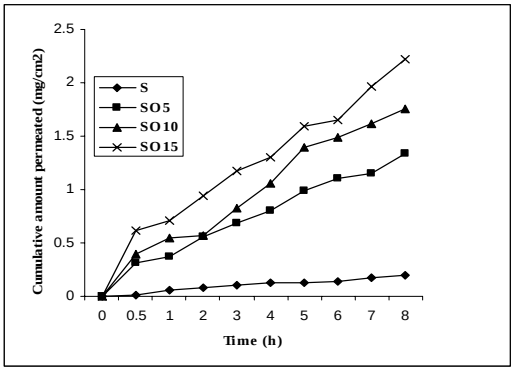
Batches with higher concentration of OA (batch SO_{15}, XO_{15} and PO_{15}) showed maximum fluxes of 0.220 ± 0.029, 0.416 ± 0.030, and 0.421 ± 0.032 mg/cm^2/h, respectively. The flux value for the marketed gel was found to be 0.159 ± 0.014 mg/cm^2/h which was three fold less than the PO_{15}.

The addition of OA increased the amount of drug permeated across the rat abdominal skin in all the bioadhesive gels. The increase in the concentration of penetration enhancer from 5%v/w to 15%v/w, as expected, increased the amount permeated and the flux (table 3 and fig. 2).

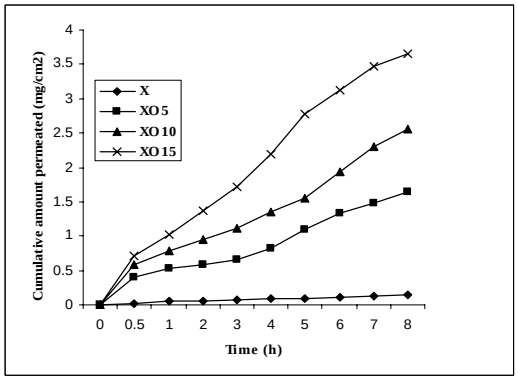
Anti-inflammatory study of selected bioadhesive gels

The *in vivo* pharmacodynamic studies were carried out by rat paw edema test on three selected bioadhsive gel formulations (batch SO_{15}, XO_{15} and PO_{15}) and the inhibition of swelling was compared with controls (without penetration enhancer) and the marketed gel. The gels without penetration enhancer (OA) showed less inhibition of the swelling or inflammation (fig 3). A significant inhibition ($P<0.05$) of inflammation was found with the gels containing OA in comparison to the gels without OA. There was no significant ($P>0.05$) difference in the inhibition of inflammation in between the gel SO_{15} and XO_{15}. However inhibition of inflammation shown by

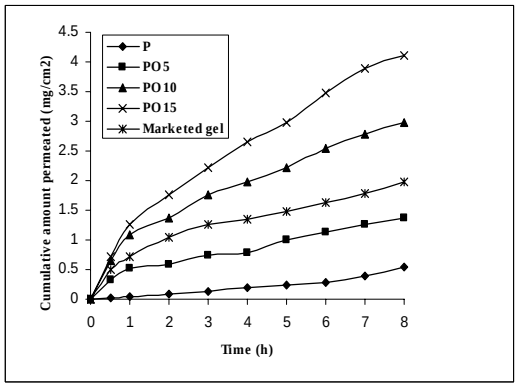
the gel PO_{15} was significantly greater ($P<0.05$) than the both SO_{15} and XO_{15}. The inhibition of inflammation was studied for the marketed gel and compared with the prepared gels containing OA. It was found that the inhibition of inflammation by the marketed gel was significantly less ($P<0.05$) than the all three selected bioadhsive gel formulations. The *in vivo* results were found to be in correlation with the *in vitro* permeation results.



(a)



(b)



(c)

Fig. 2: Cumulative amount of ketoprofen permeated from the gels containing sodium carboxymethylcellulose (a), xanthan gum (b), poloxamers 407 (c) through the rat abdominal skin

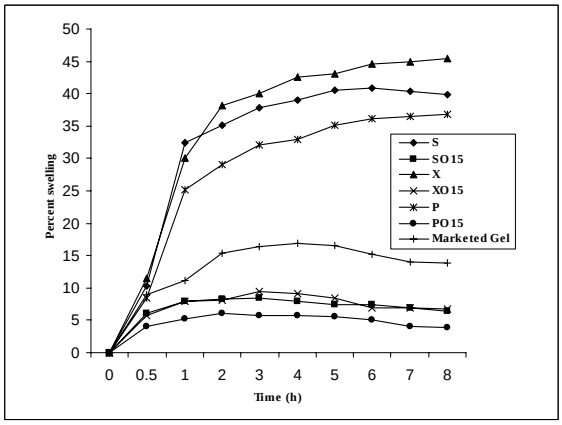


Fig. 3: Percentage swelling of carrageenan induced rat paw inflammation showing anti-inflammatory effect of ketoprofen bioadhesive gels and marketed gel.

CONCLUSIONS

This study demonstrated that incorporating KP into bioadhesive gels containing a penetration enhancer in various concentrations enhanced the drug permeation through rat skin and *in vivo* performance (table 3 and figs. 2-3). Bioadhesive gels of KP containing OA may offer promise as an anti-inflammatory dosage form, ensuring more effective therapy, but additional experiments should be performed before the formulation is used in humans.

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