



## Influence of Dimethyl Sulfoxide as a Penetration Enhancer of Piroxicam Gel Through Biological Skin

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### Abstract

Piroxicam is a non-steroidal anti-inflammatory agent which has an extensive use in rheumatic disorders. Since its skin penetration is still a subject for research, the aim of this study was to evaluate the effect of dimethyl sulfoxide on percutaneous penetration of piroxicam gel formulation through skin. In this study, as a model, two types of 0.5% piroxicam new gels, so called red and green gel, were prepared using 5% (w/w) DMSO only for the red gel. Water, ethanol and propylene glycol were the solvent composition specified by a triple phase diagram, in addition to carbomer P934 and hydroxypropyl methylcellulose as the gel bases. The release and dermal penetration of the drug were measured and compared with a commercial brand using static diffusion cells and hairless rat skin as a biological membrane, by UV spectrophotometer. Also, piroxicam serum level was measured after application of 1 g gel on the deltoid muscle, twice daily for two weeks, in three groups of healthy male volunteers. The results of all physico-chemical controls for the gels indicated an acceptable criteria. The penetration of piroxicam through animal skin showed a good linearity between the square root of time and amount of piroxicam released from the gels. The *in vitro* study showed that application of DMSO had no significant effect on percutaneous penetration of the drug through animal skin. In human study, the red gel containing DMSO had the highest piroxicam serum level with a relatively meaningful difference between the results compare to the green and commercial gels ( $p < 0.05$ ). But the green and commercial gels had no statistical difference. This preference might be related to the *in vivo* DMSO positive effect as a penetration enhancer, contrary to the *in vitro* results. Therefore, relying on laboratorial data is not always sufficient.

**Keywords:** Dimethyl sulfoxide; Gel; Piroxicam; Skin penetration.

**Received:** March 1, 2006; **Accepted:** July 14, 2006

### Introduction

Piroxicam is a non-steroidal anti-

inflammatory drug used in musculoskeletal and joint disorders. The most frequent adverse effects of piroxicam are reported at gastrointestinal track, on the other hand, local irritation and bleeding may occur with piroxicam suppositories. Also there may be

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**Table 1:** Formulation of piroxicam gels.

Materials	Functions	Green gel (%)	Red gel (%)
Piroxicam	Active ingredient	0.5	0.5
Carbamer 934	Jelling agent	0.6	0.6
Ethanol	Solvent	25	25
Propylene glycol	Co-solvent	10	10
Hydroxypropyl	Viscosity agent	0.2	0.2
$\alpha$ -methyl cellulose			
Sodium editate	Chelating agent	0.05	0.05
Benzyl alcohol	Preservative	1	1
<b>Dimethyl sulfoxide* (DMSO)</b>	<b>Penetration enhancer</b>	<b>0</b>	<b>5</b>
Di-isopropanol amine	Alkaling agent	qs to pH adjustment	qs to pH adjustment
Water	Vehicle	qs to 100	qs to 100

\*Penetration enhancer

pain and occasionally tissue damages for the injections of piroxicam [1-4]. The gels often provide a faster release of drug substance compared to creams and ointments independent from the drug's water solubility [5-8]. Gels effective as anti-inflammation and in musculoskeletal disorders are some recent products that are more formulated in pharmaceutical industries because of their oil free and water removable desirability, efficacy and harmless dermal effects. The most important goal of this research was measuring the permeability of piroxicam gels through the natural skin instead of a synthetic polymer. A necessary natural quality control test to make sure that a new gel has a sufficient efficacy with low toxicity before using in humans.

## 2. Materials and Methods

### 2.1. Materials

Piroxicam was from Esteve Quimica, Spain, and carbomer P 934 was from B.F Goodrich, USA. Disodium editate, propylene glycol, and dimethyl sulfoxide were purchased from Merck, Germany. Benzyl alcohol was from BDH chemicals, England, and hydroxypropyl methylcellulose from Shin-Etsu Chemical Co., Japan. Ethanol (HPMC) 96 was obtained from Parsian Co., Iran, and diisopropanolamine from Fluka Chemical, Switzerland. Piroxicam, carbomer

and HPMC were kindly donated by Iranian Razak, Shafa and Loghman pharmaceutical factories, respectively. All other materials were of analytical grade.

### 2.2. Preparation of the gels and pH adjustment

In the first step, benzyl alcohol and disodium editate were dissolved in deionized water. Then while the solution was agitated vigorously by an electrical mixer, carbomer powder, which had been dried for one hour at 80 °C previously, was added to the solution gradually. In the second phase, 10 g of HPMC mixture was prepared by dispersion of 0.2 g HPMC in a bout 5 ml of hot (80-90 °C) water, and an additional 5 g of 96% alcohols was added to the HPMC mixture and both solutions of the first and second steps were incorporated at this process. In the third stage, piroxicam powder which had been dispersed in 10 g of propylene glycol, was dissolved first by the required volume of 25% (w/w) diisopropanolamine solution and then water to a weight of 25 g. The third solution was added to the above incorporated solutions to prepare the final piroxicam gels. The red gel was different from the green gel just by replacement of 5% DMSO instead of the equivalent amount of water (Table 1). Gel formation in this formulation occurs by a chemical reaction which neutralizes the

**Table 2.** The volunteer's specifications.

Gels	Subjects No.	Age (year)	Weight (kg)	Height (cm)
		Mean±SD	Mean±SD	Mean±SD
Green	11	24.1±1.58	74.35±5.93	175.91±3.53
Red	9	23.2±1.10	70.00±2.74	177.20±2.28
Commercial	10	24.2±1.23	73.50±3.04	178.00±2.22

gelling agent. This pH needs to be adjusted at a range of 6.5 to 8.

### 2.3. Drug content test

The samples were provided by dissolving 1 g of each 5% piroxicam gel in 10 ml normal saline, separately. The blank also, was prepared with simple gel base and other inactive ingredients in normal saline. Samples containing drug were determined by a Jaco UV spectrophotometer (Japan), at 345 nm.

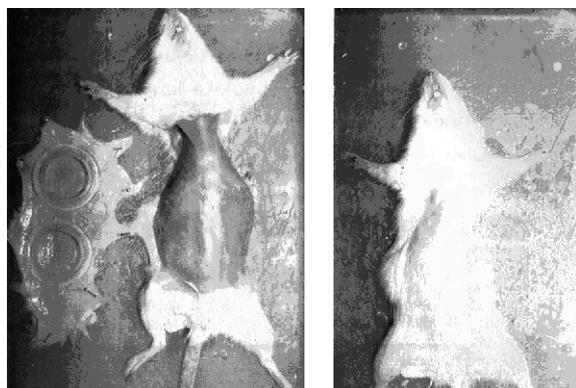
### 2.4. Drug permeation through animal skin

Static diffusion cell (made by Jihad Daneshgahi glassware of Tehran University) was used to determine the amount of piroxicam released from the biologic skin of 95-110 g weight of female N. Mari rats. The shaved skin (Figure 1) was removed from the stomach region of the animal [6].

### 2.5. Serum level study

In other to make a comparison of DMSO influence between human and animal skin, penetration of piroxicam was studied on three groups of human (male) volunteers which their specifications are given in Table 2.

The samples were analyzed spectrophotometrically following 2 weeks application of

**Figure 1.** Shaved and excised rats.

1g gels on the deltoid muscle twice daily [7]. Ishizaki method was used to prepare blood samples [8]. In this method 0.5 mL serum was added to 0.5 mL of glacial acetic acid in a stoppered tube and incubated at 108 °C for 2 h. Then 2 mL NaCl (2%) and 5 mL ethyl acetate was added to each tube and centrifugated after mixing for 20 seconds. A 4 mL sample from ethyl acetate phase was mixed to 3 mL sulfuric acid (0.1 N) for 10 seconds in another stoppered tube and centrifugated for 2 min. Finally, after aspiration of ethyl acetate part, aqueous acidic phase was measured spectrophotometrically at 354 nm.

### 2.6. Determination of preservative efficiency

A suspension of the following standard strains of microorganism containing 100 millions per mL sterile sodium chloride water was prepared:

*Asperigillus niger* (ATCC no. 16404)

*Escherichia coli* (ATCC no. 8739)

*Pseudomonas aeruginosa* (ATCC no. 9027)

*Staphylococcus aureous* (ATCC no. 6538)

*Candida albicans* (ATCC no. 10231)

Turbidimetry method and counting at solid agar medium was used to identify the amount of bacteria. Samples containing  $1 \times 10^6$  cfu/g gels were provided and incubated 24 h at 37 °C and 48 h at 25 °C for bacteria and *C. albicans*, respectively [9].

### 2.7. Statistical analysis of data

Expression of data was as mean±SD and statistical analysis performed by the student "t" test or analysis of variance (ANOVA) followed by TUKEY post HOC test. Significance level was taken at  $p < 0.05$ .

### 3. Results and discussion

#### 3.1. Formulation criteria

Applying a triple phase diagram, the most stable formulations were obtained at the ranges of 61-66, 22-27 and 8-13 g of the water phase, ethanol and propylene glycol for the solvent composition, respectively. This composition contained a bout 96 g of the gel formula and the remainder up to 100 g, was the weight of the other ingredients. The suitable pH (6.5-8) has an important role in this formulation. Not only with the view of neutralizing process of the jelling agent, but also because of the ionization of the drug molecules which decreases passing of the drug through lipid membranes [10].

Although, the horny layer of human dermis resists pH fluctuation at 3-9. In liquid preparations such as gels the pH of liquid phase is an important factor on the activity coefficient of an acid or basic drug [11-12].

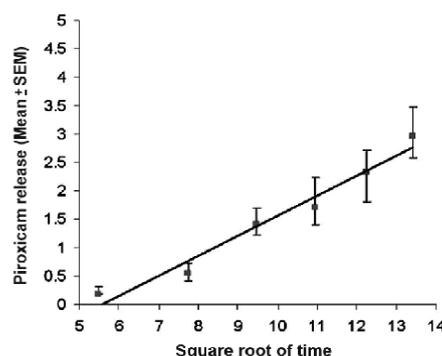
#### 3.2. Viscosity

The result of viscosity measurement showed that the gels containing 0.6% of carbomer P934 (jelling agent) and 0.2% of HPMC (viscosity modifier) provided the constant viscosity of 48485 and 51587.5 cps, for the red and green gels, respectively. It is important to have a constant viscosity and temperature degree during release study, because the rate of release and diffusion of drug from the vehicle base depend on the viscosity and have a reciprocal relationship, especially for the low solubility drugs.

Carbomer P934 provides a viscosity range between 4000-6000 cps in about 0.5-0.6% concentration and also, resists heat fluctuation. This amount is generally suitable for topical gel formulations and produces the most transparency with a desirable pH value ranges

**Table 3.** Permeability coefficient of the gels.

Type of gel	Permeability coefficient (cm/sec)
Green	$1.78 \times 10^{-7}$
Red	$3.26 \times 10^{-7}$
RZK	$1.14 \times 10^{-7}$



**Figure 2.** The release profile of green gel through rat skin.

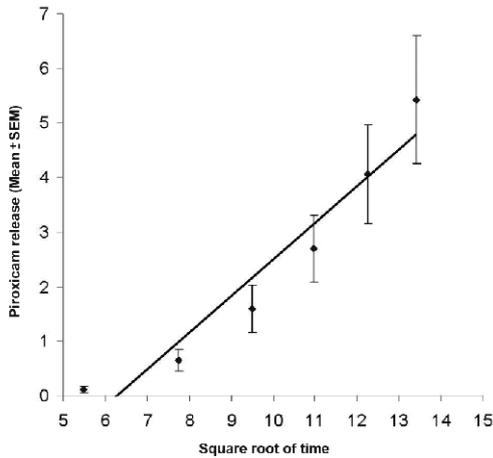
of 5 to 10. The molecular weight of a drug and also vehicle polymer as the carrier, have an important and reciprocal relationship on the molecular passing of the drug through skin [13,14].

#### 3.3. Preservative efficiency

The growth of microorganism in gels is a trouble phenomenon which occurs because of high percentage of water in medium. This problem may change the physico-chemical properties of gels such as viscosity that can affect or fluctuate the rate of surface penetration. The result of this evaluation showed a good antimicrobial activity after incubation time and the growth of the tested microorganisms were less than the reference amount (data not shown). That means applying 1% of benzyl alcohol as a microbial preservative for the gels is sufficient and therefore the efficiency of the preservative is approved qualitatively and also quantitatively.

#### 3.4. Drug content

The calibration curve of different concentrations of piroxicam in normal saline medium produced the standard equation of:  $Y=0.0444X+0.005$  with  $R=0.9993$ , by which the unknown samples were calculated for their contents. The drug content of the new



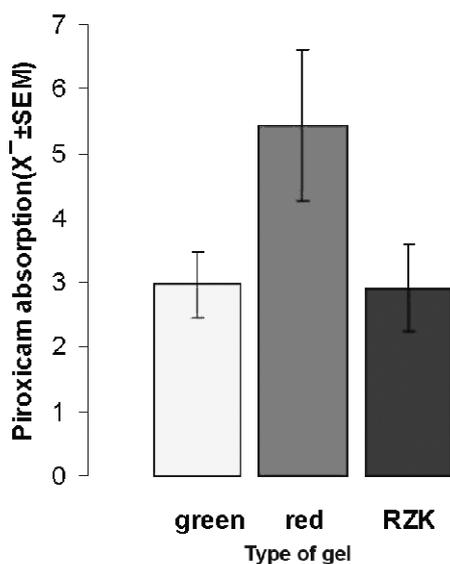
**Figure 3.** The release profile of red gel through rat skin.

and commercial gels was calculated less than  $\pm 5\%$  of the labeled amount. This result indicated an approved total drug and uniformity content with respect to the USP reference.

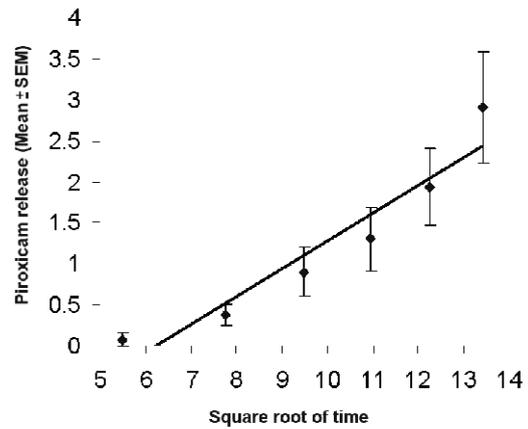
### 3.5. Rat skin penetration

The release profiles of piroxicam from the green, red and RZK gel formulations through rat skin are shown in Figures 2-4, respectively.

The figures show a relatively good linearity between the square root of time and amount



**Figure 5.** The release of piroxicam through the rat dermis after 3 h.



**Figure 4.** The release profile of RZK gel through rat skin.

of piroxicam release as compared with the other mechanisms ( $p < 0.05$ ). This result confirms that the release of piroxicam from the gel bases follows Higuchi's equation. The results also show that there are no meaningful differences among types of the gels in the release of piroxicam after 3 h (Figure 5).

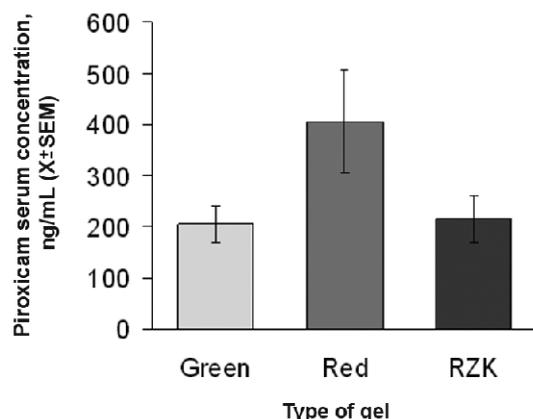
### 3.6. Piroxicam serum level

Piroxicam serum concentrations (ng/mL) in different groups of humans are shown in Figure 6.

Human serum data show the highest level of piroxicam for the red gel with a significant difference ( $p < 0.05$ ), compared to the green and RZK gels. But, the green and RZK gels have no statistical difference ( $p > 0.05$ ). Besides, comparing the results of piroxicam released from the gels (Figures 2-4) showed that the red gel had a better power in its release process.

### 3.7. Calculation of permeability coefficient

In order to make clear and interpret the reason of a higher serum level of the red gel compared to the other gels we decided to measure the permeability coefficient of the gels applying the equation of:  $q = PACot$  [10,11]. This equation defines the terms as follows:



**Figure 6.** Piroxicam concentration in human serum.

q: Amount of released drug (mg)  
 P: Permeability coefficient (cm/sec)  
 A: Diffusion area (cm<sup>2</sup>)  
 Co: Initial concentration (mg/mL)  
 t: Time (sec)

The results are shown in Table 3.

The permeability coefficient amount of the red gel containing DMSO also, shows a significant difference ( $p < 0.05$ ) compared with the green and RZK gels, that probably it might be a good reason to interpret the higher serum level of this product, too. Although, DMSO showed a different result in terms of *in vitro* study. This result was the same as Babar and Hsu results [6,15]. Also, it is possible that the reason of *in vitro* and *in vivo* opposite effects of DMSO as a penetration enhancer interprets by the different structure between human and rat skin, biologic medium or instrumental errors. In this study to omit instrumental influence, we decided to measure the samples by the same instrument. This method was also used by Ishizaki et al. [8] in the past years, although the authors had no other choice in the site and (or) feel not so important then it was a comparative study from a kinetic point of view.

### Conclusion

It can be concluded that from an *in vitro* point of view, the red and green gels are in

parallel quality in comparison to the commercial gel, even though the red gel has some superiority in term of *in vivo* study. This preference may be related to DMSO incorporation in the red gel product as a penetration enhancer. The other conclusion is that relying on *in vitro* data is not always sufficient and needs to be completed by *in vivo* results.

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