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PIROXICAM RELEASE FROM DERMATOLOGICAL BASES: IN-VITRO STUDIES USING CELLULOSE MEMBRANE AND HAIRLESS MOUSE SKIN

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ABSTRACT

Piroxicam is one of the most potent non-steroidal, anti-inflammatory agents which also exhibits antipyretic activity. Piroxicam is well absorbed following the oral administration, however, its use has been associated with a number of gastro-intestinal disorders including bleeding and ulceration. To overcome these side effects, this study was undertaken to develop diadermatic dosage form using various polymeric gel and ointment bases. Gels and ointment bases containing 1% piroxicam were prepared to study the in-vitro release of the drug. Also, a series of additive ingredients, such as, alcohol USP, polyethylene glycol-400 and dimethyl sulfoxide (DMSO) were incorporated in these formulations at various concentration levels to evaluate their effects on drug release. The general rank order for the in-vitro drug release from all the bases evaluated was: gel base > hydrophillic base > emulsion base. In general, additives had little or no effect in enhancing the drug release from these bases. The in-vitro release data were treated with various kinetic principles to assess the relevant parameters, such as, diffusion coefficient, permeability coefficient, partition coefficient, zero order and first order rate constants. Among the formulations evaluated, the gel base containing (DMSO) gave the best in-vitro drug release both through the cellulose membrane and the hairless mouse skin.

INTRODUCTION

Arthritis in one or the other form afflicts 20 million people a year, of which 5 million suffer from rheumatoid arthritis and additional 12 million have some type of osteoarthritis (1). Arthritis and rheumatism are responsible for the largest share of chronic disability in the United States where rheumatic complaints are the second greatest cause of the incapacitation (2). In general, rheumatic diseases are of several kinds (3), (a) diseases affecting only the

joints or muscles, (b) diseases affecting the musculoskeletal system, (c) systemic diseases producing arthralgias but not affecting any pathological changes in the musculoskeletal system and (d) connective tissue diseases that affect the musculoskeletal systems including skin and internal organs. Although aspirin is a remarkable drug, the incidences of side effects, especially with its long term use makes it rather unsuitable for many arthritic patients. A search for alternative therapies with minimum side effects have lead to the development of a new class of drugs: the non-steroidal anti-inflammatory drugs (NSAIDs). The effectiveness of these compounds in the management of arthritic diseases is equal to the high doses of aspirin. Among these some of the important members include: Anthranilic acid derivative (Meclomen®), Arylacetic acid derivative (Naprosyn®), Indene derivative (Clinoril®), Indole derivative (Indocin®), Oxicam, (Feldene®), Propionic acid derivatives (Nalfon® and Motrin®), Pyrazolones (Butazolidin® and Tandearil®), etc.

In a comparative study (4), it was observed that the anti-inflammatory activity of Piroxicam (Feldene®) on carrageenin edema in rats was equal by oral and rectal route, and it was twice as effective as indole derivative (Indocin®) and 20 times as effective as phenylbutazone (Butazolidin®). As an analgesic Piroxicam is more potent than aspirin, fenoprofen (Nalfon®), Ibuprofen (Motrin®), Arylacetic derivative (Naprosyn®) and phenylbutazone (Butazolidine®). The mode of action of piroxicam is not fully understood, but the mechanism of its activity may exist in its ability to inhibit the biosynthesis of prostaglandins (5). Piroxicam is effective against inflammation regardless of the etiology and exhibits enhanced antipyretic activity (6).

Piroxicam is well absorbed following oral administration. A single dose 20mg will generally produce a peak piroxicam level of 1.5 to 2ug/ml while maximum blood plasma level after repeated doses may stabilize at about 3-8 ug/ml (6,7,8). The maintenance of steady state plasma level of a drug is not only dependent on the drug's half-life, but also on the systemic clearance of the drug from the body (7,9). Piroxicam has been shown to undergo enterohepatic circulation which is demonstrated through double plasma peaks (7,9). The metabolism of piroxicam leads to the formation of several inactive metabolites including hydroxy derivative, and only about 10% of the single dose of the drug is received unchanged in the urine within the first 8 days after administration.

Although the usual oral dose of 20mg of piroxicam is well tolerated by the patients, yet several side effects have been reported including: gastrointestinal disturbances, edema, dizziness, headache and skin rash, etc. (6,10). In addition, peptic ulcer has been reported in about 9% of the patients with chronic arthritis when treated with 20mg of daily oral dose (11). In light of these side effects associated with the oral use of piroxicam, it was proposed to develop the various topical dosage forms of

TABLE 1

FORMULATIONS

INGREDIENT	% W/W (I) Gel	(II) Modified Hydrophilic Base	(III) Emulsion Base
Piroxicam	1.00	1.00	1.00
Hydroxypropyl Methylcellulose	1.25	-	-
Petrolatum White, USP.	-	20.00	-
Stearyl Alcohol	-	15.00	-
Sodium Lauryl Sulfate	-	1.50	-
Propylene Glycol	10.00	12.00	-
Mineral Oil	-	-	5.00
Isopropyl Lanolate	-	-	0.50
Stearic Acid, T.P.	-	-	3.00
Cetyl Alcohol	-	-	0.50
Glyceromono Stearate, SE.	-	-	1.00
Triethanolamine	-	-	1.50
Glycerine	-	-	5.00
Sodium Hydroxide (IN)	2.50	2.50	2.50
Methyl Paraben	0.20	0.20	0.20
Propyl Paraben	0.05	0.05	0.05
Purified Water q.s. to	100.00	100.00	100.00

the drug and to study the in-vitro release of the drug from these formulations. Also, to evaluate the roles of various additive ingredients in possibly enhancing the piroxicam release and select formulations with optimum drug delivery for in-vivo evaluations.

EXPERIMENTAL

Materials

Piroxicam[1], Hydroxypropylmethylcellulose[2] (methocel K-100), propylene glycol[3], methyl paraben[4], propyl paraben[2], sodium lauryl sulfate[3], stearyl alcohol[4], white petrolatum[5], mineral oil[4], stearic acid[4], cetyl alcohol[6], glyceromono-stearate SE[4], glycerin[3], triethanolamine[6], dimethyl sulfoxide[6], polyethylene glycol 400[4], isopropyl lanolate[7] (Amerlate-P), and alcohol USP[8].

Equipments

Double beam spectrophometer (Spectronic 200 UV)[9], Franz-chien diffusion cell apparatus[10], constant temperature water bath and circulators[11].

Preparation of Samples

1. Gel Samples: The methocel was dispersed in hot water in small increments with continuous stirring until uniformly dispersed and the solution was allowed to cool to 50°C. The other ingredients weighed in the percentage ratio described in the formulation in Table I were pre-mixed and added to the batch. Piroxicam was dissolved in small amounts of water with the aid of (IN) sodium hydroxide solution prior to incorporation of in the formulation.
2. Ointment Samples: All ingredients in the percentage ratio described in the formulations in Table I were accurately weighed. All the aqueous phase ingredients and the oil phase ingredients were placed in separate stainless steel containers and heated to 80°C + 5°C. The water phase was then added to the oil phase under continuous stirring. The ointment batches were then cooled to approximately 50°C and the piroxicam previously dissolved in water with the aid of small amounts of sodium hydroxide solution (IN), was incorporated. Any additive ingredient included in the formulation was also added at this stage. The samples were then stored in air tight glass jars until used.

Analytical Method

All samples were analyzed for the piroxicam content spectophotometrically[9] using a wavelength of 355nm.

Contents Uniformity

All samples were analyzed for piroxicam contents prior to diffusion studies. Only samples with piroxicam contents within 100 ± 10% were used for diffusion studies.

IN-VITRO RELEASE STUDIES

(a)-Using Cellulose Membrane

A 4.00 gm sample of each formulation was accurately weighed and placed in the donor part of each Franz-chien diffusion cell, and a semi-permeable membrane with a molecular weight cut-off point of 1000 was placed over the mouth of the acceptor post as shown in Figure 1. The acceptor part contained freshly prepared saline solution as a diffusion medium maintained at a constant temperature of 37°C + 1°C by means of the water jacket in each cell. All diffusion studies were carried out in triplicate. The donor part containing the sample was then placed over the semi-permeable membrane and diffusion was allowed to take place. Samples were withdrawn at 30, 60, 90, 120, 150 and 180 minute intervals and analyzed for piroxicam contents. The volume of the diffusion was maintained by replacing the amount withdrawn with an equal volume of the diffusion medium. The solution in the acceptor cell was kept well stirred with the magnetic stirrer throughout the time of the diffusion studies.

The samples withdrawn were analyzed spectrophotometerically at a wavelength of 355nm. The concentration of piroxicam in each sample was determined by using a previously constructed standard curve of known concentration of piroxicam in the same diffusion medium. Blank ointment samples were run simultaneously to check for any interference.

(b)-Using Hairless Mouse Skin

On the basis of the in-vitro release studies conducted with semi-permeable membrane sample with optimum drug release were selected to study the release of drug using the freshly excised hairless mouse skin. The mice used were six (6) weeks old, females. The skin was excised just prior to the experiments and cleaned using saline solution to remove all visceral debris. The skin was then placed over the mouth of the acceptor part containing the diffusion medium maintained at $37^{o}C \pm 1^{o}C$. The diffusion studies were carried out withdrawing samples at time intervals of 30, 60, 90, 120, 150 and 180 minutes respectively, and analyzed for piroxicam contents spectrophotometerically as described earlier.

RESULTS AND DISCUSSIONS

In-Vitro Release Through Cellulose Membrane

The results of the percentage release of piroxicam from the various bases evaluated over a three (3) hour period are exhibited in Table 2. The general rank order of piroxicam release was observed to be: Gel Base > Modified Hydrophilic Base > Emulsion Base. Also, the results of the effects of the additive ingredients on the release of the prioxicam are shown in Table 3. Analysis of the data of the release of piroxicam from the different bases were carried out using the Higuchi Equation (12), which is valid when the release of the medicament from the base is less than 30%.

$$\frac{q}{A} = 2C_o \left(\frac{Dt}{\pi}\right)^{\frac{1}{2}} \qquad \text{Eq. 1}$$

where q = amount of drug released (mg), D = diffusion coefficient (cm^2/sec), t = time in seconds, A = area of the diffusion membrane (cm^2), C_o = initial concentration of the drug in the ointment (mg/cm^3) and π = constant.

The in-vitro release data comply well with the requirements of Equation 1, namely (a) - only a single drug species is assumed to diffuse, (b) - the diffusion coefficient must be constant with respect to both time and position of ointment layer, (c) - only the drug is able to diffuse out of the layer (d) - the drug reaching the receptor site is removed rapidly.

In the present study the experimental conditions matched favorably to the above stated conditions. Equation 1 may be further simplified to:

$$q = K (t)^{\frac{1}{2}} \qquad \text{Eq. 2}$$

$K = 2C_o (D\pi)^{\frac{1}{2}}$, and since C_o, A and D are constants according to the assumptions mentioned in Higuchi's equation, therefore, the product of

TABLE 2

IN-VITRO RELEASE OF PIROXICAM FROM DIFFERENT BASES

(USING CELLULOSE MEMBRANE)

BASE TYPE	% RELEASE (± SD)					
	TIME (minutes)					
	30	60	90	120	150	180
GEL	2.03±0.06	385±0.25	535±0.19	6.60±0.53	8.25±0.64	9.70±0.60
HYDROPHILIC	1.05±0.08	1.48±0.12	1.93±0.09	2.38±0.02	2.65±0.04	2.88±0.06
EMULSION	0.76±0.01	1.15±0.07	1.47±0.48	1.70±0.04	1.93±0.02	2.21±0.02

Note: Each reading is an average of three determinations

TABLE 3

EFFECTS OF ADDITIVES ON PIROXICAM RELEASE FROM VARIOUS BASES AFTER 3 HOURS

Sample	% Release ± SD (I) (GEL)	(II) Modified Hydrophilic Base	(III) Emulsion Base
Control	9.70 ± 0.60	2.88 ± 0.62	2.12 ± 0.03
Base + PEG (400)			
5%	5.35 ± 0.30	2.17 ± 0.06	1.77 ± 0.06
10%	4.25 ± 0.14	2.00 ± 0.04	1.67 ± 0.05
15%	3.15 ± 0.12	1.98 ± 0.06	1.77 ± 0.03
Base + Alcohol, USP			
5%	8.15 ± 0.40	2.18 ± 0.06	1.88 ± 0.02
10%	6.25 ± 0.28	2.42 ± 0.05	1.98 ± 0.08
15%	6.15 ± 0.44	2.30 ± 0.05	1.87 ± 0.06
Base + DMSO			
5%	10.70 ± 0.12	2.85 ± 0.04	1.95 ± 0.04
10%	10.00 ± 0.28	2.67 ± 0.06	1.82 ± 0.06
15%	9.80 ± 0.18	2.37 ± 0.08	1.85 ± 0.02

Note: Each reading is the average of three (3) determinations

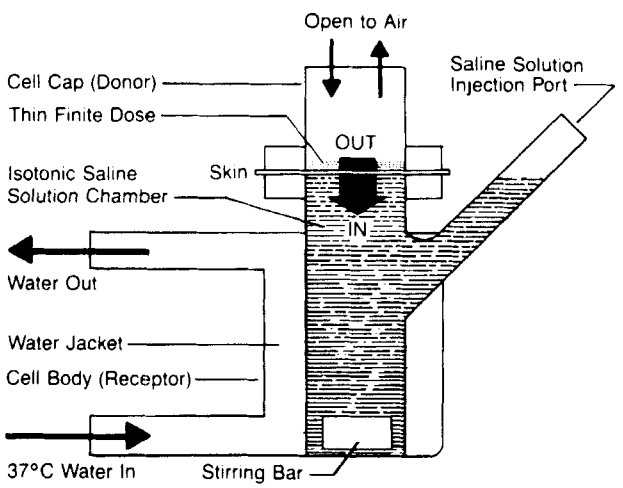

FIGURE 1

Schematic Diagram Of The Apparatus For The In-Vitro Diffusion Studies.

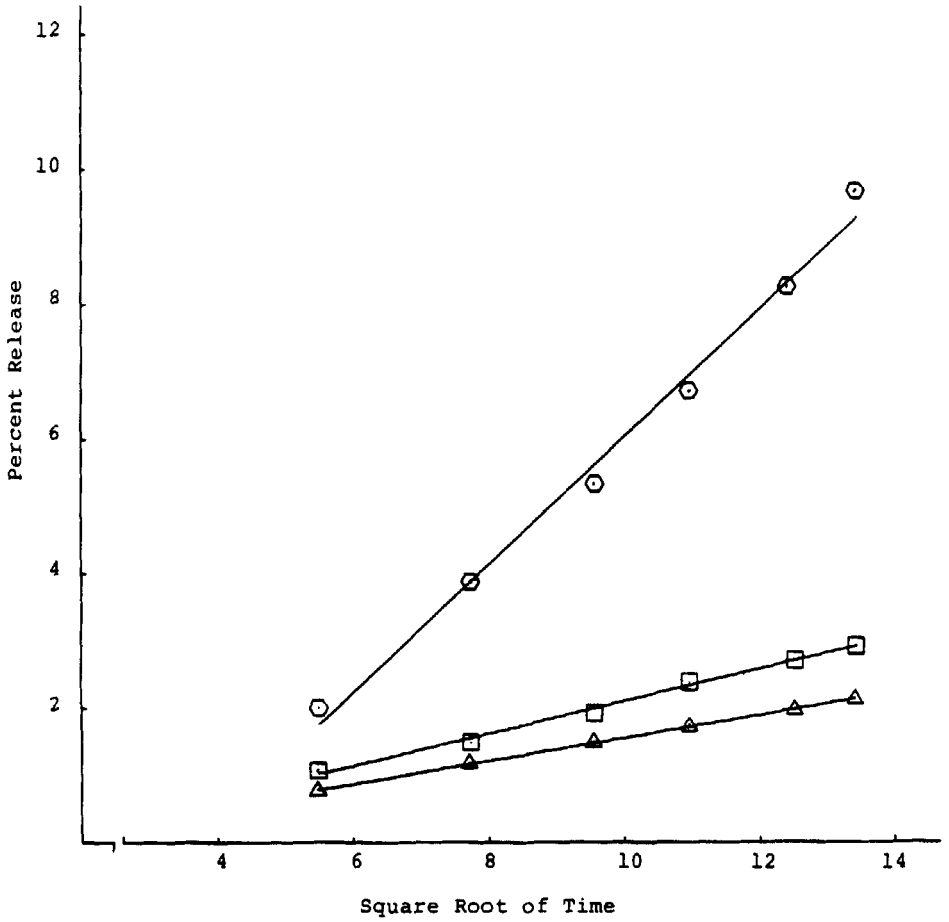

FIGURE 2

In-Vitro Release Of Piroxicam (Using Cellulose Membrane). Percentage Release Versus Square Root Of Time. ⊙ Gel Base ⊡ Hydrophilic Base △ Emulsion Base.

these constants K is also a constant. When the percentage release of piroxicam was plotted against time, curves were obtained whereas, when this was plotted against square root of time straight lines were obtained as exhibited in Figure 2, indicating that the release of piroxicam from the bases evaluated followed Higuchi's equation. This indicates a direct dependence of the release rate on the diffusion coefficient, which in turn is dependent, among other factors, on the solubility of the drug in the base. Therefore, a greater release of the drug is expected when there is less affinity of the drug for the base as in the case of the gel formulation,

TABLE 4

DIFFUSION, PERMEABILITY AND PARTITION COEFFICIENTS

AS CALCULATED FROM THE IN-VITRO DATA

(USING CELLULOSE MEMBRANE)

Base Type	Diffusion Coefficient [D] ($Dx10^{-8}$) cm^2/sec	Permeability Coefficient [P] ($Px10^{-6}$) cm/sc	Partition Coefficient [K_p]
Gel	20.000	5.10	0.153
Hydrophilic	1.928	1.50	0.467
Emulsion	1.040	1.10	0.635

which gave the highest diffusion coefficient value of the order of 2×10^{-7} (cm^2/sec). The diffusivity of the drug through any base depends on the nature and composition of the individual base and any changes in these parameters effect the release of the active medicaments.

Treating the in-vitro data in accordance with Fick's Law of diffusion, Equation 3, the permeability coefficient values for piroxicam were calcualted.

$$q = PAC_o t \qquad \text{Eq. 3}$$

where q = mg of drug diffused through the membrane at time t (seconds), P = permeability coefficient (cm/sec), A = area of diffusion membrane (cm^2) and C_o = concentration (mg/L) of the drug present in the base at time zero. The permeability coefficient values for piroxicam are exhibited in Table 4. From this one observes that the highest value of 5.1×10^{-6} was obtained for the gel formulation.

The partition coefficient factor is considered as one of the important parameters for the estimation of the drug distribution between the vehicle and the receiving medium. And, the relationship between the partition coefficient and permeability coefficient may be expressed by the following equation.

$$Kp = \frac{Ph}{D} \qquad \text{Eq. 4}$$

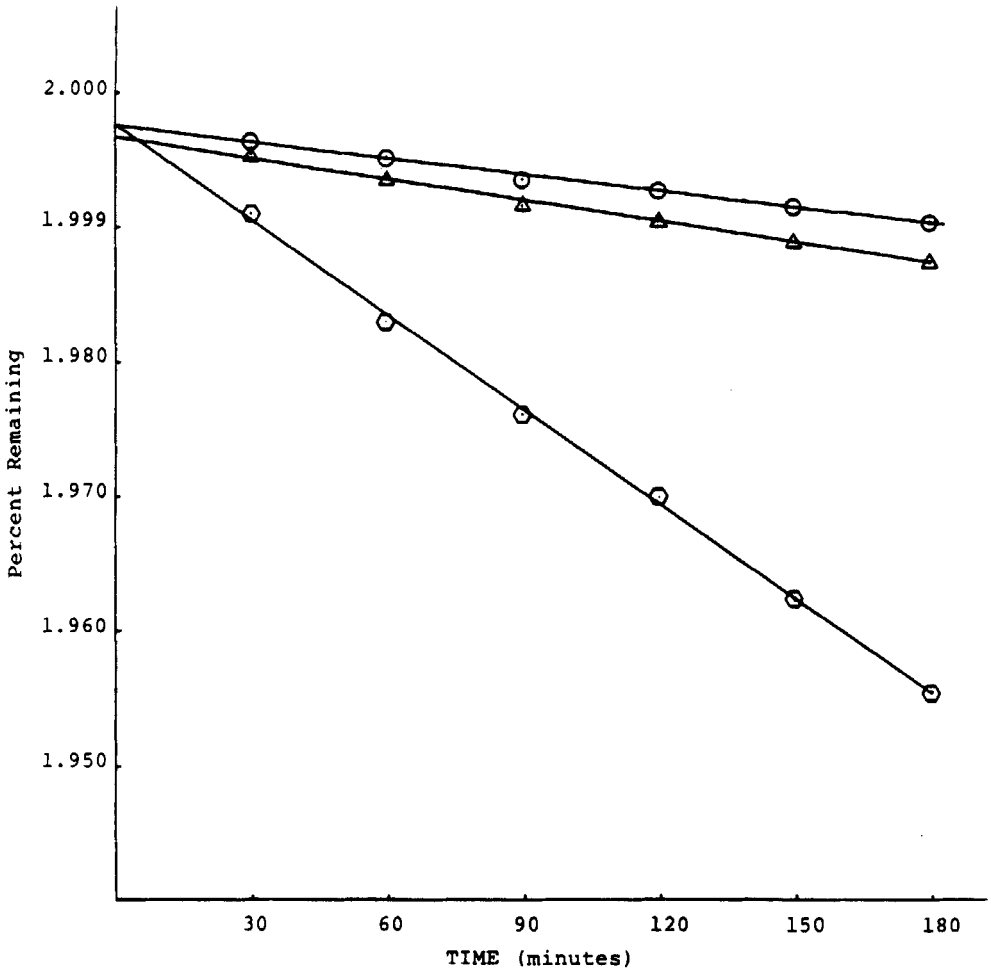

FIGURE 3

In-Vitro Release Of Piroxicam (Using Cellulose Membrane). Plotted In First Order Kinetic Fashion. ⬡ Gel Base △ Hydrophilic Base ⊙ Emulsion Base.

where K_p = partition coefficient, P = permeability coefficient (cm/sec), D = diffusion coefficient (cm^2/sec^2) and H = thickness of the barrier (cm). The partition coefficient values for the various formulations are also given in Table 4. From this table one observes that the gel formulation with the highest release of the drug yielded the low partition coefficient value (0.153), and the emulsion base formulation with the lowest drug release gave the highest partition coefficient value of 0.635.

TABLE 5

DIFFUSION RATE DATA EXPRESSED AS PARAMETERS

OF FIRST ORDER KINETICS

Base	K 10^{-4} min^{-1}	Y-intercept	STD Regression Coefficient r-value
Gel	6.12	1.9975	0.999
Hydrophilic	2.29	1.9967	0.992
Emulsion	1.70	1.9974	0.991

K = first order rate constant

In order to develop an ideal kinetic model to interpret diffusion rate data in terms of meaningful parameters, various kinetic models were applied to obtain the best fit of the data. The diffusion rate data were treated with first and zero order kinetic fashions, and when the logrithm of the percentage piroxicam remaining versus time were plotted, the straight lines were obtained as shown in Figure 3. The values of the release rate constant, y-intercept and regression coefficient calculated using the first order kinetic equation are exhibited in Table 5.

In-Vitro Release Through Hairless Mouse Skin

The formulations with optimum release through the cellular membrane, i.e., the gel base and hydrophillic base were selected for the in-vitro release of piroxicam through the hairless mouse skin. Also, these formulations with 5% of dimethylsulfoxide (DMSO) were evaluated. The percentage drug release from the various formulations are shown in Table 6. From the data, one notes that the maximum release of the drug is obtained from the gel formulation as previously observed in the study using cellular membrane. However, the inclusion of 5% DMSO adversely affected the drug release from both formulations. This could be attributed to change in solubility of the drug, complexation of the drug, changes in the permeability and partition coefficient profiles of the drug, etc.

TABLE 6

IN-VITRO RELEASE OF PIROXICAM FROM SELECTED BASES WITH ADDITIVES

(USING HAIRLESS MOUSE SKIN)

BASE TYPE	% RELEASE (±SD) TIME (minutes)					
	30	60	90	120	150	180
GEL	0.23±0.04	0.39±0.06	0.59±0.07	0.73±0.06	0.87±0.07	1.02±0.09
GEL + 5% DMSO	0.18±0.03	0.29±0.04	0.49±0.03	0.64±0.05	0.79±0.07	0.93±0.06
HYDROPHILIC	0.17±0.42	0.26±0.03	0.39±0.42	0.53±0.04	0.70±0.41	0.82±0.06
HYDRO + 5% DMSO	0.12±0.01	0.23±0.01	0.36±0.03	0.49±0.42	0.64±0.06	0.78±0.07

Note: Each reading is an average of three determinations

TABLE 7

DIFFUSION AND PERMEABILITY COEFFICIENTS FOR SELECTED BASES AND ADDITIVES FROM IN-VITRO DATA (USING HAIRLESS MOUSE SKIN)

Base	Diffusion Coefficient [D] ($Dx10^{-9}$) cm^2/sec	Permeability Coefficient [P] ($Px10^{-7}$) cm/sec
Gel	2.40	5.00
Gel + 5% DMSO	1.99	5.00
Hydrophilic	1.55	4.00
Hydrophilic + 5% DMSO	1.42	4.00

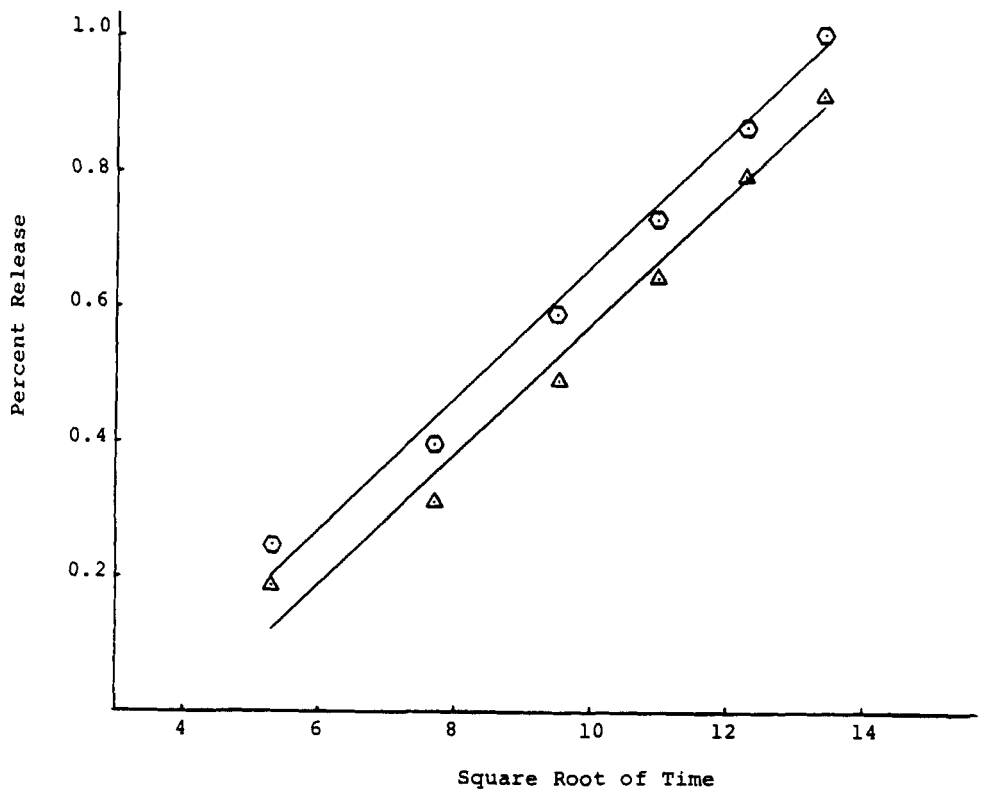

FIGURE 4

In-Vitro Release Of Piroxicam (Using Hairless Mouse Skin). Percentage Release Versus Square Root of Time. ⊙ Gel Base △ Gel Base + 5% DMSO.

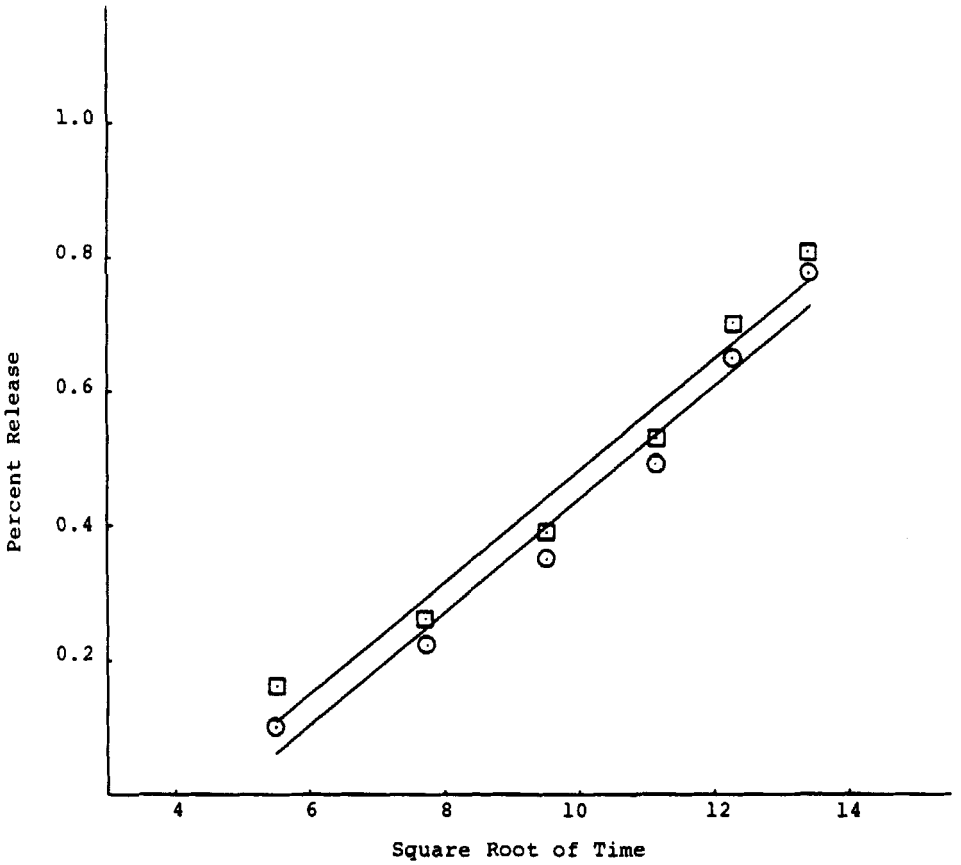

FIGURE 5

In-Vitro Release Of Piroxicam (Using Hairless Mouse Skin). Percentage Release Versus Square Root Of Time. ⊡ Hydrophilic Base ⊙ Hydrophilic Base + 5% DMSO.

Comparing the drug release from the gel formulation through two different barriers, it is observed that 9.7% of piroxicam is released using cellular membrane in contrast to 1.02% release through the hairless mouse skin. In the case of hydrophillic base, the drug release was 2.88% using the cellular membrane compared to only 0.82% with the hairless mouse skin.

The diffusion and permeability coefficient values for the different bases employed are listed in Table 7. A maximum value of (5×10^{-7} cm/sec) of permeability coefficient was obtained for gel formulation using the hairless

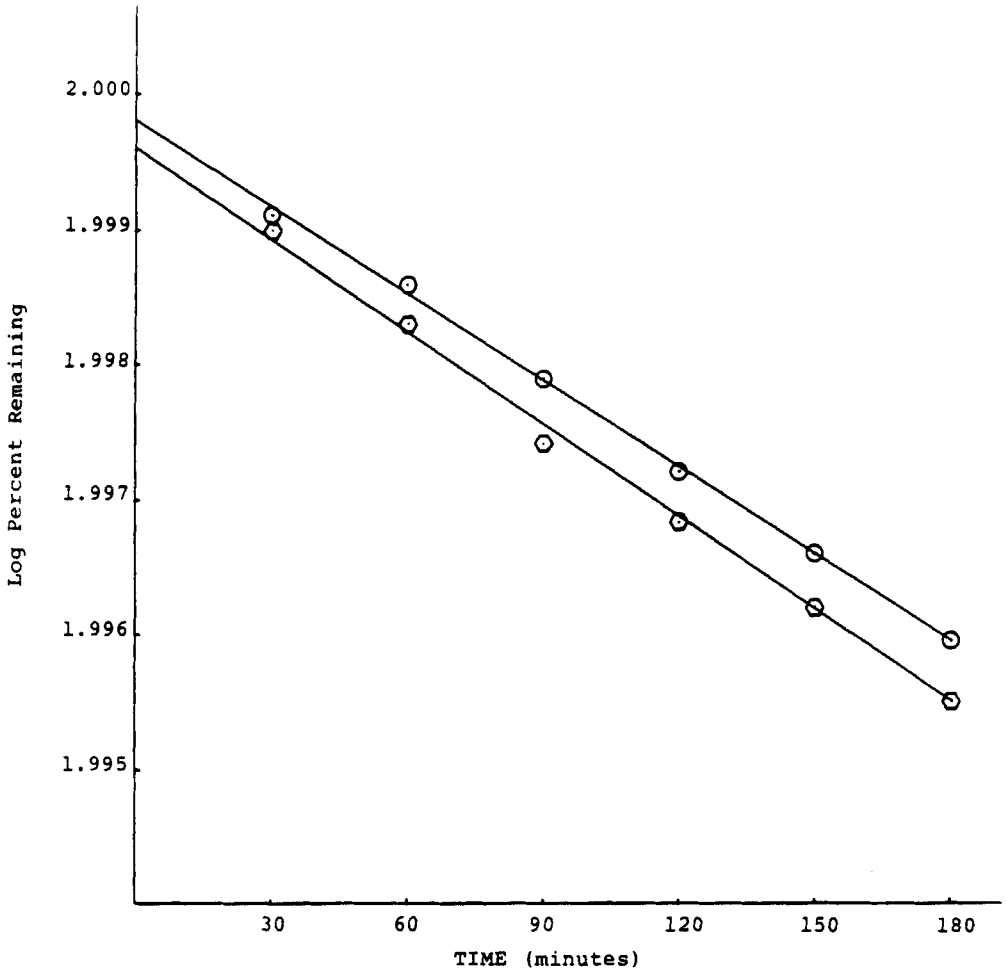

FIGURE 6

In-Vitro Release Of Piroxicam (Using Hairless Mouse Skin. Plotted In First Order Kinetic Fashion. ⊙ Gel Base ⊙ Gel Base + 5% DMSO.

mouse skin compared to (5.1×10^{-6} cm/sec) obtained when the cellulose membrane was used. Similarly, the diffusion coefficient (2.4×10^{-9} cm^2/sec) was obtained for the gel formulation with the hairless mouse skin compared to (20×10^{-8} cm^2/sec) using the cellulose membrane.

When the percentage release of piroxicam using the hairless mouse skin was plotted against the square root of time, straight lines were obtained as shown in Figure 4 and 5 respectively. This indicated that once again

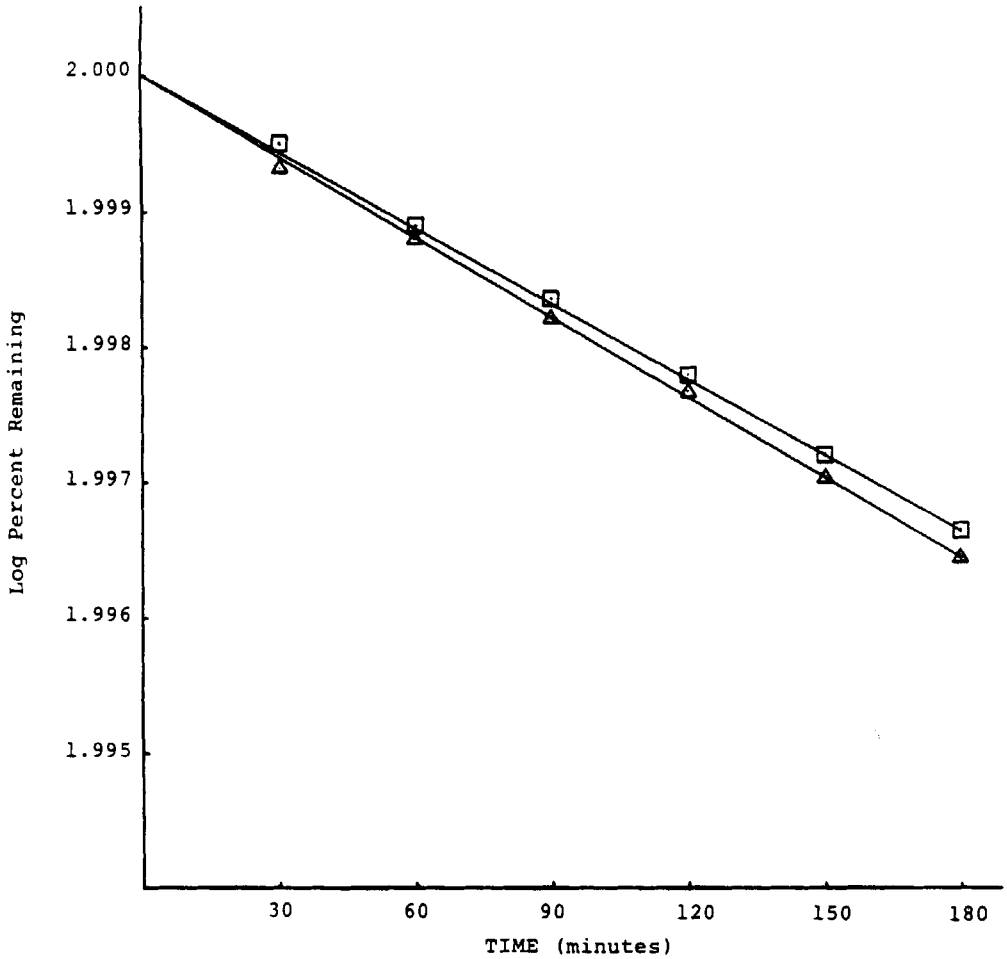

FIGURE 7

In-Vitro Release Of Piroxicam (Using Hairless Mouse Skin). Plotted In First Order Kinetic Fashion. △ Hydrophilic Base ⊡ Hydrophilic Base + 5% DMSO.

the release of the drug from various bases followed the Higuchi's equation. As the rate of piroxicam release from the selected bases remained low i.e. (1.02%), the in-vitro data can be considered to follow the first order kinetics, since the drug remaining in the donor port at any one time is essentially unchanged. A plot of logarithm of percentage drug remaining versus time gave straight lines as shown in Figures 6 and 7. The first order rate constants were calculated from the slopes of these lines and

TABLE 8

DIFFUSION RATE DATA OF SELECTED BASES EXPRESSED AS PARAMETERS OF FIRST ORDER KINETICS (USING HAIRLESS MOUSE SKIN)

Base	$K \times 10^{-5}$	Y Intercept	Standard Regression Coefficient r-value
Gel	6.44	1.9996	0.998
Gel + 5% DMSO	5.37	1.9998	0.998
Hydrophilic	4.57	2.000	0.997
Hydrophilic + 5% DMSO	4.05	2.000	0.999

the values of y-intercept and regression coefficient are exhibited in Table 8.

CONCLUSION

Diffusion studies were used to evaluate the in-vitro release of prioxicam from various topical bases with and without the additive ingredients and proved to be meaningful in screening formulations for relative availability of the active ingredient. The general rank order of piroxicam release from the bases was: Gel base > Hydrophillic base > Emulsion base. In general, the inclusion of the additive ingredients adversely affected the drug release, except for DMSO, which had a little effect in enhancing the drug release. The two formulations exhibiting higher drug release through the semi-permeable membrane were selected for further drug release studies through the hairless mouse skin. Among all the formulations screened, the gel base formulation gave the highest drug release via both the semi-permeable membrane and the hairless mouse skin. The study supports the evidence that the in-vitro diffusion method served as a useful model for screening formulations with optimum drug release for use in in-vivo evaluations. And, this

can serve as the basis for developing topical formulations of piroxicam with the best drug release profiles.

NOTES

1. Pfizer Corporation, Connecticut.
2. Dow Chemical Co., MI.
3. Amend Drug & Chemical Corp., N.J.
4. Ruger Chemical Corp., N.J.
5. Lannet Co., Inc., PA.
6. Fisher Scientific Co., Inc., N.J.
7. Amerchol Corporation, Inc., N.J.
8. Industrial Chemical Co., Inc., N.J.
9. Bausch and Lomb Corp., N.J.
10. Crown Glass Corp., N.J.
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