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MEDICAMENT RELEASE FROM OINTMENT BASES: IV. PIROXICAM: IN-VITRO RELEASE AND IN-VIVO ABSORPTION IN RABBITS

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ABSTRACT

Several ointment bases containing 1% piroxicam were studied to determine the in-vitro release of the drug. The general rank order of the drug release was found to be: Modified hydrophilic ointment > hydrophilic ointment USP > water washable base > modified U.C.H. base > hydrophilic petrolatum > U.C.H. base. Furthermore, it was found that additives such as urea, ethanol and DMSO at 1%, 3% and 5% concentration levels had no significant effect in enhancing the release of piroxicam from the modified

hydrophilic ointment base. The in-vitro release data were assessed by various kinetic principles in order to determine the relevant parameters such as diffusion, permeability and partition coefficients. The in-vitro absorption of 1% and 3% piroxicam in hydrophilic base was determined in rabbits. It was found that the addition of 5% DMSO increases the drug absorption. The statistical evaluation of the in-vivo release data was done using one-way ANOVA.

INTRODUCTION

Rheumatoid arthritis is a chronic systemic disease which afflicts about 7 million people in the United States. The common symptoms of the disease are pain, stiffness and inflammation in one or more joints (1). Rest, exercise, joint protection and anti-inflammatory drugs currently play an important role in the management of this chronic disease (2). Recently, a number of compounds, in the newest category, called "oxicams", have been developed. Approximately one hundred of the new oxicam compounds were synthesized before piroxicam was selected in January 1967 for clinical evaluation. By 1984 piroxicam was one of the most commonly prescribed non-steroidal anti-inflammatory agents in the United States (3).

Topically applied piroxicam has been shown to have activity comparable with that of the equal orally admin-

istered dose when applied the day before or 15 days after adjuvant induced arthritis, indicating that the piroxicam itself, rather than its metabolites, is responsible for the anti-inflammatory activity (4). In the carrageenan-paw edema test, rectally administered piroxicam in suspension form was found to be equally potent to the orally administered drug (5).

Percutaneous absorption of a number of non-steroidal anti-inflammatory agents has been reported (4, 6, 7). It has been found that penetration of drugs through skin can be significantly improved with the presence of promotors (6-8).

A topical preparation of piroxicam would allow the administration of piroxicam in those patients who cannot tolerate the drug orally because of its adverse gastrointestinal effects. Furthermore, topical administration of piroxicam at the inflammed site will offer the potential advantage of delivering the drug directly to the surface of the relevant area and producing locally high concentrations of the drug.

In a recent study Larson and Lombardino (4) demonstrated that topically applied piroxicam is a potent inhibitor of inflammation. They also demonstrated that the potency of topical piroxicam exceeds that of topically applied bufexamac or phenylbutazone in the rat adjuvant arthritis model. They also demonstrated that

percutaneous administration of piroxicam from the University of California Hospital base, is comparable with equal oral doses. However, they used only one base and no promotors. Therefore, the purpose of this investigation was to screen various ointment bases and to study the effects of various additives on the in-vitro and in vivo release of piroxicam and to develop an "optimum" topical dosage form for the drug.

EXPERIMENTAL

Materials - The following chemicals were used as received from the manufacturers: piroxicam[1], white petrolatum[2], stearyl alcohol[3], propylene glycol[3], sodium lauryl sulfate[3], methylparaben[3], propylparaben[3], isopropyl lanolate (Amerlate-P®)[4], myltisterol extract of lanolin (Amerchol CAB®)[4], glycerin[3], glyceromonostearate self-emulsifier[2], polyoxyethylene 40 stearate (Myrj 52®), cetyl alcohol[6], dimethylsulfoxide (DMSO)[6], urea[7], cholesterolized absorbent eucerite ointment base (Aquaphor®)[8], polyethylene glycol 400[2], polyethylene glycol 4000[2], di-butylamine phosphate (Reagent D-4®)[9], acetonitrile[6], and cholesterol[2].

EQUIPMENT

The following equipment were used: Double beam spectrophotometer, (Spectronic 200 UV)[10], high performance liquid chromatograph (HPLC) equipped with an absorbance detector (Model 440)[11], a universal inject-

or (Model U6K)[11], a solvent delivery (Model 6000A)[11], a μBondapak C18 (3.9 mm ID x 10cm) column[11], a minigrator[12], and a recorder[13].

FORMULATIONS

The in-vitro release of piroxicam was studied from the following ointment bases.

a. Emulsion bases - Hydrophilic ointment, USP, Formulation A and Modified Hydrophilic Ointment, Formulation B (Table I); Water Washable Base, Formulation C (Table II); University of California Hospital Base, Piroxicam suspended in the water phase, Formulation D; Piroxicam dissolved in the oil phase, Formulation E; Piroxicam dissolved in the water phase with propylene glycol, Formulation F; and Piroxicam dissolved in the water phase with PEG-400, Formulation G.

b. Absorption bases - 1% Piroxicam in 99% Aquaphor, Formulation H; Hydrophilic petrolatum, USP, Formulation I.

c. Water Soluble bases - Formulation K and Formulation L (Table III).

METHOD OF PREPARATION OF THE OINTMENT

Emulsion Bases - All the ingredients of the ointments were accurately weighed. The oil phase ingredients and the water phase ingredients were placed into two separate containers and heated to 80°C ± 0.5°C. The water phase was slowly mixed with the oil phase with continuous stirring until it congealed at room termperature.

TABLE I

Hydrophilic Ointments Containing 1% Piroxicam

Ingredients		Formulations (%w/w)[1] A	B
White petrolatum		25.00	25.00
Stearyl alcohol		25.00	15.00
Propyl paraben		0.06	0.06
Methyl paraben		0.15	0.15
Propylene glycol		12.00	12.00
Sodium lauryl sulfate		1.00	1.00
Sodium hydroxide (1N)		2.50 ml	2.50 ml
Piroxicam		1.00	1.00
Water	q.s.	100.00	100.00

[1] The piroxicam was dissolved in the water phase

Absorption Base - The Aquaphor® ointment was prepared by levigation using Ethyl Alcohol as the levigation agent.

Water Soluble Bases - The PEG-4000 was melted by heating at 70°C. The PEG-400 then was added. Finally, piroxicam was dissolved in the above mixture under continuous stirring.

TABLE II

Water Washable Base Containing 1% of Piroxicam

(Formulation C)

Ingredients	(%w/w)
Amerchol CAB®	5.00
Amerlate P®	2.00
White petrolatum USP	20.00
Stearyl alcohol	3.00
Glyceryl stearate SE	5.00
Myrj 52	4.00
Propylparaben	0.06
Glycerin	5.00
Polyethylene glycol 400	4.00
Tween 80	0.20
Sodium hydroxide (1N)	2.50
Piroxicam	1.00
Methylparaben	0.15
Water	q.s. 100.00

[1] Piroxicam was dissolved in the water phase

TABLE III

Water Soluble Bases Containing 1% of Piroxicam

Ingredients Trade Name	Fromulation K	(%w/w) L
Polyethylene glycol 400	29.00	39.00
Polyethylene glycol 4000	70.00	60.00
Piroxicam	1.00	1.00

In-Vitro Release - A one ounce empty plastic jar was weighed and completely filled with one of the piroxicam ointments. The excess of the ointment was removed with the edge of a spatula to produce an even surface and the total weight of the jar was determined. The open end of the jar was covered by a semipermeable membrane with a molecular weight cut-off point of 1000 and sealed with a silk thread so that the entire surface of the ointment was in contact with the membrane. The ointment jar was then immersed in a 250 ml beaker containing 100 ml of phosphate buffer (pH = 6.8) preheated to 37 ± 1°C. At each sampling interval (5, 15, 30, 45, 60, 90 and 120 min.) an aliquot (3 ml) of the diffusion medium was drawn off and replaced by equal volume of the diffusion medium. The diffusion medium was constantly stirred to avoid any development of a concentration gradient. The

samples were then assayed spectrophotometrically at a wavelength of 355 nm. The concentration of piroxicam in each sample was determined from a standard curve previously constructed from known concentrations of piroxicam. Blank ointment samples were simultaneously to check for any interferences.

IN-VIVO METHOD

Application of ointment and blood sampling - Male New Zealand white rabbits weighing 2-4 kg were used. Three rabbits were designated for each formulation. The rabbit's skin was carefully shaved using an electric clipper. An area of 19.6 square centimeters was uniformly covered with a known quantity of ointment. The rabbit was then placed in a restrainer. Blood samples were obtained from the ear veins at time intervals of 15, 30, 60, 90, 120, 180 and 240 minutes. The blood samples were allowed to clot at room temperature for approximately 15 minutes and centrifuged at 2000 rpm for 30 minutes. The serum was separated and kept in a freezer until the analysis was carried out.

Analytical method - An HPLC equipped with a UV detector (constant wavelength set at 254 nm), a solvent delivery system, universal injector and μBondapak C18 column were used. The mobile phase consisted of an acetonitrile/0.02M di-butyl amine phosphate 60/40 mixture. The flow rate was 1.5 ml/min, the opening temperature was ambient

and the operating pressure was 1700 psi. The chart speed of the recorder was 0.2 inch per minute. Indomethacin solution (30 µg/ml) was used as an inaternal standard (30 mg of indomethacin were accurately weighed, transferred into a 1000 ml volumetric flask and the volume was adjusted with acetonitrile). The calibration curve was prepared by spiking plasma with known concentrations of piroxicam and internal standard, and plotting peak area ratios vs. known concentration of piroxicam. Each point was the average of three determinations and the regression line slope was calculated to be 0.98.

Serum Assay - A 0.5 ml of serum spiked with 2 ml of the internal standard solution was transferred into a glass stoppered centrifuge tube. The mixture was shaken on a vortex mixer for 30 seconds in order to precipitate the proteins of the blood. The organic phase was then removed with pasteur pipets[1] and filtered through an organic reagent resistant filter (Acrodisc®, pore size 0.45 µm)[2]. A 20 µl portion was injected into the column of the HPLC through a stop-flow injection port.

RESULTS AND DISCUSSION

In-vitro studies - The in vitro release of 1% piroxicam from several ointment bases was determined and the re-

[1] Fisher Scientific, NJ

[2] Gelman Co., MI

lease profiles are shown in Fig. 1 and 2. The general rank order for the in-vitro release of the drug was found to be: B > A > C > G > F > E > K > I > H > D. Furthermore, the release data for the emulsion ointment indicated that the release of piroxicam was higher when the drug was incorporated into the water phase than in the oil phase in which it has greater solubility. Piroxicam was dissolved in the water phase of the emulsion ointment with the aid of 12% propylene glycol or 4% polyethylene glycol-400, 0.2% of surfactant and 1N sodium hydroxide sufficient to ensure the solution (approximately 2.5 ml).

Recently, it was reported that piroxicam incorporated in the UCH base with the aid of 12% propylene glycol, when topically applied showed a potency comparable to the orally administered drug (4). In our study, piroxicam was either suspended in the water phase of the U.C.H. base with the assistance of 12% propylene glycol or it was dissolved in the oil phase. In both cases, its release was very low. Therefore, in order to improve its release, the piroxicam was dissolved into the water phase of the U.C.H. base with the aid of polyethylene glycol-400 or propylene glycol and 1N sodium hydroxide. This modification resulted in an increased release of piroxicam, up to 80%, over the U.C.H. base.

The mathematical evaluation of the in-vitro release of the drug was done by using a simplified Higuchi equa-

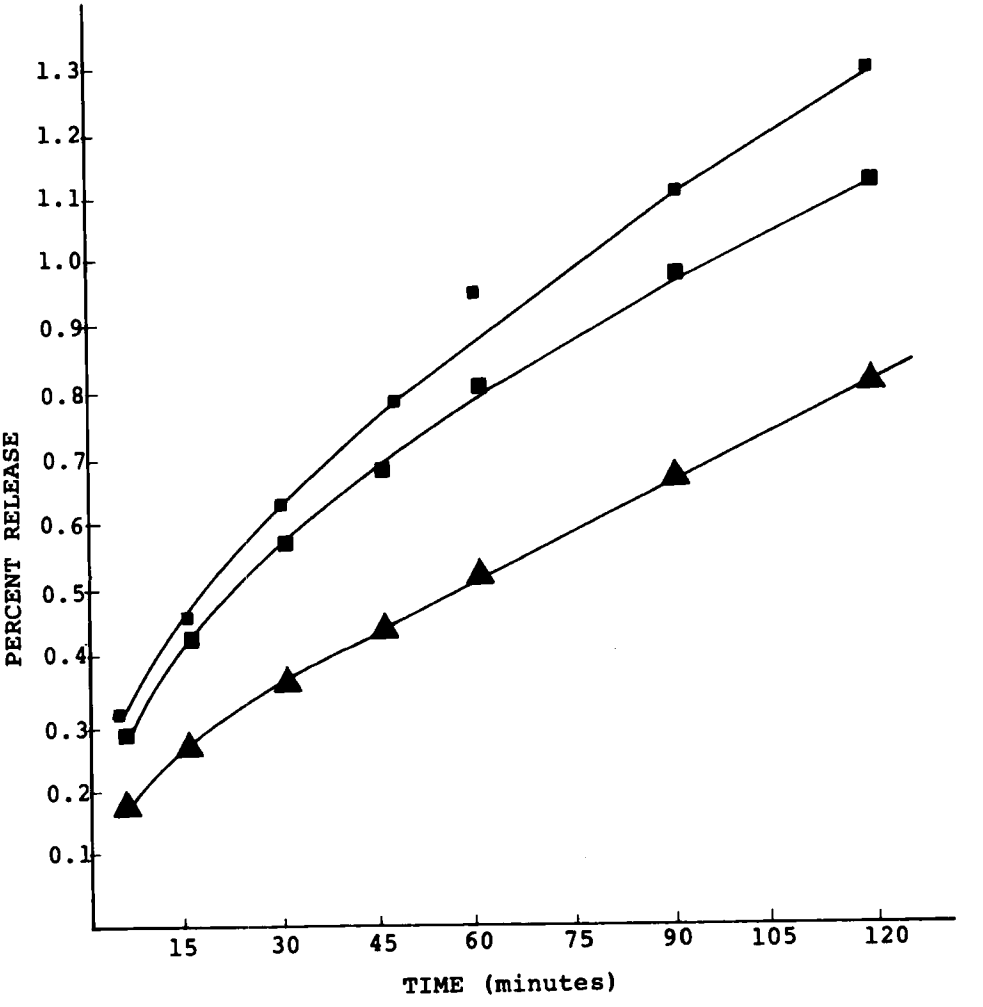

FIGURE 1

In-Vitro Release of Piroxicam from Different Ointment Bases Key: (■) Modified Hydrophilic Ointment (B); (■) Hydrophylic Ointment USP (A); (▲) Water Washable Bases (C).

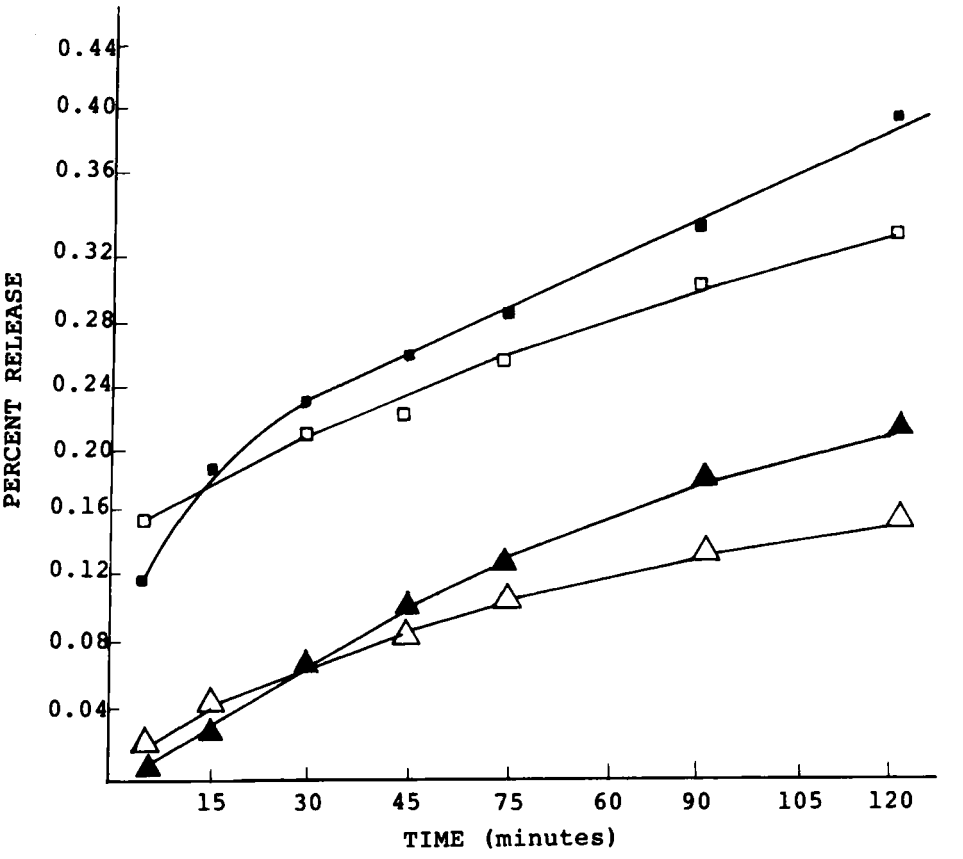

FIGURE 2

In-Vitro Release of Piroxicam from U.C.H. and Modified U.C.H. Bases. Key: (■) Modified U.C.H. Base (E); (□) Modified U.C.H. Base (F); (▲) U.C.H. Base (E); (△) U.C.H. Base (D).

tion (8,9), which is valid for drugs with release less than 30%.

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

where, q = amount of drug release (mg), A = area of application (cm^2), C_o = initial concentration of the drug in the ointment base (mg/cm^3), D = diffusion coefficient

(cm^2/sec), t = time of application (sec). Equation 1 is valid only if:

a. Only a single drug species is important in the ointment base.

b. The diffusion coefficient must be constant with respect to both time and position in the ointment layer.

c. Only the drug is able to diffuse out of the layer.

d. The drug reaching the receptor site is removed rapidly.

Since in the present study the experimental conditions appear to match favorably to the above, Equation 1 may be used.

If $K = 2 C_o A (D/\pi)^{\frac{1}{2}}$ then Eqation 1 can be further simplified to Equation 2.

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

When the percent release of piroxicam was plotted against the square root of time straight lines were obtained (Figure 3). This indicates the release of piroxicam from different ointment bases followed by Higuchi equation (Eq. 2).

Using the Higuchi equation the diffusion coefficients of piroxicam from different ointment bases were calculated and are presented in Table IV. The Higuchi equation indicates that when a drug is in solution in a vehicle, its release rate can be altered by changing its

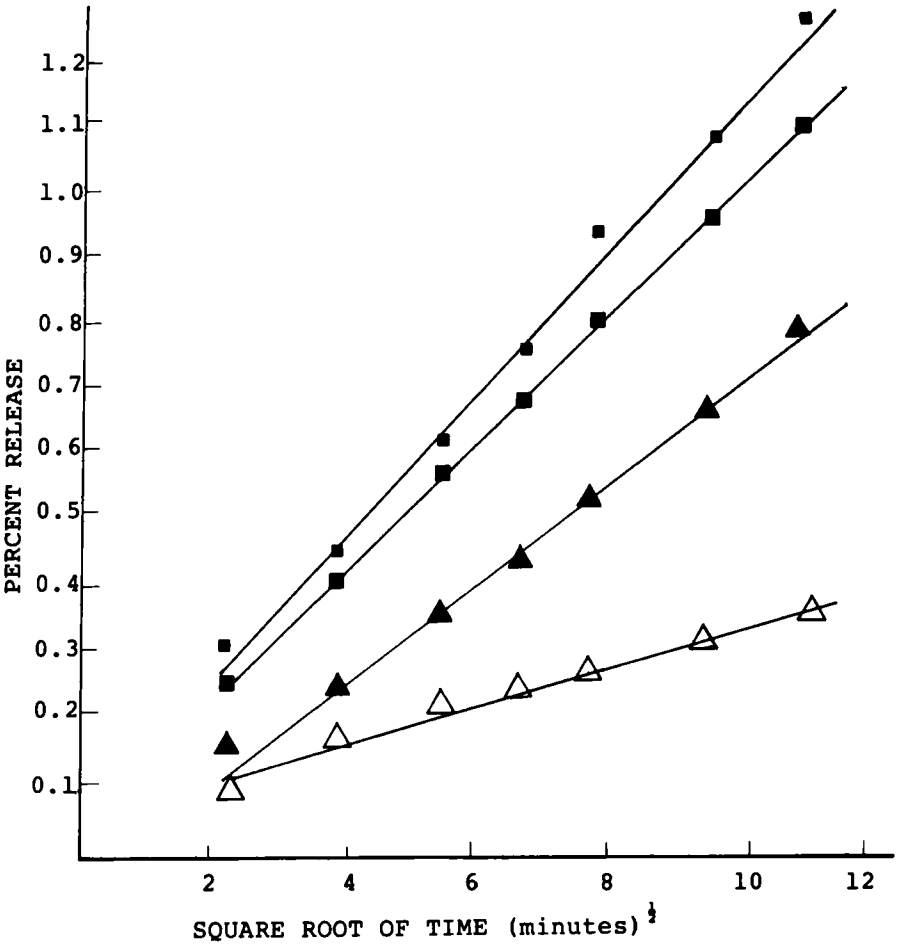

FIGURE 3

Percent Release of Piroxicam from Different Ointment Bases as a Function of the Square Root of Time. Key: (■) Modified Hydrophilic Ointment (B); (■) Hydrophilic Ointment USP (A); (▲) Water Washable Base (C); (▲) Modified U.C.H. Base (G).

diffusion coefficient. The highest value of diffusion coefficient was obtained from the modified hydrophilic ointment and the lowest from the U.C.H. base. This could be due to the fact that the diffusitivity of any drug

TABLE IV

Diffusion, Permeability and Partition Coefficients, Calculated From The In-Vitro Release Of Piroxicam From Different Ointment Bases

Ointment Base	Diffusion Coefficient [D] ($Dx10^{7}$) cm^2/sec	Permeability Coefficient [P] ($Px10^{6}$) cm/sec	Partition Coefficient [K_p]
Modified Hydrophilic Ointment	9.7	3.3	0.27
Hydrophilic Ointment (USP)	8.0	2.9	0.29
Water Washable	4.7	2.1	0.36
Modified U.C.H. Base	0.7	1.0	1.12

through different ointment bases depends on the nature and composition of the individual base and any changes in the nature and composition of the base affect the release rate of the active ingredient.

The permeability coefficient values for piroxicam were also calculated for the in-vitro data by using equation 3, which is derived from the Fick's law of diffusion

$$q = PA\ C_o\ t \qquad \text{(Eq. 3)}$$

where, q = amount of drug diffused (mg) at any given time t (seconds), P = permeability coefficient (cm/sec), A = area of the diffusion membrane (cm^2) and C_o = initial concentration of the drug in the ointment (mg/ml). The values of the permeability coefficients of piroxicam from different ointment bases are presented in Table IV. The highest value was obtained from the modified hydrophilic ointment and the lowest from the modified U.C.H. base. Comparison of the values of the permeability coefficients with those of the diffusion coefficients indicated that they were directly proportional. Thus piroxicam was readily available for diffusion from the modified hydrophilic ointment.

The partition coefficients of piroxicam between the ointment bases and the acceptor medium were calculated by using equation 4, and are shown in Table IV.

$$P = K_p\ D/h \qquad \text{(Eq. 4)}$$

where, P = permeability coefficient (cm/sec.), K_p = par-

tition coefficient, D = diffusion coefficient (cm^2/sec), h = thickness of the barrier (cm)[1].

The partition coefficient indicates the relative distribution of piroxicam between the ointment base and the acceptor medium. It was noted that piroxicam had a lower partition coefficient in hydrophilic ointment or less affinity for the base. Therefore, the drug had a faster release from this particular base.

The release of piroxicam from the various ointment bases used was very low as can be seen from the in-vitro release data in Fig. 1 and 2. Since the release of the drug was less than 5% (1.29%), the in-vitro data can be considered to follow first order kinetics.

By plotting the logarithm of the ratio of initial amount of piroxicam and the amount of piroxicam remaining versus time, straight lines obtained as shown in Fig. 4. By utilizing the concepts of first order kinetics, the release rate constnats was calculated from the slope of the line and are shown in Table V. It is apparent that the modified hydrophilic ointment has the highest release rate content.

The effects of different additives on the release of piroxicam from the modified hydrophilic ointment was studied. Three additives were used, urea, ethanol, and dimethylsulfoxide at 1%, 3% and 5% levels. The release rate data from these formulations are presented in Table

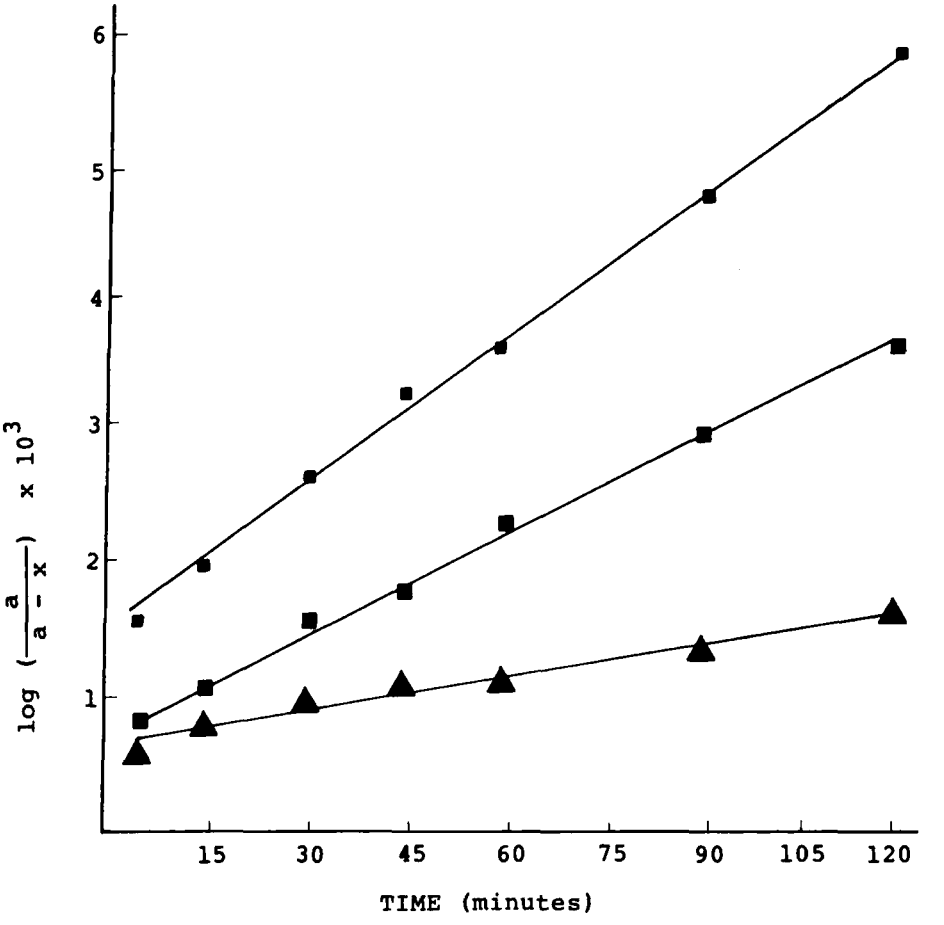

FIGURE 4

Logarithm of the Ratio of the Initial Amount of Piroxicam (a) and the Amount Remaining of Piroxicam (a-x) from Different Ointment Bases versus Time. Key: (■) Modified Hydrophilic Ointment (B); (■) Water Washable Base (C); (▲) Modified U.C.H. Base (G)

TABLE V

Release Rate[1] of Piroxicam from Different Ointment Bases

Ointment	Slope [K/2.303] x 10^5	Y-Intercept x 10^3	Standard Registration (r-value) Coefficient	K x 10^5 ($minutes^{-1}$)
Modified Hydrophilic Ointment	3.63	1.54	0.985	1.58
Hydrophilic Ointment (USP)	2.08	1.45	0.983	1.34
Water Washable Base	2.36	0.78	0.993	1.02
Modified U.C.H. Base	0.86	0.65	0.989	0.37

[1] Derived by utilizing first order kinetic equation.

TABLE VI

Effects of Different Additives on the In-Vitro Percent Release of Piroxicam From the Modified Hydrophilic Ointment After 2 Hours Period of Time

Additive	Percentage[1] Released	(± SD)
No Additive	1.29	(± 0.03)
1% Urea	1.35	(± 0.02)
3% Urea	1.35	(± 0.01)
5% Urea	1.35	(± 0.02)
1% Ethanol	1.31	(± 0.01)
3% Ethanol	1.35	(± 0.02)
5% Ethanol	1.38	(± 0.04)
1% DMSO	1.29	(± 0.03)
3% DMSO	1.28	(± 0.02)
5% DMSO	1.28	(± 0.03)

[1] Average of three determinations

VI. However, using one way ANOVA (Table VII), it was determined that none of these effects were statistically significant.

In-vivo studies - The in-vivo release of piroxicam from the modified hydrophilic ointment, which was the best in-vitro formulation, was studied in rabbits. Piroxicam

TABLE VII

Analysis of Variance of the Percent Release of Piroxicam from the Modified Hydrophilic Ointment in Presence of Urea, Ethnol and DMSO

ANOVA	
Comparison Between Formulations Containing	F-ratio
0, 1, 3 and 5% Urea	2.39[1]
0, 1, 3 and 5% Ethanol	3.78[1]
0, 1, 3 and 5% DMSO	3.37[1]

[1] No significant difference exists between the formulations since $p(F_{3,8} < 4.05) = 0.95$

was incorporated into the modified hydrophilic ointment at concentration levels of 1% and 3%. In addition, the effect of 5% DMSO on the release rate of the modified hydrophilic ointment containing 3% piroxicam was determined.

Piroxicam was detected in serum 60 minutes after the application of the formulation containing 1% drug,

TABLE VIII

Serum Concentration of Piroxicam (mcg/ml)

After Application of Different Ointments

in Rabbits

Time (min)	Serum Concentration (mcg/ml)[1] Formulations		
	B with 1% Piroxicam	B with 3% Piroxicam	B with 3% Piroxicam and 5% DMSO
30	--	0.28 (±0.07)	0.37 (±0.08)
60	0.34 (±0.09)	0.64 (±0.13)	0.64 (±0.08)
90	0.54 (±0.08)	0.88 (±0.14)	1.04 (±0.14)
120	0.61 (±0.10)	1.09 (±0.12)	1.28 (±0.18)
180	--	1.35 (±0.13)	1.56 (±0.17)

[1] Each point is the average of three determinations

while the detection time was decreased to 30 minutes when the formulation containing 3% drug was applied. The apparent delay of drug appearance in the serum is probably due to the low concentration of the drug in the ointment base or it could be due to its high lipid solubility which may delay its passage through the skin of the rabbit. Furthermore, it is apparent from Tables VIII-IX that under the present experimental

TABLE IX

Total Serum Piroxicam Concentration (mcg) After Application of Different Ointments in Rabbits

Time (min)	Total Serum Concentration (mcg)[1]		
	Formulations		
	B with 1% Piroxicam[2]	B with 3% Piroxicam	B with 3% Piroxicam and 5% DMSO
30	--	140.66 (±11.8)	177.50 (±45.18)
60	304.05 (±37.40)	299.74 (±62.6)	294.23 (±92.12)
90	210.91 (±27.62)	421.99 (±51.3)	481.68 (±89.11)
120	236.40 (±28.11)	519.79 (±28.6)	590.64 (±106.49)
180	--	643.35 (±12.6)	721.05 (±91.07)

[1] Each point is the average of three determinations.

[2] For all calculations the volume of distribution was assumed to be 0.14 l/Kg.

condition it is not possible to establish maximum blood levels, therefore, a next study is under way to establish the complete bioavailability profile of piroxicam from ointments.

Furthermore, it was calculated that the addition of 5% DMSO in the modified hydrophilic ointment containing

TABLE X

Analysis of Variance of the Serum Concentration of Piroxicam Obtained From the Modified Hydrophilic Ointment Containing 1%, 3% and 3% Piroxicam with 5% DMSO.

	ANOVA
Comparison between formulations of the modified Hydrophilic ointment containing 1%,3% and 3% Piroxicam with 5% DMSO	F-ratio = 36.38[1]

[1] Significant difference between the formulations exists since $p(F_{2,6} < 5.14) = 0.95$

3% of the drug produced a serum concentration level of 1.56 µg/ml. This value is within the reported range (1.5-2.0 µg/ml) which would be obtained from single oral dose of 20 mg of piroxicam (10).

Statistical evaluation of the in-vivo release data was done using one-way ANOVA. It was found that there are statistically significant differences on the release of piroxicam from the above mentioned formulations after a two hour period of time (Table X), since $p(F_{2,6} < 5.14) = 0.95$.

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FOOTNOTES

[1] Pfizer, Co., CN

[2] Ruger Chemical Corp., NJ

[3] Amend Drug and Chemical Corp., NJ

[4] Amerchol Corp., NJ

[5] ICI United States Inc., NJ

[6] Fisher Scientific Co., NJ

[7] J.T. Baker Chemical Corp., NJ

[8] Beiersdorf Inc., CN

[9] Waters Associates, MA

[10] Shimadzu Bausch and Lomb, NJ

[11] Waters Associates, MA

[12] Perkin-Elmer, NJ

[13] Houston Instrument, TX

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