DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 12(14), 2521-2540 (1986)

MEDICAMENT RELEASE FROM OINTMENT BASES: III. IBUPROFEN: IN VITRO RELEASE AND IN-VIVO ABSORPTION IN RABBITS

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ABSTRACT

The in-vitro release of ibuprofen from various topical bases including: water-washable base, hydrophilic base, cream, Canadian formulary base, gel, emulsion, water-soluble base and University of California Hospital base were studied. Also, the effects of the additives (ethanol, polyethylene glycol-400, urea and dimethylsulfoxide) on the release rate of the drug from the water-washable base were evaluated.

In general, the in-vitro release rate rank order of the drug was observed to be: water-washable base > hydrophilic base > Canadian formulary base > gel > PEG water washable > emulsion > cream > University of California base. The additive ingredients had a little or no effect in enhancing the release of drug from the samples studied.

The formulations with optimum in-vitro drug release were scaled up for in-vivo percutaneous absorption in rabbits. The

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blood samples were analyzed by a HPLC method. Among the candidates evaluated in-vivo, the bioavailability of the drug was significantly higher from the water-washable base when compared to the hydrophilic base and others. The addition of 10% dimethylsulfoxide to the hydrophilic enhanced the release of ibuprofen and adversely affected the release from the water-washable base.

In-vitro and in-vivo data were treated by various kinetic models and the values for diffusion coefficient, permeability coefficient and partition coefficient were calculated. Also, some pharmacokinetic parameters, such as, absorption rate constant, elimination rate constant and half-life of the drug were calculated for meaningful interpretations of the data for the release of drug from topical bases.

INTRODUCTION

Ibuprofen is a potent non-steroidal, anti-inflammatory drug used for the treatment of rheumatoid arthritis (1-5), osteoarthritis, (6,7), acute gouty arthritis (8) and more recently, in the treatment of dysmenorrhea (9,10).

Like other oral anti-inflammatory drugs, ibuprofen is also known to cause gastro-intestinal ulceration and bleeding in humans (11-14). Since, the introduction of over-the counter dosage forms of ibuprofen, these adverse effects associated with oral administration of the drug are of serious concerns to the pharmaceutical industries and to the medical community.

In light of this, a study was undertaken to evaluate the in-vitro release of ibuprofen from various topical bases and screen formulations for in-vitro percutaneous absorption in rabbits. Such investigations could possibly lead to the development of an optimum topical drug delivery system and, consequently,

overcome the untowards effects often encountered with oral administration of this drug.

EXPERIMENTAL

Materials - The following chemicals were used. Ibuprofen[1], White Petrolatum[2], Glyceryl Monosterate[3], Polyethylene Glycol 400[3], Polyethylene Glycol 4000[3], Polyoxyethylene monosterate[3], Cetyl Alcohol[4], Dimethyl Sulfoxide[4], Sodium Lauryl Sulfate[5], Propylene Glycol[5], Stearyl Alcohol[5], Glycerine[5], Acetonitrile[6], Di-Butyl Amine Phosphate[6], Multisterol extract of lanolin (Amerchol Cab)[7], Isopropyl ester of lanolin fatty acids (Amerlate P)[7], Acetylated lanolin, (Modulan)[7], Twenty mole ethoxylate of methyl glucoside (Glucam E-20)[7], Twenty mole ethoxylate of methyl glucoside sesquistearate, (Glucamate SSE-20)[7], Methyl glucoside sesquistearate, (Glucate SS)[7], Urea[8], and Cellophane Membrane[9]. All chemicals were used as received.

Preparation of Ointments - All the ointments were prepared by the fusion method as follows: All the aqueous phase ingredients and the oil phase ingredients were placed into two different beakers and heated to 75 ± 5°C. The water phase was then added slowly to the oil phase with continuous stirring until it congealed at room temperature. The following ointments (Table I) were prepared by the fusion method: Hydrophilic, (I), University of California Hospital Base (UCH), (II), water washable (III), Canadian Formulary Base (IV), PEG-water washable, (V) emulsion base (VI) Gel (VII) and cream (VIII). All formulations contained 3% Ibuprofen while formulas (I-IV) contained sodium bicarbonate.

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1 Ethyl Corporation
2 Pharmaderm Inc., NY
3 Myrj 52; Ruger Chemical Co., NJ
4 DMSO; Fisher Scientific Co., NJ
5 Amend Drug and Chemical Co., Inc., NJ
6 Water Associate, Millford, MA
7 Amerchol Corporation, NJ
8 T.J. Baker Chemical Corporation, NJ
9 Spectrum Medical Industires, Inc., LA, CA

Table I. - Formula of Different Ointments

Ingredient #	Formulation (weight in grams)							
	I	II	III	IV	V	VI	VII	VIII
White Petrolatum	25	14.3	20	--	-	--	--	--
Stearyl Alcohol	15	5.4	3	20.8	-	--	--	--
Propylene glycol	12	--	--	--	-	--	--	--
Cetyl alcohol	--	6.4	--	--	2.0	--	4	--
Mineral oil	--	21.4	--	--	6.0	--	--	--
Multisterol extract lanolin	--	--	5	--	-	--	--	--
Isopropyl ester lanolin fatty acids	--	--	2	--	-	--	--	--
GMS-SE	--	--	5	--	-	--	--	--
Polyoxyethylene monostearate	--	--	4	--	-	--	--	--
Glycerin	--	--	5	17	-	--	5	--
PEG 400	--	--	--	11.2	-	60	--	--
Methyl glucoside sesquistearate	--	--	--	--	0.8	--	--	--
Acetylylated lanolin	--	--	--	--	2.0	--	--	--
Stearic acid	--	--	--	--	2.0	--	18	--
20 mole ethoxylate of methyl glucoside sesquistearate	--	--	--	--	12	--	--	--
20 mole ethoxylate methyl glucoside	--	--	--	--	5.0	--	--	6
Bentonite	--	--	--	--	1.5	--	--	--
PEG-4000	--	--	--	--	-	30	--	--
Sodium bicarbonate	--	--	1.22	--	-	--	--	--
Span-40	--	--	--	--	-	1	--	--
Triethanolamine	--	--	--	--	-	--	2	10
Carbopol 940	--	--	--	--	-	--	--	0.5
PEG-6000	--	--	--	--	-	--	--	4
Tween-20	--	--	--	--	-	--	--	10
Triethanolone (10% in water)	--	--	--	--	-	--	--	--
Propyl paraben	0.015	--	0.067	--	-	--	--	--
Methyl paraben	0.025	--	0.015	--	-	--	--	--
Sodium Lauryl sulfate	1.00	1.5	--	1	-	--	--	--
Ibuprofen	3	3	3	3	3	3	3	3
Water q.s.	100	100	100	100	100	100	100	100

CONTENT UNIFORMITY

All samples prepared were analyzed for the ibuprofen content prior to their use by HPLC method (15). Only formulations with drug contents of 100 ± 10% were used in this study.

IN-VITRO RELEASE STUDIES

A one ounce plastic jar of known weight was filled completely with the sample formulation and the weight was determined.

The surface of the ointment was then covered by a semipermeable membrane with a molecular weight cut-off point of 1000 and secured with a rubber band and thread. The calculated contact surface area of the drug was 9.6 cm^2.

The ointment jars filled with samples were then immersed invertedly in a 250 ml beaker containing 100 ml of phosphate buffer (pH = 8) previously heated and maintained at 37° ± 1°C in a water bath. A 5 ml sample of the diffusion medium was withdrawn at 5, 15, 30, 45, 60, 95 and 120 minutes and replaced with an equal volume of the dissolution medium. The diffusion medium was continuously stirred prior to withdrawal of the sample at each time interval. The samples were analyzed for ibuprofen contents spectrophotometrically at wavelength of 264 nm.

IN-VIVO RELEASE STUDY

White male New Zealand rabbits each weighing 5-8 pounds were selected for percutaneous absorption of the drug from the selected formulations. The hair from the midback portion of the rabbit was shaved carefully, and a known amount of the sample ointment was uniformly applied to an approximately 26 square centimeters. Following application, a 2 ml sample of blood was withdrawn from the ear vein at 5, 15, 40, 60, 90 and 120 minutes time intervals. The blood samples were allowed to clot at room temperature for 15 minutes and later centrifuged at 2000 rpm for 30 minutes. The serum samples separated, were stored in a freezer up until the analysis of the drug was carried out.

ANALYTICAL PROCEDURE FOR IBUPROFEN IN SERUM

The analysis of the drug in serum was carried out by slightly modifying a previously published method by this laboratory (15). A high performance liquid chromatograph (HPLC) equipped with a universal liquid chromatographic in-

jector, a UV detector at 254 nm, a stop chart recorder and a minigrator were used.

The serum samples were chromatographed at room temerature on a microparticulate (μBondapak C-18) reversed phase HPLC column (4mm x 30 cm) with an eluting mobile phase of acetonitrile/0.02M dibutylamine phosphate mixture of (45:55 v/v) ratio. The flow rate was adjusted to 3 ml per minute with an inlet pressure of 2500 psig. The chart speed used was 0.2 inch per minute. The column effluent was continuously monitored by a UV absorption detector previously set at 254 nm. A standard curve was constructed by using known concentration of ibuprofen-spiked plasma samples for calculating the drug concentration. The ratio of the peak area of the drug to that of the internal standard was used to quantitatively measure the ibuprofen concentration in various samples.

INTERNAL STANDARD

An accurately weighed quantity of sulindac equal to 10 mg was transferred to a 10 ml volumetric flask and diluted to volume with methanol. A 2 ml of this solution was then diluted to 100 ml with methanol and used as the internal standard.

BLOOD SERUM ASSAY

A 0.5 ml of serum was mixed with 2 ml of the internal standard solution. The mixture was centrifuged for 10 minutes, and the supernatant was removed and passed through an acrodisc of an average pore size 0.45μ m. A 100 microliter of this solution was then injected into the column through a stop flow injector port for the drug analysis as described previously.

RESULTS AND DISCUSSION

IN-VITRO RELEASE STUDIES

The in-vitro release of ibuprofen from the various ointment bases is shown in Table II. From this, it is observed that

TABLE II

PERCENT RELEASE OF IBUPROFEN FROM DIFFERENT BASES WITH TIME

Ointment bases	Time (minutes)[a] % Released						
	5	15	30	45	60	90	120
Water Washable	0.745	1.220	1.950	2.560	3.050	3.950	4.670
Hydrophilic	0.365	0.479	0.696	0.852	0.977	1.269	1.400
U.C.H.	0.150	0.164	0.244	0.271	0.299	0.353	0.426
PEG Water Washable	0.084	0.130	0.183	0.314	0.435	0.624	0.863
Canadian Formulary	0.222	0.445	0.664	0.809	0.903	1.160	1.390
Gel	0.144	0.315	0.502	0.665	0.826	1.138	1.383
Cream	0.080	0.221	0.276	0.321	0.387	0.459	0.502
Emulsion	0.276	0.443	0.533	0.644	0.683	0.753	0.833

[a] each point is the average of three determinations

the release of drug was maximum from the water-washable base and the general release rate rank order was as follows: water-washable base > hydrophilic base > Canadian formulary base > gel > PEG water washable > emulsion base > cream and > University of California base.

The release of a drug from ointment can be expressed by the following equation (16).

$$Q = h\,C_o\left[1 - \frac{8}{\pi^2}\sum_{m=o}^{\alpha}\frac{1}{(2m+1)^2}\,e^{\frac{-D\,(2m+1)^2 n^2 t}{2\,h^2}}\right] \qquad \text{(Eq. 1)}$$

Where Q = amount of drug release (number of moles/unit area), h = thickness of the layer (cm), C_o = initial concentration of the drug (moles/liter) in ointment, t = time in minutes after application, and m = integer, as goes from 0 - α.

The equation above is based on the assumption that (a) one drug specie is improved in the ointment, (b) D must be constant with respect to both the time and position of the layer (c) only the drug is able to diffuse out the layer and finally, (d) the drug reaching the body is rapidly diluted.

Higuchi (17) reported a simplified version of equation 1. which is applicable to the system, where the percentage of drug release remains under 30. This is represented as follows:

$$R = \frac{D\,t^{\frac{1}{2}}}{\pi\,h^2} \qquad \text{(Eq. 2)}$$

Where R = percentage of the drug release, D = diffusion coefficient (cm^2/sec), t = time in minutes of the application of the ointment and h = thickness of the ointment layer.

In this study, since the percentage amount of ibuprofen released remained under 30, the experimental parameters appear to conform approximately to all of the above requirements of equation 2. When the percentage of drug released from the water washable base was plotted against square root of time, a straight

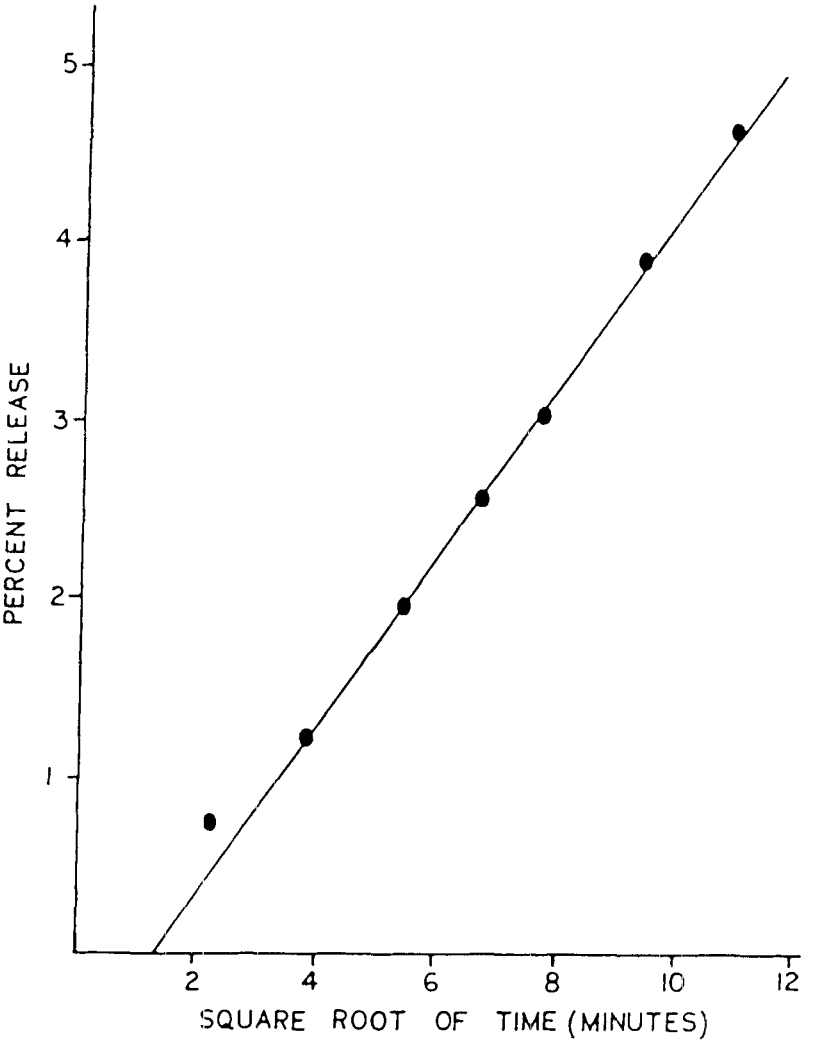

Figure 1

Square Root of Time Versus Percent Release Plot for Water Washable Base.

line was obtained as shown in Figure 1. Therefore, equation 2 was used to calculate the values for the diffusion coefficient of the drug from various experimental ointment bases and are exhibited in Table III. The Higuchi equation indicates that when the drug is dissolved in the vehicle, its release can be altered by changing its diffusion coefficient.

TABLE III

VALUES OF DIFFUSION, PERMAEABILITY, AND PARTITION COEFFICIENTS FOR DIFFERENT OINTMENTS OBTAINED FROM IN VITRO DATA

Ointments	Diffusion coefficient $(D \times 10^{7})\ cm^2/sec$	Permeability coeffieicents $(P \times 10^{5})\ cm/sec$	Parition coefficient $(K_p \times 10^{-2})$	Zero Order Rate Constants $(K_o \times 10^{4})\ \frac{moles}{l/hr}$
Water washable	5.33	2.90	0.87	1.52
Hydrophilic	0.56	0.88	2.51	0.46
Canadian formulary	0.49	0.88	2.87	0.46
Gel	0.40	0.84	3.36	0.44
Emulsion	0.26	0.61	3.75	0.32
PEG water washable	0.11	0.48	6.98	0.25
Cream	0.07	0.39	8.31	0.20
U.C.H.	0.05	0.27	8.31	0.14

From the release data, it is observed that the sodium salt of ibuprofen being less soluble in the oil phase is readily available for diffusion, which is evident from the higher diffusion coefficient value of 5.33×10^{-7} cm^2/sec. On the other hand, ibuprofen in the UCH base is more soluble due to higher contents of lipids, therefore, the amount of drug available for diffusion is decreased. Consequently the value observed for the diffusion coefficient is low (0.05×10^{-7} $cm^2/sec.$). The permeability coefficient values for the in-vitro release data were calculated by using the following equation and are shown in Table III.

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

Where q = number of moles of the drug diffused through the membrane at time t, P = permeability coefficient (cm/sec), A = area (cm^2) of the diffusion membrane, and C_o = concentration (m/l) of the drug present at time zero.

The permeability coefficient values are valid, only if the drug concentration in the vehicle remains in large excess to the amount actually released, and stays essentially unchanged during the rate measurements. The in-vitro release of ibuprofen from all formulations is observed to be very low, therefore, the values of permeability coefficient calculated by the above equation are considered valid. Since the permeability coefficient is directly proportional to the diffusion coefficient, the formulations with higher diffusion coefficient values also gave the higher permeability coefficient values, and the samples with lower diffusion coefficient values gave the lower permeability coefficient values as exhibited in Table III.

The important variable in the permeability coefficient is actually the partition coefficient factor. The diffusivity of substances of similar molecular weight and shape usually differ

only slightly (16). This relationship can be expressed by equation 4.

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

Where P = permeability coefficient (cm/sec), K_p = partition coefficient, D = diffusion coefficient (cm^2/sec) and h = thickness of the barrier (cm).

The partition coefficient is an indication of the distribution of ibuprofen between the formulation vehicle and the receiving medium. In water-washable base, where the drug is poorly soluble, the K_p value remained low (0.87×10^2) compared to the UCH base, where ibuprofen is more soluble, the K_p value was observed to be very high (8.31×10^2). The partition coefficient values the drug for all samples evaluated are also listed in Table III.

Since the amounts of ibuprofen released from various formulations evaluated were found to be extremely low and independent of drug concentration, the data were also treated by zero order kinetic using the following equation:

$$K_o = (A_o - A_t)/t \qquad \text{(Eq. 5)}$$

Where K_o = zero rate constant (moles liter $^{-1}hr^{-1}$), A_o = initial concentration (m/l), and A_t = drug concentration (moles/liter) at time t (hour). The results of the values of K_o obtained for the various formulation are exhibited in Table III.

The effect of different additives (Polyethylene glycol-400, urea, ethanol and dimethylsulfoxide) on the release of ibuprofen release from the water-washable base were also investigated. Each additive was included in these formulation at 5% and 10% concentration level. Addition of the additive ingredients had little or no effect on the enhancement of the in-vitro drug release.

IN-VIVO STUDIES

The formulations with maximum in-vitro drug release i.e., water-washable base, water-washable base with 10% dimethylsulfoxide (DMSO), hydrophilic base and hydrophilic base with 10% DMSO were selected for percutaneous absorption studies in rabbits. The blood serum ibuprofen concentration obtained for the above formulation from rabbits are shown in Table IV.

After application of the ointment sample on the skin of the rabbit the drug undergoes the process of absorption, metabolism and excretion simultaneously. The rate of appearance of the drug in blood serum at any given time can be expressed as follows (18).

$$\frac{dX}{dt} = k_a X_a - KX \qquad \text{(Eq. 6)}$$

Where k_a is the absorption rate constant, X_a is the amount of drug remaining at the site of absorption, K is the overall elimination rate constant, and X is the amount of drug absorbed. The above equation 6 can be integrated as follows:

$$X = \frac{k_a F X_o}{(k_a - K)} (e^{-Kt} - e^{-k_a t}) \qquad \text{(Eq. 7)}$$

or,

$$C = \frac{k_a F X_o}{(k_a - K)V} (e^{-Kt} - e^{-k_a t}) \qquad \text{(Eq. 8)}$$

Where F is the fraction of the administered dose, X_o is the amount absorbed following the application of ointment and V is the apparent volume of distribution. As the amount of drug remaining at the absorption sited decreases, the rate of absorption also decreases unit $e^{-k_a t} \to 0$. At this point the plasma drug concentration can be described only by the elimination rate constant of the drug, and equation 8 can thus be

TABLE IV

SERUM CONCENTRATION (mcg/ml) OF TOPICALLY APPLIED IBUPROFEN ON RABBITS FROM DIFFERENT OINTMENTS

	Hydrophilic base		Water washable base	
Time (min)	Plain[a]	10% DMSO[b]	Plain[a]	10% DMSO[b]
5	-	7.50	7.00	6.31
10	4.92	-	-	-
15	-	9.00	7.51	6.45
20	5.90	-	-	-
30	8.62	12.00	10.96	10.17
60	11.31	16.60	13.45	12.73
90	7.15	11.33	10.73	9.41
120	-	-	5.66	4.83

[a] each number is the average of two rabbits

[b] each number is the average of three rabbits

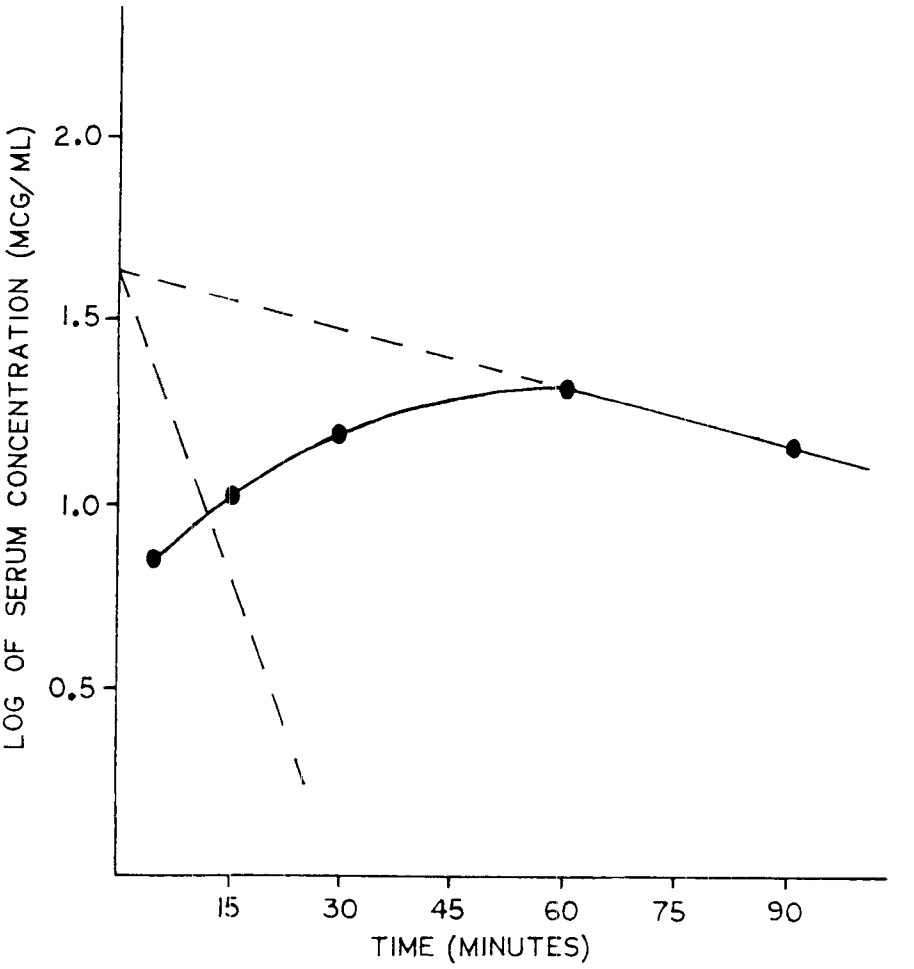

Figure 2

A Typical Plot of Log of Serum Concentration Versus Time for the Calculation of Rate Constants.

simplified as:

$$C' = \frac{k_a F X_o}{(k_a - K)V} e^{-Kt} \qquad \text{(Eq. 9)}$$

where C' is the plasma drug concentration during the post-absorption phase at any given time t.

If $A = \frac{k_a F X_o}{(k_a - K)V}$, then equation 9 can be expressed as:

$$c' = A\, e^{-KT} \qquad \text{(Eq. 10)}$$

$$\log C' = \log A - Kt/2.303 \qquad \text{(Eq. 11)}$$

It is therefore, observed that if log C' is plotted versus time, a straight line will be obtained with a slope -K/2.303. Therefore, the elimination rate constants and $t_{\frac{1}{2}}$ lives of ibuprofen after topical application to the rabbit skin were calculated and reported in Table V. From these data, it is observed that the $t_{\frac{1}{2}}$ of ibuprofen ranged from 50.2 minutes from the hydrophilic ointment to 69.9 minutes from the water-washable base with 10% DMSO. However, in human the $t_{\frac{1}{2}}$ for this drug has been reported to be approximately 2 hours. This decrease in half life of ibuprofen in rabbits may be attributed to the species variation, metabolism pattern during the percutaneous absorption, conditions of the experiments, etc.

Furthermore, equation 7 can be written as follows:

$$C = Ae^{-Kt} - Ae^{-k_a t} \qquad \text{(Eq. 13)}$$

or

$$Ae^{-Kt} - C = Ae^{-k_a t} \qquad \text{(Eq. 14)}$$

Since $Ae^{-Kt} = C'$

Equation 14, therefore, becomes:

$$C = Ae^{-K_a t} \qquad \text{(Eq. 15)}$$

or, $\log (C' - C) = \log A - K_a t/2.303$ (Eq. 16)

and, since the terminal position of the plasma concentration (log scale) versus time plots represents only the elimination phase, it can be extrapolated back to time zero on the plasma concentration axis, and C' for any time can be obtained. From the slopes of the lines, the K and k_a values were calculated as shown in Figure 2 and Table V.

The degree of percutaneous absorption of ibuprofen from the various formulations evaluated was estimated by calculating areas under the curve using trapezoidal method (19) as indicated below:

TABLE V

RELATED BIOAVAILABILITY PARAMETERS FROM SERUM LEVEL DATA

Ointment Bases	Absorption rate constant ($k_a \times 10^2$) min^{-1}	Elimination rate constant ($K \times 10^2$) min^{-1}	Half-life ($t_{1/2}$) (minutes)
	Average k_a	Average k	Average ($t_{1/2}$)
	3.89	1.38	50.2
with 10% DMSO	3.57	1.32	53.8
Water washable	3.10	1.39	69.9
Water washable with 10% DMSO	4.05	0.99	69.9

TABLE VI

COMPARATIVE BIOAVAILABILITY FROM DIFFERENT OINTMENTS

Ointment Bases	t^a_{max} (minutes)	Avergae C_{max}^b (mcg/ml)	Average AUC_{90}^5 (mcg/ml minute)
Hydrophilic	60	11.31	648.5
Hydrophilic with 10% DMSO	60	16.60	1079.3
Water washable	60	13.95	948
Water washable with 10% DMSO	60	12.74	905.11

[a] t_{max} is the time of peak serum concentration

[b] C_{max} is the peak serum concentration

$$AUC = t[(C_o + C_1)/2] + t[(C_1 + C_2)/2] \quad t[(C_{n-2} + C_{n-1})] + t[(C_{n-1} + C_n)/2]$$

Where, t is the time interval between any two samples, C_o, C_1, ... C_{n-1}, C_n are the serum concentrations at time t_o, t_2, ... t_{n-1}, t_n.

The AUC, the time of peak plasma concentration (t_{max}), and the peak plasma concentration (C_{max}) are shown in Table VI. The AUC of ibuprofen obtained from hydrophilic base and hydrophilic base with 10% DMSO were found to be 648.50 mcg/ml min and 1079.30 mcg/ml min respectively. This indicates that incorporation of DMSO almost doubled the percutaneous absorption and bioavailability of the drug. The AUC obtained from the water washable base was found to be 948 mcg/ml min and that from the same base with 10% DMSO was 905.11 mcg/ml min, indicating a decrease in bio-

availability of the drug. These findings are in agreement with the previously published studies (21-23).

Acknowledgements

Presented in part at the 36th National meeting of the Academy of Pharmaceutical Sciences, Montreal, Canada

Abstracted from a thesis submitted by A. Muktadir to the Graduate Faculty, of the Arnold and Marie Schwartz College of Pharmacy and Health Sciences, L.I.U., in partial fulfillment of the M.S. degree requirements

This work was supported in part by a Grant from the Zupko-Schwartz Endowment Fund

The authors thank Boots Pharmaceuticals, Shreveport, Louisiana, for providing ibuprofen and Amerchol Corp., N.J. for providing a number of chemical.

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