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Stability, anti-inflammatory effect and percutaneous absorption of indomethacin in absorption ointment formulations

Yi-Hung Tsai [1], Min-Yung Yang [1] and Shun-Ichi Naito [2]

[1] *School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan (R.O.C.) and* [2] *Kyoto College of Pharmacy, Kyoto 607 (Japan)*

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Summary

Both the topical and percutaneous anti-inflammatory activities of absorption ointment formulations containing indomethacin were investigated in rabbits. Indomethacin absorption ointment significantly inhibited the formation of granuloma and decreased edema on the dorsal skin induced by a subcutaneous injection of carrageenin.

The determination of indomethacin and its hydrolysis products such as *p*-chlorobenzoic acid and 5-methoxy-2-methyl-3-indole acetic acid from an ointment was proposed by using reversed-phase HPLC and UV detection. The apparent first-order rate constant can be obtained accurately and easily by using the Weibull probability paper, and was used to predict the stability of indomethacin in an ointment. The stability increased in proportion to the concentration of polysorbate 20 or polysorbate 80. However, a marked decrease in the percutaneous absorption of indomethacin occurred with the absorption ointment base containing a concentration of 4.62% polysorbate 20. But the additional concentration of polysorbate 80 below 4.62% did not produce any significant change in percutaneous absorption of indomethacin.

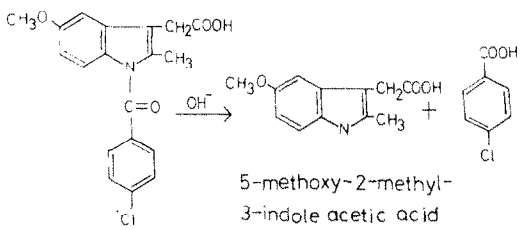
Introduction

The influence of the characteristics of the ointment bases on the percutaneous absorption of indomethacin has been described previously (Naito and Tsai, 1981). It was demonstrated that good percutaneous absorption of the drug was found with an absorption ointment at a low pH value of the indomethacin solution in the ointment (Tsai and Naito, 1982). Therefore, the composition of the ointment base is believed to affect significantly the ability of indomethacin in a topical formulation to penetrate the skin. The topical anti-inflammatory effects of percutaneous indomethacin from gel ointment (Ishihama et al., 1979; Wada et al., 1982) and poultice (Tanaka et al., 1981) have already been studied, but that from the absorption ointment base has not yet been investigated. Therefore the present study examined the anti-inflammatory activity of indomethacin in the absorption ointment in rabbits.

From the pharmaceutical application viewpoint, the stability of indomethacin in absorption ointment base is important. Indomethacin is stable in a neutral or slightly acidic medium, but decomposes under alkaline conditions and is known to hydrolyze to *p*-chlorobenzoic acid and 5-methoxy-2-methyl-3-indole acetic acid as shown in Scheme

Correspondence: S.-I. Naito, Kyoto College of Pharmacy, Kyoto 607, Japan.

1 (Hajratwala and Dawson, 1977; Krasowska, 1974; Cipiciani et al., 1983; Rowe and Carless, 1983). The stabilization of indomethacin against alkaline hydrolysis in an aqueous solution containing non-ionic surfactants had been also reported (Dawson et al., 1977; Krasowska, 1979). The in-



Indomethacin

P-chlorobenzoic acid

Scheme 1. Pathway of indomethacin hydrolysis.

domethacin absorption ointment was prepared by dissolving the drug in a slightly alkali medium as internal phase. Therefore, it was necessary to evaluate the stability of indomethacin in the absorption ointment base. From a practical point of view, the present work made an attempt to use the Weibull probability paper (Okusa, 1975) and to predict the stability of the indomethacin in the absorption ointment. In addition, the percutaneous absorption of indomethacin from the absorption ointment containing a stabilizier such as polysorbate was investigated in rabbits under the same method as before (Naito and Tsai, 1981).

Materials and Methods

Materials and ointment preparation

The reagents and the preparation of the indomethacin absorption ointments formulated in Table 1 were essentially the same as that described previously (Naito and Tsai, 1981). Polysorbates [1] or other stabilizers such as propylene glycol [2], butyl hydroxyanisole, sodium bisulfite [2], α-tocopherol [3] and β-cyclodextrin [4] were used. Carrageenin [5] is type IV, lambda fraction. The hydrolysis product of indomethacin, 5-methoxy-2-methyl-3-indole acetic acid was prepared by a method detailed in the Japanese Pharmacopoeia (1976). The material produced exhibited IR α-NMR spectra identical with those of authentic data (Pouchart, 1978).

Carrageenin-induced paw edema in rabbits

The study was conducted on male rabbits weighing 1.8–2.2 kg fasted for 18 h with water available ad libitum. Paw edema was induced by subcutaneous injecting into the right plantar aponeurosis with 0.5 ml of a 2.5% carrageenin physiologic saline solution (Winter et al., 1962). The volume for the right hindpaw was measured by means of a water–mercury plethysmometer (Lence, 1962). The time course of the anti-edemal effect of the following treatment was studied: oral indomethacin (20 mg/kg by an oesophageal tube, q.i.d.); topical indomethacin absorption ointment (no. A, weight 6 g): application to the "inflamed" hindpaw (8×7.5 cm^2) with the occlusive dressing technique (ODT); topical indomethacin absorption ointment (no. A, weight 6 g): application to the previously depilated abdominal skin (6×10 cm^2) with ODT.

Carrageenin-induced edema on the dorsal skin (punch method)

Male rabbits weighing 1.8–2.2 kg were used, and the method was a modified technique of that described in the previous report (Tanaka et al., 1981). A 0.2 ml of 2.5% carrageenin physiological saline solution and a 0.2 ml of a physiological saline solution as the control were injected subcutaneously into selected sites on either side of the dorsal midline, respectively. The skin of the treated area was excised, and disks (120 mm diameter) were punched out of the "inflamed" and contralateral "control" area after the administration of the drug at appropriate time intervals. The difference in weight between the pairs of disks from each rabbit denoted the oedema. The time course of the anti-edemal effect of the following

[1] E. Merk, F.R.G.
[2] Wako pure Chemicals, Osaka, Japan.
[3] A commercial grade.
[4] Nakarai Chemicals, Kyoto, Japan.
[5] Sigma, U.S.A.

TABLE 1

FORMULA OF THE INDOMETHACIN ABSORPTION OINTMENTS

Component	Concentration (% w/w)			
	No. A	No. B	No. C	No. D
White vaseline	33.33	33.33	33.33	33.33
Cetyl alcohol	15.0	15.0	15.0	15.0
Arlacel-C	5.0	5.0	5.0	5.0
Brij-35	0.5	0.5	0.5	0.5
Indomethacin	3.33	3.33	3.33	3.33
$NaHCO_3$	1.0	0.75	0.75	0.75
Polysorbate	–	–	1.15–15	4.62
Other stabilizers	–	–	–	0.09–18.73
Water	41.84	42.09	40.94–27.09	37.38–18.74

treatment was studied: oral indomethacin (20 mg/kg using an esophageal tube, once a day); topical indomethacin absorption ointment (no. A, weight 6 g): application to the "inflamed" area (6×10 cm^2) with ODT; topical indomethacin absorption ointment (no. A, weight 6 g): application to the previously depilated abdominal skin (6×10 cm^2) with ODT.

Cotton pellet method

The anti-inflammatory effect of the indomethacin ointment on the granuloma formation by means of the cotton pellet method in rabbits weighing 1.8–2.2 kg was measured by modifying the method described previously (Tanaka et al., 1981). Two accurately weighed pellets of cotton (70 mm diameter; weight 50 mg) were implanted, under ether anaesthesia using the sterile technique, one on either side of the dorsal midline incision. Six grams of the indomethacin absorption ointment (no. A) was applied to the previously depilated dorsal or abdominal skin (6×10 cm^2) with ODT for 10 h every other day for 17 days after the implantation of the cotton pellet. The weight of the granuloma was measured on the 17th day after the tissue was dried for 16 h at 60°C.

Stability of indomethacin in ointment

An accurately weighed quantity, 200 mg of the indomethacin ointment, was placed in a 7-ml amber vial which was closed with a rubber stopper and an aluminum seal, and then was stored in a constant-temperature heater at 40, 50 and 60°C at appropriate intervals, the vials were removed from the heater and chilled until analysis, the remaining content of indomethacin and its decomposition products were analyzed by the HPLC method.

Chromatographic condition

A Waters Associates Model 450 high-performance liquid chromatograph equipped with a UV detector, U6K universal injector and μ-Bondapak C_{18} (3.9 mm i.d. $\times$ 32 cm) column was used. The mobile phase consisted of a methanol–0.05% acetic acid (65 : 35 v/v) mixture. The operating temperature was ambient, and the flow-rate was 1.5 ml/min. The column effluent was monitored continuously at 254 nm with a 0.1 aufs and the chart speed of the recorder was maintained at 5 mm/min.

Analytical procedures

At appropriate time intervals, the vials containing the 200-mg ointment tested were withdrawn and 1 ml of tetrahydrofuran was injected directly into the ointment. The sample ointment was dissolved by vortexing for 1 min. Four ml of methanol was added, vigorously shaken for 5 min, and the sample was centrifuged at 1000 rpm for 5 min. A 0.1-ml portion of upper clear layer was accurately transferred into another tube and evaporated to dryness under nitrogen in a 40°C water bath.

The residues were redissolved in 1.8 ml of the mobile phase and 0.2 ml of the phenylbutazone methanol solution (0.4 mg/ml) was added as the internal standard and mixed for 1 min by a vortex mixer, then was filtered through a 0.45 μm filter. This filtrate amounting to 10 μl was injected into the column for HPLC through a stop-flow injection port.

The calibration curves were prepared, using a known concentration of indomethacin and its two decomposition products with a plain ointment base, by plotting the concentration of indomethacin or its decomposition products (mg/g of ointment) against the respective peak-height ratios. The analytic procedure for all blood specimen was similar to the method described previously (Tsai and Naito, 1981), except that the internal standard and the extract solvent were replaced by phenyl-

butazone and cyclohexane–ether solvent, respectively.

Results and Discussion

The indomethacin absorption ointment (no. A) applied to the abdominal site displayed significantly inhibitory effects on the carrageenin-induced paw edema at 8 h and the activity was approximately equivalent to that of the oral route (Table 2). However, there was no influence of this regarding the application of the ointment to the "inflamed" hindpaw (Fig. 1). It is possible that may be attributed to the fact that indomethacin penetrates poorly into the hindpaw from the ointment base. The time course of the anti-edemal effect by means of the punch method is shown in Fig. 2. The oral route was the least effective, but percutaneous indomethacin displayed considerable inhibitory potency; more inhibition when given on the "inflamed" dorsal skin (100%) than on the abdominal skin (78.5%) as shown in Table 3. The absorption ointment also produced similarly significant inhibitory effects on the granuloma formation of the implanted cotton pellet after being applied either to the "inflamed" dorsal skin (49.8%) or the abdominal skin (56.7%) as shown in Table 4.

The indomethacin absorption ointment (no. A) was shown to have some anti-inflammatory activity mentioned above and a good percutaneous

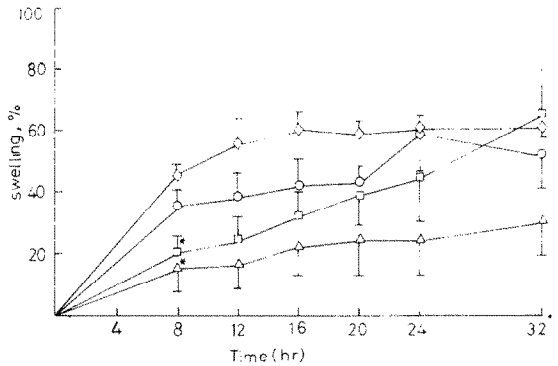


Fig. 1. Effect of indomethacin on swelling of rabbit hindpaw induced by carrageenin (2.5%, 0.5 ml). Key: ○, control; △, 20 mg/kg, p.o. at 0, 8, 16 and 24 h after carrageenin injection; □, topical application in abdomen; ◇, topical application in injected hindpaw. Means ± S.E. of 4 rabbits. * Significant difference from zero-time control value: $P < 0.05$.

absorption (Tsai and Naito, 1982). The indomethacin absorption ointment was prepared by the drug dissolved in slightly alkali medium containing 1% sodium bicarbonate. Therefore, it was necessary to evaluate the stability of indomethacin in the absorption ointment base and to develop an analytical procedure for indomethacin and its decomposition in ointment.

Fig. 3 gives typical chromatograms for 5-methoxy-2-methyl-3-indoleacetic acid, *p*-chlorobenzoic acid, phenylbutazone and indomethacin obtained from a spiked ointment sample (Fig. 3, Y) and aged absorption ointment sample (Fig. 3, Z). Under the chromatographic conditions de-

TABLE 2

THE ANTI-INFLAMMATORY EFFECT OF INDOMETHACIN OINTMENT (NO. A) ON THE CARRAGEENIN-INDUCED EDEMA IN THE HINDPAW OF RABBITS

Groups	Site of application	Applied area (cm^2)	Swelling (%) at 8 h	Inhibition (%)
Control	–	–	35.1 ± 5.93	–
Topical indomethacin	"inflamed" paw	8 × 7.5	45.3 ± 3.51	–
Topical indomethacin	abdomen	6 × 10	20.0 ± 5.87 *	43.0
Indomethacin p.o.	–	–	14.9 ± 7.96 *	57.5

* Significant difference from control value: $P < 0.05$.
Each value indicates mean ± S.E. (n = 4).

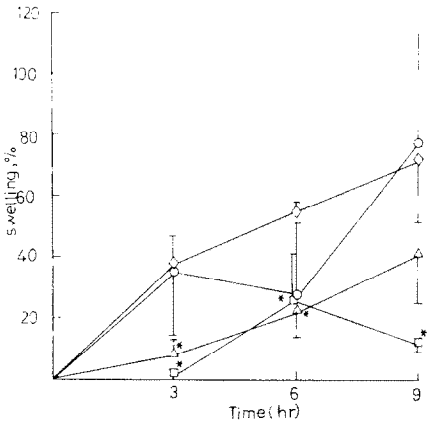


Fig. 2. Effect of indomethacin on swelling of rabbit dorsal skin induced by carrageenin (2.5%, 0.5 ml). Key: ◇, control; ○, 20 mg/kg, p.o. at 0 h after carrageenin injection; △, topical application in abdomen; □, topical application in injected dorsal skin. Means ± S.E. of 4 rabbits. * Significant difference from zero-time control value: $P < 0.05$.

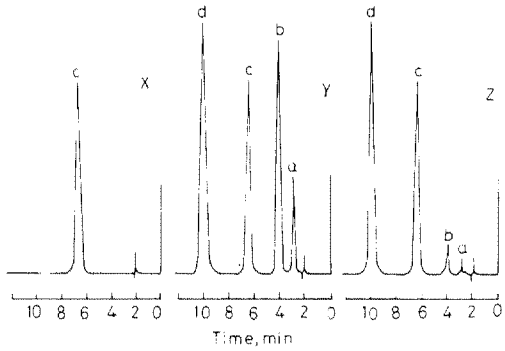


Fig. 3. High-performance liquid chromatograms of the ointment sample. Key: X, plain ointment containing phenylbutazone (c) as internal standard; Y, plain ointment containing phenylbutazone (c) as internal standard, 5-methoxy-2-methyl-3-indoleacetic acid (a), *p*-chlorobenzoic acid (b) and indomethacin (d); Z, indomethacin absorption ointment after storage for two days at 60°C.

TABLE 3

THE ANTI-INFLAMMATORY EFFECT OF INDOMETHACIN OINTMENT (NO. A) ON THE CARRAGEENIN-INDUCED EDEMA ON THE DORSAL SKIN OF RABBITS

Groups	Site of application	Applied area (cm^2)	Swelling (%) at 3 h	Inhibition (%)
Control	–	–	37.6 ± 8.91	–
Topical indomethacin	"inflamed" dorsal skin	6 × 10	0 *	100
Topical indomethacin	abdominal skin	6 × 10	8.1 ± 4.86 *	78.5
Indomethacin, p.o.	–	–	35.2 ± 20.65	6.4

* Significant difference from control value: $P < 0.05$.
Each value indicates mean ± S.E. (n = 4).

TABLE 4

THE ANTI-INFLAMMATORY EFFECT OF INDOMETHACIN OINTMENT (NO. A) ON THE GRANULOMA FORMATION BY MEANS OF THE COTTON PELLET METHOD IN RABBITS *

Groups	Site of application	Applied area (cm^2)	Granuloma dry weight (mg)	Inhibition (%)
Control	–	–	0.307 ± 0.01	–
Topical indomethacin	"inflamed" dorsal skin	6 × 10	0.154 ± 0.001 **	49.8
Topical indomethacin	abdominal skin	6 × 10	0.133 ± 0.02 **	56.7

* The absorption ointment was applied for 10 h every other day for 17 days after the implantation of the cotton pellet, and the weight of the granuloma was measured on the 17th day.
** Significant difference from control value: $P < 0.05$.

TABLE 5

ASSAY PRECISION AND REPRODUCIBILITY

Compound	Concentration in ointment (mg/g)	n	Concentration found (mg/g)		
			Mean ± S.E.	Range	CV (%)
Indomethacin	8.73	3	9.51 ± 0.20	9.17 ~ 9.85	3.58
	17.45	3	17.58 ± 0.29	17.08 ~ 18.07	2.82
	26.18	3	24.92 ± 0.08	24.78 ~ 25.07	0.58
	34.90	3	33.78 ± 0.32	33.22 ~ 34.34	1.66
	43.63	3	44.95 ± 1.22	42.83 ~ 47.06	4.71
5-Methoxy-2-methyl-3-indole-acetic acid	5	3	4.93 ± 0.02	4.89 ~ 4.97	0.83
	7.5	3	7.46 ± 0.16	7.27 ~ 7.78	3.76
	10	3	10.03 ± 0.18	9.80 ~ 10.38	3.07
	15	3	15.09 ± 0.30	14.67 ~ 15.67	3.44
	20	3	20.53 ± 0.23	20.14 ~ 20.93	1.94
p-Chlorobenzoic acid	5	3	5.03 ± 0.02	4.99 ~ 5.07	0.80
	7.5	3	7.48 ± 0.05	7.40 ~ 7.55	1.04
	10	3	9.80 ± 0.07	9.67 ~ 9.91	1.26
	15	3	14.94 ± 0.04	14.87 ~ 15.02	0.50
	20	3	19.97 ± 0.07	19.84 ~ 20.06	0.57

scribed above, the retention times of these compounds were 3, 4.2, 6.5 and 10.2 min, respectively. As indicated in Fig. 3, X, negligible interfering peaks were formed in the plain ointment. The complete separation of the components in the chromatograms permitted an accurate measurement of a small quantity of *p*-chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid in the presence of a large quantity of indomethacin.

The linearities of the calibration curves for 5-methoxy-2-methyl-3-indole acetic acid, *p*-chlorobenzoic acid and indomethacin in a plain ointment at concentrations ranging from 8.7 to 43.6 mg/g ointment were obtained from the observed values in Table 5. The assay precision and reproducibility are also summarized in Table 5. The coefficient of variation (CV) for these results was less than 5% for all the concentrations investigated.

The tetrahydrofuran solvent used can dissolve all components of the ointment base, indomethacin and its decomposition products. Most ointment bases were precipitated after adding methanol

TABLE 6

PARAMETERS K AND m OBTAINED FROM FIG. 4 FOR THE DEGRADATION RATIO OF INDOMETHACIN IN THE ABSORPTION OINTMENT (NO. B) AT VARIOUS TEMPERATURES

Temperature (°C)	m	ln K	K
40	1.00	−5.00	6.74×10^{-3}
50	1.00	−4.21	1.48×10^{-2}
60	1.00	−3.44	3.21×10^{-2}
70	1.00	−2.68	6.86×10^{-2}
80	1.00	−1.85	1.57×10^{-1}

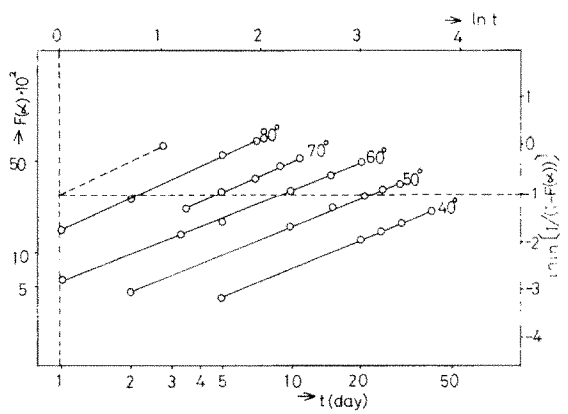


Fig. 4. Regression lines of the degradation ratio vs storage time on the Weibull probability paper for indomethacin in the absorption ointment (no. B) at various temperatures.

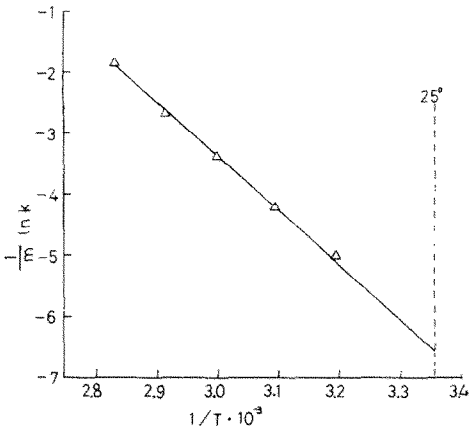


Fig. 5. Temperature dependence on (1/m)·ln K for indomethacin in the absorption ointment (no. B).

to the mixture. The method gave a good recovery of these compounds with essentially no interference from the ointment.

The typical degradation ratios of indomethacin in the absorption ointment after storage at an elevated temperature were plotted against time on the Weibull probability paper (Fig. 4), and they produced straight lines at each temperature. This relationship can be expressed by Eqn. 1:

$$\ln \ln\left(\frac{1}{1-\alpha}\right) = \ln K + m \ln t \qquad (1)$$

where α is the degradation ratio, t is the time and m and k are the parameters (Okusa, 1975). Parameters m and k obtained from Fig. 4 are listed in

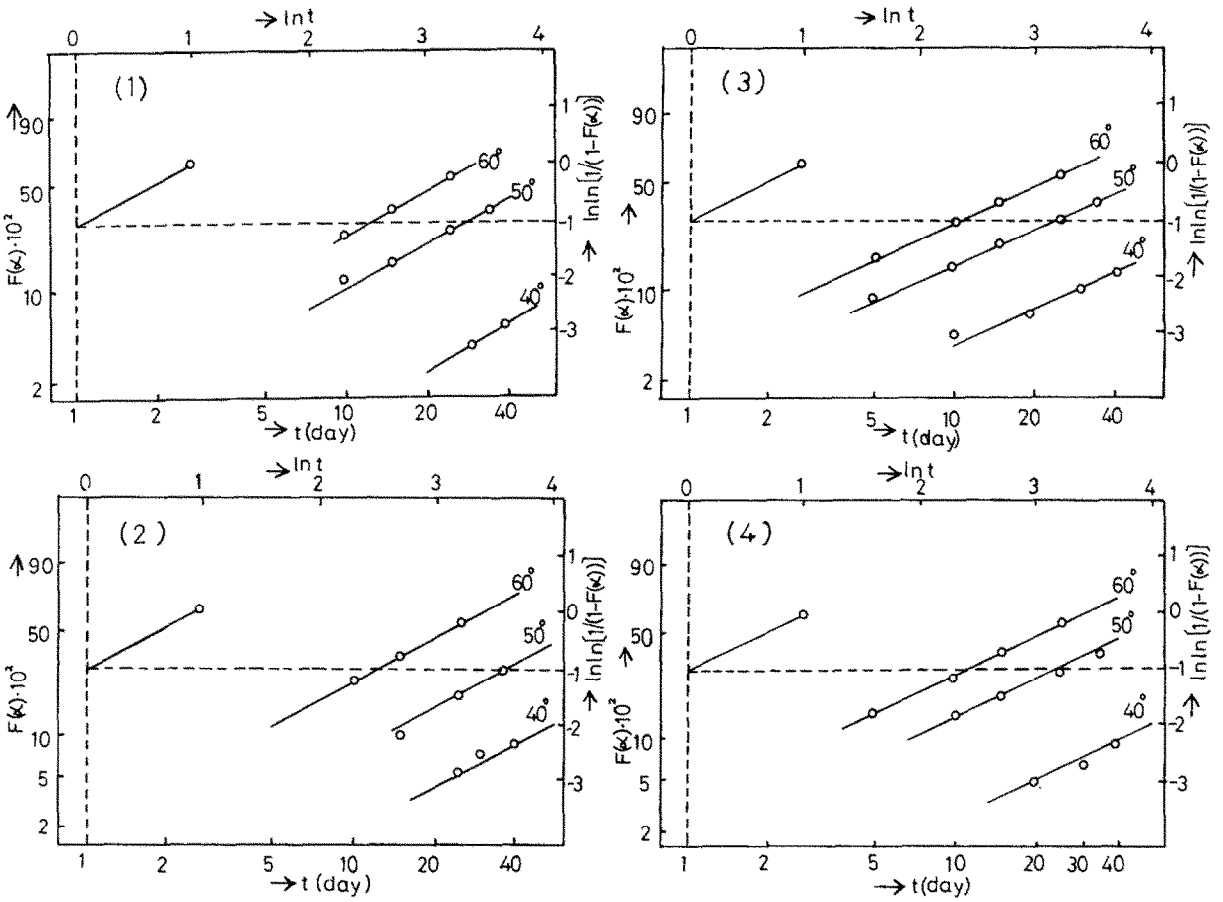


Fig. 6. Regression lines of the degradation ratio vs storage time on the Weibull probability paper for indomethacin in the absorption ointment containing 4.62% of each polysorbate. Key: (1), polysorbate 20; (2), polysorbate 40; (3), polysorbate 60; (4), polysorbate 80.

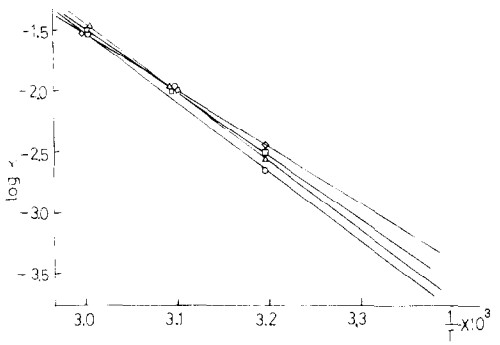


Fig. 7. Arrhenius plot showing temperature dependence of the degradation of indomethacin in the absorption ointment containing 4.62% of each polysorbate. Key: ◇, polysorbate 60; □, polysorbate 40; △, polysorbate 20; ○, polysorbate 80.

Table 6. The m values were 1.00 for each elevated temperature and indicated that the degradation ratio of indomethacin follows the first-order reaction. The prediction of the degradation ratio by means of the Weibull probability paper seemed to be possible. The typical temperature dependence of indomethacin in the absorption ointment (no. B) is shown in Fig. 5 as a regression line for $(1/m) \cdot \ln K$ vs $1/T$. The values of $(1/m) \cdot \ln K$ at 25°C were estimated by the extrapolation of this line.

In the previous report, indomethacin absorption ointment with the proportion of indomethacin to sodium bicarbonate (200 mg : 60 mg, no. A) had a good percutaneous absorption (Tsai and Naito, 1982). However, its stability was unfavorable when compared with that of the proportion of indomethacin to sodium bicarbonate (200 mg : 45 mg, no. B) as shown in Table 7. The $t_{1/2}$ increased as the content of sodium bicarbonate decreased. For example, at 40°C the $t_{1/2}$ of indomethacin in the absorption ointment with 45 mg of sodium bicarbonate was 9-fold as great as that with 60 mg

TABLE 7

EFFECT OF THE $NaHCO_3$ CONCENTRATION ON THE DEGRADATION RATIO OF INDOMETHACIN IN THE ABSORPTION OINTMENT AT VARIOUS TEMPERATURES

Concentration of $NaHCO_3$ in ointment (mg/6 g)	K (60°C)	$t_{1/2}$ (days)	K (50°C)	$t_{1/2}$ (days)	K (40°C)	$t_{1/2}$ (days)
60 mg (no. A)	17.26	4.0	8.93	7.8	6.04	11.5
45 mg (no. B)	3.21	21.6	1.48	46.7	0.67	102.8

K = apparent rate constant, 10^{-2} day^{-1}; $t_{1/2}$ = half-life.

TABLE 8

APPARENT RATE CONSTANT ($10^{-2} \cdot$ DAY^{-1}) AND RELATED PARAMETERS FOR THE DEGRADATION OF INDOMETHACIN IN THE ABSORPTION OINTMENT WITH 4.62% OF VARIOUS POLYSORBATES OR 2.31% β-CYCLODEXTRIN AT 40, 50 and 60°C

Surfactant	K (60°C)	$t_{1/2}$ (days)	K (50°C)	$t_{1/2}$ (days)	K (40°C)	$t_{1/2}$ [a] (days)	$T_{0.1}^{25}$ [b] (days)	E_a [c]	m [d]
none	3.20	21.6	1.48	46.7	0.67	102.8	73.4	16.76	1.0
polysorbate 20	3.17	21.8	1.21	57.2	0.27	259.5	291.7	25.54	1.0
polysorbate 40	3.04	22.8	1.14	60.6	0.30	231.8	245.9	23.97	1.0
polysorbate 60	3.00	23.1	1.19	58.1	0.36	189.9	163.0	21.78	1.0
polysorbate 80	2.84	24.4	1.19	58.3	0.22	317.9	369.7	26.42	1.0
β-cyclodextrin	4.28	16.2	1.80	38.5	0.87	79.4	47.1	16.51	1.0

[a] Half-life.
[b] 10% degradation time at 25°C.
[c] Activation energy, kcal/mol.
[d] Slope of the line on the Weibull probability paper.

TABLE 9

APPARENT RATE CONSTANT ($10^{-2} \cdot DAY^{-1}$) AND HALF-LIFE PERIOD FOR THE DEGRADATION OF INDOMETHACIN IN THE ABSORPTION OINTMENT CONTAINING 4.62% POLYSORBATE 80 AND VARIOUS ANTI-OXIDANTS OR PROPYLENE GLYCOL AT 40, 50 AND 60°C

Compound	Concentration (% w/w)	K (60°C)	$t_{1/2}$ (days)	K (50°C)	$t_{1/2}$ (days)	K (40°C)	$t_{1/2}$ (days)
None	–	2.84	24.4	1.19	58.3	0.22	317.9
Butyl hydroxyanisole	0.009	2.73	25.4	1.07	64.5	0.53	131.7
Sodium bisulfite	0.069	2.41	28.8	0.87	80.0	0.49	140.6
α-Tocopherol	0.462	2.65	26.1	0.96	72.0	0.63	110.0
Propylene glycol	18.73	2.58	26.9	0.92	75.3	0.53	130.8

TABLE 10

APPARENT RATE CONSTANT ($10^{-2} \cdot DAY^{-1}$), HALF-LIFE AND RELATED PARAMETERS FOR THE DEGRADATION OF INDOMETHACIN IN THE ABSORPTION OINTMENT WITH VARIOUS CONCENTRATIONS OF POLYSORBATE 20 AT 40, 50 and 60°C

Concentration (% w/w)	K (60°C)	$t_{1/2}$ (days)	K (50°C)	$t_{1/2}$ (days)	K (40°C)	$t_{1/2}$ [a] (days)	$T_{0.1}^{25}$ [b] (days)	E_a [c]	m [d]
0	3.21	21.6	1.49	46.7	0.67	102.8	73.4	16.76	1.0
1.15	2.90	23.9	1.36	50.9	0.57	121.2	80.9	16.78	1.0
2.30	3.35	20.7	1.21	57.2	0.56	123.1	87.5	18.52	1.0
4.62	3.17	21.8	1.21	57.2	0.27	259.6	291.7	25.54	1.0

[a] Half-life.
[b] 10% degradation time at 25°C.
[c] Activation energy, kcal/mol.
[d] Slope of the line on the Weibull probability paper.

TABLE 11

APPARENT RATE CONSTANT ($10^{-2} \cdot DAY^{-1}$), HALF-LIFE AND RELATED PARAMETERS FOR THE DEGRADATION OF INDOMETHACIN IN THE ABSORPTION OINTMENT WITH VARIOUS CONCENTRATIONS OF POLYSORBATE 80 AT 40, 50 AND 60°C

Concentration (% w/w)	K (60°C)	$t_{1/2}$ (days)	K (50°C)	$t_{1/2}$ (days)	K (40°C)	$t_{1/2}$ [a] (days)	$T_{0.1}^{25}$ [b] (days)	E_a [c]	m [d]
0	3.21	21.6	1.49	46.7	0.67	102.8	73.4	16.76	1.0
1.15	3.27	21.2	1.56	44.3	0.50	137.8	95.5	19.32	1.0
2.30	2.86	24.2	1.31	52.9	0.34	205.0	172.7	22.02	1.0
4.62	2.84	24.4	1.19	58.3	0.22	317.9	369.7	26.42	1.0
6.93	2.49	27.9	1.20	57.7	0.17	398.3	470.7	27.31	1.0
15	1.99	34.8	0.85	81.7	0.05	1386	3511.7	37.83	1.0

[a] Half-life.
[b] 10% degradation time at 25°C.
[c] Activation energy, kcal/mol.
[d] Slope of the line on the Weibull probability paper.

sodium bicarbonate. In addition, the effects of sodium bicarbonate at 60 mg (1%) and 45 mg (0.75%) on the percutaneous absorption of indomethacin from the absorption ointment (6 g) were compared and the $AUC_{0\text{-}14h}$ of these two ointments was not significantly different (t-test, $P > 0.05$) as shown in Table 12. Therefore, the formula (no. B in Table 1) of the indomethacin absorption ointment was used as a base throughout the study.

The effects of the various polysorbates in the 4.62% concentration regarding the degradation ratio of the indomethacin absorption ointment (no. B) vs time were plotted on the Weibull probability paper (Fig. 6) and they also produced a straight line indicating degradation following first-order reaction at each temperature. Typical plots of log K against 1/T showing the influence of temperature on the degradation of indomethacin in the presence of polysorbates were described by the Arrhenius equation (Fig. 7).

A comparison of the observed K values calculated for indomethacin in the absorption ointment alone and with 4.62% of the various polysorbates showed that the rate of the indomethacin degradation was reduced appreciably with these surfactants (Table 8). The increase in the stability was also evident from the increase in the apparent activation energies. However, increasing the length of the hydrophobic chain in the polysorbate molecule did not reflect in the same manner as the degree of protection from the decomposition of indomethacin. This finding was similar to those of Krasowska (1979). However, the 3-fold increase in the $t_{1/2}$ at 40°C for the indomethacin ointment with 4.62% polysorbate 80 was the best among the various polysorbates. It is predicted that the shelf-life ($t_{90\%}$) of indomethacin in the absorption ointment with 4.62% polysorbate 80 could be at least one year.

The modifying effect of a surfactant on the rate of degradation is usually explained on the basis of the two-phase model of the solubilized systems and the distribution of the drug between the aqueous and micellar phases. The postulation was made in this model that the rate in the micellar phase was smaller than the rate in the bulk phase, because the drug was firmly incorporated into the non-ionic micelle and protected by it from the attacking ions. Since the indomethacin absorption ointment was prepared by the drug primarily dissolved in an aqueous phase containing sodium bicarbonate, the solubilized molecule was ionized and could produce a charge on the micelle and protect it from the attacking ions.

In a previous report (Hamada et al., 1975), the rate of hydrolysis of indomethacin was inhibited with β-cyclodextrin. This result seemed to be due to the fact that β-cyclodextrin could include the whole functional group  –Cl of indomethacin. However, an inspection of Table 8 shows that the $t_{1/2}$ of indomethacin in the ointment with

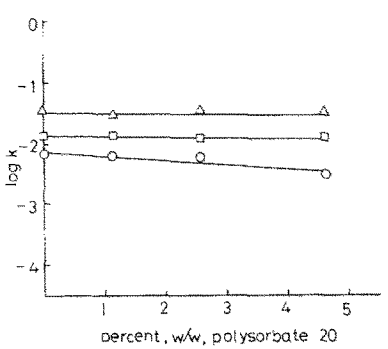


Fig. 8. Relationship of log K for the degradation of indomethacin in the absorption ointment at 40 (○), 50 (□) and 60°C (△) to concentrations of polysorbate 20.

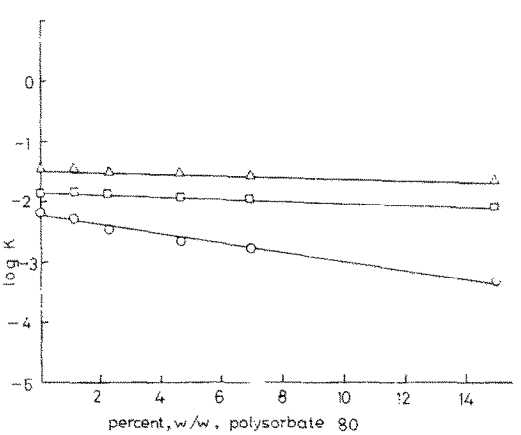


Fig. 9. Relationship of log K for the degradation of indomethacin in the absorption ointment at 40 (○), 50 (□) and 60°C (△) to concentrations of polysorbate 80.

TABLE 12

PERCUTANEOUS ABSORPTION OF INDOMETHACIN FROM THE ABSORPTION OINTMENT BASE WITH OR WITHOUT POLYSORBATES

Serial no.	Polysorbate	Concentration	Indomethacin $AUC_{0\text{-}14h}$ [a] (mcg h/ml) ± S.E. (n = 4)	Statistic
A	none	–	14.15 ± 4.57	
B	none	–	12.64 ± 3.95	NS [b]
C-1	polysorbate 20	4.62%	4.14 ± 1.21	S [c]
C-2	polysorbate 40	4.62%	7.89 ± 2.29	S [d]
C-3	polysorbate 60	4.62%	12.79 ± 3.03	NS [b]
C-4	polysorbate 80	2.30%	13.68 ± 1.68	NS [b]
C-5	polysorbate 80	4.62%	9.87 ± 1.28	NS [b]
C-6	polysorbate 80	6.93%	4.90 ± 0.87	S [c]
C-7	polysorbate 80	15.0%	3.48 ± 0.50	S [c]

[a] Obtained by the trapezoidal rule technique.
[b] Not significant difference from no. A value: $P > 0.05$.
[c] Significant difference from no. A value: $P < 0.01$.
[d] Significant difference from no. A value: $P < 0.05$.

2.31% β-cyclodextrin was slightly reduced. It would seem that the degradation of indomethacin was accelerated to a slight degree with the addition of β-cyclodextrin.

As shown in Table 9, the $t_{1/2}$ of indomethacin in the absorption ointment in 4.62% polysorbate 80 with various anti-oxidants or propylene glycol were reduced to approximately 0.5-fold at 40°C. It would seem that the degradation of indomethacin was mainly caused by hydrolysis.

As shown in Tables 10 and 11, the $t_{1/2}$ increased as the concentration of polysorbate 20 and polysorbate 80 increased, respectively. For example, at 40°C the $t_{1/2}$ of the indomethacin absorption ointment with 15% polysorbate 80 was 14 times as great as that without the surfactant. The $t_{0.1}^{25}$ calculated from the Arrhenius plot anticipated a shelf-life of about 10 years.

As shown in Fig. 8, the logarithm of the rate constant is linearly related to the concentration of polysorbate 20. This relationship may be expressed as:

$$\log K_{60} = 0.003C - 1.507 \quad (2)$$

$$\log K_{50} = -0.019C - 1.844 \quad (3)$$

$$\log K_{40} = -0.086C - 2.135 \quad (4)$$

where C is the percent of polysorbate 20. The slopes of the lines were not the same and decreased as the temperature decreased. The linear relationship shown in Fig. 9 between log K and the concentration of polysorbate 80 may be expressed as:

$$\log K_{60} = -0.014C - 1.491 \quad (5)$$

$$\log K_{50} = -0.017C - 1.823 \quad (6)$$

$$\log K_{40} = -0.073C - 2.247 \quad (7)$$

The slopes of the lines were also not equal to each other and decreased as the temperature decreased. Thus, the influence of the concentration of the polysorbates on the degradation of the indomethacin ointment may be affected by temperature. The ratio of d(log K)/dc for polysorbate 20 to that of polysorbate 80 were approximately 1.11 and 1.17 at 50 and 40°C, respectively. On a percentage basis, addition of polysorbate 20 gave the same good results as polysorbate 80 in retarding the degradation of the indomethacin ointment under the conditions studied.

The stability of indomethacin in the absorption ointment containing polysorbate could be improved to the remarkable degree described above. However, the presence of polysorbates in the ab-

sorption ointment base may increase, decrease or exert no effect on the percutaneous absorption of indomethacin as shown in Table 12. The percutaneous absorption of indomethacin was decreased significantly when 4.62% of polysorbate 20 and 40 was added to the absorption ointment, respectively. No effect in the drug percutaneous absorption occurred at a concentration of 4.62% polysorbate 60. Four different concentrations of polysorbate 80 were studied: 2.3, 4.62, 6.93 and 15% (w/w). The additional concentration of polysorbate 80 below 4.62% did not produce any significant change in the percutaneous absorption of indomethacin from the absorption ointment base.

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