CHAPTER III

EXPERIMENTS AND RESULTS

Synthesis.

A. Materials.

1. Equipments.

Analytical balance	Sartorius 1615 MR and Sartorius 1104
Melting point apparatus	Buchi Capillary Melting Point Apparatus
Infrared spectrophotometer	Shimadzu IR-400[a]
Nuclear Magnetic Resonance Spectrophotometer	Joel FX 90Q (90 MHz)[a]
Elemental Analyzer	Perkin Elmer 240 C[a]
Mass Spectrometer	Joel FX 3000 double focusing[a]
UV Spectrophotometer	Hitachi U-3200

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[a]The Scientific and Technological Research Equipment Center, Chulalongkorn University.

2. Chemicals.

Lidocaine hydrochloride	USP XXI
Adipic acid,AR	Searle
Maleic acid,AR	BDH
Malonic acid,AR	BDH
p-Toluenesulfonic acid,AR	E. Merck
Sodium hydroxide,AR	E. Merck
Sodium sulfate, anhydrous,AR	E. Merck
Chloroform,AR	E. Merck
Methanol,AR	E. Merck
Ethanol,AR	E. Merck
Benzene,AR	E. Merck
Ethyl acetate,AR	E. Merck
Glacial acetic acid,AR	E. Merck
Ethyl ether, anhydrous,AR	J.T. Baker
Hexane,AR	J.T. Baker

B. Methods and Results.

Melting points of the compounds were determined on a Buchi Capillary Melting Point Apparatus and uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Joel FX 90Q (90 MHz). Chemical shifts were reported in ppm related to the internal standard, tetramethylsilane. Infrared (IR) spectra were recorded as potassium bromide disc on a Shimadzu IR-400. Mass (Joel FX 3000 double focusing) and ultraviolet (Hitachi U-3200) spectra were determined

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for all compounds. Analytical results from elemental analyzer (Perkin Elmer 240C) obtained for all compounds were within ± 0.4% of the theoretical value unless otherwise stated.

Approximate solubilities of the compounds were determined at room temperature (32°C) by dissolving 100.0 mg of the test compound in 0.05 ml of water, ethanol, chloroform and ether individually. The mixtures were placed in ultrasonic bath for half an hour and observed for clarity. If the true solution was not obtained, a further 0.05 ml of the solvent was gradually added, and sonicated. The procedure was repeated until the true solution was obtained. Descriptive terms according to USP XXII were used to express approximate solubilities of the compounds.

Results of the syntheses of test compounds were summarized in Table 2-6; recrystallization solvent, melting point and percent yield (Table 2), approximate solubility (Table 3) and elemental analyses (Table 4). Characteristic IR and ^{1}H NMR data were shown in Table 5 and Table 6, respectively.

1. Lidocaine (I).

A solution of 5.000 g of lidocaine hydrochloride in 20 mL water was adjusted to pH 11 with 1N sodium hydroxide and then extracted with 25.0 mL of chloroform for three times. The collected

chloroform layers were washed with 2 x 15 mL of water. The chloroform was dried over anhydrous sodium sulfate and evaporated on a steam bath to dryness. The white crystals of lidocaine were obtained (3.89 g; 96% yield), m.p. 68-69°C. IR(KBr) ν : 3250(N-H), 3040 (aromatic C-H), 2950 - 2850(aliphatic C-H), 1665(C=O), 1595(aromatic C=C), 1495(N-C=O), 1200 (aliphatic C-N); δ : 760,710(3-adjacent protons of aromatic) cm^{-1} (Figure 1). λ_{max} (isotonic phosphate buffer pH 7.4) : 262.5(ϵ 463) nm. ^{1}H NMR ($CDCl_3$) : 1.14(t, J 7.18 Hz, 6H, $-CH_2CH_3$), 2.23(s, 6H, ph-CH_3), 2.65(q, J 7.18 Hz, 4H, $-CH_2CH_3$), 3.22(s, 2H, CO-CH_2-N), 7.08(s, 3H, aromatic protons), 7.25($CHCl_3$),8.93(bs,1H,exchangeable with D_2O, CO-NH) (Figure 2-3).

2. Lidocaine adipate (IV-A).

A solution of 0.937 g (4 mmole) of lidocaine in 25 mL of anhydrous ethyl ether was prepared. Then, a solution of 0.585 g (4 mmole) of adipic acid in 15 mL of acetone was added and the mixture was stirred for 10 minutes and cool at 4°C, the precipitated solid was removed by filtration, washed with anhydrous ethyl ether and then dried. The resulting solid was collected and recrystallized with ethyl acetate to give lidocaine adipate as white crystals (1.22 g; 80% yield), m.p. 119 - 120°C. IR (KBr) ν : 3450(O-H), 3200(N-H), 3050(aromatic C-H), 2950 - 2900(aliphatic C-H), 2650 - 2500($\overset{+}{N}$-H), 1690(C=O

of acid and amide), 1550(aromatic C=C), 1480 - 1460 (N-C=O), 1260 - 1220(C-O), 1200(aliphatic C-N) δ : 780, 730(3-adjacent protons of aromatic) cm^{-1} (Figure 4). λ_{max} (isotonic phosphate buffer pH 7.4) : 262.5(ϵ 457) nm. ^1H NMR (DMSO-d_6 + $CDCl_3$) : 1.15(t, J 7.18 Hz, 6H, $-CH_2CH_3$), 1.62(m, 4H, adipicβ-CH_2), 2.20(10H, overlap of ph-CH_3 and adipicα-CH_2), 2.65(q, 4H, $-CH_2CH_3$), 3.20(s, 2H, CO-CH_2-N), 7.06(s, 3H, aromatic protons), 7.65($CHCl_3$), 8.99(bs, 1H, exchangeable with D_2O, CO-NH), 10.40(bs, 2H, exchangeable with D_2O, COOH) (Figure 5-6). MS : M/E 234(56.9%), 128(9.2), 120(19.3), 100(57.2), 87(69.4), 86(100), 72(51.2), 58(68.0), 56(25.4), 43(29.2), 42(27.7) (Figure 7).

Elemental analysis for $C_{20}H_{32}N_2O_5$:

Calculated	C = 63.14;	H = 8.48;	N = 7.36
Found	C = 63.29;	H = 8.73;	N = 7.35

3. Lidocaine maleate (IV-B).

A solution of 0.937 g (4 mmole) of lidocaine in 5 mL of anhydrous ethyl ether was prepared. Then, a solution of 0.464 g (4 mmole) of maleic acid in 25 mL of anhydrous ethyl ether was added and the mixture was stirred. White precipitates were formed immediately. After standing overnight at 4^oC, the precipitated solid was removed by filtration, washed with anhydrous ethyl ether and then dried. The resulting solid was collected and recrystallized with ethyl acetate to give lidocaine maleate as white

crystals (1.12 g; 80% yield), m.p. 93 - 94°C. IR (KBr) ν : 3450(O-H), 3200(N-H), 3050(aromatic C-H, olefenic C-H), 2950 - 2900(aliphatic C-H), 2650($\overset{+}{N}$-H), 1670(C=O of acid and amide), 1570(aromatic C=C, olefinic C=C), 1470(N-C=O), 1360 - 1270(C-O), 1190 (aliphatic C-N) δ:765(3-adjacent protons of aromatic), 700(C-H cis-olefins) cm^{-1} (Figure 8). λ_{max} (isotonic phosphate buffer, pH 7.4) : 270.2(ϵ 494) nm. ^{1}H NMR ($CDCl_3$) : 1.33(t, J 7.18 Hz, 6H, $-CH_2CH_3$), 2.18(s, 6H, ph-CH_3), 3.33(q, J 7.18 Hz, 4H $-CH_2CH_3$), 4.25(s, 2H, CO-CH_2-N), 6.24(s, 2H, =CH-), 7.04(s, 3H, aromatic protons), 7.26($CHCl_3$), 9.89(s, 1H, exchangeable with D_2O, CO-NH), 13.10(bs, 1H, exchangeable with D_2O, COOH) (Figure 9-10). MS : M/E 234(39.7%), 120(19.5), 91(17.6), 87(62.5), 86(100.0), 72(49.6), 58(66.9), 56(21.9), 42(21.6) (Figure 11).

Elemental analysis for $C_{18}H_{26}N_2O_5$:

Calculated	C = 61.70;	H = 7.49;	N = 7.99
Found	C = 61.21;	H = 7.83;	N = 7.97

4. Lidocaine malonate (IV-C).

A solution of 0.937 g (4 mmole) of lidocaine in 10 mL of anhydrous ethyl ether was prepared. Then, a solution of 0.416 g (4 mmol) of malonic acid in 5 mL of anhydrous ethyl ether was added and the mixture was stirred. White precipitates were formed immediately. After standing overnight, the precipitated solid was removed by filtration,

washed with anhydrous ethyl ether and then dried. The resulting solid was collected and recrystallized with ethyl acetate to give lidocaine malonate as white crystals (0.97 g; 72% yield), m.p. 136 - 137°C. IR (KBr) ν : 3450(O-H), 3200(N-H), 3050(aromatic C-H), 2950 - 2900(aliphatic C-H),2650($\overset{+}{N}$-H), 1695(C=O of acid, amide), 1540(aromatic C=C), 1470(N-C=O), 1360 - 1270 (C-O), 1160(C-N) δ : 780, 710(3-adjacent protons of aromatic) cm^{-1} (Figure 12). λ_{max} (isotonic phosphate buffer pH 7.4) : 262.5(ϵ 457) nm. ^{1}H NMR ($CDCl_3$) : 1.26(t, J 6.84 Hz, 6H, $-CH_2CH_3$), 2.16(s, 6H, ph-CH_3), 3.13(s, 2H, CH_2COOH), 3.19(q, J 7.14 Hz, 4H, $-CH_2CH_3$), 4.14(s, 2H, CO-CH_2-N), 7.03(s, 3H, aromatic protons), 7.28($CHCl_3$), 9.84(s, 1H, exchangeable with D_2O,CO-NH), 11.87 (s, 2H, exchangeable with D_2O, COOH) (Figure 13-14). MS : M/E 234(37.6%), 120(19.7), 105(6.4), 87(62.6), 86(100.0), 72(44.1), 58(66.2), 42(38.4) (Figure 15).

Elemental analysis for $C_{17}H_{26}N_2O_5$:

Calculated	C = 60.34;	H = 7.74;	N = 8.28
Found	C = 60.00;	H = 8.02;	N = 7.93

5. Lidocaine tosylate (IV-D).

A solution of 0.937 g (4 mmole) of lidocaine in 5 mL of anhydrous ethyl ether was prepared. Then, a solution of 0.761 g (4 mmole) of p-toluenesulfonic acid (monohydrate) in 30 mL of anhydrous ethyl ether was added and the mixture was

stirred. White precipitates were formed immediately. After standing overnight, the precipitated solid was removed by filtration, washed with anhydrous ethyl ether and then dried. The resulting solid was collected and recrystallized with ethyl acetate to give lidocaine tosylate as white crystals, (1.10 g, 67% yield), m.p. 149 - 151°C. IR (KBr) ν : 3250 (N-H), 3050(aromatic C-H), 2950 - 2850(aliphatic C-H), 2850($\overset{+}{N}$-H), 1690(C=O), 1600, 1545(aromatic C=C), 1470 (N-C=O), 1210, 1030, 1010(S=O), 1180(C-N), 680(S-O) δ : 1470(NH), 820(2-adjacent protons of aromatic), 770(3-adjacent protons of aromatic) cm^{-1} (Figure 16). λ_{max} (isotonic phosphate buffer, pH 7.4) : 261.4 (ϵ 792) nm. ^{1}H NMR ($CDCl_3$) : 1.28(t, J 7.18 Hz, 6H, $-CH_2CH_3$), 2.09(s, 6H, NH-ph-CH_3), 2.30(s, 3H, SO_3-ph-CH_3), 3.34(m, 4H, $-CH_2CH_3$), 4.36(d, 2H, CO-CH_2), 6.95(s, 3H, aromatic protons of lidocaine), 7.05(d, J 8.2 Hz, 2H, aromatic protons of tosylate), 7.26 ($CHCl_3$), 7.64(d, J 8.2 Hz, 2H, aromatic protons of tosylate, meta to CH_3), 9.47(bs, 1H, exchangeable with D_2O, $\overset{+}{N}$H), 9.83(bs, 1H, exchangeable with D_2O, CO-NH) (Figure 17-18). MS : M/E 234(39.6%), 172(24.3), 120(18.5), 107(12.4), 91(42.9), 87(62.7), 86(100.0), 77(18.3), 72(43.7), 58(66.1), 56(22.5), 42(21.6) (Figure 19).

Elemental analysis for $C_{21}H_{30}N_2O_4S$:

Calculated	C = 62.04;	H = 7.44;	N = 6.89
Found	C = 62.18;	H = 7.71;	N = 6.89

Table 2 : Recrystallization solvent, melting point and percent yield of test compounds.

Test Compound[a]	Molecular Formula	M.W.	Recrystallization Solvent	m.p.[b]	% Yield
I	$C_{14}H_{22}N_2O$	234.34	n-Hexane	68-69	96
I.HCl	$C_{14}H_{22}N_2O.HCl.H_2O$	288.81	Acetone	76-77	-
IV-A	$C_{20}H_{32}N_2O_5$	380.48	EtOAc	119-120	80
IV-B	$C_{18}H_{26}N_2O_5$	350.41	EtOAc	93-94	80
IV-C	$C_{17}H_{26}N_2O_5$	338.40	EtOAc	136-137	72
IV-D	$C_{21}H_{30}N_2O_4S$	406.54	EtOAc	149-150	67

[a] I = lidocaine , I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[b] Uncorrected m.p. in ^{o}C.

Table 3 : Approximate solubility of test compounds.

Test Compound[a]	Approximate Solubility[b]			
	Water	Ethanol	Chloroform	Ether
I	slightly soluble	very soluble	very soluble	very soluble
I.HCl	freely soluble	sparingly soluble	slightly soluble	insoluble
IV-A	freely soluble	sparingly soluble	slightly soluble	very slightly soluble
IV-B	very soluble	very soluble	soluble	insoluble
IV-C	very soluble	freely soluble	soluble	very slightly soluble
IV-D	freely soluble	freely soluble	soluble	insoluble

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[b] USP descriptive terms : very soluble is less than 1 part of solvent required for 1 part of solute; freely soluble is from 1 to 10 parts of solvent; soluble is from 10 to 30; sparingly soluble is from 30 to 100; slightly soluble is from 100 to 1000; very slightly soluble is from 1000 to 10,000 and insoluble is more than 10,000.

Table 4 : Elemental analyses of test compounds.

Test Compound[a]	Elemental Analysis					
	% Carbon		% Hydrogen		% Nitrogen	
	Calcd.	Found	Calcd.	Found	Calcd.	Found
IV-A	63.14	63.29	8.48	8.73	7.36	7.35
IV-B	61.70	62.21	7.49	7.83	7.99	7.97
IV-C	60.34	60.00	7.74	8.02	8.28	7.93
IV-D	62.04	62.18	7.44	7.71	6.89	6.89

[a]IV-A = lidocaine adipate, IV-B = lidocaine maleate
IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

Table 5 : Characteristic IR data of test compounds as potassium bromide pellets.

Test Compound[a]	Wave Number (cm^{-1})								
	νO-H	νN-H	νC-H	$\nu\overset{+}{N}$-H	νC=O	νC=C, δN-H, νN-C=O	νC-O	νC-N	δaromatic C-H
I	-	3250	3040, 2950, 2800	-	1665	1595, 1495	-	1200	760, 710
I.HCl[b]	-	3400, 3200	3040, 2900	2650-2500	1670, 1650	1525, 1470	1370-1260	1200, 1160	780, 710
IV-A	3450	3200	3050, 2900	2650, 2500	1690	1550, 1480-1460	1260-1220	1200	780, 730
IV-B	3450	3200	3050, 2900	2650	1670	1570, 1470	1360-1270	1190	765, 700(δ=C-H)
IV-C	3450	3200	3050, 2900	2650	1695	1540, 1470	1360-1270	1160	780 710
IV-D	-	3250	3050, 2950	2850	1690 1545,	1600, 1545, 1470	-	1210(νS=O), 1180, 1030(νS=O), 1010(νS=O)	820, 770, 710, 680(νC-S)

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[b] Thai Pharmacopoeia.

Table 6 : Characteristic ^{1}H NMR data of test compounds.

Test Compound[a]	Solvent	Chemical Shift of Proton (ppm)							
		$-CH_2C\underline{H}_3$	$ph-C\underline{H}_3$	$N-C\underline{H}_2CH_3$	$CO-C\underline{H}_2-N$	ar-$\underline{H}$	$\overset{+}{N}\underline{H}$	CO-N$\underline{H}$	COO$\underline{H}$
I	$CDCl_3$	1.14	2.23	2.65	3.22	7.08	-	8.93	-
I.HCl[b]	$CDCl_3$	1.42	2.21	2.60	3.22	7.02	-	10.24	-
IV-A	$DMSO-d_6$ + $CDCl_3$	1.15	2.20	2.65	3.20	7.06	-	8.99	10.40
IV-B	$CDCl_3$	1.33	2.18	3.33	4.25	7.04	-	9.89	13.10[c]
IV-C	$CDCl_3$	1.26	2.16	3.19	4.14	7.03	-	9.84	11.87
IV-D	$CDCl_3$	1.28	2.09	3.34	4.36	6.95, 7.05, 7.64	9.47	9.83	-

[a]I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[b]Powell, M.F. (1986).

[c]Only one proton from two carboxylic groups was found.

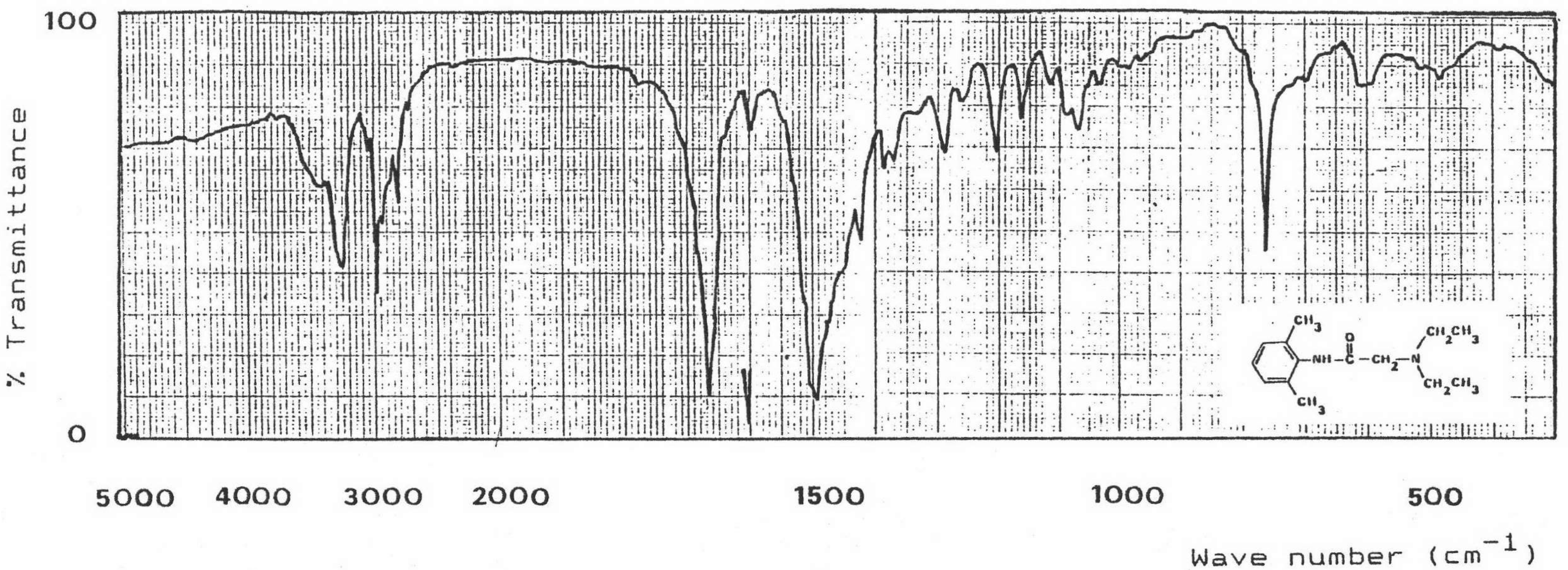

Figure 1 Infrared Absorption Spectrum of Lidocaine as a Potassium Bromide Pellet.

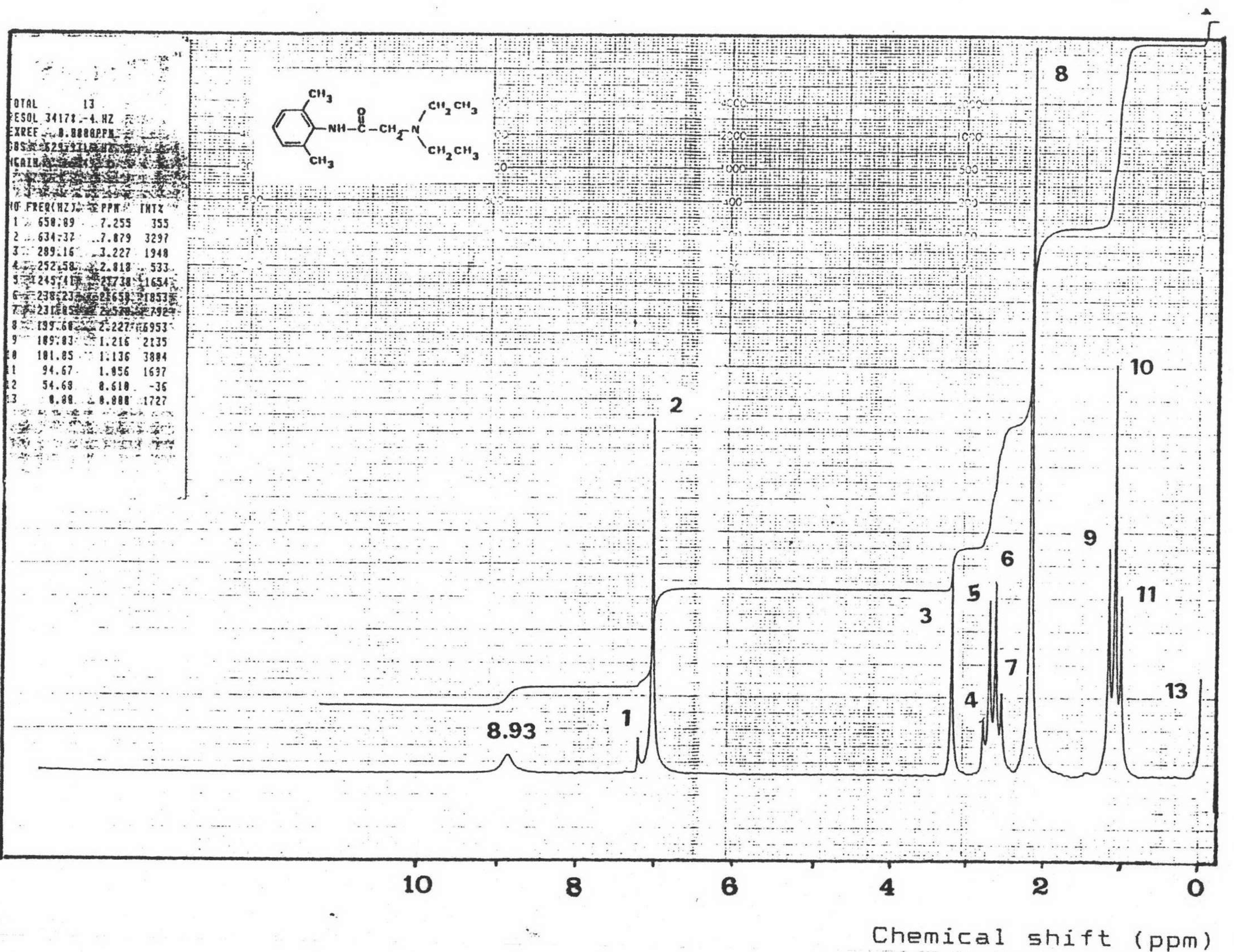

Figure 2 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine in Chloroform-d.

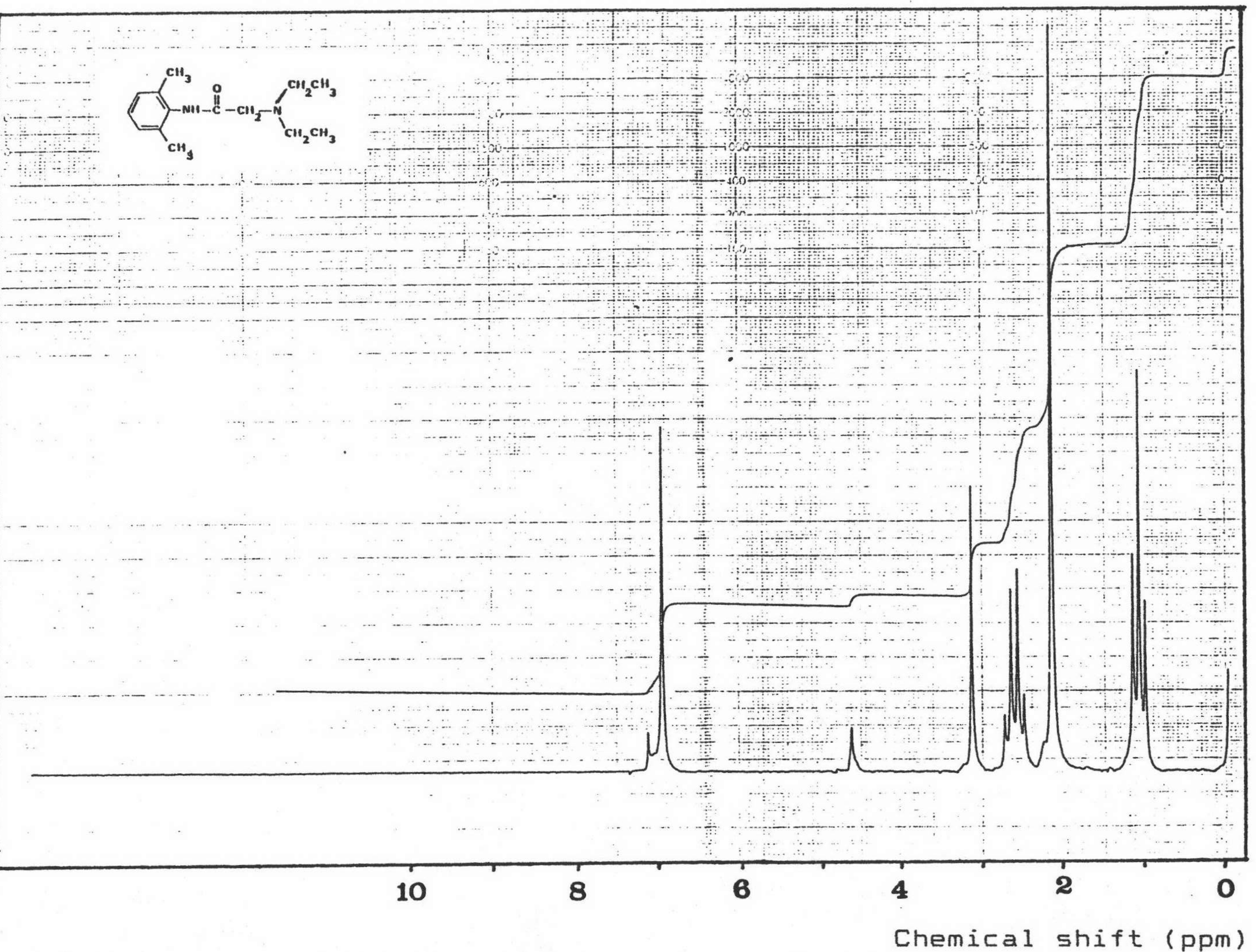

Figure 3 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine in Chloroform-d and Deuterium Oxide.

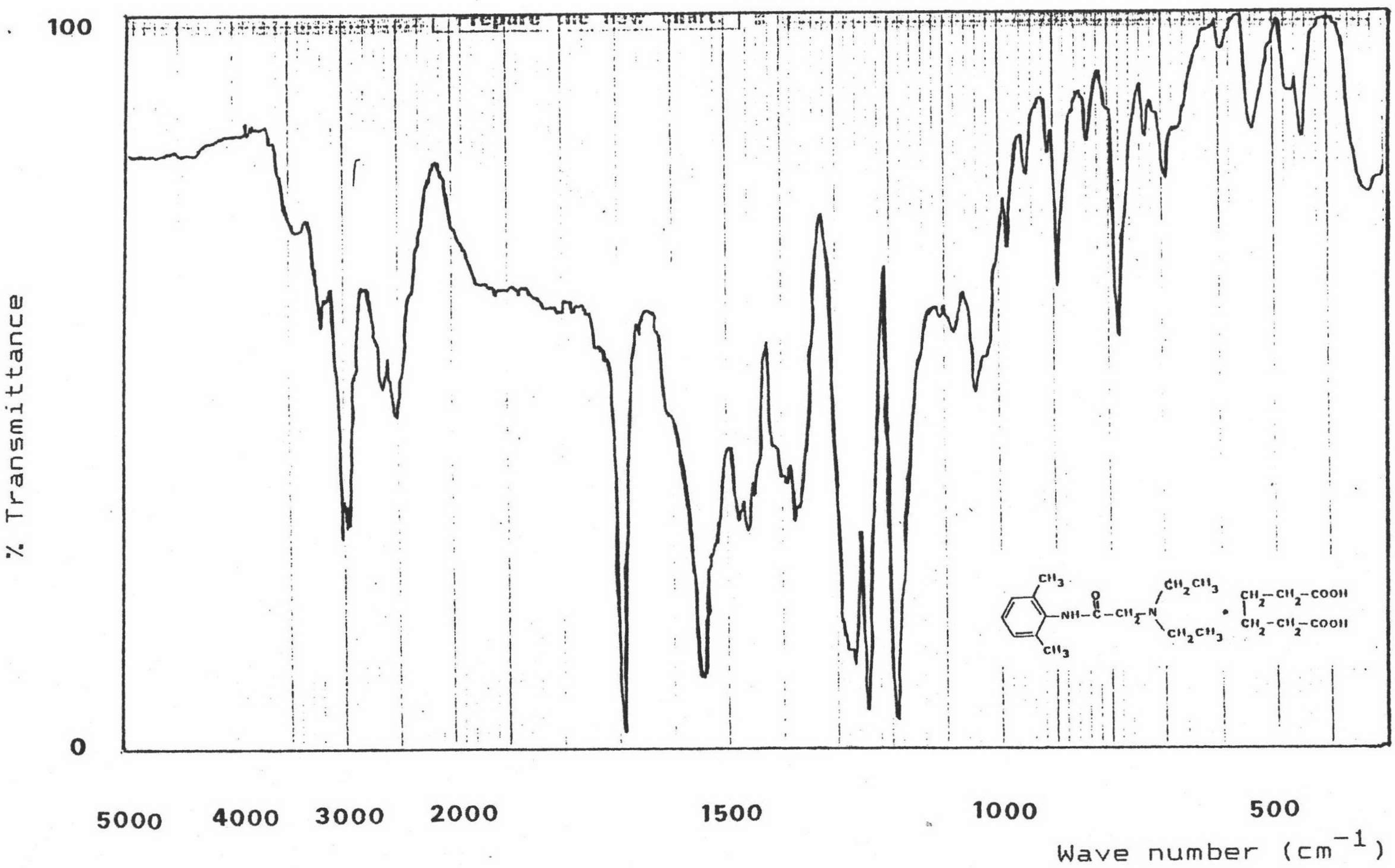

Figure 4 Infrared Absorption Spectrum of Lidocaine Adipate as a Potassium Bromide Pellet.

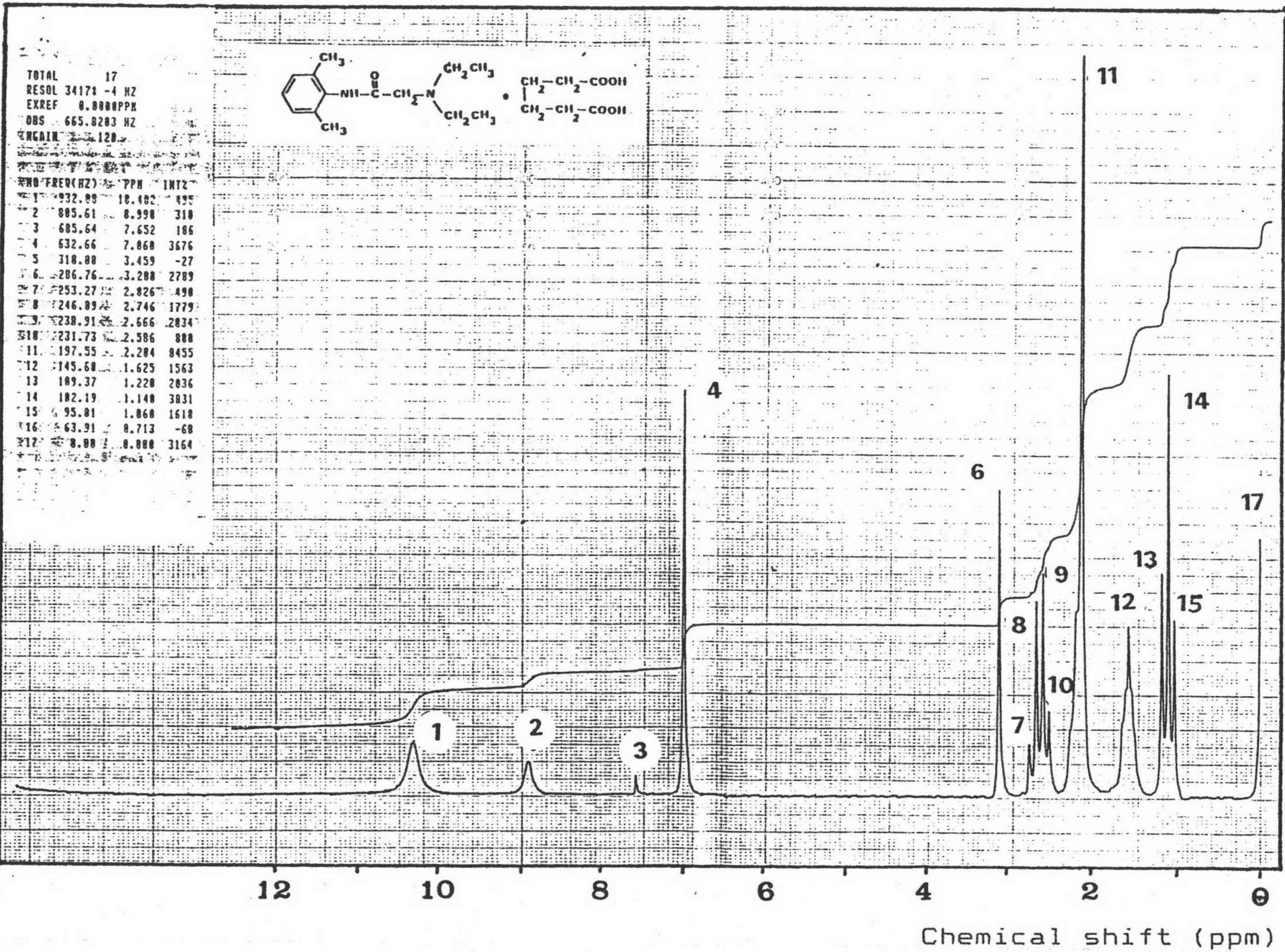

Figure 5 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Adipate in Dimethylsulfoxide-d_6 and Chloroform-d.

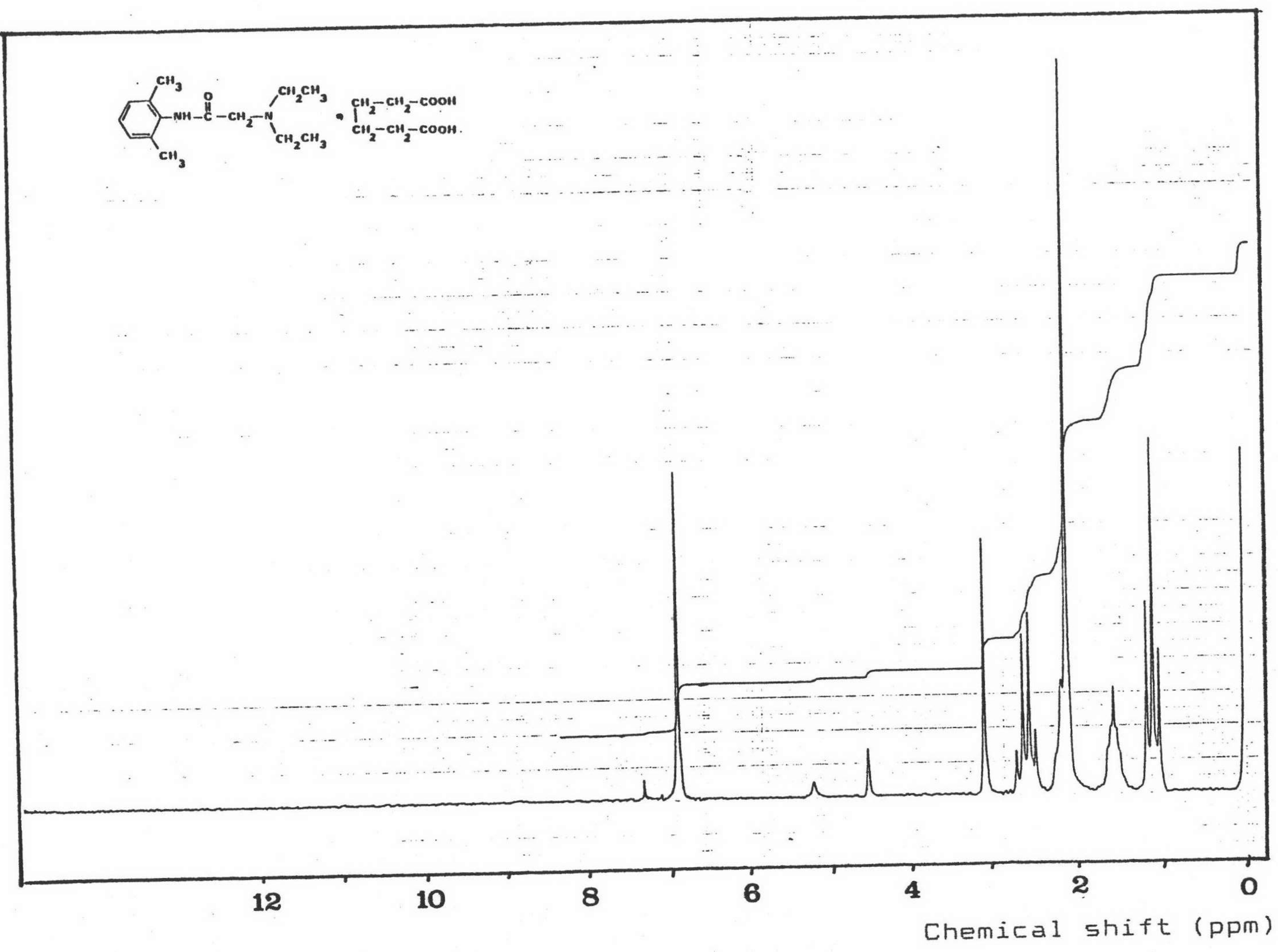

Figure 6 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Adipate in Dimethylsulfoxide-d_6, Chloroform-d and Deuterium Oxide.

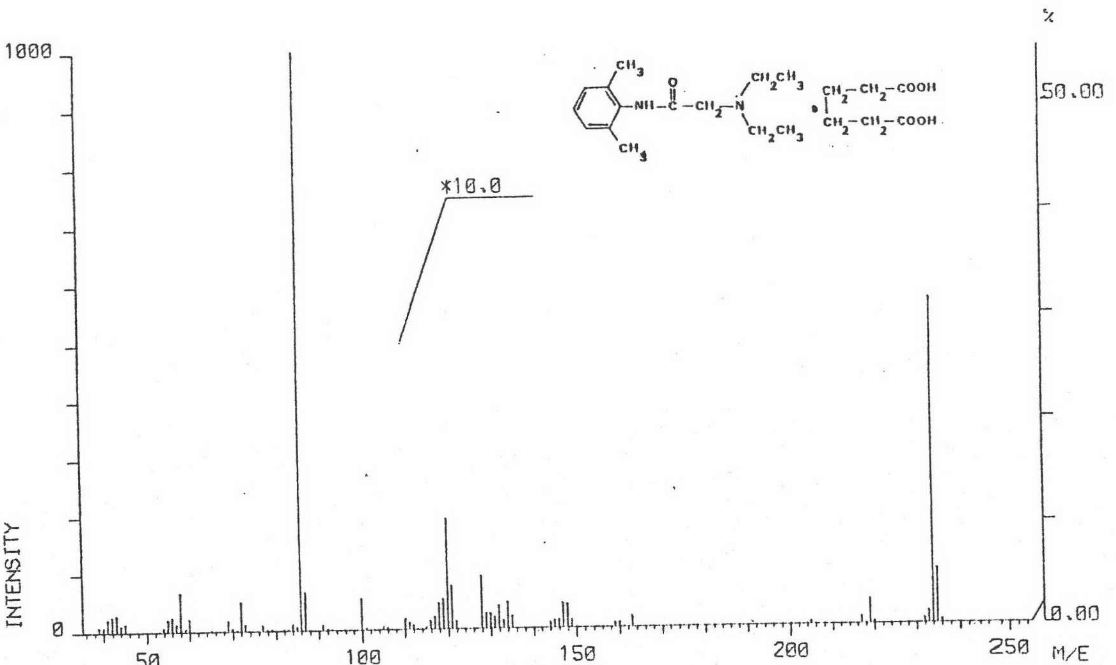

Figure 7 Mass Spectrum of Lidocaine Adipate.

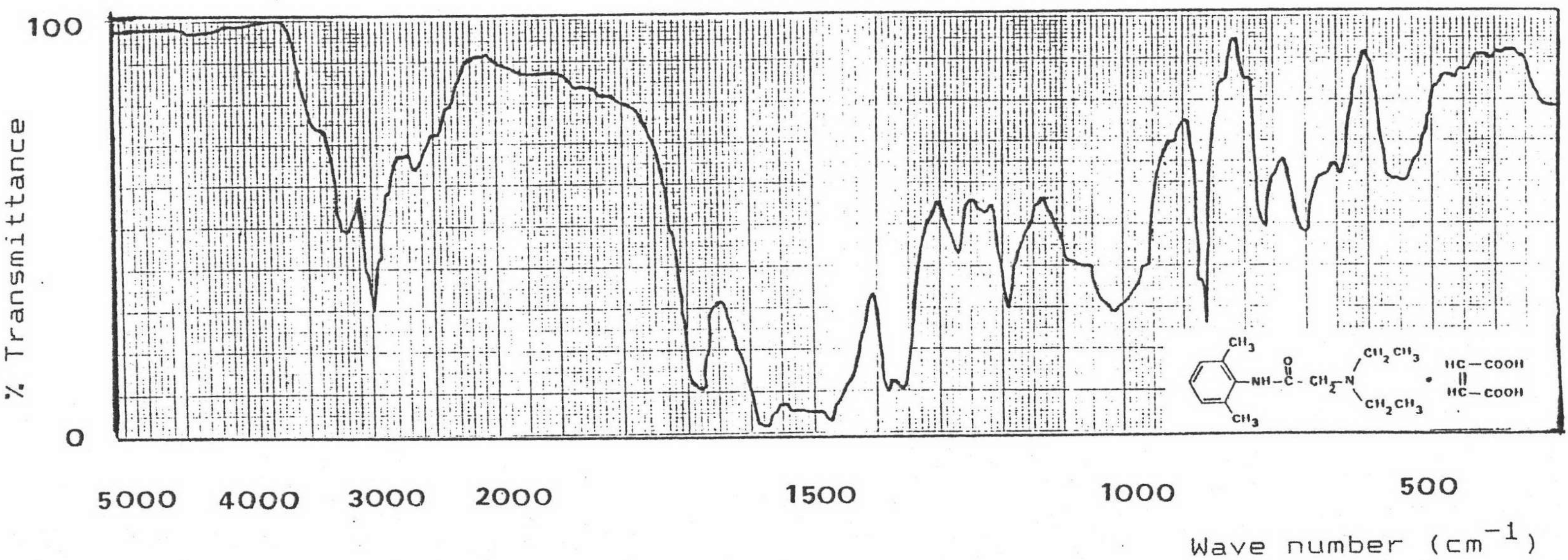

Figure 8 Infrared Absorption Spectrum of Lidocaine Maleate as a Potassium Bromide Pellet.

TOTAL 15
RESOL 3417: -4 HZ
EXREF 0.0000PPM
OBS 630.9570 HZ
NGAIN 85.

NO	FREQ(HZ)	PPM	INT%
1	886.62	9.894	582
2	651.12	7.266	527
3	630.61	7.037	2632
4	559.52	6.244	2752
5	380.76	4.249	1508
6	309.66	3.455	418
7	302.49	3.375	1214
8	295.31	3.295	1298
9	288.13	3.215	512
10	195.50	2.181	6223
11	125.78	1.403	1521
12	118.94	1.327	2912
13	111.42	1.243	1216
14	77.24	0.862	-51
15	0.00	0.000	2587

Chemical shift (ppm)

Figure 9 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Maleate in Chloroform-d.

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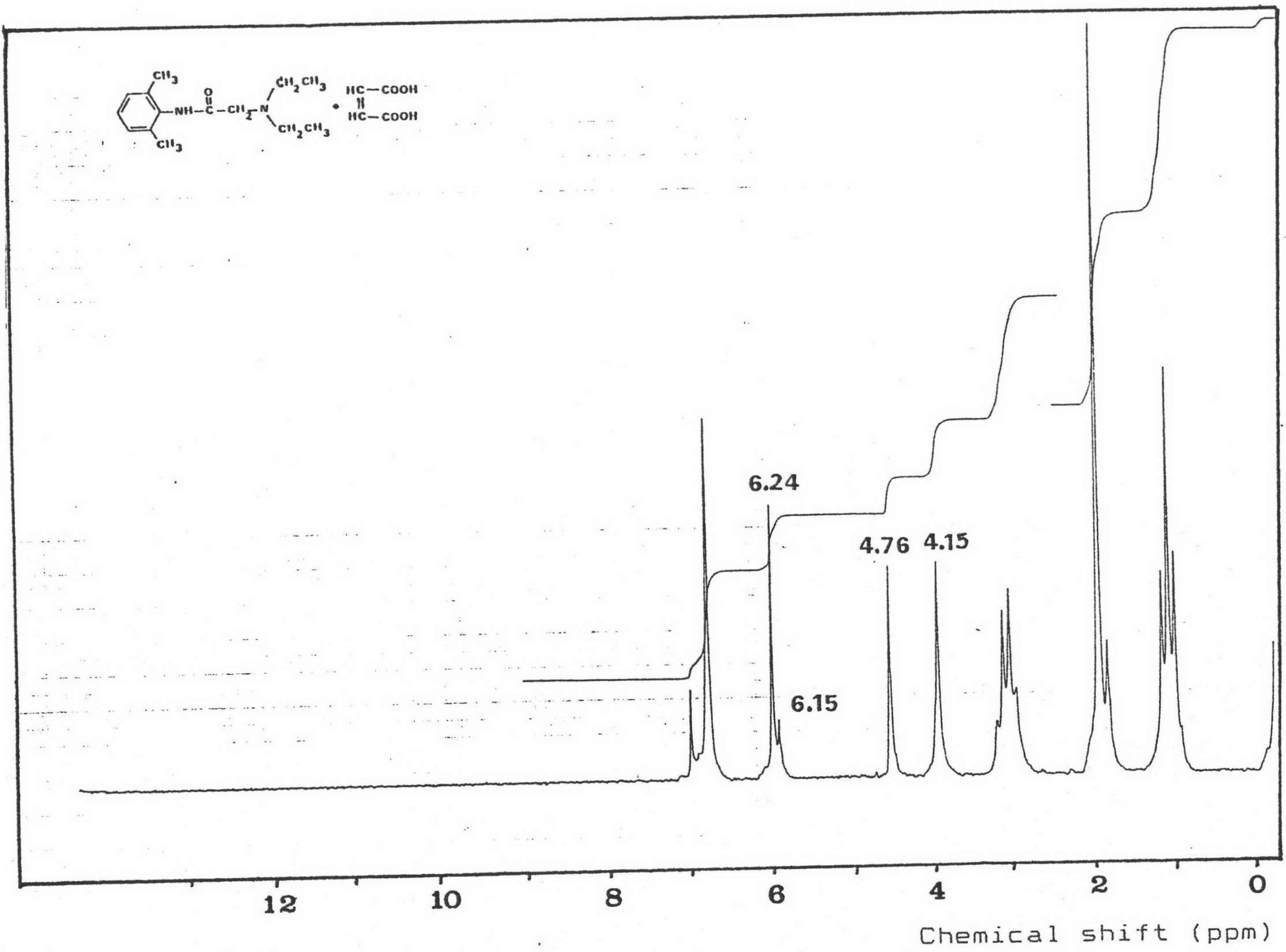

Figure 10 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Maleate in Chloroform-d and Deuterium Oxide.

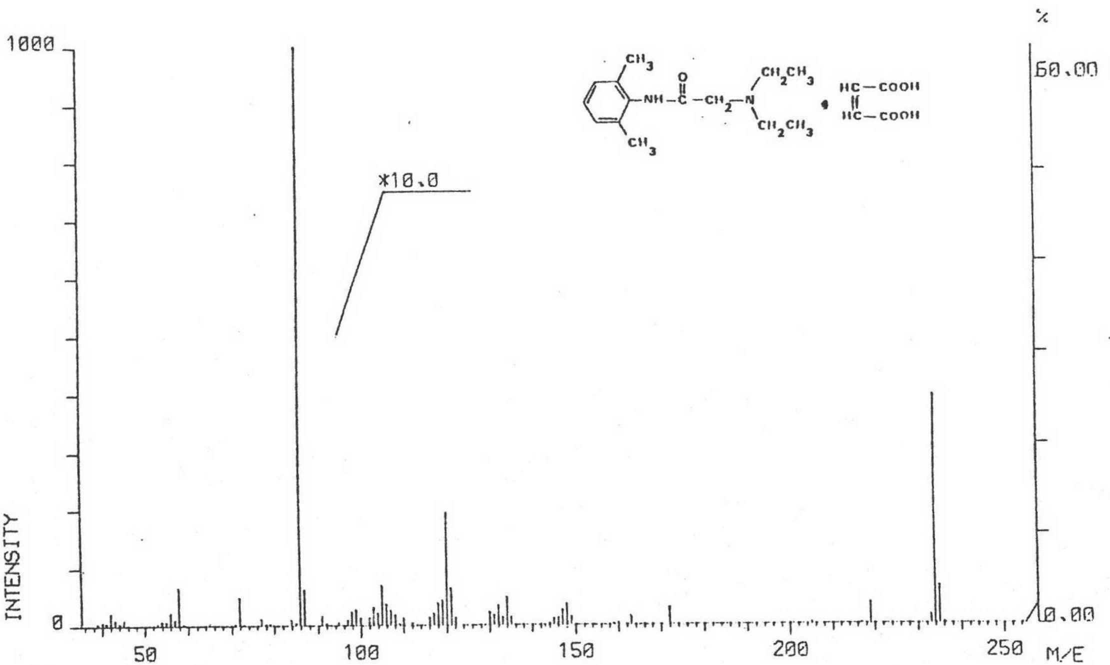

Figure 11   Mass Spectrum of Lidocaine Maleate.

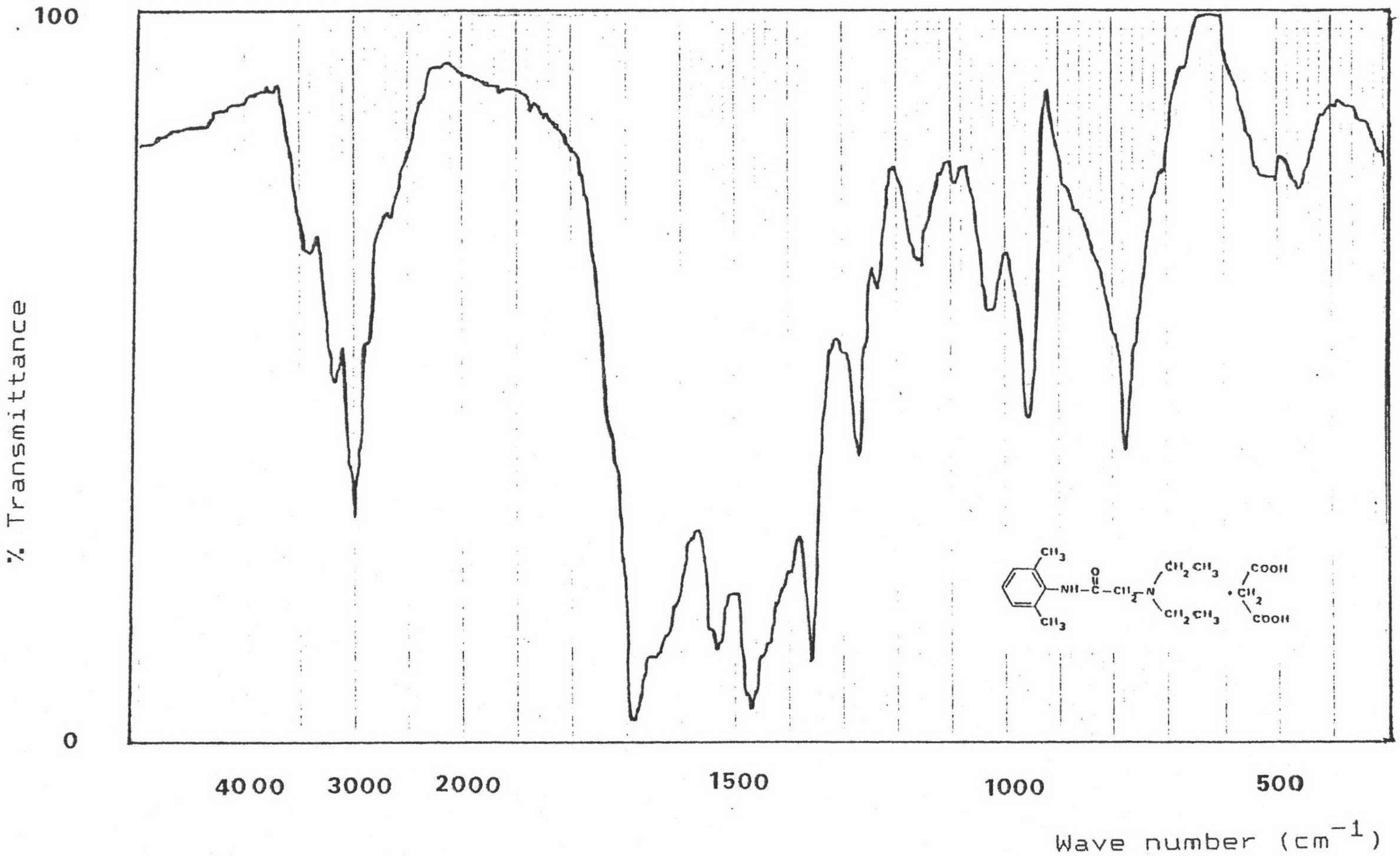

Figure 12 Infrared Absorption Spectrum of Lidocaine Malonate as a Potassium Bromide Pellet.

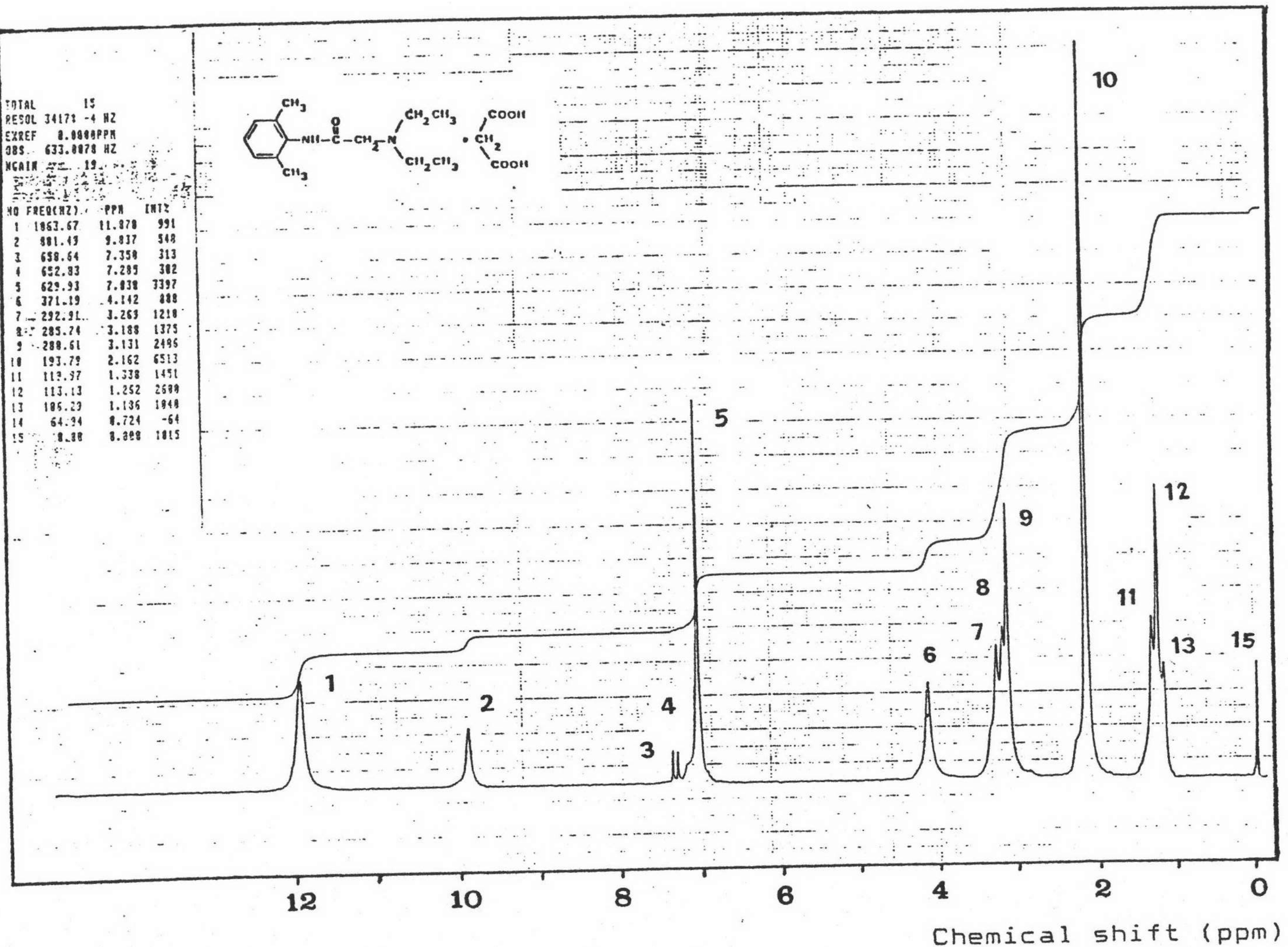

Figure 13 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Malonate in Chloroform-d.

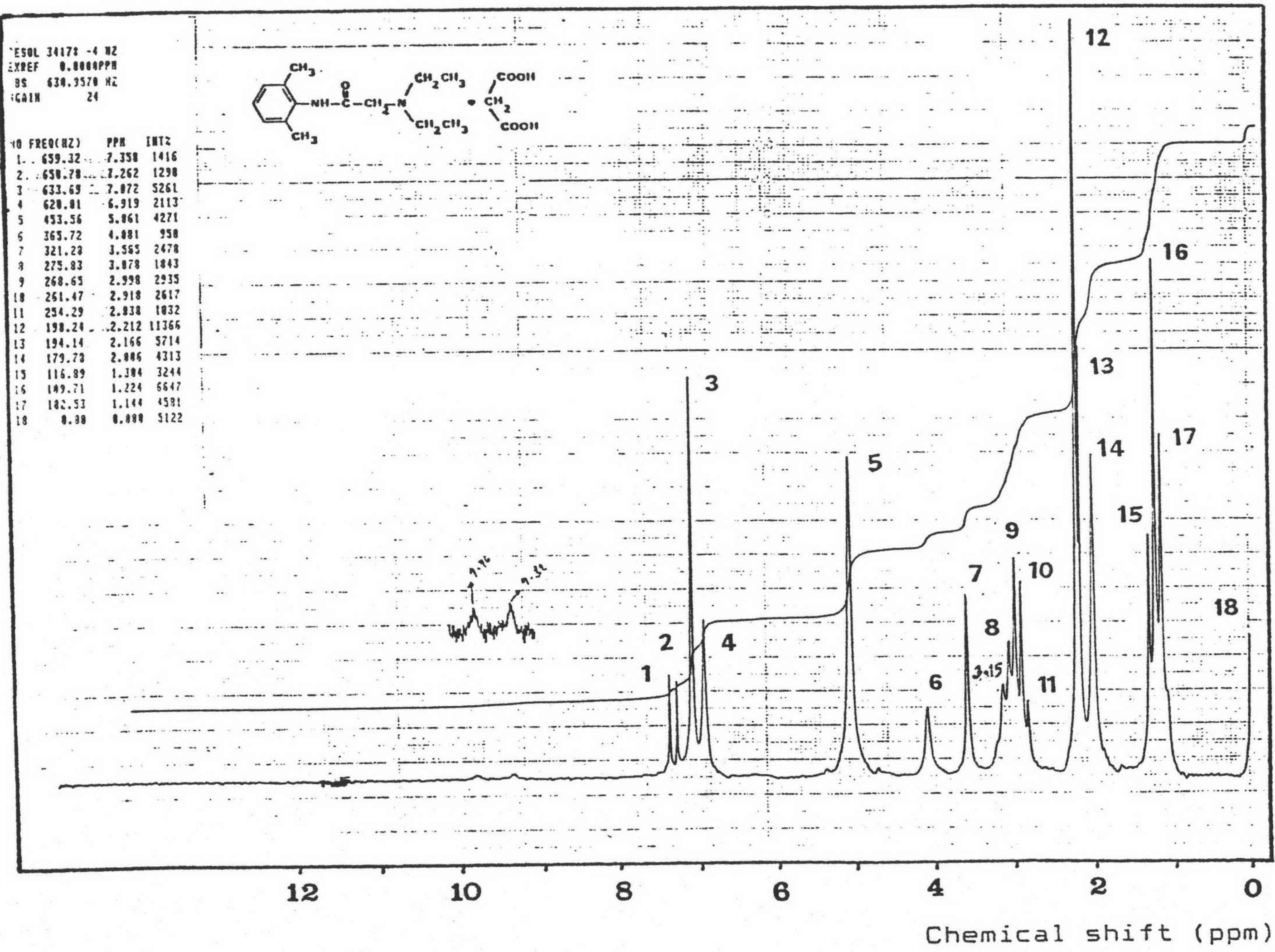

Figure 14 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Malonate in Chloroform-d and Deuterium Oxide.

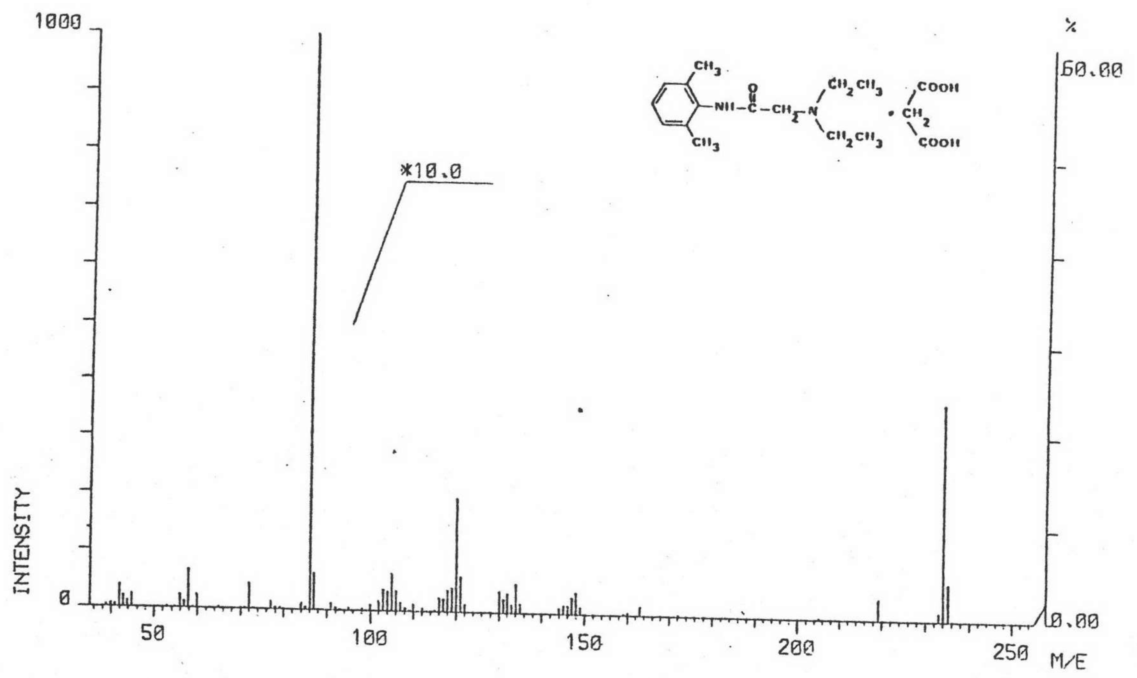

Figure 15 Mass Spectrum of Lidocaine Malonate.

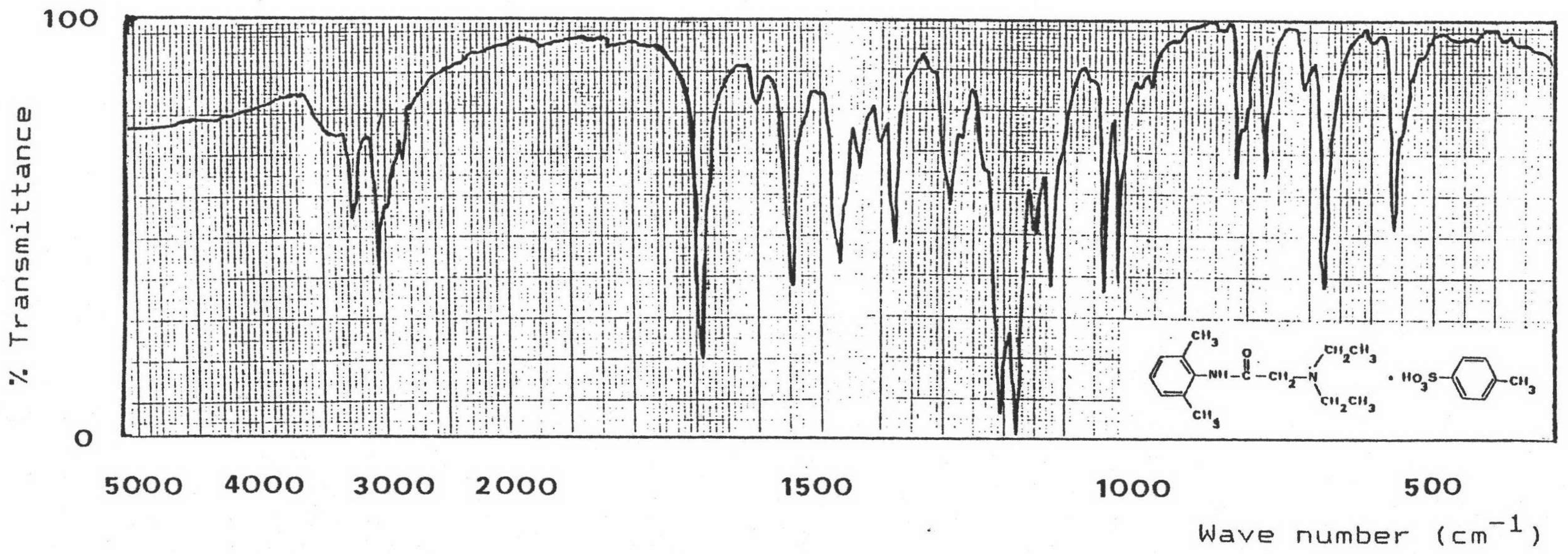

Figure 16 Infrared Absorption Spectrum of Lidocaine Tosylate as a Potassium Bromide Pellet.

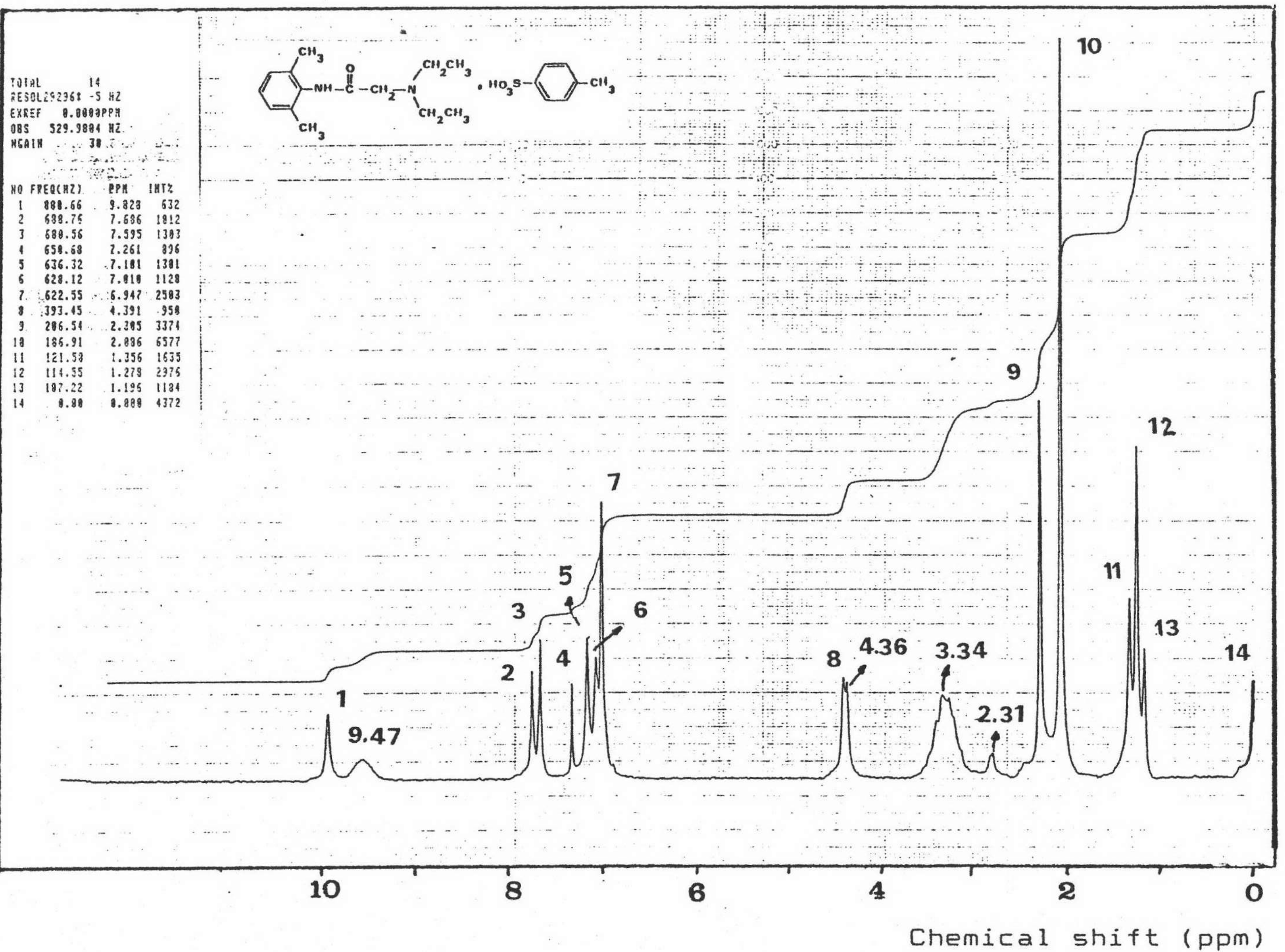

Figure 17 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Tosylate in Chloroform-d.

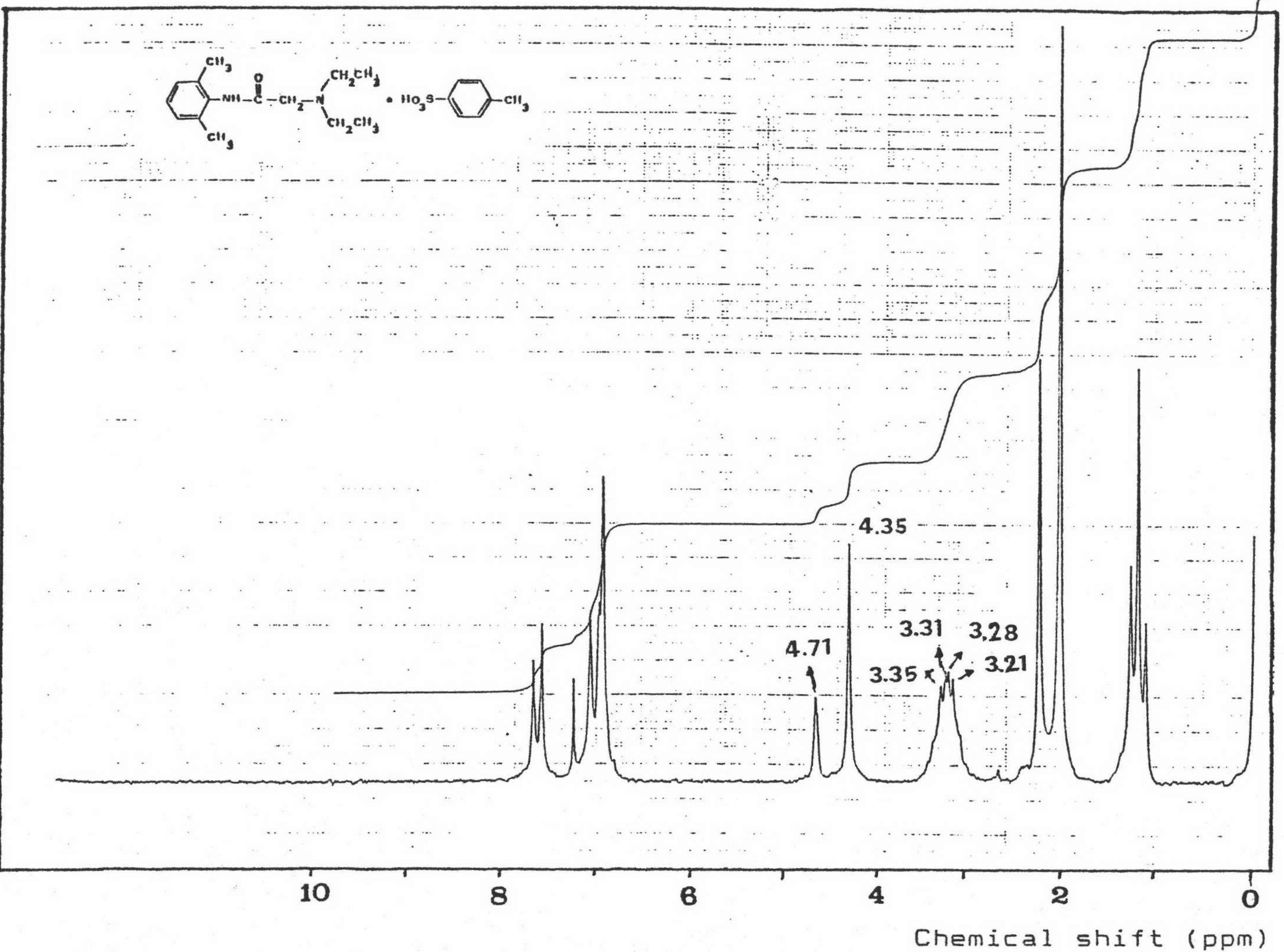

Figure 18 Proton Nuclear Magnetic Resonance Spectrum of Lidocaine Tosylate in Chloroform-d and Deuterium Oxide.

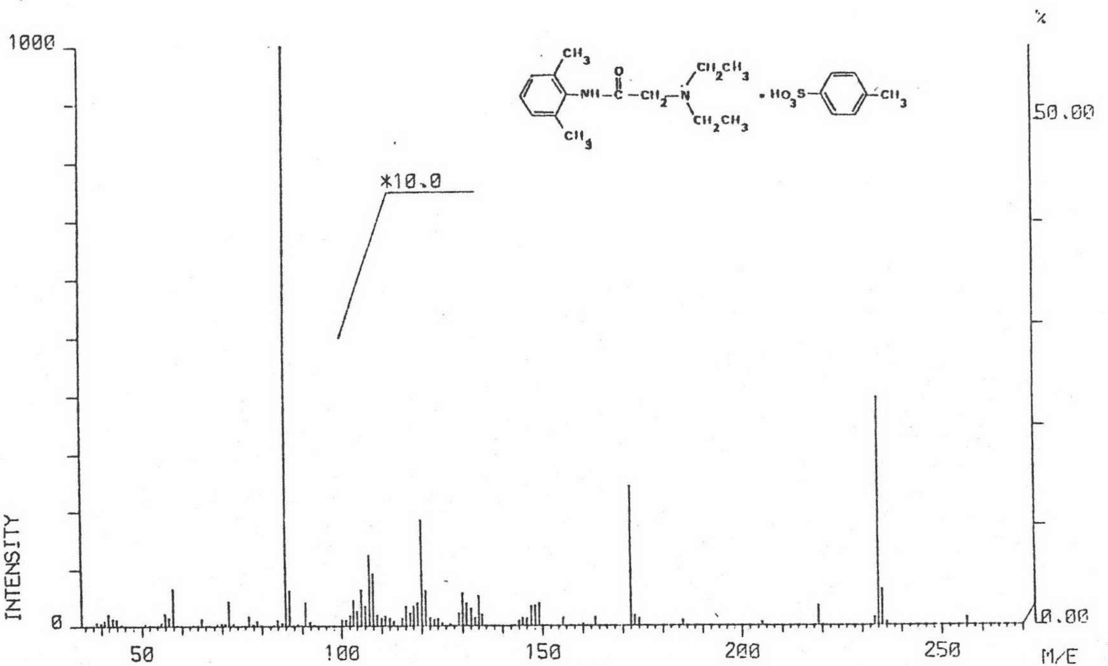

Figure 19 Mass Spectrum of Lidocaine Tosylate.

In Vitro Skin Permeation Study.

A. Materials.

1. Equipments.

UV Spectrophotometer	Hitachi U-3200
Analytical balance	Sartorius 2842
pH Meter	Radiometer PHM 61
Magnetic stirrer	Nuova II stirrer
Ultrasonic bath	Bransonic 321
Permeation cell	

2. Chemicals.

Test compounds[a]

- Lidocaine
- Lidocaine hydrochloride
- Lidocaine adipate
- Lidocaine maleate
- Lidocaine malonate
- Lidocaine tosylate

Monobasic sodium phosphate,AR	May & Baker
Dibasic sodium phosphate,AR	Mallinckrodt
Sodium chloride,AR	E. Merck
Formaldehyde,AR	May & Baker
Propylene glycol	Dow chemicals

3. Animals.

New born pigs Local farm in Nakornprathom

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[a]The compounds were prepared as described in synthesis section A.

B. Methods and Results.

1. Solutions.

a) Monobasic sodium phosphate stock solution. NaH_2PO_4 (8.00 g) was dissolved in distilled water and diluted to 1000 mL volume.

b) Dibasic sodium phosphate stock solution. Na_2HPO_4 (9.47 g) was dissolved in distilled water and diluted to 1000 mL volume.

c) Isotonic phosphate buffer pH 7.4 (NF XIV). Sodium chloride (4.40 g) and 2.70 mL of 37% formaldehyde solution (as a preservative) were added to the mixture of 200 mL of monobasic sodium phosphate stock solution and 800 mL of dibasic sodium phosphate stock solution. The prepared solution was mixed well, adjusted to pH 7.4 $\pm$ 0.1 with 10N sodium hydroxide or 18N phosphoric acid and degassed prior to use by ultrasonication.

d) Standard solutions. Standard solutions (1 mg/mL) of test compounds; lidocaine (I), lidocaine hydrochloride (I.HCl), lidocaine adipate (IV-A), lidocaine maleate (IV-B), lidocaine malonate (IV-C) and lidocaine tosylate (IV-D) were prepared by the following procedures. Stock solution of I was prepared by dissolving 50.0 mg, accurately weighed, in 1.0 mL of propylene glycol in a 25-mL beaker. The solution was transferred to a 50-mL volumetric flask

with the aid of isotonic phosphate buffer pH 7.4 and diluted to volume with the same buffer. Stock solutions of other test compounds; I.HCl, IV-A, IV-B, IV-C, and IV-D, were prepared by dissolving 50.0 mg, accurately weighed, of the test compounds in isotonic phosphate buffer pH 7.4 in an individual 50-mL volumetric flask and diluting to volume with the same buffer.

The following volumes 1.0, 2.0, 5.0, 10.0 and 20.0 mL of stock solutions were individually pipetted into 50-mL volumetric flask and diluted to volume with isotonic phosphate buffer pH 7.4 so that each flask contained a concentration of 0.020, 0.040, 0.100, 0.200 and 0.400 mg/mL, respectively. In addition 0.004 mg/mL solution of test compounds were prepared by diluting 1.0 mL of stock solutions with isotonic phosphate buffer pH 7.4 to 250.0 mL volume.

e) Test solutions. Solutions of test compounds were prepared to give a concentration of 1% w/v in propylene glycol by dissolving 100.0 mg, accurately weighed, of test compounds in propylene glycol in an individual 10-mL volumetric flask and diluting to volume with propylene glycol.

2. Permeation cell.

Skin permeation study was carried out using a permeation cell (Figure 20) modified from Franz diffusion apparatus and the apparatus in Hadgraft's study (Figure 21). The permeation cell consisted of two compartments, the donor cell in the upper and the receptor cell in the lower. The donor cell was mounted with the membrane on which the test solution was applied. The capacity of the receptor cell was 25 ml and the cross-sectional area of the donor cell which was effective permeation area was 3.8 cm^2.

3. Skin preparation.

All permeation experiments were performed with full-thickness pig skin which were excised from side of male pigs. The age of the pigs was 1 day and the weight was about 1 kilogram. The subcutaneous fat and epidermal hair was removed by blunt section. The skin was free of obvious holes or defects. The skin obtained was rinsed with isotonic phosphate buffer pH 7.4, blotted dry, wrapped in plastic, overwrapped with aluminium foil, and store frozen before use. The frozen skin were immersed in isotonic phosphate buffer pH 7.4 and used in the permeation experiment within an hour.

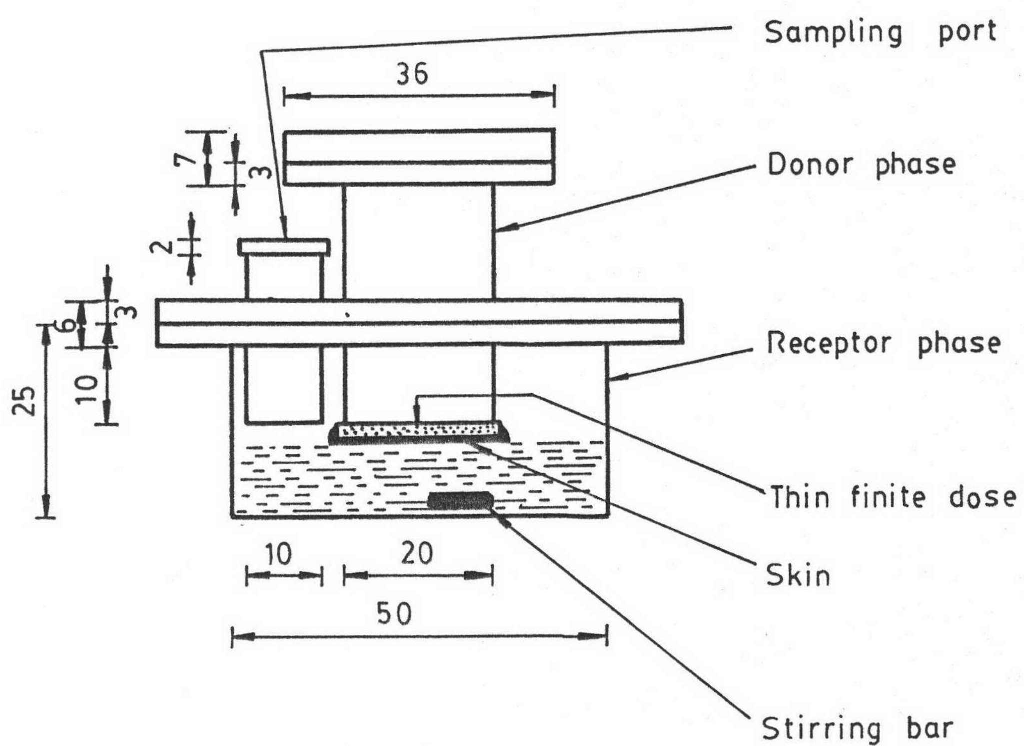
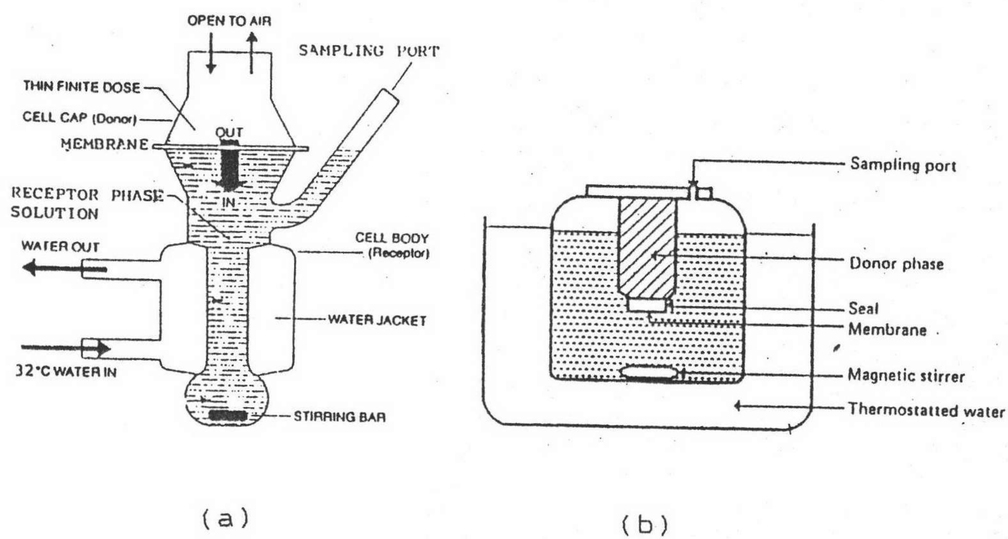


Figure 20 Permeation Cell.



(a) (b)

Figure 21 Franz (a) and Hadgraft (b) Diffusion Cell.

4. Analytical method.

First derivative UV spectrophotometry is a technique selected for the determination of the content of test compounds in receptor cell to minimize matrix interferences. Equation 1 shows the expression of first derivative value (D_1).

$$D_1 = \frac{dA}{d\lambda} \qquad (1)$$

where D_1 = first derivative of absorbance

A = absorbance

λ = wavelength

Serial dilutions of concentration 0.004, 0.020, 0.040, 0.100, 0.200 and 0.400 mg/mL of prepared standard dilutions were recorded in 1-cm quartz cells over the range of 260 - 285 nm using spectrophotometer with derivative capability. The spectra were obtained at a band pass of 2 nm and a scanning speed of 60 nm/min. The recorder response was set at fast and derivative sensitivity was 8. The first derivative mode was adjusted zero by placing cuvettes filled with isotonic phosphate buffer pH 7.4 in both reference and sample compartments. After adjustment, sample compartment was replaced with standard or sample solutions and measured. At first, the absorbance spectrum in ultraviolet region was obtained, then the first derivative mode was selected

and distinct spectrum from first derivative mode was obtained.

The wavelengths of maximum D_1 of each test compound were determined. The maximum D_1 values of standard solutions of all test compounds being examined were found to be at the same wavelength of about 272.9 nm. D_1 spectra of serial standard solutions of test compounds were shown in Figure 22 - 27. The D_1 values of the standard solutions versus concentrations were listed in Table 7. Calibration curves between D_1 values and concentrations of standard solutions were plotted (Figure 28). Each plot indicated that the relationship between D_1 value and concentration was linear (R^2 = 0.9999 to 1.0000) and conformed to Beer-Lambert's Law. The regression parameters relating D_1 value and concentration were shown in Table 8.

5. Measurement of permeabilities.

Test compounds : I, I.HCl, IV-A, IV-B, IV-C and IV-D.

A circular sheet of pig skin of approximately 4 cm^2 in area was placed, dermal side down, between the donor and receptor phase of the permeation cell. The receptor phase was 25 mL isotonic phosphate buffer pH 7.4, maintained at room

temperature (about 32 ± 2°C) and stirred with stirring rate maintained at 600 rpm. The skin was kept in contact with the receptor phase for 48 hours prior to the application of the donor phase. The receptor phase was changed every 12 hours during 48-hour preapplication leach period. After this period, a 1.0 ml aliquot of the test solution, equivalent to about 10 mg of test compound, was applied to the donor side of the skin. The receptor phase was stirred at stirring rate maintained at 600 rpm. Samples (5.0 mL) were taken from the receptor phase every 12 hours during 48 hours period after application. The volume taken was replaced by fresh isotonic phosphate buffer pH 7.4. The samples were stored in a refrigerator until assay by first derivative UV spectrophotometry as described in **in vitro** skin permeation study section B4. Four determinations of skin permeation tests were performed and two pigs were used for each test compounds.

Control tests were carried out by application of the donor vehicle, 1.0 mL propylene glycol, instead of the test solutions. D_1 values of control tests were used for correction of D_1 values from samples in order to eliminate matrix interferences from pig skin. Absorbance spectrum and D_1 spectrum of control test were displayed in Figure 29 - 30. D_1 values of matrix interferences in

receptor phase of control experiment were listed in Table 9. Percent recovery of test compounds from D_1 and corrected D_1 were determined and shown in Table 10.

The D_1 values measured from samples at 272.9 nm were listed in Table 11. The corrected D_1 value were determined by subtraction of D_1 value of the control test from D_1 value of samples. Concentrations (C_T) in μg/mL of the samples taken at various time intervals were calculated by substituting corrected D_1 values in the regression equations indicated in Table 8. The corrected D_1 values and calculated concentrations (C_T) were shown in Table 12. The cumulative amount (Q_T) which was the total amount of test compound permeated through pig skin at the time observed was calculated by using equation 2.

$$Q_T = (C_T \times 25) + (Q_{T-12} \times 0.2) \quad \text{------} \quad (2)$$

where Q_T = cumulative amount of test compound (μg) permeated at time T.

C_T = concentration of test compound (μg/mL) in sample taken at time T.

T = time of permeation (hr).

25 = volume of solution in receptor phase (mL).

0.2 = volume of sample taken (5.0 mL) divided by volume of solution in receptor phase (25 mL).

Furthermore, determination of flux (J_T) of each test compound permeated through pig skin was accomplished. The expression shown in equation 3 was used to calculate J_T.

$$J_T = \frac{Q_T}{AT} \qquad \text{------------} \qquad (3)$$

where J_T = total amount of test compound permeated per unit of skin area at time T ($\mu g/cm^2$. hr).

Q_T = cumulative amount of test compound (μg) permeated at time T.

A = skin area exposed to the donor phase (3.8 cm^2)

T = time of permeation (hr).

For permeability comparison, Q and J values were converted from μg to $\mu mole$. The obtained Q and J values of the test compounds were listed in Table 13. The Q and J values were plotted as a function of time as displayed in Figure 31 - 38. The comparison of Q and J values between the test compounds at observed time interval were evaluated by analysis of variance, Duncan multiple range test at the significant level of $\alpha < 0.05$. The results were outlined in Table 14 - 17.

Table 7 : First derivative of absorbance (D_1) of standard solution of test compounds at 272.9 nm.[a]

Concentration[b] (mg/mL)	I		I.HCl		IV-A		IV-B		IV-C		IV-D	
	D_1	%CV	D_1	%CV	D_1	%CV	D_1	%CV	D_1	%CV	D_1	%CV
0.004	-0.0064	1.80	-0.0069	1.67	-0.0054	1.72	-0.0058	1.00	-0.0058	1.00	-0.0056	2.06
0.020	-0.0258	0.59	-0.0238	1.94	-0.0164	1.96	-0.0217	1.22	-0.0184	1.37	-0.0246	0.62
0.040	-0.0515	0.62	-0.0449	1.02	-0.0332	0.46	-0.0418	0.36	-0.0360	0.16	-0.0453	0.22
0.100	-0.1284	0.44	-0.1052	0.38	-0.0777	0.32	-0.0970	0.30	-0.0886	0.45	-0.1122	0.58
0.200	-0.2580	0.63	-0.2097	0.87	-0.1557	0.06	-0.1969	0.36	-0.1790	0.23	-0.2262	1.27
0.400	-0.5083	0.38	-0.4070	0.77	-0.3122	0.20	-0.3911	0.36	-0.3500	0.16	-0.4498	0.77

[a] D_1 values are averages of three determinations; test compounds are I (lidocaine), I.HCl (lidocaine hydrochloride), IV-A (lidocaine adipate) IV-B (lidocaine maleate), IV-C (lidocaine malonate) and IV-D (lidocaine tosylate).

[b] The solvent is isotonic phosphate buffer pH 7.4.

Table 8 : Regression parameters of first derivative mode.[a]

Test Compound[b]	Regression Parameter[c] m(Slope)	z(Intercept)	R^2
I	-1.27062	-0.00126	0.9999
I.HCl	-1.01104	-0.00416	0.9999
IV-A	-0.77561	-0.00133	0.9999
IV-B	-0.97280	-0.00185	0.9999
IV-C	-0.87323	-0.00178	0.9999
IV-D	-1.12199	-0.00109	1.0000

[a]Three determinations of six concentration levels; 0.004, 0.202, 0.040, 0.100, 0.200 and 0.400 mg/mL.

[b]I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[c]$Y = mX + z$, Y and X represent D_1 value and concentration (mg/mL), respectively.

Table 9 : D_1 value of matrix interferences.[a]

Time (hr)	D_1 (272.9 nm)				Average
	Pig A	Pig A	Pig B	Pig B	
12	-0.0025	-0.0019	-0.0017	-0.0014	-0.0019
24	-0.0024	-0.0022	-0.0025	-0.0016	-0.0022
36	-0.0028	-0.0031	-0.0034	-0.0032	-0.0032
48	-0.0034	-0.0035	-0.0033	-0.0034	-0.0034

[a] D_1 value of receptor phase from control experiments described in **in vitro** skin permeation study section B5.

Table 10 : Percent recovery of test compounds.

Test Compound[a]	D_1[b]	%Recovery[c]	Corrected D_1[d]	%Recovery[e]
I	-0.1315	102.50	-0.1296	101.01
IV-A	-0.0822	104.27	-0.0803	101.82
IV-B	-0.1005	101.41	-0.0986	99.46
IV-C	-0.0911	102.29	-0.0892	100.11

[a] I = lidocaine, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate.

[b] Average D_1 value (n = 2) of 0.100 mg/mL test compound in matrix from control experiment at 12 hours after application of propylene glycol.

[c] $\% \text{ Recovery} = \frac{\text{mg/mL Found}}{\text{mg/mL Added}} \times 100$; mg/mL Found was calculated from regression equations indicated in Table 8 using D_1 and mg/mL Added was 0.1 mg/mL.

[d] Corrected $D_1 = D_1 - D_{matrix}$; D_{matrix} at 12 hours after application of propylene glycol was 0.0019.

[e] Corrected D_1 was used to calculate mg/mL Found.

Table 11 : D_1 value of receptor phase after application of test compounds.

Test Compound[a]	Time (hr)	D_1 (272.9 nm)			
		Pig A	Pig A	Pig B	Pig B
I	12	-0.0080	-0.0091	-0.0075	-0.0133
	24	-0.0100	-0.0195	-0.0142	-0.0160
	36	-0.0272	-0.0236	-0.0224	-0.0172
	48	-0.0301	-0.0298	-0.0286	-0.0236
I.HCl	12	-0.0065	-0.0062	-0.0074	-0.0061
	24	-0.0114	-0.0115	-0.0106	-0.0109
	36	-0.0126	-0.0126	-0.0122	-0.0119
	48	-0.0155	-0.0158	-0.0154	-0.0152
IV-A	12	-0.0055	-0.0108	-0.0054	-0.0048
	24	-0.0113	-0.0128	-0.0112	-0.0108
	36	-0.0121	-0.0137	-0.0123	-0.0133
	48	-0.0146	-0.0146	-0.0146	-0.0146
IV-B	12	-0.0063	-0.0084	-0.0061	-0.0045
	24	-0.0102	-0.0101	-0.0100	-0.0080
	36	-0.0111	-0.0119	-0.0142	-0.0100
	48	-0.0122	-0.0124	-0.0149	-0.0116
IV-C	12	-0.0092	-0.0075	-0.0063	-0.0073
	24	-0.0103	-0.0096	-0.0082	-0.0092
	36	-0.0113	-0.0117	-0.0100	-0.0121
	48	-0.0136	-0.0139	-0.0141	-0.0129
IV-D	12	-0.0070	-0.0067	-0.0056	-0.0082
	24	-0.0114	-0.0117	-0.0103	-0.0091
	36	-0.0132	-0.0128	-0.0134	-0.0143
	48	-0.0153	-0.0159	-0.0151	-0.0163

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, V-C = lidocaine malonate, IV-D = lidocaine tosylate.

Table 12 : Corrected D_1 value and C_T at various time intervals after application.

Test Compound[a]	Time (hr)	Pig A		Pig A		Pig B		Pig B		Average	
		D_1[b]	C_T[c]	D_1	C_T	D_1	C_T	D_1	C_T	D_1	C_T
I	12	-0.0061	3.809	-0.0072	4.674	-0.0056	3.415	-0.0094	6.406	-0.0071	4.576
	24	-0.0088	5.934	-0.0173	12.623	-0.0120	8.452	-0.0138	9.869	-0.0130	9.220
	36	-0.0240	17.896	-0.0204	15.063	-0.0149	10.734	-0.0192	14.119	-0.0196	14.453
	48	-0.0267	20.021	-0.0264	19.785	-0.0252	18.841	-0.0202	14.906	-0.0246	18.388
I.HCl	12	-0.0046	0.435	-0.0043	0.138	-0.0055	1.325	-0.0042	0.039	-0.0047	0.484
	24	-0.0092	4.984	-0.0093	5.083	-0.0084	4.193	-0.0087	4.490	-0.0089	4.688
	36	-0.0094	5.182	-0.0094	5.182	-0.0090	4.787	-0.0087	4.490	-0.0091	4.910
	48	-0.0121	7.853	-0.0124	8.150	-0.0120	7.754	-0.0118	7.556	-0.0121	7.828
IV-A	12	-0.0036	2.926	-0.0089	9.760	-0.0035	2.797	-0.0029	2.024	-0.0047	4.377
	24	-0.0091	10.018	-0.0106	11.952	-0.0090	9.889	-0.0086	9.373	-0.0093	10.308
	36	-0.0090	9.889	-0.0105	11.823	-0.0091	10.018	-0.0101	11.307	-0.0097	10.759
	48	-0.0112	12.725	-0.0112	12.725	-0.0117	13.370	-0.0115	13.112	-0.0114	12.983
IV-B	12	-0.0044	2.621	-0.0065	4.780	-0.0042	2.415	-0.0026	0.770	-0.0044	2.647
	24	-0.0080	6.321	-0.0079	6.219	-0.0078	6.116	-0.0058	4.060	-0.0074	5.679
	36	-0.0079	6.219	-0.0087	7.041	-0.0110	9.405	-0.0068	5.088	-0.0086	6.938
	48	-0.0088	7.144	-0.0090	7.349	-0.0115	9.919	-0.0082	6.527	-0.0094	7.735
IV-C	12	-0.0073	6.321	-0.0056	4.374	-0.0074	3.000	-0.0054	4.145	-0.0057	4.460
	24	-0.0081	7.237	-0.0074	6.435	-0.0060	4.832	-0.0070	5.977	-0.0071	6.120
	36	-0.0081	7.237	-0.0085	7.695	-0.0094	8.726	-0.0089	8.153	-0.0087	7.953
	48	-0.0102	9.642	-0.0105	9.985	-0.0107	10.214	-0.0095	8.840	-0.0102	9.670
IV-D	12	-0.0051	3.574	-0.0048	3.306	-0.0037	2.326	-0.0063	4.643	-0.0050	3.462
	24	-0.0092	7.228	-0.0095	7.495	-0.0081	6.247	-0.0069	5.178	-0.0084	6.537
	36	-0.0100	7.941	-0.0096	7.584	-0.0102	8.119	-0.0111	8.921	-0.0102	8.141
	48	-0.0119	9.634	-0.0125	10.169	-0.0117	9.456	-0.0129	10.525	-0.0123	9.946

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate
IV-C = lidocaine malonate, IV-D = lidocaine tosylate.
[b] Corrected D_1 value.
[c] Concentration of test compounds (μg/mL) in sample taken at time T.

Table 13 : *In vitro* permeability of test compounds.

Test Compound[a]	Time (hr)	Q^b(SEM) (μg)	J^c(SEM) (μg/cm^2.hr)	Q^b(SEM) (μM)x10	J^c(SEM) (μM/cm^2.hr)x10^3
I	12	114.4 (16.6)	2.509 (0.364)	4.88 (0.71)	10.707 (1.553)
	24	253.4 (36.5)	2.779 (0.400)	10.81 (1.56)	11.859 (1.707)
	36	412.0 (35.8)	3.012 (0.262)	17.58 (1.53)	12.853 (1.118)
	48	542.1 (32.2)	2.972 (0.177)	23.13 (1.37)	12.682 (0.755)
I.HCl	12	12.1 (7.3)	0.266 (0.160)	0.42 (0.25)	0.921 (0.554)
	24	119.6 (4.4)	1.312 (0.049)	4.14 (0.15)	4.543 (0.170)
	36	146.7 (5.0)	1.072 (0.037)	5.08 (0.17)	3.712 (0.128)
	48	225.1 (4.0)	1.234 (0.022)	7.79 (0.14)	4.273 (0.076)
IV-A	12	109.4 (45.1)	2.400 (0.990)	2.88 (1.19)	6.308 (2.602)
	24	279.6 (23.1)	3.066 (0.253)	7.35 (0.61)	8.085 (0.665)
	36	324.9 (15.2)	2.375 (0.111)	8.54 (0.40)	6.242 (0.292)
	48	389.6 (3.8)	2.136 (0.021)	10.24 (0.10)	5.614 (0.055)
IV-B	12	66.2 (20.6)	1.451 (0.451)	1.89 (0.59)	4.141 (1.287)
	24	155.2 (16.9)	1.702 (0.185)	4.43 (0.48)	4.857 (0.528)
	36	204.5 (25.0)	1.495 (0.183)	5.84 (0.71)	4.266 (0.522)
	48	234.3 (23.5)	1.284 (0.129)	6.69 (0.67)	3.664 (0.368)
IV-C	12	111.5 (17.2)	2.445 (0.378)	3.30 (0.51)	7.225 (1.117)
	24	175.3 (15.9)	1.922 (0.174)	5.18 (0.47)	5.680 (0.514)
	36	233.9 (4.8)	1.710 (0.035)	6.91 (0.14)	5.053 (0.103)
	48	288.6 (7.7)	1.582 (0.042)	8.53 (0.23)	4.675 (0.124)
IV-D	12	86.6 (11.9)	1.898 (0.261)	2.13 (0.29)	4.669 (0.642)
	24	180.8 (12.3)	1.982 (0.135)	4.45 (0.30)	4.875 (0.332)
	36	239.7 (4.9)	1.752 (0.036)	5.90 (0.12)	4.310 (0.089)
	48	296.6 (6.7)	1.626 (0.037)	7.30 (0.16)	4.000 (0.091)

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[b] Cumulative amount of test compound permeated.

[c] Flux.

Table 14 : Comparison of permeability results at 12 hours after application.

Test Compound[a]	Q(row)/Q(column) (μMx10)					J(row)/J(column) ($\mu Mx10^3/cm^2.hr$)				
	I	I.HCl	IV-A	IV-B	IV-C	I	I.HCl	IV-A	IV-B	IV-C
I.HCl	0.42/4.88*					0.92/10.71*				
IV-A	2.87/4.88	2.87/0.42*				6.31/10.71	6.31/0.92*			
IV-B	1.89/4.88*	1.89/0.42	1.89/2.87			4.14/10.71*	4.14/0.92	4.14/6.31		
IV-C	3.29/4.88	3.29/0.42*	3.29/2.87	3.29/1.89		7.23/10.71	7.23/0.92*	7.23/6.31	7.32/4.14	
IV-D	2.13/4.88*	2.13/0.42	2.13/2.87	2.13/1.89	2.13/3.29	4.67/10.71*	4.67/0.92	4.67/6.31	4.67/4.14	4.67/7.23

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

*Significantly different ($\alpha < 0.05$), Analysis of variance, Duncan's multiple range test.

Table 15 : Comparison of permeability results at 24 hours after application.

Test Compound[a]	Q(row)/Q(column) (μMx10)					J(row)/J(column) ($\mu Mx10^3/cm^2.hr$)				
	I	I.HCl	IV-A	IV-B	IV-C	I	I.HCl	IV-A	IV-B	IV-C
I.HCl	4.14/10.81*					4.54/11.86*				
IV-A	7.35/10.81*	7.35/4.14*				8.06/11.86*	8.06/4.54*			
IV-B	4.43/10.81*	4.43/4.14	4.43/7.35*			4.86/11.86*	4.86/4.54	4.86/8.06*		
IV-C	5.18/10.81*	5.18/4.14	5.18/7.35	5.18/4.43		5.68/11.86*	5.68/4.54	5.68/8.06	5.68/4.86	
IV-D	4.45/10.81*	4.45/4.14	4.45/7.35*	4.45/4.43	4.45/5.18	4.87/11/86*	4.87/4.54	4.87/8.06*	4.87/4.86	4.87/5.68

[a]I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

*Significantly different ($\alpha < 0.05$), Analysis of variance, Duncan's multiple range test.

Table 16 : Comparison of permeability results at 36 hours after application.

Test Compound[a]	Q(row)/Q(column) (μMx10)					J(row)/J(column) ($\mu Mx10^3/cm^2.hr$)				
	I	I.HCl	IV-A	IV-B	IV-C	I	I.HCl	IV-A	IV-B	IV-C
I.HCl	5.08/17.58*					3.71/11.33*				
IV-A	8.54/17.58*	8.54/5.08*				6.24/11.33*	6.24/3.71*			
IV-B	5.83/17.58*	5.83/5.08	5.83/8.54*			4.27/11.33*	4.27/3.71	4.27/6.24*		
IV-C	6.91/17.58*	6.91/5.08	6.91/8.54	6.91/5.83		5.05/11.33*	5.05/3.71	5.05/6.24	5.05/4.27	
IV-D	5.90/17.58*	5.90/5.08	5.90/8.54*	5.90/5.83	5.90/6.91	4.31/11.33*	4.31/3.71	4.31/6.24*	4.31/4.27	4.31/5.05

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

*Significantly different ($\alpha < 0.05$), Analysis of variance, Duncan's multiple range test.

Table 17 : Comparison of permeability results at 48 hours after application.

Test Compound[a]	Q(row)/Q(column) (μMx10)					J(row)/J(column) ($\mu Mx10^3/cm^2.hr$)				
	I	I.HCl	IV-A	IV-B	IV-C	I	I.HCl	IV-A	IV-B	IV-C
I.HCl	7.79/23.13*					4.27/12.68*				
IV-A	10.24/23.13*	10.24/7.79*				5.61/12.68*	5.61/4.27*			
IV-B	6.68/23.13*	6.68/7.79	6.68/10.24*			3.67/12.68*	3.67/4.27	3.67/5.61*		
IV-C	8.53/23.13*	8.53/7.79	8.53/10.24	8.53/6.68		4.67/12.68*	4.67/4.27	4.67/5.61	4.67/3.67	
IV-D	7.30/23.13*	7.30/7.79	7.30/10.24*	7.30/6.68	7.30/8.53	4.00/12.68*	4.00/4.27	4.00/5.61*	4.00/3.67	4.00/4.67

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

*Significantly different ($\alpha < 0.05$), Analysis of variance, Duncan's multiple range test.

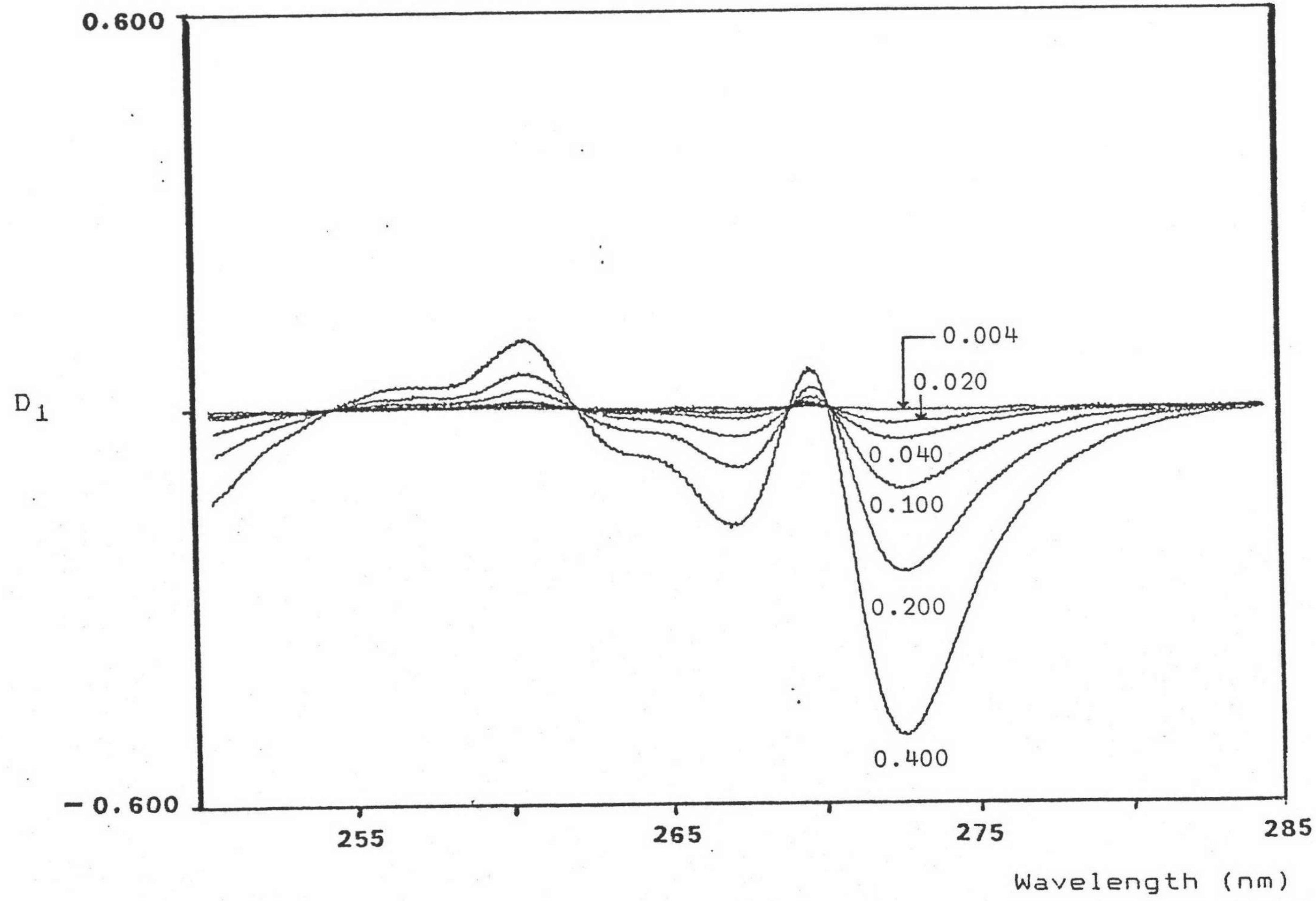


Figure 22 Ultraviolet Absorption Spectra of Lidocaine in Isotonic Phosphate Buffer pH 7.4 in First Derivative Mode.

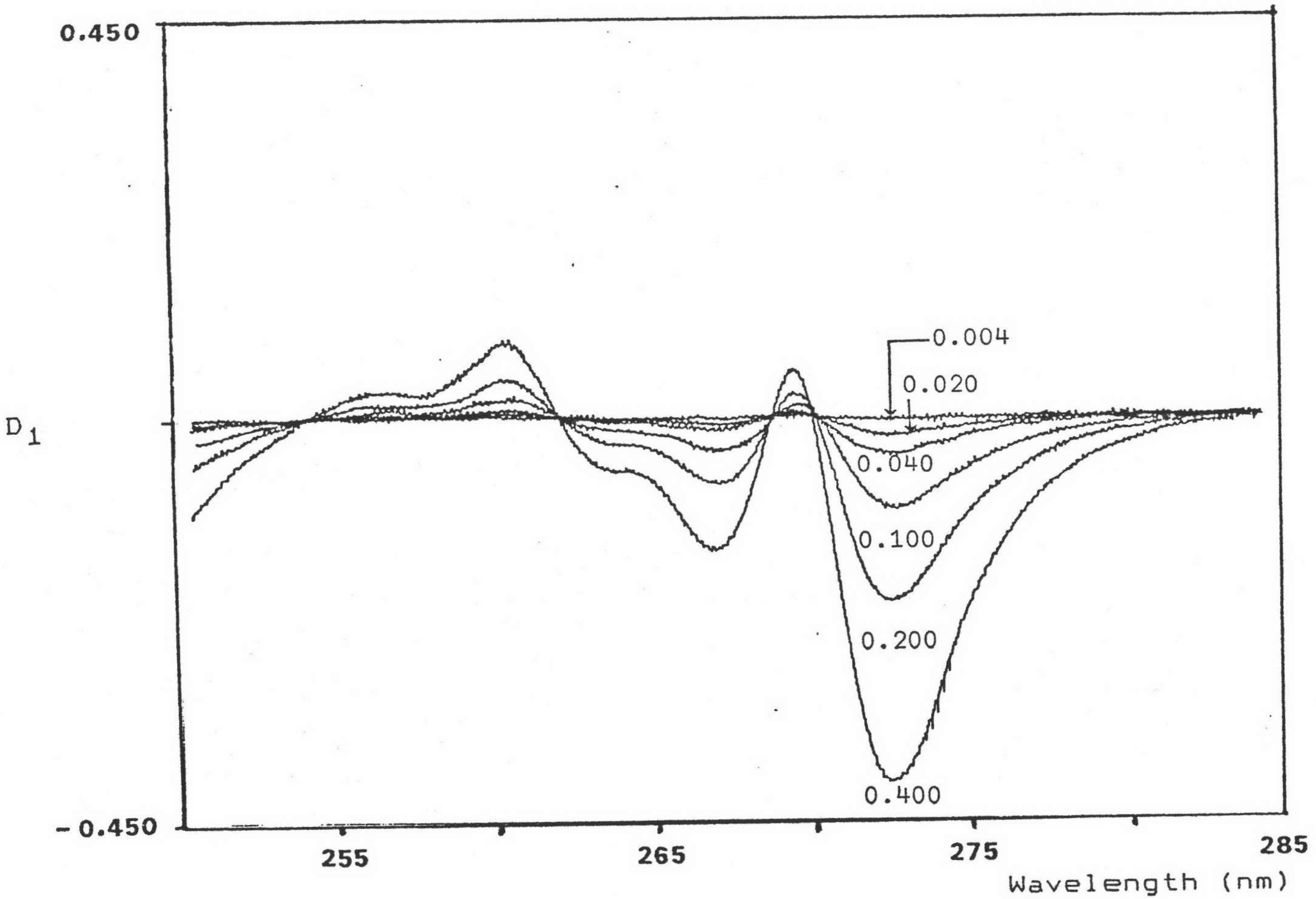


Figure 23 Ultraviolet Absorption Spectra of Lidocaine Hydrochloride in Isotonic Phosphate Buffer pH 7.4 in First Derivative Mode.

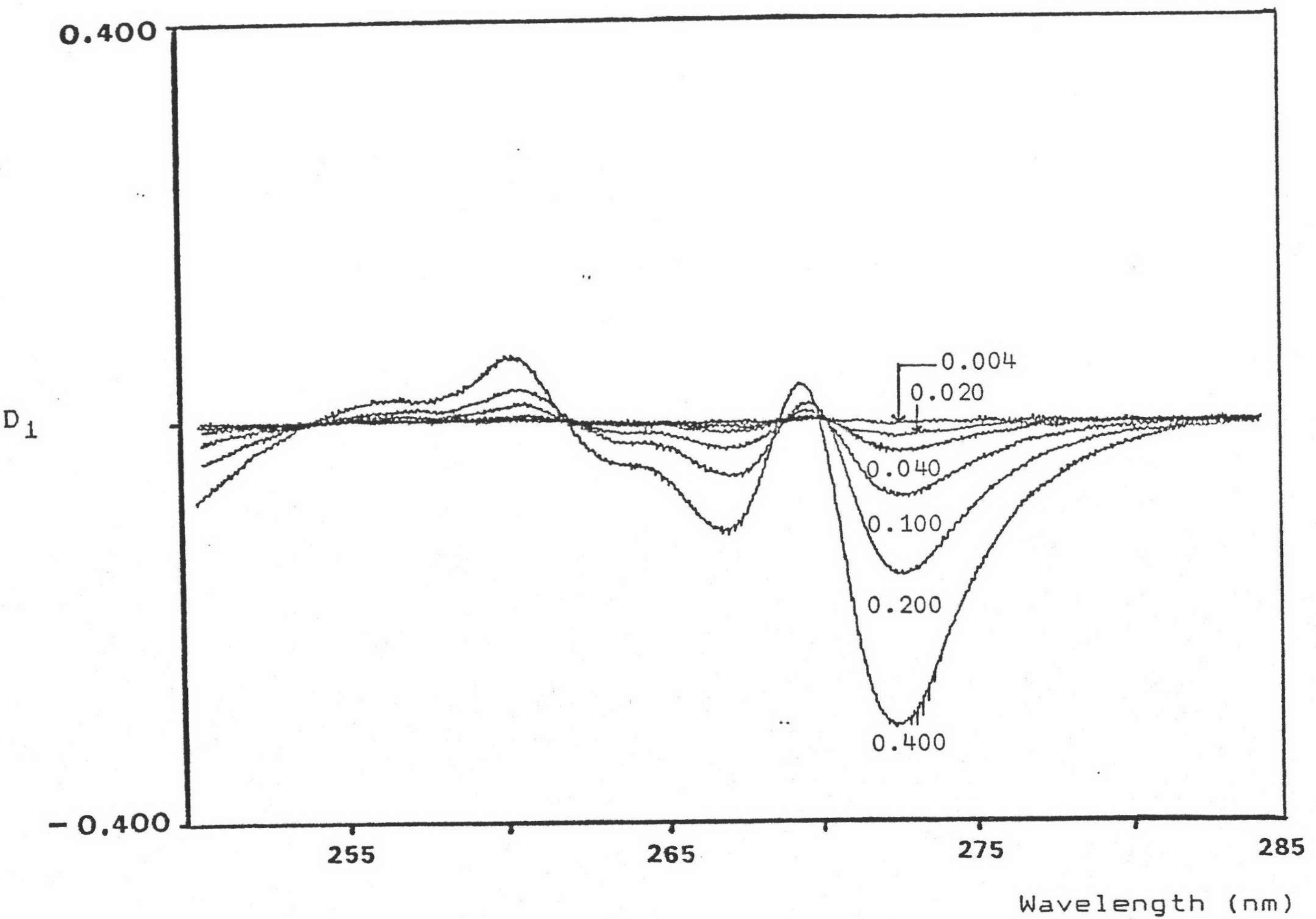


Figure 24 Ultraviolet Absorption Spectra of Lidocaine Adipate in Isotonic Phosphate Buffer pH 7.4 in First Derivative Mode.

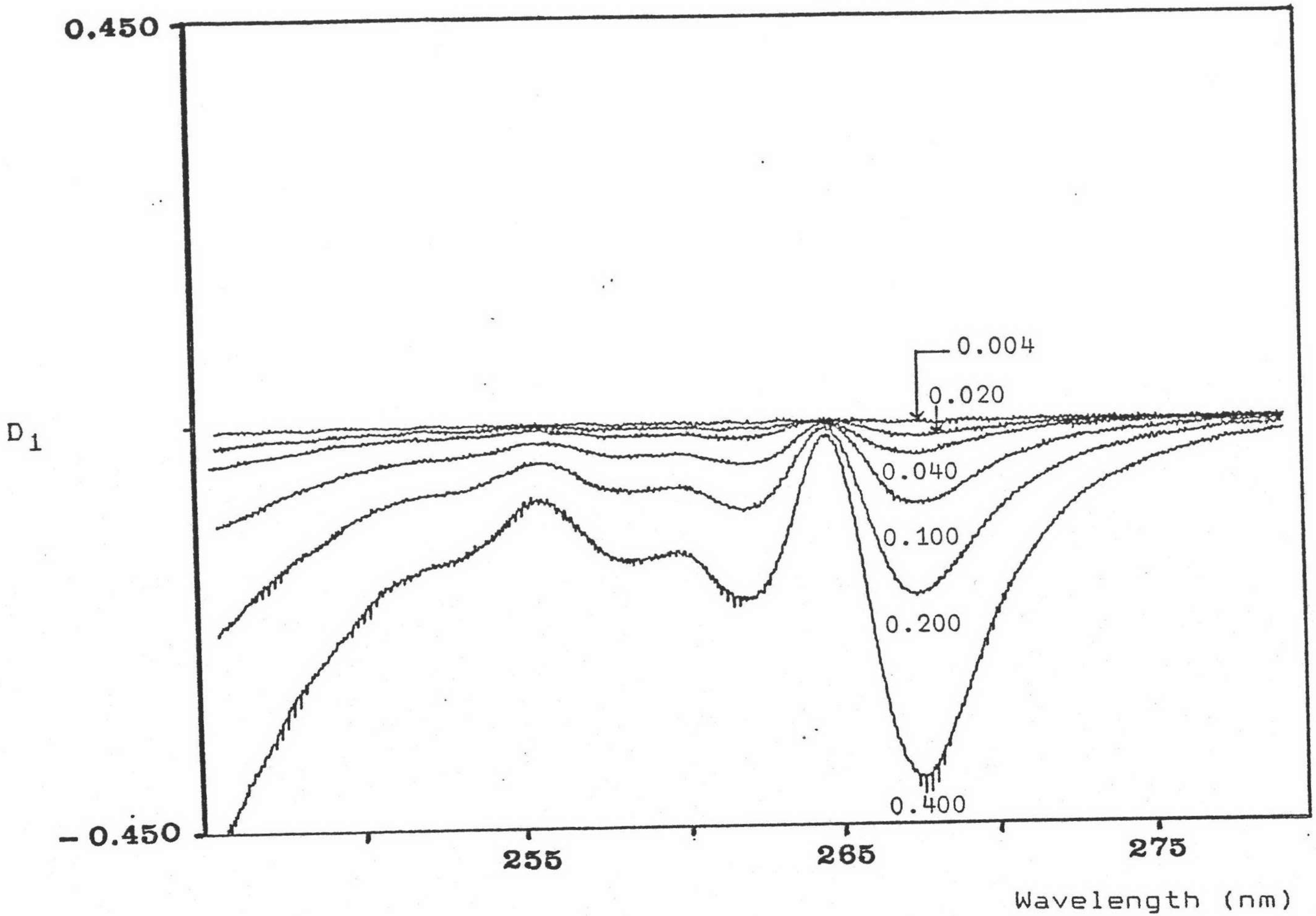


Figure 25 Ultraviolet Absorption Spectra of Lidocaine Maleate in Isotonic Phosphate Buffer pH 7.4 in First Derivative Mode.

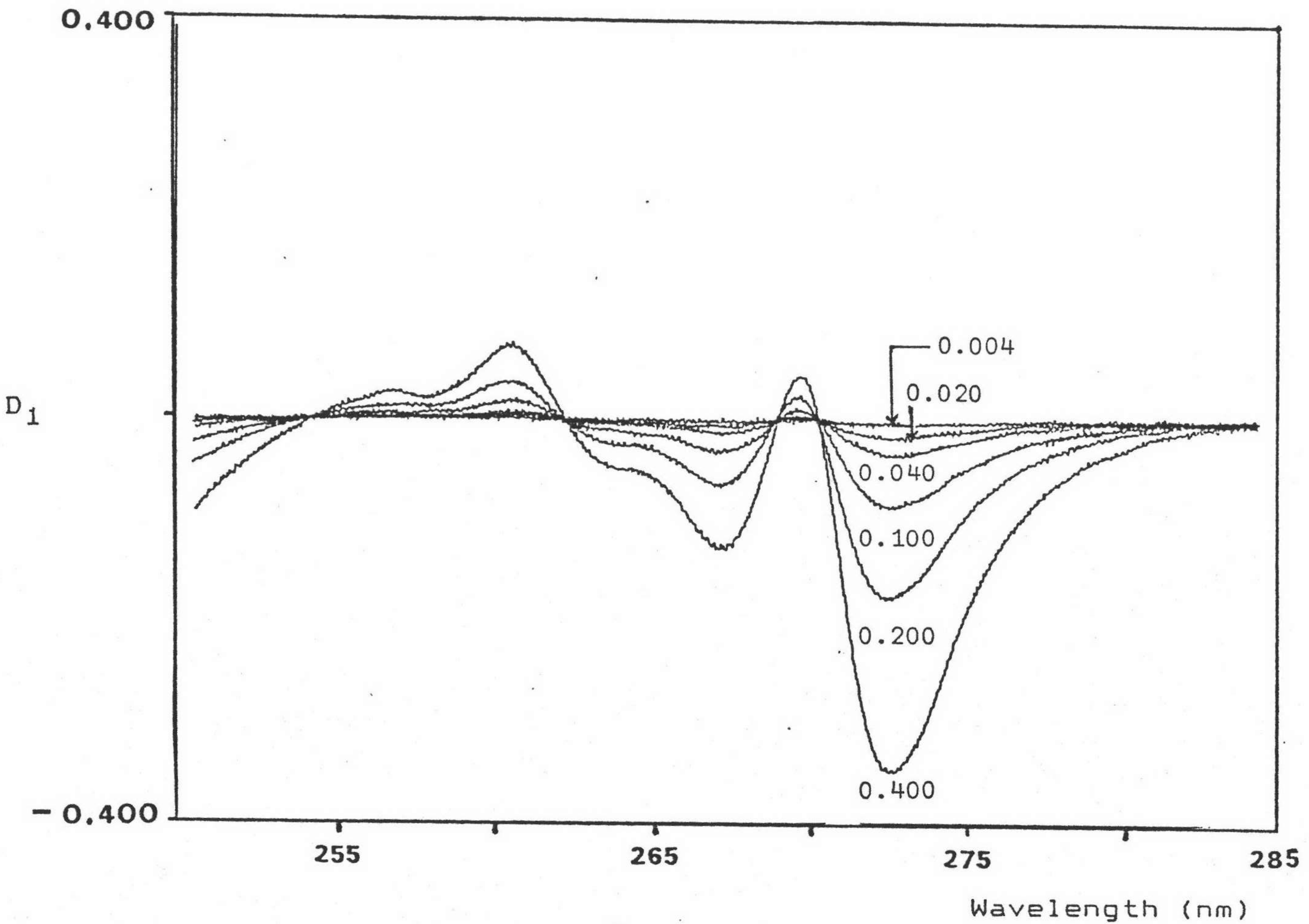


Figure 26 Ultraviolet Absorption Spectra of Lidocaine Malonate in Isotonic Phosphate Buffer pH 7.4 in First Derivative Mode.

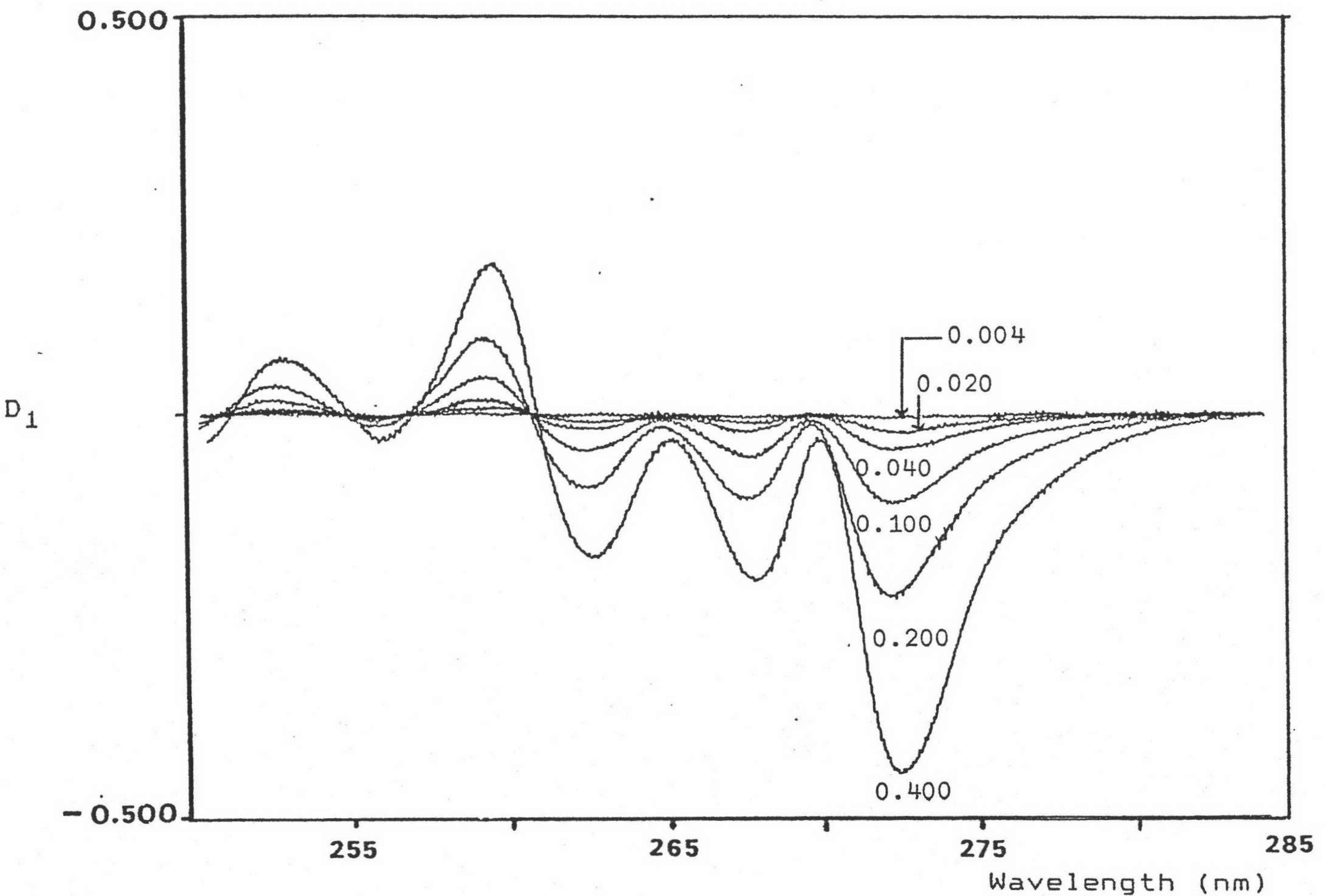


Figure 27 Ultraviolet Absorption Spectra of Lidocaine Tosylate in Isotonic Phosphate Buffer pH 7.4 in First Derivative Mode.

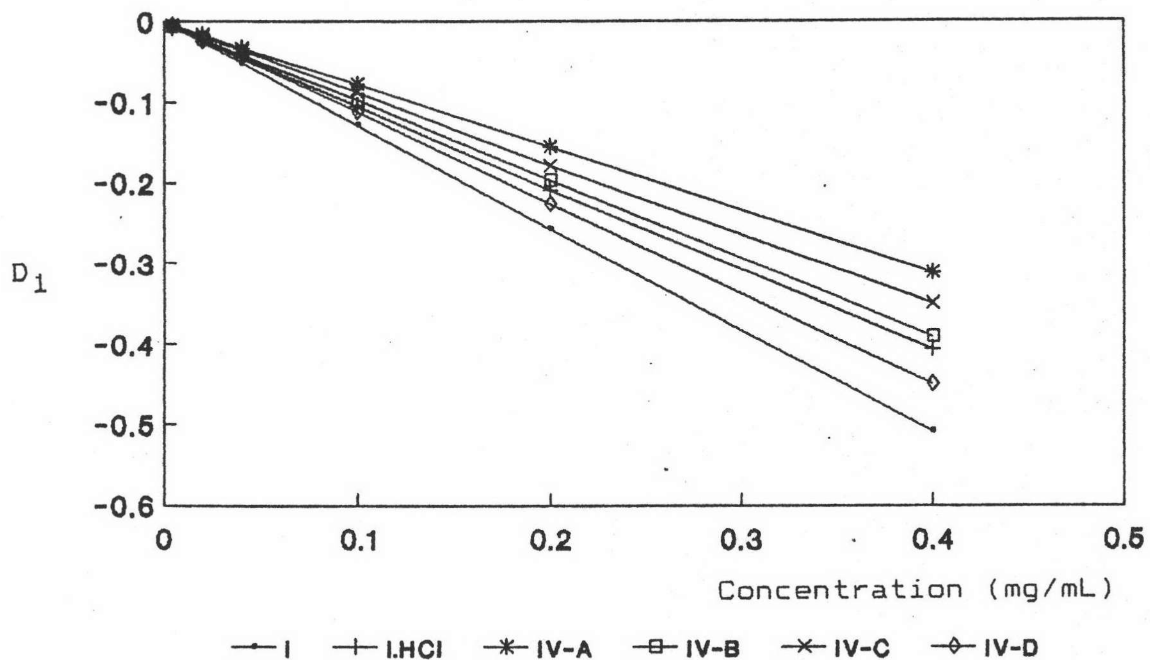


Figure 28 Calibration Curve of Test Compounds in First Derivative Mode.

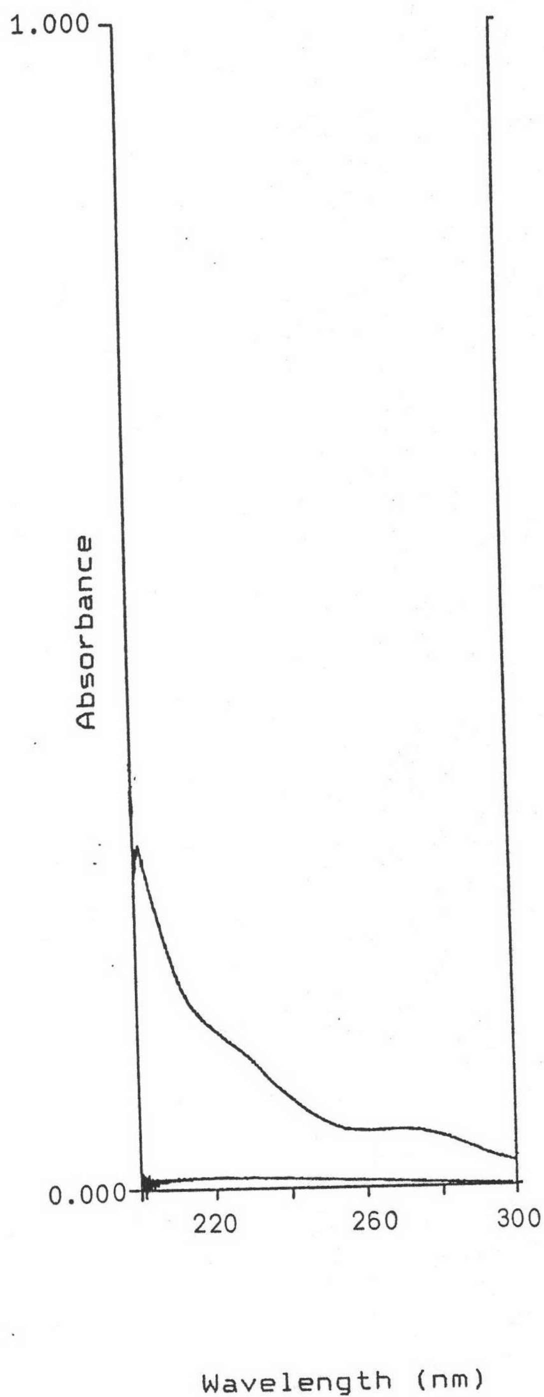


Figure 29 Ultraviolet Absorption Spectrum of Receptor Phase from Control Experiment after 12 hours.

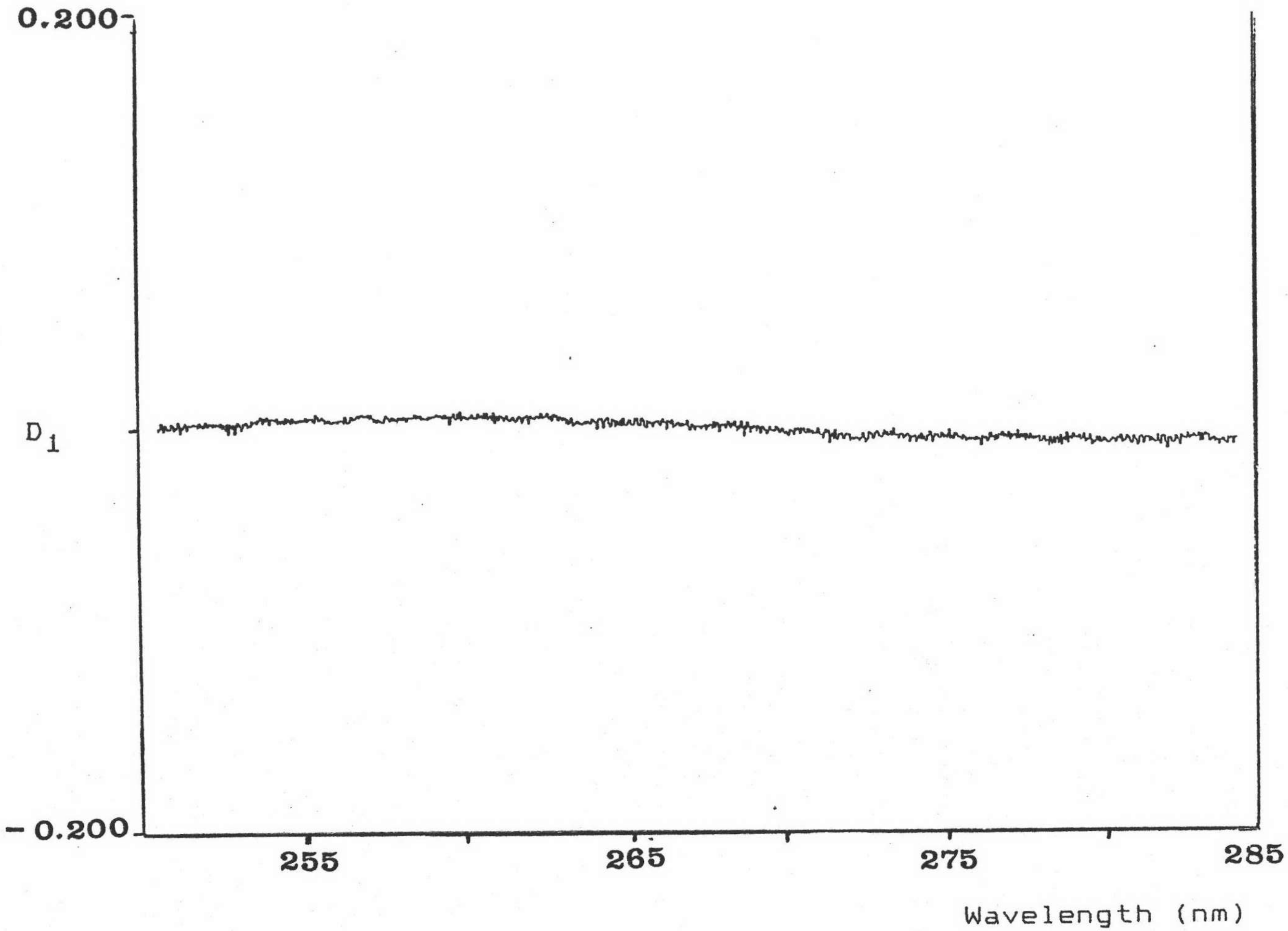


Figure 30 Ultraviolet Absorption Spectrum of Receptor Phase from Control Experiment after 12 hours in First Derivative Mode.

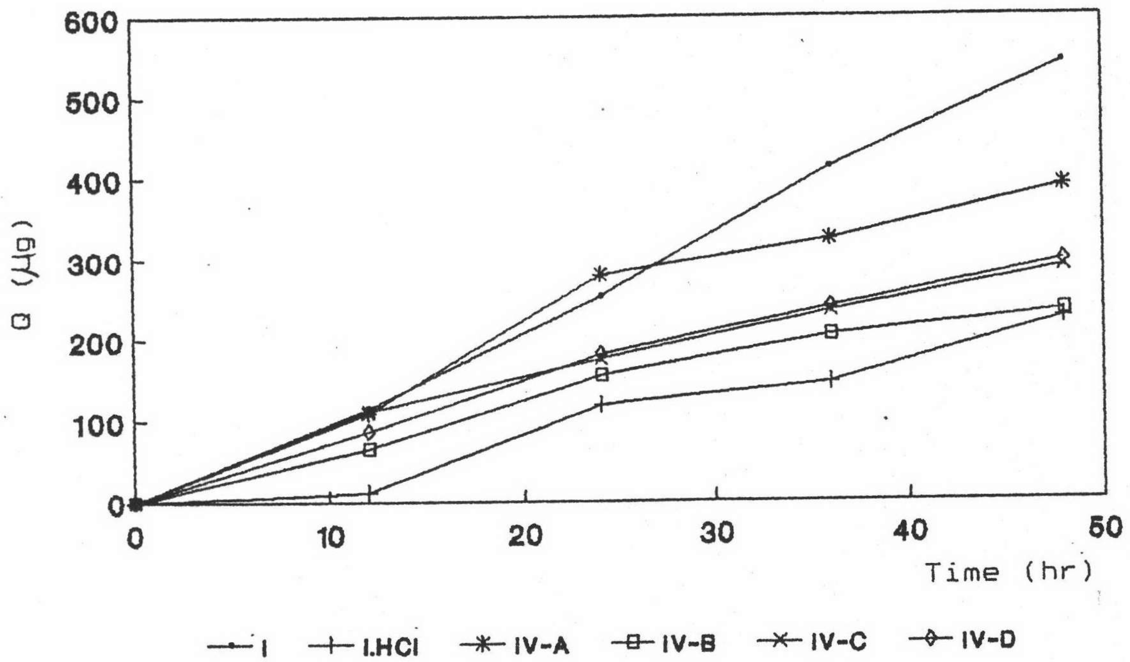


Figure 31 Cumulative Amount (Q) of Test Compounds Permeated versus Time.

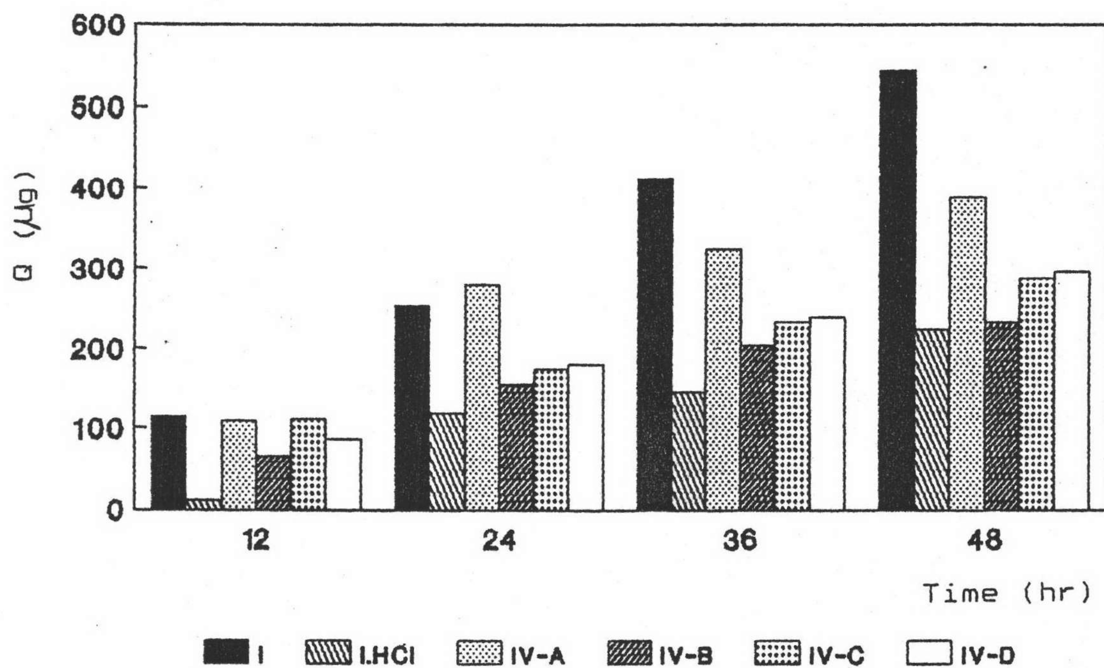


Figure 32 Cumulative Amount (Q) of Test Compounds Permeated versus Time.

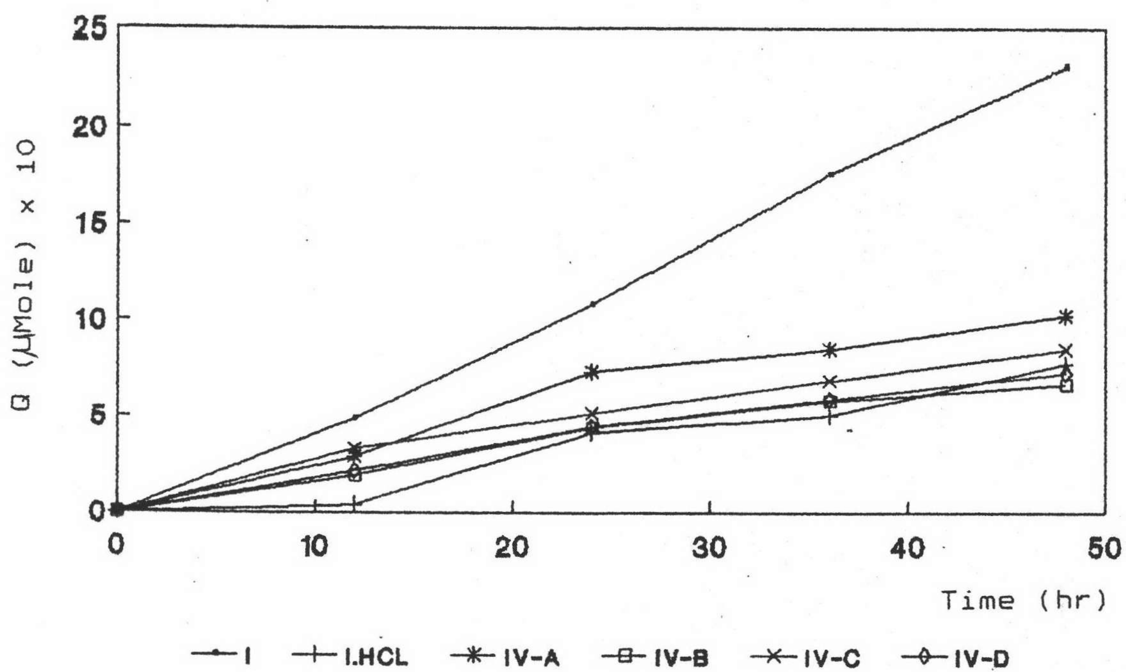


Figure 33 Cumulative Amount (Q) of Test Compounds Permeated versus Time.

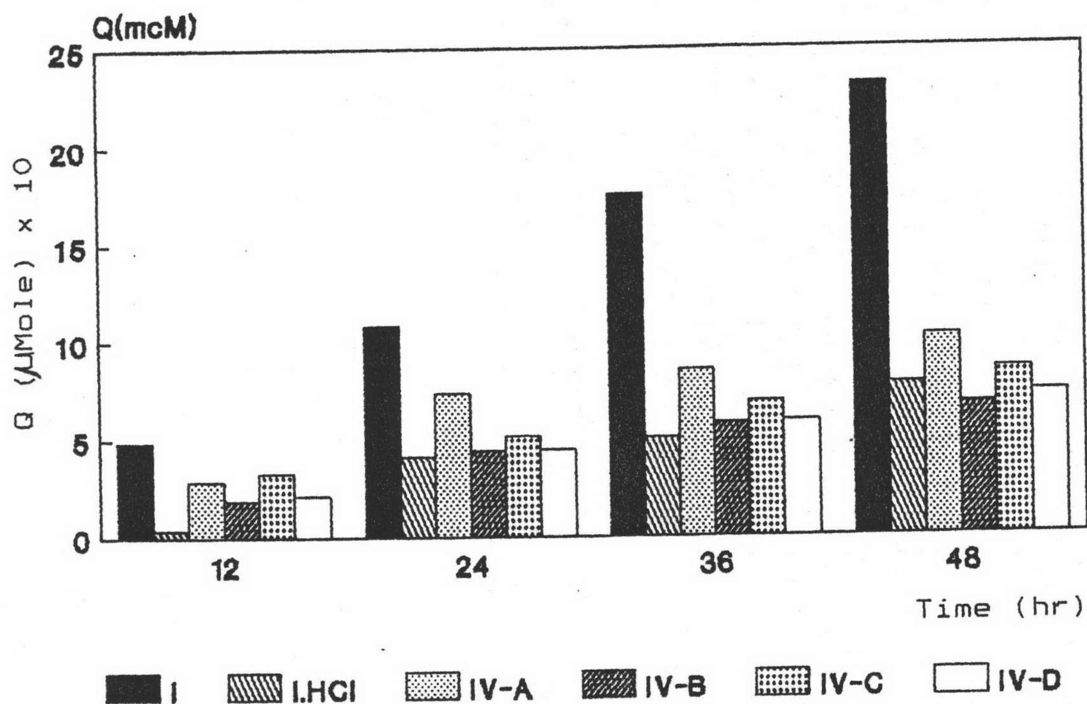


Figure 34 Cumulative Amount (Q) of Test Compounds Permeated versus Time.

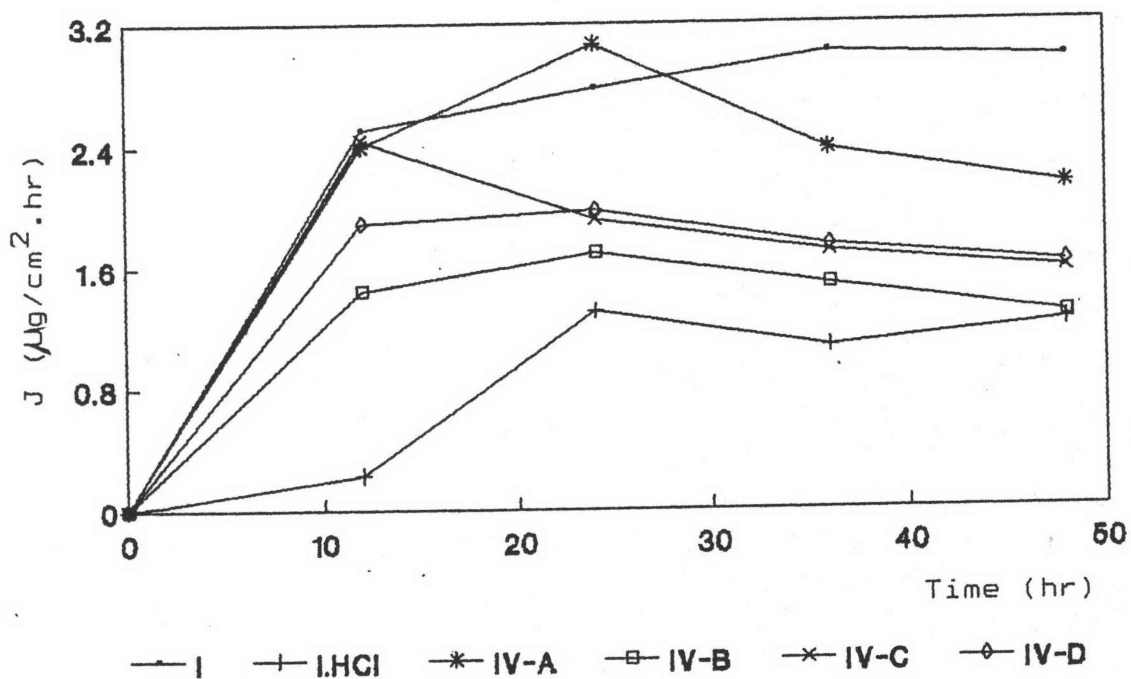


Figure 35 Flux (J) of Test Compounds versus Time.

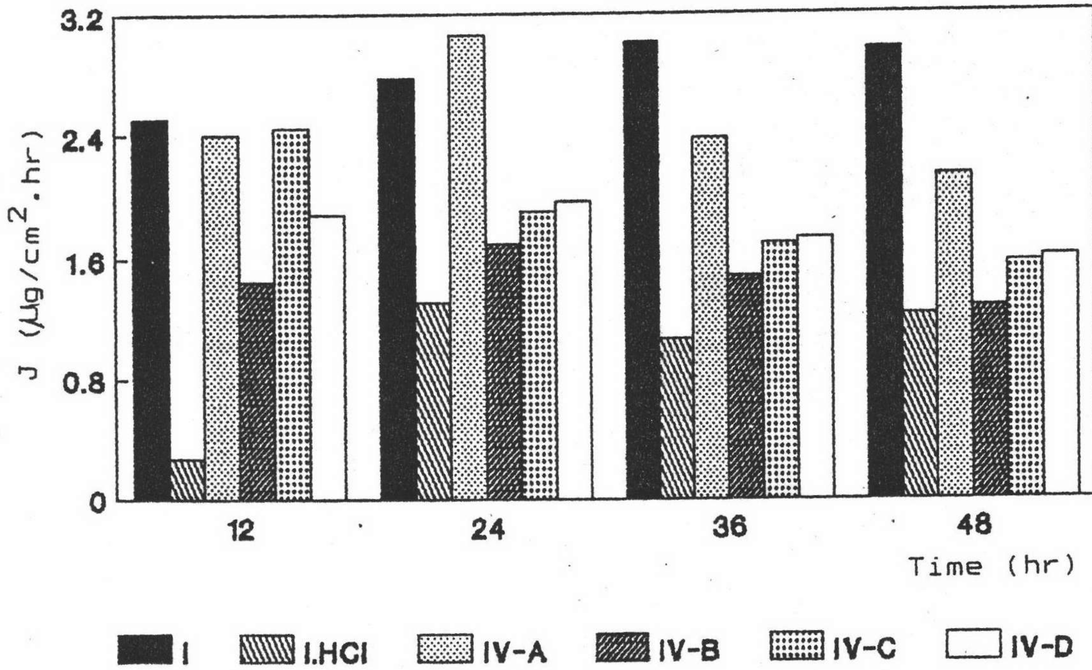


Figure 36 Flux (J) of Test Compounds versus Time.

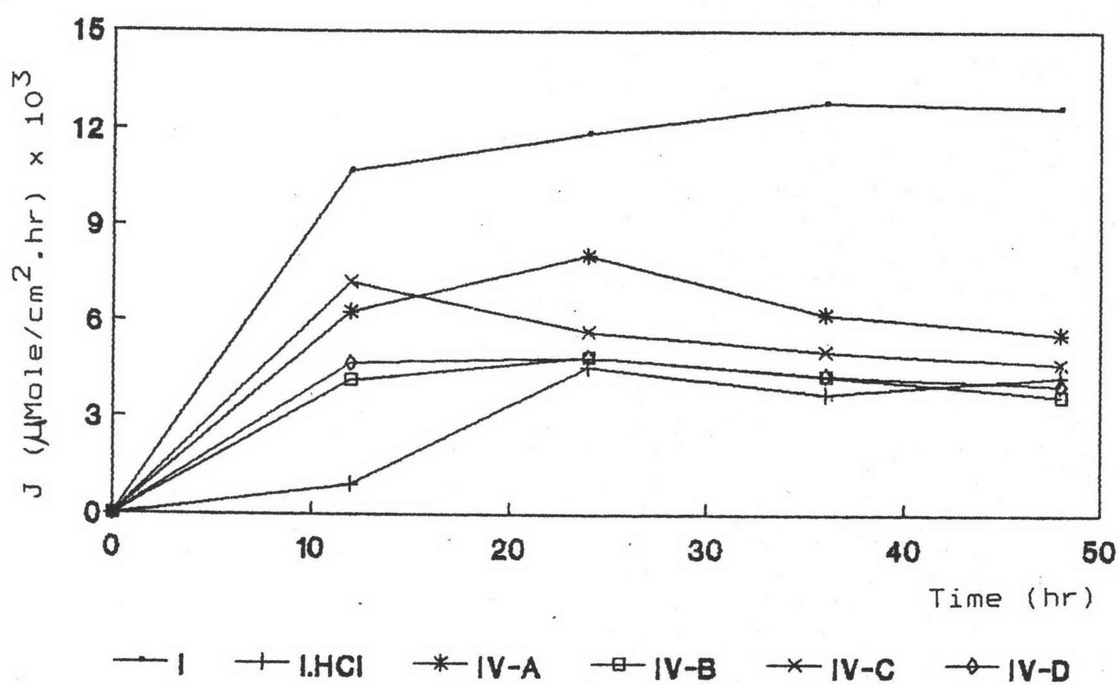


Figure 37 Flux (J) of Test Compounds versus Time.

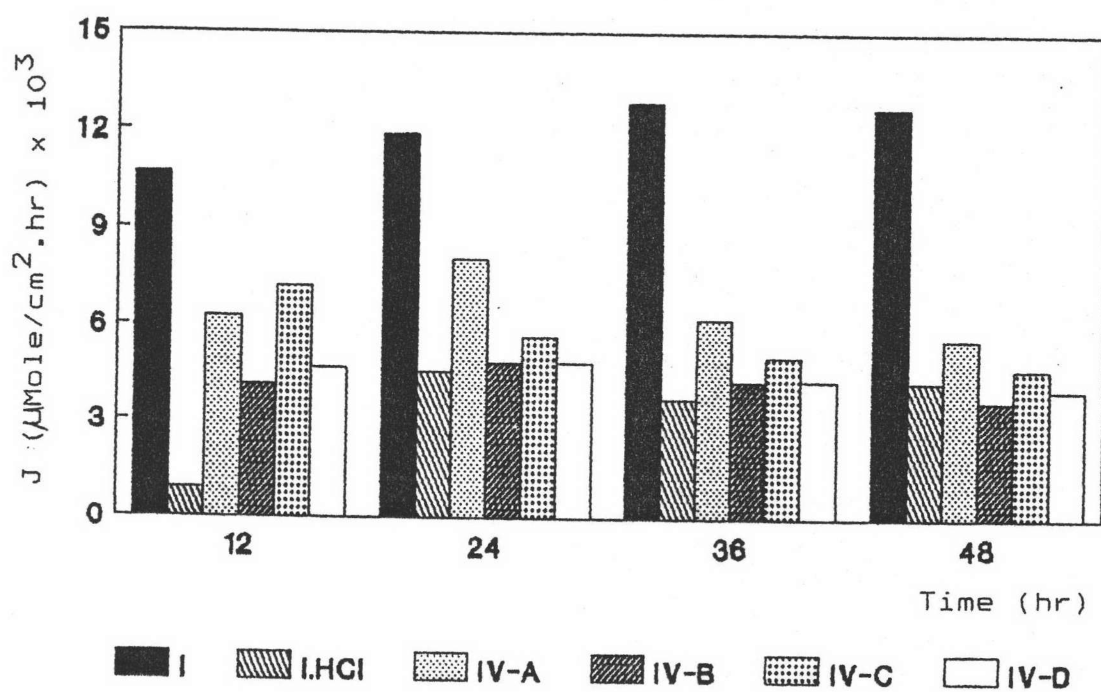


Figure 38 Flux (J) of Test Compounds versus Time.

Determination of Apparent Partition Coefficients.

A. Materials.

1. Equipments.

UV Spectrophotometer	Hitachi U-3200
Analytical balance	Sartorius 2842
pH Meter	Radiometer PHM 61
Mechanical shaker	Kotterman
Ultrasonic bath	Bransonic 321

2. Chemicals.

Test compounds[a].

- Lidocaine
- Lidocaine hydrochloride
- Lidocaine adipate
- Lidocaine maleate
- Lidocaine malonate
- Lidocaine tosylate

Monobasic sodium phosphate,AR	May & Baker
Dibasic sodium phosphate,AR	Mallinckrodt
Sodium chloride,AR	E. Merck
Octanol,AR	E. Merck

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[a]The compounds were prepared as described in synthesis section A.

B. Methods and Results.

1. Solutions.

a) Monobasic sodium phosphate stock solution. NaH_2PO_4 (8.00 g) was dissolved in distilled water and diluted to 1000 mL volume.

b) Dibasic sodium phosphate stock solution. Na_2HPO_4 (9.47 g) was dissolved in distilled water and diluted to 1000 mL volume.

c) Isotonic phosphate buffer pH 7.4 (NF XIV). Sodium chloride (4.40 g) was added to the mixture of 200 mL of monobasic sodium phosphate stock solution and 800 mL of dibasic sodium phosphate stock solution. The prepared solution was mixed well, adjusted to pH 7.4 $\pm$ 0.1 with 10N sodium hydroxide or 18N phosphoric acid.

The prepared buffer was saturated with octanol by 24 hour stirring at room temperature (32°C) and standing for phase separation in a separatory funnel before using of either phase. The lower phase was octanol-saturated isotonic phosphate buffer pH 7.4 and the upper phase was buffer-saturated octanol.

d) Standard solution. Standard solutions (1 mg/mL) of test compounds; lidocaine (I), lidocaine hydrochloride (I.HCl), lidocaine adipate (IV-A), lidocaine maleate (IV-B), lidocaine malonate (IV-C), and lidocaine tosylate (IV-D) were prepared by the following procedures. Stock solution of I was prepared by dissolving 50.0 mg, accurately weighed in 1.0 mL of propylene glycol in a 25-mL beaker. The solution was transferred to a 50-ml volumetric flask with the aid of octanol-saturated isotonic phosphate buffer pH 7.4 and diluted to volume with the same buffer. Stock solutions of other test compounds; I.HCl, IV-A, IV-B, IV-C and IV-D were prepared by dissolving 50.0 mg, accurately weighed, of the test compounds in octanol-saturated isotonic phosphate buffer pH 7.4 in an individual 50-mL volumetric flask and diluting to volume with the same buffer.

The following volumes 1.0, 2.0, 5.0, 10.0 and 20.0 mL of stock solutions were individually pipetted into 50-mL volumetric flask and diluted to volume with octanol-saturated isotonic phosphate buffer pH 7.4 so that each flask contained a concentration of 0.020, 0.040, 0.100, 0.200 and 0.400 mg/mL, respectively. In addition 0.004 mg/mL solution of test compounds were prepared by diluting 1.0 mL of stock solutions with octanol-saturated isotonic phosphate buffer pH 7.4 to 250.0 mL volume.

e) Test solutions. Solutions of test compounds were prepared at concentrations which resulted in suitable absorbances of the buffer phase before and after the distribution equilibrium. I (5.0 mg/mL) were prepared by dissolving 50.0 mg of I, accurately weighed, in buffer-saturated octanol in a 10-mL volumetric flask and diluting to volume with the same solvent. Test solution of other test compounds; I.HCl, IV-A, IV-B, IV-C and IV-D, were prepared by dissolving 30.0, 34.0, 30.0, 30.0 and 21.0 mg, accurately weighed, of test compounds, respectively, in octanol-saturated isotonic phosphate buffer pH 7.4 in an individual 50-mL volumetric flask and diluting to volume with the same solvent.

2. Analytical method.

UV spectrophotometer was employed. Serial dilutions of concentration 0.004, 0.020, 0.040, 0.100, 0.200 and 0.400 mg/mL of prepared standard dilutions were recorded in 1-cm quartz cells over the range of 200 - 300 nm. The spectrum was obtained at a band pass of 2 nm and a scanning speed of 60 nm/min. The recorder response was set at fast. Zero absorbance was adjusted by placing cuvettes filled with octanol-saturated isotonic phosphate buffer pH 7.4 in both reference and sample compartments. After adjustment, sample compartment was replaced with standard or

sample solution and measured. UV spectra of test compounds were obtained.

The wavelengths of maximum absorbance of each test compound were determined. The maximum absorbances of standard solutions of all test compounds being examined were found to be at wavelengths of 262.5 nm for I, I.HCl, IV-A and IV-C, 270.2 nm for IV-B and 261.4 nm for IV-D. UV absorbance spectra of serial standard solutions of test compounds were shown in Figure 39 - 41. The absorbances of the standard solutions versus concentrations were recorded in Table 18. Calibration curves between absorbances and concentrations of standard solutions were plotted (Figure 42). Each plotted indicated the relationship between absorbance and concentration was linear (R^2 = 0.9995 to 1.0000) and conformed to Beer-Lambert's Law. The regression parameters relating absorbance and concentration were shown in Table 19.

3. Procedure.

Test compounds : I, I.HCl, IV-A, IV-B, IV-C, and IV-D

The apparent partition coefficients (P) of the test compounds were determined in octanol-isotonic phosphate buffer pH 7.4 at room temperature (32°C). The suitable volumes of each phase were chosen so that

the absorbance of test compound in the aqueous phase (buffer), before and after distribution, could readily be measured using UV spectrophotometer. Octanol : buffer ratio were determined to eliminate saturation of test compound in either phase. The phase volume ratios used in the experiment were shown in Table 20. The octanol-buffer mixtures in glass-stoppered containers were shaken for 12 hours to reach a distribution equilibria. The mixtures were transferred to separatory funnels and stood to separate for at least two hours. The aqueous phase was separated and quantitated by spectrophotometry. The absorbances of test solutions before distribution were also recorded at the wavelengths of maximum absorbance of each test compound as described in determination of apparent partition coefficient section B2. Four determinations were performed for each test compound. Concentrations in mg/mL of the aqueous phase before and after distribution equilibrium were calculated by regression equations listed in Table 19. From the amount of test compound distributed in buffer phase, the apparent partition coefficient (P) of each test compound was determined from Equation 4. (Wells, 1988).

$$P = \frac{(C_i - C_w)}{C_w} \times \frac{V_w}{V_o} \qquad ------ (4)$$

where C_i = concentration of test compound in aqueous phase (buffer) before distribution (mg/mL)

C_w concentration of test compound in aqueous phase (buffer) after distribution (mg/mL)

$C_i - C_w$ = C_o = concentration of test compound in oil phase (octanol) after distribution (mg/mL)

V_w = volume of aqueous phase (buffer) in mL

V_o = volume of oil phase (octanol) in mL

Log P of each test compound was calculated and shown reproducible results. Data and results of the determination of P and log P of test compounds were summarized in Table 20.

Table 18 : Absorbance of standard solution of test compounds.[a]

Concentration[b] (mg/mL)	I		I.HCl		IV-A		IV-B		IV-C		IV-D	
	A[c] (262.5 nm)	%CV	A (262.5 nm)	%CV	A (262.5 nm)	%CV	A (270.2 nm)	%CV	A (262.5 nm)	%CV	A (261.4 nm)	%CV
0.004	0.0182	1.10	0.0095	1.82	0.0106	1.96	0.0166	1.74	0.0125	2.01	0.0100	2.00
0.020	0.0460	1.31	0.0283	1.62	0.0381	0.55	0.0344	0.34	0.0285	0.35	0.0481	0.67
0.040	0.0864	0.24	0.0552	1.00	0.0629	0.95	0.0635	0.40	0.0577	1.23	0.0889	1.30
0.100	0.2029	0.43	0.1273	0.29	0.1307	0.78	0.1513	0.33	0.1402	0.42	0.2088	0.42
0.200	0.4025	0.41	0.2532	0.28	0.2508	0.41	0.2888	0.55	0.2741	0.39	0.3980	0.86
0.400	0.7908	0.19	0.5005	0.31	0.4807	0.50	0.5635	0.04	0.5402	0.14	0.7797	0.22

[a]Average of three determinations, test compounds are I (lidocaine), I.HCl (lidocaine hydrochloride), IV-A (lidocaine adipate), IV-B (lidocaine maleate), IV-C (lidocaine malonate) and IV-D (lidocaine tosylate).

[b]The solvent is octanol-saturated isotonic phosphate buffer pH 7.4.

[c]Absorbance.

Table 19 : Regression parameters of absorbance mode[a].

Test Compound[b]	Regression Parameter[c]		R^2
	m (Slope)	z (Intercept)	
I	1.95765	0.008531	1.0000
I.HCl	1.24065	0.004357	1.0000
IV-A	1.175175	0.012644	0.9995
IV-B	1.387536	0.009681	0.9999
IV-C	1.340007	0.004916	0.9999
IV-D	1.931864	0.009587	0.9998

[a]Three determinations of six concentration levels; 0.004, 0.020, 0.040, 0.100, 0.200 and 0.400 mg/mL.

[b]I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[c]$Y = mX + Z$, Y and X represent absorbance and concentration (mg/mL) respectively.

Table 20 : Apparent partition coefficient (P) of test compounds.

Test Compound[a]	o/w Ratio[b]	P[c]	%CV	log P	%CV
I	1 : 20	1918.90	0.49	3.28	0.07
I.HCl	1 : 20	64.85	2.03	1.81	0.59
IV-A	1 : 20	62.63	0.55	1.80	0.15
IV-B	1 : 20	25.96	0.62	1.41	0.20
IV-C	1 : 20	58.85	1.04	1.77	0.24
IV-D	10 : 10	1.28	0.60	0.11	2.45

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[b] Volume ratio of octanol phase and isotonic phosphate buffer phase.

[c] Average of four determinations.

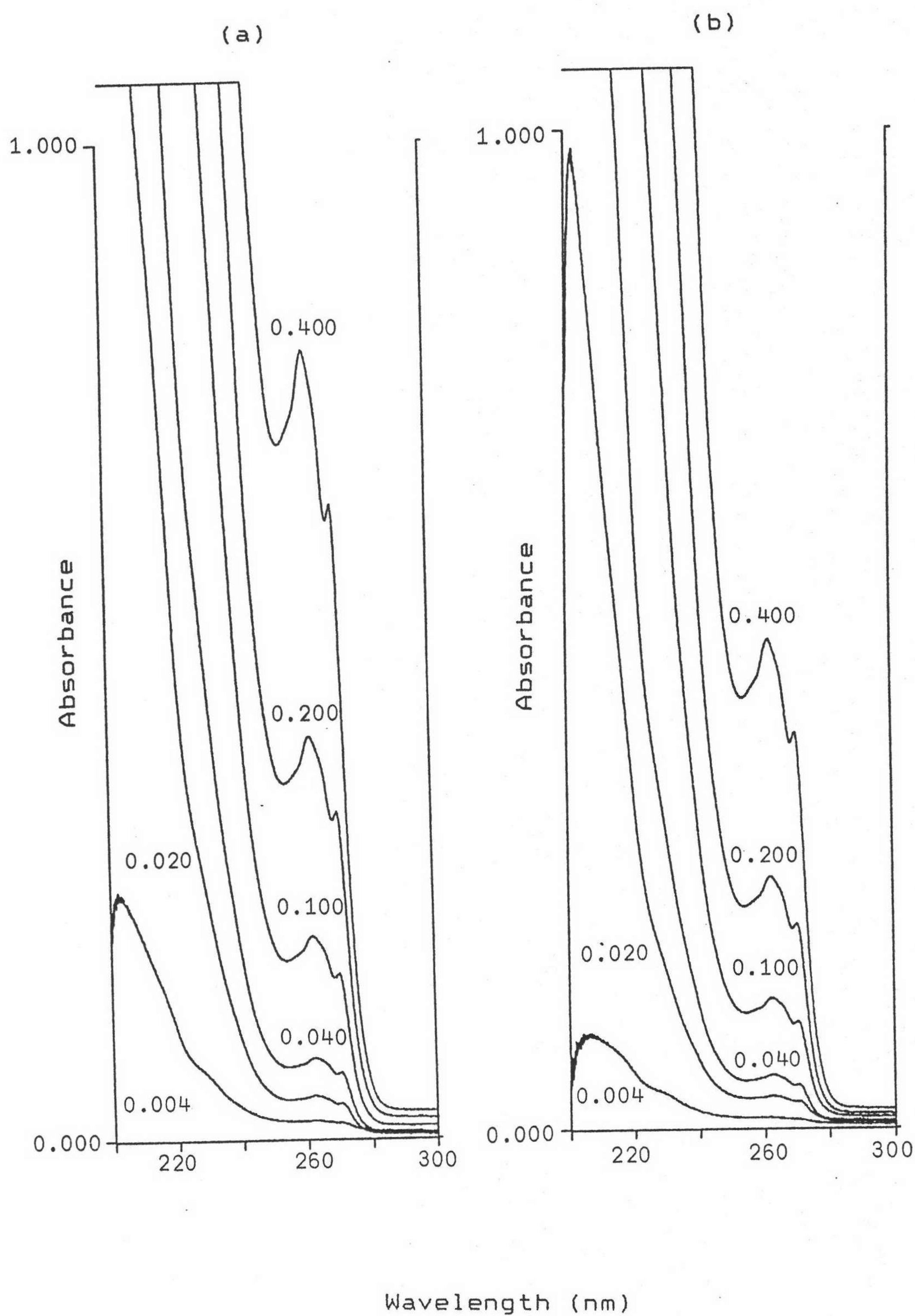

Figure 39 Ultraviolet Absorption Spectra of Lidocaine (a) and Lidocaine Adipate (b) in Octanol-saturated Isotonic Phosphate Buffer pH 7.4.

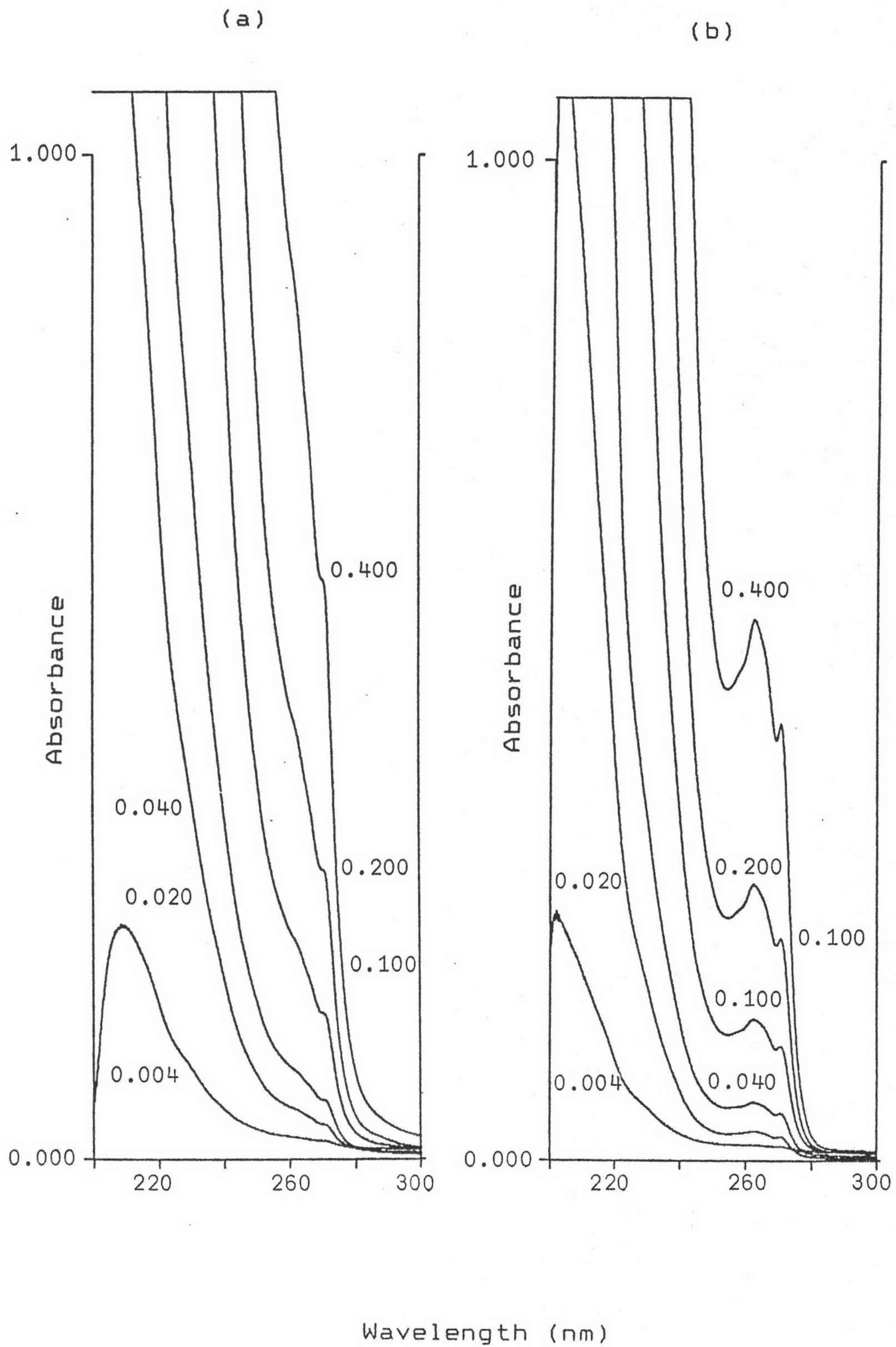

Figure 40 Ultraviolet Absorption Spectra of Lidocaine Maleate (a) and Lidocaine Malonate (b) in Octanol-saturated Isotonic Phosphate Buffer pH 7.4.

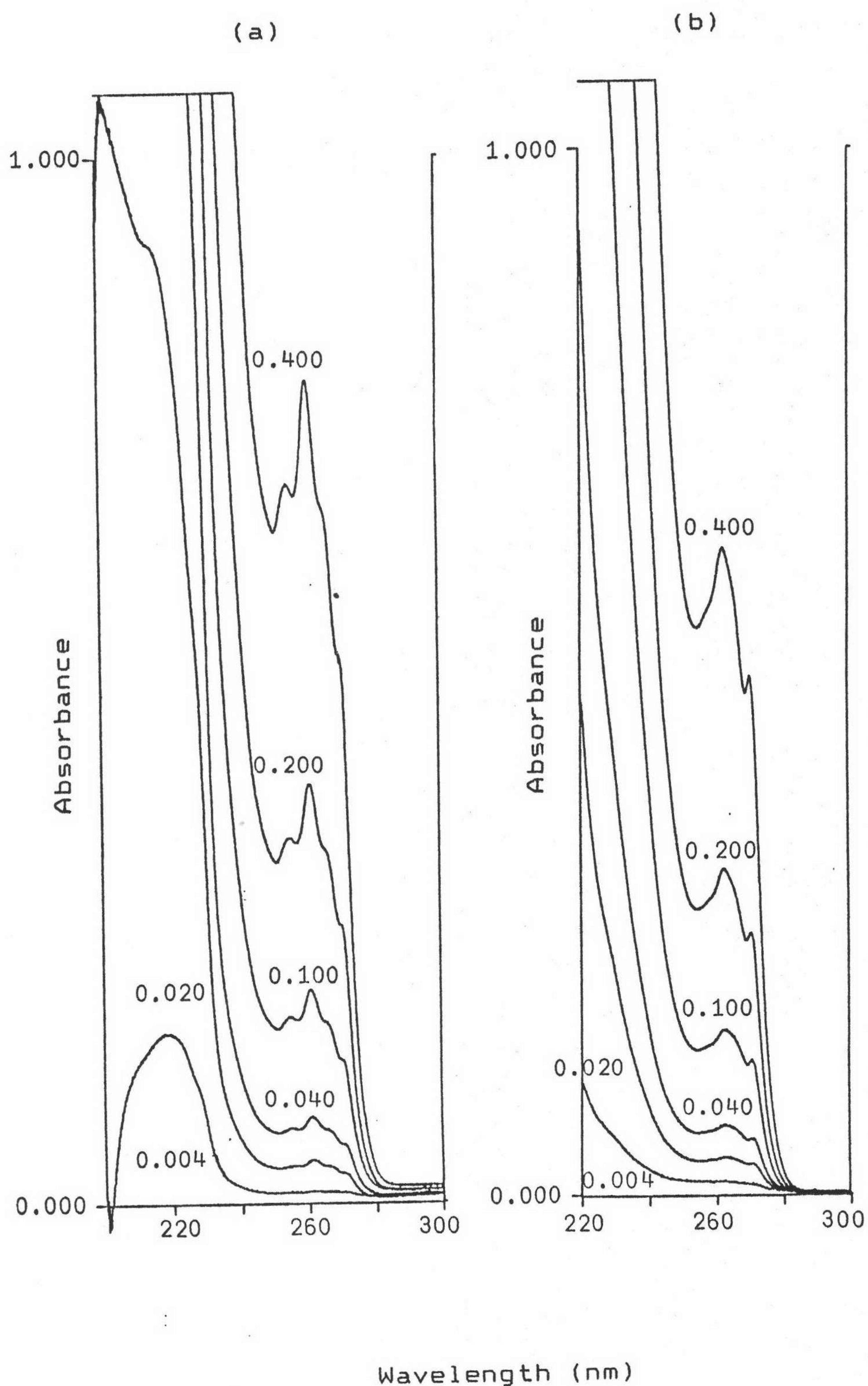

Figure 41 Ultraviolet Absorption Spectra of Lidocaine Tosylate (a) and Lidocaine Hydrochloride (b) in Octanol-saturated Isotonic Phosphate Buffer pH 7.4.

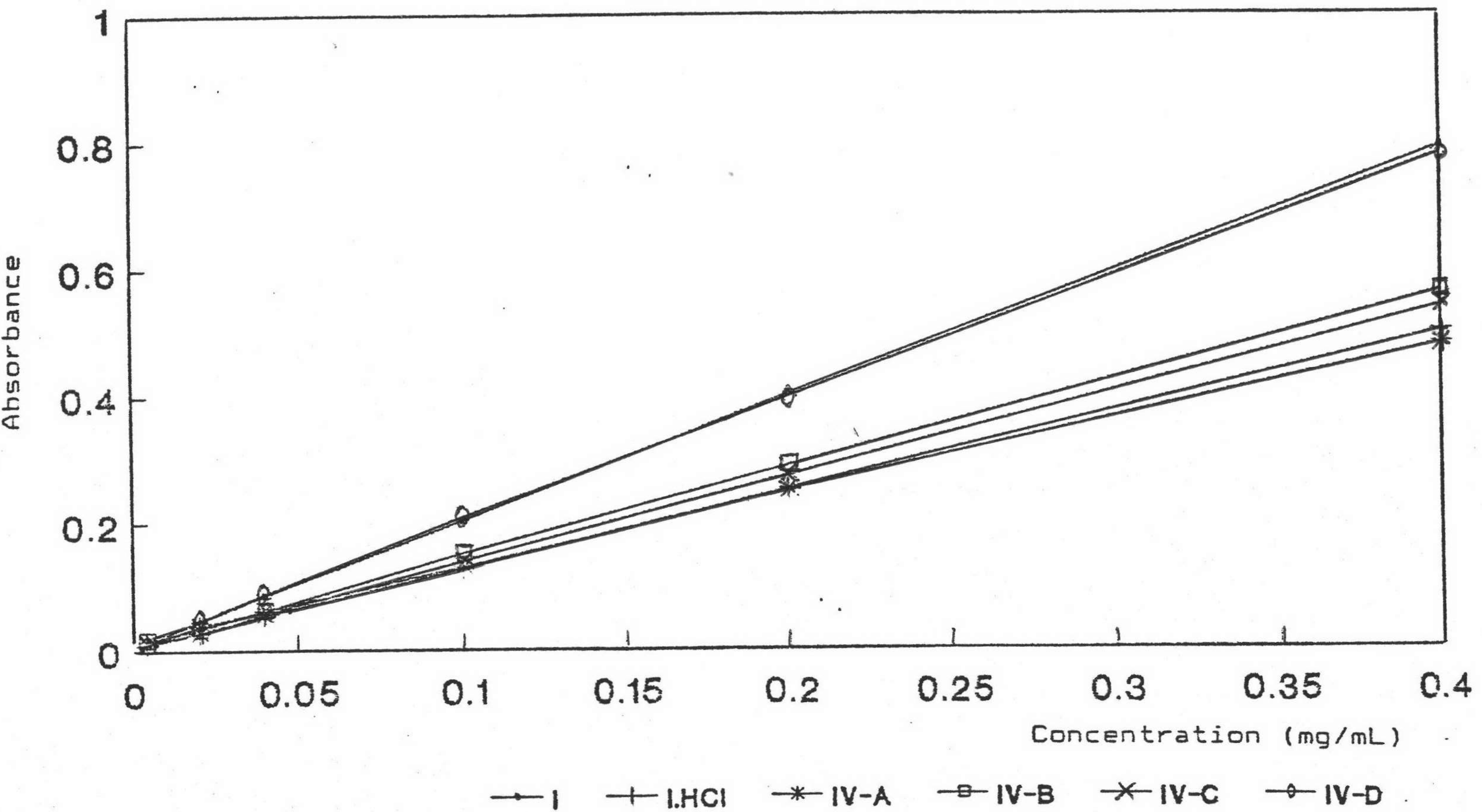

Figure 42 Calibration Curve of Test Compounds.