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Experimental Diclofenac Emulgel

	Ingredients	Amount	
1	Diclofenac Diethylammonium	2.32	gm
2	Propylene Glycol	10	gm
3	Tween60	1.048	gm
4	Span40	0.952	gm
5	Carbopol 934	2.4	gm
6	Triethanolamine	3	gm
7	Liquid Paraffin	3	gm
8	Isopropyl Alcohol	30	gm
9	Lavender Oil	0.7	gm
10	Nerolt Oil	0.7	gm
11	Purified Water	145.88	gm

Procedure

1. Dissolve diclofenac diethylammonium in isopropyl alcohol and propylene glycol
2. 60 ml of purified water was added and then stirred with magnetic stirrer
3. Carbopol 934 was added and dispersed
4. Add the triethanolamine and then stirred
5. Prepare of Emulsion
   - 5.1 1.048 gm.of tween60 was added in 86 ml of distilled water and then heat at 70 °C
   - 5.2 3 gm of liquid paraffin with 0.952 ml span 40 was added and then heat at 75 °C
   - 5.3 mix 5.1 and 5.2 until the emulsion was occured.
6. Mix the solution from 4. with 88.86 gm of emulsion
7. Adjust the emusion(from 6) with purified water to 200 gm

Appendix II

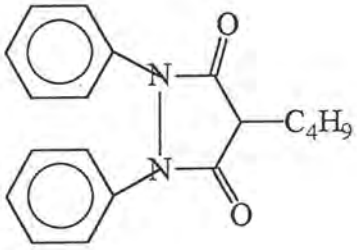
Details of Diclofenac Diethylammonium and Phenylbutazone

PHENYLBUTAZONE

(Connors et al, 1986 ; Ali, 1982)

Phenylbutazone was the first nonsteroidal anti-inflammatory agent marketed in the United States (1952) following the introduction of aspirin 40 years earlier.

Formula



Molecular formula	$C_{19}H_{20}N_2O_2$
Molecular weight	308.38
Appearance	

A white or almost white, crystalline powder, practically odorless, with a slighlty bitter taste.

Melting point	104-107 ^{0}C
Solubility	

Shows the solubility of phenylbutazone in various solvents as one part per specified parts of solvent.

Solubility of phenylbutazone at 20 ^{0}C.

Solvent	Solubility (parts)
Water	Insoluble
Ethanol	28
Methanol	18
Ether	15
Chloroform	1.3
Acetone	2.5
Benzene	3.5

Dissociation constant

Phenylbutazone has an acidic hydrogen at C-4. The pKa values between 4.5 and of 4.7 and of 4.89 have been reported. The influence of solvent on pKa values of phenylbutazone has been reported. The pKa values in ethanol, methanol and water were found to be 5.76, 5.42 and 5.07, respectively.

Stability

Aqueous decomposition of phenylbutazone occurs by hydrolysis and oxidation. The hydrolysis rate of phenylbutazone and the position of equilibrium depend on the solvent but practically not on the pH.

Stability studies of phenylbutazone solid dosage forms have revealed no evidence of instability under conditions of ambient temperature of 37^{0}C and 37^{0}C with 75% relative humidity. However at

60^0C, a significant degradation occured. Chemical degradation appears to be more significant with antacid preparations of

phenylbutazone. PEG 6000 and urea in solid dispersions acceleraterate the degradation of phenylbutazone. Temperature sppears to have a greater effect on the stability of phenylbutazone than does humidity

DICLOFENAC DIETHYLAMMONIUM

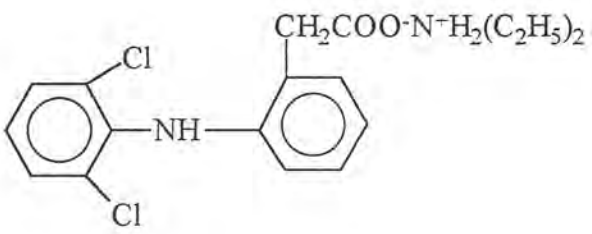
(Cocabo et al, 1992)

Trade name Voltaren emulgel (Ciba-Geigy/FE.Zuellig)

Chemical name :

Diethylammonium-{0-[(2,6-dichlorophenyl)-amino]-phenyl}-acetate

Formula



Molecular formula $C_{18}H_{22}Cl_2N_2O_2$

Molecular weight 396.30

Appearance

Diclofenac diethylammonium is the diethylamine salt of diclofenac. It is yellow crystalline powder. It melts and degrades at 154^0C.

Appendix I

Formula of Experimental Diclofenac Gel Product.

Solubility

It is soluble well in methanol, ethanol, chloroform, and acetone; sparingly soluble in acetic acid and not soluble in alkaline.

Pharmacological actions

Diclofenac emulgel is an anti-inflammatory and analgesic preparation designed for extemal application. It is contained in novel pharmaceutical dosage forms, a quantity of 1.17% diclofenac diethylammonium equivalent to 1% diclofenac sodium. The base is a fatty emulsion in an aqueous gel. The white creamy, non-greasy preparation can easily be rubbed into the skin, and thanks to its aqueous-alcoholic base. It exerts a soothing and cooling effet. 6% of the dose applied.

In patients with rheumatioid arthritis who had received repeated treatment with voltaren® emulgel, the concentrations measured in the region of the inflamed wrist, both experimental studies in animals have shown that, when applied locally, the active substance penetrates the skin, accumulates in the underlying tissue, and combats both acute and chronic inflammatory reactions.

Phamacokinetics

When the emulgel is applied locally, the active substance is absorbed through the skin. Determined by reference to the urinary excretion of diclofenac and its hydorxylated metabolites, the amount of diclofenac absorbed following local application of voltaren emugel to the skin in

healthy subjets, as compared with oral administration of voltaren coated tablets, is equivalent to approximately in the synovial fluid and in synovial tissue removed during surgery, proved to be higher than the plasma comcentrations. This finding confirms that diclofenac penetrates into the inflamed zone following local application of voltaren® emulgel.

Indications

For the local treatment of traumatic inflammation of the tendons, ligaments, muscles and joints e.g. due to sprains, strains and bruises ; localised forms of soft-tissue rheumatism e.g tendovaginitis, shoulder-hand syndrome and buritis, localised rheumatic diseases e.g. osteoarthrosis of peripheral joints and of the vertebral column, periarthropathy.

Dosage and administration

Depending on the size of the painful site to be treated, apply 2-4 g of the emulgel 3-4 times daily of the affected parts and rub-in gently.

Appendix III

Data of Percent Labelled Amount of Diclofenac diethylammonium in Four Commercial Products

Assay of Percent Labelled Amount

Standard Solution

	Abs1	Abs2
Standard solution conc.23.2 μg/ml	0.689	0.691
Average abs.	0.690	

Product	Gel weight (gm.)	conc.(μg/ml) from weight	Abs.	conc.(μg/ml.) from assay	%LA	$\overline{X}$
A1	2.066	23.97	0.701	23.37	98.37	99.01
A2	1.998	23.18	0.687	23.10	99.65	
B1	2.004	23.25	0.670	22.56	97.03	97.29
B2	2.006	23.27	0.675	22.70	97.55	
C1	2.056	23.85	0.697	23.44	98.30	97.42
C2	2.015	23.37	0.671	22.56	96.53	
D1	2.100	24.36	0.709	23.84	97.87	97.65
D2	2.005	23.26	0.674	22.66	97.43	

Appendix IV

Data on the Release of the Four Diclenac Gel Products through Cellulose acetate and Durapore® membranes.

Product A (Diclofenac diethylammonium 1.16%) release through cellulose acetate membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.010	2.020	4.040	8.080	12.12	16.16
PAR	0.1122	0.3465	0.8162	1.7553	3.6331	5.5102	7.3887

$$Y = - 0.1225 + 0.4648x$$

$$r^2 = 0.9999$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	5.1930	328.7880	4.8645	505.7864	6.6459	415.0160
0.50	2.6791	173.2917	2.7888	178.5113	2.7669	177.1685
1.00	2.9609	190.7223	2.4178	155.7628	2.5186	161.9435
1.50	1.7337	114.8144	1.9608	127.7410	1.7323	114.1475
2.00	1.4792	99.0725	1.7789	116.5876	1.6178	107.1009
2.50	1.4554	97.6003	1.6706	109.9470	1.9581	127.5755
3.00	1.9728	129.6039	1.8844	123.0565	2.2303	144.2660
4.00	3.0452	195.9367	3.9559	250.0741	3.6166	229.2693
5.00	4.0097	255.5768	3.2732	209.2862	3.9105	247.2902
6.00	4.4484	282.7313	4.3361	273.3866	3.5316	224.0573
Receptor Volume(ml)	5.75		6.70		5.70	

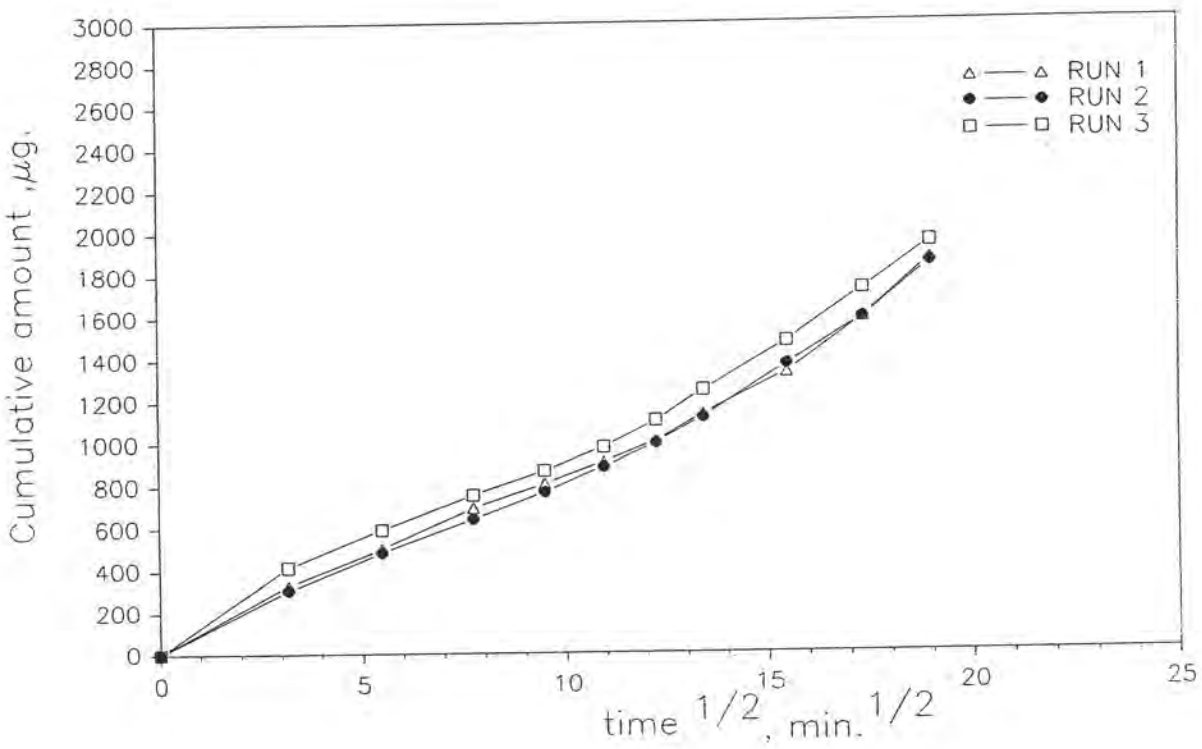
Dilution factor = 5

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Squre root of time (hr.)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
0.16	3.16	328.79	305.79	415.02
0.50	5.48	502.08	484.30	592.18
1.00	7.75	692.80	640.06	754.13
1.50	9.49	807.62	767.80	868.28
2.00	10.95	906.69	884.39	975.38
2.50	12.25	1004.29	994.34	1102.95
3.00	13.42	1133.89	1117.39	1247.22
4.00	15.49	1329.83	1367.47	1476.49
5.00	17.32	1585.41	1586.75	1723.78
6.00	18.97	1868.14	1850.14	1947.83
Release rate ($\mu g/min^{1/2}$)		97.15	99.98	101.06
r^2		0.9720	0.9781	0.9789
Membrane Thickness (cm.)		0.016	0.016	0.016

Average release rate = 99.40 $\mu g/min^{1/2}$

%CV = 2.03

Release profile of four diclofenac diethylammonium gel product through cellulose acetate membrane : Product A



Product B (Diclofenac diethylammonium 1.16%) permeate through cellulose acetate membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.010	2.020	4.040	8.080	12.120	24.240
PAR	0.0660	0.1239	0.2699	0.6117	1.5208	2.2407	4.5360

$$Y = - 0.0789 + 0.1907x$$

$$r^2 = 0.9991$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	1.2853	205.6673	1.4591	229.8532	0.9621	166.4945
0.50	1.2412	199.0187	1.2474	198.2147	1.3442	227.6064
1.00	1.0887	176.0278	0.8107	132.9502	0.9857	170.2690
1.50	0.7395	122.3094	1.0929	175.1248	0.9251	160.5768
2.00	0.7442	124.0908	0.5866	99.4586	0.6920	123.2955
2.50	0.7545	125.6437	0.7907	129.9612	0.5707	103.8951
3.00	0.5111	88.9486	0.6419	107.7231	0.4679	87.4536
4.00	0.9934	161.6603	1.0830	173.6453	1.5778	264.9678
5.00	0.7375	122.0105	1.3998	220.9908	1.3304	225.3993
6.00	1.2791	204.7326	0.4958	85.8886	0.3459	67.9413
Receptor Volume(ml)	5.75		5.7		6.1	

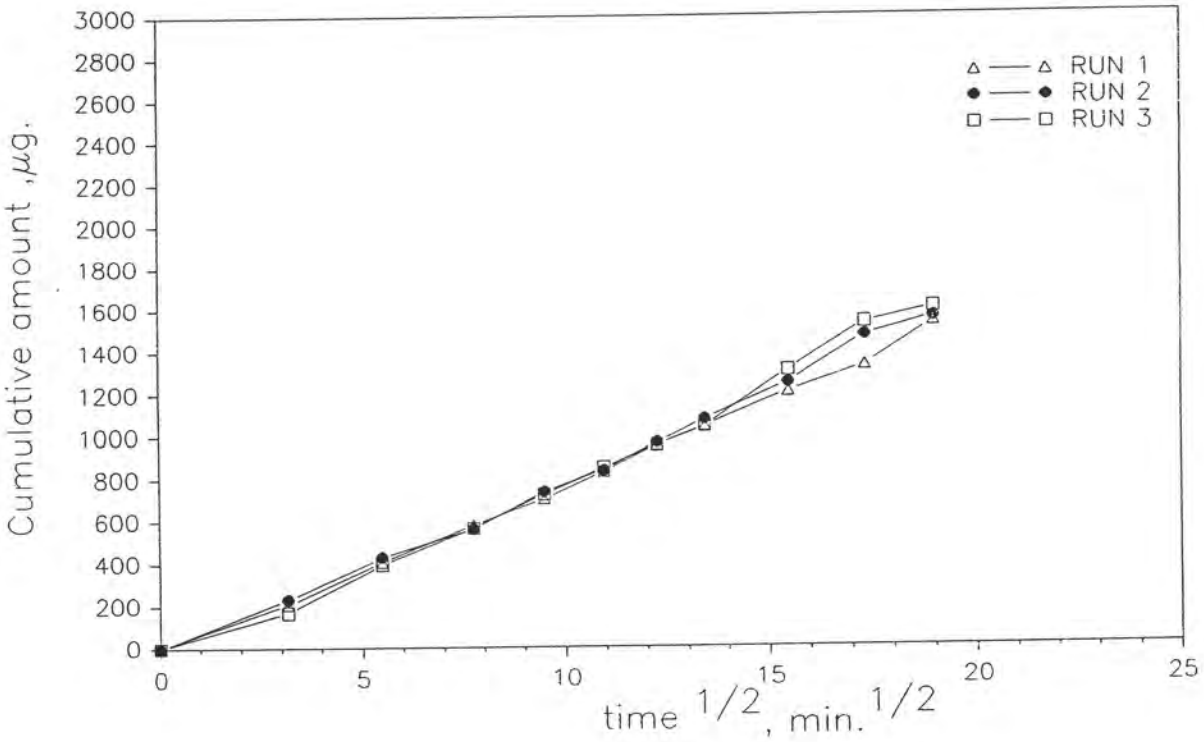
Dilution factor = 5

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Square root of Time ($min^{1/2}$)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
0.16	3.16	205.67	229.85	166.49
0.50	5.48	404.69	428.06	394.10
1.00	7.75	580.72	561.01	564.37
1.50	9.45	703.03	736.13	724.95
2.00	10.95	827.12	835.59	848.25
2.50	12.25	952.76	965.55	952.15
3.00	13.42	1041.71	1073.27	1039.60
4.00	15.49	1203.37	1246.92	1304.57
5.00	17.32	1325.38	1467.91	1529.97
6.00	18.97	1530.11	1553.80	1597.91
Release rate ($\mu g/min^{1/2}$)		81.77	85.59	93.40
r^2		0.9970	0.9990	0.9916
Membrane Thickness(cm.)		0.016	0.016	0.016

Average release rate = 86.92 $\mu g/min^{1/2}$

%CV = 6.82

Release profile of four diclofenac diethylammonium gel product through cellulose acetate membrane : Product B



Product C (Diclofenac diethylammonium 1.16%) permeate through cellulose acetate membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.01	2.02	4.04	8.08	12.12	16.16
PAR	0.1790	0.4040	0.8536	1.7542	3.555	5.3544	7.1563

$$Y = - 0.0462 + 0.4457x$$

$$r^2 = 0.9999$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	6.0783	395.0625	6.5449	421.4637	6.0330	388.7305
0.50	3.0569	200.1663	3.8794	251.0200	3.8996	252.3116
1.00	2.9072	190.5099	3.0517	198.0932	2.9775	193.3486
1.50	2.0214	133.3700	2.3934	155.9987	2.8693	186.4298
2.00	2.4901	163.6047	2.3184	151.2028	2.0614	134.7691
2.50	2.1414	141.1117	2.2631	147.6667	1.8444	120.8932
3.00	2.1543	141.9439	2.2453	146.5285	1.9531	127.8440
4.00	2.8653	187.8071	2.4562	160.0144	3.4530	223.7541
5.00	3.2074	209.8744	2.9187	189.5886	3.1384	203.6372
6.00	3.2137	210.2807	2.8224	183.4308	3.1075	201.6613
Receptor Volume(ml)	5.75		5.7		5.7	

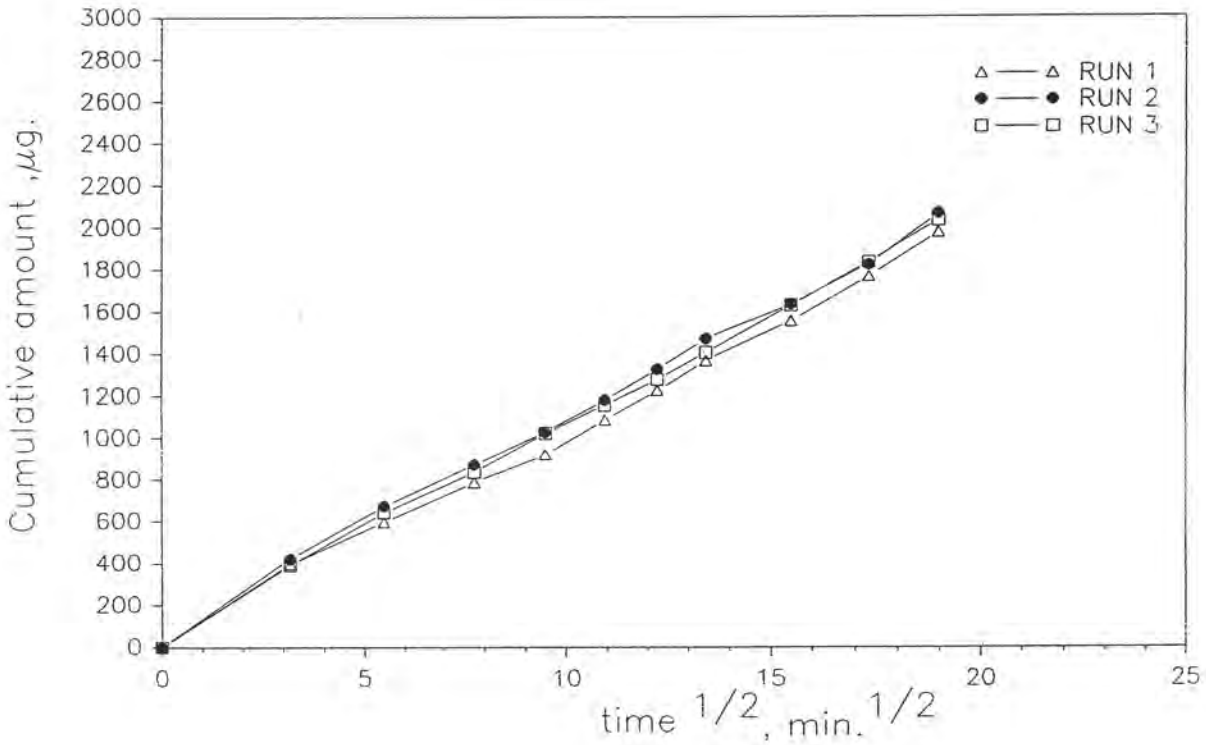
Dilution factor = 5

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Square root of Time ($min^{1/2}$)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
0.16	3.16	395.06	421.46	388.73
0.50	5.48	595.23	672.48	641.04
1.00	7.75	785.74	870.58	834.39
1.50	9.49	919.11	1026.58	1020.82
2.00	10.95	1082.71	1177.60	1155.59
2.50	12.25	1223.83	1325.27	1276.48
3.00	13.42	1365.77	1471.80	1404.33
4.00	15.49	1553.58	1631.81	1628.08
5.00	17.32	1763.45	1821.40	1831.72
6.00	18.97	1973.73	2064.83	2033.38
Release rate ($\mu g/min^{1/2}$)		102.74	102.13	103.22
r^2		0.9975	0.9960	0.9984
Membrane Thickness (cm.)		0.016	0.016	0.016

Average release rate = 102.70 $\mu g/min^{1/2}$

%CV = 0.54%

Release profile of four diclofenac diethylammonium gel product through cellulose acetate membrane : Product C



Product D (Diclofenac diethylammonium 1.16%) permeate through cellulose acetate membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.01	2.02	4.04	8.08	12.12	24.24
PAR	0.3226	0.6355	1.3320	1.6814	3.3082	5.0150	9.7448

$$Y = -0.2399 + 0.3914x$$

$$r^2 = 0.9980$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	3.9050[a]	107.6869	4.9316	136.6515	2.7788	73.9485
0.50	3.5787[a]	98.0996	2.9532	79.0282	3.6285	98.6971
1.00	3.3698[a]	91.9618	3.9181	107.1320	5.0163	139.1184
1.50	5.1145[a]	141.9786	6.0373	168.8563	5.9891	167.4524
2.00	3.6065[a]	98.9165	3.6873	100.4097	2.6965	71.5515
2.50	6.0260[a]	170.0055	3.0205	80.9883	4.3862	120.7660
3.00	4.6679[a]	128.9709	2.6394	69.8883	1.9030	48.4398
4.00	3.9043[b]	269.1658	3.1893	214.7621	5.1167	142.0427
5.00	4.1955[b]	290.5557	3.6668	249.5316	3.5531	96.5010
6.00	4.4579[b]	309.8301	4.6350	320.0316	5.4418	151.5117
Receptor Volume (ml)	5.75		5.7		5.7	

a : Diution factor = 2

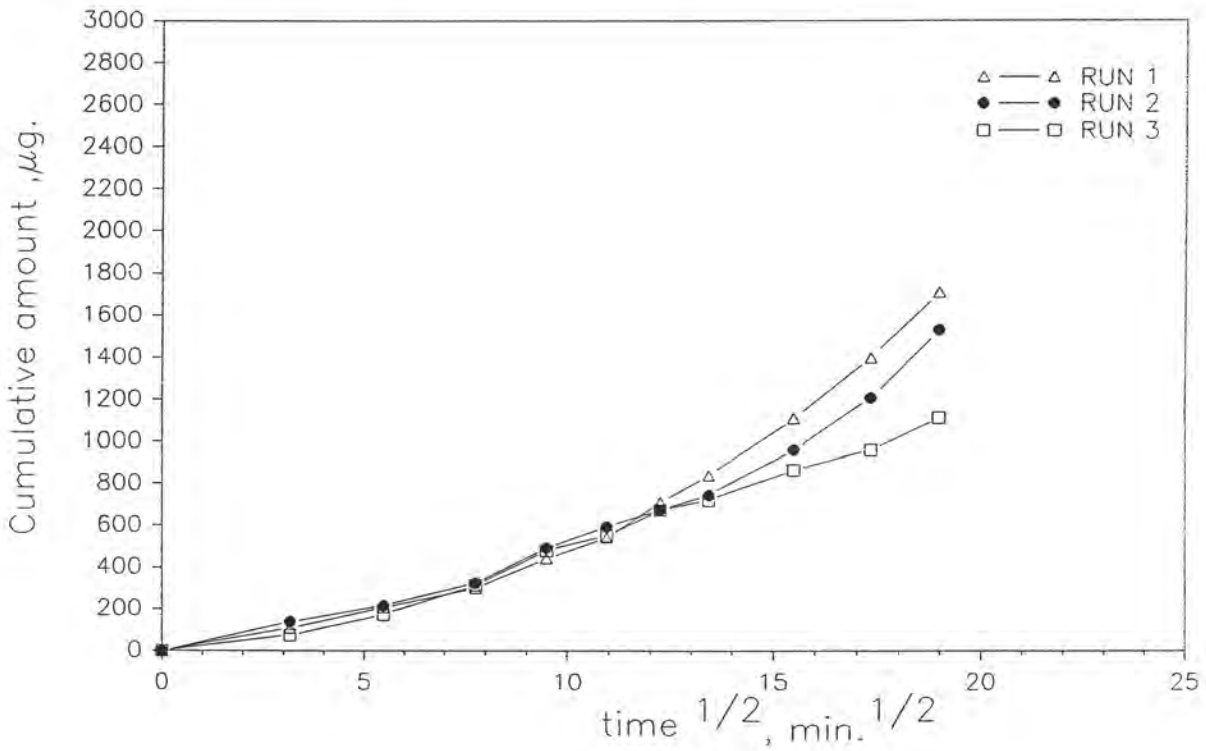
b : Dilution factor = 5

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Square root of Time ($min^{1/2}$)	Cumulation Amount (μg)	Cumulation Amount (μg)	Cumulation Amount (μg)
0.16	3.16	107.69	136.65	73.95
0.50	5.48	205.79	215.68	172.65
1.00	7.75	297.75	322.81	311.77
1.50	9.49	439.73	491.67	479.22
2.00	10.95	538.65	592.08	550.72
2.50	12.25	708.66	673.07	671.54
3.00	13.42	837.63	742.96	719.98
4.00	15.49	1106.80	957.72	862.02
5.00	17.32	1397.36	1207.25	958.52
6.00	18.97	1707.19	1527.28	1110.03
Release rate ($\mu g/min^{1/2}$)		280.50	225.87	148.41
r^2		0.9924	0.9883	0.9734
Membrane Thickness (cm.)		0.016	0.016	0.016

Average release rate = 91.58 $\mu g/min^{1/2}$

%CV = 21.53%

Releaseprofile of four diclofenac diethylammonium gel product through cellulose acetate membrane : Product D



Product A (Diclofenac diethylammonium 1.16%) release through Durapore® membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.010	2.020	4.040	8.080	12.12	32.32
PAR	0.2246	0.3788	0.6458	1.2567	3.2724	4.8896	13.2868

$$Y = 0.4149x - 0.1397$$

$$r^2 = 0.9991$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	1.5753	118.8389	2.4455	190.0424	2.1270	157.0683
0.50	1.5394	116.3512	1.5438	123.7569	1.5382	116.2681
1.00	1.7035	127.7223	1.4869	119.5741	2.0840	154.0886
1.50	1.3825	105.4790	1.3662	110.7013	1.5110	114.3833
2.00	1.4282	108.6458	1.2809	104.4307	1.1985	92.7290
2.50	1.2839	98.6467	1.0919	90.5370	1.3310	101.9104
3.00	1.1363	88.4189	1.0139	84.8031	1.2354	95.2859
4.00	1.5113	114.4041	1.4188	114.5680	1.8738	139.5231
5.00	1.9601	145.5031	1.4443	116.4425	1.7217	128.9835
6.00	1.7592	131.5820	1.1382	93.9406	1.4358	109.1724
Receptor Volume(ml)	5.75		6.10		5.75	

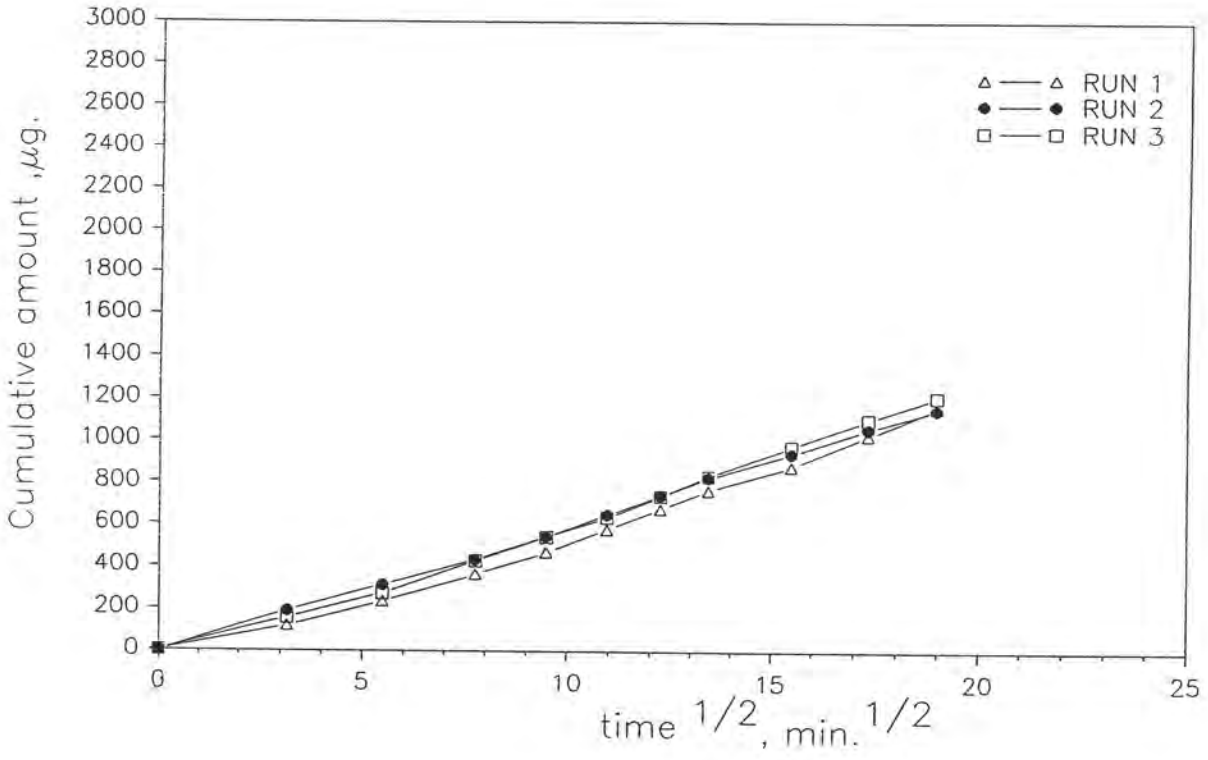
Diution factor = 5

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Square root of Time ($min^{1/2}$)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
0.16	3.16	118.84	190.04	157.07
0.50	5.48	235.19	313.80	273.34
1.00	7.75	362.91	433.37	427.43
1.50	9.49	468.39	544.07	541.81
2.00	10.95	577.04	648.50	634.54
2.50	12.25	675.69	739.04	736.45
3.00	13.42	764.11	823.84	831.74
4.00	15.49	878.51	938.41	971.26
5.00	17.32	1024.01	1054.85	1100.24
6.00	18.97	1155.59	1148.79	1209.41
Release rate ($\mu g/min^{1/2}$)		59.98	53.34	70.12
r^2		0.9983	0.9983	0.9993
Membrane Thickness (cm.)		0.016	0.016	0.016

Average release rate = 61.15 $\mu g/min^{1/2}$

%CV = 13.82%

Release profile of four diclofenac diethylammonium gel products through Durapore® membrane : Product A



Product B (Diclofenac diethylammonium 1.16%) permeate through Durapore® membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.01	2.02	4.04	8.08	12.12	24.24
PAR	0.1016	0.1524	0.3807	0.5263	1.8281	2.6393	5.5166

$$Y = 0.2291X - 0.0628$$

$$r^2 = 0.9995$$

Diffusion Run Data

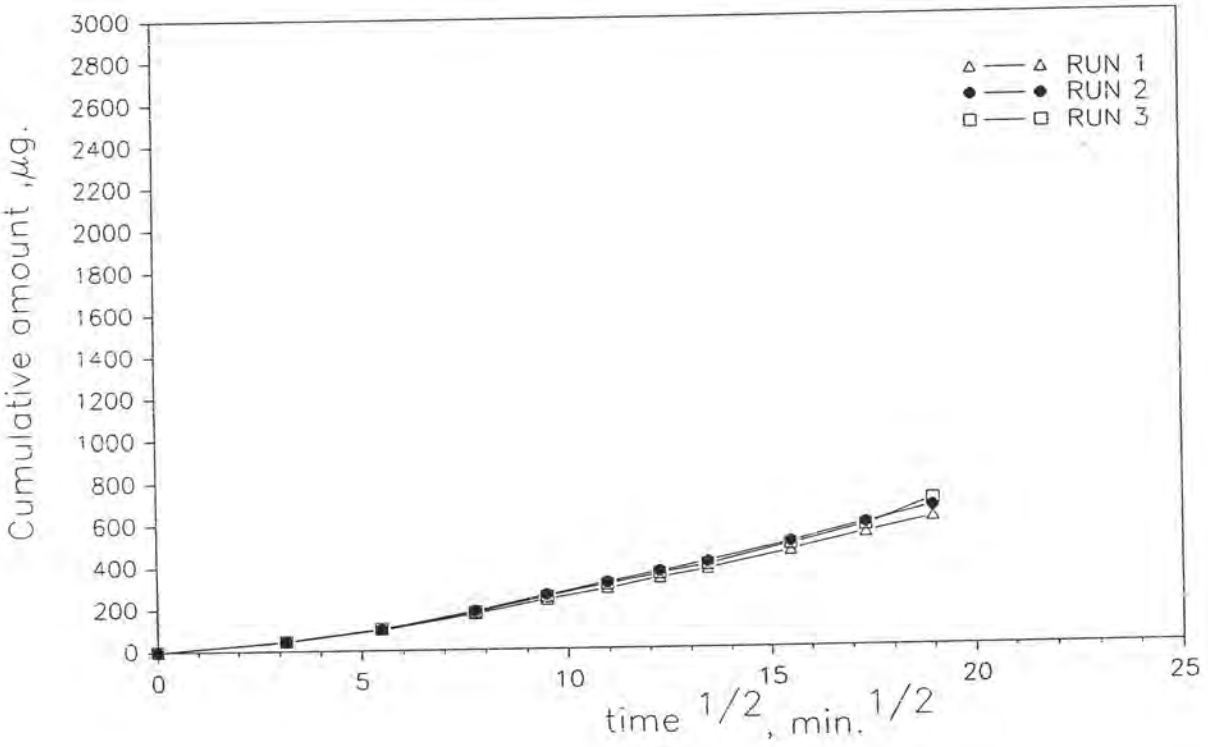
Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	0.2859	43.76	0.3059	45.87	0.3023	45.82
0.50	0.3565	52.62	0.3853	55.74	0.4031	58.47
1.00	0.5055	71.32	0.5939	81.69	0.5300	74.35
1.50	0.4636	66.06	0.5236	72.95	0.4926	69.70
2.00	0.3451	51.19	0.4024	57.87	0.3827	55.91
2.50	0.3065	46.34	0.3352	49.51	0.3183	47.82
3.00	0.2723	42.05	0.3029	45.49	0.2845	43.58
4.00	0.6103	84.47	0.6535	89.11	0.6699	91.95
5.00	0.5827	81.00	0.6447	88.01	0.6181	85.45
6.00	0.4937	69.84	0.5334	74.17	0.9014	121.00
Receptor Volume(ml)	5.75		5.70		5.75	

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Square root of Time ($min^{1/2}$)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
0.16	3.16	43.76	45.87	45.82
0.50	5.48	96.38	101.61	104.29
1.00	7.75	167.70	183.30	178.68
1.50	9.49	233.76	256.25	248.38
2.00	10.95	284.95	314.12	304.29
2.50	12.25	331.29	363.63	352.11
3.00	13.42	373.34	409.12	395.69
4.00	15.49	457.81	498.23	487.64
5.00	17.32	538.81	586.24	573.09
6.00	18.97	608.65	660.41	694.09
Release rate ($\mu g/min^{1/2}$)		38.18	41.44	42.39
r^2		0.9985	0.9984	0.9856
Membrane Thickness(cm.)		0.016	0.016	0.016

Average release rate = 40.67 $\mu g/min^{1/2}$

%CV = 5.43%

Release profile of four diclofenac diethylammonium gel products through Durapore® membrane : Product B



Product C (Diclofenac diethylammonium 1.16%) permeate through Durapore® membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.01	2.02	4.04	8.08	12.12	24.24
PAR	0.1200	0.2122	0.3880	0.9036	1.7990	2.6544	6.5350

$$Y = -0.1819 + 0.2670x$$

$$r^2 = 0.9906$$

Diffusion Run Data

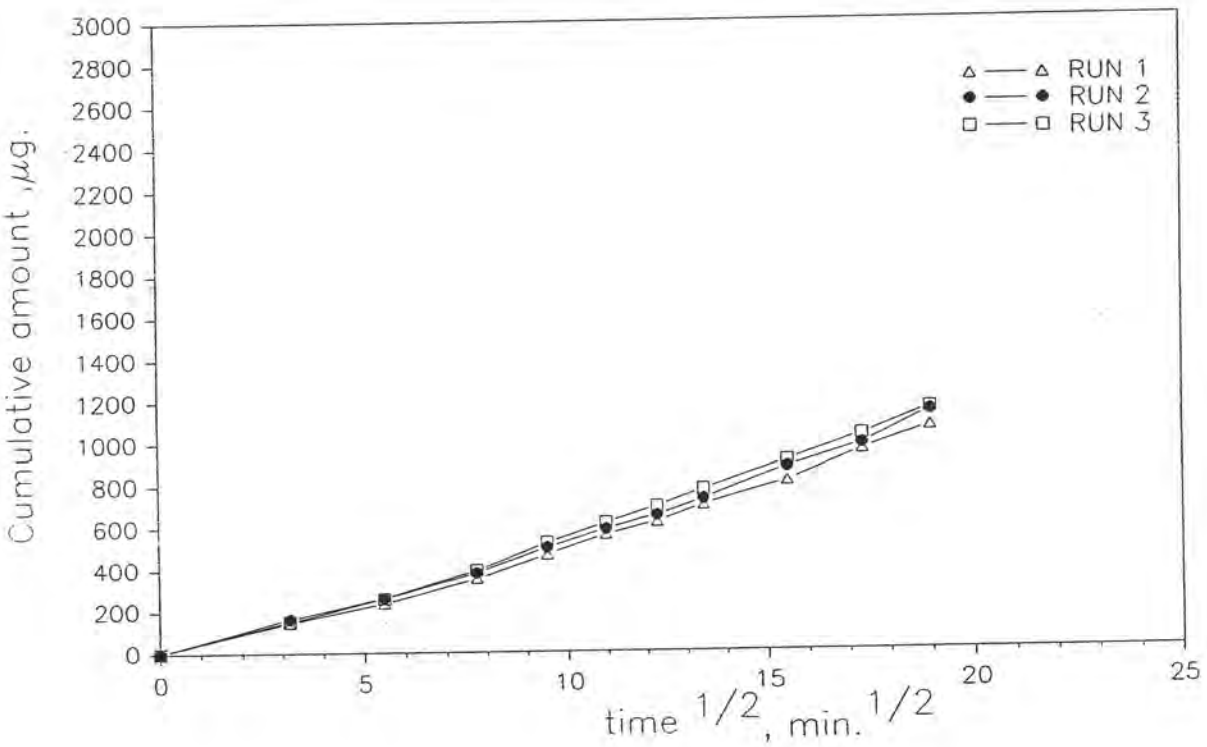
Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	1.1508	143.50	1.3468	163.18	1.1361	150.56
0.50	0.6628	90.96	0.7131	95.53	0.7634	107.98
1.00	0.9062	117.16	0.9469	120.49	0.9831	133.08
1.50	0.8857	114.96	0.9358	119.31	0.9699	131.57
2.00	0.6981	94.76	0.6427	88.02	0.6294	92.68
2.50	0.3563	57.95	0.4231	64.58	0.4974	77.60
3.00	0.5638	80.30	0.5287	75.85	0.5030	78.24
4.00	0.8282	108.77	1.2039	147.92	0.9810	132.84
5.00	1.1674	145.29	0.8157	106.49	0.9021	123.83
6.00	0.8401	110.05	1.2840	156.47	0.8922	122.70
Receptor Volume(ml)	5.75		5.7		6.10	

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Square root of Time ($min^{1/2}$)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
0.16	3.16	143.50	163.18	150.56
0.50	5.48	234.46	258.71	258.54
1.00	7.75	351.62	379.20	391.62
1.50	9.49	466.58	498.51	523.19
2.00	10.95	561.34	586.53	615.87
2.50	12.25	619.29	651.11	693.47
3.00	13.42	699.59	726.96	771.71
4.00	15.49	808.36	874.88	904.55
5.00	17.32	953.65	981.37	1028.38
6.00	18.97	1063.70	1137.84	1151.08
Release rate ($\mu g/min^{1/2}$)		61.36	64.17	66.03
r^2		0.9973	0.9953	0.9999
Membrane Thickness (cm.)		0.016	0.016	0.016

Average release rate = 63.85 $\mu g/min^{1/2}$

%CV = 3.68%

Release profile of four diclofenac diethylammonium gel products through Durapore® membrane : Product C



Product D (Diclofenac diethylammonium 1.16%) permeate through Durapore® membrane

Calibration Curve Date

conc (μg/ml)	0.505	1.01	2.02	4.04	8.08	12.12	24.24
PAR	0.0645	0.1013	0.3998	0.8232	1.5183	2.2422	4.2089

$$Y = 0.1752x + 0.0351$$

$$r^2 = 0.9971$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
0.16	0.7164	55.90	0.7308	56.59	0.7406	61.41
0.50	1.2489	99.59	1.3002	102.90	1.2965	109.80
1.00	1.3087	104.50	1.3147	104.08	1.3088	110.87
1.50	1.1897	94.73	1.3293	105.26	1.2609	106.70
2.00	1.1012	87.47	1.1733	92.58	0.9914	83.24
2.50	0.6311	48.90	0.8903	69.56	0.9345	78.29
3.00	0.6998	54.54	0.8710	67.99	0.8797	73.52
4.00	1.5529	124.53	1.5831	125.91	1.5440	131.34
5.00	1.4078	111.65	1.5263	121.29	1.4740	125.25
6.00	1.1271	89.60	1.3237	104.81	1.1869	100.26
Receptor Volume (ml)	5.75		5.7		6.1	

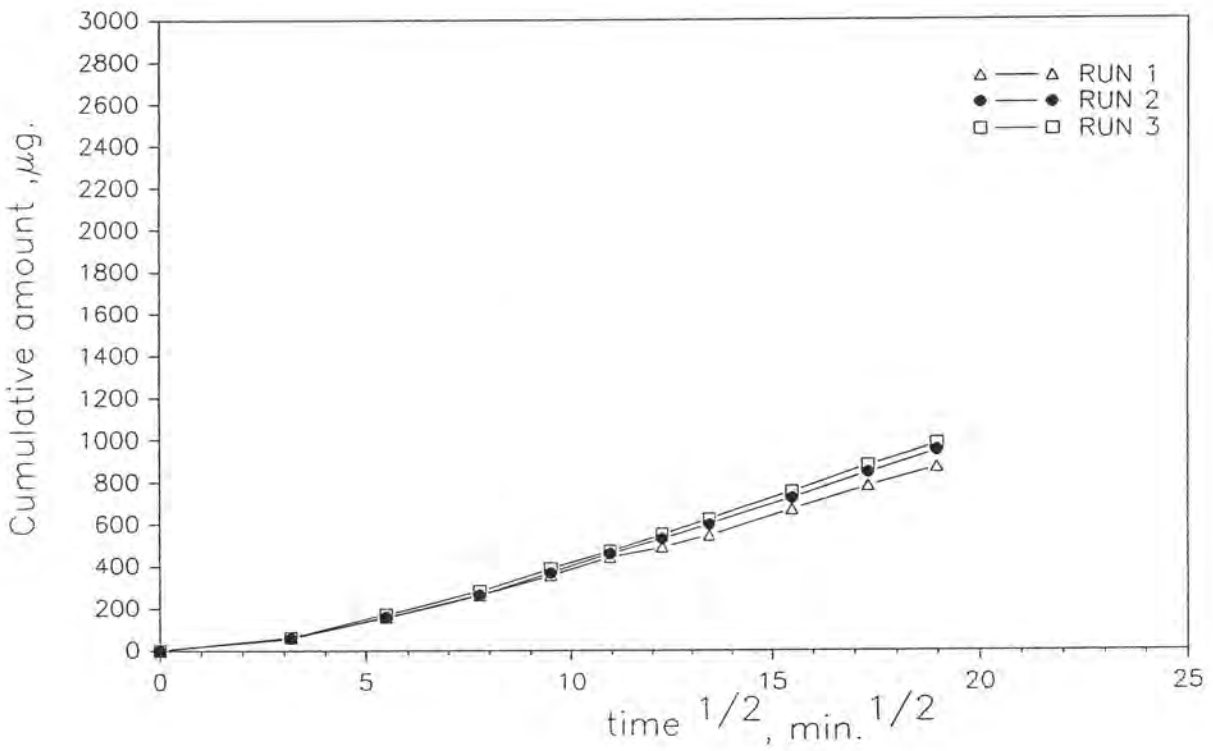
Diution factor = 2 : 5

Diffusion Run		Run I	Run II	Run III
Time (hr.)	Square root of Time ($min^{1/2}$)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
0.16	3.16	55.90	56.59	61.41
0.50	5.48	155.49	159.49	171.21
1.00	7.75	259.99	263.57	282.08
1.50	9.49	354.72	368.83	388.78
2.00	10.95	442.19	461.41	472.02
2.50	12.25	491.09	530.97	550.31
3.00	13.42	545.63	598.96	623.83
4.00	15.49	670.16	724.87	755.17
5.00	17.32	781.81	846.16	880.42
6.00	18.97	871.41	950.97	980.68
Release rate ($\mu g/min^{1/2}$)		53.33	59.43	61.01
r^2		0.9975	0.9990	0.9981
Membrane Thickness (cm.)		0.016	0.016	0.016

Average release rate = 57.92+4.06 $\mu g/min^{1/2}$

%CV = 7.00

Release profile of four diclofenac diethylammonium gel products through Durapore® membrane : Product D



Appendix V

Statistical Analyses of the Release Rate and Cumulative Amount Released of Four Diclofenac Gel Products Through Cellulose Acetate and Durapore® membranes

1. Comparison of cumulative amount of four diclofenac gel products through cellulose actate membrane at 6 hours.

Means	Product
Mean # 1 = 1888.703	A
Mean # 2 = 1560.607	B
Mean # 3 = 2023.98	C
Mean # 4 = 1448.167	D

Source	df	SS	S	F	Prob.
Between	3	659200	219733.3	8.800598	.0070
Within	8	199744	24968		
Total	11	858944			

Duncan's new multiple range test

MS Error = 24968 , df Error = 8

Significance level = .05

Least Significant Ranges

LSR where P = 2 -----297.4054

LSR where p = 3 -----368.5638

LSR where p = 4 -----413.2659

	Means	Product
Mean # 1	= 1448.167	D
Mean # 2	= 1560.607	B
Mean # 3	= 1888.703	A
Mean # 4	= 2023.98	C

Result : D < B < A < C

2. Comparison of the cumulative amount of four diclofenac gel products released through Durapore® membrane at 6 hour

Means			Product
Mean # 1	=	1171.263	A
Mean # 2	=	654.3834	B
Mean # 3	=	1117.54	C
Mean # 4	=	934.3532	D

Source	df	SS	MS	F	Prob.
Between	3	489473	163157.7	78.02388	.0001
Within	8	167294	2091.125		
Total	11	506202			

Duncan's new multiple range test

MS Error = 2091.125 df Error = 8

Significance level = .05

Least Significant Ranges

LSR where P = 2 -----149.08

LSR where p = 3 -----155.02

LSR where p = 4 -----158.68

Means		Product
Mean # 1 =	654.3834	B
Mean # 2 =	934.3532	D
Mean # 3 =	1117.54	A
Mean # 4 =	1171.263	C

Result :

Product B < D < A < C

3. Release rate comparison of four diclofenac gel products through cellulose acetate membrane

Means		Product
Mean # 1 =	99.40	A
Mean # 2 =	86.92	B
Mean # 3 =	102.70	C
Mean # 4 =	91.58	D

Source	df	SS	MS	F	Prob.
Between	3	466.336	155.445	1.2359	.3591
Within	8	1006.188	125.773		

From the data $p > .005$. ' . there is no significance different in release rate of diclofenac from four products through cellulose acetate membrane.

4. Release rate comparison of four diclofenac gel products

Durapore® membrane

Means		Product
Mean # 1 =	61.147	A
Mean # 2 =	40.67	B
Mean # 3 =	63.85	C
Mean # 4 =	57.92	D

Source	df	SS	MS	F	Prob.
Between	3	980.488	326.829	13.304	0.0023
Within	8	196.5234	24.565		
Total	11	1177.0114			

Duncan's new multiple range test

MS Error = 24.565 df Error = 8

Significance level = 0.05

Least Significant Ranges

LSR where	P = 2 -----	16.16
LSR where	p = 3 -----	16.80
LSR where	p = 4 -----	17.20

Means			Product
Mean # 1	=	40.67	B
Mean # 2	=	57.92	D
Mean # 3	=	61.15	A
Mean # 4	=	63.85	C

Result : B < D < A < C

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Appendix VI

Data on the Permeation of the Four Diclofenac Gel Products Through Shed Snake Skins.

Product A (Diclofenac diethylammonium 1.16%) permeate through shed snake skin

Calibration Curve Date

conc(μg/ml)	0.505	1.010	2.020	8.08	16.16	32.32	48.48
PAR	0.1643	0.3103	0.4958	3.2594	6.4466	13.0079	19.4698

$$Y = -0.0558 + 0.4031x$$

$$r^2 = 0.9998$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time	PAR	Amount	PAR	Amount	PAR	Amount
(hr.)		(μg)		(μg)		(μg)
1.0	0.7479[a]	22.9287	0.6752	20.6739	0.1904	7.4512
2.0	1.6299[a]	48.0913	1.0378	31.0126	0.5699	18.9368
3.0	0.4746[a]	15.1317	1.4931	43.8039	0.6914	22.6145
4.0	0.5059[a]	16.0247	1.5014	44.0388	0.7409	24.1127
5.0	0.5121[a]	16.2018	1.5184	44.5199	0.7225	23.5558
6.0	0.5849[a]	18.2787	1.4933	43.8096	0.7574	24.6117
8.0	1.3512[a]	40.1402	1.9668	57.2006	1.5899	49.8077
10.0	1.2045[a]	35.9548	2.1327	61.8923	1.4242	44.7868
12.0	1.3646[a]	40.5226	1.6642	48.6432	3.1759	97.8086
16.0	1.1005[b]	82.4700	1.5311	112.1971	1.5747	123.3695
20.0	1.0750[b]	80.9364	1.1281	83.7039	2.0338	158.1065
24.0	1.1198[b]	83.8465	1.3825	101.6909	1.9633	152.7721
Receptor Volume(ml)	5.75		5.7		6.1	

a : dilution factor = 2

b : dilution factor = 5

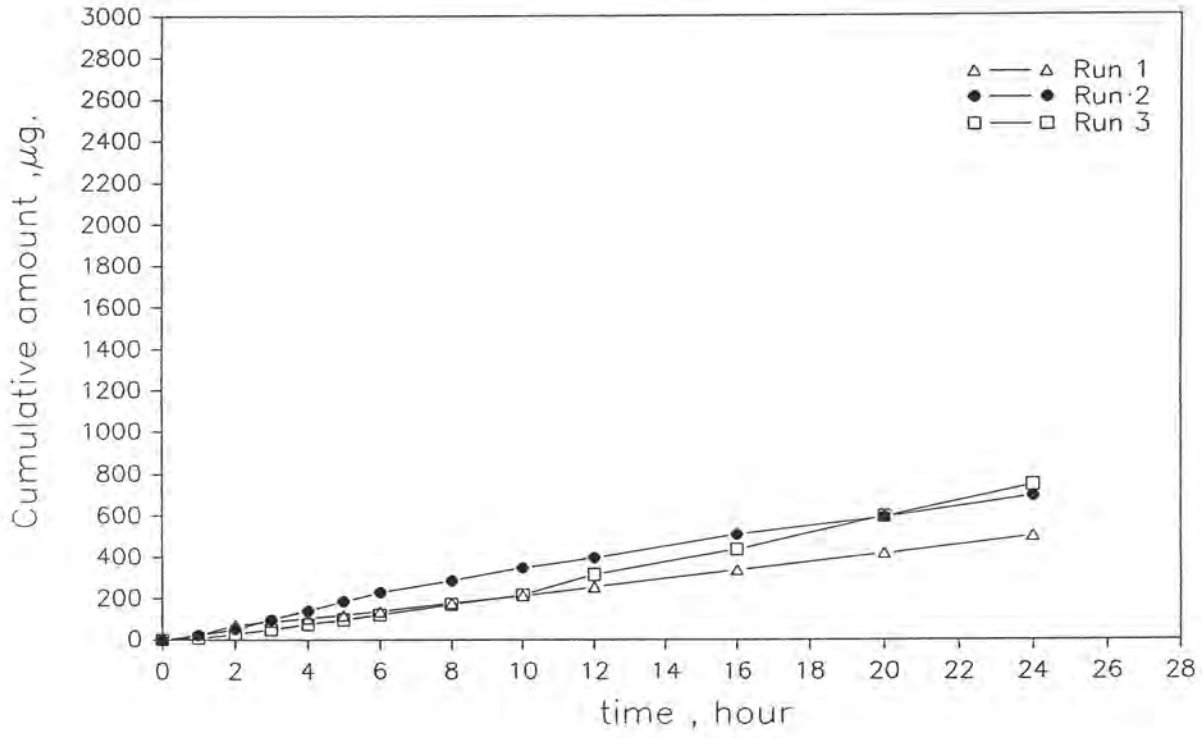
Diffusion Run	Run I	Run II	Run III
Time (hr.)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
1.0	22.93	20.74	7.45
2.0	71.02	51.75	26.39
3.0	86.16	95.55	49.00
4.0	102.18	139.59	73.12
5.0	118.38	184.11	96.67
6.0	136.66	227.92	121.28
8.0	176.80	285.12	171.09
10.0	212.75	347.01	215.88
12.0	253.27	395.65	313.69
16.0	335.74	507.85	437.06
20.0	416.68	591.55	595.16
24.0	500.53	693.24	747.93
(8.0-24.0 hr.) Steady-state Slope(μg/hr)	20.33	25.19	36.12
Jss (μg/hr.cm^2)	13.20	16.36	23.45
r^2	0.9998	0.9977	0.9973
Membrane Thickness(cm.)	0.024±0.005	0.024±0.03	0.025±0.003
Normalized Jss (μg/hr.cm^3)	550.00	581.67	938.00

Average normalized flux = 723.233 μg/hr.cm^3

Average flux (Jss) = 17.67±5.25 μg/hr.cm^2

%CV = 29.0

Permeation profile of diclofenac diethylammonium gel products through shed snake skin : Product A



Product B (Diclofenac diethylammonium 1.16%gel) permeate through shed snake skin

Calibration Curve Data

conc (μg/ml)	0.202	1.01	2.02	8.08	12.12	16.16	24.24
PAR	0.076	0.1761	0.4508	1.2619	1.8332	2.8923	4.5645

$$Y = -0.0553 + 0.1824x$$

$$r^2 = 0.9878$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
1.0	0.4751[a]	33.150	0.1910	16.4740	0.3505	25.5850
2.0	0.6062[a]	41.3438	0.4400	33.1286	0.4891	34.3235
3.0	1.3694[a]	89.0438	0.6613	47.9305	1.0429	69.2396
4.0	1.1985[a]	78.3625	1.0330	72.7920	0.9631	64.2083
5.0	1.2265[a]	80.1125	1.0820	76.0694	0.9823	65.4189
6.0	1.0172[a]	67.031	0.7833	56.0906	0.9053	60.5641
8.0	1.8786[a]	120.868	1.5288	105.9541	1.7429	113.3733
10.0	1.4709[a]	95.3875	1.3182	91.8679	1.6971	110.4794
12.0	1.4235[a]	92.4250	1.3867	96.4496	1.8606	120.8130
16.0	0.8711[b]	144.750	0.7335	131.8990	0.7906	133.3313
20.0	0.9662[b]	159.609	0.9901	174.8060	1.0500	174.2181
24.0	0.9558[b]	115.798	1.0329	181.9633	0.9631	160.5208
Receptor Volume(ml)	5.70		6.10		5.75	

a : dilution factor = 2

b : dilution factor = 5

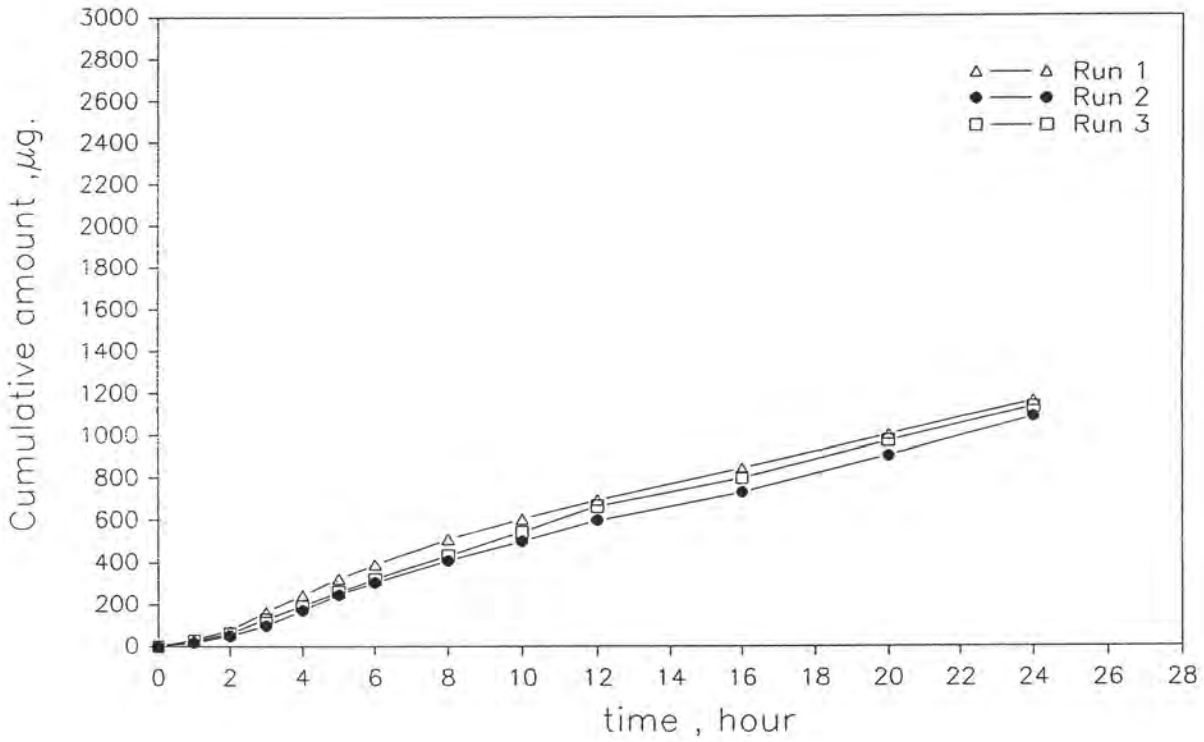
Diffusion Run	Run I	Run II	Run III
Time (hr.)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
1.0	33.15	16.47	25.59
2.0	74.49	49.60	59.91
3.0	163.54	97.53	129.15
4.0	241.90	170.33	193.36
5.0	322.01	246.39	258.78
6.0	389.04	302.49	319.34
8.0	509.91	408.44	432.71
10.0	605.30	500.31	543.19
12.0	693.73	596.76	664.00
16.0	842.48	728.66	797.34
20.0	1002.09	903.46	971.56
24.0	1160.03	1085.43	1132.08
(6.0-24.0 hr.) Steady-state Slope(μg/hr)	41.62	42.25	44.26
Jss (μg/hr.cm^2)	27.03	27.44	28.74
r^2	0.9950	0.9972	0.9927
Membrane Thickness(cm.)	0.025±0.004	0.024±0.003	0.025±0.004
Normalized Jss (μg/hr.cm^3)	1081.20	1055.38	1149.60

Average Normalized flux = 1095.39 μg/hr.cm^3

Average flux (Jss) = 27.74±0.89 μg/hr.cm^2

%CV = 3.21

Permeation profile of diclofenac diethylammonium gel products through shed snake skin : Product B



Product C (Diclofenac diethylammonium 1.16%gel) permeate through shed snake skin

Calibration Curve Date

conc (μg/ml)	0.505	1.010	2.020	4.040	8.080	12.120	32.320
PAR	0.2246	0.3788	0.6458	1.2567	3.2724	4.8896	13.2868

$$Y = -0.1837 + 0.4169x$$

$$r^2 = 0.9991$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
1.0	0.3097[a]	13.6185	0.9311	32.7086	0.2407	11.6967
2.0	0.3568[a]	14.9183	1.0417	36.1092	0.4600	17.7407
3.0	0.5242[a]	19.5389	1.3895	46.1583	0.6225	22.2193
4.0	0.7959[a]	27.0380	1.2803	42.9543	0.6538	23.0820
5.0	0.7822[a]	26.6601	1.2394	41.7543	0.6992	24.3332
6.0	0.6975[a]	24.3223	1.1974	40.5220	0.7428	25.5319
8.0	1.4169[a]	44.1787	2.0151	64.5136	1.5421	47.5640
10.0	2.4933[a]	73.8888	2.4028	75.8889	1.6699	51.0863
12.0	1.3288[a]	41.7194	2.8910	90.2128	2.0314	61.0494
16.0	2.8758[b]	211.112	4.6459	354.2556	4.5003	322.7342
20.0	2.6754[b]	197.28	4.3434	332.2138	3.6474	263.9682
24.0	2.1068[b]	158.05	3.8534	296.1250	2.0430	153.4228
Receptor Volume (ml.)	5.75		6.10		5.75	

a : dilution factor = 2

b : dilution factor = 5

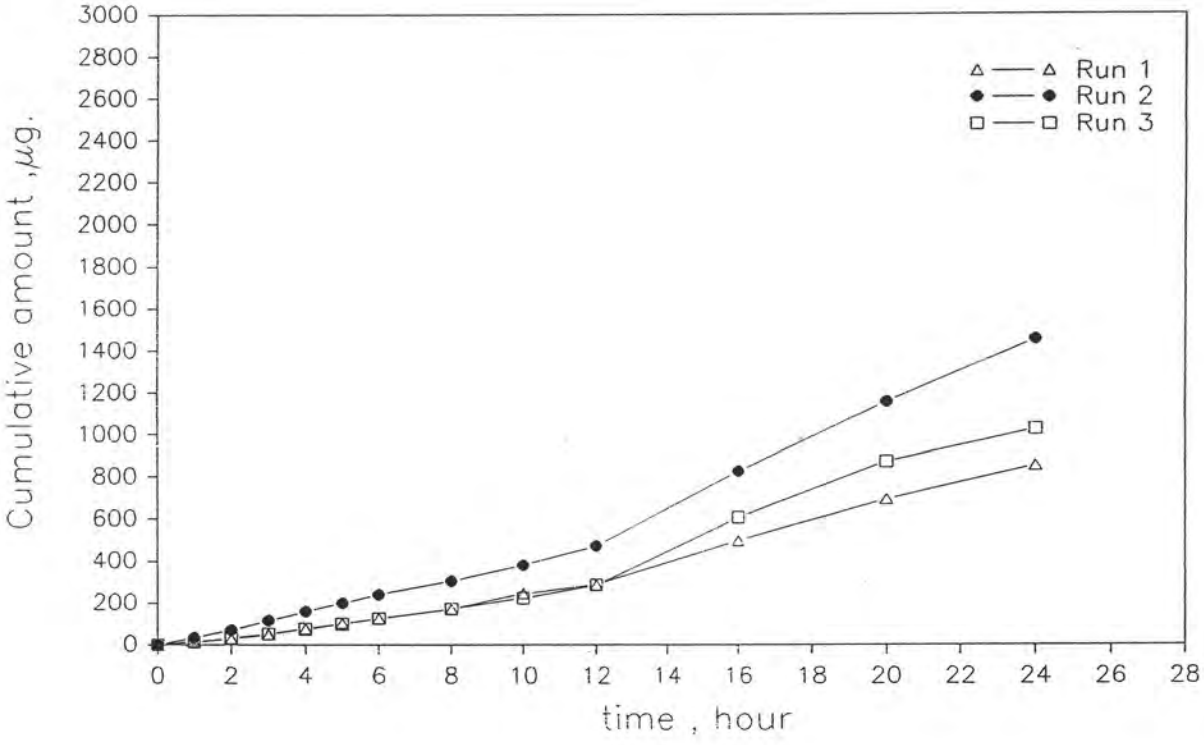
Diffusion Run	Run I	Run II	Run III
Time (hr.)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
1.0	13.62	32.71	11.70
2.0	28.54	68.82	29.44
3.0	48.08	114.97	51.66
4.0	75.11	157.93	74.74
5.0	101.77	199.68	99.07
6.0	126.09	240.21	124.60
8.0	170.27	304.72	172.17
10.0	244.16	380.61	223.26
12.0	285.88	470.82	284.31
16.0	496.99	825.08	607.04
20.0	694.28	1157.29	871.01
24.0	852.33	1453.42	1024.43
(6.0-24.0 hr.) Steady-state Slope (μg/hr)	47.42	82.00	62.11
Jss (μg/hr.cm^2)	30.79.	53.25	40.33
r^2	0.9959	0.9984	0.9769
Membrane Thickness(cm.)	0.022±0.003	0.023±0.004	0.021±0.003
Normalized Jss (μg/hr.cm^3)	1399.55	2315.22	1920.48

Average Normalized flux = 1878.42 μg/hr.cm^3

Average flux (Jss) = 41.46±11.27 μg/hr.cm^2

%CV = 27.18

Permeation profile of diclofenac diethylammonium gel products through shed snake skin : Product C



Product D (Diclofenac diethylammonium 1.16%gel) permeate through shed snake skin

Calibration Curve Date

conc (μg/ml)	0.505	1.01	2.02	4.04	8.08	12.12	16.16
PAR	0.3038	0.5849	1.3150	2.6704	5.9488	9.1995	11.0485

$$Y = - 0.0638 + 0.7174x$$

$$r^2 = 0.9945$$

Diffusion Run Data

Diffusion Run	Run I		Run II		Run III	
Time (hr.)	PAR	Amount (μg)	PAR	Amount (μg)	PAR	Amount (μg)
1.0	0.3879[a]	7.2404	0.3554	6.344	0.3536	6.6328
2.0	0.5716[a]	10.186	0.4255	7.775	0.5532	9.8046
3.0	0.8340[a]	14.392	0.6736	11.718	0.7458	11.2852
4.0	07176[a]	12.524	0.5508	9.766	0.7074	12.2549
5.0	0.7509[a]	13.060	0.6997	12.133	0.7252	12.5378
6.0	0.8765[a]	15.073	0.7460	12.868	0.8011	13.7439
8.0	1.8666[a]	30.945	1.4164	23.522	1.6542	27.3003
10.0	1.1975[a]	20.225	1.5117	25.036	1.4234	23.6327
12.0	2.5810[a]	102.954	1.4761	24.470	2.5231	41.1077
16.0	2.0584[b]	85.048	1.6173	66.785	1.8119	74.5155
20.0	0.9503[b]	40.640	1.0059	42.496	1.0572	44.5338
24.0	1.2260[b]	51.689	1.2531	52.316	1.3212	55.0216
Receptor Volume(ml)	5.75		5.7		5.7	

a = dilution factor = 2

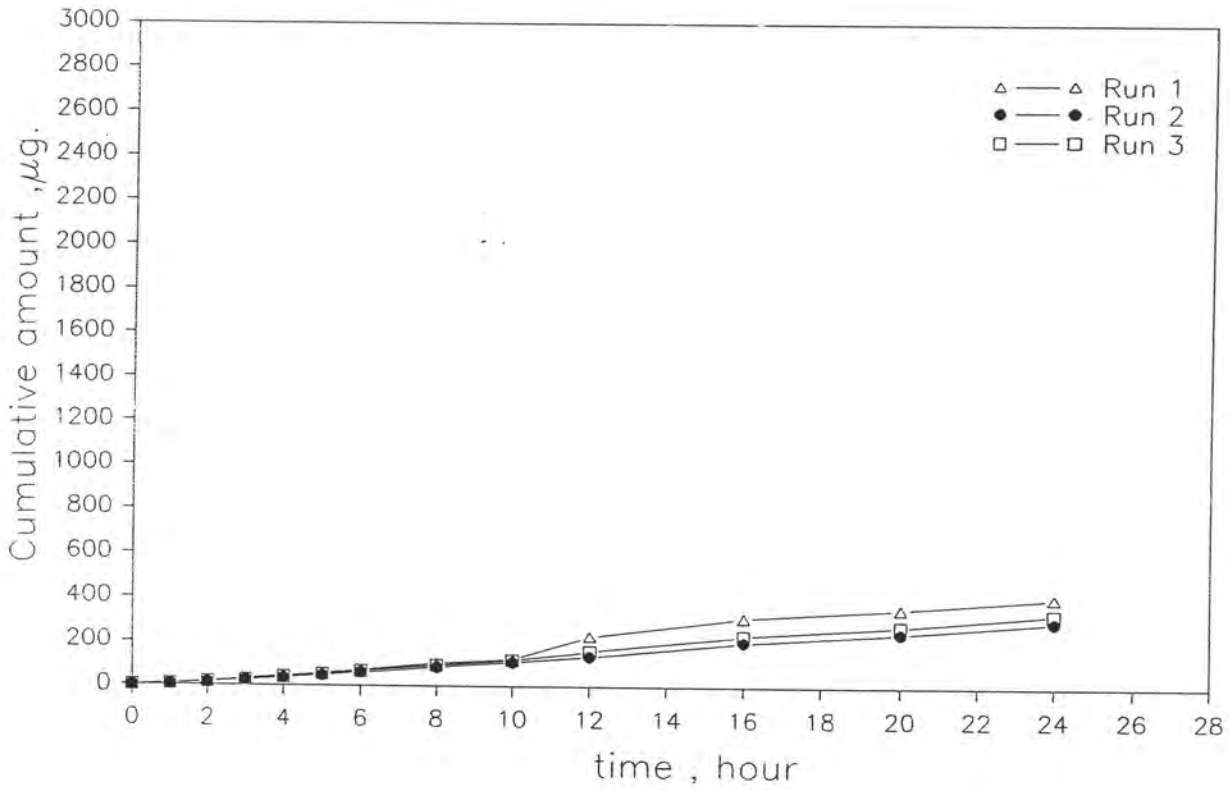
b = dilution factor = 5

Diffusion Run	Run I	Run II	Run III
Time (hr.)	Cumulative Amount (μg)	Cumulative Amount (μg)	Cumulative Amount (μg)
1.0	7.24	6.34	6.63
2.0	17.43	14.12	16.44
3.0	31.82	25.84	27.72
4.0	44.34	35.60	39.98
5.0	57.40	47.74	52.52
6.0	72.48	60.61	66.26
8.0	103.42	84.13	93.56
10.0	123.15	109.16	117.19
12.0	226.60	133.63	158.30
16.0	311.65	200.42	229.82
20.0	352.29	242.91	274.35
24.0	403.98	295.23	329.37
(6.0-24.0 hr.) Steady-state Slope(μg/hr)	18.51	13.35	14.88
Jss (μg/hr.cm^2)	12.02	8.67	9.66
r^2	0.9177	0.9950	0.9892
Membrane Thickness(cm.)	0.025±0.004	0.024±0.003	0.025±0.004
Normalized Jss (μg/hr.cm^3)	546.36	394.09	460.00

Average Normalized flux = 466.82 μg/hr.cm^3

Average flux (Jss) = 10.12±1.72 μg/hr.cm^3

Permeation profile of diclofenac diethylammonium gel products through shed snake skin : Product D



Appendix VII

Statistical Analyses of the Permeation Flux of Four Diclofenac Gel Products through Shed Snake Skin.

Comparison of cumulative amount of four Diclofenac gel products diffused through shed snake skin after 24 hours.

		Means	Product
Mean # 1	=	647.23	A
Mean # 2	=	1125.85	B
Mean # 3	=	1110.06	C
Mean # 4	=	342.86	D

Source	df	SS	MS	F	Prob.
Between	3	13.3378	434459.2	14.82429	.0017
Within	8	234458	29307.25		
Total	11	1537836			

Duncan's new multiple range test :

MS Error = 29307.25 df Error = 8

Significance level = 0.05

Least Significant Ranges

LSR where P = 2 -----	322.2141
LSR where p = 3 -----	399.3082
LSR where p = 4 -----	447.7392

Means		Product
Mean # 1	= 342.86	D
Mean # 2	= 647.2333	A
Mean # 3	= 1110.06	C
Mean # 4	= 1125.847	B

Result : D < A < C < B

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Steady state flux comparision of four diclofenac gel products permeate through shed snake skin after 24 hours.

Means			Product
Mean # 1	=	17.67	A
Mean # 2	=	27.74	B
Mean # 3	=	41.46	C
Mean # 4	=	10.12	D

Source	df	SS	MS	F	Prob.
Between	3	1653.822	551.274	13.9232	0.0021
Within	8	316.752	39.594		
Total	11	1970.574			

Duncan's new multiple range test :

MS Error = 39.594 df Error = 8

Significance level = 0.05

Least Significant Ranges

LSR where P = 2 -----	11.8433
LSR where p = 3 -----	14.6769
LSR where p = 4 -----	16.4571

Means		Product
Mean #1	= 10.12	D
Mean #2	= 17.67	A
Mean #3	= 27.74	B
Mean #4	= 41.46	C

Result : D < A < B < C

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Appendix VIII

Preliminary Data on Skin -Stripping Sequence and Optimum Occlusion Time.

Subject Number 1

Calibration Curve Data :

Conc (μg/ml)	2.0	4.0	6.0	8.0	12.0	16.0	24.0
PAR	1.1737	2.4752	3.7320	4.879	7.4430	10.1273	15.7273

$$y = -0.260+0.6580x$$

$$r^2 = 0.999$$

Occlusion time = 1 hour

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	3.1615	2.4592	1.0706	1.1589	1.0321
2	5.1419	2.1185	1.6142	1.0893	0.9594
3	4.4479	4.1953	3.7298	2.5441	1.7698

Occlusion time = 3 hours

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	11.7306	4.4413	3.4403	2.7885	1.7351
2	9.9618	5.6232	5.6496	3.3694	2.4137
3	15.3011	6.5565	8.2222	3.0625	1.8960

Occlusion time = 6 hours

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	9.8280	4.4456	2.3491	1.9111	2.3167
2	6.1129	4.3053	2.4169	2.0768	1.0552
3	11.8332	3.3120	2.2677	1.3696	0.9143

Subject number 1:

Occlusion time = 1 hour

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip 3+4 Amount (μg)	Strip 5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip9+10 Amount (μg)
Spot 1	5.20	4.13	2.02	2.16	1.96
Spot 2	8.21	3.61	2.85	2.05	1.85
Spot 3	7.16	6.77	6.06	4.26	3.09
$\bar{X}$	6.86	4.84	3.64	2.82	2.30
SD	1.53	1.69	2.13	1.25	0.69

Occlusion time = 3 hours

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip3+4 Amount (μg)	Strip 5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip9+10 Amount (μg)
Spot 1	18.22	7.15	5.62	4.63	3.03
Spot 2	15.53	8.94	8.98	5.52	4.06
Spot 3	23.65	10.36	12.89	5.05	3.28
$\bar{X}$	19.13	8.82	9.16	5.06	3.46
SD	4.14	1.61	3.64	0.45	0.54

Occlusion time = 6 hours

Strip Number / Area Number	Strip 1+2 Amount (μg)	Strip3+4 Amount (μg)	Strip5+6 Amount (μg)	Strip7+8 Amount (μg)	Strip9+10 Amount (μg)
Area 1	15.33	7.15	3.97	3.30	3.92
Area 2	9.69	6.94	4.07	3.56	2.00
Area 3	18.38	5.43	3.84	2.48	1.78
$\bar{X}$	14.47	6.51	3.64	3.11	2.57
SD	4.41	0.94	2.13	0.56	1.18

Subject Number 2

Calibration Curve Data :

Conc (µg/ml)	1.0	2.0	4.0	8.0	16.0	20.0
PAR	0.2878	0.6437	1.4856	2.9701	6.1101	11.6325

$$y = -0.708+0.5369x$$

$$r^2 = 0.9329$$

Occlusion time = 1 hour

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	2.7775	1.6646	0.9137	0.5987	0.6902
2	2.4374	1.3103	0.9271	0.8105	0.7674
3	3.6365	1.3670	0.7571	0.8976	1.2587

Occlusion time = 3 hours

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	3.7683	2.1095	0.7268	0.6670	0.5657
2	3.3532	1.3472	1.3603	0.8885	0.8786
3	5.0984	3.0962	2.0353	1.7785	1.3020

Occlusion time = 6 hours

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	3.9015	1.5337	1.8480	1.727	1.6107
2	5.0236	2.3250	2.9595	1.9657	0.7816
3	2.9423	2.3778	2.0285	2.1180	1.1570

Subject Number 2

Occlusion time = 1 hour

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip 3+4 Amount (μg)	Strip5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip 9+10 Amount (μg)
Spot 1	6.49	4.42	3.02	2.43	2.61
Spot 2	5.86	3.76	3.05	2.83	2.75
Spot 3	8.09	3.87	2.73	3.00	3.66
$\bar{X}$	7.14	4.02	2.93	2.75	3.01
SD	1.15	0.35	0.18	0.29	0.57

Occlusion time = 3 hours

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip 3+4 Amount (μg)	Strip 5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip 9+10 Amount (μg)
Spot 1	8.34	5.25	2.67	2.56	2.37
Spot 2	7.56	3.83	3.85	2.97	2.96
Spot 3	10.82	7.09	5.11	4.63	3.74
$\bar{X}$	8.913	5.39	3.88	3.39	3.02
SD	1.70	1.63	1.22	1.10	0.69

Occlusion time = 6 hours.

Strip Number / Spot Number	Strip 1+2 Amount (μg)	Strip3+4 Amount (μg)	Strip 5+6 Amount (μg)	Strip 7+8 Amount (μg)	Strip 9+10 Amount (μg)
Spot 1	8.59	4.18	4.76	4.54	4.33
Spot 2	10.68	5.56	6.83	4.98	2.78
Spot 3	6.80	5.75	5.10	5.26	3.47
$\bar{X}$	8.69	5.19	5.56	4.93	3.53
SD	1.94	0.88	1.11	0.36	0.78

Preliminary Strip Data :

Subject Number 3

Calibration Curve Data :

Conc (μg/ml)	0.25	0.50	1.00	2.00	4.00	8.00	16.00
PAR	0.1590	0.2820	0.5921	1.1737	2.4752	4.8798	10.1273

$$y = -0.0560+0.6325x$$

$$r^2 = 0.9996$$

Occlusion time = 1 hour

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	3.1343	1.1831	0.7476	0.4732	0.5434
2	2.3931	1.2730	0.7684	0.4726	0.3829
3	4.1373	1.2309	1.9230	1.3488	0.8157

Occlusion time = 3 hours

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	3.6460	1.4044	0.7818	0.5443	0.3248
2	4.3756	2.8722	1.8515	1.1602	0.6789
3	5.6254	2.5583	1.7089	1.4015	0.7826

Occlusion time = 6 hours

Spot No.	PAR Strip 1+2	PAR Strip 3+4	PAR Strip 5+6	PAR Strip 7+8	PAR Strip 9+10
1	1.0584	0.8977	0.3549	0.3252	0.4215
2	1.3994	0.7814	0.6839	0.4961	0.3112
3	0.8040	0.2999	0.1784	0.2145	0.1367

Subject Number 3:

Occlusion time = 1 hour

Strip Number Spot Number	Strip 1+2 Amount (µg)	Strip 3+4 Amount (µg)	Strip5+6 Amount (µg)	Strip7+8 Amount (µg)	Strip 9+10 Amount (µg)
Spot 1	5.04	1.96	1.27	0.84	0.95
Spot 2	3.87	2.10	1.30	0.84	0.69
Spot 3	6.63	2.03	3.13	2.22	1.38
$\overline{X}$	4.01	2.03	1.90	1.30	1.01
SD	0.40	0.07	1.06	0.80	0.35

Occlusion time = 3 hours

Strip Number Spot Number	Strip 1+2 Amount (µg)	Strip 3+4 Amount (µg)	Strip5+6 Amount (µg)	Strip7+8 Amount (µg)	Strip 9+10 Amount (µg)
Spot 1	5.85	2.31	1.32	0.95	0.60
Spot 2	7.01	4.63	3.02	1.92	1.16
Spot 3	8.41	4.13	2.79	2.30	1.33
$\overline{X}$	7.09	3.69	2.38	1.73	1.03
SD	1.28	1.22	0.92	0.70	0.38

Occlusion time = 6 hours.

Strip Number Spot Number	Strip 1+2 Amount (µg)	Strip3+4 Amount (µg)	Strip5+6 Amount (µg)	Strip7+8 Amount (µg)	Strip9+10 Amount (µg)
Spot 1	1.76	1.51	0.65	0.60	0.75
Spot 2	2.30	1.32	1.17	0.87	0.58
Spot 3	0.56	0.56	0.37	0.43	0.31
$\overline{X}$	1.54	1.13	0.73	0.63	0.55
SD	0.89	0.50	0.41	0.22	0.23

Appendix IX

Preliminary Data on the Testing of Difference Area between Left and Right Forearm.

Right Forearm

Spot No	Row 1		Row 2		Row 3		Row 4		Ro
	PAR*	Amount (μg)*	PAR*	Amount (μg)*	PAR*	Amount (μg)*	PAR*	Amount (μg)*	PAR*
1	2.3263	6.633	2.283	6.498	2.1398	6.024	3.0909	9.174	3.4069
2	2.0667	5.782	2.347	6.713	2.1694	6.122	2.8299	8.309	2.8794
3	2.2759	6.475	3.392	10.173	2.3003	6.556	2.7617	8.083	3.0571

* dilution factor = 2

Left Forearm

Spot No.	Row 1		Row 2		Row 3		Row 4		Row
	PAR*	Amount (μg)*	PAR*	Amount (μg)*	PAR*	Amount (μg)*	PAR*	Amount (μg)*	PAR*
1	3.3013	9.870	3.333	9.975	3.3880	10.157	2.727	7.969	2.2738
2	3.1892	9.499	2.193	6.201	1.9554	5.414	2.261	6.426	2.2544
3	3.4369	10.319	3.0424	9.013	3.0065	8.894	2.449	7.048	2.6767

* dilution factor = 2

ANOVA result on the effect of right and left forearm

Mean #1	= 15.24	Right forearm
Mean #2	= 16.19	Left forearm

ANOVA Table

Source	df	SS	MS	F	Prob.
Between	1	6.8071	6.8071	0.6580	0.5705
Within	28	289.658	10.3449		
Total	29	296.465			

Result : There is not significant difference between left and right forearm

($p > 0.05$)

ANOVA results on the effect of different rows in the same forearm(Left)

Mean of row 1	= 19.79
Mean of row 2	= 16.79
Mean of row 3	= 16.31
Mean of row 4	=14.30
Mean of row 5	=13.79

ANOVA Table

Source	df	SS	MS	F	Prob.
Between	4	67.9771	16.9943	1.8884	0.1889
Within	10	89.9937	8.9994		
Total	14	157.9707			

Result: There is not significant difference among five rows in each forearm ($p > 0.05$)

Appendix X

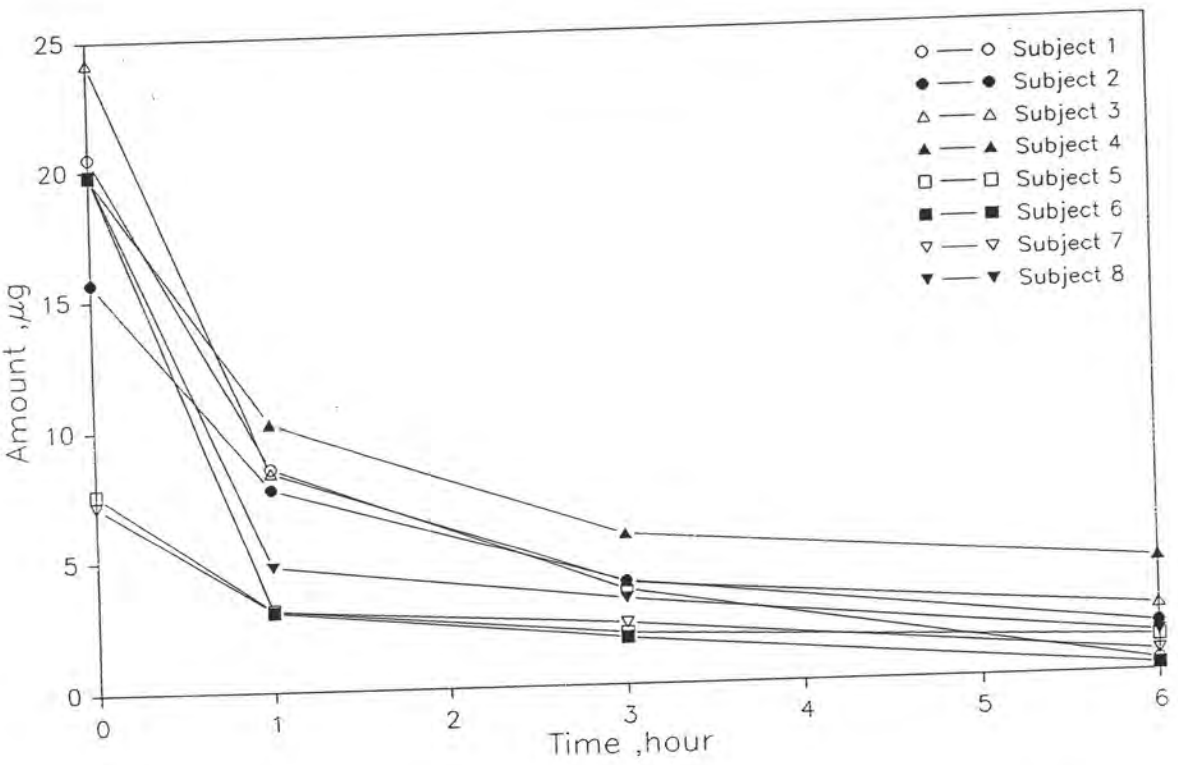
Data on *In Vivo* Percutaneous Absorption of Four Diclofenac Gel Products using Skin Stripping Technique

Amount of diclofenac remaining in stratum corneum:Product A

Time (hr)	0		1		3		6	
Subject Number	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B
1	16.06	24.91	7.39	9.63	6.34	0.88	0.81	0
2	15.56	15.74	6.84	8.59	4.63	3.20	1.20	2.47
3	29.00	19.36	7.02	9.70	3.27	4.48	1.68	3.35
4	18.04	21.46	9.52	10.88	5.13	6.29	2.85	5.76
5	9.06	6.15	2.27	3.91	2.00	1.91	1.67	0.94
6	21.69	17.91	2.35	3.64	1.15	2.40	0.46	0
7	7.89	6.43	2.42	3.69	3.05	1.63	0.72	0.80
8	18.52	21.24	4.35	5.15	2.56	3.97	1.04	1.90
Average amount* (μg)	16.81±6.61		6.28±3.13		3.31±1.70		1.60±1.46	

* n = 16, mean ± SD

Amount of diclofenac remaining in stratum corneum of 8 subjects at various times : Product A



(Each point represent average amount of two spots)

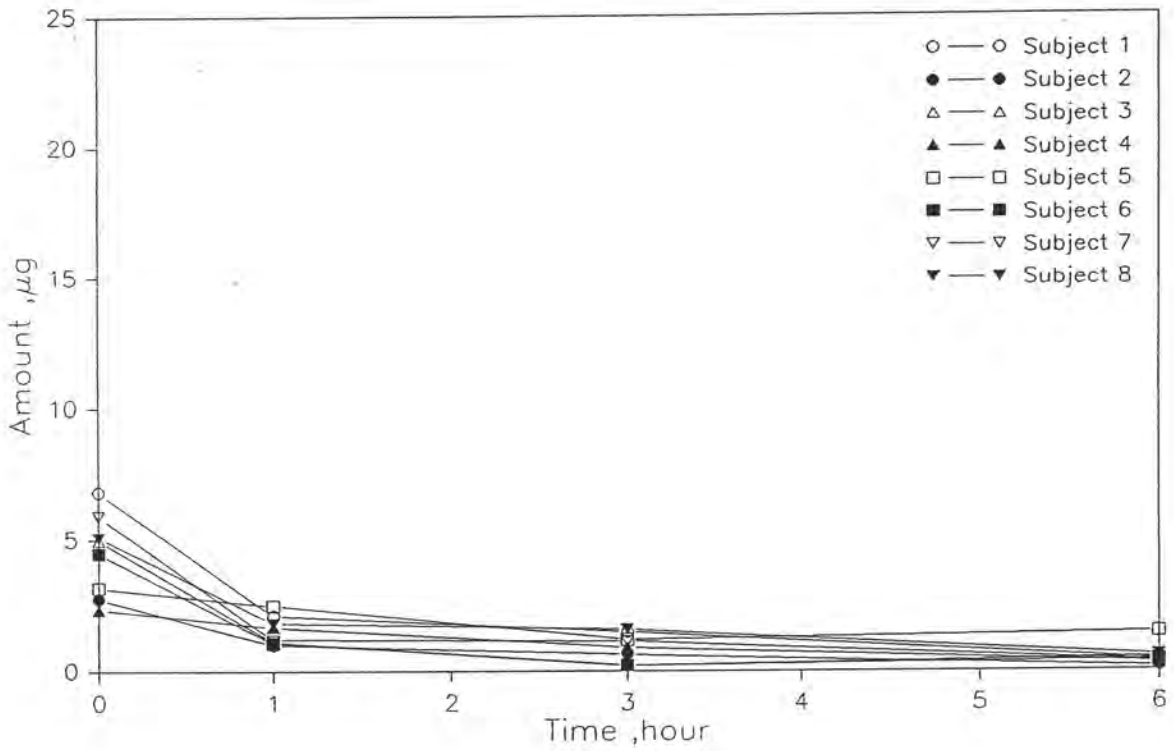
Amount of diclofenac remaining in stratum corneum :Product B

Time (hour)	0		1		3		6	
Subject Number	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B
1	7.66	5.97	1.88	2.33	1.88	1.10	0.39	0.44
2	2.77	2.74	0.97	1.01	0.60	0.66	0.30	0.30
3	5.09	4.84	1.22	0.98	0.21	-	0.67	0.67
4	1.91	3.57	1.44	1.89	0.19	1.60	0.22	0.37
5	7.32	5.38	2.88	2.10	1.70	0.70	1.51	1.41
6	3.22	5.74	1.90	1.23	0.17	0.80	0.30	0.47
7	4.39	7.45	1.24	1.16	0.58	1.70	0.63	0.10
8	5.32	4.85	1.87	1.81	0.99	1.22	0.81	0.34
Average amount (μg)	4.89_1.74		1.62_0.55		0.91_0.6**		0.52_0.42	

*n = 16, mean ± SD

*n =15, mean ± SD

Amount of diclofenac remaining in stratum corneum of 8 subjects at various times : Product B



(Each point represent average amount of two spots)

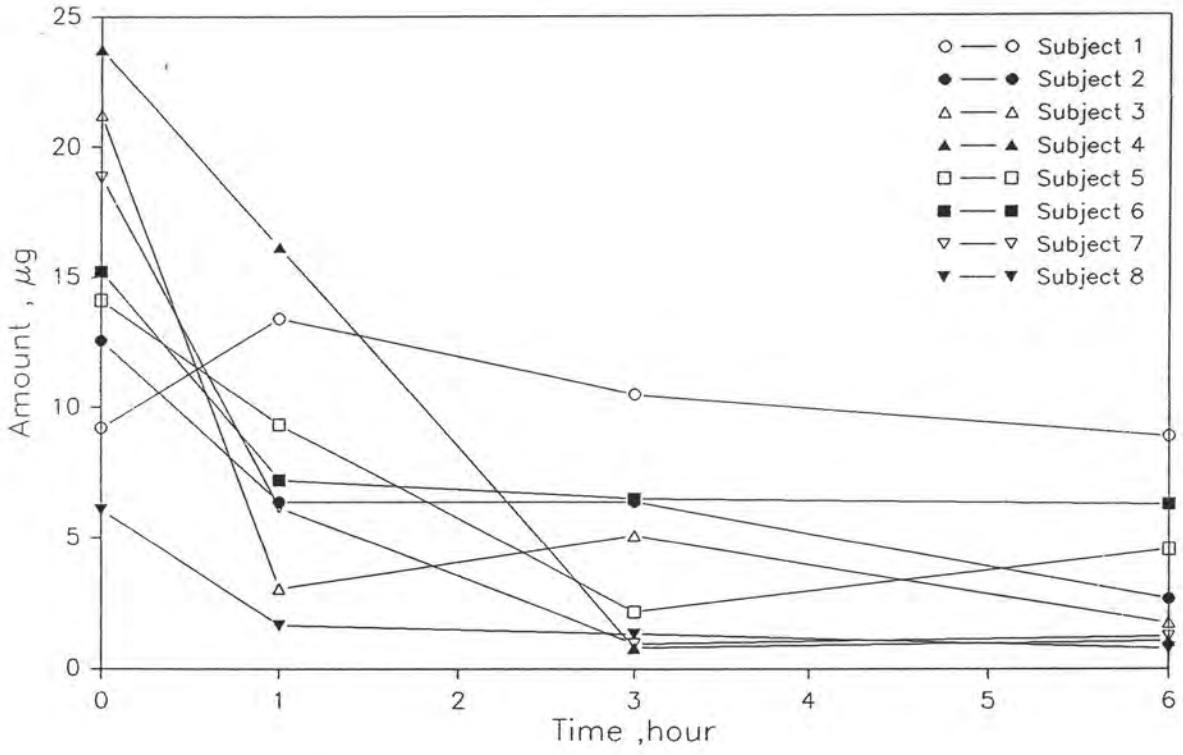
Amount of diclofenac remaining in stratum corneum:Product C

Time (hr)	0		1		3		6	
Subject Number	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B
1	17.39	15.03	16.66	10.16	11.80	9.16	10.70	7.04
2	11.00	14.13	5.93	6.78	6.00	6.73	1.93	3.40
3	21.53	20.87	4.84	1.26	5.25	4.90	1.83	1.67
4	21.55	25.89	12.15	20.12	0.89	0.69	2.12	0
5	14.10	14.12	9.36	9.27	1.71	2.62	4.55	-
6	16.05	14.36	9.05	5.35	6.17	6.78	5.69	6.85
7	21.66	16.01	9.22	3.09	0.87	1.02	1.45	0.98
8	5.48	6.66	1.85	1.45	0.51	2.14	0.50	1.05
Average amount* (μg)	15.99±5.50		7.53±5.42		4.20±3.42		3.32±3.10**	

* n = 16, mean ± SD

**n = 15, mean ± SD

Amount of diclofenac remaining in stratum corneum of 8 subjects at various times : Product C



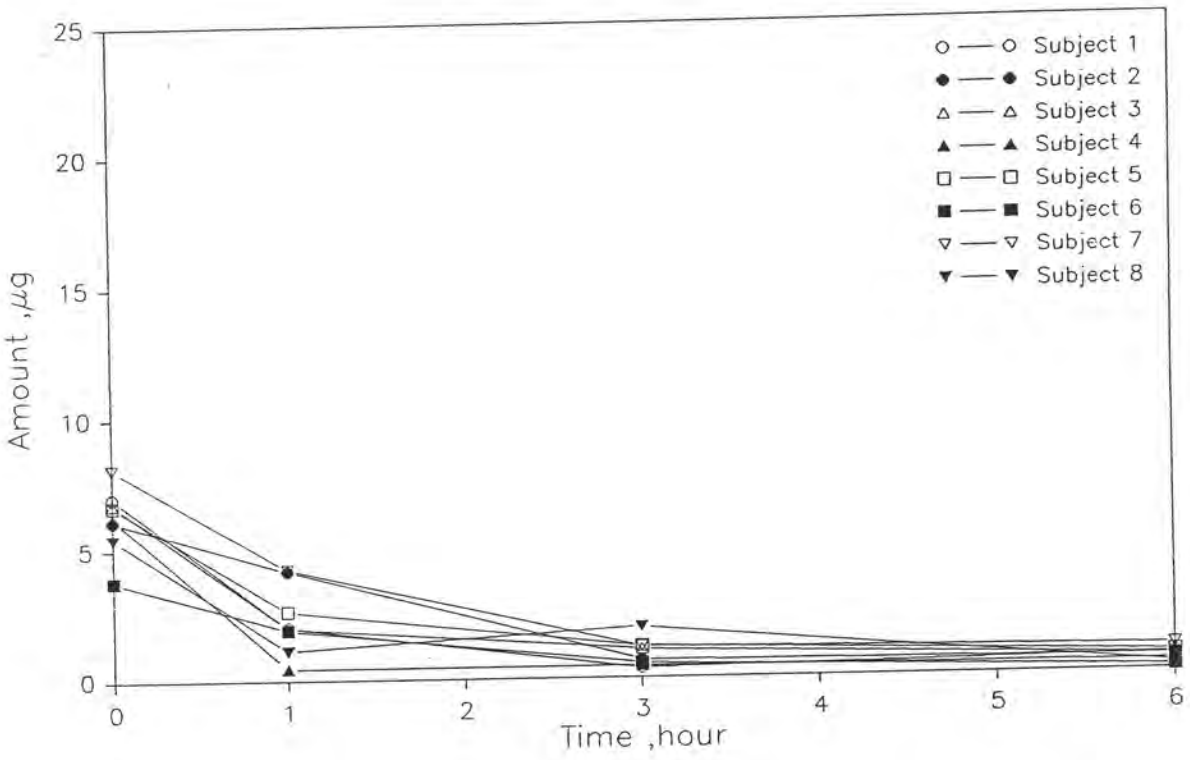
(Each point represent average amount of two spots)

Amount of diclofenac remaining in stratum corneum :Product D

Time (hr)	0		1		3		6	
Subject Number	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B	Spot A	Spot B
1	8.15	5.88	2.45	1.56	0.42	0.27	0.80	0.43
2	6.89	5.37	4.49	3.85	0.83	0.48	0.69	0.42
3	7.20	6.32	2.16	1.84	1.20	0.93	0.61	0.55
4	4.62	7.91	0.84	0.03	0.50	0.37	0.26	0.44
5	6.43	6.95	2.15	3.11	1.30	1.10	0.35	1.02
6	3.10	4.50	1.34	2.48	0.56	0.52	0.19	0.07
7	9.04	7.21	2.60	7.33	1.15	1.18	0.31	1.60
8	4.65	6.22	1.05	1.21	1.03	2.82	0.21	0.32
Average amount* (μg)	6.28±1.55		2.41±1.73		0.92±0.62		0.52±0.38	

* n = 16, mean ± SD

Amount of diclofenac remaining in stratum corneum of 8 subjects at various times : Product D



(Each point represent average amount of two spots)

Appendix XI

Statistical Comparision of the Amount of Diclofenac Remaining in the Stratum corneum at Different Times after Administration of the Four Gel Products.

Appendix XI a

Comparison of Diclofenac Diethylammonium Remaining in the Stratum Corneum at 0 hour

Subject Number	Product A		Product B		Product C		Product D	
	Spot 1	Spot 2	Spot 1	Spot 2	Spot 1	Spot 2	Spot 1	Spot 2
1	16.06	24.91	7.66	5.97	17.39	15.03	8.15	5.88
2	15.56	15.74	2.77	2.74	11.00	14.13	6.89	5.37
3	29.00	19.36	5.09	4.84	21.53	20.87	7.20	6.32
4	18.04	21.46	1.91	3.57	21.55	25.89	4.62	7.91
5	9.06	6.15	7.32	5.38	14.10	14.12	6.43	6.95
6	21.69	17.91	3.22	5.74	16.05	14.36	3.10	4.50
7	7.89	6.43	4.39	7.45	21.66	16.01	9.04	7.21
8	18.52	21.24	5.32	4.85	5.48	6.66	4.65	6.22

Two-way ANOVA

Grand mean = 10.99281, Total number of observations =64

Treatment means

Product A = 16.82 µg

Product B = 4.89 µg

Product C = 15.99 µg

Product D = 6.28 µg

Subject mean Subject No.1 = 12.63 µg Subject No.5 = 8.69 µg

No.2 = 9.27 µg No.6 = 10.82 µg

No.3 = 14.28 µg No.7 = 10.01 µg

No.4= 13.12 µg No.8 = 9.12 µg

Source	df	SS	MS	F	Prob
treatment (A)	3	1893.835	631.2781	17.0848	.0001
subject (B)	7	246.2534	35.17906	6.6615	.0002
AB	21	775.9448	36.94975	6.9968	.0001
Error	32	168.9902	5.280945		
Total	63	3085.023			

Duncan's Test :

MS Error = 5.2809 df Error = 32

Significance level = 0.05

Least Significant Ranges

LSR where P = 2 ----- 5.385

LSR where p = 3 ----- 6.482

LSR where p = 4 ----- 7.151

Means

Mean of Product A = 16.82 μg

Product B = 4.89 μg

Product C = 15.99 μg

Product D = 6.28 μg

Result : B < D < C < A

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Appendix XI b

Comparison of Diclofenac Diethylammonium Remaining in the Stratum Corneum at 1 hour

Subject Number	Product A	Product B	Product C	Product D	
	Spot 1	Spot 2	Spot 1	Spot 2	Spot 1
1	7.39	9.63	1.88	2.33	16.66
2	6.84	8.59	0.97	1.01	5.93
3	7.02	9.70	1.22	0.98	4.84
4	9.52	10.88	1.44	1.89	12.15
5	2.07	3.91	2.88	2.10	9.36
6	2.35	3.64	1.90	1.23	9.05
7	2.42	3.69	1.24	1.16	9.22
8	4.35	5.15	1.87	1.81	1.85

Two-way ANOVA : 1 hour

Grand mean = 4.5045, Total number of observations = 64

Treatment means

Product A = 6.08 μg

Product B = 1.62 μg

Product C = 7.91 μg

Product D = 2.41 μg

Subject means

Subject No.1 = 6.51 μg

No.2 = 4.81 μg

No.3 = 3.81 μg

No.4 = 7.11 μg

Subject No.5 = 4.38 μg

No.6 = 3.42 μg

No.7 = 3.84 μg

No.8 = 2.34 μg

Source	df	SS	MS	F (mix model)	Prob.
treat(A)	3	429.0269	143.00	8.5980	.0001
subj (B)	7	143.7017	20.52881	5.9029	.0004
AB	21	349.2901	16.63286	4.7826	.0002
Error	32	111.2878	3.477745		
Total	63	1033.306			

Duncan's Test :

MS Error = 3.48 df Error = 32

Significance level = 0.05

Least Significant Ranges

LSR where P = 2 ----- 5.02

LSR where p = 3 ----- 6.04

LSR where p = 4 ----- 6.66

Means

Mean of Product A = 6.08 μg

Product B = 1.62 μg

Product C = 7.91 μg

Product D = 2.41 μg

Result : B < D < A < C

Appendix XI c

Comparison of Diclofenac Diethylammonium Remaining in the Stratum Corneum at 3 hour

Subject Number	Product A		Product B		Product C		Product D	
	Spot 1	Spot 2	Spot 1	Spot 2	Spot 1	Spot 2	Spot 1	Spot 2
1	6.34	0.88	1.88	1.10	11.8	9.16	0.42	0.27
2	4.63	3.20	0.60	0.66	6.00	6.73	0.83	0.48
3	3.27	4.48	0.21	-	5.25	4.90	1.20	0.93
4	5.13	6.29	0.19	1.60	0.89	0.69	0.50	0.37
5	2.00	1.91	1.70	0.70	1.71	2.62	1.30	1.19
6	1.15	2.40	0.17	0.28	6.19	6.78	0.56	0.52
7	3.05	1.63	0.58	1.70	0.87	1.02	1.15	1.18
8	2.56	3.97	0.99	1.22	0.51	2.14	1.03	2.82

Two-way ANOVA : 3 hour

Grand mean = 2.54, Total number of observations = 63

Treatment means

Product A = 3.31 μg

Product B = 0.91 μg

Product C = 4.20 μg

Product D = 0.92 μg

Subject means

Subject No.1= 3.98 μg Subject No.5 = 1.64 μg

No.2 = 2.89 μg No.6 = 2.26 μg

No.3 = 2.89 μg No.7 = 1.40 μg

No.4 = 1.96 μg No.8 = 1.91 μg

Source	df	SS	MS	F	Prob.
treat(A)	3	119.9337	39.9779	5.26688	.0042
subj (B)	7	40.4945	5.78493	5.95997	.0004
AB	21	159.399	7.59043	7.82011	.0001
Error	31	34.6275	0.97063		
Total	62	354.4547			

Duncan's Test :

MS Error = 0.97 df Error = 31

Significance level = 0.05

Least Significant Ranges

LSR where P = 2 ----- 1.41

LSR where p = 3 ----- 1.71

LSR where p = 4 ----- 1.89

Means

Mean of Product A = 3.31 μg

Product B = 0.91 μg

Product C = 4.20 μg

Product D = 0.92 μg

Result : B < D < A < C

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Appendix XI d

Comparison of Diclofenac Diethylammonium Remaining in the Stratum Corneum at 6 hour.

Subject	Product A		Product B		Product C		Product D	
Number	Spot 1	Spot 2	Spot 1	Spot 2	Spot 1	Spot 2	Spot 1	Spot 2
1	0.81	0	0.39	0.44	10.7	7.04	0.80	0.43
2	1.20	2.47	0	0.30	1.93	3.40	0.69	0.42
3	1.68	3.35	0.32	0.67	1.83	1.67	0.61	0.55
4	2.85	5.76	0.22	0.37	2.12	0*	0.26	0.44
5	1.67	0.94	1.51	1.41	4.55	-**	0.35	1.02
6	0.46	0	0.30	0.47	5.69	6.85	0.19	0.07
7	0.72	0.80	0.63	0.10	1.45	0.98	0.31	1.60
8	1.04	1.90	0.81	0.34	0.50	1.05	0.21	0.32

* = undetectable , ** = missing value

Two-way ANOVA : 6 hour

Grand mean = 1.56, Total number of observations = 63

Treatment means Product A = 1.60 µg

Product B = 0.52 µg

Product C = 3.31 µg

Product D = 0.52 µg

Subject means:Subject No.1= 2.58 µg Subject No.5 = 1.64 µg

No.2= 1.30 µg No.6 = 1.75 µg

No.3= 1.34 µg No.7= 0.82 µg

No.4= 1.50 µg No.8 = 0.77 µg

Source	df	SS	MS	F	Prob.
treat (A)	3	70.0586	23.35287	3.45771	.0067
subj (B)	7	27.68707	3.95529	3.81775	.0036
AB	21	141.8311	6.75386	6.42101	.0001
Error	31	32.11685	1.03603		
Total	62	271.6936			

Duncan's Test :

MS Error = 1.036 , df Error = 31

Significance level = 0.05

Least Significant Ranges

LSR where P = 2 ----- 1.471

LSR where p = 3 ----- 1.771

LSR where p = 4 ----- 1.954

Means

Mean of Product A = 1.60 μg

Product B = 0.52 μg

Product C = 3.36 μg

Product D = 0.52 μg

Result : B < D < A < C

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Percent of absorbed diclofenac of four products at 1 hour

Percent absorbed of Subject Number	A	B	C	D
1	58.47	69.14	-	71.44
2	53.18	64.14	49.44	31.97
3	65.43	77.87	85.62	70.55
4	48.36	28.85	31.98	93.06
5	60.71	22.19	33.98	60.69
6	84.88	65.07	52.67	49.74
7	57.33	79.73	67.33	38.93
8	76.11	63.85	72.82	76.23
mean $\pm$ SD	63.06$\pm$13.40	58.85$\pm$21.74	56.26$\pm$22.16	61.95$\pm$23.80

*percent absorbed at 1 hr = $\frac{\text{Average amount in SC. at 0 hr.- Amount in SC. at 1 hr}}{\text{Average amount in SC. at 0 hr.}} \times 10$

Percent of absorbed diclofenac of four products at 3 hours

Percent absorbed of Subject Number	A	B	C	D
1	82.24	78.18	35.35	95.09
2	74.99	78.15	0.49	89.32
3	83.82	95.77	49.37	84.31
4	71.09	61.15	76.07	93.07
5	74.31	62.27	84.66	81.39
6	91.04	94.98	57.36	85.79
7	67.32	80.74	94.99	85.67
8	83.58	78.29	78.17	64.62
mean ± SD	78.56±7.91	74.96±15.23	71.55±22.09	85.15±9.48

*percent absorbed at 3 hr= $\frac{\text{Average amount in SC. at 0 hr.- Amount in SC. at 3 hr}}{\text{Average amount in SC. at 0 hr.}} \times 100$

Percent of absorbed diclofenac of four products at 6 hours

Percent absorbed of Subject Number	A	B	C	D
1	98.03	93.92	23.48	91.24
2	88.28	94.57	78.80	90.86
3	89.60	90.04	91.75	91.46
4	78.21	87.40	95.53	94.42
5	82.86	54.09	67.75	89.76
6	98.84	91.41	58.78	96.58
7	89.39	93.84	93.55	88.26
8	92.61	91.79	87.23	95.13
mean $\pm$S D	89.72$\pm$7.82	87.12$\pm$13.50	78.75$\pm$20.83	92.21$\pm$15.89

*percent absorbed at 6 hr= $\frac{\text{Average amount in SC. at 0 hr.- Amount in SC. at 6 hr}}{\text{Average amount in SC. at 0 hr.}} \times 100$

1. Comparision of percent percutaneously absorbed of four diclofenac gel products in stratum corneum at 1 hour

Percent absorbe of Product Subject Number	**A**	**B**	**C**	**D**
1	58.47	69.14	-	71.44
2	53.18	64.14	49.44	31.97
3	65.43	77.87	85.62	70.55
4	48.36	28.85	31.98	93.06
5	60.71	22.19	33.98	60.69
6	84.88	65.07	52.67	49.74
7	57.33	79.73	67.33	38.93
8	76.11	63.85	72.82	76.23

Two-way ANOVA

Source	df	SS	MS	F	
Treatment (A)	3	176.5424	58.84748	0.1896	($F_{3,20}$= 3.10)
Subject (B)	7	3394.35	484.76459	1.5623	($F_{7,20}$= 2.51)
Residual	20	6205.9684	310.29842		
Total	30	9776.86			

Result : P Value of this test is greater than 0.05. Therefore,there is not significance different in percent absorption of four products in stratum corneum
at 1 hour.

2. Comparison of percent absorption of four diclofenac gel products in stratum corneum at 3 hour

Percent absorbe of Produc Subject Number	A	B	C	D
1	82.24	78.15	0.43	95.09
2	74.99	77.18	49.37	89.32
3	83.82	95.77	76.07	84.31
4	71.09	61.75	96.67	93.07
5	74.31	62.27	84.66	81.39
6	91.04	94.98	57.36	85.79
7	67.32	80.74	94.99	85.67
8	83.58	78.29	78.17	64.62

Two-way ANOVA

Source	df	SS	MS	F
Treatment (A)	3	1303.4916	434.4972	1.1544 ($F_{3,21}$=3.07)
Subject (B)	7	1286.3759	183.7680	0.4883 ($F_{7,21}$=2.49)
Residual	21	7904.0445	376.3831	
Total	31	10493.9120		

Result : P Value of this test is greater than 0.05 .Therefore,there is not significance different in percent absorption of four products in stratum corneum at 3 hour.

3.Comparision of percent absorption of four diclofenac gel products in stratum corneum at 6 hour

Percent absorbe of Produc Subject Number	A	B	C	D
1	98.03	93.92	23.48	91.24
2	88.28	94.57	78.80	90.95
3	89.60	90.04	91.75	91.46
4	78.21	87.40	95.53	94.42
5	82.86	54.09	67.75	89.76
6	98.84	91.41	58.78	96.58
7	89.39	93.84	93.55	88.26
8	92.61	91.75	87.23	95.13

Two-way ANOVA

Source	df	SS	MS	F
Treatment (A)	3	1420.2975	473.4325	2.2323 ($F_{3,21}$=3.08)
Subject (B)	7	1444.7960	206.3994	0.9732 ($F_{7,21}$= 2.49)
Residual	21	4453.6670	212.0794	
Total	31	7318.7605		

Result : P Value of this test is greater than 0.05: therefore , there is not significance different in percent absorption of four products in stratum corneum at 6 hour.

Appendix XIII

Correlation Results between *In Vitro* Release/Permeation and *In Vivo* Skin-Stripping Data

Appendix XIII a (Cellulose acetate membrane)

Correlation data of four diclofenac gel products release through cellulase acetate membrane and amount remaining in the stratum corneum at 0 hour

Product	Amount in SC. at 0 hour, X (μg)	Release rate, Y (μg/min$^{1/2}$)
A	16.81	99.40
B	4.89	86.92
C	15.99	102.70
D	6.28	91.58

Test of Zero correlation :

Ho : $\rho = 0$ Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9548

$$\therefore t_{n-2} = \left| \frac{0.9548\sqrt{4-2}}{\sqrt{1-(0.9548)^2}} \right|$$

$$= 4.543$$

From the correlation coefficient at 5% level of significance, $t_{0.025}$ (df=2) is 4.30. Therefore, there is a correlation between release rate of drug through cellulose acetate membrane and amount of drug remaining on stratum corneum at 0 hour.($p<0.05$)

Correlation data of cumulative amount of four diclofenac gel products released through cellulase acetate membrane and amount remaining in the stratum corneum at 0 hour

Product	Amount in SC at 0 hr, X (μg)	Cumulative amount, Y (μg)
A	16.81	1888.70
B	4.89	1560.60
C	15.99	2023.98
D	6.28	1448.17

Test of Zero correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9326

$$\therefore t_{n-2} = \left| \frac{0.9326\sqrt{4-2}}{\sqrt{1-(0.9326)^2}} \right|$$

$$= 3.654$$

From the correlation coefficient at 5% level of significance, $t_{0.025}$(df=2) is 4.30. Therefore, there is no correlation between amount of drug release through cellulose acetate membrane and amount of drug remaining in the stratum corneum at 0 hour($p > 0.05$)

Correlation data of release rate of four diclofenac gel products through cellulose acetate membrane and amount remaining on stratum corneum at 1 hour.

Product	Amount in SC. at 1 hour, X (μg)	Release rate, Y ($\mu g/min^{1/2}$)
A	6.28	99.40
B	1.62	86.92
C	7.53	102.70
D	0.52	91.58

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9878

$$\therefore t_{4-2} = \left| \frac{0.9878\sqrt{4-2}}{\sqrt{1-(0.9878)^2}} \right|$$

$$= 8.978$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance at the 5% level. Therefore, there is a correlation between release rate of diclofenac through cellulose acetate membrane and amount remaining on corneum at 1 hour is significance correlate ($p < 0.05$).

Correlation data of cumulative amount of four diclofenac gel products release through cellulose acetate membrane and amount remaining in the stratum corneum at 1 hour.

Product	amount in SC. at 1 hour, X (μg)	cumulative amount, Y (μg)
A	6.28	1888.70
B	1.62	1560.60
C	7.53	2023.98
D	0.52	1448.17

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9599

$$\therefore t_{4-2} = \left| \frac{0.9599\sqrt{4-2}}{\sqrt{1-(0.9599)^2}} \right|$$

$$= 4.843$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level.Therefore, there is a correlation between cumulative amount of diclofenac released through cellulose acetate membrane and amount remaining in the stratum corneum at 1hour ($p<0.05$).

Correlation data of release rate of four diclofenac gel products through cellulose acetate membrane and amount remaining in the stratum corneum at 3 hour.

Product	amount in SC. at 3 hour, X (μg)	release rate, Y ($\mu g/min^{1/2}$)
A	3.31	99.40
B	0.91	86.92
C	4.20	102.70
D	0.92	91.58

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9649

$$\therefore t_{4-2} = \left| \frac{0.9649\sqrt{4-2}}{\sqrt{1-(0.9649)^2}} \right|$$

$$= 5.1965$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is a correlation between release rate of diclofenac through cellulose acetate membrane and amount remaining on stratum corneum at 3 hour ($p < 0.05$).

Correlation data of cumulative amount of four diclofenac gel products through cellulose acetate membrane and amount remaining in the stratum corneum at 3 hour.

Product	amount in SC. at 3 hour, X (μg)	cumulative amount, Y (μg)
A	3.31	1888.70
B	0.91	1560.60
C	4.20	2023.98
D	0.92	1448.17

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when $r = 0.9851$

$$\therefore t_{4-2} = \left| \frac{0.9851\sqrt{4-2}}{\sqrt{1-(0.9851)^2}} \right|$$

$$= 8.10$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is a correlation between cumulative amount of diclofenac through cellulose acetate membrane and amount remaining on stratum corneum at 3 hour ($p < 0.05$).

Correlation data of the release rate of four diclofenac gel products through cellulose acetate membrane and amount remaining in the stratum corneum at 6 hour.

Product	amount in SC. at 6 hour, X (μg)	release rate, Y ($\mu g/min^{1/2}$)
A	1.60	99.40
B	0.91	86.92
C	3.60	102.70
D	0.92	91.58

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.8458

$$\therefore t_{4-2} = \left| \frac{0.8458\sqrt{4-2}}{\sqrt{1-(0.8458)^2}} \right|$$

$$= 2.2421$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between release rate of diclofenac through cellulose acetate membrane and amount remaining in the stratum corneum at 6 hour ($p > 0.05$) .

Correlation data of the cumulative amount of four diclofenac gel products through cellulose acetate membrane and amount remaining in the stratum corneum at 6 hour.

Product	amount in SC. at 6 hour, X (μg)	cumulative amount, Y (μg)
A	1.60	1888.70
B	0.52	1560.60
C	3.31	2023.98
D	0.52	1448.17

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9258

$$\therefore t_{4-2} = \left| \frac{0.9258\sqrt{4-2}}{\sqrt{1-(0.9258)^2}} \right|$$

$$= 3.464$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not correlation between cumulative amount of diclofenac through cellulose acetate membrane and amount remaining in the stratum corneum at 6 hour ($p > 0.05$).

Correlation data of release rate of four diclofenac gel products through cellulose acetate membrane and AUC (0-6 hr) in the stratum corneum at 1 hour.

Product	AUC(0-6 hr), X ($\mu g.cm^2$)	Release rate, Y ($\mu g/min^{1/2}$)
A	27.72	99.40
B	7.66	86.92
C	35.04	102.70
D	9.69	91.58

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9785

$$\therefore t_{4-2} = \left| \frac{0.9785\sqrt{4-2}}{\sqrt{1-(0.9785)^2}} \right| = 6.7081$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is a correlation between release rate of diclofenac released through cellulose acetate and AUC(0-6 hr) in the stratum corneum ($p < 0.05$).

* AUC = Area under the concentration - time curve

Correlation data of cumulative amount release of four diclofenac gel products through cellulose acetate membrane and AUC (0-6 hr) in the stratum corneum .

Product	AUC(0-6 hr), X ($\mu g.cm^2$)	Cumulative Amount Y ($\mu g/min^{1/2}$)
A	27.72	1888.70
B	7.66	1560.60
C	35.04	2023.98
D	9.69	1448.17

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9731

$$\therefore t_{4-2} = \left| \frac{0.9731\sqrt{4-2}}{\sqrt{1-(0.9731)^2}} \right|$$

$$= 5.9730$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is a correlation between cumulative amount released of diclofenac through cellulose acetate and AUC(0-6 hr) in the stratum corneum ($p < 0.05$).

* AUC = Area under the concentration - time curve

Appendix XIII b (Durapore® membrane)

Correlation data of four diclofenac gel products release through Durapore® membrane and amount remaining in the stratum corneum at 0 hour

Product	Amount in SC. at 0 hour, X (μg)	Release rate, Y (μg/min$^{1/2}$)
A	16.81	64.46
B	4.89	40.64
C	15.99	63.85
D	6.28	57.92

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.8268

$$\therefore t_{n-2} = \left| \frac{0.8268\sqrt{4-2}}{\sqrt{1-(0.8268)^2}} \right|$$

$$= 2.079$$

From the correlation coefficient at 5% level of significance, $t_{0.025}$(df=2) is 4.3. Therefore, there is not a correlation between release rate of drug through Durapore® membrane and amount of drug remaining in the stratum correum at 0 hour ($p > 0.05$).

Correlation data of cumulative amount of four diclofenac gel products release through Durapore® membrane and amount remaining in the stratum corneum at 0 hour.

Product	Amount in SC. at o hour, X (μg)	Cumulative amount, Y (μg)
A	16.81	1171.26
B	4.89	654.38
C	15.99	1117.54
D	6.28	934.35

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.9111

$$\therefore t_{n-2} = \left| \frac{0.9111\sqrt{4-2}}{\sqrt{1-(0.9111)^2}} \right|$$

$$= 3.126$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significonce at the 5% level. Therefore, there is not a correlation between cumulative amount release through Durapore® membrane and amount remaining in the stratum corneum at 0 hour ($p > 0.05$).

Correlation data of release rate of four diclofenac gel products through Durapore® membrane and amount remaining in the stratum coneum at 1 hour.

Product	amount in SC. at 1 hour, X (μg)	release rate, Y (μg/min$^{1/2}$)
A	6.28	64.46
B	1.62	40.64
C	7.53	63.85
D	2.41	57.92

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.8224

$$\therefore t_{4-2} = \left| \frac{0.8224\sqrt{4-2}}{\sqrt{1-(0.8224)^2}} \right|$$

$$= 1.280$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between release rate of diclofenac through Durapore® membrane and amount remaining in the stratum corneum at 1 hour ($p > 0.05$).

Correlation data of cumulative amount of four diclofenac gel products release through Durapore® membrane and amount remaining in the stratum corneum at 1 hour.

Product	amount in SC. at 1 hour, X (μg)	cumulative amount, Y (μg)
A	6.28	1171.26
B	1.62	654.38
C	7.53	1117.54
D	2.41	934.35

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.8855

$$\therefore t_{4-2} = \left| \frac{0.8855\sqrt{4-2}}{\sqrt{1-(0.8855)^2}} \right|$$

$$= 2.695$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac release through Durapore® membrane and amount remaining in the stratum corneum at 1 hour ($p > 0.05$) .

Correlation data of the release rate of four diclofenac gel products through Durapore® membrane and amount remaining in the stratum corneum at 3 hour.

Product	amount in SC. at 3 hour, X (μg)	release rate, Y ($\mu g/min^{1/2}$)
A	3.31	64.46
B	0.91	40.64
C	4.20	63.85
D	0.92	57.92

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.7509

$$\therefore t_{4-2} = \left| \frac{0.7509\sqrt{4-2}}{\sqrt{1-(0.7509)^2}} \right|$$

$$= 1.608$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between release rate of diclofenac through Durapore® membrane and amount remaining in the stratum corneum at 3 hour($p > 0.05$) .

Correlation data of cumulative amount of four diclofenac gel products through Durapore® membrane and amount remaining in the stratum corneum at 3 hour.

Product	amount in SC. at 3 hour, X (μg)	cumulative amount, Y (μg)
A	3.31	1171.26
B	0.91	654.38
C	4.20	1117.54
D	0.92	934.35

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.8269

$$\therefore t_2 = \left| \frac{0.8269\sqrt{4-2}}{\sqrt{1-(0.8269)^2}} \right|$$

$$= 2.0793$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac release through Durapore® membrane and amount remaining in the stratum corneum at 3 hour ($p > 0.05$) .

Correlation data of the release rate of four diclofenac gel product through Durapore® membrane and amount remaining in the stratum corneum at 6 hour.

Product	amount in SC. at 6 hour, X (μg)	release rate, Y ($\mu g/min^{1/2}$)
A	1.60	64.46
B	0.52	40.64
C	3.31	63.85
D	0.52	57.92

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.6433

$$\therefore t_{4-2} = \left| \frac{0.6433\sqrt{4-2}}{\sqrt{1-(0.6433)^2}} \right|$$

$$= 1.188$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between release rate of diclofenac through Durapore® membrane and amount remaining in the stratum corneum at 6 hour ($p > 0.05$) .

Correlation data of cumulative amount of four diclofenac gel products through Durapore® membrane and amount remaining in the stratum corneum at 6 hour.

Product	amount in SC. at 6 hour, X (μg)	cumulative amount, Y (μg)
A	1.60	1171.26
B	0.52	654.38
C	3.31	1117.54
D	0.52	934.35

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when $r = 0.6851$

$$\therefore t_{4-2} = \left| \frac{0.6851\sqrt{4-2}}{\sqrt{1-(0.6851)^2}} \right|$$

$$= 1.330$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac release through Durapore® membrane and amount remaining in the stratum corneum at 6 hour ($p > 0.05$).

Correlation data of release rate of four diclofenac gel products through Durapore® membrane and AUC (0-6 hr) in the stratum corneum at 1 hour.

Product	AUC(0-6 hr), X ($\mu g.cm^2$)	Release rate, Y ($\mu g/min^{1/2}$)
A	27.72	64.46
B	7.66	40.64
C	35.04	63.85
D	9.69	57.92

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.7859

$$\therefore t_{4-2} = \left| \frac{0.7859\sqrt{4-2}}{\sqrt{1-(0.7859)^2}} \right| = 1.7972$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between release rate of diclofenac released through Durapore® and AUC(0-6 hr) in the stratum corneum

($p > 0.05$).

* AUC = Area under the concentration - time curve

Correlation data of cumulative amount release of four diclofenac gel products through Durapore® membrane and AUC (0-6 hr) in the stratum corneum .

Product	AUC(0-6 hr), X ($\mu g.cm^2$)	Cumulative Amount Y ($\mu g/min^{1/2}$)
A	27.72	647.23
B	7.66	1125.85
C	35.04	1110.06
D	9.69	342.86

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.2715

$$\therefore t_{4-2} = \left| \frac{0.2715\sqrt{4-2}}{\sqrt{1-(0.2715)^2}} \right|$$

$$= 0.3989$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount released of diclofenac through Durapore® and AUC(0-6 hr) in the stratum corneum ($p > 0.05$)

* AUC = Area under the concentration - time curve

Appendix XIIIc (Shed snake skin)

Correlation data of steady state flux of four diclofenac gel products through shed snake skin and amount remaining in the stratum corneum at 0 hour.

Product	amount in SC. at 0 hour, X (μg)	steady state flux, Y ($\mu g/hr.cm^2$)
A	16.81	17.67
B	4.89	27.74
C	15.99	41.46
D	6.28	10.12

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.3643

$$\therefore t_{4-2} = \left| \frac{0.3643\sqrt{4-2}}{\sqrt{1-(0.3643)^2}} \right|$$

$$= 0.5532$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between steady state flux of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 0 hour ($p > 0.05$).

Correlation data of steady state flux of four diclofenac gel products through shed snake skin and amount remaining in the stratum corneum at 1 hour.

Product	amount in SC. at 1 hour, X (μg)	steady state flux, Y (μg/hr.cm^2)
A	6.28	17.67
B	1.62	27.74
C	7.53	41.46
D	2.41	10.12

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.5103

$$\therefore t_{4-2} = \left| \frac{0.5103\sqrt{4-2}}{\sqrt{1-(0.5103)^2}} \right|$$

$$= 0.8392$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between steady state flux of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 1 hour ($p > 0.05$).

Correlation data of steady state flux of four diclofenac gel products through shed snake skin and amount remaining in the stratum corneum at 3 hour.

Product	amount in SC. at 3 hour, X (μg)	steady state flux, Y ($\mu g/hr.cm^2$)
A	3.31	17.67
B	0.91	27.74
C	4.20	41.46
D	0.92	10.12

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.5959

$$\therefore t_{4-2} = \left| \frac{0.5959\sqrt{4-2}}{\sqrt{1-(0.5959)^2}} \right|$$

$$= 1.0495$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between steady state flux of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 3 hour ($p > 0.05$).

Correlation data of steady state flux of four diclofenac gel products through shed snake skin and amount remaining in the stratum corneum at 6 hour.

Product	amount in SC. at 6 hour, X (μg)	steady state flux, Y ($\mu g/hr.cm^2$)
A	1.60	17.67
B	0.52	27.74
C	3.31	41.46
D	0.52	10.12

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.7646

$$\therefore t_{4-2} = \left| \frac{0.7646\sqrt{4-2}}{\sqrt{1-(0.7646)^2}} \right|$$

$$= 2.6031$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between steady state flux of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 6 hour ($p > 0.05$).

Correlation data of cumulative amount of four diclofenac gel products permeate through shed snake skin after 24 hours and amount remaining in the stratum corneum at 0 hour.

Product	amount in SC. at 0 hour, X (μg)	cumulative amount, Y (μg)
A	16.81	647.23
B	4.86	1125.85
C	15.99	1110.06
D	6.28	342.86

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.1153

$$\therefore t_{4-2} = \left| \frac{0.1153\sqrt{4-2}}{\sqrt{1-(0.1153)^2}} \right|$$

$$= 0.1642$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 0 hour ($p > 0.05$).

Correlation data of cumulative amount of four diclofenac gel products permeate through shed snake skin after 24 hours and amount remaining in the stratum corneum at 1 hour.

Product	amount in SC. at 1 hour, X (μg)	cumulative amount, Y (μg)
A	6.28	647.23
B	1.62	1125.85
C	7.53	1110.06
D	2.41	342.86

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.2080

$$\therefore t_{4-2} = \left| \frac{0.2080\sqrt{4-2}}{\sqrt{1-(0.2080)^2}} \right|$$

$$= 0.301$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 1 hour ($p > 0.05$).

Correlation data of cumulative amount of four diclofenac gel products permeate through shed snake skin after 24 hours and amount remaining in the stratum corneum at 3 hour.

Product	amount in SC. at 3 hour, X (μg)	cumulative amount, Y (μg)
A	3.31	647.23
B	0.91	1125.85
C	4.20	1110.06
D	0.92	342.86

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.3191

$$\therefore t_{4-2} = \left| \frac{0.3191\sqrt{4-2}}{\sqrt{1-(0.3191)^2}} \right|$$

$$= 0.476$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 3 hr ($p > 0.05$).

Correlation data of cumulative amount of four diclofenac gel products permeate through shed snake skin after 24 hours and amount remaining in the stratum corneum at 6 hour.

Product	amount in SC. at 6 hour, X (μg)	cumulative amount, Y (μg)
A	1.60	647.23
B	0.52	1125.85
C	3.31	1110.06
D	0.52	342.86

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.4488

$$t_{4-2} = \left| \frac{0.4488\sqrt{4-2}}{\sqrt{1-(0.4488)^2}} \right|$$

$$= 0.710$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac permeate through shed snake skin and amount remaining in the stratum corneum at 6 hour ($p > 0.05$).

Correlation data of cumulative amount of four diclofenac gel products permeate through shed snake skin after 24 hours and AUC*(0-6 hr) in the stratum corneum

Product	AUC*(0-6 hr), X (μg)	cumulative amount, Y (μg)
A	27.72	647.23
B	7.66	1125.85
C	35.04	1110.06
D	9.69	342.86

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.2715

$$t_{4-2} = \left| \frac{0.2715\sqrt{4-2}}{\sqrt{1-(0.2715)^2}} \right|$$

$$= 0.3990$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between cumulative amount of diclofenac permeate through shed snake skin and AUC(0-6 hr) in the stratum corneum ($p > 0.05$)

* AUC = Area under the concentration - time curve

Correlation data of steady state flux of four diclofenac gel products permeate through shed snake skin after 24 hours and AUC*(0-6 hr) in the stratum corneum

Product	AUC*(0-6 hr), X (μg)	Steady state flux, Y ($\mu g/hr.cm^2$)
A	27.72	17.67
B	7.66	27.74
C	35.04	41.46
D	9.69	10.12

Test of Zero Correlation :

Ho : $\rho = 0$, Ha : $\rho \neq 0$

where ρ is the true correlation coefficient, estimate by r

$$t_{n-2} = \left| \frac{r\sqrt{N-2}}{\sqrt{1-r^2}} \right|$$

when r = 0.5670

$$t_{4-2} = \left| \frac{0.5670\sqrt{4-2}}{\sqrt{1-(0.5670)^2}} \right|$$

$$= 0.9735$$

A value of $t_{0.05}$, df2 equal to 4.3 is needed for significance correlation at 5% level. Therefore, there is not a correlation between steady state flux of diclofenac permeate through shed snake skin and AUC(0-6 hr) in the stratum corneum ($p > 0.05$).

* AUC = Area under the concentration - time curve

Appendix XIV

Comparison of Area Under the Concentration-Time Curve of Four Diclofenac Diethylammonium Gel Products at 0-6 hour

Area under the Concentration-time Curve of Diclofenac Diethylammonium in the stratum Corneum at 0-6 hour

Product Subject Number	A	B	C	D
1	32.65	10.93	64.22	8.33
2	31.97	4.67	35.76	11.81
3	38.11	5.41	30.50	9.94
4	45.92	6.38	39.64	5.42
5	15.31	10.52	33.29	11.33
6	19.20	5.01	44.04	6.31
7	15.16	8.17	22.87	9.63
8	27.45	10.18	10.01	0.27
mean**± SD	27.72±11.11	7.66±2.62	35.04±15.82	9.70±3.04

Two-way ANOVA

Grand means

Treatment means

Product A = 27.72

Product B = 7.66

Product C = 35.04

Product D = 9.69

Subject means

Subject No. 1 = 29.03 μg No. 5 = 17.61 μg

No. 2 = 21.05 μg No. 6 = 17.64 μg

No. 3 = 20.99 μg No. 7 = 15.25 μg

No.4 = 24.34 μg No. 8 = 14.31 μg

Source	df.	SS	MS	F	Prob.
Treatment (A)	3	4354.387	1451.462	14.2375	0.0001
Subject (B)	7	674.875	96.4107	0.9457	0.5056
Error	21	2140.883			
Total	31	7170.145			

Duncan's test

MS Error = 96.4107 , df Error = 21

Significance level = 0.05

Least Significance Ranges

LSR where p = 2.....10.504

LSR where p = 3.....12.735

LSR where p = 4.....14.083

Result : B < D < A < C

VITA

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