CHAPTER 2

N I F E D I P I N E

Experimental

Materials : Nifedipine (Nfd., courtesy Sarabhai Research Centre, Baroda, India), Polyethylene glycol 6000 (s.d. Fine Chemicals, Boisar, India), Citric acid (s.d. Fine Chemicals, Boisar, India), Urea (Sarabhai M. Chemicals, Baroda, India), Mannitol (Sarabhai M. Chemicals, Baroda, India) and Poloxamer 188 and 407 (BASF WyandotteCorp., U.S.A), were used as supplied. All other chemicals were either of pharmacoepoeial or reagent grade.

Methods : Particle size and size distributions - Particle size (length mean diameter) and particle size distribution were determined by optical microscopy.

Preparation of calibration curve - About 0.02 g of Nifedipine was dissolved in 50 ml. of methanol and diluted with dist. water to make 100 ml. Aliquots were withdrawn from this and diluted appropriately to get a range of concentrations (0-20 µg/ml), the absorbance of which were measured at 340 nm using Spectronic 20 (Bausch & Lomb visible spectrophotometer), The Beer's plot is shown in Fig. 2.

Preparation of dispersion systems - Physical mixtures - The physical mixtures were prepared by mechanically mixing together weighed quantities of preseived fraction (150 - 250 µm) of drug and excipients, in a cylindrical laboratory mixer for about 10 minutes.

Fused mixtures - The fused mixtures were prepared by heating the corresponding ground physical mixture in a porcelain dish

to about 5–10℃ above the melting point of excepient, with continuous mixing for 60 sec., as adopted by Kaur et al, (1980). The samples were immediately quenched to 4°C, the resulting solid was scrapped out and stored in a desiccator at room temperature for 48 hrs. The samples were ground using a glass pestle and mortar and fraction of 150–250 μm was collected prior to conducting dissolution studies. The drug excipient ratio was analytically confirmed in both physical mixtures and solid dispersion systems, the drug content being between 90–110% w/w. The composition of systems prepared and evaluated are shown in Table : 1.

Infrared spectral studies - IR spectra of pure drug, excipients, physical mixtures and solid dispersions of two selected systems were scanned in nujol on Shimadzu recording IR spectrophotometer (Shimadzu Corporation, Japan).

Solubility determinations - To determine any possible interaction between drug and excipients in aqueous solution, solubility studies were performed. An excess amount of drug was placed in 30 ml. glass vials equipped with aluminium seals, containing 20 ml. of an aqueous solution of excipients in varying concentrations. The contents in the vials were shaken in a gyratory incubator shaker at 37° for 12 hrs. and then allowed to equilibrate overnight, then the aliquots were withdrawn, filtered through a G–4 sintered funnel, suitably diluted and analysed at 340 nm using Spectronic - 20

(Bausch & Lomb visible spectrophotometer). The aliquots were diluted in such a way, if needed, to avoid any interference because of excipient.

Dissolution studies - The dissolution rate studies were conducted by two different methods viz. U.S.P. method II and Tape method (Goldberg et al, 1965).

Dissolution rate studies using U.S.P. method II - Dissolution rates of pure drug, physical mixtures and solid dispersion systems were studied in 900 ml. of distilled water at 37 ± 1°C. The paddle was rotated at 100 r.p.m. Test preparations containing equivalent of 10 mg. of Nifedipine, were added on the surface of dissolution media. Five ml. of samples were removed as a function of time and analysed for drug content after filtering them through a G-4 sintered funnel. The volume was replaced by fresh dissolution medium. A cumulative correction was made for the previously removed samples. Nifedipine content was analysed at 340 nm and no dilution of the samples was necessary. There was no or negligible absorption due to the presence of excipients at this wavelength.

Tape method - This procedure involves accurately weighing and quantitatively transferring the screened material to be tested to a taut, adhesive surface and in turn, introducing the resulting monoparticulate layer to 400 ml. of distilled water in a 500 ml beaker maintained at 37°. Stirring rate was maintained at 100 r.p.m. by means of constant speed motor.

The stir paddle was having the following dimensions (55x23 mm) which was more than the exposed surface of Tape (40x25 mm) (Goldberg et al, 1965). The increased speed and wider stir paddle was necessitated by the extremely low solubility of Nifedipine.

Two selected systems were evaluated by the tape method. Sampling times were 1,2, 3, 5, 10 & 15 min. At each time interval 5 ml. samples were withdrawn and filtered through a G-4 sintered funnel. The volume was immediately replaced by 5 ml. of fresh distilled water. Nifedipine concentration was determined spectrophotometrically at 340 nm. All values were corrected to account for drug removed by prior sampling. All dissolution experiments were carried out with the aid of sodium lamp.

Solid - solid interaction - Phase diagrams of two selected systems were constructed in order to gain an insight into the mechanism of enhanced dissolution. The phase diagrams were constructed employing hot stage microscopy.

Hot stage microscopy - About 1 mg. of sample was placed between a microscope slide and a cover slide and heated at 1-5°/min under a hot stage microscope(Richert, Austria) fitted with polarizers. The onset of melting was characterized by the first appearance of liquid and disappearance of solid was considered as the completion of melting.

X-ray diffraction studies - X-ray diffraction (Philips x-ray diffractomer, Phillips, Eindhowen, Netherlands) patterns

were obtained from two selected test preparations, physical mixtures, excipients and drug. X-ray diffraction patterns were obtained on powder samples using nickel filtered copper radiation at the scanning speed of 2°/min. in the form a 2θ angle.

Photomicrographic studies - Photomicrographs of various samples were taken on polorising microscope (Leitz Laborlux, W. Germany).

Stability studies - In order to study the drug stability, test preparations selected for in-vivo studies, were kept under normal temperature and humidity conditions in dark. These preparations were analysed for the intact drug at frequent intervals for a period of two years, by spectrophotometry at 340 nm.

Aging studies - This was carried out with the view to assess the effect of storage time on dissolution behaviour of test preparations selected for in-vivo studies. The test preparations were stored under normal ambient conditions with time to time analysis of their dissolution behaviour over a period of twelve months.

Bioavailability study - a) Four preparations namely, Nfd-Poloxamer 188 solid dispersion, Nfd-PEG 6000 solid dispersion, Adalat capsule (Bayer) and a test tablet (Sarabhai Chemicals Ltd., Baroda, India) were administered orally to healthy male volunteers. All these dosage had dose equivalent to 10 mg. of Nifedipine.

b) Subjects - A four way cross over study was carried out in six healthy male volunteers aged between 34 and 43 years (mean 38.2 yrs) and weighing between 54 and 81 kg. (mean 64.5 kg). The volunteers gave their voluntary written consent after the object and the procedure of the trial had been fully explained to them. No abnormalities were found on clinical examination, in the results of hematological and biochemical profiles of the subjects.

c) Trial design - A preparation was administered to each subject with 200 ml. of water following an overnight fast. The volunteers continued fasting for 4 hrs. after the dose was given. Blood samples were taken by venipuncture into centrifuge tubes containing heparin at ½ , 1, 2, 4 & 6 hrs. after administration and stored at 4°C in dark till analysed. A wash out period of ten days was allowed between various treatments.

d) Assay of the plasma level - The plasma samples were assayed for Nifedipine by the method of Jakobsen et al (1979) using gas chromatography. The slope of Beer's law plot [conc. (ng/0.5 ml) vs. height of peak (mm)] was 1.121 with correlation coefficient r = 0.997. All samples were analysed with the aid of sodium vapour lamp.

Results & Discussion

Nifedipine (mean length dia. 88.3 μm) did not show any degredation during the preparation (Fig. 1), which was checked by monitoring the drug content in solid dispersions which were within the compenedial limits (90-110% w/w) and measuring the λ max in ethanol - water system which did not show any shift (238 nm & 338 nm).

Most of the solid dispersion systems did show considerable improvement in their dissolution in comparision to drug alone. The change in dissolution rate varied from as low as 7.6 times to about 29 times at 60 min. amongst solid dispersion systems (test preparations) of varying compositions with respect to drug. This was 14.3 and 4 times at 120 min. (Table : 2-7 & Fig. 3-8).

Table 1 - Compositions of various solid dispersion systems & physical mixtures of Nifedipine alongwith in-vitro method employed.

Systems	Ratio (w/w) (Nfd:Excipient)	USP Method II	Tape Method
Solid dispersions			
Nfd-Urea	1 : 49	*	
Nfd- citric acid	1 : 49	*	
Nfd - Mannitol	1 : 49	*	
Nfd - Mannitol	1 : 19	*	
Nfd - PEG 6000	1 : 49	*	*

Systems	Ration w/w (Nfd: Excipient)	USP Method II	Tape Method
Nfd - PEG 6000	1 : 19	*	*
Nfd - Poloxamer 188	1 : 49	*	*
Nfd - Poloxamer 188	1 : 19	*	*
Nfd - Poloxamer 407	1 : 49	*	
Nfd - Poloxamer 407	1 : 19	*	
Physical mixtures			
Nfd - Urea	1 : 49	*	
Nfd - Citric acid	1 : 49	*	
Nfd - Mannitol	1 : 49	*	
Nfd - PEG 6000	1 : 49	*	*
Nfd - Poloxamer 188	1 : 49	*	*
Nfd - Poloxamer 407	1 : 49	*	
Nifedipine	100 : 0	*	*

* Systems evaluated.

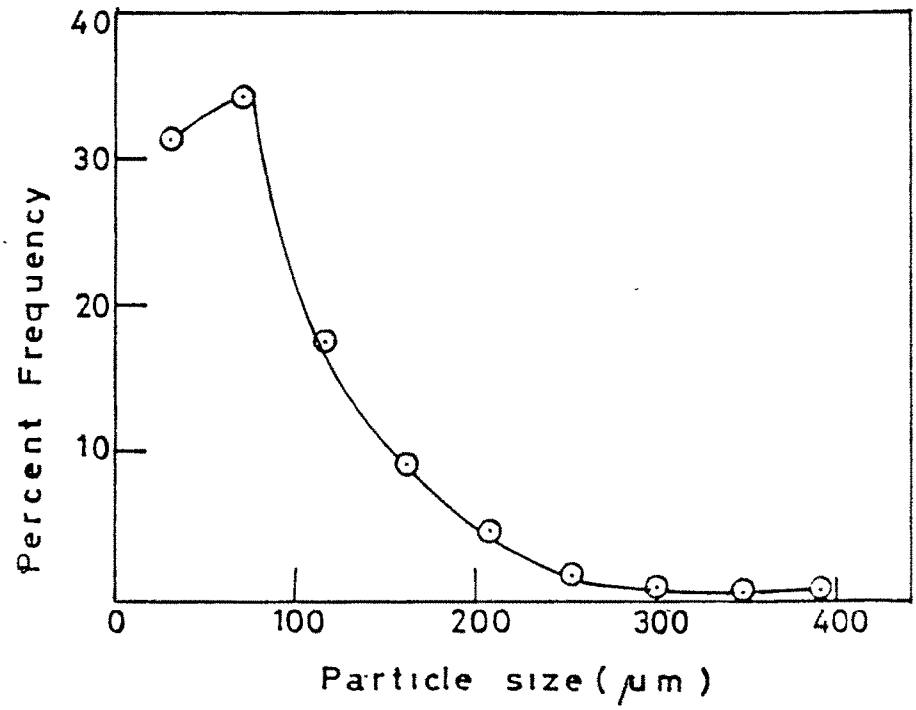

Fig. 1 PARTICLE SIZE DISTRIBUTION OF NIFEDIPINE.

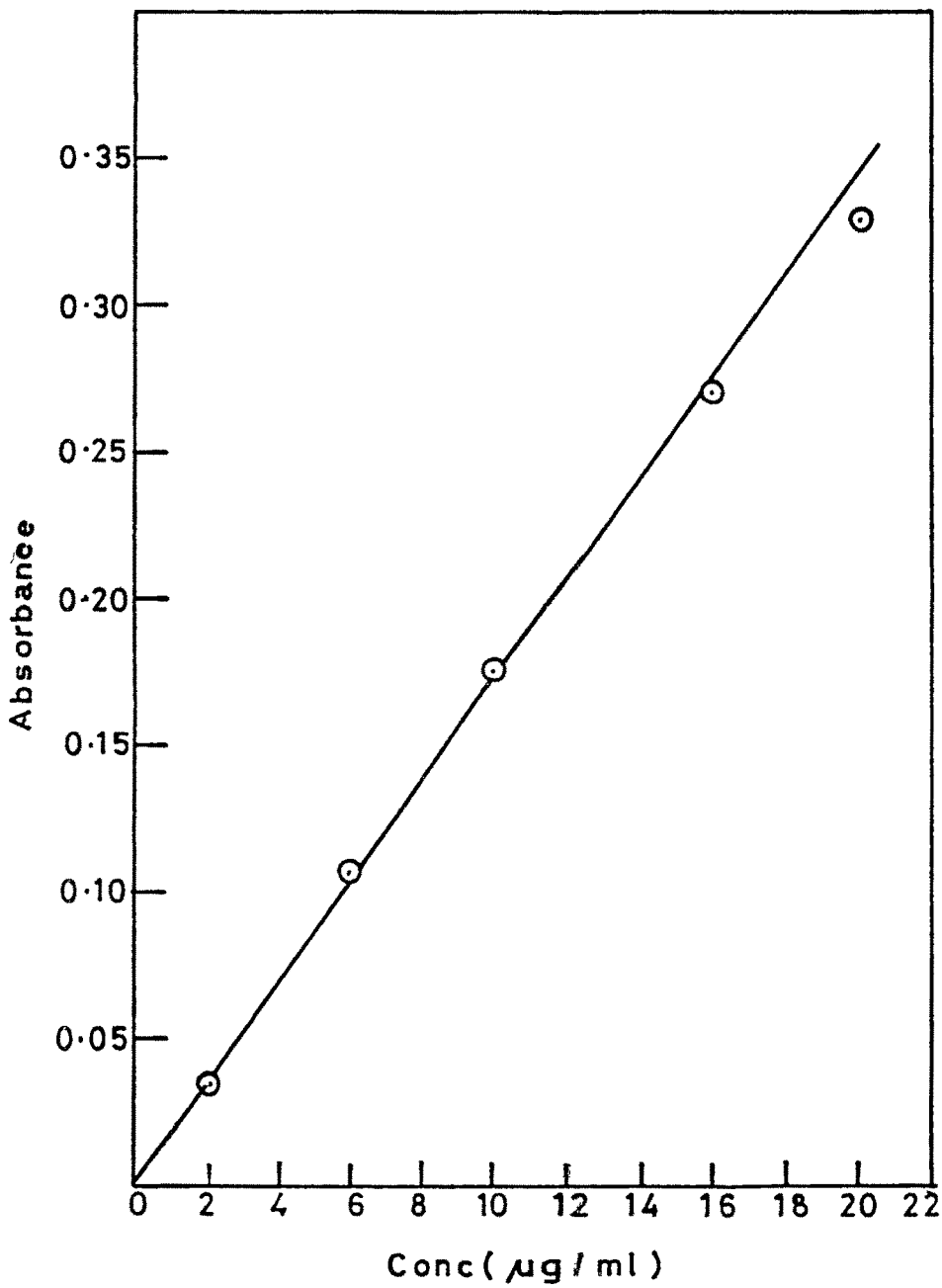

Fig 2 CALIBRATION CURVE OF NIFEDIPINE.

Table : 2 - Dissolution rates of Nifedipine from test preparations.

Systems	Average amount of Nifedipine released (mg/900 ml).						
	5	10	20	40	60	90	120 (min)
Solid dispersions							
Nfd-Urea(1:49)	2.21	2.83	3.51	4.08	4.35	4.60	5.01
Physical mixture							
Nfd-Urea(1:49)	0.46	0.25	0.25	0.41	0.46	0.61	0.77
Nfd.	0.31	0.15	0.10	0.36	0.36	0.61	0.72

Table : 3 - Dissolution rates of Nifedipine from test preparations.

Systems	Average amount of Nifedipine released (mg/900 ml).						
	5	10	20	40	60	90	120 (min)
Solid dispersion							
Nfd-Citric acid (1:49)	4.42	5.22	5.22	6.04	5.89	5.95	6.21
Physical mixture							
Nfd-Citric acid (1:49)	0.61	0.51	0.61	0.72	0.82	1.03	1.19
Nfd.	0.31	0.15	0.10	0.36	0.36	0.61	0.72

Table : 4 - Dissolution rates of Nifedipine from test preparations.

Systems	Average amount of Nifedipine released (mg/900 ml)						
	5	10	20	40	60	90	120 (min)
Solid dispersions							
Nfd - Mannitol (1:49)	2.62	3.36	4.03	4.70	4.91	5.22	5.63
Nfd-Mannitol (1:19)	1.75	1.81	2.22	2.63	2.95	3.26	3.56
Physical mixture							
Nfd-Mannitol (1:49)	0.12	0.16	0.23	0.20	0.50	0.47	0.88
Nfd.	0.31	0.15	0.10	0.36	0.36	0.61	0.72

Table : 5 - Dissolution rates of Nifedipine from test preparations.

Systems	Average amount of Nifedipine released (mg/900 ml)						
	5	10	20	40	60	90	120 (min)
Solid dispersions							
Nfd-PEG 6000 (1:49)	8.17	9.76	9.41	10.08	10.34	10.23	10.29
Nfd - PEG 6000 (1:19)	3.45	5.06	6.09	7.13	7.33	7.69	7.76
Physical mixture							
Nfd - PEG 6000 (1:49)	0.67	1.65	0.98	1.14	1.14	1.50	1.39
Nfd.	0.31	0.15	0.10	0.36	0.36	0.61	0.72

Table : 6 - Dissolution rates of Nifedipine from test preparations.

Systems	Average amount of Nifedipine released (mg/900 ml)						
	5	10	20	40	60	90	120(min)
Solid dispersions							
Nfd - Poloxamer 188 (1: 49)	6.17	6.97	6.97	7.49	7.40	7.34	7.34
Nfd - Poloxamer 188 (1 : 19)	3.13	4.49	5.07	5.32	5.79	6.09	6.16
Physical mixture							
Nfd - Poloxamer 188 (1 : 49)	0.46	0.36	0.51	0.61	0.71	0.94	1.17
Nfd.	0.31	0.15	0.10	0.36	0.36	0.61	0.72

Table : 7 - Dissolution rates of Nifedipine from test preparations.

Systems	Average amount of Nifedipine released (mg/900 ml)						
	5	10	20	40	60	90	120(min)
Solid dispersions							
Nfd-Poloxamer 407 (1:49)	1.90	2.99	2.84	2.23	2.74	2.69	2.90
Nfd-Poloxamer 407 (1:19)	2.67	4.23	4.86	5.27	5.48	5.63	5.58
Physical mixture							
Nfd-Poloxamer 407 (1:49)	No detectable release						
Nfd.	0.31	0.15	0.10	0.36	0.36	0.61	0.72

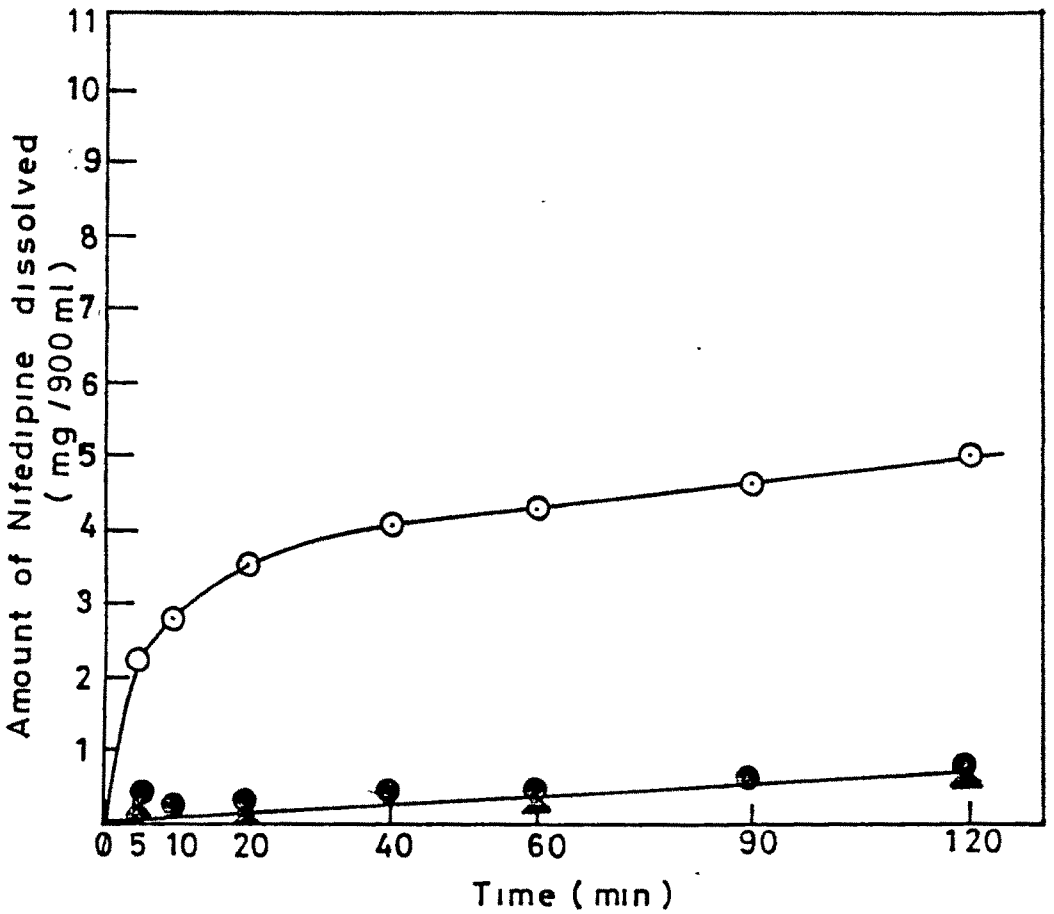

Fig_3 DISSOLUTION PROFILE OF Nfd, Nfd-UREA SD AND Nfd-UREA PM (USP method II).

KEY : ⊙ Nfd-UREA (1:49) SD.

● Nfd-UREA (1:49) PM.

▲ Nfd.

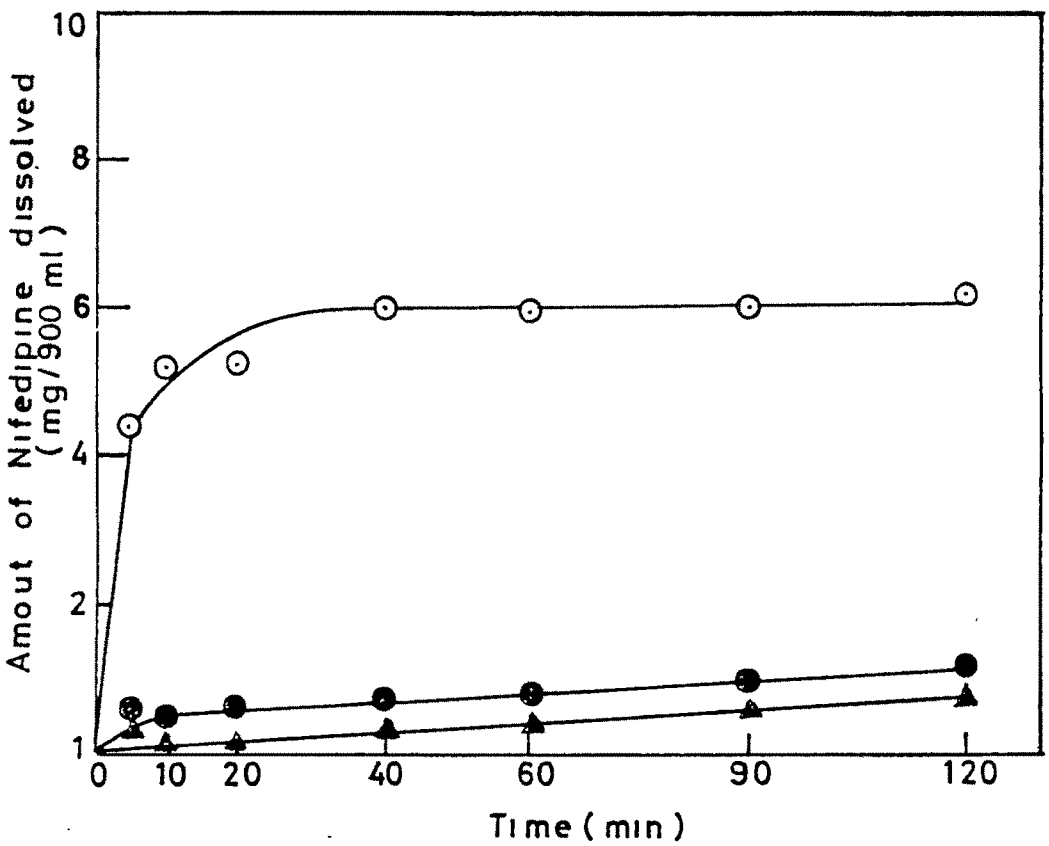

Fig. 4 DISSOLUTION PROFILE OF Nfd-CITRIC SD, Nfd-CITRIC ACID PM AND Nfd (USP METHOD II).

KEY : ⊙ Nfd-CITRIC ACID (1:49);
● Nfd-CITRIC ACID (1:49) PM;
▲ Nfd.

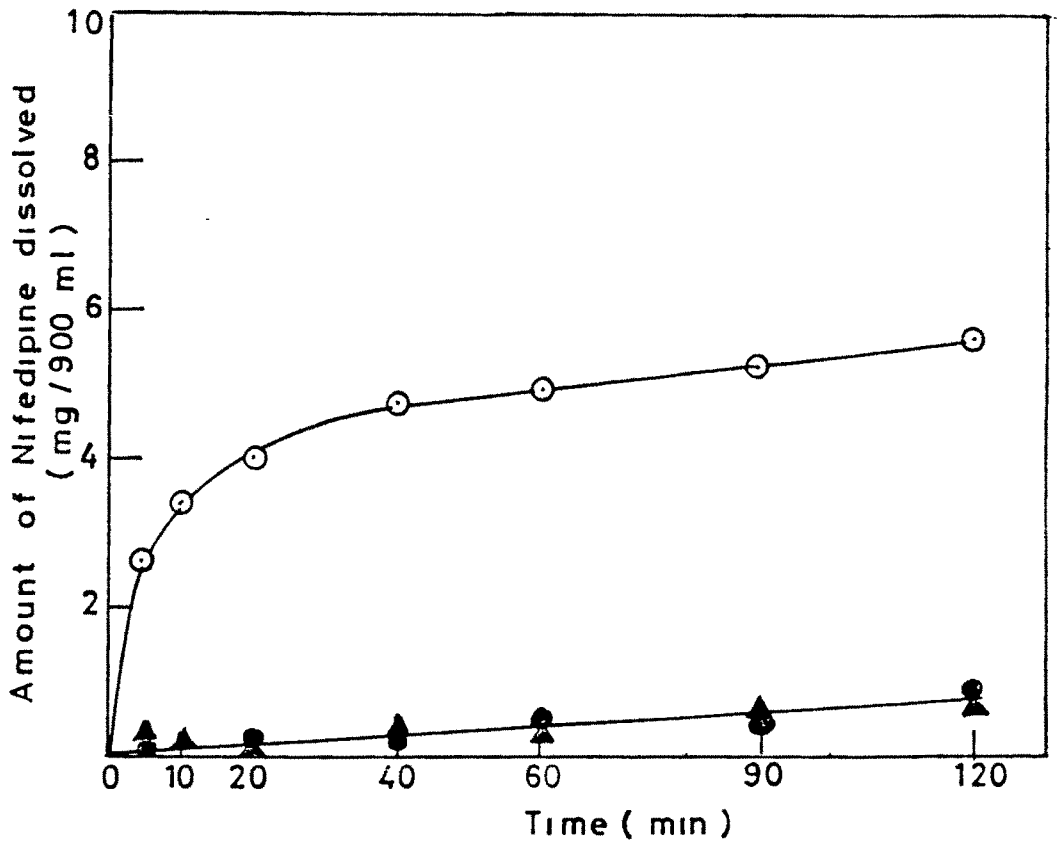

Fig 5 DISSOLUTION PROFILE OF Nfd-MANNITOL SD, Nfd-MANNITOL PM AND Nfd (USP METHOD II)

KEY : ⊙ Nfd-MANNITOL (1:49) SD;

● Nfd-MANNITOL (1:49) PM;

▲ Nfd.

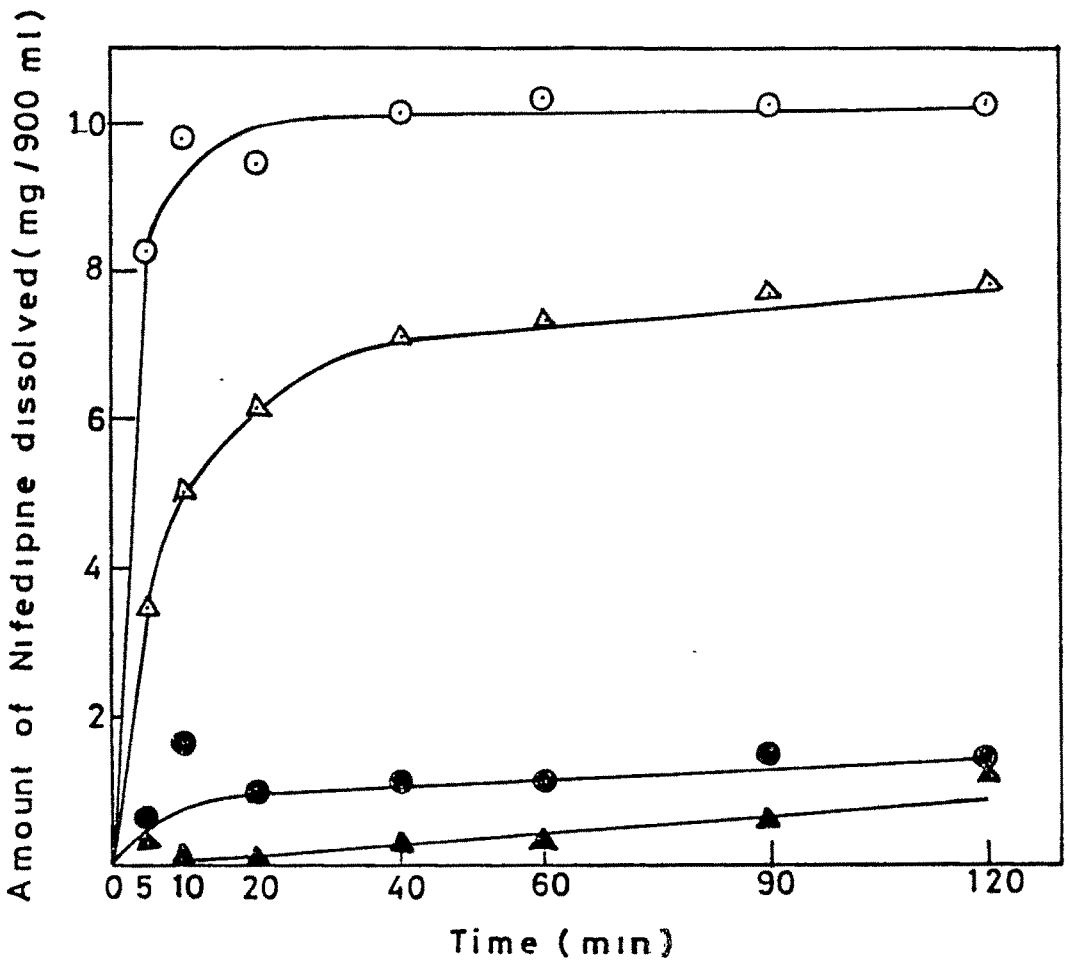

Fig. 6 DISSOLUTION PROFILES OF Nfd - PEG6000 SDS, Nfd - PEG6000 PM AND Nfd (USP method II)

KEY : ⊙ Nfd - PEG6000 (1:49) SD;
△ Nfd - PEG6000 (1:19) SD;
● Nfd - PEG 6000 (1:49) PM; ▲ Nfd.

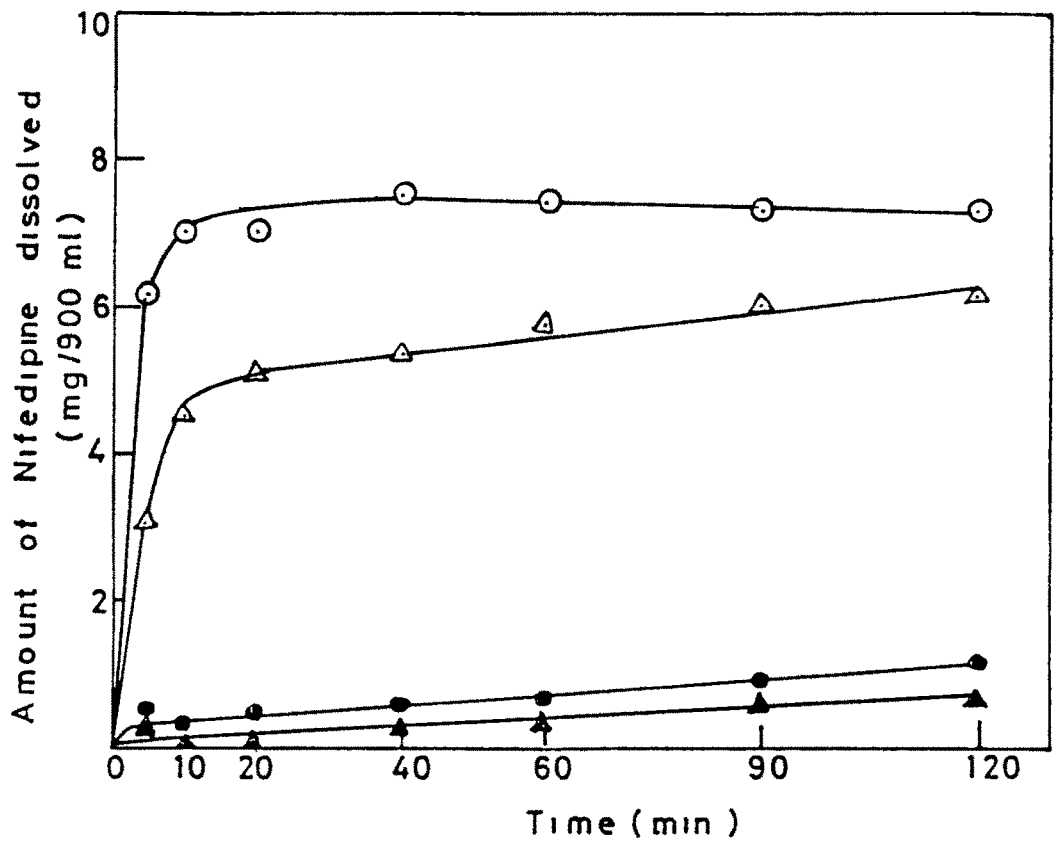

Fig 7 DISSOLUTION PROFILES OF Nfd-POLOXAMER 188 SDS, Nfd-POLOXAMER 188 PM AND Nfd (USP method II).

KEY: ⊙ Nfd-POLOXAMER 188 (1:49) SD;
△ Nfd-POLOXAMER 188 (1:19) SD;
● Nfd-POLOXAMER 188 (1:49) PM;
▲ Nfd.

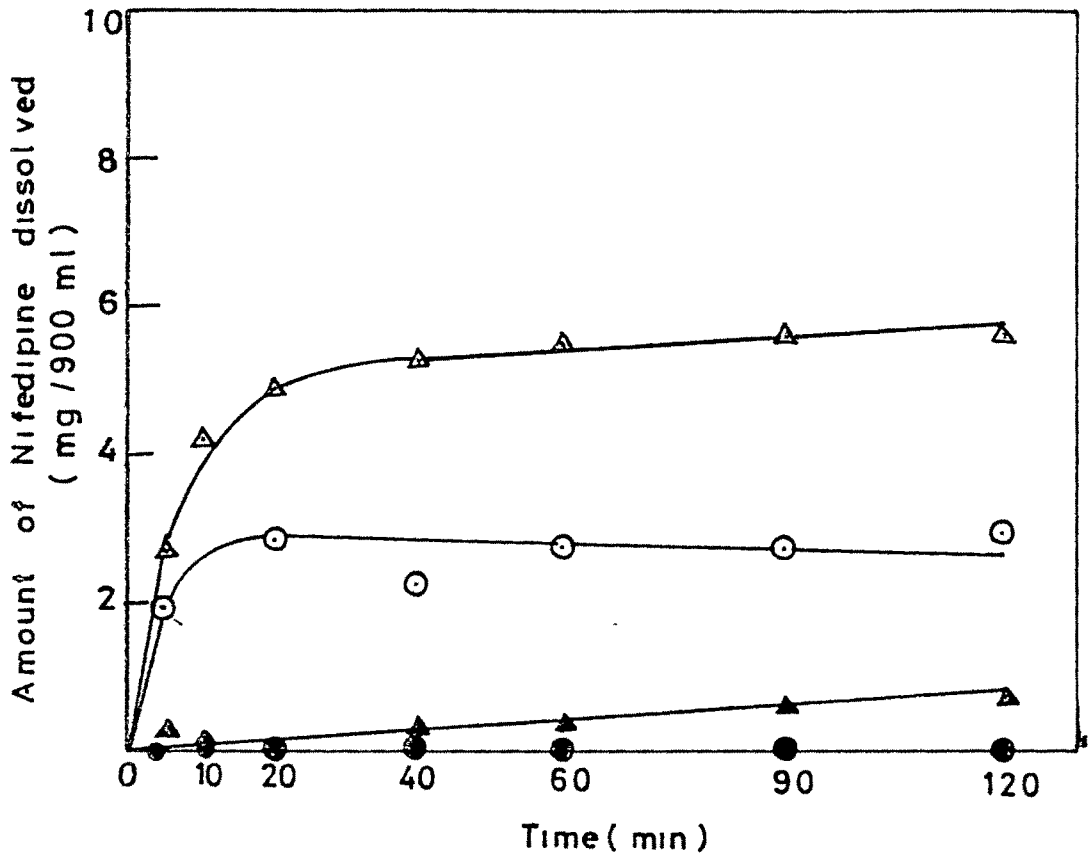

Fig 8 DISSOLUTION PROFILES OF Nfd - POLOXAMER 407 SDS, Nfd-POLOXAMER 407 PM AND Nfd (USP method II)

KEY : ⊙ Nfd - POLOXAMER 407 (1:49) SD ,
△ Nfd - POLOXAMER 407 (1:19) SD,
● Nfd - POLOXAMER (1:49) PM
▲ Nfd .

The order of release at 2% Nifedipine concentration (60 min) was PEG 6000 > Poloxamer 188 > Citric acid > Mannitol > Urea > Poloxamer 407 whereas at 5% Nifedipine concentration it was PEG 6000 > Poloxamer 188 > Poloxamer 407 > Mannitol. From these observations, it is very much evident that PEG 6000 and Poloxamer 188 exerted strongest in improving the dissolution rate of Nifedipine.

The dissolution profile of physical mixtures having 2% Nifedipine, did not show any appreciable improvement in the dissolution rate of drug, the maximum being about 3 fold increase in case of Nfd - PEG 6000 system.

Therefore, the possibility of the local effect of excipients in substantially improving the dissolution rates may not be holding. Looking at these figures, it is noticeable that there exists large differences in dissolution rates amongst various solid dispersion systems. In order to reason out for the differences among various systems, the possible effects of different concentrations of excipients, on the aqueous solubility of Nifedipine were investigated. Most of the excipients did show an increase in the solubility of Nifedipine showing their solubilizing power to varying degree (Fig. 9). Poloxamer 407 was most effective followed by Poloxamer 188, Mannitol did not produce any effect on the aqueous solubility of Nifedipine. The excipient concentration in dissolution fluid at maximum is 0.05% w/v

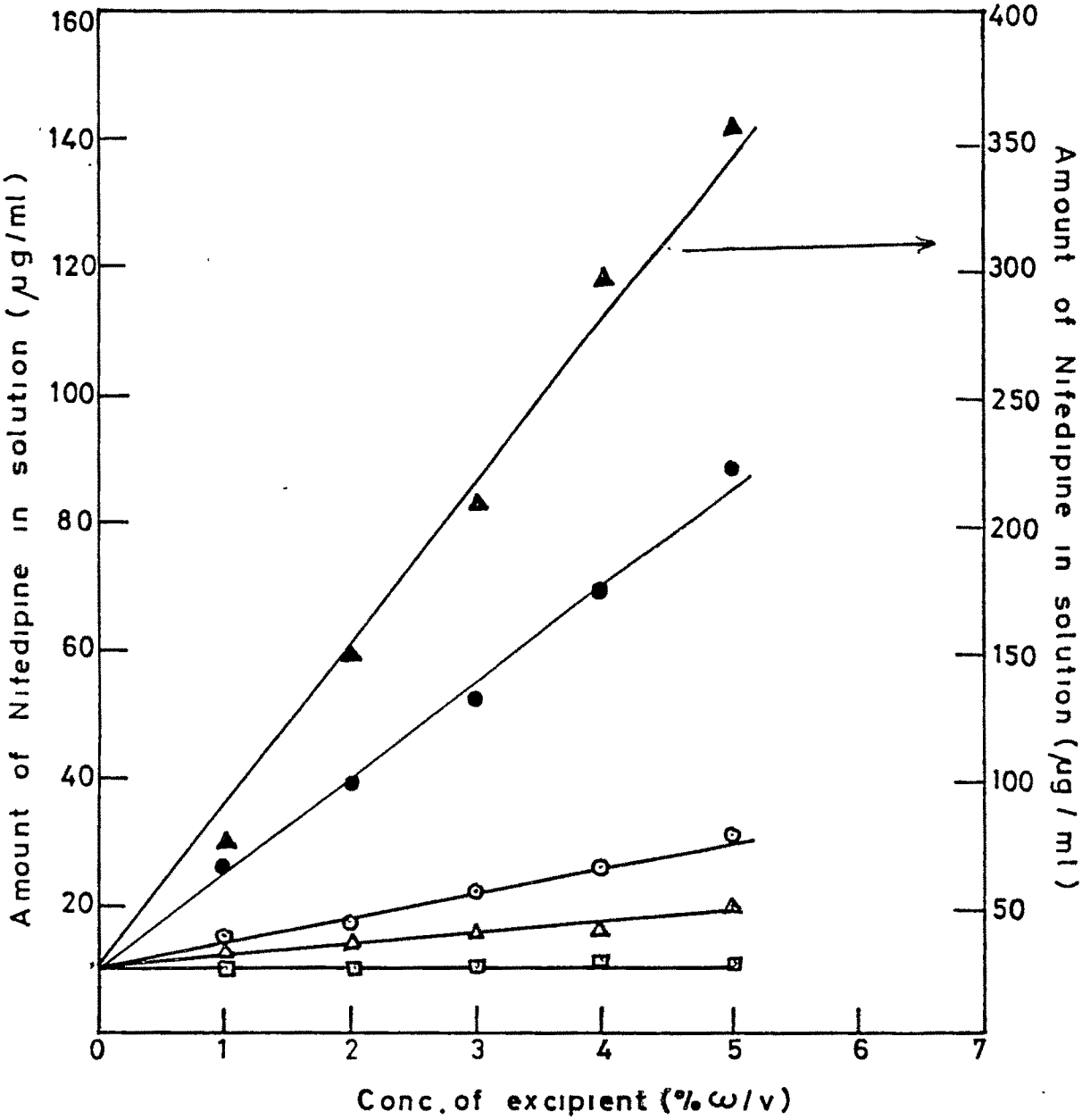

Fig. 9 EFFECT OF EXCIPIENTS ON THE SOLUBILITY OF NIFEDIPINE IN WATER AT 37 ±1°C.

KEY : ▲ POLOXAMER 407; ● POLOXAMER 188.
⊙ PEG6000; △ UREA ; ▣ MANNITOL .

and minimum is 0.02% w/v. There may not be sufficient reason to presume that increased dissolution rates are solely due only to solubilization. This may further be substantiated by dissolution study of physical mixtures at 2% drug composition. Even at 2% drug composition, there is no appreciable effect on the dissolution rate of drug, during the study period.

Fig. 3 to 8, also reflect a common feature (except Nfd-Poloxamer 407 solid dispersion system) that larger the ratio of excipients to drug, the greater the dissolution of drug from these solid dispersion systems improved. The probable reason for hindering the dissolution rate in case of Nfd-Poloxamer 407 system may be the increased viscosity of dissolution fluid as a result of using higher concentration of Poloxamer 407 in relation to the system having lower concentration of excipient.

Two of these systems were also evaluated using Tape method (Goldberg et al, 1965) meant for monodisperse particles. There was no interference in the special measurements due to the adhesive used.

The dissolution data recorded (Table - 8, 9 and Fig : 10, 11 so far also show a great degree of improvement in the dissolution rates of solid dispersion systems. The degree of improvement of the dissolution rates from such solid dispersion could not be quantitated as there was virtually no release from drug alone as well as physical mixtures. Still solid

Table : 8 - Dissolution rates * of Nifedipine from test preparations by Tape method.

Systems	Average amount of Nifedipine released (mg/400 ml)					
	1	2	3	5	10	15 (min)
Solid dispersion						
Nfd - PEG 6000 (1:49)	0.320	0.651	0.834	1.005	1.142	1.234
Nfd - PEG 6000 (1:19)	0.176	0.187	0.205	0.251	0.343	0.394
Physical mixture						
Nfd - PEG 6000 (1:49)	No release					
Nfd.	No release					

* Average of five determinations.

Table : 9 - Dissolution rates* of Nifedipine from test preparations by Tape method.

Systems	Average amount of Nifedipine released (mg/400 ml)					
	1	2	3	5	10	15 (min)
Solid dispersions						
Nfd - Poloxamer 188 (1:49)	0.480	0.960	1.257	1.394	1.440	1.441
Nfd - Poloxamer 188 (1:19)	0.514	0.571	0.765	0.914	1.097	1.188
Physical mixture						
Nfd - Poloxamer 188 (1:49)	No release					
Nfd.	No release					

* Average of five determinations.

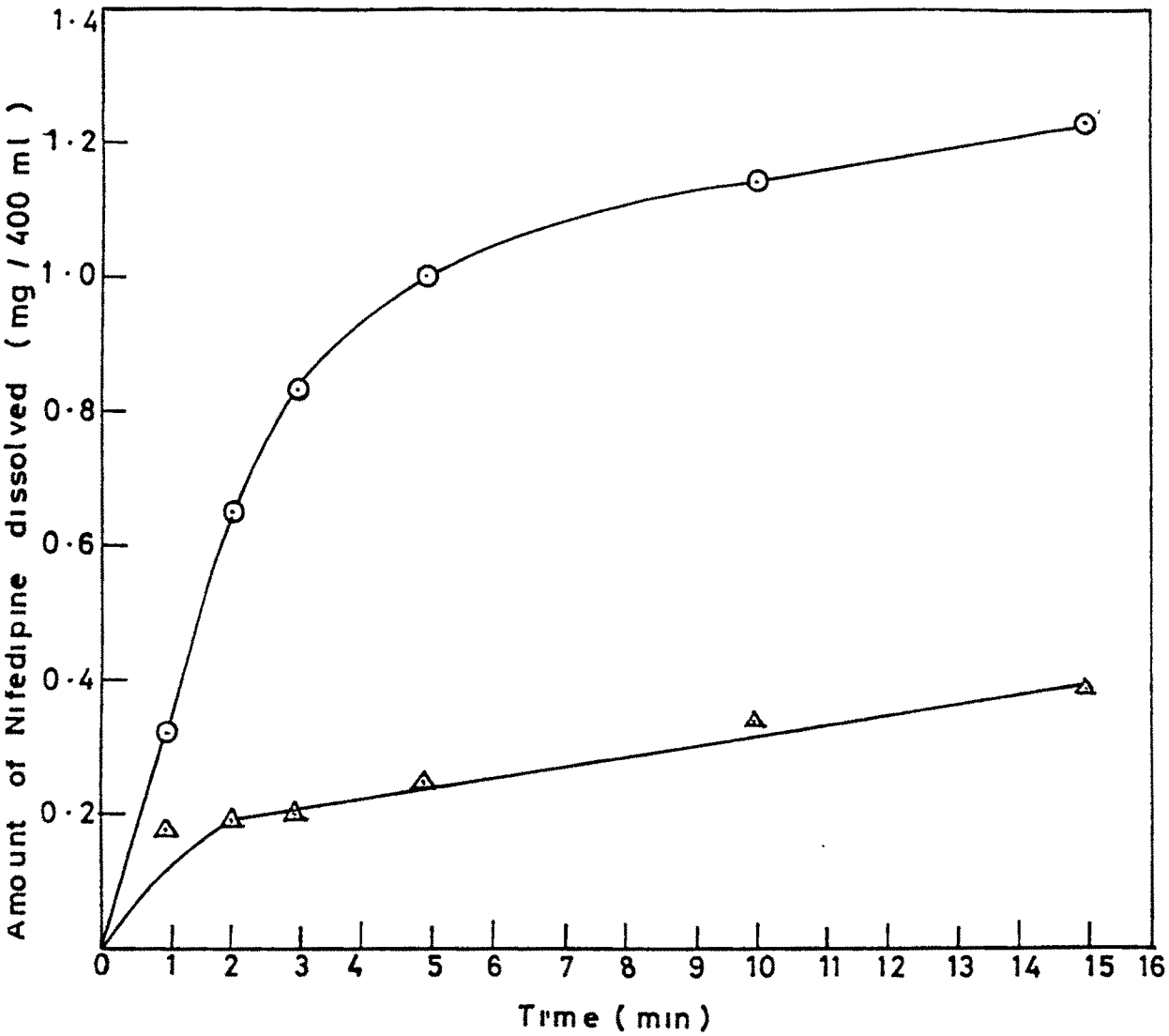

Fig 10 DISSOLUTION PROFILES OF Nfd – PEG6000 SDS (Tape method)

KEY : ⊙ Nfd - PEG6000 (1:49) SD.

△ Nfd - PEG6000 (1:19) SD.

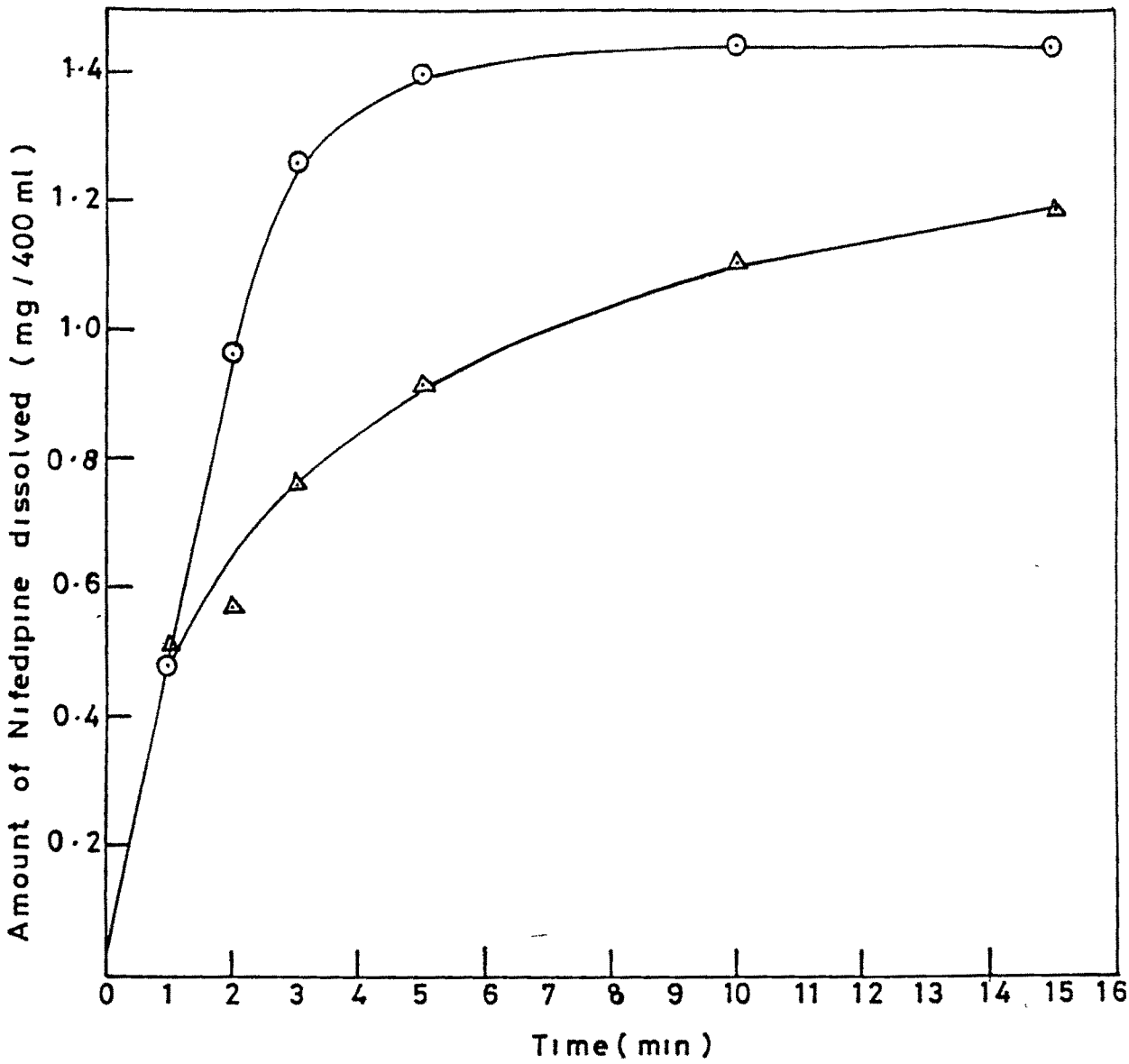

Fig 11 DISSOLUTION PROFILE OF Nfd - PÔLOXAMER 188 SDS (tape method).

KEY: ⊙ Nfd - POLOXAMER 188 (1:49) SD;

Δ Nfd - POLOXAMER 188 (1:19) SD.

dispersions of different compositions can be evaluated in terms of their drug releasing capacities in apparent sink conditions, relative to each other.

In case of Nfd-PEG 6000 solid dispersion systems, the test preparation containing (1:49, 2% w/w) drug exhibited about 4 times the dissolution rate to that of test preparation containing (1:19, 5% w/w) drug at 3 min. While it was about 3 times at 15 min.

In Nfd-Poloxamer 188 system, the test preparation containing (2% w/w, 1:49) showed 1.6 times, the dissolution rate shown by test preparation containing (5% w/w, 1:19) drug at 3 min., whereas it was 1.2 times at 15 min.

While comparing the dissolution rates from these two systems Nfd-Poloxamer 188 system was observed to yield better dissolution rates than Nfd-PEG 6000 system. This is quite contrary to earlier observations of dissolution studies in the preceeding part of this section. This may be due to difference in the volume of dissolution fluid in relation to amount of drug used and the agitational conditions.

The dissolution rate data was analysed by appropriately fitting these data in various mathematical models. While it did not fit zero order, yet initial dissolution rates upto 5 min fitted in Hixson Crowell's model and cube root dissolution rate constants were computed (Table : 10 - 13).

Table : 10 - Cube root dissolution rate constants of solid dispersion system

Nfd - PEG 6000 (1:49)

Time (min)	Amt. dissolved (mg)	Amt. undisso-lved (mg)	Amt. undisso-lved (g).	$w^{1/3}$	$wo^{1/3} - w^{1/3}$	K
0	0	2.0	0.002	0	0	0
1	0.320	1.68	0.0017	0.122	0.0066	0.0066
2	0.651	1.349	0.0013	0.111	0.0176	0.0088
3	0.834	1.166	0.0012	0.108	0.0206	0.0069
5	1.005	0.995	0.0010	0.102	0.0266	0.0053

K (Average) = 0.0069 $g^{1/3}$ min^{-1}

Table : 11 - Cube root dissolution rate constants of solid dispersion system Nfd - PEG 6000 (1:19)

Time (min)	Amt. dissolved (mg)	Amt. undissolved(mg)	Amt. undissolved (g).	$w^{1/3}$	$wo^{1/3} - w^{1/3}$	K
0	0	2.0	0.002	-	0	0
1	0.176	1.824	0.00182	0.1246	0.004	0.0040
2	0.187	1.813	0.00181	0.1244	0.0042	0.0021
3	0.205	1.795	0.00179	0.1250	0.0046	0.0015
5	0.251	1.749	0.00175	0.1230	0.0056	0.0011

K (Average) : 0.0022 $g^{1/3}$ min^{-1}

Table : 12 - Cube root dissolution rate constants of solid dispersion system. Nfd - Poloxamer 188 (1:49)

Time (min)	Amt. dissolved (mg)	Amt. undissolved (mg)	Amt. undissolved (g)	$W^{1/3}$	$W_o^{1/3} - W^{1/3}$	K
0	0	2.0	0.002	-	0	0
1	0.48	1.52	0.0015	0.116	0.0126	0.0126
2	0.96	1.04	0.0010	0.102	0.026	0.013
3	1.257	0.743	0.0074	0.092	0.0366	0.0122
5	1.394	0.606	0.006	0.086	0.0426	0.0085

K (Average) : 0.0115 $g^{1/3}$ min^{-1}

Table : 13 - Cube root dissolution rate constant of solid dispersion system Nfd - Poloxamer 188 (1:19).

Time (min)	Amt. dissolved (mg)	Amt. undissolved (mg)	Amt. undissolved (g)	$w^{1/3}$	$wo^{1/3} - w^{1/3}$	K
0	0	2.0	0.002	-	0	0
1	0.514	1.486	0.00148	0.116	0.0126	0.0126
2	0.571	1.429	0.00143	0.115	0.0136	0.0068
3	0.765	1.235	0.00123	0.109	0.0196	0.0065
5	0.914	1.086	0.00108	0.105	0.0236	0.0047

K (Average) : 0.0076 $g^{1/3}$ min^{-1}

The value of cube root dissolution rate constant for test preparation containing (2% Nfd, 1:49) was about 3 times the value for test preparation containing (5% w/w Nfd, 1:19). These results are in general agreement of earlier observations regarding excipient to drug ratio.

Likewise, Nfd-Poloxamer 188 system had also shown somewhat similar results but the value of cube root dissolution rate constant for test preparation containing (2% Nfd, 1:49) drug was about 1.5 times the other test preparation containing (5% Nfd, 1 : 19) drug.

In order to investigate the reason for the enhancement of the dissolution rate of two selected test preparations namely Nfd-PEG 6000(1:19) and Nfd-Poloxamer 188 (1:19), the x-ray diffraction patterns of fine granules were measured to examine the crystallanity of Nifedipine. Peaks attributed to PEG 6000 and Poloxamer 188 were discernable from peaks of Nifedipine. The diffraction patterns of fine granules using PEG 6000 and Poloxamer 188 did not show diffraction peaks attributed to Nifedipine crystals (Fig. 12, 13). But the peaks attributed to PEG 6000 & Poloxamer 188 were visible in solid dispersion system also. These results suggest that amorphous Nifedipine is dispersed in PEG 6000 and Poloxamer 188. The amorphous Nifedipine is expected to have faster dissolution rate in comparison to crystalline Nifedipine as the amorphous phase possesses a higher surface free energy.

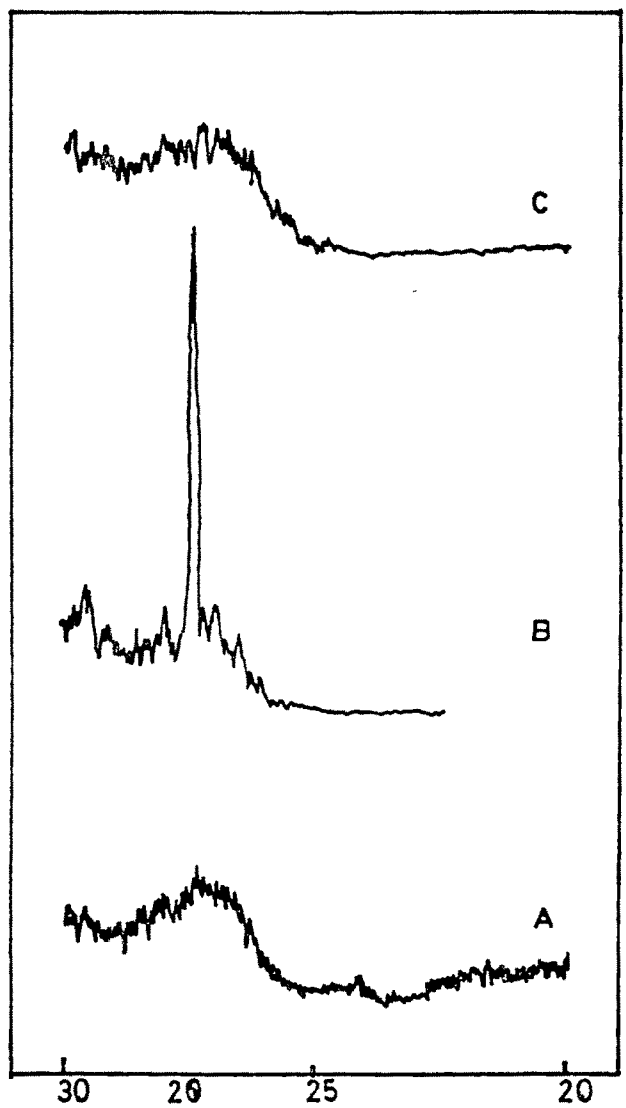

Fig 12 X-RAY DIFFRACTION PATTERNS OF(A) PEG 6000;
(B) Nfd:(C) Nfd PEG 6000 SD

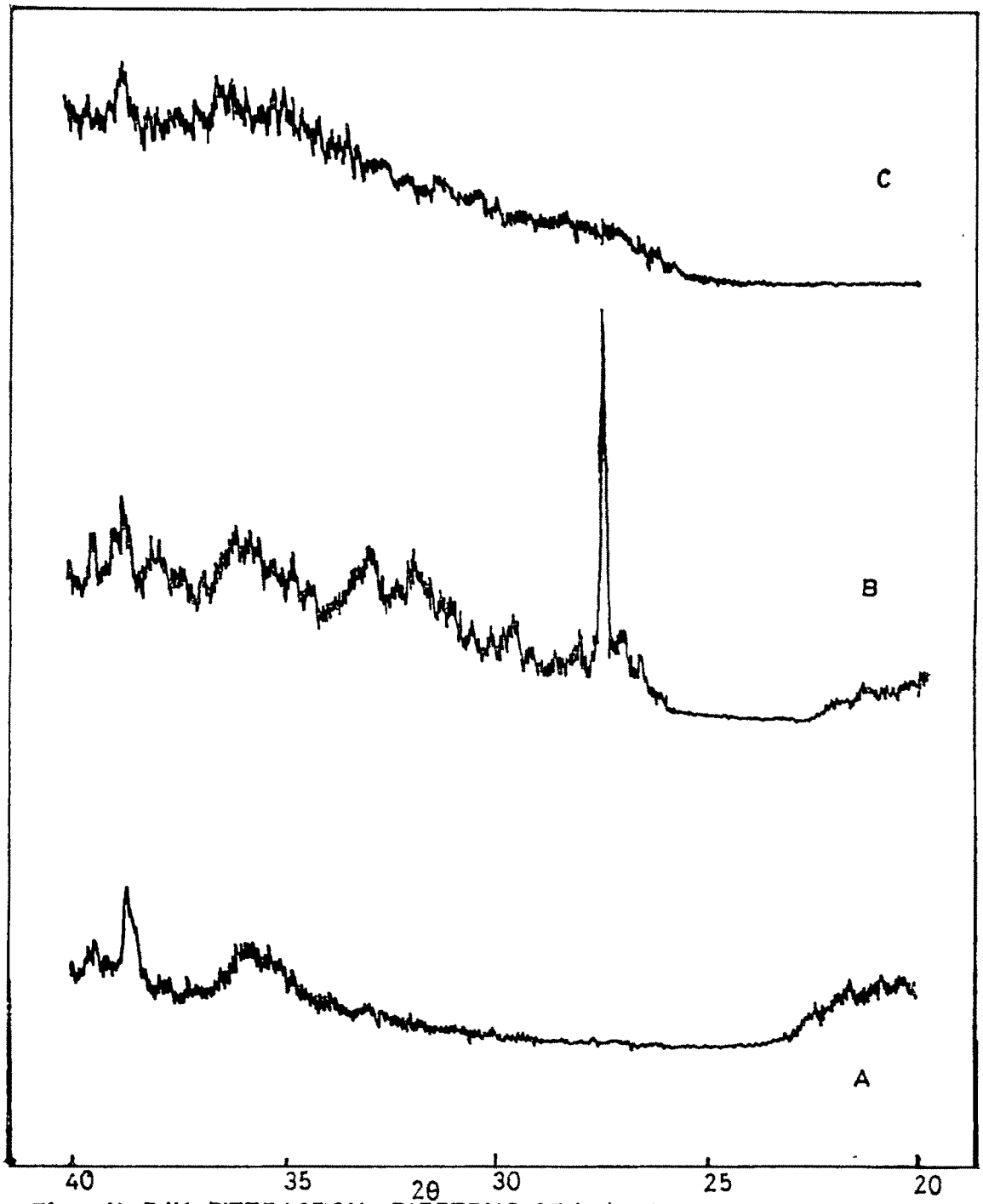

Fig.13 X-RAY DIFFRACTION PATTERNS OF (A) POLOXAMER 188 :

(B) Nfd ; (C) Nfd POLOXAMER 188 (1:19) SD

The above results were further corroborated using photomicrographs of the test preparations, excipients and Nifedipine. First, photomicrographs of PEG 6000 and Poloxamer 188 revealed the lamellar structure. It is likely that these lamellae are lamellar fibrils of a larger spherulite, as large spherulites are the predominant structural forms in bulk crystallized polyethylene glycol. (Sharples, 1966). This may also hold true for Poloxamer 188. (Fig.14, 17).

Fig. 16 shows the photomicrographs of single Nifedipine crystal. It shows needle shaped crystal with straight edges compared to those of excipients which are having striations due to lamellar nature of excipients. This difference in morphology is used as a means of distinguishing excipients from Nifedipine in dispersions.

The photomicrograph of Nfd-PEG 6000 (1:19) (Fig. 15) does not reveal the presence of crystalline drug, substantiates our x-ray diffraction data, suggesting the presence of amorphous form of Nifedipine.

Photomicrograph of Nfd-Poloxamer 188 (1:19) showed the growth of needle shaped projections on the solid amorphous surface (Fig. 18) . Surprisingly, even when some surface growth was evident from photomicrograph, the sample appeared amorphous by the x-ray diffraction method. Whisker like growth is not very common in pharmaceutical systems; whisker

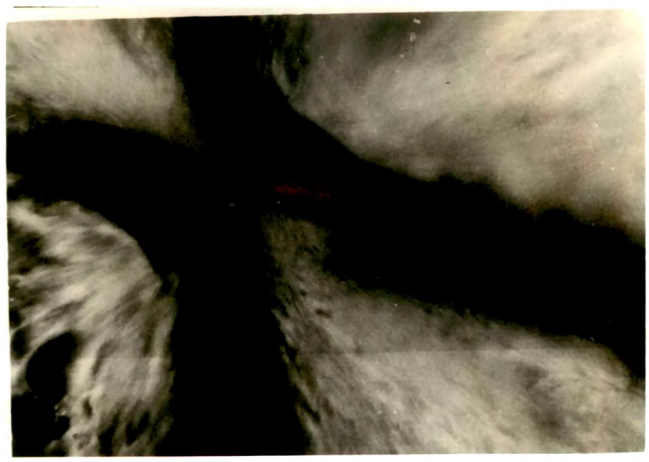

Fig 14 PHOTOMICROGRAPH OF PEG6000 (x300)

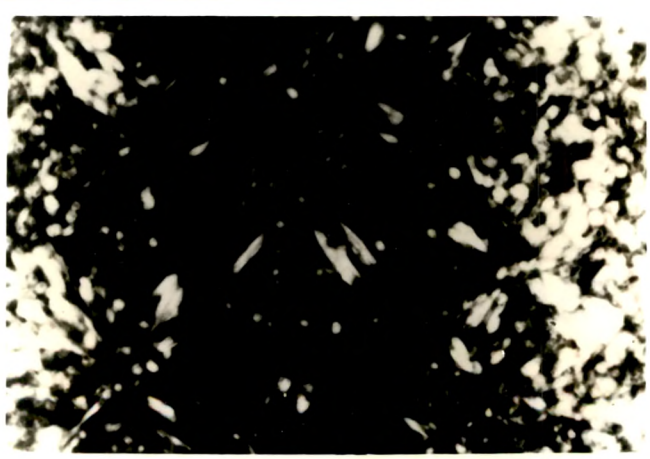

Fig 15 PHOTOMICROGRAPH OF Nfd-PEG6000 (1:19)SD (x300)

16 PHOTOMICROGRAPH OF Nfd CRYSTAL (× 300)

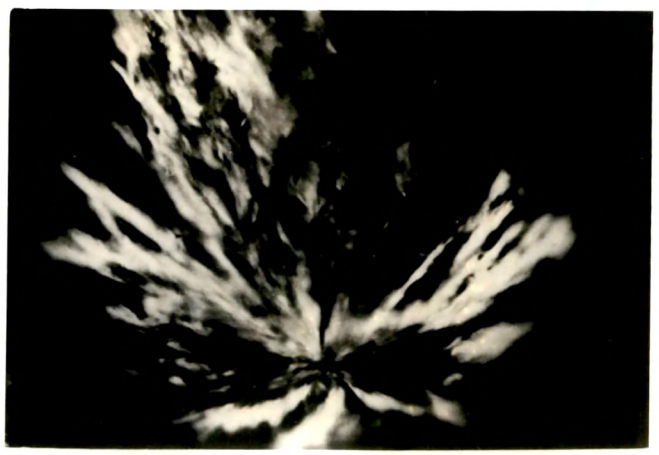

Fig 17 PHOTOMICROGRAPH OF POLOXAMER188 (x300)

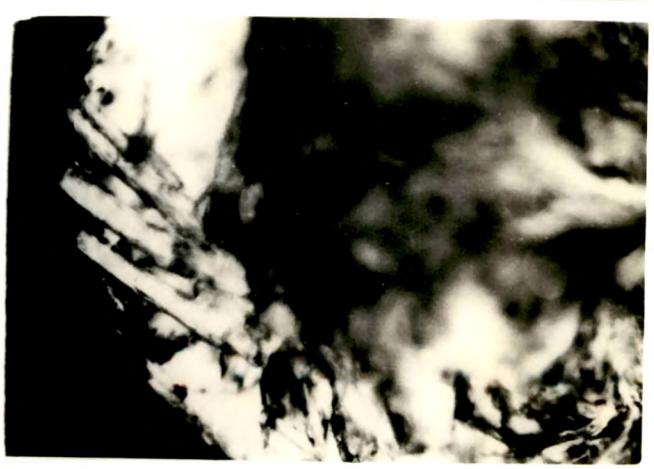

Fig 18 PHOTOMICROGRAPH OF Nfd-POLOXAMER188(1:19)SD (x300)

growth on the surface of ethenzamide and caffeine anhydride tablets has been reported (Yuasa et al, 1981). Recently Corrigan et al (1985) reported the existence of similar phenomenon in the work on Indomethacin cospraydried with polyvinylpyrrolidone.

Furthermore, the infrared spectra of Nifedipine - PEG 6000 and Nifedipine - Poloxamer 188 physical mixture, are the summation of Nifedipine and corresponding excipient spectrum; and exact replica of corresponding solid dispersion thereby indicating no chemical interaction during the method of preparation.

Phase diagrams of two selected systems were characterized by features common to eutectic behaviour (Fig. 19, 20). The phase diagrams for both the systems can be considered to be eutectic systems in which the liquidus (freezing point curve of Nifedipine) and the solidus (melting point curve of excipients) are superimposed. Both excipients were capable of dissolving 10% (w/w) Nifedipine, with eutectic composition of systems contained 10% w/w Nifedipine and 90% w/w excipients (10 : 90).

The presence of a small amount of excipients with drug causes little depression in the melting point of the drug and, conversely, the presence of a small amount of drug hardly affects the melting point of excipients. These phase diagrams did not reveal the formation of solid solution to any appreciable extent.

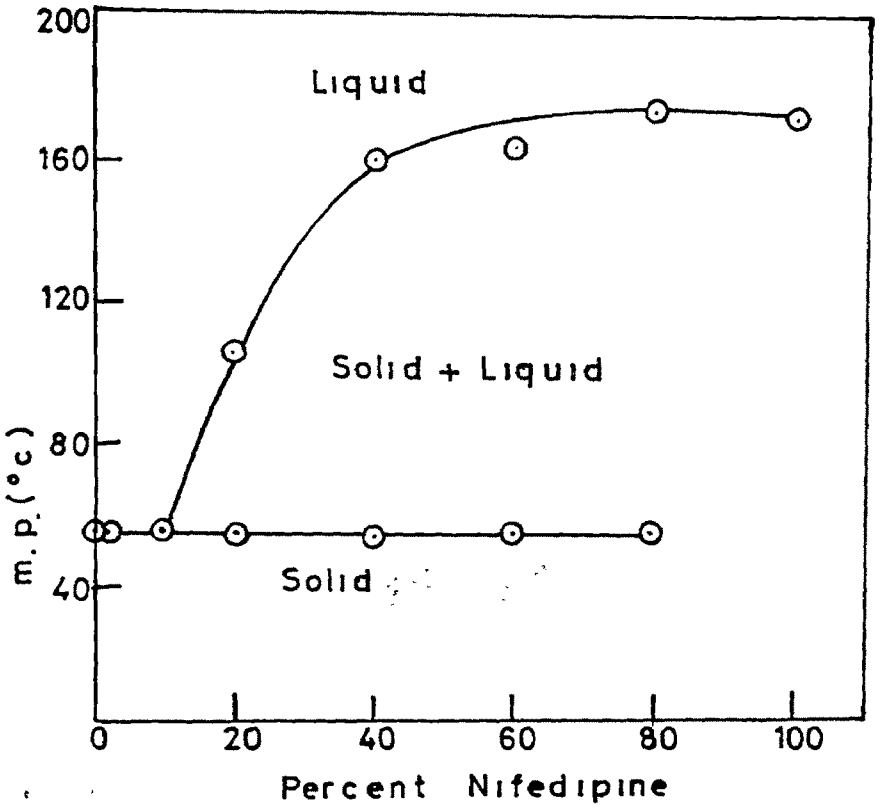

Fig 19 PHASE DIAGRAM OF Nfd - PEG6000 SD.

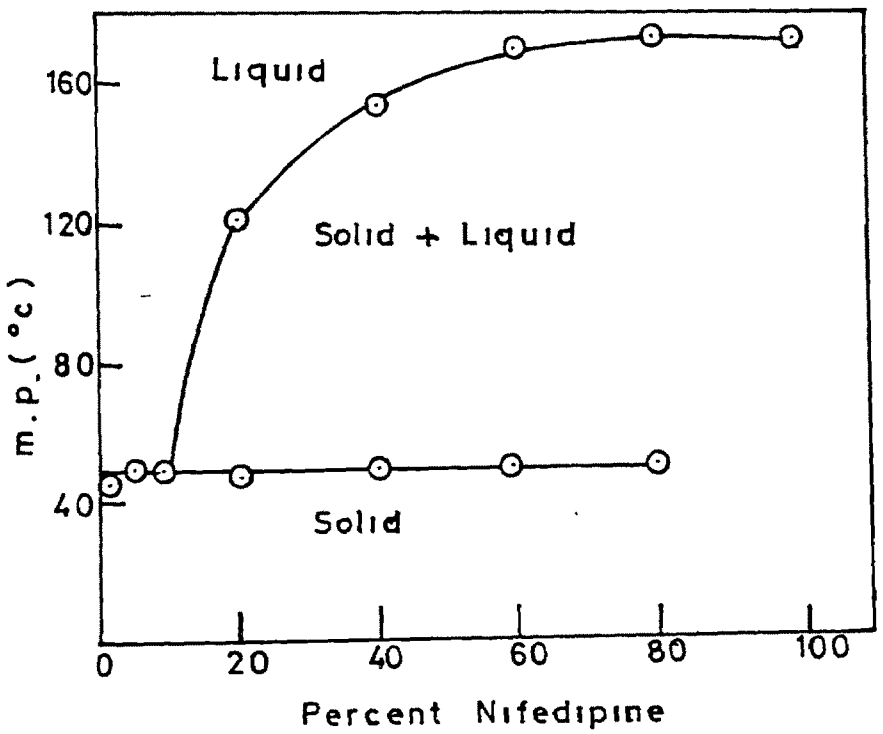

Fig. 20 PHASE DIAGRAM OF Nfd - POLOXAMER 188 SD.

The compositions used in this study are higher than the eutectic composition. The formation of eutectic, is likely to result in the increased dissolution rate. When tested, the preparations corresponding to eutectic composition yielded an increased dissolution rate of drug in relation to Nifedipine alone but were found to be inferior to other preparations having higher composition than the eutectic. But had it been the only efficacious composition than, it should have at least equal or superior efficacy than the other higher compositions which is not the case (except Nfd.- Poloxamer (1:9) solid dispersion which almost proved equal to Nfd - Poloxamer 188 (1:19) solid dispersion in terms of drug releasing efficiency). The much enhanced dissolution of drug from the compositions studied, than the eutectic mixture, may probably be due to the presence of additional higher concentrations of highly water soluble excipients around the drug particle.

Therefore, apart from above reason, other factors discussed earlier may be playing significant role in enhancing the dissolution and ultimately increasing the bioavailability of the drug.

Chemical stability - The fine granules of the two selected test preparations viz. Nifedipine - PEG 6000 (1:19) solid dispersion (1:19) and Nifedipine - Poloxamer 188 (1:19) solid dispersions, were stored in dark under ambient

conditions for two years. Nifedipine content in the stored samples were periodically determined by uv measurement at 340 nm (Table : 14). A decrease of Nifedipine content was observed but it remained within the compendial limits at the end of study period. The samples also became slightly darker in appearance when compared to freshly made solid disperions.

Aging Study - The importance of aging studies has been mentioned elsewhere in this report. Two selected test preparations as designated above were subjected to aging study by storing under ambient conditions for a period of 12 months. The samples were periodically analysed for their dissolution behaviour. No significant change in the dissolution behaviour was observed till the end of study period (Fig. 21, 22). Therefore, it may be concluded that these test preparations, are not susceptible to normal temperature and humidity conditions.

Bioavailability Study - The selected composition (for bioavailability study) of solid dispersions (1:19) was compromise between relatively good dissolution behaviour and volume of the dose in a single unit. It has been reported that there exists a good relationship between dissolution behaviour and bioavailability of various Nifedipine preparations. The test preparations using PEG 6000 (Nfd - PEG 6000, 1:19), Poloxamer 188 (Nfd - Poloxamer 188, 1:19), Adalat capsule (Bayer) and a tablet (Sarabhai Chemicals Ltd., Baroda, India) were chosen to be candidates

Table : 14 - Stability of Nifedipine (Percent residual amount) in samples stored under normal temperature and humidity conditions in dark.

Sample	Storage Period		
	Initial	12 mo	24 mo
Nfd-PEG 6000(1:19)SD	100.00	95.53	91.81
Nfd-Poloxamer 188 (1:19) SD	100.00	93.73	90.37

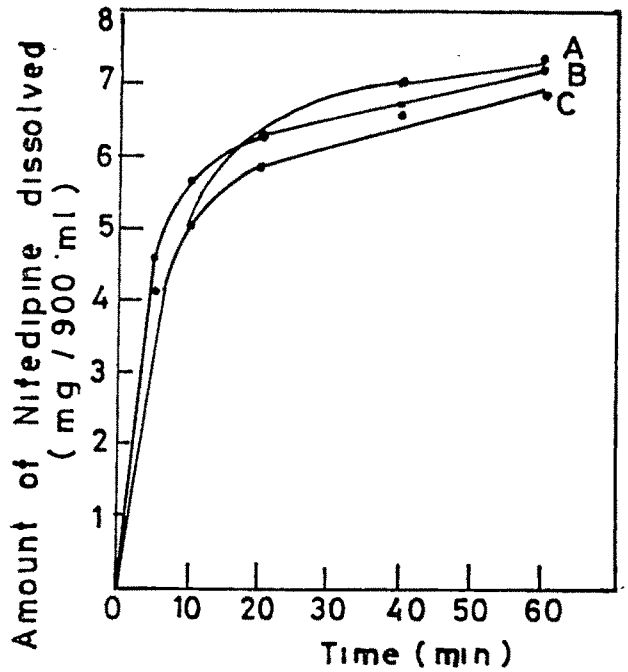

Fig 21 EFFECT OF STORAGE UNDER AMBIENT CONDITIONS ON THE DISSOLUTION BEHAVIOUR OF Nfd-PEG6000 (1:19) SD; STORAGE PERIOD; (A) INITIAL, (B) 6 mo, (C) 12 mo.

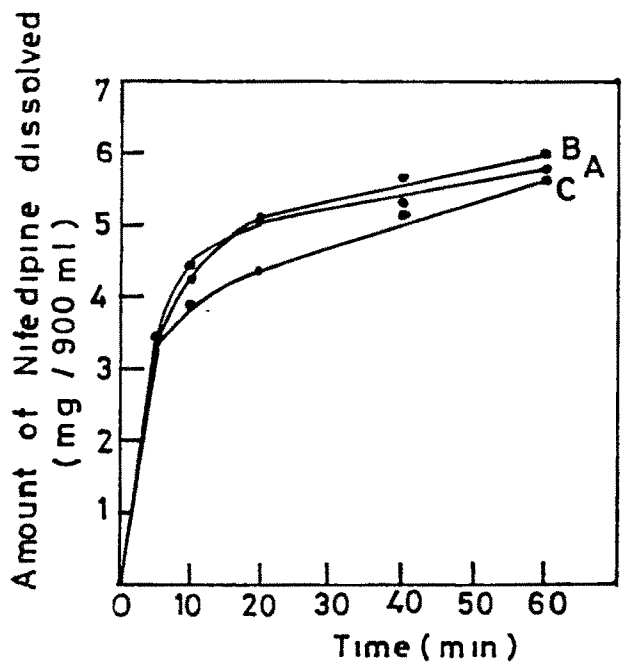

Fig 22 EFFECT OF STORAGE UNDER AMBIENT CONDITIONS ON THE DISSOLUTION BEHAVIOUR OF Nfd-POLOXAMER 188 (1:19) SD; STORAGE PERIOD; (A) INITIAL, (B) 6 mo, (C) 12 mo.

for bioavailability study. Nifedipine powder was not taken for comparative study since it did not show any detectable plasma level of drug in a preliminary study. Thus, a tablet preparation manufactured by a well known multinational company was included as a reference dosage form. Mean plasma concentrations of Nifedipine after oral administration of these test preparations to six normal human volunteers are reported in Table 15 & Fig. 23. Three parameters were examined to assess bioavailability from the plasma concentration data (Table : 16). They were peak plasma level (C max), time required to attain Cmax (Tmax) and area under the time plasma concentration (AUC) from 0 to 6 hr.

Bioavailability parameters of Nfd. tablet were found to be inferior (Cmax 44.03 ng/ml, Tmax 1 hr, and AUC 0-6 hr .. 141.79 ngh/ml) to those of other test preparations. Tmax of solid dispersion containing PEG 6000 was 2 hr. whereas it was 1 hr. for both Adalat capsule and solid dispersion containing poloxamer 188. Cmax values of Adalat capsule were about 2.33 times that due to Nfd. tablet. Solid dispersions containing PEG 6000 and Poloxamer 188 too exhibited about twice and 1.7 times the value of Nfd. Tablet, though it was at 2 hr. in case of solid dispersion containing PEG 6000. AUC values of test preparations were 1.5 to 2.0 fold larger than those of Nfd. Tablet.

Table : 15 - Mean plasma levels of Nifedipine from test preparations.

Time	Plasma level[a] (ng/ml)			
	Nfd. Tablet	Adalat Caps.	Nfd-PEG 6000 solid dispersion	Nfd-Poloxamer 188 solid disp.
0.5 hr.	26.58 ± 23.81	68.33 ± 47.89	35.93± 32.17	38.95±23.11
1 hr.	44.03 ± 12.25	102.83 ± 25.77	69.68± 21.01	75.08±17.31
2 hr.	31.36 ± 5.61	50.23 ± 19.40	84.96± 52.38	50.13±15.20
4 hr.	19.30 ± 4.41	28.6 ± 12.07	35.35± 22.05	24.66±11.13
6 hr.	9.83 ± 2.72	13.66 ± 7.86	24.06± 13.74	17.98±11.01

(a) mean ± SD (b) Dose : 10 mg/body.

Table : 16 - Mean time to peak, peak plasma level & AUC following oral administration of various test preparations having dose equivalent to 10 mg. of Nifedipine.

Test preparation	Time to peak (hrs).	Peak plasma level[a] (ng/ml)	AVC[a] (ng.h/ml)
Nfd. Tablet (Sarabhai Chem. Ltd., India).	1	44.03 ± 12.25	141.79± 12.12
Adalat Capsule (Bayer)	1	102.83 ± 25.77	257.50± 68.94
Nfd.- PEG 6000 Solid dispersion	2	84.96 ± 52.38	292.44± 118.61
Nfd - Poloxamer 188 Solid dispersion	1	75.08 ± 17.31	218.30± 58.54

(a) mean ± SD

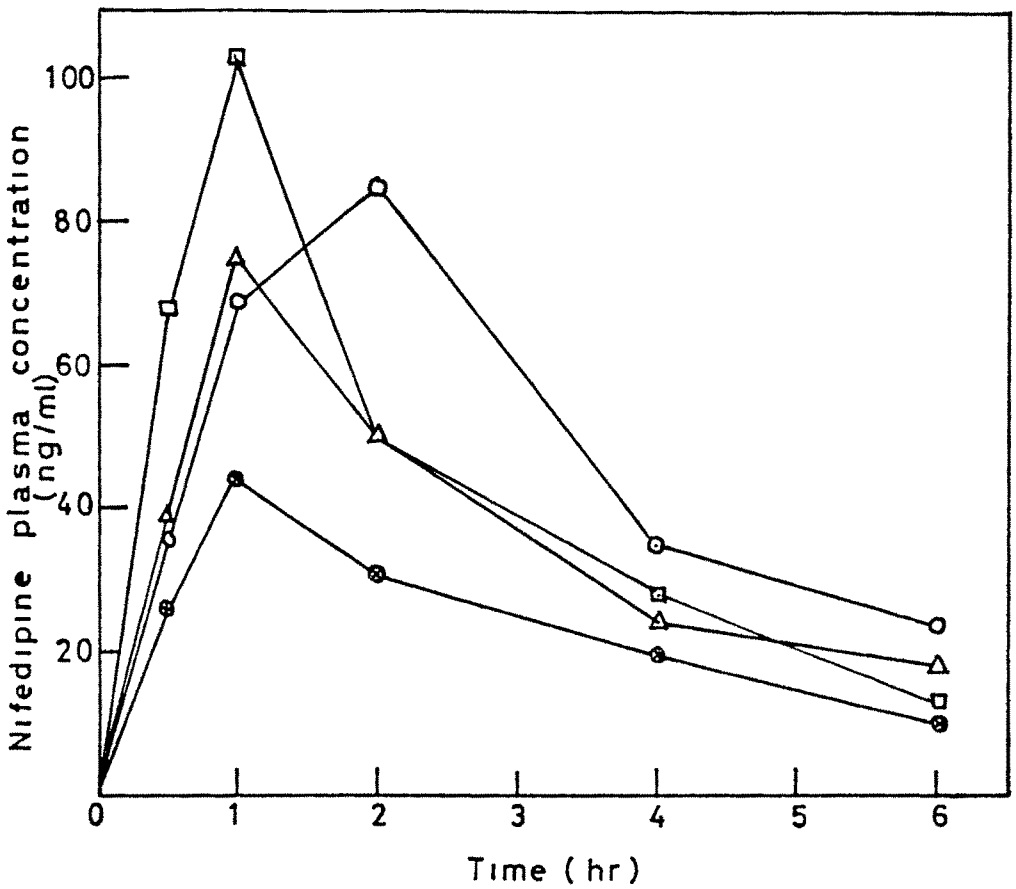

Fig 23 AVERAGE PLASMA CONCENTRATION OF NIFEDIPINE AFTER ADMINISTRATION OF TEST PREPARATIONS CONTAINING EQUIVALENT OF 10 mg OF NIFEDIPINE.

KEY : ▢ ADALAT CAP.
O Nfd - PEG 6000 SD CAP
Δ Nfd - POLOXAMER 188 SD CAP.
⊗ Nfd TAB

Multiple variance test of significance was applied to plasma concentration data at each time interval to check whether (i) there exists a significant difference among various test preparations, if yes then (ii) which of these test preparations differ significantly at each time interval (Tables - 17-21; P = 0.05).

There is a significant difference between Nfd. Tablet and Adalat capsule at 0.5 hr. Rest of the test preparations do not differ significantly either amongst themselves or with Nfd. tablet.

At one hr, Nfd. tablet differs from Adalat capsule and solid dispersion containing Poloxamer 188 but there is no significant difference with solid dispersion containing PEG 6000. Adalat capsule differs with both solid dispersions but there is no significant difference between two solid dispersions.

At second hour, all these test preparations differ with each other i.e. they are not taken out from the same pool (as indicated by the calculated F value which is higher than tabulated value at P = 0.10 but lower than at 5% level). When subjected to t-test further, Nfd - tablet differs from test preparation containing PEG 6000 and rest of the test preparations do not differ significantly either amongst themselves or with Nfd. Tablet.

Table : 17 - Analysis of variance at 0.5 hr.

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Among regimen s	t-1=3	9423.24	3141.08	4.01
Within regimen s	(ni-1)=20	15647.94	s^2=782.39	
Total	N-1 = 23	25071.18		

4.01 > 3.86 at P = 0.025

Ranked means :

Nfd Tabs.	Nfd-PEG 6000 SD	Nfd-Poloxamer 188 SD	Adalat Caps.
26.58	35.93	38.95	68.33

Any two means underscored by the same line do not differ statistically at P = 0.05

Statistically significant different preparations ($P \leq 0.05$)

Nfd. Tab vs. Adalat Caps.

Table : 18 - Analysis of variance at 1 hr.

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Among regimens	t-1=3	10466.41	3488.80	F = 8.26
Within regimens	(ni-1)=20	8446.13	s^2=422.30	
Total	N-1 =23	18912.54		

8.26 > 4.94 at P = 0.01

Ranked means

Nfd Tab.	Nfd-PEG 6000 SD	Nfd-Poloxamer 188SD	Adalat Cap.
44.03	69.68	75.08	102.83

Any two means underscored by the same line do not differ statistically at P = 0.05

Statistically significant different preparations ($P \leq 0.05$)

Nfd. Tab. vs. Adalat cap.

Nfd. Tab. vs. Nfd-Poloxamer 188 SD

Nfd-PEG 6000 SD vs. Adalat Cap.

Nfd-Poloxamer 188 SD vs. Adalat Cap.

Table : 19 - Analysis of variance at 2 hr.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F ratio
Among regimens	t-1 = 3	7701.11	2567.03	F = 2.96
Within regimens	(ni-1)=20	17297.92	s^2=864.89	
Total	N-1= 23	24999.03		

2.96 > 2.38 at P = 0.10

Ranked means

Nfd. Tab.	Nfd-Poloxamer 188 SD	Adalat Cap.	Nfd - PEG 6000 SD
31.36	50.13	50.23	84.96

Any two means underscored by the same time do not differ statistically at P = 0.05.

Statistically significant different preparations ($P \leq 0.05$)

Nfd. Tab. vs. Nfd - PEG 6000 SD.

Table : 20 - Analysis of variance at 4 hr.

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Among regimens	t-1 = 3	817.08	272.36	F= 1.36
Within regimens	(ni-1) = 20	4016.92	s^2=200.84	
	N-1 = 23	4834.00		

No significant difference even at P = 0.10

Table : 21 - Analysis of variance at 6 hr.

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Among regimens	t-1=3	671.26	223.75	F=2.28
Within regimens	(ni-1)=20	1960.20	s^2=98.01	
Total	N-1 = 23	2631.46		

No significant difference even at P = 0.10

There does not seem to be significant difference amongst and within various test preparations at fourth as well as sixth hour.

From the above analysis, it may be concluded that (i) there exists significant differences amongst and within test preparations(plasma level upto 2 hr), (ii) in most instances Nfd. tablet differs significantly from either of solid dispersion systems or Adalat capsule. (iii) Solid dispersions do not seem to differ significantly at any given time interval.

Therefore, it may be assumed that solid dispersion systems are bioequivalent though a trial involving large number of volunteers or equal clinical efficacy may give further conclusive evidence. Summing up the conclusions drawn from the above discussion, one may conclude that the bioavailability of three test preparations namely Adalat capsule and two solid dispersion systems were superior to that of Nfd. Tablet. The extent of absorption as reflected by AUC data, may not differ significantly among the above three preparations except in terms of rate of absorption wherein plasma concentration of Adalat capsule differs significantly from solid dispersion systems at one time interval(1 hr)as evident from the results cited above. Probably two solid dispersions are slightly inferior to Adalat capsule in terms of the rate of absorption .

The overall above discussion highlights the utility of these two solid dispersions in enhancing the gastro-intestinal absorption of the drug in question.