CHAPTER 4

K E T O P R O F E N

Experimental

Materials : Ketoprofen (Keto, Courtesy May & Baker India Limited, Bombay, India), Polyethylene glycol 6000 (s.d. Fine Chemicals, Boisar, India), Poloxamer 188 (BASF Wyandotte Corp., U.S.A.) were used as received. All other reagents were either pharmacoepoeial or reagent grade.

Methods :

Particle size and size distribution - Particle size (average diameter) and particle size distribution were measured employing optical microscopy.

Preparation of Calibration Curve - About 0.05 g. of Ketoprofen was dissolved in 25 ml. of methanol and diluted to 100 ml. with water or 0.1N Hcl. Aliquots were withdrawn from this and diluted appropriately to get a range of concentrations, the absorbance of which were measured at 260 nm in a uv spectrophotometer (vsu - 2P, CZ Jena, G.D.R). The Beer's plot is shown in Fig.: 2.

Preparation of dispersion systems - Physical mixtures and solid dispersions were prepared in the same way as mentioned in previous section dealing with Nifedipine except that no attempt was made to increase the particle size of Ketoprofen in physical mixture. The solid dispersion system contained 10% w/w of Ketoprofen in PEG 6000 and Poloxamer 188. Only these excipients were used in the current study as they showed promising results in earlier studies with Nifedipine

and some other drugs. The solid dispersion systems were stored in desiccator at room temperature for 48 hr. before subjecting to dissolution studies. The particle size used for dissolution studies was # 16/35 (500-1190 µm) as it was extremely difficult to pass these granules through finer seives. The higher concentration of excipients used is justified by the fact that use of lower concentration of excipients did not yield granules having sufficient texture to be passed through these seives. Therefore, the study was restricted to just one ratio each i.e. Drug - excipient (10 : 90).

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

Solubility determinations - Aqueous solubility studies were performed in order to check any possible interaction between drug and excipients. An excess amount of drug was placed in 30 ml. glass vials equipped with aluminium seals, containing 20 ml. of acidic aqueous media of excipients adjusted to pH 1.2 with Hcl. The contents in the vials were shaken in a gyratory incubator shaker at 37°C for 8 hr. and then allowed to equilibrate overnight before aliquots were withdrawn, filtered through a G4 sintered funnel, suitably diluted and analysed at 260 nm using uv spectrophotometer. The aliquots

were diluted in a way, if needed, to eliminate any interference because of excipient.

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

Dissolution rate studies using USP method II - The method adopted was the same as had been used for Nifedipine except a few modifications i.e. in this case, the dissolution media was 500 ml. of 0.1N Hcl and the amount of test preparations added to the dissolution media was 1000 mg. representing 100 mg. of Ketoprofen. The aliquots withdrawn so far, were filtered through a G4 sintered funnel suitably diluted with 0.1N Hcl and analysed by uv spectrophotometry at 260 nm. There was no or negligible absorption due to the presence of excipients at this wavelength and dilution.

Tape method - This method was same as the one used for such study in case of Nifedipine. The differences were (i) the use of 400 ml 0.1N Hcl as the dissolution media (ii) the stir paddle speed was maintained at 50 rpm (iii) the amount of test preparations used was equivalent to 4 mg. of Ketoprofen.

Solid solid interaction - Phase diagrams of these solid dispersions were constructed in a similar way as in case of Nfd - PEG 6000 & Nfd - Poloxamer i.e. by Hot Stage Microscopy.

X-ray diffraction studies - Same method and instrument were used to obtain x-ray diffraction patterns, as mentioned in

the earlier section dealing with Nifedipine.

Stability studies - Chemical stability of test preparations selected for bioavailability trials, was conducted on samples stored under ambient conditions for a period of two years.

Aging studies - The aim and method for this study was same as in the case of Nifedipine.

Bioavailability study - Urinary excretion study was undertaken to assess the bioavailability of the selected preparations.

a) Formulation and dose - Keto - PEG 6000 physical mixture, Keto-Poloxamer 188 solid dispersion (both filled in capsules); Ketoprofen capsule containing drug only and Ketoprofen capsules (2 Caps. of 50 mg. each May & Baker, India Ltd), in the dose equivalent to 100 mg. of Ketoprofen were administered orally to healthy male volunteers.

b) Subjects : A total cross over study was conducted in four male healthy volunteers aged between 24 - 28 years (mean 25.6 yrs) and weighing between 52 - 65 kg. (mean 56 kg). The study was initially planned with five human volunteers, but due to the last minute withdrawal of one volunteer, the study was continued with four volunteers only.

The volunteers gave their written consent after the object and procedure of the trial had been fully explained to them. No abnormalities were found on clinical examination.

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 hr. after the dose was given, however water (100 ml/hr) was allowed to maintain the normal volume of urine. Urine samples were collected at 1, 2, 3, 4 & 6 hr. after administration and stored at 4°C till analysed. The samples were analysed the next day. Following a washout period of five days, the volunteers received another preparation and series of samples were taken following the same schedule as described above.

d) Assay of urinary level - The urinary concentration of total Ketoprofen was estimated by modified method of Populaire et al (1973), in which concentration of Ketoprofen was measured by uv measurement (at 252 nm in hexane) instead of measuring by colorimetric method, used by authors, as it could not be reproduced in our laboratory. It was found that the method used could quantitate Ketoprofen well with good recovery. The slope of Beer's plot (0-20 ug/ml) was 0.0388 with correlation coefficient, r = 0.997.

Results & Discussion

Ketoprofen (average diameter 1.8 μm) did not show any degredation during preparation (Fig: 1), checked by measuring the drug content in solid dispersions which were in the compendial limit (92.5 - 107.5% w/w) and scanning the solid dispersion for λmax (260 nm) in methanol water system which did not show any shift.

Both the solid dispersions were found to be superior to drug alone, in terms of their release characteristics (Table : 1, 2 & Fig : 3 & 4). In Keto - PEG 6000 (1:9) system, solid dispersion exhibited about 7.3 fold larger dissolution rate at 10 min. when compared with Ketoprofen only. This ratio gradually decreased with time and it was 1.6 times the dissolution rate of Ketoprofen at 60 min. Physical mixture too showed marked increase in the dissolution rate when compared with Ketoprofen. In fact, at 5 min. it was even higher than that shown by solid dispersion and at 10 min. the dissolution rate was about 6.5 times the rate of Ketoprofen. This ratio was reduced to about 1.5 times at 60 min.

Thus, it became very much evident that physical mixture was comparable to the solid dispersion with respect to the drug releasing efficiency. This served as an alternative to solid dispersion to be used in bioavailability trials later.

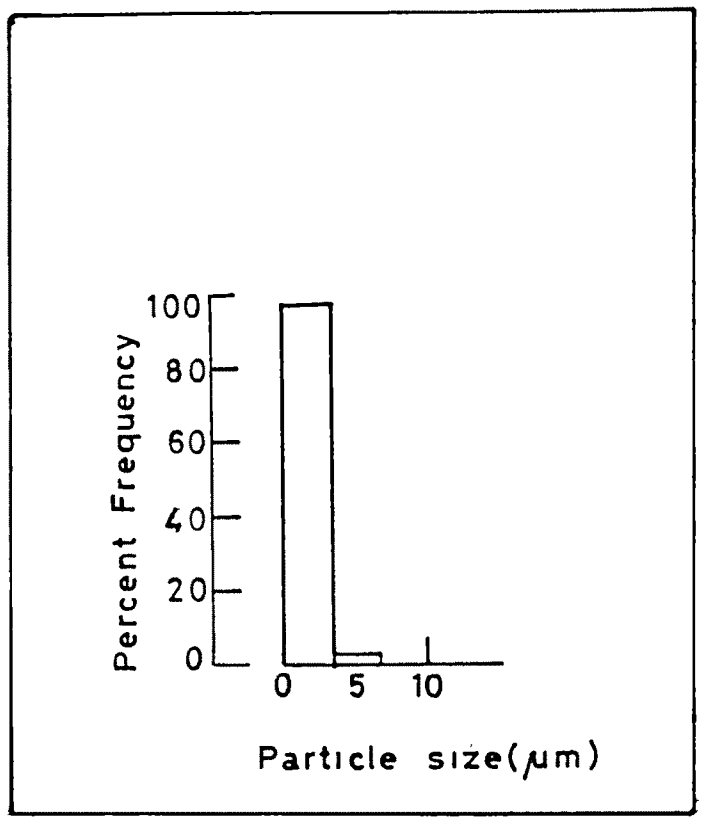

Fig1 PARTICLE SIZE DISTRIBUTION OF KETOPROFEN.

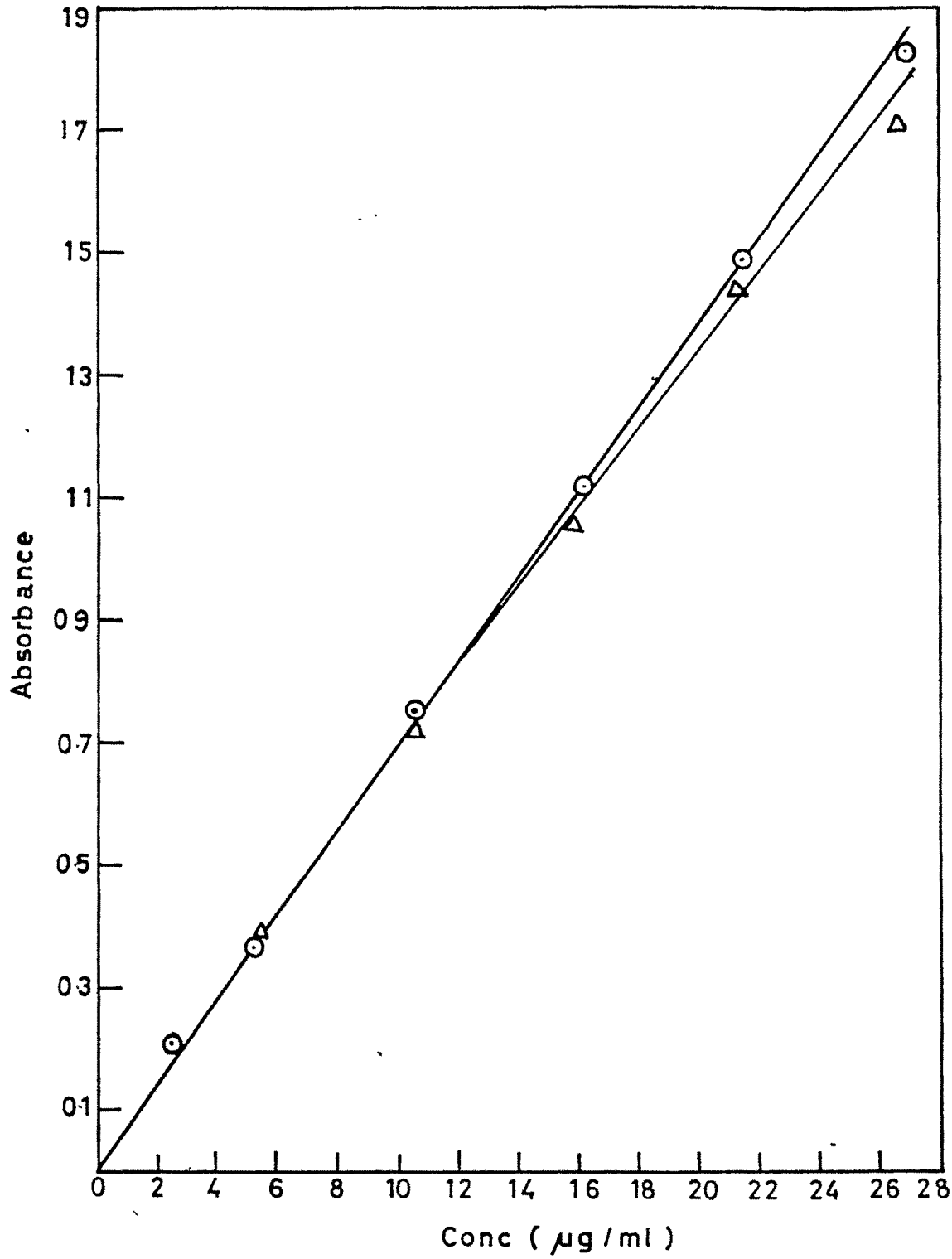

Fig 2 CALIBRATION CURVE OF KETOPROFEN AT 260 nm.
KEY : 0·1 N HCl; Δ DISTILLED WATER.

Table : 1 - Dissolution rates of Ketoprofen from test preparations.

Systems	Average amount of Ketoprofen released (mg/500 ml)				
	5	10	20	40	60 (min)
Solid dispersion					
Keto-PEG 6000 (1:9)	15.77	55.20	65.72	69.97	72.88
Physical mixture					
Keto-PEG 6000 (1:9)	26.97	49.70	59.92	66.93	69.35
Ketoprofen	2.67	7.54	17.77	33.24	44.28

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

Systems	Average amount of Ketoprofen released (mg/500 ml)				
	5	10	20	40	60 (min)
Solid dispersion					
Keto-Poloxamer 188 (1:9)	25.77	61.29	74.40	81.77	87.43
Physical mixture					
Keto-Poloxamer 188 (1:9)	11.97	52.15	64.00	68.84	72.88
Ketoprofen	2.67	7.54	17.77	33.24	44.28

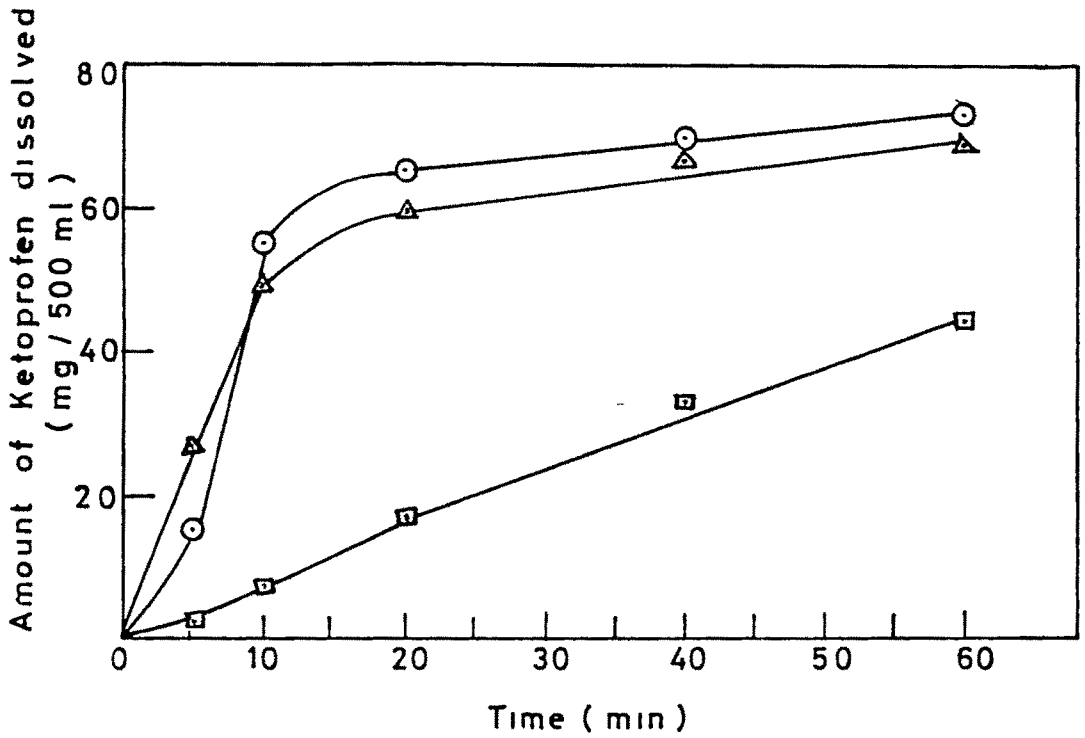

Fig 3 DISSOLUTION PROFILE OF KETO - PEG6000 SD, KETO - PEG600 PM AND KETO (USP method II)

KEY : ⊙ KETO - PEG (1:9) SD ,
△ KETO - PEG (1:9) PM,
⊡ KETO.

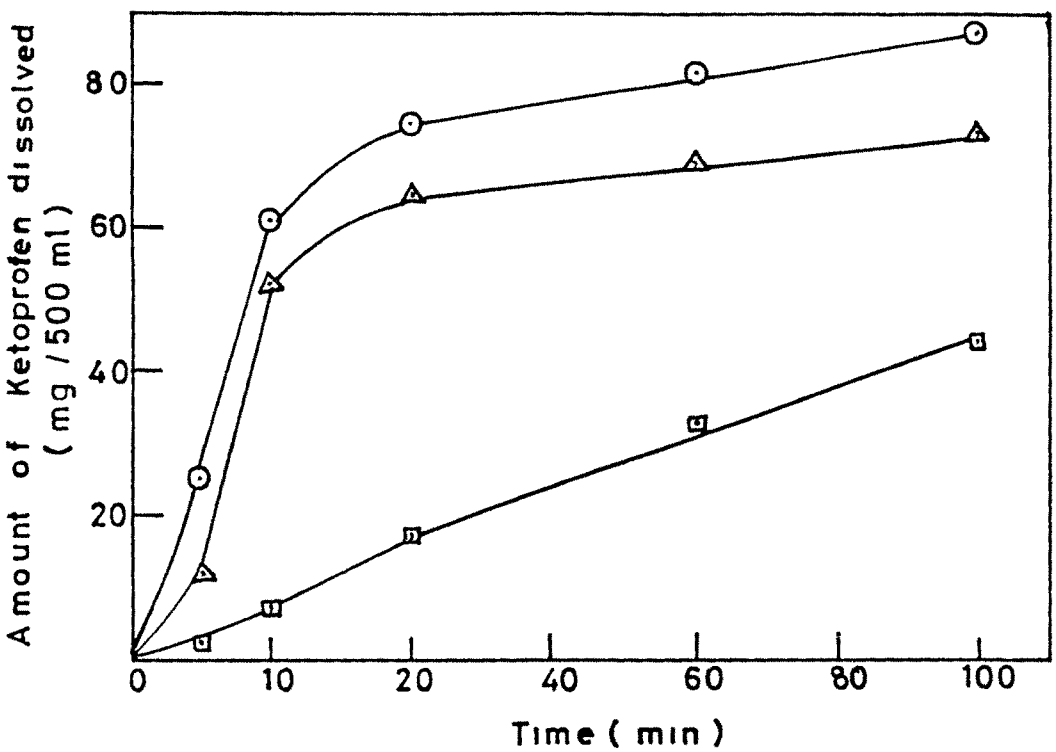

Fig 4 DISSOLUTION PROFILE OF KETO-POLOXAMER SD, KETO-POLOXAMER 188 PM AND KETO (USP method II)

KEY : ⊙ KETO-POLOXAMER 188 (1:9) SD.

▲ KETO-POLOXAMER 188 (1:9) PM.

⊡ KETO.

Keto-Poloxamer 188 solid dispersion also did show marked improvement in the dissolution rate of drug in comparision to Ketoprofen powder. At 10 min; the dissolution rate of drug from solid dispersion was about 8.1 times higher than the drug whereas it remained about twice higher at 60 min. Physical mixture, like in the previous system exhibited much better dissolution when compared to drug. The dissolution rate was about 7 times higher than the drug at 10 min. and it was 1.6 times at 60 min.

The above results suggest some role of local action of excipients on the dissolution rate of drug from these systems.

To analyse this factor, aqueous solubility studies of Ketoprofen employing different concentrations of two excipients, were undertaken. The results revealed the solubilizing power of these two excipients; Poloxamer 188 being found superior to PEG 6000. (Fig-5)

A careful inspection of results revealed that all the test preparations in two systems except Ketoprofen achieved the supersaturation of drug in solution (observed solubility of Ketoprofen in 0.IN Hcl at 37°C is 120 ug/ml). It is well known that reducing the particle size of a material to a very fine state of subdivision often results in supersaturation. (May and Kolthoff, 1948). But a drug whose average particle size is very small (1.8 um), therefore can it further undergo

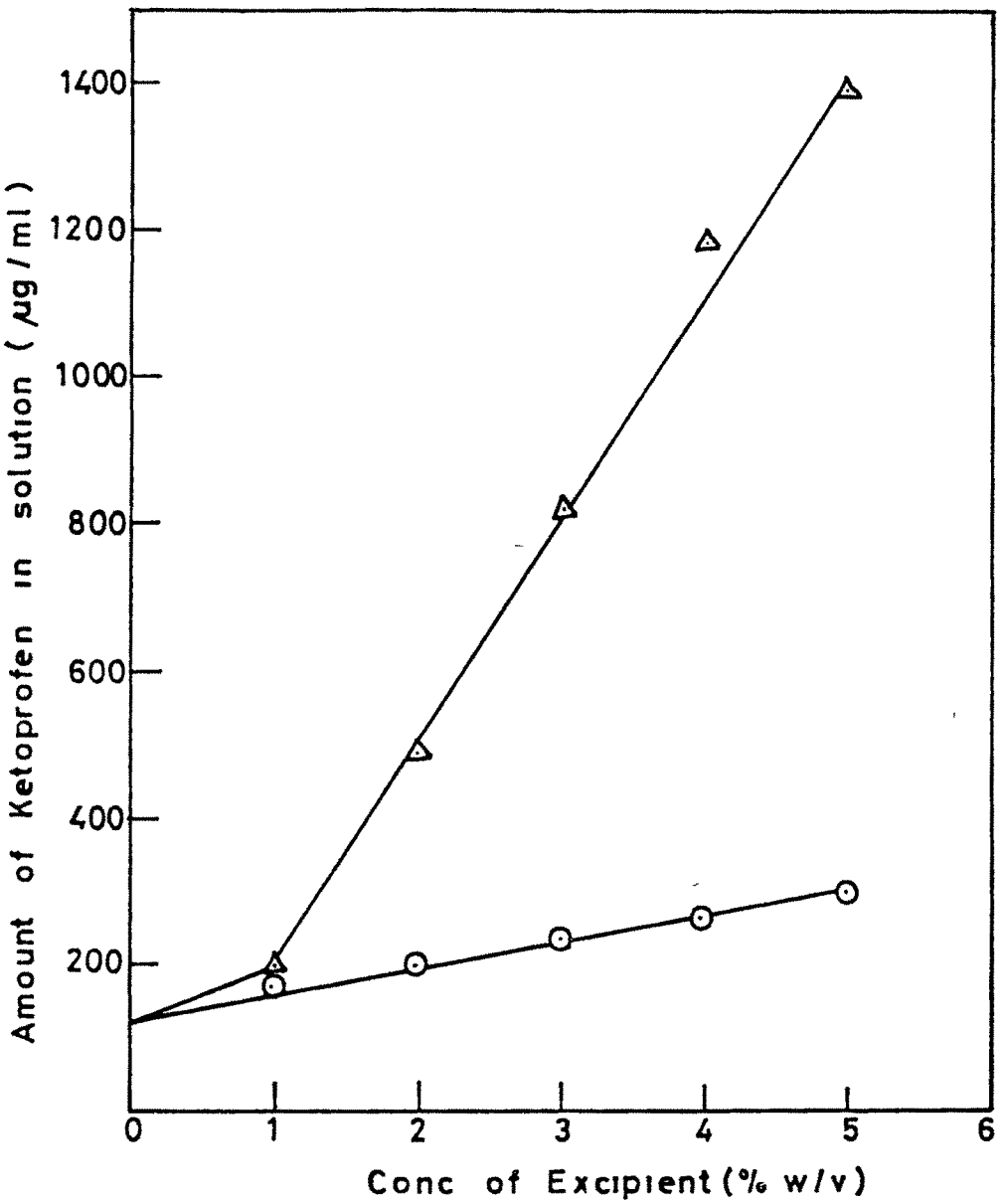

Fig 5 THE EFFECT OF EXCIPIENTS ON THE SOLUBILITY OF KETOPROFEN AT 37 ± 1 °C.

KEY : O PEG6000
Δ POLOXAMER 188.

reduction in particle size as a result of incorporation in solid dispersion. But physical mixtures where the particle size may not be considered reduced further also show an improvement in the dissolution rates. Had it been solely due to the effect of excipients than in both the cases, the dissolution rates of physical mixture should have been similar to solid dispersion, which is probably not the case.

The other possibilities for a fine state of subdivision are the presence of eutectic mixture. But in systems studied so far, the composition of solid dispersions is more than their eutectic compositions (reported elsewhere in this section). Therefore, reduction in particle size may hitherto play a limited role in improving the dissolution behaviour of drug from these solid dispersions as has been reflected in Keto-PEG 6000 SD, the peak attributed to drug is suppressed whereas in Keto-Poloxamer 188 SD, it is even stronger. Further, the observed improvement in the dissolution characteristics of solid dispersions may possibly be attributed to a composite of local factors like (i) reduction in aggregation and agglomeration of the drug (ii) the existence of high concentrations of excipients in the vicinity of drug particle, so that the solubility of the drug at the surface is increased and (iii) role of micellar solubilization at a concentration higher than the critical micelle concentration of both excipients (observed CMC : 0.1% w/v, Fig : 6).

Another noteworthy observation is that there is no recrystallization of drug from supersaturated solution but the dissolution of drug is still progressing. The absence of

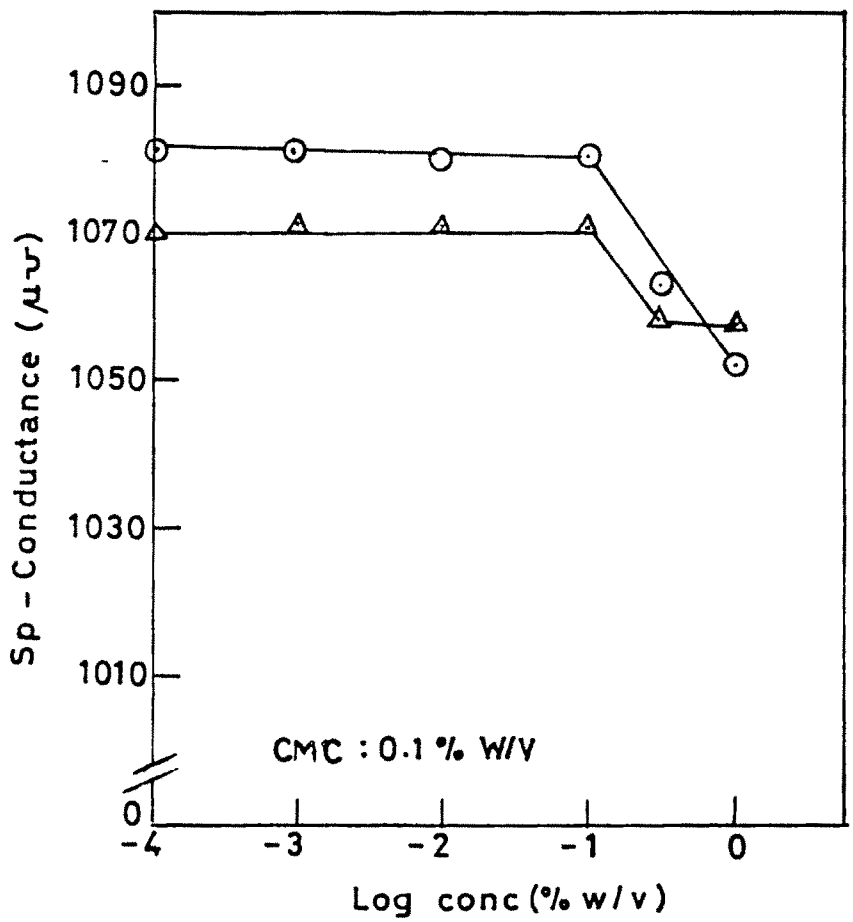

Fig. 6 EFFECT OF CONCENTRATION (log scale) OF PEG 6000 (⊙) AND POLOXAMER 188 (△) ON THE SPECIFIC CONDUCTANCE OF WATER AT 37 °C.

recrystallization is probably due to the retardation effect of excipient molecules on the crystallization of drug. PVP also retarded the recrystallization of drug from supersaturated solution of a sulfonamide (Sekikawa et al, 1979). Both systems were also evaluated using tape method (Goldberg et al, 1965) meant for monodisperse particles.

There was no interference in the spectral measurements due to adhesive used on tapes. The dissolution data recorded (Table : 3, 4 & Fig : 7, 8) also revealed improvement in the dissolution rates of drug from solid dispersions in comparision to Ketoprofen.

Keto-Poloxamer 188 solid dispersion showed dissolution rate 11 fold larger than Ketoprofen at 3 min. and it was 5.5 folds at the end of 15 min.

Physical mixture too indicated faster dissolution of drug. At 3 min, Ketoprofen from physical mixture dissolved about 5.5 times faster than Ketoprofen whereas it dissolved 3.3 times faster at the end of 15 min.

There seems to be an excellent correlation between the dissolution data (Ketoprofen - Poloxamer 188 system) by these two methods apart from the fact that there is a larger difference between dissolution rate shown by solid dispersion and physical mixture, in tape method.

In case of Keto-PEG 6000 physical mixture, the dissolution rate of drug from physical mixture is about 2.5 times the dissolution rate of Ketoprofen(cond.....)

Table : 3 - Dissolution rates* of Ketoprofen from test preparations by tape method.

Systems	Average amount of Ketoprofen released (mg/400 ml)					
	1	2	3	5	10	15(min)
Solid dispersion						
Keto-Poloxamer 188 (1:9)	0.85	1.91	2.42	3.68	3.67	3.84
Physical mixture						
Keto-Poloxamer 188 (1:9)	0.34	0.77	1.21	1.70	2.20	2.31
Ketoprofen	0.15	0.17	0.22	0.31	0.55	0.70

* Average of five determinations.

Table : 4 - Dissolution rates* of Ketoprofen from test preparations by tape method.

Systems	Average amount of Ketoprofen released (mg/400 ml)					
	1	2	3	5	10	15(min)
Physical mixture						
Keto-PEG 6000 (1:9)	0.25	0.37	0.55	0.78	1.19	1.56
Ketoprofen	0.15	0.17	0.22	0.31	0.55	0.70

* Average of five determinations.

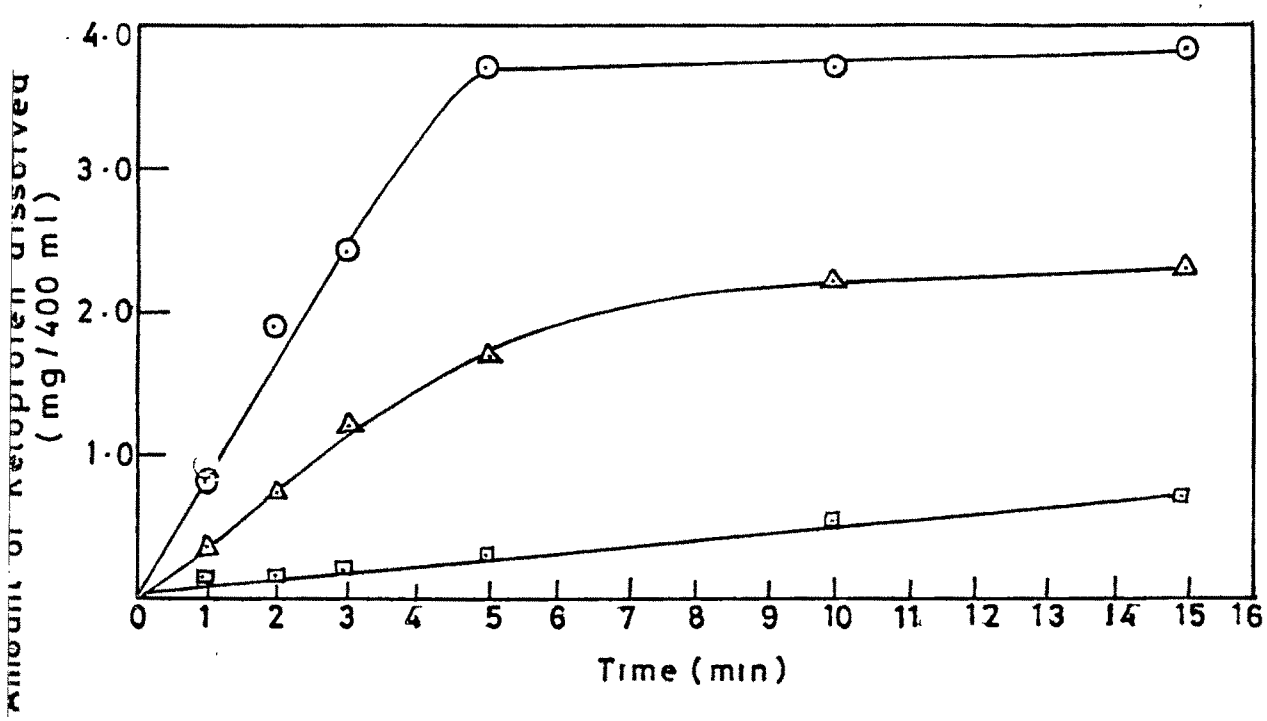

Fig.7 DISSOLUTION PROFILE OF KETO-POLOXAMER 188 AND SD, KETO-POLOXAMER 188 PM AND KETO (tape Method)

KEY : ⊙ KETO-POLOXAMER 188 (1:9) SD;
△ KETO-POLOXAMER-188 (1:9) PM;
⊡ KETO.

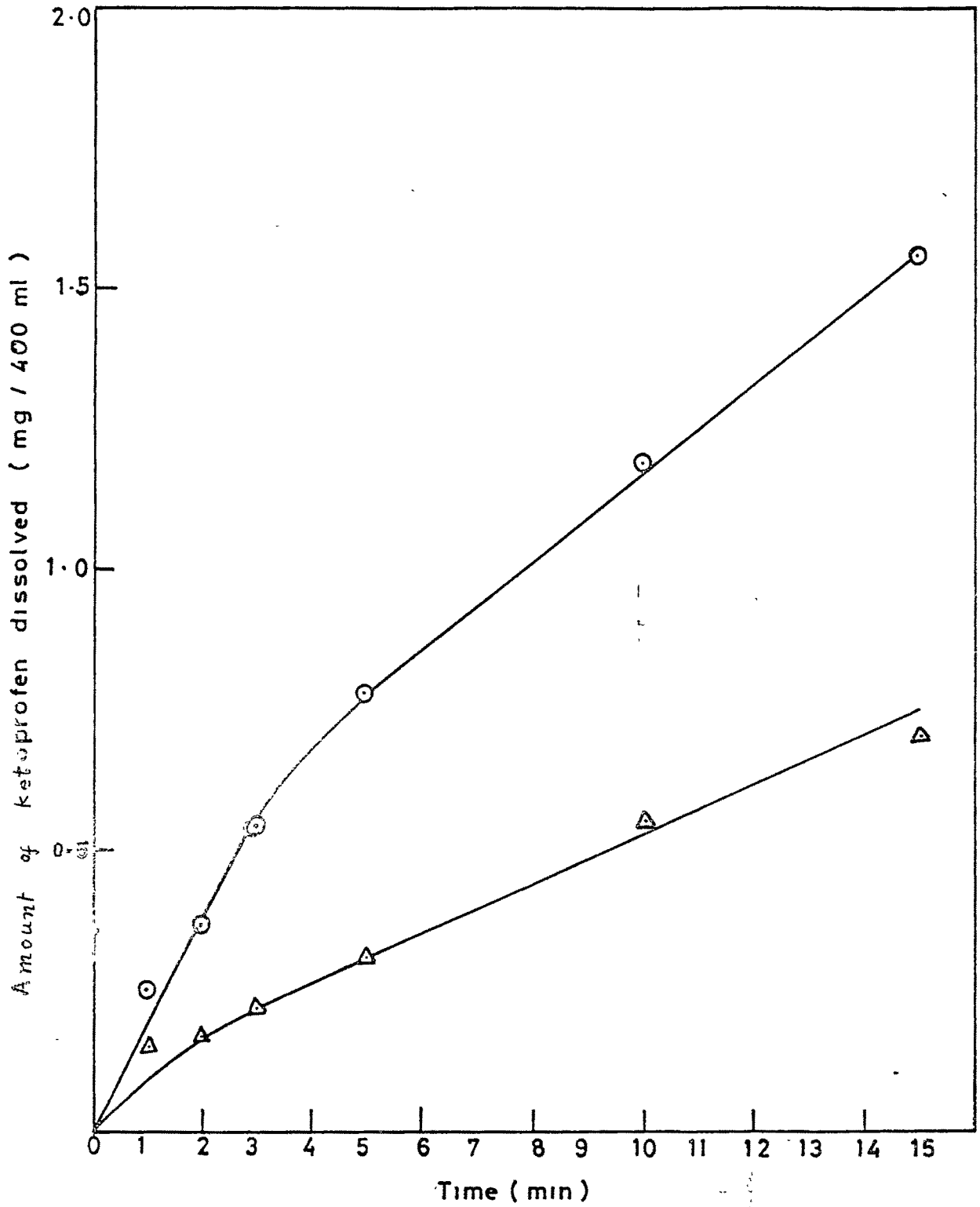

Fig. 8 DISSOLUTION PROFILE OF KETOPROFEN AND KETOPROFEN PEG6000 (1:9) PM (Tape method)

KEY : ⊙ KETO -PEG 6000 (1:9) PM.

Δ KETOPROFEN.

at 3 min. and it is 2.2 times at the end of 15 min. These ratios are much less than the dissolution data obtained by USP method.

Another observation was that Keto-Poloxamer 188 solid dispersion appears superior to the Keto-PEG 6000 physical mixture whereas no such remarkable superiority is reflected in USP dissolution method. This may probably be accounted for, in terms of the difference in the degree of agitation in the two months.

The data when fitted into Hixson's Crowells equation for particulate dissolution showed deviation from the cube root law after 5 min.

According to the Noyes Whitney equation, the dissolution rate of a solid is a function of the surface area presented to the dissolution medium as well as the concentration gradient existing between the solid-liquid interface and the bulk of the dissolution medium. Under conditions of constant surface area, as observed in the dissolution from a planar surface, the dissolution rate should follow zero order kinetics provided the concentration gradient is maintained essentially constant by keeping the dissolution medium sufficiently dilute. On the other hand, in those situations where only the concentration gradient may be maintained constant eg. in particulate dissolution, the dissolution rate is proportional to the surface area. Accordingly, the dissolution of particulate solids has been found to adhere to the "Cube root law" which represents a mathematical model to account for the

decrease in surface area during the dissolution process.

As may be noted in Fig. 7 & 8, dissolution curves were of two distinct types : either linear or near linear and biphasic, consisting of two linear segments. Sample of Ketoprofen followed zero order kinetics over the 15 min. period but data for entire time period did not fit well in the "cube root law", however cube root dissolution rate constant has been calculated to assess the enhancing effect of solid dispersion in relation to drug alone.

It has been theorized that the dissolution process involves the formation of a thin layer or film of saturated solution at the solid liquid interface and the diffusion of molecules from this layer to the bulk solution. Applying this concept to the dissolution of particulates from the tape, it is apparent that as long as the particles are farther apart than the thickness of diffusion layer, the cube root law would be obeyed. However, if the particulate density is increased so that the diffusion layer of each particle "overlaps" with that of its neighbouring particle, it is reasonable to expect dissolution to approach a planar surface model which is observed in case of pure Ketoprofen.

When particles of Ketoprofen were physically mixed with particles of excipients, a biphasic dissolution curve was obtained (Fig. 7 & 8). Theoretically in a system measuring

only particulate dissolution the excipients would not influence the dissolution rate of the Ketoprofen since each particle would dissolve independently. However, the initial dissolution rate of Ketoprofen (from zero to 5 min) from both physically mixed samples were significantly greater than the dissolution rate of Ketoprofen alone. These findings support the assumption that diffusion layers of excipients and Ketoprofen particles "overlap". When the sample is first introduced into the dissolution medium, the excipients dissolve rapidly and quickly attain a very high microscopic environmental concentration in the "mixed" diffusion layer of both particles. The presence of excipients increases the solubility of Ketoprofen in diffusion layer and thereby increases the dissolution rate of the drug. When the solid dispersion (Keto-Poloxamer 188) was studied, a biphasic curve again resulted, demonstrating an increased initial dissolution rate of Ketoprofen.

These findings indicate that dissolution process, under these experimental conditions probably adheres to a mixed mathematical model. This however, does not underscore the value of the technique for comparing the relative dissolution rate of Ketoprofen from various samples as such and in terms of cube root dissolution constants.

The value of cube root dissolution rate constants are about 10 and 4 times the dissolution rate constant of Ketoprofen for Keto-Poloxamer 188 solid dispersion and its physical mixture respectively. Likewise, it is approximately twice for Keto-PEG 6000 physical mixture. (Table 5 - 8)

Table : 5 - Cube root dissolution rate constants of solid dispersion system

Keto-Poloxamer 188 (1:9)

Time (min)	Amt. dissolved (mg)	Amt. undissolved (mg)	Amt. undissolved (g)	$W^{1/3}$	$Wo^{1/3} - W^{1/3}$	K
0	0	4	0.004	-	0	0
1	0.837	3.17	0.00317	0.149	0.0126	0.0126
2	1.910	2.1	0.0021	0.130	0.0316	0.0158
3	2.428	1.6	0.0016	0.119	0.0426	0.0142
5	3.683	0.32	0.0032	0.070	0.0916	0.0183

K(average) : 0.0152 $g^{1/3}$ min^{-1}

Table : 6 - Cube root dissolution rate constants of physical mixture Keto-Poloxamer 188 (1:9)

Time (min)	Amt. dissolved (mg)	Amt. undissolved (mg)	Amt. undissolved (g)	$W^{1/3}$	$Wo^{1/3}-W^{1/3}$	K
0	0	4	0.004	-	0	0
1	0.346	3.654	0.0036	0.156	0.0056	0.0056
2	0.776	3.224	0.0032	0.150	0.0116	0.0058
3	1.214	2.786	0.00278	0.143	0.0186	0.0062
5	1.709	2.291	0.00229	0.134	0.0276	0.0055

K(average) : 0.0057 $g^{1/3}$ min^{-1}

Table 7 - Cube root dissolution rate constants of physical mixture, Keto - PEG 6000 (1:9)

Time (Min)	Amt. dissolved (mg.)	Amt. Undissolved (mg.)	Amt. Undissolved (g.)	$W^{1/3}$	$W_o^{1/3} - W^{1/3}$	K
0	0	4	0.004	–	0	0
1	0.256	3.7	0.0037	0.157	0.0046	0.0046
2	0.371	3.6	0.0036	0.156	0.0056	0.0028
3	0.559	3.4	0.0034	0.153	0.0086	0.0028
5	0.788	3.21	0.00321	0.150	0.00116	0.0022

$K(\text{average}) = 0.0031\ g.^{1/3}\ min.^{-1}$

Table 8 - Cube root dissolution rate constants of Ketoprofen

Time (Min)	Amt. dissolved (mg.)	Amt. Un-dissolved (mg.)	Amt. Un-dissolved (g.)	$W^{1/3}$	$W_a^{1/3} - W^{1/3}$	K
0	0	4	0.004	-	0	0
1	0.156	3.84	0.00384	0.159	0.00268	0.0026
2	0.171	3.829	0.00382	0.159	0.00268	0.0018
3	0.225	3.775	0.00377	0.158	0.00368	0.0012
5	0.314	3.686	0.00368	0.157	0.00468	0.0009

$$K(\text{average}) = 0.0015 \ g.^{1/3} \ min^{-1}$$

To have an insight into the possible reason for the increased dissolution rate of drug, the samples were subjected to x-ray diffraction analysis (Fig : 9,10). On examination, x-ray diffractions of two selected solid dispersions, firstly, peaks attributed to excipients could be differentiated from the peak attributed to Ketoprofen. Keto-Poloxamer 188 solid dispersion, reflected the peak attributed to Ketoprofen, in fact, the intensity of peak being stronger than the original peak of drug at 2θ value of about 23.5°. Therefore, Ketoprofen in this solid dispersion retains the crystallanity. On the other side, in Ketoprofen - PEG 6000 solid dispersion, the peak attributed to Ketoprofen at 2θ value of about 23.5°, is suppressed which may indicate a reduction in crystallite size almost approaching amorphous form. This may partially account for the observed increase in dissolution rate of drug from Keto-PEG 6000 solid dispersion, in addition to the factors discussed previously. However, Keto-PEG 6000 physical mixture reflected crystallanity.

IR spectra of Ketoprofen-PEG 6000 and Ketoprofen Poloxamer 188 physical mixture were the summation of Ketoprofen and corresponding excipients spectrum and were idential to the corresponding solid dispersions spectra respectively, indicating no chemical alteration during the method of preparation.

Phase diagrams of both the solid dispersions reflected features common to eutectic mixture (Fig : 11, 12). The melt

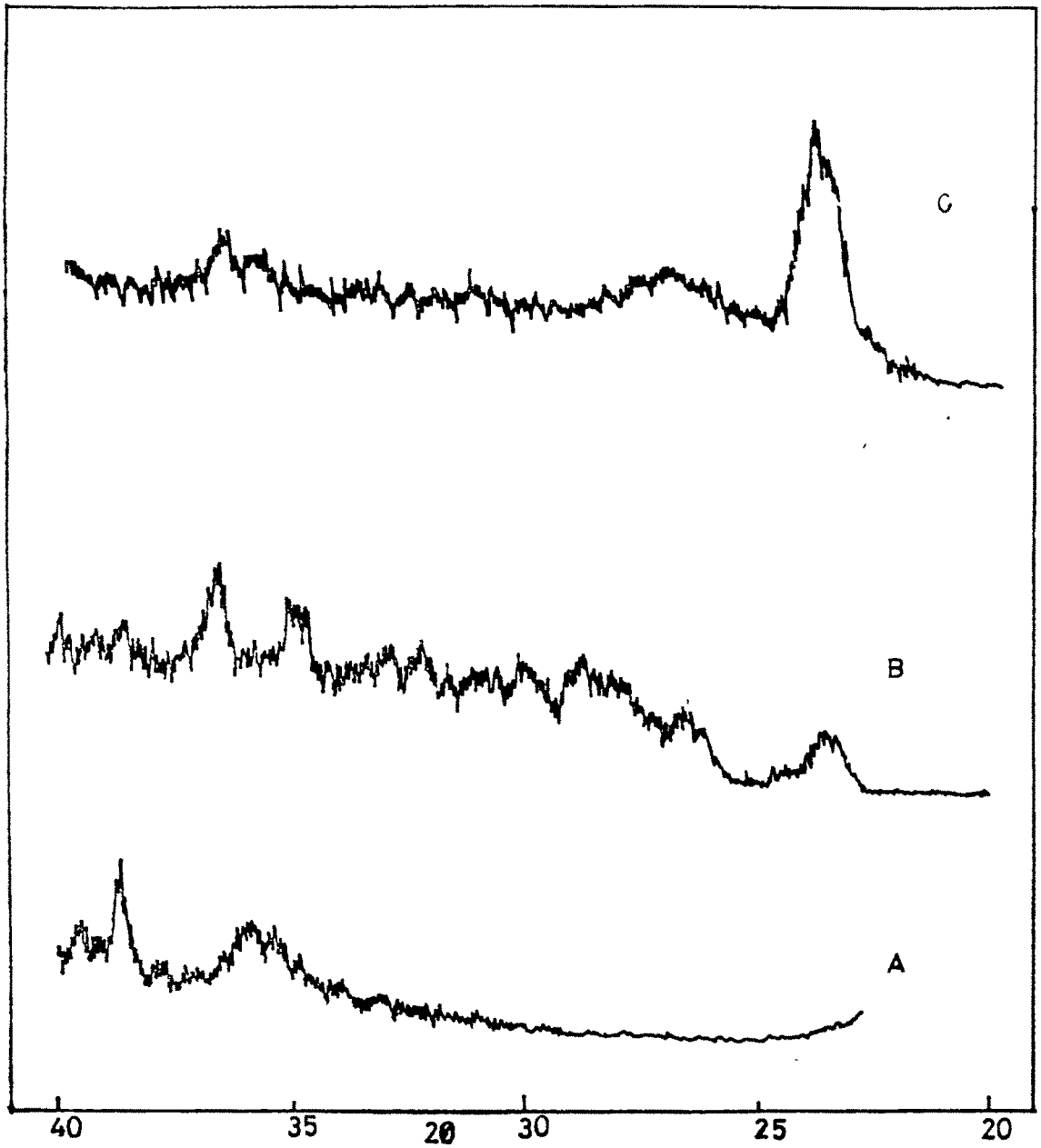

Fig.9 X - RAY DIFFRACTION PATTERNS OF

(A) POLOXAMER 188 : (B) KETO :

(C) KETO - POLOXAMER 188 S D

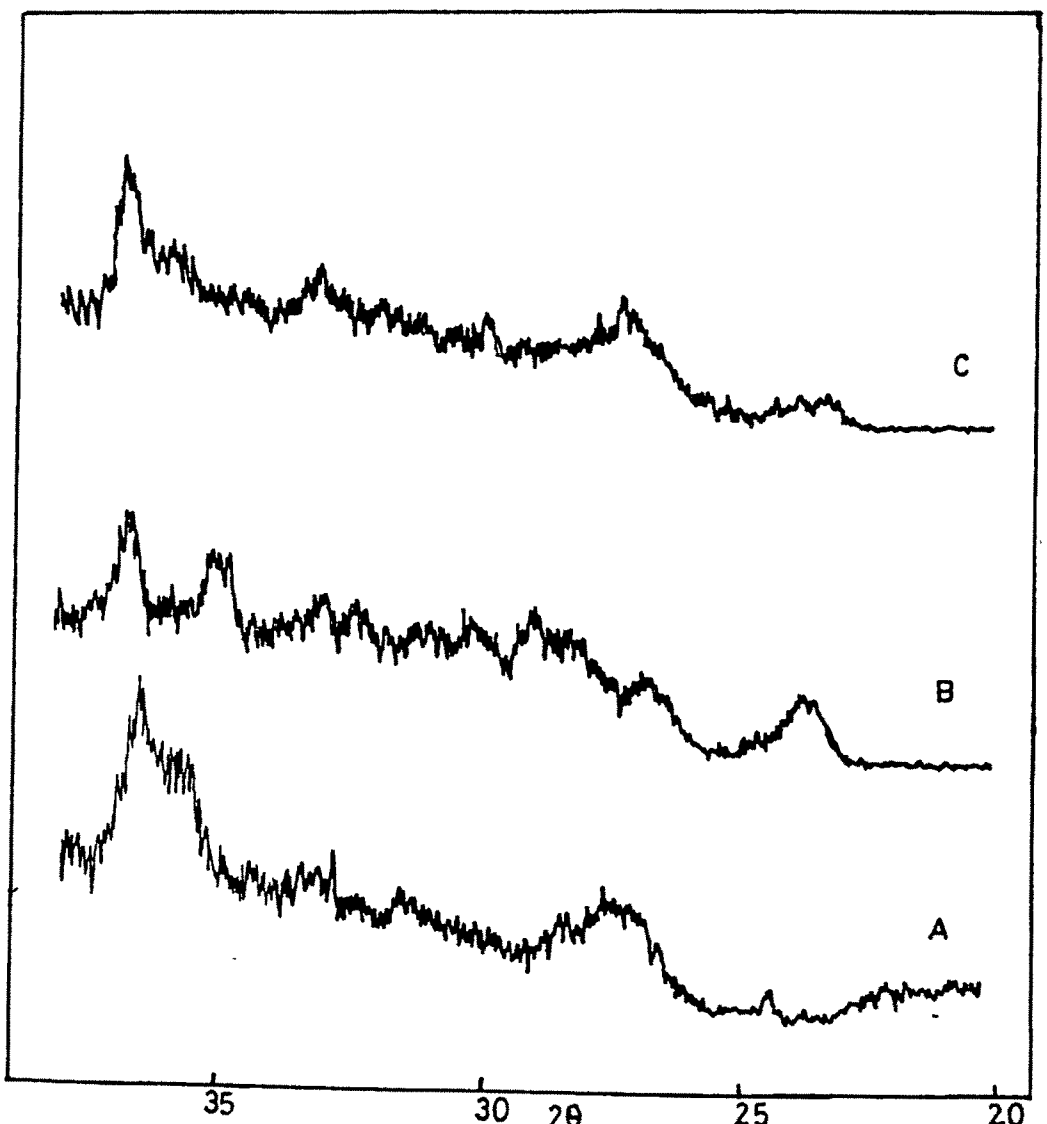

Fig.10 X-RAY DIFFRACTION PATTERNS OF (A) PEG 6000 : (B) KETO : (C) KETO-PEG 6000 SD

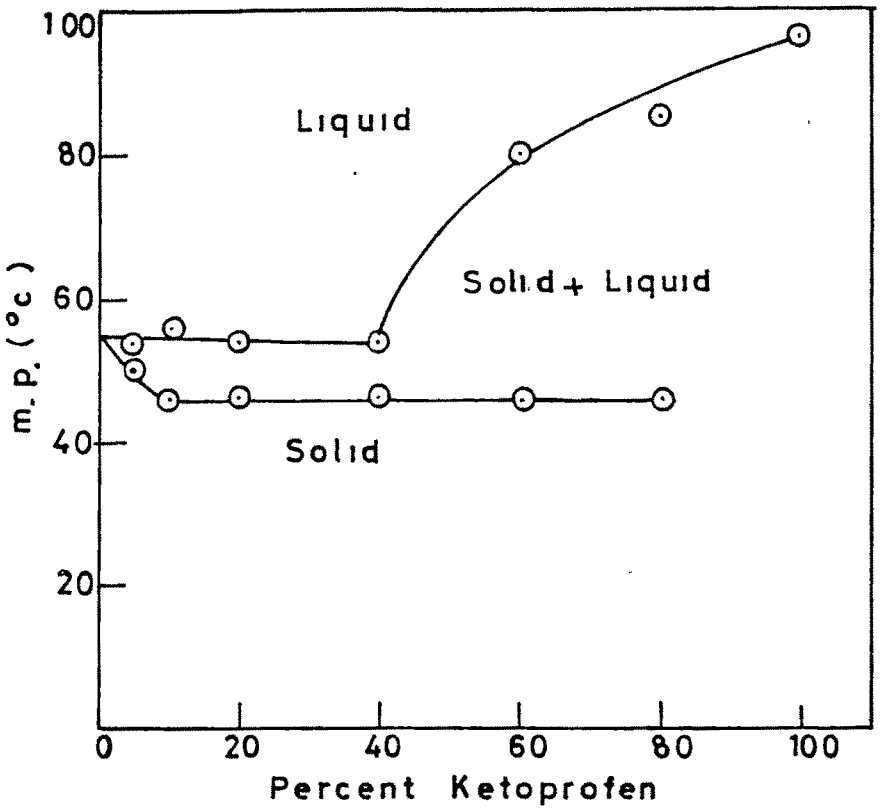

Fig 11 PHASE DIAGRAM OF KETO - PEG6000 SD.

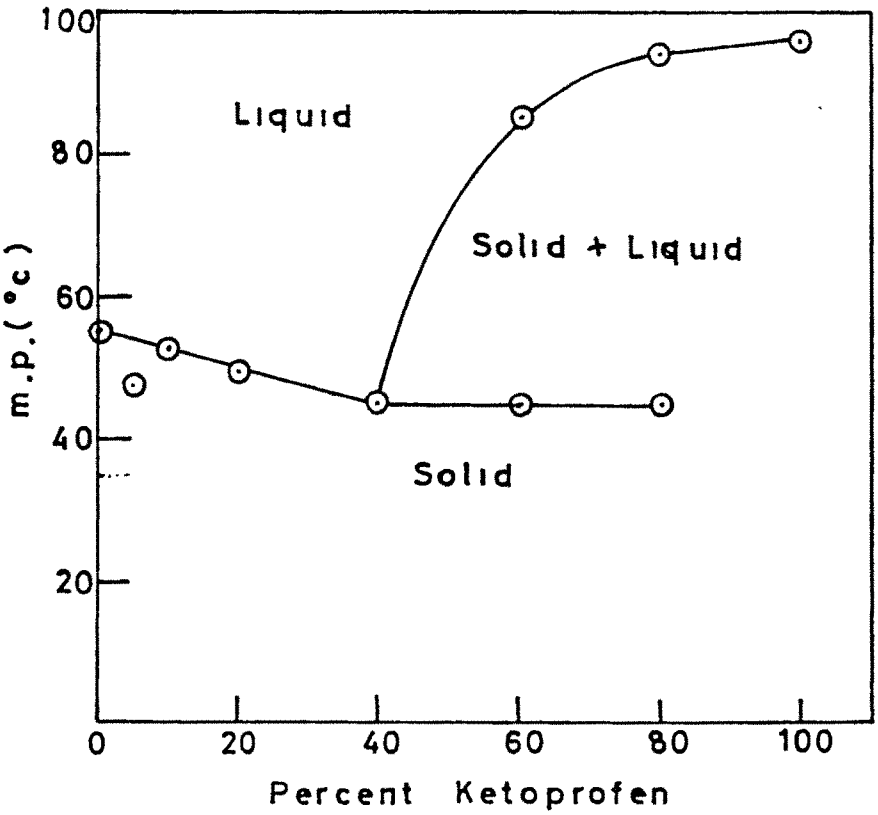

Fig. 12 PHASE DIAGRAM OF KETO-POLOXAMER 188 SD.

of both the excipients was capable of dissolving about 40% w/w of drug with eutectic composition consiting of 40% w/w drug and 60% w/w excipients.

Keto-PEG 6000 solid dispersion revealed no evidence suggesting the formation of solid solution. However, in Keto-Poloxamer 188 solid dispersion, solid dispersions in the left arm of the curve seemed to have little higher m.p. than the eutectic temperature. But the rise in m.p. is relatively low, therefore, it is rather difficult to predict the existence of solid solution. In any case, the role of either eutectic mixture or solid solution, seemed to be limited in explaining the observed much enhanced dissolution behaviour of drug from these solid dispersions; as solid dispersions corresponding to eutectic composition, on testing exhibited an increase in the dissolution rate in comparision to drug alone but was inferior to solid dispersions in this study i.e. Keto-excipient (1:9). Further, improved dissolution behaviour of corresponding physical mixtures (1:9) too may outline a greater role for local factors than the formation of eutectics or eutectic with solid solution, even if the later possibility exists.

Chemical stability - Two test preparations viz. Keto-PEG 6000 (1:9) physical mixture and Keto-Poloxamer 188 (1:9) solid dispersion were stored under ambient conditions for two years. Ketoprofen content in the stored samples was determined by uv measurement at 260 nm. There was no

Table : 9 - Stability of Ketoprofen (Percent residual amount) in samples stored under normal temperature and humidity conditions.

Sample	Storage Period		
	Initial	12 mo	24 mo
Keto-PEG 6000PM (1:9)	100.00	98.26	97.59
Keto-Poloxamer SD 188 (1:9)	100.00	97.56	100.74

significant change in drug content in these preparations (Table : 9). There was also no change in physical appearance of samples.

Aging study - Two selected test preparations mentioned above were subjected to aging study when stored under ambient conditions for a period of 12 months. The samples were periodically analysed for their dissolution behaviour. There was no significant change in the dissolution behaviour till the end of 12 months (Fig : 13, 14).

Therefore, it may be concluded that these test preparations are stable at room temperature and humidity conditions, with respect to any chemical change and dissolution behaviour for a reasonable length of time.

Bioavailability study - Keto-Poloxamer 188 solid dispersion, Keto-PEG 6000 physical mixture (both filled in capsules), Keto capsules (M & B) and Keto capsule (containing Ketoprofen only) representing equivalent to 100 mg. of Ketoprofen were orally administered to human volunteers. The mean urinary excretion rates of total Ketoprofen time to peak, peak excretion rates and area under the excretion rate time curves are shown in Table : 10, 11 & Fig. 15.

Following the administration of Ketoprofen alone, the maximum value of mean urinary excretion rate of total Ketoprofen appeared in 3-4 hr. period whereas it was between 1-2 hrs for Keto-Poloxamer 188 solid dispersion and Keto-PEG 6000 physical mixture. Keto capsule (M&B) showed

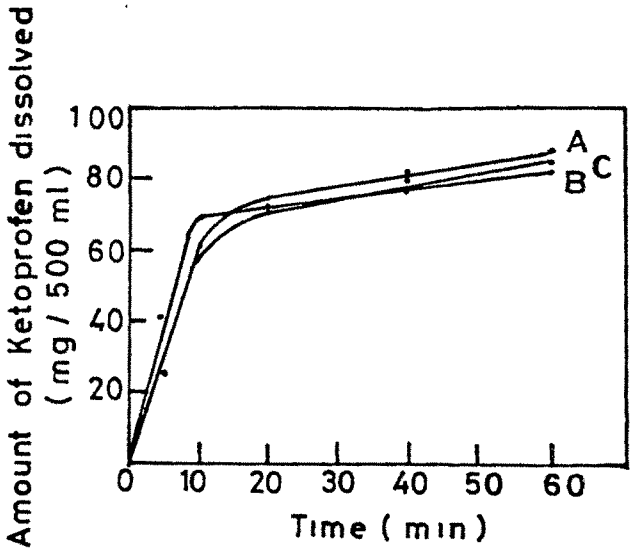

Fig 13 EFFECT OF STORAGE UNDER AMBIENT CONDITIONS ON THE DISSOLUTION BEHAVIOUR OF KETO-POLOXAMER SD STORAGE PERIOD: (A) INITIAL, (B) 6 mo. (C) 12 mo.

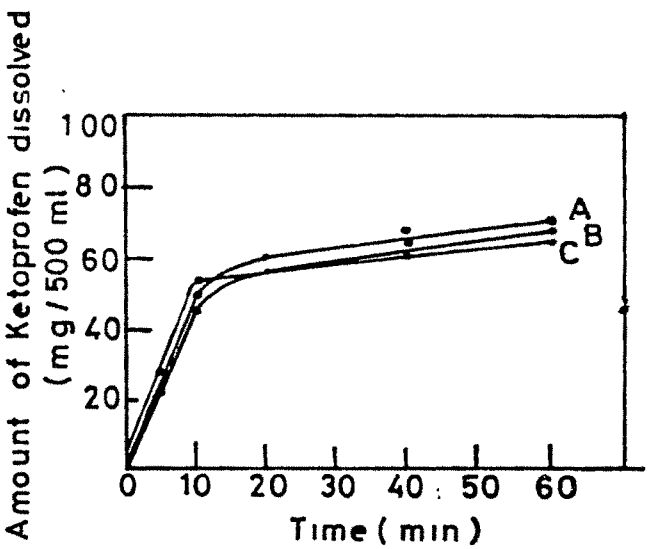

Fig 14 EFFECT OF STORAGE UNDER AMBIENT CONDITIONS ON THE DISSOLUTION BEHAVIOUR OF KETO-PEG6000 PM. STORAGE PERIOD: (A) INITIAL, (B) 6 mo, (C) 12 mo.

Table : 10 - Mean excretion rate of Ketoprofen from test preparations.

Time (Midpoint of time interval, in hr)	Excretion rate[a] mg hr^{-1}			
	A	B	C	D
0.5	8.79±1.88	2.47±1.95	0.95±0.88	9.74±1.88
1.5	18.92±8.15	7.64±3.86	7.51±3.27	13.68±3.49
2.5	11.95±5.39	2.91±2.05	4.77±2.32	14.69±3.41
3.5	9.12±0.66	3.56±3.31	10.75±5.80	9.87±3.22
5	5.26±3.60	3.89±1.19	2.93±1.61	3.28±1.71

a) Mean ± SD (A) Keto-Poloxamer 188 SD (B) Keto-PEG 6000 PM

(C) Keto Caps (M&B) (D) Ketoprofen Capsule

Table : 11 - Mean time to peak, peak excretion rate and AUC of excretion data following oral administration of 100 mg. of Ketoprofen, Ketoprofen Capsule (M&B) and two test preparations.

	Time to peak	Peak excretion rate (mg hr^{-1})[a]	AUC[a] mg. hr.
Keto-Poloxamer 188 SD.	1.5(1-2 hrs)	18.92 ± 8.15	58.53 ± 16.36
Keto-PEG 6000 PM.	1.5(1-2 hrs)	7.64 ± 3.86	22.26 ± 3.22
Keto Capsule (M&B).	3.5(3-4 hrs)	10.75 ± 5.80	32.11 ± 5.21
Keto Capsule	2.5(2-3 hrs)	14.69 ± 3.41	55.94 ± 5.91

a) Mean ± SD

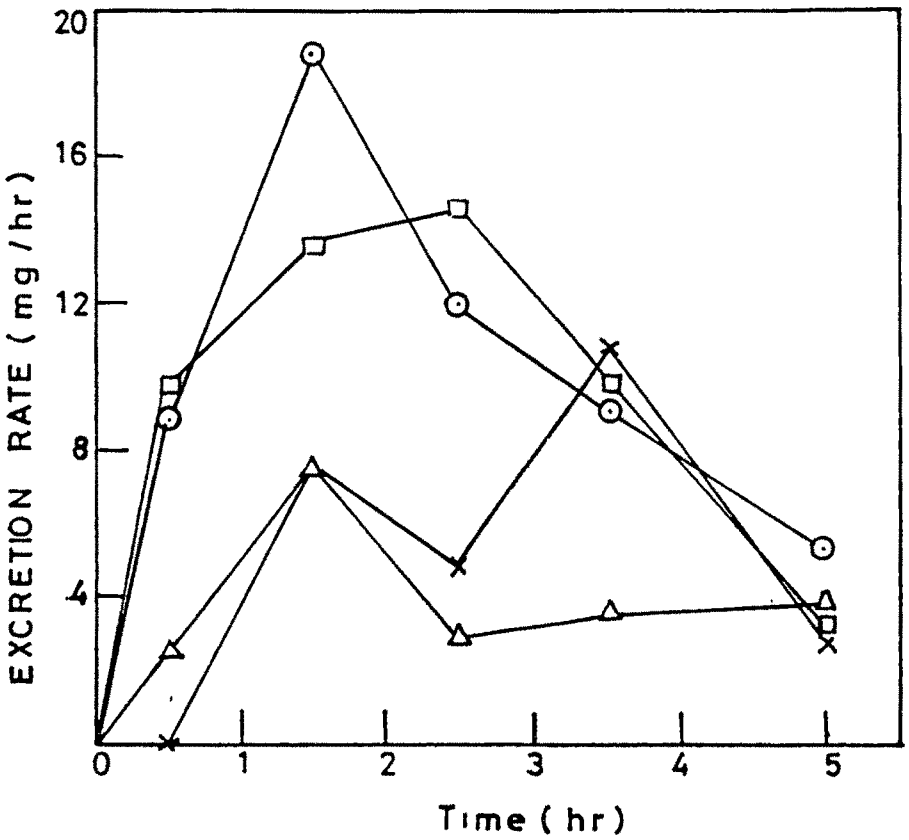

Fig15 URINARY EXCRETION RATE OF TOTAL KETOPROFEN FOLLOWING THE ORAL ADMINISTRATION OF VARIOUS TEST PREPARATION CONTAINING EQUIVALENT OF 100 mg. OF KETOPROFEN.

KEY : O KETO-POLOXAMER 188 CAPS.
Δ KETO-PEG6000 PM CAPS.
□ KETO CAP.
X KETO CAPS (M AND B)

the maximum value of mean excretion rate between 2-3 hrs. Mean excretion rate at this maximum corresponded to 1.28 times as great as Ketoprofen alone, for Keto-Poloxamer 188 SD. But it was 0.52 and 0.73 times the mean excretion rate of Ketoprofen alone, for Keto-PEG 6000 physical mixture and Keto Caps. (M&B) respectively.

The bioavailability of Ketoprofen from Keto-Poloxamer 188 solid dispersion was 1.04 times greater than Ketoprofen alone, the difference being not statistically significant. It was about 0.4 and 0.51 times for Keto-PEG 6000 physical mixture and Keto Capsule (M&B) respectively with reference to Ketoprofen alone.

Multiple variance test of significance was applied to the mean excretion rate data at the midpoint of each urine collection time interval to check if (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 (Table : 12-16 ; P=0.05).

At 0.5 hr, Ketoprofen-Poloxamer 188 solid dispersion differs from Keto-PEG 6000 physical mixture and Keto Caps (M&B). Keto Capsule (containing Ketoprofen alone) differs significantly from Keto-PEG 6000 physical mixture and Keto Caps. (M&B). While Keto-PEG 6000 physical mixture and Keto Caps (M&B) on one hand and Keto-Poloxamer 188 solid dispersion and Ketoprofen alone on the other hand, do not

Table : 12 - Analysis of variance at 0.5 hr. (midpoint of urine collection time).

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Among regimens	t-1 = 3	229.6	76.53	7.63
Within regimens.	(ni-1)=12	120.4	s^2=10.03	
Total :	N-1 = 15	350.0		

7.63 > 6.55 at P = 0.01

Ranked means

Keto Caps (M&B)	Keto-PEG 6000 PM	Keto-Poloxamer 188 SD	Keto Cap.
0.95	2.47	8.79	9.74

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

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

Keto Caps (M&B) vs. Keto-Poloxamer SD

Keto-PEG 6000 PM vs. Keto-Poloxamer SD

Keto-Caps (M&B) vs. Keto Cap.

Keto-PEG 6000 PM vs. Keto Cap.

Table : 13 - Analysis of variance at 1.5 hr. (midpoint of urine collection time).

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Among regimens	t-1 = 3	359.51	119.83	4.59
Within regimens	(ni-1)=12	312.75	s^2=26.06	
Total	N-1 =15	672.26		

4.59 > 3.71, at P=0.05

Ranked means

Keto Caps(M&B)	Keto-PEG 6000 PM	Keto Cap	Keto-Poloxamer 188 SD
7.51	7.64	13.68	18.92

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

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

Keto Caps (M&B) vs. Keto-Poloxamer 188 SD

Keto-PEG 6000 PM vs. Keto-Poloxamer 188 SD

Table : 14 - Analysis of variance at 2.5 hrs. (midpoint of urine collection time).

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Among regimens	t-1 = 3	380,98	126.99	10.07
Within regimens	(ni-1)=12	151.29	s^2=12.60	
Total	N-1 =15	532.27		

10.0 > 6.55 at P = 0.01

Ranked means

Keto-PEG 6000 PM	Keto Caps (M&B)	Keto-Poloxamer 188 SD	Keto Cap.
2.91	4.77	11.95	14.69

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

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

Keto - PEG 6000 PM vs. Keto-Poloxamer 188 SD

Keto - PEG 6000 PM vs. Keto Cap.

Keto - Caps (M&B) vs. Keto-Poloxamer SD

Keto Caps (M&B) vs. Keto Cap.

Table : 15 - Analysis of variance at 3.5 hrs. (midpoint of urine collection time).

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Among regimens	t-1 = 3	126.58	42.19	3.04
Within regimens	(ni-1)=12	166.36	13.86	
Total	N-1 = 15	292.94		

3.04 > 2.73 at P = 0.10

Ranked means

Keto-PEG 6000 PM	Keto-Poloxamer 188 SD	Keto-Cap	Keto Caps (M& B)
3.56	9.12	9.87	10.75

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

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

Keto-PEG 6000 PM vs rest of preparations.

Table : 16 - Analysis of variance at 5 hrs. (midpoint of urine collection time).

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Among regimens	t-1=3	12.60	4.20	0.84
Within regimens	(ni-1)=12	59.90	s^2=4.99	
Total	N-1 = 15	72.50		

$0.84 < 2.73$ at P = 0.10

No significant difference exists.

show any significant difference.

At 1.5 hr. Keto-Poloxamer 188 solid dispersion differs significantly from Keto-PEG 6000 physical mixture and Keto Caps. (M&B).

At 2.5 hr, Keto-PEG 6000 physical mixture differs significantly from Keto-Poloxamer 188 solid dispersion and Ketoprofen alone. Keto Caps (M&B) is significantly different from Keto-Poloxamer 188 solid dispersion and Ketoprofen alone.

At 3.5 hr, firstly all these test preparations do not differ from each other (either among regimens or within regimens) at $P = 0.05$ as is clearly indicated from the calculated value of 3.04 which is less than the tabulated value of 3.71 but they differ from each other at $P = 0.10$. When further tested for the significant difference amongst various test preparations at $P = 0.05$, it can be seen that Keto-PEG 6000 physical mixture differs significantly from rest of the test preparations.

At 5 hr, there is no significant difference among various test preparations.

From the above analysis, it may be concluded that (i) there exist significant differences amongst and within test preparations(upto 3.5 hr) (ii) in all instances Keto-Poloxamer 188 solid dispersion differ significantly from Keto-PEG 6000 physical mixture and Keto Caps (M&B) (ii) Keto-Poloxamer 188 solid dispersion and Ketoprofen

alone do not differ significantly from each other.

Summing up the conclusions, it may be presumed that bioavailability of Ketoprofen from Keto-Poloxamer 188 solid dispersion is superior to either Keto-PEG 6000 physical mixture or Keto Caps (M&B). Keto-Poloxamer 188 solid dispersion and Ketoprofen alone may be considered to be bioequivalent preparations, however equal clinical efficacy or broad based bioavailability trial, might provide a further conclusive evidence.

The results of bioavailability study deviate from the expected behaviour based on in-vitro results. The unexpected lower bioavailability of Keto-PEG 6000 physical mixture is inexplicable at this stage.

Lower bioavailability of Keto Caps (M&B) was on the expected lines, as was evident from the lower dissolution rate of Ketoprofen from these capsules. This may be explained by the fact that the powder mix in these capsules was under some compression and possible role of formulation factors. As the dissolution proceeded, two small plugs of powder were seen, therefore, the lower surface area of powder exposed to dissolution medium may be one of the reasons for decreased dissolution rate.

As expected, there was good bioavailability of Ketoprofen from Ketoprofen-Poloxamer 188 solid dispersion. But at the

same time, equivalent bioavailability of Ketoprofen was achieved from capsule containing Ketoprofen alone. The in-vitro results did not suggest such a behaviour.

However, use of such solid dispersions could lead to faster absorption of drug while at the same time minimising the bioinequivalency to certain extent.