Chapter - 4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSIONS

4.1 ANALYTICAL METHODS

i) Calibration Curve for the Estimation of 5-Fluorouracil

Calibration curve for the estimation of 5-fluorouracil was plotted in phosphate buffered saline in the concentration range of 5 to 50 µg/ml (Table 6). The Beer's curve was found to be rectilinear upto 30 µg/ml (Fig. 5).

ii) Calibration Curve for the Estimation of Amino Acids

Calibration plots of the amino acids using the Ninhydrin method were found to be rectilinear in the concentration range of 1 to 10 µg/ml (Table 7, Fig. 6).

iii) Partition Coefficient of Amino Acids between Chloroform : Cyclohexane and Sodium carbonate

Table 8 gives the partition coefficients of the amino acids between the organic and the aqueous phases used in the preparation of polyterephthalamide microcapsules viz. chloroform : cyclohexane (1:3) and 0.45M sodium carbonate solution.

Table : 6

Data for Calibration Curve of 5-Fluorouracil in Phosphate Buffered Saline

Concentration (μg/ml)	Absorbance at 266 nm (average of 3 readings)
5.0	0.268
10.0	0.524
15.0	0.797
20.0	1.13
25.0	1.32
30.0	1.62
40.0	> 2
50.0	> 2

Regression line : $Y_c = 0.054X + 0.003$

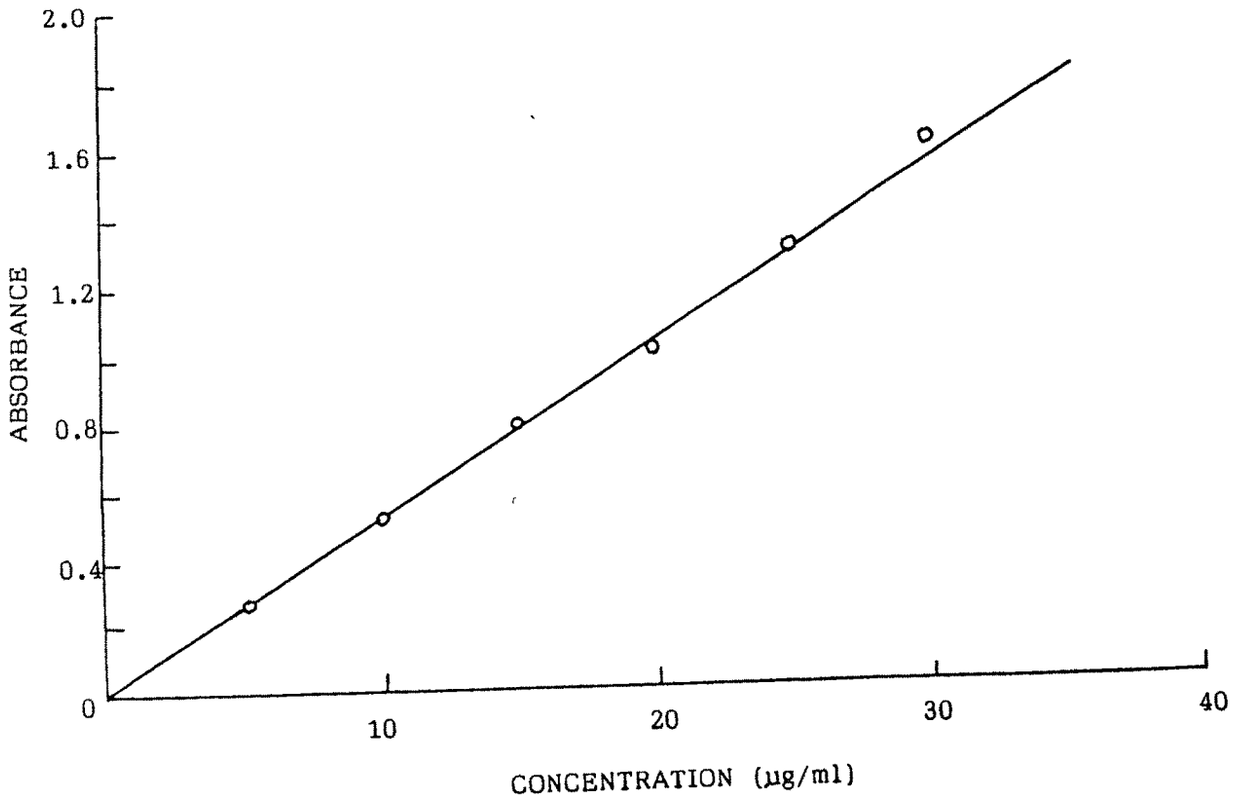


Fig. 5 : Calibration curve for estimation of 5-FU in Phosphate Buffered Saline

Table : 7

Data for Calibration Curves of Various Amino Acids

Concentration µg/ml	Amino Acid	Absorbance						
		Arginine	Asparagine	Citrulline	Cystine	Glutamine	Lysine	Ornithine
1		0.095	0.18	0.084	0.365	0.08	0.22	0.125
2		0.205	0.36	0.18	0.63	0.13	0.47	0.25
4		0.310	0.82	0.27	1.20	0.265	1.05	0.45
6		0.45	1.15	0.43	1.86	0.35	1.37	0.71
8		0.65	1.45	0.55	> 2	0.47	1.9	0.95
10		0.82	1.78	0.69	-	0.58	> 2	1.22
12		0.96	> 2	0.83	-	0.76	-	1.48
Regression line Y_c =		0.08X + 0.008	0.183X + 0.011	0.069X + 0.009	0.308X + 0.013	0.061X + 0.001	0.238X + 0.002	0.121X - 0.002

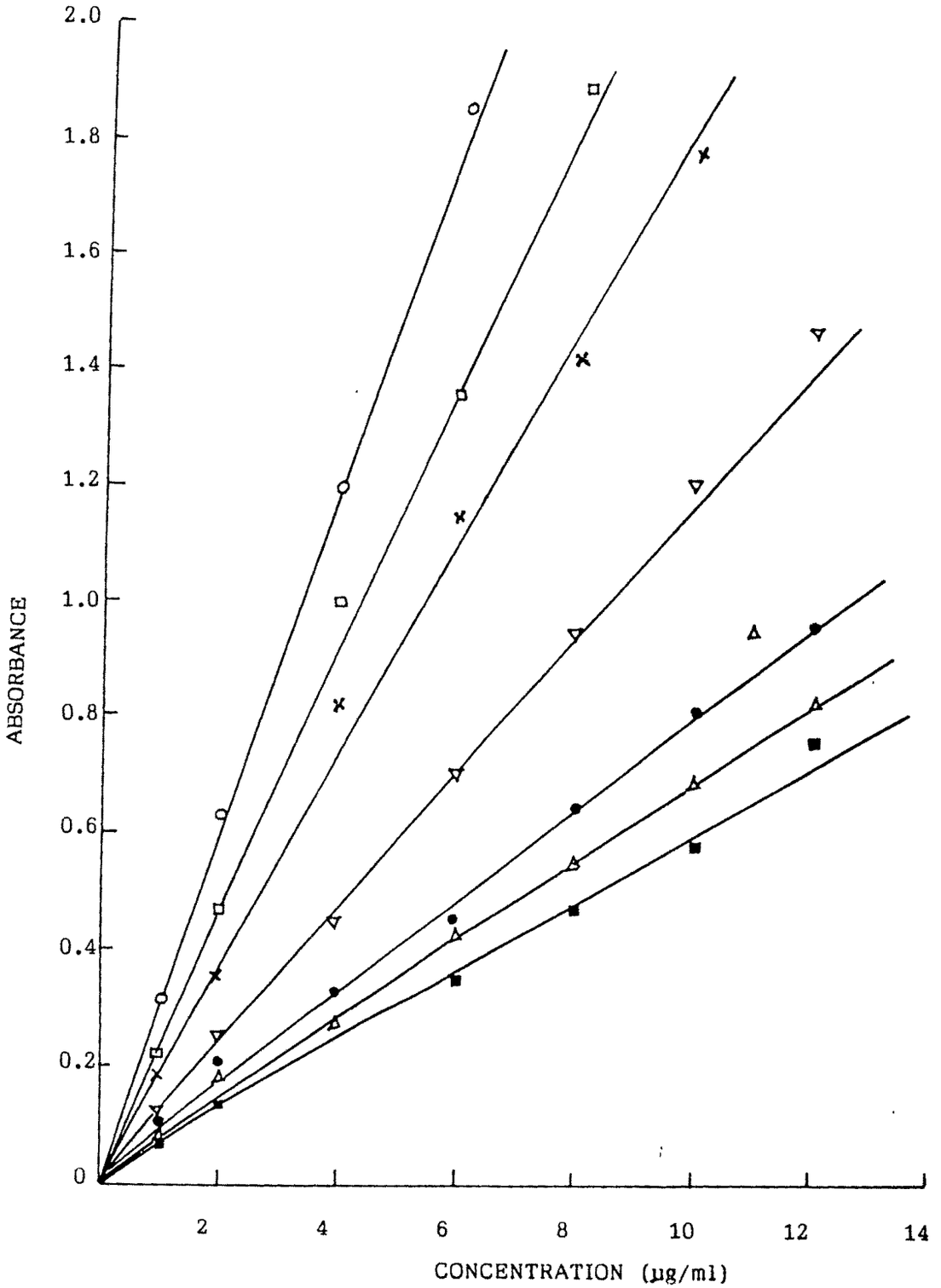


Fig. 6 : Calibration curve for estimation of Amino acids

• - Arginine; x - Asparagine; Δ- Citrulline
o - Cystine; ■ - Glutamine; □ - Lysine; ∇ - Ornithine

TABLE : 8

Partition Coefficient $K_{o/w}$ of Amino Acids

Amino Acid	Amount in aqueous phase (mg)	Amount in organic phase (mg)	$K_{o/w} = \frac{\text{amt (org)}}{\text{amt (aq)}}$
Arginine	62.89 ± 2.12	17.11 ± 2.12	0.27
Asparagine	34.49 ± 0.61	45.50 ± 0.61	1.32
Citrulline	68.57 ± 1.49	11.43 ± 1.49	0.17
Cystine	7.81 ± 0.88	32.19 ± 0.88	4.12
Glutamine	70.50 ± 2.82	9.50 ± 2.82	0.13
Lysine	49.57 ± 1.89	30.43 ± 1.89	0.61
Ornithine	57.55 ± 5.23	22.45 ± 5.23	0.39

iv) Extraction of 5-Fluorouracil from Aqueous Phase

Assay of microcapsules had to be carried out indirectly by extracting the drug from the aqueous supernatant and washings. A reported solvent system comprising of ethyl acetate : propanol (7:3) was used for the purpose. The data for the calibration plot for the estimation of 5-FU by extraction is given in Table 9 and Fig. 7. The calibration curve was rectilinear in the concentration range of 4 to 100 µg/ml.

v) Reliability of Extraction Procedure for Assay of 5-FU from Microcapsules

To validate the applicability of the proposed extraction procedure involving ethyl acetate : propanol (7:3), the calibration curve for 5-FU was carried out in the same solvent mixture in the concentration range of 1 to 50 µg/ml (Fig. 8). Thereafter, standard solutions of 5-FU in phosphate buffered saline were extracted with the solvent mixture and the two layers were collected separately. The absorbance of each layer was read at 272 nm and the amount of 5-FU in the two phases was calculated from their respective calibration plots. Finally, the percent drug recovery in the combined phases was also estimated.

TABLE : 9

Data for Calibration Curve of 5-Fluorouracil after extraction with Ethyl Acetate : Propanol

Concentration (µg/ml)	Absorbance at 272 nm (average of 3 readings)
4.0	0.085
10.0	0.142
20.0	0.353
30.0	0.529
40.0	0.649
50.0	0.840
60.0	1.099
80.0	1.407
100.0	1.780

Regression line : $Y_c = 0.018X + 0.006$

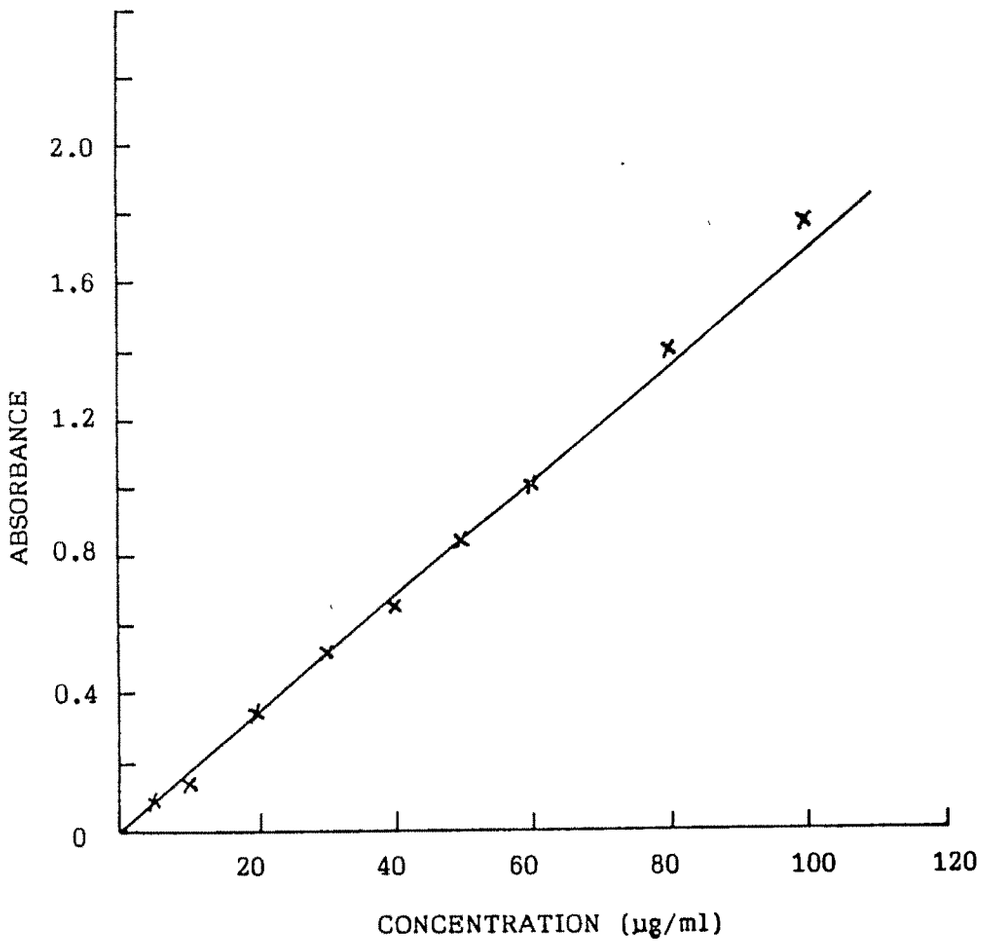


Fig. 7 : Calibration curve for estimation of 5-FU by extraction with Ethyl Acetate : Propanol

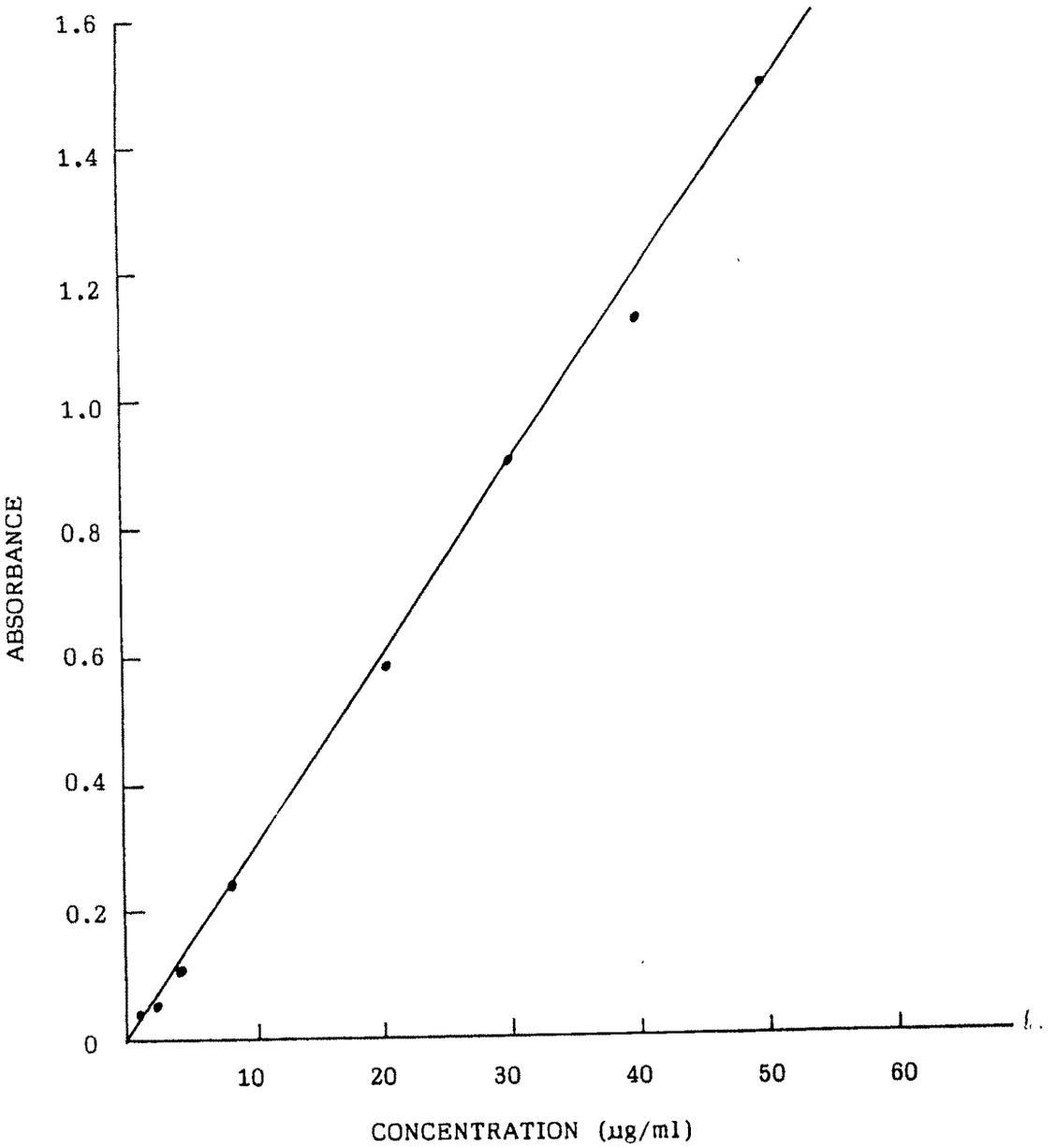


Fig. 8 : Calibration curve for estimation of 5-FU in Ethyl Acetate : Propanol (7:3)

The results (Table 10) show that the percent drug recovery at all concentration levels studied ranged from 96% to 104%, giving an error margin of 4% only. This indicates that the procedure adopted for extracting 5-FU from the aqueous solution by means of the chosen solvent system is a reliable one.

vi) Calibration Curve for Estimation of 5-FU in Various Organs

Table 11 gives the data for the calibration of 5-FU in various organs, alongwith their regression lines. The corresponding calibration plots are illustrated in figures 9-13. In general, the calibration curves were linear in the concentration range of 1 to 50 µg/ml.

4.2 PREPARATION AND OPTIMIZATION

4.2.1 Polyamide Microcapsules

A] PREPARATION OF MICROCAPSULES

Polyamide microcapsules were prepared by an w/o interfacial polymerisation reaction between a few diamino acids and terephthaloyl chloride. Terephthaloyl chloride was selected as an ideal diacid chloride for the preparation of microcapsules for incorporating 5-fluorouracil, based on

TABLE : 10

Data showing the Reliability of the Extraction Procedure for Assay of the Microcapsules

Concentration (µg)	Amount of drug in Aqueous phase 1 (µg)	Amount of drug in Organic phase 2 (µg)	Total Amount of drug recovered 1 + 2 (µg)	% drug recovery
1	0.77	0.26	1.03	103.00
2	1.52	0.51	2.03	101.50
4	3.08	0.93	4.01	100.03
8	6.11	2.06	8.17	100.21
10	8.10	2.77	10.87	108.70
20	16.06	4.73	20.79	103.85
30	22.08	6.97	29.05	96.83
40	30.05	9.83	39.88	99.20
50	37.81	13.23	51.04	102.08

TABLE : 11

Data for Calibration Curve of 5-FU in Various Organs

Concentration (µg/ml)	Absorbance				
	Lungs	Liver	Kidneys	Intestine	Spleen
1	0.011	0.045	0.023	0.050	-
2	0.021	0.105	0.043	0.122	0.041
4	0.042	0.151	0.085	-	0.085
6	-	0.183	0.101	0.171	-
8	0.104	-	0.161	-	0.172
10	0.143	0.304	0.252	0.304	0.224
20	0.275	0.611	0.505	0.510	0.481
30	0.380	0.905	0.721	0.718	0.611
40	0.540	1.296	0.877	0.967	0.825
50	-	1.512	-	-	1.154
60	0.872	-	-	-	-
Regression Line Y=	0.014X - 0.008	0.031X + 0.007	0.023X - 0.001	0.025X + 0.017	0.022X - 0.008

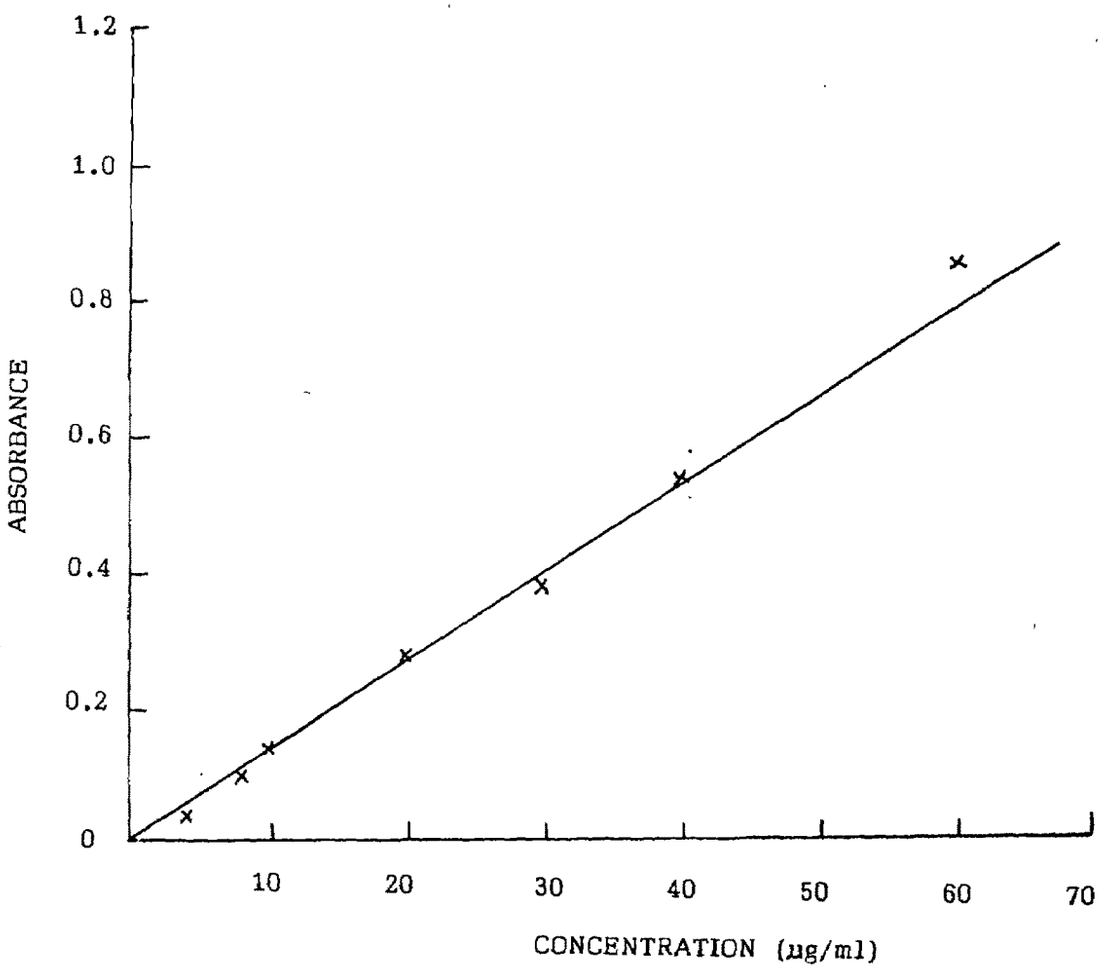


Fig. 9 : Calibration curve for estimation of 5-FU in Lungs

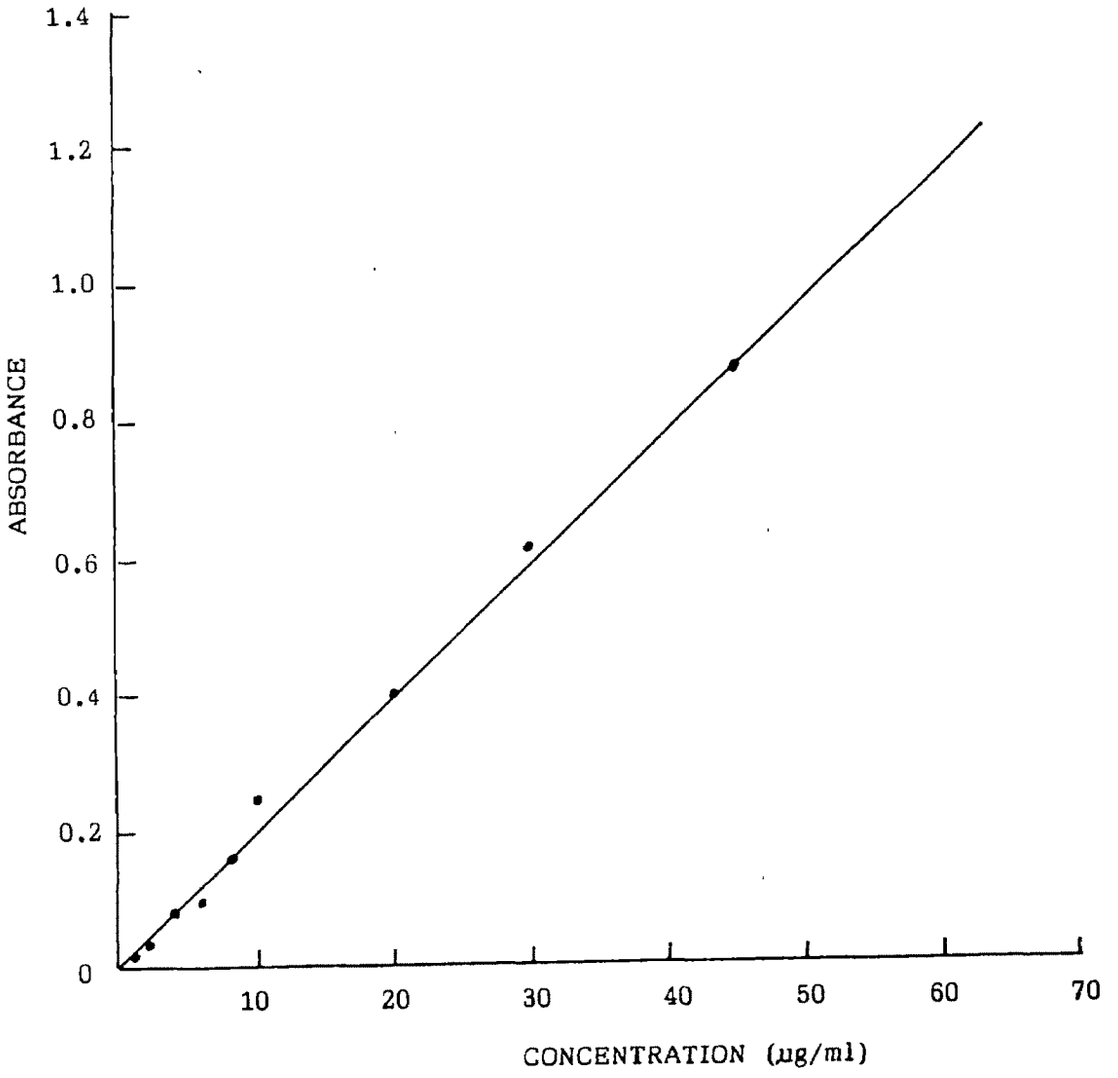


Fig. 10 : Calibration curve for estimation of 5-FU in Kidneys

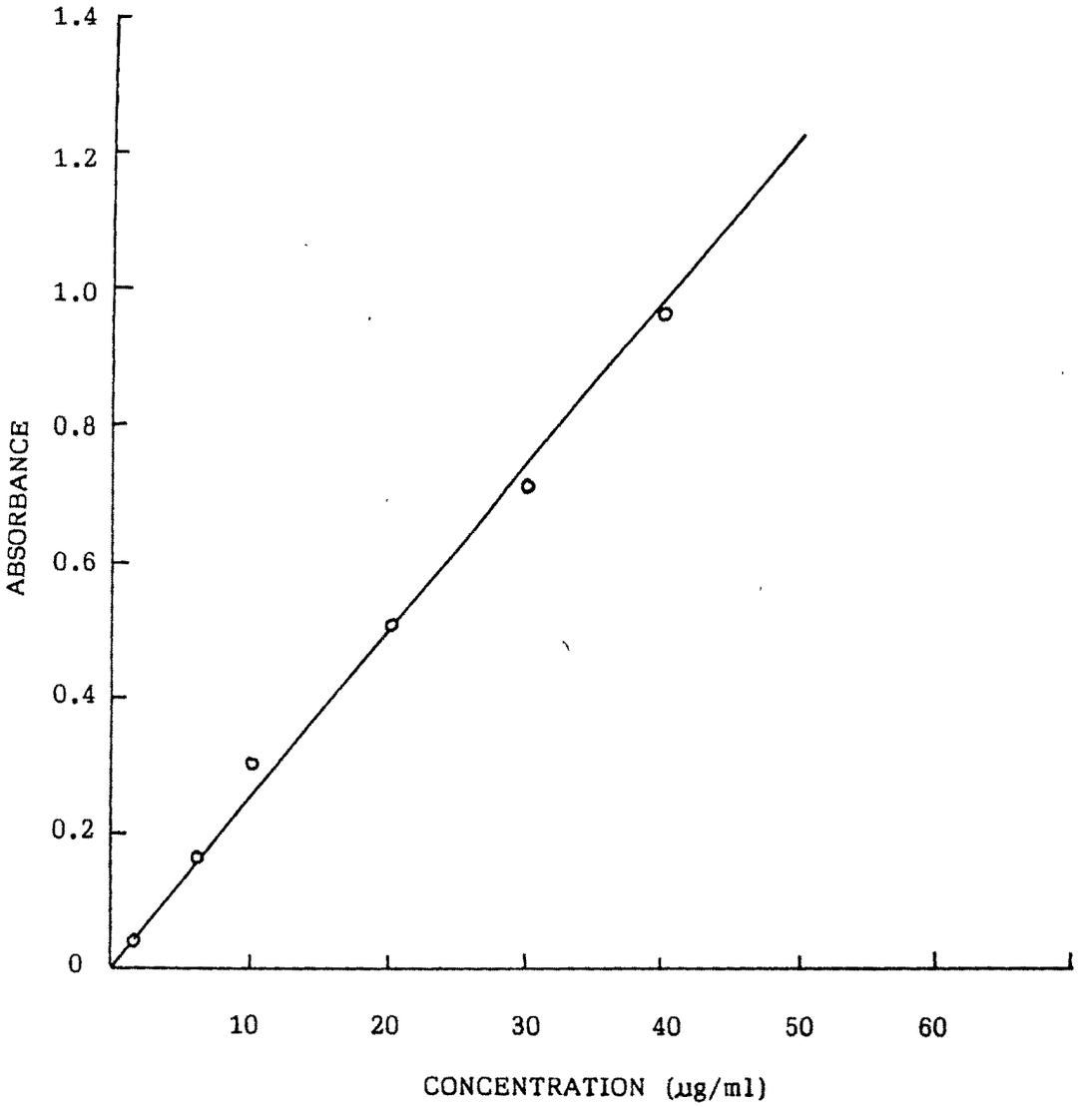


Fig. 11 : Calibration curve for estimation of 5-FU in Intestine

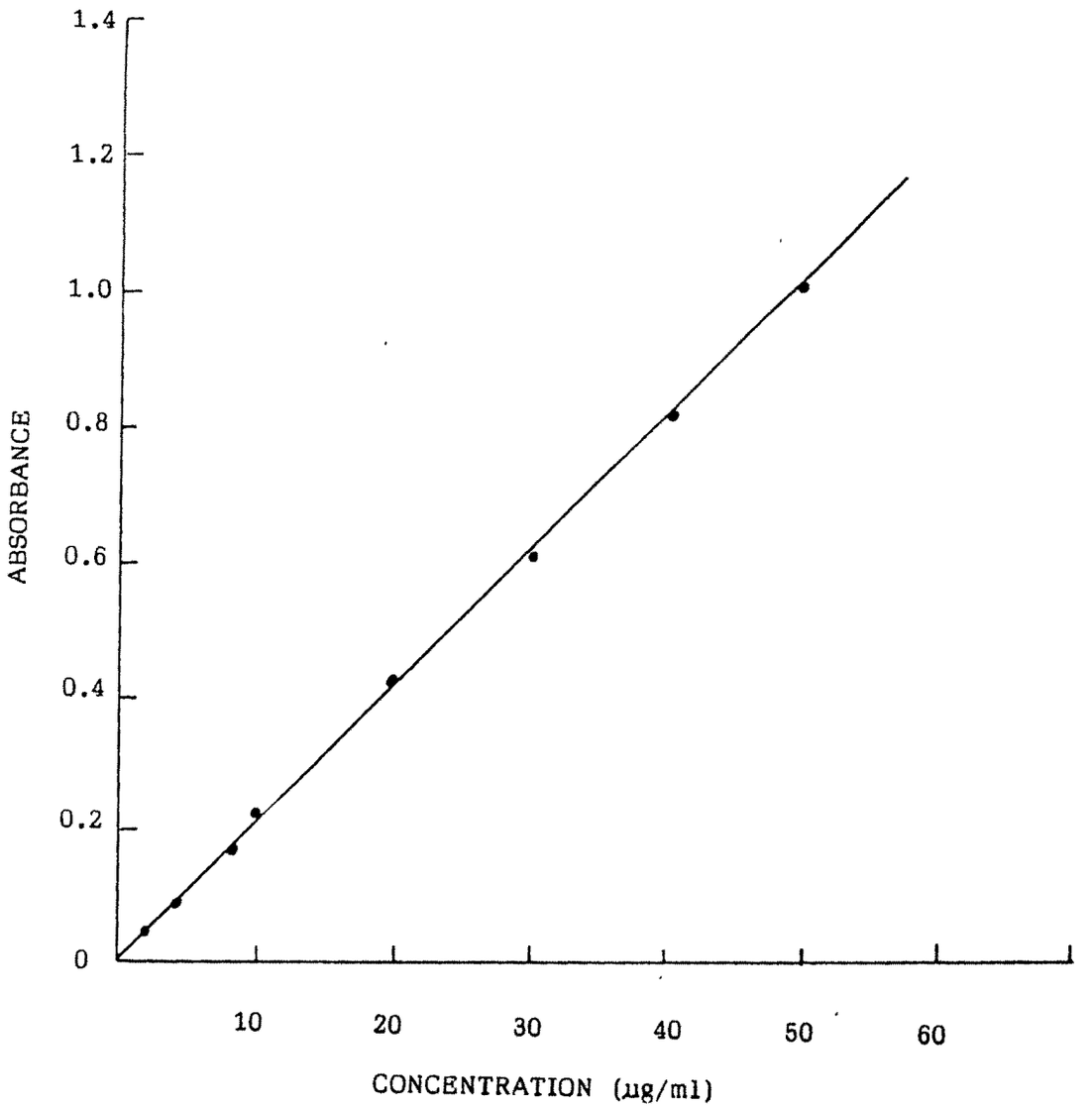


Fig. 12 : Calibration curve for estimation of 5-FU in Spleen

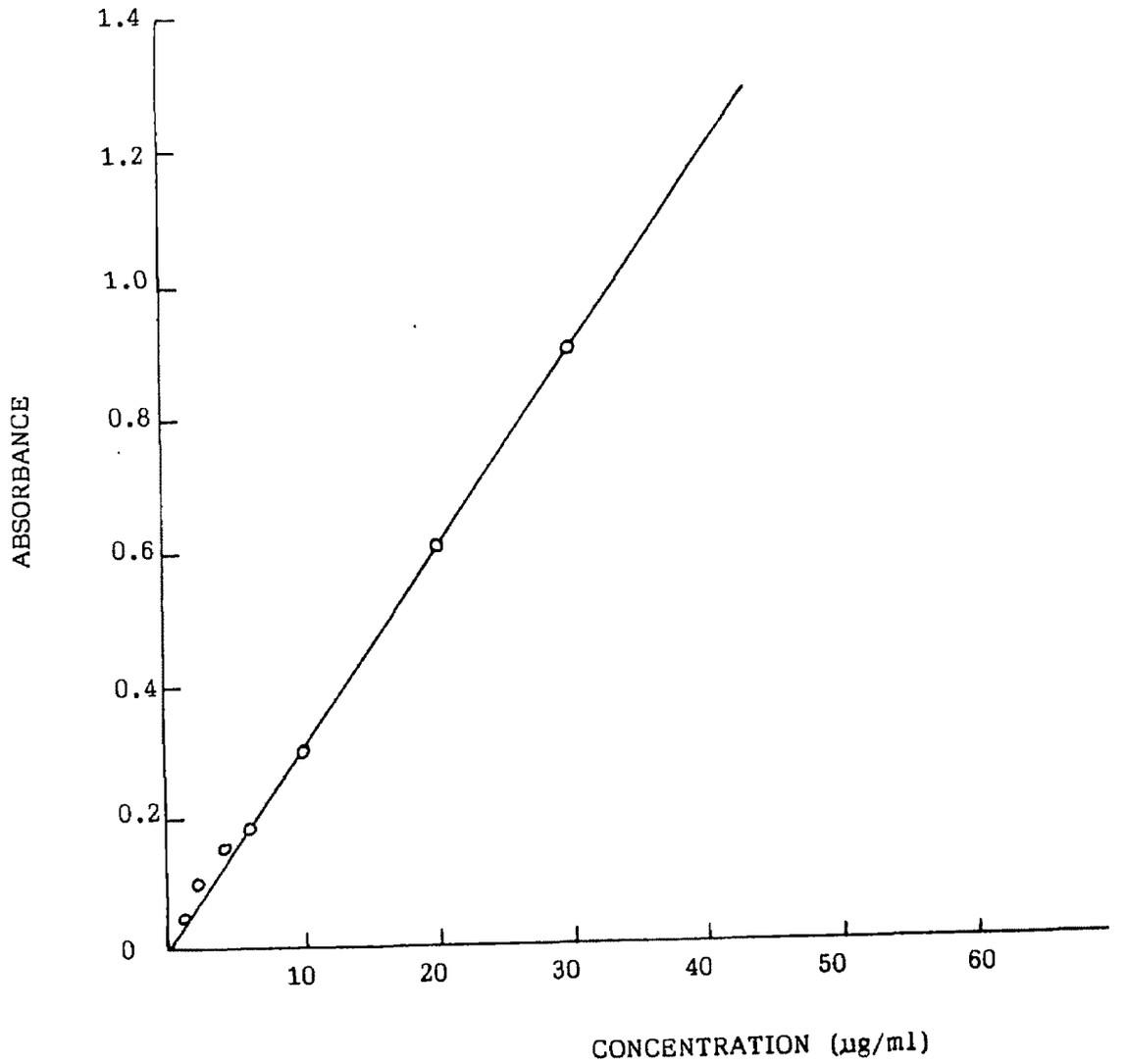


Fig. 13 : Calibration curve for estimation of of 5-FU in liver

reports describing the stability and suitability of polyterephthalamide microcapsules as drug carriers :

Madan[105] reported that adipoyl chloride and succinyl chloride, which had less than eight carbon atoms, did not produce satisfactory microcapsules whereas the microcapsules prepared using sebacoyl chloride were highly permeable and would have resulted in drug leakage.

Wood and Whateley[117] reported a very low permeability of the drug from polyterephthalamide microcapsules. Moreover, microcapsules prepared using terephthaloyl chloride were found to be stable and resistant to rupture during centrifugation.[118]

The selection of diamino acids was mainly based on their bio-compatibility, non-antigenicity, bio-degradability, ease of availability and their ability to form strong microcapsule walls with a high degree of cross-linking when reacted with diacyl chlorides.

The reaction of a diamine with a diacid chloride in the formation of a polyamide is a stoichiometric reaction and hence theoretically, the quantities of these two reactants should be taken on an equimolar basis. However, reports[101] describe an improvement in the quality of the polyamide when

the diacid chloride was taken in a slight excess as compared to the diamine. Therefore, the diamino acid to diacid chloride ratio was kept as 0.01 to 0.012 moles.

B] OPTIMISATION OF FORMULATION CONDITIONS

Table 12 summarizes the various formulation parameters and their effect on the average size of the polytereph-thalamide microcapsules.

i) Choice of Equipment for Emulsification OR Mode of Emulsification

Most of the reported procedures for the preparation of polyamide microcapsules have mentioned the use of either a simple three-blade stirrer or a magnetic stirrer for emulsification. But the effect of the type of equipment used for emulsification on the particle size and size of distribution of the microcapsules has not been reported.

Several batches of microcapsules were prepared using a simple stirrer (Remi) at maximum speed, magnetic stirrer (Remi) at highest speed and ultrasonic vibrator (Vibronics) at 120W.

Table - 12

Effect of Variable Parameters on the Particle Size of Polyterephthalamide Microcapsules

Batch No.	Parameter studied	Level of Study	Mean diameter ± Standard deviation (µm)
1a	Mode of	3-blade stirrer	7.5 to 60 µm
1b	emulsification	Magnetic stirrer	11.55 ± 4.84
1c		Ultrasonic vibrator*	7.9 ± 2.71
4a	Time of	1 minute	6.9 ± 4.71
4b	emulsification	3 minutes	4.75 ± 2.39
4c		5 minutes*	3.43 ± 0.93
5a	Concentration	1%	5.21 ± 0.35
5b	of Span 85	5%*	3.65 ± 2.08
5c		10%	3.34 ± 1.01
6a	Temperature	Room temperature (25°C)	4.36 ± 2.72
6b	of reaction	Ice bath temperature* (4°C)	3.38 ± 0.97
7a	Time of	1 minute	2.25 ± 0.61
7b	polymerisation	3 minutes*	3.38 ± 0.57
		5 minutes	4.72 ± 2.09
10a	Stabilizers	Egg Albumin	17.41 ± 5.53
10b		Bovine Serum Albumin	33.74 ± 10.89
10c		Casein	15 to 250 µm
10d		Gelatin	20 to 300 µm
10e		P.E.G. - 400	13.84 ± 6.51

* - optimum level

Fig. 14 indicates the particle size distribution of microcapsules in these batches. Out of these batches, only the batch of microcapsules prepared using ultrasonic vibrator showed particle size distribution suitable for formulation of an injectable suspension of the microcapsules.

ii) Choice of Aqueous Phase

Based on reported literature, solutions of sodium hydroxide, sodium bicarbonate, sodium carbonate and sodium carbonate buffer (pH 9.8) were selected as the aqueous phase for the study. However, the yield of microcapsules was found to be very less when 0.1N sodium hydroxide and sodium bicarbonate buffer (pH 9.8)[202] were used. As satisfactory yield was obtained when 0.45M sodium carbonate solution was used, it was selected as the aqueous phase for further studies.

iii) Choice of Organic Phase

Ether, chloroform, carbon tetrachloride, and various combinations thereof were tried as the organic phase for the reaction. While use of ether alone failed to yield any polymer, chloroform and carbon tetrachloride alone gave poor yield.

Madan[106] had recommended the use of mineral oil as the non-aqueous phase because it was found to improve the quality and flow properties of the product. However, we found that the mineral oil-based system could not be emulsified effectively using the Ultrasonic Vibrator, resulting in low yield, larger particle size and difficulty in product isolation. Hence, the use of mineral oil was thought to be unsuitable.

Satisfactory yield was obtained when chloroform and cyclohexane were used in 1:3 and 1:4 ratio. Hence, a mixture of these solvents in the ratio of 1:3 was chosen as the organic phase for the formulation of microcapsules.

iv) Time of Emulsification

Table 1 indicates that the time required for emulsification ranges from 20 seconds to 5 minutes. The time of emulsification is a crucial factor in any interfacial polymerisation reaction as it ultimately influences the size of the microcapsules formed. Hence, the effect of emulsification time on the particle size distribution of polyamide microcapsules was studied and is shown in Fig. 15. It was observed that the particle size decreased with an increase in the emulsification

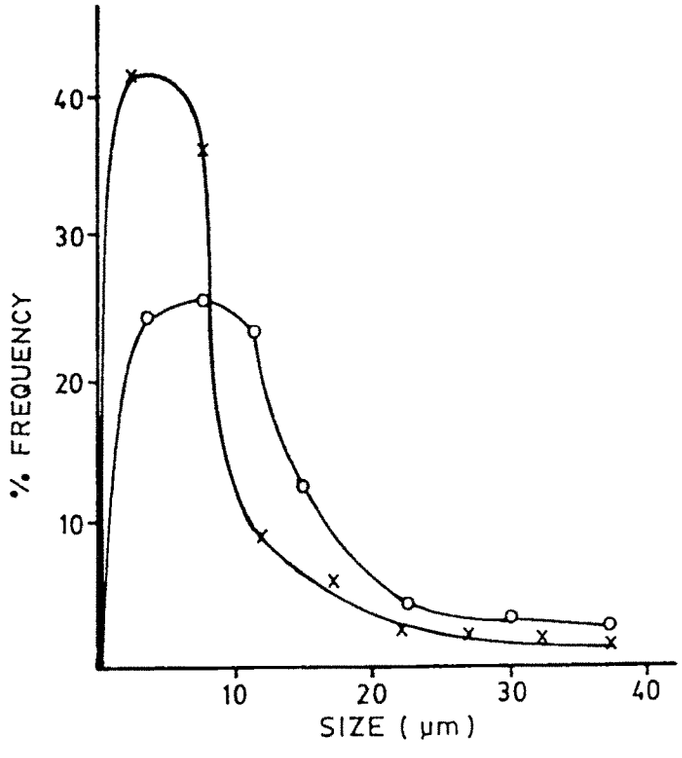


Fig. 14 : Effect of Mode of Emulsification on the particle size of polyterephthalamide microcapsules

o - Magnetic stirrer; x - Ultrasonic vibrator

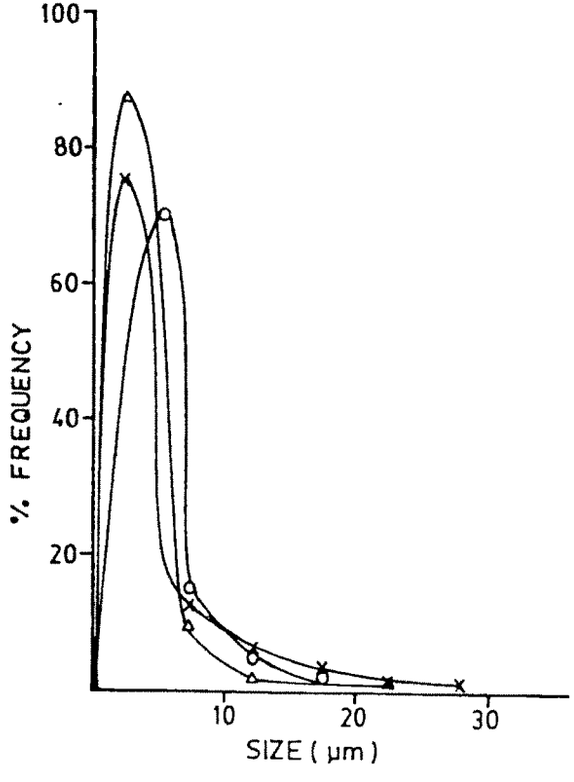


Fig. 15 : Effect of Time of Emulsification on the particle size of polyterephthalamide microcapsules

o - 1 min.; x - 3 min.; △ - 5 min.

time. Emulsification for 1 minute gave large particles with broad size distribution and partial separation of the aqueous phase also occured from the emulsion, indicating inefficient emulsification. It was concluded that emulsification for 5 minutes was optimum as it gave microcapsules with small particle size, narrow size distribution and minimum standard deviation.

v) Concentration of Span 85

All reports on polyamide microcapsules indicate that the presence of an emulsifier is necessary for the formation and stability of the polymer membrane. The emulsifier helps in formation of the microcapsule wall by aiding the diffusion of the diamine from the aqueous phase into the organic phase and also influences the microcapsule size by affecting the emulsion droplet size. Span 85 is universally used as the emulsifier for this purpose, but its concentration has been highly variable (1% to 20%). Hence, it was necessary to optimize the concentration of Span 85 required for the present study.

Low concentration of Span (1%) was found to produce large particles and was accompanied by partial separation of the aqueous phase, indicating inefficient

emulsification. Higher concentrations viz. 5% and 10% of Span produced microcapsules with comparable particle size and size distribution (Fig. 16). These findings are in conformation with the reports that above 5%, Span has no significant effect on the particle size distribution of the microcapsules. However, the batch of microcapsules prepared using 10% Span 85 was difficult to separate even upon prolonged centrifugation, which might rupture the polymeric wall. Hence, a Span 85 concentration of 5% was chosen as optimum even though the standard deviation was less for the batch prepared using 10% Span 85.

vi) Temperature of Reaction

Contrasting reports exist regarding the effect of temperature on the particle size of polyamide microcapsules.

Shigeri et al[104] reported that an increase in the temperature of reaction decreased the particle size of the microcapsules whereas a number of other investigators have reported the use of ice bath or 0°C temperature for the same purpose. Hence, we conducted our studies at both, ice bath (4°C) as well as room (25°C) temperatures.

We found that ice bath temperature was required during the polyamide microcapsule formation in order to minimize the evaporation of the organic phase, reduce the heat of reaction, minimize the hydrolysis of the diacid chloride and to render the rate of reaction slower. Reaction at room temperature will not only hasten the rate of reaction leading to formation of coarse and uneven films but it will also increase the rate of evaporation of the organic phase, thus increasing the phase volume ratio, which in turn may reduce the stability of the emulsion and ultimately decrease the payload efficiency of the microcapsules.

It is seen that there is a decrease in the particle size (and size distribution) with a decrease in the temperature of reaction (Fig. 17).

These contrasting results with those of Shigeri et al may be due to the fact that they had used a protective protein as a stabilizer in the aqueous phase. The presence of this protein may have increased the viscosity of the emulsion and the beneficial effect of temperature may be due to a reduction in the viscosity, which will ultimately reduce the size of the emulsion droplet.

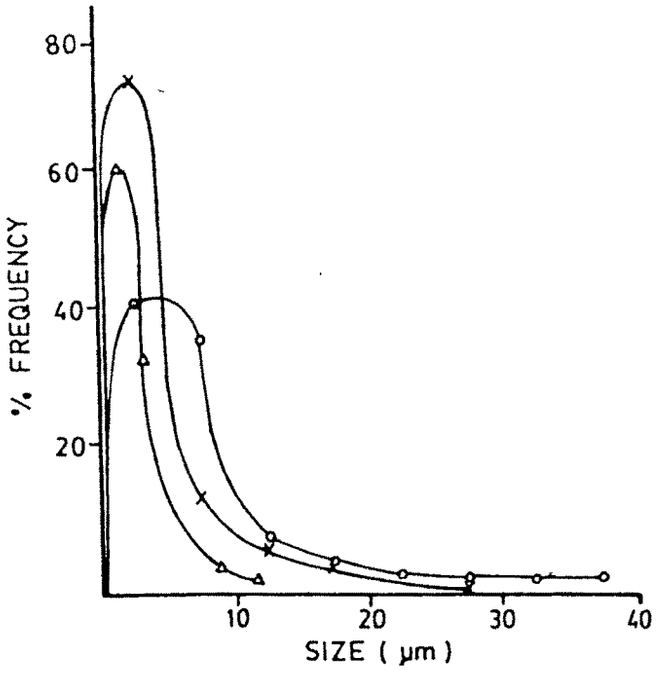


Fig. 16 : Effect of Concentration of Span 85 on the particle size of polyterephthalamide microcapsules

o - 1%; x - 5%; Δ - 10%

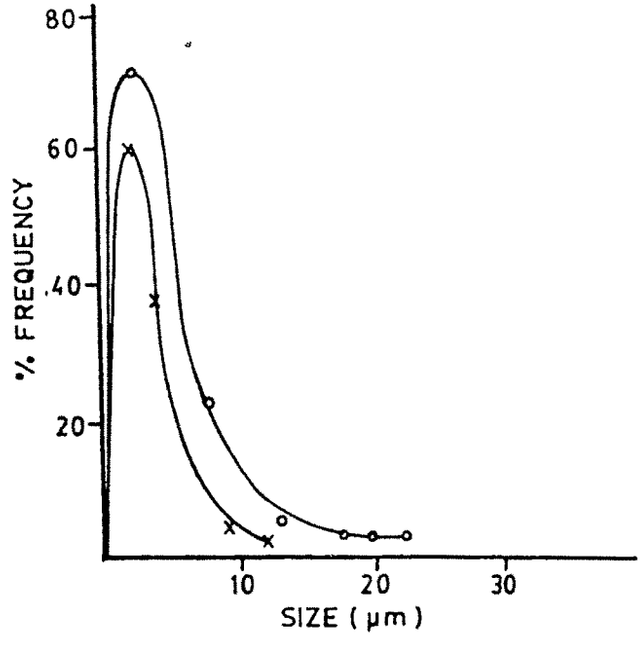


Fig. 17 : Effect of Temperature of Reaction on the particle size of polyterephthalamide microcapsules

o - Room temp.; x - Ice bath temp.

vii) Time of Polymerisation

The time of polymerisation is a critical factor in the interfacial polycondensation reaction because the molecular weight of the resulting polymer, the degree of cross-linking and the thickness and porosity of the polymer film depend on it. If the polymerisation time is too less, the resulting polymer film will be thin, fragile and will not be able to resist the stress during separation and centrifugation. On the other hand, if the reaction time is too high, a thick, coarse, uneven polymer film of broad range of molecular weight will be formed.

Studies conducted to optimize the time of polymerisation showed that a polymerisation time of 1 minute produced very small particles with fragile films which broke down upon centrifugation (Fig. 18). On the other hand, a polymerisation time of 5 minutes gave particles with almost double the size of the original microcapsules, having a broad size distribution and a high value of standard deviation. This indicates excessive degree of polymerisation, resulting in formation of coarse, thick films.

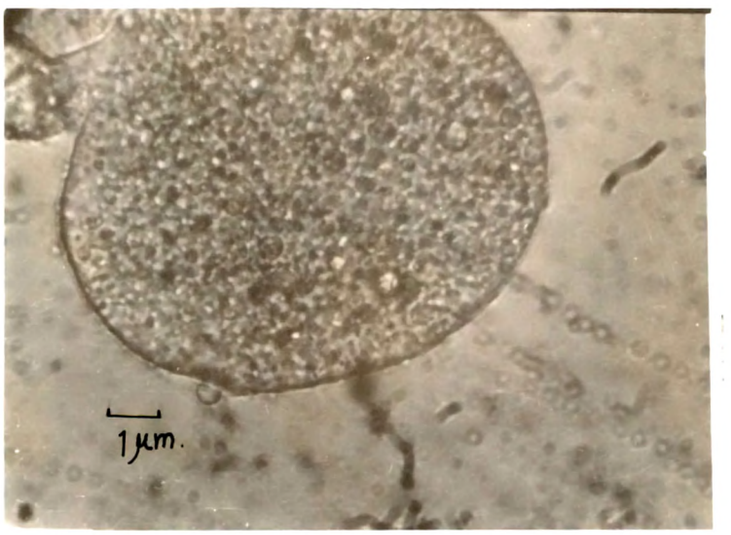


Fig.18 : Photomicrograph of a Polyterephthalamide microcapsule showing ruptured wall.

Fig. 19 depicts the effect of polymerisation time on the particle size distribution of polyamide microcapsules. It shows that the particle size distribution increases with increase in the polymerisation time. A polymerisation time of 3 minutes was fixed as optimum as it produced microcapsules with small average diameter, low standard deviation (3.38 ± 0.57 μm) and narrow size distribution.

viii) Phase Volume Ratio

The reported phase volume ratio of 1:5 amounted to only 20% of the internal phase whereas theoretically upto 50% of the internal phase can be accomodated in an emulsion without affecting its stability. Hence, we also studied higher phase volume ratios of 1:3 and 1:4. However, at the higher aqueous to organic phase ratios, the average size of the microcapsules increased and some amount of the aqueous phase separated out from the emulsion, which would lead to a proportionate reduction in the amount of drug incorporated into the microcapsules. Hence, it was concluded that a minimum phase volume ratio of 1:5 was necessary for obtaining maximum drug loading efficiency.

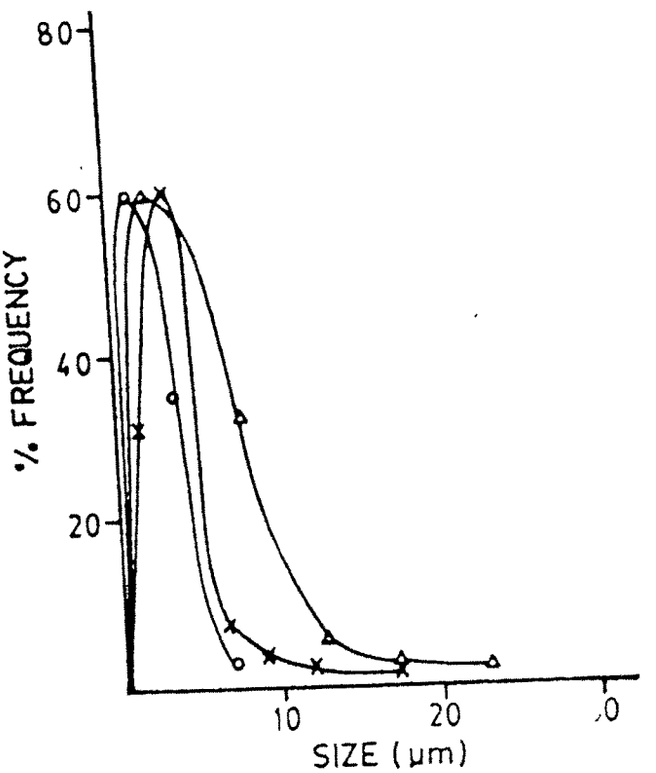


Fig. 19 : Effect of Time of Polymerisation on the particle size of polyterephthalamide microcapsules

o - 1 min.; x - 3 min.; Δ - 5 min.

ix) Use of Stabilizers

The microcapsules tended to break down due to the excessive and prolonged centrifigation required during the microcapsule separation and collection procedure. A number of workers have recommended the use of various proteins or polyethylene glycol (PEG-400) as stabilizers for maintaining the turgor and improving the stress resistance of the microcapsules. So we studied the effect of proteins like egg albumin, bovine serum albumin (BSA), casein, gelatin and PEG-400 on the stress durability of the microcapsules. But incorporation of these substances drastically increased the particle size and size distribution of the microcapsules (Fig. 20), probably by increasing the viscosity of the internal phase, leading to an increase in the emulsion droplet size. Hence, no stabilizers were used for further studies.

x) Method of Collection of Microcapsules

Separation and collection of the microcapsules is the most tedious and time consuming process in the preparation of microcapsules by the interfacial polycondensation procedure. As the polymerisation takes place essentially in the organic phase of the water-in-

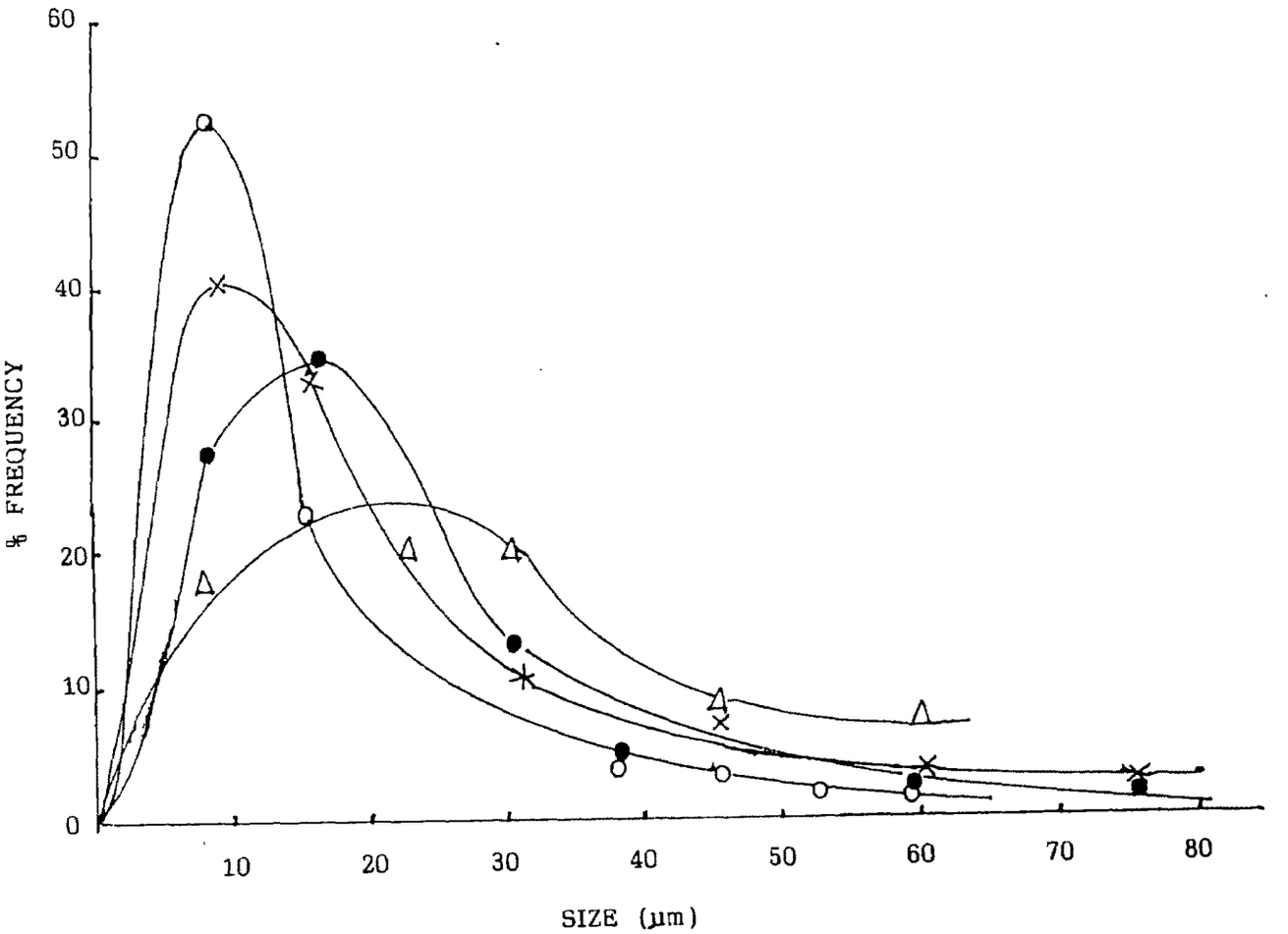


Fig. 20 : Effect of Stabilizers on particle size of polyterephthalamide microcapsules

o - Egg Albumin; • - Bovine Serum Albumin;
x - Casein; Δ - PEG-400

oil emulsion, the resultant product tends to be hydrophobic and cannot be collected in the aqueous phase using ordinary separation methods. The small size of these microcapsules is a limiting factor in their separation by conventional filtration technique. Various approaches like washing with ethanol, aqueous or alcoholic Tween 20 solution or mixture of glycerin and Tween 20 have been reportedly used by various workers to impart hydrophilicity to the microcapsules.

We first tried alcohol for washing and separation of the microcapsules. Though reasonable separation was obtained, the resultant microcapsules were found to flocculate when suspended in phosphate buffered saline (Fig. 21).

Although the use of a 50% aqueous solution of Tween 20 gave excellent separation, the product had to be washed repeatedly at least five to six times with saline to remove the excess Tween from its surface. However, subjecting the microcapsules to repeated centrifugation after each washing increased their vulnerability towards rupture.

Finally, the use of a mixture of glycerin and Tween 20 in a ratio of 3:1 proved to be equally effective for

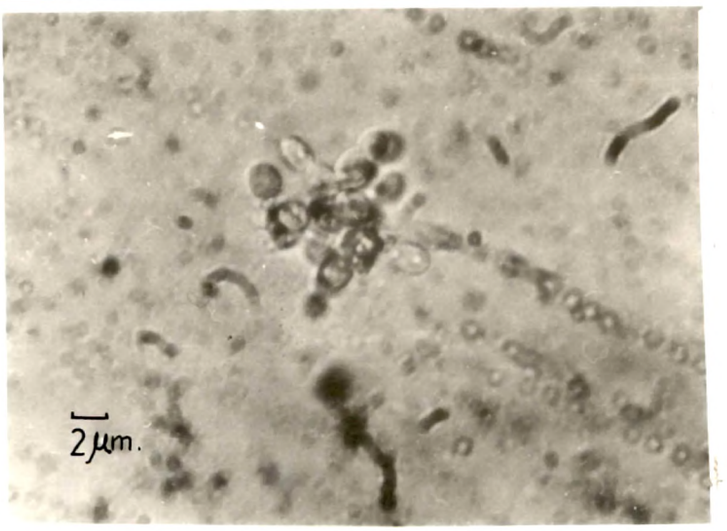


Fig.21 : Photomicrograph showing flocculated polyterephthalamide microcapsules.

separating the microcapsules. And as the Tween content in the dispersion medium was reduced, the number of washings required to remove excess Tween also reduced to two to three washings.

Hence, glycerin-Tween mixture was considered optimum for separation and collection of the microcapsules.

Final formulation conditions for preparation of polyterephthalamide microcapsules are :

1)	Mode of Emulsification	-	Ultrasonic Vibrator (120W)
2)	Aqueous Phase	-	0.45M sodium carbonate solution
3)	Organic Phase	-	Chloroform : Cyclohexane (1:3)
4)	Diamino acid Concentration	-	0.01 moles
5)	Terephthaloyl chloride Concentration	-	0.012 moles
6)	Aqueous to Organic Phase Ratio	-	1:5
7)	Span 85 Concentration	-	5%

8) Time of Emulsification - 5 minutes

9) Temperature of Polymerisation - Ice bath (4°C)

10) Time of Polymerisation - 3 minutes

11) Method of Separation - Dispersion in glycerin : Tween 20 mixture (3:1)

4.2.2 Cross-linked Haemoglobin Microcapsules

A] OPTIMISATION OF FORMULATION CONDITIONS

i) Concentration of Span 85

The batch of cross-linked haemoglobin microcapsules prepared using 5% Span 85 had large particles with a wide range of size distribution (d_{mean} = 15.84 μm). Hence, more batches were prepared using higher concentrations of the emulsifier. Although an increase in the Span concentration upto 10% reduced the average microcapsules size and narrowed its size distribution (Fig. 22), a further increase in the concentration was found essential to improve the drug entrapment efficiency. Thus, at a Span 85 concentration of 15%, the payload capacity of the microcapsules increased almost two times (70% to 80%) as compared to the batch prepared using 10% Span.

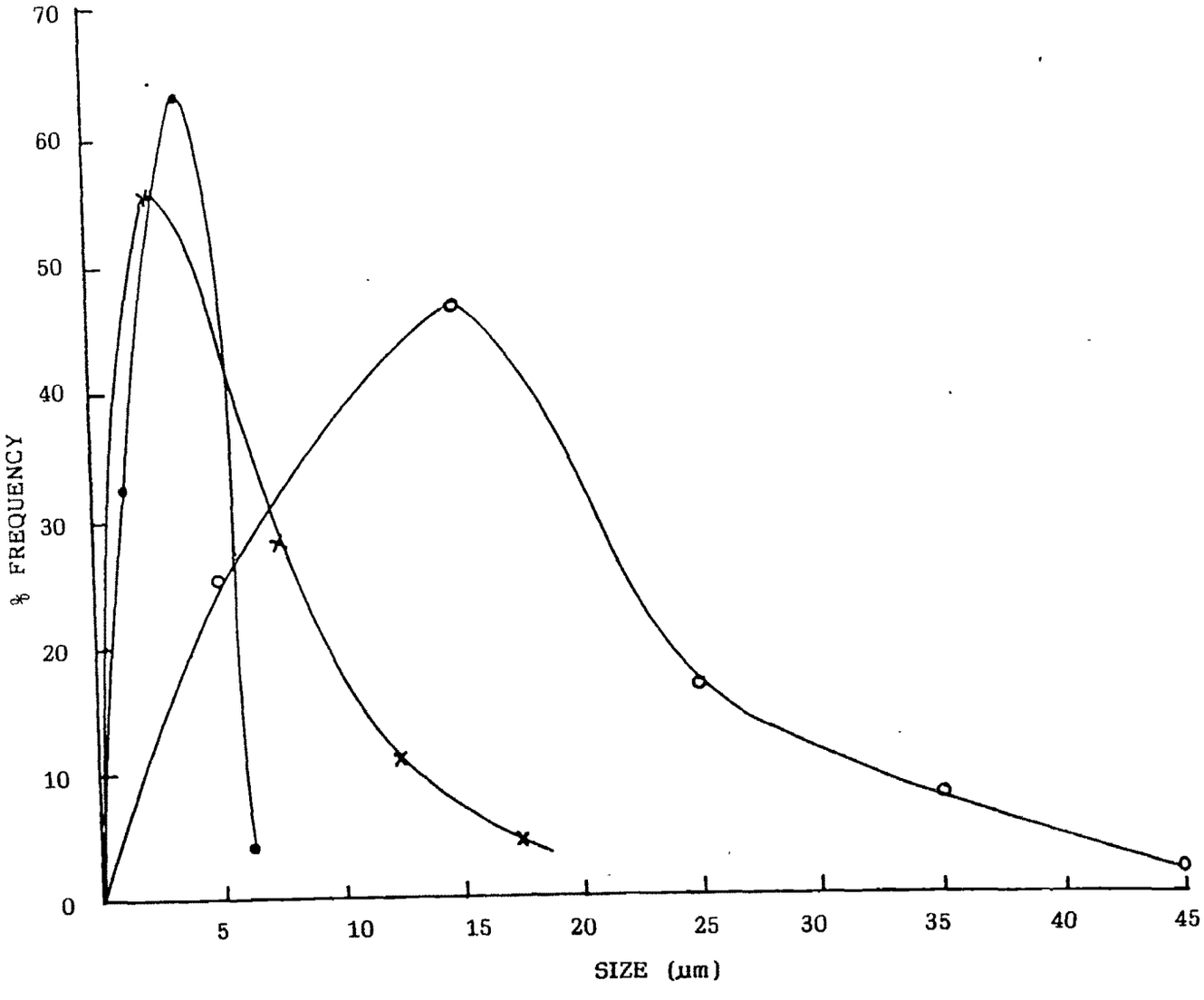


Fig. 22 : Effect of Concentration of Span 85 on the particle size of Hb-Tc microcapsules

o - 5%; x - 10%; • - 15%

ii) Temperature of Reaction

It was observed that the yield of cross-linked haemoglobin microcapsules increased appreciably when the temperature of reaction was elevated from 4°C (ice bath) to 25°C (ambient temperature). The necessity of a higher reaction temperature in this case may be due to the reduced reactivity of the amino groups in a high molecular weight protein like haemoglobin as compared to the free amino acids used in the preparation of polyamide microcapsules. A reduction in the temperature of reaction may further hinder the reticulation process by reducing the rate of partitioning of the protein between the two phases. Thus, a higher temperature was required to increase the effectiveness of the emulsification reticulation process for a protein like haemoglobin.

iii) Aqueous to Organic Phase Volume Ratio

The aqueous to organic phase volume ratio of 1:5, which was found optimum in case of polytereph-thalamide microcapsules, was not suitable for preparing cross-linked haemoglobin microcapsules because haemoglobin precipitated out during the emulsification step (Fig. 23). This may be due to a reduction in its

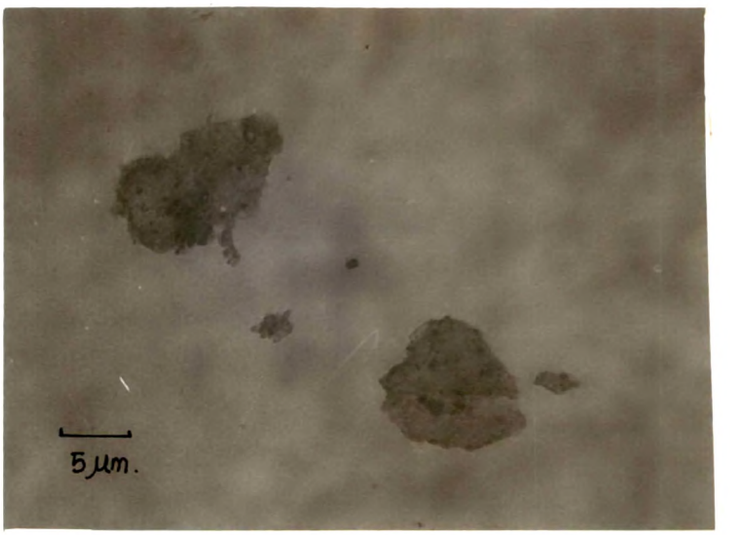


Fig.23 : Photomicrograph showing precipitated haemoglobin in Hb-Tc microcapsules.

solubility upon addition of excess quantity of the organic phase. However, a reduction in the volume of the organic phase was found to minimize precipitation, and a phase volume ratio of 1:3 produced most satisfactory results.

4.2.3 Polyacrylamide Microcapsules

A] METHOD OF PREPARATION

Theoretically, the gel polymerisation technique gave 100% drug entrapment efficiency because the entire system polymerised into a gel. But when the gel was homogenized, a reduction in the gel size simultaneously increased the surface area available for drug diffusion from the matrix. Consequently, almost 50% to 60% of the entrapped drug was lost during size reduction and washing, so that the effective drug entrapment efficiency was only 40% to 50%. Moreover, there are chances of small quantities of monomers, insoluble oligomers and trace quantities of water-insoluble initiator remaining behind in the product. Furthermore, even the use of a tissue homogenizer could not effectively reduce the particle size of the gel to the desired level. One additional drawback of the gel polymerisation technique is the formation of a highly cross-linked polymer with a high molecular weight distribution due to the greater extent of polymerisation.

Considering these factors, emulsion polymerisation procedure was considered more suitable for preparing polyacrylamide microparticles.

Fig. 24 shows a polyacrylamide microparticle obtained after homogenizing the gel.

B] OPTIMISATION OF FORMULATION CONDITIONS **(by Emulsion Polymerisation Technique)**

The effect of variable parameters on the particle size of polyacrylamide microparticles is summarised in Table 13 .

i) Pluronic F 68 Concentration

Pluronic F 68 was chosen as the emulsifier because majority of the reports recommended its use for emulsification. But its concentration has been highly variable and has ranged from 0.03% to 1.25%. However, during initial trials, we could not observe satisfactory polymerisation upto Pluronic concentrations of 0.1%. The polymerisation did take place at 0.5% concentration but the particle size range obtained was unsuitable for parenteral administration (Fig. 25). Increasing the level upto 1.0% greatly reduced the particle size and narrowed down the size distribution.

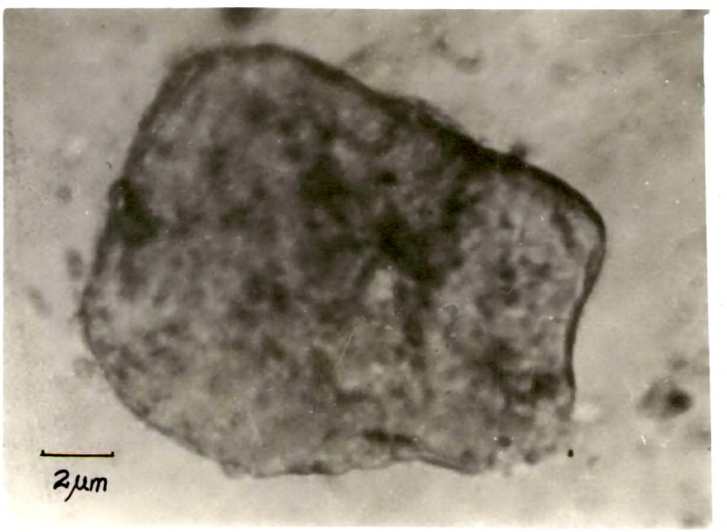


Fig.24 : Photomicrograph of a polyacrylamide particle prepared by gel polymerization method.

Table - 13

Effect of Variable Parameters on the Particle Size of Polyacrylamide Microcapsules

Batch No.	Parameter studied	Level of Study	Mean diameter ± Standard deviation (µm)
c1	Pluronic F 68	0.5 %	18.80 ± 7.70
d1	concentration	0.75%	2.26 ± 0.40
e1		1.0 %*	≃ 2 µm
f1		2.0 %	2.86 ± 0.35
b2	Phase volume	1 : 5	8.36 ± 1.26
c2	ratio	1 : 4	5.27 ± 1.91
d2		1 : 3*	3.02 ± 0.88
		1 : 2	difficulty in isolation
a4	Time of	5 minutes	15 to 250 µm
b4	emulsification	10 minutes*	2.4 ± 0.48
c4		15 minutes	2.9 ± 0.63
d4		20 minutes	3.33 ± 1.07
c5	Time of	30 minutes	2.65 ± 0.37
d5	polymerisation	1 hour*	3.54 ± 1.06
e5		2 hours	4.02 ± 0.69
f5		3 hours	5.05 ± 2.61
a6	Temperature	Ice bath (4°C)	≃ 2 µm
b6	of reaction	Room temperature* (25°C)	2.3 ± 0.42

* - indicates optimum level

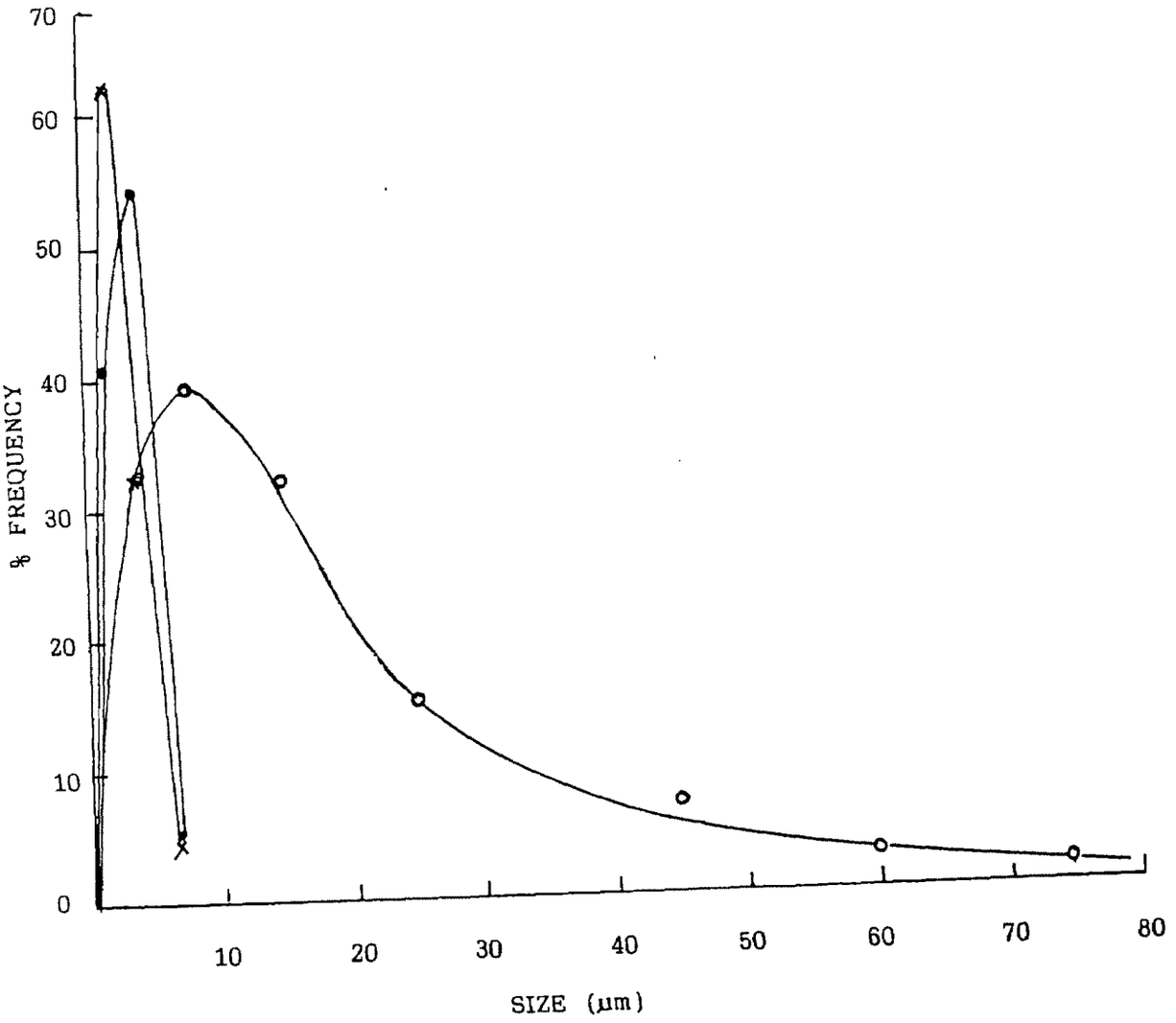


Fig. 25 : Effect of Concentration of Pluronic F 68 on the particle size of polyacrylamide microcapsules

o - 0.5%; x - 0.75%; • - 2%

However, a further increase in the emulsifier concentration was found to increase the viscosity of the emulsion, which in turn increased the particle size and also caused difficulty in separation of the emulsion. Hence, a Pluronic F 68 concentration of 1% was considered to be optimum.

Note : The particle size analysis of the batch of polyacrylamide microparticles prepared with 1% Pluronic F 68 could not be carried out because the particles were lying below the counting range of the microscope. Hence, the particle size of this batch was assumed to be $\hat{=}$ 2 µm.

ii) Aqueous to Organic Phase Volume Ratio

A wide range of phase volume ratios from 1:60 to 1:5 have been used by various workers for preparing polyacrylamide microparticles. Oxygen has been found to reduce the rate of polymerisation due to inactivation of the initiator. Hence, the reported procedures recommend the use of nitrogen atmosphere for preparing polyacrylamide microparticles. Under the conditions of our experimentation where polymerisation was conducted in presence of atmospheric oxygen, a low phase volume ratio failed to yield the product. It was found that a

minimum phase volume ratio of 1:5 was required to obtain satisfactory results. The average particle size decreased with a further increase in the phase volume ratio upto 1:4 and 1:3 (Fig. 26). However, a still greater phase volume ratio of 1:2 was unsuitable as it increased the viscosity of the system, leading to problems in separation of the microcapsules. Hence, an aqueous to organic phase volume ratio of 1:3 was fixed as optimum.

(iii) Mode of Emulsification

The significantly small particle size obtained by using ultrasonic vibrator while preparing polyamide microcapsules prompted us to use the same for preparing polyacrylamide microparticles. However, it proved to be ineffective in preventing gel formation. When the reaction was carried out using a 3-blade stirrer at highest speed, excessive evaporation of the organic phase was observed, leading to an increase in the viscosity of the emulsion and a consequent increase in the emulsion droplet size. This drawback was overcome by using a magnetic stirrer wherein gelling was avoided, viscosity of the emulsion was controlled and particles with mean diameter between 2.5 to 2.8 μm were obtained.

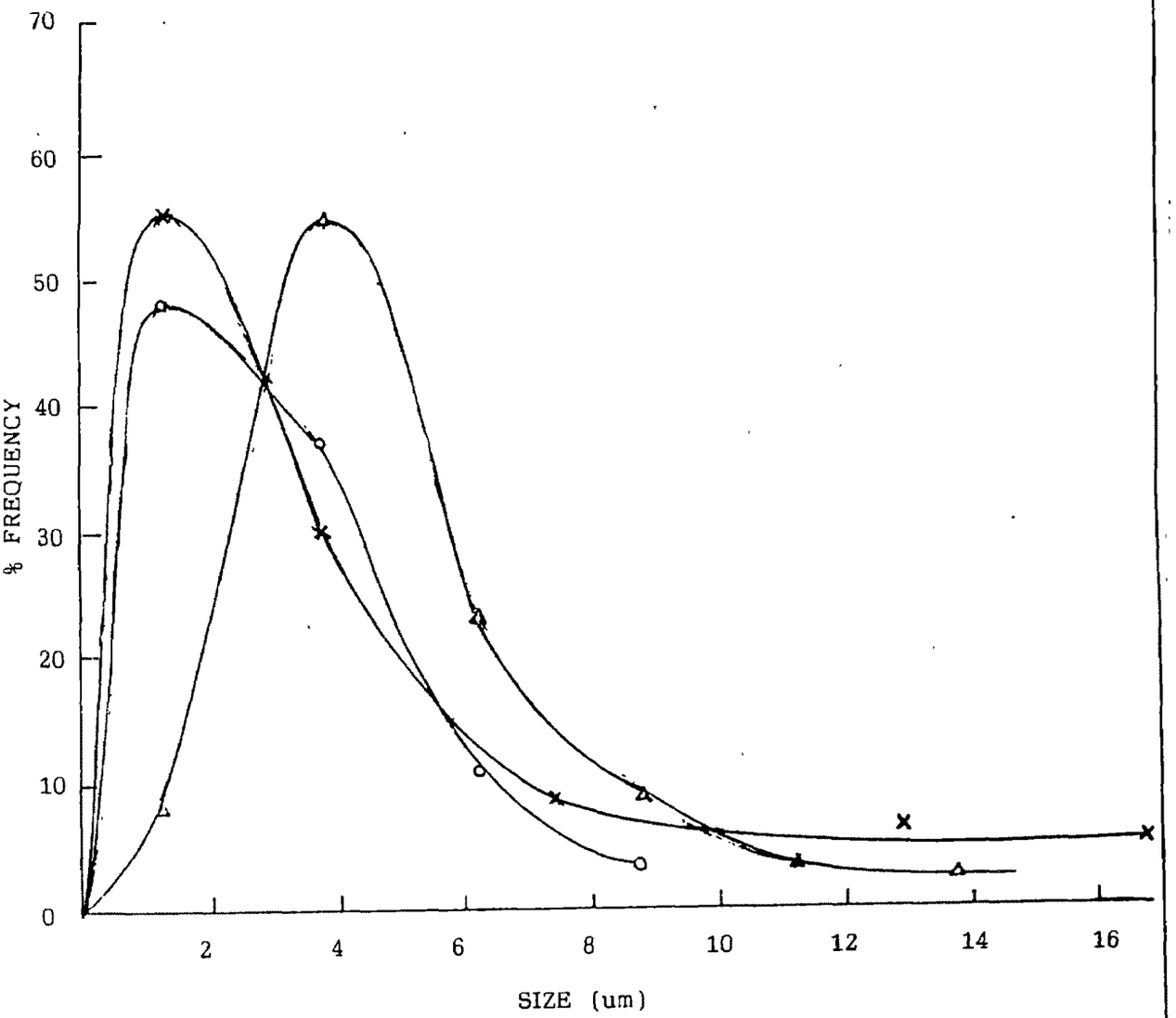


Fig. 26 : Effect of Phase Volume Ratio on the particle size of polyacrylamide microcapsules

o - 1:3; x - 1:4; Δ - 1:5

iv) Time of Emulsification

Only a few of the reports screened have mentioned the time of emulsification required for preparing polyacrylamide microparticles. Hence, we optimised this parameter by allowing the emulsification to take place for 5, 10, 15 and 20 minutes. Emulsification time of 5 minutes gave large particles in the size range of 15 to 250 μm. Increasing the time upto 10 minutes reduced the particle size and narrowed down the size distribution. But a further increase had no significant influence on the particle size distribution (Fig. 27). Hence, an emulsification time of 10 minutes was found to be optimum.

v) Time of Polymerisation

The interfacial polymerisation reaction is an instantaneous reaction whereas the acrylamide polymerisation reaction is an initiator-mediated process, requiring a much longer duration of time. As per reports, 5 to 30 minutes were required for polymerisation when the reaction was conducted in absence of oxygen. It followed that a greater time would be required when the same was carried out in presence of oxygen. Hence, various batches of

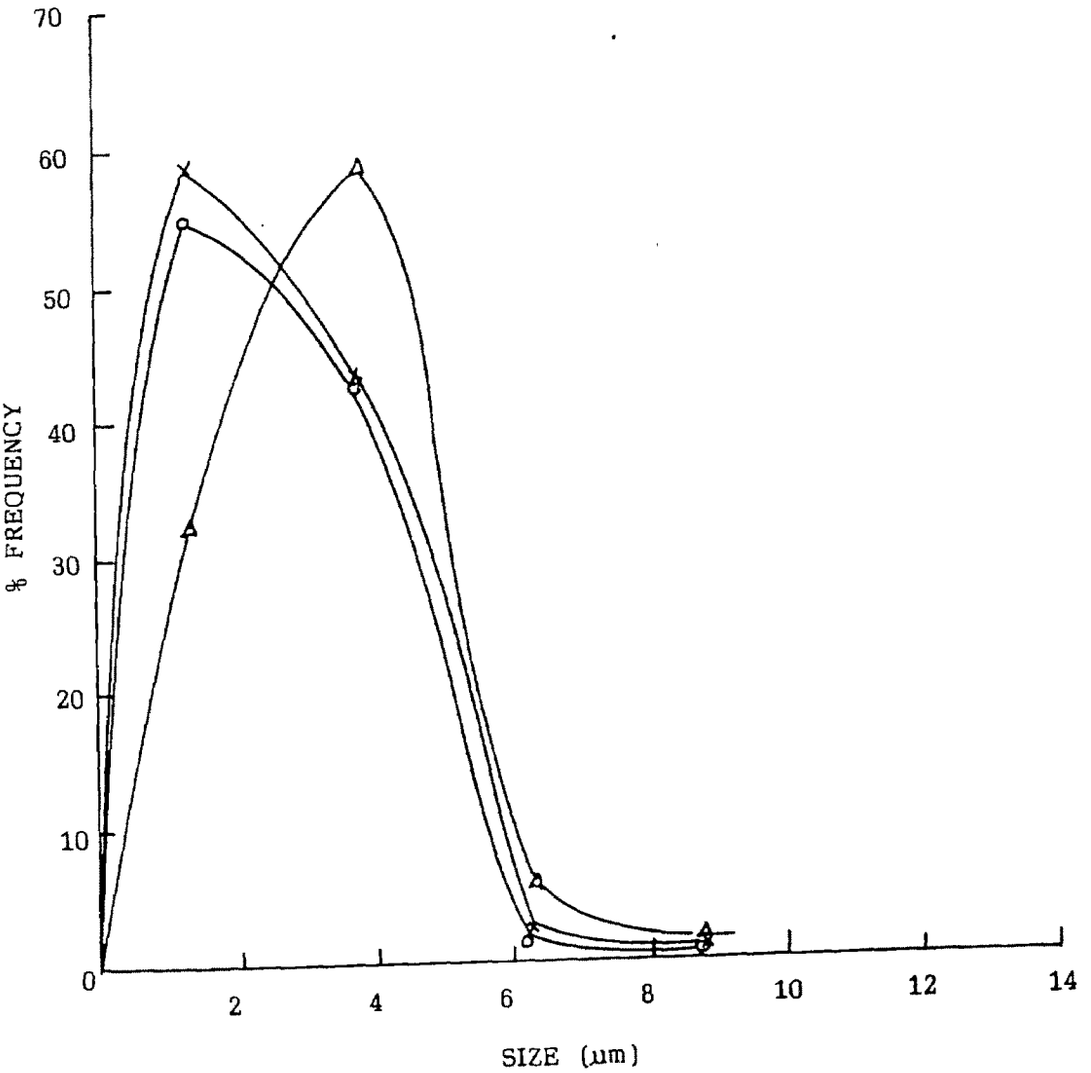


Fig. 27 : Effect of Time of Emulsification on the particle size of polyacrylamide microcapsules

o - 10 min.; x - 15 min.; Δ - 20 min.

microcapsules were prepared by allowing polymerisation to proceed for half hour to three hours.

It was observed that the particle size increased with an increase in the time of polymerisation (Fig. 28), confirming that the polymerisation proceeds progressively. However, an increase in the extent of polymerisation beyond a certain time may result in a greater degree of cross-linking, thereby decreasing the in vivo degradation rate of the injected polymer. Hence, a polymerisation time of 1 hour was considered to be optimum.

vi) Temperature of Polymerisation

As per reports, the polyacrylamide preparation was conducted at low (4°C) as well as ambient (25°C) temperature. It was found that the temperature of reaction influenced the yield, particle size and drug entrapment efficiency of the microparticles. Very small particles were obtained at a reaction temperature of 4°C (ice bath), but the yield was low and the drug entrapment efficiency was only 15% to 25% whereas all these problems were overcome when the reaction was carried out at ambient temperature (25°C). The poor results obtained at ice bath temperature may be due to

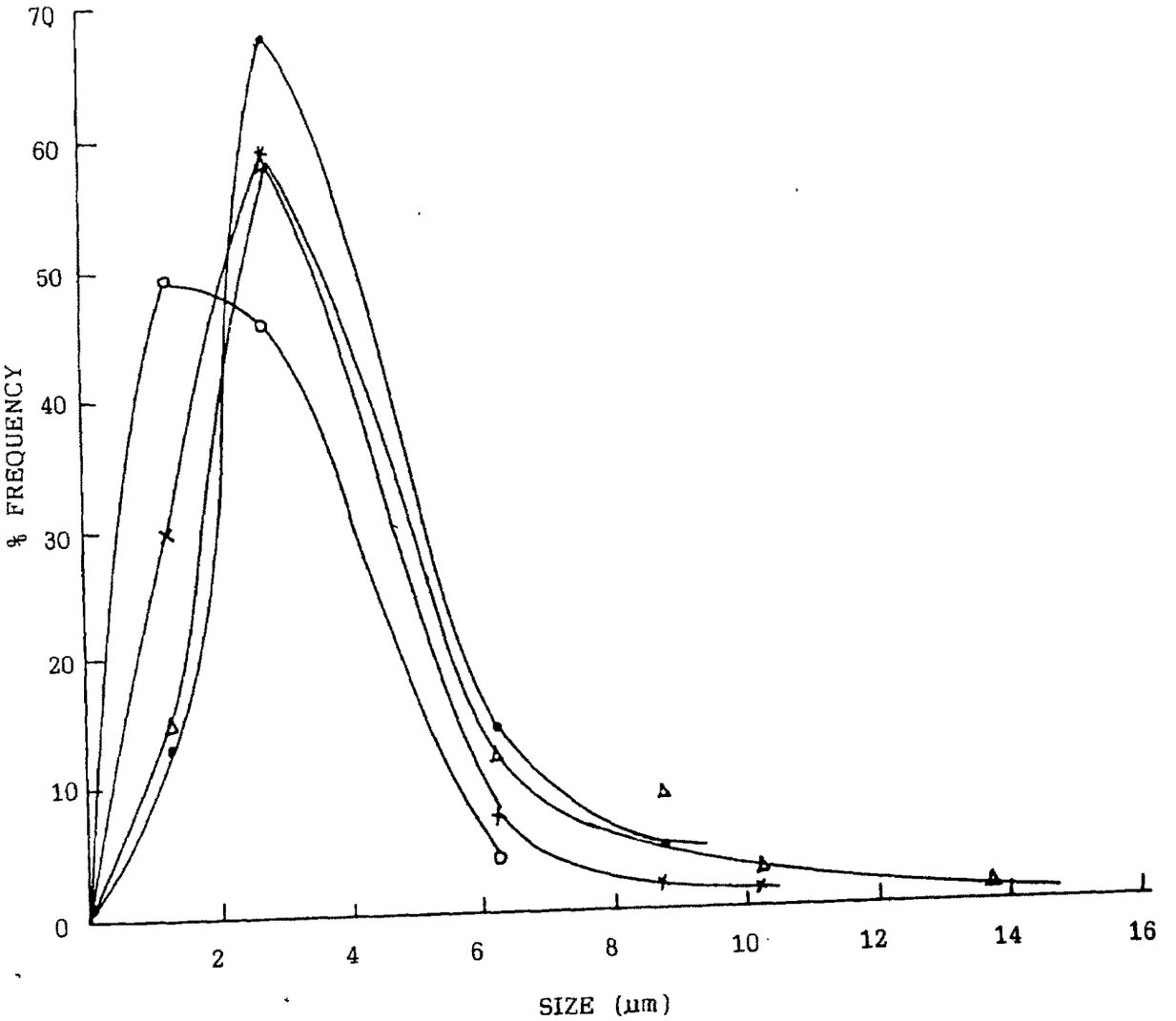


Fig. 28 : Effect of Time of Polymerisation on particle size of polyacrylamide microcapsules

o - ½ hr.; x - 1 hr.; • - 2 hrs.; Δ - 3 hrs.

a reduced rate and extent of initiator propagation at lower temperature. Hence, further reactions were carried out at room temperature.

vii) Method of Collection

Many reports suggest the use of phosphate buffer (pH 7.4) for washing and collecting polyacrylamide microparticles. However, we could not obtain satisfactory recovery of the particles by using phosphate buffer for isolation of the product. Out of the other washing media used, alcohol gave excellent dispersion and separation of the microcapsules. Hence, in the present study, the polyacrylamide microparticles were collected by dispersion in 2 x 10 ml alcohol followed by centrifugation and washing with 4 x 40 ml phosphate buffered saline to remove unreacted monomers and traces of catalyst, before finally suspending the product in phosphate buffered saline.

viii) Addition of Catalyst and Initiator

Variations were observed in the cited literature regarding the point of addition of the catalyst (ammonium peroxodisulphate) and the initiator (temed).

When both substances were added simultaneously to the aqueous phase before emulsification as reported by Johansson and Ekman et al,[133-134,135-138] the system immediately polymerised into a gel.

When the catalyst was added to the aqueous phase and the initiator added to the organic phase before emulsification, partial gel formation was observed.

Proper microparticle formation was observed only when the catalyst was first emulsified and then the initiator was added in a small volume of continuous phase to the water-in-oil emulsion as suggested by Edman[144] and Samaligy et al.[151]

Optimised formulation conditions for preparation of polyacrylamide microparticles are :

1) Aqueous Phase : phosphate buffer (pH 7.4)

2) Organic Phase : chloroform : toluene (1:3)

3) Acrylamide : N,N' Methylene bisacrylamide Ratio : 5:1

4) Aqueous to Organic Phase Ratio : 1:3

5) Pluronic F 68 Concentration : 1% w/v

6) Mode of Emulsification : Magnetic stirrer (Remi) at highest speed

7) Temperature of Reaction : Room temperature (25°C)

8) Catalyst : 0.1 ml of a 0.5 gm/ml solution of ammonium peroxodisulphate

9) Initiator : 0.1 ml (100 µl) of tetra ethyl methylene diamine

10) Time of Emulsification : 10 minutes

11) Time of Polymerisation : 1 hour

12) Concentration of 5-Fluorouracil : 10 mg/ml

13) Method of Collection : dispersion in ethanol followed by washing with phosphate buffered saline.

4.2.4 Polyisobutylcyanoacrylate Microparticles

i) Method of Preparation

Polyisobutylcyanoacrylate (PiBCA) microparticles were prepared by dispersion polymerisation and emulsion polymerisation methods. Irregular, large particles in the size range of 7.5 to 75 μm were obtained by the earlier method whereas spherical, relatively small microparticles were obtained by the latter method (Fig. 29). However, the drug entrapment efficiency was found to be in the range of 30 to 35% in both cases. This was acceptable, considering the fact that only 20 μl of the monomer was used for polymerisation.

The emulsion polymerisation method was selected as the method of choice for PiBCA microparticles.

ii) Mode of Emulsification

The monomer tended to gel into an irregular mass immediately upon addition to the emulsion when an ultrasonic vibrator was used. Hence, all further batches of PiBCA microparticles were prepared using a magnetic stirrer at its highest speed.

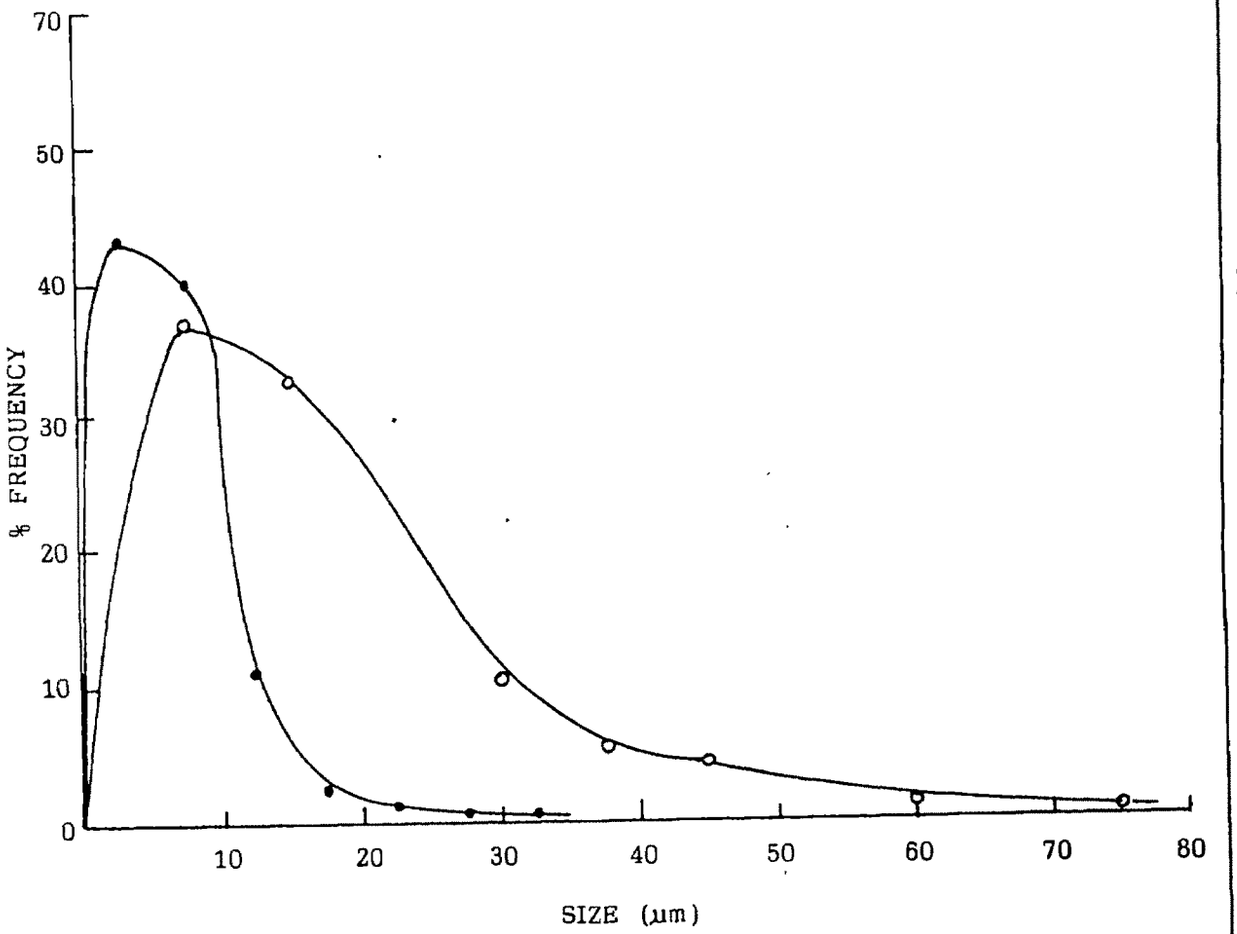


Fig. 29 : Effect of Method of Preparation on the particle size of PiBCA microparticles

● - Emulsion polymerisation;

o - Dispersion polymerisation

4.3 EVALUATION OF MICROCAPSULES

4.3.1 Polyamide Microcapsules

i) Particle Size Analysis

The mean diameter and the particle size distribution of various batches of polyamide microcapsules is given in Table 14 and Figs. 30-37.

The data indicates that although all batches of polytereph-thalamide microcapsules had a size distribution in the range of 1.25 to 30 μm, there was a small but significant difference in their average particle size, indicating a difference in the reactivity of the individual amino acids. It was observed that the particle size was a function of the partition coefficient ($K_{O/W}$) of the amino acid between the organic and aqueous phases. Thus, the batches containing cystine and asparagine, which had the highest $K_{O/W}$ values, also had highest average diameters whereas the batches prepared from citrulline and glutamine, having least $K_{O/W}$ values, had the lowest average particle size.

It can be concluded that the particle size of the microcapsules is a function of the partitioning of the diamino acids, which enhances the rate and extent of polymerisation.

Table : 14

Mean Diameter and Drug Entrapment Efficiency of Various Batches of Polyterephthalamide Microcapsules

diamine moiety	Batch Code	$K_{o/w}$ of amino acid	Mean diameter ± S. D. (μm)	% drug entrapment* ± S. D.
Arginine	Ar-Tc	0.27	4.64 ± 1.92	58.33 ± 3.03
Asparagine	As-Tc	1.32	5.83 ± 2.96	80.67 ± 5.07
Citrulline	Ci-Tc	0.17	3.27 ± 0.60	45.01 ± 5.10
Cystine	Cy-Tc	4.12	5.74 ± 3.03	74.00 ± 4.50
Glutamine	Gl-Tc	0.13	3.25 ± 0.63	40.50 ± 4.54
Lysine	Ly-Tc	0.61	4.50 ± 1.73	65.51 ± 4.19
Ornithine	Or-Tc	0.39	3.95 ± 1.95	52.67 ± 3.40
Haemoglobin	Hb-Tc	-	3.50 ± 1.01	73.00 ± 6.13

* - average of three assays

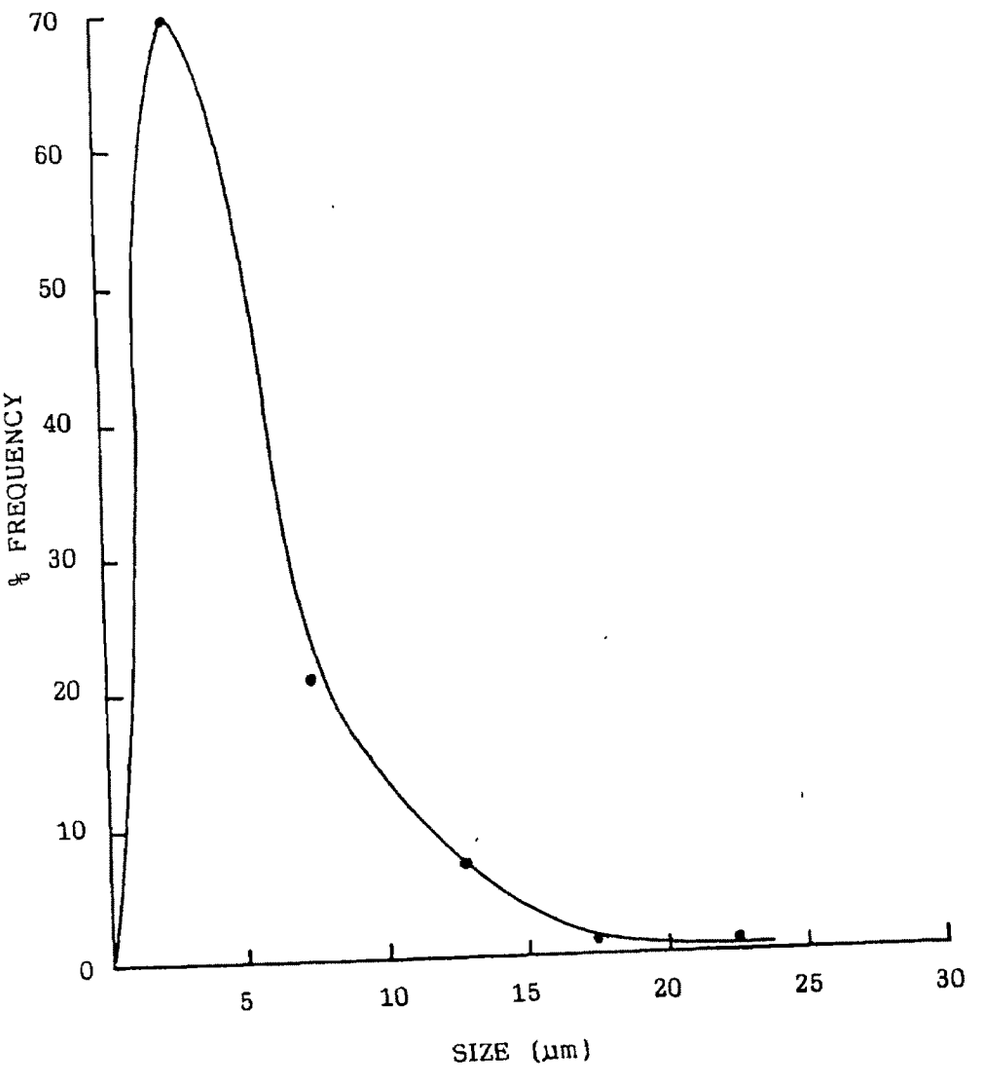


Fig. 30 : Frequency Distribution Plot of Ar-Tc microcapsules

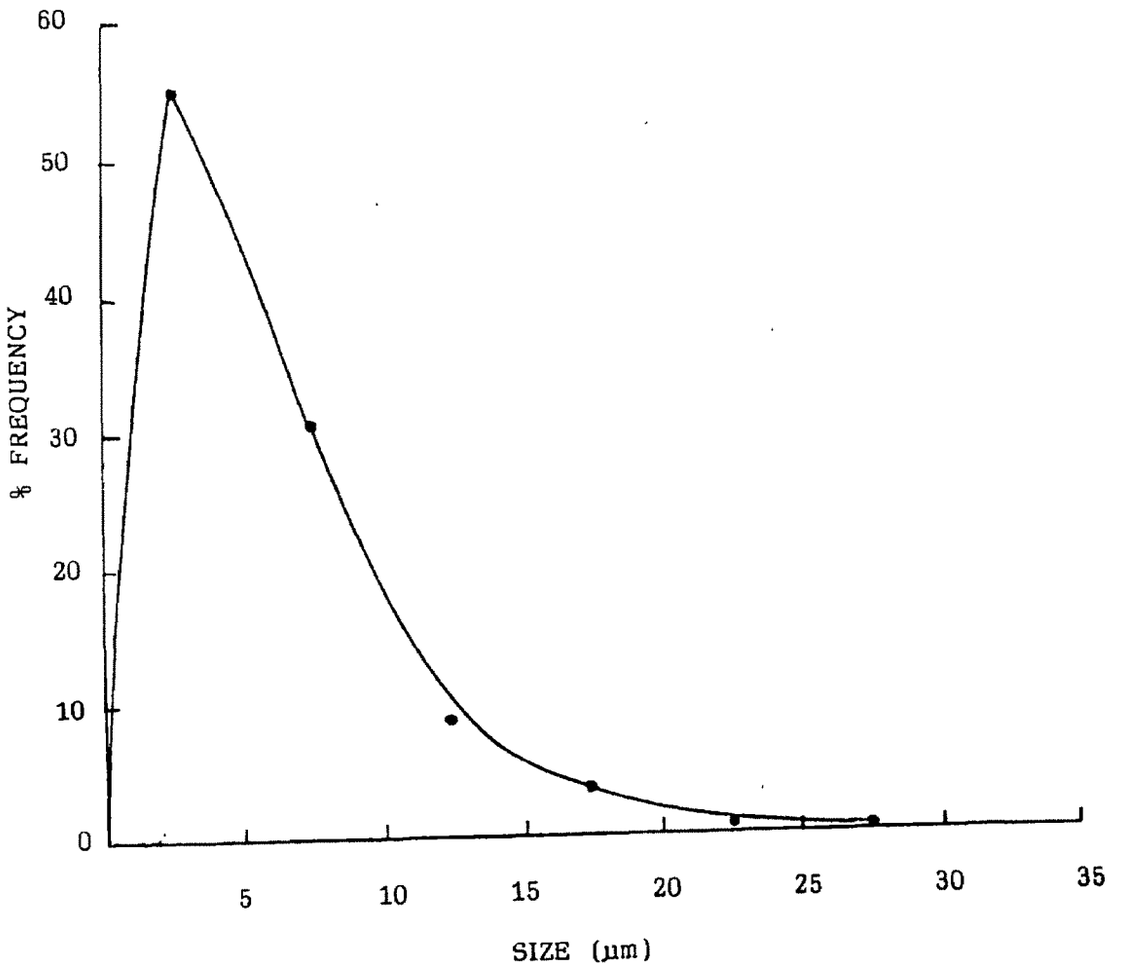


Fig. 31 : Frequency Distribution Plot of As-Tc microcapsules

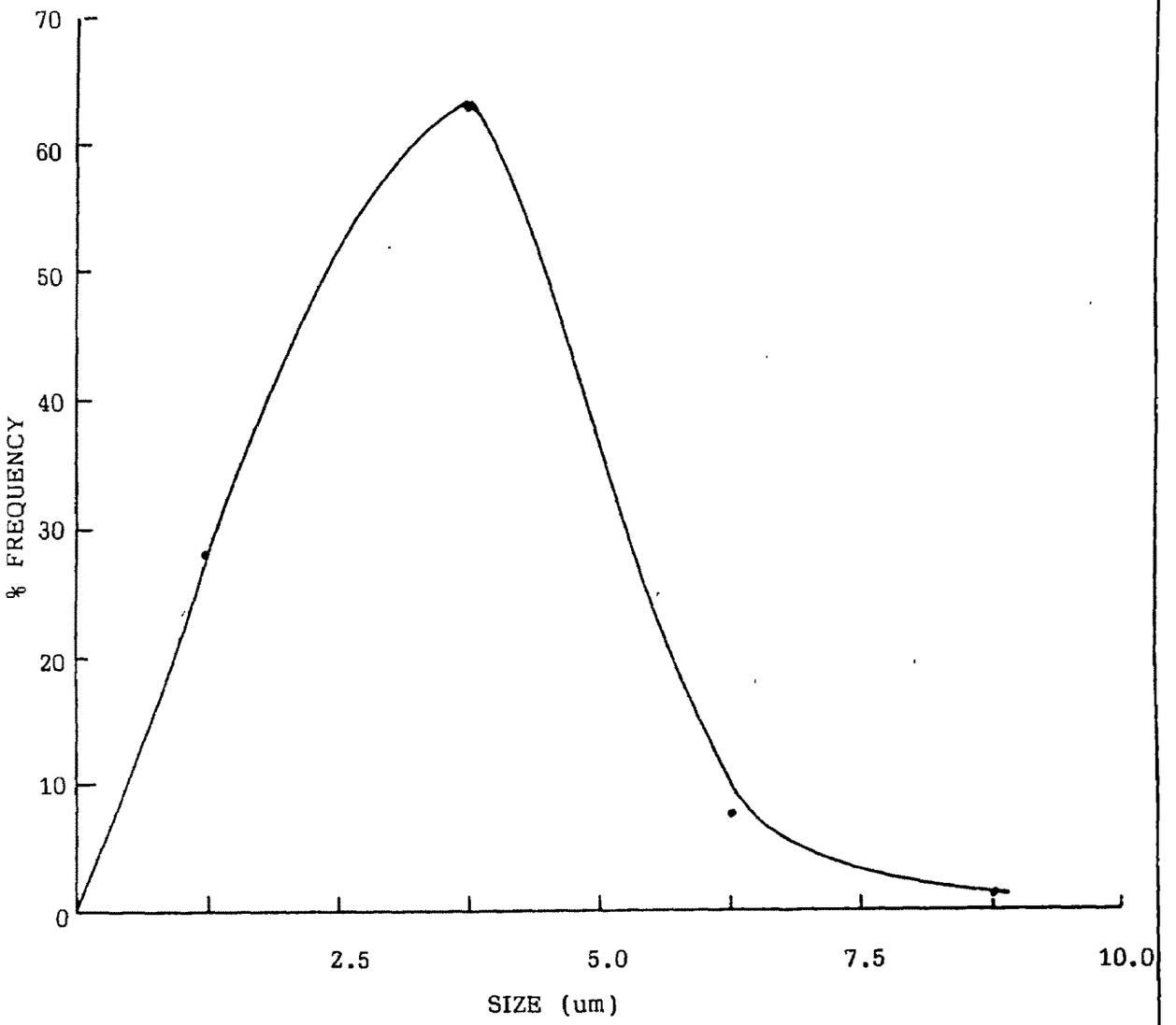


Fig. 32 : Frequency Distribution Plot of Ci-Tc microcapsules

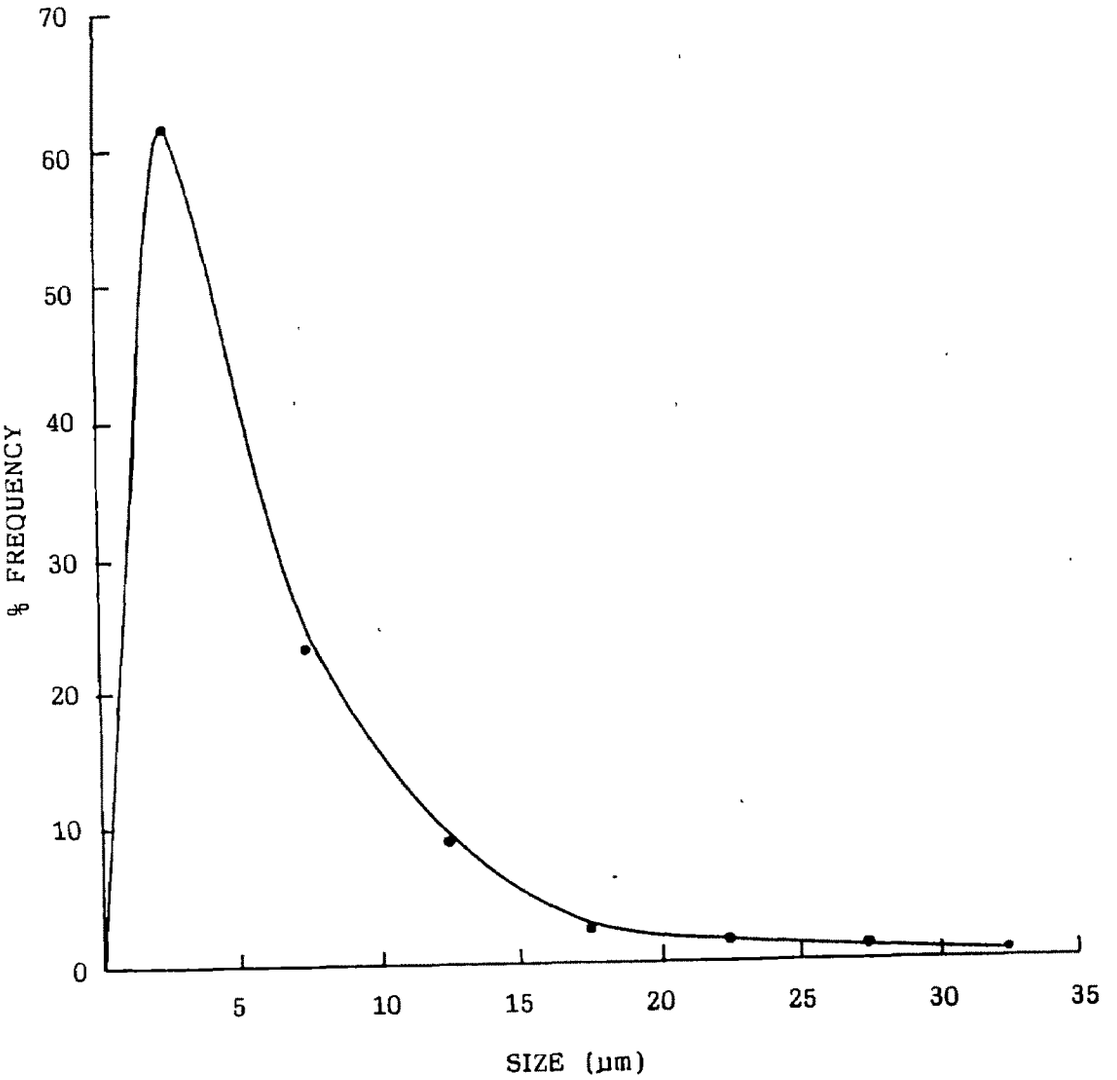


Fig. 33 : Frequency Distribution Plot of Cy-Tc microcapsules

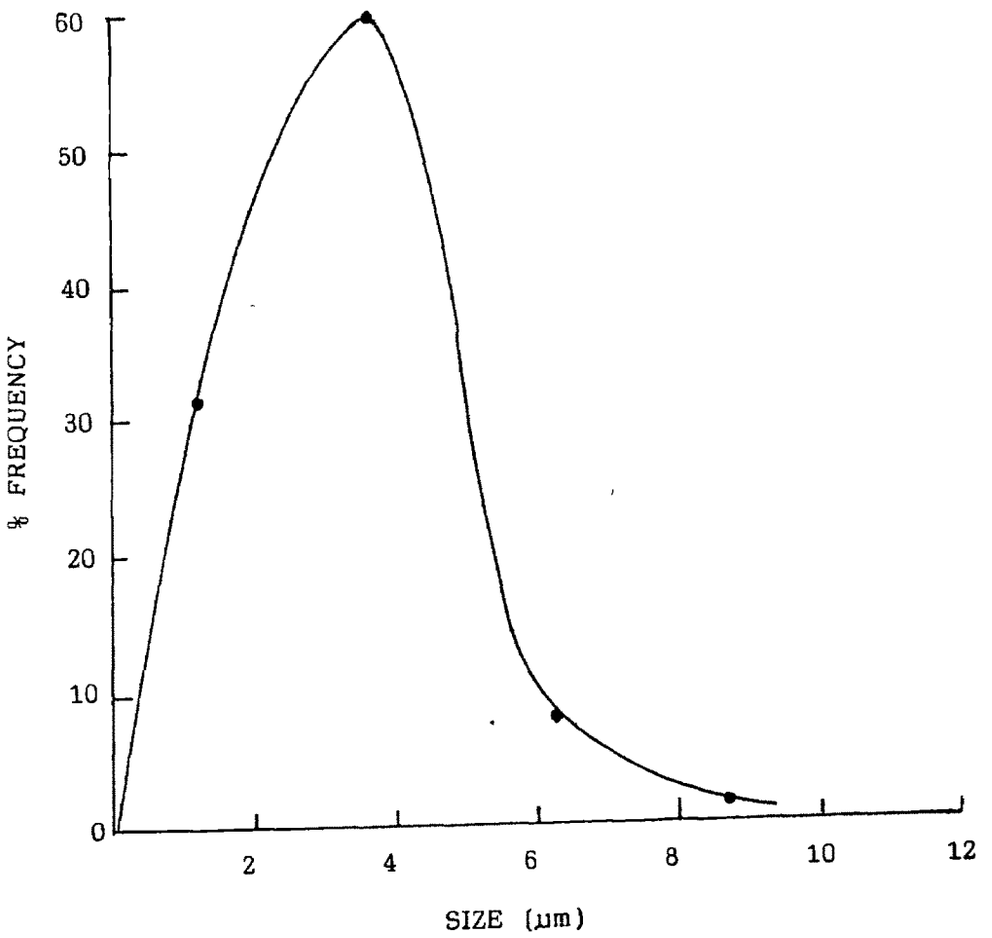


Fig. 34 : Frequency Distribution Plot of Gl-Tc microcapsules

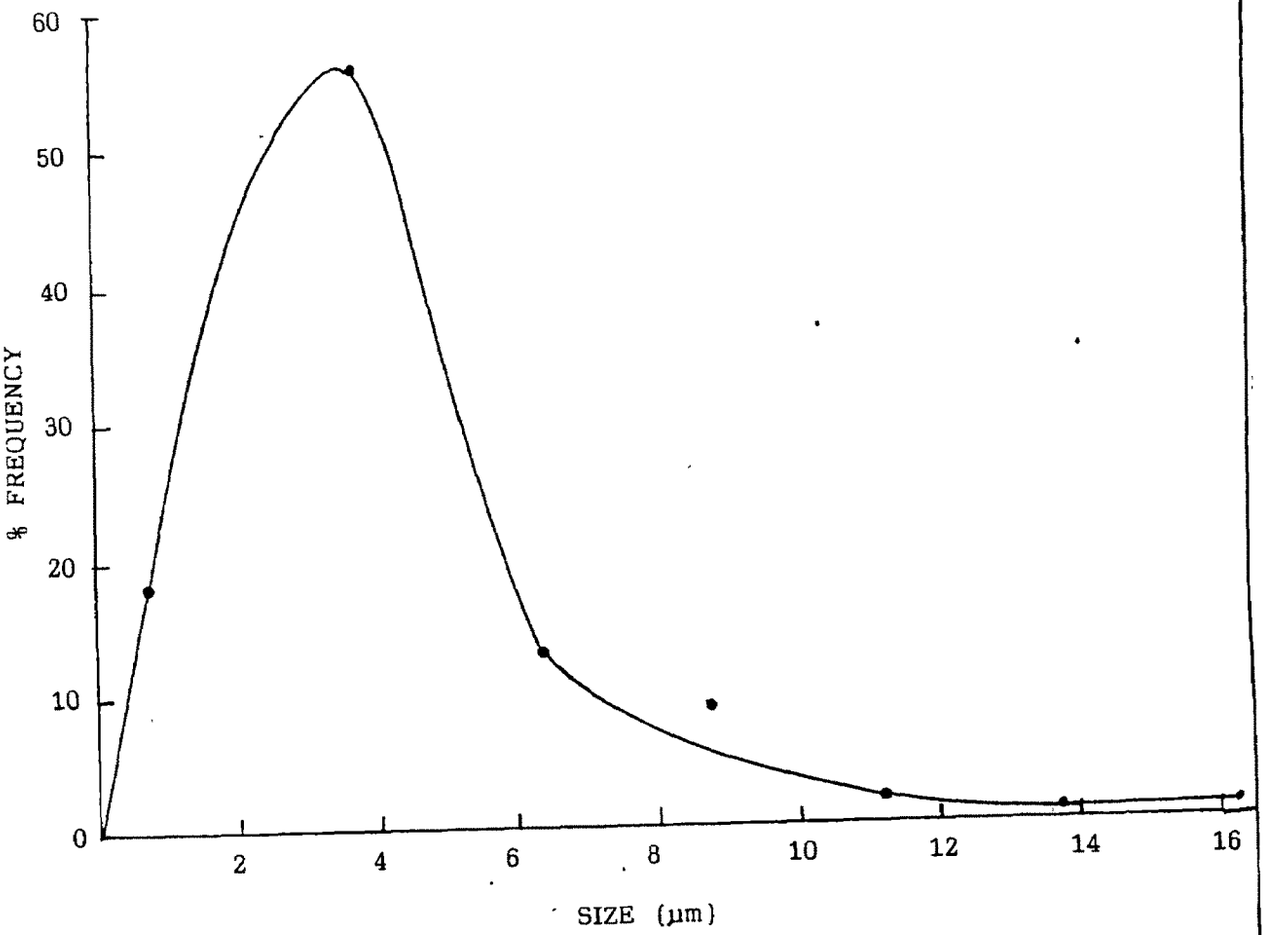


Fig. 35 : Frequency Distribution Plot of Ly-Tc microcapsules

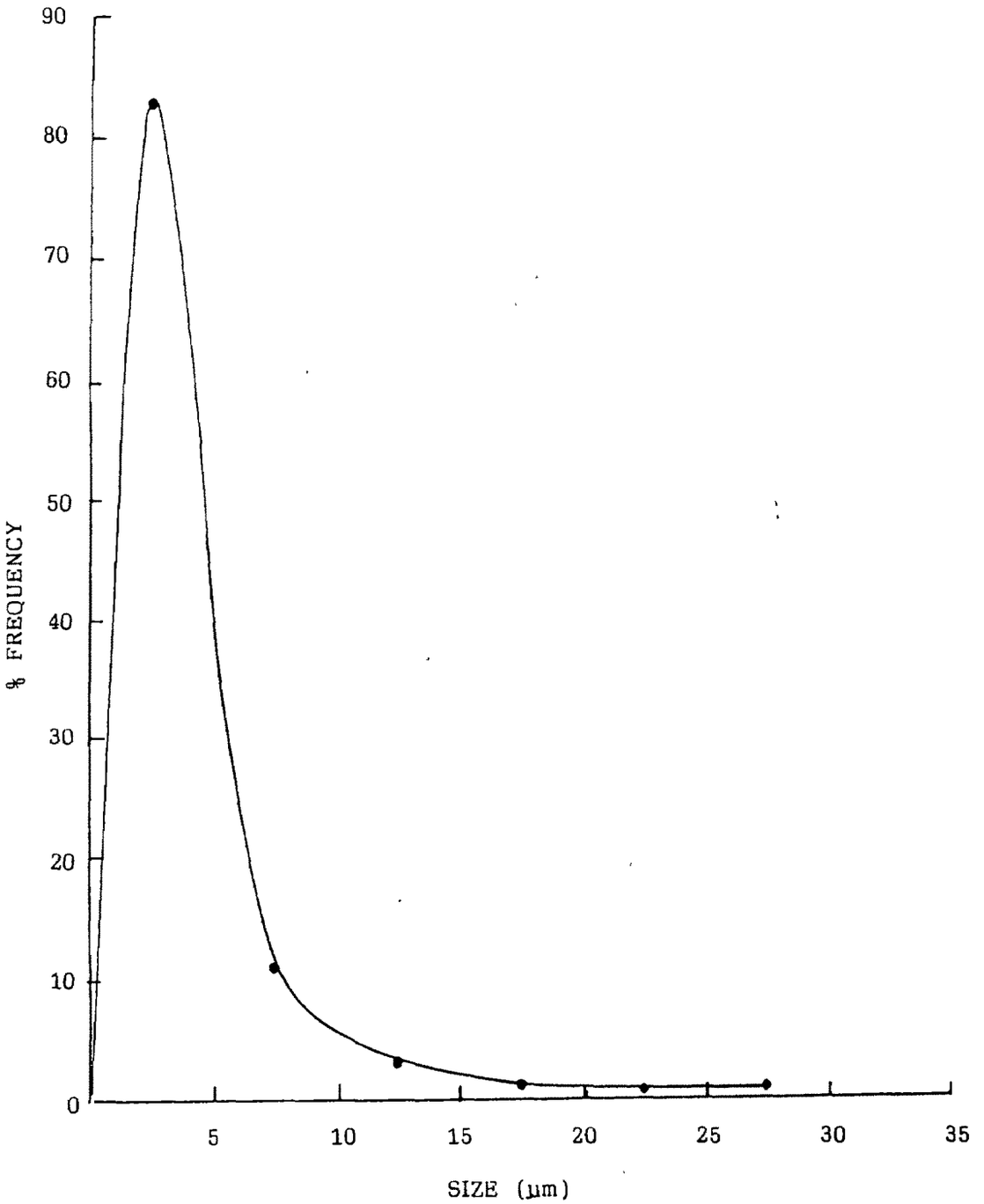


Fig. 36 : Frequency Distribution Plot of Or-Tc microcapsules

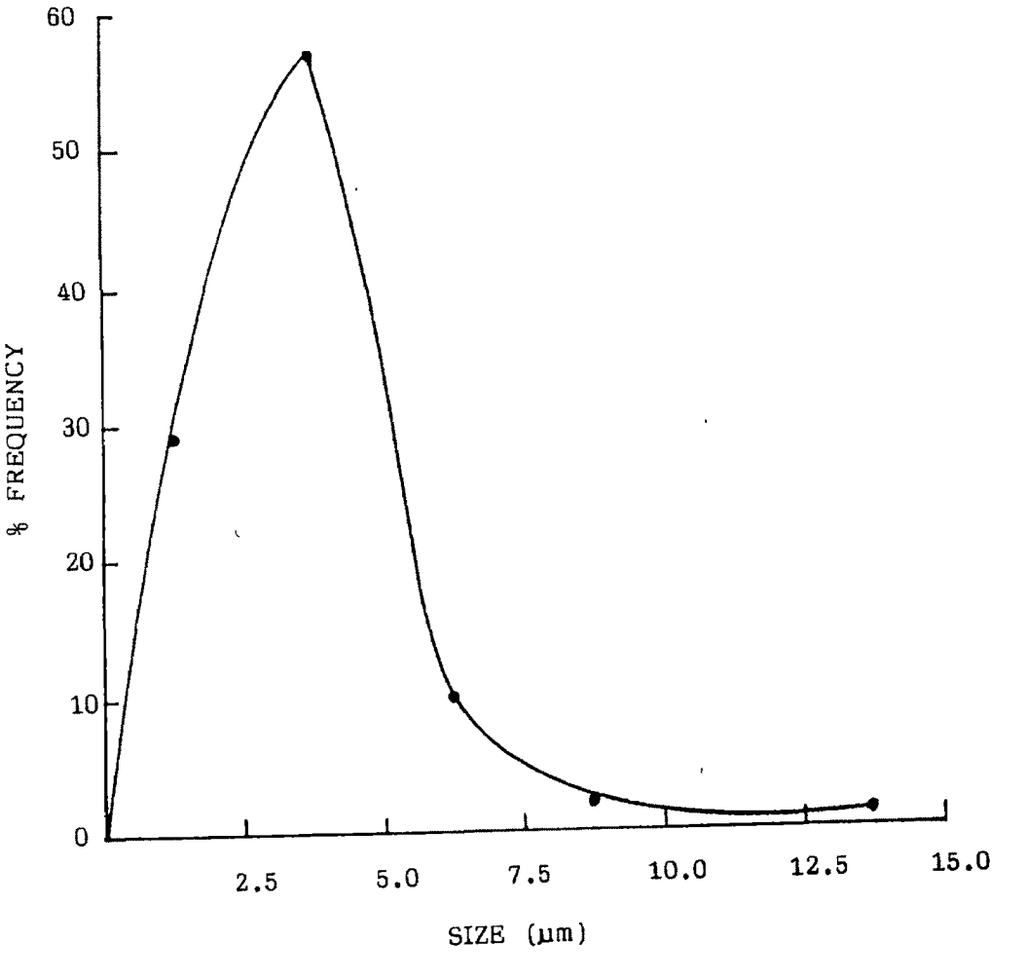


Fig. 37 : Frequency Distribution Plot of Hb-Tc microcapsules

ii) Photomicrographs of Microcapsules

The photomicrographs of various batches of polytereph-thalamide microcapsules are shown in Figs. 38 to 44.

The photographs show that in general, all the polytereph-thalamide microcapsules are spherical particles with a distinct boundary encapsulating the core material.

A careful examination of the figures will indicate a difference in the membrane thickness of the various types of micro-capsules. Thus, the Cy-Tc, As-Tc, Ly-Tc and Ar-Tc batches show thick, well-defined walls whereas the other batches have relatively thinner boundaries. This is due to the differing reactivities of the amino acid entities of the polymer, which in turn is a function of the partition coefficient ($K_{o/w}$) of the amino acid. Thus, the batches showing a thicker polyamide wall are the ones having amino acid with a high $K_{o/w}$ and vice versa.

Another noteworthy feature in case of polyterephthalamide microcapsules was the presence of particles showing multiple droplet formation, especially in case of batches prepared from amino acids with low $K_{o/w}$ values. Such a phenomenon has been reported by Shigeri[104] and Shiba[108] et al. They attributed the formation of multiple droplets to the reduced

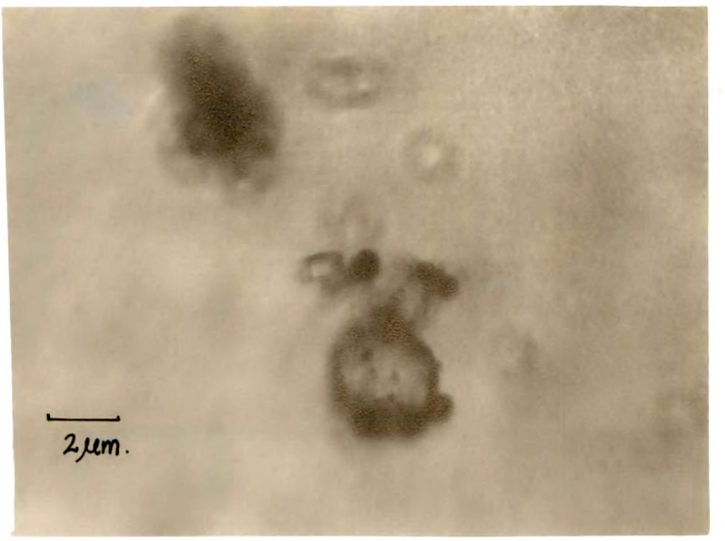


Fig.38 : Photomicrograph of Ar-Tc microcapsules

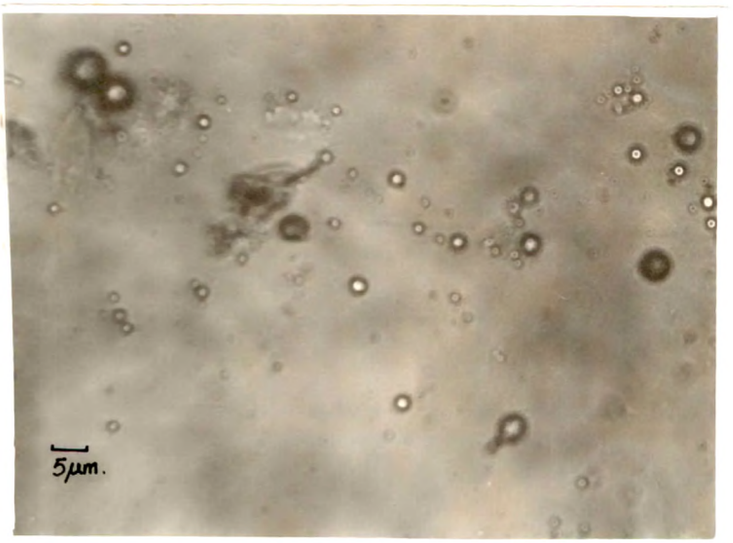


Fig.39 : Photomicrograph of As-Tc microcapsules

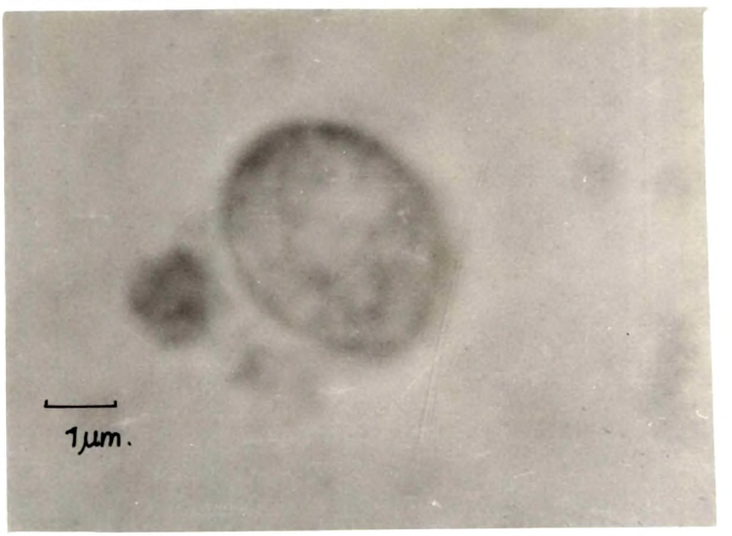


Fig.40 : Photomicrograph of Ci-Tc microcapsules

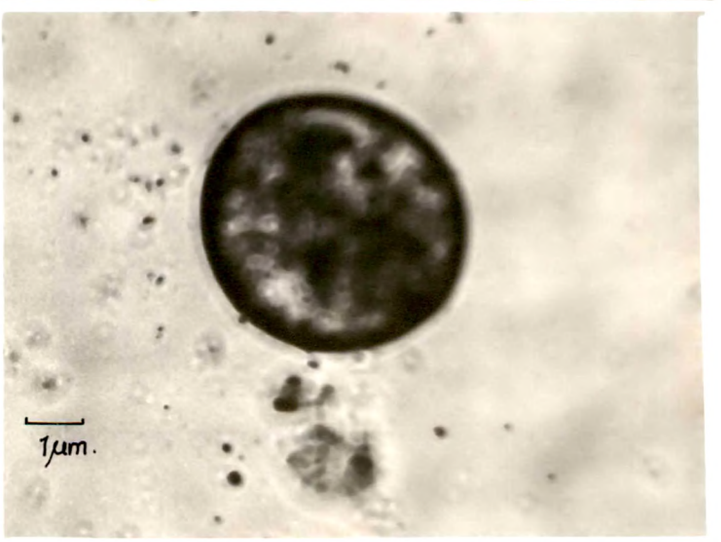


Fig.41 : Photomicrograph of Cy-Tc microcapsules

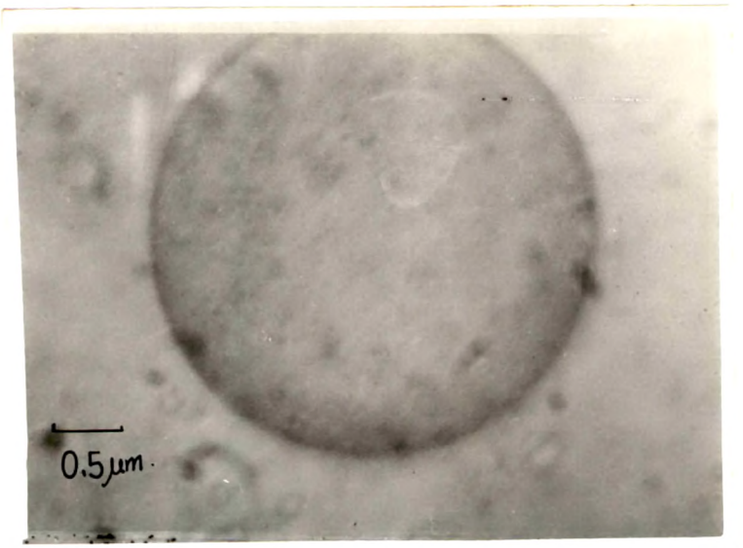


Fig.42 : Photomicrograph of Gl-Tc microcapsules

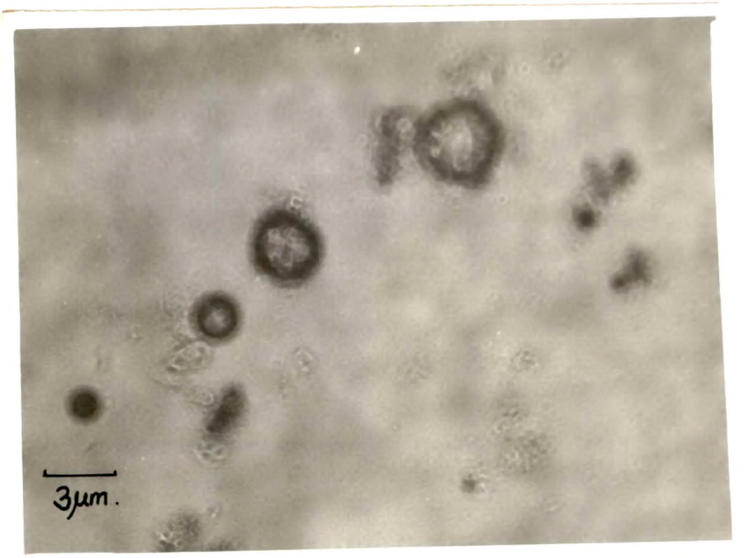


Fig.43 : Photomicrograph of Ly-Tc microcapsules

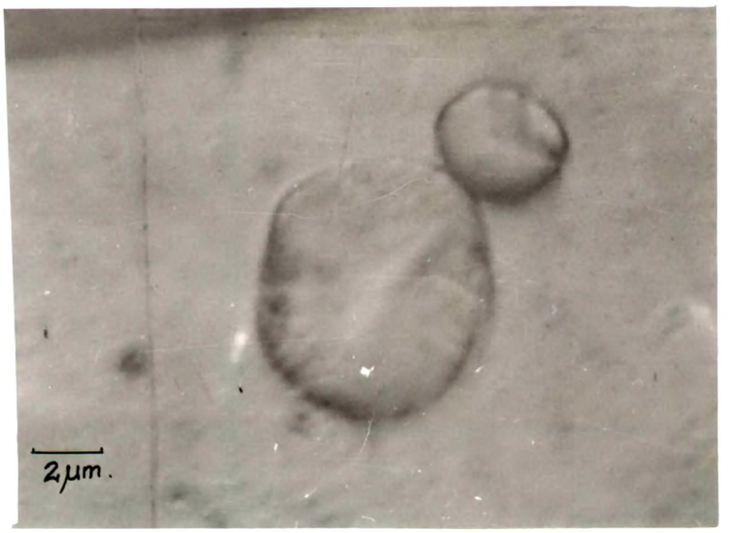


Fig.44 : Photomicrograph of Or-Tc microcapsules

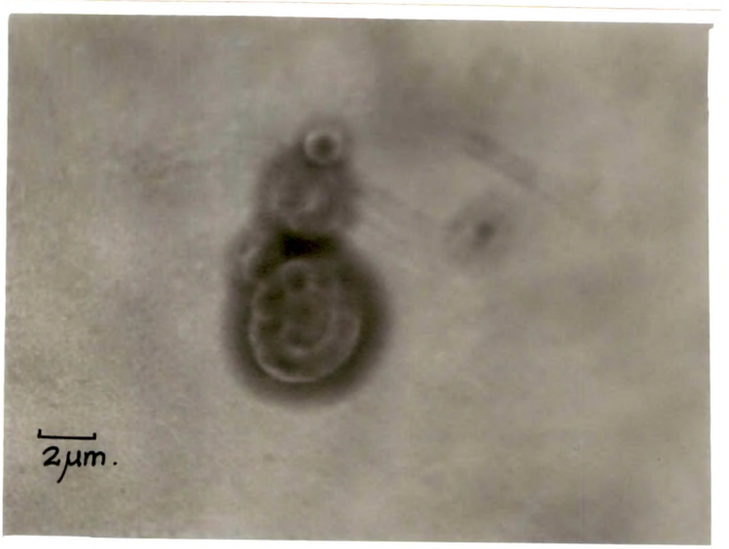


Fig.45 : Photomicrograph of Hb-Tc microcapsules

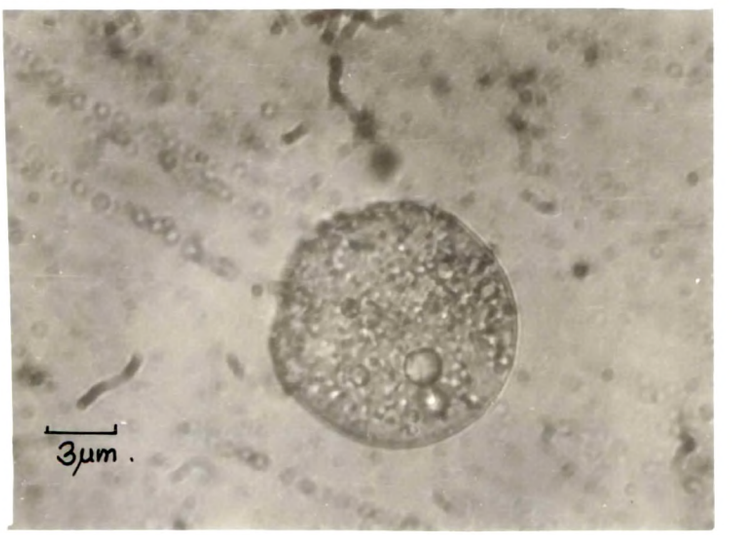


Fig.46 : Photomicrograph of a polyterephthalamide microcapsule showing multiple droplet formation.

rate of diffusion of the diamine from the aqueous to the organic phase. In this case, such an occurrence was observed in batches of microcapsules prepared from readily water soluble amino acids viz. citrulline, ornithine and glutamine, whose lower oil/water partition coefficients would have reduced their rate of diffusion into the organic phase so that many small, fully formed microcapsules would have penetrated the ill-formed boundaries of bigger droplets, giving rise to multiple microcapsule formation (Fig. 46).

Fig. 45 is a photomicrograph of a cross-linked haemoglobin microcapsule of spherical shape with a clearly visible polymeric wall encapsulating it.

iii) Drug Entrapment in Microcapsules

The extent of drug incorporation in the microcapsules was estimated indirectly by assaying the aqueous supernatant and washings for the drug content.

The percent drug entrapment in various batches of microcapsules is shown in Table 14. The values indicate a wide variation in drug entrapment efficiency of various batches of microcapsules. This also was found to be a function of the partition coefficient ($K_{o/w}$) of the amino acid.

iv) Infrared Spectroscopy of Microcapsules

Figures 47 to 54 show infrared spectra of various batches of polyterephthalamide microcapsules. The characteristic peaks and their corresponding functional groups have been tabulated in Tables 15 to 22.

In general, in all the batches of polyterephthalamide microcapsules, there is an absence of peaks corresponding to free amine groups (3400 or more, 2220-2000, 1550 cm^{-1}) and acid halide moiety (3080, 1800, 1740, 865 cm^{-1}) whereas there is an appearance of secondary amide peaks, confirming the formation of a polyamide (R'CONHR) as a result of reaction between the RNH_2 group of the diamino acid and the R'COCl group of the diacid chloride.

In some cases (Ar-Tc, As-Tc, Or-Tc etc.), few acid anhydride bands were also observed. This may be due to an anhydride linkage (C-CO-O-CO-C) between the free carboxylic acid groups of different diamino acid molecules, which is reported to increase the strength of the polyamide wall.

IR spectra of a few batches of microcapsules (Ci-Tc, Gl-Tc) show bands corresponding to free acid halide, indicating the presence of unreacted diacid chloride in the microcapsules. This may be due to the reduced reactivity of the monomers

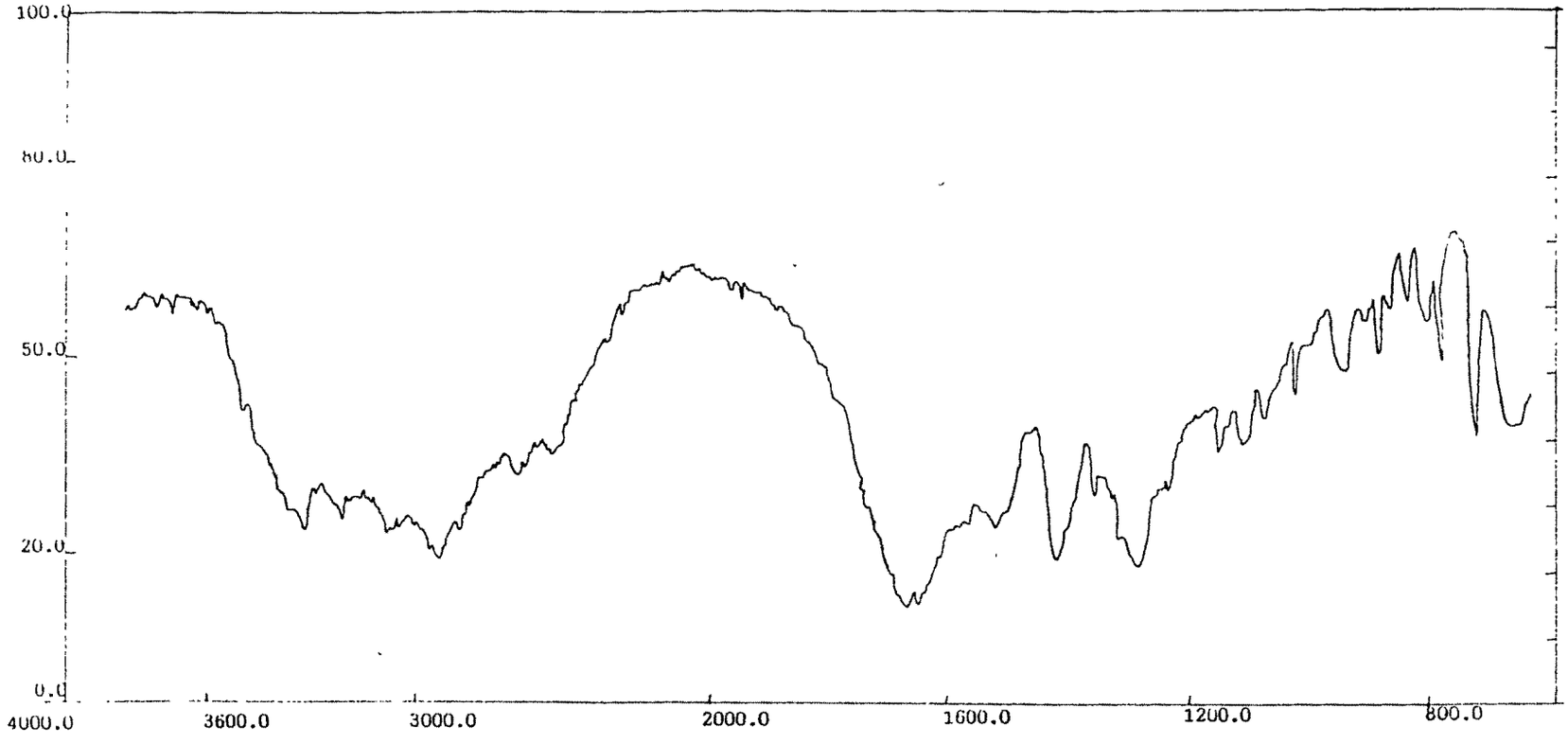


Fig. 47 : Infrared Spectrum of Ar-Tc Microcapsules

Table : 15

Infrared Spectral Assignments for
Ar-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
3390	-	Bonded N-H stretch of amide
1525	-	N-H deformation of amide II
3260	-	H-bonded N-H stretch of sec. amide
2920	-	CH_2 asymmetric stretch
1655, 1670	-	C=O stretch of $RCONH_2$ due to resonance
1290	-	N-H deformation of sec. amide, overlapping C-N stretch
785	-	H-bonded N-H deformation of sec. amide
670-690	-	H-bonded skeletal deformation of amide V
1020	-	Skeletal deformation of amide
935-950, 1150	-	Acid anhydride peaks
880	-	Resonance due to C=O stretch
1425	-	C-N stretch of amide overlapping O-H bending (amino acid)

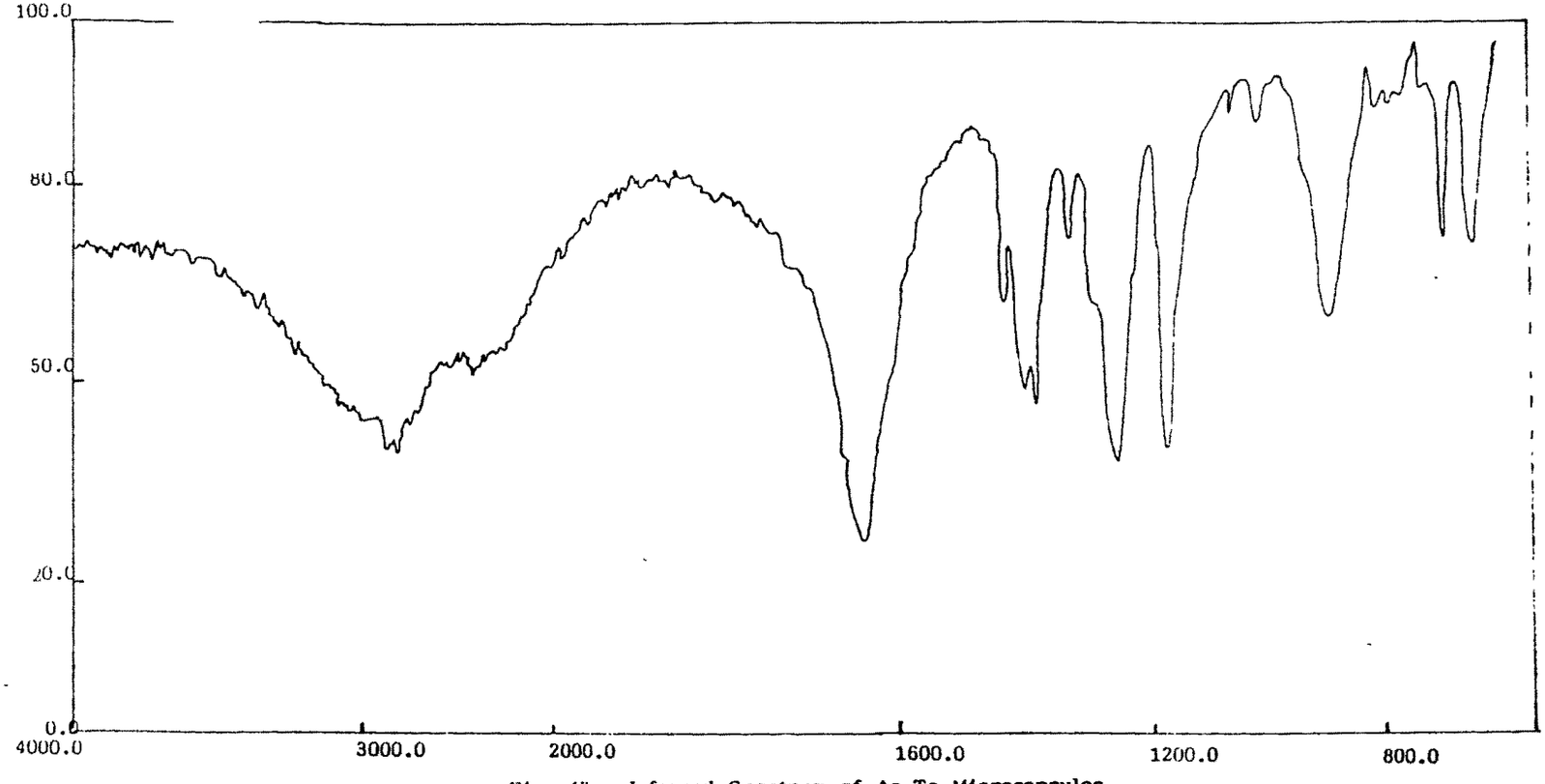


Fig. 48 : Infrared Spectrum of As-Tc Microcapsules

Table : 16

Infrared Spectral Assignments for
As-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
1680	-	C=O stretch of sec. amide
1275	-	N-H deformation of amide II, overlapping acid anhydride peak
2910, 2940	-	CH_2 asymmetric stretch
1405-1425	-	C-N stretching vibrations of amides
1460	-	-CONH in-form deformation
1040	-	-CONH in-form rocking, overlapping acid anhydride peak
1190	-	C=O ester stretch, overlapping acid anhydride peak
730	-	H-bonded N-H deformation of sec. amide
685	-	Skeletal deformation of amide V
920	-	Acid anhydride peak

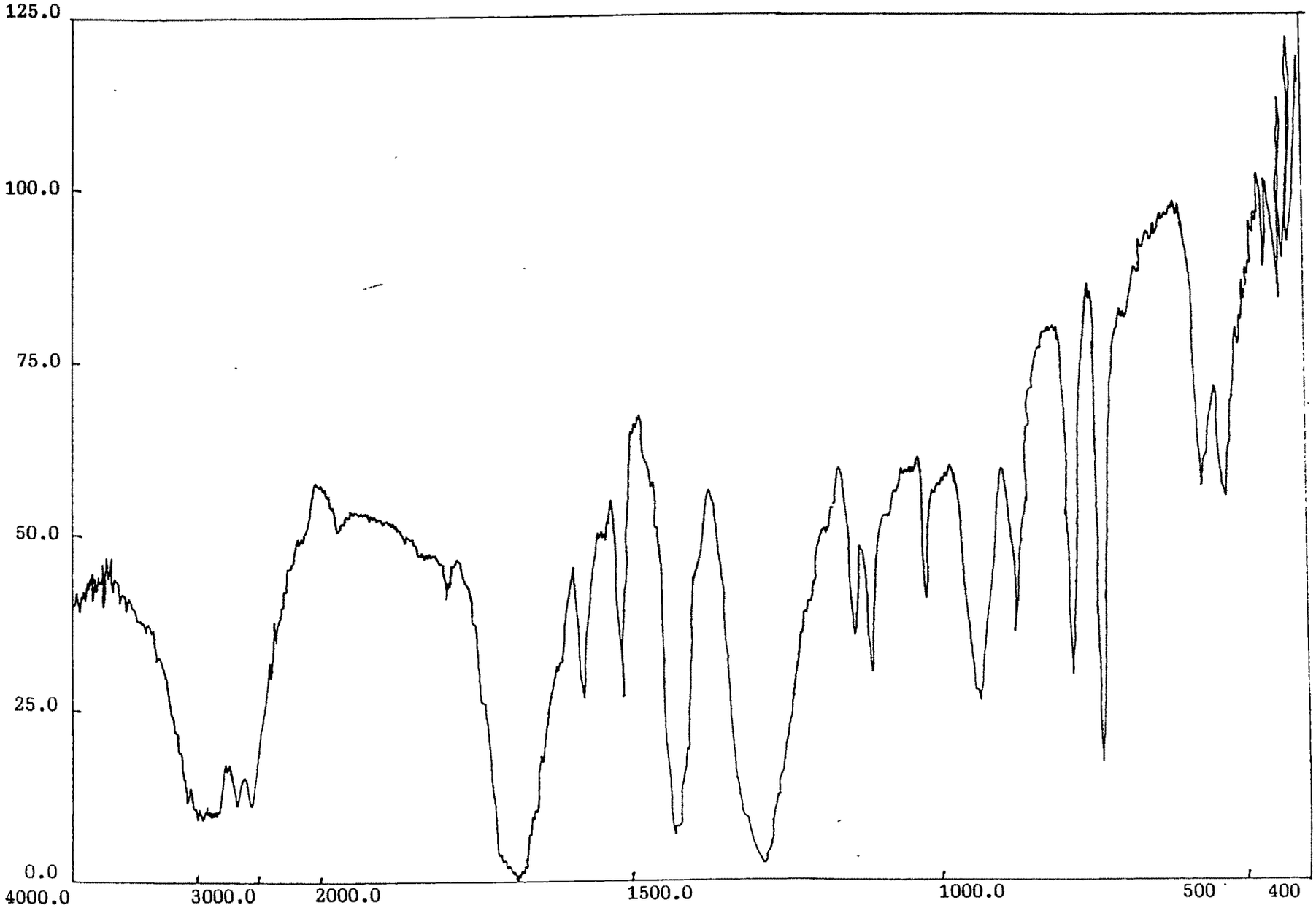


Fig.48a : Infrared Spectrum of 5-FU loaded As-Tc Microcapsules

Table : 16a

Infrared Spectral Assignments for 5-FU-loaded
As-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
1575	-	N-H deformation of amide II
1280	-	N-H bending of amide II
1015	-	In plane -CONH stretch
700	-	H-bonded N-H deformation of sec. amide
565	-	Skeletal deformation of amide VI
780, 530	-	H-bonded N-H deformation
1670-1720	-	C=O stretch of 5-FU
400-500, 930, 1510	-	Other peaks of 5-FU

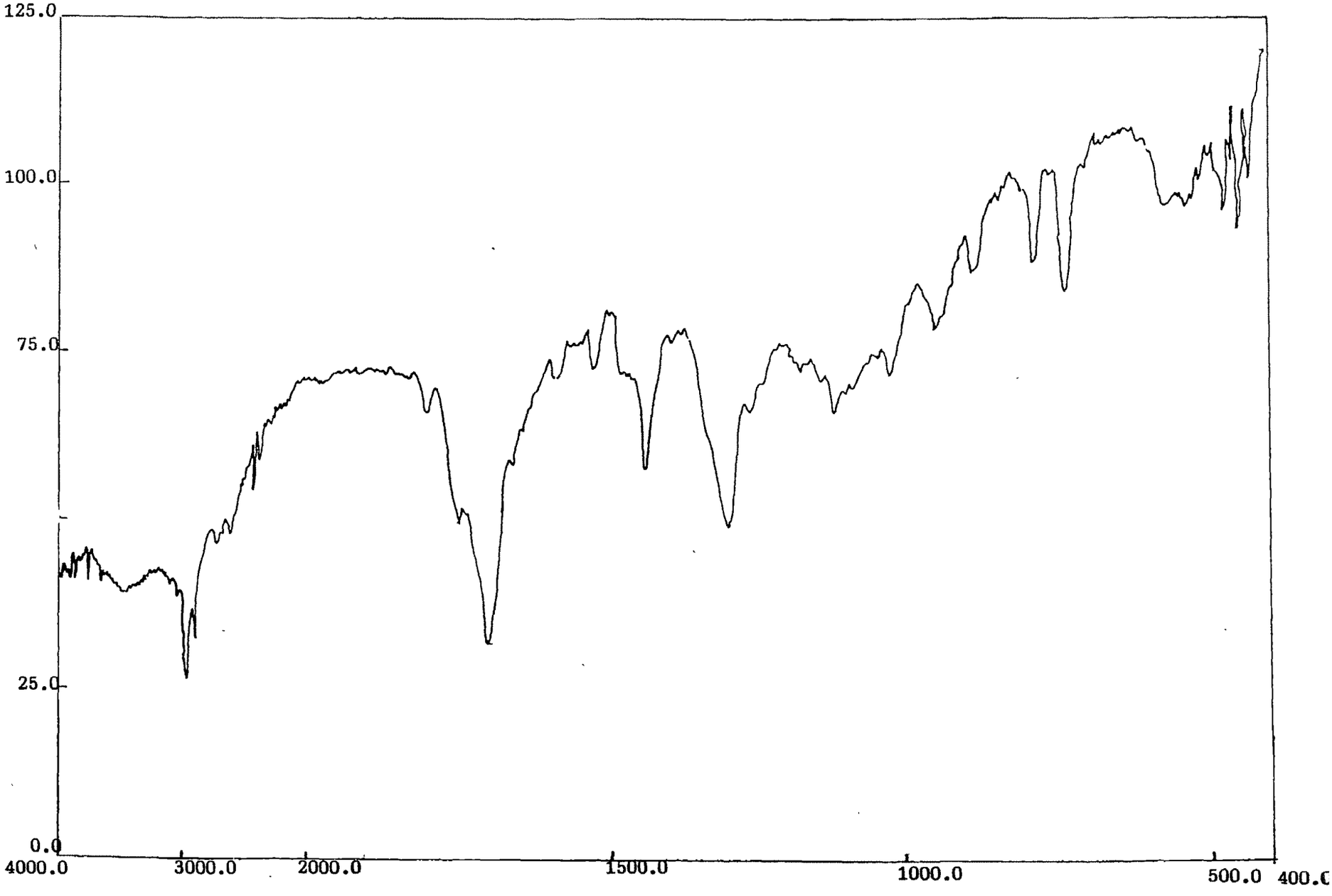


Fig.49 : Infrared Spectrum of Ci-Tc Microcapsules

Table : 17

Infrared Spectral Assignments for
Ci-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
2920	-	CH_2 asymmetric stretch
2850	-	C=O of amide II, overlapping C-H stretch
1285	-	N-H bending of amide III
1015	-	-CONH in plane deformation of amide
780	-	H-bonded N-H deformation
530-560	-	Skeletal deformation of amide VI
1690	-	C=O stretch
1790	-	C=O stretch of aromatic acid halide (Tere. chloride)
880, 1740	-	Fermi resonance due to C=O stretch of aromatic acid halide (Tere. chloride)
1740	-	C=O stretch of carboxylic acid (amino acid)

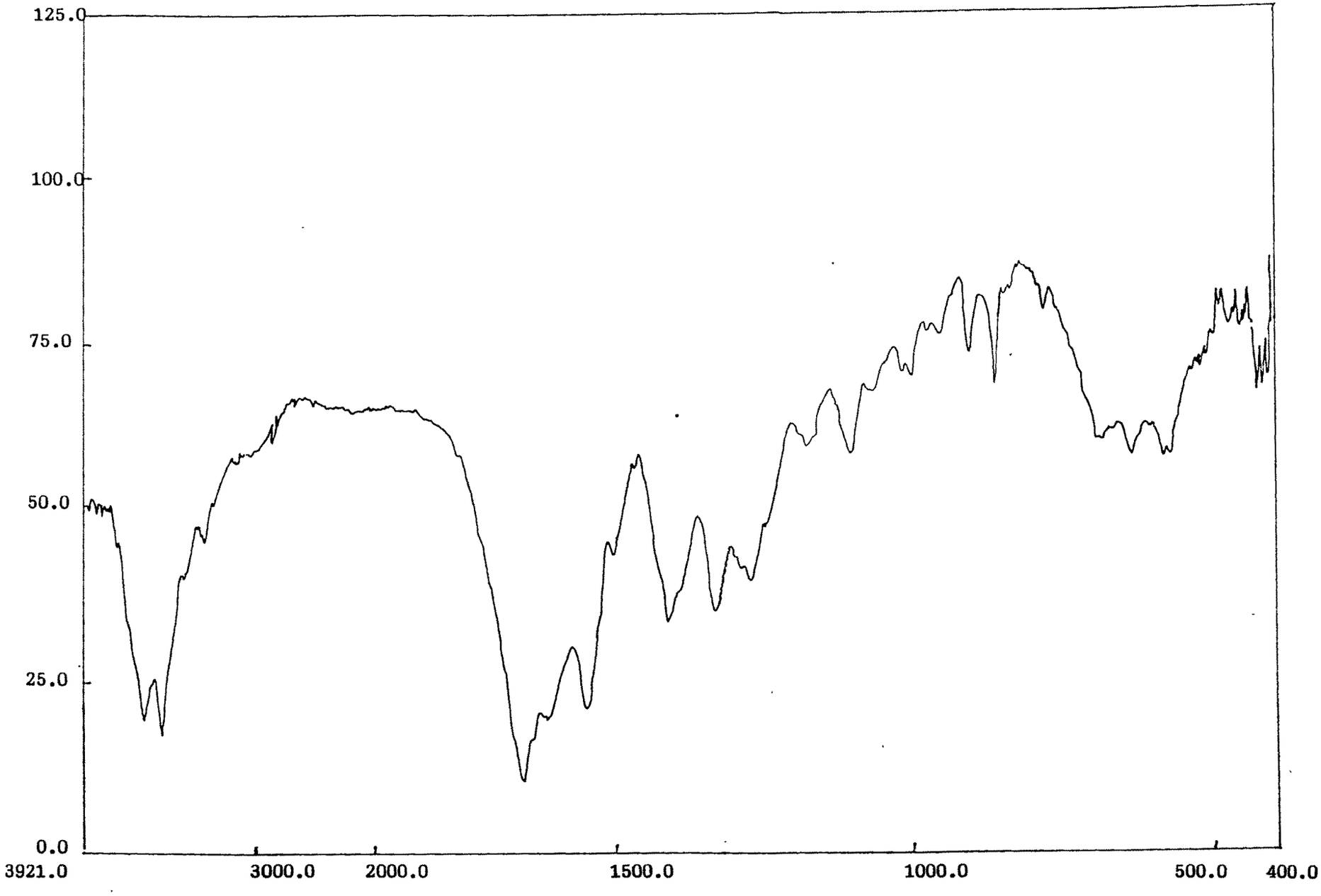


Fig.50 : Infrared Spectrum of Cy-Tc Microcapsules

Table : 18

Infrared Spectral Assignments for
Cy-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
3400	-	Free N-H group
3290	-	H-bonded N-H stretch
1655	-	C=O stretch of amide I
1550	-	N-H deformation of amide II
1265	-	N-H bending of amide III
1015	-	-CONH in plane deformation
570-580	-	Skeletal deformation of amide VI
860	-	-CONH stretch
635	-	H-bonded N-H deformation
1410	-	COOH bending (amino acid)
1335	-	Peak of dicarboxylic acid (i.e. cystine)

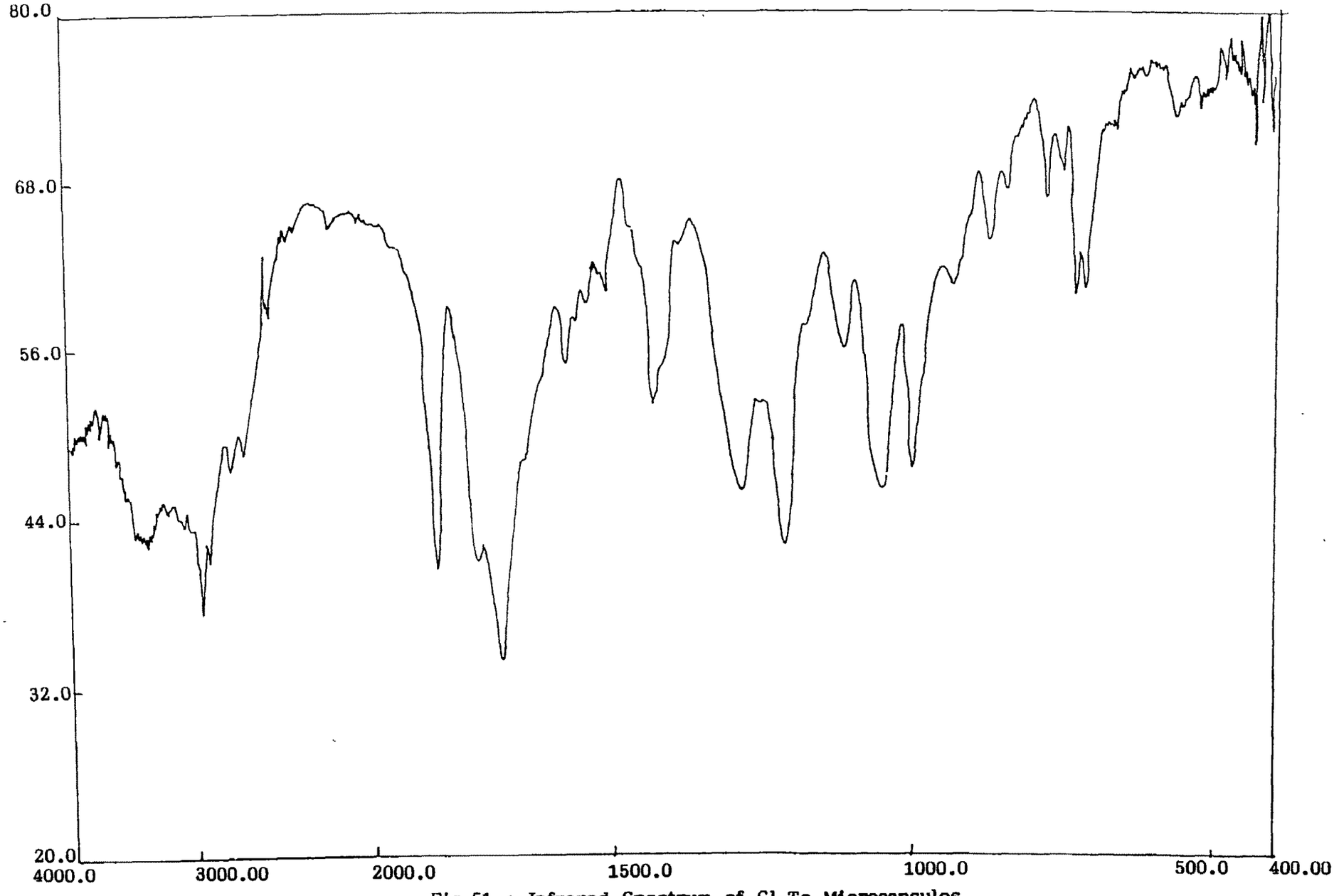


Fig.51 : Infrared Spectrum of Gl-Tc Microcapsules

Table : 19

Infrared Spectral Assignments for
Gl-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
2850	-	C=O of amide II, overlapping C-H stretch
1530, 1570	-	N-H deformation of amide II
1280	-	N-H bending of amide III
780	-	H-bonded N-H deformation
560	-	Skeletal deformation of amide VI
1690, 1720	-	C=O ester stretch of sec. amide due to resonance
1790	-	C=O stretch of aromatic acid halide (Tere. chloride)
1210	-	C=O stretch of carboxylic acid (amino acid)

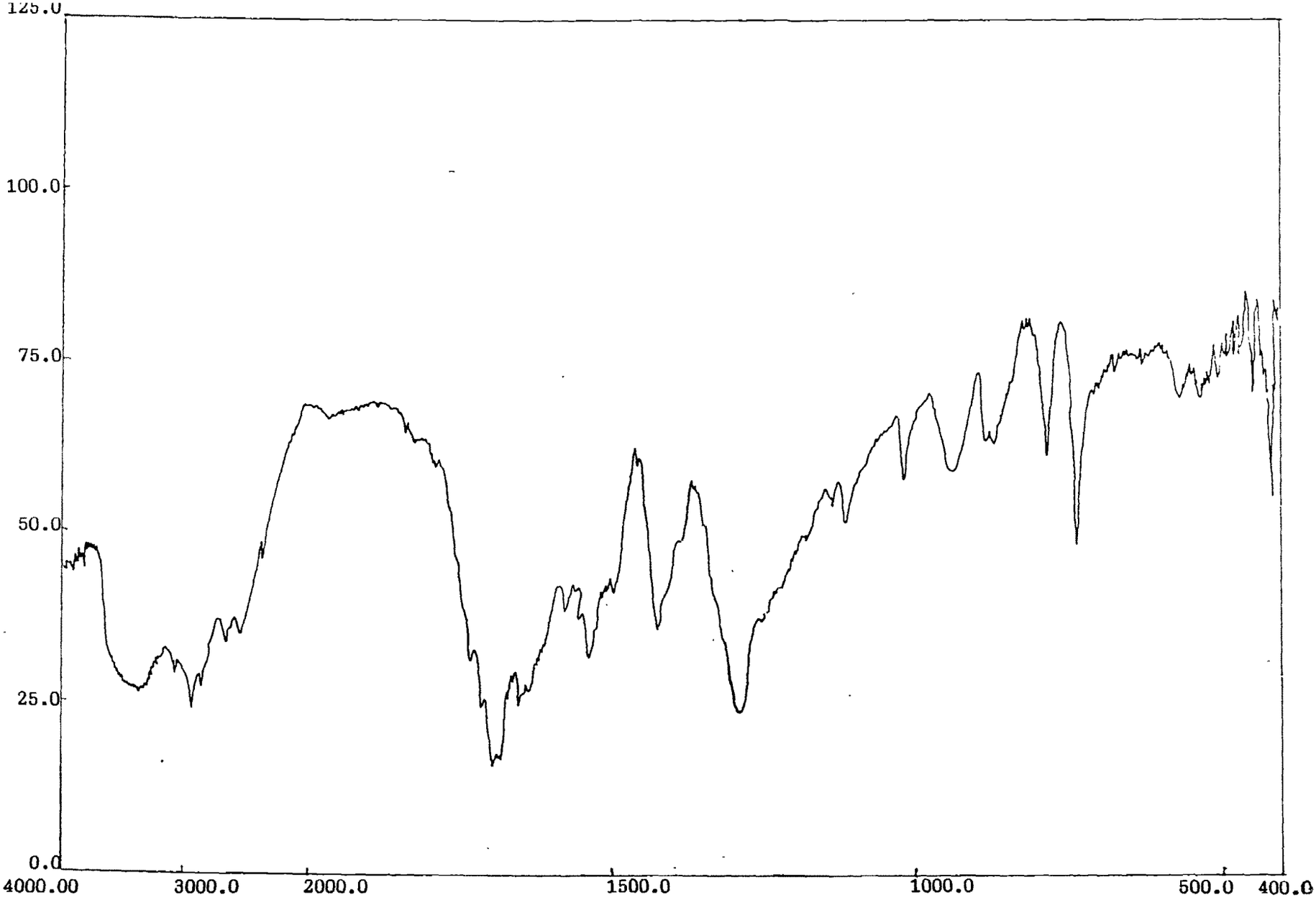


Fig.52 : Infrared Spectrum of Ly-Tc Microcapsules

Table : 20

Infrared Spectral Assignments for
Ly-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
2930	-	CH_2 asymmetric stretch
2850	-	C=O of amide II, overlapping C-H stretch
1290	-	N-H bending of amide III
780, 530	-	H-bonded N-H deformation
565	-	Skeletal deformation of amide VI
1535, 1645	-	N-H deformation of amide II
1685-1695	-	C=O stretch of $RCONH_2$ due to resonance
880-865	-	-CONH stretch
1015	-	-CONH in plane stretch

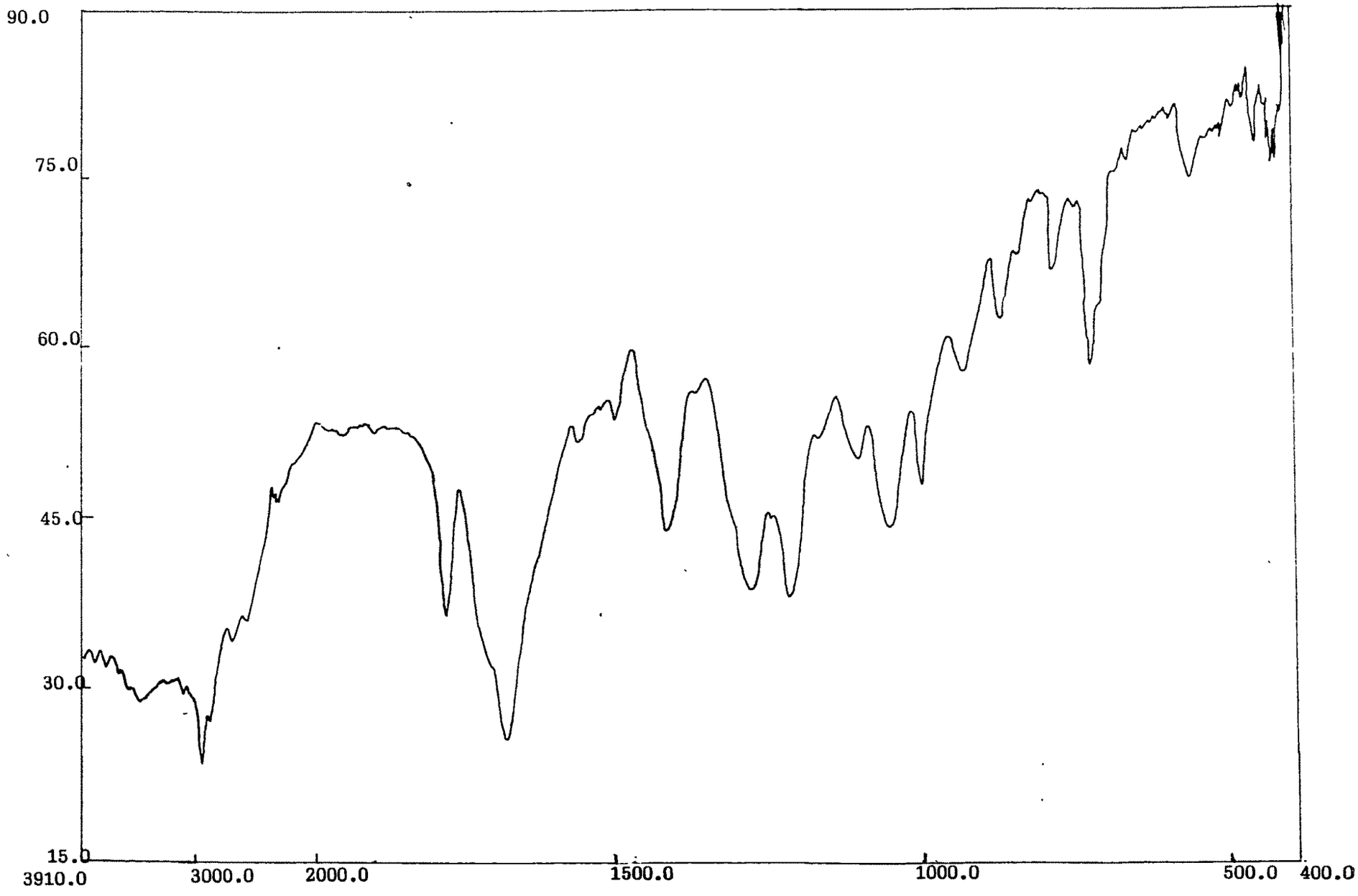


Fig.53 : Infrared Spectrum of Or-Tc Microcapsules

Table : 21

Infrared Spectral Assignments for
Or-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
2900	-	CH_2 asymmetric stretch
1560	-	N-H deformation of amide II
1280	-	N-H bending of amide III
560	-	Skeletal deformation of amide VI
720	-	Out of plane N-H wagging of amide V
775	-	H-bonded N-H deformation
1050	-	CO-O-CO stretch of acid anhydrides
1680	-	C=O stretch of amide due to resonance
1420	-	O-H bending of COOH group (amino acid)
865	-	Resonance of C=O stretch

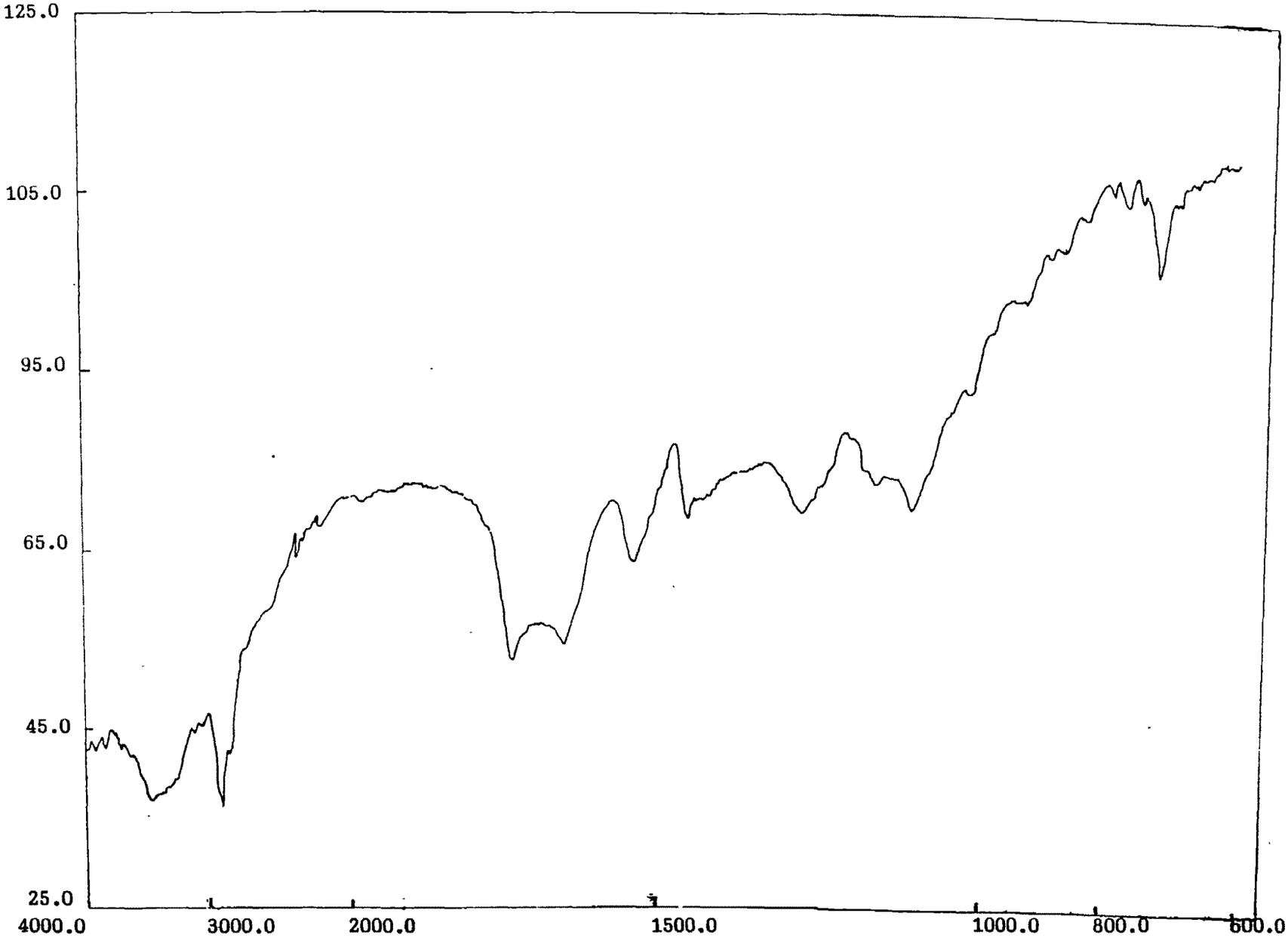


Fig.54 : Infrared Spectrum of Hb-Tc Microcapsules

Table : 22

Infrared Spectral Assignments for
Hb-Tc Batch of Microcapsules

Frequency (cm^{-1})		Functional Group
3300-3500	-	Free N-H stretch
1645	-	C=O stretch of amide I
2900	-	CH_2 asymmetric stretch
1540	-	N-H deformation of amide II
1270	-	N-H bending of amide III
720	-	Skeletal deformation of amide V
1730	-	C=O ester stretch
1450	-	C-H deformation, overlapping C-C skeletal stretch
1100	-	C-H deformation of disubstituted benzene (Tere. chloride)

owing to low values of $K_{o/w}$ partition coefficients of the readily water soluble amino acids like citrulline and glutamine.

Fig. 48a is an infrared spectrum of a 5-fluorouracil-loaded batch of polyterephthalamide microcapsules. Presence of some characteristic peaks of 5-FU (Table 16a) indicate that some drug is associated with the surface of the polyamide wall.

Note : The infrared spectra have been interpreted using standard books on spectrometric identification of organic compounds[203-204] and specific literature on polyamides.[99, 205]

v) In vitro Drug Leaching Studies

The extent of drug loss due to leaching from the microcapsules during storage in a refrigerator upto 2 months is represented in Table 23 and Figs. 55-57.

The results indicate a wide variability in the extent of drug leaching from various batches of microcapsules. Thus, while only 1.4 to 2.9% of the encapsulated drug leached out from batches Gl-Tc and Ci-Tc, as high as 18 to 19.24% escaped from Hb-Tc, Cy-Tc and As-Tc batches.

Table : 23

Extent of Drug Leaching from Various Batches of Polyterephthalamide Microcapsules

B A T C H															
Ar-Tc		As-Tc		Ci-Tc		Cy-Tc		Gl-Tc		Ly-Tc		Or-Tc		Hb-Tc	
Time in days	Cumulative % drug leached	Time in days	Cumulative % drug leached	Time	Cumulative % drug leached	Time in days	Cumulative % drug leached	Time in days	Cumulative % drug leached	Time in days	Cumulative % drug leached	Time in days	Cumulative % drug leached	Time in days	Cumulative % drug leached
3	1.49	1	2.26	2	0.68	1	7.61	1	0.77	2	6.59	2	1.55	3	6.95
6	2.75	3	4.82	5	1.49	3	13.82	3	0.83	4	10.96	4	1.78	6	10.12
12	3.82	5	5.79	9	2.09	6	15.83	6	1.05	7	11.81	8	3.87	9	11.11
16	4.44	10	7.43	14	2.42	9	16.31	10	1.11	18	12.56	16	6.49	17	13.07
23	4.57	17	9.63	21	2.53	16	17.13	14	1.24	25	12.74	20	6.89	28	14.28
31	5.21	29	10.90	35	2.56	20	17.50	22	1.25	32	13.42	29	8.27	35	15.06
38	5.63	36	13.50	41	2.62	25	17.75	36	1.30	41	14.58	32	9.33	43	16.14
45	6.15	44	15.84	48	2.72	31	18.41	42	1.36	47	14.76	38	9.91	59	17.47
52	6.33	53	17.71	58	2.93	38	18.52	56	1.39	55	14.95	45	10.57	64	18.03
60	6.48	60	19.24			45	18.58	61	1.41	62	15.03	52	10.82		
						51	18.63					60	10.99		
						58	18.66								
						63	18.70								

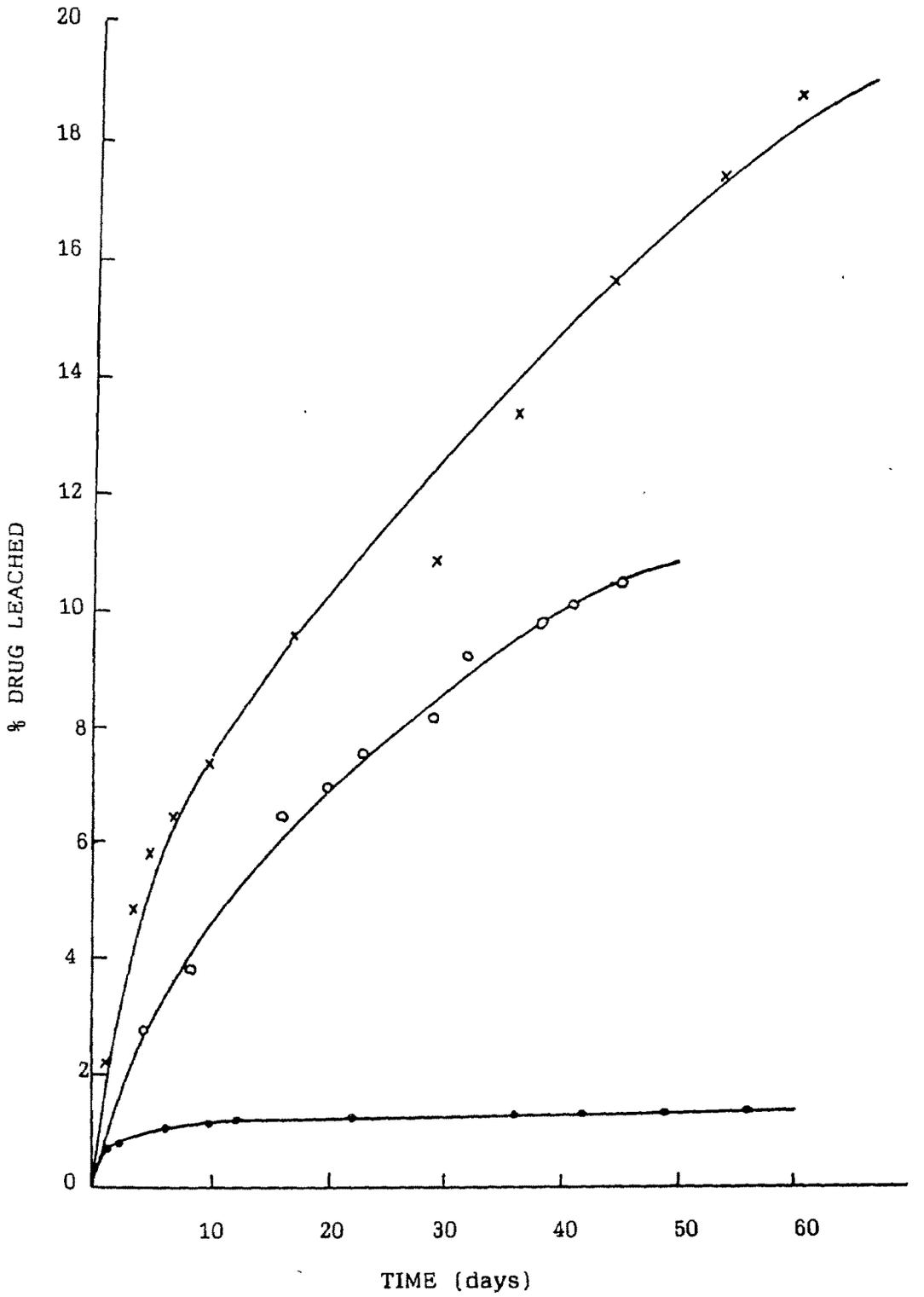


Fig. 55 : In vitro Leaching Profile of 5-FU from polyterephthalamide microcapsules

x - As-Tc; o - Or-Tc; • - Gl-Tc

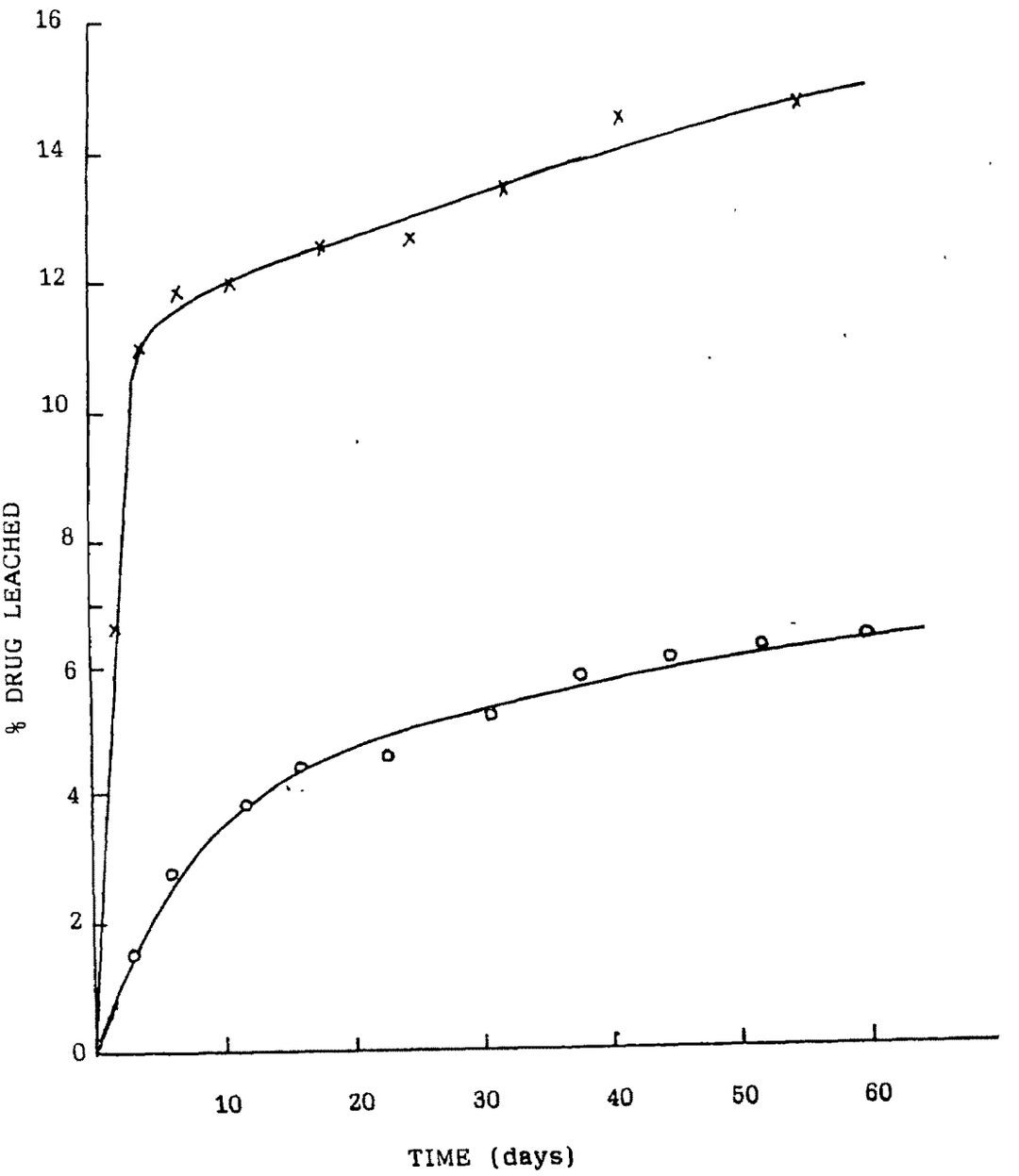


Fig. 56 : In vitro Leaching Profile of 5-FU from polyterephthalamide microcapsules

x - Ly-Tc; o - Ar-Tc

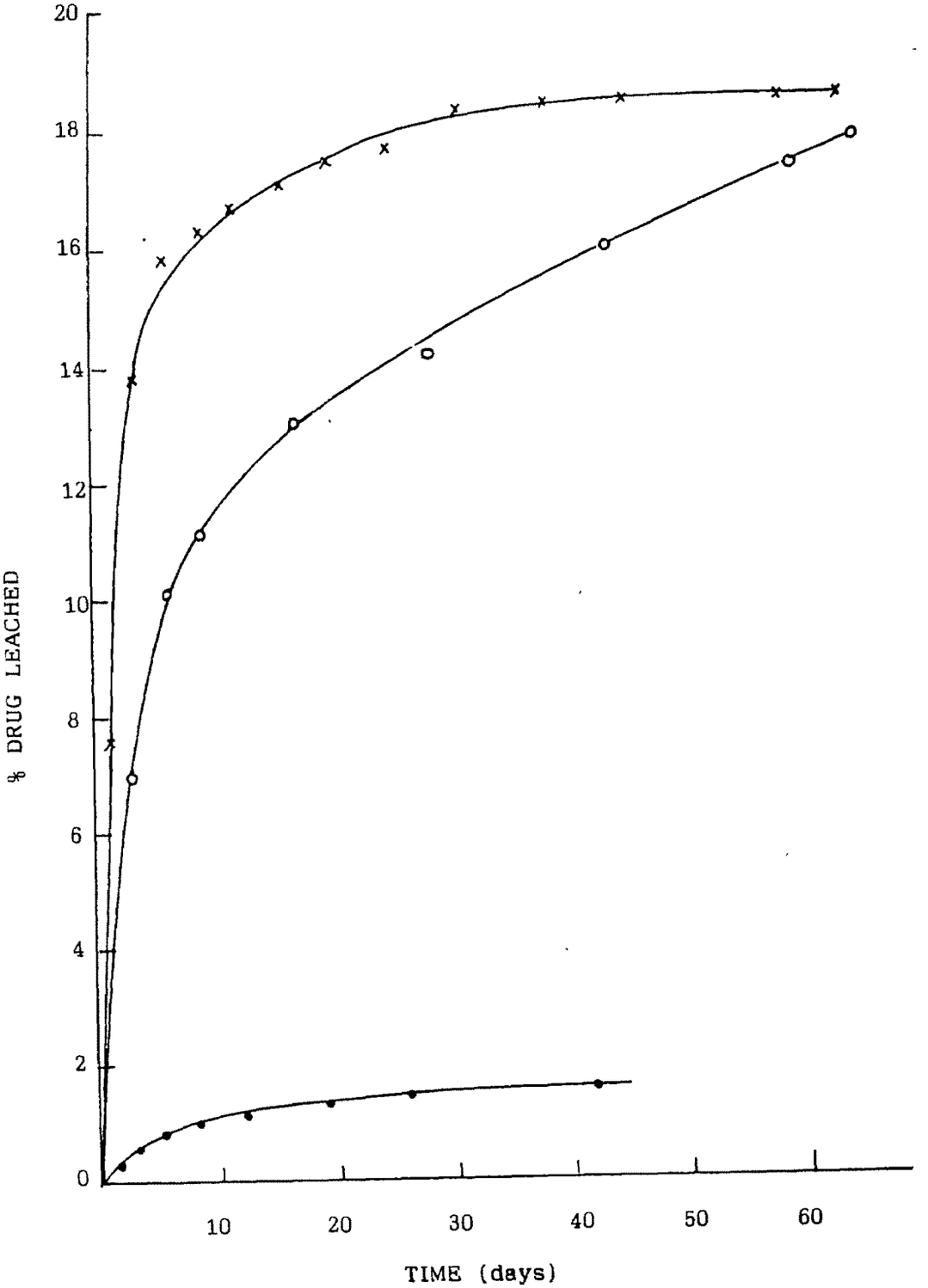


Fig. 57 : In vitro Leaching Profile of 5-FU from polyterephthalamide microcapsules

x - Cy-Tc; o - Hb-Tc; • - Ci-Tc

This data further strengthens the theory that the variation in the properties of the microcapsules is influenced by the diamine entity. It can be inferred that the polyamide wall of the microcapsules prepared from cystine, asparagine, haemoglobin and lysine showed higher permeability to 5-FU than the batches prepared from citrulline and glutamine.

The initial rate of drug leaching from all batches of microcapsules was high upto 5 - 10 days and thereafter, it either reduced or attained equilibrium (Ar-Tc, Ci-Tc and Gl-Tc batches). This may be due to a reduction in the concentration gradient of the drug or saturation of the suspending fluid respectively, because of the non-sink conditions.

vi) In vitro Drug Release Studies

The percentage of drug released from various batches of microcapsules is given in Table 24 and Figs. 58-60.

The results indicate that there is a significant difference in the rate and extent of drug release from various batches of polyterephthalamide microcapsules. Drug release was appreciably higher from Cy-Tc (73%), Hb-Tc (68.4%), As-Tc (53.4%) and Ly-Tc (47.4%) batches while relatively less drug release was observed from Ci-Tc (7.4%), Gl-Tc (7.4%) and Ar-

Table : 24

Percentage of 5-Fluorouracil Released from Various Batches of Polyterephthalamide Microcapsules

Time in Hours	B A T C H							
	Ar-Tc	As-Tc	Ci-Tc	Cy-Tc	Gl-Tc	Ly-Tc	Or-Tc	Hb-Tc
0.5	6.83	14.38	3.42	23.04	4.39	35.04	24.98	29.42
1.0	8.61	22.63	4.59	29.11	5.22	40.77	32.41	39.19
2.0	11.84	31.57	5.61	39.58	5.44	43.18	35.21	47.10
3.0	12.39	37.41	6.18	46.62	5.59	44.62	36.59	51.22
4.0	14.62	46.78	6.62	57.37	6.21	46.19	39.08	55.41
5.0	15.18	49.22	6.98	65.21	6.63	46.82	39.41	57.56
6.0	16.21	53.36	7.43	73.02	7.36	47.37	42.77	68.38

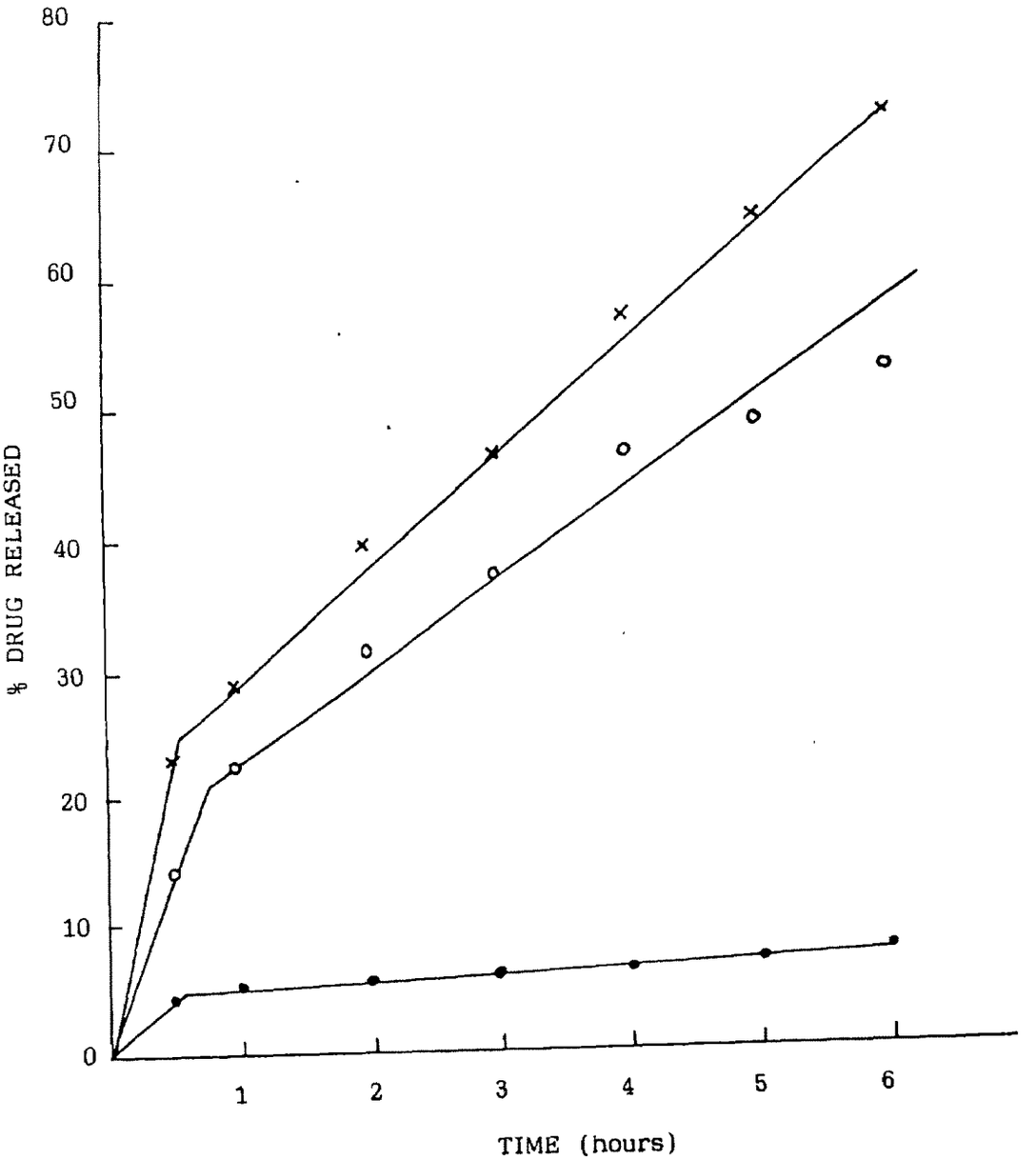


Fig. 58 : In vitro Release Profile of 5-FU from polyterephthalamide microcapsules

x - Cy-Tc; o - As-Tc; • - Gl-Tc

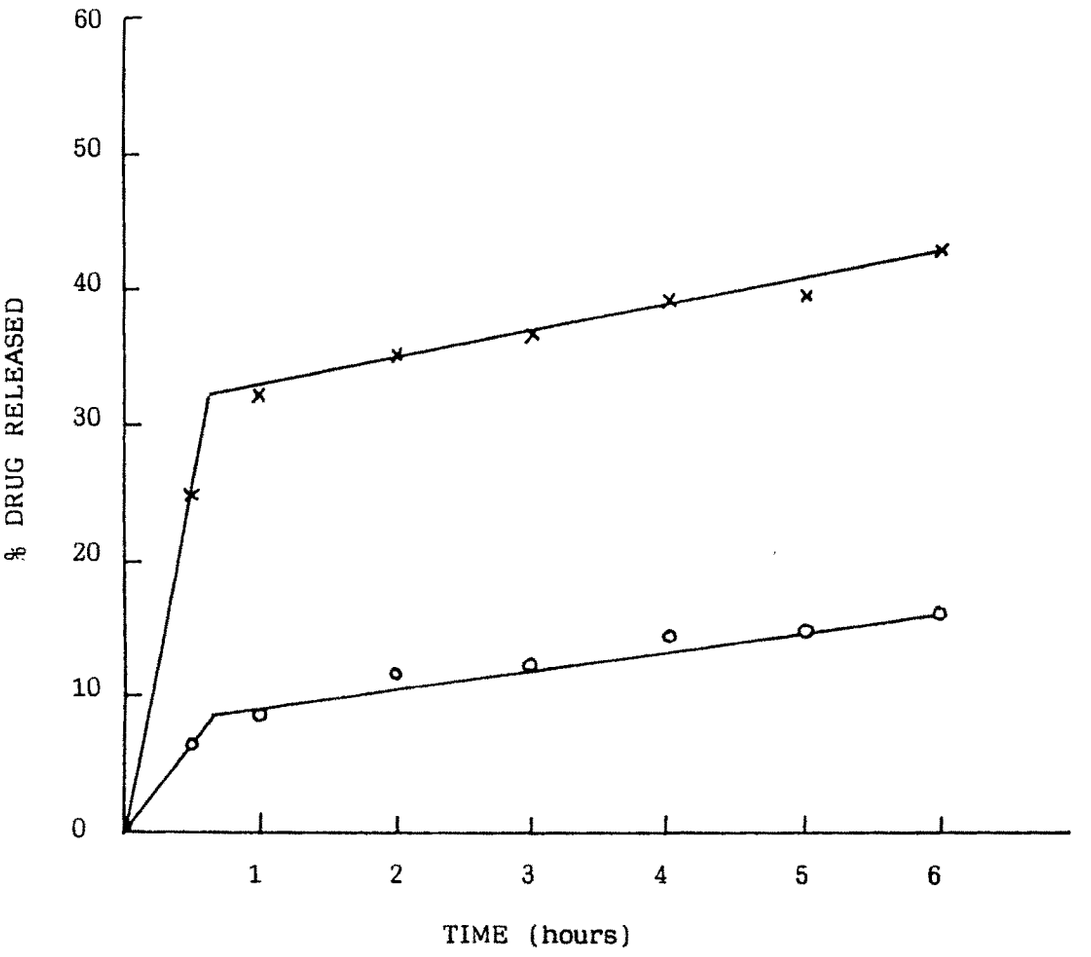


Fig. 59 : In vitro Release Profile of 5-FU from polyterephthalamide microcapsules

x - Or-Tc; o - Ar-Tc

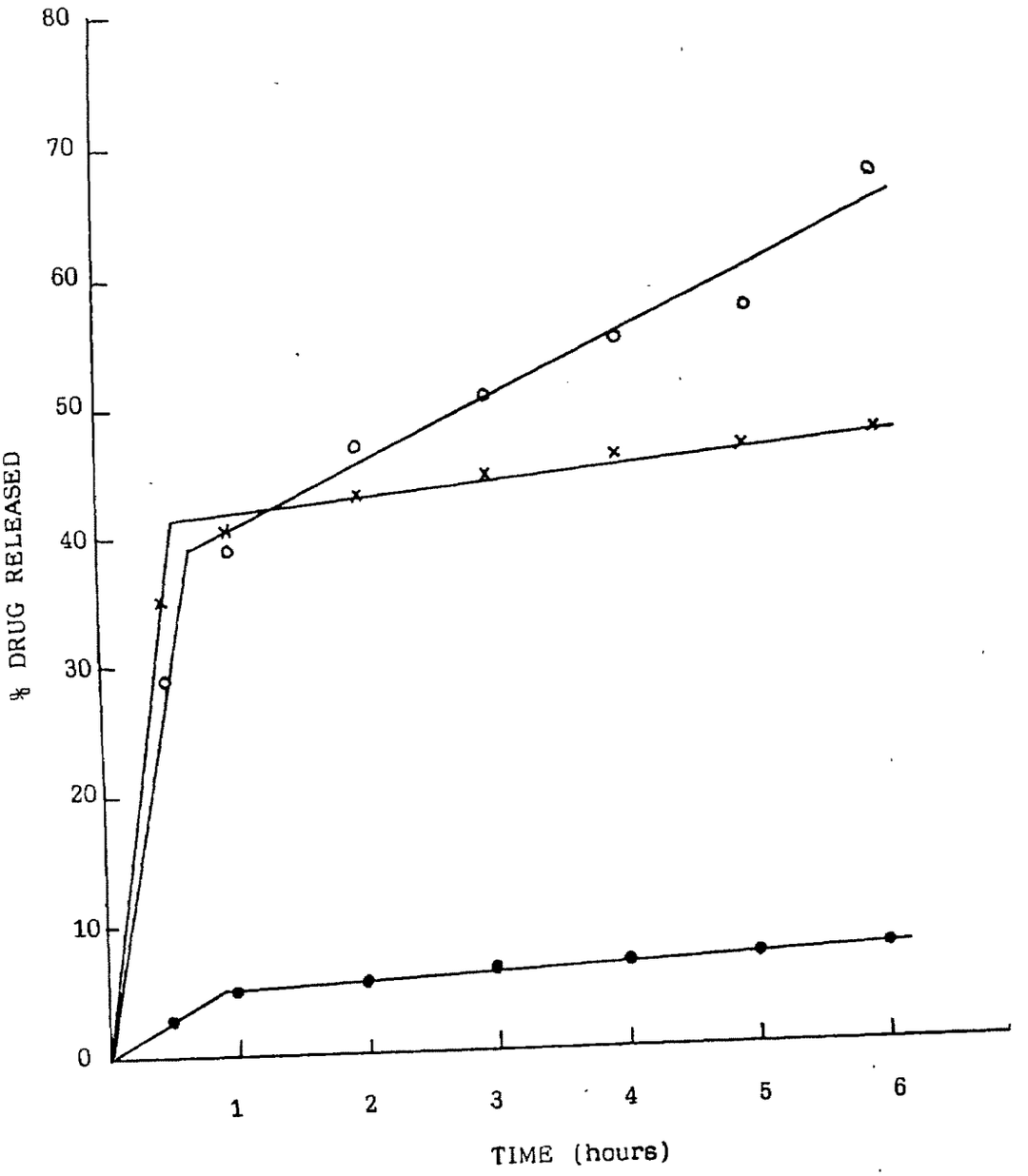


Fig. 60 : In vitro Release Profile of 5-FU from polyterephthalamide microcapsules

o - Hb-Tc; x - Ly-Tc; • - Ci-Tc

Tc (16.2%) batches of microcapsules. The figures show that all the batches of microcapsules showed zero-ordered release kinetics over a period of 6 hours after an initial burst effect upto 1 hour.

Again, as in case of earlier results, the in vitro release studies also confirm the hypothesis that the permeability/ porosity of the polymer depends on the partitioning of the diamino acid between the two phases. Higher the $K_{o/w}$ value, greater is the monomer reactivity which will result in the formation of a coarser and therefore more permeable membrane.

vii) In vivo Organ Distribution Studies

Any foreign body or particle is immediately removed from the blood stream by the macrophages of the reticuloendothelial system (RES) by a process known as opsonization.[206] When macromolecular or colloidal particles are injected into the blood stream, they are also treated as foreign bodies and meet the same fate at the hands of the macrophages. It is this appetite of the organs of the RES viz. liver, spleen, bone marrow and lungs, which has been successfully used to deliver drugs in microencapsulated particulate form when the target organ is a member of the RES. This process of drug targeting is called natural or passive targeting and is the

proposed method of delivering 5-FU to target organs like liver, kidneys and intestine, in this study.

Hence, the pharmacokinetic disposition of 5-FU from various batches of microcapsules was studied in healthy rats in comparison to free drug to determine the potential of these polymeric microcapsules as carriers for achieving site-specific delivery of 5-fluorouracil.

The amount of drug per gram of organ (µg/gm) and the percentage of administered dose (% Ad) in each organ at various times from each batch of microcapsules is given in Tables 25 to 32 and Figures 61 to 68.

The data on the pharmacokinetic disposition of 5-FU from various batches of polyterephthalamide microcapsules shows a wide variation in the extent of drug distribution in various organs from different batches of microcapsules. While 70% of the administered dose was detected in the liver, lungs, kidneys and intestine during 4 hours after administration of Cy-Tc batch of microcapsules, only 16% of the administered dose was deposited in these organs in case of Gl-Tc batch of microcapsules.

TABLE : 25

Organ Distribution of 5-FU from Free 5-FU Solution

Organs	TIME			
	½ hours		2 hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	82.72 ± 14.18	8.38 ± 1.89	35.27 ± 10.49	5.60 ± 0.75
Lungs	138.54 ± 10.94	3.95 ± 0.31	72.76 ± 9.71	1.78 ± 0.16
Kidneys	115.31 ± 10.20	2.66 ± 0.21	11.36 ± 2.35	0.25 ± 0.03
Intestine	75.46 ± 17.60	6.56 ± 0.66	22.83 ± 3.85	1.81 ± 0.26
Spleen	94.81 ± 13.91	0.95 ± 0.21	n.d.	-
Total		22.5 ± 1.62		9.45 ± 0.86

n.d. - not detectable

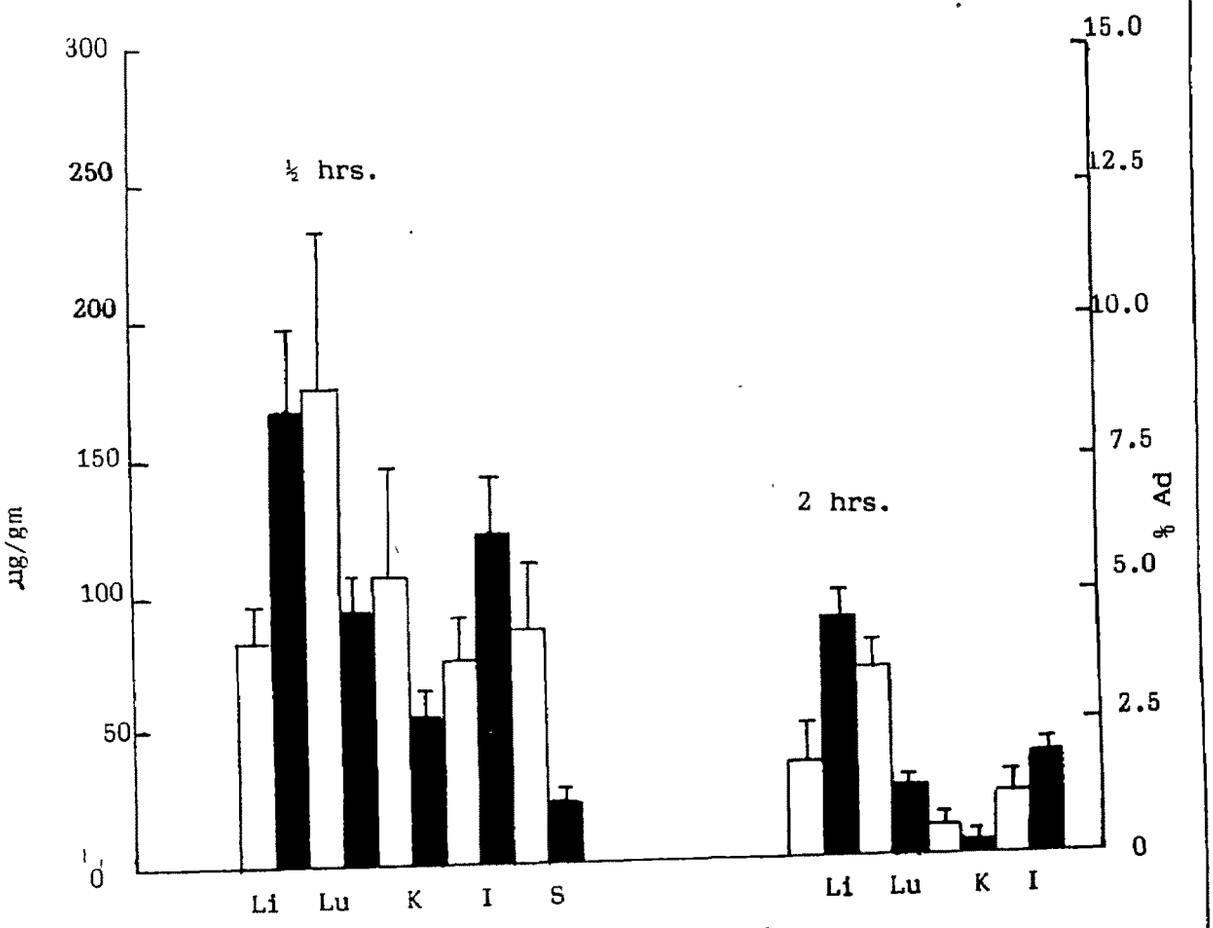


Fig. 61 : Organ distribution of 5-FU from free drug solution

Table : 26

Organ Distribution of 5-FU from Ar-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	24.13 ± 5.03	2.82 ± 0.56	64.06 ± 16.44	8.00 ± 2.29	42.39 ± 5.76	4.11 ± 0.92
Lungs	183.66 ± 30.65	8.05 ± 2.30	535.15 ± 130.09	15.88 ± 2.34	219.50 ± 44.78	7.04 ± 1.07
Kidneys	54.77 ± 10.77	1.19 ± 0.06	63.07 ± 12.32	1.67 ± 0.32	10.88 ± 3.21	0.27 ± 0.06
Intestine	9.93 ± 1.01	1.15 ± 0.12	13.85 ± 2.85	1.10 ± 0.17	3.33 ± 0.59	0.30 ± 0.07
Total		12.92 ± 2.30		26.66 ± 4.64		12.50 ± 1.16

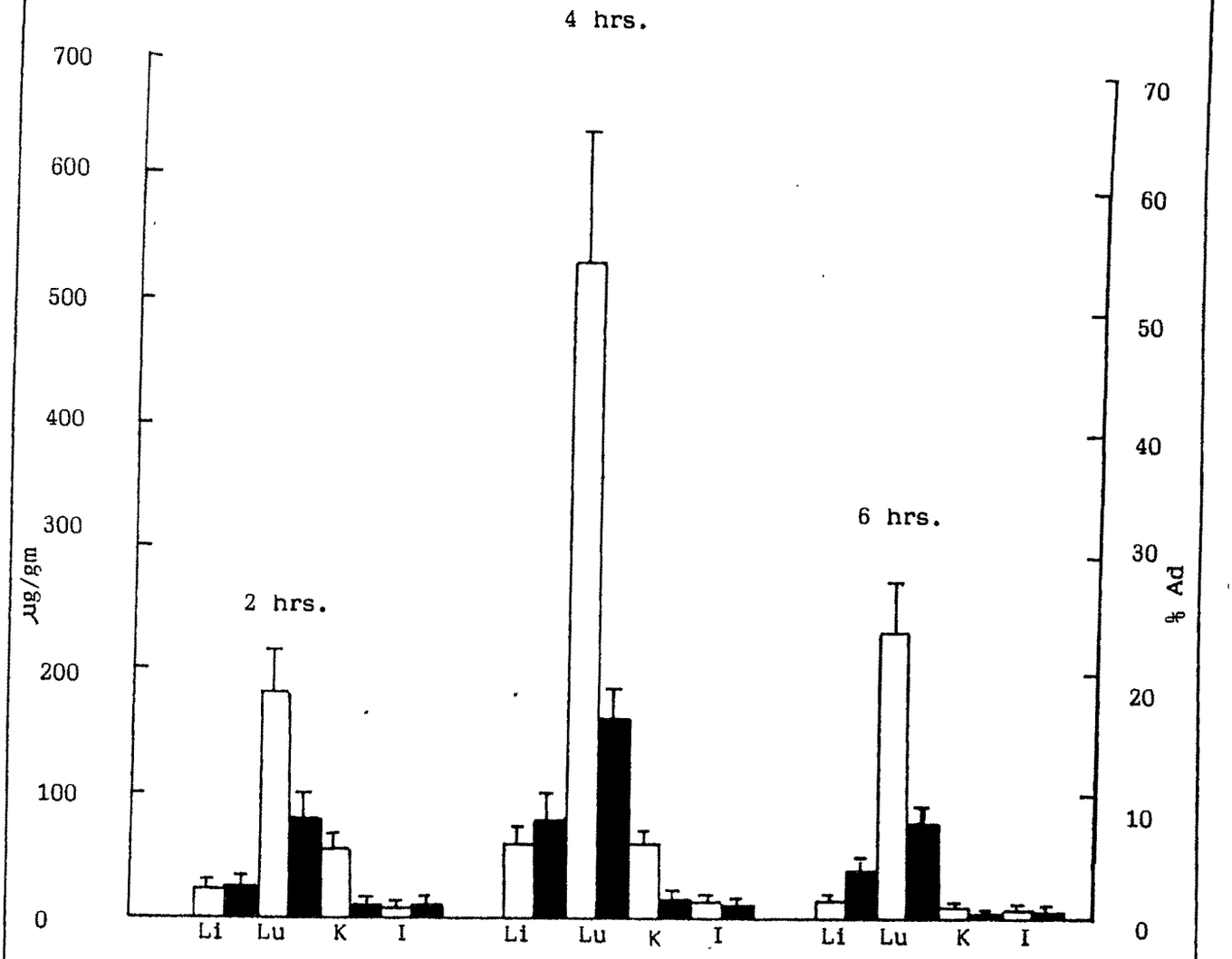


Fig. 62 : Organ distribution of 5-FU from Ar-Tc microcapsules

TABLE : 27

Organ Distribution of 5-FU from As-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	58.22 ± 3.36	6.40 ± 2.03	109.51 ± 17.75	13.79 ± 1.76	29.6 ± 4.76	3.32 ± 0.92
Lungs	560.90 ± 83.56	20.53 ± 0.50	1179.41 ± 207.07	31.17 ± 2.88	441.12 ± 115.51	12.43 ± 1.00
Kidneys	145.47 ± 11.24	3.56 ± 0.16	145.18 ± 42.19	3.38 ± 0.38	14.93 ± 1.05	0.39 ± 0.04
Intestine	49.46 ± 5.04	5.45 ± 0.88	46.26 ± 11.54	3.70 ± 0.52	19.73 ± 3.83	1.31 ± 0.18
Total		35.92 ± 2.00		52.03 ± 4.76		17.45 ± 1.63

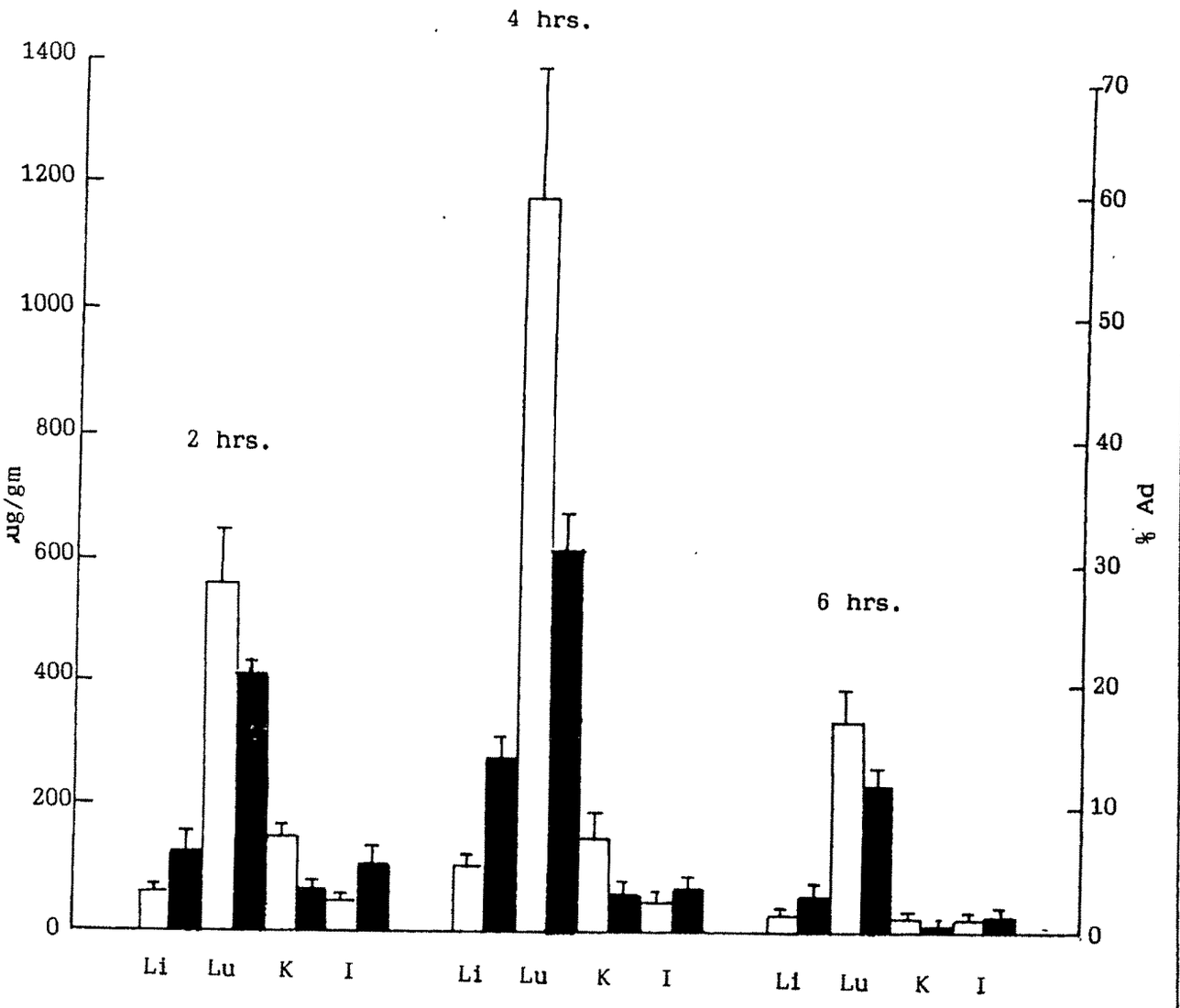


Fig. 63 : Organ distribution of 5-FU from As-Tc microcapsules

Table : 28

Organ Distribution of 5-FU from Ci-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	60.35 ± 12.39	8.00 ± 2.73	18.15 ± 2.26	2.63 ± 0.15	13.19 ± 1.32	2.26 ± 0.52
Lungs	211.64 ± 24.53	8.08 ± 1.36	71.20 ± 18.56	2.25 ± 0.19	33.42 ± 1.96	1.23 ± 0.21
Kidneys	143.52 ± 16.65	3.51 ± 0.19	14.47 ± 4.41	0.43 ± 0.14	8.30 ± 2.00	0.26 ± 0.06
Intestine	14.94 ± 0.88	1.39 ± 0.20	15.38 ± 2.92	1.43 ± 0.12	5.43 ± 1.12	0.59 ± 0.11
Spleen	212.99 ± 56.15	2.87 ± 0.21	n.d.	-	n.d.	-
Total		23.86 ± 1.70		6.75 ± 0.27		5.55 ± 1.28

n.d. - not detectable

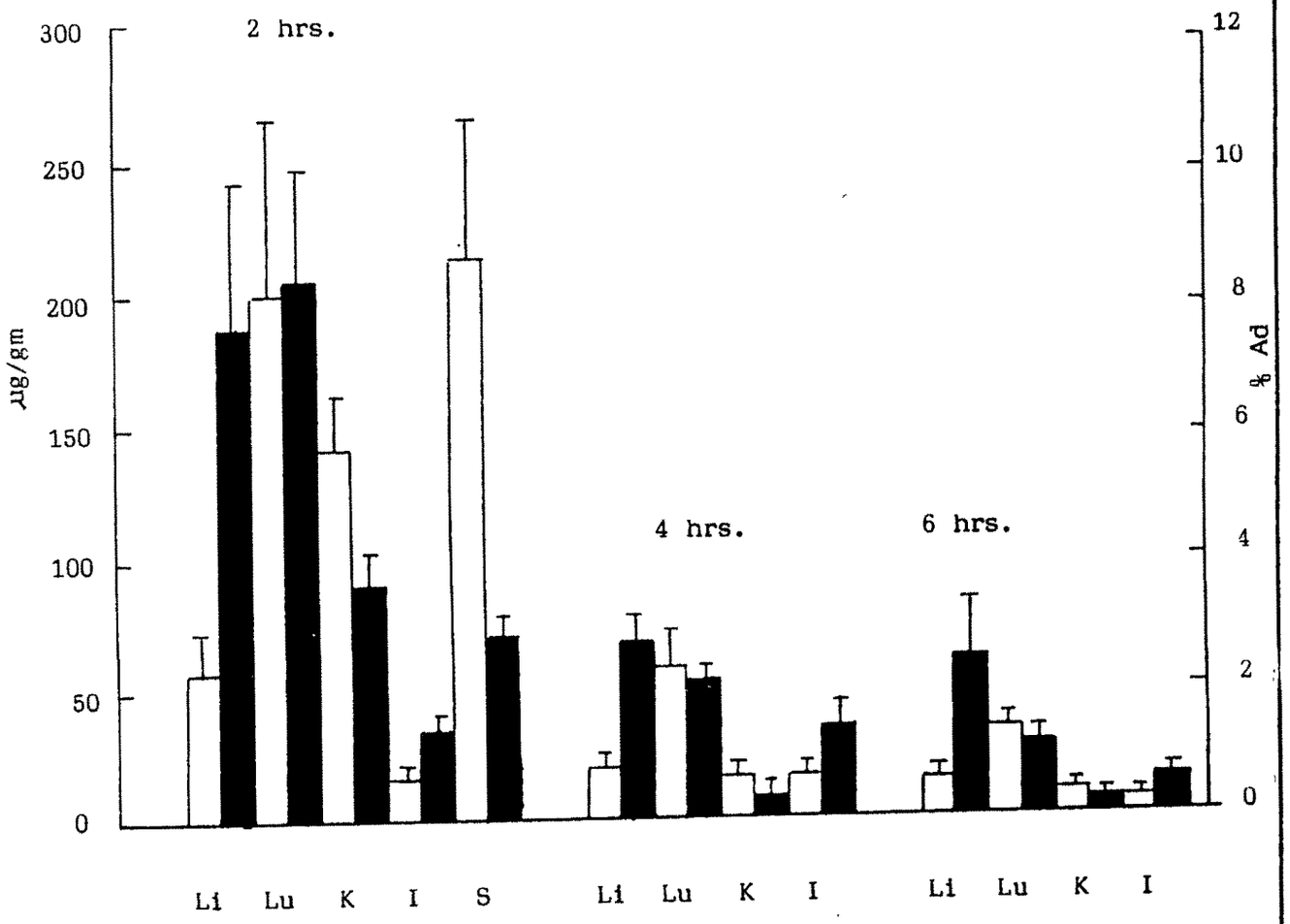


Fig. 64 : Organ distribution of 5-FU from Ci-Tc microcapsules

Table : 29

Organ Distribution of 5-FU from Cy-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	118.91 ± 16.45	1.48 ± 0.16	165.56 ± 14.34	19.57 ± 2.68	6.93 ± 2.19	0.83 ± 0.28
Lungs	836.03 ± 126.51	24.60 ± 3.80	1278.59 ± 134.68	36.73 ± 2.48	193.13 ± 40.90	7.48 ± 2.15
Kidneys	146.01 ± 14.95	4.41 ± 1.18	95.82 ± 15.61	6.36 ± 0.42	17.45 ± 6.36	0.42 ± 0.12
Intestine	70.78 ± 8.80	6.06 ± 2.05	223.59 ± 35.53	7.13 ± 0.47	8.37 ± 3.29	0.77 ± 0.27
Spleen	n.d.	-	n.d.	-	212.65 ± 34.86	2.37 ± 0.34
Total		36.55 ± 5.96		69.79 ± 5.52		11.56 ± 2.04

n.d. - not detectable

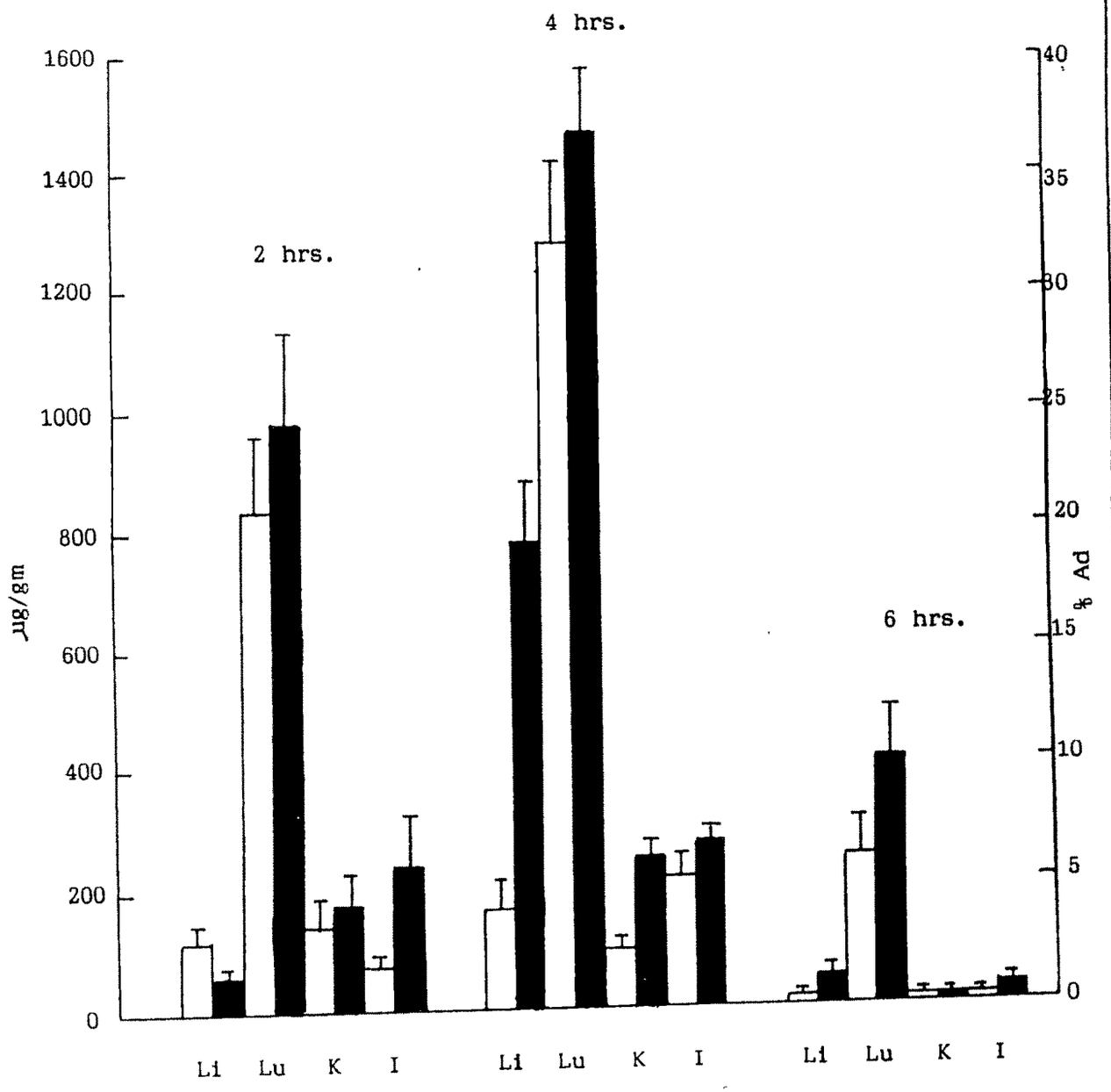


Fig. 65 : Organ distribution of 5-FU from Cy-Tc microcapsules

Table : 30

Organ Distribution of 5-FU from Gl-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	42.88 ± 6.12	4.27 ± 0.46	6.26 ± 0.49	0.99 ± 0.18	4.93 ± 0.78	0.67 ± 0.09
Lungs	256.37 ± 32.24	7.37 ± 0.57	417.90 ± 81.14	12.09 ± 2.00	117.73 ± 12.66	3.93 ± 0.50
Kidneys	9.48 ± 0.65	0.23 ± 0.02	57.58 ± 9.39	1.74 ± 0.46	10.17 ± 3.18	0.31 ± 0.10
Intestine	18.37 ± 4.58	1.48 ± 0.38	8.41 ± 3.41	0.68 ± 0.04	4.29 ± 0.81	0.37 ± 0.10
Spleen	80.83 ± 36.14	1.20 ± 0.49	40.55 ± 7.94	0.63 ± 0.06	n.d.	-
Total		14.56 ± 0.59		16.13 ± 2.27		5.26 ± 0.71

n.d. - not detectable

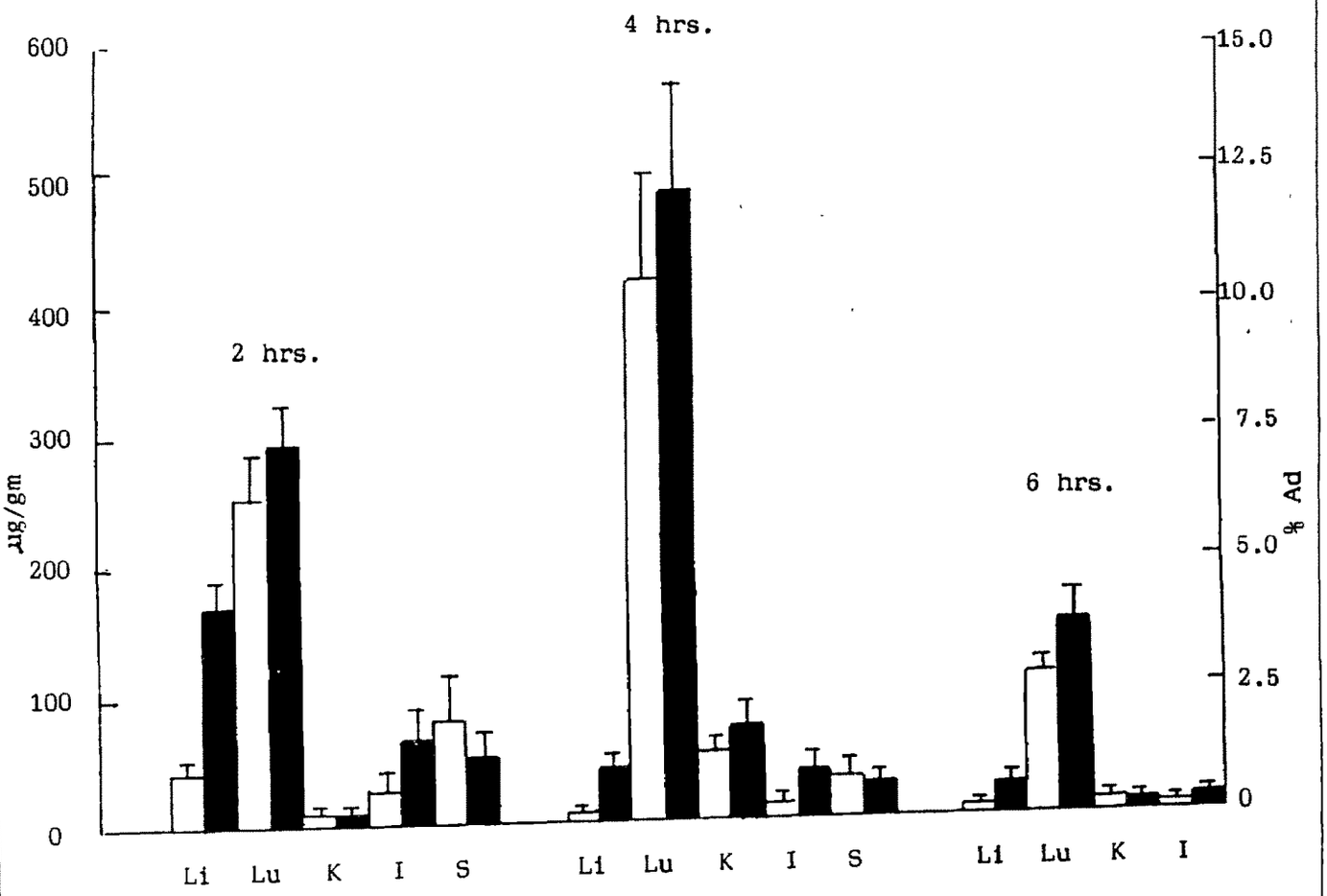


Fig. 66 : Organ distribution of 5-FU from Gl-Tc microcapsules

Table : 31

Organ Distribution of 5-FU from Ly-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	134.76 ± 20.51	15.08 ± 2.26	21.89 ± 7.10	1.78 ± 0.52	16.25 ± 4.64	1.24 ± 0.28
Lungs	633.47 ± 38.34	19.93 ± 2.00	370.88 ± 51.84	8.12 ± 1.44	259.11 ± 86.73	5.36 ± 1.32
Kidneys	373.11 ± 29.80	8.55 ± 0.31	15.72 ± 2.56	0.32 ± 0.05	8.94 ± 1.36	0.15 ± 0.08
Intestine	7.19 ± 1.04	0.60 ± 0.10	8.43 ± 1.30	0.61 ± 0.10	4.29 ± 1.30	0.23 ± 0.06
Total		44.04 ± 4.24		10.83 ± 1.87		7.31 ± 0.87

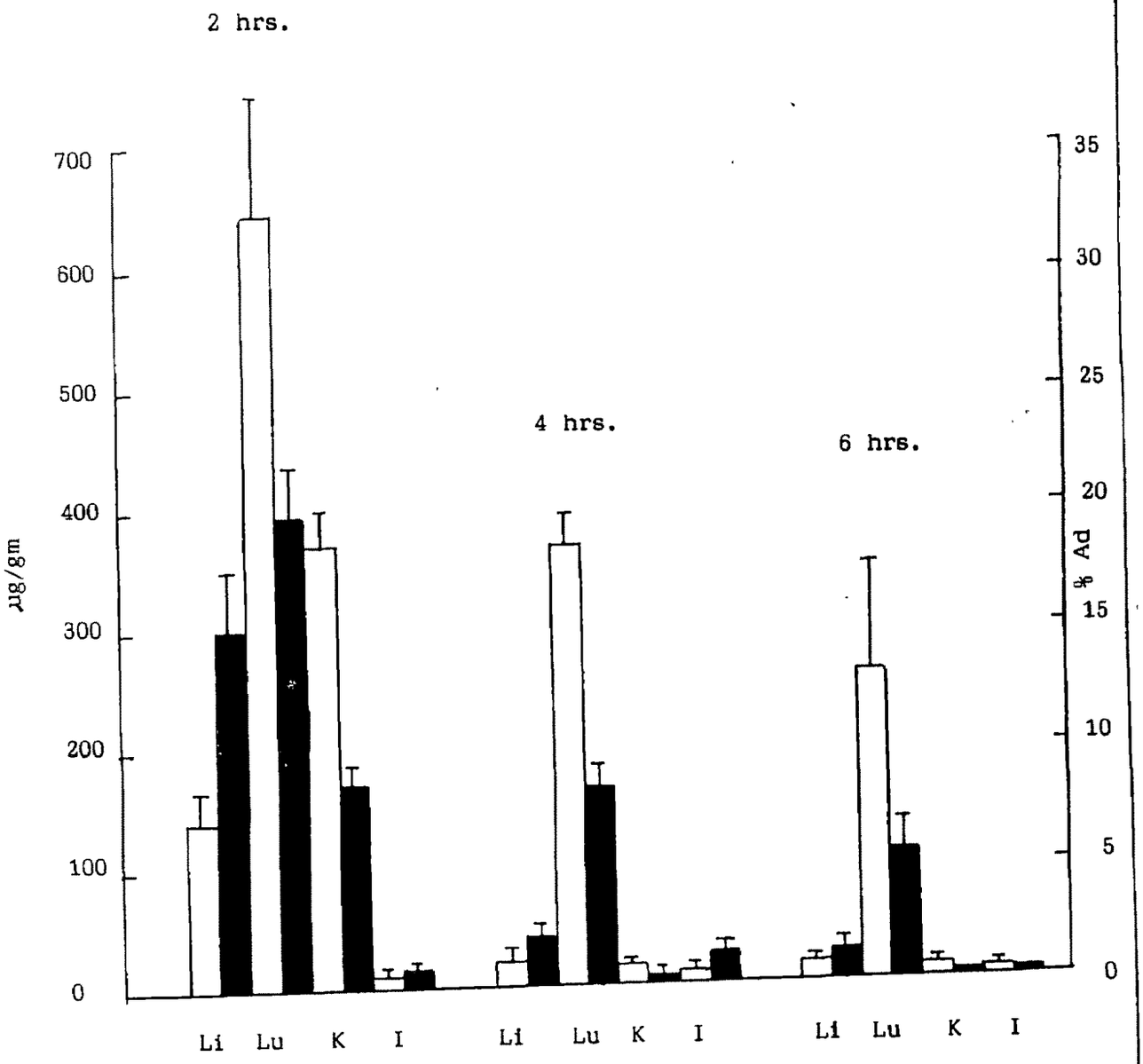


Fig. 67 : Organ distribution of 5-FU from Ly-Tc microcapsules

Table : 32

Organ Distribution of 5-FU from Or-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	58.31 ± 14.61	8.23 ± 1.83	67.04 ± 21.12	8.89 ± 2.59	23.77 ± 1.35	2.33 ± 0.12
Lungs	339.30 ± 28.17	12.47 ± 0.50	459.74 ± 57.07	21.38 ± 10.99	204.75 ± 8.41	5.43 ± 0.54
Kidneys	81.99 ± 15.74	2.69 ± 0.11	41.46 ± 6.49	1.23 ± 0.12	9.29 ± 2.57	0.23 ± 0.05
Intestine	9.74 ± 0.42	0.76 ± 0.16	29.63 ± 6.76	3.41 ± 0.80	5.54 ± 1.51	0.41 ± 0.06
Total		24.15 ± 2.11		34.91 ± 13.92		8.40 ± 0.64

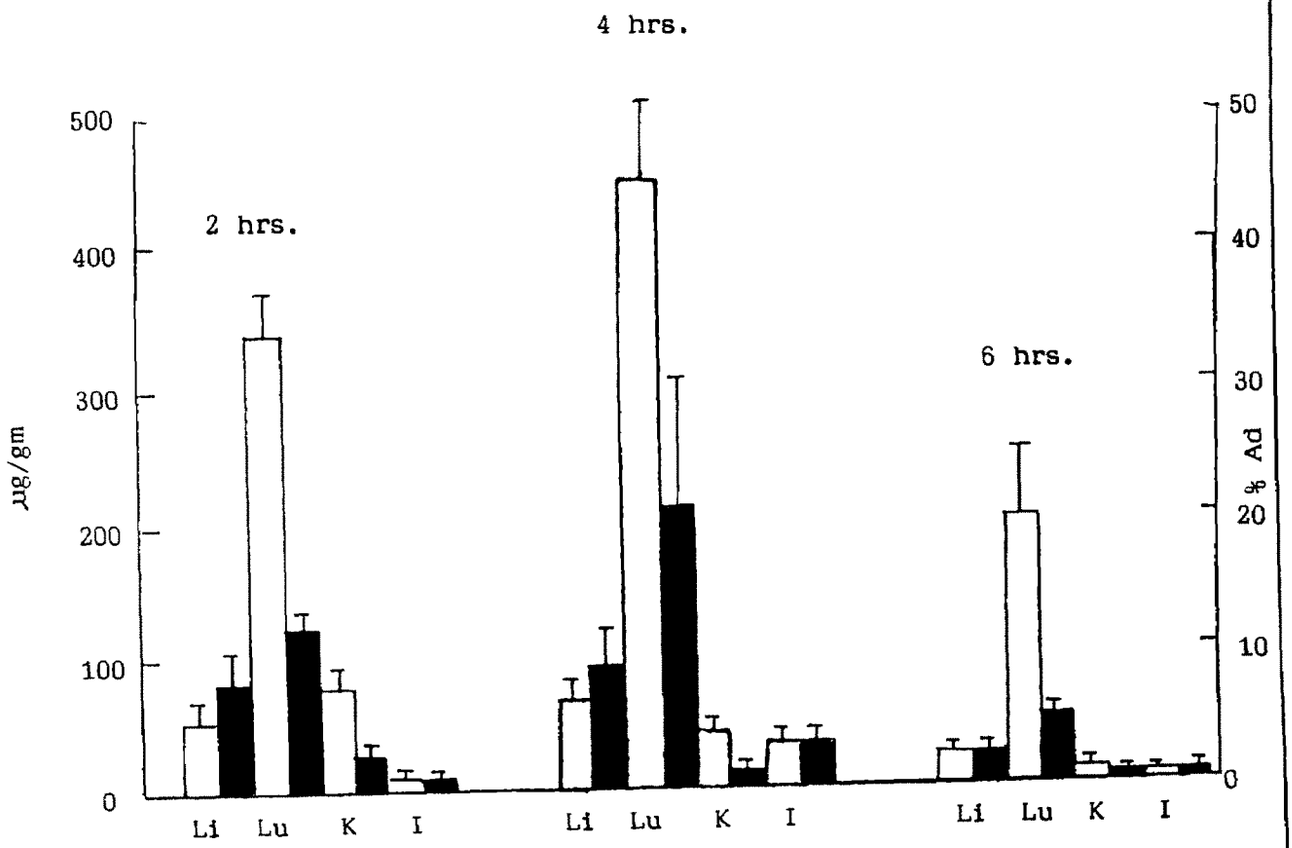


Fig. 68 : Organ distribution of 5-FU from Or-Tc microcapsules

A careful examination of the results obtained in the present study highlights the following salient features :

1) The amount of 5-FU deposited per gram of tissue (µg/gm) is significantly higher in the lungs compared to all the other organs studied from all batches of polyterephthalamide microcapsules.

2) The percentage of administered dose (% Ad) was also found to be maximum in case of lungs as compared to all the other organs studied.

The above results indicate that the disposition of the microcapsules in the lungs is due to the size dependent distribution of intravenously injected particles into various organs of RES.

Various workers have shown that intravenously administered particles are taken up by the macrophages of various organs depending on their particle size.

Kante et al[205] had first demonstrated the effect of particle size on the blood clearance and organ distribution of polystyrene microparticles in dogs. They reported that particles of 3 to 12 µm size were localized initially into the lungs from where only the

smaller particles (3-5 μm) were distributed into the other organs like the liver and spleen, whereas the bigger particles (7-12 μm) were retained in the lungs throughout the duration of the study.

Illum et al[208] demonstrated that still smaller particles (1.27 μm) were taken up preferentially by the liver whereas the bigger particles (> 5 μm) were retained in the lungs.

Yoshioka et al[207] reported that gelatin microspheres (d_{mean} = 14.9 μm) containing mitomycin C distributed mainly into the lungs whereas their nanospheres (d_{mean} = 280 nm) selectively accumulated into the liver and spleen.

The particle size range of our microcapsules is 2 to 20 μm, with the average particle size being in the range of 2 to 6 μm. Based on the above cited reports, it is logical to conclude that the particles above the critical size range of 2 to 5 μm would have been mechanically filtered by the capillary beds of the lungs, where they would have released their drug payload. Hence, a greater percentage of the administered dose was accounted for in the lungs from all batches of microcapsules. The smaller particles

which had circumvented the lung-barrier would have been phagocytosized by the remaining organs of the RES, including the liver, bone marrow and spleen. However, in most of the cases, no drug could be detected in the spleen. This might be due to the fact that the spleen is a very small organ and the quantity of drug accumulated therein was below the sensitivity limits of the method adopted for drug assay in the tissues.

3) The in vivo distribution data also reveals that the peak levels of 5-FU in the body occured at 4 hours from almost all batches of polyterephthalamide microcapsules. Exception was observed in case of Ci-Tc and Ly-Tc batches of microcapsules, from which maximum amount of drug was released in 2 hours.

It was also observed that in most of the organs, the amount of drug accumulated increased upto 4 hours and then reduced steeply by 6 hours.

4) Statistical Significance

The Student's t test, Analysis of variance (ANOVA) and Dunnett's test were applied to the data generated. The statistical parameters and the levels of significance for

the various batches of polyterephthalamide microcapsules with respect to the free drug solution is shown in Tables 33 to 35.

The data reveals that there is a significant modification in the biodistribution pattern of 5-FU when administered in microencapsulated form. The total amount of 5-FU in the organs studied was significantly higher from almost all batches of polyterephthalamide microcapsules as compared to the free drug. Of the seven batches of microcapsules, Cy-Tc, As-Tc and Ly-Tc batches were most significant ($P < 0.05$).

The organ-wise distribution data indicates that except for Ci-Tc, all the other batches of polyterephthalamide microcapsules had a significantly higher accumulation in the lungs ($P < 0.05$) as compared to free 5-FU. However, only the Ci-Tc batch of microcapsules produced a significantly higher drug level in the spleen as compared to free 5-FU injection. Higher levels of the drug were observed in the liver from Cy-Tc, Ly-Tc ($P < 0.05$) and As-Tc ($P < 0.1$) batches while only Cy-Tc batch showed significantly higher distribution in the intestine, with all the other batches showing significantly lesser levels ($P < 0.05$) in the intestine as compared to that of the free drug.

Table : 33

Data for Application of ANOVA and Dunnett's Test for comparing the 'ug Drug per gram Organ' Values obtained by Administration of Polyterephthalamide Microcapsules and Free 5-FU Solution.

Value	Formulation	5-FU	Ar-Tc	As-Tc	Ci-Tc	Cy-Tc	Gl-Tc	Ly-Tc	Or-Tc
Total :(F = 27.98)	$\bar{X}$	507.22	676.53	1480.30	643.40	1763.52	530.71	1160.97	597.91
5% allowance = 354.83		Std.	n.s.	**	n.s.	**	n.s.	**	n.s.
Ranked means		8	4	2	5	1	7	3	6
Liver :(F = 16.76)	$\bar{X}$	82.72	64.06	109.51	60.35	165.56	6.26	134.76	67.04
5% allowance = 47.18		Std.	n.s.	**	n.s.	**	**(↓)	**	n.s.
Ranked means		4	6	3	7	1	8	2	5
Lung :(F = 42.21)	$\bar{X}$	138.54	535.50	1179.41	211.64	1278.59	417.90	633.47	459.74
5% allowance = 255.82		Std.	**	**	n.s.	**	**	**	**
Ranked means		8	4	2	7	1	6	3	5
Kidney :(F = 59.87)	$\bar{X}$	115.81	63.07	145.18	143.52	95.82	57.58	373.11	41.46
5% allowance = 58.36		Std.	*(↓)	n.s.	n.s.	n.s.	*(↓)	**	**(↓)
Ranked means		4	6	2	3	5	7	1	8
Intestine :(F = 41.47)	$\bar{X}$	75.46	13.85	46.26	14.94	223.59	8.41	7.19	29.63
5% allowance = 43.38		Std.	**(↓)	n.s.	**(↓)	**	**(↓)	**(↓)	**(↓)
Ranked means		2	6	3	5	1	7	8	4
Spleen :(F = 13.78)	$\bar{X}$	94.81	n.d.	n.d.	212.99	n.d.	40.55	n.d.	n.d.
5% allowance = 89.96		Std.	-	-	**	-	n.s.	-	-
Ranked means		2	-	-	1	-	3	-	-

n.s. - not significant, n.d. - not detected, ↓ - less than standard,

N = 27, $(n_1-1) = 19$, $F_{0.01} = 2.55$, ** - $P < 0.05$, * - $P < 0.1$

Table : 34

Data for Application of ANOVA and Dunnett's Test for Comparing '% of Administered Dose' Values obtained by Administration of Polyterephthalamide Micrcapsules and Free 5-FU Solution.

Values	Formulation	5-FU	Ar-Tc	As-Tc	Ci-Tc	Cy-Tc	Gl-Tc	Ly-Tc	Or-Tc
Total :(F = 22.55)	$\bar{X}$	22.50	26.65	52.03	23.86	69.79	16.13	44.04	34.91
5% allowance = 14.51		Std.	n.s.	**	n.s.	**	n.s.	**	n.s.
Ranked means		7	6	2	5	1	8	3	4
Liver :(F = 14.95)	$\bar{X}$	8.38	8.00	13.79	8.00	19.57	0.99	15.08	8.89
5% allowance = 5.71		Std.	n.s.	**	n.s.	**	**(↓)	**	n.s.
Ranked means		5	6	3	6	1	7	2	4
Lungs :(F = 10.07)	$\bar{X}$	3.95	15.88	31.17	8.08	36.73	12.09	19.93	21.38
5% allowance = 13.36		Std.	*	**	n.s.	**	n.s.	**	**
Ranked means		8	5	2	7	1	6	4	3
Kidneys :(F = 8.43)	$\bar{X}$	2.66	1.67	3.38	3.51	6.36	1.74	8.55	1.23
5% allowance = 0.85		Std.	**(↓)	n.s.	*	**	**(↓)	**	**(↓)
Ranked means		5	7	4	3	2	6	1	8
Intestine :(F = 75.31)	$\bar{X}$	6.56	1.10	3.70	1.39	7.13	0.68	0.60	3.41
5% allowance = 1.18		Std.	**(↓)	**(↓)	**(↓)	n.s.	**(↓)	**(↓)	**(↓)
Ranked means		2	6	3	5	1	7	8	4
Spleen :(F = 101)	$\bar{X}$	0.95	n.d.	n.d.	2.87	n.d.	0.63	n.d.	n.d.
5% allowance = 0.42		Std.	-	-	**	-	n.s.	-	-
Ranked means		2	-	-	1	-	3	-	-

n.s. - not significant, n.d. - not detected, ↓- less than standard,

N = 27, $(n_1-1) = 19$, $F_{0.01} = 2.55$, ** - $P < 0.05$, * - $P < 0.1$

Table : 35

Statistical Summary of Various Polyterephthalamide Microcapsules showing a Significantly ($P < 0.05$) Different Organ Distribution as compared to Free Drug Solution

Organ	µg of 5-FU per gram of organ	% of Administered dose
Total	Cy-Tc > As-Tc > Ly-Tc	Cy-Tc > As-Tc > Ly-Tc
Liver	Cy-Tc > Ly-Tc	Cy-Tc > Ly-Tc > As-Tc*
	Gl-Tc (↓)	Gl-Tc (↓)
Lungs	Cy-Tc > As-Tc > Ly-Tc >	Cy-Tc > As-Tc > Or-Tc >
	Ar-Tc > Or-Tc > Gl-Tc	Ly-Tc > As-Tc*
Kidneys	Ly-Tc	Ly-Tc > Cy-Tc > Ci-Tc
	Ar-Tc > Gl-Tc & Or-Tc (↓)	Gl-Tc > Ar-Tc & Or-Tc (↓)
Intestine	Cy-Tc	none
	Or-Tc, Ci-Tc, Ar-Tc	As-Tc, Or-Tc, Ci-Tc,
	Gl-Tc & Ly-Tc (↓)	Ar-Tc, Gl-Tc & Ly-Tc (↓)
Spleen	Ci-Tc	Ci-Tc
		Gl-Tc* (↓)

* - $P < 0.10$

↓ - values are lower as compared to standard.

Preferential distribution of 5-FU was observed from Ly-Tc batch of microcapsules in terms of amount of drug deposited per gram organ weight but a significant increase in percentage of administered dose was also found in case of Cy-Tc ($P < 0.05$) and Ci-Tc ($P < 0.01$) batches.

viii) Cross-linked Haemoglobin Microcapsules

A significant feature in the organ distribution pattern of cross-linked haemoglobin microcapsules was a relatively higher drug accumulation in the liver and kidneys (Table 36, Fig. 69). Thus, the maximum amount of 5-FU per gram (µg/gm) in the kidneys was two and half times greater than the liver and two-third of the lung values. However, the percentage of the accumulated dose was highest for the liver (45% of the accounted dose), followed by the kidneys (26% of the accounted dose) while it was only 17% for the lungs.

Appreciable levels of 5-FU were observed in the spleen after administration of cross-linked haemoglobin microcapsules. The µg/gm of the drug in the spleen was higher than that in the liver, kidneys and intestine at 2 hours and accounted for 2.56% of the administered dose but it reduced to 0.87% at 4 hours.

Table : 36

Organ Distribution of 5-FU from Hb-Tc Microcapsules

Organs	TIME					
	2 Hours		4 Hours		6 Hours	
	µg drug/gm organ ± S.D.	% Administered dose ± S.D.	µg drug/gm organ S.D.	% Administered dose ± S.D.	µg drug/gm organ ± S.D.	% Administered dose ± S.D.
Liver	92.26 ± 11.09	12.49 ± 1.22	148.28 ± 28.89	20.70 ± 5.56	83.17 ± 11.24	10.45 ± 2.55
Lungs	452.98 ± 151.40	10.14 ± 1.39	246.74 ± 50.50	7.67 ± 2.20	409.78 ± 139.38	9.12 ± 3.31
Kidneys	155.75 ± 37.29	4.08 ± 0.32	366.30 ± 93.80	11.74 ± 1.83	219.76 ± 97.40	7.37 ± 2.54
Intestine	28.64 ± 6.17	2.64 ± 0.58	44.88 ± 8.59	5.01 ± 1.25	46.16 ± 8.88	4.15 ± 0.66
Spleen	215.99 ± 36.20	2.56 ± 0.32	71.64 ± 19.65	0.87 ± 0.21	n.d.	-
Total		31.89 ± 2.53		45.97 ± 7.44		30.06 ± 6.67

n.d. - not detectable

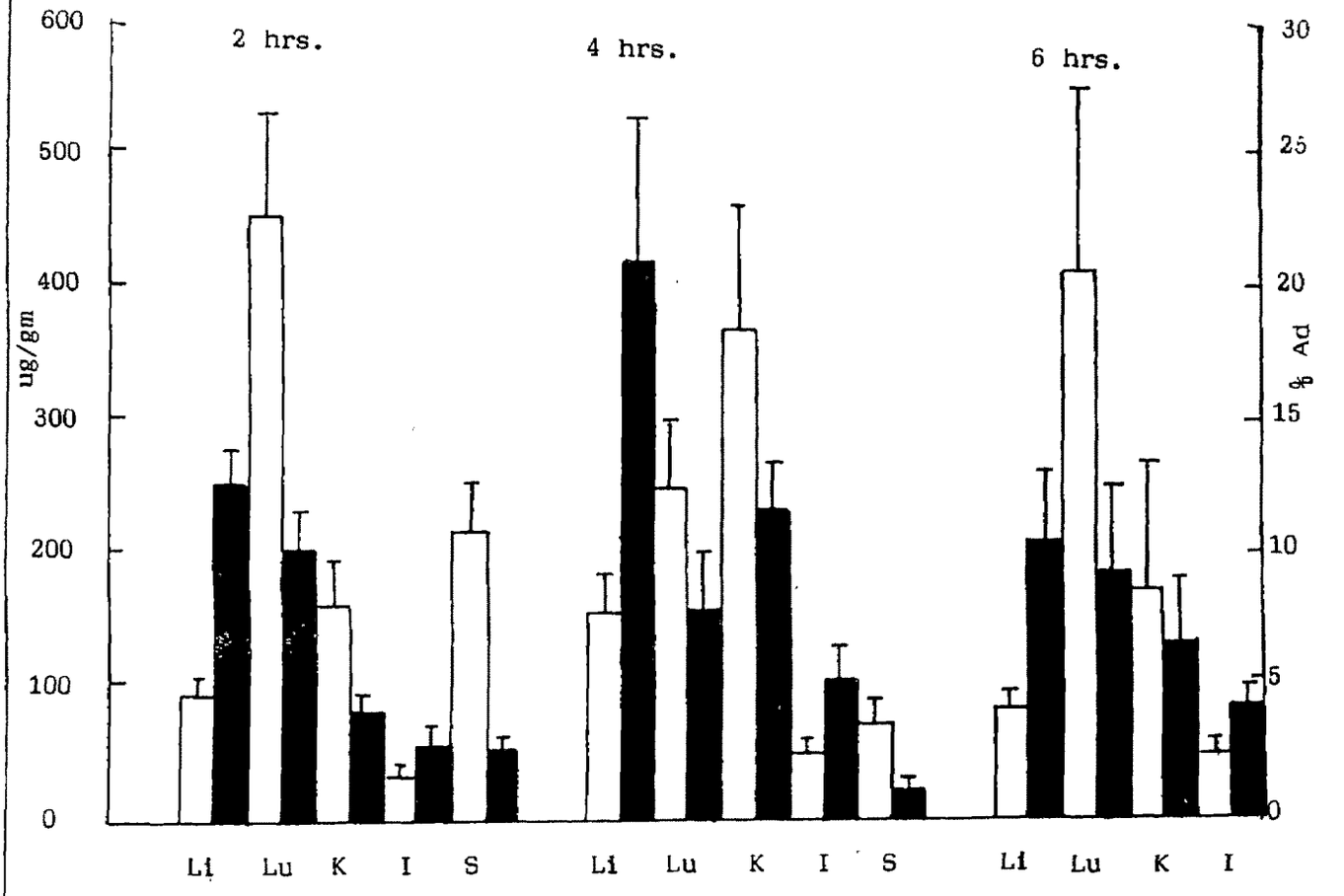


Fig. 69 : Organ distribution of 5-FU from Hb-Tc microcapsules

Another noteworthy feature in case of cross-linked haemoglobin microcapsules was that, as high as 30% of the administered dose was detected upto 6 hours, with appreciable drug levels in all the organs tested except the spleen. This may be attributed to a higher resistance of the polyamide to enzymatic degradation either due to a greater degree of cross-linking achieved during polymerisation or the presence of the high molecular weight protein moiety which metabolises slowly into amino acids.

Student's t test was applied to estimate the statistical significance of the in vivo drug distribution data generated from cross-linked haemoglobin microcapsules with respect to the free drug solution. The t values and the significance levels are shown in Table 37. The data clearly indicates a highly significant increase ($0.001 < P < 0.01$) in the total drug accumulation from cross-linked haemoglobin microcapsules as compared to the free drug injection. There was also a significant increase in the amount of 5-FU released in the liver, lungs ($0.05 < P < 0.10$), and kidneys ($0.001 < P < 0.02$) from these microcapsules. However, the drug distribution in the intestine was slightly less ($0.1 < P < 0.2$) than the free drug injection and that in the spleen was not significantly affected after encapsulation ($0.2 < P < 0.6$).

Table : 37

Application of Student's t Test for comparing Organ Distribution Pattern of Cross-linked Haemoglobin Microcapsules and Free 5-FU

Organ	µg drug/gm organ		% Administered dose	
	t-value	Significance level	t-value	Significance level
Total	10.59	$P < 0.001$	4.54	$P < 0.001$
Liver	2.54	$P < 0.10$	3.11	$P < 0.05$
Lungs	3.08	$P < 0.05$	2.45	$P < 0.10$
Kidneys	3.89	$P < 0.02$	7.21	$P < 0.001$
Intestine	1.76	n.s.	1.65	n.s.
Spleen	1.50	n.s.	0.58	n.s.

DF=5, $t_{0.1}=2.02$, $t_{0.05}=2.57$, $t_{0.02}=3.37$, $t_{0.01}=4.032$, $t_{0.001}=6.87$, n.s.=not significant

4.3.2 Polyacrylamide Microcapsules

i) Particle Size Analysis

The particle size analysis of polyacrylamide microcapsules obtained after optimising the formulation parameters showed a size distribution range upto 10 um, with the average diameter of 2.65 ± 0.37 μm. The frequency distribution plot of a typical batch of polyacrylamide microcapsules is shown in Fig. 70.

ii) Photomicrographs of Microcapsules

The photomicrograph of polyacrylamide microcapsules (Fig. 71) shows well-formed, spherical microcapsules with a distinct polymer membrane enclosing the core material.

iii) Drug Entrapment in Microcapsules

Assay of 5-FU in the aqueous supernatant and washings revealed that 55 to 65% of the drug was entrapped in the microcapsules (average = 60.33 ± 3.68%).

iv) Infrared Spectroscopy of Microcapsules

The IR spectrum of polyacrylamide microcapsules (Fig. 72,

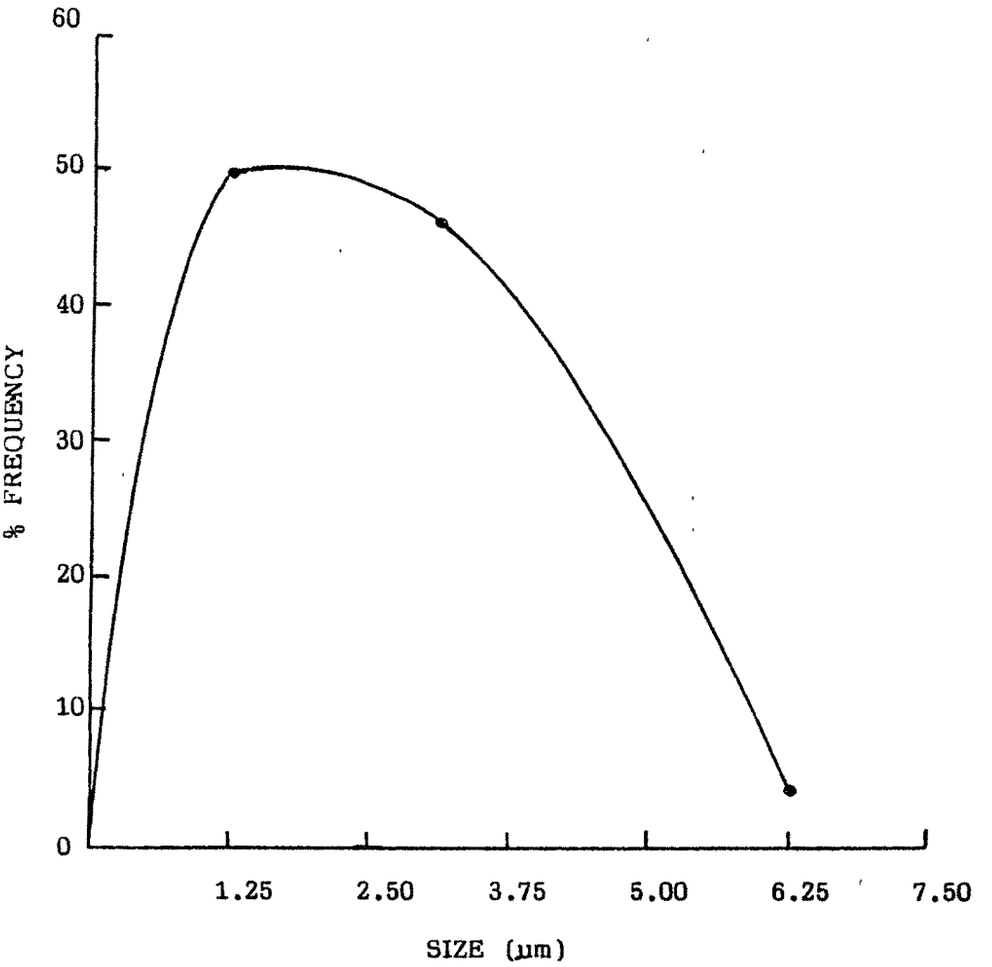


Fig. 70 : Frequency Distribution Plot of Polyacrylamide microcapsules

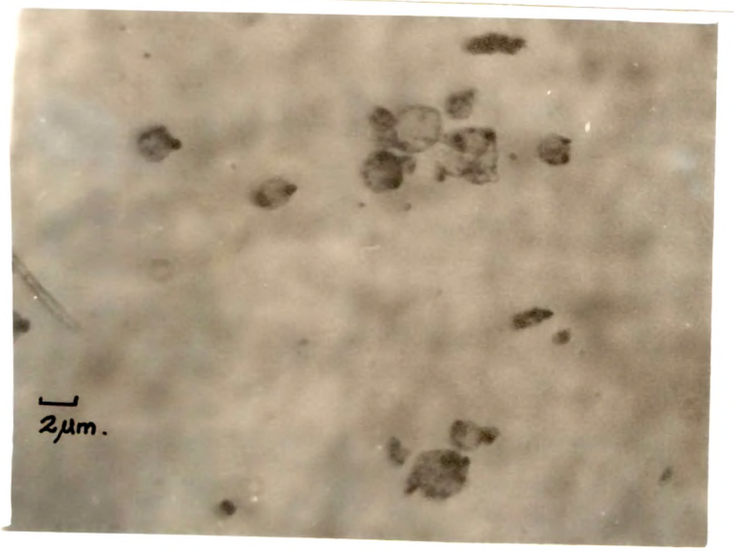
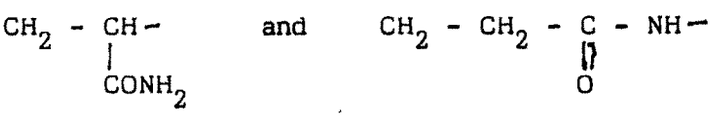


Fig.71 : Photomicrograph of Polyacrylamide microcapsules.

Table 38) shows peaks corresponding to both, the primary as well as the secondary amides. At the same time, peaks characteristic of C=C group of acrylamide have disappeared, indicating conversion of the monomer into a polymer. As both types of amides are observed, the resulting acrylamide polymer probably will have both types of structures :



primary amide secondary amide

Note : The IR spectrum was interpreted with the help of standard text on organic spectroscopy[203-204] and specific literature on identification of polyacrylamide.[210]

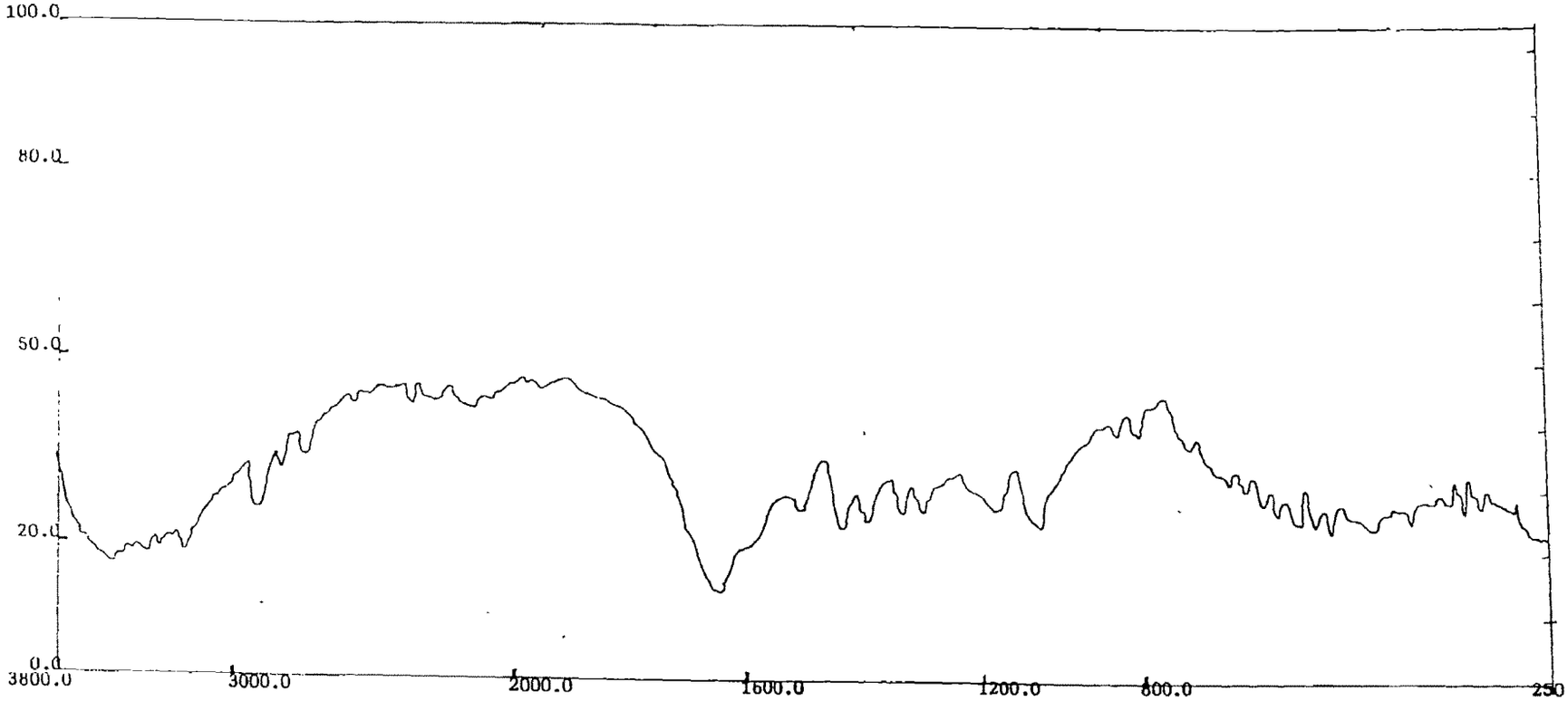


Fig. 72 : Infrared Spectrum of Polyacrylamide Microcapsules

Table : 38

Infrared Spectral Assignments for
Polyacrylamide Microcapsules

Frequency (Cm^{-1})		Functional Group
3440-3450	-	Free N-H stretch of primary amide
3200	-	H-bonded N-H stretch of primary amide
2920-2960	-	CH_2 asymmetric stretch
1400, 1440	-	Vibratory modes of primary amide
1310, 1170-1190	-	N-H deformation and C-N stretch of sec. amide
600-800 (multiple peaks)	-	H-bonded N-H deformation of sec. amide
1650-1670	-	Carbonyl stretch of primary amide

v) In vitro Drug Leaching Study

The rate of drug leaching from polyacrylamide micro-capsules during storage is shown in Fig. 73. The plot reveals that the loss of 5-FU from polyacrylamide microcapsules occurs at a very slow rate as compared to the polyamide micro-capsules. The leaching profile shows a slight burst effect initially and thereafter, the drug leaching rate follows zero-ordered kinetics, giving a straight line. The fact that only 6.54% of the entrapped drug was leached out of the microcapsules after 2 months indicates that the drug must be entrapped in the highly cross-linked polymer net work. These results indicate the possibility of storing the polyacrylamide microcapsules as a suspension without appreciable drug loss occurring due to leaching.

vi) In vitro Drug Release Studies

The in vitro drug release rate profile of polyacrylamide microcapsules is depicted in Fig. 74. It shows a zero-ordered release pattern, after an initial burst effect upto 1 hour. The drug release rate is very slow indicative of the high degree of cross-linking and low permeability of 5-FU from the polyacrylamide wall. Thus, only 9.4% of the entrapped 5-FU was released from the microcapsules in 6 hours.

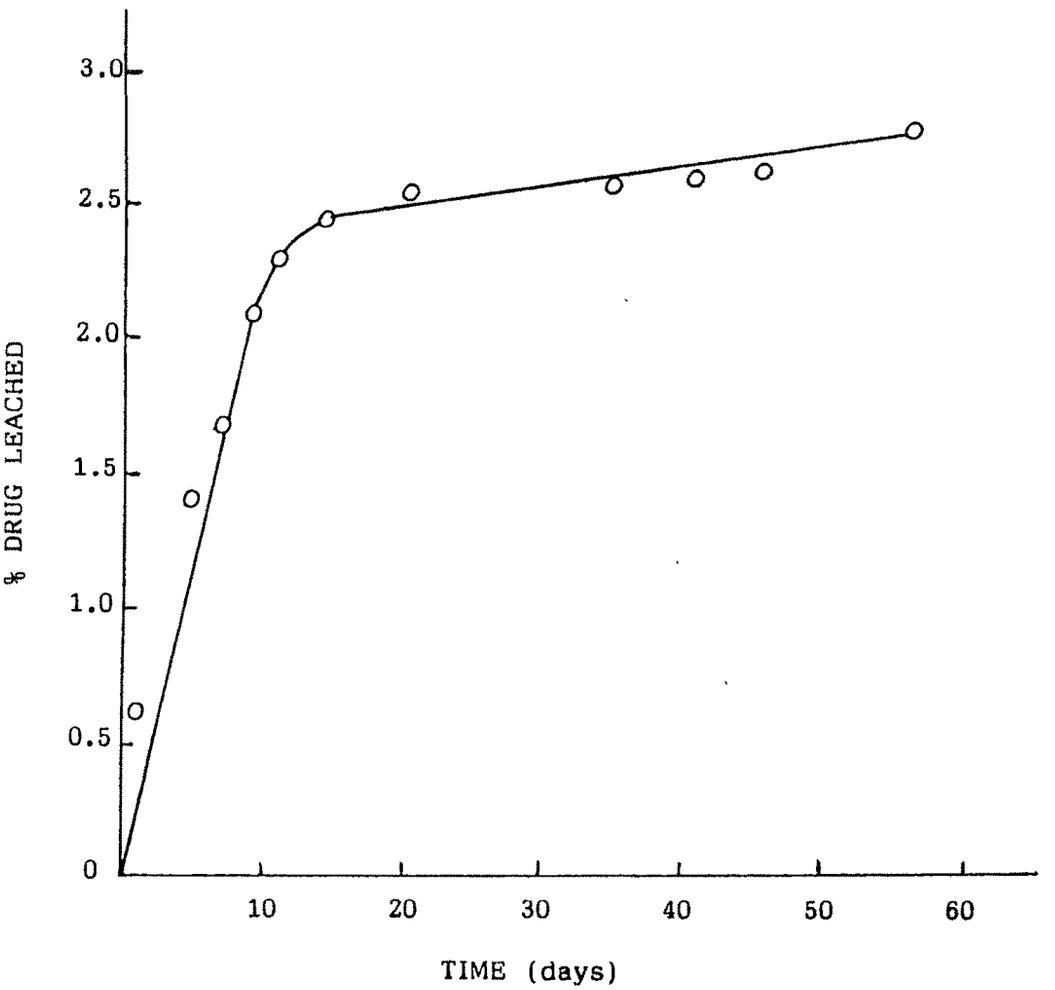


Fig. 73 : In vitro Leaching Profile of 5-FU from polyacrylamide microcapsules

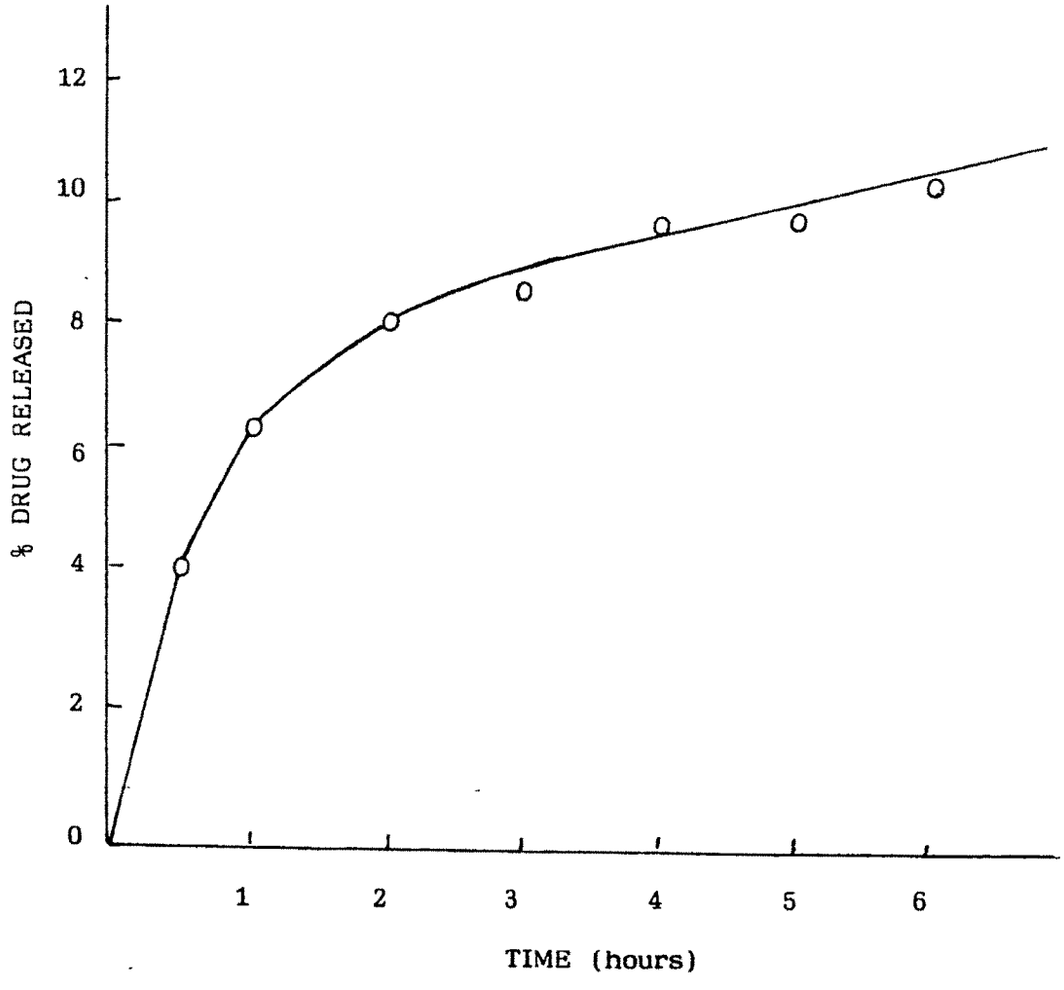


Fig. 74 : In vitro Release Profile of 5-FU from polyacrylamide microcapsules

vii) In vivo Organ Distribution Studies

Organ distribution of 5-FU after intravenous administration of a 5 mg/ml suspension of polyacrylamide microcapsules in healthy rats was studied upto 48 hours in liver, lungs, kidneys, intestine and spleen. The data is tabulated in Tables 39-40 and Fig. 75.

The results confirm the slow rate of elimination of polyacrylamide. Thus, 2 hours postinjection, only small quantities of the drug, equivalent to about 2.2% of the administered dose was detected in the lungs and spleen.

Thereafter, the drug levels were detected in the other organs also and their levels were found to increase progressively. Peak drug levels were found in the liver, lungs and kidneys at 24 hours. But after 8 hours, the drug could no longer be detected from the intestine. The drug could no longer be detected from the liver 48 hours postinjection and its levels in the lungs and kidneys also diminished, indicating the elimination phase.

As expected, maximum drug levels were found in the lungs followed by the liver and kidneys. Although the levels in the intestine increased progressively upto 8 hours, it hardly accounted for 1% of the administered dose and amounted to 8.65 µg drug per gram of organ.

Table : 39

µg of 5-FU Distributed per gram of Organ from Polyacrylamide Microcapsules

Organs	TIME						
	2 hrs.	4 hrs.	6 hrs.	8 hrs.	16 hrs.	24 hrs.	48 hrs.
Liver	n.d.	28.24 ± 11.32	12.43 ± 1.82	14.11 ± 3.04	54.85 ± 17.26	44.18 ± 12.49	n.d.
Lungs	51.35 ± 6.43	55.17 ± 20.23	39.29 ± 8.22	92.10 ± 21.03	408.10 ± 45.67	585.47 ± 88.37	260.07 ± 70.30
Kidneys	n.d.	8.82 ± 2.75	28.34 ± 4.02	18.83 ± 2.64	72.57 ± 20.50	231.14 ± 36.16	171.46 ± 48.94
Intestine	n.d.	2.14 ± 1.41	6.13 ± 2.01	8.65 ± 1.42	n.d.	n.d.	n.d.
Spleen	42.21 ± 16.62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable

Table : 40

Organ Distribution of 5-FU as Percentage of Administered Dose from Polyacrylamide Microcapsules

Organs	TIME						
	2 hrs.	4 hrs.	6 hrs.	8 hrs.	16 hrs.	24 hrs.	48 hrs.
Liver	n.d.	2.06 ± 0.40	1.47 ± 0.18	1.95 ± 0.46	6.56 ± 1.97	6.56 ± 1.47	n.d.
Lungs	1.90 ± 0.28	2.27 ± 0.25	1.44 ± 0.23	3.07 ± 0.76	10.70 ± 1.51	11.99 ± 2.70	5.53 ± 1.59
Kidneys	n.d.	0.24 ± 0.10	0.62 ± 0.19	0.59 ± 0.17	1.86 ± 0.65	4.62 ± 0.72	5.08 ± 1.67
Intestine	n.d.	0.20 ± 0.10	0.60 ± 0.23	0.95 ± 0.32	n.d.	n.d.	n.d.
Spleen	0.42 ± 0.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	2.20 ± 0.40	4.72 ± 0.14	4.11 ± 0.46	6.55 ± 1.52	19.08 ± 3.45	24.35 ± 2.32	10.06 ± 2.21

n.d. = not detectable

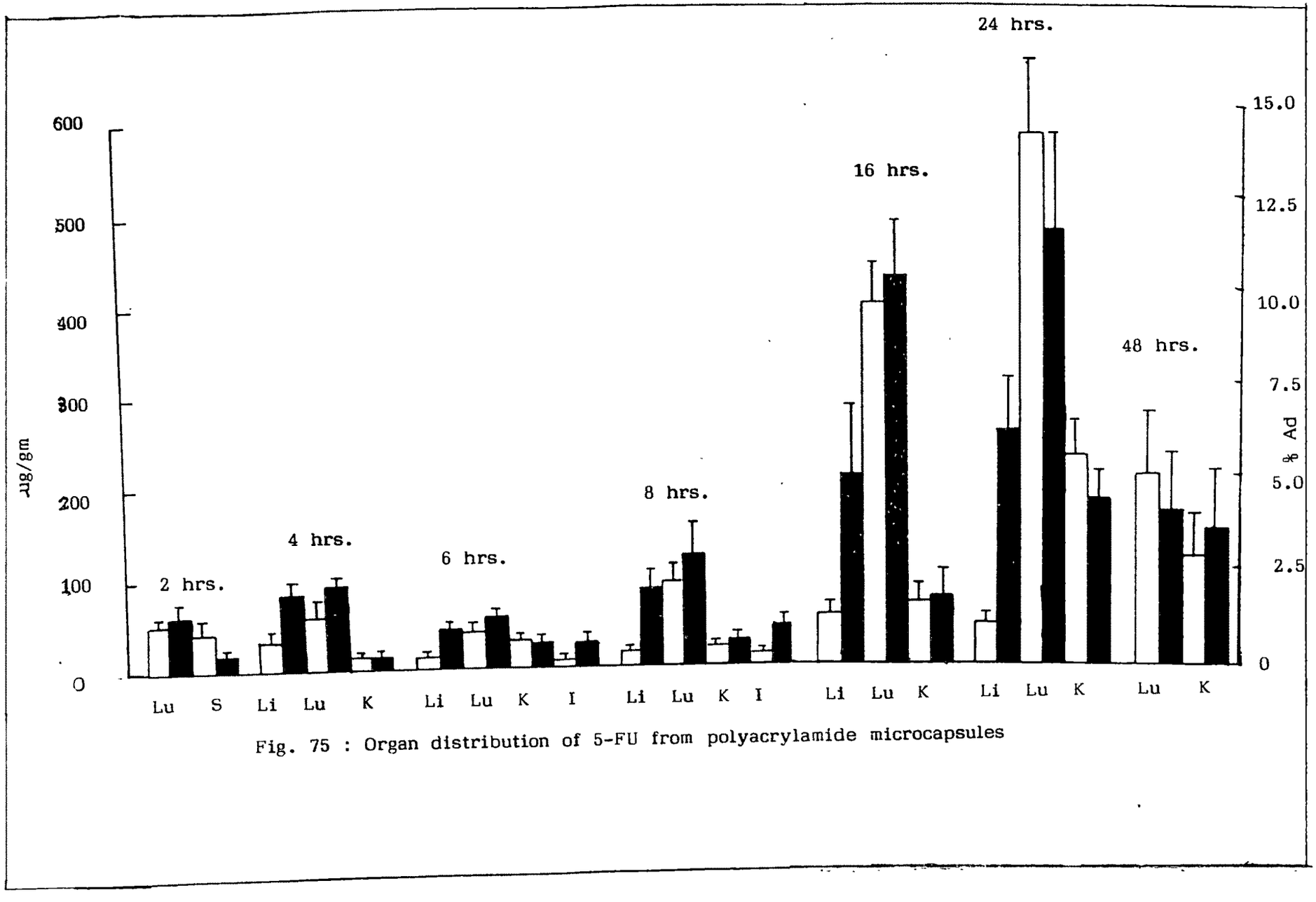


Fig. 75 : Organ distribution of 5-FU from polyacrylamide microcapsules

Edman and Sjoholm et al, in a series of reports[144, 150] demonstrated that polyacrylamide microcapsules were predominantly taken up by the organs of the RES immediately after intravenous injection. After initial uptake in the lungs, the microparticles were found to distribute in favour of the liver and spleen. This was due to the smaller size range of the particles used by them (0.2 to 0.5 μm). However, when they used larger polyacryldextran particles (0.5 to 1.5 μm), significant uptake in the lungs was observed.

Thus, the preferential uptake of 5-FU from polyacrylamide microcapsules observed in our study is due to the greater size range of the particles (2 to 15 μm), which will lead to their mechanical filtration by the capillary beds of the lungs.

Application of Student's t test for comparing the data with that obtained after administration of free 5-FU indicates that the drug accumulation per gram of organ was significantly higher ($P < 0.01$) from polyacrylamide microcapsules. Comparison of peak levels obtained from both treatments in various organs showed that there was a significant increase in drug accumulation in the lungs ($P < 0.001$) and kidneys ($P < 0.01$) whereas it was significantly lower in the liver ($P < 0.1$) and intestine ($P < 0.001$). The data is represented in Table 41.

Table : 41

Application of Student's t Test for comparing Organs Distribution Pattern of Polyacrylamide Microparticles and Free 5-FU

Organs	µg drug/gm organ		% Administered dose	
	t-value	Significance level	t-value	Significance level
Total	7.41	$P < 0.01$	1.02	n.s.
Liver	2.06	$P < 0.10$(↓)	1.20	n.s.
Lungs	16.56	$P < 0.001$	11.20	$P < 0.001$
Kidneys	4.54	$P < 0.01$	5.68	$P < 0.01$
Intestine	4.08	$P < 0.01$(↓)	12.67	$P < 0.001$(↓)
Spleen	3.51	$P < 0.02$(↓)	7.32	$P < 0.001$(↓)

↓ - Less than standard, n.s.-not significant

DF-5, $t_{0.1}=2.02$, $t_{0.02}=3,37$, $t_{0.01}=4.03$, $t_{0.001}=6.87$

4.3.3 Polyisobutylcyanoacrylate Microparticles

i) Particle Size Analysis

Using the emulsion polymerisation technique, spherical, uniform microcapsules with mean diameter of 6.60 ± 3.24 μm were obtained. The frequency distribution plot of a typical batch of PiBCA microcapsules is shown in Fig. 76.

The particle size of these microparticles was larger as compared to the other types of microparticles formulated. This may be due to the instantaneous reactivity of the alkylcyanoacrylate monomer in undergoing polymerisation.

ii) Photomicrographs of PiBCA Microparticles

Photomicrographs of PiBCA microparticles (Fig. 77) show that unlike the other types of microcapsules, these products are 'solid' microparticles and not microcapsules. They are nearly spherical in nature and do not have any distinct boundary enclosing the core material. Hence, they have been referred to as 'microparticles'. The instantaneous reactivity of the alkylcyanoacrylate monomer leading to complete polymerisation in a fraction of time may be the reason for the formation of a solid microparticle wherein the surface-associated monomer would have polymerised as a solid block and in doing so, would have occupied the core of the emulsion droplet.

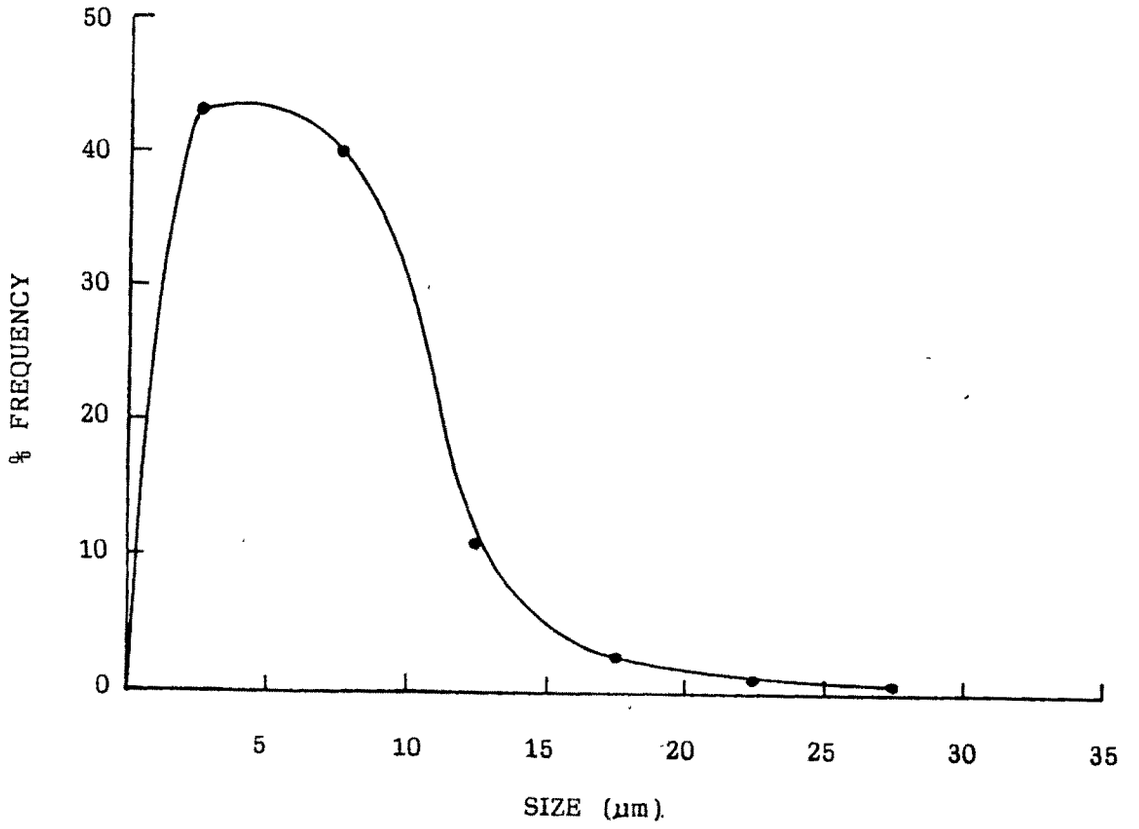


Fig. 76 : Frequency Distribution Plot of PiBCA microparticles

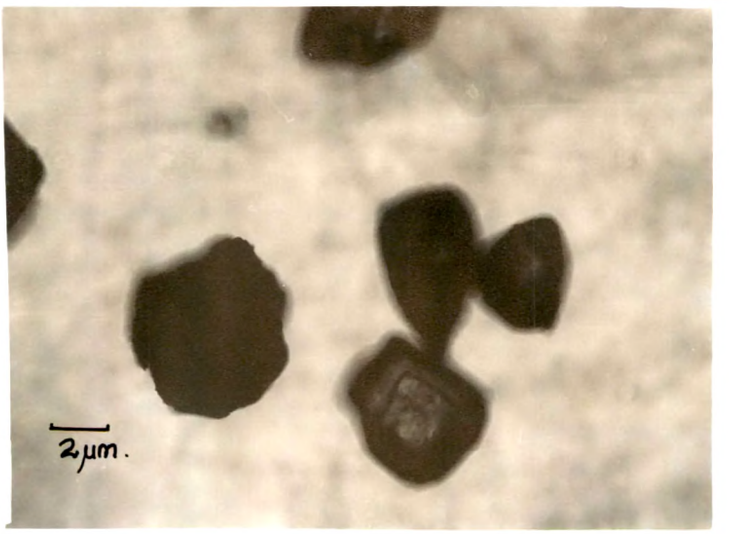


Fig.77 : Photomicrograph of PiBCA microparticles

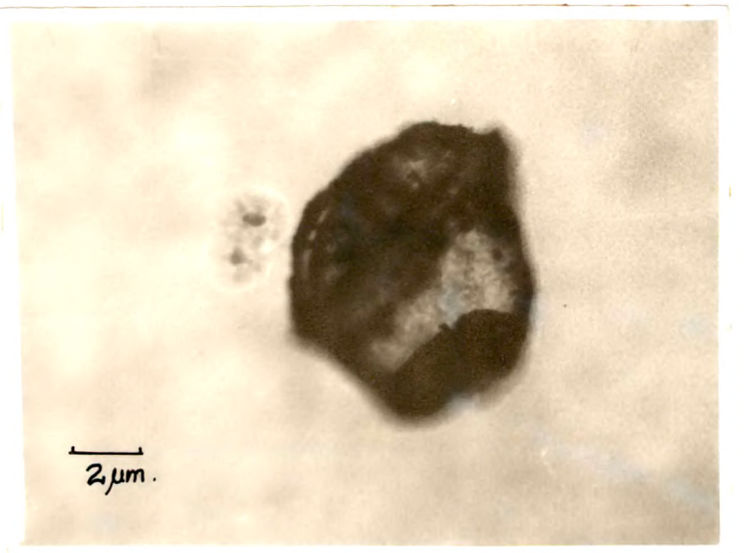


Fig.78 : Photomicrograph of 5-FU-loaded PiBCA microparticles

These results are in accordance with those obtained by Couvreur[170] and Rollot[166] et al, who reported the formation of solid PiBCA microparticles without a surrounding boundary, using emulsion polymerisation procedure.

Fig. 78 shows a photomicrograph of a 5-FU-loaded PiBCA microparticle. The drug crystal is clearly seen against the dark solid background of the polymer.

iii) Drug Entrapment in PiBCA Microparticles

The drug entrapment efficiency of PiBCA microparticles was found between 30 to 40% with the mean entrapment of 33.33 ± 4.52%.

iv) In vivo Organ Distribution Studies

In vivo organ distribution studies of 5-FU from PiBCA microparticles were conducted on healthy rats upto 2 hours following intravenous injection of 1 ml of a microcapsule suspension containing 5 mg of 5-FU. The data is presented in Tables 42-43 and Fig. 79.

The results confirm the rapidly biodegradable nature of polyalkylcyanoacrylates. Thus, appreciable levels of 5-FU could be detected in all organs studied within half-hour postinjection, accounting for 28% of the administered dose.

Table : 42

µg of 5-FU Distributed per gram of Organ from PiBCA Microcapsules

Organs	T I M E			
	½ hr.	1 hr.	1½ hr.	2 hrs.
Liver	79.93 ± 12.37	72.66 ± 13.92	n.d.	n.d.
Lungs	471.60 ± 47.81	510.08 ± 73.92	22.25 ± 7.98	82.56 ± 13.16
Kidneys	80.35 ± 10.82	218.63 ± 39.15	49.85 ± 14.90	n.d.
Intestine	43.39 ± 5.96	22.16 ± 3.24	n.d.	n.d.
Spleen	189.95 ± 14.24	165.48 ± 36.62	3.53 ± 1.57	34.68 ± 5.62

n.d. - not detectable

Table : 43

Organ Distribution of 5-FU as Percentage of Administered Dose from PiBCA Microcapsules.

Organs	TIME			
	½ hr.	1 hr.	1½ hr.	2 hrs.
Liver	8.52 ± 1.34	8.51 ± 1.35	-	-
Lungs	11.79 ± 1.25	11.07 ± 2.06	0.52 ± 0.14	1.89 ± 0.29
Kidneys	1.89 ± 0.34	4.77 ± 0.53	1.10 ± 0.44	-
Intestine	4.10 ± 0.70	1.95 ± 0.45	-	-
Spleen	1.91 ± 0.14	1.45 ± 0.15	0.04 ± 0.02	0.37 ± 0.05
Total	28.31 ± 2.38	27.77 ± 2.03	1.66 ± 0.39	2.26 ± 0.25

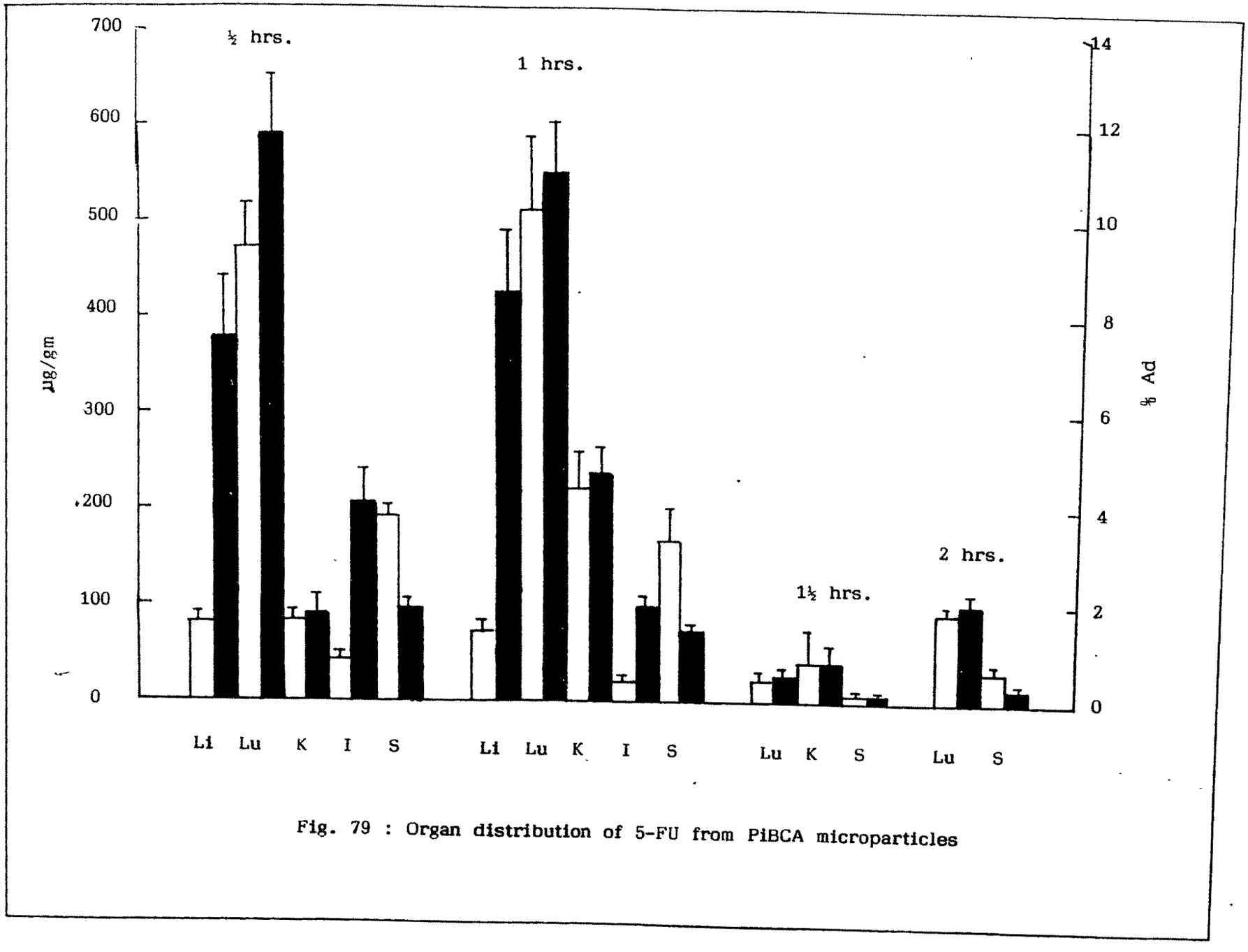


Fig. 79 : Organ distribution of 5-FU from PiBCA microparticles

The drug levels in the liver, lungs and spleen remained almost constant upto 1 hour and fell rapidly within 1½ hours. The drug levels in the intestine peaked in ½ hour and then reduced to half of the original value by 1 hour.

The drug could not be detected in the liver, kidneys and intestine 2 hours postinjection whereas appreciable quantities were found in the lungs and spleen. In fact, the drug levels in both these organs were much higher than the values found at 1½ hours postinjection. This might be due to a redistribution of the microparticles from the other organs or due to drug release as a result of biodegradation of the intact microcapsules.

As in case of earlier studies, the results from this study also showed maximum drug accumulation in the lungs. But a noteworthy feature of the in vivo data generated from these PiBCA microcapsules is that appreciable drug levels were found in the spleen upto 2 hours. In fact, the drug levels in the spleen were 2.5 times as high as those of the liver and kidneys and 4.5 times as high as that of the intestine on weight basis, and accounted for almost 2% of the administered dose.

The preferential drug uptake by the lungs and the spleen observed in this study is in accordance with the findings of

Couvreur et al[172] who reported that vinblastine levels were significantly higher in the spleen, lungs, kidneys and liver when administered in polyalkylcyanoacrylate encapsulated form as compared to the free drug. They also reported a peak drug level in ½ hour after administration.

Preferential distribution of actinomycin D in the spleen, liver and lungs was also reported by Kante et al[173] following injection of drug-loaded PiBCA nanoparticles in rats.

Similar results of preferential drug distribution in the organs of the RES viz. liver, lungs and spleen after administration of polyalkylcyanoacrylate nanoparticles were also reported by Waser et al,[180] Gipps et al[183-184] and Simeonova et al.[191]

Student's t test was applied to the data generated from the pharmacokinetic study of PiBCA microparticles. It showed that there was a significantly higher total distribution of 5-FU on weight basis ($P < 0.001$) from PiBCA microparticles as compared to free 5-FU. But the total percentage of administered dose was not significantly different in both cases ($P < 0.05$) (Table 44).

Organ-wise distribution of 5-FU was significantly higher ($P < 0.001$) in the lungs and spleen with PiBCA microcapsules as compared to free 5-FU. However, the drug distribution in

Table : 44

Application of Student's t Test for comparing Organ Distribution Pattern of PiBCA Microcapsules and Free 5-FU

Organ	µg drug/gm organ		% Administered dose	
	t-value	Significance level	t-value	Significance level
Total	10.00	$P < 0.001$	3.08	$P < 0.05$
Liver	0.15	n.s.	0.04	n.s.
Lungs	10.00	$P < 0.001$	9.00	$P < 0.001$
Kidneys	3.72	$P < 0.02$(↓)	2.86	$P < 0.05$(↓)
Intestine	1.91	n.s.	3.99	$P < 0.02$(↓)
Spleen	7.65	$P < 0.001$	6.95	$P < 0.001$

n.s. - not significant, ↓ - less than standard

D.F. = 5, $t_{0.05} = 25.70$, $t_{0.02} = 3.37$, $t_{0.01} = 4.03$, $t_{0.001} = 6.87$

the kidneys and intestine was significantly lower ($0.02 < P < 0.05$), while that in the liver was not changed after microencapsulation in polyisobutylcyanoacrylate particles.