CHAPTER II

DESIGNING OF NEW CONTROLLED DRUG DELIVERY SYSTEM EMPLOYING LASER

The conventional hard gelatin capsules normally disintegrate rapidly exhibiting a cube-root dissolution pattern of the encapsulated drug (1). Exposure to formalin vapours causes the cross linkage of the gelatin molecules resulting in an unpredictable decrease in solubility of these capsules (2).

The possibility of using laser in designing controlled release dosage form employing laser has been recently reported (3). Theeuwes et al. (4) used automated laser drilling in making accurate and precise exit pores for indomethacin in designing an elementary osmotic pump.

In view of the above reports and the hypothesized controlled drug delivery system, the objectives of this part of the study were to :

i) prepare GIT resistant hard gelatin capsules by formalin vapour treatment and confirm their resistance by in vitro and in vivo tests;

ii) make minute pores on the hardened wall of the capsule by laser drilling employing a CO_2 gas laser so as to make the contents of the capsule

release at a controlled rate through these minute pores;

iii) study the probable mechanism of drug release from the capsule; and

iv) standardize the system designed with respect to laser drilling technique and other parameters which are likely to influence the preparation of GIT resistant, laser drilled capsules, and the factors likely to influence the release of the drug from the capsule by *in vitro* dissolution tests; using tetracycline hydrochloride as the model drug.

EXPERIMENTAL

Preparation of GIT Resistant Capsules - The conventional hard gelatin capsules[1] were arranged in a specially prepared capsule stand after separating the cap and the body and the stand was then placed in a formalin treatment chamber[2], as shown in Figure II.1. The capsules were exposed to formalin vapours for 15, 30, 45, 60, 75, 90, 120, 150 and 180 min in the chamber at the end of which the capsules were subjected to *in vitro* GIT resistance, residual formalin content,

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1. Hard gelatin capsules, Associated Capsules Pvt. Ltd., Kandivali (West), Bombay, INDIA.
2. Histometer, INCO, Ambala, INDIA.

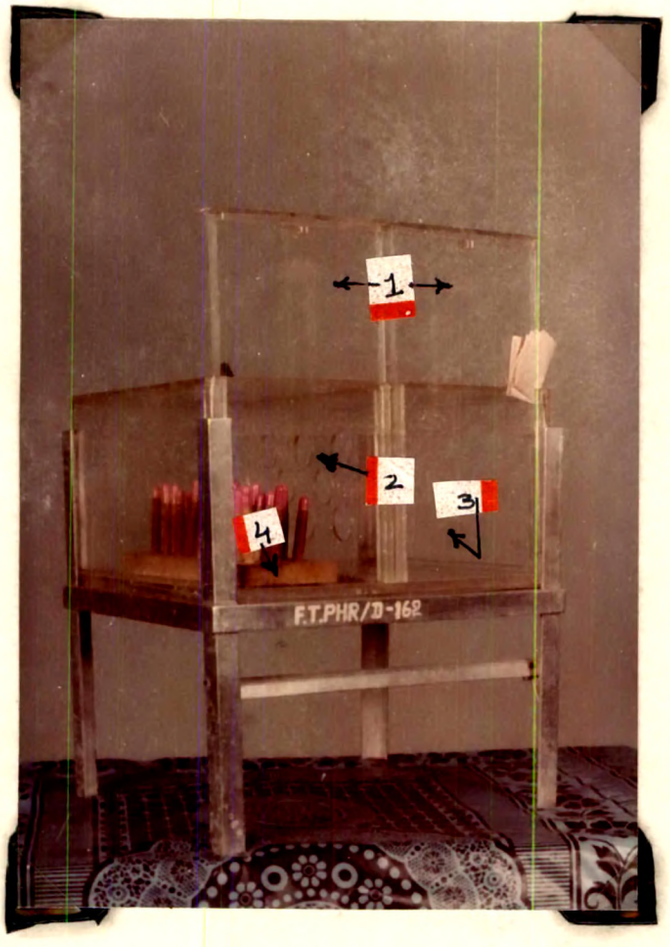

Figure II.1 - Formalin Treatment Chamber.

Key : 1. Sliding doors, 2. Perforated central partition,
3. Formalin gas generation chamber, and
4. Capsule placed in a capsule stand.

physical characteristics and suitability to laser drilling tests (as explained in the sections to follow), in order to evaluate the formalin treated capsule, with respect to these control parameters. The capsules exposed to formalin vapours for 90 min were then subjected to drying at 40, 50, 60 and 70°C for 15, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min under each specified drying temperature, in an oven. Further the process of formalin treatment was repeated under some selected rational combinations of treatment conditions like 60:40, 75:40, 75:50, 90:50 corresponding to formalin contact time (min) and drying temperature (°C) respectively, with drying time of 30 min, maintained constant in all these trials.

Residual Formalin Content - The analytical procedure used for the formalin content determination is based on the method of Macfadyan (5). The method involves the photo-colorimetric measurement of violet colour, produced on heating 1.0 ml of the sample (prepared as described below) containing about 2 to 3 µg of formalin with 9.0 ml of 0.2% (w/v) solution of chromotropic acid[3] in 80% (v/v) sulphuric acid on a boiling waterbath for 30 min, at 490 nm.

The sample was prepared by placing one formalin treated capsule in each of the three 20.0 ml clean and dry

3. Chromotropic acid, Loba Chemie, Indaustrimale Co., P.B.No. 6136, Bombay (INDIA).

glass vials containing 10.0 ml of purified water. The vials after closing with rubber stopper and crimping with aluminium seals, were subjected to agitation on a mechanical shaker for 1.0 hr. The saturation solubility of formalin from each capsule in 10.0 ml of purified water with respect to shaking time was earlier optimised by trial experiments. 1.0 ml content of each vial was then withdrawn separately employing a syringe and was used for the determination of residual formalin content. The average content of the three vials is reported as residual formalin content.

Standard Curve - Formaldehyde solution[4], 37-41% w/v (considered as 40% w/v) was diluted to give 400 ug/ml solution (solution I), using purified water. 0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 ml of solution I equivalent to 0, 40, 80, 120, 160, 200 and 400 ug of formaldehyde respectively, was placed in seven clean and dry graduated glass test tubes, marked from 0 to 6. The content of each test tube was made up to 1.0 ml by adding 1.0, 0.9, 0.8, 0.7, 0.6, 0.5 and 0.0 ml of purified water to test tubes marked 0 to 6, respectively. 9.0 ml of 0.2% (w/v) solution of chromotropic acid was then added to each test tube and the test tubes were heated in a boiling waterbath for 30 min. The violet colour developed was measured in a photocolorimeter[5] at 490 nm, using the solution

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4. Formaldehyde solution, SD'S Lab. Chem. Industries, BOMBAY 400 067 (INDIA).

5. Spectronic 20, Bousch and Lamb, U.S.A.

in test tube marked '0' as the blank. Figure II.2 shows the Beer's plot which was used as the standard curve for the determination of residual formalin content in test solutions.

In vitro GIT Resistance Test - The tests were carried out in an USP XIX (6) dissolution apparatus, using a rotating basket assembly. A flask containing 900.0 ml of gastric fluid (7) as the dissolution medium was immersed in a constant temperature bath at 37±0.5°C. The empty formalin treated capsule with laser drilled pores on the body was then placed in the basket, and was rotated at 100 rpm, for 4.0 hr. At the end of 4.0 hr, 900.0 ml of simulated gastric fluid was replaced by an equal quantity of simulated intestinal fluid (7) and the experiment under similar conditions was continued for another 4.0 hr.

In vivo GIT Resistance Test - The in vivo GIT resistance test was carried out by X-ray studies in human subjects. Two volunteers, male subjects, 27 and 29 years in age and 65.5 and 71.5 kg in weight were selected for the study. The subjects had no history of any GI disease and none admitted the use of any medication regularly. Both volunteers were asked to abstain from alcoholic beverages 48.0 hr preceding each experiment and advised overnight fasting. Beginning at 8.30 a.m. on the day of the experiment, one capsule containing 250.0 mg of Barium sulphate[6] and band sealed with

6. Barium sulphate X-ray Quality, Magneta Chemicals, Kurla, Bombay 400 058 (INDIA).

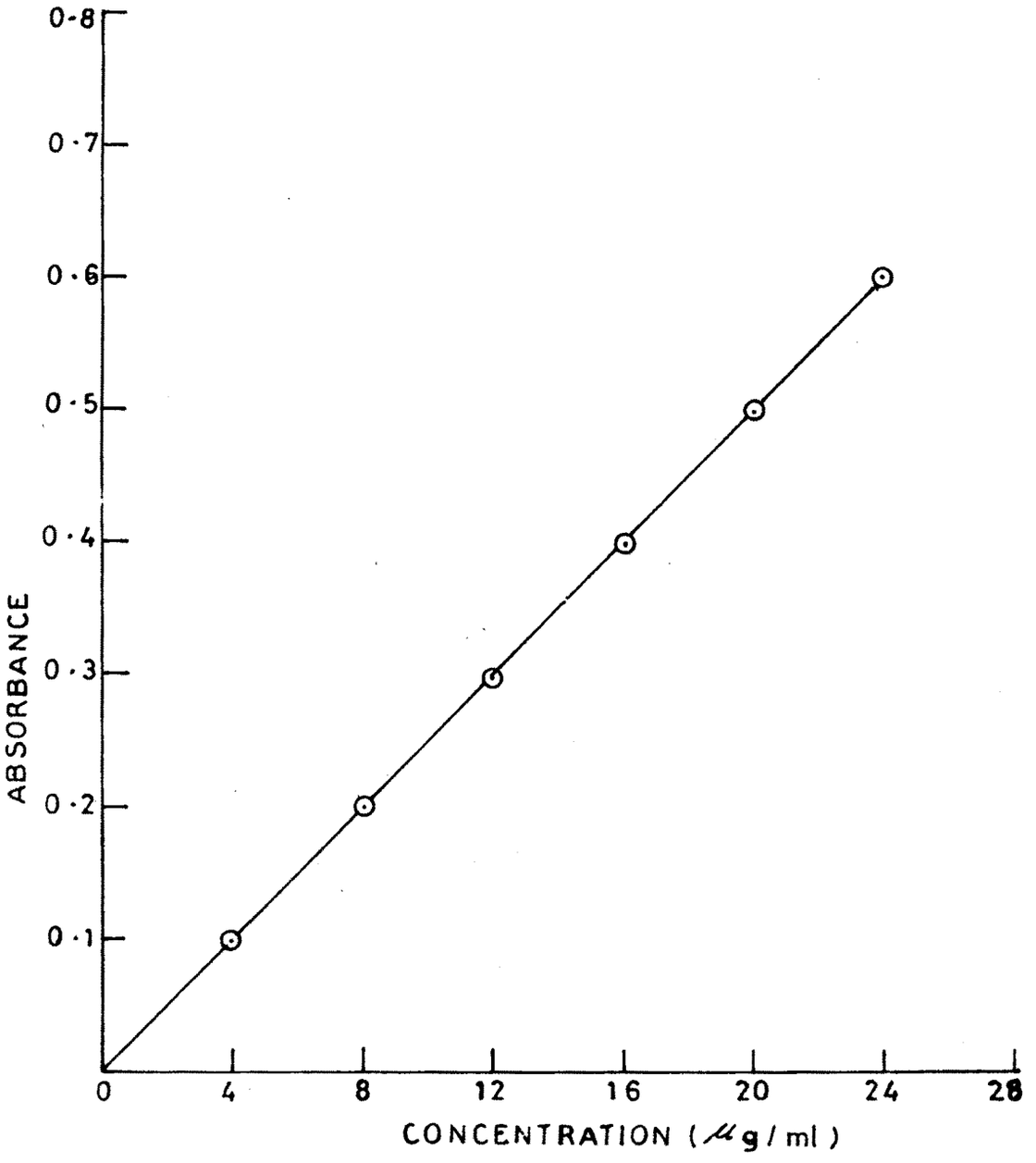

Figure II.2 - Beer's plot For Residual Formalin Content Determination.

a suitable GIT resistant material, was administered orally with about 25.0 ml of water. X-ray photographs were taken at 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 hr interval of time, following the administration of the capsule.

Laser Drilling - 50 W CO_2 (CW/pulsed) laser[7], Figure II.3, was used for the purpose of making small pores on the hardened shells of the capsules. This laser can be operated in continuous as well as pulsed modes. In pulsed mode the pulse width of laser can be varied from 1 pps to 1000 pps. Variation in pulse width and frequency offer a provision for changing the diameter and the number of drilled pores respectively. The laser was internally pulsed through electronically controlled trigger switch. This type of pulsing provides an output power of four to five times the average output power depending on pulse width selected.

Well insulated high voltage terminals, automatic switch to ground the capacitor bank on switching off or tripping off power supply, and interlocking the power supply with coolant flow; are few salient features of this laser system.

In the preparation of GIT resistant capsules, the formalin vapour contact time, drying temperature and

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7. The CO_2 laser Model JLS-C-101. Designed and developed by Laser Division, Jyoti Limited, Baroda 390 007 (INDIA).

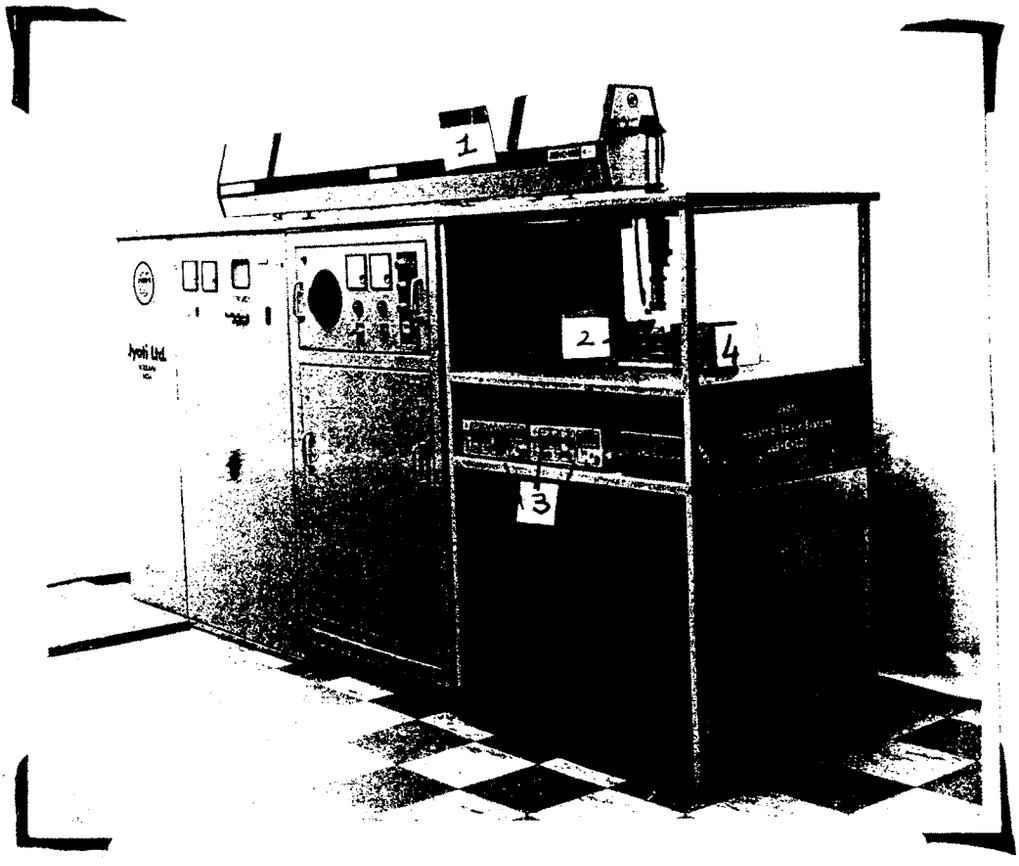


Figure II.3 - CO_2 Gas Laser Along With Linear Drive.

Key : 1. Discharge tube, 2. Laser beam and
3. Laser Parameter Control Switches.
4. Linear Drive.

duration of drying were optimised with respect to laser drilling requirements by trial experiments. Photomicrographs of laser drilled minute pores made on the wall of the GIT resistant capsules, prepared under optimised conditions, using a CO_2 gas laser are shown in Figure II.4.

Study of the Probable Mechanism of Drug Release - The study was carried out using a laser drilled GIT resistant capsule encapsulated with the die methylene blue[8] and band sealed with a suitable GIT resistant material. The capsule was then placed in water, contained in a 1.0 liter beaker in a static condition and was observed carefully. Once the capsule started releasing the dye, after it stabilized, the release pattern was photographed.

Study of the Factors Likely to Influence The Release Rate of the Drug - The different factors which are likely to influence the release rate of the drug from the capsule studied include variation in :

i) laser drilling parameters, and
ii) some physical properties of the encapsulated drug and formulatory additives.

(i) Variation in Laser Drilling Parameters - Laser drilled capsules were prepared with variation in : (a) number of

8. Methylene Blue, Burogoyne Burbidges & Co., Lower Parel Bombay 400 003 (INDIA).

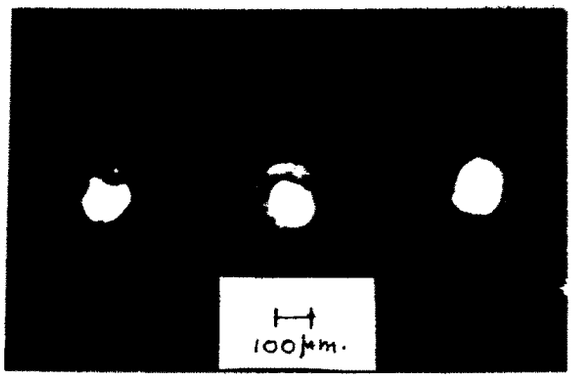
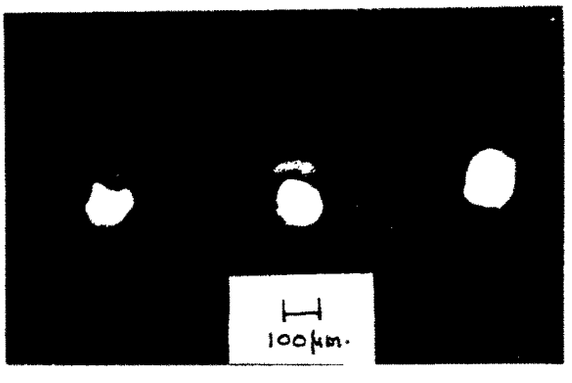


Fig. II.4 - Different Sizes of Laser Drilled Pores on the Capsule Shell.



drilled pores; (b) diameter of drilled pores; and (c) pattern of drilling.

For variation in number of drilled pores, the capsule was mounted on a linear drive (speed 2 mm/sec), with the help of a specially made capsule holder. By changing the laser frequency and keeping the power and pulsewidth constant at 220 u sec) 25, 50, 75 and 100 number of pores were drilled on the body of the capsule shell. The pores were placed equidistant, in three parallel lines, with almost equal numbers.

For diameter variation 50 drilled pores with SD_1, SD_2, SD_3 and SD_4 distribution of pore sizes (Table II.1) having different average diameters (determined by optical microscopic method (8) were obtained by varying the pulse-width and keeping the frequency of the laser constant at 1 pps.

Variation in pattern of drilling the pores was studied using capsules with 50 drilled pores of SD_2 distribution on the body only and on cap and body both as shown in Figure II.5.

The studies on effect of these variations in laser drilling parameters on release rate of the encapsulated drug were made by filling these capsules with tetracycline hydrochloride[9] as a model drug and subjecting them to

9. Tetracycline hydrochloride, Synbiotics, Wadi Wadi, Baroda 390 007 (INDIA).

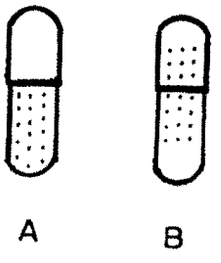


Figure II.5 - Schematic Representation of
Variation in Patterr of Drilling.

Table II.1 : Pulsewidth Variations with Corresponding Pore size and Average Diameters.

No.	Pore Size Distribution	Pulsewidth pps	Average Pore Diameter (μ)	
			d_{ln} [a]	$d_g \pm$ SD [b]
1.	SD_1	100	83.88	78.50 ± 1.57
2.	SD_2	220	103.60	100.00 ± 1.41
3.	SD_3	300	160.20	155.00 ± 1.52
4.	SD_4	420	207.80	205.00 ± 1.38

a = Statistical length-number mean diameter (d_{ln})

b = Geometric mean diameter (dg) $\pm$ Standard deviation obtained from the log-probability plot, Figure II.10.

in vitro dissolution studies after band sealing the capsules with a suitable GIT resistant material.

(ii) Variation In Some Physical Properties of the Encapsulated Drug and the Formulatory Additives - GIT resistant capsules having 50 laser drilled pores on the body with SD_2 size distribution of pores filled with tetracycline hydrochloride (as a model drug) and band sealed with suitable GIT resistant material were used for the study. Average weight of filled capsules was determined by weighing 10 capsules in each case. In order to ensure that there is no loss of the encapsulated drug through the laser drilled pores, the capsules were subjected to friability testing in a Roche Friabilator (9).

To study the effect of diluents, capsules each containing 250.0 mg of tetracycline hydrochloride, using lactose and microcrystalline cellulose as the diluents were prepared separately. Differences in bulk density between drug and diluents were taken into consideration in calculating the fill weight of the individual capsules.

The effect of powder properties like particle size and size distribution and bulk density was studied using capsules encapsulated with three powder samples, sample I, sample II and sample III separately, having different powder characteristics. The original sample of tetracycline hydrochloride

(sample I) was screened through 360 # standard sieve. The oversize fraction (sample II) and the undersize fraction which was further pulverised in a pestle and mortar (sample III) were used for the study. Bulk density of each sample was determined by the standard three tap method, suggested by Butler and Ransey(10). The particle size and size distribution analysis was made by optical microscopic method (8) by preparing a suspension of the powder sample in glycerin or propylene glycol.

Tetracycline hydrochloride capsules prepared by filling tetracycline hydrochloride with 2.0% talc or 1.0% magnesium stearate separately were used to study the effect of common lubricants.

Saturated solutions of dioctyl sodium sulfosuccinate[10] and polysorbate-80[11] were sprayed separately on tetracycline hydrochloride, so as to contain 0.5% (w/w) and 1.0% (w/w) respectively, of these wetting agents, uniformly distributed in the dried (40°C) tetracycline hydrochloride.

These samples were then encapsulated in GIT resistant laser drilled capsules to study the effect of presence of welting agents.

In vitro Dissolution Rate Tests - The dissolution rate

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10. Doxinate, Hoechst Aktiengesel Schaft, Badvilpel, GERMANY.
11. Tween 80, Atlas Chemical Co., Wilmington Del., U.S.A.

tests were carried out by a method similar to that used in in vitro GIT resistance test, but in this case the dissolution medium used was 0.1N HCl and 5.0 ml samples were withdrawn at 30 min interval of time and the volume of dissolution medium was maintained by replacement with an equal volume of fresh medium after each withdrawal. The samples were analysed at 353 nm using a spectrophotometer[12]. The noninterference of the soluble additives like dioctyl sodium sulfosuccinate and polysorbate-80 on λ max was confirmed separately.

Beer's Plot of Tetracycline Hydrochloride - About 100.0 mg of tetracycline hydrochloride was accurately weighed into a clean and dry 100.0 ml volumetric flask and was dissolved in and diluted to 100.0 ml with 0.1N HCl. The resulting 1.0 mg/ml solution was further diluted to obtain 50 µg/ml solution of tetracycline hydrochloride (solution I), using 0.1N HCl. 2, 4, 6, 8, 10 and 12 ml of solution I was transferred into six, clean and dry, 50.0 ml volumetric flasks marked 1 to 6 and the volume of each flask was made upto 50.0 ml using 0.1N HCl again to get 2, 4, 6, 8, 10 and 12 µg/ml solutions of tetracycline hydrochloride respectively. Aliquots from each flask were then analysed at 353 nm using a spectrophotometer[12]. Figure II.6 shows the Beer's plot of tetracycline hydrochloride, which was used as the standard curve in the analysis of test samples.

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12. Model VSU-2 P spectrophotometer, GERMANY.

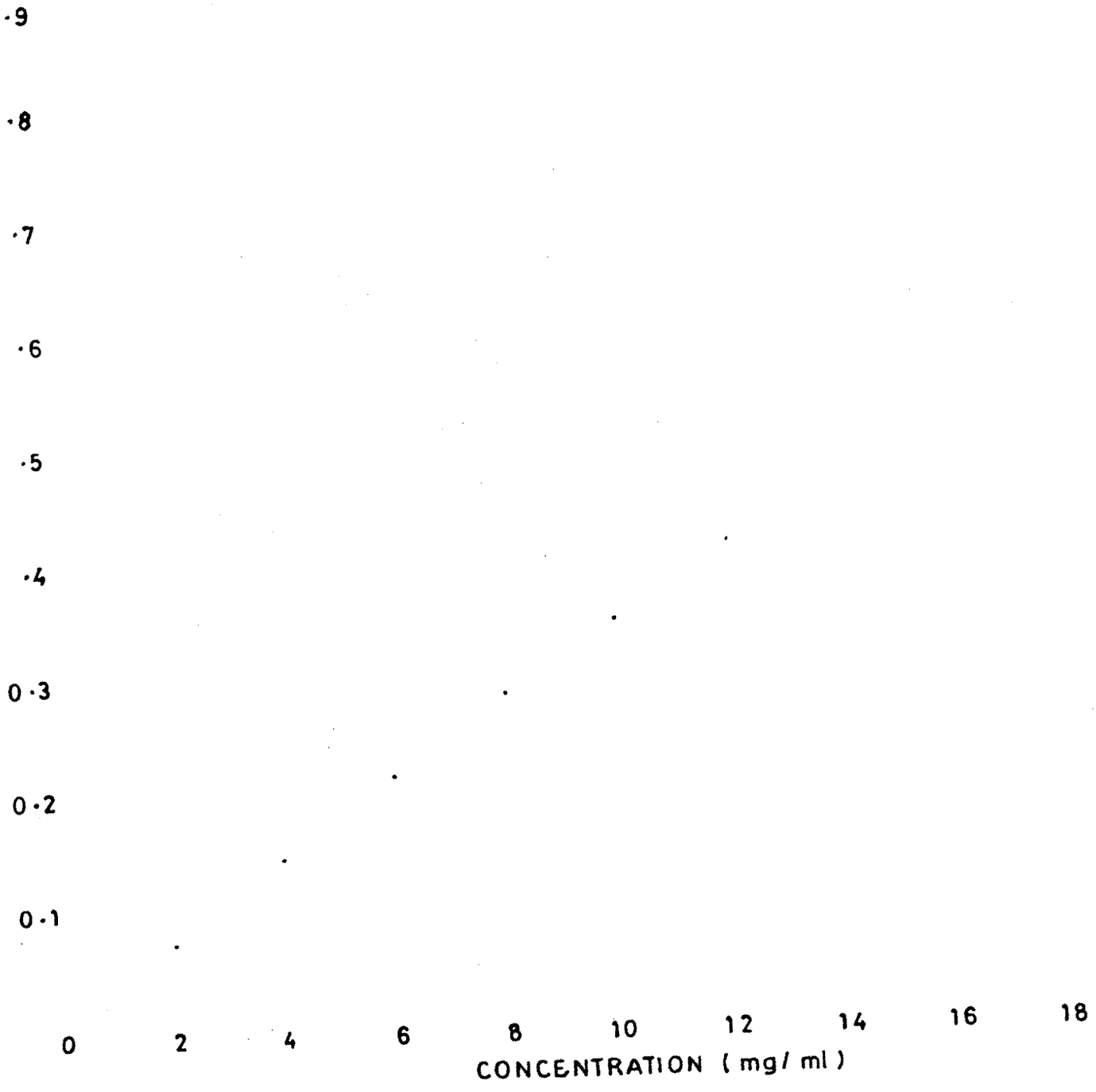


Figure 6 - Beer's plot of Tetracycline Hydrochloride.

RESULTS AND DISCUSSION

In the preparation of GIT resistant capsules, when the conventional hard **gelatin** capsules were exposed to formalin vapours for different length of time ranging between 5 and 180 min, they showed different characteristics with respect to different control parameters evaluated i.e. GIT resistance, residual formalin content, physical properties and laser drilling requirements as given in Table II.2. Formalin vapour contact time of 60 min and above was found to be satisfactory to get the required GIT resistant capsules as observed by the *in vitro* GIT resistance test. However, it was also observed that as the formalin contact time increased, residual formalin content also increased correspondingly. The capsules in all the cases remained wet and soft, after the treatment, making it unsatisfactory for subsequent laser drilling operation. Hence it was thought appropriate to dry the capsule to usual 10-15% humidity level so as to get the required physical characteristic, and also simultaneously bring down the excess residual formalin content and maintain it in optimum range of 50-100 µg/capsule. Capsules which were exposed to formalin vapours for 90 min with a residual formalin content less than 200 µg/capsule were selected for the drying process with a view to have shortest feasible drying time and at the same time have the moisture and

Table II.2 : Standardization of Formalin Contact Time for the Preparation of GIT Resistant Capsules.

Formalin Gas Contact Time (min)	Control Parameters			
	GIT Resistance	Residual Formalin content	Laser Drilling[x] Requirements	Physical Characteristics[y]
5	-	+[a]	-	Wet and soft capsules
10	-	+	-	
15	-	+	-	
30	-	+	-	
45	-	+	-	
60	+	"[b]	-	
75	+	++	-	
90	+	++	-	
120	+	+++[c]	-	
150	+	+++	-	
180	+	+++	-	

(-) = Unsatisfactory
(+) = Satisfactory

a 50 µg/capsule
b 200 µg/capsule
c 200 µg/capsule

(-)=Unsatisfactory
(x)=Brittleness
Bulging
Uniformity of pores.

y=appearance
hardness
Brittleness
Colour

residual formaldehyde content within the required level. Table II.3 shows characteristics of these capsules with respect to the control parameters tested, when subjected to drying at different temperatures for different length of time. Notably lowest temperature i.e. 40°C required longer length of time to bring down the formalin and moisture contents to the required level. Drying for more than 120 min at 40°C reduced the formalin content below 50 µg/capsule, adversely affecting the GIT resistance of the capsule. At 50°C satisfactory formalin and moisture levels were obtained between 30 and 60 min of drying time. Drying for more than 60 min adversely affected the GIT resistance and physical characteristics of the capsules. Drying at higher temperatures such as 60 and 70°C though gave satisfactory capsules within 15 min, there was a risk of capsule becoming brittle, with very little margin of time. Also drying at 60 and 70°C for more than 15 min affected the GIT resistance by reducing the formalin content below 50 µg/capsule level.

Based on the observations made, as given in Table II.2 and Table II. 3 five rational combinations of formalin contact time, drying time and drying temperature were selected to work out the optimum condition for preparation of GIT resistant capsules. Table II.4 shows the observations made with respect to the control parameters studied, under five rational

Table II.3 : Standardization of Drying Conditions for the Preparation of GIT Resistant Capsules.

Drying Temperature (°C)	Control Parameters	Drying Time (min)							
		15	30	45	60	90	120	150	180
40	1	+	+	+	+	+	+	-	-
	2	-	-	-	-	+	+	-	-
	3	-	-	-	+	+	+	+	+
	4	+	+	+	+	+	+	+	+
50	1	+	+	+	+	-	-	-	-
	2	-	+	+	+	-	-	-	-
	3	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	-	-
60	1	*	+	-	-	-	-	-	-
	2	+	+	-	-	-	-	-	-
	3	+	+	+	+	+	+	+	+
	4	+	-	-	-	-	-	-	-
70	1	+	+	-	-	-	-	-	-
	2	+	+	-	-	-	-	-	-
	3	+	+	+	+	+	+	+	+
	4	+	-	-	-	-	-	-	-

1. GIT Resistance; 2. Residual Formalin Content; 3. Laser Drilling Requirement; 4. Physical Characteristics.

(+) = Satisfactory (-) = Unsatisfactory.

Table II.4: Optimization of Conditions for the Preparation of GIT Resistant Capsules.

Rational Treatment	Combination of Conditions		Control Parameters			
Formalin Contact time (min)	Drying Temperature (°C)	Drying Time (min)	GIT Resistance (*in vitro*)	Residual formalin content (50-100 µg/capsule)	Laser drilling requirements[x]	Physical characteristics[y]
60	40	30	-	-	+	+
75	40	30	-	-	+	+
75	50	30	-	-	+	+
90	50	30	+	+	+	+
90	60	30	-	-	+	+

(+) = Satisfactory

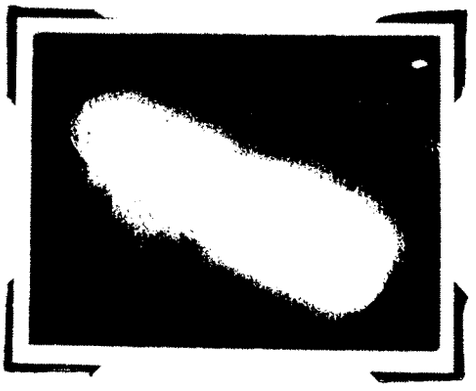
(-) = Unsatisfactory

x=Brittleness
Bulging
Uniformity of pores.

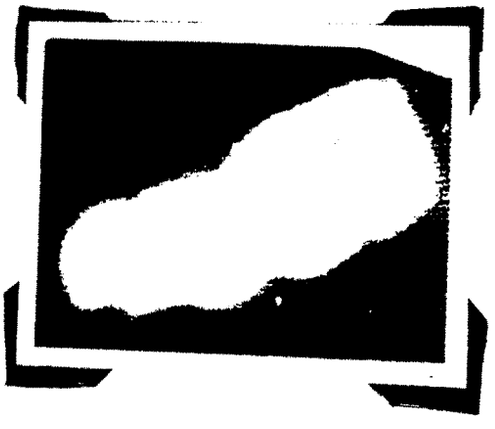
y=Appearance
Hardness
Brittleness
Colour

combinations of conditions studied. The capsules exposed to formalin vapours only for 60 and 75 min reduced the formalin content below 50 µg/ml, when subjected to drying at 40 and 40 & 50°C respectively, making it unsatisfactory with respect to its GIT resistance, like capsules with 90 min formalin contact time dried at 60°C; for 30 min. However, capsule with 90 min formalin contact time when dried at 50°C for 30 min gave satisfactory and reproducible results with respect to desired capsule characteristics. Hence the condition 90:50:30 corresponding to formalin vapour contact time (min), drying temperature (°C) and drying time (min) respectively, was selected as set of optimum condition for preparation of GIT resistant capsules. The residual formalin content 24.0 hr after the treatment under this set of optimum condition was found to be 80 µg/capsule, which further reduced down to 68 ug and 60 µg/capsule after 15 and 30 days of storage respectively.

All the GIT resistant capsules prepared under the set of optimum conditions of treatment, when tested for <u>in vitro</u> GIT resistance, showed resistance both in simulated gastric and intestinal fluids. The capsules remained intact throughout the GIT in X-ray studies, confirming its <u>in vivo</u> resistance in the subjects tested. Figure II.7 shows the X-ray photograph of the intact capsule in the GIT of one of the subjects. The



(a)



(b)

Figure II.7 - Intact Capsules Traced in the GIT of One of the Human Volunteers by X-ray Photograph After the Capsules were Administered orally.

Key : (a) after 0.5 hr and (b) after 4.0 hr.

deshaping of the capsule as seen in the figure may be attributed to the shape of barium sulphate core inside the capsule. Figure II.8 shows the position of the capsule in the GIT at 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 hr interval of time after the capsule was administered in one of the subjects. The capsule remained in the stomach for more than 1.0 hr and, 2.0 hr after the administration it was in the duodenal part of the small intestine. Further, the capsule was traced intact in ileum after 4.0 hr, in colon after 6.0 hr and finally in the rectum at the end of 8.0 hr.

After having prepared the GIT resistant capsules an attempt was made to make minute laser drilled pores on the wall of the capsule using CO_2 gas laser. The photomicrographs of different sizes of laser drilled pores on the wall of the capsule (Figure II.4) and the variation in diameter of drilled pores obtained by varying the pulsewidth of the laser, keeping the frequency constant at 1 pps (Table II.1) indicate that laser drilling technique can be successfully used in making accurate and precise minute pores in designing the proposed new controlled drug delivery system.

Capsules encapsulated with methylene blue when placed in water (Figure II.9) in a static condition, showed instant depression on the capsule surface along the drilled portion, indicating imbibing of water through the minute laser drilled

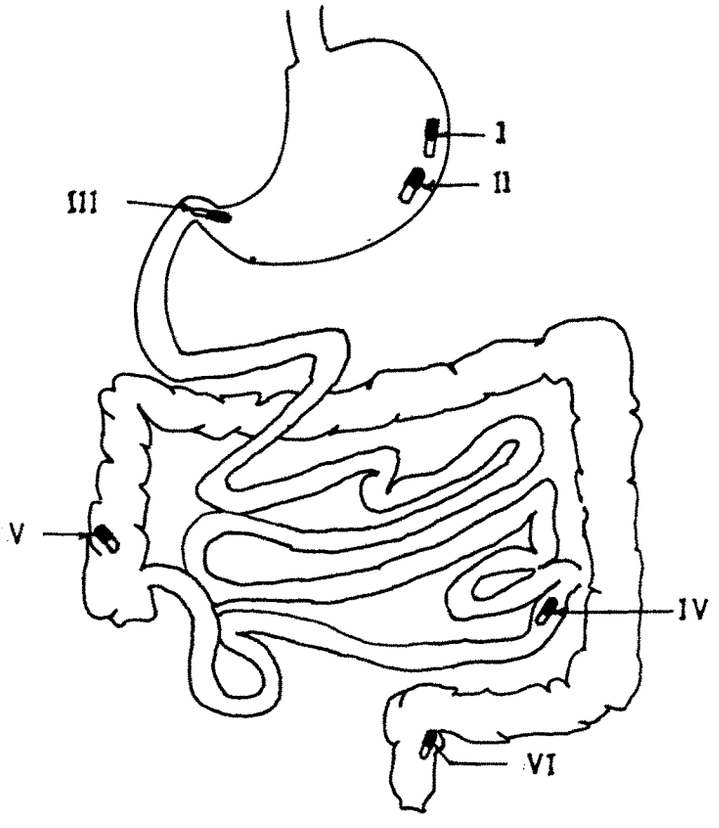


Figure II.8 - Schematic representation of the position of the capsule in the GIT. (Detected by X-ray photography) at different intervals of time after the capsule administration. Key : I, 0.5 hr; II. 1.0 hr; III. 2.0 hr; IV, 4.0 hr; V, 6.0 hr; and VI, 8.0 hr.

Figure II.9 - Release Pattern of Methylene Blue from GIT Resistant Laser Drilled Capsules.

1. GIT Resistant Capsule with 3 laser drilled pores,
2. Water in static condition and 3. Laminar pattern of drug release.

pores. The driving force for such a movement of water may be due to the capillary action of the encapsulated methylene blue powder. The observed slight swelling of the capsule, followed by slow release of the dye through the laser drilled pores indicate that considerable amount of water has entered into the capsule, subsequently dissolving the contents to form a saturated solution, creating a positive concentration gradient inside the capsule, which may be responsible for the subsequent release of the dye solution from the capsule by diffusion. The time taken for imbibition of water into the capsule and the dissolution of the dye in the imbibed water might have contributed to the observed initial lag period of about 20 min. Once the process of diffusion was stabilized, the release of the dye from the capsule was in a laminar fashion, in an unagitated condition of the dissolution medium as seen in Figure II.9, where the flow of drug solution from each pore is seen distinctly separate. This experimental finding, hence, supported the proposed mechanism of working of the hypothesized system (Scheme 15, Chapter I) i.e. the system works on the combined principle of dissolution and diffusion.

Variation in number of drilled pores showed a considerable influence on the release rate of tetracycline hydrochloride from the capsule, Table II.5 (Figure II.10).

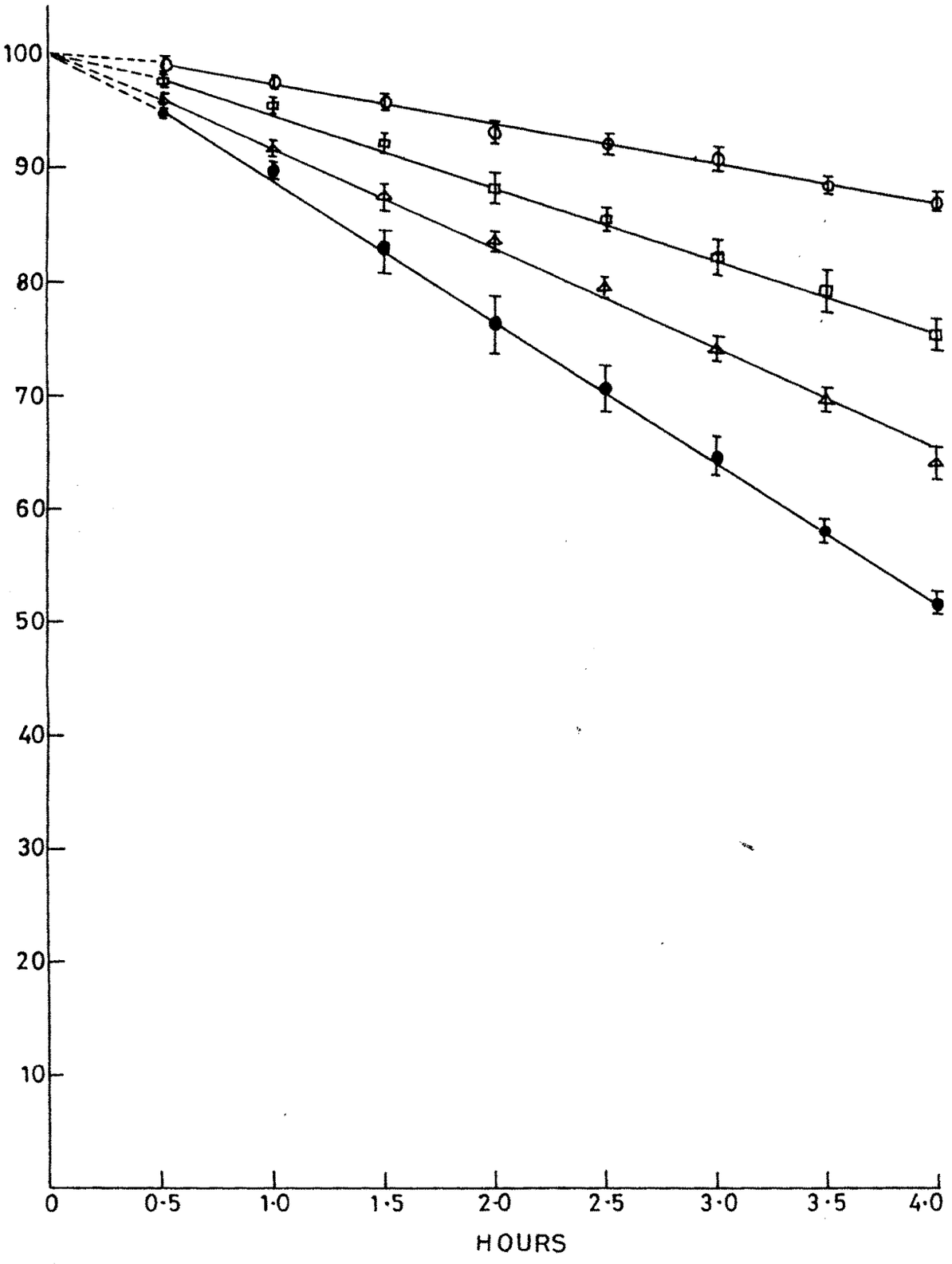


Figure II.10 - Effect of Variation in Number of Drills, n, on Percent Drug Retained vs Time. Key: O, n= 25; □ ,n = 50; △ n = 75; and ● n = 100.

Table II.5 : Effect of Variation in Number of Drills, n, on Percent Drug Retained as a Function of Time.

Sr. No.	Time, x, (hr)	Percent Drug Retained, y,				Release Rate (Kn)=slope= $\alpha = \frac{\{\Sigma xy - \Sigma x \Sigma y\}}{\{\Sigma x^2 - [\Sigma x]^2\}}$ (% hr^{-1})			
		n = 25	n = 50	n = 75	n = 100	n=25	n=50	n=75	n=100
1.	0.0	100.00	100.00	100.00	100.00				
2.	0.5	99.21	98.20	96.23	95.98				
3.	1.0	97.51	95.53	91.52	90.01				
4.	1.5	95.67	92.07	87.25	82.66	3.30	6.32	8.92	12.27
5.	2.0	93.21	88.80	83.85	76.19				
6.	2.5	92.14	85.25	79.78	70.84				
7.	3.0	90.57	81.84	73.92	64.77				
8.	3.5	88.84	79.30	69.49	58.16				
9.	4.0	87.35	75.35	63.84	51.91				
		$\Sigma x=18$	$\Sigma y=744.50$	$\Sigma y=796.34$	$\Sigma y=745.88$	$\Sigma y=690.52$			
			$\Sigma xy=1639.43$	$\Sigma xy=1497.94$	$\Sigma xy=1358.01$	$\Sigma xy=1196.98$			

A plot of percentage drug retained against time in hr being a straight line, the figure clearly indicates that the drug release in all the cases follows a zero order pattern after an initial lag period of 30 min. This lag period may be attributed to the time required for the dissolution fluid to penetrate into the capsule, wet, dissolve the drug and finally make the drug pass through the pores. The slope values were calculated based on Eq. 1 (11).

$$\alpha = \frac{\{\Sigma xy - (\Sigma x \cdot \Sigma y/n)\}}{\{\Sigma x^2 - [\Sigma (x)^2/n]\}} \qquad \text{Eq. (1)}$$

where, α, is the slope, x, is the time in hr and y, is the percentage drug retained at a particular interval of time. Since the release follows a zero-order kinetics the slope of the lines represents the rate of release, Kn, where n, indicates the number of pores drilled and the calculated values of K_{25}, K_{50}, K_{75}, K_{100} were 3.30, 6.32, 8.92 and 12.27 per cent/hr respectively. The variation in number of drilled pores being in the ratio of 1:2:3:4, the release rate constant Kn also showed almost a similar ratio indicating a proportionate increase in release rate of the drug with increase in number of drills.

The effect of pore size and size distribution on release rate constant of tetracycline hydrochloride is shown in

Table II.6, Figure II.11 for the different size distribution ranges SD_1, SD_2, SD_3 and SD_4 studied. The statistical mean diameter of these different size distributions studied were calculated based on the Eq. 2 (12).

$$\text{mean} = \left(\frac{\sum nd^{p+f}}{\sum nd^{f}} \right)^{1/p} \qquad \text{Eq. (2)}$$

Where n is the number of pores in a size range whose mid point d, is one of the equivalent arithmetic, geometric or harmonic mean diameters, 'p' is the index related to size of individual pore since, d, raised to the power (p=1, p=2) is an expression of pore length or surface respectively. The frequency with which the pores in a certain size range occur is expressed by nd^f. When the frequency index has the values of 0, 1 or 2 then the size frequency distribution is expressed in terms of total number, length or surface of pores respectively. The frequency distribution and the log probability plots of SD_1, SD_2, SD_3 and SD_4 pore size distributions studied are shown in Figure II.12 and Figure II.13 respectively drawn based on the data compiled in Table II.7. The arithmetic length-number mean diameters d_{ln}, calculated based on Eq. 3 (13), were found to be equal to 83.58, 103.60, 160.20 and 207.80 respectively. These values

$$d_{ln} = \frac{\sum nd}{\sum n} \qquad \text{Eq. (3)}$$

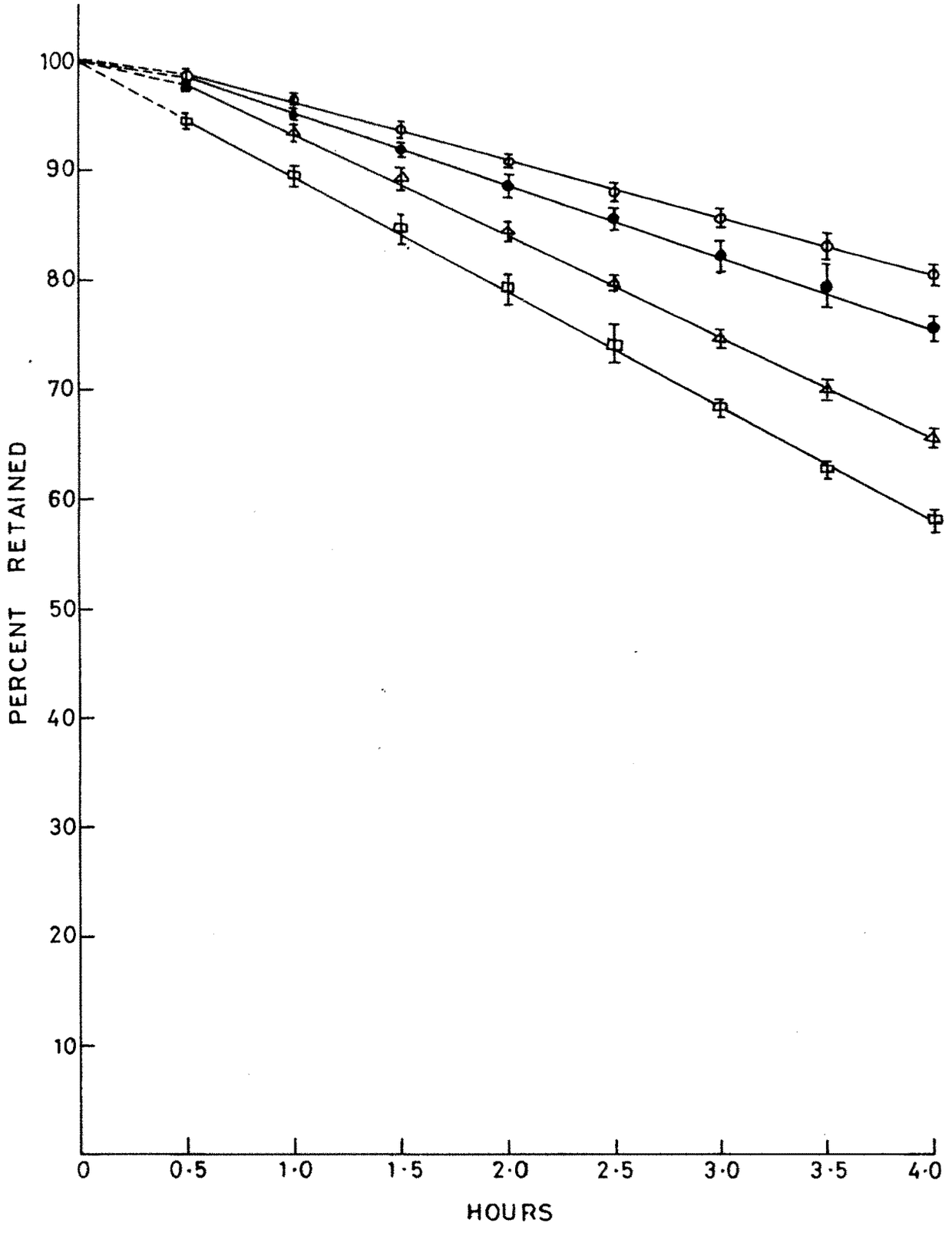


Figure II.11- Effect of Variation in Size Distribution of peres on percent Drug Retained Vs. Time. Key: ○ , SD_1, ● , SD_2, △ , SD_3 and □ , SD_4.

Table II.6 : Effect of Variation in Size Distribution of Pores on Percent Drug Retained Versus Time

Sr. No.	Time, x, (hr)	Percent Drug Retained, y,				Release Rate(K)=slope= $\alpha = \frac{\{\Sigma xy - \frac{\Sigma x \Sigma y}{n}\}}{\{\Sigma x^2 - \frac{(\Sigma x)^2}{n}\}}$ (% hr^{-1})			
		SD_1	SD_2	SD_3	SD_4	SD_1	SD_2	SD_3	SD_4
1.	0.0	100.00	100.00	100.00	100.00				
2.	0.5	98.77	98.20	97.70	94.50				
3.	1.0	96.46	95.53	93.70	87.73	5.04	6.32	8.96	10.60
4.	1.5	93.50	92.07	89.37	84.52				
5.	2.0	90.85	88.80	84.43	79.09				
6.	2.5	88.11	85.25	79.63	74.13				
7.	3.0	85.52	81.84	74.61	68.35				
8.	3.5	83.16	79.30	70.08	62.34				
9.	4.0	80.73	75.35	65.44	57.82				
$\Sigma x=18$		$\Sigma y=817.10$	$\Sigma y=796.34$	$\Sigma y=754.96$	$\Sigma y=710.28$				
		$\Sigma xy=1558.62$	$\Sigma xy=1497.94$	$\Sigma xy=1375.42$	$\Sigma xy=1261.59$				

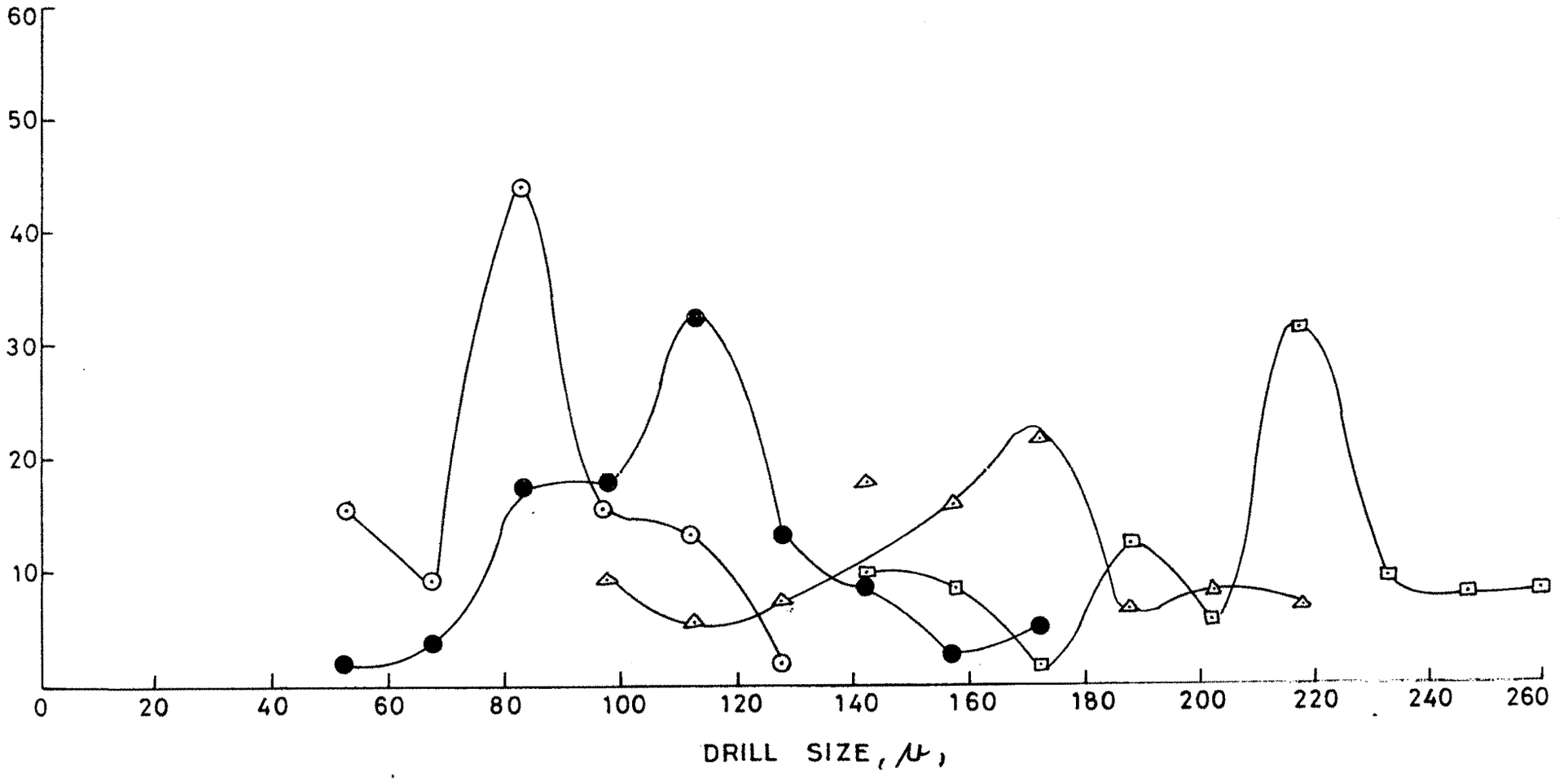


Figure II.12 - Frequency Distribution plots of Different Size Ranges of Drilled Pores.

Key : O, SD_1; ● , SD_2; △ ,SD_3 and □ ,SD_4.

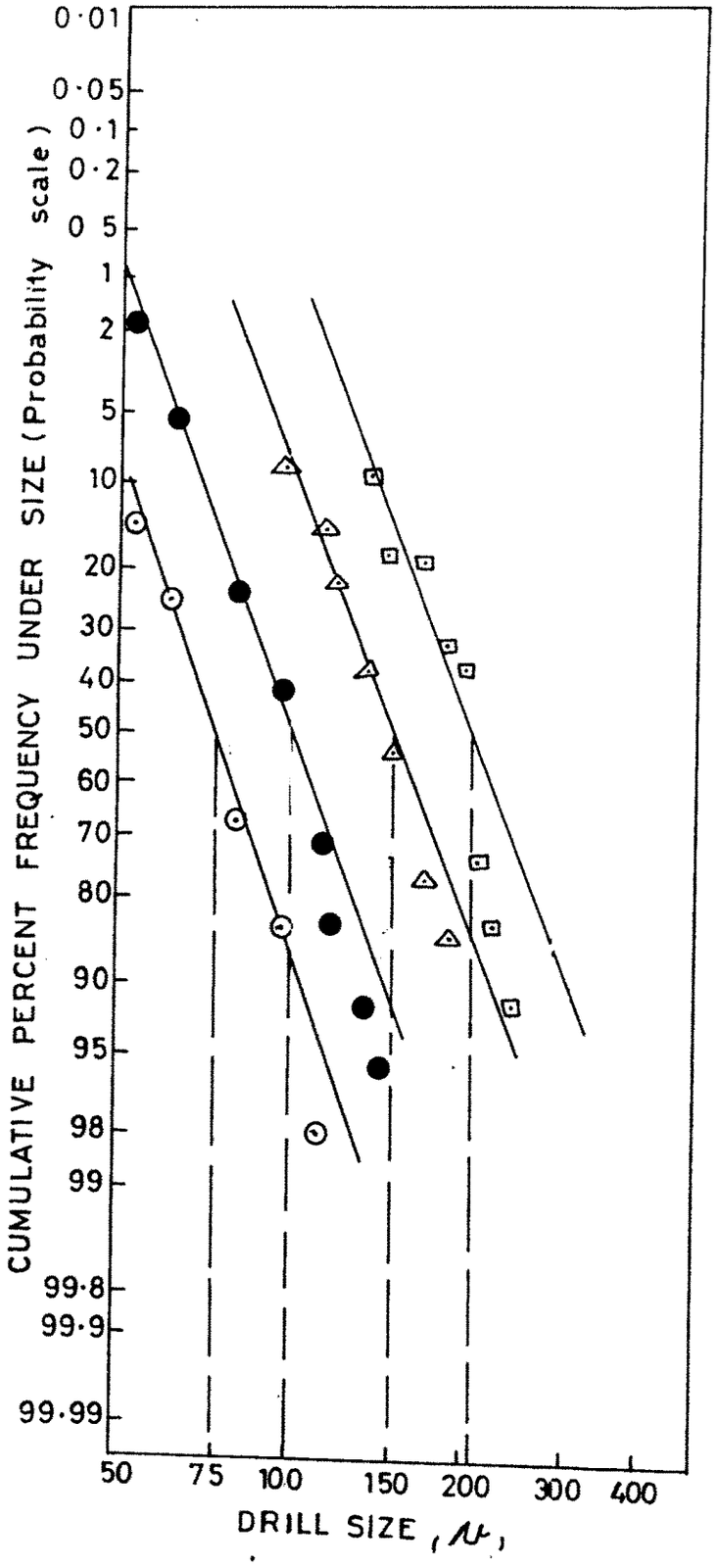


Figure II.13 - Log-probability plots of Different size Ranges of Drilled pores. Key : O, SD_1; ● , SD_2; △ , SD_3 and □ , SD_4.

of average diameters calculated statistically, correlated well with, geometric mean diameters of 78.50, 100.00, 155.00 and 205.00 μ, those which were obtained from the log probability plot (Figure II.13) with standard deviations calculated from the slope of the lines equal to 1.57, 1.41, 1.52 and 1.38 respectively. Considering the drills as circles, the total surface area available for the release of the drug with 50 pores on the body of the capsule for corresponding average diameter of the pores was equal to 1.10, 1.68, 4.03 and 6.78 cm^2/capsule and the calculated values of release rate constants indicate that drug is being released at the rate of 5.04, 6.32, 8.96 and 10.60 per cent/hr for the respective release area available. The release rate followed zero-order kinetics in all the cases after an initial lag-period of 30 min, except in the case of capsules having SD_4 type of size distribution where the lag period was not seen. Such a deviation in the case of bigger sizes of pores may be attributed to the minimum time required for the dissolution fluid to penetrate into the capsule with a larger surface area available. The capsules with pores of SD_1 and SD_2 size distribution showed a loss of less than 0.8% of the contents per capsule when subjected to friability test, unlike the capsule with SD_3 and SD_4 size distribution of pores where the loss was more than 1.%/capsule, indicating that the laser drilled pores with SD_1 and SD_2 size

distribution are suitable for designing controlled drug delivery system while pores with SD_3 and SD_4 size distribution are unsatisfactory.

The variation in pattern of drilling did not show statistically significant difference ($t=0.037$, $d=16$ $P > 0.01$) on release rate of the drug, Figure II.14. The higher standard deviations observed in the individual value of the cap and body drilling may be attributed to possible incomplete drilling when comparatively thicker cap and body surface was simultaneously drilled with same laser parameter.

The release of the drug from the capsule was considerably affected by the powder characteristics like bulk density and particle size distribution of the encapsulated drug samples I, II and III, with bulk densities 0.61, 0.60 and 0.73 gms/c.c. and average length number mean diameter d_{ln}, equal to 51.20, 95.74 and 23.30 μ, respectively. The corresponding release rate from capsules encapsulating sample I, II and III were 6.32, 5.00 and 8.11 %/hr respectively, as shown in Table II.8, Figure II.15, clearly indicating an increase in release rate with decrease in particle size and size distribution of the encapsulated drug sample. The frequency distribution and log-probability plot of the three samples studied are shown in Figure II.16 and Figure II.17 respectively, drawn based on the data compiled in Table II.9.

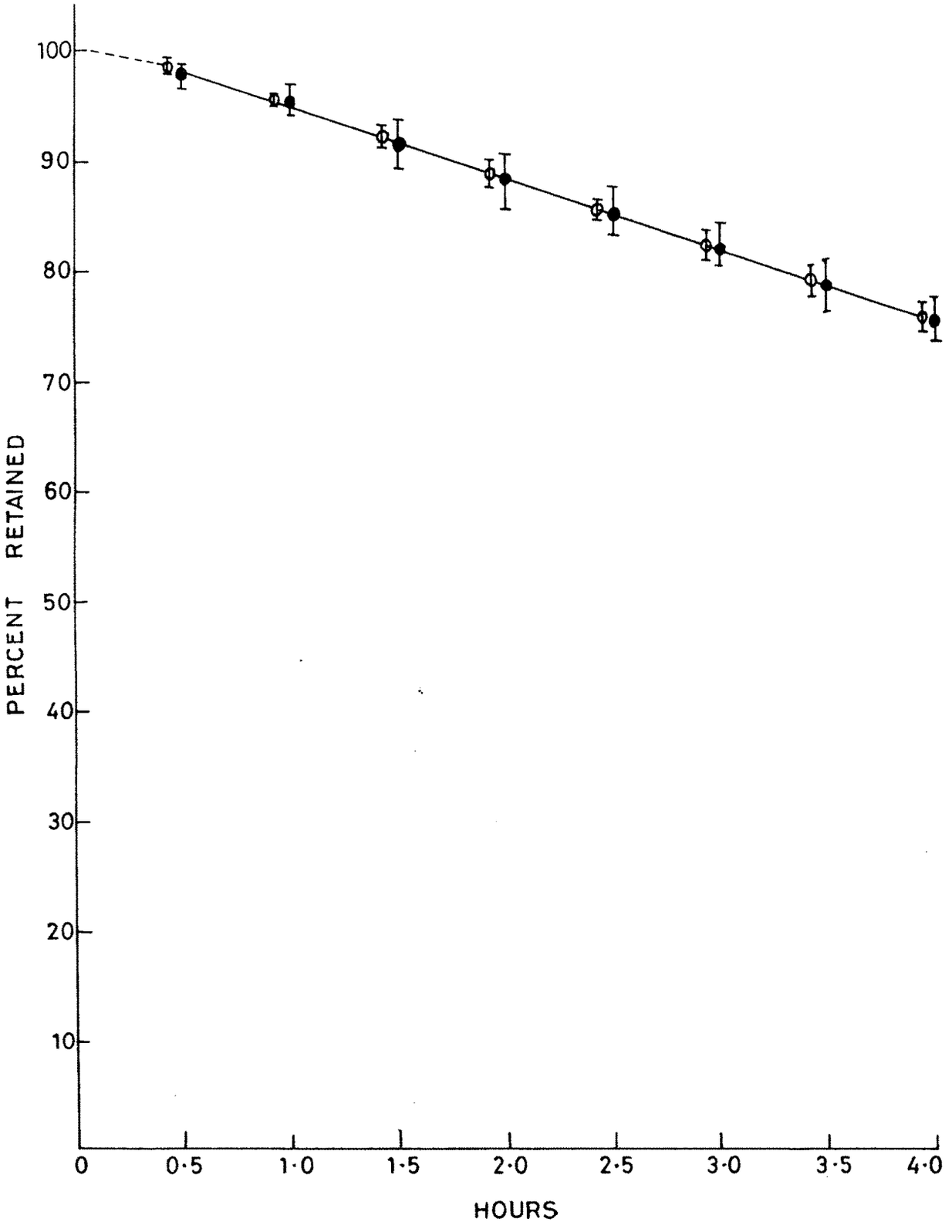


Figure II.14- Influence of Variation in Pattern of Drilling on Percent Drug Retained vs. Time. Key : O, Only body drilling and ●, cap and body drilling.

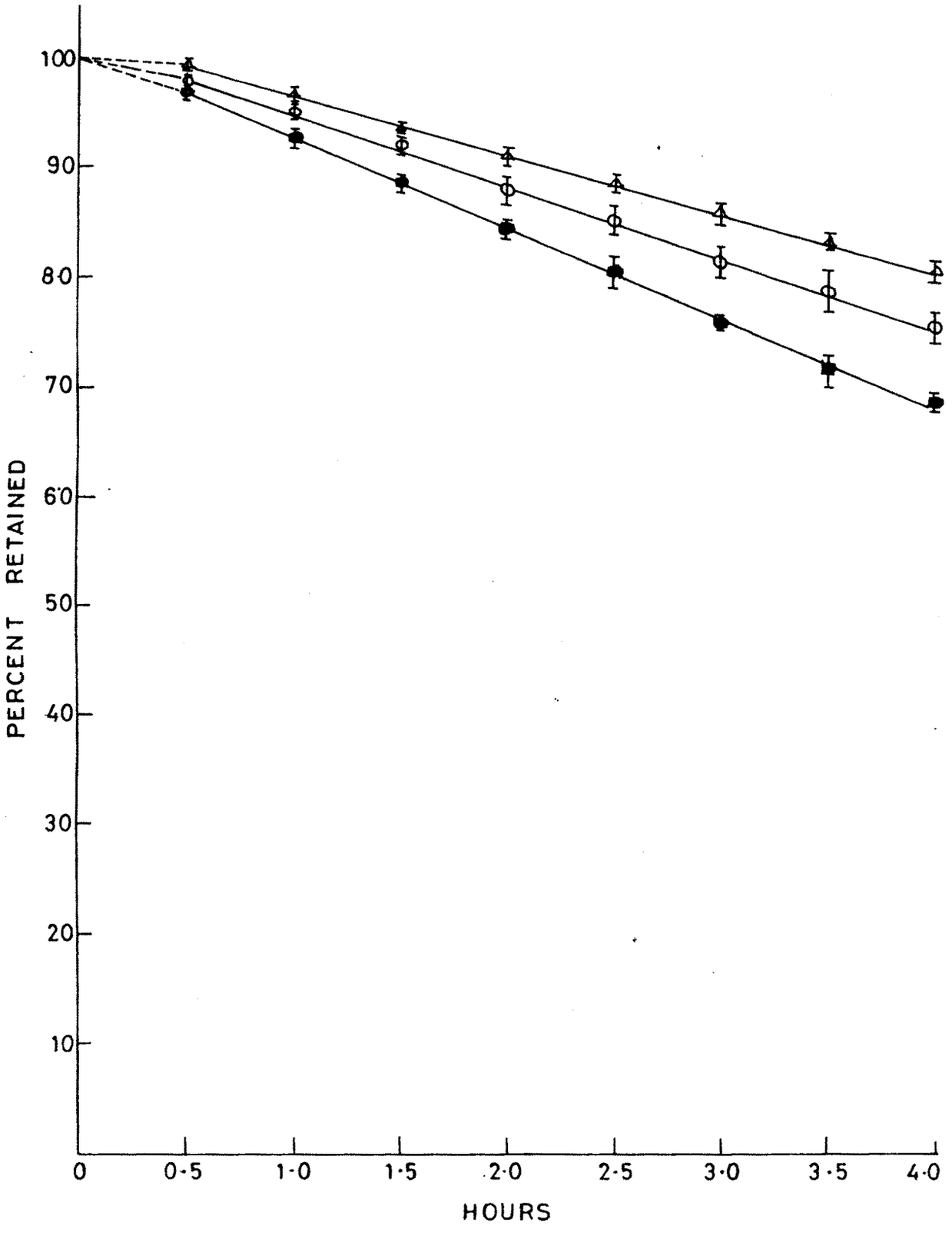


Figure II.15- Effect of particle Size and Size Distribution of the Encapsulated Drug Sample on Percent Drug Retained vs. Time. Key : O, Sample I; △, Sample II and ●, Sample III.

Table II.8 : Effect of Variation in Particle Size of the Drug Encapsulated . on Percent Drug Retained versus Time

Sr. No.	Time, x, (hr)	Percent Drug Retained, y,			Release Rate (K)=Slope= $\alpha = \frac{\{\Sigma xy - (\Sigma x \cdot \Sigma y/n)\}}{\{\Sigma x^2 - [(\Sigma x)^2/n]\}}$ ($\% \ hr^{-1}$)		
		Sample I	Sample II	Sample III	Sample I	Sample II	Sample III
1.	0.0	100.00	100.00	100.00			
2.	0.5	97.04	98.20	98.20			
3.	1.0	92.94	95.53	96.68	6.32	5.00	8.11
4.	1.5	88.68	92.07	93.35			
5.	2.0	84.56	88.80	91.32			
6.	2.5	80.49	85.25	88.52			
7.	3.0	76.31	81.24	85.63			
8.	3.5	71.89	79.30	83.61			
9.	4.0	68.36	75.35	80.88			
$\Sigma x = 18$		$\Sigma y=760.27$	$\Sigma y=796.34$	$\Sigma y=818.19$			
		$\Sigma xy=1398.82$	$\Sigma xy=1497.94$	$\Sigma xy=1562.80$			

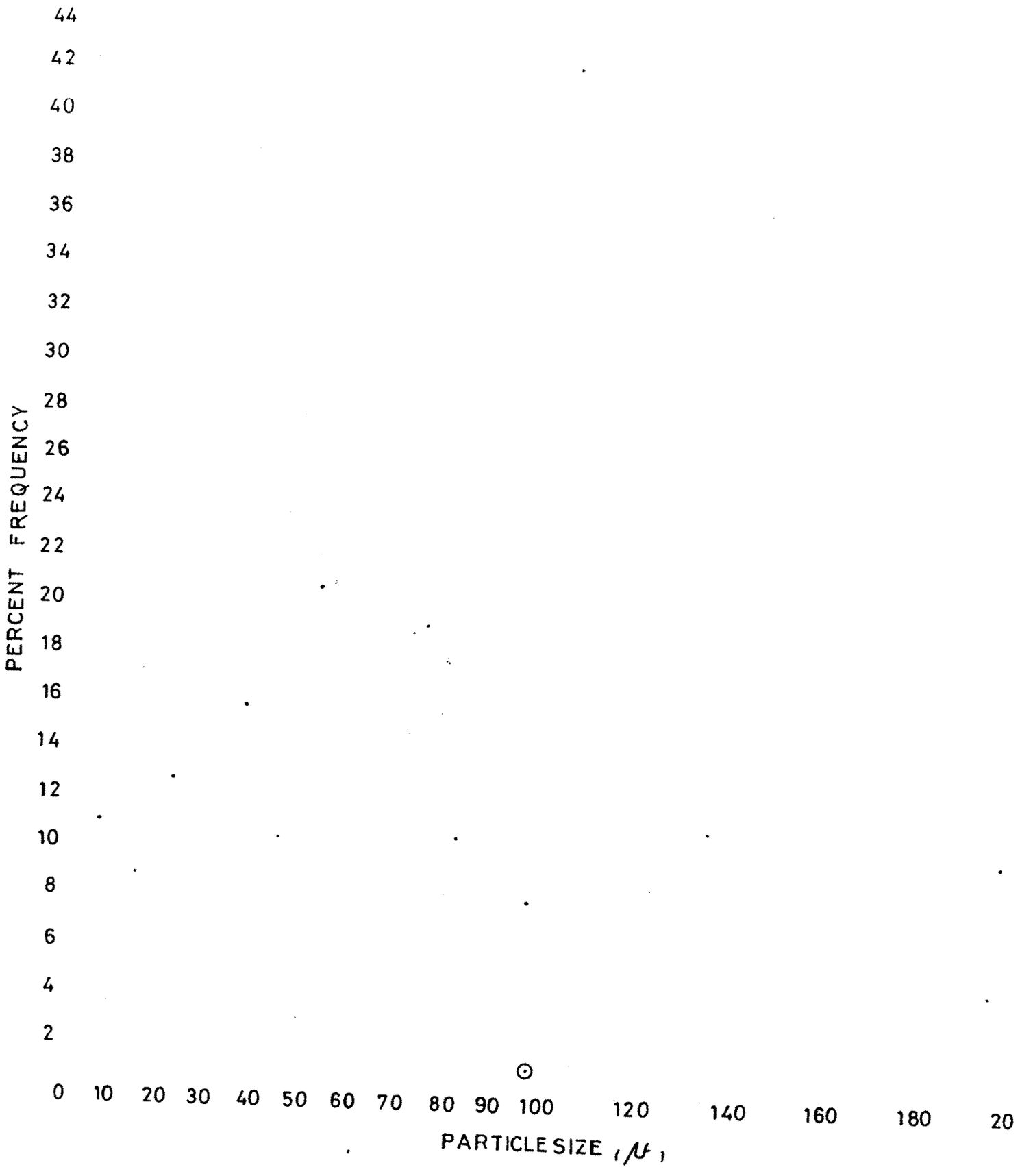


Figure II.16- Frequency Distribution plots of Different Samples of Powder, Key : O, Sample I; △, Sample II and ●, Sample III.

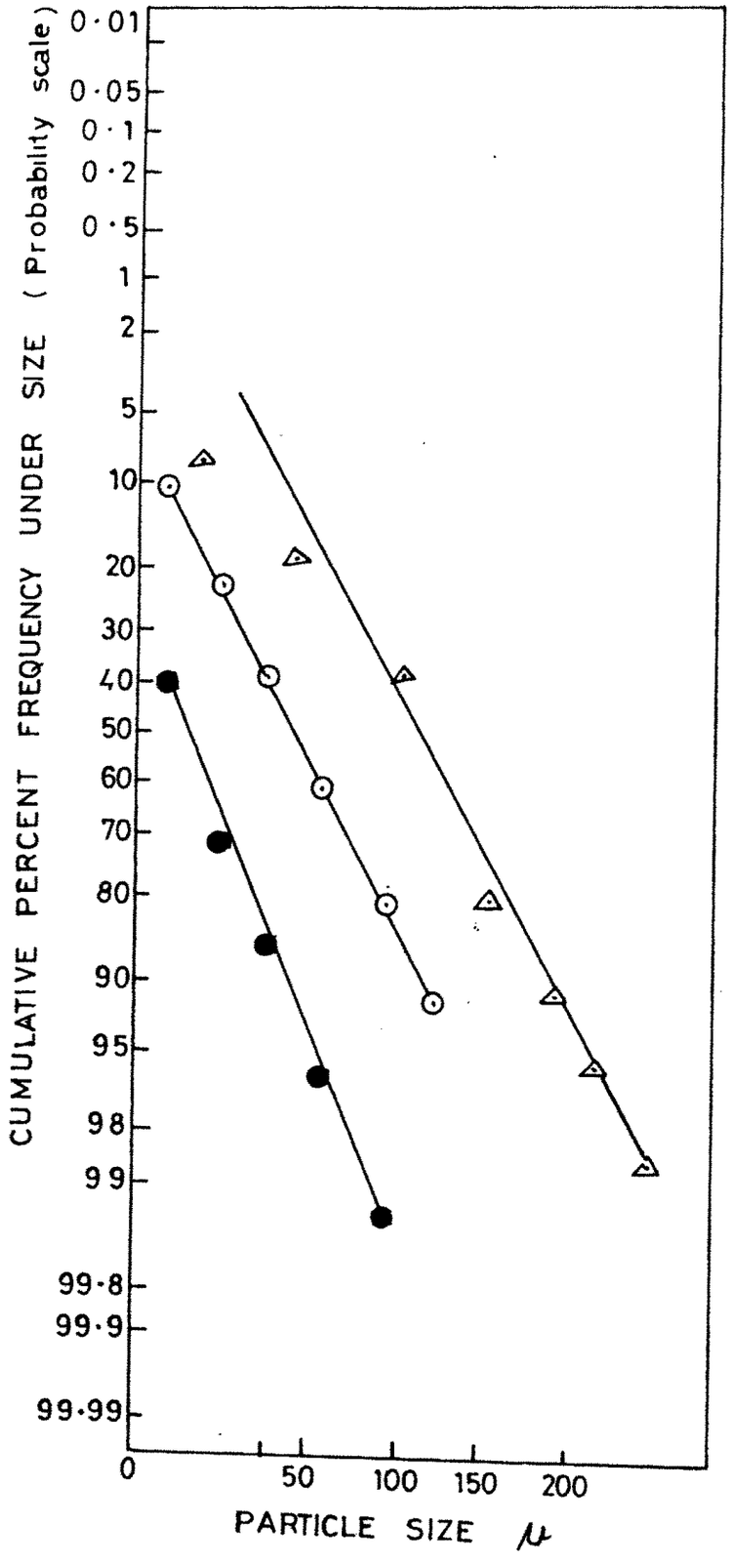


Figure II.17- Log-Probability Plot of Different Samples of Powder.

Key : O, Sample I; △, Sample II and ●, Sample III.

The mean statistical diameters of sample I, II and III correlated well with the geometric mean diameters dg, equal to 48.50, 85.0 and 17.5 μ, respectively, with corresponding standard deviations, calculated from the slope of the curve equal to 3.03, 1.67 and 2.8 μ as obtained from the log-probability plot, Figure II.17.

Figures II.18 and II.19 show the effect of different formulatory additives used on the release rate of the drug from the capsule. The use of lactose and microcrystalline cellulose as diluents, and magnesium stearate and talc as lubricants, did not significantly effect the release rate of the drug. Of the two wetting agents used, dioctyl sodium sulfosuccinate showed a statistically significant enhancement of the release rate, unlike polysorbate 80, which though showed some enhancement of the release rate, was not statistically significant. Figure II.20, clearly indicates the noninterference of dioctyl sodium sulfosuccinate and polysorbate-80 with λ max of tetracycline hydrochloride.

Table II.10 shows the analysis of variance, a sort of multiple t-test (14) of the above study where the effect of common formulatory additives on release rate of the drug, i.e. mean percentage drug retained at different interval of time, is being compared. The calculated values of F ratio being more than F value, obtained from F table (14) at $F_1 = 6$,

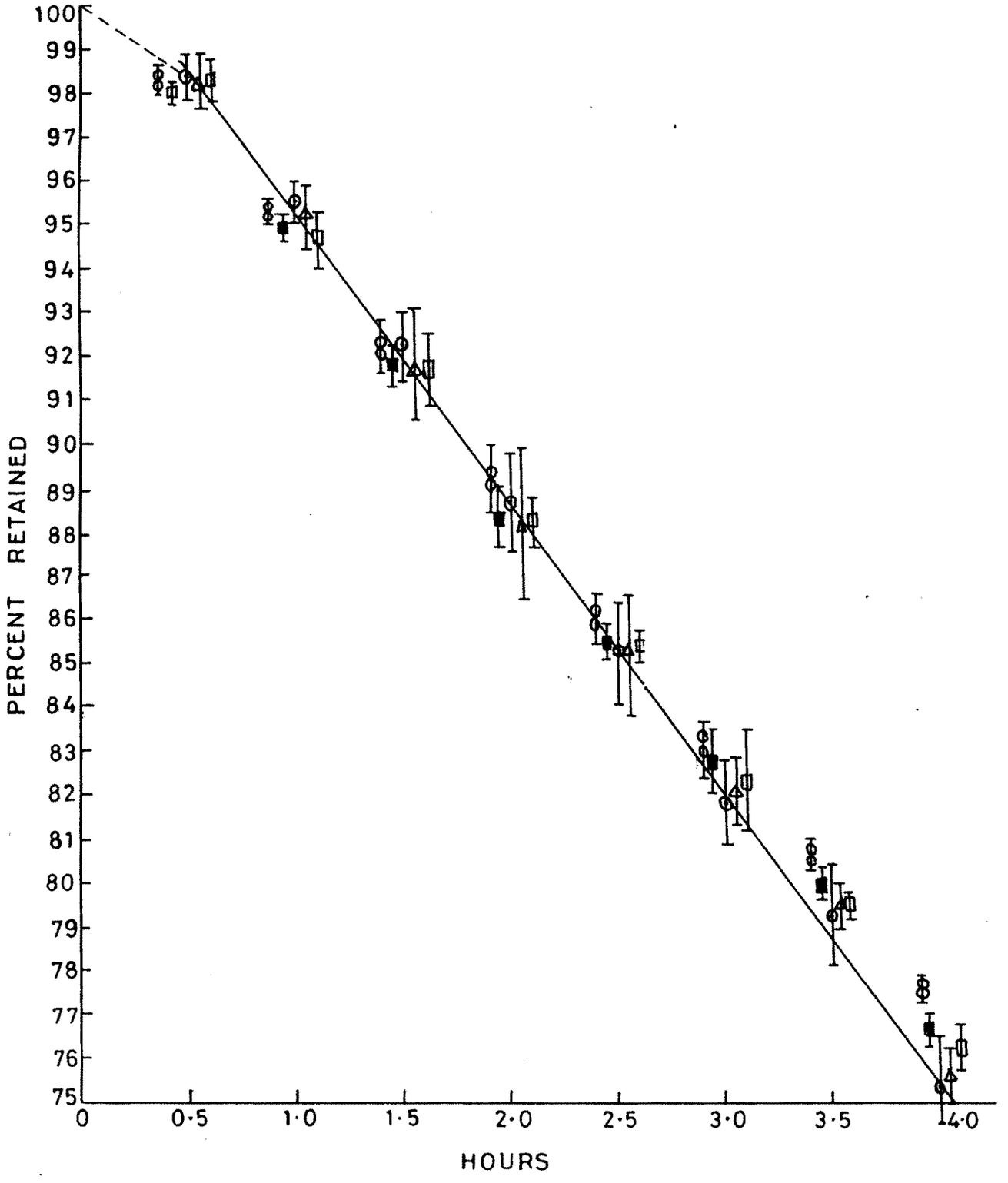


Figure II.18- Effect of Common Capsule Additives on Percent Drug Retained Vs. Time. Key : O, only drug : and in the presence of △, MCC; □, lactose; ●, Talc and ⚷, Magnesium stearate.

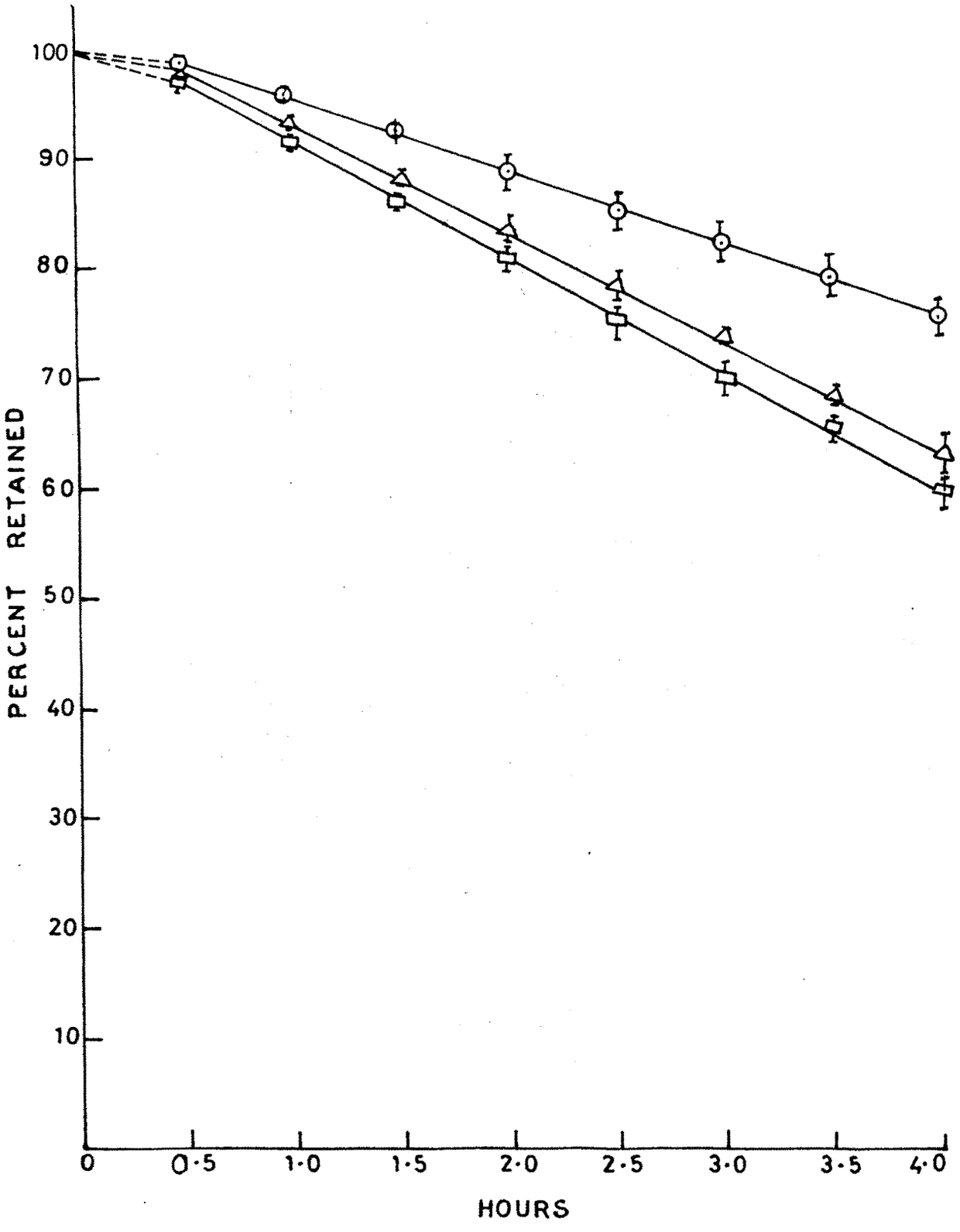


Figure II.19- Effect of Wetting Agents on Percent Drug Retained vs. Time.

Key : O, Only Drug and in the presence of □, Dioctyl Sodium Sulfosuccinate and △ Polysorbate 80.

F_2 = 56, p = 0.05, indicate that all the samples do not have the same release rate i.e. they have statistically significant difference.

Further to determine, which of the samples are different from each other, and which are not, least significant procedure (14) was applied. Table II.11 shows the ranked means; the calculated value of 5% allowance being 7.05, dioctyl sodium sulfosuccinate indicates a statistically significant difference in release rate ($P < 0.05$) at $t = 2.0$, $S_2 = 55.99$, $n_1 = 9$ and DF = 56, from the other additives studied.

CONCLUSIONS

GIT resistant capsule can be prepared by formalin vapour treatment under optimum set of conditions i.e. 90:50:30 corresponding to formalin gas contact time (min), drying temperature (°C) and drying time (min), respectively. The GIT resistance is confirmed by *in vitro* and *in vivo* studies. The CO_2 gas laser can be used to make accurate and precise minute pores for release of the contents. Variation in number and diameter of the drilled pores varies the rate of release of the drug from the capsule. Laser drilled pores with SD_1 and SD_2 type of size distribution are found satisfactory since the loss of encapsulated

Table II.11 : Ranked Means (Reference Table II.10).

Drug	Talc	Magnesium stearate	Microcrystalline Cellulose	Lactose	Polysorbate-80	Dioctyl sodium sulfo-succinate
88.48	88.70	89.10	83.54	88.38	82.78*	80.53

$t = 2.00$, $S_2 = 55.99$, ni, n = 9, 9; DF = 56, P = 0.05

5% Allowance = $t \quad S^2(\frac{1}{ni} + \frac{1}{nj}) = 7.05^*$

* Any two means not underscored by the same line are statistically significantly different.

content, when the capsules are subjected to friability test, is not more than 0.8%. The observation and the findings of the test carried out using methylene blue encapsulated capsule indicate that the drug release from this new system is based on the combined principles of dissolution and diffusion. The common capsule additives like lactose, microcrystalline cellulose, magnesium stearate and talc do not significantly effect the rate of release of the drug and so also the variation in pattern of drilling. Presence of dioctyl sodium sulfosuccinate significantly effects the release rate unlike polysorbate-80 which though enhanced the release, was not statistically significant.

From the results of this part of the study therefore, it can be concluded that the laser drilling technique could be successfully employed in designing the proposed controlled drug delivery system.

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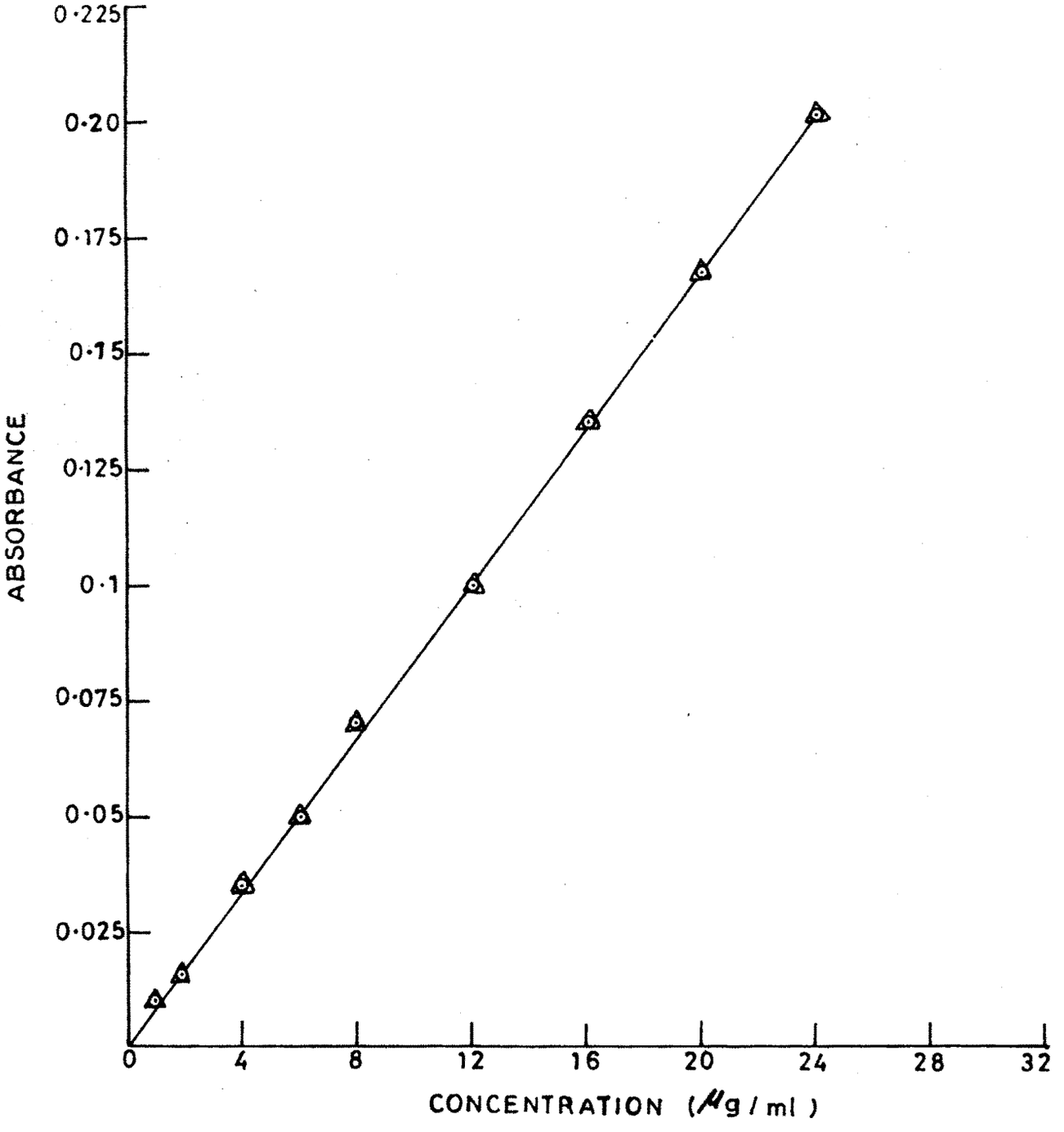

Figure III.1 - Beer's Plot for Colorimetric Determination of Aspirin.
Key: ○, Colour Developed after hydrolysis of Aspirin and
△, Colour developed using pure Salicylic acid.

the resultant solution to 50.0 ml with purified water, the red-colour produced was measured at 540 nm.

Complete hydrolysis of aspirin to salicylic acid with respect to alkaline hydrolysis time (30 min) was confirmed by comparing the concentrations vs. absorbance values obtained using pure salicylic acid[7] under similar experimental conditions (Figure III.1).

(b) Spectrophotometric Determination of Tetracycline - Figure III.2 shows the Beer's plot of tetracycline hydrochloride in different buffer solutions used in the study, at 353 nm.

(c) Spectrophotocolorimetric Determination of Tetracycline in urine - The analytical procedure used for the determination of tetracycline content in urine was based on the method of Mahgaub et al. (6). The method involves spectrophotocolorimetric measurement of colour of uranium-tetracycline complex, produced by heating pretreated urine samples (7) (prepared as described below), containing about 5 to 30 µg of tetracycline with 0.2 ml of 0.1% (w/w) uranyl acetate[8] solution in 1:1 mixture of dioxane : water, at 420 nm. λ max of uranium-tetracycline complex was determined by measuring the absorbance of 10 µg/ml solution at

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7. Salicylic Acid I.P., Hansa Chemical Works, Sion, Bombay 400 071 (INDIA).
8. Uranium Acetate, BDH, ENGLAND.

different wavelengths (Figure III.3). Beer's plot of uranium-tetracycline complex is shown in Figure III.4.

The urine sample was prepared by heating 1.0 ml aliquot with 1.0 ml of dioxane, 2.0 ml of 0.1 N HCl and 0.5 ml of 0.1% (w/v) solution of β-thiopropionic acid[9], in a waterbath for 30 min (polyethylene balls over the top of each tube were used to minimize the loss by evaporation during heating). The tubes were then cooled to room temperature, before the addition of uranyl acetate solution.

RESULTS AND DISCUSSION

A plot of percentage aspirin retained as a function of time as shown in Figure III.5 indicates that the slow release aspirin capsules failed to release the drug under the experimental conditions for 2.0 hr, unlike conventional capsules which released 50% of the drug in less than 30 min. The failure of slow release capsule to release aspirin may be attributed to the poor solubility of aspirin in water thus hindering the process of dissolution, the first step in the proposed mechanism of drug release from the new drug delivery system designed. Hence good solubility of the encapsulated drug may be an essential property of the drug if it has to be successfully encapsulated in the proposed

9. Thiolactic acid, Fluka AG. Chem. Fabrick, CH 9470, Bucks, ~~ENGLAND.~~ Switzerland

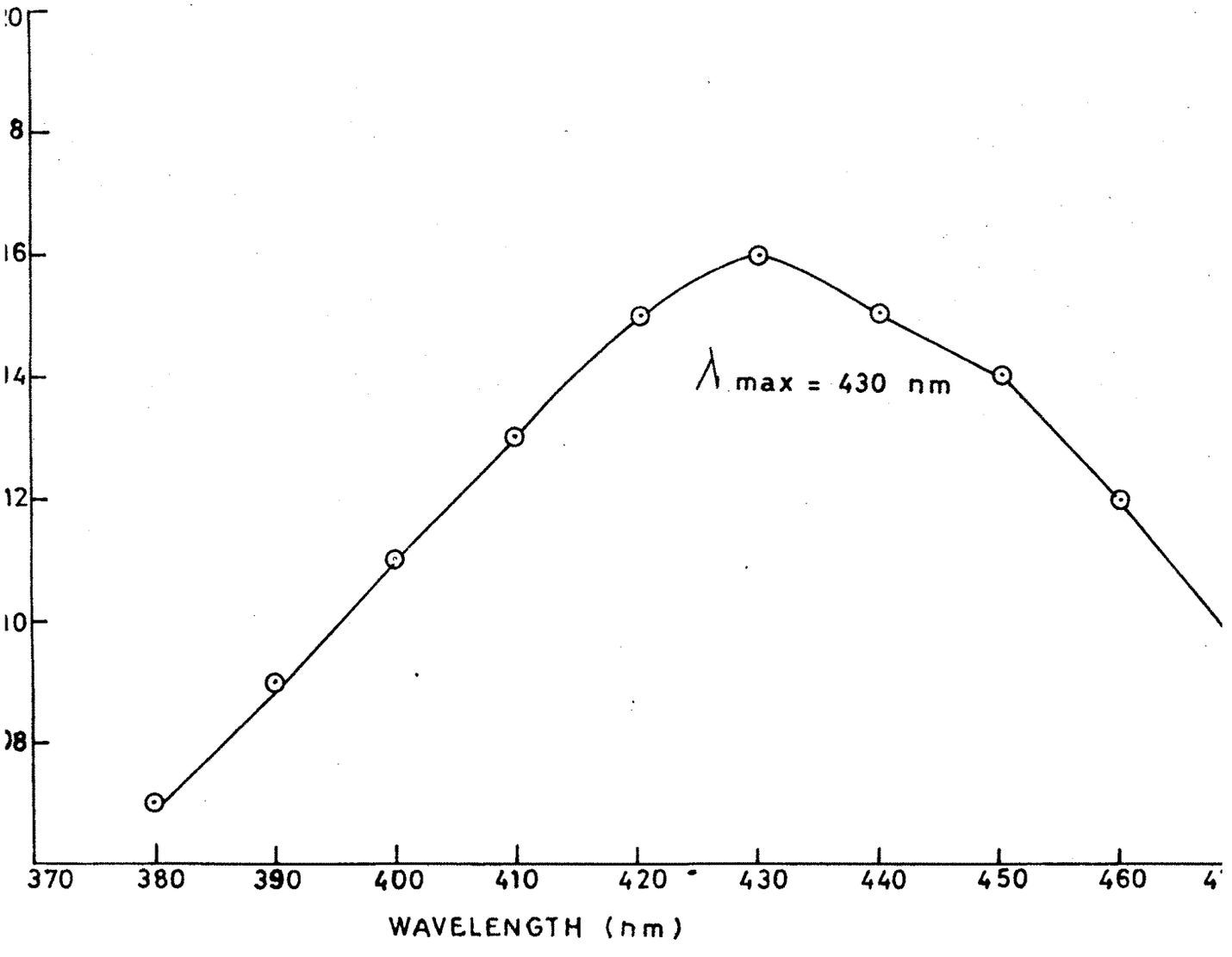

Figure III.3- λ_{max} of Uranium Tetracycline Complex.

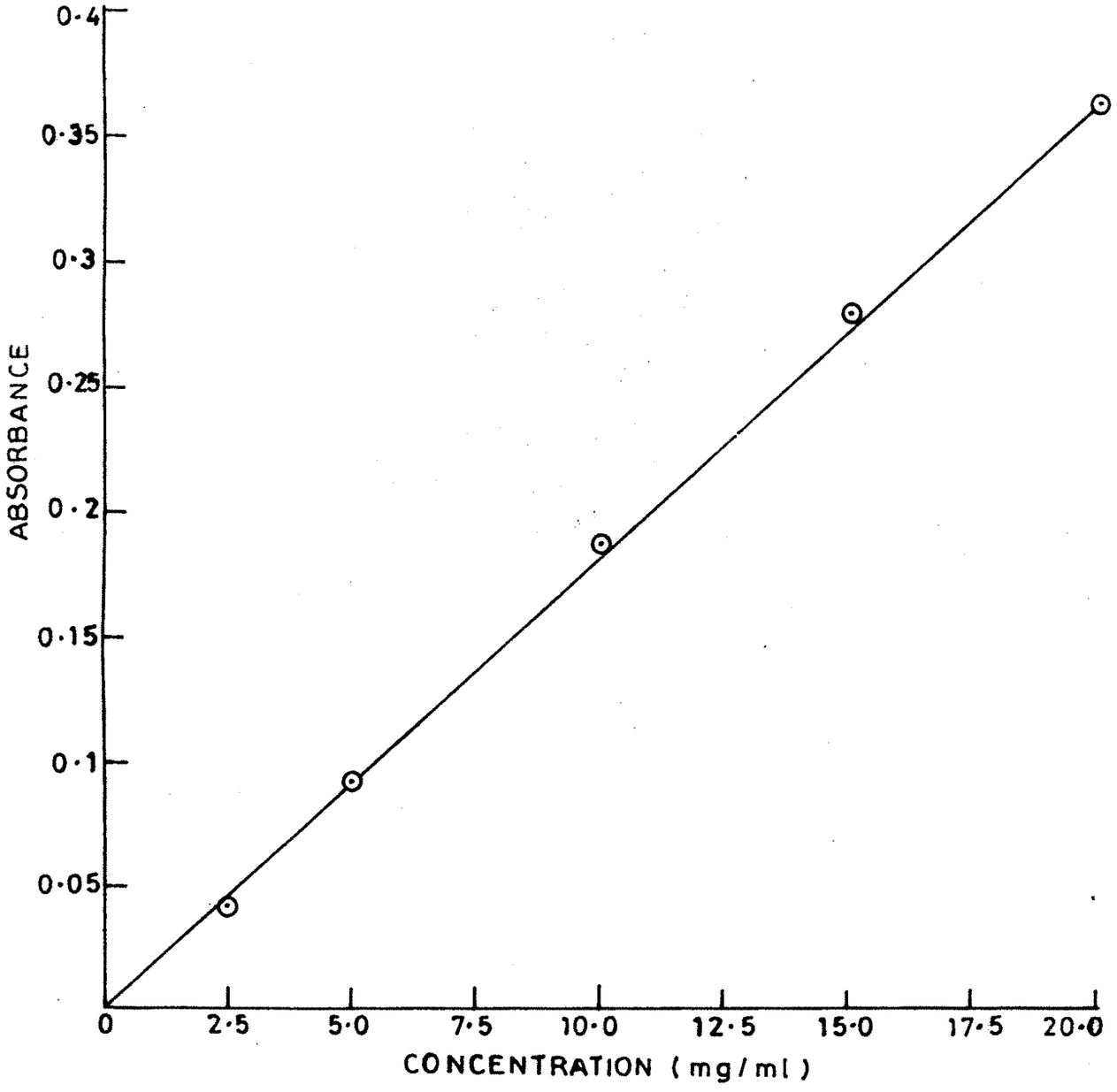

Figure III.4- Beer's Plot of Tetracycline-Uranium Complex for Colorimetric Determination of Tetracycline in Urine samples.

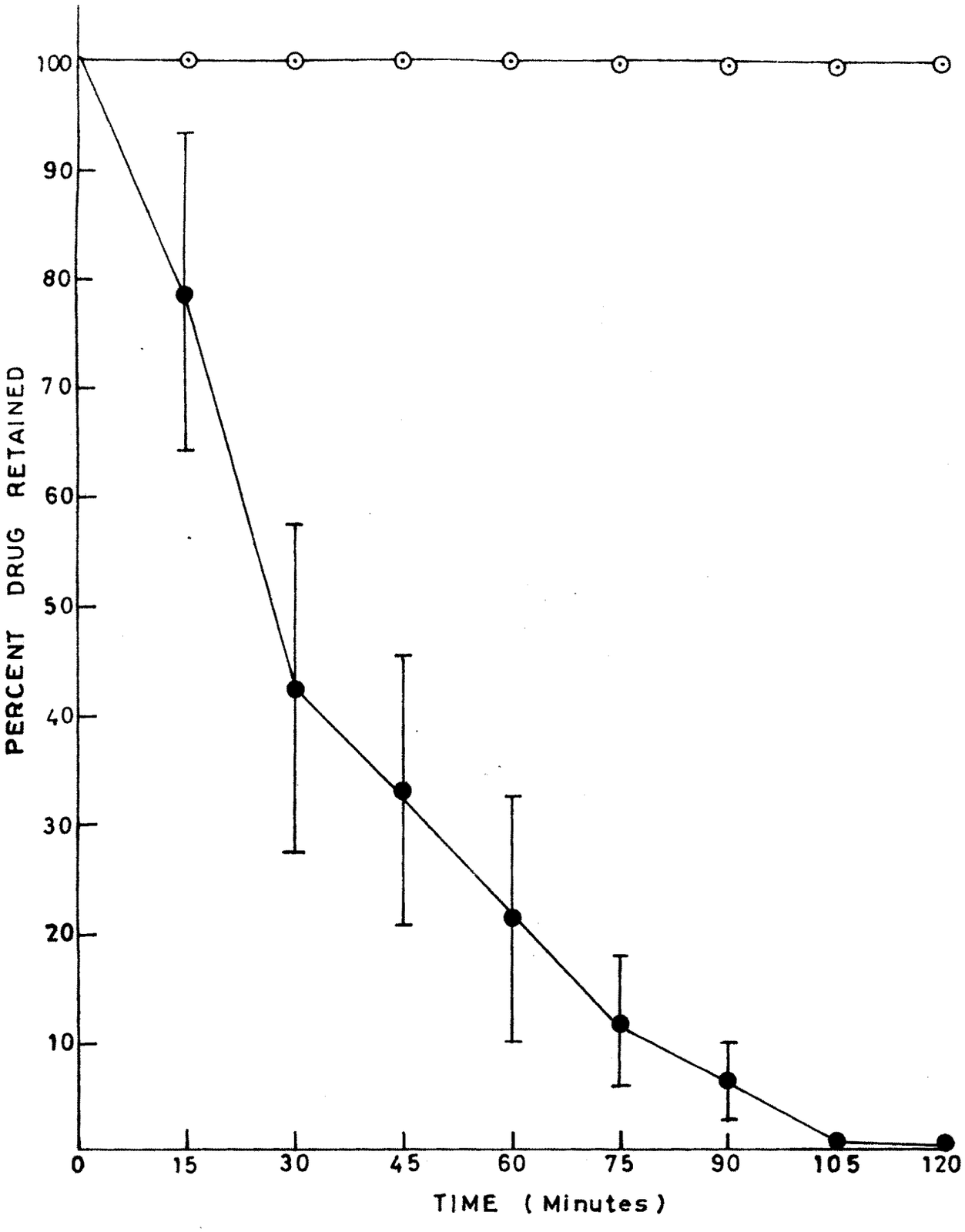

Figure III.5- Percent Aspirin Retained vs. Time for Slow Release Capsules Compared with Conventional Capsules,
Key : ○ , Slow Release Capsules and
● , Conventional capsules.

new drug delivery system (Scheme I, page 41). Figure III.6(a) shows the comparative *in vitro* dissolution plots of slow release and conventional tetracycline capsules. Slow release capsule released only 21.8% of the drug slowly at different rates under different pH conditions over a period of 8.0 hr, with the highest dissolution rate at pH 2.5 and 4.5, followed by pH 1.2 and 7.0, while, the rate was very slow in the alkaline pH. Unlike the slow release capsule, the conventional capsule showed a rapid dissolution rate at pH 1.2 with a $t_{50\%}$ value of less than 0.1 hr.

When subjected to urinary excretion rate study in human volunteers, the amount of tetracycline excreted through urine in the case of slow release capsules as shown in Figure III.6(b) is almost negligible, indicating that slow release capsule has failed to release the drug in the GIT after the capsule was administered orally. Unlike slow release capsule, in the case of conventional capsule, about 65.7 mg of the drug was excreted through urine in 48.0 hr. The failure of slow release capsule to release the drug in the GIT may be attributed to the failure of the GIT fluid to make an entry into the capsule through the pores due to low magnitude of attractive force offered by the core material encapsulated.

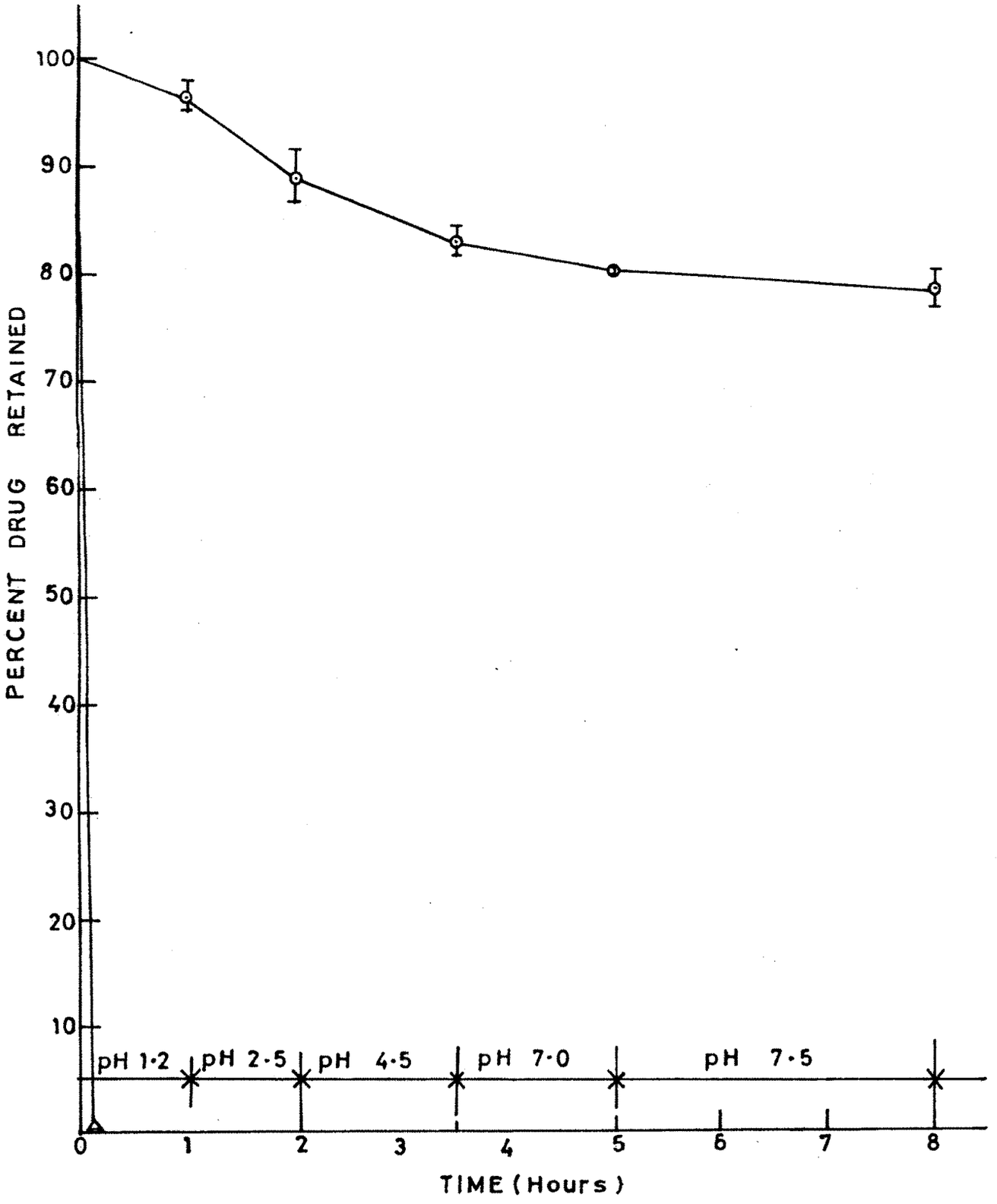

Figure III.6 (a)-Percent Tetracycline Hydrochloride Retained vs. Time for slow Release Capsules compared with Conventional Capsules. Key : ○ , Slow Release Capsules, and △ , Conventional Capsules.

CONCLUSIONS

Failure of aspirin to be released from the slow release capsule when subjected to *in vitro* dissolution studies indicates that the new drug delivery system may not be suitable for slow release capsule preparation of a slightly soluble drug. This finding also supports proposed mechanism of drug release from the new controlled drug delivery system i.e. the first step of drug release from the system is dissolution. The release of drug from such a system does not follow zero-order kinetics, under different conditions of pH. The failure of slow release tetracycline capsule in *in vivo* performance test suggests that the modification in core formulation to improve the attractive force imparted by the core material and thus facilitate the entry of GIT fluid in to the capsule, could be a study worth trial.