C H A P T E R I I I B

B. USE OF NEW LASER DRILLED CONTROLLED DRUG DELIVERY SYSTEM IN DESIGNING SLOW RELEASE CAPSULE

The earlier experimental findings of our study (Chapter II and III-A) indicate that :

i) *in vitro* release of tetracycline hydrochloride from the slow release capsule is pH dependent;

ii) when subjected to *in vivo* studies, the slow release capsule administered orally failed to release the encapsulated tetracycline hydrochloride in the GIT of human volunteers, may be because the GIT fluid failed to make an entry into the capsule as a result of low attractive force offered by the encapsulated core material; and

iii) variation in number of drilled pores considerably influences the release rate of encapsulated tetracycline hydrochloride from the laser drilled capsules. Increase in number of laser drilled pores from 50 to 100, keeping the diameter same, almost doubled the release rate of tetracycline hydrochloride in 0.1 N HCl (Chapter I, p.71-72).

The use of channeling agent has been reported (8), to facilitate the attraction of gastric fluid into an inert

plastic matrix having thousands of small passages, encapsulated with drug in solid form, in designing prolonged action pharmaceuticals, based on the method of diffusion of drug from porous inert carriers. Succinic acid was used in sustained release tablet matrix formulations to overcome the effect of pH on the release of tetracycline hydrochloride, since succinic acid maintains acid media within the tablet matrix, even when the tablet matrix is exposed to an environment of varying pH (9).

In view of the above experimental findings and reported information, the objectives of this part of the study were to :

i) work on the possibility of using sucrose as a channeling agent to improve the attractive force of the core material to imbibe the surrounding fluid, to use succinic acid as an acidifier to overcome the effect of pH on tetracycline release from the capsule and optimize the core formulation with these additives along with lactose, by preliminary studies;

ii) prepare laser drilled slow release capsule using the best formulation, based on the results of the preliminary studies and carry out comparative *in vitro* and *in vivo* test on these laser drilled slow

release capsules using similarly formulated conventional capsule as the control; and

iii) compare the stability of laser drilled slow release capsule with that of conventional capsules under different conditions of storage.

EXPERIMENTAL

Selection of Formulation - Preliminary studies were carried out on eight formulations listed in Table III.1 encapsulated separately, in No. 0, GIT resistant laser drilled capsules with 50 minute pores of 100 um size made on the body of the capsule. The capsules were sealed with a suitable GIT resistant material and then subjected to *in vitro* dissolution studies.

Preparation of Slow Release Capsules - No. 0, GIT resistant, laser drilled capsules with 100 minute laser drilled pores of an average diameter of about 100 um made on the body of the capsules were encapsulated with contents prepared as per formulation 3 (Table III.2), which showed the best *in vitro* performance in the preliminary studies. The capsules were then sealed with a suitable GIT resistant material.

Preparation of Conventional Capsules - Conventional No. 0

Table III.1 : Eight Different Formulation Variations Studied to Optimize The Core Formulation in Preparing Laser Drilled Slow Release Capsules.

Formulation No.	Name and Quantity (mg) of Ingredients			
	Tetracycline hydrochloride with 0.5% (w/w) dioctyl sodium sulfo-succinate	Sucrose[1]	Succinic Acid[2]	Lactose
1	Equivalent to 250.0 mg of tetracycline hydrochloride	-	-	175.0
2	"	25.0	-	153.0
3	"	50.0	-	120.0
4	"	100.0	-	80.0
5	"	191.0	-	-
6	"	-	25.0	153.0
7	"	50.0	12.5	110.0
8	"	100.0	25.0	45.0

1. Saccharose pure, P.P.H. Polaskie, odczynniki Chem-Gliwice, POLAND

2. Acid Succinic, Burgoyne-Burbidges & Co., 25, Dalal Street, Bombay-400 001, (INDIA).

Table III.2 : Percent Drug Retained Versus Time (*in vitro*) under Different pH Conditions For Eight Formulatory Variations Studied in Preparing Laser Drilled Slow Release Capsules.

Time hr	pH	Percent Drug Retained For Different Formulations							
		1	2	3	4	5	6	7	8
1.0	1.2	96.5	95.2	86.0	83.6	86.0	97.6	88.0	88.8
2.0	2.5	89.5	87.0	65.2	66.8	72.4	80.8	71.6	77.8
3.5	4.5	83.0	77.0	45.4	53.2	59.2	64.0	62.8	57.6
5.0	7.0	80.5	73.8	36.8	46.0	54.4	55.0	56.8	47.6
8.0	7.5	76.0	71.0	32.4	40.8	51.4	50.6	50.8	44.4

hard gelatin capsules[1] were filled with same contents as used in the preparation of slow release capsules.

In Vitro Dissolution Test - Dissolution tests were carried out using a basket stirrer of USP XX (3) dissolution apparatus at a stirrer speed of 100 rpm and the dissolution medium temperature was held at 37±0.5°C. 300.0 ml each of pH 1.2, 2.5, 4.5, 7.0 and 7.5 dissolution media were prepared and changed at different interval of time as per the method recommended in NF XIV (4) under Timed Release Tablets and Capsules In Vitro Test Procedure. 5.0 ml samples were withdrawn at the end of 1.0, 2.0, 3.5, 5.0 and 8.0 hr intervals, filtered and analysed at 353 nm, in a spectrophotometer[2].

In Vivo Study - A crossover study was carried out with one week washout period in between, on four healthy human volunteers weighing between 55.0 and 70.0 kg, 26-34 years old as the subjects. The treatments consisted of slow release capsules (Treatment I) and conventional capsules (Treatment II). The subjects were advised fasting 3.0 hr before and after receiving each treatment. Capsules were administered orally with 200.0 ml of water. Urine samples

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1. Hard Gelatin capsules, Associated Capsules Pvt. Ltd., Kandivali, Bombay 400 067 (INDIA).
2. Model VSU 2-P, C.Z.Spectrophotometer, GERMANY.

were collected at 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 18.0, 24.0, 30.0, 36.0, 42.0 and 48.0 hr post administration. The urine volumes were measured and recorded after each collection and aliquots were frozen until assayed.

Spectrophotocolorimetric Determination of Tetracycline in Urine - The method used was similar to spectrophotocolorimetric determination of tetracycline in urine as described in Chapter III.A page .

Stability Studies - Three batches each of slow release and conventional capsules, assayed, packed and sealed in colourless glass vials, were stored at four different storage conditions, such as refrigerator, room temperature & humidity, 35°C-65% RH, and 50°C, for three months. Samples were withdrawn periodically at 15, 30, 45, 60, 75 and 90 days time intervals and assayed by the method described in USP XX (10) under determination of 4-epianhydro tetracycline in tetracycline dosage forms. The residual formalin content was measured (11) after 90 days and the capsules were also subjected to <u>in vitro</u> and <u>in vivo</u> GIT resistance test (Chapter II, page 55).

RESULTS AND DISCUSSION

The results of the preliminary studies as shown in Figure III.7, Table III.2 indicate that the addition of sucrose as a channeling agent, in combination with lactose (Formulation 2, 3 and 4) in an optimum concentration considerably influences the release rate of the drug from the slow release capsule. 50.0 mg of sucrose mixed with 120.0 mg of lactose as the diluent per capsule gave the best results as compared to the other formulations studied, releasing about 67.6% of the drug (*in vitro*), in 8.0 hr. However, addition of succinic acid did not considerably increase the release rate of the drug in the alkaline pH as expected, on the contrary, use of succinic acid in combination with sucrose reduced the amount of drug released in the acidic pH, may be because succinic acid makes the environment highly acidic (below 2.5) affecting the solubility of the tetracycline hydrochloride. The dissolution profile of laser drilled slow release capsule; prepared as per formula 3 i.e. tetracycline hydrochloride. 250.0 mg with a mixture of 50.0 mg of sucrose and 120.0 mg of lactose as the diluent (which gave the best result in preliminary studies), as compared to conventional capsule, Figure III.8 indicates that slow release capsules with 100 laser drilled pores on the body released about 74.4% of the drug slowly at different pH conditions over a period of 8.0 hr and the

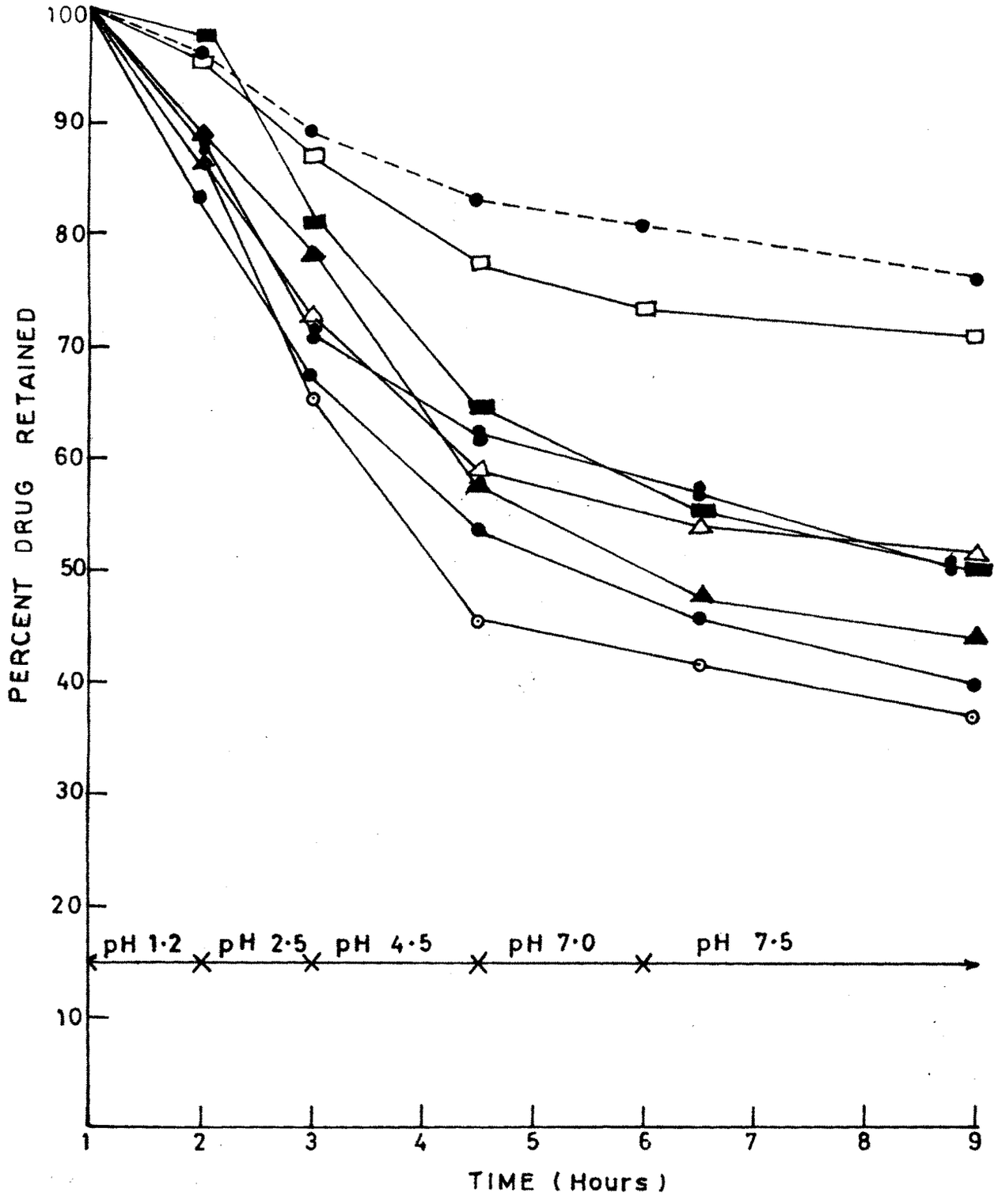

Figure III.7 - Percent Drug Retained vs. Time for the Eight Formulations Studied to Optimise the Core Formulation. Key : ●–● Formulation 1, □ , Formulation 2; ○ , Formulation 3; ● , Formulation 4; △ , Formulation 5; ■ , Formulation 6; ⁞ , Formulation 7 and ▲ , Formulation 8.

time required for the capsule to release 50% of the contents ($t_{50\%}$) was about 2.5 hr. The laser drilled slow release capsules showed 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 7.5. Unlike the slow release capsule the conventional capsule showed a rapid dissolution rate at pH 1.2 with a $t_{50\%}$ value less than 0.1 hr.

A plot of logarithm of excretion rate of unchanged tetracycline in urine versus time (determined at the mid point of urine collection time) was linear (Figure III.9) indicating that tetracycline was eliminated following first-order kinetics. The elimination half-lives, the time required for the excretion rate to decrease to one half on the descending part of the plot (12) were calculated from the resultant slope and elimination constants (K_e) were calculated based on the equation $K_e = \frac{0.693}{t_{0.5}}$. No significant difference ($P > 0.01$) in the value of plasma half-life ($t_{0.5}$), 8.6 hr and elimination constant (K_e), 0.083 mg/hr were obtained with Treatment I as compared to the values $t_{0.5}$, 7.75 hr and K_e 0.09 mg/hr obtained with Treatment II.

Barr *et al.* (13) reported the value of $t_{0.5}$ for tetracycline as 9.0 hr after oral administration of

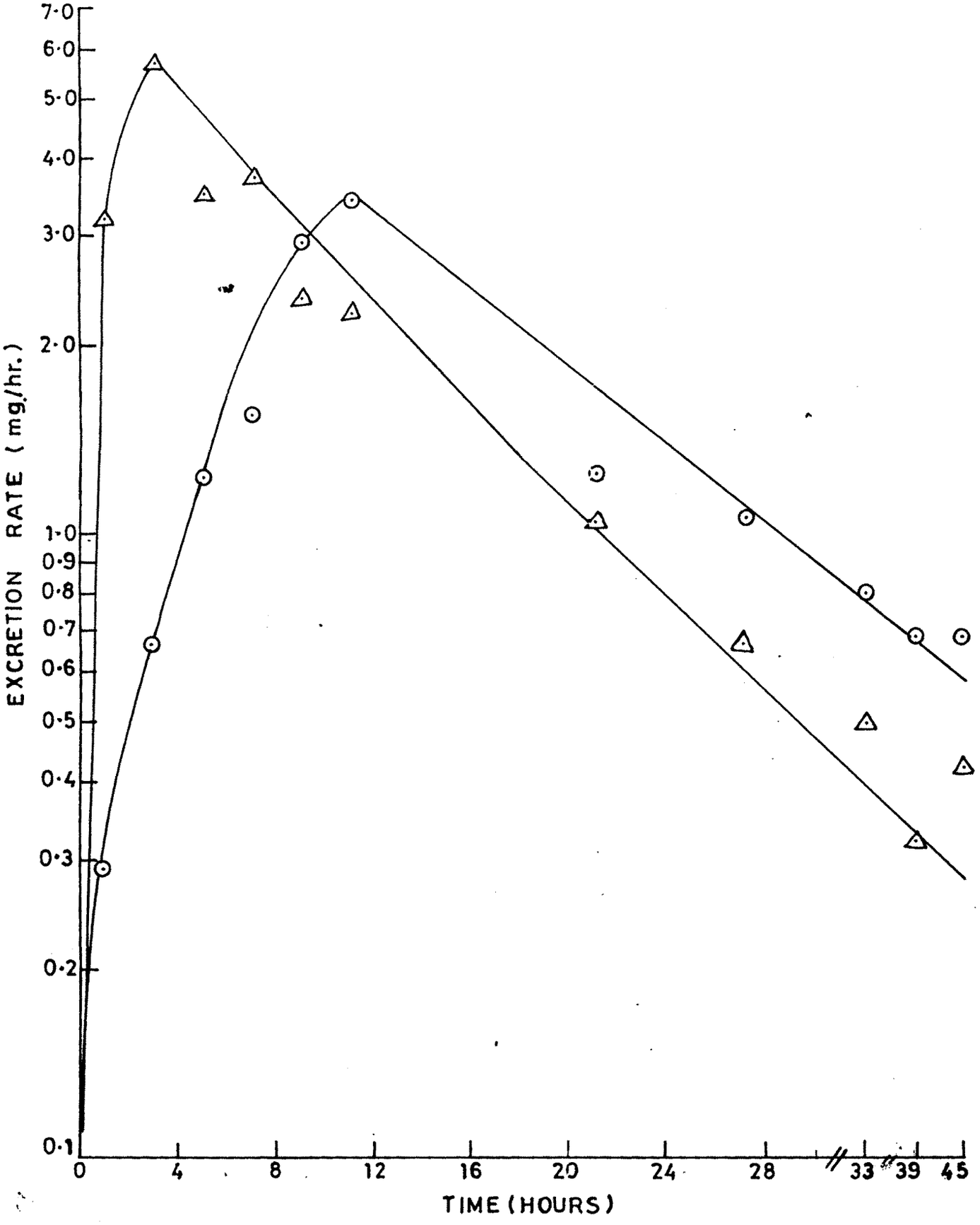

Figure III.9- Semilogarithmic Plot of Average Excretion Rate vs. Time (Determined at the Points of Each Urine Collection Time) For the Two Treatments Studied. Key : ○, Treatment I and △, Treatment II.

250.0 mg of tetracycline hydrochloride. Approximate serum level concentrations of tetracycline at different intervals of time as shown in Figure III.10 were predicted, based on the relationship between urinary excretion rates and serum concentrations (determined at the midpoint of each urine collection time) reported by Barr et al. (13). The predicted values of t_{max}, 11.0 hr and C_{max}, 0.067±0.02 µg/ml for Treatment I were considerably different from predicted t_{max}. 3.0 hr and C_{max} 1.10±0.20 µg/ml values for Treatment II. Kunin and Finland (14) and Lovering et al. (15) reported the value of t_{max}, 2.5 hr and C_{max} 1.33 µg/ml respectively, after oral administration of 250.0 mg tetracycline hydrochloride. Table III.3 illustrates the comparison of urinary excretion rate of tetracycline from Treatment I and Treatment II by paired t-test at the midpoint of each urine collection time. The mean excretion rate of Treatment I at 1.0, 3.0, 5.0, 7.0 and 9.0 hr was significantly lower ($P < 0.01$, 0.01, 0.2, 0.05, and 0.2 respectively, than that of Treatment II. The high excretion rate of Treatment II between 1.0 and 9.0 hr may be attributed to the immediate release of contents from the conventional capsules in comparison with Treatment I, whereas the observed low excretion rate of Treatment I could be because of the slow release of the contents from the slow release capsule under GIT pH conditions as apparent from

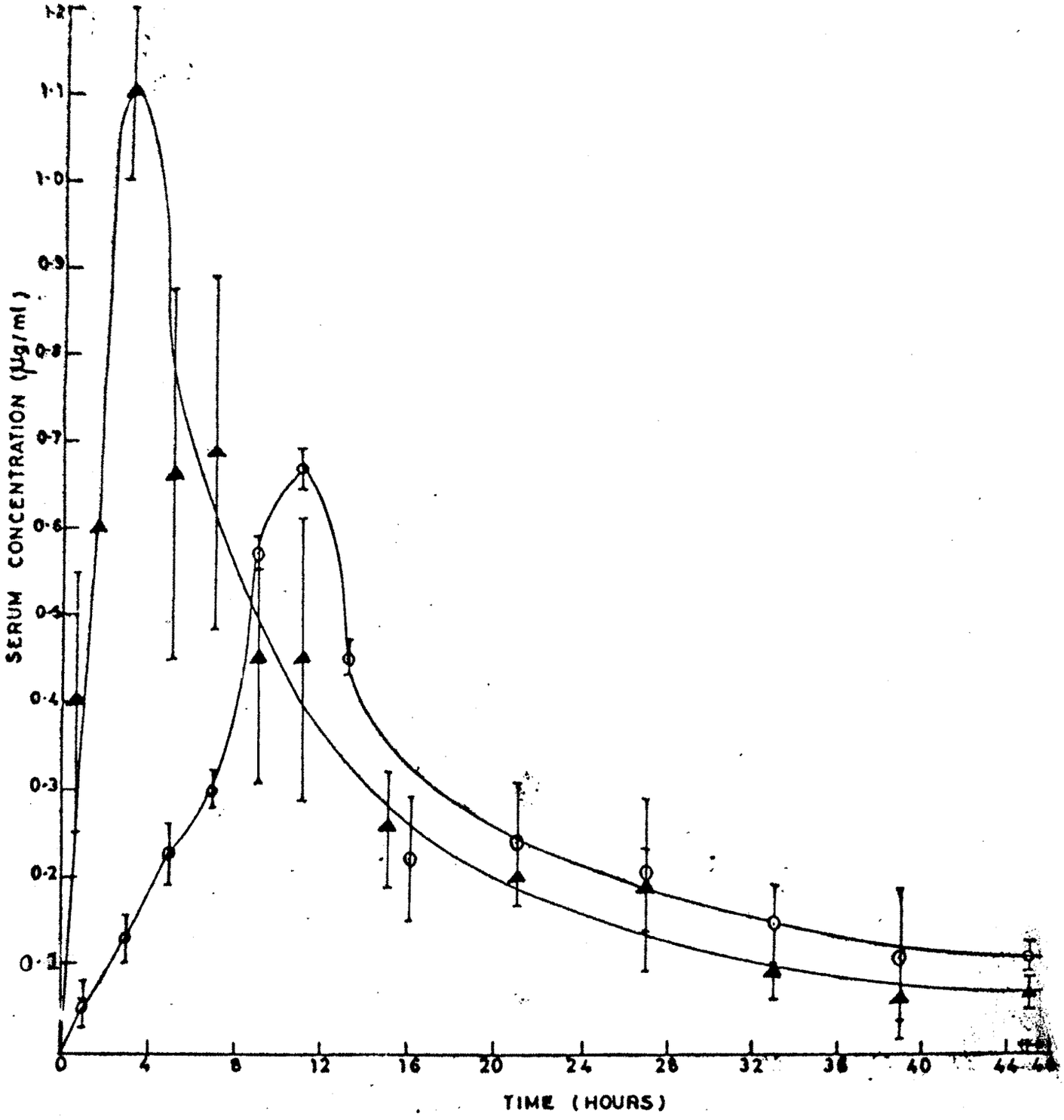

Figure III.10- Predicted Average Serum Level Concentration (±SE) As a Function of Time for the Two Treatments.
Key : O , Treatment I and ▲ Treatment II.

Table III.3 : Mean Urinary Excretion Rate Compared by Paired t-test at the Midpoint of Each Urine Collection Time.

Time (hr)	Urinary Excretion Rate Mean ± SE		Calculated t-value	Significance level@
	Treatment I	Treatment II		
1.0	0.28±0.08	3.14±0.39	t = 14.30	P <0.01
3.0	0.67±0.26	5.76±0.87	t = 6.28	P <0.01
5.0	1.23±0.31	3.47±0.46	t = 2.23	P <0.2
7.0	1.55±0.21	3.63±0.57	t = 3.5	P <0.05
9.0	2.98±0.21	2.37±0.43	t = 2.01	P <0.2
11.0	3.46±0.2	2.29±0.47	t = 2.71	P <0.1
21.0	1.25±1.1	1.05±0.61	t = 1.68	P <0.2
27.0	1.08±0.8	0.97±0.56	t = 2.44	P <0.1
33.0	0.81±0.37	0.50±0.08	t = 2.44	P <0.1
39.0	0.59±0.27	0.32±0.16	t = 3.17	P <0.1
45.0	0.59±0.29	0.43±0.05	t = 4.15	P <0.05

@ t value, DF = 3.

the *in vitro* dissolution results (Figure III.8). Further the mean excretion rates from Treatment I at 11.0, 21.0, 27.0, 33.0, 39.0 and 45.0 hr were significantly higher ($P < 0.1$, 0.2, 0.1, 0.1, 0.1 and 0.05 respectively) than that obtained for Treatment II. The higher mean excretion rate of Treatment I at later hours could be because the slow release capsule continues to release the drug at duodenal and small intestinal region of the GIT, under the prevailing pH conditions as apparent from the *in vitro* dissolution results, giving a delayed peak plasma level (t_{max}, 11.0 hr) as seen in Figure III.10). In the mean time in the case of conventional capsules, more than 50.0% of the drug administered would have been already excreted resulting in low excretion rates from Treatment II in the later hours.

The observed considerably high t_{max} value and C_{max} value; significantly low excretion rate in the initial hours and subsequent significantly high excretion rate in the later hours and insignificant difference in $t_{0.5}$ and K_e value with Treatment I as compared with Treatment II, indicate that slow release capsule must have released the contents slowly at different rates depending on the site at which it is present in the GIT and the favourable condition existing therein for dissolution and absorption of tetracycline, thus avoiding dose dumping in the stomach and maintaining high

blood levels for a longer duration of time, without loss of bioavailability, possibly giving more time for the drug in the blood circulation to interact with the site of action.

Figure III.11 illustrates the logarithm of per cent drug retained under different storage conditions, as a function of storage time. Storage of slow release and conventional capsules at refrigerated and room temperature and humidity conditions for 3 months did not show apparent degradation, while storage under accelerated conditions such as 50°C and 35°C-65% RH showed degradation of tetracycline following first-order kinetics. However, slow release capsules did not show any apparent differences in the rate and extent of degradation in comparison with the conventional capsules indicating that making minute pores on the walls of the capsules does not affect the stability of the contents. Slow release capsule stored at accelerated conditions also showed considerable reduction in residual formalin content, slightly affecting the *in vitro* and *in vivo* GIT resistance of the capsules, however, storage under refrigerated condition did not affect the residual formalin content.

CONCLUSIONS

Laser drilled slow release tetracycline hydrochloride capsules with 50 drilled pores on the body, prepared using

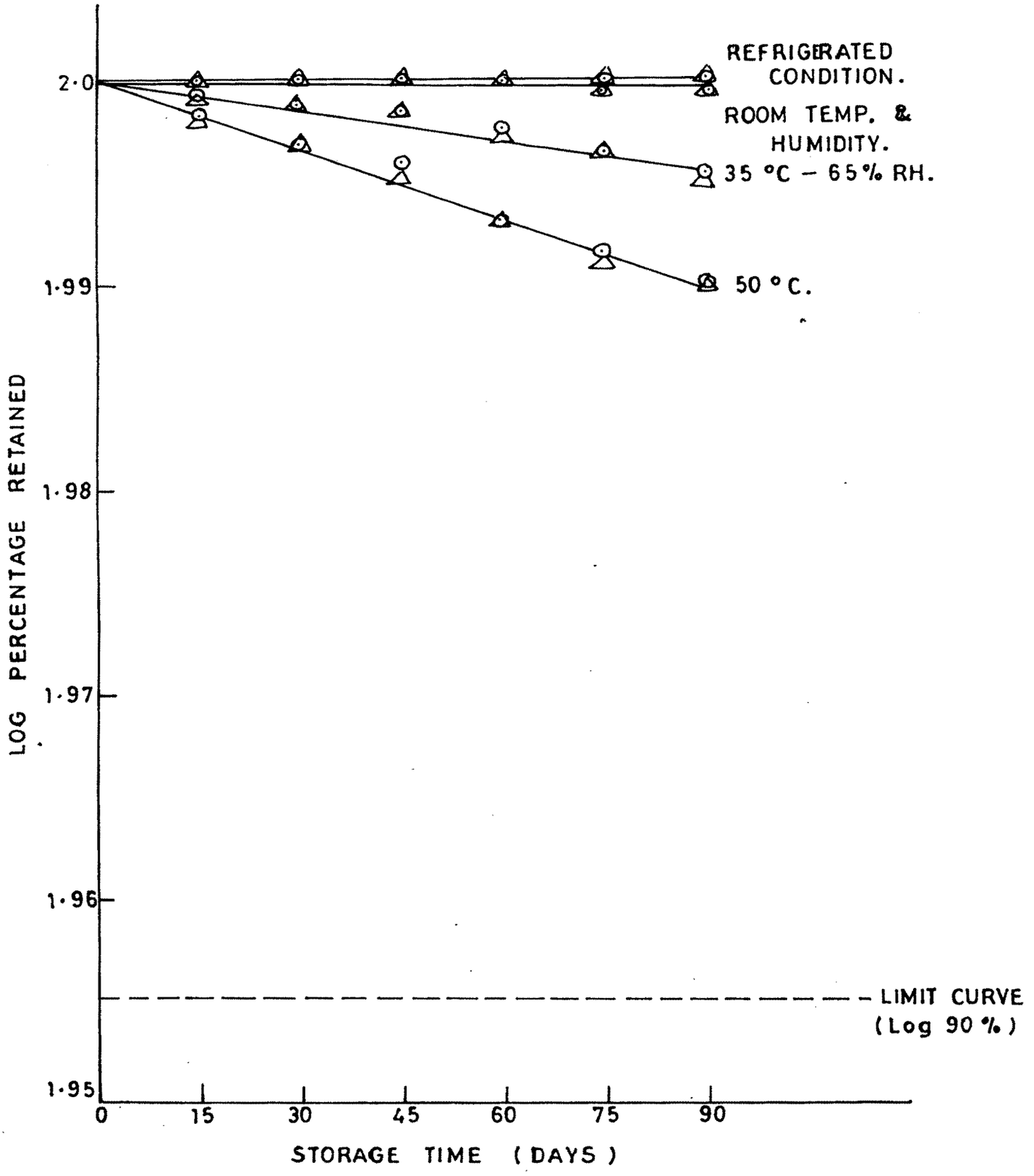

Figure III.11- Log-Percentage of Tetracycline Retained as a Function of Time for the Two Capsules Studied; Under Different Conditions of Storage.
Key : ○, Slow Release Capsules and △, Conventional Capsules

lactose as the diluent released only 21.8% of the drug in 8.0 hr, when subjected to *in vitro* dissolution studies while it failed to release the drug *in vivo*, when administered orally to human volunteers. Use of 50.0 mg sucrose as the channeling agent in combination with lactose as the diluent increased amount of the drug released *in vitro* from 21.8% to 67.6%. Further increase in number of drilled pores from 50 to 100 increases the amount of the drug released *in vitro* from 67.6% to 74.4%. The release of the drug in all the above cases is pH dependent and use of succinic acid to overcome the effect of pH on release of tetracycline hydrochloride from these capsules has no effect, however, it increases the amount of the drug released in neutral and alkaline pH, but, simultaneously reduces the amount of the drug released in the acidic pH, with no overall increase in the amount of drug released. Hence a drug having pH dependent solubility characteristics does not follow zero-order release pattern, when encapsulated in laser drilled capsules.

The conventional capsule releases 100.0% of the drug in less than 10 min in comparison to slow release capsule which releases only 74.41% of the drug in 8.0 hr, when subjected to *in vitro* dissolution studies. The slow release capsules performed well when subjected to *in vivo* studies giving low C_{max} and high t_{max} with significantly similar $t_{0.5}$

and K_e values as compared to conventional capsule. Hence use of sucrose as a channeling agent, as desired, helps the release of the drug *in vivo* possibly by increasing the attractive force of the core material and facilitating the imbibition of surrounding gastric fluid into the capsule.

The possible advantage of such laser drilled slow release capsule could be :

i) The potential hazard associated with encapsulation of highly soluble drug in conventional hard gelatin capsule, due to sudden release of such compounds resulting in localized irritating concentrations of the drug (16), could be overcome.

ii) The drug with narrow therapeutic range could be administered by the oral route safely because of relatively low C_{max} value.

iii) The therapeutic efficacy of the drugs may be increased and the frequency of administration may be reduced since slow release capsules maintain blood levels of the drug for a longer duration of time, thus allowing better and longer interaction of the drug at the active sites.

The minute pores made on the wall of the slow release capsule do not considerably affect the stability of the

encapsulated drug under the storage conditions as compared to conventional capsules. Considerable reduction in the residual formalin content at 35°C-65% RH and 50°C affecting the *in vitro* and *in vivo* GIT resistance indicated that slow release capsule should be advised to be stored under refrigerated conditions, as storage under refrigerated condition did not affect the residual formalin content.

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