DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 15(5), 825-844 (1989)

CONTROLLED RELEASE CAPSULE OF TETRACYCLINE HYDROCHLORIDE

N.K. Jain* and A.N. Misra**
Pharmaceutics Laboratory, Pharmacy Department
Faculty of Technology and Engineering
M.S. University of Baroda
Baroda (INDIA) - 390001

ABSTRACT

Controlled release capsules of tetracycline hydrochloride were prepared by coating of beads with eudragits employing spray technique. Effect of various factors influencing drug release from beads was evaluated. In vitro dissolution study was carried out following the N.F.XIV procedure of testing "Timed Release Tablets and Capsules In Vitro Test Procedure" using USP XX dissolution apparatus. Promising products were evaluated in vivo on human volunteers. Selected products were also subjected to stability studies.

INTRODUCTION

Controlled release drug delivery systems are being designed to optimise their pharmacokinetic activity. The approach basically envisages the deve-

* Correspondence

**Present address : Quality Control,
Sarabhai Chemicals, Wadi Wadi,
Baroda (India) 390 007.

lopment of a dosage form which could deliver the drug to the target area at a rate and concentration that maximise therapeutic effects and minimise side effects.

Incomplete absorption (1), peaks and troughs in its blood levels due to high and frequent dosing (2), incidence of side effects due to high dosing (3) and prolonged therapy necessitate formulating controlled release drug delivery system of tetracycline hydrochloride.

EXPERIMENTAL

Preparation of Beads

Weighed amounts of tetracycline hydrochloride[1], succinic acid[2], microcrystalline cellulose[3] were mixed thoroughly. To the mix required amount of polyvinylpyrrolidone[4] in isopropyl alcohol[2] (25% w/v solution) was added as a binder and a mass suitable for pelletting was prepared by adding extra amount of isopropyl alcohol, if required. The wet mass was then passed through a pelletter[5] and pellets so obtained were rotated in a marumeriser[5] for required time (30 seconds - three times) to obtain spherical beads of suitable size. Beads were dried at 45°, screened and 16/40 mesh fraction was taken for further studies. Finally beads had composition of Tetracycline hydrochloride, 100% (650 g), Succinic acid (65 g), Microcrystalline cellulose (205 g) and Polyvinylpyrrolidone (80 g). The resulting beads had a yield of 85 to 95%.

Coating of Beads

About 200 g of beads were placed in a small coating pan (7.5" diameter) attached to Erweka unit[6]

and coated differentially with eudragit RL 100 and/or RS 100[7] solutions employing a spray gun (Pilot type 59)[8]. The composition of different coating solutions was as follows: Eudragit RL100/RS100/RL100-RS100 (1:1) 12.0 g, Magnesium stearate[9] 0.5 g, Dibutyl phthalate[10] 0.5 g, Methylene chloride[2] and Isopropyl alcohol (1:1) q.s. to 100 ml.

Quantities of drug, excipients and coating materials were altered and effect of these alterations on release of tetracycline hydrochloride was studied. In another set of experiments microcrystalline cellulose was partially (10%) replaced with eudragit RSPM[7] and release of the drug was studied after coating of the beads of this composition.

Beads equivalent to 500 mg of tetracycline hydrochloride were filled in capsules and subjected to *in vitro* evaluation.

Dissolution studies

Dissolution studies were carried out using a basket stirrer assembly of USP XX (4) dissolution test apparatus at stirrer speed of 100 rpm at 37 ± 0.5°. 900 ml of each of pH 1.2, 2.5, 4.5, 7.0 and 7.5 dissolution media were prepared and changed at different intervals of time as per the method recommended in N.F. XIV (5), under "Timed Release Tablets and Capsules *In Vitro* Test Procedure". Aliquots were withdrawn at 1.0, 2.0, 3.5, 5.0, 7.0 and 9.0 hr and absorbance of the suitably diluted solution was measured at maximum of 353 nm on a spectrophotometer (Hitachi Perkin-Elmer-139 UV-visible)[11] after adjusting the pH of the solution to 1 (6).

Observations on release of the drug from products are shown graphically in Figures 1 to 5.

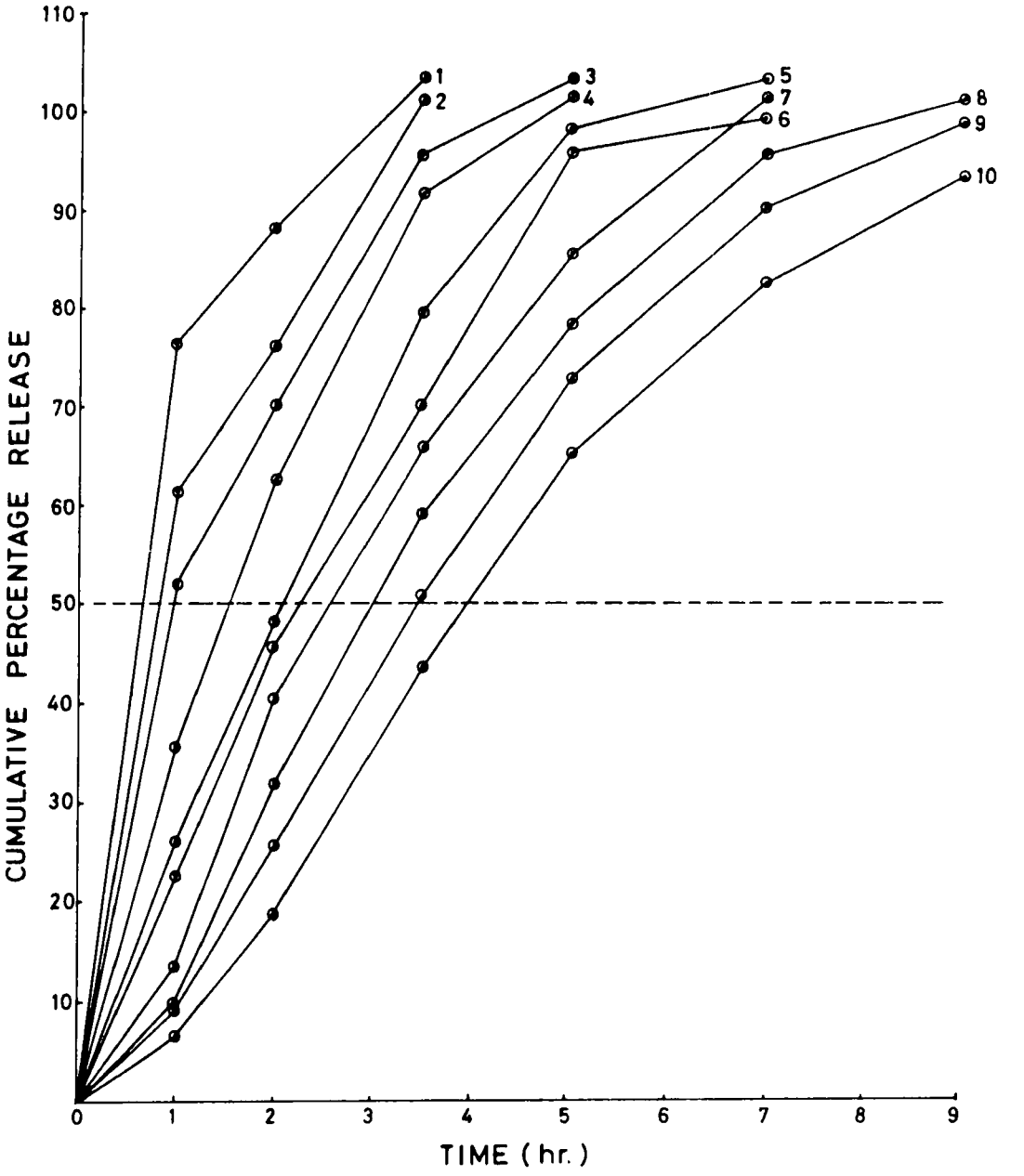

FIGURE 1

Cumulative percentage release of tetracycline hydrochloride from eudragit RL-100 coated beads

Key : 1,2,3.... 10 represents 5,10,15....50% coating respectively.

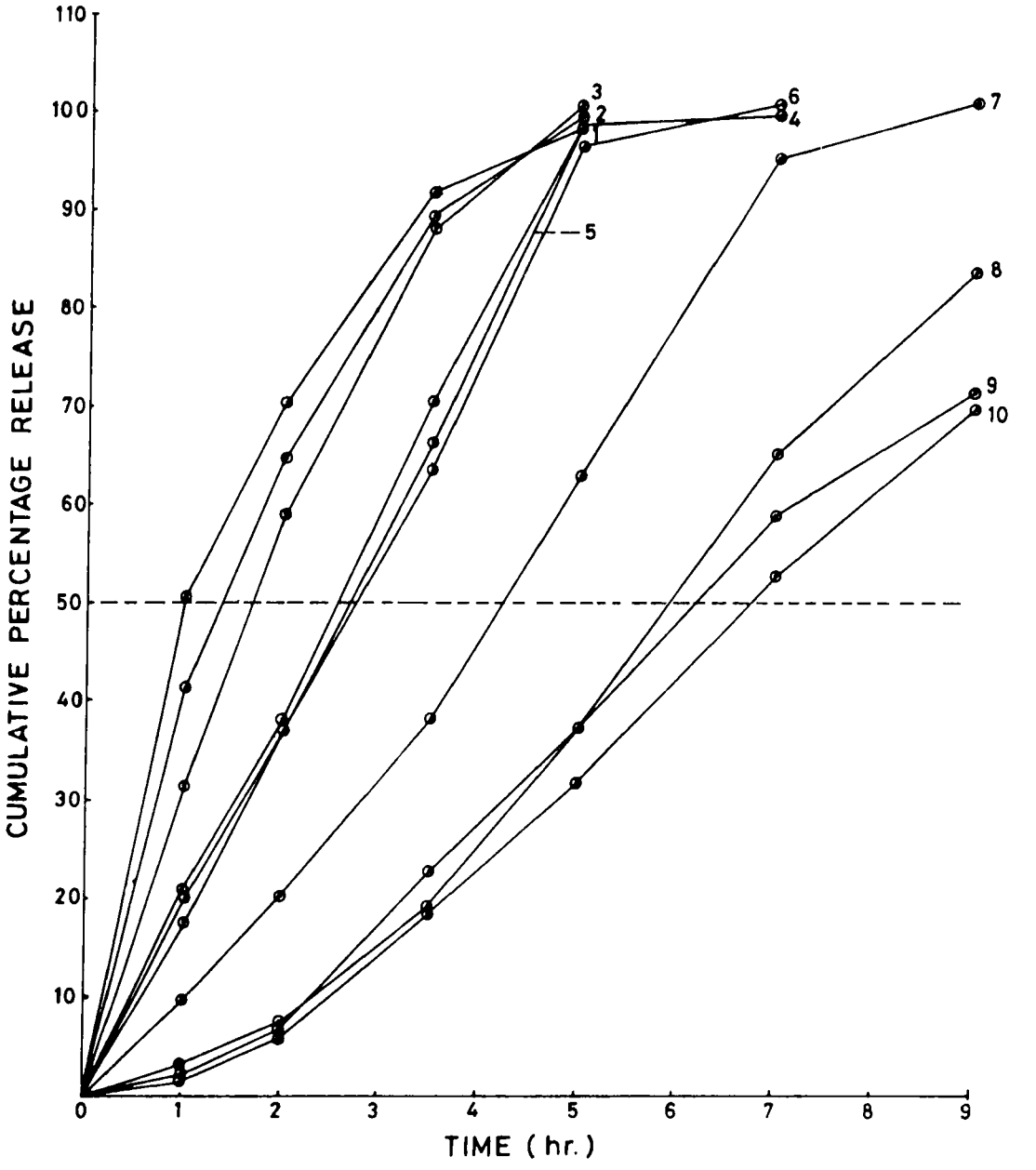

FIGURE 2

Cumulative percentage release of tetracycline hydrochloride from eudragit RS-100 coated beads

Key : 1,2,3....10 represents 5,10,15....50% coating respectively.

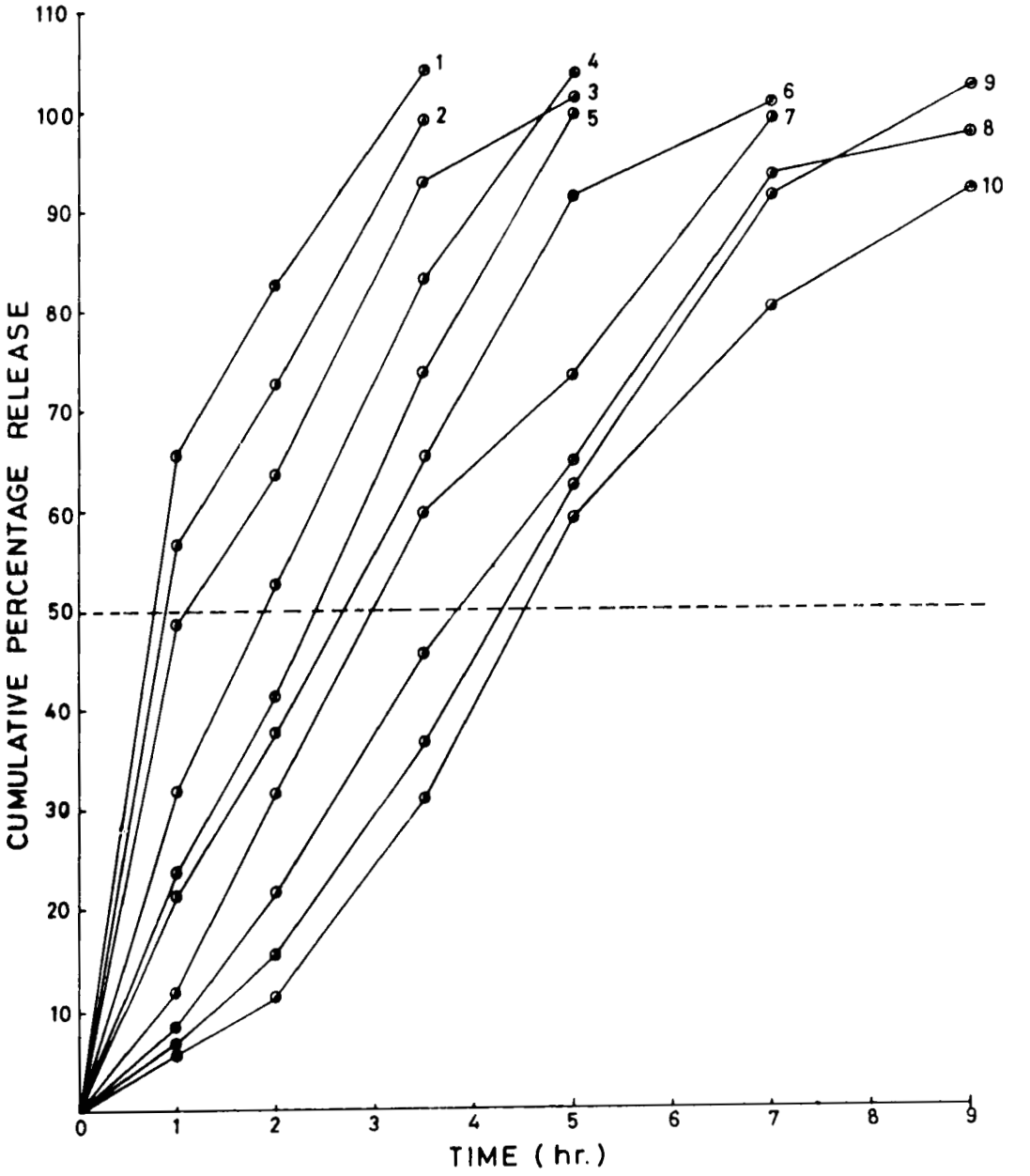

FIGURE 3

Cumulative percentage release of tetracycline hydrochloride from eudragit RL100-RS100 (50:50) coated beads

Key : 1,2,3....10 represents 5,10,15....50% coating respectively.

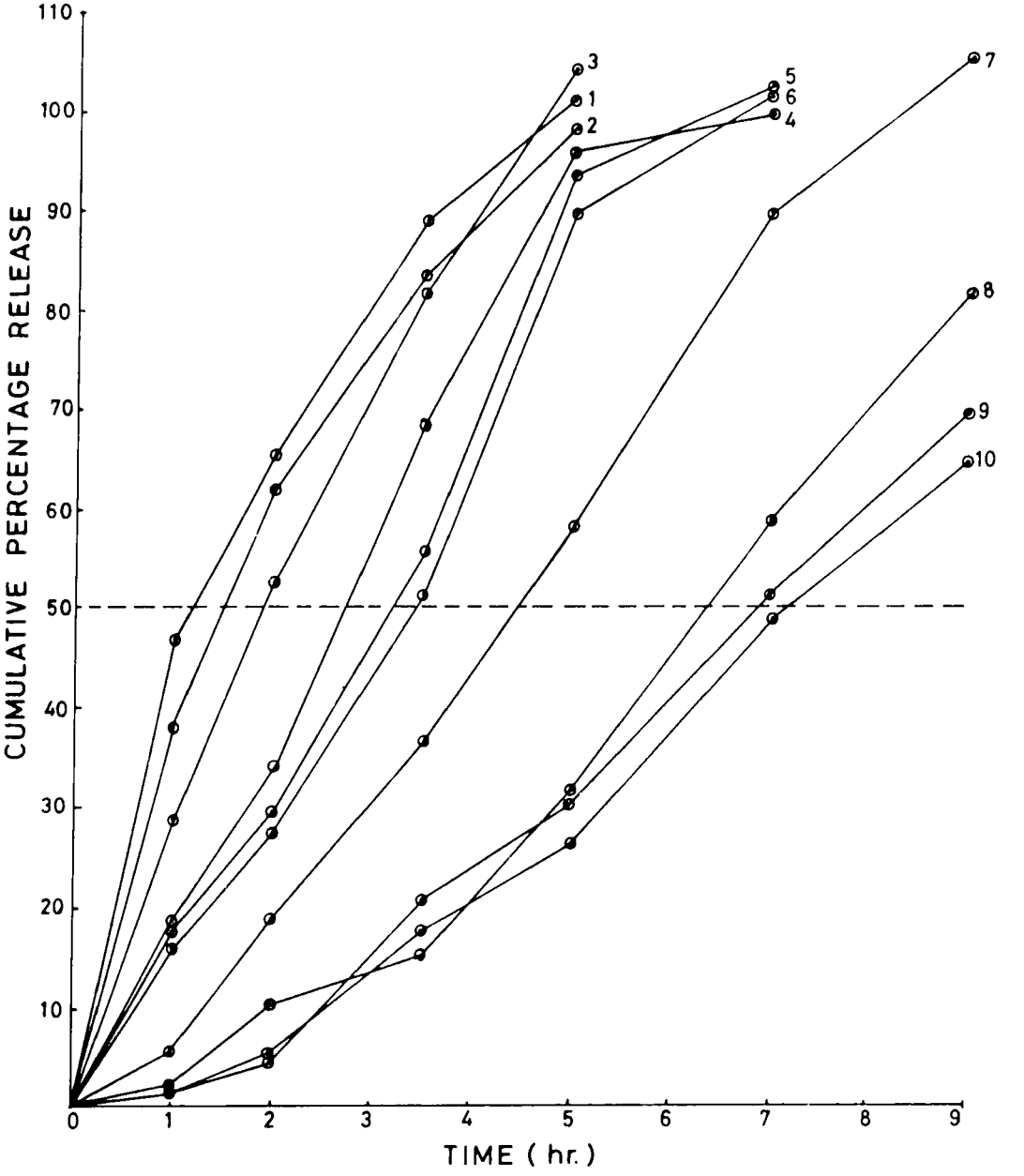

FIGURE 4

Cumulative percentage release of tetracycline hydrochloride from eudragit RS100 coated beads*

Key : 1,2,3....10 represents 5,10,15....50% coating respectively.

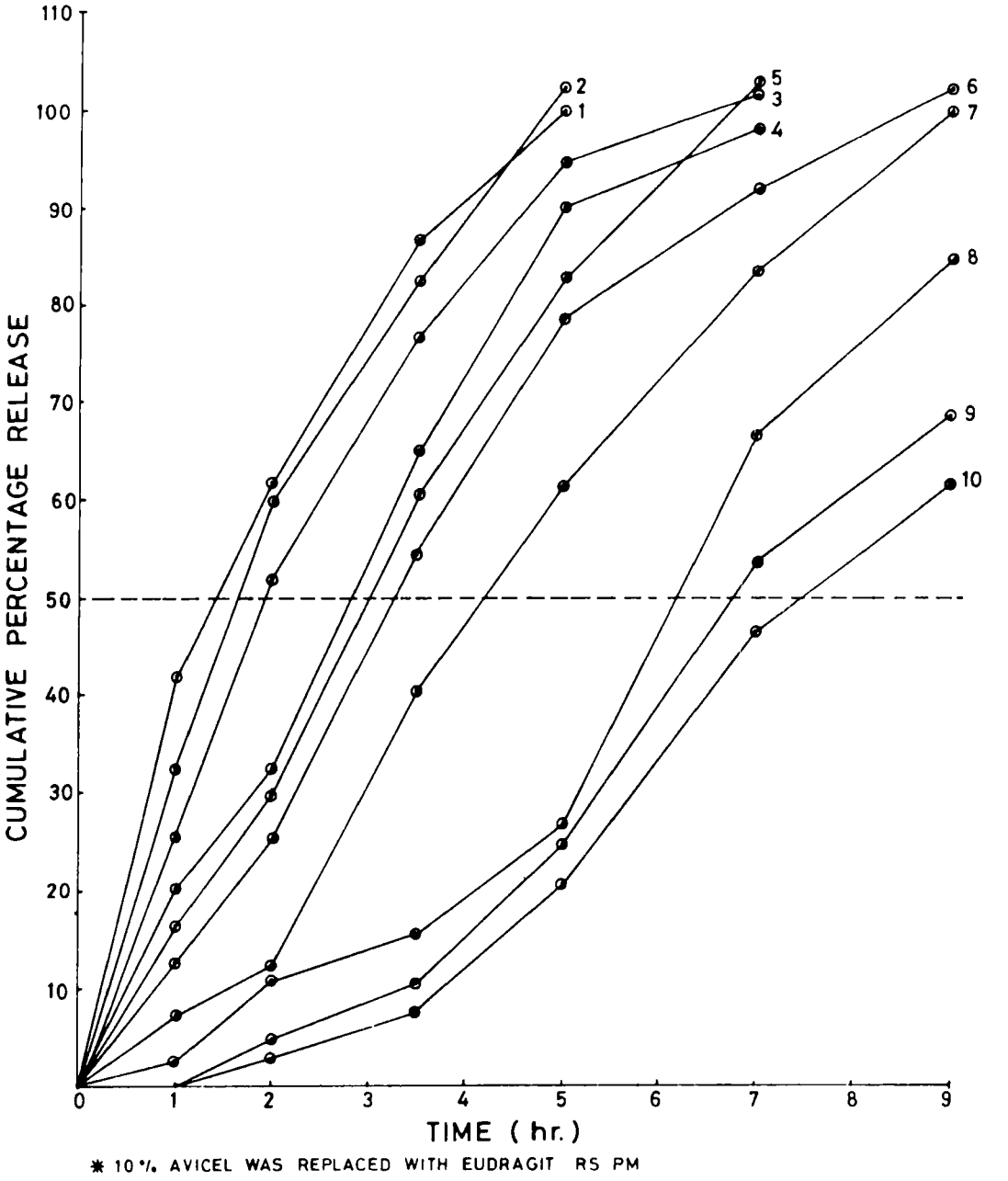

FIGURE 5

Cumulative percentage release of tetracycline hydrochloride from eudragit RS100 coated beads*

Key : 1,2,3....10 represents 5,10,15....50% coating respectively.

In Vivo Evaluation

Urinary excretion data was employed to assess the controlled release and conventional products of tetracycline hydrochloride after oral administration. Four healthy male human volunteers weighing 55-75 kg, 160-170 cm in height and between 26-34 years of age were selected. Experiment was carried out in a cross-over design allowing one week washout period in between. Volunteers were advised not to consume any drug during the period of study. They also had not consumed any drug in the preceding two months. Volunteers were fasted overnight and second urine in the morning was collected as blank without consuming anything except water. After collection of blank, a capsule was administered orally with about 200 ml of water. Food was withheld for atleast 4 hr after drug administration. Milk and dairy products were strictly forbidden during the first day of study. Urine samples were collected at 1,2,4,6,8,12,18,24,36 and 48 hr after administration of the sample. The urine volume was measured and recorded after each collection, aliquots were frozen till analyzed. Methods reported by Mahgoub et al. (7) and Hall (8) were utilized to develop spectrophotometric method for estimation of tetracycline in human urine for present investigation. The method is based on formation of yellow complex, uranyl - tetracycline, with an absorption maximum at 430 nm. Results are recorded in Tables 1 to 3.

Stability Studies

Controlled release capsules were subjected to stability studies. Capsules were assayed and packed in amber coloured vials and stored at air condition

TABLE 1. Comparative Urinary Excretion Rates and Total Amount Excreted of Tetracycline Hydrochloride after Oral Administration of Controlled Release and Conventional Products

Time (Midpoint of urine collection time) (hr)	Mean Excretion Rates ($mg\ hr^{-1}$)							
	a		b		c		d	
	Mean	± S.E.	Mean	± S.E.	Mean	± S.E.	Mean	± S.E.
0.5	5.74	0.325	2.09	0.370	4.41	0.290	2.26	0.310
1.5	6.74	0.345	3.94	0.395	6.67	0.340	2.87	0.280
3.0	7.34	0.375	5.76	0.313	8.33	0.395	3.75	0.355
5.0	6.55	0.405	2.30	0.365	5.91	0.360	3.63	0.260
7.0	2.80	0.160	1.37	0.390	7.22	0.260	3.75	0.315
10.0	1.96	0.040	0.73	0.075	5.08	0.280	2.50	0.130
15.0	0.47	0.005	0.38	0.055	1.80	0.090	0.98	0.090
21.0	0.08	0.010	0.06	0.005	0.92	0.065	0.13	0.030
30.0	-	-	-	-	0.30	0.030	-	-
42.0	-	-	-	-	-	-	-	-
Total amount of tetracycline excreted in 48 hr (mg)	129.79		68.13		166.49		81.32	

Key
a 500 mg conventional capsule
b 250 mg conventional capsule
c 500 mg controlled release capsule
d 250 mg controlled release capsule

S.E. = Standard Error

TABLE 2. Predicted Serum Levels of Tetracycline after Oral Administration of Conventional and Controlled Release Products and their Comparison

Time (Mid point of urine collection time) (hr)	Mean Serum Level ($\mu g\ ml^{-1}$)								Mean Serum levels compared by paired t-test (level of significance)	
	a		b		c		d			
	Mean	± S.E.	Mean	± S.E.	Mean	± S.E.	Mean	± S.E.	a vs b	b vs d
0.5	1.14	0.070	0.42	0.080	0.88	0.065	0.46	0.070	S	N.S.
1.5	1.34	0.075	0.80	0.080	1.32	0.075	0.58	0.060	N.S.	S
3.0	1.46	0.080	1.14	0.070	1.66	0.080	0.74	0.075	S	S
5.0	1.30	0.090	0.46	0.080	1.16	0.075	0.72	0.060	S	S
7.0	0.56	0.040	0.28	0.080	1.42	0.060	0.74	0.070	S	S
10.0	0.40	0.010	0.16	0.020	1.02	0.060	0.52	0.030	S	S
15.0	0.10	0.020	0.08	0.020	0.36	0.020	0.22	0.020	S	S
21.0	0.02	0.005	0.02	0.005	0.18	0.020	0.04	0.010	S	N.S.
30.0	-	-	-	-	0.08	0.010	-	-	S	-
42.0	-	-	-	-	-	-	-	-	-	-

Key a 500 mg Conventional capsule
b 250 mg Conventional capsule
c 500 mg Controlled release capsule
d 250 mg Controlled release capsule

S.E. - Standard Error
S. - Significant at the 0.05 level

TABLE 3. Pharmacokinetic Parameters after Oral Administration of Conventional and Controlled Release Products of Tetracycline Hydrochloride

Product Pharmacokinetic Parameter	a Mean	± S.E.	b Mean	± S.E.	c Mean	± S.E.	d Mean	± S.E.
T_{max} (hr)	3.00	0.250	3.00	0.250	3.00	0.250	3.00	0.250
C_{max} ($\mu g\ ml^{-1}$)	1.46	0.080	1.14	0.070	1.66	0.080	0.74	0.075
Peak to trough ratio	3.65	0.805	7.13	1.512	1.63	0.182	1.42	0.243
Bioavailability (%)	-		-		128.28		119.36	
AUC_{0-30} ($\mu g\ hr\ ml^{-1}$)	11.29	0.730	5.80	0.845	16.36	1.330	9.06	0.810

Key a 500 mg Conventional capsule
b 250 mg Conventional capsule
c 500 mg Controlled release capsule
d 250 mg Controlled release capsule

S.E. : Standard Error
T_{max} : Time required to achieve peak serum level
C_{max} : Peak serum concentration
Peak to trough ratio : C_{max}/serum concentration at 10 hr

Bioavailability: $\frac{\text{Amount excreted by controlled release capsule in 48 hrs}}{\text{Amount excreted by conventional product in 48 hrs}}$

AUC_{0-30} : Area under serum level curve 0-30 hr.

TABLE 4 Degradation Rate Constant (K) and Shelf life of Controlled Release Capsules of Tetracycline Hydrochloride at Various Conditions of Storage

Product	Condition of storage	Degradation rate constant	Shelf life (days)
Eudragit RL100-RS100 coated Beads capsules (500 mg)	I	1.407×10^{-4}	739
	II	1.637×10^{-4}	635
	III	4.068×10^{-4}	256
	IV	5.092×10^{-4}	204
Eudragit RL100-RS100 coated Beads capsules (250 mg)	I	1.305×10^{-4}	797
	II	1.563×10^{-4}	665
	III	4.100×10^{-4}	253
	IV	5.560×10^{-4}	187

Key : I = A.C. (20±2°, 45±5% R.H.); II = Room temperature; III = 37° , 65% R.H.; IV = 50°.

(20 ± 2°, 45 ± 5% R.H.), room temperature, 37°-65% R.H. and 50° for 90 days. Samples were withdrawn at the end of 15,30,60 and 90 days and subjected to both physical and chemical examination. Tetracycline hydrochloride was assayed by the method described in USP XX (9), under determination of 4-epi-anhydrotetracycline and anhydrotetracycline in tetracycline hydrochloride capsules. Results of shelf life at various conditions of exposure are shown in Table 4.

RESULTS AND DISCUSSION

Dissolution Studies

Tetracycline hydrochloride beads consist of tetracycline hydrochloride, microcrystalline cellulose, an organic acid and excipients in core and different

amount of eudragit RL100 and/or RS100 in coating. Observations on release of the drug from different beads have been shown graphically in Figures 1 to 5.

(a) $T_{50\%}$ values, as shown in figures, increase with increase in percentage of the coating material applied to beads in each case. However, extent to prolongation varies from one coating material to the other and was found to follow the order eudragit RS100 > eudragit RS100 and eudragit RL100 (1:1) > eudragit RL100.

(b) Eudragit RL100 and/or eudragit RS100 coated beads show good sustained action with less inter-capsule variation in release of the drug. The release of the drug from these beads was found to be independent of the pH of the dissolution medium. Yield of the product was also between 85 to 95%. Photomicrograph of eudragit RL100-RS100 coated beads of tetracycline hydrochloride shows nearly spherical beads with uniform coating (Figure 6). Hence the capsules prepared with eudragit RL100-RS100 coating were selected for *in vivo* studies.

(c) Replacing part of microcrystalline cellulose of beads core with eudragit RS (as RSPM) incorporated during the preparation of beads followed by coating with eudragit RS100 helps in further delaying the release of the drug.

But larger amount of eudragit RS in total is required to obtain similar results as that for coating of plain beads. However, the results were found to be reproducible. This procedure can be used when larger amount of eudragit RS is to be applied by coating to obtain desired release pattern and application of that much amount of eudragit RS by coating is not feasible due to problems of sticking, solvent use etc.

(d) Amount of tetracycline hydrochloride in plain beads was increased from 65 to 75% by weight and then

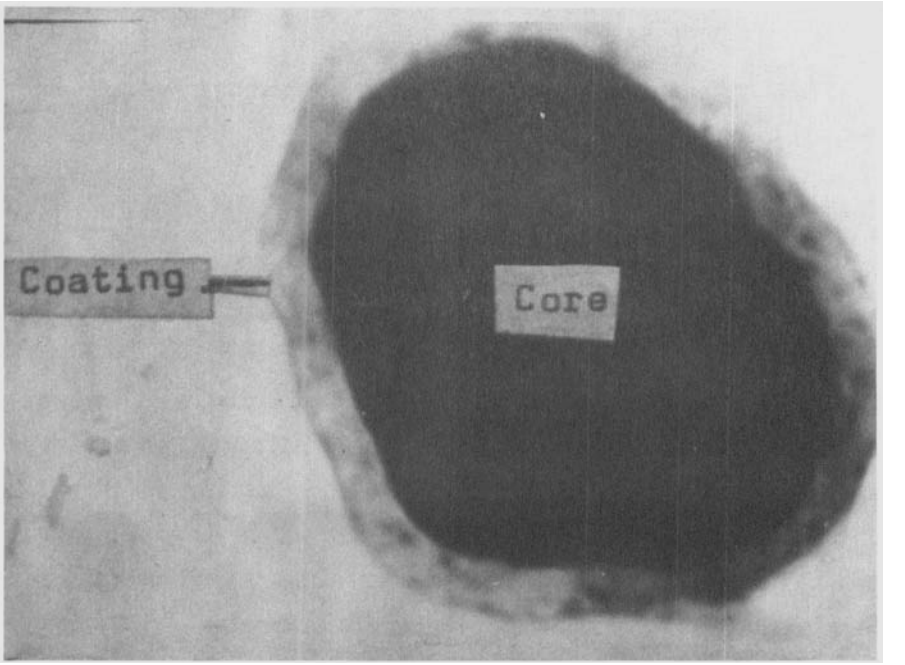

FIGURE 6

Photomicrograph of eudragit RS100-RL100 coated beads of tetracycline hydrochloride showing core and coating (X 100)

beads were coated with eudragit RS100. Cbservations show decrease in the release of the tetracycline hydrochloride even after application of same amount of eudragit RS100 by coating. This may be attributed to reduction in surface area available for diffusion per unit weight due to an increase in the amount of tetracycline hydrochloride. Succinic acid was included in matrix to prevent the hydrolysis of tetracycline hydrochloride at pH value of 3 or more (as upon leaving the stomach) and resultant precipitation of the drug as base. This is necessary to make the drug available for absorption throughout gastro-intestinal tract.

Urinary Excretion Rates

The urinary excretion rates of tetracycline at the mid-point of urine collection time after administration of each of the products were computed (10). Bioavailability of controlled release products were calculated from total amount of the drug excreted in 48 hr after administration of controlled release and conventional capsules.

These studies have shown that controlled release capsules have significantly higher bioavailability in comparison to conventional capsules. As an amphoteric substance tetracycline forms salts with acids as well as with bases. With hydrochloric acid it forms a hydrochloride, the solutions of which are strongly subjected to hydrolysis in a neutral medium. Tetracycline bases which precipitate above pH 3 are very difficultly soluble (11). Therefore, absorption of tetracycline hydrochloride is limited to small area of gastrointestinal tract. In case of controlled release capsules of present investigation, as soon as they come to a region with a pH value of 3 or more, such as upon leaving the stomach; the medium is influenced by the organic acid (succinic acid) in such a way that the pH of the surrounding fluid never exceeds a value which would permit hydrolysis of the tetracycline hydrochloride and thereby precipitation of the free base. This makes tetracycline hydrochloride available throughout the gastrointestinal tract.

Serum levels

Approximate serum levels of tetracycline at the mid-point of urine collection time were predicted based on relationship between urinary excretion rates and serum levels reported by Barr et al. (12). Analysis of

the data at time point by paired t-test revealed the following significant differences ($P < 0.05$).

(a) The mean peak tetracycline serum level was significantly higher in case of 500 mg controlled release capsule than 500 mg conventional capsule. However, C_{max} value with 250 mg controlled release capsule was lower as compared to C_{max} attained with 250 mg conventional capsule.

(b) The mean tetracycline serum levels attained between 3 to 30 hr with 500 mg controlled release capsule were higher than those achieved with the 500 mg conventional capsule.

(c) The mean tetracycline serum levels between 5-21 hr were higher with 250 mg controlled release capsule when compared with the serum levels obtained after administration of 250 mg conventional capsule.

(d) Peak to trough ratio was calculated for both conventional and controlled release capsule from C_{max} value and serum level at 10 hr. Peak to trough ratio was higher with conventional capsule. Smaller is the peak to trough ratio better is sustained action.

(e) Areas under the serum level curves after administration of each capsule were calculated using trapezoidal rule (10). Controlled release capsules have larger areas under the curves compared to conventional capsule. This may be due to comparatively faster changes in serum concentrations of the drug following the administration of the conventional capsule.

From these studies, the controlled release capsules appear to be promising with regard to making medication simpler to the patients and to reduce fluctuation in tetracycline levels throughout the therapy. Controlled release capsules have shown comparatively better

bioavailability. Further, the incidence of side effects should be established through clinical trials. However, prolonged action tetracycline hydrochloride products (13,14) were reported to be equally effective as compared to the conventional tablet in the treatment of acne vulgaris and gonorrhoea. One of the prolonged action products (13) was reported to be effective even when one half the daily dosage compared to the conventional tablet was administered, hence reducing the possible incidence of undesirable side effects.

Stability Studies

The controlled release capsule of tetracycline hydrochloride show little change in colour of beads at 37°-65% R.H. and 50° in three months. However, the change is not visible if capsule shells are opaque. Plots of log percentage drug retained versus time show that tetracycline hydrochloride in the controlled release capsule follow first order degradation kinetics. Controlled release capsules have shelf-life of more than 1½ years at room temperature and at other stress conditions capsules have sufficient shelf life. No change was observed in release pattern of tetracycline hydrochloride from capsules kept on stability at different conditions. The studies show that controlled release capsules have sufficiently high stability at room temperature and other stress conditions.

ACKNOWLEDGEMENTS

Abstracted in part from a thesis submitted by A.N. Misra to the M.S.University of Baroda in fulfilment of the Doctor of Philosophy degree requirements. Authors are thankful to M/s. Sarabhai Chemicals, Baroda for providing a gift sample of drug and other help in carrying out present investigation.

FOOT NOTES

1. Synbiotics Ltd., Baroda, India.
2. Sarabhai M. Chemicals, Baroda, India.
3. Cellulose Products of India, Ahmedabad, India.
4. S. D. Fine Chem. Pvt. Ltd., Boisar, India.
5. Fuji Paudal Co. Ltd., Osaka, Japan.
6. Erweka Apparatebau, West Germany.
7. Rohm Pharma GmbH, West Germany.
8. Manik Mfg. Pvt. Ltd., Bombay, India.
9. Chemical Supply Corpn., Bombay, India.
10. Riedel - Dehaen AG Seelize - Hannover.
11. Hitachi Ltd., Tokyo, Japan.

REFERENCES

1. J.V.Bennett, J.S. Mickelwait, J.S.Barrett, J.L.Brodie and W.M.M.Kirby, "Antimicrob. Agents Chemother.", 1965, p.180.
2. C.M.Davis, J.V. Vandersarl, E.W. Kraus, Amer. J. Med. Sci., 265, 69 (1973).
3. "Martindale, The Extra Pharmacopoeia (J.E.F. Reynolds Ed.)", 28th Ed., The Pharmaceutical Press, London, U.K., 1982, p.1217.
4. "The United States Pharmacopoeia", 20th rev., U.S. Pharmacopoeial Convention, Inc., Rockville, Md., U.S.A., 1980, p. 959.
5. "The National Formulary" 14th Ed., American Pharmaceutical Association, Washington, DC., U.S.A., 1975, p. 985.
6. "British Pharmacopoeia", Vol. II, Her Majesty's Stationery office, London, 1980, p. 826.

7. A.E.Mahgoub, E.M.Khairy and A.Kasem, J. Pharm. Sci., 63, 1451 (1974).

8. D. Hall, J. Pharm. Pharmacol., 28, 420 (1976).

9. "The United States Pharmacopoeia" 20th Rev., U.S. Pharmacopoeial Convention, Rockville, Md., U.S.A., 1980, p. 1288.

10. M. Gibaldi, D. Perrier, "Pharmacokinetics", Marcel Dekker, Inc. New York, 1975, p. 7, 153.

11. W.H.Barr, J. Adir and L. Garrettson, Clin. Pharmacol. Ther., 12, 799 (1971).

12. W.H.Barr, L.M.Gerbracht, K. Letcher, M. Plant and N. Strahl, Clin. Pharmacol. Ther., 13, 97 (1972).

13. C.C.Lim, D.G.C. Presbury and J. Adamson, Practitioner, 212, 728 (1974).

14. P.S. Silver, Brit. J. Vener. Dis., 51, 48 (1975).