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IN VITRO EVALUATION OF ALBUMIN MICROSPHERES CONTAINING ACTINOMYCIN D.

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ABSTRACT

Albumin microspheres containing actinomycin D were prepared by the heat stabilization method. The compatability of the drug with magnetite and the optimum stability of the drug in different pH were studied. Drug loaded albumin microspheres containing magnetite showed good magnetic response. Release of the drug was slow and continued for 7 days exhibiting sustained release property. The difference as regards to the size, shape, drug content and release rate from freshly prepared and freeze dried drug loaded albumin microspheres was negligible.

INTRODUCTION

The need to divert cytotoxic anti-cancer angents away from organs and tissues in which toxicity arises and towards the desired site of action has long been recognised. Albumin microspheres were first suggested as drug delivery system for cytotoxics by Kramer (1974). The original concept was that micron sized particles could be "targeted" to solid tumours in a manner analogous to the suggested for liposomes. The intervening years have seen this concept founder on the prodigious capacity of the reticuloendothelial system to sequester particles of this size, essen-

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tially restricting their deposition to phagocytic cells of organs such as liver and spleen (1).

Several investigators have successfully prepared albumin microspheres using a variety of drugs to determine the microsphere size, shape and drug release (2-7).

Effect of pH & Buffer System on the Stability of Actinomycin D

Five portions (100 ml each) of the stock solution of actinomycin D (5 ug/ml) were further taken in alkali free, amber coloured glass bottles. To each portion 40 mg of magnetite (Ferrofluidics corp.Burlington, Mass, U.S.A.) was added and all were marked serially from 1 to 5. The pH of the respective drug solution was adjusted to 5.5, 6.5, 6.8, 7.0 and 8.0 with phosphate buffer (8) and checked on a digital pH meter (Systronics, India). These solutions were kept in an incubator at $28\pm1^{o}C$ and the absorbance was measured daily for a week. The pH of the solution showing minimum degradation of the drug was considered to be the optimum for actinomycin D. The pH of each portion was adjusted to 7.0 by addition of acetate buffer (9), Clark and Lub's buffer (10) and Mcllavine buffer (11) respectively. The observations are recorded in Table I & II.

Preparation of Albumin Microspheres

An aqueous internal phase (30 ml) of an emulsion (w/o type) containing 400 mg bovine serum albumin, 10 mg aclinomycm D and 75 mg magnetite was emulsified with 250 ml of pure olive oil under constant stirring at 1200 rpm while the temperature was maintained at $4^{o}C$. This emulsion was added gradually (10-15 drops/min) to preheated ($120^{o}C$) pure

TABLE - I

Effect of pH on the Aqueous Stability of Actinomycin D in Presence of Magnetite at 28±1°C

Time (days)	Residual drug %				
	pH 5.5	6.5	6.8	7.0	8.0
0	100.00	100.00	100.00	100.00	100.00
1	88.25	89.65	92.05	96.20	94.90
2	77.60	79.30	84.85	91.60	87.40
3	67.45	68.00	76.75	86.00	80.50
4	56.20	58.40	67.50	81.70	73.60
5	44.00	49.20	59.25	76.00	66.20
6	32.60	37.15	52.15	72.80	59.00
7	21.85	27.10	43.20	67.30	53.35

TABLE - II

Effect of Different Buffer Systems (pH 7.0) on the Aqueous Stability of Actinomycin D in Presence of Magnetite at 28±1°C

Time (days)	Residual drug %		
	Buffer Systems I	II	III
0	100.00	100.00	100.00
1	95.10	92.56	94.80
2	89.10	86.50	89.76
3	84.74	78.50	84.90
4	79.20	72.25	79.40
5	74.10	65.20	74.86
6	68.80	58.10	69.74
7	63.44	51.20	64.72

I-Acetate; II-Clark and Lub's; III- Mcllavine Buffer

olive oil under constant stirring at 750r.p.m for 15 minutes and mixture was allowed to cool down to room temperature. The heat stabilized albumin microspheres were separated by centrifugation at 3000 rpm for 5 minutes. The separated microspheres were washed with 60 ml of anhydrown ether (Qualigens, India) thrice and separated by centrifugation (Remi Research Centrifuge R-24 India). Finally the micro-

spheres were suspended in 10 ml of ether and stored at -4°C in an air tight container. The microspheres were observed under scanning electron microscope (Phillips EM 515,Holland) and noted to be spherical in shape with an average diameter of 4.5 um.

Analysis of Drug Content in Microspheres

The actinomycin D content associated with microspheres was analysed for the surface and entrapped drug.

(a) Surface Drug - To a portion of ether suspension of actinomycin D representing 5 mg of microspheres, 2 drops of Tween 80 (Hico Products Ltd., India) was added and shaken gently. Ether was then evaporated under vacuum and 1 ml of phosphate buffer (pH 7.0) was added to the residue. The suspension was then centrifuged at 3000 g for 5 min and the supernatant, was collected. Such three washings were pooled and analysed for drug content by spectrophotometric method.

(b) Entrapped Drug - Microspheres obtained after four washings were digested overnight in 5 ml of 0.5 M acetic acid and then 5 ml of 0.5 M glacial acetic acid was added to it. A white precipitate was obtained which was centrifuged to get a clear supernatant. The supernatant following appropriate dilution with phosphate buffer (pH 7.0) was assayed for actinomycin D spectrophotometrically. The same homogenate was again digested and analysed for drug content. The observation are recorded in Table IV.

TABLE - III

Effect of Magnetite Concentration on Magnetic Responsiveness of Actinomycin D Loaded Albumin Microspheres

S. No.	Concentration of magnetite(mg)	Concentration of drug(mg/ml)	Drug loaded albumin microspheres entrapped (%)
1.	75	40	93.94
2.	50	40	91.38
3.	25	40	90.52

Determination of Magnetite Content from Drug Loaded Albumin Microspheres

Accurately weighed drug loaded albumin microspheres (5 mg) were taken in a boiling tube and conc. HCl (1 ml)was added to denature the protein. The contents of tube were heated and then cooled and centrifuged at 5000 g for 10 min. A bar magnet of 7500 oersted was kept under the tube and the supernatant decanted. After decanting the supernatant under the influence of magnetic field, whole of the sediment was taken in an accurately weighed steel crucible and heated for 30 min to burn off the protein. The crucible was weighed again after cooling and the amount of magnetite calculated (16.75% w/w).

Study of Magnetic Responsiveness of the Fabricated Product

The apparatus described by Lee et al. (13) was used for determining the magnetic responsiveness. Three batches of drug loaded albumin microspheres containing different concentrations of magnetite were used. The percentage of drug loaded albumin microspheres retained by the magnet from different batches is shown in Table III.

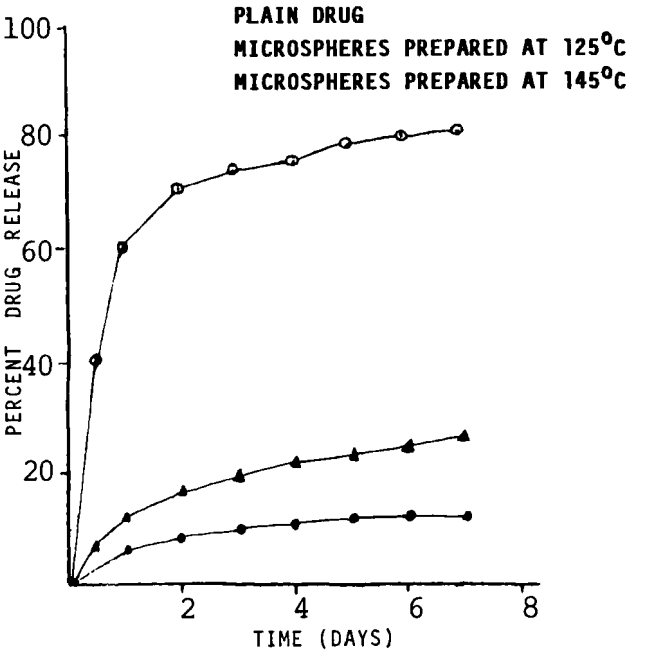

FIGURE - I
Release Pattern of Actinomycin D from Albumin Microspheres

In-vitro Drug Release from Albumin Microspheres

Drug release from the loaded albumin microspheres determined by means of a dynamic dialysis system employing cellulose tubing(14).Thirty ml of phosphate buffer (pH 7.0) was taken in a cellulose visking tube and 84 mg of albumin microspheres (1 mg of the drug) were suspended in it. Ten ml of the fluid was removed from the beaker every 24 hr and replaced by the same amount of fresh fluid. The experiment was carried out for a week and the drug content of the sample was determined spectrophotometrically at 445 nm. The drug release from albumin microspheres prepared at 145°C and plain drug was also studied in the same manner. The observations are shown graphically in fig. 1.

TABLE - IV

Comparative Study of Freshly Prepared and Freeze Dried Drug Loaded Albumin Microspheres

Characteristics	Drug loaded albumin microspheres	
	Freshly prepared	Freeze dried
Shape	Spherical	Spherical
Colour	White	White
Odour	Odourless	Odourless
Size	4.88 um	4.94 um
Drug Content		
(a) Surface drug	7.20 ug/mg	7.18 ug/mg
(b) Entrapped drug	11.93 ug/mg	11.86 ug/mg
(c) Magnetite Concentration	16.75%	16.75%
Release rate on		
1st day	5.20%	5.20%
5th day	20.70%	20.70%
7th day	24.50%	24.87%
Sability in glass and plastic container	Stable	Stable
Stability at 4°C for 4-5 weeks	Stable	Stable
Stability at 4°C for 6-8 weeks	Unstable	Stable

Drug loaded albumin microspheres (840 mg) were then freeze dried and compared with freshly prepared drug loaded albumin microspheres. The observations are given in Table IV.

RESULTS AND DISCUSSION

In the present investigation, the first study was undertaken to assess the compatability of magnetite in presence of drug in an aqueous solution and it was found that the drug and magnetite were compatable with each other. Degradation studies for the plain drug and the one containing magnetite ruled out any complex formation. The degradation patterns were identical.

The effect of pH on the stability of the drug was studied by incubating the drug and magnetite with phosphate buffers of different pH. After seven days, it was the pH 7.0 in which the drug was most stable. There was a marginal decrease in the stability of drug containing magnetite with phosphate buffer at pH 6.8 and 8.0. In order to determine whether only the phosphate buffer is suitable to this combination (drug and magnetite) acetate, Clark and Lub's and Mcllavine buffers of pH 7.0 were also used. Although there was only a slight difference in the stability in Mcllavine and phosphate buffers, yet the drug was more stable in phosphate buffer. Hence, phosphate buffer (pH 7.0) was used throughout the investigation for the estimation of drug.

It has been reported that in drug associated albumin microspheres, about 40% of the total drug released rapidly and the remaining 60% release slowly (2). The rapidly released drug is due to the desoprtion of the surface drug. To characterize the drug release at target site the drug release from washed microspheres was studied. It can be seen from table IV that nearly 40% of actinomycin D associated with unwashed microspheres was rapidly removed from the surface. Magnetite content 16.75% w/w of albumin microspheres is safe and well below toxic level (4).

The amount of actinomycin D released from the albumin microspheres in phosphate buffer (pH 7.0) was determined with a dialysis cell. Fig. 1 shows plot of data expressed as cumulative amount of drug released versus time. The in vitro actinomycin D release from albumin microspheres continued for one week, the level of drug release depending on the temperature of the microsphere preparation suggesting difference in microsphere structure and hardness level.

It was observed that freeze drying had no substantial effect on size and shape of the microspheres. However, freshly prepared microspheres were 4.80 um in diameter as compared to the freeze dried microspheres which were 4.94 um. This slight increase in microsphere size after freeze drying can be attributed to the fact that the freeze dried microspheres have a tendency to swell in aqueous media.

It was noted that drug loaded albumin microspheres stored at 4°C were stable for only 4-5 weeks, while the freeze dried microspheres retained their stability for several months. Hence, the microspheres need not be absolutely fresh at the time of administration as long as they are stored in air tight container and at low temperature (2°C or even less) after preparation.

It can thus be concluded that the procedure for preparing albumin microspheres can withstand quite a large number of variables, which might alter the drug release and other studies undertaken.

REFERENCES

1. N.Willmott and P.J. Harrison, **Int. J.Pharmaceutics, 43,** 161 (1988).
2. K.J. Widder, G. Glouret and A.E. Senyei, **J.Pharm.Sci., 68,** 79 (1979).
3. K.J. Widder, A.E. Senyei and D.F. Ranney, **Cancer Research, 40,** 3512 (1980).
4. A. Ibrahim, P. Couvreur, M. Roland, P.Speiser, **J. Pharm.Pharmacol., 35,** 59 (1983).
5. J.J. Birger, E. Tomilson, J.E. Deroo and J.Palmer, **Methods in Enzymology, 43,** 112 (1985).
6. M. Shen, M.A. Moustafa and T.D. Sokoloski, **J.Parenteral Sci.Tech., 40,** 253 (1986).
7. S. Benita, O. Zonai and J.P. Benoit, **J.Pharm.Sci., 75,** 847 (1986).
8. "British Pharmacopocia", Vol.**II**, University Press, Cambridge, 1980, p. Appendix A-52.
9. E.L. Parrot, 'Pharmaceutical Technology', Burgess Publishing Co., Minnespolis, 1970, p. 232.
10. P.J. Elving, J.M.Markowitz and I.Rosenthal, **Anal.Chem., 28,** 1179 (1956).
11. J.M. Gallo, C.T. Hung and D.G. Perrier, **Int.J.Pharmaceutics, 22,** 63 (1984).
12. K.C. Lee, I.B. Koh, W.B. Kim and Y.J. Lee, **Int.J.Pharmaceutics, 44,** 49 (1988).
13. K.Sugibayshi, M.Akimoto, Y.Morimoto, T.Nadai and Y.Kato, **J.Pharm. Dyn., 2,** 350 (1979).