Preparation and Evaluation of Albumin – Chitosan Microsphere Containing Theophylline

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Albumin–chitosan beads were prepared by heat stabilization method in the presence of Span 80. Theophylline was used as the model drug. The chemical interactions between the drug, albumin and chitosan were evaluated using IR and HPLC. It was found that there was no interaction between them. Microspheres with seven drug to carrier ratios were prepared and thermally cross-linked. Drug to carrier ratio 1:1:2 showed maximum yield and highest entrapment. The particle size range increased approximately from 542 to 1078 μm with a peak between 779 to 948 μm. The maximum encapsulation efficiency was found to be 78.2±2.1% w/w. *In vitro* release studies were carried out in different pH for a period of 8 h and compared with the pure drug. These albumin-chitosan microspheres are, thus, suitable for oral sustained release of theophylline.

Theophylline is a xanthine bronchodilator. It relaxes directly the smooth muscle of the bronchial airways and pulmonary blood vessels. So, theophylline is used to relieve and/or prevent symptoms of asthma[1]. It has a narrow therapeutic range and its short half-life is influenced by a number of known variables such as chronic alcoholism, impaired hepatic function, renal failure and age. Thus, large fluctuations in theophylline blood concentrations following oral administration of the drug in conventional dosage forms are frequently reported[2]. Therefore, theophylline has received a considerable amount of attentions in sustained release formulations.

The main objective of any drug therapy is to achieve a desired concentration of the drug in blood or tissue, which is therapeutically effective and non-toxic for an extended period of time. This goal can be achieved by proper design of the sustained release dosage regimen[3]. Of the various biodegradable polymers used for the development of sustained release formulations, albumin and chitosan have been reported to be advantageous since they are natural products and are biocompatible[4]. It has been also reported that albumin microspheres and chitosan microspheres provide a potentially useful means of delivering drugs because they are both physically and chemically stable, amenable to preparation in large batches, non-antigenic, metabolize within the body and capable of accommodating a wide variety of drug molecules in a relatively non-specific fashion[5]. The present study deals with the design and evaluation of albumin-chitosan microspheres of theophylline for sustained release.

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Albumin, chitosan and gelatine were obtained from S.D. Fine Chemicals, Mumbai. Theophylline was a gift sample from Cipla Pharmaceuticals, Mumbai. Span 80 and sunflower oil were procured from Loba Chem. Pvt. Ltd., Mumbai.

Albumin-chitosan microspheres containing theophylline were prepared as per the method of Friedman[6,7]. Theophylline anhydrous was dispersed in the mixture containing 5 ml of 1% w/v albumin solution and 5 ml of 2% w/v chitosan in acetic acid, to this 5 ml of 20% w/v gelatine solution was added and mixed. This mixture was dropped through a syringe into 20 ml of sunflower oil containing 0.5% w/v of span 80 with gently stirred for 10 min and was maintained at 60-70°. The resulting (w/o) emulsion was stirred well for 10 min using a Remi stirrer at 1000 rpm and then was cooled to 5° for 30 min by keeping it in an ice bath. Dehydration was carried out by adding 50 ml of butanol. The formed beads were separated and washed three times with petroleum ether. The washings were analysed for the presence of drug and the washing continued until the oil was removed completely. The microspheres were then dried under vacuum and stored in a desiccator until used for further

studies.

To study the shape of the microspheres, the microspheres were dispersed in liquid paraffin and observed under 200X magnification using an optical microscope. The size distribution was carried out by optical microscope. An average of about 300 particles were counted and determined.

Theophylline was extracted from the microspheres with phosphate buffered saline (pH 7.4) and absorbance was measured using an UV/Vis spectrophotometer at 271 nm. The amount of theophylline in the microspheres was estimated with the help of a standard graph. Albumin-chitosan microspheres containing theophylline were prepared using seven different drug to carrier ratios, as shown in the Table 1, by phase separation emulsification technique as reported by Friedman[5].

The surface morphology and the internal textures of albumin-chitosan microspheres were observed under a scanning electron microscope (fig. 1). The size of the microspheres varied approximately from 542 to 1078 μm with a peak value between 779 to 948 μm. The bulk volume and weight, tapping volume and weight, fluidity, angle of repose, weight deviation, relative deviation, density and porosity of microspheres were studied. To determine flowability, Hausner ratio and consolidation index was also calculated from the results obtained, which are presented in Table 2.

The *in vitro* release profile of theophylline from albumin-chitosan microspheres were determined by using an Erlenmeyer flask containing dissolution medium consisting of 500 ml of biofluids such as either simulated gastric fluid (0.1 M HCL buffer, pH 1.2) or simulated intestinal fluid (phosphate buffer, pH 7.4). Microspheres equivalent to 50 mg of drug were suspended in the dissolution medium and the medium was maintained at 37±2°. Five millilitres of samples were withdrawn periodically at intervals of 1 h and same volume of fresh medium was replaced in to the flask. The concentration of the drug released at different time intervals was then determined using an UV/Vis spectrophotometer at 271 nm and with the help of a standard graph.

Among the seven drug to carrier ratios used, the ratio 1:1:2 showed maximum yield of 82 % at pH 1.2 and 92.4% at pH 7.4 after 8 h and 1:1:2 ratio also showed highest drug entrapment of 78.2±2.1% w/w, as shown in the Table 2. As shown in figs. 2 and 3, when the release of a pure drug and

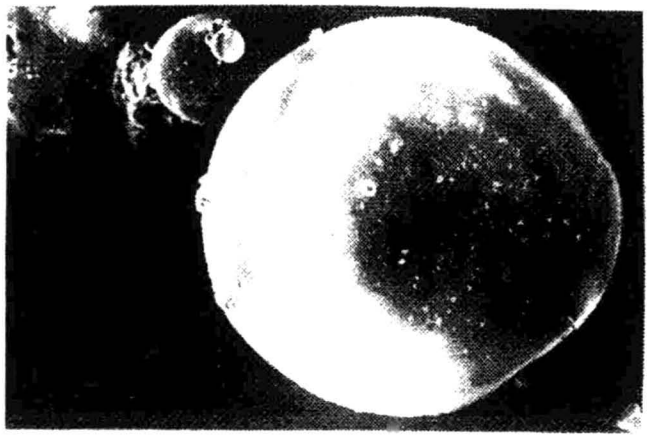

Fig. 1: Scanning Electron Micrograph of Microsphere.

TABEL 1: YIELD, DRUG ENTRAPMENT AND AVERAGE PARTICLE SIZE OF ALBUMIN-CHITOSAN MICROSPHERES OF THEOPHYLLINE

Formulation code	Drug:Albumin:Chitosan	Percent yield* (mean±s.d)	Drug Entrapment (% w/w)	Average Particle size (μm)
F1	1:1:1	67.8±1.47	34.8	842
F2	1:1:2	92.4±2.41	78.2	926
F3	1:1:3	82.5±0.11	42.4	542
F4	1:2:1	63.7±1.80	58.0	693
F5	1:2:2	58.3±1.91	31.4	1038
F6	1:2:3	72.5±1.12	64.9	642
F7	1:3:2	60.8±2.52	47.2	589

*Average of three preparations.

TABLE 2: MICROMETRIC PROPERTIES OF ALBUMIN-CHITOSAN MICROSPHERES

		Pure Drug	Formulation (F2)* 779-948 μm
Bulk Volume (ml/g)		4.218±0.052	5.426±0.027
Bulk Weight (g/ml)		0.389±0.006	0.272±0.001
Tapping Volume (g/ml)		2.007±0.024	3.875±0.074
Angle of Repose (o)	1 g	36.78±0.39	20.66±2.15
Weight Deviation (±mg)	Volume (1 ml)	12.018	9.762
Relative Deviation (± %)	Volume (1 ml)	4.113	3.981
True Density (g/ml)		1.496± 0.050	0.987± 0.009
Porosity		0.77	0.601±0.005
Hausner Ratio		1.72	1.08
Consolidation Index (Carr)		36.84%	7.84%

*F2 is Microsphere containing Drug, albumin and chitosan ratio of 1:1:2.

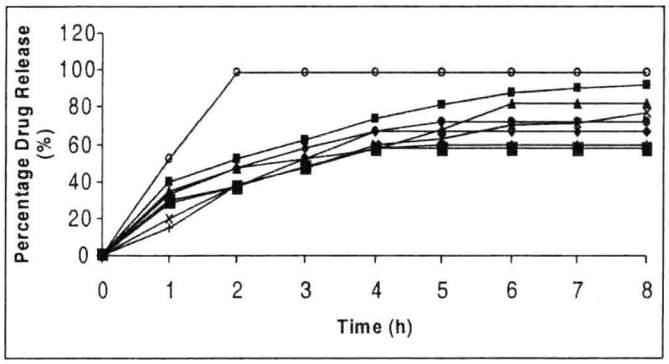

Fig. 2: *In vitro* drug release of theophylline from albumin-chitosan microspheres at pH 7.4.

***In vitro* dissolution profiles of theophylline from albumin-chitosan microspheres formulations F1 (-△-), F2 (-♦-), F3 (-●-), F4 (-○-), F5 (-□-), F6 (-▲-), F7 (-■-) and pure drug (-◊-) were studied in phosphate buffer (pH 7.4), samples drawn at regular time intervals and theophylline content was measured at 271 nm.**

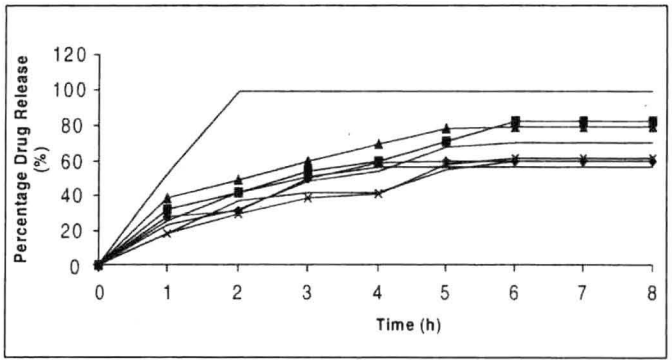

Fig. 3: *In vitro* drug release of thyophylline from albumin-chitosan microspheres at pH 1.2.

***In vitro* dissolution profiles of theophylline from albumin-chitosan microspheres formulations F1 (-△-), F2 (-♦-), F3 (-●-), F4 (-○-), F5 (-□-), F6 (-▲-), F7 (-■-) and pure drug (-◊-) were studied in simulated gastric fluid (pH 1.2), samples drawn at regular time intervals and theophylline content was measured at 271 nm.**

microspheres were compared, the pure drug was entirely released within 2 h, where as in case of microspheres the release pattern was in the following order, 1:1:1 ratio showed maximum release within 4 h, 1:1:3 ratio showed within 6 h and 1:1:2 ratio showed within 8 h. These results indicated that microspheres prepared with the drug to carrier ration of 1:1:2 showed a better pattern of sustained release.

This method of preparation of albumin-chitosan microspheres of theophylline was found to be simple and reproducible. The carriers used, albumin and chitosan are easily available, biocompatible and biodegradable. From the above data, it may be concluded that drug-loaded microspheres appear to be a suitable delivery system for theophylline, and may help to reduce the dose of the drug and frequency of administration.

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RP-HPLC Estimation of Cefdinir in Capsules

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A simple efficient and reproducible method for the determination of cefdinir in capsules has been developed using reversed phase high performance liquid chromatographic method. The elution was done using a mobile phase consisting of 0.01 N KH_2PO_4 (pH 6.9) and methanol (80:20% v/v) on Water's- Spherisorb ODS 4.6x150 mm analytical column with flow rate of 1 ml/min with detection at 285 nm. An external standard calibration method was employed for quantitation. The elution time was 2 min. The linearity range was 5-10 µg/ml for cefdinir.

Cefdinir[1] is a cephalosporin antibiotic. Chemically the drug is (6R-[6α,7β(Z)]]-7-[[2-aminothiazolyl)(hydroxyimino) acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2ene-2-carboxylicacid. A capsule formulation is available, which contains 300 mg cefdinir. HPLC-based analytical methods have been reported for the estimation of cefdinir in biological fluids[2,3]. These methods describe the determination cefdinir and related substances or estimation of cefdinir in plasma. No method has so far been reported for the estimation of cefdinir from pharmaceutical dosage forms. The present paper aims at reporting an isocratic RP-HPLC method for the determination of cefdinir in capsules.

The apparatus used was a Shimadzu HPLC SPD 10-A chromatograph equipped with fixed wavelength UV detector and model 7725i Rheodyne injector with 20 µl external loop. The column used was Water's Spherisorb ODS 4.6x150 mm analytical column the elution was carried out isocratically at the flow rate of 1 ml/min using KH_2PO_4 (0.1 N) at pH 6.99 and methanol 80:20% v/v as mobile phase. The detector was set at wavelength of 285 nm. Responses of peak areas were recorded and integrated using software.

Cefdinir was obtained from Unichem Pharmaceutical Limited, Mumbai with certificate of analysis. Methanol HPLC grade and potassium dihydrogen orthophosphate AR grade were obtained from S. D. Fine Chemicals Ltd., Mumbai.

Standard stock solutions of the drug were prepared by dissolving 25 mg of cefdinir in KH_2PO_4, pH 6.99 and made up to 25 ml with the same (solution A, 1000 µg/ml). From the above solution 1 ml was taken and made up to 10 ml (100 µg/ml.). From the above solution 2.5 ml was taken and made up to 25 ml (10 µg/ml) (solution B). The gradient dilutions were prepared by taking 5, 6, 7, 8 and 9 ml of solutions B and made up to 10 ml in a standard flask with mobile phase. These standard solutions were injected and peak area was obtained. A calibration graph was constructed.

Not less than 20 capsules (Sefdin, Unichem laboratories Ltd., Mumbai) were weighed and emptied. A quantity of

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