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COMPARATIVE EVALUATION OF THEOPHYLLINE MICROSPHERE PREPARED USING VARIOUS BIODEGRADABLE POLYMERS

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ABSTRACT

The present investigation was to formulate theophylline loaded microspheres using different grade of chitosan, chitosan - sodium alginate and chitosan-albumin by the following methods such as phase separation emulsification, modified ionotropic gelation and heat stabilization method. The prepared microspheres were evaluated in terms of drug content, incorporation efficiency, micromeritic studies, moisture content and *in vitro* drug release profile. Chitosan –sodium alginate combination produced microspheres with spherical, smooth surface and frees flowing. It exhibited incorporation efficiency above 75% and size range between 999-994 μm. The drug release from the microspheres follows first order kinetics and the mechanism is Higguchi's diffusion. Theophylline loaded microspheres prepared from Chitosan–sodium alginate combination exhibited good sustained release characteristics and was found suitable for chronic obstructive pulmonary disease (COPD) and nocturnal asthma.

Key words: Microsphere, Chitosan, Sodium alginate, Albumin, Theophylline, Nocturnal Asthma, *in vitro* release.

INTRODUCTION

Microspheres are widely accepted and become one of the successful carriers in overcoming the problems caused by conventional therapy and also widening the therapeutic margin of the drugs currently used in clinical practice[1]. An important requirement of biodegradable polymers is that the degradation products should be non-toxic because they enter systematic circulation or result in tissue deposition. Biodegradable carrier matrices can be desired to deliver the therapeutic agent for prolonged time[2]. Theophylline, methyl xanthenes has proven efficacy in chronic obstructive pulmonary

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disease and nocturnal asthma but it is difficult to maintain relative constant concentration of theophylline in plasma throughout the day without multiple doses. [3]

The present work was aimed to formulate theophylline microspheres as an effort to achieve initial onset of action followed by a sustained release profile to overcome the above mentioned problems. The microsphere was prepared with chitosan alone and in combination with sodium alginate and egg albumin. A comparative evaluation of the prepared microspheres was carried out to achieve the above mentioned aim.

EXPERIMENTAL

Materials

Theophylline was a gift sample from M/S Micro Labs Ltd., Hosur, India. Chitosan (grade 33 cps, 50 cps, 106 cps, 191 cps) were procured from India sea foods, Kochi. Sodium alginate (Rolex), Egg albumin powder (Ottokami), Hexane (Reachem), Butanal (Sd fine) was procured from commercial scale. All other materials used were of pharmacopoeial grade.

Methods

Preparation of microspheres

Microsphere containing theophylline was prepared by three different methods employing chitosan alone and in combination with sodium alginate and albumin as biodegradable polymers.

Preparation of chitosan microsphere

Chitosan microspheres were prepared by phase separation emulsification technique[4]. 25 mL of glacial acetic acid and 1% w/v of chitosan were used to disperse the drug (Theophylline 250 mg). Then 75 mL of sunflower oil containing 5000 mg of span was added and stirred for 45 min .To the above, 5 mL of 25% formaldehyde was added and stirring was continued. In another beaker same quantity of sunflower oil was taken and stirred at 80-90°C .The emulsion, which was prepared in another beaker, was transferred to this and stirred at for 45 min at 500 rpm. The microspheres were mixed with n-hexane and separated by ultra-centrifugation at 20000 rpm for 45 min, washed with diethyl ether and dried.

Preparation of sodium alginate–chitosan microspheres

Sodium alginate –chitosan microspheres were prepared by modified ionotropic gelation method[5]. Theophylline anhydrous (250 mg) was added to different concentrations of aqueous solution of sodium alginate (0.5, 5.5, 6.0, 6.5% w/v) and stirred. This is dropped using hypodermic syringe into solution containing calcium chloride (1% w/v) and chitosan (1% w/v) which was previously dissolved in 15 mL acetic acid solutions and kept for 15 min for complete gelification. The microspheres were separated, washed and dried at room temperature.

Preparation of albumin–chitosan microspheres

Albumin–chitosan microspheres were prepared by heat stabilization method[6]. Theophylline anhydrous (250 mg) was dispersed in 25 mL of albumin with various concentrations (1, 2, 3, 4% w/v) and 25 mL of chitosan (1% w/v). This mixture was dropped into 75 mL of sunflower oil containing 500 mg of span 20 with gentle stirring (1000 rpm) at 60-70°C for 10 min. Then cooled to 5°C for 30 min in an ice bath and the microsphere were separated, washed with hexane and dried at room temperature.

Estimation of theophylline

Theophylline microsphere was estimated by UV-VIS spectrophotometrie method[6] based on the measurement of absorbance at 270 nm in phosphate buffer pH 7.4. The method was validated for linearity, accuracy and precision. The method obeyed the Bee's law in the concentration range of 0-12 μg/mL.

Characterization of microsphere

Quantitative analysis

To determine the homogeneity, absence of drug degradation in microsphere and also to know whether there is an interaction between the drug and carrier, TLC [7] and FTIR[8] studies were done.

Micromeritic studies

Shape and morphologic analysis by SEM

The shape and surface morphology of the microspheres were observed by a scanning electron microscope (SEM –Hitachi, S–450). The sample was coated with gold to

a thickness of 100 Å using Hitachi vacuum evaporator (Mahel HUS 5GB).

Particle size measurement[9]

Particle size distribution and the arithmetic mean size was determined by using sieving method. 20 g of microspheres were mechanically shaken and the amounts retained on different sieves were weighed and particle size was calculated.

Bulk density, true density, porosity, Carr's consolidation index and angle of repose were calculated for the prepared microsphere[1°].

Incorporation efficiency[11]

Incorporation efficiency of the microspheres was determined from the ratio of theophylline incorporated to the weighed theophylline initially taken.

Moisture absorption studies

Moisture absorption studies were carried out to see the hygroscopic nature of the microspheres. 3 g of the microspheres (x) was weighed in a butter paper and placed in a hot air oven at 105°C for 4 hours. Then it was weighed (x^1) and difference in weight gives the moisture absorbed ($Y = X^1 - X$).

$$\% \text{ Moistured absorbed} = (Y/X \times 100)$$

***In vitro* release studies[12]**

The drug release was performed for prepared theophylline microspheres using USP dissolution apparatus 2. The test was studied for first two hrs in acidic buffer at pH 1.2 and then for 10 hrs in phosphate buffer pH at 7.4. Samples were withdrawn at 30 min intervals, filtered and analyzed by UV – VIS spectrophotometer at 271 nm.

RESULTS AND DISCUSSION

From the results observed from quantitative analysis such as TLC and FTIR confirmed that the R_f values, peaks and patterns of spectra were similar in all the three combinations. It indicates true homogeneity, absence of drug degradation and interaction between the drug and polymers. The microsphers prepared from chitosan exhibited good sphericity with chitosan 191 cps, which showed somewhat distorted shape. This might be

due to the increased viscosity of the droplets leading to difficulty in dispersion and subdivision of droplets during the formulations. The particle size of chitosan microspheres showed a general increase as the concentration and viscosity of chitosan, where as the combination of chitosan - sodium alginate produced microspheres with good sphericity and smooth surface. This is due to polyelectrolyte complex that has the negatively charged carboxylic acid groups of alginates, where binds with positively charged amino group of chitosan acting as a cross linking agent; thus, improving the sphericity and hardness of microspheres. The particle size of these combinations was in the range of 900-994 μm. In the second combination of albumin with chitosan produced the microspheres with spherical shape but uneven surface might be due to the invate solubility of albumin that might have loosened the network of matrix. The particle size of this combination was comparatively small in the range of 45-50 μm. This might be due to the decrease viscosity of polymer solutions, which enabled easy dispersion, and subdivision of the droplets during the process of preparation.

Table 1. Charecterization of theophylline microspheres

Batch code	Size of microsphere	Incorporation efficiency (%)	Carr's index	Moisture uptake (%)	Drug content (mg)
A1	45.83	80.50	14.7	0.53	201.25
A2	69.44	80.76	14.21	0.40	201.90
B1	55.55	78.21	10.8	0.73	195.52
B2	57.77	77.13	12.8	0.40	172.83
C1	61.11	85.00	15.0	0.60	212.50
C2	133.33	83.70	10.10	0.60	209.25
D1	111.11	73.00	12.5	0.60	182.5
D2	133.33	70.71	15.02	0.63	176.77
E1	900.00	83.20	10.09	0.73	208.00
E2	947.00	85.38	14.70	0.56	213.45
E3	990.00	86.92	14.75	0.46	217.30
E4	994.00	91.85	15.47	0.33	229.63

Cont…

Batch code	Size of microsphere	Incorporation efficiency (%)	Carr's index	Moisture uptake (%)	Drug content (mg)
F1	45.83	75.56	10.36	0.80	188.65
F2	46.83	79.63	12.06	0.43	199.08
F3	46.98	84.29	10.83	0.56	210.73
F4	47.02	87.02	13.33	0.60	217.55

Bulk density, true density, porosity, compressibility index and angle of repose were found enough for all batches so that they can be easily encapsulated. Compressibility index value was less than or equal to 15% in all the batches and hence, these were expected to have good flow property. The angle of repose was 25-30^{o} in chitosan and the combination of chitosan- albumin microspheres indicates the good flow property. But sodium alginate-chitosan microspheres were having θ values less than 25^{o} and hence, it can be expected to have a excellent flow property. This is due to good sphericity of microspheres. All the batches of microspheres were found to satisfy the limit (0.5-1.0% moisture) and hence, the prepared microspheres can be considered as relatively non-hygroscopic.

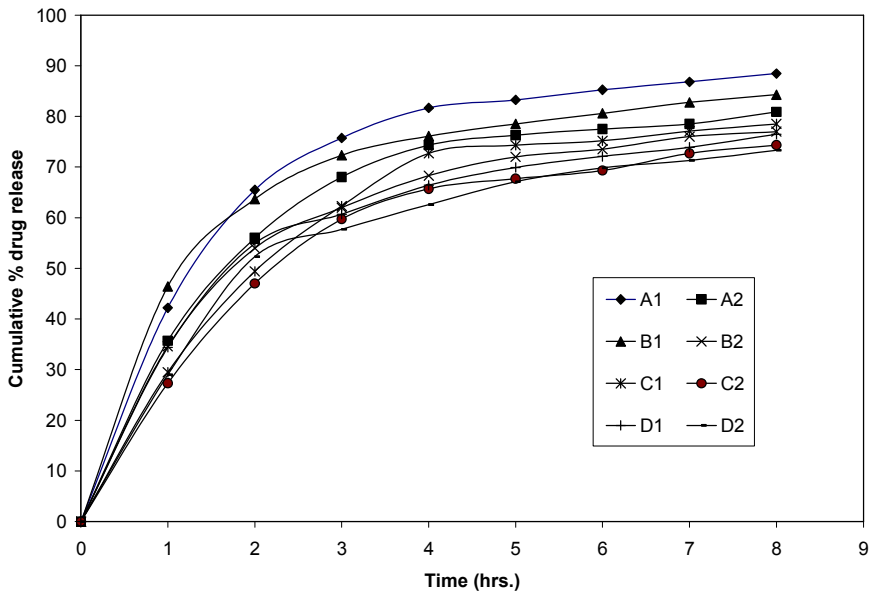

Fig. 1: *In vitro* release profile of chitosan microspheres containing theophylline

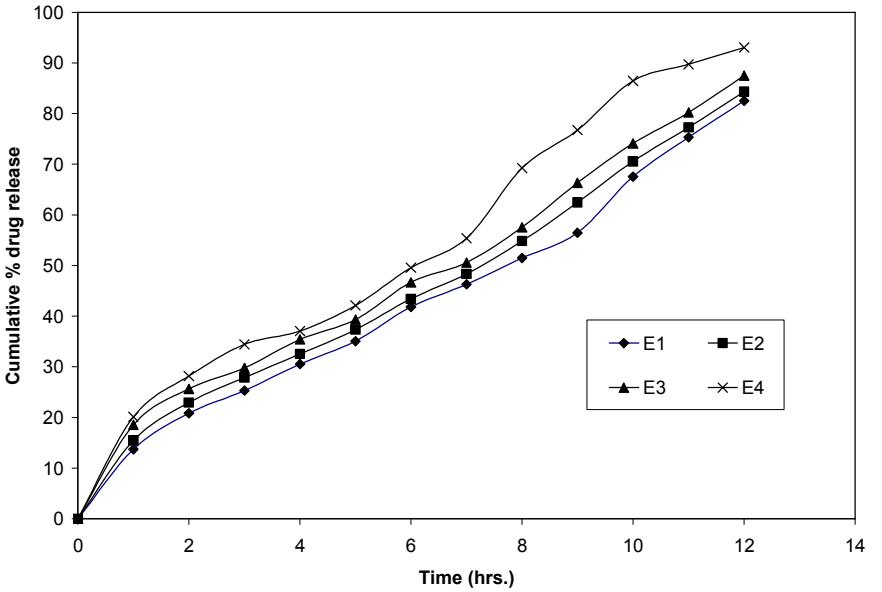

Fig 2: *In vitro* release profile of chitosan – sodium alginate microspheres containing theophylline

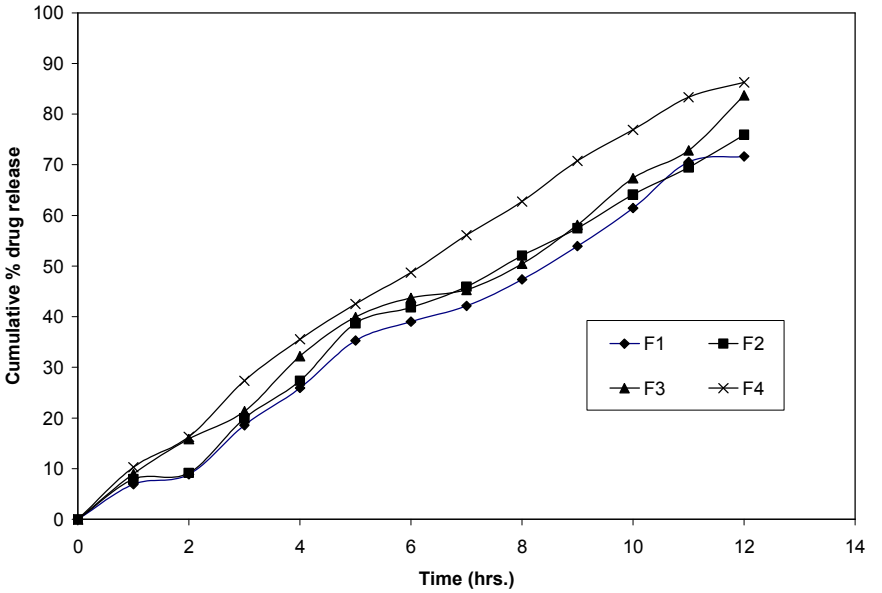

Fig 3: *In vitro* release profile of chitosan - albumin microspheres containing theophylline

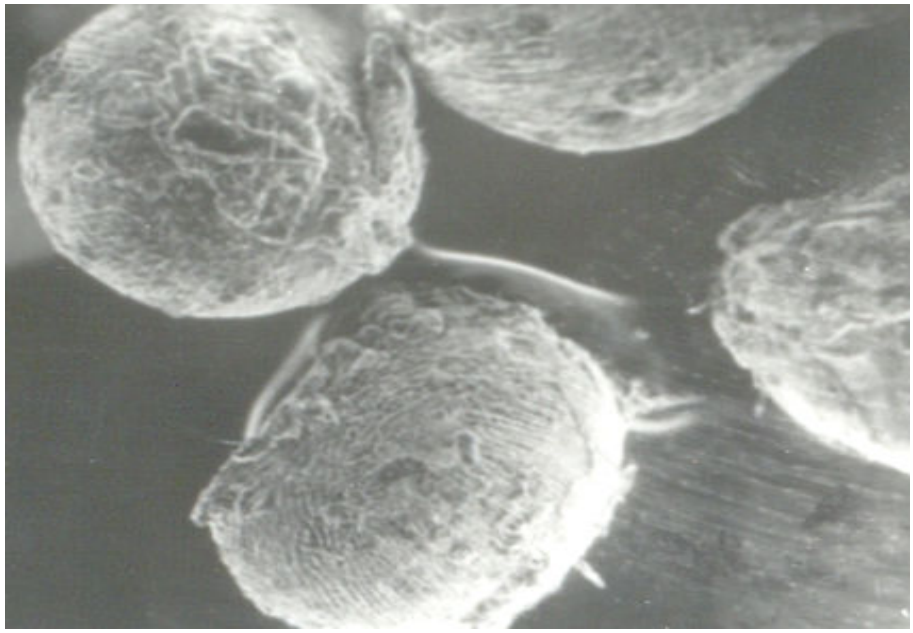

Fig 4: SEM photograph of theophylline loaded chitosan sodium alginate microspheres (1 : 1 : 6.5) batch E_4– magnification 24 X

For fulfilling the present study, the drug release profile of the microspheres prepared from different polymers was studied. The drug release of the microspheres prepared from chitosan (grade 33 cps, 50 cps, 106 cps and 191 cps) was affected by pH of the medium, which was evident from the *in vitro* dissolution profiles of the batches A_1, A_2, B_1, B_2, C_1, C_2, D_1 and D_3. Around 40-60% of theophylline was released within the 2 hours in acid buffer. This may due to the fact that pH can affect the ionization of glucosamine residues of chitosan. Out of the four grades chitosan, 106 cps released 30% in 105 min and 80 % at the time of 380 min. This might be due to the fact that at lower pH, the D glucosamine residues are ionized and triggered the drug release from the initial period and slow the release in phosphate buffer.

To study the effect of sodium alginate with chitosan, four batches (E_1, E_2, E_3 and E_4) were formulated. From the *in vitro* drug release profiles, it was evident that all the formulations released around 25 % of drugs within first two hours and extended the release profile up to 720 min. while formulation E_4 released 28 % drug at the two hours and extended to 720 min, which will be beneficial for the management of nocturnal asthma. This was due to deprotonation of alginic acid, which causes the disintegration of the microspheres.

To study the effect of albumin, four different formulations (F_1, F_2, F_3 and F_4) were formulated. The *in vitro* release profile revealed that the addition of albumin microspheres has reduced the burst release and also it has sustained the drug release upto 12 hours, which will be suitable for sustained release; but it was fulfilled to provide initial onset of action.

The results obtained state that all the formulations followed first order kinetics and the mechanism of drug release from the polymer matrix is by Higuchi's equations.

From the comparative evaluation, the formulation E_4, gave an initial onset of action as well as sustained the drug release up to 12 hours, which is expected to be more effective in the treatment of chronic obstructive pulmonary disease (COPD) and nocturnal asthma.

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