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# Formulation and evaluation of albumin–chitosan floating microsphere containing clarithromycin and estimation by spectrophotometric method

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**ABSTRACT**

The objective of the present study was to develop albumin- chitosan based floating mucoadhesive microsphere of clarithromycin to provide prolonged contact time for drug delivery of antibiotics to treat stomach ulcers, increase the gastric residence time, decrease the diffusional distance, and also act locally at the infectious site. Microspheres prepared by heat stabilization method in the presence of span 80 were optimized by varying different formulation and processes parameters like drug to polymer ratio. It was subjected to evaluation for particle size, incorporation efficiency, in vitro buoyancy and in *vitro* –drug release. The kinetics of release was determined and fitted to an empirical equation. Drug release from microsphere was found to be First order release, F7 which shows high percentage drug release. It was concluded that drug-loaded microsphere appear to be a suitable delivery system for clarithromycin, and since no visible spectrophotometric method is reported for the analysis of clarithromycin from microspheres.

**Keywords:** albumin, clarithromycin, spectophotometric, microsphere

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## INTRODUCTION

Clarithromycin, chemically 6-o-methyl erythromycin is a semi-synthetic macrolide antibiotic used in the treatment of respiratory tract infection and in skin and soft tissue infections, eradication of H.pylori, anti-diarrhoea, anti-pneumonic, whooping cough,sometimes in heart infections, in sinusitis,anti-tubercle [1,2]. It is administerd in the dose range of 250-500mg given as twice daily regimen associated with side effects of Diarrhoea, Nausea, vomiting and Dyspepsia. Biological half life of drug is about 3-4 hours. As it is required frequent dosing to maintain the therapeutic effect, it was chosen as a model drug for present study; therefore, clarithromycin 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-chitosan microspheres provide a potentially useful means of delivering drugs because they are both physically and chemically stable, amenable to separation in large batches, non-antigenic, metabolize within the body and capable of accommodating non-specific fashion [5]. The present study deals with the design and evaluation of albumin-chitosan microspheres of clarithromycin for sustained release.

### Materials

Albumin, chitosan and gelatin were obtained from S.D Fine Chemicals, Mumbai. Clarithromycin was a gift sample from Kopran Limited,Mumbai. Span 80 and sunflower oil were procured from Loba Chem. Pvt.Ltd., Mumbai.

## EXPERIMENTAL METHODS

### Preparation of floating-mucoadhesive microsphere

Albumin and chitosan microsphere were prepared by heat stabilization technique.[6]The clarithromycin was dispersed in the mixture containing 5 ml of 1% w/v albumin solution and 5ml of 2% w/v chitosan in acetic acid as rate controlling polymer, to this 5 ml of 20 %w/v gelatin solution and 1.5% of calcium carbonate solution was added and mixed to aid floating character. The mixer was dropped through syringe into 25 ml of sunflower oil containing 0.5% w/v span 80, gently stirred for 10 min and was maintained at 60-70$^0$c. The resulting (w/o) emulsion then stirred well for 10min using remi stirrer at 1000 rpm and then was cooled to 5$^0$c for 30 min by keeping in 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. The formula for prepared Microsphere were given in Table.1

### CHARACTERIZATION OF MICROSPHERES

### Morphology

The Morphology of the Microspheres was examined by scanning electron Microscopy. The shape and surface morphology of various batches of microspheres prepared, were determined by scanning electron microscopy (SEM-JEOL Model 8404, Japan at Magnification 100x and 250x) (fig.2&3). The size of the microspheres varied approximately from 256.8 to 286.3 µm. The Bulk volume, tapped volume, porosity and angle of repose of microspheres were studied and to determine flowability, consolidation index was also calculated which are presented in Table 2.

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**In vitro buoyancy efficiency**

The floating character of the prepared formulations was evaluated in SGF (pH 2.0). The time the formulation took to emerge on the medium surface (floating lag time) and the percentage of the microspheres that floated on the dissolution medium surface were evaluated. The calcium carbonate effervesced, releasing carbon dioxide and the released carbon dioxide is entrapped in the gel network producing buoyant formulation for prolonged periods and all the microsphere starts to float within a short time, and remained floating until the completion of drug release. The buoyancy lag time for this system was in the range of 10-15 min. The *in vitro* floating test clearly showed that most of the microspheres floated for around 12 hrs. The microspheres with the higher concentration of polymer were more floatable than those with lower concentrations of polymer [6]. This may be attributed to a decrease in density of microspheres with an increase in polymer concentration as shown in Table.3

$$\text{Buoyancy \%} = \frac{\text{wt of microsphere floated on medium}}{\text{Wt of µ.sphere floated on medium + Wt of µ.sphere settled at bottom of flask}} \times 100$$

**ENTRAPMENT EFFICIENCY**

To determine the total drug content of microspheres a known amount of microspheres were ground to fine powder. Accurately weighed 50mg of microspheres were soaked in 50ml of distilled water and sonicated using probe sonicator (UP 400s, Dr.Hielscher GmbH, Germany) for 2 hrs. The whole solution was centrifuged to remove the polymeric debris. Then the polymeric debris was washed twice with fresh solvent (water) to extract any adhered drug. The clear supernatant solution was filtrated through a 0.45 µm syringe filter then estimated by uv method [7, 8].

Entrapment efficiency = b/ a

a = theoretical drug content

b = drug entrapped

*IN VITRO* **RELEASE PROFILE**

The in-vitro drug release studies of clarithromycin were carried out by using USP dissolution apparatus type I, at 100 rpm and maintained temperature at 37º c ±0.5º c using 900 ml of 0.1N HCL (pH 2.0 ) as a dissolution medium . From each batch, 250 mg of clarithromycin loaded microsphere were taken and subjected to dissolution studies. Samples were taken at appropriate time intervals and replaced with equal volume of fresh dissolution medium, the collected samples were extracted with 10 ml of chloroform until complete recovery of drug.The separated solvent layer was shaken for 5 minutes with 5ml of bromophenol blue and clarithromycin content were analysed spectrophotometrically at 414 nm [9].

## RESULTS AND DISCUSSIONS

The size of Microspheres ranged between 256.8 to 286.3 µm. The particle size distribution was uniform and spherical. The results obtained for the measurement of flow properties by angle of repose indicated good packing and flow properties as shown in table 2. It was found that incorporation efficiency of clarithromycin was between 49.34 to 68.94 % depending on core and coat ratio as shown in table 3. The floating ability of the prepared formulations was evaluated in SGF (pH 2.0) and the percentage of the microspheres that floated on the dissolution medium surface were evaluated and are shown in Table 3. In FT-IR study, the characteristic peaks due to pure clarithromycin have appeared in microspheres, without any change in their position after successful

encapsulation, indicating no chemical interaction between clarithromycin and polymers used, and the stability of drug during microencapsulation process.

Drug release from all formulations was slow and sustained over 12 hours (Fig.1). The drug release rate was decreased on increasing the polymer ratio. By the end of 12 hours F7,F8 and F9 released 83.35%, 76.33% and 73.53% loaded drug respectively. The polymer drug ratio (1:1.5:0.5) showed better drug entrapment and release pattern. It controlled the drug release over 12 hours and was found to be suitable among all formulations. These results indicated that microspheres prepared with drug to carrier ratio of ( 1:1.5:0.5) showed a better pattern of sustained release as shown in Table.4

In order to understand the mechanism and kinetics of drug release, the result of the in*vitro* dissolution study of microspheres were fitted with various kinetic equations like zero order (% Release versus time), Higuchi (% drug release versus square root of time). The linear regression analysis is summarized in table.5.The examination of coefficient determination $r^2$ value indicated that drug release followed the diffusion control mechanism from the microsphere, further to understand the drug release mechanism the data were fitted with peppas exponent model and 'n' is the release exponent which characterize the drug transport mechanism . The value of 'n' is in the range of 0.50 and above which indicates the drug release followed by diffusion control mechanism

## CONCLUSION

Thus, microsphere of albumin-chitosan which is biocompatible and biodegradable were prepared by heat stabilization method was able to sustain the drug release effectively. SEM photograph of sample which proves micron size of clarithromycin loaded microsphere. The microsphere having 3:1 polymer ratio F7 provides best sustained and higher drug release among all. The *in vitro* floating test clearly showed that most of the microspheres floated for around 12 hrs. The microspheres with the higher concentration of polymer were more floatable than those with lower concentrations of polymer. From the above data, it may be concluded that drug-loaded microsphere appear to be a suitable delivery system for clarithromycin, and since no visible spectrophotometric method is reported for the estimation of clarithromycin loaded microsphere, the method developed in the present investigation may perhaps be used for the analysis of clarithromycin from microspheres.

**Table 1: Formulation composition of floating-bioadhesive microspheres of clarithromycin**

| Batch no. | Amt of clarithromycin used(mg) | Amt of albumin and chitosan ratio | |
|---|---|---|---|
| F1 | 250 | 1 | 1 |
| F2 | 250 | 1 | 2 |
| F3 | 250 | 1 | 3 |
| F4 | 250 | 2 | 1 |
| F5 | 250 | 2 | 2 |
| F6 | 250 | 2 | 3 |
| F7 | 250 | 3 | 1 |
| F8 | 250 | 3 | 2 |
| F9 | 250 | 3 | 3 |

Note: All the formulation containing 15 % w/v of gelatin and 1.5% of calcium carbonate solution.

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**Table 2: Micrometrics studies**

| Batch no. | Angle of repose(θ) | Bulk volume(ml/g) | Tapped volume(ml/g) | True density(gm/cm $^3$) | Porosity | Consolidation index (%) |
|---|---|---|---|---|---|---|
| F1 | $31^0$ 22" | 5.248 | 3.5635 | 0.857 | 0.582 | 11.96 |
| F2 | $31^0$ 56" | 4.9658 | 3.5745 | 0.9745 | 0.6214 | 12.25 |
| F3 | $32^0$ 34" | 5.0168 | 3.2869 | 0.895 | 0.5932 | 11.26 |
| F4 | $31^0$ 47" | 5.1783 | 3.2149 | 0.995 | 0.6745 | 12.56 |
| F5 | $32^0$ 30" | 5.246 | 3.0145 | 0.9124 | 0.6823 | 12.15 |
| F6 | $32^0$ 28" | 4.8624 | 3.2105 | 0.9356 | 0.601 | 12.05 |
| F7 | $32^0$ 45" | 5.214 | 3.1428 | 0.9745 | 0.7012 | 12.86 |
| F8 | $33^0$ 42" | 5.1452 | 2.8954 | 0.8931 | 0.5864 | 13.59 |
| F9 | $32^0$ 34" | 5.2381 | 3.157 | 0.8965 | 0.5961 | 11.95 |

**Table.3. Physico-chemical characteristics of the floating-mucoadhesive microspheres of clarithromycin**

| Batch code | Mean particle size in µm | Incorporation efficiency (%) | Buoyancy (%) |
|---|---|---|---|
| **F1** | 175.23 | 54.23 | 70.26 |
| **F2** | 169.1 | 52.91 | 65.3 |
| **F3** | 156.8 | 49.36 | 85.2 |
| **F4** | 174.26 | 61.25 | 72.12 |
| **F5** | 171.29 | 66.26 | 76.23 |
| **F6** | 170.6 | 64.3 | 83.56 |
| **F7** | 186.3 | 68.86 | 68.36 |
| **F8** | 184.96 | 67.55 | 80.96 |
| **F9** | 170.3 | 68.94 | 89.1 |

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**Table.4. In*vitro* release profile of the floating-mucoadhesive microspheres of clarithromycin**

| Time (hrs) | Cummulative percentage of drug release | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
| **0** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **2** | 36.69 | 36.16 | 36.36 | 36.29 | 33.62 | 35.13 | 36.08 | 33.57 | 33.29 |
| **4** | 48.55 | 49.22 | 47.71 | 46.63 | 47.42 | 46.73 | 52.76 | 47.42 | 46.75 |
| **6** | 55.61 | 55.82 | 53.95 | 52.91 | 53.07 | 53.04 | 58.85 | 55.83 | 54.96 |
| **8** | 64.16 | 64.50 | 63.87 | 64.22 | 64.13 | 63.93 | 65.06 | 66.93 | 67.44 |
| **12** | 78.76 | 81.81 | 81.62 | 79.27 | 76.58 | 79.45 | 83.35 | 76.33 | 73.53 |

**Table.5. *In vitro* drug release kinetic of floating-mucoadhesive microspheres of clarithromycin**

| Batch No. | correlation coefficients ($r^2$) | | | Release Exponent ´n ' |
|---|---|---|---|---|
| | Zero Order | Higuchi | Peppas | |
| F1 | 0.9787 | 0.9965 | 0.9971 | 0.5310 |
| F2 | 0.9846 | 0.9974 | 0.9950 | 0.5012 |
| F3 | 0.9840 | 0.9965 | 0.9903 | 0.5224 |
| F4 | 0.9833 | 0.9965 | 0.9900 | 0.5017 |
| F5 | 0.9807 | 0.9979 | 0.9962 | 0.4985 |
| F6 | 0.9856 | 0.9976 | 0.9933 | 0.5226 |
| F7 | 0.9818 | 0.0.9958 | 0.9920 | 0.5612 |
| F8 | 0.9777 | 0.9976 | 0.9976 | 0.4819 |
| F9 | 0.9818 | 0.9945 | 0.9930 | 0.5045 |

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**Fig:1**

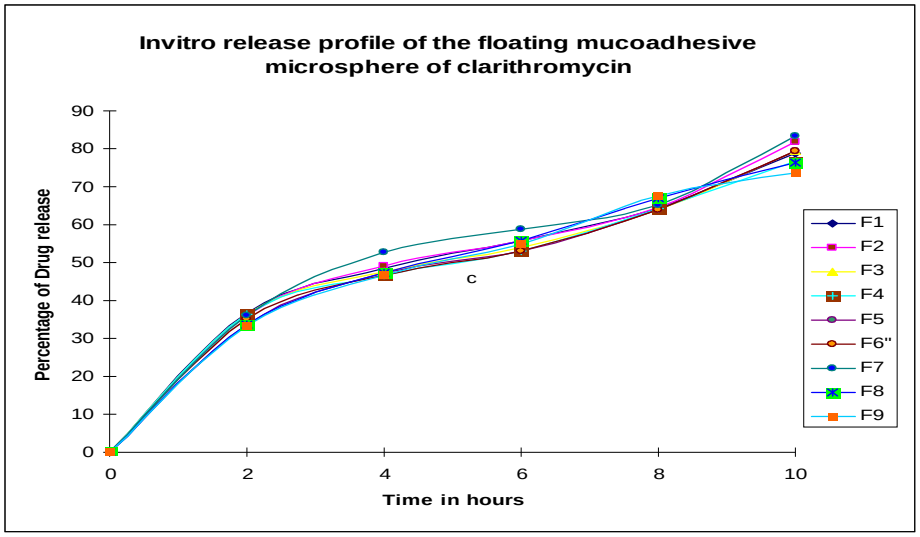


**Fig 2.SEM photograph (X250) of floating-mucoadhesive microspheres of clarithromycin**

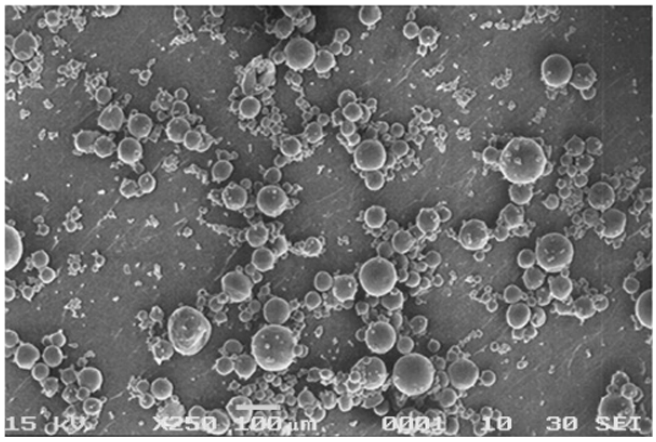

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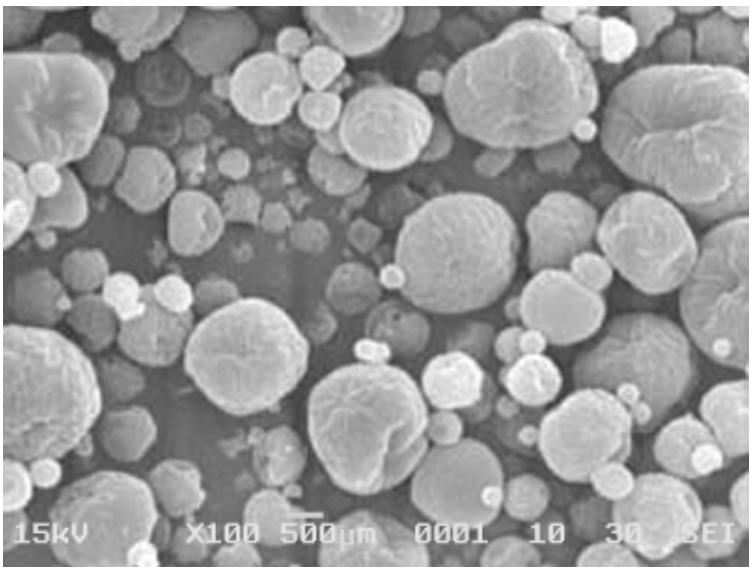
**Fig 3. SEM photograph (100X) of floating-mucoadhesive microspheres of clarithromycin**

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