

## CONTROLLED RELEASE OF A WATER SOLUBLE DRUG, METOPROLOL SUCCINATE, BY $\alpha$ -LACTALBUMIN MICROPARTICLES

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### ABSTRACT

**Objective:** The present study was aimed to investigate the controlled release of a water soluble drug, metoprolol, from  $\alpha$ -lactalbumin microparticles.

**Methods:** The microparticles were prepared by steric-stabilization process using lactic acid as the stabilizing agent. Metoprolol succinate loaded  $\alpha$ -lactalbumin particles were characterized using particle analyzer, Fourier transform infra red spectroscopy, X-ray diffraction and scanning electron microscopy.

**Results:** It was found that the size of the particles was in the range of 1-2  $\mu$ m and had encapsulation efficiency of 70 – 80%. The *in vitro* release studies of the encapsulated drug were performed under two simulated conditions of body fluids (SGF and SIF). The results indicated controlled release of about 70% of the encapsulated drug over a period of 24 hours, compared to the release of more than 90% of the drug (within two hours) from a physical mixture of  $\alpha$ -lactalbumin and metoprolol succinate.

**Conclusions:** The results thus offer hope for the design of drug delivery system using lactic acid-stabilized  $\alpha$ -lactalbumin microparticles for the controlled release of metoprolol succinate. Using lactic acid to prepare  $\alpha$ -lactalbumin microparticles provides improved biocompatibility in comparison to chemically cross-linked microparticles.

**Keywords:** Encapsulation, Lactic acid, Metoprolol succinate,  $\alpha$ -lactalbumin, Microparticle, Controlled release.

### INTRODUCTION

Design of appropriate drug delivery systems have tremendous importance for the controlled release of drugs and the choice of the method used for drug delivery can have significant effect on its efficacy. To this effect, extensive investigations are being carried out to unravel new and potential drug carriers. The design of such stable drug carriers, logistically, is followed by engineering the carriers to achieve targeted delivery. Concentration of the drugs delivered / released also has greater impact on the therapeutic benefit of the drugs. Therefore there is a need for multidisciplinary approach for the efficient delivery of therapeutics to specific targets in tissues [1]. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability various drug delivery and drug targeting systems are currently under development. Controlled delivery systems possess many advantages like immediate distribution, more constant plasma levels, better dose by dose reproducibility and smaller decrease in bioavailability when compared with single dose systems [2].

Several proteins such as albumin, casein, gelatin has been used as carriers for the sustained release of drugs [3, 4]. However, there seems to be no carrier that can cater to the release of a wide range of drugs. The encapsulation efficiency and sustained release of drugs differ on the type of protein used and the physico-chemical property of the drug under consideration. To this effect, we have embarked on the study of the delivery of water soluble drugs by  $\alpha$ -lactalbumin based microparticles. Recently we have shown that  $\alpha$ -lactalbumin could encapsulate methotrexate with high efficiency and is also capable of sustained release of the drug [5].  $\alpha$ -Lactalbumin is the second most abundant protein in milk, making up approximately 20-25% of the whey protein and is biocompatible. In the present study we embarked on investigating the encapsulation efficiency and release of a water soluble drug with a different physico-chemical property, namely metoprolol succinate (a cardiac  $\beta$   $\beta$  blocker), using  $\alpha$ -lactalbumin microparticles stabilized by lactic acid.

### MATERIALS AND METHODS

$\alpha$ -lactalbumin was received as a gift from Davisco Foods International Inc, Minnesota, USA. Metoprolol succinate and Ethyl cellulose (Low viscosity grade) was provided by Cipla Research center, Mumbai, India and Lupin Research Park, Pune, India

respectively. Chloroform, lactic acid and acetone were purchased from Ranchem, Mumbai, India. All other chemicals used were of high quality analytical grade.

### Formulation of $\alpha$ -Lactalbumin microparticles

Steric stabilization is a well-known phenomenon for the hindrance of agglomeration and therefore for the stabilization of particles [6]. In short, 4 ml of 10%  $\alpha$ -lactalbumin solution were dispersed in 50 ml of chloroform (containing 1% Ethyl Cellulose). The emulsion obtained was homogenized using a homogenizer (Kinematica A.G) at 20,000 rpm for a period of 30 minutes. One ml of 3% lactic acid was added during the homogenization process for stabilizing the microparticles. The suspension containing the particles thus formed was stirred for 12 hours and then centrifuged at 8000 rpm for 10 minutes to remove the organic phase. The pellet was washed repeatedly with acetone to remove excess ethyl cellulose and chloroform. The microparticles obtained were dried at room temperature. In a similar way, metoprolol succinate loaded  $\alpha$ -lactalbumin microparticles were prepared using  $\alpha$ -lactalbumin solution containing the known quantity of the drug.

### Particle Size Distribution

Microparticle size was analyzed by laser scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) at 20°C. The average particle size was expressed in volume mean diameter.

### Encapsulation efficiency of $\alpha$ -lactalbumin microparticles

The drug content in the particles was determined by pulverizing the air dried drug-loaded  $\alpha$ -lactalbumin microparticles (10 mg) followed by dispersing them in 100 ml of 0.5 mM phosphate buffer (pH 7.4). The suspension was agitated at various speeds (100-500 rpm) at 30°C for 10 min. The drug concentration was determined by measuring the absorbance (UV-Vis Spectrophotometer –Shimadzu Model) at 275 nm. All samples were analyzed in triplicates and the encapsulation efficiency was calculated according to the following equation [7]:

$$\% \text{ Encapsulation efficiency} = (W_A / W_T) * 100$$

where  $W_A$  and  $W_T$  represents actual and theoretical drug content, respectively, in the microparticles.

### Morphological characterization using Scanning Electron Microscopy

The size and the surface morphology of the prepared microparticles were analyzed using scanning electron microscopy (Jeol, JSM-5200, Japan, 15 KV). The particles were sprinkled on adhesive aluminum stub and then surface coating was done with gold to a thickness of ~300 Å using a sputter coater. (SPI Sputter™ Coating Unit, SPI Supplies, Division of Structure Probe, Inc., PA USA).

### X-ray Powder Diffraction Studies

The X-ray diffractograms of the microparticles and the metoprolol succinate loaded microparticles were obtained from a D8 Advance Model X-Ray Diffractometer (Bruker, Germany) using Ni filtered radiation ( $\lambda = 15.4$  nm, 40 kV and 30 mA). The measurements were carried out using PMMA sample holder and lynx eye detector.

### Fourier transform infrared spectroscopic analysis

The Fourier transform infrared (FTIR) spectrum of the microparticle was recorded in the range of 400–4000  $\text{cm}^{-1}$  using KBr pellet with a FTIR spectrophotometer (Nicolet Avatar 330) at room temperature.

### In vitro release of metoprolol succinate

*In vitro* release of metoprolol succinate from  $\alpha$ -lactalbumin microparticles was performed both in simulated intestinal fluid (SIF, pH 7.4) as well as simulated gastric fluid (SGF, pH 1.2). Known amount of drug loaded  $\alpha$ -lactalbumin micro particles (100 mg) was dispersed in 0.5 ml of the simulated fluids (SIF / SGF) and was subsequently dialyzed (10kDa cut off membrane) against 50 ml of the same fluid. The system was agitated at 50 rpm at 37°C. Aliquots (0.5 ml) were collected at predetermined time points and equal volume of buffer was added to maintain the sink conditions throughout the experiment. The amount of metoprolol succinate in the aliquot was quantified by UV-Vis spectrophotometer at 275 nm (as described above).

## RESULTS

### Particle Size Distribution

Particle size analysis revealed that the microparticles prepared by steric stabilization method followed Gaussian distribution and were

in the range of 0.5-2  $\mu\text{m}$ , with an average size of 0.98  $\mu\text{m}$ , with a volume distribution of 26% (Figure 1). In order to address the issue of the stability of the microparticles, the size of the microparticles were measured periodically over a period of six months (Figure 1). The results indicate that the microparticles were relatively stable with respect to size. The size of the particles obtained is similar to those of albumin microparticles reported in literature [8].

### Encapsulation efficiency of $\alpha$ -lactalbumin microparticles

Depending on the dry weight of the  $\alpha$ -lactalbumin and metoprolol succinate used for the preparation of the microparticles, the conditions representing 100% encapsulation efficiency was calculated. Since the solution containing 40 mg of the drug and 100 mg of  $\alpha$ -lactalbumin was used for the preparation of the microparticles, it is expected that 100% encapsulation efficiency is obtained if 0.13 mg of the drug is contained per milligram of the microparticles. However estimation of the amount of drug revealed that only 0.094 mg was encapsulated in per milligram of  $\alpha$ -lactalbumin microparticles. This indicates that the encapsulation efficiency is 72% under the conditions used in this study.

### Morphological characterization using scanning electron microscopy

The results obtained from SEM studies confirmed spherical structure and roughened surface structure of the microparticles (Figure 2). The diameter of the microparticles was obtained from scanning electron micrographs and was found that majority of the particles were in the range of 1 to 2  $\mu\text{m}$ . The size of the particles is comparable with the results obtained from particle size analysis by light scattering studies.

### X-ray Powder Diffraction

XRPD (X-Ray Power Diffraction) was used to determine if metoprolol succinate is encapsulated with in the  $\alpha$ -lactalbumin microparticles. The XRPD patterns for metoprolol succinate (Figure 3) correspond to those obtained for crystalline samples. However, such sharp lines are absent after encapsulation within the  $\alpha$ -lactalbumin microparticles, thus confirming the physical dispersion of the drug molecules within the  $\alpha$ -lactalbumin microparticle framework. The patterns were similar to those observed for the methotrexate encapsulated  $\alpha$ -lactalbumin [5].

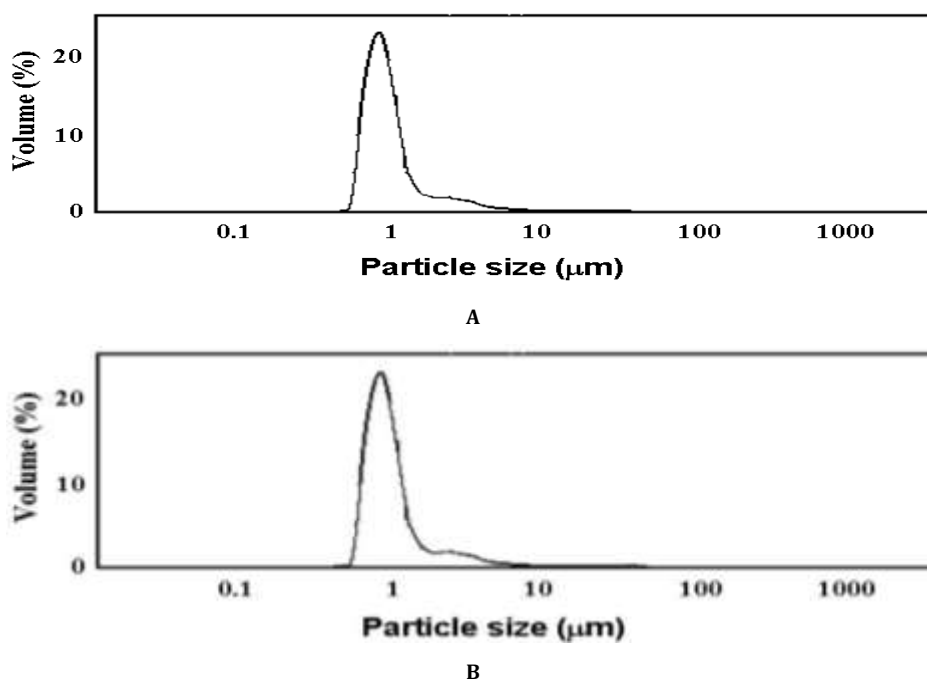


Fig. 1: Particle size distribution of Metoprolol succinate loaded  $\alpha$ -lactalbumin microparticles immediately after preparation (A) and after 6 months (B). The mean particle size is 0.98  $\mu\text{m}$ .

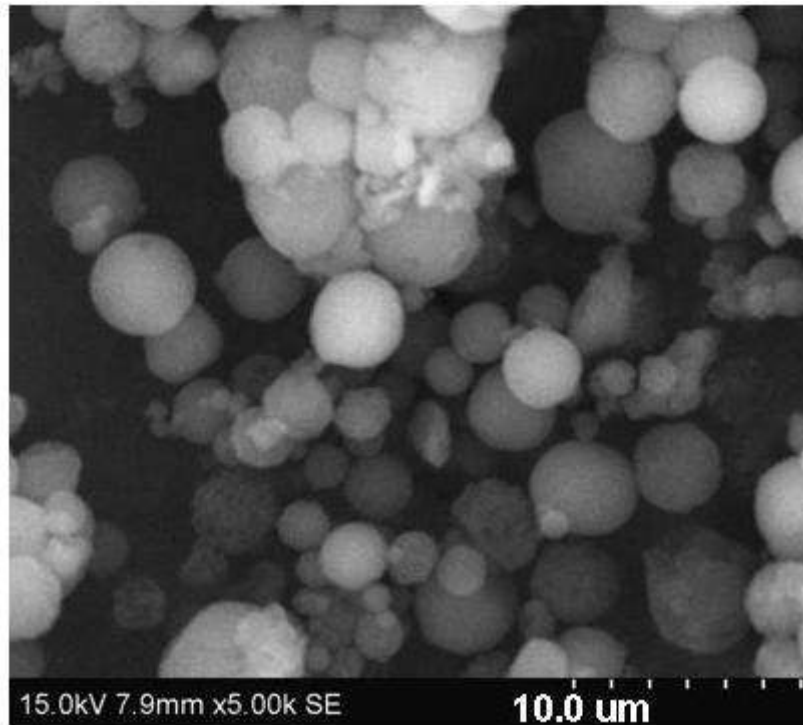


Fig. 2: Scanning electron micrograph of metoprolol succinate loaded  $\alpha$ -lactalbumin microparticles

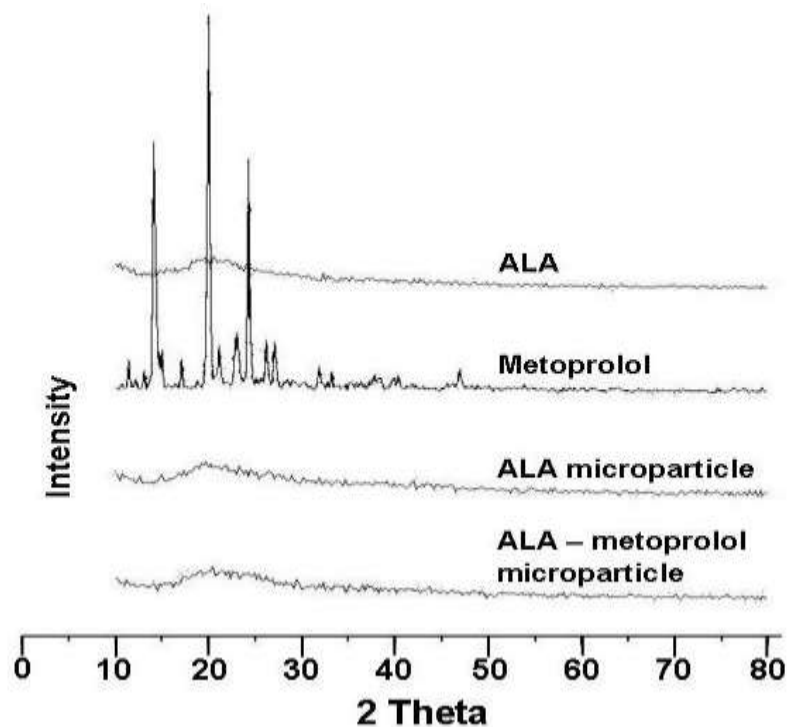


Fig. 3: X-ray Powder Diffraction analysis of free and encapsulated metoprolol succinate.

#### Fourier transform infrared spectroscopy

Infrared spectra (Figure 4) were also used to infer the interaction between the polymer and the drug in microparticles. The spectra of raw materials (free drug +  $\alpha$ -lactalbumin) showed their characteristic vibrational and rotational bands. When this polymer is ionized, the

carbonyl band corresponding to the anti-symmetrical vibration shifts from 1728 to 1560  $\text{cm}^{-1}$  [9, 10, 11]. The bands observed in the microparticles spectrum did not show any such shift, suggesting that no new chemical bond was formed involving the functional groups after preparing the formulation. This result confirmed that the drug is physically dispersed within the polymer matrix.

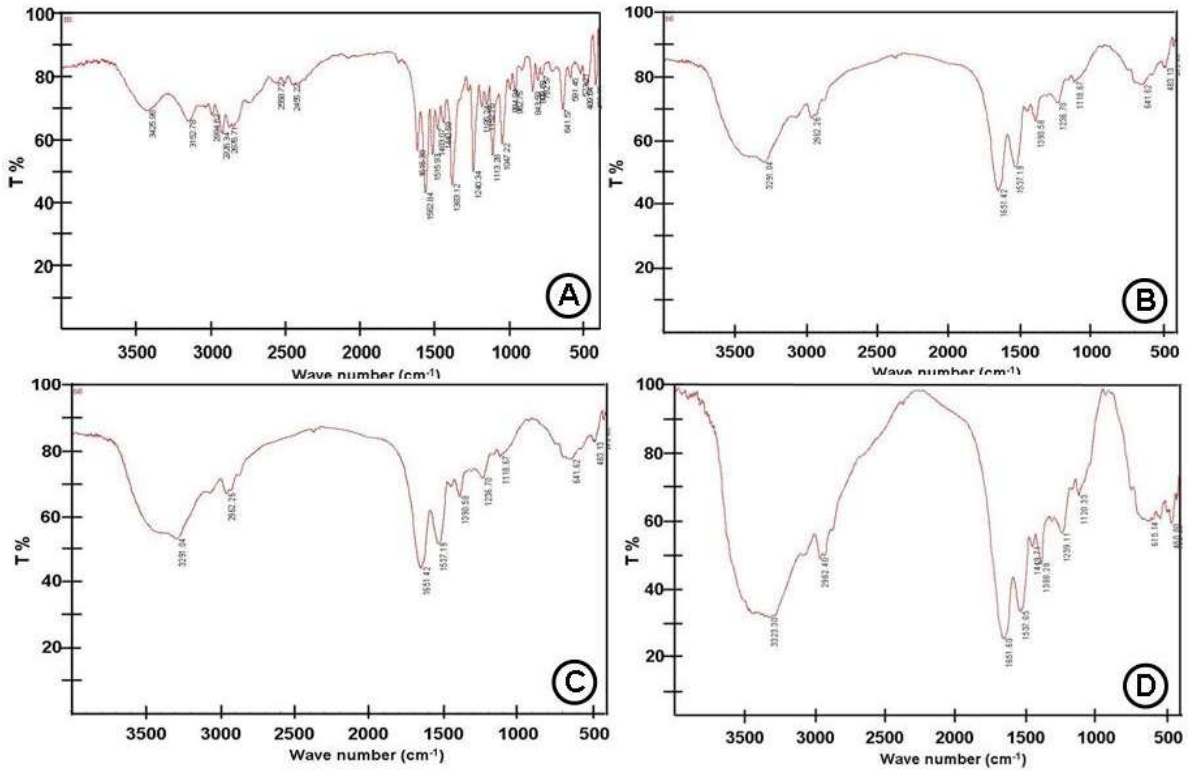


Fig. 4: FTIR spectra of (A) metoprolol succinate, (B) metoprolol succinate loaded α-lactalbumin microparticle, (C) α-lactalbumin and (D) α-lactalbumin micro particles.

**In-vitro release of metoprolol succinate**

In vitro release of the drug from α-lactalbumin microparticles was studied in simulated intestinal fluid (SIF, pH 7.4) and simulated gastric fluid (SGF, pH 1.2). The percentage of drug released in the simulated intestinal fluid was 65 ± 2% and in the simulated gastric fluid was 70 ± 1% at the end of 24 hour. In contrast the physical mixture of metoprolol succinate and α-lactalbumin releases 85% of the drug within two hours (Figure 5).

The in vitro release of the formulation in SIF was as follows: 1 hour (11±2% release), 2 hour (21±1% release), 3 hour (30±1% release), 4 hour (39±2% release), 6 hour (50±2% release) and 8 hour (57±1% release).

The in vitro release of the formulation in simulated gastric fluid was as follows: 1 hour (22±2% release), 2 hour (30±3% release), 3 hour (40±1% release), 4 hour (44±2% release), 6 hour (56±2% release) and 8 hour (66±2% release). The initial release is more in SGF than in SIF (1 h), the percentage of drug release at the end of 8 h is same in both SGF and SIF.

In order to understand the role of lactic acid on the in vitro release of the encapsulated drug, microparticles were formulated in the presence of different concentration of lactic acid and the release of the drug was monitored. Figure 6 depicts the observed differences in the release of encapsulated drug after one hour of incubation. It could be observed that the amount of drug released decreased with increased in concentration of lactic acid.

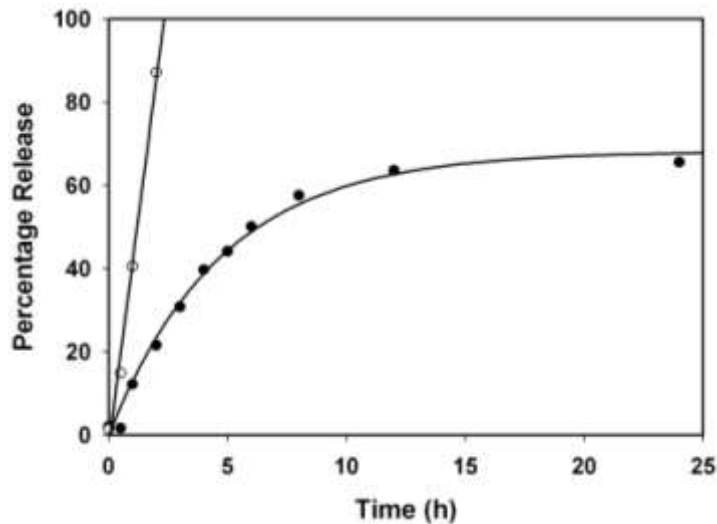


Fig. 5: In-vitro release profile of Metoprolol succinate in SIF. Release of the drug from a physical mixture is given for reference.

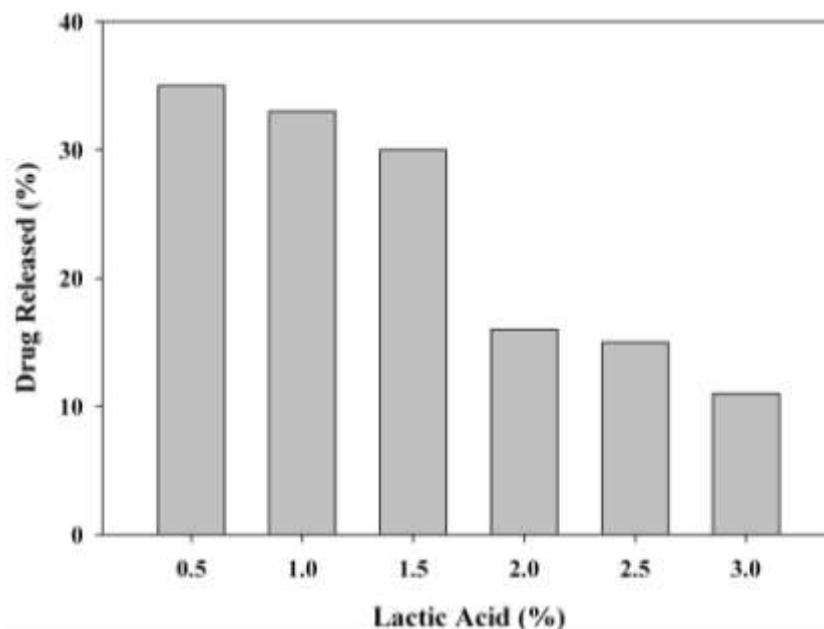


Fig. 6: *In vitro* release of the encapsulated metoprolol from lactalbumin particles at 1 hour. The lactalbumin was stabilized by various concentrations of lactic acid (in the range 0.5 % to 3.0%).

The above mentioned release data confirms that the formulations are found to be a suitable candidates for sustained release of the drug, metoprolol succinate.

#### DISCUSSION

The results obtained in this study is part of our research effort to synthesis  $\alpha$ -lactalbumin microparticles which can accommodate range of drugs with varied physiochemical properties and also to study the effects of processing conditions on particles size, drug loading and sustained release. In the past different controlled release formulations such as matrix tablets [12] multiple emulsions [13] iontophoretic application [14] osmotic tablets [15] electrolyte induced peripheral stiffening matrix system [16] and other hydrophilic polymers [17] have been used for the encapsulation and sustained release of water soluble drug, metoprolol succinate. In this article  $\alpha$ -lactalbumin microparticles were prepared by a modified steric stabilization process in which lactic acid is used a stabilizing agent instead of crosslinking the particles. The process variables such as concentration of  $\alpha$ -lactalbumin and ethyl cellulose, stirring rate, volume of  $\alpha$ -lactalbumin solution, were optimized in our earlier study [5]. Optimization was performed for the concentration of lactic acid used in the formulation. Six different formulations of the microparticles were made with different lactic acid concentrations between 0.5% and 3%. There was direct correlation noted between lactic acid concentration and encapsulation of microparticles. The encapsulation was highest when the lactic acid percentage is 3% beyond that percentage there was no more increase in encapsulation. Between 0.5% to 3% lactic acid the release of drug seemed to be more controlled with higher concentration of lactic acid. Also, when the release of the drug was monitored for an extended period of time, it was observed that most of the encapsulated drug could be released irrespective of the concentration of the lactic acid used. However, the time taken for the release was proportional to the lactic acid concentration (in the range 0.5 % to 3.0%), thus indicating controlled release of the drug by these formulations (Figure 6). The comparison study of physical mixture formulation and microparticles revealed that the microparticle formulation gave a sustained pH independent release profile of metoprolol succinate.

It is observed that the release of the drug follows a biphasic pattern as reported with other delivery systems. However, the metoprolol sustained release formulations reported in literature have indicated a burst phase, with close to 50% of the drug being released within 4

h [17, 18, 19, 20]. The encapsulation of metoprolol with a very highly hydrophilic carrier, poly(methylglyoxalate) was studied by Brachais et al (1988). The authors report that the encapsulation efficiency is proportional to the amount of drug, as inferred from the release profiles [21]. Moreover, at lower concentration of the drug close of 20% of the encapsulated drug is released in the burst phase (30 min). Such release is a common phenomenon and has been attributed to higher solubility of metoprolol in PBS at pH 7.4 [22]. It is rationalized that the diffusion rates of such water soluble drugs encapsulated within the water soluble hydrophilic polymers depend on the polymer level. Higher packing of the polymers leads to dense materials and therefore retards the release of the drug [17]. However, the present study clearly indicate that under the similar *in vitro* release conditions (PBS, pH 7.4)  $\alpha$ -lactalbumin microparticle could exhibit a more controlled release of this highly water soluble drug.

The particles size was characterized by SEM and was found to be in the range of 1- 2 $\mu$ m. The unique XRD and FTIR data denotes the dispersion of drug within the polymers. The release of the drug Metoprolol Succinate was found to be steadily increasing till 8 hours. This may represent that drug can be released within the therapeutic window. The metoprolol succinate loaded  $\alpha$ -lactalbumin microparticles found to be stable in simulated gastric fluid (SGF) and drug release was comparable with the simulated intestinal fluid.

#### CONCLUSION

Effective drug delivery requires successful navigation of carriers through complex biological processes in the body. Hence, particles whose properties can be controlled in real time open new opportunities. Control over particle properties including size, surface chemistry and shape provide timely control over their interactions with cells or subcellular compartments [23]. With the current process it is feasible to produce customized  $\alpha$ -lactalbumin microparticles for optimal drug delivery.

Using lactic acid to prepare  $\alpha$ -lactalbumin microparticles provides improved biocompatibility in comparison to chemically cross-linked albumin microparticles. This property also favours use of lactic acid stabilised-albumin microparticles for drug delivery. Thus, the present study demonstrates that  $\alpha$ -lactalbumin microparticles can be prepared with predictable and reproducible size, in a size range between, 1- 2 $\mu$ m by steric stabilization process using lactic acid as stabilizing agent. Encapsulation efficiency as high as 70% was

achieved combined with a much sustained release up to 24 hours in these formulations in *in vitro* studies. Based on these findings we conclude that  $\alpha$ -lactalbumin microparticle is a potential candidate for sustained release of metoprolol succinate.

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