

Alginate-Poloxamer Beads for Controlled Release of Metoprolol Succinate

Rabinarayan PARHI*, Podilam SURESH

GITAM University, GITAM Institute of Pharmacy, Gandhi Nagar Campus, Rushikunda, Visakhapatnam-530045, Andhra Pradesh, INDIA

The aim of this present study is to develop micro-beads for controlled release of Metoprolol succinate (MS). Different combinations of alginate and poloxamer 407 (PL) were taken to prepare beads by employing ionotropic gelation method, where CaCl_2 used as gelling agent. FTIR technique was used to study the drug and polymers interaction. The prepared beads were evaluated for drug loading, entrapment efficiency, particle size and morphology, *in vitro* drug release and drug release kinetics. There were no shift in the major peaks of the pure drug was observed from the FTIR spectra, which indicates the absence of interaction between drug and polymers. The highest drug loading and entrapment efficiency were found to be 3.11 % and 35.67 %, respectively for the formulation FB1. The particle size was measured by sieving method and it was found that the particles were in the micron range for all the formulations. The morphology of the formulation FB4 was studied by SEM and the image showed irregular surface. The *in vitro* drug release study was carried out in pH 6.8 phosphate buffer for 12 hr at 37°C and the results demonstrate cumulative percentage of drug release was decreased with the increase in PL ratio in the composition that indicates controlled drug release. To know the mechanism of drug release the *in vitro* drug release data were fitted to zero order, first order, Higuchi and Korsmeyer-Peppas equation. The formulation FB4 showed highest correlation coefficient (r^2) of 0.97 for Higuchi equation due to which it was selected as best formulation. The release exponent (n) of all the formulation were below 0.45 except FB4 (0.48) demonstrating Fickian diffusion is the drug release mechanism, whereas the formulation FB4 indicated non-Fickian diffusion i.e. diffusion and erosion were the mechanism.

Key words: Metoprolol succinate, Micro-beads, Alginate, Poloxamer, Fickian diffusion.

Metoprolol Süksinat'ın Kontrollü Salımı İçin Aljinat-Poloksamer Boncuklar

Bu çalışmanın amacı Metoprolol süksinat (MS)'ın kontrollü salım yapan mikro boncuklarının geliştirilmesidir. CaCl_2 'nin jelasyon ajanı olarak kullanıldığı iyonotropik jelasyon metodu ile boncukları hazırlamak için aljinat ve poloksamer 407 (PL)'nin farklı kombinasyonları kullanıldı. Etkin madde ve polimer etkileşimi için FTIR tekniği kullanıldı. Hazırlanan boncuklar, ilaç yükleme, etken madde yükleme verimi, partikül büyüklüğü ve morfoloji, *in vitro* etken madde salımı ve etken madde salım kinetikleri açısından değerlendirildi. FTIR spekturmunda saf etken maddenin major piklerinden hiç kayma olmadığını tespiti, etken madde ile polimer arasında bir etkileşim olmadığını gösterdi. Formülasyon FB1 için en yüksek etken madde yüklemesi ve yükleme verimi sırasıyla %3.11 ve %35.67 olarak bulundu. Partikül büyüklüğü elek metodu ile ölçüldü ve tüm formülasyonlar için partiküllerin mikron aralığında olduğu bulundu. FB4 formülasyonunun morfolojisi SEM ile çalışıldı, görüntü düzensiz bir yüzey olduğunu gösterdi. *In vitro* etken madde salım çalışması pH 6.8 fosfat tamponunda 37°C de 12 saat yapıldı ve sonuçlar yüzde kümülatif etken madde salımının, kompozisyondaki PL oranının artması ile azaldığını ve kontrollü salım olduğunu gösterdi. Etkin madde salım mekanizmasını bilebilmek için *in vitro* etken madde salım verileri sıfır derece, birinci derece Higuchi and Korsmeyer-Peppas eşitliklerine uygulandı. FB4 formülasyonu en yüksek korelasyon katsayısını (r^2) 0.97, Higuchi eşitliği ile gösterdi. Tüm formülasyonların salım katsayısı (n) FB4 hariç (0.48), 0.45'in altında olması, etken madde salım mekanizmasının Fick difüzyonu olduğunu gösterirken, FB4 non-Fick difüzyon, yani difüzyon ve erezyon mekanizması olduğunu gösterdi.

Anahtar kelimeler: Metoprolol süksinat, Mikro boncuklar, Aljinat, Poloksamer, Fick Difüzyon

*Correspondence: E-mail: bhu_rabi@rediffmail.com; Tel: 09052983544

INTRODUCTION

Hypertension is the most common cardiovascular disease and its prevalency increases with advanced age (1). There are various classes of drugs such as angiotensin converting enzyme inhibitors, angiotensin blockers, calcium channel blockers, diuretics and β -adrenergic blockers with different mechanisms are available for the treatment of hypertension. Among all, β -adrenergic receptor antagonists are mainstays of antihypertensive therapy. MS is a cardioselective β -blocker which acts preferentially on β_1 -adrenoceptors (Competitive antagonism of β_1 -adrenoceptors) in the heart, which results in decrease of hypertension by a number of mechanisms, including a reduction in myocardial contractility, heart rate, cardiac output and systolic blood pressure (2).

Immediate release dosage form releases the entire drug dose rapidly, which often results in variable plasma drug level. In order to control this effect the concept of controlled release is being used, where the drug is generally incorporated into the polymeric matrix which releases the drug in controlled manner for longer period of time. As a result the frequency of dosing is reduced, thereby patient convenience and drug efficiency improved and drug toxicity decreased.

Beads are one of the multiparticulate systems, which ensure more uniform drug release by avoiding the uncertainty of gastric emptying, chances of dose failure due to non-disintegration and variable transit rates through the gastro-intestinal tract as compared to single unit dosage form (3, 4). The other common advantages the beads can offer are limited drug fluctuation within therapeutic range, which reduces side effects and improving patient compliance by decreasing dosing frequency (5).

Naturally occurring polysaccharides are more preferred over synthetic one in the development of controlled release dosage forms due to their abundance, low price, nontoxic and biodegradable nature (4). Alginate is such a linear unbranched polysaccharides obtained from various species of brown algae and is consisting of varying

amounts of β -D-mannuronic acid and α -L-guluronic acid residue that are linked by 1,4'-linkage. Sodium alginate (SA) undergoes gelation and cross-linking with various divalent cations (i.e. Ca^{2+} , Sr^{2+} , or Ba^{2+}), which bind to the guluronic acid unit of alginate to form large unit (6). It is widely used as matrix-former in various drug delivery systems. However, high gel porosity of alginate matrix in aqueous medium leads to high diffusion of drugs followed by burst release of the loaded drug (7).

Poloxamer 407 is a non-toxic copolymer consisting 70% of hydrophilic ethylene oxide units and 30% of hydrophobic propylene oxide units. It forms clear solution between 20 to 30% (w/w) concentration at refrigerator temperature (4°C) and upon warming to room temperature or above it undergoes sol-gel transition (8, 9). In addition to above reverse thermal gelling property, it also provides excellent wetting, antifoaming and solubilizing action because it is classified as surfactant. However, excess of aqueous medium causes the dissociation of packed PL micelles resulting in the loss of gel integrity (10).

Keeping in mind the above problems associated with both SA and PL, we proposed to develop sustained release beads of MS by taking both the polymers in combination. The main intension is to fill the pores in the SA matrix by PL which acts as barrier in diffusion of drug. This results in sustained release of MS from the beads.

MATERIALS AND METHODS

Metoprolol succinate was supplied by Cadila Healthcare Ltd., Ahmedabad, India. Pluronic F-68 (Poloxamer 127) and sodium alginate were obtained from Loba Chem Pvt. Ltd., Mumbai, India. Trisodium citrate was purchased from Merck, Mumbai. All other ingredients used in the study were of analytical grade.

FT-IR spectrophotometry

FT-IR analysis was performed on MS, alginate and poloxamer individually, and in physical mixture (1:1) of MS with alginate and poloxamer to investigate the polymer

drug interaction. A FT-IR spectrophotometer (Alpha-FT-IR, Bruker Optics, Germany) was used for recording the spectra of the samples in nujol mull.

Preparation of beads

Beads of alginate treated poloxamer containing MS were prepared by ionotropic gelation method (11, 12). Total polymer concentration of 2 % w/v were dissolved in 100 mL of cool (10°C) distilled water under gentle stirring using magnetic stirrer (Remi instruments, Mumbai). A weighed amount of MS was added to above polymer solution and was stirred to obtained homogenous mixture. The obtained mixture was kept overnight for deaeration in room condition. The mixture was introduced into a 10 mL syringe and then extruded dropwise into 100 mL of 6% (w/v) CaCl₂ solution through the 23 gauge needle. The formed drug loaded beads were allowed to harden for 30 min in the solution. Then, beads were filtered and washed three times with distilled water to remove CaCl₂ from beads surface followed by drying at 40°C in tray dryer for 15 hr. The distance between edge of the needle and the surface of the solution was maintain at 10 cm.

Determination of drug loading and encapsulation efficiency

Accurately weighed amount (~ 10 mg) of drug loaded beads were dissolved in 10 mL trisodium citrate solution (3% w/w) under horizontal shaking (100 rpm, water bath shaker, Remi, India) for 24 hr at 37°C. Then, the solutions were taken and MS content was determined spectrophotometrically (UV-1700, Shimadzu, Japan) at 224 nm (13, 14) after suitable dilution. The drug loading content and encapsulation efficiency were calculated by using Equation 1 and 2.

Drug loading (%) =

$$\frac{\text{Drug recovered in beads (mg)}}{\text{Beads recovered (mg)}} \times 100$$

(Eq.1)

Encapsulation efficiency (%) =

$$\frac{\text{Drug found in beads (mg)}}{\text{Drug initially added to the formulation (mg)}} \times 100$$

(Eq.2)

Particle size distribution

The size distribution of beads was evaluated by sieve analysis, using mechanical sieve shaker and five standard sieves of 22 (710 μm), 30 (500 μm), 44 (355 μm), 60 (250), 85 (180), 100 (150). Around 200 beads were taken and sieve shaker operated for 10 min (15). Then, the particle size distribution was measured depending on the retention of beads on respective screen.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM, 5SM-5800, JEOL, Tokyo, Japan) was used to study the surface morphology of beads. Samples were coated with platinum using auto fine coater for 75 sec with thickness 25 nm at a 40 mA operating current prior to viewing.

In vitro drug release

The *in-vitro* drug release study was carried out in 500 mL of phosphate buffer pH 6.8 at 37°C by using USP I dissolution apparatus (Electrolab-model TDT-06L) (16, 17). The known quantity of beads (100 mg) from each formulation was placed into basket. The rotational speed of basket was kept at 75 rpm for 12 hr. At pre-determined time intervals (0.5, 1, 2, 3, 4, 6, 8, 12 hr) aliquots of 10 mL from the dissolution medium were withdrawn and was replaced with same volume of phosphate buffer pH 6.8 maintained at 37°C. The withdrawn sample was filtered and drug content was measured by using UV-Vis spectrophotometry at 224 nm.

Drug release kinetic and mechanism

In order to understand drug release pattern, the *in-vitro* drug release data were fitted to different kinetic models such as zero order, first order, Higuchi model. Further to conform the mechanism of drug release, Power law (equation 3) was used:

$$M_t / M_\infty = kt^n \quad (\text{Eq.3})$$

where M_t and M_∞ are the mass of drug released at a time t and the total mass of released drug, respectively. k is a constant and n is the release exponent which conforms the mechanism of drug release. For spherical systems (in this case beads): n ≤ 0.43 for

purely Fickian diffusion, $0.43 < n < 0.85$ for anomalous (non-Fickian transport), $n = 0.85$ for zero-order release systems, and values > 0.85 indicate super case-II transport (18).

Analysis of variance (ANOVA) with Benferroni multiple comparison tests was used to measure statistical significant differences between different *in vitro* drug releases at 5% significance level. It was performed using trial version of GraphPad Instat software.

RESULT AND DISCUSSION

FT-IR spectrophotometry

In order to know the interaction between selected polymer and drug, FT-IR spectra (Figure 1) of pure MS, PL, and alginate and in combinations were taken. The spectra of pure MS present the characteristic peaks at 3397.63 cm^{-1} (O-H stretching), 3148.66 cm^{-1} (C-H aromatic stretching), 2923.97 cm^{-1} (C-H alkyl stretching), 1613.86 cm^{-1} , 1563.74 cm^{-1} , 1536.72 cm^{-1} (C=C aromatic stretching vibration), 1242.23 cm^{-1} (C-N stretching), 1114.24 cm^{-1} (C-O stretching, 2° alcohol) and 1051.82 cm^{-1} (C-O-C stretching). There were no major shifts in peaks evident from the spectra of the physical mixture of MS with alginate and MS with PL, which conforms that no interaction between drug and polymer had taken place.

Determination of drug loading content and encapsulation efficiency

The drug loading and encapsulation efficiency values were mentioned in the Table 1. Drug/polymer ratio was kept constant at 1:10 for all the formulation. The highest drug loading and EE was found to be 3.11% and 35.67% for the formulation FB1 and both the values are decreased as the concentration of PL increases from 0.25% for the formulation FB2 to 1% for formulation FB5. These results indicate that the EE not only depends on the alginate content but also on the alginate/PL ratio (10).

Particle size distribution and SEM

Particle size of the beads was determined by sieve method and the finding is presented in Table 1. The smaller particle size (249 ± 38

μm) in case of FB1 was because of higher degree of cross-linking in the absence of PL (19). The size of beads was decreased as the PL ratio increased in the subsequent formulations (FB2 to FB5) from poloxamer:alginate ratio of 1:7 to 1:1. The SEM of formulation FB4 was presented in Figure 2. The surface of the bead is irregular which could be due to higher PL content.

In vitro drug release

In vitro drug release from all the prepared formulations was performed in phosphate buffer of pH 6.8 and the data are represented in Figure 3. It was observed from the graph that highest amount (96.19%) of drug released from the formulation FB1 after 12 hr, which is containing only alginate (2%). This can be explained by the fact that alginate dissociates at higher pH due to de-linking (deprotonation) of alginate molecules by non-gelling ions present in the phosphate buffer (20). Furthermore, alginate beads, not reinforced by PL, had probably insufficient cross-linking density to prevent drug molecules to diffuse out. In addition, metoprolol being a small molecule (mol. wt. of 267.36 and hydrophilic (freely soluble in water) in nature, has the tendency to diffuse out easily (21). It was also seen that with increase in PL concentration in the ratio the cumulative percentage of MS release decreases from 93.13% in formulation FB2 to 76.56% in FB5 during the same period of 12 hr. This result attributed to the reduction of gel porosity (as mentioned above) by PL along with longer diffusion path length formed with higher concentration of PL. In addition, as a common phenomenon smaller the particle sizes more is the drug release from it, which is clearly evident from the *in-vitro* MS release.

Drug Release Kinetic and Mechanism

The experimental data were fitted to different kinetic models such as zero order, first order, Higuchi model and Korsmeyer-Peppas and the obtained co-relation coefficient (r^2) and release exponent (n) values were presented in the Table 2. From the r^2 values, it is evident that all the formulation except FB3 shown adequate fit to Higuchi model. This indicates diffusion is the

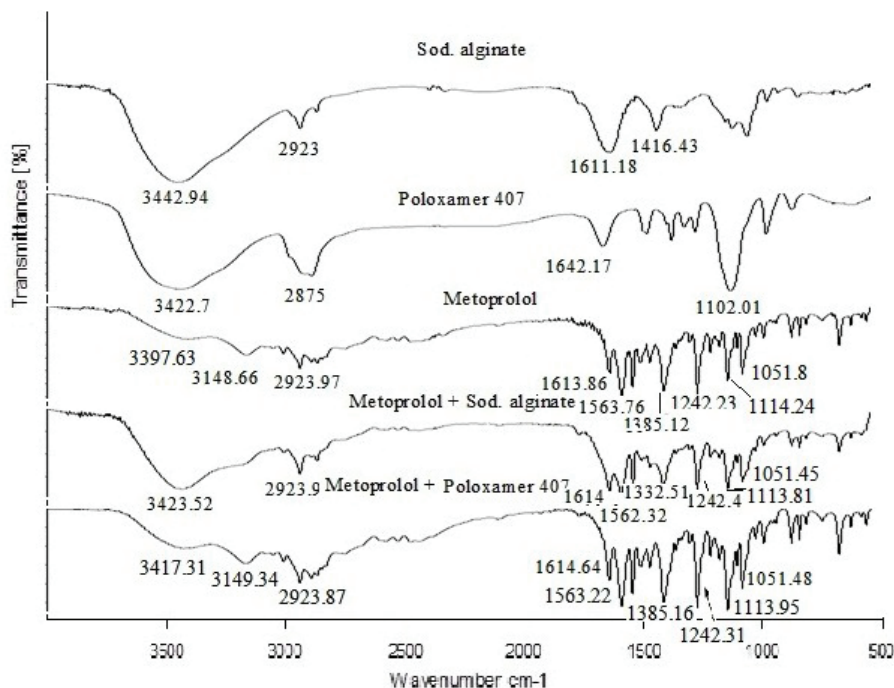


Figure 1. The FTIR spectra of MS, SA, PL and physical mixture of MS with SA and PL.

drug release mechanism. The *n* values were either equal to or less than 0.43 (except formulation FB4, where the *n* value is 0.48), which clearly demonstrates diffusion of the drug from the formulations followed Fickian type. The formulation FB4 demonstrates increased *n* value which may be due to swelling of the matrix (22).

One-way ANOVA data represented that there were significant difference of *in-vitro* drug release ($P < 0.05$ level) between batches except FB1-FB2, FB1- FB3, FB2- FB3, and FB4- FB5 combination.

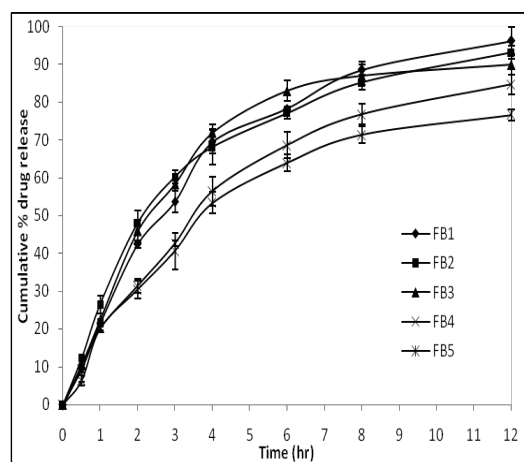


Figure 3. The dissolution profile of different bead formulations in pH 6.8 phosphate buffer (mean \pm SD, *n*=6).

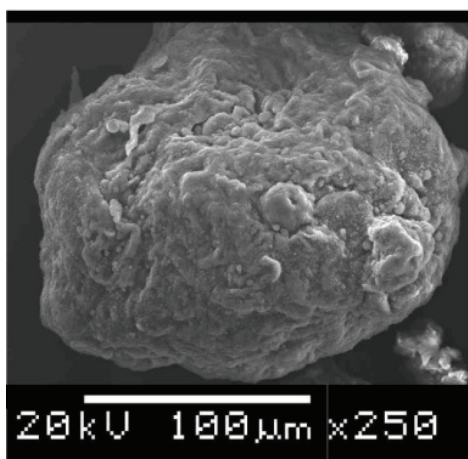


Figure 2. The SEM image of formulation FB4

CONCLUSION

In the present investigation, it was tried to develop controlled release micro-beads of MS as it is a highly water soluble drug. Further to ensure controlled release of MS for longer period of time (in this case 12 hr), PL is added

Table 1. Different beads composition, drug loading, encapsulation efficiency and mean diameter

| Form code | Metoprolol succinate (mg) | Sodium alginate (%) | Poloxamer (%) | Drug loading (%) | Encapsulation efficiency (%) | Mean diameter (μm) ($\pm\text{S.D.}$, n=6) |
|-----------|---------------------------|---------------------|---------------|------------------|------------------------------|---|
| FB1 | 200 | 2 | --- | 3.11 | 35.67 | 249 \pm 38 |
| FB2 | 200 | 1.75 | 0.25 | 3.08 | 33.03 | 267 \pm 24 |
| FB3 | 200 | 1.5 | 0.5 | 3.01 | 30.05 | 319 \pm 31 |
| FB4 | 200 | 1.25 | 0.75 | 2.98 | 25.36 | 345 \pm 54 |
| FB5 | 200 | 1 | 1 | 2.96 | 20.63 | 410 \pm 39 |

Table 2. Values of co-relation coefficient (r^2) and release exponent (n) of all formulations

| Form. code | Zero order (r^2) | First order (r^2) | Higuchi (r^2) | Korsmeyer-Peppas | | |
|------------|----------------------|-----------------------|-------------------|------------------|-------|------------------------|
| | | | | (r^2) | n | Drug release mechanism |
| FB1 | 0.833 | 0.623 | 0.95 | 0.933 | 0.4 | Fickian Diffusion |
| FB2 | 0.801 | 0.596 | 0.931 | 0.988 | 0.315 | Fickian Diffusion |
| FB3 | 0.749 | 0.575 | 0.896 | 0.858 | 0.307 | Fickian Diffusion |
| FB4 | 0.877 | 0.685 | 0.97 | 0.935 | 0.48 | Non-Fickian Diffusion |
| FB5 | 0.846 | 0.603 | 0.955 | 0.91 | 0.437 | Fickian Diffusion |

to alginate (generally used to prepare beads) in different proportion and found out 1.25:0.75 (alginate:poloxamer) combination in formulation FB4 was the best formulation. This could be due to reverse thermal gelling property of PL, which in turn filled pores formed in beads having only alginate. To concrete the findings, *in vivo* studies has to be undertaken in the future.

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