RESEARCH ARTICLE

# Extended Release of Metoprolol Succinate from HPMC Reinforced Alginate Microparticle

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**Extended Release of Metoprolol Succinate from HPMC Reinforced Alginate Microparticle**

**Summary**

The aim of the present investigation was to prepare and characterize hydroxypropyl methylcellulose K100LV (HPMC K100LV) reinforced sodium alginate microparticles of Metoprolol succinate (MS) in order to reduce burst release and extend the drug release. Microparticles were prepared by w/o-emulsification external gelation technique. The microparticles were characterized by X-ray analysis, FT-IR study, particle size and scanning electron microscopy (SEM). The in vitro drug release was carried out in phosphate buffer pH 6.8 as dissolution medium for 8 h. Irregular surface texture with particle size less than 7μm were obtained from SEM and particle size analysis. FT-IR spectra gave evidence of interaction between N-H group of MS and C = O group of alginate. MS was dispersed in the molecular form in the polymer matrix as indicated by X-ray diffraction pattern. More than 87% drug release was found in all the batches after 8 hr of dissolution study. Burst release was evident (65.08% drug release in 1 hr) in microparticles (MP1) with only alginate. A significant reduction in the burst effect and extended drug release were observed as the concentration of HPMC increased. Release rate exponent (n) values were less than 0.43, indicating Fickian diffusion could be the mechanism of drug release.

**Key Words:** Extended release, Metoprolol succinate, Burst release, Hydroxypropyl methylcellulose, Alginate.

**HPMC ile Kuvvetlendirilmiş Aljinat Mikrokürelerinden Metoprolol Suksinatın Uzatılmış Salımı**

**Özet**

Sunulan bu araştırmanın temel amacı; ilk hızlı salımı azaltarak salımı uzatabilmek için Metoprolol suksinat (MS) içeren hidroksipropil metil selüloz K100LV (HPMC K100LV) ile kuvvetlendirilmiş sodyum aljinat mikrokürelerinin hazırlanması ve karakterizasyonunun gerçekleştirilmesidir. Mikropartiküller S/O emülsifikasyon – eksternal jelasyon tekniği ile hazırlanmıştır. Mikropartiküllerin karakterizasyonu, X-ray analizi, FT-IR çalışmaları, partikül boyutu analizi ve taramalı elektron mikroskopisi (SEM) kullanılarak gerçekleştirilmiştir. In-vitro salım çalışmaları çözünme hızı ortamı olarak pH 6,8 fosfat tamponu kullanılarak 8 saat boyunca devam ettirilmiştir.

SEM ve partikül boyutu analizleri sonucunda düzensiz yüzeye sahip 7 μm'den küçük partiküllerin oluştuğu gözlenmiştir. FT-IR spekturumu ile metoprolol suksinatın N-H grupları ile aljinatın C = O grupları arasında etkileşim olduğu kanıtlanmıştır. Etkin maddenin (MS) polimer matriks içinde moleküler formda disperse olduğu X-ray difraksiyonu ile kanıtlanmıştır.

Tüm formülasyon gruplarında 8 saatlik çözünme hızı çalışmalarının sonunda %87'den daha fazla salım elde edilmiştir. Sadece aljinat ile hazırlanmış mikropartiküllerde (MP1) ilk hızlı salım elde edildi. (1 saatte %65,08 etkin madde salımı) Formülasyonlara artan konsantrasyonlarda ilave eden HPMC ile ilk hızlı salımın azaldığı ve uzatılmış salımın elde edildiği gözlendi. Salım hızı üssel katsayısı (n) değeri 0,43'den küçük olduğundanetkin madde salımının Fick difüzyon mekanizması ile olduğu düşünülmektedir.

**Anahtar Kelimeler:** Uzatılmış Salım, Metoprolol suksinat, İlk hızlı salım, Hidroksipropil metil selüloz, Aljinat.

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## INTRODUCTION

Oral route is one of the most widely used routes of drug administration because of its advantages of ease of administration, improved patient compliance, convenience, least sterility constraints and flexibility in designing the dosage form (1). In immediate-release dosage forms, there are little or no control over release of drug from the dosage form, which most often result in unpredictable and often sub- or supra-therapeutic plasma concentration (2). Developing oral controlled release systems for drugs with high water-solubility have always challenged the pharmaceutical technologist. Most of these drugs with high water-solubility, if not formulated properly, are released at high rates and are likely to produce toxic effects (3).

Metoprolol succinate is widely used in the treatment of hypertension, angina pectoris, and arrhythmias, due to its β-selective adrenoceptor blocking property (4). The drug is freely soluble in water and is administered at a dose of 100 mg daily, the half-life of MS is about 3-4 h, and its oral bioavailability has been reported to be about 50% (5). Several oxidative pathways including a-hydroxylation, O-demethylation and N-dealkylation extensively metabolize the drug (6). The therapeutic efficacy of MS by providing constant rate input and maintenance of steady-state blood levels (7, 8). In addition, it was reported that MS showing good absorption in the entire GI tract, rapid elimination and a well defined relationship between the β-blocking effect and plasma drug concentration (9). Combination of the above properties make MS a suitable candidate for the development of an extended-release (ER) formulation. Different types of controlled release formulations such as matrix tablets (10), multiple emulsion (11), iontophoretic application (12), osmotic tablets (13), electrolyte induced peripheral stiffening matrix system (14) and three layered tablets (1, 15) were reported.

Polymer-based drug delivery systems, such as microspheres/microparticles, are relatively simple to produce and can be administered by various routes, including oral, pulmonary and parenteral injection. Microspheres/Microparticles using a single polymer generally lead to high initial drug release (burst effect), due to which it is impossible to sustain the release for long periods. Burst release may occur due to a number of phenomena, including the presence of the drug on the microsphere surface, porosity of the microparticles and physical-chemical nature of the polymeric matrix (16, 17). Therefore, it is proposed to develop microparticles with a combination of polymers, in order to reduce initial burst release and prolong the drug release.

Alginates are naturally occurring, linear unbranched polysaccharides which contain various amounts of 1,4-linked β-D-mannuronic and α-L-guluronic acid units arranged as blocks along the chain, where homopolymeric regions are interdispersed with regions of alternating structure. They are able to form water-insoluble gels by cross-linking with divalent cations (e.g., $Ca^{2+}$) (18). HPMC has been extensively used as a rate controlling polymer in oral ER dosage forms because its non-toxic nature, availability in different chemical substitution forms, high hydration rates and different viscosity grades. Rapid formation of a viscous gel layer upon hydration and the viscosity of the gel layer of HPMC govern its performance in an ER matrix system (19). Upon formation of the gel layer, viscosity of the gel layer regulates the overall rate of drug release. In addition, variations in HPMC concentration from low to high (10–50%), results in broad differences in dissolution profiles (20).

The combination of HPMC and calcium alginate was seleceted as polymers in developing the microparticulate system. The idea is to reinforce the HPMC gel via a calcium cross-linked alginate network. Hence, the rapid dissociation of HPMC can be reduced. At the same time, HPMC could fill the pores of the alginate gel upon gelation and act as diffusion barrier for entrapped drug. Thereby sustained release of the drug can be achieved.

## MATERIALS AND METHODS
### Materials

The drug, metoprolol succinate, was supplied by Cadila Healthcare Ltd., Ahemedabad, India. HPMC K100LV and Sodium alginate were obtained from Loba Chem Pvt. Ltd., Mumbai, India. Tween 80 and span 80 (polysorbate 80 and sorbitan monooleate 80) were purchased from Merck, India. All other ingredients used throughout the study were of analytical grade.

**Microparticle Preparation**

Microparticles were prepared by w/o/emulsion external cross-linking method (21). In detail, twenty gram aqueous solution of 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 g (w/w) sodium alginate with 0, 0.1, 0.2, 0.3, 0.4, 0.5 g (w/w) of HPMC, respectively were prepared. To the above solutions 100 mg of the drug was added. Then, this aqueous phases were dispersed in 30 g peanut oil (organic phase) containing 3.3% w/w Span 80 (1 g) using a magnetic stirrer (Remi, India) at 600 rpm for 10 min. Two grams aqueous solution of 25% Tween 80 (0.5 g, resulting in a ratio of Span 80: Tween 80 = 2:1) was added, and the emulsion was stirred for another 5 min. The system was heated to 40–45°C under continuous stirring on a magnetic heating plate. Then, 8 g of an aqueous $CaCl_2$ solution (25% w/w) was added drop wise (during 4 min) using a syringe with needle no. 23. Stirring was continued for another 15 min. The system was then allowed to cool down for 10 min (under stirring at 200 rpm). Subsequently, the microparticles were recovered by vacuum filtration, further washing three times (3x30 ml) with isopropanol. After the washing, microparticles were dried in oven at 60°C for 6 hrs.

**Characterization of Microparticles**

*Determination of drug loading content and encapsulation efficiency*

Microparticles (10 mg, accurately weighed) were dissolved under horizontal shaking (100 rpm, water bath shaker, Remi, India) for 24 hrs at room temperature in 10 mL sodium citrate solution (3% w/w). This medium was selected because it contains calcium chelating ions, which displaces the drug associated to alginate (22, 23). The MS content was determined spectrophotometrically (UV-1700, Shimadzu, Japan) at $\lambda max = 274$ nm. Then, the drug loading content and encapsulation efficiency were calculated, using Equation 1 and 2

$$\text{Drug loading content (\%)} = \frac{\text{Drug recovered in microparticles (mg)}}{\text{Microparticles recovered (mg)}} \times 100 \quad (1)$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Drug found in the microparticles (mg)}}{\text{Drug initialy added to the formulation (mg)}} \quad (2)$$

*Size distribution of microparticles*

The size distribution of microparticles was evaluated by nanoparticle analyser (SZ-100, Horiba, Japan). Microparticles were suspended in isopropanol by sonication (Bath sonicator) for 1 min prior to measurement.

*Scanning Electron Microscopy (SEM)*

The morphology of microparticles was carried out by Scanning Electron Microscopy (SEM, 5SM-5800, JEOL, Tokyo, Japan). Samples were coated with platinum using auto fine coater for 75 sec with thickness 25 nm at a 40 mA operating current.

*Powder X-Ray Diffractometry (PXRD)*

PXRD analysis was carried out to study the change of state of MS from crystalline to amorphous state in the polymer matrix. PXRD study was performed over the range $2\theta$ of 5 to 70°, using PANlytical X'Pert PRO X-ray diffractometer (MPD PW3040/60 XRD, Almelo, The Netherland) with Ni-filtered CuKα radiation.

*FT-IR spectrophotometry*

FT-IR analysis was performed on MS, HPMC and Alginate in bulk, MS in a physical mixture with both alginate and HPMC (1:1) and on the microparticles. An FT-IR spectrophotometer (Alpha-FT-IR, Bruker Optics, Germany) was used for recording the spectra of the samples in nujol mull.

*In-Vitro drug release*

The *in-vitro* drug release study was carried out in a phosphate buffer of pH 6.8 at 37 ±0.5°C, using USP I dissolution apparatus (Electrolab-model TDT-06L). A total amount of microparticles equivalent to 10 mg of drug was placed in the basket. The basket was rotated at 100 rpm for 8 h. After pre-determined time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8 h) aliquots of 10 ml from the dissolution medium were withdrawn and the same volume of phosphate buffer pH 6.8 maintained at 37 ±0.5°C were replaced immediately. The samples were

then analyzed by UV-Vis spectrophotometry (λmax = 274nm) and the amount of the released drug was calculated.

## Drug Release Kinetic Modelling

To investigate the effect of the degree of cross-linking and different proportions of polymers on the mechanism of MS release from microparticles, a semi-empirical model, known as the power law was used (24). It is presented as Equation 3:

$$M_t / M_\infty = kt^n \quad (3)$$

where $M_t$ is the mass of drug released at time t, $M_\infty$ is the amount released at time $t = \infty$, thus $M_t/M_\infty$ is the fraction of drug released at time t, provided that t is limited to times where the fraction of drug release is more than 60%, k is the kinetic constant, and n is the release exponent, which is used to characterize the mechanism of the drug release (25).

Analysis of variance (ANOVA-one way) was performed to find out if there was any significant difference of effect of the content of polymer (Sod. Alginate and HPMC), at different concentration levels, on drug release from different formulation at 5% confidence level. The statistical analysis was performed using Microsoft Excel software.

## RESULTS AND DISCUSSION

In this study, alginate microparticles loaded with MS were developed using emulsification and external gelation methods. Aqueous emulsion droplets, containing both polymers and drug, converted into solid microparticle system by gelling agent $CaCl_2$. Calcium ions are bound to guluronic unit of the alginate, thereby forming water insoluble gel particles.

Microparticles of different formulations showed different drug loading and encapsulation efficiency, as shown in Table 1. This can be explained by the fact that polyelectrolyte complex could be formed between polyanionic alginate and the oppositely charged MS, having a cationic group. As a result, MS could be entrapped in higher concentration in the matrices having higher proportion of alginate in the formulations. Furthermore, the ratios of alginate to HPMC in the microparticle formulations were different. Since the molecular weight of alginate (Mw = $2.85 \times 10^5$ g/mol) was much higher than that of HPMC K100LV (Mw = $2.5 \times 10^4$ g/mol), the drug loading and encapsulation efficiency increased with increasing alginate content from formulation MP6 to MP1. The highest encapsulation efficiency was found to be 60.1% and it decreased substantially as the concentration of HPMC increased. This is likely due to the higher exposure times to ionic solution (24, 26).

The SEM images of the surface of the tray-dried microparticles (MP1 and MP4) are presented in Figure 1. The alginate:HPMC ratio significantly affected the morphology and the particle shape. Microparticles with HPMC (MP4) were distorted in shape, exhibiting an uneven (but not porous) surface. This result is attributed to higher viscosity of the peanut oil (18). The

**Table 1.** Different microparticle compositions and its effect on drug loading and encapsulation efficiency

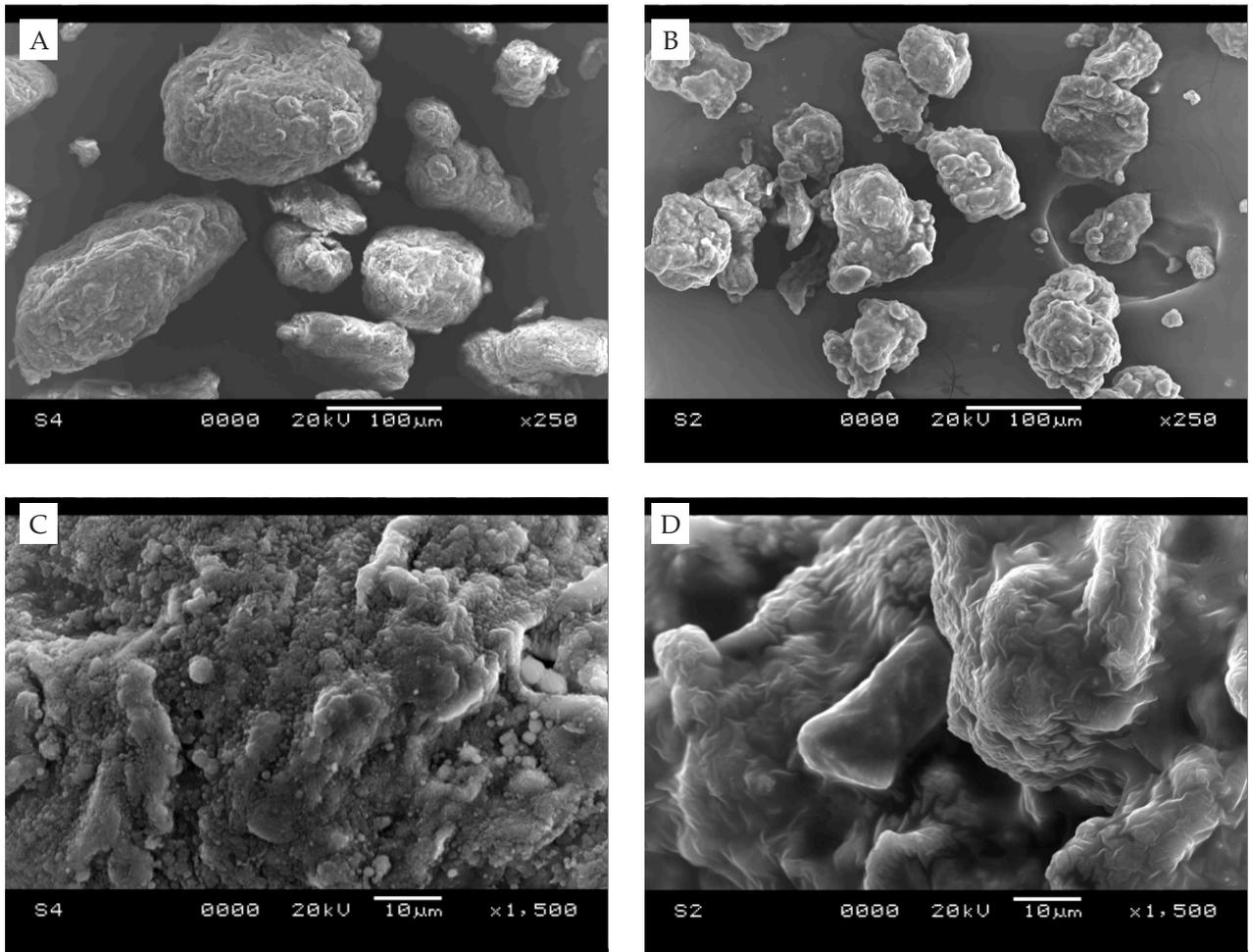| Formulation Code | Drug (mg) | Sod. alginate (mg) | HPMC (mg) | Drug Loading (% wt) | Drug encapsulation efficiency (%) |
|---|---|---|---|---|---|
| MP1 | 100 | 600 | —— | 5.08 ± 0.24 | 60.1 ±3.09 |
| MP2 | 100 | 500 | 100 | 4.24 ± 0.31 | 37.97 ±3.34 |
| MP3 | 100 | 400 | 200 | 3.76 ± 0.07 | 22.94 ±2.1 |
| MP4 | 100 | 300 | 300 | 3.22 ± 0.12 | 14.67 ±0.56 |
| MP5 | 100 | 200 | 400 | 2.77 ± 0.23 | 9.87 ±1.35 |
| MP6 | 100 | 100 | 500 | 2.68 ± 0.06 | 6.76 ±0.63 |

n = 3

**Figure 1.** SEM micrographs: (A & C) Sample-MP1 and (B & D) Sample- MP4

expanded SEM image of microparticles MP1 shows irregular surface with a number of pores. However, the formulation MP4 in the expanded image revealed no cracks, indicating that the reinforcement was effective. Furthermore, it is expected denser cross-linked calcium alginate gel to retain more amounts of drug, leading to formation of a tough membrane, which is particularly evident in microparticle MP1 (27).

The microscopy observation is in good agreement with the data obtained by the particle size analysis. With increasing alginate content, microparticles became smaller in size as depicted in Figure 2. The average size of the formulation MP1 was determined as 4.63 μm, which is less, when compared to formulation MP4 (6.08 μm). These differences in average sizes of microparticles can be attributed to the fact that cross-linking is effective in the absence

of HPMC. In addition, the polydispersive index (PI), which is a measure of the width of the particle size distribution, is higher in both formulations, which could be due to slow speed (600 rpm) in stirring and in tray-drying process.

The XRD pattern of MS, HPMC, sodium alginate individualy and formulation MP4 are shown in the Figure 3. The diffraction pattern of MS showed numerous characteristic peaks at $2\theta$ = 13.85, 19.19, 22.44, 23.6, 25.59, 26.57 and 43.63°, which are not present in the formulation MP4. This is an indication that the drug dissolved in the carrier state carrier matrices in amorphous form rather than the original crystalline form. This was obvious because of the solubility of drug in water initially, and followed by encapsulation of it in the polymeric matrix as molecularly dispersed form. This indicates that
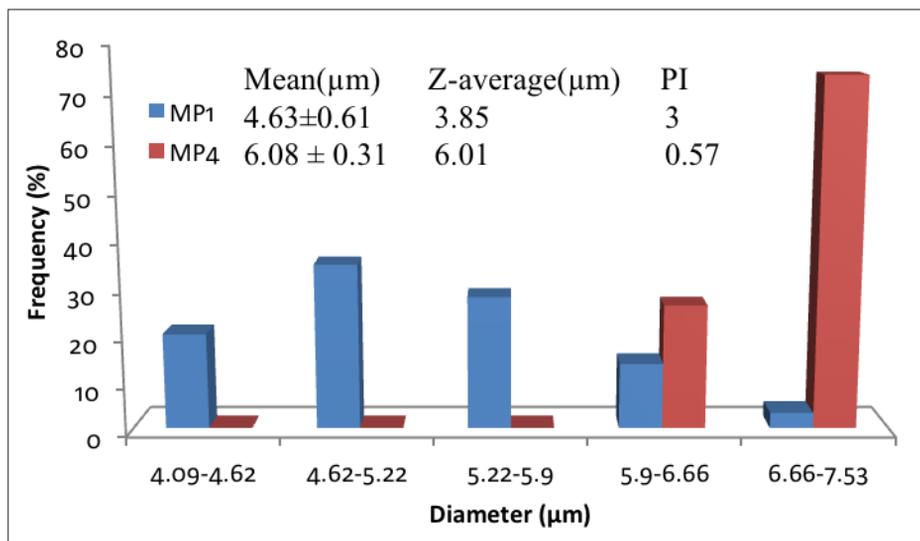
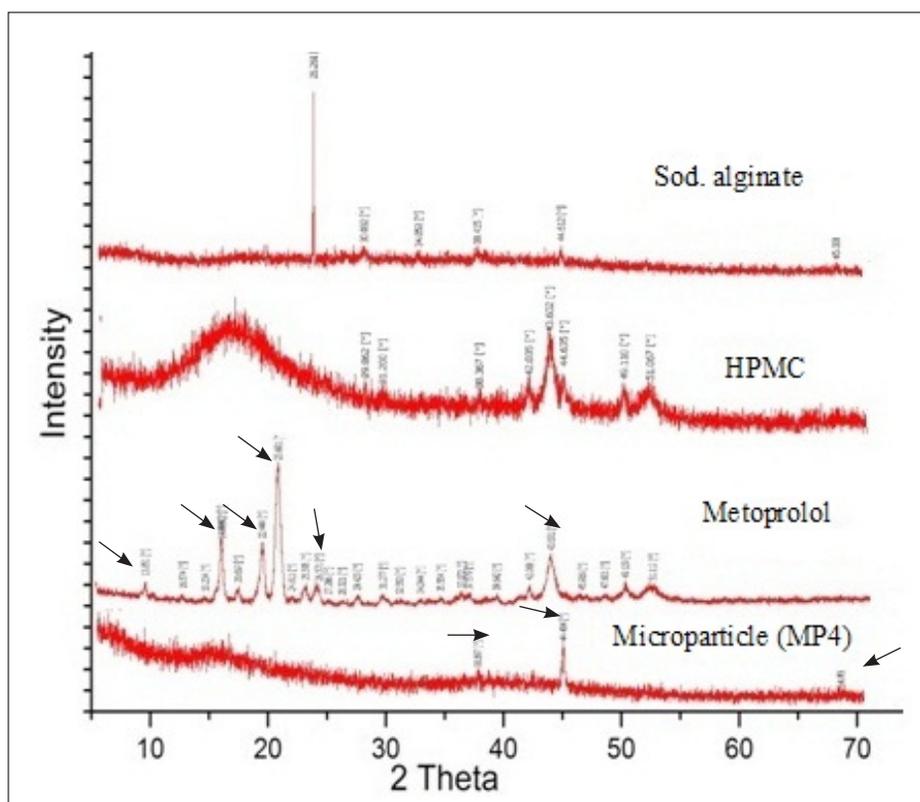**Figure 2.** Microparticle size distribution: Formulation MP1 and MP4 (n = 3)



**Figure 3.** Comparison of XRD diffraction pattern of Sod. Alginate, HPMC, Metoprolol and Microparticles.

drug was encapsulated within the polymer matrix effectively. Three peaks at $2\theta$ = 38.41, 43.6 and 65º were present, which could be due to the presence of the polymer in microparticles.

FT-IR spectroscopy was carried out to investigate the possibility of interaction between MS and polymers. MS has the characteristic broad peaks at 3397.63 and 3148.66 cm⁻, attributable to its vibrational stretching

of O-H and functional N-H bond, respectively. Other peaks are; C-O-C stretching vibration at 1114.24 cm⁻; C-N stretching at 1242.23 cm⁻; C = C aromatic stretching vibration at 1563.76 cm⁻; C-H streching at 2923.97 cm⁻ and C-O streching at 1385.12 cm⁻. The used carriers have lots of O-H groups (both HPMC and alginate) and C = O groups (alginate) that may react with the above groups of MS. Such types of interactions result from the formation of hydrogen bonding between drug and polymers that will lead to frequency shifts or splitting in absorption peaks. As can be seen from the spectra (Figure 4) of physical mixture of MS and alginate and microparticles, the peaks at 3148.66 cm⁻ (functional peak for N-H bond) and 1611.18 cm⁻ (C = O group) disappeared, indicating that there could be an interaction involved between C = O group of alginate and N-H group of MS. In addition, there was no evidence of interaction in the spectra of physical mixture of drug and HPMC.

The dissolution study was conducted in a phosphate buffer of pH 6.8 for 8 hrs and the release profile is presented in Figure 5. It was evident from the graph that the formulation MP1 showed initial burst release in which more than 65.08% of MS delivered in 1 hr. Other formulations showed biphasic profile, initial fast release phase until more than 80% of drug release in 5 hrs, followed by a sustained release.

The initial burst release in formulation MP1 is attributed to the fact that alginate with carboxylate groups dissolves at higher pH (28). In addition, the presence of the drug on the surface of microparticles also contributes to the effect. The subsequent phase in formulation MP2 to MP6 could be related to de-linking of polymer(s) by non-gelling ions contained in the phosphate buffer and diffusion of drug through reinforced HPMC along with the above mechanism (alginate dissolution at higher pH). In addition, by increasing the HPMC concentration in
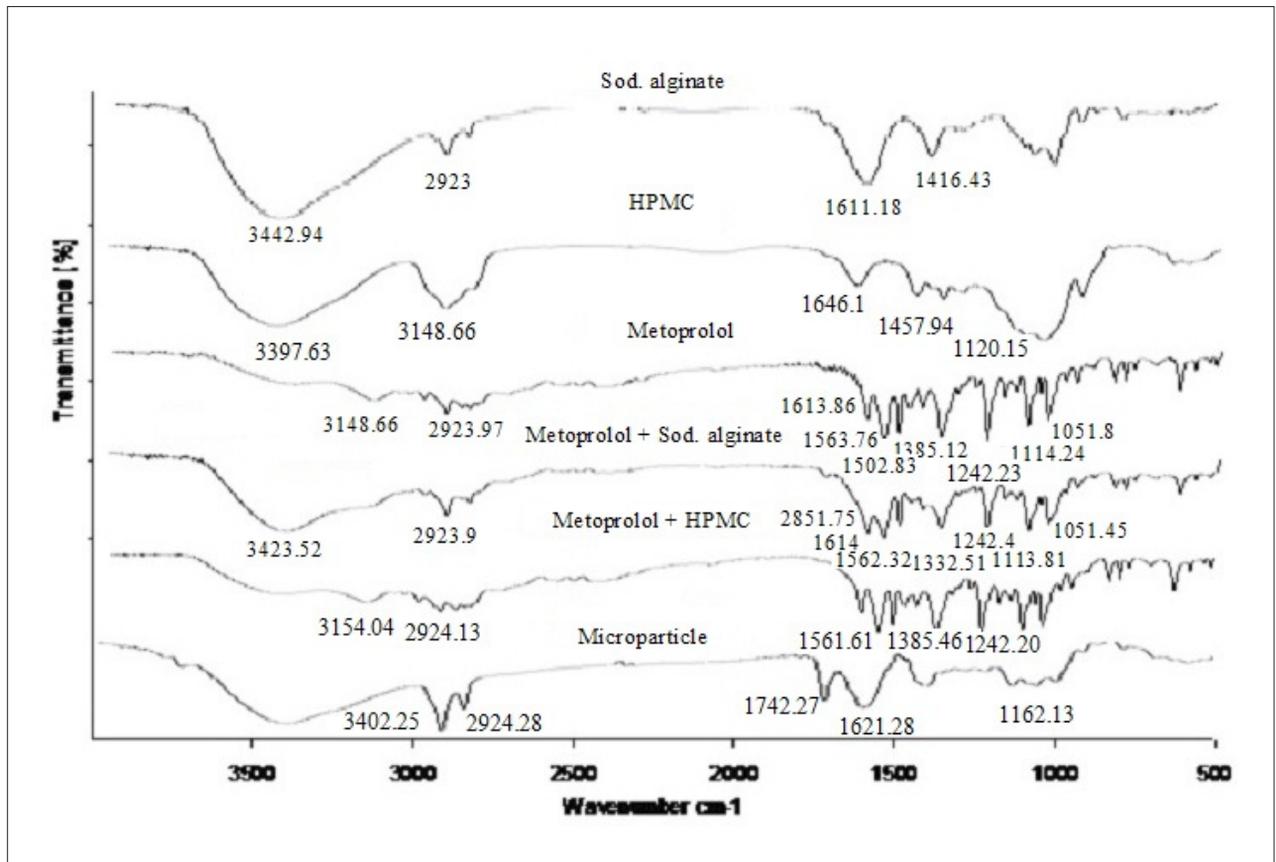


**Figure 4.** Comparison of FTIR spectra of sod. alginate, HPMC, Metoprolol, physical mixture of Metoprolol with sod. alginate and HPMC and Microparticles.
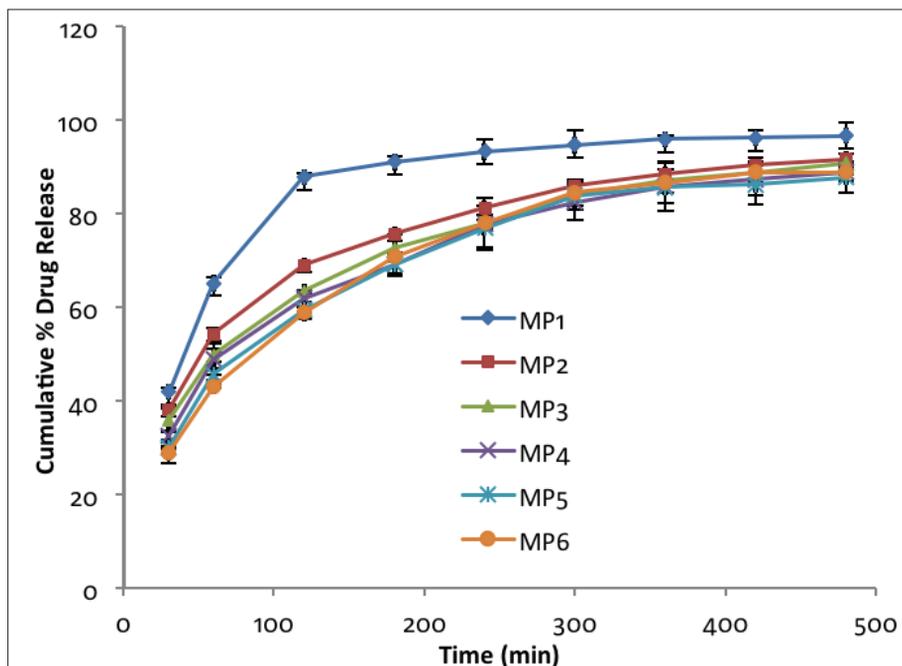
**Figure 5**. *In vitro* drug release of microparticles in phosphate buffer pH 6.8.

ratio, the rate of drug release decreases, owing to the long diffusional path length. In case of microparticles without HPMC, the drug release was 96.62% after 8 hrs. During the same period 88.92% of MS released from the formulation MP6, where the ratio of HPMC to alginate was 5:1.

The release data was studied in function of the power law. The $r^2$ values of all formulations were above 0.96, except for MP1, indicating a well fit to Korsmeyer-Peppas equation. Furthermore, the values of kinetic exponent 'n' in all formulations were below 0.43, which indicates the release mechanism is Fickian diffusion (29, 30).

**Table 2.** Values of kinetic exponent (n) and coefficient of regression ($r^2$)

| Formulation Code | Power law | | |
|---|---|---|---|
| | **n** | **$r^2$** | **Mechanism** |
| MP1 | 0.27 | 0.85 | Fickian Diffusion |
| MP2 | 0.30 | 0.96 | Fickian Diffusion |
| MP3 | 0.33 | 0.98 | Fickian Diffusion |
| MP4 | 0.35 | 0.97 | Fickian Diffusion |
| MP5 | 0.38 | 0.97 | Fickian Diffusion |
| MP6 | 0.40 | 0.97 | Fickian Diffusion |

Analysis of variance (ANOVA-one way) reports showed no significant difference of effect of ratio of polymers on drug release (P <0.05 level) among formulations containing combination of polymer (MP2 to MP6), but there were significant differences between the formulation MP1 with rest of the formulations at 8 hrs.

**CONCLUSIONS**

Microparticle drug delivery systems based on HPMC reinforced alginate were developed by emulsifying external gelation technique. MS was loaded into the microparticle drug delivery system. The *in vitro* release shows that HPMC addition could effectively extended the release of the drug. Further work has to be undertaken in order to ascertain the acceptability of interaction between MS and Sod. alginate.

**REFERENCES**

1. Krishnaiah, Y.S.R., Karthikeyan, R.S., Satyanarayana, V., A three-layer guar gum matrix tablet for oral controlled delivery of highly soluble metoprolol tartrate, *Int J Pharm*, 2002, 241: 353-366.

2. Kumaravelrajan, R., Narayanan, N., Suba, V., Bhaskar, K., Simultaneous delivery of Nifedipine

and Metoprolol tartarate using sandwiched osmotic pump tablet system, *Int J Pharm,* 2010, 399: 60–70.

3. Ye, Z-W., Rombout, P., Remon, J.P., Vervaet, C., Mooter, G.V., Correlation between the permeability of metoprolol tartrate through plasticized isolated ethylcellulose/hydroxypropyl methylcellulose films and drug release from reservoir pellets, *Eur J Pharm Biopharm,* 2007, 67: 485–490.

4. Al-Saidan, S.M., Krishnaiah, Y.S.R., Satyanarayana, V., Bhaskar, P., Karthikeyan, R.S., Pharmacokinetic evaluation of guar gum-based three-layer matrix tablets for oral controlled delivery of highly soluble metoprolol tartrate as a model drug, *Eur J Pharm Biopharm,*2004, 58: 697–703.

5. Kendall, M.J., Maxwell, S.R., Westergren, S.A., Controlled release metoprolol. Clinical pharmacokinetic and therapeutic implications. *Clin Pharmcokinet,* 1991, 5: 319–330.

6. Lennard, M.S., Silas, J.H., Freestone, S., Trevethick, J., Defective metabolism of metoprolol in poor hydroxylators of debrisoquine, *Br J Clin Pharmacol,* 1982, 14: 301–303.

7. Sandberg, A., Ragnarsson, G., Johnson, U.E., Sjogren, J., Design of a new multiple unit controlled release formulation of metoprolol-metoprolol CR, *Eur J Clin Pharmacol,* 1998, 33: S3–S7.

8. Kendall, M.J., Metoprolol-controlled release, zero order kinetics, *J Clin Pharm Ther,* 1989, 24: 159–179.

9. Abrahamsson, B., Alpsten, M., Jonsson, U.E., Lundberg, P.J., Sandberg, A., Sundgren, M., Svenheden, A., To11i, J., Gastro-intestinal transit of a multiple-unit formulation (metoprolol CR/ZOK) and a non-disintegrating tablet with emphasis on colon, *Int J Pharm,* 1996, 140: 229-235.

10. Eddington, N.D., Marroum, P., Uppoor, R., Hussain, A., Development and internal validation of an *in vitro–in vivo* correlation for a hydrophilic metoprolol tartrate extended-release tablet formulation, *Pharm Res,* 1998, 15: 466–473.

11. Ghosh, L.K., Sairam, P., Gupta, B.K., Development and evaluation of an oral controlled release

multiple emulsion drug delivery system of a specific beta blocker metoprolol tartrate, *Bulletino Chemico Farmaceutico,*1996, 135: 468–471.

12. Okabe, K., Yamoguchi, H., Kawai, Y., New iontophoretic transdermal administration of the beta-blocker-metoprolol, *J Control Release,* 1987, 4: 79–85.

13. Breimer, D.D., Gastrointestinal drug delivery systems, *Pharm Tech,* 1983, **7**: 18–23.

14. Pillay, V., Fassihi, R., A novel approach for constant rate delivery of highly soluble bioactives from a simple monolithic system, *J Control Release,* 2000, 67: 67–78.

15. Narendra, C., Srinath, M.S., Rao, B.P., Development of three layered buccal compact containing metoprolol tartrate by statistical optimization technique, *Int J Pharm,* 2005, 304: 102–114.

16. Hasan, A.S., Socha, M., Lamprecht, A., Gazhouani, F., Sapin, A., Hoffman, M., Maincent, P., Ubrich, N., Effect of the microencapsulation of nanoparticles on the reduction of burst release, *Int J Pharm,* 2007, 344: 53-61.

17. Bazzo, G.C., Lemos-Senna, E., Pires, A.T.N., Poly (3-hydroxybutyrate) /chitosan /ketoprofen or piroxicam composite microparticles: preparation and controlled drug release evaluation, *Carbohydrate Polymers*, 2009, 77: 839-844.

18. Moebus, K., Siepmann, J., Bodmeier, R., Alginate–poloxamer microparticles for controlled drug delivery to mucosal tissue, *Eur J Pharm Biopharm,* 2009, 72: 42-53.

19. Nellore, R.V., Rekhi, G.S., Hussain, A.S., Tillman, L.G., Augsburger, L.L., Development of metoprolol tartrate extended-release matrix tablet formulations for regulatory policy consideration, *J Control Release,* 1998, 50: 247–256.

20. Rekhi, G.S., Nellore, R.V., Hussain, A.S., Tillman, L.G., Malinowski, H.J., Augsburger, L.L., Identification of critical formulation and processing variables for metoprolol tartrate extended-release (ER) matrix tablets, *J Control Release,* 1999, 59: 327–342.

21. Chan, L.W., Heng, P.W.S., Effects of aldehydes and methods of cross-linking on properties of calcium alginate microspheres prepared by emulsification, *Biomaterials*, 2002, 23: 1319–1326.

22. Coppi, G., Iannuccelli, V., Alginate/chitosan microparticles for tamoxifen delivery to the lymphatic system, *Int J Pharm,* 2009, 367: 127–132.

23. Stockwell, A.F., Davis, S.S., Walker, S.E., In vitro evaluation of alginate gel system as sustained release drug delivery system, *J Control Release,* 1986, 3: 167–175.

24. Jay, S.M., Saltzman, W.M., Controlled delivery of VEGF via modulation of alginate microparticle ionic crosslinking, *J Control Release,* 2009, 134: 26–34.

25. Siepmann, J., Siepmann, F., Mathematical modeling of drug delivery, *Int J Pharm,* 2008, 364: 328–343.

26. Morch, Y.A., Donati, I., Strand, B.L., Skjak-Braek, G., Effect of Ca2+, Ba2+, and Sr2+ on

27. alginate microbeads, *Biomacromol,* 2006, 7: 1471-1480

28. Hurteaux, R., Edwards-Lévy, F., Laurent-Maquin, D., Levy, M.-C., Coating alginate microspheres with a serum albumin-alginate membrane: application to the encapsulation of a peptide, *Eur J Pharm Sci,* 2005, 24: 187–197.

29. Yu, C.-Y., Zhang, X.-C., Zhou, F.-Z., Zhang, X.-Z., Cheng, S.-X., Zhuo, R.-X., Sustained release of antineoplastic drugs from chitosan-reinforced alginate microparticle drug delivery system, *Int J Pharm,* 2008, 357: 15-21.

30. Ritger, P.L., Peppas, N.A., A simple equation for description of solute release. I. Fickian and non-Fickian release fromnon-swellable devices in the formof slabs, spheres, cylinders or discs, *J Control Release,* 1987a, 5: 23–36.

31. Ritger, P.L., Peppas, N.A., A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices, *J Control Release,* 1987b, 5: 37–42.