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ORIGINAL ARTICLE

Design and *In Vitro* Evaluation of Eudragit-Based Extended Release Diltiazem Microspheres for Once- and Twice-Daily Administration: The Effect of Coating on Drug Release Behavior

Günde Bir ve İki Kez Uygulama için Eudragit Esaslı Uzatılmış Salımlı Diltiazem Mikrokürelerin Tasarımı ve *İn Vitro* Değerlendirilmesi: Kaplamanın Etken Madde Salım Şekline Etkisi

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ABSTRACT

Objectives: The aim of this investigation was to develop an extended release formulation of diltiazem hydrochloride (DL) for once- and twice-daily administration, based on Eudragit (Eud) RL and RS microspheres using emulsion solvent evaporation.

Materials and Methods: Formulations with different drug–polymer concentrations were produced and characterized in terms of yield, encapsulation efficiency (EE), particle size, and surface morphology. The drug release and thermal behavior of the microspheres were also investigated. Selected microspheres were then coated with Eud RS by continuous solvent evaporation, in order to modify the microspheres' properties and burst release.

Results: According to the results, the EE was in the range of 56%-93% for uncoated microspheres. The mean particle size of microspheres was different from 470 to above 1000 µm, based on various formulation variables. No difference was observed between the mean size of particles prepared with Eud RL and Eud RS. Microspheres showed sustained release behavior, which was affected by the drug:polymer ratio as well as particle size. Coating the microspheres not only improved the EE values (82%-92%) but also reduced the mean dissolution rate as well as the burst release.

Conclusion: Microspheres prepared with DL:Eud RL ratios of 1:3 and 1:4 showed release profiles in accordance with the USP criteria for a DL extended release product for dosing every 12 and 24 h, respectively.

Key words: Coating, diltiazem hydrochloride, Eudragit RL and RS, extended release, microspheres

ÖZ

Amaç: Bu araştırmanın amacı, emülsiyon çözücü buharlaştırma kullanarak hazırlanan Eudragit (Eud) RL ve RS mikro küreleri ile günde bir ve iki kez uygulama için diltiazem hidroklorürün (DL) uzatılmış salım formülasyonunu geliştirmektir.

Gereç ve Yöntemler: Farklı etken madde-polimer konsantrasyonlarına sahip formülasyonlar üretilmiş ve verim, enkapsülasyon etkinliği (EE), partikül büyüklüğü ve yüzey morfolojisi açısından karakterize edilmiştir. Mikrokürelerin etken madde salınımı ve termal davranışı da incelenmiştir. Seçilen mikro küreler daha sonra mikro kürelerin özelliklerini modifiye ve hızlı ilk salınımını değiştirmek için sürekli çözücü buharlaştırma yoluyla Eud RS ile kaplanmıştır.

Bulgular: Sonuçlara göre, kaplanmamış mikroküreler için EE %56 -%93 aralığındadır. Mikrokürelerin ortalama partikül büyüklüğü, çeşitli formülasyon değişkenlerine bağlı olarak 470 ila 1000 um'nin üzerinde olmuştur. Eud RL ve Eud RS ile hazırlanan partiküllerin ortalama ortalama partikül büyüklüğü arasında bir fark gözlenmemiştir. Mikroküreler, etken madde: polimer oranının yanı sıra partikül boyutundan etkilenen sürekli salım davranışı göstermiştir. Mikrokürelerin kaplanması sadece EE değerlerini iyileştirmemiş (%82 -%92), aynı zamanda hızlı ilk çıkış yanı sıra ortalama çözünme oranınıda (MDR) azaltmıştır.

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Sonuç: 1:3 ve 1:4 oranlarında DL:Eud RL mikroküreler, sırasıyla her 12 ve 24 saatte bir dozlama için DL uzatılmış salım ürünü için USP kriterlerine uygun salım profilleri göstermiştir.

Anahtar kelimeler: Kaplama, diltiazem hidroklorür, Eudragit RL ve RS, uzatılmış salınım, mikroküreler

INTRODUCTION

Diltiazem hydrochloride (DL) is a highly soluble calcium channel blocker drug that is used in the treatment of high blood pressure and angina pectoris.[1] Due to its short elimination half-life of 2-5 h, the conventional oral dosage forms are administered 3-4 times a day, to maintain an effective plasma concentration, which results in low and variable bioavailability.[2] Using a sustained release form of this medication is vital for its efficacy by achieving relative constant blood concentrations and improving the clinical efficacy of the drug, as well as patient compliance.

However, along with the benefits of using extended release single-unit tablets, there are some limitations for these systems, such as the dose adjustment problem and the effect of food on drug release. Moreover, breaking the tablets before taking them could cause different release behavior and serious side effects.[3] The above-mentioned problems could be overcome using microspheres as multiple-unit dosage forms. Microspheres are uniformly distributed in the gastrointestinal tract and result in more uniform drug absorption, limited fluctuation within a therapeutic range, decreased dose frequency, and reduced patient-to-patient variability.[4]

The physical properties and release behavior of microspheres are dependent on different factors such as drug and polymer nature as well as the method of manufacturing. According to the literature, the existence of drug particles on the surface and particles embedded in the surface layers as well as high porosity of microspheres are considered the main reasons for initial burst release. Coating of microspheres is one of the approaches to reduce the burst release and modify the drug release behavior.[5] Preparation and separation of initial microspheres and then using them in a separate coating process would be time and cost consuming. Therefore, application of a continuous preparation and coating process for microspheres seems to be preferable.

To date, modified-release microspheres of DL using different polymers and methods were developed in order to extend its clinical effects.[2,6-11] Only a few studies were performed on the preparation of Eudragit (Eud) RS-based DL microspheres by solvent evaporation.[12-14]

The objective of the present research was to design and evaluate DL-loaded Eud RL and RS matrix-type microspheres as extended release systems for both once- and twice-daily administration, in order to reduce its dosing frequency. With the aim of achieving both systems, different drug–polymer concentrations were applied and examined. In addition, the effect of coating of microspheres by Eud RS on drug release behavior and the burst effect was also evaluated. Emulsion solvent evaporation was used for microsphere preparation as well as continuous coating. This is a simple method that has been used to prepare microspheres of different soluble and insoluble compounds.[15-17]

MATERIALS AND METHODS

Materials

DL powder (Zambon Group SPA, Italy), Eud RL and RS 100 (Röhm Pharma GmbH, Germany), span 60 (Sigma-Aldrich, St. Louis, MO, USA), n-hexane (Carlo Erba, France), and liquid paraffin (Merck, Germany) were used in this study. The materials and excipients used in preparing the microspheres were of pharmacopoeial grade.

Microsphere preparation

DL-loaded Eud RL and RS microspheres were produced by emulsion solvent evaporation.[18] Different amounts of drug and polymer were dissolved in 3 mL of ethanol (dispersed phase), which was then slowly (at the rate of 1 mL/min) added to a beaker containing a mixture of 50 mL of liquid paraffin and 0.1% w/v span 60 (continuous phase) with stirring at 500 rpm using a mechanical stirrer (IKA, Germany). The mixture was stirred until the organic solvent evaporated completely. The prepared microspheres were collected by filtration and washed three times with n-hexane until all the paraffin was removed. Finally, the microspheres were dried at room temperature for 24 h and kept in air-tight containers for further studies.

Coating of microspheres

A one-step continuous solvent evaporation technique was used for the coating process. Primary microspheres were prepared by the above-mentioned method, but before completing the process and collecting the microspheres a 3.3% w/v Eud RS ethanolic solution was added dropwise to the continuous phase and stirred until complete solvent evaporation.[9] The other steps were similar to the previous method.

Characterization of microspheres

The prepared microspheres were characterized in terms of yield value, encapsulation efficiency (EE), morphology, drug release, particle size, and thermal analysis. The yield value of each formulation was calculated by the following equation:[19]

Yield value(%) = (weight of dried microspheres/total solid material amount in the dispersed phase) × 100

Drug content

Ten milligrams of dried microspheres was accurately weighed and transferred to a beaker containing 10 mL of methanol and stirred for 15 min to dissolve the microspheres completely. The solution was analyzed for DL content by a UV spectrophotometer (Shimadzu UV1201, Japan) at 240 nm after dilution. The drug loading and EE were calculated using the following equations:[20]

Drug loading (%) = (weight of drug in microspheres/weight of microspheres) × 100

Drug EE (%) = (actual drug loading/theoretical drug loading) × 100

In vitro drug release

Drug release of all microspheres was carried out using a USP type II dissolution test apparatus (Erweka DT6R, Germany) in 900 mL of phosphate buffer solution (pH 7.2) at 37±0.5°C at 50 rpm (in accordance with the USP test number 5 for DL extended release form dosing every 12 h). Then 3 mL of the medium was withdrawn at predetermined time intervals and replaced with the same amount of fresh dissolution medium after each sampling. The sample solutions were analyzed for drug content at 240 nm by a UV spectrophotometer.

The dissolution test was also performed on selected microspheres in compliance with the USP test number 2 for DL extended release form dosing every 24 h, using an apparatus II at 100 rpm and 900 mL of dissolution medium (distilled water) for 15 h. All experiments were performed in triplicate for each formulation.

All formulations were compared using different dissolution parameters.[21] Mean dissolution time (MDT), which was applied to analyze dissolution profiles, was calculated arithmetically by the following equation:

$$MDT = \frac{\sum_{i=1}^{n} ti\ \Delta Mi}{\sum_{i=1}^{n} \Delta Mi}$$

where ΔM_i is the fraction of drug released in time t_i (calculated by $(t_i+t_{i-1})/2$) and i is the sample number.

In addition, the area under the dissolution curve [dissolution efficiency (DE)] was calculated by the formula below:

$$DE = \frac{\int_o^t y.dt}{y100.t} \times 100$$

where y is the percentage of drug dissolved at time t. Mean dissolution rate (MDR) was also calculated based on the following equation:

$$MDR = \frac{\sum_{i=1}^{n} \Delta Mi/\Delta t}{n}$$

where Δt is the time at the midpoint between t and t_{-1} and n is the number of dissolution sample times.

Particle size

The mean particle size of the DL microspheres was determined by optical microscopy. At least 200 microspheres were analyzed for each preparation and the mean diameter was calculated.

Surface morphology

The appearance and surface morphology of microspheres were evaluated by scanning electron microscopy [scanning electron microscopy (SEM), Philips XL30, the Netherlands]. The microspheres were attached to a specimen holder with double-sided adhesive tape and coated under vacuum by gold sputter coater (Bal-Tec SCD 005, Switzerland) prior to observation.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis of the drug, polymer, selected DL-loaded microspheres, and related physical mixture was conducted. After calibrating the apparatus (Shimadzu DSC-60, Japan) by indium standard, accurately weighed samples (5 mg) were placed in sealed aluminum pans. The containers were placed in the DSC apparatus and heated at a constant rate of 10/min over a temperature range of 25 to 300°C. An empty standard aluminum pan was used as reference.

Statistical analysis

Statistical analysis of the different variables was carried out using ANOVA followed by Tukey's *post hoc* test. Significance was tested at the 0.05 level of probability.

RESULTS AND DISCUSSION

DL microspheres were successfully prepared by emulsion solvent evaporation using ethanol as the drug–polymer solvent (dispersed phase) and a liquid paraffin–span 60 mixture as the continuous phase. The yield value was in the range of 62.8%-92.4% for the initial microspheres and 81.3%-97.6% for the Eud RS-coated microparticles.

Characterization of microspheres

Encapsulation efficiency and particle size

Table 1 shows the composition and properties of the Eud RL- and RS-based microspheres prepared with different drug:polymer ratios. Increasing the amount of Eud RL from 300 to 800 mg led to a 25% enhancement in the EE values. In fact, the size of emulsion droplets was increased due to the higher viscosity of the polymeric solution, which in turn decreased the surface area and also drug molecule transport from dispersed to continuous phases.[22] The particle size of those microspheres was also increased significantly ($p<0.001$), which was expected. Similar results were obtained for the microspheres prepared with higher DL concentrations. Based on the results (Table 1), there is a significant difference between the EE values of M8L and M4L ($p<0.05$). In addition, using higher drug:polymer ratios resulted in significantly ($p<0.001$) increased particle size. Although the effect of polymer concentration on particle size seemed to be more than that of the drug, the results revealed that in certain drug concentrations its effect on particle size cannot be neglected.

The application of various drug:polymer concentrations with the same ratio (M1L, M2L, and M7L) resulted in microspheres with different EEs and mean particle sizes. An increase of 20% was found for the EE value of M7L (higher DL-Eud RL concentration) compared to M1L and M2L. In other words, an appropriate simultaneous increase in drug and polymer concentrations led to more drug entrapment in the microspheres. The same trend was also observed for microparticles size, which could be attributed to the higher viscosity and emulsion droplet size of this formulation. However, the difference observed between M2L and M1L was far smaller.

By changing the polymer type from Eud RL to Eud RS (Table 1), no difference was observed in EE % for lower drug:polymer concentrations (M1L and M1S). However, the opposite was found for the formulations prepared with higher drug:polymer concentrations, in which M2S showed an improved EE value compared to M2L, which is in accordance with some reports in the literature.[23,24] Eud RL is more permeable and the diffusion of drug molecules from the droplets to the surrounding medium during the preparation process is more probable than with Eud RS. In addition, the repulsion between the quaternary ammonium groups of Eud RL and the cationic drug could facilitate DL removal to the external phase and reduce the EE.

According to the results, coating of microspheres improved the EE % significantly ($p<0.001$) compared to the uncoated microspheres (Table 2). It is probable that application of the Eud RS coating on the surface of the initial microspheres prevents the drug molecules' transport to the emulsion external phase during the preparation process. Meanwhile, no difference was observed in the EE values of the coated microspheres with different inner polymers. As was expected, the mean particle size of the microparticles was increased following the coating process. The higher mean particle size of M2LS and M2SS compared to M1LS and M1SS was related to the higher inner polymer concentration used to prepare the initial microspheres.

SEM

The SEM micrographs (Figure 1) show that the microspheres prepared in the presence of a lower polymer concentration (M2L) were more spherical with wrinkled surfaces compared to M5L (higher polymer amount). Using a higher DL concentration in the formulations, did not affect the microspheres' shape, but increased their roughness mainly due to the existence of drug crystals on the surface layers of the microspheres. No difference was observed between the microspheres prepared with Eud RL and RS (M1L and M1S) in terms of shape or surface properties, which was in accordance with previous research.[24]

Based on the results, following the coating of microspheres, they were still spherical with more uniform surfaces compared to the initial uncoated microparticles. The study of the surface morphology of M1LS and M1SS (Figure 1) confirmed the absence of drug crystals on the surface of the microparticles and suitable coverage of the initial microspheres during the continuous coating process.

Drug release studies

The release profiles of DL from microspheres prepared with different formulations are presented in Figure 2. Based on the results, the drug release rate decreased apparently with increasing polymer concentration (Figure 2a). The DL released after 3 h of the experiment for M2L and M5L was 73.28% and 26.09%, respectively. This trend was also observed in MDR and DE values (Table 1). Furthermore, decreasing the drug release rate led to an increase in MDT values. In fact, a higher polymer concentration resulted in larger particle size with less surface

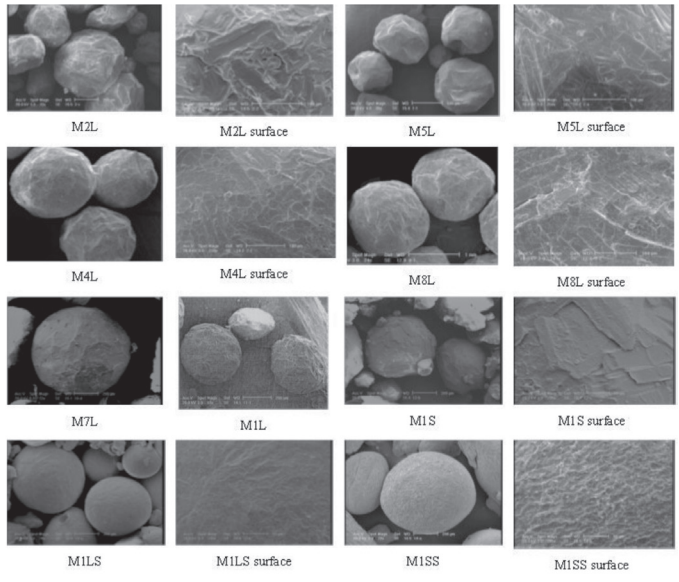

Figure 1. Scanning electron microscopy micrographs of different microspheres and their surfaces

Table 1. Composition and physicochemical properties of diltiazem hydrochloride microspheres (mean ± standard deviation, n=3)

Formulation	Drug (mg)	Polymer (mg)	EE[c] (%)	Mean particle size (μm)	MDT[d] (min)	DE[e] (%)	MDR[f] (%min^{-1})
M1L[a]	100	150	61.43±1.33	452.9±7.29	82.45±4.17	83.78±0.98	0.331±0.005
M2L	200	300	62.15±1.09	513.8±10.09	94.38±4.05	75.29±0.58	0.210±0.004
M3L	200	500	56.62±3.75	620.0±5.82	150.70±6.77	74.96±0.54	0.174±0.007
M4L	200	600	81.46±2.60	665.7±6.71	197.11±5.62	61.43±1.11	0.086±0.004
M5L	200	800	87.70±3.57	720.1±12.58	210.27±2.34	54.00±1.19	0.068±0.006
M6L	300	600	82.18±0.84	745.9±5.58	235.95±7.86	58.29±1.09	0.064±0.002
M7L	400	600	84.97±3.53	813.2±2.33	201.50±4.26	66.98±0.43	0.147±0.005
M8L	500	600	92.86±3.90	1027.3±6.50	114.94±6.45	80.78±1.91	0.246±0.007
M1S[b]	100	150	59.53±1.19	463.5±4.05	116.74±5.05	65.00±1.93	0.211±0.005
M2S	200	300	77.09±1.05	528.6±3.35	122.57±1.71	63.95±0.36	0.224±0.011

[a]L: Eudragit RL, [b]S: Eudragit RS, [c]EE: Encapsulation efficiency, [d]MDT: Mean dissolution time, [e]DE: Dissolution efficiency, [f]MDR: Mean dissolution rate

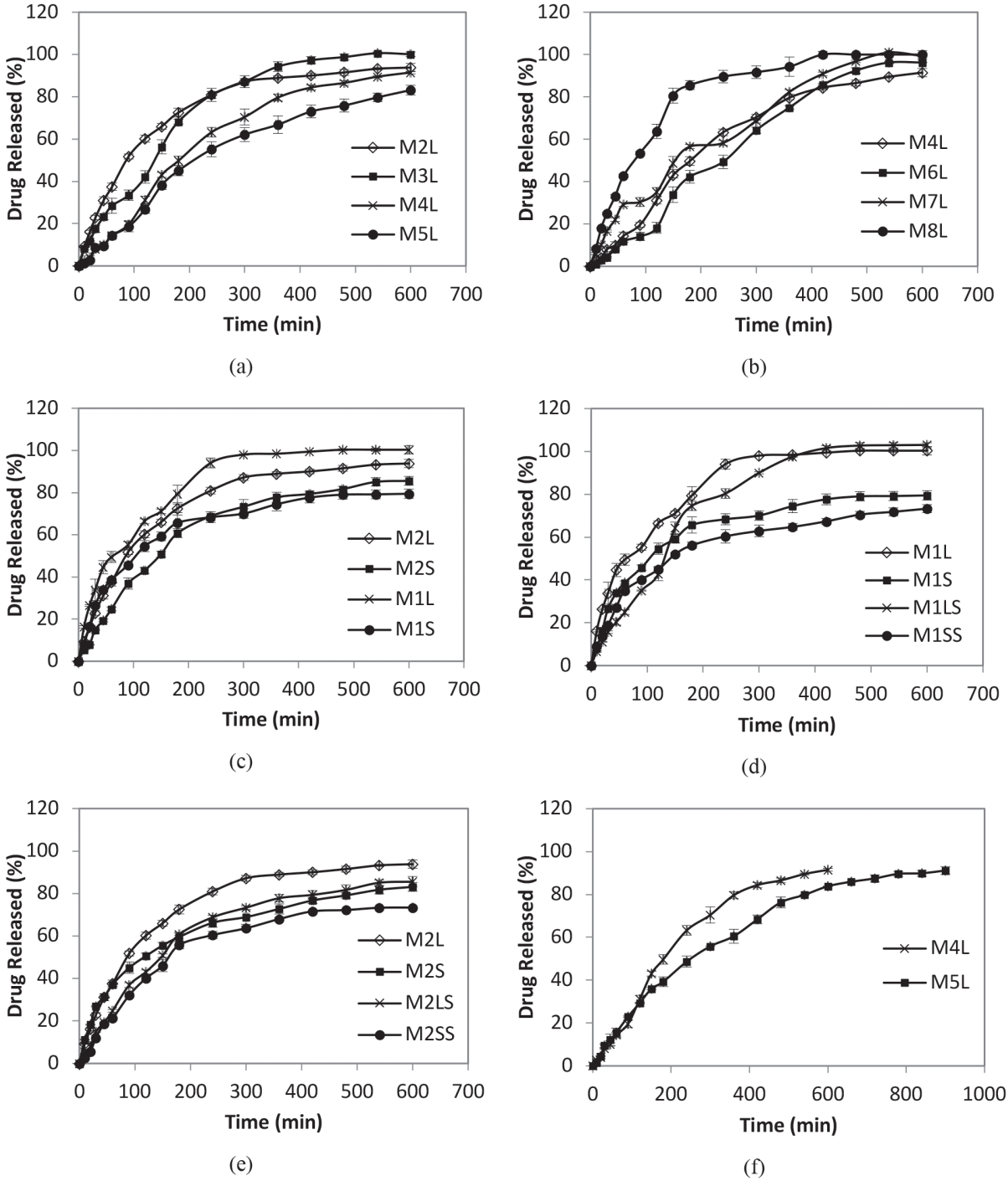

Figure 2. Release profiles of DL from (a-c) microspheres with different formulation variables in phosphate buffer (pH 7.2), (d, e) coated versus uncoated microspheres, (f) M4L in phosphate buffer (pH 7.2) and M5L in water (n=3)

area and therefore a lower release rate. A burst release of about 37% was observed for M2L during the first hour of the study, which could be attributed to the lower polymer content and particle size, as well as more drug particles on the surface layers of the microspheres.

Using higher drug concentrations with a fixed amount of polymer enhanced the drug release apparently (Figure 2b) and about 90% of DL was dissolved over 5 h from M8L (DE=80.78±1.91%). Table 1 shows that MDR was significantly increased in the formulations prepared with higher drug concentrations ($p<0.0001$). In addition, the burst release of these microspheres was in the range of 11.88%-42.70%. It seems that the presence of more drug particles on the surface layers of the microspheres prepared with higher drug levels enhanced the drug release rate in spite of the larger particle size.[15] In fact, reduction of the drug diffusion pathway is possible in microspheres with higher drug loading.[25] Moreover, removal

of drug particles from microspheres leads to the formation of a more porous structure, which plays an important role in accelerating drug release.[26]

Using various drug:polymer concentrations with the same ratio also led to microspheres with different release behavior. Based on Table 1, M7L prepared with a higher drug:polymer concentration extended the drug release more than M2L and M1L ($p<0.001$), mainly due to its larger particle size. The significant decrease in the MDR value for M7L ($p<0.001$) corresponds to an increase of more than 110 min in MDT of this formulation in comparison to M1L. All those three microspheres showed a burst release in the range of 30%-50%.

Figure 2c shows the release profiles of DL from microspheres prepared with Eud RL and RS. It is obvious that the drug release from the Eud RS-based microspheres was slower than that of the particles made with Eud RL. The difference observed between M1L and M1S was more evident. Based on Table 1, a reduction of more than 18% in DE and about 1.5-fold in MDR was observed for M1S compared to M1L. The MDT values for M1S and M2S were also significantly greater than for M1L and M2L ($p<0.001$). Since the mean particle size was not affected very much by the polymer type, the results obtained could be attributed to the lower permeability of Eud RS.

The effect of coating microspheres on the DL release profile is illustrated in Figures 2d and 2e. The drug release from all coated particles decreased clearly compared to the uncoated microspheres. Based on Table 2, a significant reduction in MDR and DE values was observed for coated particles. The lowest MDR was for the formulation M2SS, which was about half that of M2S. The lowest DE % was also obtained for M2SS. A decreasing release rate was observed with increasing MDTs. A significant difference was observed between the MDTs of M1L and M1LS and also M2L and M2LS ($p<0.01$). Following the coating process, MDT of the microspheres with Eud RL as inner polymer was enhanced more than that of Eud RS. Furthermore, although the burst release declined for all coated microspheres, this was more noticeable for the microspheres with Eud RL as core polymer.

The results revealed that although coating of microspheres was helpful in decreasing the drug release rate, it was not as effective as using an appropriate drug:polymer concentration in the preparation process, without any coating. Formulations M5L and M6L showed the lowest MDRs and the highest MDT values among all coated and uncoated microspheres. It seems that the drug particles in the mass of microspheres were much more than the particles in the surface layers and controlling their diffusion was more important in achieving the desirable extended release behavior. However, application of a higher polymer concentration in the coating process could cause different effects.

DL microspheres for once- and twice-daily administrations

Figure 2f shows the release profiles of two selected formulations (M4L and M5L) in phosphate buffer solution (pH 7.2) (USP test number 5) and water (USP test number 2), respectively. The results indicated that the microspheres prepared with DL: Eud RL ratios of 1:3 (M4L) and 1:4 (M5L) were in accordance with the USP test for DL extended release form dosing every 12 and 24 h, respectively, without any further treatment.

The release kinetics of these formulations was investigated using three different models, i.e. zero order, first order, and the Higuchi equation. Based on the squared correlation coefficient (R^2), the release profile of M4L was best fitted with zero order (R^2=0.989) compared with first order and the Higuchi model (R^2=0.973 and 0.944, respectively). Although the R^2 values calculated for M5L based on first order (0.991) and the Higuchi equation (0.994) were higher than that of zero order (0.954), there is no evidence to specify the dominant kinetics model for this formulation.

DSC

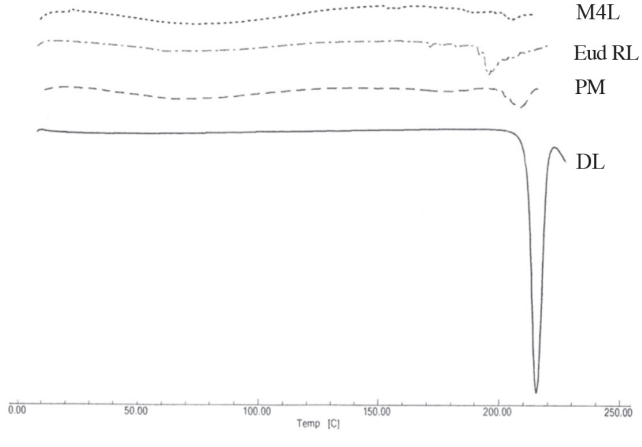

Figure 3. Differential scanning calorimetry thermograms of DL, Eudragit RL, M4L, and related physical mixture

PM: Physical mixture

Table 2. Composition and physicochemical properties of diltiazem hydrochloride microspheres coated with Eud RS (mean ± standard deviation, n=3)

Formulation	Core drug (mg)	Core polymer (mg)	Type of core polymer	EE[a] (%)	Mean particle size (μm)	MDT[b] (min)	DE[c] (%)	MDR[d] (%min^{-1})
M1LS	100	150	Eudragit RL	91.99±0.82	510.4±5.85	118.04±3.27	77.42±0.69	0.195±0.004
M1SS	100	150	Eudragit RS	88.32±1.31	500.2±3.05	131.16±5.71	57.25±1.48	0.184±0.007
M2LS	200	300	Eudragit RL	82.08±2.05	641.1±7.81	125.18±5.32	63.92±1.53	0.146±0.004
M2SS	200	300	Eudragit RS	83.84±1.92	610.5±3.52	138.87±2.30	56.33±0.20	0.106±0.002

[a]EE: Encapsulation efficiency, [b]MDT: Mean dissolution time, [c]DE: Dissolution efficiency, [d]MDR: Mean dissolution rate

The DSC thermograms of DL, Eud RL, selected microsphere (M4L), and related physical mixture (PM) are depicted in Figure 3. A characteristic endotherm appeared for the drug at the onset temperature of 210.08°C, which could be attributed to the melting of DL.[27] A broad peak in the range of 50-60°C was observed in the thermogram of Eud RL, which is related to its glass transition temperature.[28] The DSC curve obtained for the microspheres presented the same thermal profile as that of the physical mixture, both containing a drug melting peak with a slight shift toward lower temperatures. These minor changes in the drug endotherm could be attributed to the presence of polymer, which lowers the drug purity.[29] This result suggests no interaction between the drug and the polymer during the preparation process.

CONCLUSIONS

DL:Eud RL extended release microspheres for once- and twice-daily administration for the treatment of hypertension and angina pectoris were successfully produced in this study by a simple method of solvent evaporation using suitable formulation variables. The results confirmed that a one-step continuous emulsion solvent evaporation process was a practical technique to prepare coated microspheres with improved physical properties (especially EE %) and reduced burst release. Using suitable drug:polymer ratios and external coating polymer concentration could modify the particle size, surface morphology, porosity, and the amount of drug particles on the surface layers, which are essential to obtain desirable results.

Conflict of Interest: No conflict of interest was declared by the authors.

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