AAPS PharmSciTech, Vol. 10, No. 2, June 2009 (© 2009)
DOI: 10.1208/s12249-009-9240-3

Research Article

Investigations on the Physical Structure and the Mechanism of Drug Release from an Enteric Matrix Microspheres with a Near-Zero-Order Release Kinetics Using SEM and Quantitative FTIR

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Received 16 January 2009; accepted 9 April 2009; published online 15 May 2009

Abstract. The objectives of this study were to evaluate the physical structure and the release mechanisms of theophylline microspheres made of Eudragit S 100 polymer as an enteric polymer, combined with a nonerodible polymer, Eudragit RL 100. In the preparation process, polymer combinations (1:1) were dissolved in an organic solvent mixture composed of acetone and methanol at a specific ratio containing a theoretical drug loading of approximately 15%. Two microsphere formulations (LS1 and LS2) were prepared at two different total polymer concentrations (10% in LS1 and 12.7% in LS2). Dissolution studies were carried out using US Pharmacopeia Dissolution Apparatus II in an acidic medium for 8 h and in an acidic medium (2 h) followed by a slightly basic-buffered medium for 10 h. Both LS1 and LS2 microsphere formulations produced particles that were spherical in shape and had very narrow size distributions with one size fraction comprising 70–80% of the yield. Scanning electron microscopy and quantitative Fourier transform infrared were used for microsphere physical structure evaluation. Except for the absence of drug crystals, photomicrographs of both LS microspheres after dissolution in pH 1.2 and 7.2 buffer solutions were similar to those before dissolution. Dissolution results indicated the ability of LS microspheres to minimize drug release during the acid stage. However, in the slightly basic medium that followed the acidic stage, the drug release was sustained and controlled in its kinetics and data fitted to Peppas equation indicated a case II transport suggesting that the drug release is mainly through swelling/erosion mechanism.

KEY WORDS: enteric; FTIR; microspheres; narrow size; SEM.

INTRODUCTION

Theophylline, a methylxanthine alkaloid that is used in the treatment of asthma as a bronchodilator, has a narrow therapeutic index in the range of 5–20 μg/ml and has therefore received a considerable amount of attention in oral sustained release formulations (1–4). The development of a dosage form such as a microsphere is believed to enhance tolerance and to control the release of theophylline to achieve a safe therapeutic concentration in the blood. Controlled-release multiple-unit dosage forms, such as microspheres, have advantages over single-unit ones (5–7). The emulsion–solvent evaporation method is one of these methods and has been extensively studied to prepare such microspheres (8).

When using the emulsion–solvent evaporation method for preparing microspheres from binary polymers, one can achieve either matrix structures where both polymers may be homogeneously or heterogeneously dispersed in each other or otherwise one polymer will engulf the other polymer resulting in a double-wall spherical matrix structure. Of course, systems in between can also be expected (9).

In this study matrix, microspheres made of polymer mixtures are considered for purposes of achieving a controlled release of theophylline. Matrix microspheres are very rugged and can be prepared for many drugs (10). Microsphere characteristics are greatly affected by processing and formulation variables (2,11,12).

Eudragit S 100 is an anionic copolymer based on methacrylic acid and methacrylate. It is a polyacrylic resin that has been suggested to be used in microencapsulation for controlled-release applications due to its unique solubility profile (3,13,14). The free carboxylic acid groups make the polymer pH sensitive, being soluble at pH 6–7.5 (15).

In a previous work (4), because microspheres composed of an enteric or pH-sensitive hydrophilic polymer (Eudragit S 100) and a nonerodible polymer hydrophobic (CAB-551-0.01) could not be produced as usual using a common solvent (s) due to the difficulty of selecting a solvent or mixture of solvents to dissolve both polymers, Eudragit S 100 was

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dispersed (not dissolved) in CAB-551-0.01/acetonitrile polymer solution to produce microspheres for purposes of achieving controlled drug release. Although the microspheres could modify drug release rates compared to release rates from CAB-551-0.01 microspheres alone, high temperature (65°C up to 4 h) was required which would be undesirable for heat-sensitive drugs. In addition, the microspheres possessed wrinkled nonsmooth surfaces.

The first aim of this study was to prepare and evaluate theophylline microspheres made of Eudragit S 100 polymer as a pH-sensitive enteric hydrophilic polymer combined with a nonerodible hydrophilic polymer (other than CAB-551-0.01) such as Eudragit RL 100 using suitable common organic solvent(s). After reviewing the prior art, we could not find such combinations of these specific polymers. Thus, this work provides solutions for producing microspheres from such combination and elucidates the composition of such microspheres. Therefore, an essential second aim of this work was to study the distribution of polymers within the microspheres and the microscopic shape of the entire microspheres using scanning electron microscopy and Fourier transform infrared (FTIR) spectroscopy.

EXPERIMENTAL

Materials

Materials used are: Eudragit S 100 (Röhm), Eudragit RL 100 (Röhm), theophylline (Sigma-Aldrich Co.), magnesium stearate (CDH, Ltd., Bombay, New Delhi, India), heptane (GFS Chemicals, Inc., Columbus, OH, USA), mineral oil (Ruger Chemical Co. Inc., Irvington, NJ, USA), acetone (Carlo Erba Reactifs-SDS), methanol (Carlo Erba Reactifs-SDS), potassium phosphate tribasic, and hydrochloric acid 50% and sodium hydroxide 50% *w*/*w* solution (J. T. Baker Inc., Phillipsburg, NJ, USA).

Instruments

Instruments used are: stirrer (Lab. Stirrer DLH, VELP Scientifica, Europe), US Pharmacopeia (USP) Dissolution Apparatus II (ERWEKA, DT-D6, Fed. Rep. of Germany), UV-visible spectrophotometer CE (Cintra 5, GBC Scientific Equipment, Bausch & Lomb, Rochester, NY, USA), SCHOTT pH meter (CG 843, Germany), standard sieves series, differential scanning calorimeter (DSC-50 Shimadzu, Japan), scanning electron microscope, and FTIR spectrophotometer (Shimadzu 8400S, Japan) with KBr pellets.

Preparation of Microspheres

Two microsphere formulations (LS1 and LS2) were prepared using Eudragit S 100 and Eudragit RL 100 polymer combinations at a 1:1 ratio and with two different total polymer concentrations (10% in LS1 and 12.7% in LS2). In the preparation process, both Eudragit S 100 and Eudragit RL 100 polymer combinations were dissolved in a solvent mixture composed of acetone and methanol (4.5:1). Then, the model drug, manually micronized theophylline, was dispersed in the polymer solution phase at a theoretical loading of 15% before it was emulsified with five times its volume of mineral oil containing 1% magnesium stearate. While incubated in a water bath at 32±1°C, the dispersion system was continuously stirred for 2–3 h at an agitation speed adjusted for each formulation to yield an approximately similar particle size distribution. The stirrer consisted of two propellers on a single shaft. After the formation of microspheres and the evaporation of solvent, the microspheres were separated from the oil phase, washed with *n*-heptane, and dried at 50°C. Blank LS1 and LS2 microspheres (without drug) were also prepared using the same method and conditions described above.

Particle Size Distribution

The size distributions were evaluated by sieve analysis using a set of standard USP sieves from 125 to 825 μm. The microspheres were placed at the topmost sieve (825 μm) and tapped by hand. The weight of microspheres retained on each individual sieve was recorded.

Drug Loading

Drug content analysis was performed by the following procedure. An accurately weighed sample of microspheres was placed in a 50-ml volumetric flask and a solvent mixture composed of methanol and phosphate buffer (pH 7.2) at 1:1 ratio was added to dissolve the polymers and the drug. After the proper dilution and filtration using micropore filter (0.45 μm), the drug concentration was determined spectrophotometrically at 274 nm. At the specified wavelengths, no spectrophotometric interferences were observed for blank microspheres (without drug) dissolved in the same solvent mixture.

In Vitro Dissolution Analysis

In vitro dissolution studies were carried out on microsphere samples under two different procedures: (1) an accurately weighed sample of microspheres (100 mg) was suspended in 900-ml acidic medium (pH 1.2±0.2) and dissolution was followed to determine the effect of the enteric polymer on drug release and (2) a two-stage dissolution procedure where the first stage is at pH 1.2±0.2 for 2 h followed by pH 7.2 media for 10 h or until the drug was depleted from the microspheres. For the two-stage dissolution, an accurately weighed sample of microspheres (100 mg) was suspended in the dissolution medium consisting of 500 ml of 0.1 N (pH 1.2±0.2) hydrochloric acid without enzymes and dissolution was performed for the first 2 h. At the end of the 2 h, 400 ml of 0.1 M tribasic sodium phosphate was added to all dissolution vessels; the pH was adjusted to 7.2 (±0.2) and the dissolution was continued for ten more hours for a total of 12 h.

All the dissolution studies were carried out on the microspheres at 37°C (±0.5°C) and 100 rpm with USP Dissolution Apparatus II. Aliquots of dissolution fluid were withdrawn at specified time intervals to assay the released drug spectrophotometrically at 271 nm in both dissolution experiments. Three batches of each formulation were prepared and evaluated. Three samples were tested from each batch and each graphical data point was an average of dissolution data from all three batches. Corrections were made for the removal of samples.

Table I. Composition, Agitation Intensities, and Microsphere Size Fractions for the Two Microsphere Formulations (LS1 and LS2)

Formulation	Weight of ERL 100 (g)	Weight of ES 100 (g)	Weight of theophylline (g)	Agitation intensity (rpm)	Microsphere size fraction (μm)	Percent from the yield
LS1	0.75	0.75	0.265	1,450	300–425	25.8±3.2
					425–600	74.2±5.7
LS2	0.90	0.90	0.320	2,000	300–425	19.8±7.1
					425–600	80.2±6.6

Scanning Electron Microscopy

Scanning electron microscope was utilized to study the shape of the whole microspheres in terms of size uniformity, surface smoothness and roughness before and after dissolution in the two dissolution media. In addition, cut or cleaved microspheres were evaluated. Micrographs were taken for microspheres of different sizes of both microsphere preparations (LS1 and LS2). Blank microspheres (without drug) were also viewed before and after dissolution in phosphate buffer (pH 7.2±0.2). Microspheres were prepared and mounted on a conductive carbon adhesive attached to aluminum stubs. Microspheres were coated with gold (Bio-Rad, Polaron division E6100). Gold was evaporated onto the specimen to a thickness of approximately 15 nm. The sample images were digitally captured using the FEI Quanta 200 scanning electron microscope.

Quantitative FTIR

To investigate the way the two polymers (Eudragit S 100 and Eudragit RL 100) mix or are deposited in the microspheres during production, four main groups of samples were examined by FTIR. Each group contains 12 replicates of the same type of sample. Group 1 contains pure Eudragit S 100 raw material; group 2 contains pure Eudragit RL 100; group 3 contains 1:1 mixture of both polymers before their subjection to the slightly basic buffer dissolution and group 4 contains the same mixture in group 3 but after buffer dissolution. Each sample in the four groups was obtained by grinding LS1 or LS2 blank microspheres and then scanned by FTIR from 850 to 300 cm^{-1}. The spectrum for each sample was recorded after background correction. The data of each spectrum were saved in an Excel file to form a column matrix. Then, the data from all samples from the entire four groups were concatenated using MATLAB 7.0.4 in one Excel file to form a two-dimensional data matrix with dimensions of (48×1,800). Each column represents the FTIR data collected for one sample. The columns in the matrix were arranged randomly. Principal component analysis (PCA) was applied to the resulting data matrix which was mean-centered prior to PCA application.

RESULTS AND DISCUSSION

Size Distribution

At the conditions used in this work, both LS1 and LS2 microsphere formulations were produced in very narrow size distributions with one size fraction comprising 70–80% of the yield. Composition and agitation intensities for microsphere preparations and microsphere size fractions are shown in Table I for the two microsphere formulations (LS1 and LS2).

Drug Loading

Generally, the efficiency of drug loading varied from 88% (±5) for 425–600-μm particles to 92% (±3) for 300–425-μm particles in LS1 and LS2 preparations. No significant differences in loadings were observed between LS1 and LS2 microsphere formulations.

Scanning Electron Microscopy

Blank Microspheres. Blank LS1 and LS2 microsphere formulations were viewed after production as whole microspheres as well as cleaved ones and compared with micrographs after dissolution in the phosphate buffer solution (pH 7.2±0.2). Both LS1 and LS2 microsphere formulations showed basically the same characteristics, so the results of scanning electron microscopy presented in this section are for LS1 preparation only.

The shape and size uniformity of blank LS1 microspheres are shown in Fig. 1. Figure 2a, b shows the surface of LS1 microspheres before and after buffer dissolution, respectively. Microspheres were similar in terms of surface uniformity, smoothness, and homogeneity. In addition, photomicrographs

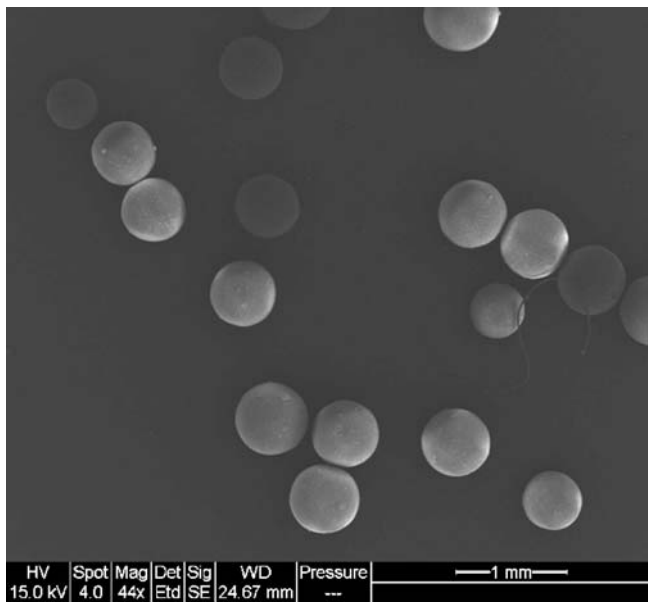

Fig. 1. Scanning electron micrographs of blank LS1 microsphere preparation from a 425–600-μm sieve fraction just after production showing the shape and size uniformity

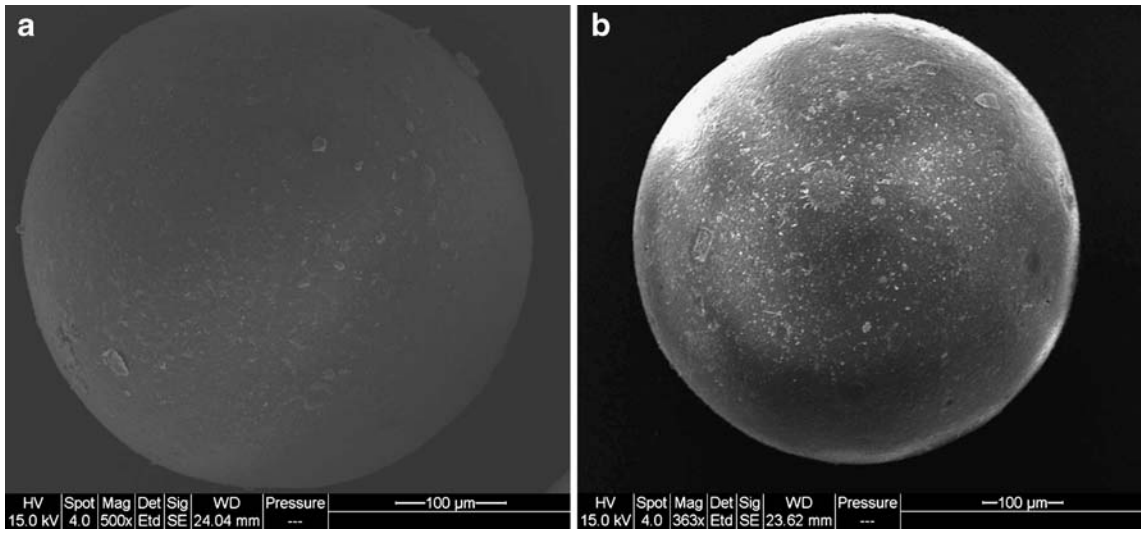

Fig. 2. **a** Scanning electron micrographs of the surface of blank LS1 microsphere preparation from a 425–600-µm sieve fraction before buffer dissolution. **b** Scanning electron micrographs of the surface of blank LS1 microsphere preparation from a 425–600-µm sieve fraction after buffer dissolution

of microspheres after buffer dissolution did not show evidence of nonuniform distribution of Eudragit S 100 (such as grooves or cavities on the surface) that would be seen if it was not evenly distributed in the matrix. For further investigation, cut or cleaved microspheres before and after buffer dissolution were also viewed. The microspheres were similar in shape with the absence of clusters of any of the polymers within the microspheres before dissolution and the absence of empty spaces or cavities within the microspheres after dissolution. Figure 3 shows a cross-sectional view of a cleaved LS1 blank microsphere after dissolution.

Drug-Containing Microspheres. LS1 and LS2 microsphere formulations were viewed after production as whole microspheres as well as cut or cleaved ones and compared with micrographs after dissolution in an acidic medium (pH 1.2±0.2) and in the slightly basic buffer solution (pH 7.2±0.2) as well. Figure 4 shows the shape and size uniformity of LS1 microspheres containing theophylline after production. In addition, Fig. 5a, b shows the surfaces of LS1 and LS2 microspheres containing theophylline, respectively, where drug crystals are present on the surfaces of both microsphere formulations with a relatively less concentration in LS2 microspheres compared to LS1. In both acid and slightly basic buffer dissolution tests, LS1 and LS2 microsphere formulations showed basically the same characteristics, so the results of scanning electron microscopy presented in this section are for LS1 preparation only.

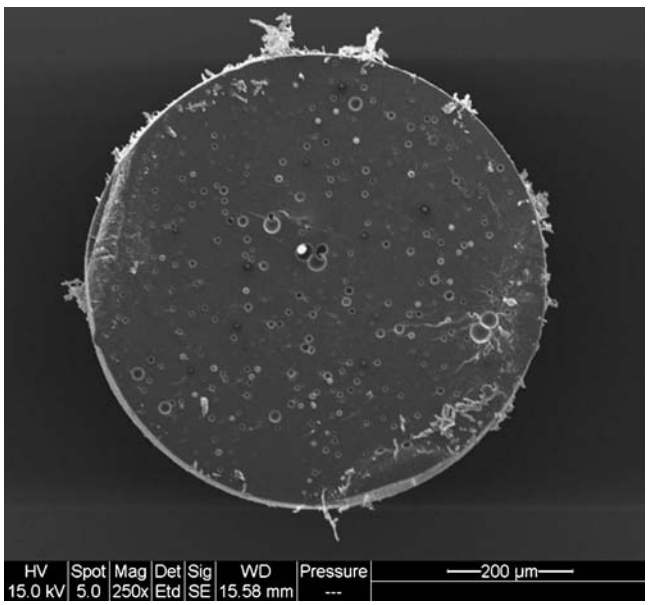

Fig. 3. Cross-sectional view of a cleaved LS1 blank microsphere preparation from a 425–600-µm sieve fraction after buffer dissolution

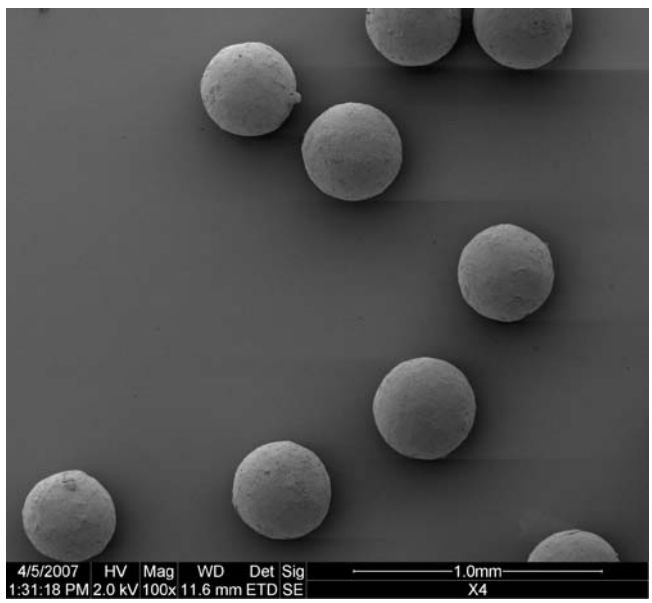

Fig. 4. Scanning electron micrographs of drug-containing LS1 microsphere preparation from a 300–425-µm sieve fraction just after production showing the shape and size uniformity

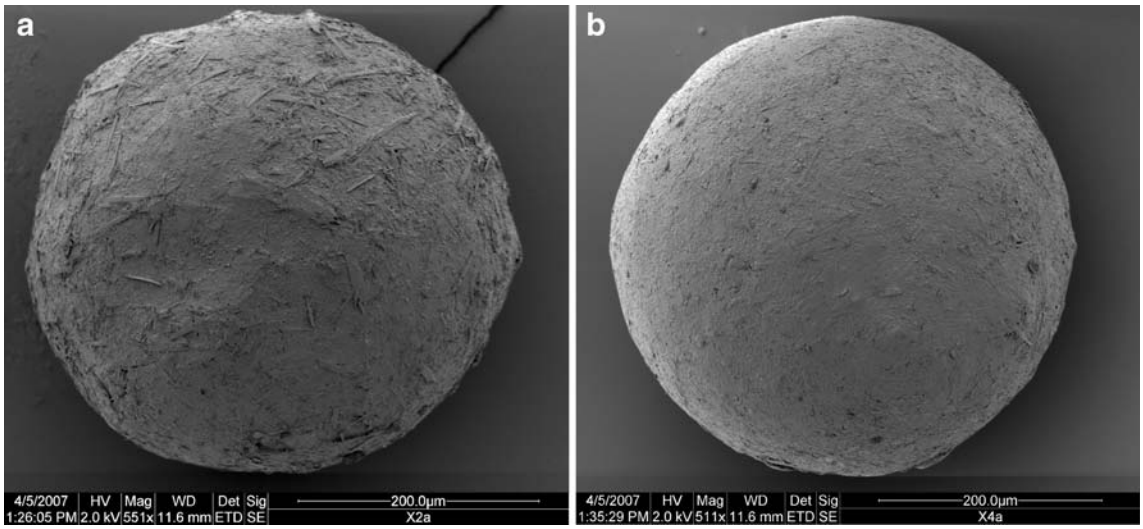

Fig. 5. **a** Scanning electron micrographs of drug-containing LS1 microsphere preparation from a 300–425-µm sieve fraction just after production showing drug crystals on the surface. **b** Scanning electron micrographs of drug-containing LS2 microsphere preparation from a 300–425-µm sieve fraction just after production showing less drug crystals on the surface

Figure 6a, b shows the shape of LS1 microspheres after acid and buffer dissolutions, respectively. After acid and buffer dissolution tests, theophylline dissolved out of the microsphere surfaces. As for blank ones, drug-containing LS1 and LS2 microspheres were similar in terms of surface uniformity, smoothness, and homogeneity. In addition, similar to blank microspheres in buffer medium, photomicrographs of drug-loaded microspheres after buffer dissolution did not show evidence of nonuniform distribution of the Eudragit S 100 in the microspheres (such as grooves or cavitations on the surface) that the Eudragit S 100 would leave behind when it dissolves out. Cleaved microspheres were also viewed before and after buffer dissolution tests. Except for the presence of drug crystals before dissolution, photomicrographs were similar in shape with the absence of clusters of any of the polymers within the microspheres before dissolution and the absence of empty spaces or cavitations within the microspheres after dissolution. Figure 7 shows a cross-sectional view of a cleaved LS1 blank microsphere after dissolution.

Dissolution Characteristics of LS1 and LS2 Microspheres

In this study, the two microsphere formulations LS1 and LS2 were fabricated using equal proportion of Eudragit S 100 and Eudragit RL 100 polymers, with LS2 microspheres containing higher total polymer concentrations/viscosities.

Two different size fractions of microspheres (300–425 and 425–600 µm) from both LS1 and LS2 formulations, each containing about 15.35% theoretical drug loadings of theophylline, were subjected to dissolution at 37°C (±0.5°C) and 100

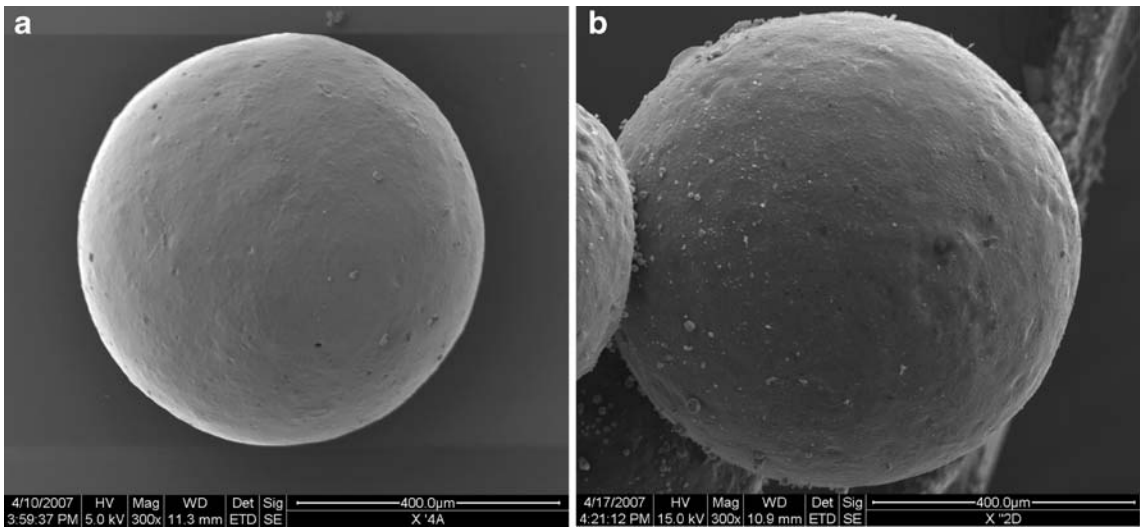

Fig. 6. **a** Scanning electron micrographs of the surface of drug-containing LS1 microsphere preparation from a 425–600-µm sieve fraction after acid dissolution. Surface drug crystals dissolved during dissolution. **b** Scanning electron micrographs of the surface of drug-containing LS1 microsphere preparation from a 425–600-µm sieve fraction after buffer dissolution

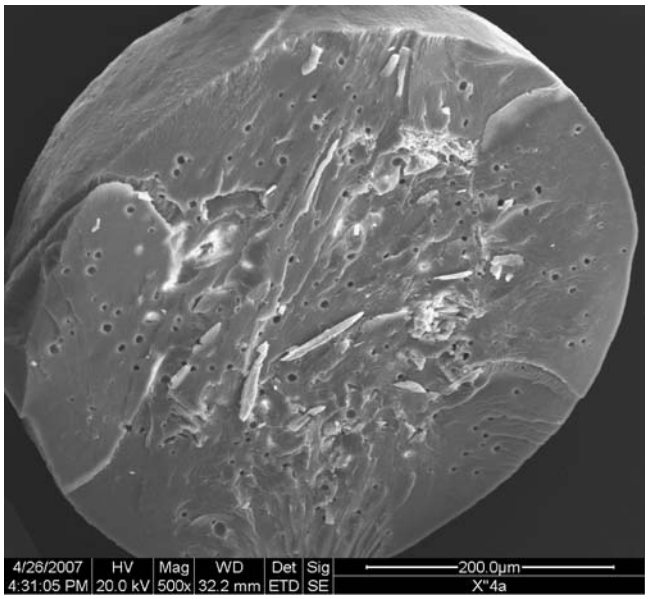

Fig. 7. Cross-sectional view of a cleaved LS1 microsphere preparation from a 300–425-µm sieve fraction after buffer dissolution

rpm with USP Dissolution Apparatus II as described in the "EXPERIMENTAL" section for *in vitro* dissolution.

Figure 8 shows the dissolution profile of the two size fraction microspheres of LS1 and LS2 in an acidic medium (pH 1.2±0.2). It is apparent that the cumulative amounts of drug released at the end of this stage (8 h) were about 30%, while it was less than 18% during the first 2 h. These results indicated the ability of LS microspheres to minimize the drug release during the acid stage, thus having the potential to be used as enteric oral dosage form. Although amount of drug released is insufficient to draw a conclusion of the drug release model, a nearly constant drug release rate from microsphere formulations in this acidic stage can be seen which could be due to the presence of the drug in a relatively more localized way in the core of the microspheres. And neither Eudragit S 100 nor Eudragit RL 100 polymers dissolved in the acidic medium (pH 1.2±0.2); however, they swelled to various degrees allowing the creation of pores and channels from which theophylline leached out (16,17).

A representative dissolution profiles of the same size fraction microspheres (300–425 and 425–600 µm) from LS1 and LS2 preparations in the two-stage media (pH 1.2±0.2) for 2 h followed by a slightly basic buffer medium (pH 7.2±0.2) for 10 h or until the depletion of drug release from microspheres is shown in Fig. 9.

When the pH of the dissolution medium was increased to the slightly basic buffer medium (pH 7.2±0.2) after subjecting the microspheres to dissolution for 2 h in the acidic medium, the cumulative amount of drug released increased substantially with the progressing times of dissolution. Since theophylline has similar solubility in acidic and basic media (18), it was suggested that the increase of drug release rates in the slightly basic medium in Fig. 9 was due to the increase of the porosity of the microspheres due to the enhanced solubility of Eudragit S 100 (17) in this medium and its subsequent gradual removal from the microsphere matrix structure. Additionally, Eudragit RL 100 swelled, allowing further increase in drug release rates. The combination of the swelling of Eudragit RL 100 and the erosion (dissolution) of Eudragit S 100 within the microspheres resulted in a nearly constant drug release rate during the dissolution in the pH 7.2 buffer medium. Models such as Higuchi model, first-order model, and the power law equation of Ritger and Peppas for the drug release were fitted to the data obtained. The

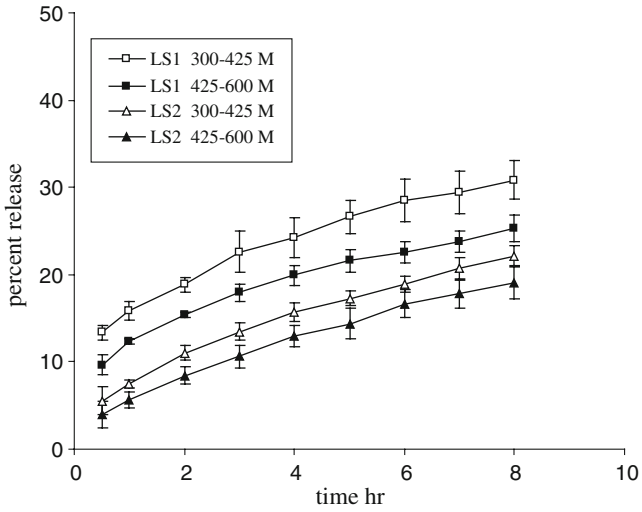

Fig. 8. Release profiles of theophylline in an acidic medium (pH 1.2±0.2) at 37°C from different size fractions of microspheres from LS1 and LS2 formulations. *Error bars* represent the standard deviation

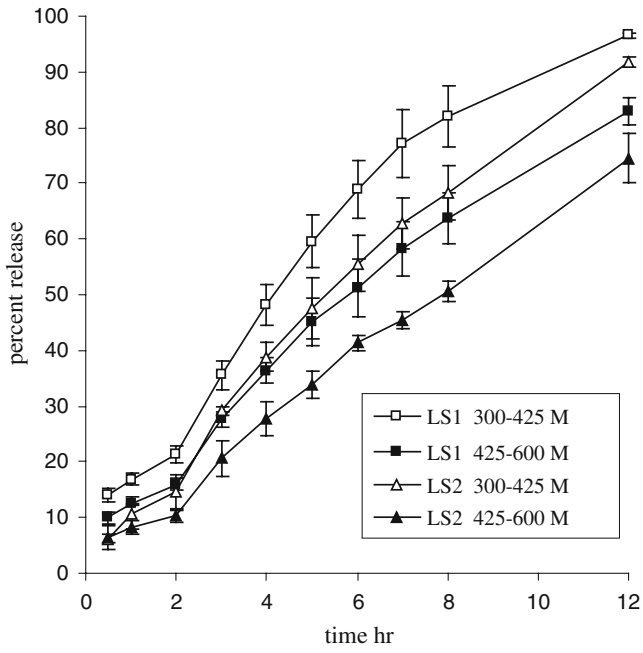

Fig. 9. Release profiles of theophylline in the two-stage media; (pH 1.2±0.2) for 2 h followed by a slightly basic buffer medium (pH 7.2±0.2) at 37°C from different size fractions microspheres from preparations LS1 and LS2. *Error bars* represent the standard deviation

Table II. Values of n Exponents (Slopes), k (Intercepts), and Regression Coefficients Obtained from Fitting Peppas Equation to Drug Release Data in the Slightly Basic Buffer Medium

Microsphere formulation	Microsphere size fraction, μm	n exponent (slope) in Peppas equation	Intercept k in Peppas equation	Regression coefficient
LS1	300–425	0.8607	−0.3734	0.9882
LS1	425–600	0.8536	−0.4748	0.9954
LS2	300–425	0.8721	−0.4915	0.9959
LS2	425–600	0.8712	−0.6262	0.9928

best fit was to Ritger and Peppas model (the power law) (19).

$$M_t/M_\infty = k\,t^n$$

Where M_t/M_∞ is the fraction of drug released at time t; k is the coefficient constant which accounts for the structural and geometrical properties of the matrix, and n is the diffusional exponent indicative of the mechanism of drug release. The resulting n values were found to be close to 0.85 (as shown in Table II) indicating a case II transport (near-zero-order drug release kinetics) suggesting that the drug release mechanism could be through swelling/polymer relaxation/erosion with insignificant or negligible contribution of Fickian diffusion in the drug transport process. At the end of dissolution, the release rate decreased with time because of the increase in the diffusion path length of the drug. The change in diffusion path length was not only because of the gradual depletion of the drug from the matrix but also because of the moving boundaries such as swelling and erosion.

This explanation of the mechanism of drug release was not based only on the results of best fit to the suggested models but also on the results we have from other studies such as the quantitative FTIR and the scanning electron microscopy studies. In these studies, the two polymers were found to be molecularly dispersed in the microsphere matrix structure. Eudragit S 100 and Eudragit RL 100 polymers swell in the acidic medium (16,17) before being exposed to the buffer medium where the swelling then becomes accompanied with the major portion of drug being released. In addition, Eudragit S 100 is pH sensitive that dissolves (erodes) after swelling. Thus, these results and explanations would support our results of the proposed mechanism of drug release.

It was evident from the results of the dissolution profiles in the two-stage media that the drug release was controlled in both kinetics and duration. These properties of such a dosage form are of high importance. The use of controlled-release technology in the formulation of pharmaceutical products has become increasingly important during the last few years since they have made significant potential to enhance clinical efficacy and reduce total treatment costs, thereby providing economic value compared to conventional immediate-release dosage forms, even when the initial acquisition costs are higher (20). In this study, both LS1 and LS2 microsphere formulations with the size fractions 300–600 μm would have the potential to provide a controlled and sustained drug release. In addition, both microsphere formulation possessed enteric properties in the acidic medium (pH 1.2±0.2) where only small percentage of the drug was released. This property can be utilized in oral controlled-release dosage forms to protect drugs from the low pH of the stomach and/or to protect the gastrointestinal tract from the irritating effects of drugs.

Effect of Microsphere Size and Polymer Concentration/Viscosity on the Dissolution Profile

The dissolution profiles of LS1 and LS2 microspheres in the acidic medium (pH 1.2±0.2) and in the slightly basic buffer medium (pH 7.2±0.2) in Figs. 8 and 9, respectively, showed that the drug release rate increased as the microsphere size decreased for both microsphere formulations. Also, it was evident that the increase in drug release rate with decreasing particle size was more pronounced for microspheres of low polymer concentration or viscosity (LS1 microspheres) in both of the dissolution media.

These observations could be attributed to the fact that an increase in organic phase viscosity would hold the drug firmly inside the microsphere so that the diffusion of the drug is slowed (2).

Also, an increase of the viscosity of the polymer solution phase could decrease the porosity of the microspheres. It was apparent from the dissolution profiles in Figs. 8 and 9 that the initial release was decreased substantially as the polymer solution viscosity was increased. The effect of viscosity on the initial release could be due to the holding and the prevention of drug particle migration to the surfaces during the solvent evaporation

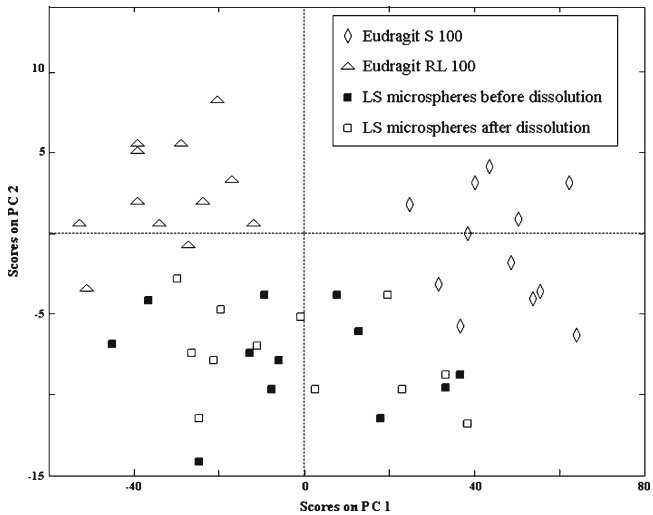

Fig. 10. Two-dimensional PCA score plot. Each point represents a single spectrum acquired for each replicate from each sample

(migration) and during the spinning centrifugal forces created by shearing procedure throughout the microsphere preparation. This could be clearly seen by comparing the scanning electron micrographs for LS1 (low concentration/viscosity) in Fig. 5a and LS2 (high concentration/viscosity) in Fig. 5b.

Quantitative FTIR Studies

It was thought from the results of the dissolution studies in the acidic medium (pH 1.2±0.2), where microspheres exhibited minimal drug release, that double-wall microspheres were produced wherein Eudragit S 100 polymer engulfs Eudragit RL 100 polymer or at least dominates the surface of the microsphere as a separate layer. To investigate this thought, a series of experiments including surface tension measurements, solubilities of both polymers in the common solvent mixture, and rheological studies were conducted. Unfortunately, except for the rheological properties, all other results were similar for both polymer solutions and were insufficient to draw a conclusion about the location of polymers within the microspheres. In addition, as shown earlier, the scanning electron microscopy studies did not reveal the presence of polymer layers or double-wall microspheres before dissolution in the slightly basic medium; neither did it reveal the presence of empty cavities after the dissolution.

To investigate the way the two polymers (Eudragit S 100 and Eudragit RL 100) do mix and whether a layer of Eudragit S 100 could exist on the surface of the microspheres, samples were examined by the FTIR as described in the "EXPERIMENTAL" section. Among the samples tested were the LS microspheres before and after dissolution in the slightly basic buffer medium. Thus, in this study, scanning electron microscopy and quantitative FTIR were used because of their availability with the useful software to analyze the data, convenience in conducting the experiments, and the high-precision results they can provide.

PCA is a multidimensional data analysis tool for investigating differences and similarities among sample's spectra through recognizing the pattern in a data set. PCA relies upon extracting the eigenvectors for the covariance or correlation matrix of the original data matrix containing the measured variables. The first principal component (PC) which accounts for as much of the variability in the data as possible has the same direction as the eigenvector associated with the largest eigenvalue. And each succeeding component accounts for as much of the remaining variability as possible (21).

In this study, the first two components are identified as PC1 and PC2. They are mutually orthogonal. The greater the separation in this two-dimensional principle component space among the four types of samples, the greater is the statistical difference between sample spectra (22).

The resulted PCA model accounts for more than 92% of the total variation in the data using the first two PCs as shown in Fig. 10. From this model, it could be seen that pure Eudragit S 100 polymer (diamond) and pure Eudragit RL 100 polymer (triangles) cluster in different spaces of the PCA model. The distance between the center of the two clusters means that they have different spectral signatures. In the same model, it could be seen that polymer mixtures of LS microspheres before the dissolution (filled squares) lie between the two clusters of the pure Eudragit S 100 and Eudragit RL 100 which means that the LS microspheres were composed of the pure Eudragit S 100 and pure Eudragit RL 100 in a comparable ratio. To investigate the composition of the same mixture of LS microspheres after dissolution in the slightly basic buffer, the spectral data of the mixture were also included in the same PCA model (empty squares). Data from the polymer mixture of LS microspheres after dissolution clustered at the same space of the mixture before the dissolution (filled squares) as shown in Fig. 10. This indicated an almost similar spectral signature of the LS microspheres before and after dissolution and hence similar composition. Thus, upon prolonged dissolution in the pH 7.2 buffer medium (48 h), it seems that Eudragit S 100 slowly but incompletely dissolved out of the microspheres. If Eudragit S 100 was dominating the surface of the microspheres, it would come out faster leaving only Eudragit RL 100 polymer and the spectral data would be close to pure Eudragit RL 100 cluster. This was supported by the gravimetric studies (data not shown) where only a few percentage of the microsphere weight was lost with the prolonged dissolution. These results suggested that Eudragit S 100 could be molecularly dispersed and distributed all through the microspheres including the surfaces that gave rise to their enteric properties.

CONCLUSIONS

Microspheres of narrow size distributions were produced with one size fraction comprising 70–80% of the yield. LS microspheres seemed to be composed of a homogeneous molecular dispersion of Eudragit S 100 and Eudragit RL 100. This was indicated to some degree by the quantitative FTIR studies. In addition, the absence of double-walled or layered microspheres before dissolution and the absence of cavities after dissolution in pH 7.2 buffer medium was confirmed by scanning electron microscopy studies.

ACKNOWLEDGEMENT

The author would like to thank Professor James Price at UGA for his remarks.

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