Influence of Aluminum Tristearate and Sucrose Stearate as the Dispersing Agents on Physical Properties and Release Characteristics of Eudragit RS Microspheres

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ABSTRACT

The purpose of this research was to investigate the effects of different concentrations of polymer and sucrose stearate, aluminum tristearate as dispersing agents on microsphere properties and performance. The yield values of microspheres were over the 78%, and the encapsulation efficiencies were found to be ~73%. Particle sizes of microspheres prepared with aluminum tristearate were between 76 and 448 μm, while that of the microspheres containing sucrose stearate were between 521 and 2000 μm. Morphological and physicochemical properties of microspheres were investigated by scanning electron micrography and differential scanning calorimetry (DSC). DSC analysis indicated that verapamil hydrochloride formed a solid solution with acrylic polymers. In vitro release studies were performed using the flow-through cell method. While ~80% of drug was released from the microspheres containing aluminum tristearate in 480 minutes, the same amount of drug was released from microspheres containing sucrose stearate in only 60 minutes. Chemical structures and concentrations of the dispersing agents were clearly effective on the physical properties of microspheres and their drug-release characteristics.

KEYWORDS: aluminum tristearate, sucrose stearate, Eudragit RS 100, solvent evaporation method, verapamil HCl.

INTRODUCTON

Verapamil hydrochloride (VRP), a calcium channel blocker, is widely used for the treatment of hypertension, angina, and myocardial infarction. VRP has a very low bioavailability of ~10% to 20% when administered by oral/intravenous (IV) routes. The low bioavailability is owing to the rapid biotransformation in the liver with a biological half life of 4.2 hours. Because of its relatively short half-life, the formulation of a controlled-release dosage form is considered to be very useful.[1,2]

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Several studies concerning the preparation of sustained-release systems containing VRP have been published; one of the most popular approaches being the incorporation of this drug into polymeric microspheres.[1] The solvent evaporation method is one of the preparation techniques for microspheres widely used for sustained-release applications. This method involves using a suitable dispersing agent to emulsify a solution containing polymer and drug into another medium in which the drug and polymer cannot be dissolved.[3,4] Dispersing agents can be various polymeric materials, proteins, or surfactants, which simplify the formation of microspheres by decreasing the interfacial tension between the lipophilic and hydrophilic phases of the emulsion.[5-8] The dispersing agent forms a thin protective layer around the droplets and hence reduces the extent of their collision and coalescence.[9] VRP is a highly water-soluble drug and the water/oil (w/o) emulsion systems are generally preferred for use with drugs that have high solubility in water. Dispersing agents used in w/o systems are especially metallic soaps (eg, magnesium stearate, aluminum tristearate), sorbitan fatty esters (eg, Spans, Tweens, Arlacels), and polyoxyethylene fatty ethers (eg, Brijs).[10-13] Sucrose esters have also been proposed as dispersing agents because of the advantages of low toxicity and biodegradation. Some of the polymers preferred in the preparation of VRP microspheres by the solvent evaporation method are different cellulose esters,[14,15] hydrogels,[2] and polymethacrylates (eg, Eudragits) .[13,16] Eudragit RS 100, a copolymer synthesized from acrylic and methacrylic acid esters with quaternary ammonium groups, is widely used as a coating material in the pharmaceutical industry. Since a Eudragit RS 100 film is slightly permeable, drug release through the film is retarded.[17,18]

The purpose of this study was to determine the effects of the variations of dispersing agent types (aluminum tristearate and sucrose stearate) and concentration as well as polymer concentration on the microspheres prepared by the solvent-evaporation method. The effect of these parameters on microsphere properties such as morphology, average particle size, size distribution, and drug content has been investigated. In vitro drug release studies were performed to evaluate the effects of dispersing agents on the release of VRP from the microspheres. To make the differences between the formulations more obvious, a pH 4.5 phosphate-citrate

buffer was selected as the dissolution medium. The interactions of drug with dispersing agents and with the polymer were investigated by differential scanning calorimetry (DSC).

Materials and Methods

Materials

The materials used were verapamil HCl (Knoll, AG, Ludwigshafen, Germany), Eudragit RS 100 (Röhm Pharma GmbH, Weiterstadt, Germany), aluminum trisrearate (Merck, Darmstadt, Germany), and sucrose stearate (Crodesta F 160, HLB 15, Croda GmbH, Mettelal, Germany). All other chemicals were of analytical grade.

Preparation of Microspheres

Verapamil HCl and Eudragit RS 100 were dissolved in an acetone-methanol mixture. The dispersing agent was added, and the mixture was stirred at 500 rpm in a water bath on a magnetic stirrer at 10°C. The mixture was then poured rapidly into liquid paraffin, previously cooled to 10°C while being stirred at a speed of 400 rpm (model RZR-2000, Heidolph-Elektro, Kelheim, Germany). The resulting emulsion was mixed at 35°C for 4 hours, and the organic solvent, acetone-methanol, was completely removed by evaporation. The solidified microspheres were filtered, washed 6 times with an aliquot of 50 mL n-hexane, dried under vacuum at room temperature overnight, and stored in a desiccator.[19,20] Formulations of microspheres are provided in Table 1.

Preparation of Physical Mixtures

Physical mixtures were prepared based on the solid content of formulations (Table 1) by blending in an agate mortar. Eudragit RS 100 was then milled by using a microgrinder (Janke and Kunkel KG, Staufen, Germany) before addition to the mixtures.

Percentage Yield Value of Microspheres

The percentage yield value of microspheres was determined from the ratio of amounts of solidified total microsphere to total solid material used in the inner phase, multiplied by 100.

Determination of Encapsulation Efficiency of Microspheres

Microspheres containing ~10 mg verapamil HCl were weighed and dissolved in methanol. Drug concentration was determined by UV spectrophotometry (Shimadzu UV-Visible 1202, Kyoto, Japan) at 279 nm (n = 5). The encapsulation efficiencies were calculated by using the following relationship:

$$\text{Encapsulation efficiency} = \left(\frac{\text{Drug entrapped}}{\text{Theoretical drug content}}\right) \times 100. \quad (1)$$

Particle Size Analysis of Microspheres

Average particle diameter and size distribution of microspheres were determined by laser diffractometry using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Approximately 10 mg of microspheres were dispersed in 2 to 3 mL distilled water containing 0.1% Nonidet P40 for several minutes using an ultrasonic bath. Then, an aliquot of the microsphere suspension was added into the small volume recirculation unit,[21] which was subsequently circulated 3500 times per minute. Each sample was measured in triplicate for the analysis. Particle size was expressed as the weighted mean of the volume distribution.

Scanning Electron Micrography

The shape and surface characteristics of microspheres were observed by a scanning electron microscope (SEM, Jeol JSM-6400, Tokyo, Japan). Microspheres were dusted onto double-sided carbon tape, which was placed onto a cylindrical sample carrier (height, 5 mm; diameter, 10 mm). The

Table 1. Formulations of Verapamil HCl Microspheres*

Content	F1 F10	F2 F11	F3 F12	F4 F13	F5 F14	F6 F15	F7 F16	F8 F17	F9 F18
Eudragit RS 100† (%)	20	20	20	14.3	14.3	14.3	11.1	11.1	11.1
Dispersing agent†,‡ (%)	1	3	5	1	3	5	1	3	5
Methanol (mL)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Acetone (mL)	26.5	26.5	26.5	38.5	38.5	38.5	50.5	50.5	50.5
Liquid paraffin (mL)	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0

*Each formulation contained 1.5 g of verapamil HCl.

†The concentrations of dispersing agents and polymer were calculated from dispersed inner phase volume (wt/vol %).

‡Dispersing agents are aluminum tristearate for F1 to F9 and sucrose stearate for F10 to F18.

samples were coated with Au-Pd mixture under vacuum (100 mTorr) with a sputter coater (Hummer VII-Hummer II, Alexandria, KY) to thickness of 50 nm. The samples were imaged using a 5 to 15 kV electron beam.

Differential Scanning Calorimetry

Thermal analysis was performed on the drug, polymer, aluminum tristearate, sucrose stearate, physical mixtures, and microspheres using DSC (Netzch Geatebau, DSC 204, Selb, Germany). Samples (5 mg) were accurately weighed into aluminum pans and then sealed. The thermograms of the samples were obtained at a scanning rate of 10°C/min conducted over a temperature range of 25°C to 230°C.

In Vitro Release Studies

Drug release from microspheres was determined using a flow-through cell (Desaga, Heidelberg, Germany). Citrate-phosphate buffer (pH 4.5), containing polysorbate 20 (0.02% wt/vol) in order to improve the wetting of the microspheres, was used as the dissolution medium. During the dissolution test, the flow rate of the dissolution fluid was adjusted to 8 mL/min in order to maintain sink conditions. The test was continued for 8 hours. The amount of drug released was determined spectrophotometrically at 278 nm.

RESULTS AND DISCUSSION

The yield values of microspheres prepared with aluminum tristearate and sucrose stearate were in the range of 78.1% to 90.6% and 79.2% to 118%, respectively (Table 2). Because the inner phase could not be entirely removed from inside of the microspheres prepared with sucrose stearate, the yield values were over 100%.

The encapsulation (drug loading) efficiencies for the microsphere formulations are summarized in Table 2. The drug-loading efficiency for the microspheres prepared with aluminum tristearate was generally higher than the theoretical yield (100%). Aluminum tristearate, known to dissolve in liquid paraffin,[22] is lost from the inner phase during the preparation process. As a smaller amount of aluminum tristearate was incorporated into microspheres, the actual drug content was found to be higher than theoretically expected. Mateovic et al[23] have also calculated incorporation efficiency values over 100% with microspheres containing magnesium stearate as a dispersing agent because the magnesium stearate was partly lost from the inner phase. The encapsulation efficiencies for the microspheres containing sucrose stearate varied between 73.4% and 100%. Standard error values for the encapsulation efficiencies of microspheres prepared with aluminum tristearate were smaller than the others, suggesting a more homogeneous distribution of drug in these microspheres.

When the data in Figure 1 and Table 2 were examined, it was discovered that the particle sizes of microspheres prepared with aluminum tristearate were quite smaller than those of microspheres prepared with sucrose stearate. The smaller particle size of microspheres containing aluminum tristearate was attributed to the better stabilizing effect of this dispersing agent on the emulsion droplets. Aluminum tristearate provided this stabilizing effect by preventing electrification and flocculation during the preparation of Eudragit RS 100

Table 2. Physical Properties of Microspheres*

		Microspheres Prepared With Aluminum Tristearate				Microspheres Prepared With Sucrose Stearate			
$C_{polymer}$	$C_{dispersing\ agent}$	YV (%)	EE ± SE (%)	d (0.5)†	Span‡	YV (%)	EE ± SE (%)	d (0.5)	Span
20	1	-	-	-	-	114	80.4 ± 3.05	>2000	-§
	3	86.7	103 ± 0.395	448	1.52	116	77.9 ± 1.53	603	0.937
	5	90.6	100 ± 0.801	363	1.43	102	100 ± 4.79	775	1.25
14.3	1	83.6	107 ± 1.48	110	2.27	97.0	83.9 ± 3.40	968	0.873
	3	88.6	105 ± 1.83	85.3	4.50	118	77.9 ± 1.59	956	0.797
	5	89.1	104 ± 1.21	155	2.83	104	91.7 ± 2.60	1070	1.25
11.1	1	78.1	101 ± 1.58	82.3	1.27	79.2	89.0 ± 1.42	912	1.07
	3	81.4	98.4 ± 0.841	76.4	1.88	110	73.4 ± 2.05	640	0.953
	5	80.8	95.0 ± 1.37	135	4.20	107	86.5 ± 1.84	521	1.98

*YV indicates yield value; EE, encapsulation efficiency (n = 5); and C, concentration.

†Values shown represent the volume median diameter (μm).

‡Values shown represent the width of distribution. It is calculated by Span = (D90 − D10)/D50.

§The particle size of microspheres greater than 2000 μm could not be measured by laser diffractometry.

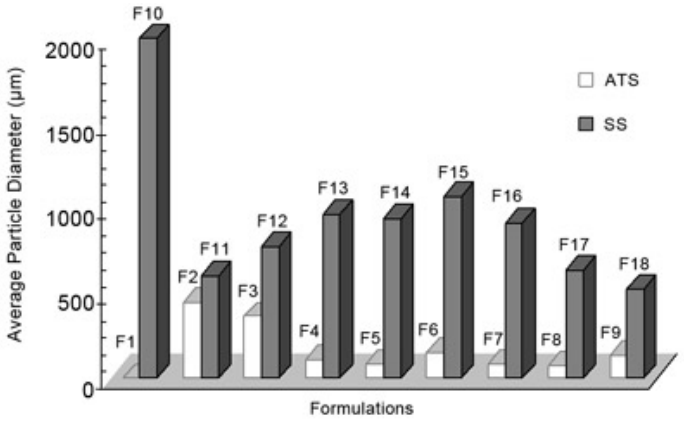

Figure 1. Average particle diameter of microsphere formulations. ATS indicates aluminum tristearate; and SS, sucrose stearate.

microspheres.[22] The variations of the concentrations of aluminum tristearate did not affect the particle size of microspheres, but increasing polymer concentrations led to an increase in particle size. However, when the concentration of sucrose stearate was increased, particle size of microspheres was not influenced. On the other hand, increasing amounts of aluminum tristearate resulted in accumulation of free aluminum tristearate particles on the surfaces of microspheres (Figure 2, F3 and F9). Examination of the photomicrographs from the F17 formulation revealed that the surface of the microspheres containing sucrose stearate was smooth and nonporous, resulting from higher solubility of this dispersing agent in the inner phase. As shown in Table 2, the Span values (width of the size distribution) of microspheres containing aluminum tristearate were larger than those of microspheres containing sucrose stearate.

DSC analysis was run on each dispersing agent, drug, and polymer, and on the F3 and F12 formulations and physical mixtures of these formulations. Thermograms are presented in Figure 3. A sharp endothermic peak corresponding to the melting of crystalline drug was found at 147.52°C. For pure polymer, the thermal transition at 64.8°C was attributed to the glass transition temperature (T_g) of polymer. The endotherm at 62.72°C corresponded to the melting of aluminum tristearate in crystalline form (Figure 3A). The thermogram of the physical mixture of the F3 formulation showed almost the same melting peaks at 143.81°C and 62.85°C with some depressions for VRP and aluminum tristearate, indicating their crystalline structures (Figures 3A and 3B). The small peaks at 169.82°C and 167.62°C (Figure 3B) could have arisen from aluminum tristearate, since a transition at 125°C to 150°C (Figure 3A) was also seen in the thermogram of the pure aluminum tristearate. It was thought that this transition had shifted to higher temperatures due to an interaction between the polymer and aluminum tristearate. The absence of the VRP crystalline peak, which should have been appeared at ~147.52°C, proved that the drug was in an amorphous state in this formulation. The melting peak of pure sucrose stearate was observed at 56.24°C (Figure 3A), while it was observed at the temperatures of 57.0°C and 51.57°C in the thermograms of the physical mixture and microspheres (Figure 3B). The VRP peak with some depressions at 137.54°C was seen in the thermogram of the physical mixture of the F12 formulation. No endothermic peak confirming the presence of crystalline drug was observed in the microspheres prepared from the F12 formulation. The absence of the crystalline peaks of VRP in the thermograms of the 2 formulations indicated that VRP and polymer interacted at the molecular level; possibly the drug formed a solid solution with polymer (Figure 3B).[24]

To compare the formulations with each other, it was decided to choose a dissolution medium that would emphasize the differences amongst the formulations. Therefore, VRP release from the F10, F11, and F12 formulations including 1%, 3%, and 5% sucrose stearate, respectively, was examined at pH 1.2 in simulated gastric fluid, pH 4.5 phosphate-citrate buffer, and pH 6.8 phosphate buffer. Figure 4 shows that the pH 4.5 phosphate-citrate buffer enhances the differences amongst the formulations in the best way. At pH 1.2, over 60% of drug was released within ~60 minutes from all formulations; at pH 6.8, the drug

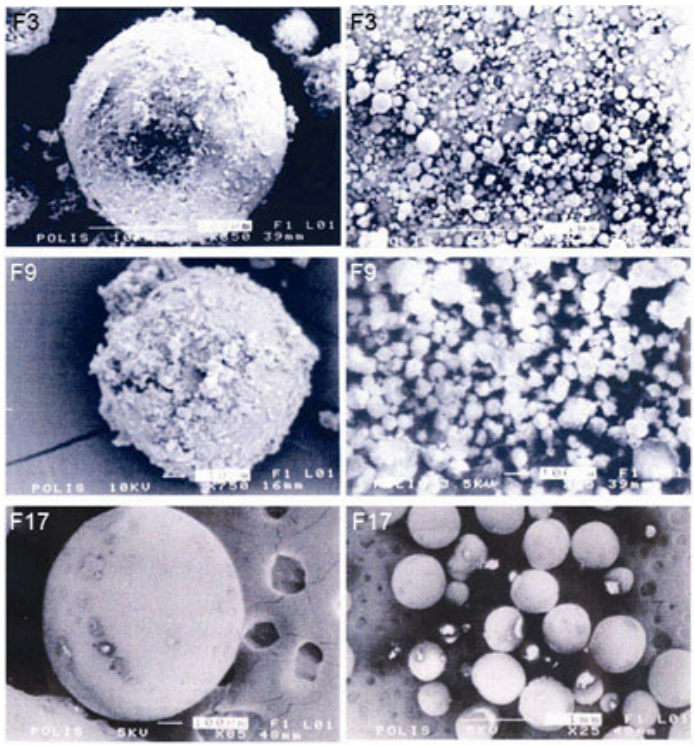

Figure 2. Scanning electron photomicrographs of microspheres. Percentages of polymer and dispersing agents are given in Table 1. Magnification: F3 left, ×350; F3 right, ×37; F9 left, ×750; F9 right, ×85; F17 left, ×85; F17 right, ×25.

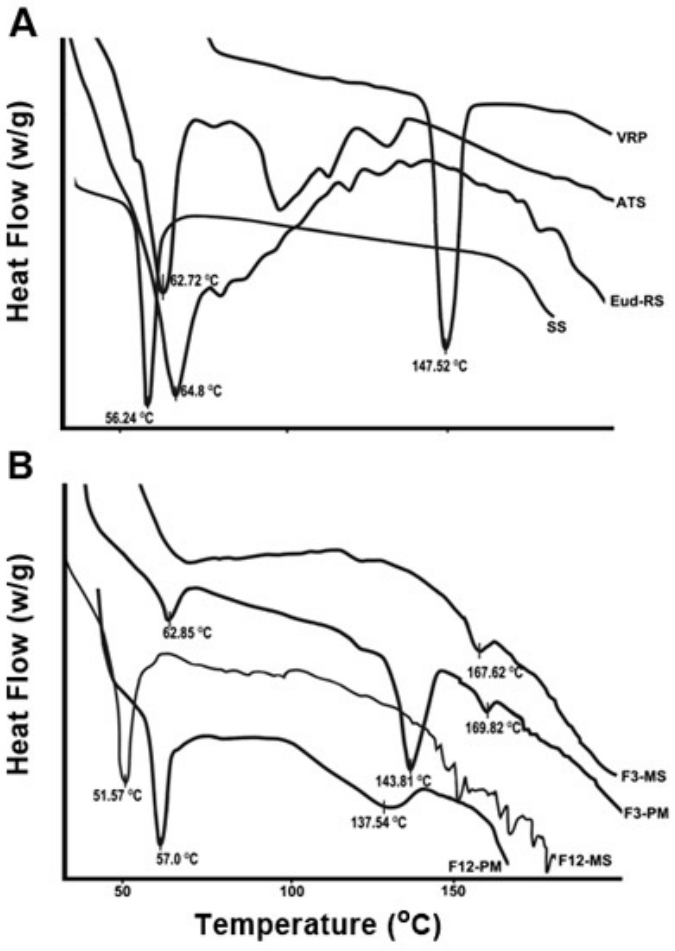

Figure 3. DSC thermograms of (A) verapamil HCl (VRP), Eudragit RS 100 (Eud-RS), aluminum tristearate (ATS), sucrose stearate (SS); and (B) physical mixture (PM) and microspheres (MS) of F3 and F12 formulations.

release from the formulations was significantly delayed, although no differences between the formulations F10 and F12 were observed.

Figure 5 shows the release profiles of VRP from the microspheres containing different dispersing agents at different concentrations. Smaller microspheres were expected to have higher release rates owing to a supposedly larger total surface area. When release profiles were examined, it was seen that although particle size of the microspheres prepared with sucrose stearate was greater than that of microspheres with aluminum tristearate, the release of the drug was generally faster at all polymer:solvent ratios. This result was owing to the hydrophilic character of sucrose stearate. Sucrose stearate has a high hydrophilic-lipophilic balance (HLB) value (15), showing promise as a candidate to increase the dissolution rate of drugs.[25] In contrast, accumulation of the hydrophobic aluminum tristearate particles onto the microspheres made the release difficult even though its microspheres had a smaller particle size.[20] The initial burst of VRP from microspheres including sucrose stearate was higher particularly at the lower polymer concentrations, which might be the result of the higher concentration of drug near and/or on the surface of the microspheres. Eighty percent of drug was released from the microspheres containing sucrose stearate in ~60 minutes at a polymer concentration of 11.1%. Decreasing the polymer concentration led to a faster release of drug in these formulations. At polymer concentrations of 14.4% and 11.1%, no difference among the drug-release profiles of microspheres containing sucrose

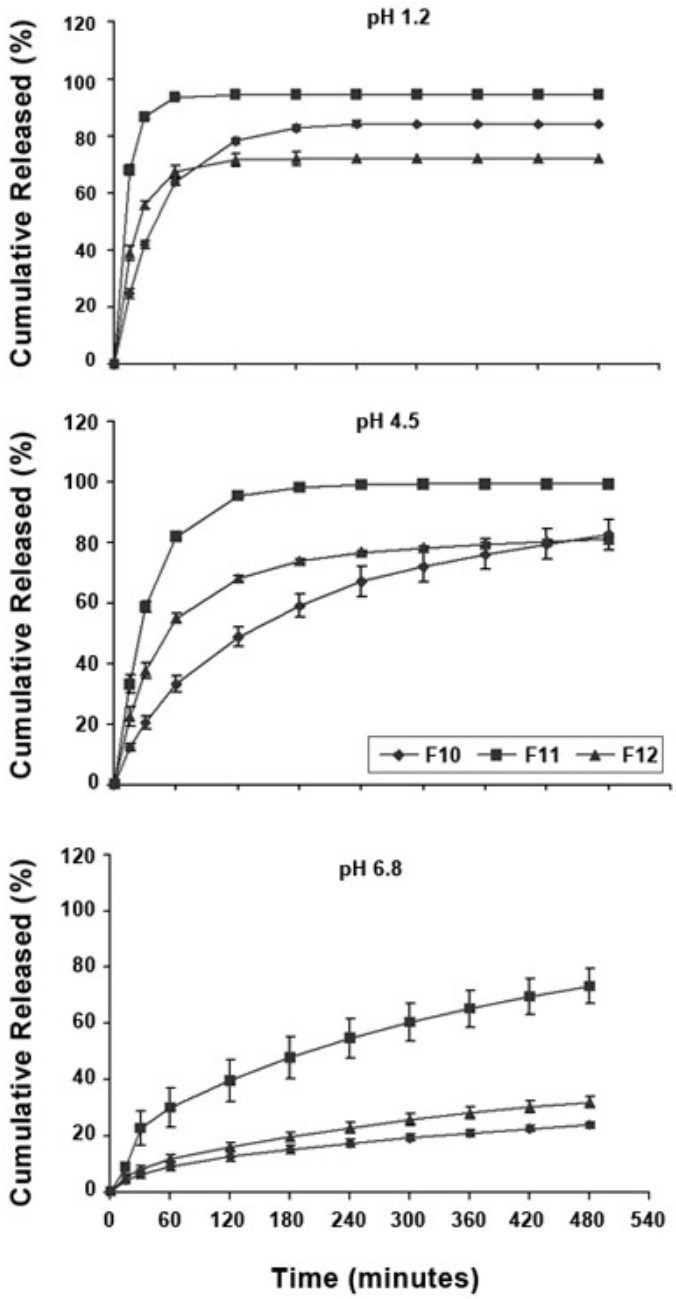

Figure 4. Release profiles of F10, F11, and F12 formulations in different pH media.

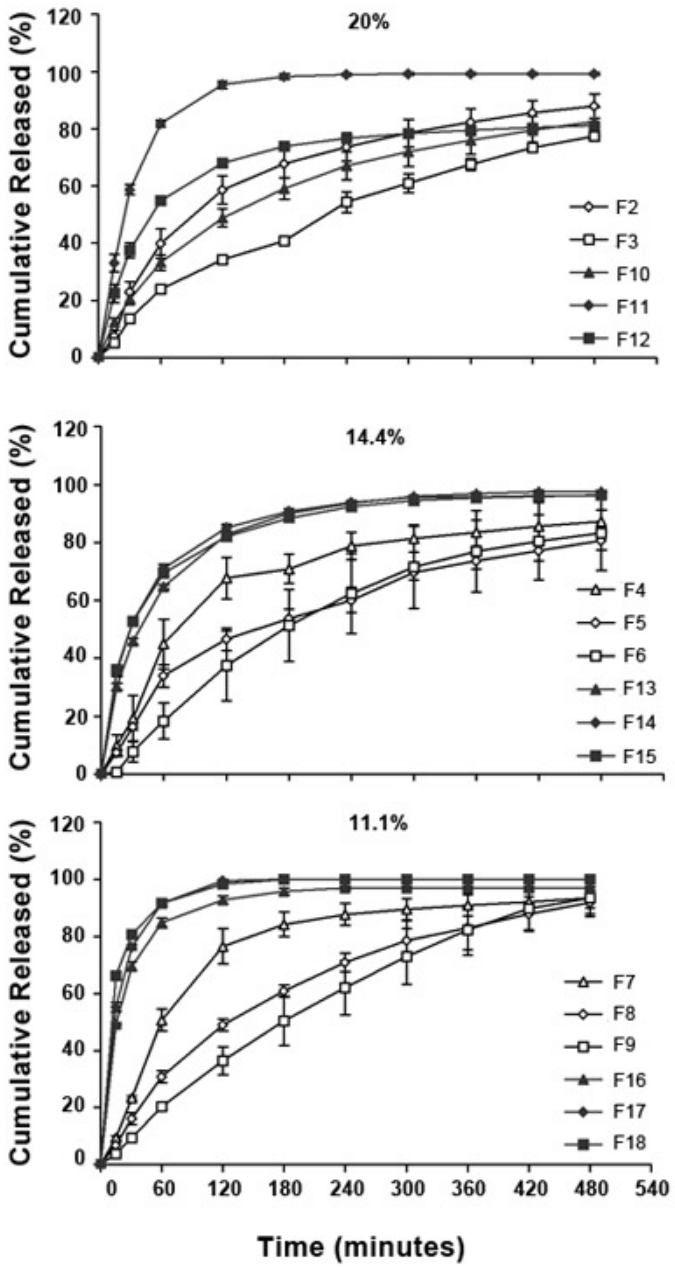

Figure 5. Release profiles of microspheres prepared with aluminum tristearate (△:1%, ◊:3%, □:5%) and sucrose stearate (▲:1%, ♦:3%, ■:5%) (n = 3) from Eudragit RS at different concentrations (20%, 14.4%, 11.1%).

stearate was seen, indicating that the different concentrations of sucrose stearate did not affect the release rate of drug. However, at the highest polymer concentration of 20%, the effect of the concentration of sucrose stearate on drug release could easily be observed. The slowest release of VRP from microspheres was observed with formulations prepared incorporating 5% aluminum tristearate at all polymer concentrations, emphasizing the effect of the hydrophobicity of the dispersing agent on the drug release. About 80% of drug was released from the microspheres in 480 minutes.

Conclusion

Dispersing agents used in this study (aluminum tristearate and sucrose stearate) were clearly effective on the average particle diameter and size distribution of microspheres. The microspheres were produced with a high yield value and encapsulation efficiency. Aluminum tristearate retarded the drug release from microspheres because of its hydrophobic structure, while sucrose stearate with a high HLB value accelerated the drug release.

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