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The preparation and evaluation of salbutamol sulphate containing poly(lactic acid-co-glycolic acid) microspheres with factorial design-based studies

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Abstract

Microspheres of salbutamol sulphate (SS) with poly(lactic acid-co-glycolic acid) (PLGA 85115) were prepared by the modified solvent evaporation method using a double emulsion. In the preparation of the formulations, we used a 2^3 factorial design based on three independent variables: drug loading, amount of gelatin and concentration of PVA. The dependent variables are particle size of the microspheres and entrapment ratio % in the microspheres. The effect of the three independent variables on the particle size and entrapment ratio % were evaluated with analysis of variance and response surface graphs. The in vitro release studies were carried out by shaking in isotonic phosphate buffer solution, pH 7.4. The particle sizes of microspheres were determined by infrared particle size apparatus (IPS). The interaction between PLGA and drug was investigated by DSC and FT-IR analysis. Extended release was obtained for 96 h with F8 formulation microspheres. The best release profile (F8 formulation) fitted the dissolution model proposed by Baker and Lonsdale. In vitro degradation of the best formulation was investigated using scanning electron microscopy. The pore size increased with time and then degraded becoming empty holes.

Keywords: Poly(lactic acid-co-glycolic acid); Biodegradable microspheres; Salbutamol sulphate; Factorial design

1. Introduction

Polylactic acid, polyglycolic acid and their copolymers are used in a variety of medical applications. Because they degrade slowly by hydrolysis to lactic acid and glycolic acid, which are body metabolites, they are biocompatible and produce little or no local and systemic toxicity on administration (Brophy and Deasy, 1990). These polymers are the most widely used biodegradable artificial polymers for sustained release preparations and may be administered intramuscularly or by other parenteral routes. The solvent evaporation method has been used by many researchers to incorporate water insoluble drugs into biodegradable microspheres. But the loading efficiency of water soluble drugs into PLA and PLGA microspheres is relatively low when a conventional o/w emulsion system is used for the solvent evapora-

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Table 1
(a) The independent variables and their levels; (b) the contents of SS-PLGA microspheres
(a)

Independent variable	Low level	High level
Drug loading (x_1)	0.1 g	0.2 g
PVA concentration (x_2)	0.1% w/v	0.5% w/v
Amount of gelatin (x_3)	0.05 g	0.1 g

(b)

Formulation	Drug loading (g)	Concentration of PVA (%)	Amount of gelatin (g)
F1	0.1	0.1	0.05
F2	0.2	0.1	0.05
F3	0.1	0.1	0.1
F4	0.2	0.1	0.1
F5	0.1	0.5	0.05
F6	0.2	0.5	0.05
F7	0.1	0.5	0.1
F8	0.2	0.5	0.1

tion process, since such drugs will readily diffuse into the aqueous outer phase of the emulsion system. To overcome this problem, PLGA microspheres containing water soluble compounds were prepared by a modified solvent evaporation method using a double emulsion (Iwata and McGinity, 1992).

Salbutamol sulphate (SS) was chosen as a model drug. It is water soluble, and a well known bronchodilator with a short half life 3–8 h and is an appropriate candidate for controlled release formulations (Cullum et al., 1969).

The purpose of the present study was: (a) to prepare and characterize salbutamol sulphate microspheres using PLGA as a biodegradable polymer by the modified solvent evaporation method using a double emulsion; (b) to utilize 2^3 factorial design experiments; (c) to investigate the effect of drug loading, PVA concentration and amount of gelatin on particle size and entrapment ratio % of microspheres; (d) to investigate what conditions affect the drug release and (e) to fit the data to various postulated drug release kinetic models.

2. Materials and methods

2.1. Materials

Salbutamol sulphate (Ilsan Pharm. Comp. Turkey) and PLGA 85/15 (inherent viscosity 0.78 dL/g, Medisorb, Cincinnati, USA, MW: 153 000) were kindly supplied. Polyvinyl alcohol (PVA) (28/10 Wacker, Germany), gelatin (B type 250 bloom) and dichloromethane (Merck, Darmstadt) were also used in this study.

2.2. Factorial design of experiments

The experiments were performed with a 2^3 factorial design. In this investigation, the independent variables are drug loading (x_1), PVA concentration (x_2) and amount of gelatin (x_3). Particle size and entrapment ratio % are the dependent variables. The independent variables and their levels investigated in the preparation of SS microspheres are shown in Table 1a. The data were evaluated using the computer program, Statgraphics and response surface graphs were plotted.

2.3. Preparation of microspheres

PLGA microspheres containing SS were prepared by the modified solvent evaporation method using w/o/w technique. As shown in Table 1, a known amount of SS and gelatin were dissolved in 1 ml of distilled water to make the inner water phase and the solution gradually poured over 1 g of PLGA in 5 ml of dichloromethane (oil phase), to make a w/o emulsion. The emulsion obtained was poured into a 400-ml solution of PVA in water to make a w/o/w emulsion and continuously stirred for 2 h at 2500 rev./min until the dichloromethane evaporated, leaving solid microspheres. The microspheres collected by filtration were dried for 48 h. The contents of SS-PLGA microspheres are shown in Table 1b.

2.4. Determination of the drug content

Microspheres (30 mg) were dissolved 3 ml dichloromethane and then SS extracted in 15 ml isotonic phosphate buffer solution. Extracted SS were assayed by UV spectroscopy at 277 nm. The experiments were done in triplicate.

2.5. In vitro release studies

The microspheres were suspended in a test tube containing 10 ml of isotonic phosphate buffer solution, pH 7.4. The tubes were shaken in a shaker bath at 37°C. Samples were withdrawn at predetermined intervals. The SS content of each sample was assayed by UV spectroscopy (Perkin-Elmer, Hitachi 200) at 277 nm. The experiments were done in triplicate. The release data were evaluated kinetically using a computer program (DISSOL) (Ağabeyoğlu, 1984).

2.6. Determination of particle size

The particle sizes of microspheres were measured by infrared particle size apparatus (IPS). The measuring range of the analyser is from 10–1200 μm. The IPS particle analyser is a measurement instrument for quantity and size analysis of solid particles in air.

2.7. Surface morphology of microspheres

The surface morphology of microspheres was examined with a scanning electron microscopy (SEM) (ASID 10) at 70 kV. Microspheres were coated with gold to a thickness of 150 Å.

2.8. In vitro degradation of microspheres

In vitro degradation of microspheres (F8 formulation) was performed using an isotonic phosphate buffer solution of pH 7.4. Following addition of microsphere suspensions, the samples were shaken in a shaker bath at 37°C. After set periods (7, 14 and 21 days), degradation was assessed using SEM (ASID 10, 70 kV). Microspheres were coated with gold to a thickness of 150 Å.

2.9. Interaction of salbutamol sulphate with PLGA 85/15

The interaction between drug the PLGA 85/15 was investigated by differential scanning calorimetry (DSC) and fourier-transform infrared spectroscopy (FT-IR).

3. Results and discussion

3.1. In vitro release studies

The release profile of all microsphere formulations was characterized by a typical initial burst effect due to SS release from the surface of the microspheres. The rapid release was attributed to leaching of drug through pores and channels after water imbibition. Following the initial SS burst, a slow release is observed for formulations F6 and F8.

The effects of the independent variables (drug loading, PVA concentration and amount of gelatin) on in vitro release of SS from microspheres were investigated.

3.1.1. Effect of drug loading

There is no difference in vitro release profiles between F1 and F2 and between F3 and F4 formulations (Fig. 1).

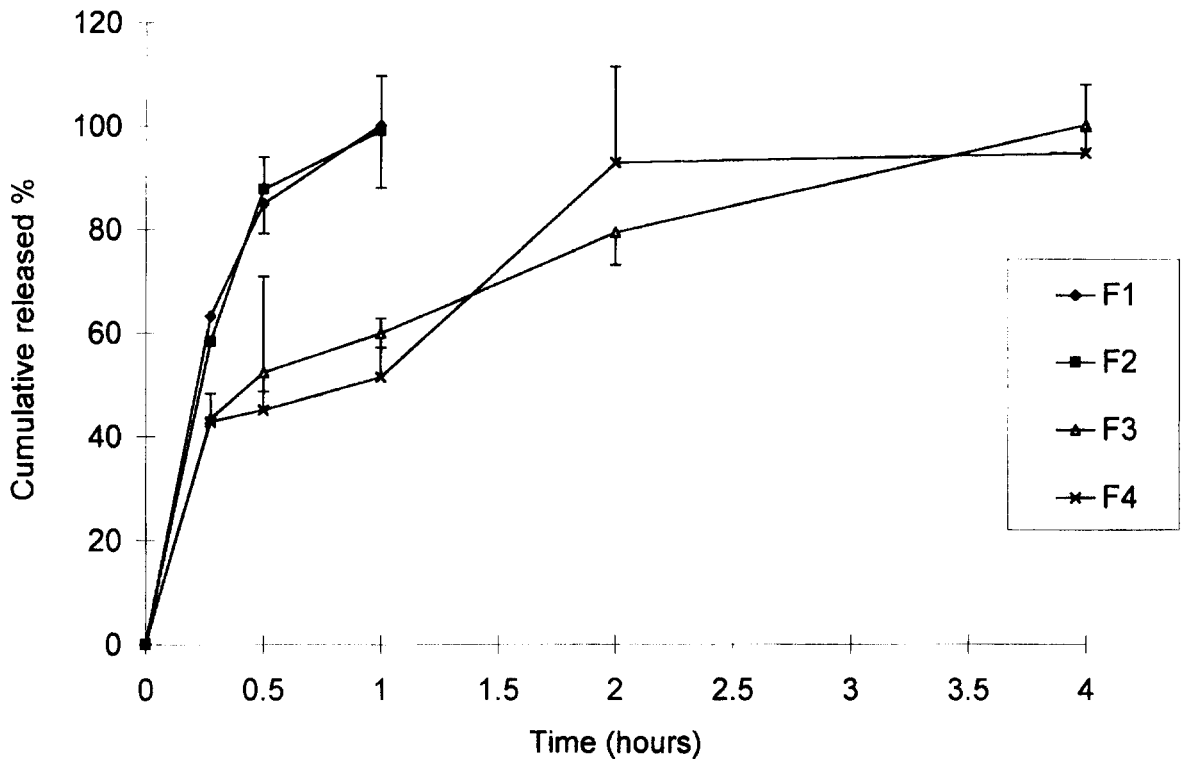


Fig. 1. The effect of drug loading and amount of gelatin on in vitro release of SS from microspheres (F1, F2, F3 and F4 formulations).

F6 and F8 which have high levels of drug loading released more slowly than F5 and F7 formulations, respectively (Fig. 2). The in vitro release of SS from formulation F8 was very slow (96 h), whereas that from formulation F7 was very rapid (1 h). As shown in Fig. 2, formulation F5 released 100% of drug for 1 h while formulation F6 released 100% of drug for 8 h. Consequently at 0.5% w/v PVA concentration, at a high level of drug loading, formulations F6 and F8 have slower in vitro release profiles.

3.1.2. Effect of amount of gelatin

Microspheres (F3, F4, F7 and F8) which were prepared with 0.1 g of gelatin had slower release profiles than those of the microspheres (F1, F2 and F8) in 0.05 g of gelatin, except F7 formulation. There is a significant difference in release profiles between F6 and F8. As shown in Fig. 2, extended release was obtained for 96 h with microspheres from F8 which contain 0.1 g of gelatin. However formulation F6 which contains 0.05 g of gelatin released drug for 8 h.

3.1.3. Effect of PVA concentration

Although F1 and F5 have different particle sizes, they have similar release patterns (Figs. 1 and 2). Microspheres of F5 have a smaller particle sizes than microspheres of F1. This can be attributed to the increase in the concentration of PVA in microspheres of F5. There is a difference between formulations F3 and F7 in vitro release profiles. F3 released fourfold longer than F7 because it has a smaller particle size than F3 (Table 2). This can be attributed to the increase in the concentration of PVA in microspheres of F7.

Consequently, formulations of F6 and F8 released more slowly than all other formulations. As shown in Fig. 2, extended release was obtained for 8 h with F6 and for 96 h with F8.

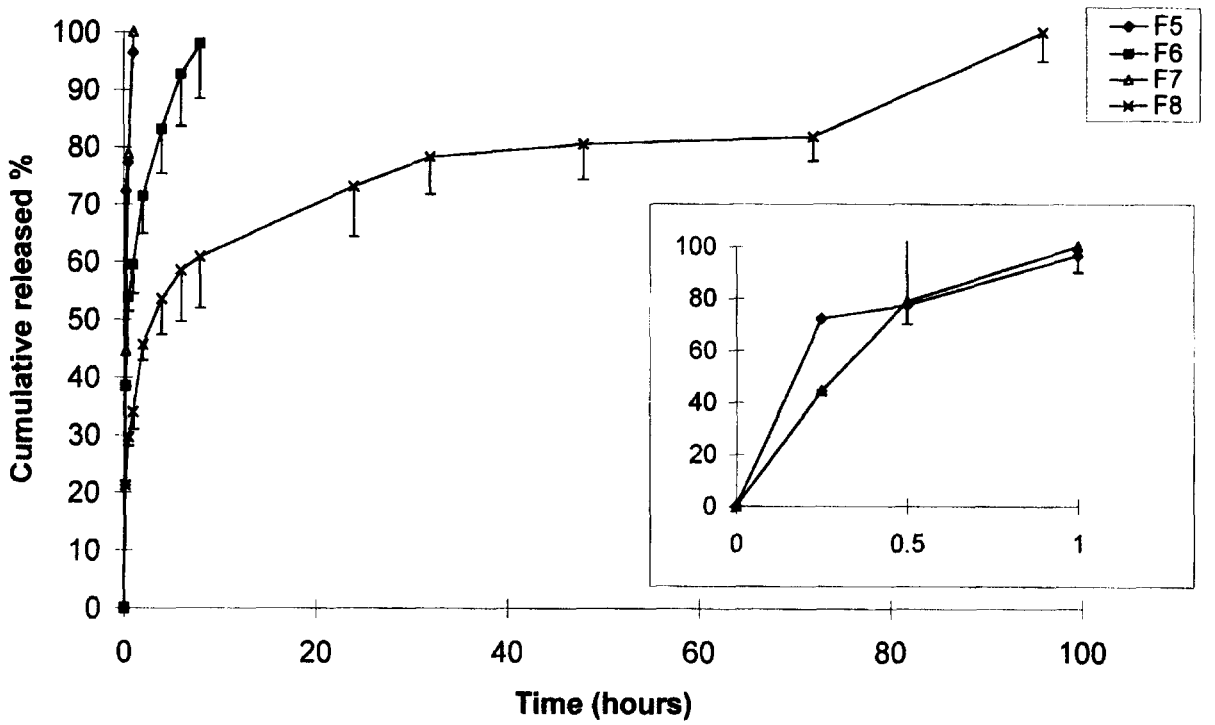


Fig. 2. The effect of drug loading and amount of gelatin on in vitro release of SS from microspheres (F5, F6, F7 and F8 formulations).

3.2. Assessment of in vitro release kinetics

According to the computer program, DISSOL, all formulations except F6, F7, F2 and F5 show $Q\sqrt{t}$ kinetics and on the other hand, Higuchi's model.

It was found that the microsphere formulations might conform to Higuchi's matrix dissolution model (Higuchi, 1963). After reviewing the equation for Higuchi's model, it was found that it was originally derived for a planar matrix system, but not for the spherical formulation. On the other hand, the equation for drug release from a spherical matrix was proposed by Baker and Lonsdale (Jun and Lai, 1983; Seki et al., 1990).

$$3/2[1-(1-F)^{2/3}]-F = kt$$

where F is the fraction of drug released, and k is the constant rate of the release.

In this study, we investigated whether drug release conformed to the Baker and Lonsdale model. For this purpose, $3/2[1-(1-F)^{2/3}]-F$ was plotted as a function of time. Interestingly, a linear relationship was found for each model. Table 3 shows the determination coefficients, SWSD (sum of the weighted squared deviation) and SSD (sum of the squared deviation) values. Although a slightly smaller determination coefficient was found with the dissolution model of Baker and Lonsdale, we might say that the release

Table 2
Characteristics of SS-PLGA microspheres

Formulation	Entrapment ratio (%) ± S.D.	Particle size (μm ± S.D.)
F1	16.3 ± 0.1	225 ± 2.54
F2	2.06 ± 0.34	250 ± 5.13
F3	10.2 ± 0.8	201 ± 3.93
F4	4.17 ± 0.17	192 ± 3.95
F5	12.2 ± 0.1	66 ± 2.92
F6	6.21 ± 0.32	117 ± 3.04
F7	22.0 ± 0.3	126 ± 2.95
F8	10.3 ± 0.9	164 ± 3.20

Table 3
Determination coefficients, SSD and SWSD values for Higuchi's model and Baker and Lonsdale model

Parameter	Higuchi's model	Baker and Lonsdale model
Determination coefficients (r^2)	0.895	0.889
SSD	677	2.97×10^{-2}
SWSD	18.5	0.249

profile of microspheres from F8 conform to the dissolution model of Baker and Lonsdale because it was found that SWSD and SSD values are significantly low when obtained by the dissolution model of Baker and Lonsdale (Table 3). Since SWSD and SSD values are more reliable than the determination coefficients we may say that microspheres of F8 show the dissolution model of Baker and Lonsdale.

3.3. Evaluation of factorial design results

The effect of the independent factors on particle size and entrapment ratio % was evaluated with analysis of variance (ANOVA). Multiple regression is applied to dependent variables against independent variables. The polynomial equations for entrapment ratio % and particle size are given in Eqs. (1) and (2), respectively.

$$y = 53.9225 - 280.225\,x_1 - 110.325\,x_2 - 396.1\,x_3 + 553.25\,x_1x_2 + 2346\,x_1x_3 + 1491\,x_2x_3 - 6960\,x_1x_2x_3 \quad (1)$$

$$y = 398.25 + 127.5\,x_1 - 132.5\,x_2 - 4015\,x_3 - 1375\,x_1x_2 + 4150\,x_1x_3 + 3150\,x_2x_3 + 10500\,x_1x_2x_3 \quad (2)$$

The results of analysis of variance indicated that the three independent variables were effective on the drug entrapment ratio. The most effective factor was drug loading. The response-surface graphs based on ANOVA results are shown in Fig. 3a–c. Fig. 3c shows the three-dimensional response-surface diagrams for entrapment ratio % with variations of drug loading (g) and concentration of PVA (%). This figure indicates that, as the drug loading and the concentration of PVA decrease, or as the concentration of PVA increases, entrapment ratio % values increase. However, as the drug loading increases, entrapment ratio % decreases. Actually, formulations F1, F3, F5, and F7 which have low level drug loadings had a high entrapment ratio %. Also Ogawa et al. (1988) found that the entrapment ratio decreased as the loading of leuprolide acetate increased.

As can be seen from Fig. 3b, an increase in the amount of gelatin and a decrease in the concentration of PVA increase entrapment ratio %. In contrast, formulations which have 0.1% w/v concentration of PVA (F1, F2, F3, F4) have a lower entrapment ratio % than formulations which have 0.5% w/v concentration of PVA (F6, F7, F8) except formulations F1 and F5 (Table 2). This is because at high concentrations of PVA, the increased viscosity of the aqueous phase prevents the diffusion of SS into the aqueous phase.

As shown in Fig. 3b and Fig. 3c, an increase in the amount of gelatin increases entrapment ratio % values. Ogawa et al. (1988) also obtained similar results. They found that the entrapment ratios into microspheres without gelatin were from 1.9 to 6.7%, whereas those into microspheres with gelatin were from 32.0 to 70.7%. Formulations which contain 0.1 g of gelatin (F3, F4, F7, F8) had a higher entrapment ratio % than formulations which contain 0.05 g gelatin (F1, F2, F5, F6) except formulations F1 and F3. This is because at high levels of gelatin, the increased viscosity of the inner phase of primer emulsion prevents the diffusion SS into the aqueous phase.

In addition the amount of gelatin could change the particle size of the microspheres. The results of particle size of microspheres are also reported in our previous studies (Erden and Çelebi, 1994). In fact, the results of analysis of variance of particle size of microspheres indicated that the most effective factor was the amount of gelatin. Fig. 3b shows the three-dimensional response-surface diagrams for particle size with variations of drug loading (g) and concentration of PVA (w/v %). As shown in Fig. 3b, an increase in drug loading increases the particle size of microspheres. However, concentration of PVA was

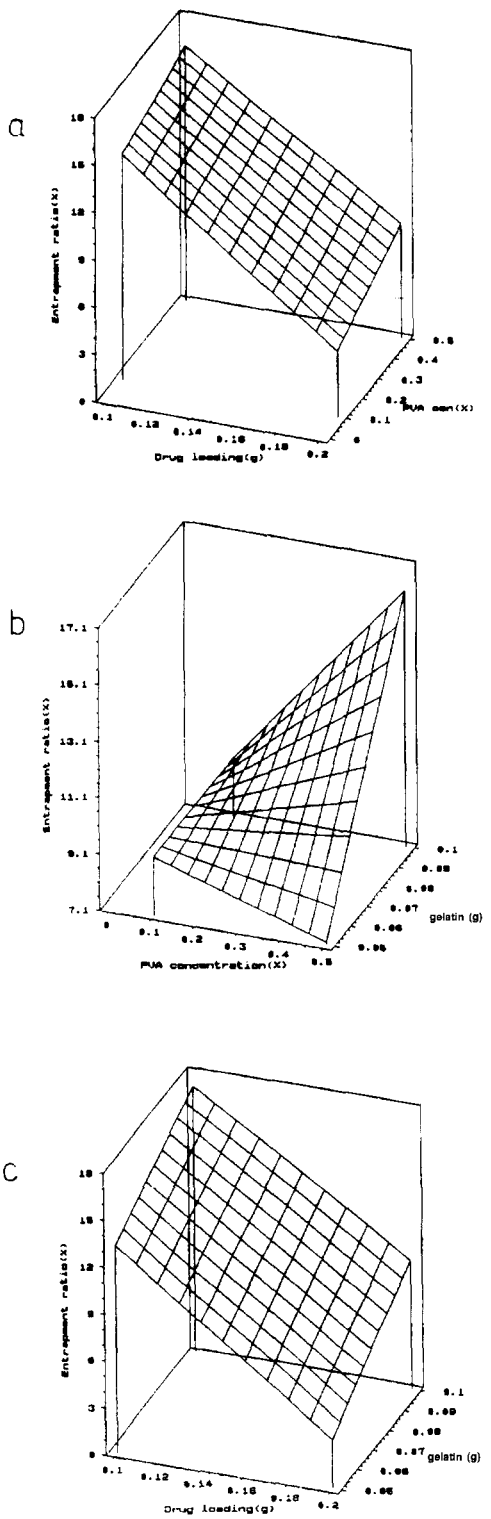


Fig. 3. Response surface graphic showing the effects on entrapment ratio; (a) drug loading and concentration of PVA (% w/v); (b) concentration of PVA and amount of gelatin; (c) drug loading and amount of gelatin.

not correlated with particle size. In contrast, Benita et al. (1984) found that microsphere size increased as the PVA concentration increased due to an increase in viscosity of the aqueous phase.

Fig. 4b shows that an increase in amount of gelatin decreases the particle size of microspheres, and a decrease in the amount of gelatin increases the particle size of microspheres.

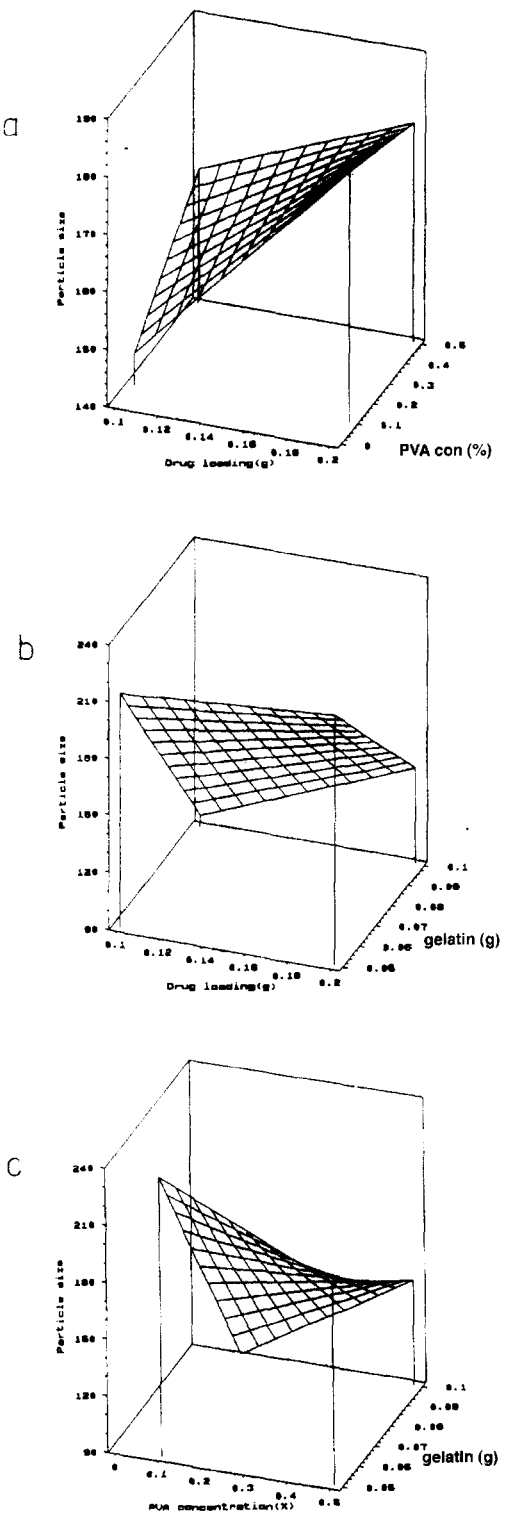
Fig. 4c indicates that the smallest particle size results from a low level PVA concentration and a high level of gelatin. In addition, we found an interaction between concentration of PVA and amount of gelatin according to analysis of variance. In formulations which contain 0.1% w/v concentration of PVA (F1, F2, F3 and F4), the particle size decreased with increasing amount of gelatin. This could be because gelatin has a surfactant effect. In contrast, it was expected that in formulations which contain 0.5% concentration of PVA (F5, F6, F7 and F8), particle size increased although amount of gelatin also increased.

3.4. Evaluation of in vitro degradation of microspheres

In vitro degradation of microspheres was investigated using SEM for F8 microspheres. SEM observation revealed that, before release, the surface of microspheres was round and very smooth (Fig. 5a). It was found that the pore size increased with time and then degraded, becoming empty holes (Fig. 5b–d).

3.5. Evaluation of interaction between PLGA 85/15 and SS

The physical state of drug in the microspheres has been examined (Benoit and Puisieux, 1986; Hartas et al., 1992). In addition, interaction has been observed between drug and polymer (Agarwal et al., 1993). DSC analysis of the polymer, SS and drug loaded microspheres was performed in order to characterize the physical state of the polymer



and drug before and after microsphere preparation. The interaction between PLGA 85/15 and SS was investigated for F8 formulation which shows the best release profile. Fig. 6 shows DSC curves for the microspheres. An endothermic peak was observed for SS at 202°C, corresponding to its melting phase transition, and the T_g of PLGA 85/15 could be detected at 60°C. An endothermic peak for SS at 202°C, was not detectable in thermograms of drug loaded microspheres (Fig. 6). This event suggests that SS was in a non-crystalline state in the microspheres. The T_g of PLGA 85/15 was detectable in thermograms of drug loaded microspheres. This finding suggest that SS exists in the amorphous state in the microspheres.

Recently the interaction between biodegradable polymers and especially drugs containing amine groups and other nucleophil groups has been reported (Maulding et al., 1986; Maulding, 1987). SS also contains amine groups so we investigated the interaction between SS and PLGA 85/15 by FT-IR (Fig. 7). The peak due to the carbonyl bond of SS was observed at 1620 cm^{-1} for crystalline SS. This peak was found to shift to 1650 cm^{-1} for the F8 microspheres. In addition the peak due to C-N for SS at 1510 cm^{-1} was found to shift to 1550 cm^{-1} for the F8 microspheres.

Consequently, it was observed that SS-PLGA microspheres were amorphous according to thermal analysis results. Microspheres of amorphous polymer matrices exhibited a much slower drug release than those of crystalline polymer matrices (Izumikawa et al., 1991; Aso et al., 1992).

4. Conclusions

From the present results, the following conclusions can be drawn:

1. After a typical initial burst effect, extended release (96 h) for salbutamol sulphate was achieved with biodegradable microspheres with PLGA 85/15 by an emulsion solvent evaporation w/o/w technique.

Fig. 4. Response surface graphic showing the effects on particle size; (a) drug loading and concentration of PVA (% w/v); (b) drug loading and amount of gelatin; (c) concentration of PVA and amount of gelatin.

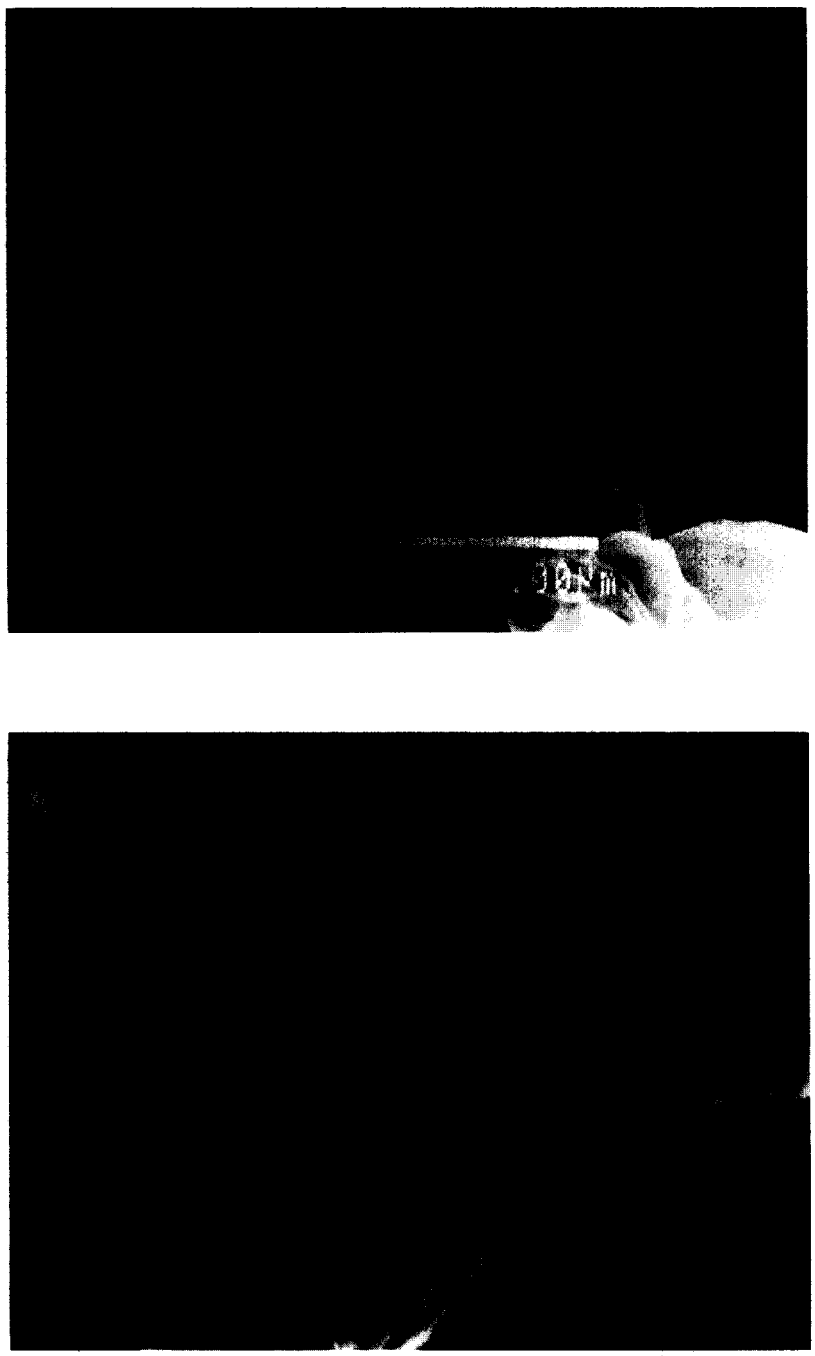


Fig. 5. Scanning electron micrographs of F8 formulation, (a) before release; (b) 7th day of in vitro degradation; (c) 14th day of in vitro degradation; (d) 21st day of in vitro degradation.

2. The release profiles for the F8 formulation fitted the dissolution model proposed by Baker and Lonsdale.

3. The results of analysis of variance indicated that the three independent variables (drug loading, amount of gelatin and concentration of

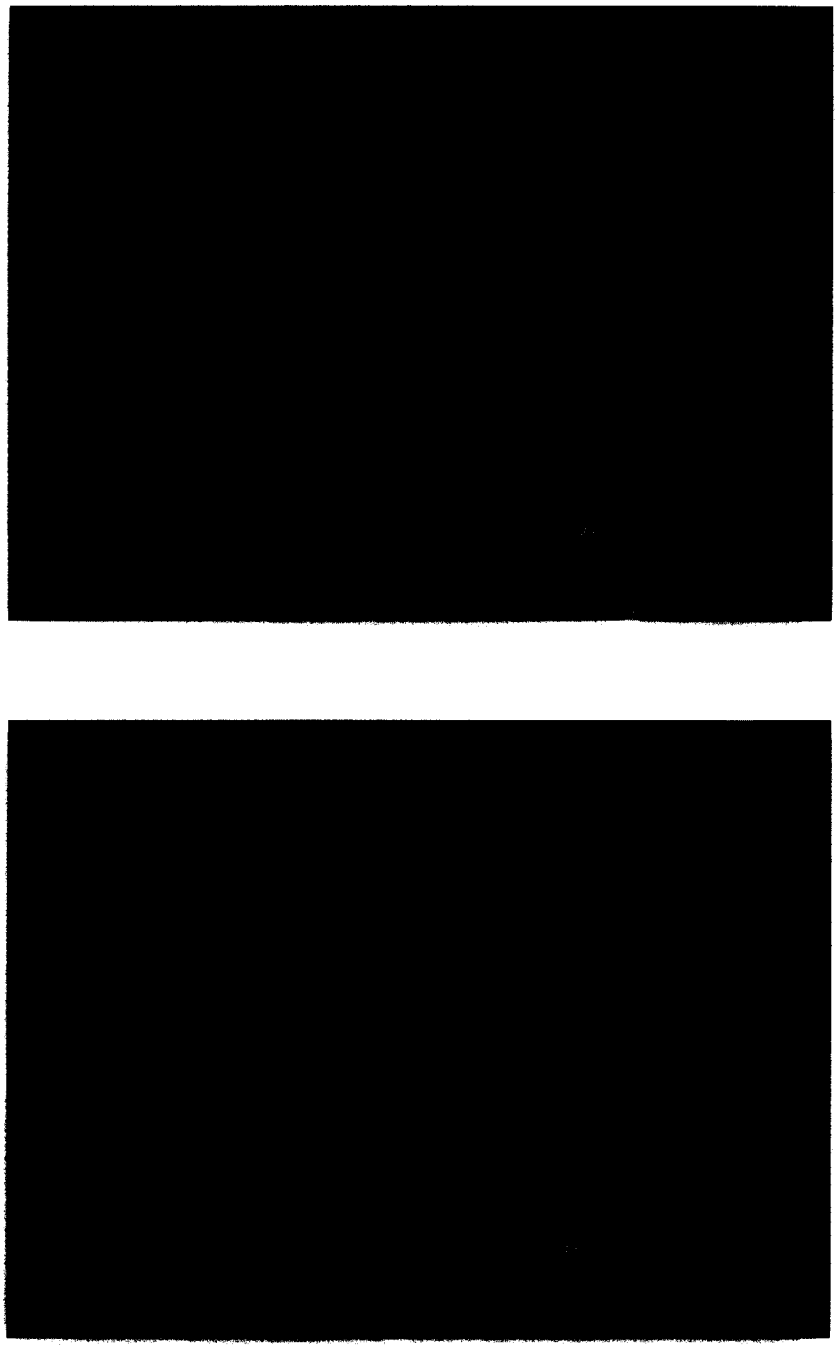


Fig. 5 (C,D).

PVA) were effective on drug entrapment ratio. The most effective factor was drug loading.

4. The results of analysis of variance of particle size of microspheres indicated that the most effective factor was amount of gelatin.

5. It was found that SS exists in an amorphous state in the microspheres.

6. In vitro degradation of microspheres (F8

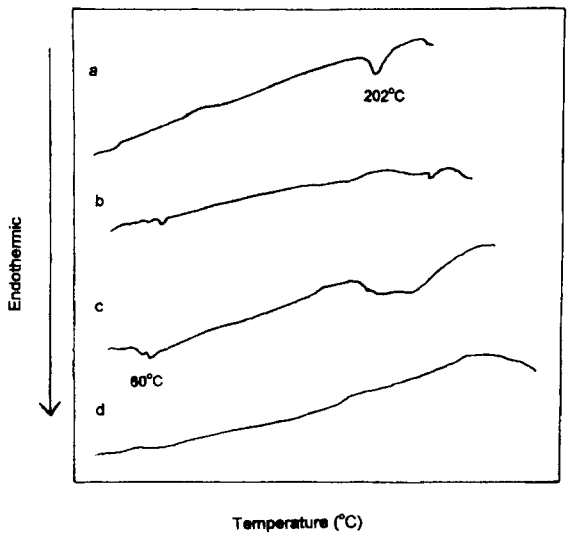


Fig. 6. DSC thermograms of (a) SS; (b) SS-PLGA (F8) microspheres; (c) PLGA 85/15; (d) blank microspheres.

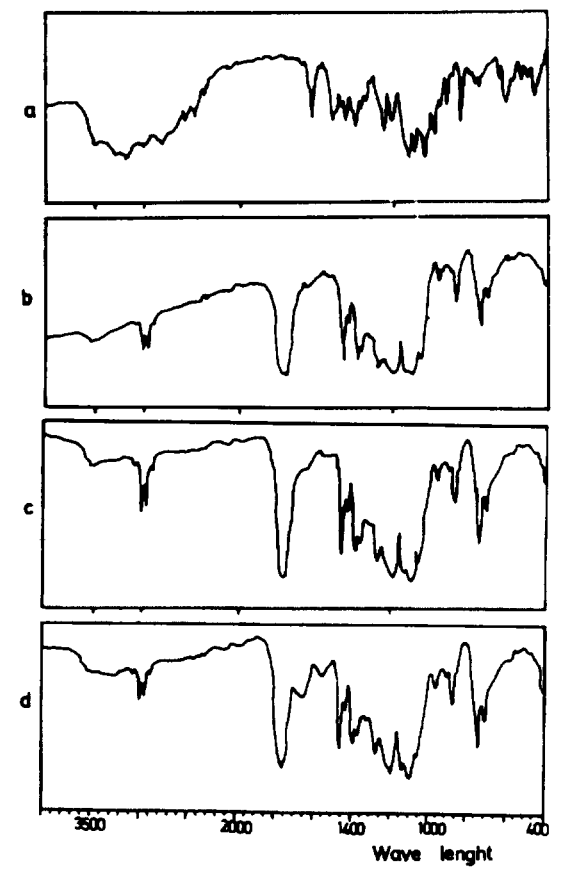


Fig. 7. FT-IR spectra of (a) SS; (b) 85/15 PLGA; (c) blank microspheres; (d) PLGA 85/15 (F8) microspheres.

formulation) was investigated using SEM. It was found that pore size increased with time and then degraded, becoming empty holes.

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