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Factors influencing release of salbutamol sulphate from poly(lactide-co-glycolide) microspheres prepared by water-in-oil-in-water emulsion technique

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Abstract

Biodegradable microspheres containing salbutamol sulphate were prepared using water-in-oil-in-water (w/o/w) emulsion technique. Biodegradable polymers of two different molecular weights were used: PLGA 50/50 and PLGA 75/25. In the preparation of the formulations, we used 2^3 factorial design based on three independent variables which are drug loading, amount of gelatin and concentration of PVA. The dependent variables are particle size of the microspheres and entrapment ratio %. The in-vitro release of PLGA microspheres was studied in buffer solution of pH 7.4 at 37°C. A biphasic release behavior of salbutamol sulphate from microspheres was observed. Initially, a burst effect and then slow release was observed. It was found that the biodegradable microspheres of salbutamol sulphate were prepared with PLGA 75/25 by w/o/w emulsion technique obtained extended release obtained for 8 h. PVA concentration was effective on the particle size of microspheres prepared with PLGA 75/25 ($p < 0.05$).

Keywords: Salbutamol sulphate; Poly(lactide-co-glycolide); Factorial design; w/o/w emulsion technique

1. Introduction

Polylactic acid (PLA) and copolylactic acid (PLGA) are biocompatible, biodegradable materials that are widely used in many controlled-release systems for pharmaceutical agents such as narcotic antagonists, local anesthetics and steroid hormones (Ogawa et al., 1988a). However, most of the drugs used in these controlled release systems were water insoluble.

For drugs with high water solubility, emulsification into an aqueous phase is generally unsuccessful in producing entrapment of drug, as the drug will rapidly partition from the more hydrophobic polymer solution phase into the aqueous surroundings (Watts et al., 1990). However, the problem can be avoided by the use of a water-in-oil-in-water (w/o/w) technique. For example, the highly water soluble luteinzing hormone-releasing hormone analog leuprolide acetate has been successfully encapsulated using this method (Ogawa et al., 1988b).

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The main objective of this study was to encapsulate water soluble drug within PLGA microspheres by using PLGA 50/50 and PLGA 75/25. In order to encapsulate a highly water soluble drug such as salbutamol sulphate (SS) was chosen as a model drug.

SS is an potent B-2 adrenoceptor stimulant used for the treatment of bronchial asthma (Perreault et al., 1992). As it has a short elimination half life of 3–8 hours (Raghuvanshi et al., 1992), frequent dosing is necessary to maintain therapeutic plasma levels. Therefore, there is a need for controlled-release formulation of SS.

In the present study, the preparation and release characteristics in-vitro PLGA microspheres containing SS were investigated. The experiments are performed with 2^3 factorial design. The use of factorial design minimizes the number of experiments and permits us to know the influence of these factors and of their interactions (Armstrong and James, 1990; Vercammen and De Neve, 1980).

2. Materials and methods

2.1. Materials

Salbutamol sulphate (Ilsan Pharm. Comp. Turkey), PLGA 50/50 (inherent viscosity 0.74 dL / g, MW: 83.000), PLGA 75/25 (inherent viscosity 0.76 dL/g, MW: 151.000), (Medisorb, Cincinnati, USA), polyvinyl alcohol (PVA) (28/10 Wacker, Germany), gelatin (B type 250 bloom), and dichlorometan (Merck, Darmstadt) were used in this study.

2.2. Factorial design of experiments

The experiments are performed with 2^3 factorial design. In this investigation, the independent variables are drug loading (a), PVA concentration (b) and amount of gelatin (c). 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 1.

2.3. Preparation of microspheres

PLGA microspheres containing SS were prepared by modified solvent evaporation method using w/o/w technique as described previously (Ogawa et al., 1988b). As shown in Table 2, certain amount of SS and gelatin were dissolved in 1 ml of distilled water to make the inner water phase and the solution gradually poured 1 g of PLGA in 5 ml of dichloromethan (oil phase) to make a w/o emulsion. The emulsion obtained was poured into 400 ml solution of PVA in water to make a w/o/w emulsion and continuously stirred for 2 h at 2500 rpm until the dichlorometan evaporated leaving solid microspheres. The microspheres collected by filtration were dried for 48 h. The preparation of microspheres is illustrated Scheme 1. The dried microspheres were sieved through a standard sieve and stored in a desicator at room temparature.

2.4. Determination of drug content

Drug content of microspheres was determined by UV spectrophotometry. A weighed amount (about 30 mg) of drug-loaded PLGA microspheres were dissolved in dichlorometan and extracted in isotonic phosphate buffer solution (pH 7.4) and then analyzed at 277nm. The experiments were done in triplicate.

2.5. Particle size measurements

The particle size of microspheres were carried out by infrared particle size apparatus (IPS). The measuring range of the analyser is 10–1200 μm.

Table 1
The independent variables and their levels

Independent variables	Low level	High level
Drug loading (a)	0.1 g	0.2 g
PVA concentration (b)	0.1% w/v	0.5% w/v
Amount of gelatin (c)	0.05 g	0.1 g

Table 2
The contents of SS-PLGA microspheres

Formulations of code	Polymer type	Drug loading (g)	Concentration of PVA (%)	Amount of gelatine (g)
F1	PLGA 50/50	0.1	0.1	0.05
F2	PLGA 50/50	0.2	0.1	0.05
F3	PLGA 50/50	0.1	0.1	0.1
F4	PLGA 50/50	0.2	0.1	0.1
F5	PLGA 50/50	0.1	0.5	0.05
F6	PLGA 50/50	0.2	0.5	0.05
F7	PLGA 50/50	0.1	0.5	0.1
F8	PLGA 50/50	0.2	0.5	0.1
F9	PLGA 75/25	0.1	0.1	0.05
F10	PLGA 75/25	0.2	0.1	0.05
F11	PLGA 75/25	0.1	0.1	0.1
F12	PLGA 75/25	0.2	0.1	0.1
F13	PLGA 75/25	0.1	0.5	0.05
F14	PLGA 75/25	0.2	0.5	0.05
F15	PLGA 75/25	0.1	0.5	0.1
F16	PLGA 75/25	0.2	0.5	0.1

IPS particle analyser is a measurement instrument for quantity and size analyser of the solid substances particles feeded in the air.

2.6. In-vitro release of SS from PLGA microspheres

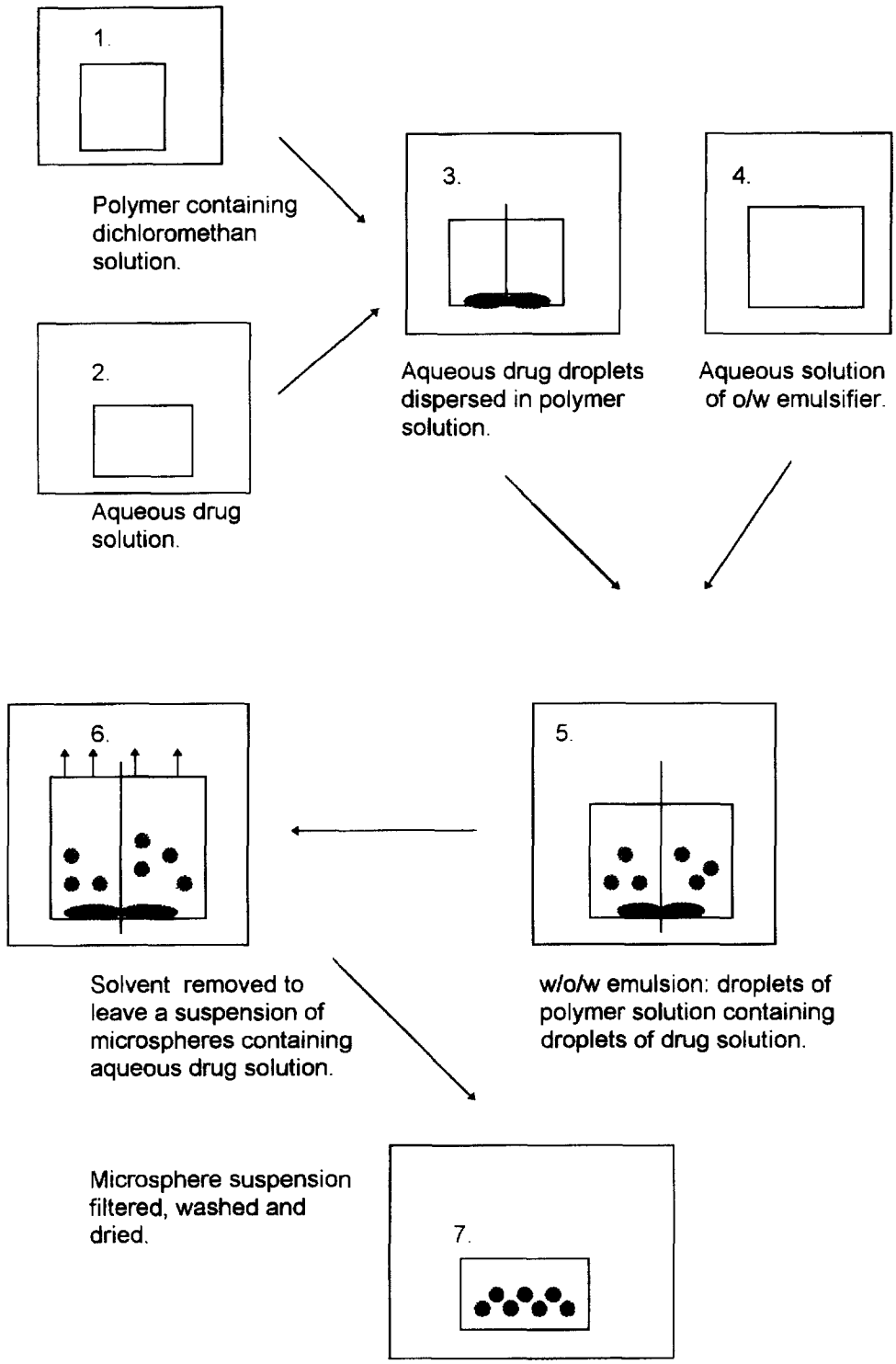
A weighed amount of microspheres was placed into a test tube containing 10 ml of isotonic phosphate buffer solution (pH 7.4). The tubes were shaken in a horizontal shaker bath at 37°C. Samples were withdrawn at predetermined intervals. The SS content of each sample was assayed by UV spectroscopy (Perkin-Elmer,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

3. Results and discussion

3.1. Preparation of SS-PLGA microspheres by w/o/w emulsion technique

The solvent evaporation method is a popular microencapsulation technique for the preparation of drug-containing matrix particles from water insoluble polymers. Various modified techniques based on aqueous or nonaqueous external phases allow the encapsulation of lipophilic as well as hydrophilic drugs. Modified solvent evaporation methods based on an external aqueous phase were developed for the encapsulation of highly water soluble drugs. The techniques include a multiple emulsion method whereby an aqueous drug solution is emulsified into the organic polymer solution to form a primary water-in-oil emulsion which is then emulsified into the external aqueous phase. The organic polymer solution acts as a barrier between the internal aqueous and external aqueous phase, thereby allowing the entrapment of water soluble drugs (Bodmeier, 1994).

As SS is water soluble drug, while PLGA is soluble only in the organic phase, we have searched for methods of matrix preparation that will combine both organic and water phases. Thus, the w/o/w emulsion method for preparation of PLGA microspheres was adopted. The resulting microspheres were sphericals, with diameter $92 \pm 4.44 - 207 \pm 4.30$ μm and $125 \pm 2.53 - 196 \pm 3.44$ μm for PLGA 50/50 and PLGA 75/25, respectively. Although we obtained large



Scheme 1. Schematic diagram of microsphere formation by a w/o/w emulsion technique (Watts et al., 1990)

Table 3
Characteristics of SS-PLGA microspheres

Code	Yield (%)	Entrapment ratio (%) ± S.D.	Particle size μm ± S.D.
F1	37.7	19.8 ± 0.5	111 ± 2.96
F2	35.5	6.84 ± 0.73	181 ± 3.73
F3	44.5	36.0 ± 0.2	92 ± 4.44
F4	48.5	9.20 ± 0.24	175 ± 4.20
F5	42.9	41.0 ± 0.1	207 ± 4.30
F6	46.0	21.6 ± 0.2	130 ± 2.62
F7	47.4	89.0 ± 0.2	186 ± 3.40
F8	45.2	8.40 ± 0.10	189 ± 3.94
F9	10.5	8.05 ± 0.12	125 ± 2.53
F10	14.5	1.63 ± 0.27	161 ± 3.25
F11	9.27	14.0 ± 0.4	196 ± 3.44
F12	21.5	7.62 ± 0.17	170 ± 3.04
F13	25.5	2.80 ± 0.04	146 ± 3.11
F14	41.0	9.75 ± 0.07	139 ± 3.61
F15	21.2	36.4 ± 0.3	149 ± 2.34
F16	17.4	5.72 ± 0.55	175 ± 3.24

particle size of microspheres, they are in an acceptable range (Table 3). Polymer molecular weight, which also has a direct relationship with the solution viscosity, has been found to affect microsphere size. High molecular weight polymers (in our study PLGA 75/25) produce larger microspheres. Although smaller size microspheres were expected from low molecular weight PLGA 50/50, there was an insignificant difference between PLGA 50/50 microspheres and PLGA 75/25 microspheres. It could be attributed to the aggregation observed during the preparation of microspheres.

3.2. *In-vitro release of SS from PLGA microspheres*

We observed a biphasic release behavior of SS from microspheres in our study (Figs. 1–4). Initially, a burst effect was observed due to release of drug located near the microsphere surface. Then there was a period of slow release. The burst effect could be explained by rapid drug diffusion through dissolution media-filled pores and channels. The slower release phase was attributed to the partion of the drug which had to be released from the microspheres by diffusion through the polymer.

Various factors can affect the drug release from the microspheres. They include the particle size and morphology, the drug loading and solubility of the drug, the physical state of the drug in the polymer, and type of polymer or additive. The release characteristics of PLGA microspheres are influenced by degradation rate of the polymer. By varying the molecular weight or the ratio of glycolic acid in the polymer, the degradation rate can be varied. PLGA 50/50 has low molecular weight

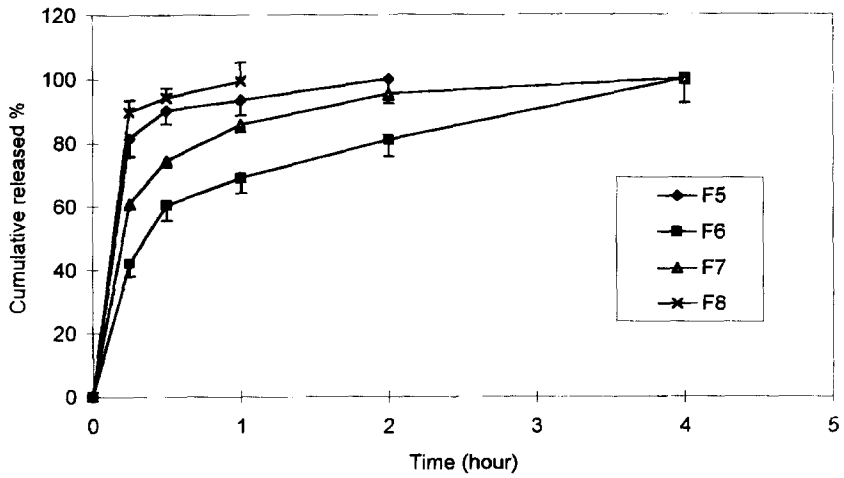


Fig. 1. The effect of drug loading and amount of gelatin on in-vitro release of SS from PLGA 50/50 microspheres (F5, F6, F7 and F8 formulations).

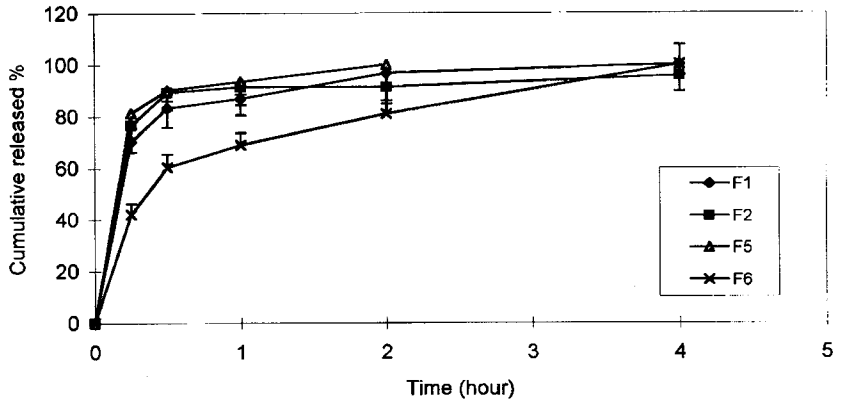


Fig. 2. The effect of concentration of PVA on in-vitro release of SS from PLGA 50/50 microspheres (F1, F2, F5 and F6 formulations).

than PLGA 75/25. Microspheres prepared from high molecular weight polymers PLGA 75/25 released SS slower in dissolution media investigated than PLGA 50/50 which has smaller molecular weight. This may be due to the larger particle size of the PLGA 75/25 microspheres which consequently have a lower surface area than PLGA 50/50 microspheres. A lower molecular weight results is a higher rate of degradation, since low molecular weight polyesters are accompanied by elevated amounts of carboxylic end of hydrophilicity and, therefore, an increased degradation rate (Hutchinson and Furr, 1990; Sturesson et al., 1993).

In this study, the effects of the independent variables (drug loading, PVA concentration and amount of gelatin) were investigated. Entrapment ratio %, production yield % and particle size of microspheres are listed in Table 3. There was no significant effect of particle size on in-vitro release of SS from PLGA microspheres which might be attributed to the sieving through a standard sieve. Also, Chang et al. (1986) obtained similar results. They reported that the swelling property of the microspheres and the agglomerate nature of the sieve fractions might complicate the drug release kinetics and obscure the particle-size effect on the dissolution rate.

3.3. Assesment of release kinetics

The fit of the release data was tested with the following kinetics model (a) zero order kinetics, (b) first order kinetics, (c) spherical erosion, and (d) square-root of time equation (Higuchi equation). The release data were evaluated by a computer programme (DISSOL) (Ağabeyoğlu, 1984). According to the determination coefficients and SWSD (sum of the weighted squared deviation), for the different release kinetics models for SS-PLGA microspheres: F1, F4, F5, F7, F12, F15 and F16 show spherical erosion; F2, F3, F11 and F14 show first orderkinetics; F6, F8, F9 and F10 $Q\sqrt{t}$ and F13 show zero order kinetics. The Higuchi equation suggests that drug release is controlled by the diffusion of drug through the pores and not through the swollen polymer.

3.4. Evaluation of microspheres prepared with PLGA 50/50

3.4.1. Effect of drug loading

Drug loading also affects the release mechanism and release rate of microspheres. It was found that with formulations (F1, F2, F3 and F4) which contain 0.1% w/v concentration of PVA, drug loading was not effective on the drug release. Formulations which contain 0.5% w/v concentra-

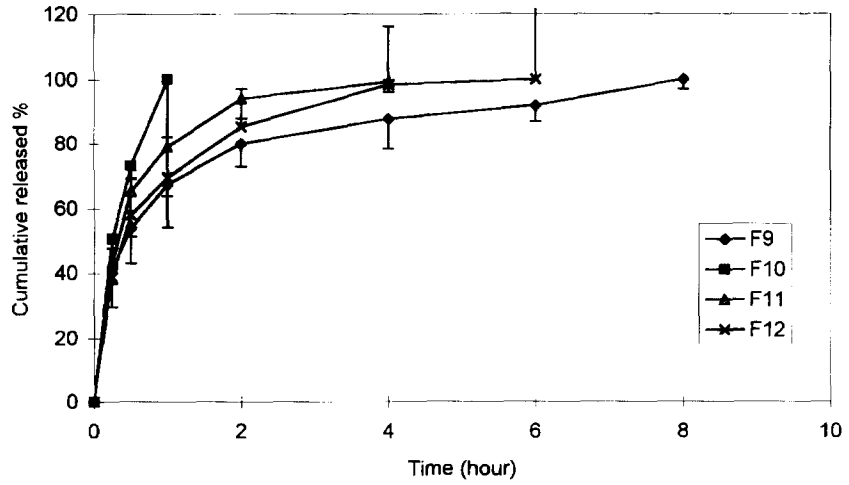


Fig. 3. The effect of drug loading and amount of gelatin on in-vitro release of SS from PLGA 75/25 microspheres (F9, F10, F11 and F12 formulations).

tion of PVA, that at high level drug loading, F6 formulation was obtained slower release than F5 formulation (Fig. 1). In contrast, F7 formulation which contains 0.5% w/v concentration of PVA, at low level drug loading, slower release was obtained than with F8 formulation which contain 0.5% w/v concentration of PVA at high level drug loading (Fig. 1). This could be explained when the drug loading is low and the drug, loaded as small (solid) crystals, is finally dispersed in the polymer matrix with no contact between the crystals. Solvent and drug molecules must diffuse through the polymer matrix to allow drug release which consequently leads to slow release.

3.4.2. Effect of amount of gelatin

The amount of gelatin was effective on the SS release all formulations. Formulations which contain 0.1% w/v concentration of PVA and 0.05 g gelatin (F1 and F2) released slower than those which contain 0.1 g of gelatin (F3 and F4). Also, the formulation which contain 0.5% w/v concentration of PVA and 0.05 g gelatin (F6) released slower than F8 which contain 0.1 g gelatin. In contrast, F7 formulation which contains 0.1 g gelatin released slower than F5 which contains 0.05 g gelatin (Fig. 1). The drug release increased with increasing amount of gelatin, except for the F7 formulation.

3.4.3. Effect of concentration of PVA

Although F2 and F6 released for 4 h, F2 shows faster burst effect than F6, whereas F2 shows larger particle size than F6 (Table 3 and Fig. 2).

3.5. Evaluation of microspheres prepared with PLGA 75/ 25

3.5.1. Effect of drug loading

There is a significant difference between F9 (at low level drug loading) and F10 (at high level drug loading) in-vitro release profiles. F9 released for 8 h while F10 released for 1 h (Fig. 3). Microspheres of F11 which comprised 10% SS released for 4 h whereas microspheres of F12 which comprised 20% SS released for 6 h (Fig. 3). There is no difference between F13 (10% drug loading) and F14 (20% drug loading) of in-vitro release profile; both released for 1 h. There is a significant difference between F15 (at low level loading) and F16 (at high level loading) of in-vitro release profiles.

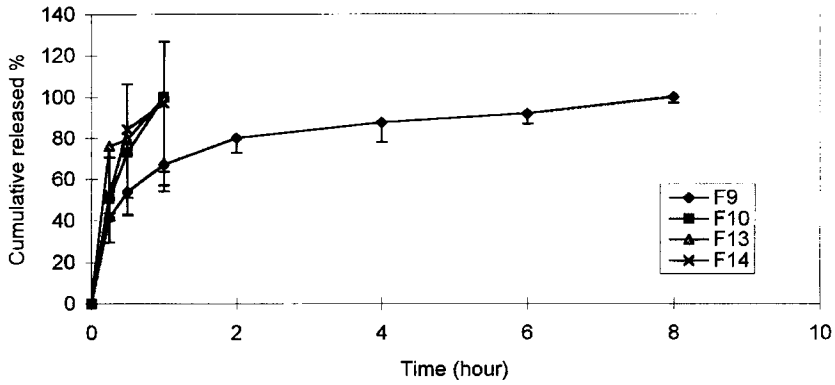


Fig. 4. The effect of concentration of PVA on in-vitro release of SS from PLGA75/25 microspheres (F9, F10, F13 and F14 formulations).

3.5.2. Effect of amount of gelatin

It was found that amount of gelatin was effective on the release profile. Formulations which contain 0.1% w/v concentration of PVA and 10% SS (F9 and F11) obtained slower release with F9 which contains 0.05 g of gelatin (Fig. 3). Formulations which contain 0.1% w/v concentration of PVA and 20% SS (F10 and F12) obtained slower release with F12 which contains 0.1 g of gelatin (Fig. 3).

3.5.3. Effect of concentration of PVA

Microspheres (F9, F10, F11 and F12) (Fig. 3) which included 0.1% w/v concentration of PVA released slower than microspheres (F13, F14, F15 and F16) (Fig. 4) which included 0.5% w/v concentration of PVA. In contrast, F11, which comprised 0.1% wv concentration of PVA, released faster than F15 which comprised 0.5% wv concentration of PVA.

3.6. Evaluation of factorial design experiment

We investigated effects of three independent variables on the dependent variables (entrapment ratio and particle size) utilized by factorial design.

3.6.1. Evaluation of microspheres prepared with PLGA 50/50

It was found that drug loading was effective on the entrapment ratio % ($P < 0.1$) (Table 4). At high drug level, microspheres (F2, F4, F6 and F8) decreased entrapment ratio % according to at low drug level microspheres (F1, F3, F5 and F7, respectively) (Table 3). We found that the amount of the gelatin was the most important independent variable on the particle size of microspheres prepared with PLGA 50/50 ($P < 0.1$) (Table 5). Particle size decreased as the amount of gelatin increased, except F6 and F8 microspheres (Table 3).

3.6.2. Evaluation of microspheres prepared with PLGA 75/25

The effects of independent variables on the entrapment ratio % of microspheres prepared with PLGA 75/25 could not be determined. It was found that concentration of PVA was effective on the particle size of microspheres prepared with PLGA 75/25 ($p < 0.05$) (Table 6).

The biodegradable microspheres of SS were prepared with PLGA 75/25 by w/o/w emulsion technique and obtained extended release for

Table 4
The results of analysis of variance for entrapment ratio (%) of F1–F8 formulations

Source of variation	Entrapment ratio (%)	Degrees of freedom	Mean square	F ratio
(1)	19.8	1	—	—
a	6.84	1	2441	74*
b	36.0	1	356	10.8
ab	9.20	1	704	21.3
c	41.0	1	972	29.5
ac	21.6	1	454	13.8
bc	89.0	1	33	—
abc	8.40	1	280	8.48

*$P < 0.1$.

hours. This formulation contains 0.1 g SS, 0.05 g gelatin and 0.1% w/v PVA concentration.

In conclusion, there was no significant effect of particle size on the drug in-vitro release profiles from the microspheres. The fact that particle size has no effect on the in-vitro dissolution might be explained by the sieved from a standart sieve. It was found that drug loading was effective on the entrapment ratio % and that the amount of gelatin was the most important independent vari-

Table 5
The results of analysis of variance for particle size of F1–F8 formulations

Source of variation	Particle size (μm)	Degrees of freedom	Mean square	F ratio
(1)	111	1	—	—
a	181	1	780	37
b	92.0	1	21.1	—
ab	175	1	1081	51.2*
c	207	1	2926	139*
ac	130	1	6441	305**
bc	186	1	496	23.5
abc	189	1	561	26.6

*$P < 0.1$.
**$P < 0.05$.

Table 6
The results of analysis of variance for particle size of F9–F16 formulations

Source of variation	Particle size (μm)	Degrees of freedom	Mean square	F ratio
(1)	125	1	—	—
a	161	1	105	10.4
b	196	1	1770	175*
ab	170	1	105	10.4
c	146	1	231	22.8
ac	139	1	10.1	—
bc	149	1	210	20.8
abc	175	1	1128	111

*$P < 0.05$.

able on the particle size of microspheres prepared with 50/50 PLGA ($P < 0.1$).

It was found that the concentration of PVA affected on the particle size of microspheres prepared with PLGA 75/25 ($P < 0.05$). The effects of independent variables on the entrapment ratio % of microspheres prepared with PLGA 75/25 could not be determined.

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