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Effect of ethanol as a processing co-solvent on the PLGA microsphere characteristics

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ABSTRACT

The effect of ethanol, as an organic phase co-solvent, on the characteristics of dexamethasone containing poly(lactide-co-glycolide) (PLGA) microspheres prepared *via* the emulsion–solvent evaporation/extraction process has been evaluated. This study allowed determination of the maximum amount of ethanol that can be used to prepare radio-labeled dexamethasone microspheres without affecting their performance. Different ethanol co-solvent concentrations (0–43.75% (v/v)) were investigated and changes in the physicochemical properties and *in vitro* release profiles were determined. A significant decrease in particle size and drug loading was observed at 12.50% and 25% (v/v) ethanol concentrations, respectively. Morphological evaluation revealed the presence of drug crystals on the microsphere surfaces prepared using ethanol co-solvent concentrations of 18.75% (v/v) and higher. A high burst release was observed with these formulations due to the presence of drug crystals on microsphere surfaces. Various competing factors such as interfacial tension between methylene chloride and water, solubility of drug in the organic phase and the viscosity of the polymer phase contributed to the changes observed in microspheres characteristics at different ethanol co-solvent concentrations. It was determined that radio-labeled dexamethasone solution (in ethanol) can be used at concentrations up to 12.5% (v/v) of organic phase without any significant change in microsphere characteristics.

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1. Introduction

Poly(lactide-co-glycolide) (PLGA) microspheres are under intensive investigation as drug delivery systems, especially for controlled and targeted parenteral drug delivery. There are currently seven FDA approved PLGA microsphere products and many others are in the pipeline (Burgess and Hickey, 2005; Chaubal, 2002; D'Souza and DeLuca, 2006). Drug release profiles ranging from days to months can be achieved with PLGA microspheres by modifying various parameters such as polymer molecular weight, copolymer ratio, particle size and preparation conditions (Igartua et al., 1997; Li et al., 1995; Mao et al., 2007; Soppimath and Aminabhavi, 2002; Yang et al., 2001; Zolnik et al., 2006). The initial phase of drug release from PLGA microspheres (also known as burst phase) occurs by diffusion of surface and pore associated drug. Once polymer degradation starts, both polymer erosion and drug diffusion contribute to drug release (Faisant et al., 2002; Yeo and Park, 2004).

PLGA microspheres are usually prepared using emulsion–solvent evaporation/extraction method (Freitas et al., 2005; Yeo and Park, 2004). The polymer is dissolved in an organic solvent and the drug is either dissolved or dispersed in this organic phase, which is then emulsified in an aqueous solution. The organic solvent evaporates or partitions into the external aqueous phase, increasing the concentration of the polymer and hence the viscosity of the emulsified droplets, resulting in phase separation. Spherical polymeric particles entrapping the drug are formed when the entire organic phase either partitions into the external aqueous phase or evaporates. Various process parameters such as solvent–co-solvent system, ratio of continuous and dispersed phase, polymer, drug and surfactant concentrations can influence the characteristics of microspheres prepared by the solvent evaporation/extraction method (Jeyanthi et al., 1997; Mao et al., 2008; Yeo and Park, 2004).

Co-solvents have been reported to affect the rate of the organic phase partitioning into the external aqueous phase and thus influence the physicochemical properties and release kinetics of PLGA microspheres (Al-Maaieh and Flanagan, 2001; Bodmeier and McGinity, 1988; Graves et al., 2005; Mandal, 1999). Water miscible co-solvents have been used to increase the encapsulation efficiency of hydrophilic drugs when microspheres are prepared by the o/w emulsion–solvent evaporation/extraction method. A water

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miscible co-solvent in the polymer phase causes faster phase separation by increasing the rate of internal phase partitioning into the external phase. Faster precipitation of polymer droplet results in higher drug loading in this case because less time is available for the hydrophilic drug to partition out into the external aqueous phase. Al-Maaieh and Flanagan (2001) used ethanol as a co-solvent to solubilize the drug quinidine sulfate in polymer/organic phase and hence achieve a homogeneous drug distribution in the microspheres. As a result of homogeneous drug distribution in microspheres, the initial burst release was minimized and a near zero order drug release profile obtained. In contrast, a biphasic release profile (high initial burst release) was obtained when drug was dispersed in the polymer phase (in the absence of co-solvent) (Al-Maaieh and Flanagan, 2001). Therefore, the release kinetics of microspheres can be changed using appropriate co-solvents.

According to the literature, the effect of co-solvents has only been studied for hydrophilic drugs in an attempt to increase their drug loading. However, in the present study, we have evaluated the effect of ethanol as an organic phase co-solvent on the characteristics of PLGA microspheres encapsulating the hydrophobic drug dexamethasone.

Dexamethasone encapsulating PLGA microspheres have been used to control inflammation and fibrosis in response to subcutaneous implants such as biosensors (Bhardwaj et al., 2007; Hickey et al., 2002a,b; Patil et al., 2007). *In vivo* assessment of drug pharmacokinetics is often performed using radio-labeled drugs in formulations. Therefore, incorporation of radioactive dexamethasone in the microspheres would be useful in understanding the pharmacokinetics of dexamethasone microspheres *in vivo*. Preparation of radio-labeled dexamethasone microspheres requires the addition of radio-labeled dexamethasone (1 mCi/ml solution in ethanol, Perkin Elmer) solution along with the non-radio-labeled drug (Patil et al., 2007). As discussed before, the presence of the co-solvent 'ethanol' in addition to the polymer (PLGA) solvent 'methylene chloride' and the non-solvent 'water' can affect the physicochemical characteristics of microspheres and could result in a different release pattern. This might lead to a difference in *in vivo* performance of formulations with and without radio-labeled drugs.

Therefore, this study was carried out with the objectives of: (1) understanding the effect of ethanol as organic phase co-solvent on microspheres properties, and therefore exploring the possibility of designing microspheres with specific release kinetics using different amounts of co-solvent (ethanol); and (2) determining the optimum amount of organic solvents in radio-labeled drug solutions (such as ethanol in radio-labeled dexamethasone solution) that can be used to prepare PLGA microspheres without affecting their *in vitro*/*in vivo* performance. This will be useful for future work with PLGA microspheres encapsulating radio-labeled dexamethasone and the knowledge gained for dexamethasone containing PLGA microspheres will help in designing fabrication processes for other PLGA microsphere systems.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA) polymer, PLGA Resomer® RG503H 50:50 (MW: 25 kDa) was a gift from Boehringer–Ingelheim. Methylene chloride, tetrahydrofuran (optima grade), ethanol (ACS grade, 200 proof) and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Pittsburg, PA). Dexamethasone and poly(vinyl alcohol) (PVA) (MW: 30–70 kDa) were obtained from Sigma–Aldrich (St. Louis, MO). Nanopure™ quality water (Barnstead, Dubuque, IA) was used for all studies.

Table 1
Composition of organic phase used for microsphere preparation ($n = 3$).

% (v/v) ethanol	Ethanol (ml)	Methylene chloride (ml)
0	0	4.00
3.13	0.125	3.875
6.25	0.25	3.75
12.50	0.50	3.50
18.75	0.75	3.25
25.00	1.00	3.00
31.25	1.25	2.75
37.50	1.50	2.50
43.75	1.75	2.25

Polymer: PLGA 50:50 (average molecular weight 25 kDa) 25% (w/v); solvent: methylene chloride; co-solvent: ethanol; drug: dexamethasone 10% (w/w) of the polymer.

2.2. Methods

2.2.1. Preparation of microspheres

Dexamethasone encapsulating PLGA microspheres were prepared by oil-in-water (o/w) emulsion–solvent extraction/evaporation technique (Zolnik et al., 2006). 1 g of PLGA was dissolved in 4 ml methylene chloride (or methylene chloride–ethanol combinations as indicated in Table 1). 100 mg dexamethasone was dispersed in the PLGA solution using a homogenizer (PowerGen 700 D, Fisher Scientific) at 10,000 rpm for 30 s. This organic phase was then added slowly to 20 ml of a 1% (w/v) aqueous poly(vinyl alcohol) (PVA) and homogenized at 10,000 rpm for 2 min. This emulsion was added to 250 ml of a 0.1% (w/v) aqueous PVA solution and stirred at 600 rpm under vacuum for 4 h at 25 °C. The resulting microspheres were filtered (Durapore® membrane filter, 0.45 μm, Fisher Scientific), washed three times with de-ionized water and vacuum dried for 24 h. All the formulations were prepared in triplicate.

2.2.2. Characterization of microspheres

2.2.2.1. High performance liquid chromatography (HPLC). The concentration of dexamethasone was determined using a Perkin Elmer HPLC system (series 200) with a UV absorbance detector (Perkin Elmer) set at 242 nm. The mobile phase was acetonitrile:water:phosphoric acid (30:70:0.5% (v/v/v)). A Zorbax® C18 (4.6 mm × 15 cm) analytical column was used with the flow rate set at 1 ml/min. The chromatographs were analyzed using PeakSimple™ Chromatography System (SRI Instruments, Torrace, CA). This method is a stability indicating HPLC assay.

2.2.2.2. Dexamethasone solubility. Dexamethasone solubility in ethanol, methylene chloride and de-ionized water was determined. Excess drug was added to 5 ml of solvent in 20 ml vials. The vials were shaken at 200 rpm and 25 °C using a C76 water bath shaker (New Brunswick Scientific) for 72 h. 1 ml of sample was withdrawn at 24, 48 and 72 h using syringe filters (Millex® PVDF 0.45 μm). The filtered samples were diluted with the respective solvents and analyzed using the HPLC method described in Section 2.2.2.1. All measurements were conducted in triplicate and the results are reported as the mean ± SD (standard deviation).

2.2.2.3. Drug loading. 5 mg of dexamethasone encapsulating PLGA microspheres were dissolved in 10 ml tetrahydrofuran (THF). The solution was filtered (Millex® HV, PVDF 0.45 μm syringe filter) and the dexamethasone concentration was determined *via* HPLC as described before using an injection volume of 2.5 μl. Drug loading was determined as: percent drug loading = (weight of drug entrapped/weight of microspheres used) × 100. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.4. Particle size. An AccuSizer 780A autodiluter particle sizing system was used to determine the mean particle diameter. About 50 mg of microspheres was dispersed in 2 ml of 0.1% (w/v) PVA solution. 200 μl of the dispersion was used for particle size analysis. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.5. Interfacial tension. Interfacial tension between de-ionized water and methylene chloride was determined at various ethanol concentrations used for preparation of different microsphere formulations as shown in Table 1. A Kruss K12 tensiometer, which is based on DuNoy ring principle, was used to determine the interfacial tension. 40 ml of water and 40 ml of methylene chloride/ethanol solution were used for interfacial tension measurement at 25 °C. Ethanol was added to the methylene chloride (maintaining a total volume of 40 ml) in the same concentration as used for the preparation of the various microsphere formulations. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.6. Glass transition temperature. The glass transition temperature of the prepared microspheres was analyzed using a TA instrument Q1000 differential scanning calorimeter (DSC). Samples were heated to 100 °C and cooled to −40 °C at a rate of 10 °C/min. The first cycle of the thermograms was used to determine the glass transition temperature (T_g) of the microspheres. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.7. Morphology. The morphology of the microspheres was characterized using scanning electron microscopy. Samples were mounted on carbon taped aluminum stubs and gold coated in a sputter coater for 1 min at 6 mA. The samples were analyzed using a scanning electron microscope (DSM982 Gemini, Carl Zeiss, Inc.) at an accelerating voltage of 2.0 kV.

2.2.2.8. In vitro release. A modified USP apparatus 4 (Sotax CE7 smart with CY 7 piston pump, Sotax, Horsham, PA) was used for *in vitro* release testing (Zolnik et al., 2005, 2006). Flow through cells (12 mm diameter) packed with glass beads (1 mm diameter) were used in a closed system to achieve laminar flow of media and prevent microsphere agglomeration. The system was temperature controlled at 37 °C. Approximately 40 mg of microspheres were dispersed in the flow through cells (fitted with fiberglass filters 0.7 μm) and 250 ml of 0.1 M phosphate buffer saline with 0.1% sodium azide was circulated. 1 ml samples were withdrawn and replaced with fresh media at suitable time intervals. The samples were analyzed using HPLC as described before using an injection volume of 20 μl. 125 ml of the media was replaced when the drug concentration reached 10% (w/v) of dexamethasone saturation solubility in water (*i.e.* 80 μg/ml) to maintain sink conditions. The drug degradation and media replacement during the release study was taken into account in the calculation of cumulative percentage release.

2.2.2.9. Statistical analysis. Statistical analysis to evaluate significant differences between different microsphere formulations was performed using JMPIN® software. Results were analyzed using one-way analysis of variance (ANOVA). Any significant difference was further analyzed by Tukey–Kramer HSD (honestly significantly different) post hoc test, a multiple range test to determine significant difference between more than two groups. The level of significance was accepted at $p < 0.05$.

3. Results

3.1. Solubility of dexamethasone

Dexamethasone solubility was determined in the solvents used for microsphere preparation *i.e.* ethanol, methylene chloride and de-ionized water. The saturation solubility of dexamethasone was 12.00 ± 0.13, 0.30 ± 0.03 and 0.076 ± 0.001 mg/ml in ethanol, methylene chloride and de-ionized water, respectively.

3.2. Drug loading

The mean drug loading of the microspheres prepared without ethanol (as co-solvent) was 6.93% (w/w). There was no significant change in the drug loading of the microspheres up to 18.75% (v/v) ethanol concentration as shown in Table 2. At 25% (v/v) ethanol concentration, there was a significant decrease in drug loading to 4.8% (w/w) ($p < 0.05$). Increase in ethanol concentration to 31.25%, 37.50% and 43.75% did not result in any further change in the drug loading.

3.3. Particle size

The number average mean diameter was determined for all microsphere formulations. The mean particle size of the microspheres prepared with only methylene chloride as the organic phase was approximately 7 μm (Table 2). The particle size did not change significantly with the addition of ethanol as a co-solvent up to a concentration of 6.25% (v/v) organic phase. A significant decrease in mean particle diameter of the microspheres was observed at 12.5% (v/v) and higher ethanol concentrations in organic phase (*e.g.* particle size of 6.24 ± 0.27 μm at 12.5% (v/v) ethanol concentration) ($p < 0.05$). The particle size stabilized at ethanol co-solvent concentrations above 25% (v/v).

3.4. Interfacial tension

The interfacial tension between methylene chloride and water was 26.68 ± 0.11 mN/m at 25 °C. The interfacial tension decreased with increase in ethanol concentration (ethanol % (v/v) of methy-

Table 2
Drug loading, mean particle size and glass transition temperature of microspheres prepared with different concentrations of ethanol as an organic phase co-solvent ($n = 3 \pm$ SD).

Microsphere formulations (% (v/v) ethanol)	Drug loading (% (w/w))	Mean particle size (μm)	Glass transition temperature (°C)
0.0	6.93 ± 0.27	7.00 ± 0.49	42.27 ± 1.76
3.13	6.79 ± 0.22	6.94 ± 0.21	40.59 ± 1.78
6.25	6.87 ± 0.34	6.94 ± 0.21	40.54 ± 1.50
12.50	6.88 ± 0.38	6.24 ± 0.27	43.53 ± 0.56
18.75	6.16 ± 0.56	5.82 ± 0.31	42.40 ± 1.11
25.00	4.83 ± 0.67	5.31 ± 0.26	42.55 ± 1.44
31.25	4.51 ± 0.54	5.30 ± 0.25	42.81 ± 0.20
37.50	5.44 ± 0.22	5.18 ± 0.20	43.37 ± 0.37
43.75	5.38 ± 0.19	5.35 ± 0.11	42.50 ± 0.34

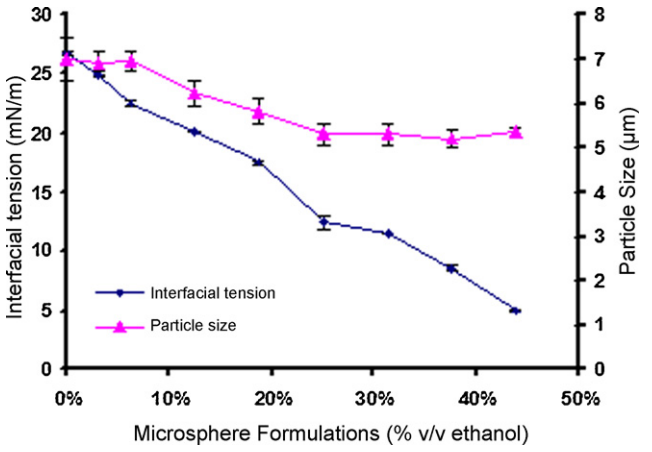

Fig. 1. Microsphere particle size and interfacial tension between water and methylene chloride at different ethanol concentrations ($n = 3 \pm SD$) at 25 °C.

lene chloride) as shown in Fig. 1. The interfacial tension decreased by approximately 2–2.5 mN/m with every 6.25% (v/v) increase in ethanol concentration up to 18.75% (v/v). A sharp decrease (*i.e.* 5.1 mN/m) in the interfacial tension was observed when the ethanol concentration was increased from 18.75% to 25% (v/v). With further increase in ethanol concentration, there was a 1–3 mN/m decrease in interfacial tension. A similar trend was observed in the microsphere particle size with decrease in particle size occurring between 12.5% and 25% (v/v) ethanol concentration. However, the particle size remained relatively constant as the ethanol concentration was increased beyond 25% (v/v).

3.5. Glass transition temperature

The glass transition temperature of microspheres prepared without ethanol was approximately 42 °C. There was no significant change in the glass transition temperature of the microspheres with the addition of ethanol as an organic phase co-solvent at any of the concentrations studied (Table 2).

3.6. Morphology

Fig. 2 shows SEM micrographs of microspheres prepared with different concentrations of ethanol as the organic phase co-solvent. Morphological analysis revealed that microspheres prepared without ethanol and with 3.13%, 6.25% and 12.5% (v/v) ethanol concentrations were smooth and spherical. Fig. 2a and b shows SEM pictures of the microspheres prepared without ethanol (only methylene chloride as organic phase) and with 12.5% (v/v) ethanol concentration.

Some surface imperfections were visible when the ethanol concentration was increased to 18.75% (v/v) (Fig. 2c). As the concentration of ethanol was further increased to 25%, 31.25% and 37.5% (v/v), the surface imperfection of the microspheres became more prominent as shown in Fig. 2d, f and g. These surface imperfections were speculated to be drug (dexamethasone) crystals. This was confirmed by preparing blank microspheres (without drug) with 25% (v/v) ethanol co-solvent. These microspheres were smooth and spherical (Fig. 2e). Fig. 2i is a magnification (10,000×) of the microsphere surface showing structures similar to dexamethasone crystals (Fig. 2j). Dexamethasone crystals were prepared by vacuum evaporation of dexamethasone solution in ethanol (Fig. 2j). At 43.75% (v/v) ethanol concentration irregular particles with rough surface were formed (Fig. 2h).

3.7. In vitro release

Fig. 3 shows the *in vitro* release profiles of microsphere formulations prepared without ethanol and with 12.5%, 18.75%, 25% and 43.75% (v/v) ethanol concentrations. Formulations prepared without ethanol and with 12.5% and 18.75% (v/v) ethanol exhibited typical triphasic release profiles. The release profiles of microspheres prepared with 12.5% (v/v) ethanol were similar to those of microspheres prepared without ethanol. The burst release of these formulations was approximately 40%. However, at 18.75% (v/v) ethanol concentration, the burst release increased from 40% to approximately 60% and the lag phase was prolonged. At 25% (v/v) ethanol concentration there was a huge burst release of approximately 75%. A lag phase was not observed for this formulation and complete release was achieved within 6 days. The release profiles of microsphere formulations prepared with 31.25% and 37.5% (v/v) ethanol were similar to that of the 25% (v/v) formulation and hence only the 25% (v/v) release profile is shown in Fig. 3. The release profile at 43.75% (v/v) ethanol was intermediate between formulations containing 25% (v/v) and 12.5% (v/v) ethanol. At 43.75% (v/v) ethanol concentration, the burst release was approximately 60% which was similar to the 18.75% (v/v) formulation. While the microsphere formulations prepared with 18.75% and 43.75% (v/v) ethanol showed similar burst release phases, there was a difference in their release patterns. At 18.75% (v/v) concentration, a typical triphasic release pattern (*i.e.* burst release, lag phase and secondary zero order release) was observed, whereas in the case of the formulation prepared with 43.75% (v/v) ethanol, the burst release was followed by zero order release kinetics.

4. Discussion

The characteristics of microspheres prepared using the solvent evaporation/extraction method depend to a great extent on the interaction between the drug, polymer and solvent system. The presence of co-solvents can alter this interaction and influence microspheres properties. Therefore, in order to determine the effect of ethanol as a co-solvent, microsphere formulations were prepared using different combinations of methylene chloride (solvent) and ethanol (co-solvent) as the organic phase (Table 1). The formulations were characterized for any change in their physicochemical properties and *in vitro* performance.

4.1. Drug loading

The decrease in microsphere drug loading with increase in ethanol concentration to 25% (v/v) is speculated to be related to change in dexamethasone solubility in the organic phase. Dexamethasone solubility is approximately 40 times higher in ethanol compared to methylene chloride. The increased dexamethasone solubility at 25% (v/v) ethanol co-solvent together with the water miscibility of ethanol is likely to have resulted in a diffusional loss of drug to the external aqueous phase (during the emulsification and/or solvent evaporation/extraction) giving a low drug loading. Increase in ethanol concentration above 25% (v/v) ethanol did not further change drug loading and this is considered to be due to increased polymer phase viscosity. At ethanol concentrations higher than 31.25% (v/v), rapid extraction of organic phase solvents into the external aqueous phase occurs resulting in increased viscosity of the polymer/organic phase. This in turn restrains the movement of drug within the polymeric microsphere droplets, therefore counteracting the diffusional loss of drug to the external aqueous phase. Various studies have reported that faster particle solidification rates increase microsphere drug loading which is in agreement with the results reported here at high ethanol concen-

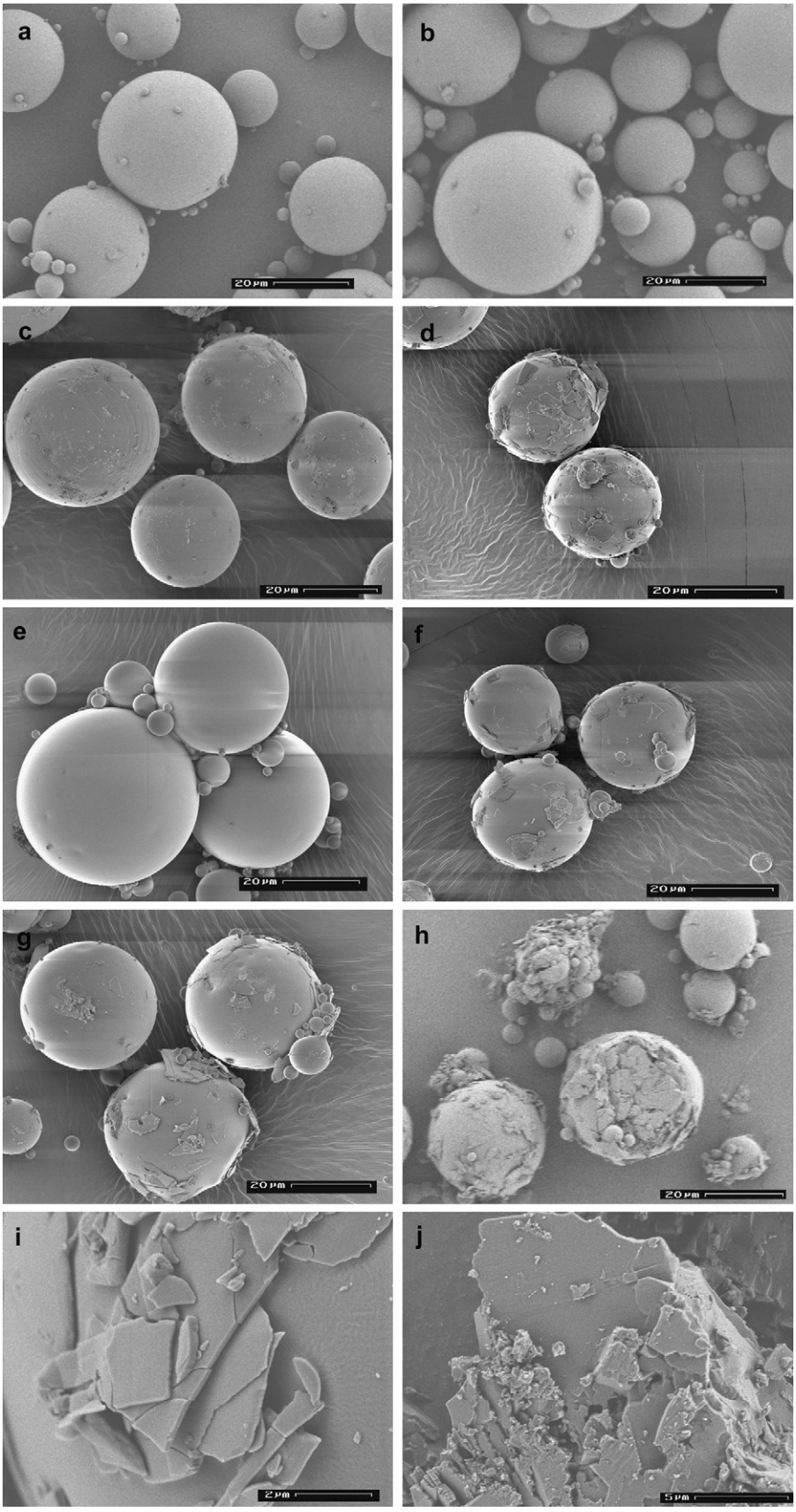

Fig. 2. SEM pictures of microspheres prepared with (a) only methylene chloride, (b) 12.5% (v/v) ethanol, (c) 18.75% (v/v) ethanol, (d) 25% (v/v) ethanol, (e) 25% (v/v) ethanol (blank), (f) 31.25% (v/v) ethanol, (g) 37.5% (v/v) ethanol and (h) 43.75% (v/v) ethanol. (i) Dexamethasone crystals on microsphere surface and (j) dexamethasone crystals prepared by vacuum evaporation of dexamethasone solution in ethanol. (a–h) is 1000×, (i) is 10,000× and (j) is 5000×.

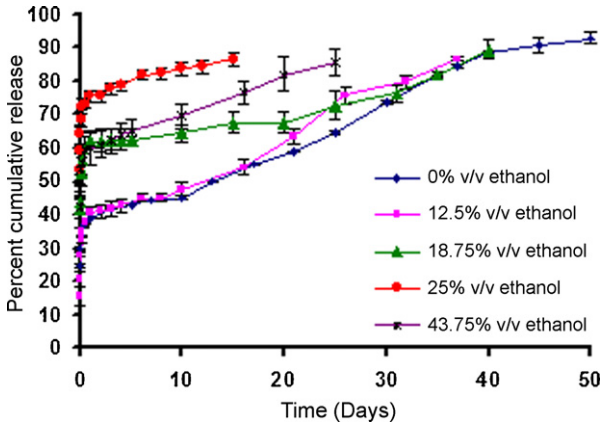

Fig. 3. *In vitro* release profiles of microsphere formulations prepared with different ethanol concentrations ($n = 3 \pm$ SD) using USP apparatus 4 in closed loop system maintained at 37 °C and 20 ml/min flow rate.

trations (≥31.25% (v/v)) (Li et al., 1995; Rosca et al., 2004; Yeo and Park, 2004). Similarly, attempts have been made to increase drug loading of hydrophilic drugs using water miscible organic phase co-solvents to increase partitioning of organic phase into the external aqueous phase resulting in faster phase separation and entrapment of the drug inside the viscous polymeric matrix (Al-Maaieh and Flanagan, 2001; Bodmeier and McGinity, 1988).

4.2. Particle size

The decrease in microsphere particle size at ethanol co-solvent concentrations of 12.50%, 18.75% and 25% (v/v) correlates with the decrease in interfacial tension between the organic (methylene chloride) and aqueous phases (Fig. 1). Reduction in interfacial tension favors formation of smaller droplets during emulsification and hence smaller sized microspheres. This observation is supported by the work of Ito et al. (2009) who formulated microspheres using various solvents (*e.g.* methylene chloride, chloroform and ethyl acetate) to dissolve PLGA and determined that those solvents with lower interfacial tension with water formed smaller sized microspheres. In the present study, the particle size did not change significantly after 25% (v/v) ethanol concentration. This might be due to the increased viscosity of the polymer phase that counteracted the effect of interfacial tension. Thomasin et al. (1998) and Jeyanthi et al. (1997) have observed an increase in particle size with increase in polymer phase viscosity. A linear increase in particle size of PLGA microspheres prepared with polymer phase viscosity in the range of 2–10 cps has been reported (Jeyanthi et al., 1997).

4.3. Morphology

Partitioning of drug (dexamethasone) dissolved in ethanol from the organic to the aqueous phase also resulted in microsphere morphological changes. The microsphere surface roughness observed at 18.75% (v/v) and higher ethanol concentrations were speculated to be due to drug (dexamethasone) crystallization on the microsphere surfaces (Fig. 2). It is speculated that during particle solidification, diffusion of ethanol from the organic to the aqueous phase caused precipitation of dissolved dexamethasone at the polymer–water interface due to the poor aqueous solubility of dexamethasone (aqueous solubility of dexamethasone: 0.076 ± 0.001 mg/ml). SEM micrographs of blank microspheres prepared with 25% (v/v) ethanol revealed that these microspheres were smooth and spherical, confirming that the surface imperfections in the corresponding drug loaded microspheres were due to dexamethasone crystals. In addition, the magnified SEM images of drug-containing microsphere prepared with 25% (v/v) ethanol (10,000×) show similar structures to that of dexamethasone crystals prepared separately by vacuum evaporation of dexamethasone solution in ethanol (5000×) (Fig. 2i and j).

At 43.25% (v/v) ethanol concentration, irregular microsphere aggregates were formed (Fig. 2h). This can be attributed to the high viscosity of the polymer phase which hinders the formation of smooth and spherical particles. Li et al. (1995) have reported that sol–gel conversion during microsphere formation occurs at a volume fraction of approximately 0.41 methylene chloride in the polymer phase. At 43.25% (v/v) ethanol concentration in the organic phase, the volume fraction of methylene chloride is approximately 0.57. This volume fraction (0.57) is close to the critical gelation point (0.41) reported by Li et al. (1995). Therefore, it appears that polymer solidification occurred rapidly preventing normal droplet formation.

4.4. Glass transition temperature

The glass transition temperature (T_g) is an important feature that helps in understanding the release pattern of PLGA microspheres. Above the glass transition temperature, the polymer is in a rubbery state and drug diffusion through the polymeric matrix is faster than that in the glassy state (*i.e.* below glass transition temperature). Solvents (such as ethanol, methylene chloride, *etc.*) used in the fabrication of polymeric devices often have a plasticizing effect on the polymer (lowering T_g of the polymer). However, in the present study, the glass transition temperature of the microspheres was not affected by the presence of ethanol as organic phase co-solvent at any concentration (Table 2).

4.5. In vitro release

The *in vitro* release profiles reflected the trend observed with the physicochemical properties *i.e.* particle size, drug loading and morphology. The release profiles of all the formulations prepared with ethanol up to 12.5% (v/v) concentration were similar which is in accordance with other physicochemical properties. As evident from the SEM micrographs, the drug crystals present on the microsphere surface resulted in a higher burst release (approximately 60%) for the formulation prepared with 18.75% (v/v) ethanol co-solvent concentration. The burst release further increased (approximately 75%) at ethanol co-solvent concentrations of 25%, 31.25% and 37.5% (v/v) due to an increase in drug crystals observed on the surface of these microspheres. At 43.75% (v/v) ethanol co-solvent, the diffusional loss of drug to the external aqueous phase during processing is restricted by the higher polymer phase viscosity as explained before. Accordingly, the release profile at 43.75% (v/v) ethanol co-solvent was intermediate between the formulations containing 25% (v/v) and lower ethanol concentrations *i.e.* 12.5% (v/v) (Fig. 3).

5. Conclusions

It is evident from results that the presence of ethanol as an organic phase co-solvent affected the performance of dexamethasone containing PLGA microspheres. The changes in the *in vitro* release profiles of these microspheres can be explained on the basis of their physicochemical properties. The changes observed in drug loading, particle size and morphology were dependent on many competing factors such as interfacial tension between methylene chloride and water, solubility of drug in the organic phase and the viscosity of the polymer phase. The change in the *in vitro* release profile observed was not due to any change in thermal behavior of the microspheres as determined from T_g data. An understanding of co-solvent effects is therefore critical in microsphere formulation

and process design as such physicochemical changes are likely to affect product performance.

This work also provides important information on the formulation of microspheres using drug solutions such as solutions of radioactive drugs in organic solvents. Based on the physicochemical properties and *in vitro* release profiles, it is speculated that radio-labeled dexamethasone solution (1 mCi/ml solution in ethanol, Perkin Elmer) can be used at concentrations up to 12.5% (v/v) of the organic phase without changing the microsphere characteristics. This information will be utilized for formulation of radio-labeled dexamethasone encapsulating PLGA microspheres in future for *in vivo* assessment of drug pharmacokinetics.

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References

Al-Maaieh, A., Flanagan, D.R., 2001. Salt and cosolvent effects on ionic drug loading into microspheres using an O/W method. J. Control. Release 70, 169–181.

Bhardwaj, U., Sura, R., Papadimitrakopoulos, F., Burgess, D.J., 2007. Controlling acute inflammation with fast releasing dexamethasone–PLGA microsphere/PVA hydrogel composites for implantable devices. J. Diabetes Sci. Technol. 1, 8–17.

Bodmeier, R., McGinity, J.W., 1988. Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by solvent evaporation method. Int. J. Pharm. 43, 179–186.

Burgess, D.J., Hickey, A.J., 2005. Microspheres: design and manufacturing. In: Burgess, D.J. (Ed.), Injectable Disperse Systems. Taylor & Francis.

Chaubal, M., 2002. Polylactides/glycolides – excipients for injectable drug delivery and beyond. Drug Deliv. Technol. 5, 34–36.

D'Souza, S.S., DeLuca, P.P., 2006. Methods to assess *in vitro* release from injectable polymeric particulate systems. Pharm. Res. 23, 460–474.

Faisant, N., Siepmann, J., Benoit, J.P., 2002. PLGA-based microparticles: elucidation of mechanisms and a new, simple mathematical model quantifying drug release. Eur. J. Pharm. Sci. 15, 355–366.

Freitas, S., Merkle, H.P., Gander, B., 2005. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. J. Control. Release 102, 313–332.

Graves, R.A., Freeman, T., Pamajula, S., Praetorius, N., Moiseyev, R., Mandal, T.K., 2005. Effect of co-solvents on the characteristics of enkephalin microspheres. J. Biomater. Sci. Polym. Ed. 17, 709–720.

Hickey, T., Kreutzer, D., Burgess, D.J., Moussy, F., 2002a. Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices. Biomaterials 23, 1649–1656.

Hickey, T., Kreutzer, D., Burgess, D.J., Moussy, F., 2002b. In vivo evaluation of a dexamethasone/PLGA microsphere system designed to suppress the inflammatory tissue response to implantable medical devices. J. Biomed. Mater. Res. 61, 180–187.

Igartua, M., Hernández, R.M., Esquisabel, A., Gascon, A.R., Calvo, M.B., Pedraz, J.L., 1997. Influence of formulation variables on the *in-vitro* release of albumin from biodegradable microparticulate systems. J. Microencapsul. 14, 349–356.

Ito, F., Fujimori, H., Honnami, H., Kawakami, H., Kanamura, K., Makino, K., 2009. Study of types and mixture ratio of organic solvent used to dissolve polymers for preparation of drug-containing PLGA microspheres. Eur. Polym. J. 45, 658–667.

Jeyanthi, R., Mehta, R.C., Thanoo, B.C., DeLuca, P.P., 1997. Effect of processing parameters on the properties of peptide-containing PLGA microspheres. J. Microencapsul. 14, 163–174.

Li, W.-I., Anderson, K.W., Mehta, R.C., DeLuca, P.P., 1995. Prediction of solvent removal profile and effect on properties for peptide loaded PLGA microspheres prepared by solvent extraction/evaporation method. J. Control. Release 37, 199–214.

Mandal, T.K., 1999. Effect of solvent on the characteristics of pentamidine loaded microcapsules. J. Biomater. Sci. Polym. Ed. 10, 1–17.

Mao, S., Shi, Y., Li, L., Xu, J., Schaper, A., Kissel, T., 2008. Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method. Eur. J. Pharm. Biopharm. 68, 214–223.

Mao, S., Xu, J., Cai, C., Germershaus, O., Schaper, A., Kissel, T., 2007. Effect of WOW process parameters on morphology and burst release of FITC-dextran loaded PLGA microspheres. Int. J. Pharm. 334, 137–148.

Patil, S.D., Papadmitrakopoulos, F., Burgess, D.J., 2007. Concurrent delivery of dexamethasone and VEGF for localized inflammation control and angiogenesis. J. Control. Release 117, 68–79.

Rosca, I.D., Watari, F., Uo, M., 2004. Microparticle formation and its mechanism in single and double emulsion solvent evaporation. J. Control. Release 99, 271–280.

Soppimath, K.S., Aminabhavi, T.M., 2002. Ethyl acetate as a dispersing solvent in the production of poly(DL-lactide-co-glycolide) microspheres: effect of process parameters and polymer type. J. Microencapsul. 19, 281–292.

Thomasin, C., Merkle, H.P., Gander, B., 1998. Drug microencapsulation by PLA/PLGA coacervation in the light of thermodynamics: 2. Parameters determining microsphere formation. J. Pharm. Sci. 87, 269–275.

Yang, Y.Y., Chung, T.S., Ng, N.P., 2001. Morphology, drug distribution, and *in vitro* release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 22, 231–241.

Yeo, Y., Park, K., 2004. Control of encapsulation efficiency and initial burst in polymeric microparticle systems. Arch. Pharm. Res. 27, 1–12.

Zolnik, B.S., Leary, P.E., Burgess, D.J., 2006. Elevated temperature accelerated release testing of PLGA microspheres. J. Control. Release 112, 293–300.

Zolnik, B.S., Raton, J.L., Burgess, D.J., 2005. Application of USP apparatus 4 and *in situ* fiber optic analysis to microsphere release testing. Dissolut. Technol., 11–14.