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Research paper

Controlled release of drugs from injectable in situ formed biodegradable PLGA microspheres: effect of various formulation variables

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Abstract

A novel in situ method for the preparation of injectable biodegradable poly(lactide-co-glycolide) (PLGA) microspheres for the controlled delivery of drugs is described here. A stable dispersion of PLGA microglobules ('premicrospheres' or 'embryonic microspheres') in a vehicle mixture on injection, comes in contact with water from aqueous buffer or physiological fluid, thereby hardening the microglobules into solid matrix type microparticles entrapping the drug (in situ formed microspheres). The drug is then released from these microspheres in a controlled fashion. The effect of the following formulation variables on the characteristics of the novel drug delivery system (NDDS) was investigated: (i) the concentrations of polyethylene glycol 400 (PEG 400), the encapsulated drug, and the hydrophilic excipient (mannitol); and (ii) the types of encapsulated drug (micromolecules and macromolecules such as protein) and vehicles (replacing triacetin and Miglyol 812 by triethyl citrate and soybean oil respectively). Also, the effect of formulation, process, and storage (15 days/4°C) conditions on the physical stability of the encapsulated protein was evaluated. The in vitro drug release was enhanced with decrease in the PEG 400 concentration and increase in the drug and mannitol concentration. The drug release was retarded with increase in the molecular weight of the encapsulated drug. Substitution of triacetin by triethyl citrate and miglyol 812 by soybean oil resulted in variation in the release of the drug from the in situ formed microspheres. A preliminary investigation of the physical stability of the myoglobin revealed that the α-helical structure was unaffected by the formulation, process, and the storage conditions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: In situ; Microspheres; Controlled release; Protein; Poly(lactide-co-glycolide) (PLGA)

1. Introduction

There is a particular interest in controlled delivery of macromolecules such as peptides and proteins through poly(-lactide-co-glycolide) (PLGA) (the copolymer of lactic and glycolic acids) microspheres and a wealth of literature has been generated on this issue [1–17]. PLGAs have shown to be biocompatible and they degrade to toxicologically acceptable lactic and glycolic acids that are eventually eliminated from the body [2]. Also, they have been approved by the FDA as controlled drug release microspheres [3,4].

The NDDS is the same as described earlier [18] (Fig. 1) and it involves a stable dispersion of PLGA microglobules ('premicrospheres' or 'embryonic microspheres') in a continuous phase consisting of acceptable vehicle mixture. This on injection, comes in contact with water from aqueous buffer or physiological fluid, thereby hardening the microglobules into solid matrix type microparticles entrapping the drug (in situ formed microspheres) [18]. The drug is then released from these microspheres in a controlled fashion.

In this study, we have investigated the effect of the following formulation variables on the characteristics of the NDDS: (i) the concentrations of polyethylene glycol 400 (PEG 400), the encapsulated drug, and the hydrophilic excipient (mannitol); and (ii) the types of encapsulated drug and the vehicles. The effect of formulation, process, and storage (15 days/4°C) conditions on the physical stability of the encapsulated protein was also evaluated.

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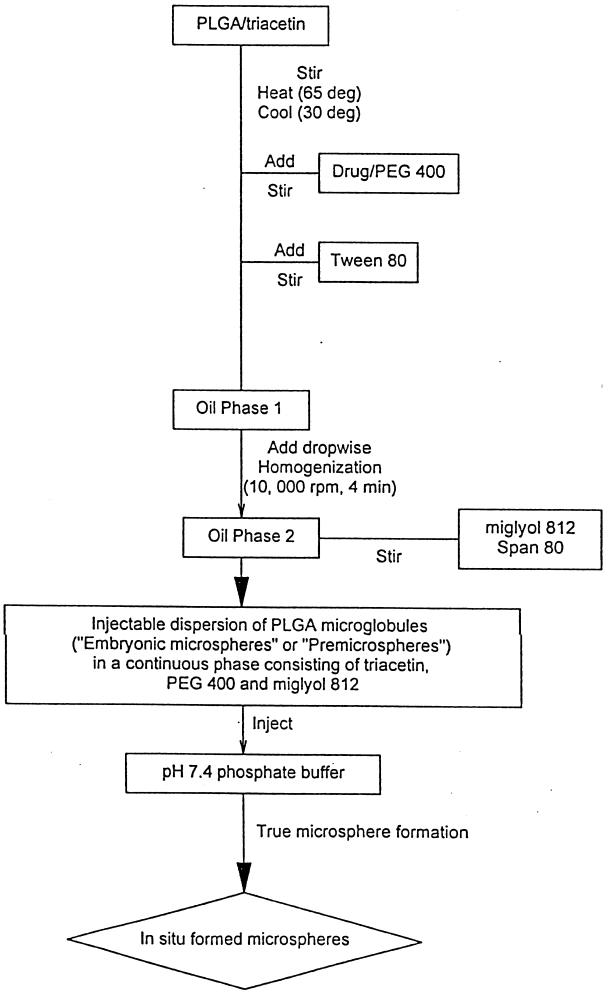

Fig. 1. Schematic representation of the novel in situ PLGA microsphere formation process.

2. Materials and methods

2.1. Materials

All the chemicals/reagents were used as purchased. Polyvinyl alcohol (PVA) (87.0–89.0% partially hydrolyzed, molecular weight: 11 000–31 000) from J.T. Baker (Phillipsburg, NJ); sodium azide, bovine heart cytochrome *c* (molecular weight: 12 327), and heart skeletal muscle myoglobin (molecular weight: 16 950) from Sigma Chemical Co. (St. Louis, MO); red D&C #33 dye (red dye) from Pylam Products Corp. (Garden City, NY); PLGA RESOMER grade RG 502 H from Boehringer Ingelheim (Ingelheim, Germany); triacetin from Eastman Fine Chemicals (Rochester, NY); triethyl citrate from Morflex Chemical Co. (Greensboro, NC); PEG 400 from Union Carbide Corp. (Danbury, CT); miglyol 812 from Hüls America, Inc. (Piscataway, NJ); soybean oil from Welch, Holme & Clark Co., Inc. (Newark, NJ); mannitol from Roquette America, Inc. (Keokuk, IA); Span 80 and Tween 80 from I.C.I. (Wilmington, DE); methylene chloride, *n*-hexane, and 2-propanol from Fisher Scientific (Fair Lawn, NJ).

2.2. Preparation of injectable PLGA microglobules and subsequent in situ formation of microspheres

PLGA was dissolved in triacetin by magnetic stirring and heating to approximately 65°C in a glass vial. The solution was cooled under ambient condition and to it was added a solution of the drug in PEG 400. This mixture was magnetically stirred for 5 min followed by addition of Tween 80 and further stirring for 5 min to form Oil Phase 1. Span 80 was dissolved in Miglyol 812 in a glass vial to form Oil Phase 2. Oil Phase 1 was added dropwise to Oil Phase 2 with continuous homogenization at 10 000 rev./min, using a Biohomogenizer (Biospec Products Inc., Bartlesville, OK) for a period of 4 min, to form the NDDS (injectable dispersion of PLGA microglobules in a continuous phase consisting of triacetin, PEG 400 and Miglyol 812 and stabilized by Tween 80 and Span 80). A typical example of the theoretical concentration of the materials used to prepare the microglobules is shown in Table 1.

The ability of the NDDS to form microspheres in situ was performed in an aqueous phosphate buffer. The NDDS in 1 ml quantity was injected in 10 ml of 10 mM, (pH 7.4) aqueous phosphate buffer contained in a 20 ml capacity glass vial. The phosphate buffer also contained 0.02% w/v PVA as an anti-adherent and 0.02% w/v sodium azide as a preservative (bacteriostatic).

2.3. In vitro drug release

The glass vials containing the in situ formed microspheres were subjected to agitation at 100 rev./min at 37°C in a G24 Environmental Incubator Shaker from New Brunswick Scientific Co., Inc. (Edison, NJ) (this step was also followed for blank in situ formed PLGA microspheres). Samples in 1 ml aliquots from each vial were withdrawn at predetermined time intervals using a 0.5 μm filter (Millipore Products Division, Bedford, MA) assisted 18G1 needle/1 cc plastic syringe assembly (Beckton Dickinson & Co., Rutherford, NJ). The vials were replenished with 1

Table 1
A typical example of the theoretical concentration of the materials used to prepare PLGA microglobules[a]

Formulation	Quantity added (% w/w)
PLGA	2.39
Triacetin	13.55
Miglyol 812	80.80
Cytochrome *c*/PEG 400 (10 mg/2000 mg)	3.08
Tween 80	0.09
Span 80	0.09

[a] During the PLGA microglobule formation process, most of the triacetin and PEG 400 goes to the continuous phase (which contains chiefly of Miglyol 812) and very little is retained in the microglobules.

ml of fresh aqueous phosphate buffer by passing it through the same filter assisted needle/syringe assembly. The amount of the drug released from the in situ formed microspheres into the aqueous phosphate buffer was quantitatively determined by using a UV-Vis Spectrophotometer from Varian (Varian Australia Pty Ltd.). The wave length used for the analysis of the red dye was 530 nm while the proteins, cytochrome *c* and myoglobin were analyzed at 409 nm. A standard concentration curve was used to determine the concentration of the drugs in the sample vials. The results were reported as mean of two experiments and for each experiment the mean of three sample analysis were determined.

2.4. Extraction of the encapsulated drug from the in situ formed PLGA microspheres

The vials containing the in situ formed microspheres were transferred to plastic centrifuging tubes, which were then centrifuged at 10 000 rev./min for 10 min using a Hermle Z382K centrifuging machine from Denville Scientific, Inc. (Metuchen, NJ). The PLGA microspheres were washed twice, each time with 5 ml of *n*-hexane:2-propanol (1:1). This was then followed by washing with 5 ml of distilled water (twice). The tubes were then dried for 24 h in a 282A Isotemp vacuum oven from Fisher Scientific and the weight of the dried PLGA microspheres determined. This was followed by addition of 5 ml of DCM to the tubes to dissolve the PLGA copolymer and subsequent extraction of the cytochrome *c* into 5 ml of distilled water. The aqueous phase containing the recovered drug was then subjected to percentage encapsulation efficiency study and circular dichroism (CD) spectroscopy.

2.5. Percentage drug encapsulation efficiency

The amount of drug in the aqueous phase was quantitatively determined by its absorbance at 409 nm. The percentage encapsulation efficiency was calculated by relating the practical drug entrapment to the theoretical drug entrapment: practical drug entrapment (% w/w)/theoretical drug entrapment (% w/w) $\times 100$. The results were reported as mean of two experiments and for each experiment the mean of three sample analysis were determined.

2.6. CD spectroscopy

The physical stability of the encapsulated proteins was investigated by CD spectroscopy. The CD spectra were measured using a J-710 spectropolarimeter from Jasco, Inc. (Easton, MD). The conditions of analysis were as follows: pathlength, 0.1 cm (quartz cell); scan speed, 50 nm/min; step resolution, 1 nm/data; sensitivity, 50 mdeg; bandwidth, 1 nm; wavelength range, 190–250 nm (far UV). For each sample an average of two scans were recorded using an attached SPL-430A X-Y plotter from Sekonic Company Ltd. (Japan).

3. Results and discussions

3.1. In situ formation of PLGA microspheres

In our previous paper, we had shown by optical microscopy the process of transformation of the PLGA microglobules into microspheres, which occurred in approximately 17 min [18]. The rubbery PLGA microglobules on coming in contact with water harden to form solid matrix type microparticles entrapping the drug (in situ formed microspheres). Also, due to the extraction of the vehicles associated with the microglobules by water, they subsequently shrink in size during their transformation to microspheres [18].

3.2. In vitro release of drugs from the in situ formed PLGA microspheres

The in situ formed microspheres from the PLGA microglobules exhibited a controlled cytochrome *c* release from few days up to 2 weeks, depending upon various formulation variables [18]. The burst effect (amount of cytochrome *c* released by the end of day 1) was mainly due to the unencapsulated cytochrome *c* [18]. Proteins are too large to diffuse through the polymer matrix and their release occurs via diffusion through the interconnecting channels and pores formed by them in the matrix [1–18]. The proteins are also released by the hydrolytic degradation (erosion) of the matrix. We suspect that the drug release from these microspheres is a combination of the two mechanisms – diffusion through the microsphere matrix and its hydrolytic degradation (which is controlled by factors like PLGA molecular weight, crystallinity, and lactide:glycolide ratio). The release of drug from these microspheres is associated with formation of pores and they increase in number and size as the PLGA degradation proceeds, resulting in subsequent faster release rates of the drug. Kissel et al.

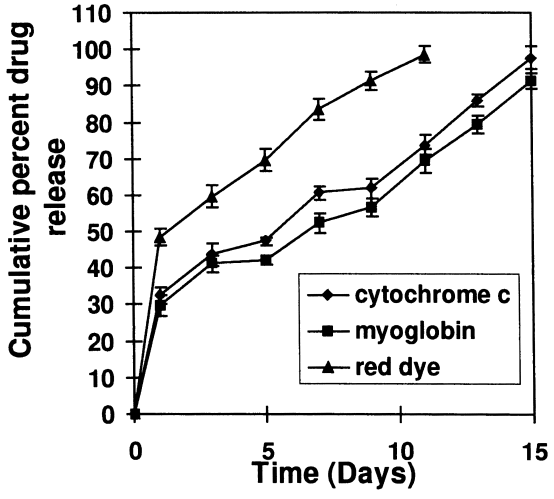

Fig. 2. Controlled release of different drugs from the in situ formed PLGA microspheres.

[19] have reported a decrease in the release of the encapsulated proteins with increase in their molecular weights. Fig. 2 shows the in vitro drug release profile of the red dye, cytochrome c and myoglobin from the in situ formed PLGA microspheres. As shown, the release of myoglobin (molecular weight: 16 950) is slightly delayed as compared to cytochrome *c* (molecular weight: 12 327). Also, the red dye (a micromolecule) is released rapidly from the PLGA matrix due to its faster diffusion from the matrix (Fig. 2). Thus, depending upon their molecular weights, the drugs exhibit variation in their burst effects and the overall release from the in situ formed PLGA microspheres.

3.3. Effect of PEG 400 and drug loading

In our previous paper, we had indicated that due to the extraction of most of the triacetin and PEG 400 in water, the microglobules harden and form microspheres (entrapping the drug) [18]. The concentration of PEG 400 used in the formulation was of great significance and was determined based on the concentration of the PLGA-triacetin solution (i.e. PLGA-triacetin/PEG 400 ratio). Decrease in the concentration of PEG 400 (ratio of 4.52) produces a concentrated PEG 400-cytochrome *c* solution. Thus more drug is available to be encapsulated by the PLGA microglobules, resulting in higher percentage cytochrome *c* encapsulation efficiency (Fig. 3a). This increased concentration of the encapsulated cytochrome *c* results in an increase in the interconnecting channels and pores in the PLGA matrix, leading to a relatively faster drug release profile (Fig. 3a). An increase in the concentration of cytochrome *c* also produces a concentrated PEG 400–cytochrome *c* solution. Thus more drug is encapsulated by the PLGA microglobules, resulting in higher percentage cytochrome *c* encapsulation efficiencies and accelerated release profile (Fig. 3b).

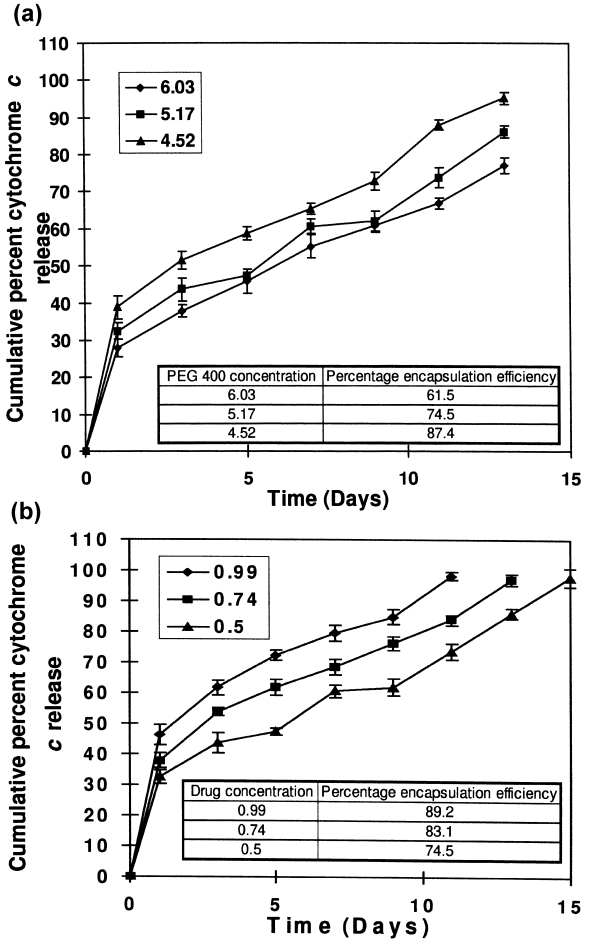

Fig. 3. (a) Effect of PEG 400 concentration on the controlled release and percentage encapsulation efficiency of cytochrome *c* (PEG 400 concentration expressed as different ratios of PLGA-triacetin/PEG 400). (b) Effect of cytochrome *c* concentration on its controlled release and percentage encapsulation efficiency (cytochrome *c* concentration expressed as % w/w of PEG 400).

3.4. Effect of addition of an hydrophilic excipient (mannitol)

Fig. 4 shows the release of myoglobin in presence of the hydrophilic excipeint, mannitol. Various groups have reported the effect of different additives/excipients on the drug release from the PLGA microparticles [20,21]. Proteins are released from the PLGA matrix by diffusion from the interconnecting channels and pores formed by them in the matrix and by the hydrolytic degradation (erosion) of the matrix. Addition of co-diffusants like mannitol imparts porosity to the PLGA matrix, thereby changing the matrix structure and providing increased diffusion through interconnecting channels for the release of the proteins. Thus, an increase in the concentration of mannitol leads to subsequent a higher protein release profile due to formation of highly porous PLGA matrix (Fig. 4). We suspect that an increase in the mannitol concentration

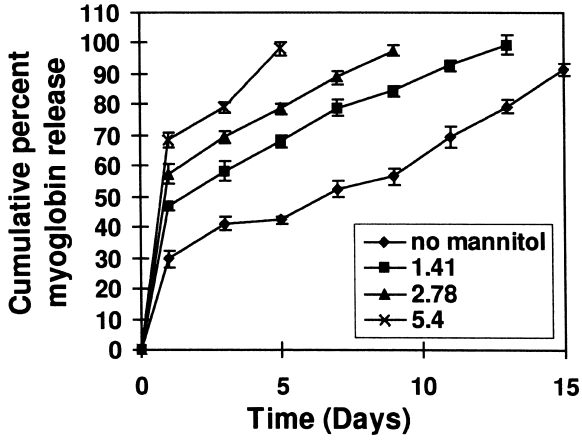

Fig. 4. Controlled release of myoglobin from the in situ formed PLGA microspheres prepared from NDDS containing different concentrations of the hydrophilic excipient, mannitol (mannitol concentration expressed as % w/w of myoglobin-PEG 400 solution).

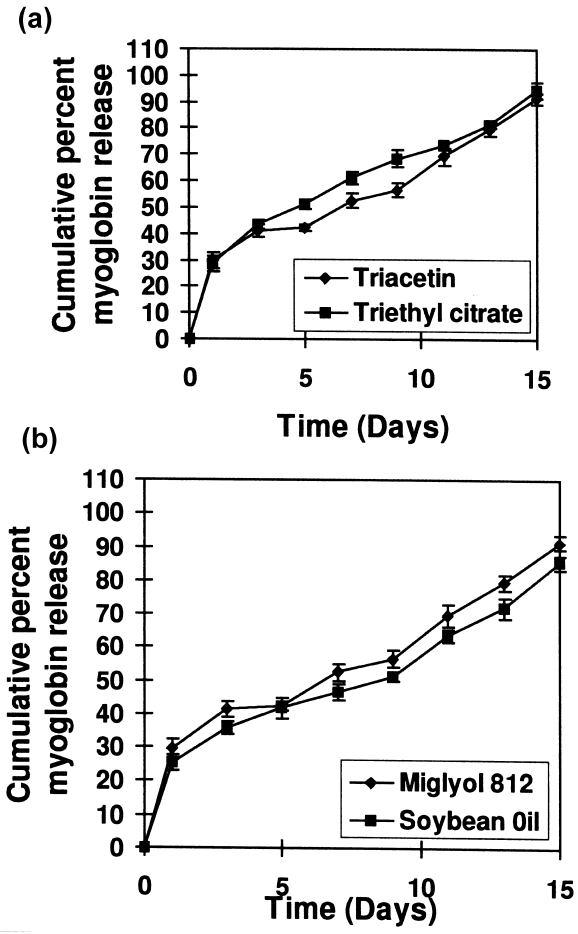

Fig. 5. (a) Controlled release of myoglobin from the in situ formed PLGA microspheres prepared from NDDS containing different plasticizers. (b) Controlled release of myoglobin from the in situ formed PLGA microspheres prepared from NDDS containing different oils.

would lead to subsequent lower percentage encapsulation efficiencies, as mannitol would compete with myoglobin to be encapsulated by the PLGA microglobules.

3.5. Effect of using different vehicles

Sah and Chien have explained the relationship between plasticization and the hydrolytic degradation of the PLGA polymer [22]. The plasticizer, triacetin (solubility in water: 77.8 mg/ml) is relatively more hydrophilic than triethyl citrate (solubility in water: 55.4 mg/ml) [23]. Thus, the two plasticizers may plasticize the PLGA polymer differently, thereby causing variation in their hydrolytic degradation. This may explain the difference in the release profiles of the in situ formed microspheres produced using triacetin and triethyl citrate (Fig. 5a). Soybean oil (mixed triglyceride of unsaturated and saturated fatty acids) is relatively more hydrophobic than miglyol 812 (mixed C_8–C_{10} triglyceride of saturated fatty acids) [24,25]. Hence use of soybean oil produces relatively more hydrophobic PLGA microglobule dispersion than those formulated using miglyol 812, thereby delaying the dissolution of the released myoglobin (Fig. 5b).

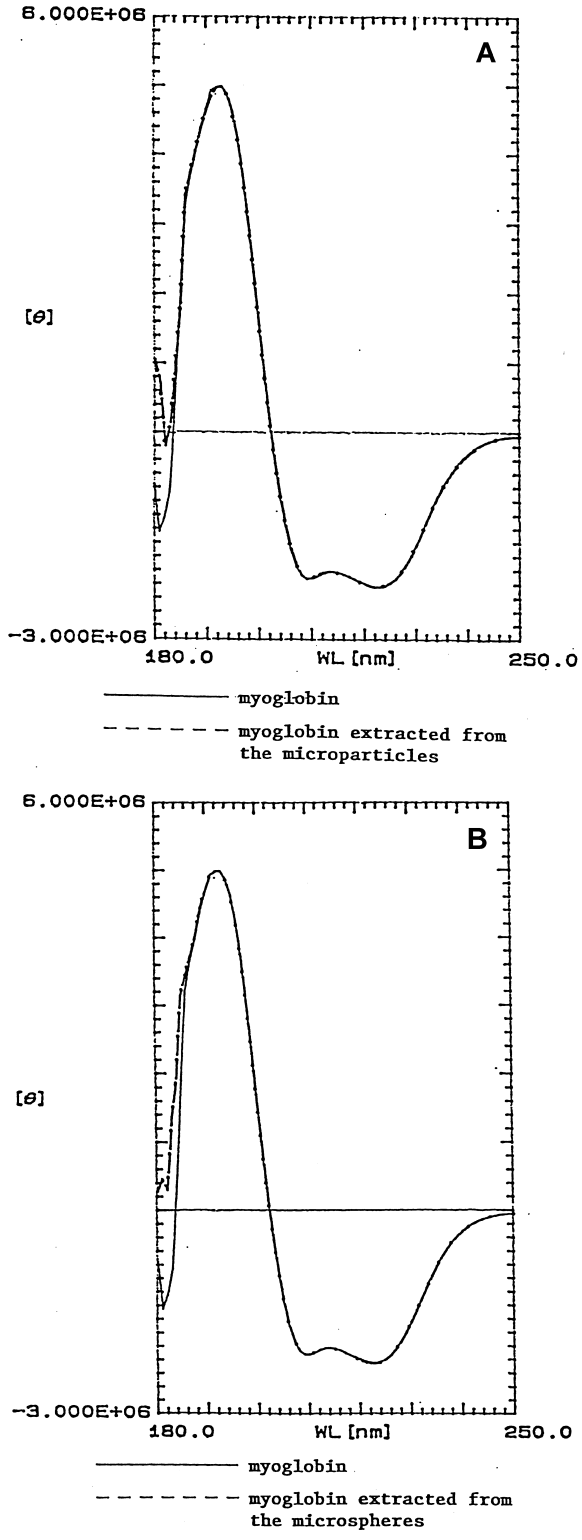

Fig. 6. (a) Effect of formulation and process conditions on the physical stability of the encapsulated myoglobin. (b) Effect of 15 days/4°C storage condition on the physical stability of the encapsulated myoglobin.

3.6. Stability of the encapsulated myoglobin

It is important to maintain the native protein conformation and its stability during the encapsulation process. In our previous paper, we had shown by CD spectroscopy that the formulation, process and the 15 days/4°C storage conditions did not adversely affect the physical stability of the encapsulated cytochrome *c* [18].

The CD profile of myoglobin in the far UV range (which provided the secondary structure of the protein) has been shown in Fig. 6a,b. As evident from these figures, the ellipticity around 222 nm was unaffected by the formulation/process and the 15 days/4°C storage conditions. This suggested that the protein maintained its α-helical structure and hence its physical stability.

4. Conclusion

Controlled release of a micromolecule and proteins from the in situ formed PLGA microspheres produced from a novel coacervation process is described here. The in vitro drug release was enhanced with decrease in the PEG 400 concentration and increase in the drug and mannitol concentration. The drug release was retarded with increase in the molecular weight of the encapsulated drug. Substitution of triacetin by triethyl citrate and miglyol 812 by soybean oil resulted in variation in the release of the drug from the in situ formed microspheres. A preliminary investigation of the physical stability of the myoglobin revealed that the α-helical structure was unaffected by the formulation, process, and the storage conditions.

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