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Development of New Reverse Micellar Microencapsulation Technique to Load Water-Soluble Drug into PLGA Microspheres

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The objective of this study was to develop a new reverse micelle-based microencapsulation technique to load tetracycline hydrochloride into PLGA microspheres. To do so, a reverse micellar system was formulated to dissolve tetracycline hydrochloride and water in ethyl formate with the aid of cetyltrimethylammonium bromide. The resultant micellar solution was used to dissolve 0.3 to 0.75 g of PLGA, and microspheres were prepared following a modified solvent quenching technique. As a control experiment, the drug was encapsulated into PLGA microspheres *via* a conventional methylene chloride-based emulsion procedure. The microspheres were then characterized with regard to drug loading efficiency, their size distribution and morphology. The reverse micellar procedure led to the formation of free-flowing, spherical microspheres with the size mode of 88 μm. When PLGA microspheres were prepared following the conventional methylene chloride-based procedure, most of tetracycline hydrochloride leached to the aqueous external phase: A maximal loading efficiency observed our experimental conditions was below 5%. Their surfaces had numerous pores, while their internal architecture was honey-combed. In sharp contrast, the new reverse micellar encapsulation technique permitted the attainment of a maximal loading efficiency of 63.19 ± 0.64%. Also, the microspheres had smooth and pore-free surfaces, and hollow cavities were absent from their internal matrices. The results of this study demonstrated that PLGA microspheres could be successfully prepared following the new reverse micellar encapsulation technique.

Key words: Reverse micelles, PLGA, Microspheres, Microencapsulation

INTRODUCTION

The encapsulation of bioactive materials into biocompatible, biodegradable poly-*d,l*-lactide-co-glycolide (PLGA) microspheres can provide their controlled release over a wide range of periods. Therefore, a microsphere dosage form can help reduce the frequency of drug administration and improve patient compliance. A number of methods useful for preparing PLGA microspheres are well known: Examples include emulsion-based solvent evaporation, solvent extraction, spray drying, phase separation, coacervation, and interfacial polymerization (Benoit *et al.*, 1996). At present, emulsion-based microencapsulation procedures are commonly used to load bioactive agents into PLGA microspheres. The types of emulsions used are oil-in-water, oil-in-oil, water-in-oil-in-water, and water-in-oil-in-oil (McGinity and Iwata, 1994; Scholes *et al.*, 1997; Hamont *et al.*, 1998; Lee *et al.*, 2000; Chen *et al.*, 2002). Among them, the water-in-oil-in-water ($W_1/O/W_2$) technique is the usual choice for microencapsulating water-soluble drugs into PLGA microspheres. In most cases, methylene chloride is used as a dispersed solvent to dissolve PLGA polymers. However, this method requires very strong shear to emulsify W_1 in O phases. Since shear is detrimental to fragile materials such as DNA and proteins, the $W_1/O/W_2$ method is sometimes inappropriate for them (Farrar *et al.*, 2002). Also, low molecular weight bioactive agents diffuse into the W_2 phase during the microencapsulation process, thereby resulting in poor loading efficiencies (Mandal, 1999).

Reverse micelles are very tiny water droplets stabilized in an organic solvent with the aid of surfactants. They are commonly used to solubilize polar solutes (e.g., amino acids and proteins) in aprotic media. A typical three-component reverse micellar system consists of water,

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bis(2-ethylhexyl)sodium sulfosuccinate (AOT) as surfactant, and iso-octane or hexane. Reverse micellar solutions are simply prepared by dissolving 25~300 mM AOT in the hydrocarbon solvent and then adding the desired amount of water (typically in the range of 0.1~5%). In general, it is unnecessary to perform sonication to make reverse micelles, while shaking by hand alone gives a clear solution. Reverse micelle-containing organic solvents have been used as effective extractants for the recovery, purification, and concentration of biomaterials (Pires *et al.*, 1996; Jarudilokkul *et al.*, 2000).

So far, only a single study has been reported to prepare microspheres by using a reverse micellar solution (Hayashi *et al.*, 1994). Proteins were dissolved in chloroform or methylene chloride with the aid of surfactants such as AOT and sucrose esters of fatty acids. Microspheres were then prepared following a conventional solvent evaporation process. Under their experimental conditions, however, the total amount of proteins dissolved in reverse micelles was so small that ca. 1 mg of superoxide dimutase was dissolved in 4 mL of the organic solvents. On recognition of these situations, our present work has aimed to develop a new reverse micelle-based microencapsulation technique to incorporate water-soluble drugs into PLGA microspheres. To do so, a reverse micellar system has been formulated to dissolve a model drug tetracycline hydrochloride (TH) in ethyl formate used as an oil phase. Established on the reverse micellar solution, PLGA microspheres have been prepared *via* a modified solvent quenching technique. The properties of the PLGA microspheres are characterized in terms of TH loading efficiency, size distribution pattern, and external and internal morphology. In addition, the results attained with our new microencapsulation process have been compared with those observed with a conventional methylene chloride-based double emulsion process.

MATERIALS AND METHODS

Materials

PLGA with a lactide:glycolide ratio of 75:25 (inherent viscosity = 0.69 dL/g in $CHCl_3$ at 30°C, lot number D01066) was purchased from Birmingham Polymers, Inc. (Birmingham, AL, USA). The polymer was abbreviated as PLGA75:25 in the text. Tetracycline hydrochloride (TH) and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyvinyl alcohol with a molecular weight of 25,000 (88% mol hydrolyzed) was obtained from Polysciences, Inc. (Warrington, PA, USA). Analytical grade ethyl formate was supplied from Aldrich (Milwaukee, WI, USA). HPLC grade methylene chloride was procured from Fisher Scientific (Malvern, PA, USA).

Preparation of TH loaded microspheres *via* a reverse micelle-based encapsulation process

TH (20 mg), CTAB (30 mg), and water (0.15 mL, W_1) were added into a vial containing 3 mL of ethyl formate. When kept at a stand still overnight inside an oven at 30°C, the mixture became a clear solution. This solution was used to dissolve 0.3 to 0.75 g of PLGA75:25. The polymeric solution was then poured into 20 mL of a 1% polyvinyl alcohol solution presaturated with ethyl formate (W_2). During this addition, the aqueous external phase was stirred at 475 rpm using a 400 HPS magnetic plate stirrer (VWR Scientific, West Chester, PA, USA). After 5 minutes, an additional 60 mL of a 0.5% polyvinyl alcohol solution (W_3) was added to the emulsion. This so-called "quenching step" led to a quick extraction of ethyl formate out of the polymeric phase into the aqueous external phase. Afterwards, the microsphere suspension was stirred for 40 minutes, collected by filtration, and redispersed in 80 ml of a 0.5% polyvinyl alcohol solution. After 1 hour-stirring, microspheres were obtained by filtration, and dried under a vacuum overnight.

Preparation of TH loaded PLGA microspheres *via* a methylene chloride-based double emulsion process

TH (20 mg) was dissolved in water (0.3 mL, W_1), which was emulsified in 7 mL of methylene chloride containing 0.3 to 0.75 g of PLGA75:25. Emulsification was performed on the mixture at 16,500 rpm for 1 minute using a VirtiShear homogenizer. The primary water-in-oil emulsion was poured into 200 mL of a 1% polyvinyl alcohol solution (W_2) to produce a water-in-oil-in-water emulsion. During the addition, the continuous phase was stirred at 475 rpm using the 400 HPS magnetic plate stirrer. The double emulsion was stirred for further 3 h at room temperature inside a hood. Microspheres were then collected by filtration, washed with distilled water, and dried under a vacuum overnight.

Determination of the size distribution of microspheres

The size distribution of microspheres was measured by laser diffraction analysis using a unified scatter technique (Microtrac X100 particle size analyzer; Microtrac, Inc., Montgomeryville, PA, USA). Aliquots of microsphere samples were dispersed in water by gentle stirring, which was loaded into the particle size analyzer.

Determination of TH encapsulation efficiency

A known amount of dried microspheres (18.4~24.4 mg) was dissolved in 3 mL of methylene chloride. Nine mL of a 20 mM phosphate buffer at pH 7 were added into this organic solution. The mixture was then vortexed for 2

minutes to extract TH back into the aqueous solution. After dilution, aliquots of the sample solutions were subject to spectrophotometric measurement at 380 nm (Utraspec 2100 pro UV/Visible spectrophotometer; Amersham Biosciences UK Limited, Buckinghamshire, England). Calculation of TH concentration was based on the standard calibration curve constructed with 6.25 to 50 μg/mL concentrations. Encapsulation efficiency (EE %) was then calculated as follows: EE % = {actual TH loading (wt.%)/ theoretical TH loading (wt.%)} × 100.

Data report

Each set of the experiments described so far was repeated at least three times. Results were reported as the mean ± standard deviation in the text.

Scanning electron microscopy

The morphology of PLGA75:25 microspheres was investigated using a Hitachi scanning electron microscope (SEM; Model S-4100, Ibaraki-Ken, Hitachi High-Corp., Japan). Microsphere samples were coated to a thickness of 100 nm by use of an ion sputter (Model E-1030, Ibaraki-Ken, Hitachi High-Technologies Corp., Japan). To observe their internal structure, microspheres were embedded in epoxy resin and then sliced with a blade before sputter-coating.

RESULTS AND DISCUSSION

The reverse micellar microencapsulation process reported in this study led to the formation of free-flowing, spherical microspheres. Fig. 1 illustrates the frequency distribution curves of the microspheres made of 0.45 and 0.6 g of PLGA75:25. Analyses of their size distribution indicated that their size mode was 88 μm, which was considered suitable for delivering bioactive agents *via* intramuscular or subcutaneous injections.

As far as the manufacturing process of the new microencapsulation technique is concerned, it is worth mentioning that a clear solution appears after CTAB, TH, water, and ethyl formate are simply mixed and then left still. This phenomenon substantiates that the formation of our reverse micelles occurs spontaneously. As a consequence, any mechanical stirring or shaking is not required. Such a feature gives the reverse micellar procedure an advantage over the conventional $W_1/O/W_2$ microencapsulation procedure: the latter procedure requires intensive dynamic or static emulsification to disperse an aqueous phase in an organic solvent in which PLGA polymer is dissolved.

Our earlier study has also corroborated that, despite its considerable water miscibility, ethyl formate is a useful solvent in preparing PLGA microspheres *via* a solvent

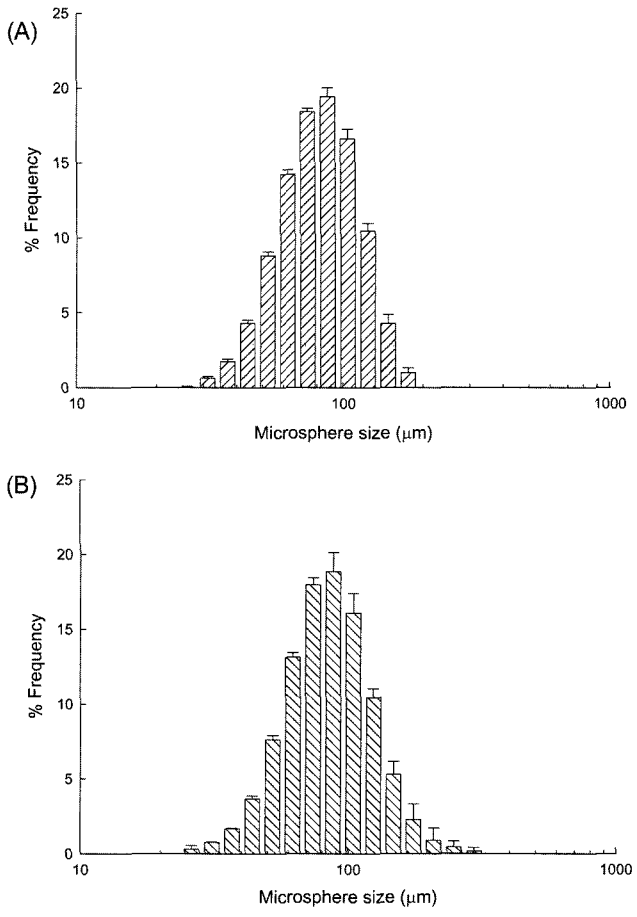

Fig. 1. The size distribution patterns of the microspheres made of (A) 0.45 and (B) 0.60 g of PLGA75:25 *via* the reverse micellar microencapsulation procedure

evaporation and quenching processes (Sah, 2000). The solvent is nonchlorinated and is not classified as a carcinogen. In addition, TH could not be dissolved in methylene chloride with the aid of CTAB under our experimental conditions. These are the reasons why our reverse micellar encapsulation process prefers ethyl formate to methylene chloride.

The conventional methylene chloride-based $W_1/O/W_2$ microencapsulation method yielded very poor microencapsulation efficiencies. In this study the loading efficiency of TH ranged only from 1.83 ± 0.10 to 3.45 ± 0.92% (Fig. 2A). Increases in the amount of PLGA75:25 from 0.3 to 0.75 g did not contribute to improving its encapsulation efficiency. Such poor encapsulation efficiencies attained in this study are consistent with those reported elsewhere (Esposito *et al.*, 1997). The substitution of TH with a more hydrophobic tetracycline free base also resulted in the attainment of low encapsulation efficiencies of less than 3%. Such poor encapsulation data might be caused by drug partition to the aqueous external phase during the process of microsphere hardening. Because the diffusion

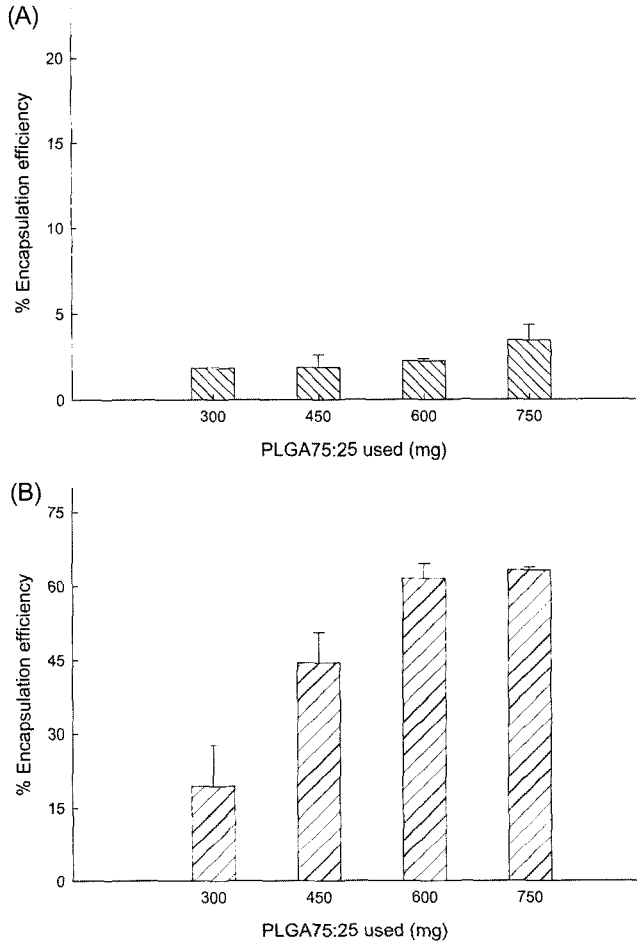

Fig. 2. Percentage of TH encapsulation efficiency observed with PLGA75:25 microspheres. (A) The methylene chloride-based double emulsion procedure was used to encapsulate TH into the microspheres. (B) The new reverse micellar encapsulation technique was used to load TH into the microspheres.

of methylene chloride out of the dispersed phase and evaporation to air occurred slowly, bioactive agents tended to leach to the W_2 phase. Such a poor loading efficiency could be considered one of the major obstacles preventing the successful loading of water-soluble drugs into PLGA microspheres *via* the double emulsion-based microencapsulation procedure.

Compared to the conventional methylene chloride-based procedure, however, the new reverse micellar procedure improved TH encapsulation efficiency to greater extents. To investigate the effect of PLGA75:25 upon the degree of its encapsulation efficiency, microspheres were prepared after various amounts of PLGA75:25 were dissolved in the reverse micellar ethyl formate solution. When microspheres were prepared with 0.3 g of PLGA75:25, 19.4 ± 8.31% of TH was loaded in the microspheres. Increases in PLGA75:25 content to 0.45, 0.6, and 0.75 g further enhanced its encapsulation efficiency to 44.42 ± 6.25, 61.53 ± 3.05, and 63.19 ± 0.64%, respectively (Fig. 2B).

In literature, several strategies have been suggested to improve the degree of microencapsulation efficiency of water-soluble drugs, when PLGA microspheres were prepared by emulsion-based microencapsulation processes. Examples of such attempts are either dissolving various electrolytes in an external phase or incorporating hydrophilic polymers into an inner aqueous phase in which a drug is dissolved (Ogawa *et al.*, 1988; Joly *et al.*, 1994; Freytag *et al.*, 2000; Al-Maaieh and Flanagan, 2001; Jiang *et al.*, 2002). Therefore, TH encapsulation efficiencies were examined with various amounts of NaCl dissolved in the W_2 phase. When PLGA75:25 microspheres were prepared under such experimental conditions, unanticipatedly extreme results were observed: TH loading efficiency declined considerably as an aqueous NaCl concentration increased (Fig. 3). For instance, when the aqueous NaCl concentration was increased from 0 to 7.5%, TH encapsulation efficiency was decreased from 63.19 ± 0.64 to 17.24 ± 3.76%, respectively. These results indicate that NaCl affects the transfer of TH across ethyl formate between two aqueous solutions, thereby influencing its encapsulation efficiency. It was previously reported that polar solutes solubilized in reverse micelles were transferred back into an external aqueous phase, when the nature of a salt in the aqueous phase was changed (Lesser *et al.*, 1986; Bausch *et al.*, 1992). It was also observed that ionic strength provided an important thermodynamic impact on their partitioning behavior: In case when reverse micelles were made of AOT, increasing the ionic strength of an external aqueous phase led to distortion of their structure. As a consequence, polar solutes tended to diffuse from the reverse micelles to the

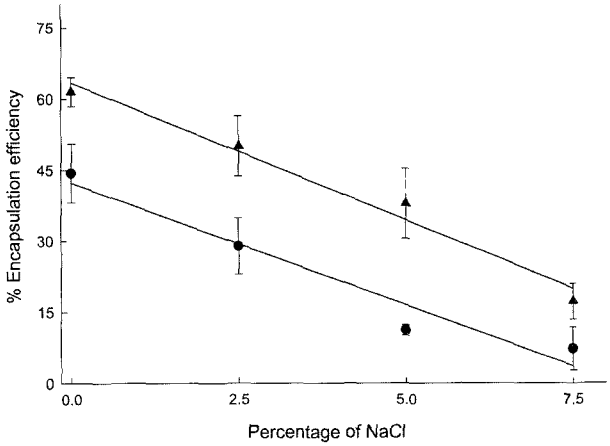

Fig. 3. Effect of an aqueous NaCl concentration upon TH encapsulation efficiency. As NaCl concentration increased, its encapsulation efficiency declined in a proportional manner. The microspheres were prepared from (●) 0.45 and (▲) 0.60 g of PLGA75:25, according to the new reverse micellar technique.

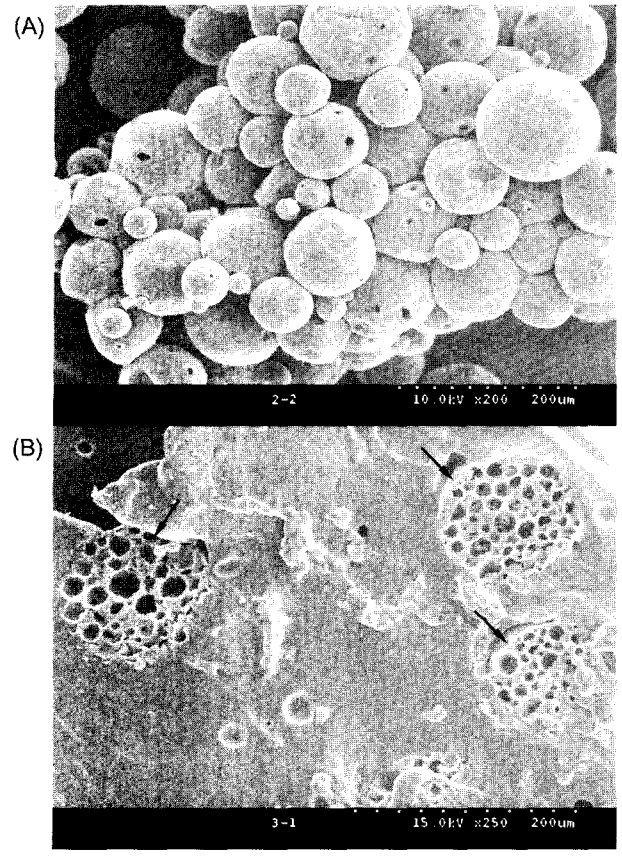

Fig. 4. SEM micrographs of the morphology of PLGA75:25 microspheres prepared following the methylene chloride-based emulsion procedure. Their surface has numerous pores (A), while their internal architecture is honey-combed (B).

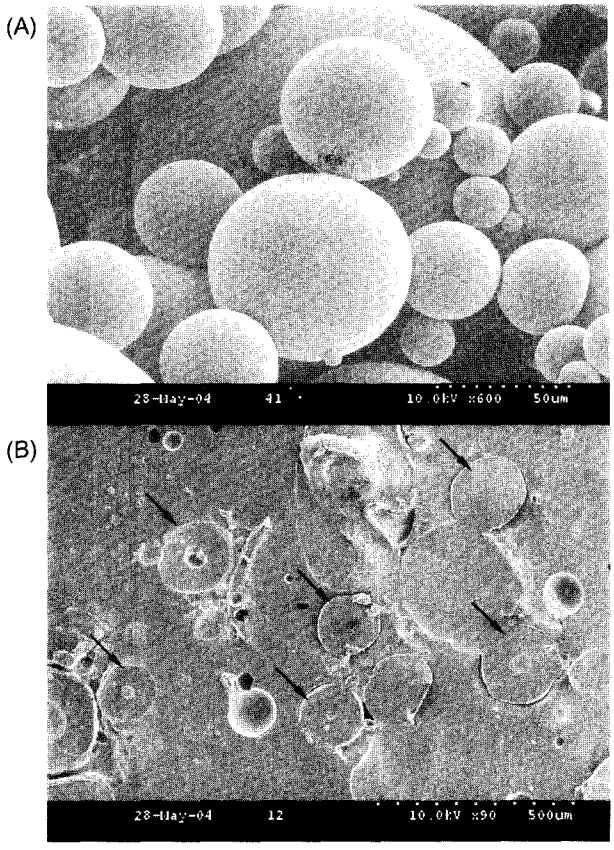

Fig. 5. SEM micrographs of the morphology of PLGA75:25 microspheres prepared via the reverse micellar encapsulation procedure. They had smooth and pore-free surfaces (A), and hollow cavities were absent from their internal matrices (B).

outer aqueous phase. Our encapsulation data also are in good agreement with these observations. It is postulated that high salt concentrations in the W_2 phase might generate osmotic pressure, thereby affecting the structure of reverse micelles. It is likely that this event serves as a driving force to facilitate the diffusion of TH to the surrounding W_2 phase. A detailed, relevant mechanism deserves further exploitation.

Fig. 4 illustrates the external and internal morphology of PLGA75:25 microspheres prepared following the conventional methylene chloride-based double emulsion process. The SEM micrographs demonstrate that their surface has numerous pores (Fig. 4A) and their internal architecture is honey-combed (Fig. 4B). Many hollow cavities present inside the microspheres originate from aqueous microdroplets (W_1) dispersed in the PLGA polymeric phase as a result of the first emulsification step. The external pores and internal cavities might be accountable for the poor encapsulation efficiencies shown in Fig. 2A: they function as pathways of TH leaching to the W_2 phase. By sharp contrast, when prepared following the ethyl formate-based reverse micellar procedure, PLGA75:25 microspheres possessed very smooth, pore-free surfaces (Fig. 5A). In addition, numerous hollow cavities observed in Figure 4 were absent from their internal matrices (Fig. 5B). Such morphology is understandable, if we consider that the reverse micelle-containing polymeric solution is single phasic. The absence of pores and interconnected cavities seems to be desirable qualities from the viewpoint of TH encapsulation efficiency.

CONCLUSIONS

The reverse micellar procedure reported in this study contributes to the successful microencapsulation of tetracycline hydrochloride into PLGA75:25 microspheres. Several attributes, being associated with manufacturing variables and microsphere characteristics, distinguish the ethyl formate-based reverse micellar technique from the conventional methylene chloride-based emulsion technique. In particular, the spontaneous formation of a reverse micellar ethyl formate solution possesses ramifications for the degree of TH loading efficiency and microsphere morphology. It is anticipated that the new reverse micellar

encapsulation technique might enable the incorporation of other water-soluble bioactive agents into PLGA microspheres.

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