Original Article

A new method of preparing TRH derivative-loaded poly(dl-lactide-co-glycolide) microspheres based on a solid solution system

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ABSTRACT: We investigated a new method of preparing peptide-loaded poly(dl-lactide-co-glycolide) microspheres with high encapsulation efficiency, low initial burst, and long-term sustained release by dissolving a peptide in a polymer by applying a solid solution system to the preparation of an oil phase. Solid solutions were prepared by dissolving a polymer (poly(dl-lactide-co-glycolide)) and a peptide (TRH derivative) in mixed solvents and then evaporating the solvents. Microspheres were prepared by an O/W emulsion solvent evaporation method, using the solution of the solid solution in dichloromethane as an oil phase. The state of the peptide in the solid solution and in the microspheres was evaluated by X-ray diffraction analysis. Release of the peptide from the microspheres was evaluated by an *in vitro* drug release test. Observation of the oil phase, X-ray diffraction analysis, and DSC analysis revealed that the peptides were dispersed in a molecular state in the solid solution and in microspheres with peptide loading of up to 15%. Encapsulation efficiency was over 90% for microspheres with peptide loading of up to 15%. The release of the peptide from the microspheres lasted over 21 days at least with the limited initial burst *in vitro*. High encapsulation efficiency, low initial burst, and long-term sustained release can be accomplished with microspheres prepared by a method based on a solid solution system.

Keywords: Microspheres, Peptide, Poly(dl-lactide-co-glycolide), Solid solution, Controlled release

Introduction

The technologies of long-term sustained release systems are useful in maximizing the efficacy of peptides, which have very short biological half-lives. Among the long-term sustained release systems, implantable microspheres made from biodegradable polymers such as polylactide and poly(lactide-co-glycolide) have been investigated by many researchers because of the ease of administration and their complete disappearance in the body. Although the polymers are insoluble in water, the polymers gradually degrade and erode in the body, releasing the microencapsulated drug.

Common techniques of microencapsulation are a phase separation method (*1*,*2*), a solvent evaporation method (*3*,*4*), and a spray-drying method (*5*,*6*). For phase separation, a poor solvent for polymers is added into the polymeric solution containing drug particles (oil phase); consequently, microspheres are prepared by precipitating polymers around the drug particles. For solvent evaporation, a polymeric solution containing drug particles (oil phase) is emulsified into another liquid (*e.g.* water, oil); the microspheres are prepared by evaporating the solvent from the oil phase. For spray-drying, the polymeric solution containing drug particles (oil phase) is sprayed through a nozzle; the microspheres are prepared by drying the oil droplets in the air. Regardless of the technique chosen, preparation of the oil phase that serves as the polymeric solution containing the drug is crucial.

The preparation of an oil phase is one of the most important processes in the microencapsulation process. The state of a drug in an oil phase influences its state in microspheres. If fine particles are dispersed well in an oil phase, microspheres with a uniformly dispersed drug can be obtained. If large particles or aggregates are dispersed in an oil phase, microspheres with an ununiformly distributed drug may be obtained. The distribution of a drug in microspheres is responsible for the property of those microspheres. Microspheres with large particles or aggregates of a drug often have low encapsulation efficiency and a high initial burst (*7*,*8*).

For most peptide drugs, the preparation of an oil phase is complicated in terms of the insolubility in the solvents used for the oil phase. Two major methods were reported for the preparation of an oil phase

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containing peptides; the peptide particles were dispersed in organic solvents (*9,10*) or a peptide aqueous solution was emulsified in organic solvents (*11,12*). Whichever method is chosen, the particles or droplets must be reduced in size in order to obtain microspheres with high encapsulation efficiency and a preferable release profile (*13,14*). There are, however, few methods of reducing the size of peptide particles, such as the crystallization method. Moreover, fine particles easily aggregate, which leads to an increase in apparent particle size. With a peptide aqueous solution, particle size can be reduced by higher-speed emulsification, but the resulting droplets readily join with one another, which leads to an increase in droplet size.

In the field of the drug dissolution and absorption, an approach involving a solid dispersion system has been investigated for several decades in order to reduce drug particle size. Solid dispersion refers to the dispersion of one or more active ingredients in an inert carrier or matrix in a solid state (*15*). A solid dispersion system can suppress the growth of crystals to provide fine particles. Specifically, dispersing a drug in a molecular state in a matrix refers to a "solid solution" (*15*). In other words, a solid solution is the state in which a drug is dissolved in a matrix. If a solid solution system can be used to prepare an oil phase, then peptides should be dispersed as extremely fine particles (a molecular state) in an oil phase because the viscosity of the polymer hampers aggregation.

This study sought to develop a new method of preparing peptide-loaded microspheres by focusing on the use of a solid solution system. This study sought to demonstrate that a method of preparation based on a solid solution system would be useful in preparing peptide-loaded microspheres with high encapsulation efficiency, low initial burst, and long-term sustained release of a drug. Taltirelin, a TRH derivative (Figure 1), was chosen as a model peptide. Poly(dl-lactide-co-glycolide) (PLGA) served as a biodegradable polymer.

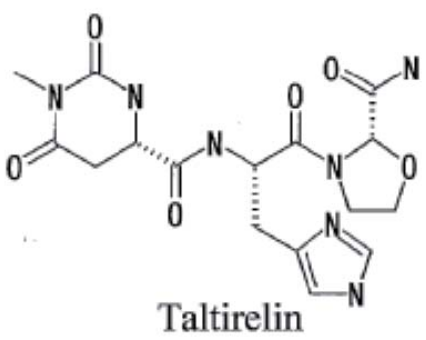

Figure 1. Structure of Taltirelin.

Materials and Methods

Materials

Poly(dl-lactide-co-glycolide) (PLGA, MW20,000, lactide:glicolide = 1:1; Wako Pure Chemicals Industries Ltd., Japan) was used as a polymer. Poly(vinyl alcohol) (PVA, EG-40; Nihon Synthetic Chemical Industries Ltd., Japan) was used as an emulsifier. Taltirelin was synthesized at Tanabe Seiyaku Co. Ltd., Japan. All other materials or solvents were of reagent grade.

Selection of mixed solvents for preparation of solid solution

Taltirelin and polymer (total amount: 0.5 g or 1.0 g) were dissolved in 2-2.5 mL of the mixed solvents, which consisted of a good solvent for the polymer and a good solvent for Taltirelin. The obtained solutions were evaporated under a nitrogen stream at 40°C and dried completely under vacuum overnight.

Preparation of microspheres

The procedure for preparation of microspheres is shown in Figure 2. Microspheres were basically prepared by an O/W emulsion solvent evaporation method. Briefly, the solid solution (0.5-1 g) [prepared from a mixture of the solvents dichloromethane and ethanol (4/1)] was dissolved in dichloromethane (1-2 g) to obtain an oil phase. The oil phase was emulsified into the PVA solution (150 mL or 400 mL) by a homogenizer (Polytron®, Kinematica Ag Littau, Switzerland) for 3 min at 8,000 rpm at 15°C. Then, the temperature was gradually increased to 30°C with stirring to remove dichloromethane. The hardened microspheres were washed with deionized water and collected by centrifugation. The obtained microspheres were re-dispersed in deionized water and lyophilized.

X-ray diffraction analysis

The intensity of X-ray diffraction from the solid solution and the microspheres was measured as a function of diffraction angles by X-ray powder diffraction (MXP3VA, Mac Science Ltd.).

Measurement of glass transition (Tg) of microspheres

The Tg of microspheres was measured by Differential Scanning Calorimetry (DSC, Thermo Flex, Rigaku Corporation) with a temperature elevation rate of 10°C.

Microscopic observation

The cross section of microspheres was prepared by slicing microspheres with razor-edge manually. The microspheres and the cross section of the microspheres were coated with gold-palladium by sputtering at 15

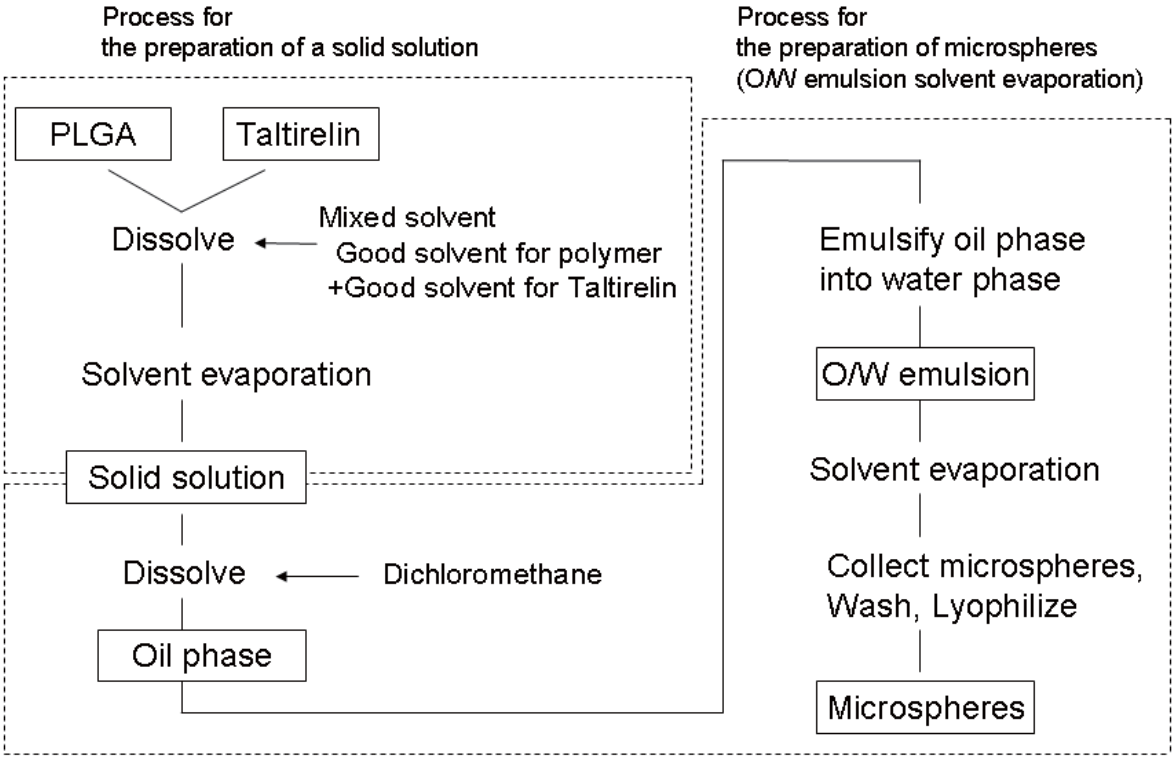

Figure 2. Procedure of the preparation for microspheres based on solid solution system.

mA for 3 min. The resulting samples were observed with a scanning electron microscope (S-2250N, Hitachi Ltd., Japan).

Determination of microsphere size

The size of microspheres was determined using a laser diffraction particle size analyzer (SALD-1100, Shimadzu Co. Ltd., Japan).

Determination of peptide contents in microspheres

The microspheres containing Taltirelin (10 mg) were dissolved in dichloromethane (2 mL) and extracted with phosphate buffer (μ = 0.15, pH 7.4) (5 mL) for 30 min. The aqueous layer was assayed for the Taltirelin concentration by the HPLC method described in "Determination of peptides by the UV-HPLC method."

In vitro release study

Microspheres containing Taltirelin were weighed out in test tubes. The test tubes were filled with phosphate buffer (μ = 0.15, pH 7.4) (10 mL) and then stirred at 25 rpm in an air chamber kept at 37 ± 1°C. Each test tube was taken out at the predetermined interval. The buffer was removed and the residual Taltirelin in microspheres was determined by the method described in "Determination of peptide contents in microspheres." Although the recovery of Taltirelin remained almost 100% during the release study, the release of Taltirelin in this study was calculated based on the difference between initial peptide content and residual peptide content.

Determination by the UV-HPLC method

Samples were analyzed with a 5C18 Nucleosil analytical column (4.6 × 150 mm, GL science). HPLC conditions for the determination of Taltirelin were as follows: flow rate, 1.0 mL/min; injection volume, 50 μL; detection wavelength, 210 nm; column temperature, 40°C; eluent, a mixture of acetonitril and 50 mM phosphate buffer (pH 2.5) (5:40) containing 0.13% sodium 1-octansulphonic acid.

Results

Preparation of solid solutions and oil phases

Solid solutions were prepared from a mixture of solvent A (good solvent for the drug) and solvent B (good solvent for the polymer). Table 1 shows the solvents mixed to prepare samples and the state of the samples in dichloromethane (oil phase). At 10% of Taltirelin loading, all samples, except for those prepared from ethyl acetate-ethanol (4:1) and dichloromethane alone, appeared transparent when

Table 3. Effect of the composition of the mixed solvents on the state of oil phase

Composition of mixed solvebts	The mixed solvents (Taltirelin + PLGA)	Loading % of Taltirelin	State of oil phase
Ethanol/Dichloromethane (1/4)	2.5 mL/0.5 g	10%	transparent
Water/Acetonitrile (1/10)	2.5 mL/0.5 g	10%	transparent
Methanol/Acetonitrile (1/4)	2.5 mL/0.5 g	10%	transparent
Ethanol/Acetonitrile (1/4)	2.5 mL/0.5 g	10%	transparent
Ethanol/Ethyl acetate (1/4)	2.5 mL/0.5 g	10%	Suspension
Dichloromethane*	2.0 mL/1.0 g	10%	Suspension
Ethanol/Dichloromethane (1/4)	2.0 mL/1.0 g	2.5%	transparent
Ethanol/Dichloromethane (1/4)	2.0 mL/1.0 g	15%	transparent
Ethanol/Dichloromethane (1/4)	2.0 mL/1.0 g	20%	Suspension
Ethanol/Dichloromethane (1/4)	2.0 mL /1.0 g	30%	Suspension
Ethanol/Dichloromethane (1/4)	2.0 mL /1.0 g	50%	Suspension

* not dissolve Taltirelin.

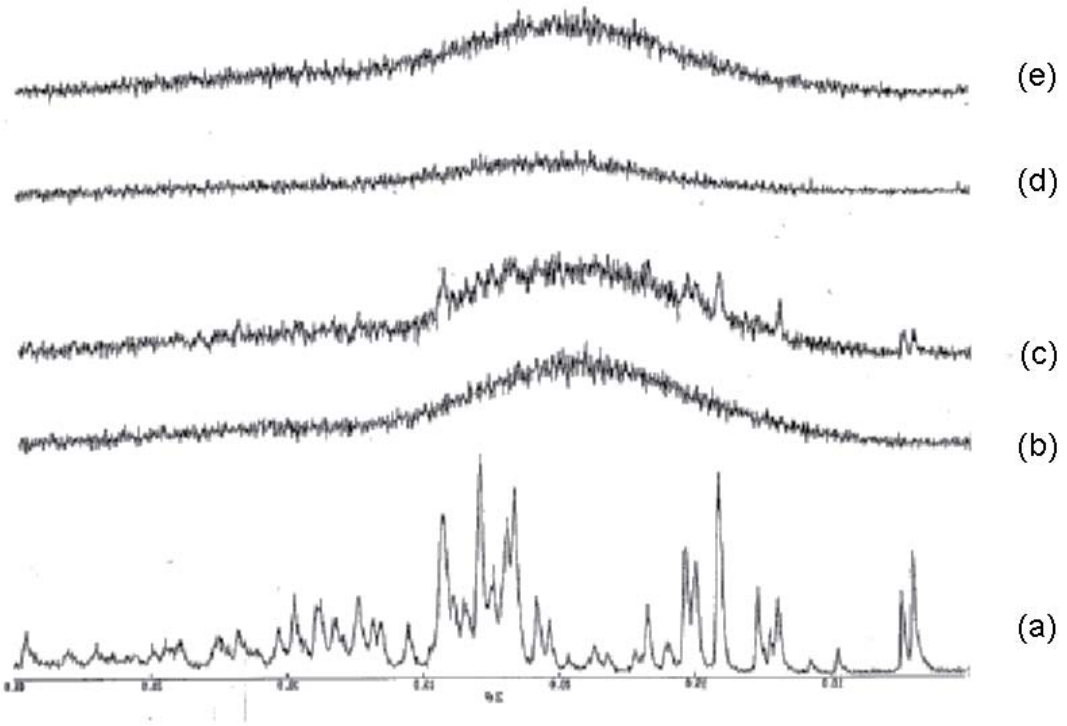

Figure 3. X-ray diffraction spectra. (a) Taltirelin, (b) PLGA, (c) physical mixture (Taltirelin: PLGA5020 = 1:9), (d) solid solution (Taltirelin: PLGA = 1:9), (e) Taltirelin-loaded microspheres (Taltirelin: PLGA = 1:9).

dissolved in dichloromethane. The resultants prepared from dichloromethane and ethanol (4:1) provided a transparent oil phase at a Taltirelin loading of up to 15%. The obtained sample prepared by dichloromethane and ethanol (4:1) at 10% of Taltirelin loading was analyzed by X-ray powder diffraction. No peaks of Taltirelin were detected for the solid solution although peaks of Taltirelin were detected for the physical mixture, as shown in Figure 3.

Characteristics of microspheres

The morphological features of microspheres prepared from the transparent oil phase, are shown in Figure 4. No drug particles were found on the surface and the inside of the microspheres. X-ray powder diffraction analysis showed no peak in Taltirelin, as shown in Figure 3.

The Tg of the microspheres increased for Taltirelin loading of up to 15% (Figure 5). The microspheres have 50% greater size of approximately 15 μm (Figure 6).

Encapsulation efficiency and in vitro release of Taltirelin

The efficiency of Taltirelin encapsulation in the microspheres prepared from the oil phase of the solid solutions was over 90% for Taltirelin loading of up to

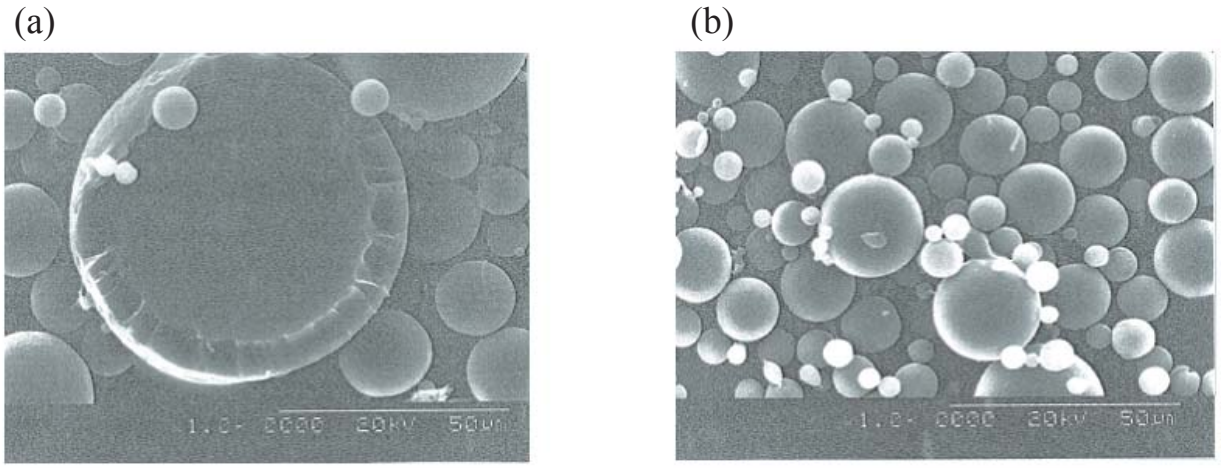

Figure 4. Scanning electron photomicrograph of Taltirelin-loaded microspheres; (a) cross section, (b) surface.

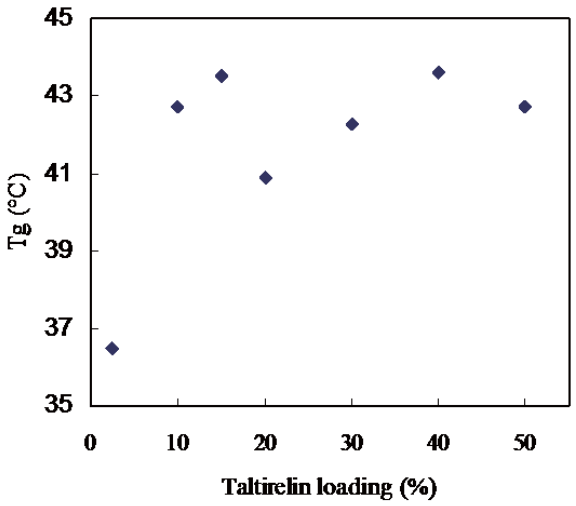

Figure 5. Effect of Taltirelin-loading on the Tg of Taltirelin-loaded microspheres.

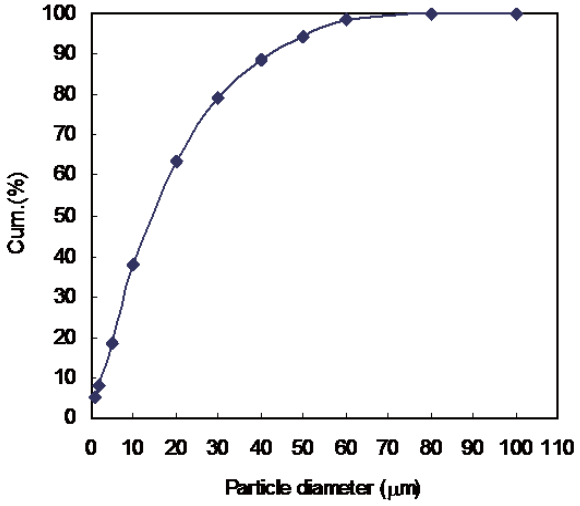

Figure 6. Particle diameter of Taltirelin-loaded microspheres (Taltirelin: PLGA = 1:9).

Table 2. Effect of drug loading on encapsulation efficiency

Loading of Taltirelin (%)	Encapsulation efficiencies
2.5	103.5
10	90.1
15	101.7
20	62.9
30	43.0
50	18.3

15% (Table 2), while that in microspheres from the conventional oil phase (dispersing peptide in solid) was 46.7% for 10% loading. The *in vitro* release of Taltirelin from microspheres is shown in Figure 7. The amount of Taltirelin released on day 1 was 7.7 ± 5.7% of microencapsulated Taltirelin (n = 3 batch). The release of Taltirelin from microspheres lasted over 21 days.

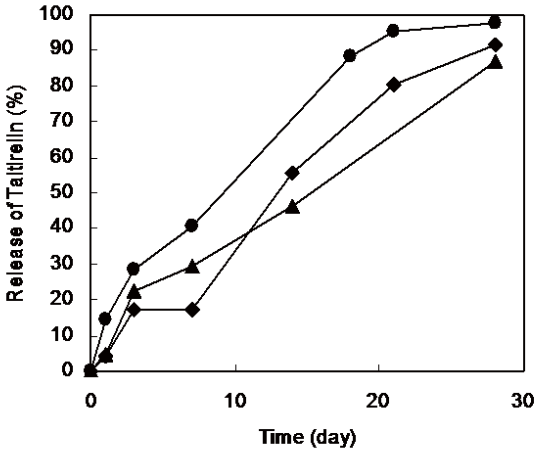

Figure 7. Release profiles of Taltirelin from Taltirelin-loaded microspheres (Taltirelin: PLGA = 1:9). ●:batch1, ♦:batch2, ▲:batch3.

Discussion

Preparation of solid solutions and oil phases

In the field of microencapsulation, some problems still remain despite the amount of research conducted. One problem is low encapsulation efficiency. Another is 'initial burst,' which is the phenomenon whereby a large portion of the encapsulated drug is released in a short time just after administration. Low encapsulation efficiency leads to an increase in manufacturing costs and initial burst is suspected of leading to side effects. Dispersing fine particles in an oil phase without aggregation is believed to be an effective way of avoiding these problems (*13,14*). The current study investigated the used of a solid solution system in the preparation of microspheres. A solid solution system is a type of solid dispersion system. A solid dispersion system is thought to be able to provide reduced particle size for a drug in a polymer because it can suppress the growth of drug crystals because of the viscosity of the polymer. Among solid dispersion systems, a solid solution system is thought to result in a dispersed drug in a polymer in a molecular state. Solid dispersions / solid solutions can be obtained by the following processes: melting drugs and excipients and then cooling and solidifying (melting method), or dissolving drugs and excipients in solvents and then evaporating the solvents (solvent method) (*15*). The melting method requires high temperatures. In contrast, the solvent method can provide solid dispersions/solid solutions even at low temperatures. Here, a solvent method was used to prepare solid solutions because this method is thought to be suitable for peptides in terms of avoiding their thermal decomposition.

In this study, the resultants from the mixtures of dichloromethane or acetonitril (good solvent for polymer and poor solvent for Taltirelin) and water, methanol, or ethanol (good solvent for Taltirelin and poor solvent for polymer) appeared transparent when dissolved in dichloromethane, which is a poor solvent for Taltirelin. This is the most important finding of this study. Namely, the peptide was apparently dissolved in an oil phase, of which dichloromethane was a poor solvent for the peptide. This indicates that Taltirelin can be dispersed in a molecular state in PLGA by a solid solution system. This is confirmed by the fact that X-ray diffraction analysis did not detect Taltirelin particles in resultants.

Production of the Taltirelin-PLGA solid solution was limited by the choice of the mixed solvents and the % of drug loading. A mixture of solvents using ethyl acetate did not provide resultants that appeared to be transparent when dissolved in dichloromethane. With regard to drug loading, a preparation with Taltirelin loading of over 20% did not provide resultants that appeared to be transparent when dissolved in dichloromethane.

Characteristics of microspheres, encapsulation efficiency, and in vitro release of Taltirelin

In the microspheres prepared from the Taltirelin-PLGA solid solution, microscopic observation and X-ray diffraction analysis revealed that Taltirelin was dispersed in a molecular state in the matrix of the microspheres. The state of Taltirelin in the solid solution reflected the state of the peptide in the microspheres.

Drugs are often reported to precipitate during the evaporation process of microencapsulation, even if the drugs are dissolved in an oil phase (*16*). This phenomenon is due to the removal of the solvents that dissolve the drugs. In the case of the oil phase of the solid solution, the precipitation of Taltirelin did not occur as a result of the removal of solvent dichloromethane because it was unable to dissolve Taltirelin. Although Taltirelin apparently dissolved in the oil phase in this study, this is believed to be due to peptide-polymer interaction and not to dissolution of Taltirelin in dichloromethane. Regarding peptide-loaded microspheres, the following points have been noted: (a) the interaction between a peptide and a PLGA occurs through $-NH_2$ and -COOH, and (b) the interaction between the peptide and polymer contributes to long-term sustained release (*17*). The finding that the Tg of microspheres increased for Taltirelin loading of up to 15% supports the interaction between Taltirelin and PLGA.

Regarding the characterization of microspheres, the present study revealed that the obtained microspheres show high encapsulation efficiencies and long-term sustained release without initial burst *in vitro* as a result of use of the solid solution system in the microencapsulation of Taltirelin. Efficient interaction is believed to have led to the high encapsulation efficiency, low initial burst, and long-term sustained release.

Advantages of the current method

In light of its use in the current study, there are several major advantages of a method based on a solid solution system. Peptides that can only be dissolved in water and water-miscible solvents (*e.g.* ethanol) can be microencapsuled in water-insoluble polymers such as polylactide and PLGA by an O/W emulsion solvent evaporation method. Another advantage is that an oil phase with a high viscosity can be obtained. An oil phase consisting of mixed solvents [a good solvent for the peptide (*e.g.* ethanol) and a good water-immiscible solvent for the polymer (*e.g.* dichloromethane)] can be used in an O/W emulsion solvent evaporation method for microencapsulation. However, the oil phase of the mixed solvent may have lower viscosity because

a larger amount of the mixed solvents is needed to dissolve both the peptide and polymer. An oil phase with a low viscosity tends to leak its drug during the manufacture of microspheres (*18*). Moreover, a large amount of solvents results in longer time for the emulsion to harden. Therefore, the oil phase of the mixed solvents may provide the microspheres with lower encapsulation efficiency. In contrast, the new method based on a solid solution system requires less solvent to prepare an oil phase to dissolve both the polymer and drug. This leads to higher encapsulation efficiency because an oil phase with higher viscosity can be obtained.

The new method is useful because it takes advantage of the properties of peptides, namely (i) solubility in solvents that solve polymers and (ii) stability in solvents. This preliminary study confirmed that several peptides (*e.g.* LH-RH and calcitonin) can be used in microencapsulation via the new method.

Conclusion

Results indicated that a solid solution system substantially reduced the particle size of Taltirelin and resulted in its apparent dissolution in the oil phase. Using a Taltirelin-PLGA solid solution to prepare microspheres provided peptide-loaded microspheres with high encapsulation efficiency, low initial burst, and long-term sustained release. These findings suggest that a method of preparation based on a solid solution system should prove useful in the microencapsulation of peptides.

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References

1. Deasy P. Microencapsulation *via* coacervation-phase separation. National Industrial Research Conference, Land O'Lakes, WI, 1966. June.
2. Ruiz JM, Tissier B, Benoit JP. Microencapsulation of peptide: A study of the phase separation of poly(dl-lactic acid-co-glycolic acid) copolymers 50/50 by silicone oil. Int J Pharm 1989; 49:69-77.
3. Beck LR, Cowsar DR, Lewis DH, Cosgrove RJ, Riddle CT, Lowry SL, Epperly T. A new long-acting injectable microcapsule system for the administration of progesterone. Fertil Steril 1979; 31:545-551.
4. Bodmeier R, McGinity JW. The preparation and evaluation of drug containing poly(dl-lactide) microspheres formed by the solvent evaporation. Pharm Res 1987; 4:465-471.
5. Bodmeier R, McGinity JW. Preparation of biodegradable polylactide microspheres using a spray-drying technique. J Pharm Pharmacol 1988; 40:754-757.
6. Nielsen F. Spray drying pharmaceuticals. Manuf Chem 1982; 53:38-39.
7. Yang YY, Chung TS, Ng NP. Morphology, drug distribution, and *in vitro* release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 2001; 22:231-241.
8. Wischke C, Borchert HH. Influence of the primary emulsification procedure on the characteristics of small protein-loaded PLGA microparticles for antigen delivery. J Microencapsul 2006; 23:435-448.
9. Kwong AK, Chou S, Sun AM, Sefton MV, Goosen MFA. *In vitro* and *in vivo* release of insulin from poly(lactic acid) microbeads and pellets. J Control Rel 1986; 4:47-62.
10. Bao W, Zhou J, Luo J, Wu D. PLGA microspheres with high drug loading and high encapsulation efficiency prepared by a novel solvent evaporation technique. J Microencapsul 2006; 23:471-479.
11. Gombotz W, Healy M, Brown. L. Very low temperature casting of controlled release microspheres. 1991; US Patent 5019400.
12. Okada H, Ogawa Y, Yashiki T. Prolonged release microcapsule and its production. 1987; US Patent 4652441.
13. Ogawa Y, Yamamoto M, Okada H, Yashiki T. Shimamoto T. A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly(lactic/glycolic) acid. Chem Pharm Bull 1988; 36:1095-1103.
14. Shukla AJ, Price JC. Effect of drug (core) particle size on the dissolution of Theophylline from microspheres made from low molecular weight cellulose acetate propionate. Pharm Res 1989; 6:418-421.
15. Chiou WL, Riegelman S. Pharmaceutical applications of solid dispersion system. J Pharm Sci 1971; 60:1281-1302.
16. Kyo M, Hyon SH, Ikada Y. Effects of preparation conditions of cisplatin-loaded microspheres on the *in vitro* release. J Control Release 1995; 35:73-82.
17. Heya T, Okada H, Tanigawara Y, Ogawa Y, Toguchi H. Effect of counteranion of TRH and loading amount on control of TRH release from copoly(dl-lactic/glycolic acid) microspheres preared by an in water drying method. Int J Pharm 1991; 69:69-75.
18. Herrmann J, Bdmeier R. Biodegradable, somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods. Eur J Pharm Biopharm 1998; 45:75-82.

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