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Research article

Biodegradable, somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods

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Abstract

Somatostatin, a therapeutic peptide drug, was entrapped within polymeric microspheres made from high molecular weight poly (D,L-lactide/glycolide) (PLGA) or low molecular weight poly (D,L-lactide) (PLA) by various modifications of the O/W-solvent evaporation method. The drug was either dispersed as solid (dispersion method), dissolved with the aid of a co-solvent (co-solvent method) or emulsified as an aqueous solution (W/O/W-multiple emulsion method) in the organic polymer solution prior to emulsification into an external aqueous phase. Additionally, a non-aqueous O/O-method was evaluated for the formation of the microspheres. Acceptable encapsulation efficiencies were obtained with all methods, regardless of the physical state of drug and the polymer type. The total volume of organic solvent and the co-solvent content were found to be important preparation factors of the O/W-co-solvent method. A more lipophilic solvent system appeared to favor efficient drug encapsulation. Replacing the widely used but toxic methylene chloride with ethyl acetate resulted in significantly lower drug loadings. The preparation method substantially affected the morphology of the microspheres and the drug release. © 1998 Elsevier Science B.V.

Keywords: Biodegradable polymers; Microencapsulation; Microparticles; Peptide delivery system; Polylactide; Solvent evaporation method; Somatostatin

1. Introduction

Considerable efforts are currently being invested in the development of drug delivery systems for peptide and protein drugs, a fact which is illustrated by the large number of contributions published in this field [1]. Despite the progress that has been achieved with nasal, pulmonary, rectal, and gastrointestinal delivery of short chain peptides, the parenteral route remains the predominant way to achieve sustained or controlled systemic delivery of these drugs, at least for the near future [2]. The most promising technical approaches are microparticles (preferably biodegradable), hydrogels, monolithic (self-diffusion) systems, and self-regulated devices [3]. Microspheres made from polylactic acid and poly-lactic-co-glycolic acid polymers containing LH–RH analogs have reached the highest degree of commercialization due to the favorable regulatory status of the matrix polymers, the well characterized method of fabrication and the pharmacodynamic/pharmacokinetic profile of these agonists, requiring low and long-lasting serum concentrations for therapeutic success. Somatostatin (somatotropin release-inhibiting factor, SRIF), the drug used in this study, is a peptide and gastrointestinal hormone with high therapeutic potential but very short in vivo half-life (1.1–3.0 min) [4]. Attempts have been made to develop long-acting analogs of somatostatin and depot forms of these molecules in order to increase the duration of action [5,6].

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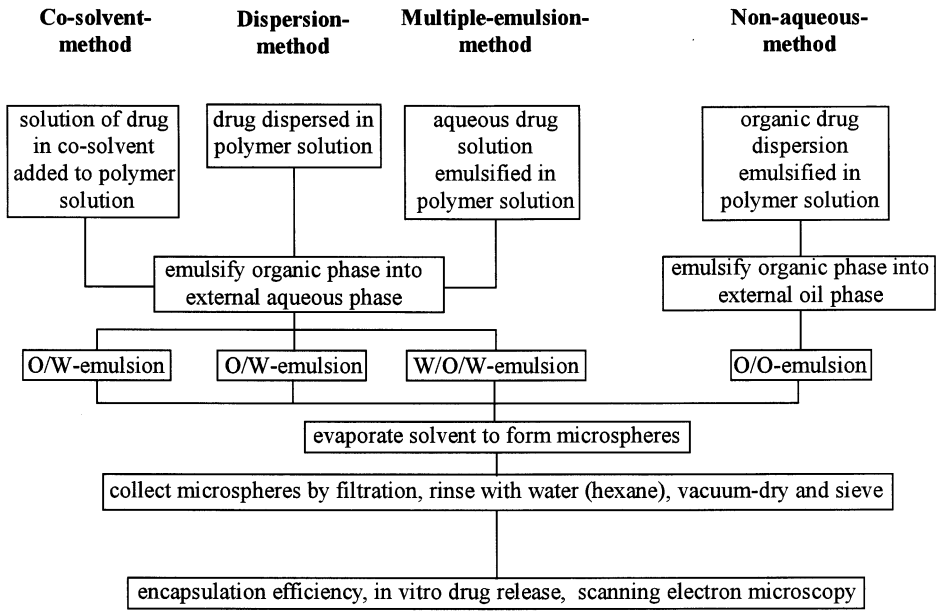

Fig. 1. Schematic diagram of the preparation of somatostatin microspheres with various types of solvent evaporation methods.

Several different methods for the preparation of microspheres are described in the literature, but the suitability of a particular technique is mainly determined by the solubility of the polymer and the drug which are usually fixed. The choice is further complicated when peptides and proteins have to be encapsulated. The potentially detrimental effect of several formulation and process parameters, such as type of organic solvent, shear forces, and temperature has to be taken into account [7]. The most popular methods for the preparation of microspheres from hydrophobic polymers are organic phase separation and solvent removal techniques. Depending on the way the solvent is removed, the latter can be subdivided into solvent evaporation, solvent extraction, spray-drying, and supercritical fluid technology [8–11]. In the classical solvent evaporation technique, a drug-containing organic polymer solution is emulsified into an aqueous phase containing a suitable stabilizer. Depending on the solubility properties of the drug, it can be either dissolved, dispersed or emulsified as an aqueous solution into the organic polymer solution [12,13]. The physical state of the drug in the polymer solution should have a major influence on the encapsulation efficiency and other properties of the microspheres (e.g. structure, drug release). Water soluble drugs can also be encapsulated by using a non-aqueous external phase of low drug solubility (e.g. oils) (O/O-solvent evaporation methods) [14,15].

Residual organic solvents in drug products for human use remain another important topic in the pharmaceutical field. Therefore, it would be desirable to replace methylene chloride, which is listed in the first draft of the ICH guidelines [16] as 'Class 2 solvent' ('Solvents to be limited'), with less toxic solvents like ethyl acetate, a 'Class 4 solvent' ('less toxic solvents').

From the above, it is apparent that more information is needed when preparing controlled release microspheres in order to choose the optimal method and preparation parameters. The objective of this study was to investigate various aqueous and non-aqueous solvent evaporation methods with respect to their suitability to entrap somatostatin acetate within biodegradable polyesters.

2. Experimental

2.1. Materials

The following materials were used as received: Somatostatin acetate (M_w of the free base 1638 D; Dr Willmar Schwabe, Karlsruhe, Germany), D,L-polylactide-co-glycolide (D,L-PLGA 85/15, $M_w = 87\,000$ D; Medisorb, Cincinnati, OH), D,L-PLA (Resomer R202; $M_w = 6000$ D, Boehringer Ingelheim, Ingelheim, Germany), poly(vinyl alcohol) (PVA, 88 mol% hydrolyzed, M_w 125 000 D, Polysciences, Worthington, PA), sorbitan monooleate (Span 80, HLB 4.3, ICI, Wilmington, DE), cottonseed oil (USP/NF, Gardena, US), methanol, methylene chloride, phosphoric acid, acetic acid, (EM, Gibbstown, NJ), ethanol (AAPER, Shelbyville, KY), ethyl acetate (Mallinckrodt, Paris, KY), acetonitrile, sodium acetate (Fisher, Fair Lawn, NJ).

2.2. Methods

Four modifications of the solvent evaporation method mainly differing in the type of organic solvent

Table 1
Preparation conditions of the standard formulations[a]

Preparation parameter	W/O/W-multiple emulsion method	O/W-co-solvent method	O/W-dispersion method	O/O-method
Internal aqueous phase (0.1 ml)	10 mM acetate buffer pH 5.9	—	—	—
Internal organic phase	CH_2Cl_2	CH_2Cl_2/methanol	CH_2Cl_2	Acetonitrile/methanol
Volume of internal organic phase (ml)	3.0	3.0	3.0	2.0
Co-solvent concentration (%v/v)	—	17	—	10
External phase	Water (800 ml)	Water (800 ml)	Water (800 ml)	Cottonseed oil (80 ml)
Stabilizer	0.25% w/v PVA	0.25% w/v PVA	0.25% w/v PVA	0.5% w/v sorbitan monooleate

[a] Polymer-150 mg, drug-3.0 mg, theoretical drug loading-2.0%.

and the physical state of the drug during microencapsulation (Fig. 1) were used. The standard formulation parameters are shown in Table 1. In the O/W-dispersion-method, the drug was dispersed as a solid in the organic polymer solution by probe sonication (sonicator, Heat Systems, Plainview, NY). In the O/W-co-solvent method, the peptide was dissolved in a suitable alcohol (methanol or ethanol) and added to the polymer solution resulting in a stable clear solution. In the W/O/W-multiple-emulsion method, the drug was dissolved in a small volume of acetate buffer, and this aqueous solution was emulsified into the polymer solution by probe sonication (30 s, ice-cooling). The resulting suspension, solution or W/O-emulsion was then emulsified into the external aqueous phase containing poly(vinyl alcohol) as an emulsifier with a magnetic stirrer (Corning PC-351, Corning, NY) to form the microspheres. The microspheres were collected by vacuum filtration after 30 min, rinsed with 30 ml water, vacuum dried for 3 days at room temperature, sieved (US Standard Sieve Series, Dual MFG, Chicago, IL) and stored in a desiccator.

In the O/O-method, the drug was first dissolved in methanol and then added to a polymer solution in acetonitrile, yielding a finely dispersed drug suspension. The dispersion was emulsified into the external cottonseed oil phase (containing sorbitan monooleate as an emulsifier) and continuously stirred for 45 min at room temperature and ambient pressure. The resulting microspheres were collected by vacuum filtration, thoroughly rinsed with hexane to remove the oil, dried and sieved as described above.

The drug content of the microspheres was determined by dissolving the microspheres (75–150 μm, approx. 4 mg accurately weighed) in 1.0 ml methylene chloride, followed by the addition of 1.0 ml of 1 M acetic acid and gentle agitation for 12 h at room temperature. The upper acetic acid phase was removed and analyzed with an HPLC method (see below). The compound recovery of this extraction method was 95–103%. The drug was stable at the relatively low pH of the acetic acid phase [17]. The drug concentration of the extraction solutions was determined with a stability sensitive HPLC procedure: LC-600-HPLC-pump, SIL-9A autoinjector, SPD-6A UV-detector, CR-601 integrator (Shimadzu, Kyoto, Japan), ET 250/8/4, Nucleosil 300-5 C18 column (Macherey and Nagel, Düren, Germany) equipped with a Vydac I-218TP guard column (Vydac, Hesperia, CA); mobile phase 75.5% v/v water, 24.0% v/v acetonitrile, 0.5% v/v phosphoric acid; flow rate 0.9 ml/min; UV-detection at 210 nm. Somatostatin solutions of known concentrations (0.01–0.2 mg/ml) were used to generate calibration curves. This method was checked with respect to linearity ($r > 0.99$), sensitivity (5×10^{-3} mg/ml), precision ($\pm$ 3% RSD) and accuracy (approx. 10% RSD) [18].

In vitro drug release profiles (up to 7 days) were obtained by incubating the microspheres (75–150 μm, 3–4 mg, accurately weighed, $n = 2$) in 1.0 ml isotonic pH 7.4 phosphate buffered saline (containing 0.05% w/v sodium azide as a preservative) at 37°C in Type-I glass vials in a horizontal shaker (Lab-Line, Mellrose Park, IL). Due to the instability of the peptide in the release medium and the small amounts released by certain formulations, the amount of drug remaining in the microspheres rather than the amount of drug released was determined. The microsphere suspension was centrifuged at each time point and the supernatant was carefully removed. The microspheres were then dried overnight at 37°C and extracted as described above. The peptide extracted from the microspheres after drug release was intact, no degradation products were detected in the extraction solution with the stability sensitive HPLC method.

To study the surface morphology of the particles, the microspheres were coated for 70 s with gold palladium (Pelco Model 3 Sputter Coater) and observed with a scanning electron microscope (SEM, Jeol JSM 35C).

Table 2
O/W-dispersion and O/W-co-solvent method

Solvent	Co-solvent	Physical state of the drug	Encapsulation efficiency (%)
Methylene chloride	None	Dispersed	82.5
Methylene chloride	Ethanol	Dissolved	82.7
Methylene chloride	Methanol	Dissolved	76.6
Ethyl acetate	None	Dispersed	63.7
Ethyl acetate	Ethanol	Dispersed	66.2
Ethyl acetate	Methanol	Dispersed	67.8

Effect of solvent and co-solvent type on the encapsulation efficiency of somatostatin acetate.

3. Results and discussion

Microencapsulation by the solvent evaporation method is, in principal, quite simple and involves two major steps, the formation of stable droplets of the drug-containing polymer solution and the subsequent removal of solvent from the droplets. In practice, however, the reproducible manufacturing of microspheres with the desired properties (good encapsulation efficiency, suitable release profile and particle distribution, acceptable solvent residuals), can be difficult, due to the large number of factors influencing the outcome, such as solvent composition, total volume and phase volume ratio of the phases, polymer concentration, type of stabilizer, stirring speed, stirring time etc. The effect of each of these parameters has to be determined empirically, predictions and scale up remain a problem. Therefore, more information is needed in order to identify the relevant parameters and save development resources.

Somatostatin is a hydrophilic, basic peptide drug with 14 amino acids (M_w of the free base 1638 D; isoelectric point pH 9.5). The drug was supplied as a sterile, freeze-dried solid, sealed in ampoules (acetate salt, purity > 97%, HPLC). Due to the high potency of the drug, the theoretical drug loading used in this study was low (2.0% w/w). The solubility properties of somatostatin were assessed in a previous study showing a high solubility in aqueous buffer solution pH 5.9 and methanol [19].

Three aqueous and one non-aqueous methods based on the concept of solvent evaporation were evaluated to prepare somatostatin containing microspheres (schematic representation of the methods, Fig. 1). The preparation conditions are summarized in Table 1. Because of the low solubility of somatostatin in methylene chloride, the drug was finely dispersed in the polymer solution with the aid of a probe sonicator, followed by emulsification of this dispersion into the external aqueous phase (O/W-dispersion method). The microspheres obtained with this method had an encapsulation efficiency of 82.5% (Table 2). Replacing methylene chloride with the more biocompatible ethyl acetate resulted in a significantly lower encapsulation efficiency of 63.7%. This could be caused by the higher solubility of ethyl acetate in water when compared with methylene chloride. Water could have diffused from the external aqueous phase into the droplets (at least at the droplet surface) causing a higher loss of drug. Scanning electron micrographs revealed that the type of solvent also affects the morphology of the microspheres. With methylene chloride, the particles are spherical and more uniform (Fig. 2a), while the use of ethyl acetate results in particles which appear to be partly collapsed (Fig. 2b). Both types of microspheres have a porous surface structure probably caused by the dissolution of drug particles located close to or embedded in the particle surface by the external aqueous phase during stirring.

The easiest way to encapsulate somatostatin would be to dissolve the drug in the polymer solution. Unlike, pure methylene chloride, a clear organic solution of the peptide and the polymer could be prepared by first dissolving the drug in methanol or ethanol and adding this solution to a polymer solution in methylene chloride (O/W-co-solvent method). Adding the peptide first to the alcohol was important since the peptide did not dissolve in a mixture of methylene chloride and alcohol. The peptide was probably solvated by the hydrophilic, hydrogen-bond forming alcohol clustering around the molecule, which could not be displaced by an excess of methylene chloride. Reversing the order of the addition (polymer solution added to drug solution) gave the same result (encapsulation efficiency = 76.8%). When ethyl acetate was used as the polymer solvent, the drug was precipitated from the alcoholic solution resulting in a turbid suspension. The more hydrophilic solvent, ethyl acetate, interacted with the alcohol and desolvated the peptide, which is practically insoluble in ethyl acetate.

Up to 50% v/v of methanol could be used as a co-solvent, higher concentrations lead to a precipitation of the polymer prior to droplet formation. The encapsulation efficiency decreased with increasing methanol content (Fig. 3). In general, a more lipophilic solvent or solvent mixture was favorable in preventing drug loss to the external aqueous phase. The encapsulation efficiency of microspheres prepared with ethyl acetate was lower than with microspheres prepared with methylene

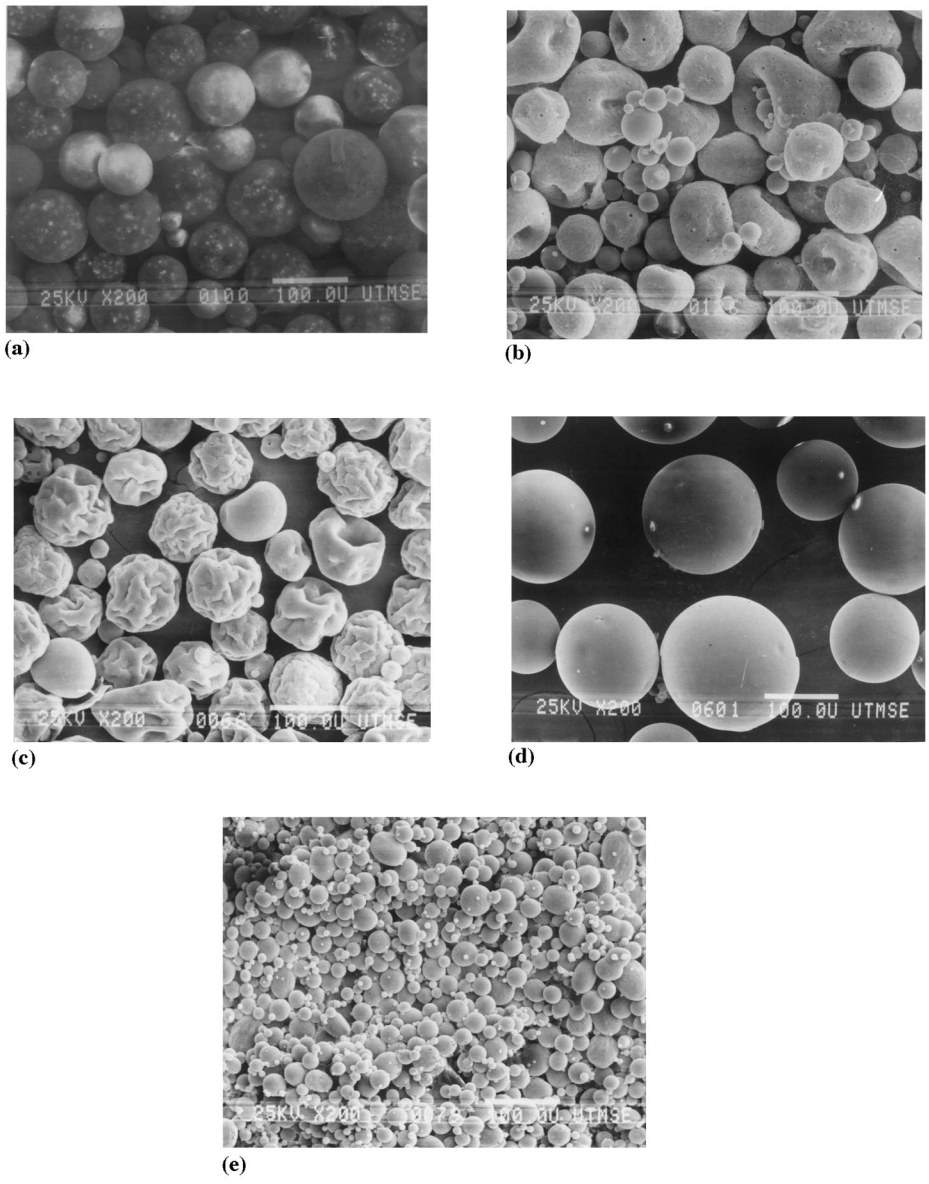

Fig. 2. Scanning electron micrographs of somatostatin containing microspheres prepared by various solvent evaporation methods (magnification 200 ×). Polymer: PLGA 85/15. (a) O/W-dispersion method, solvent-methylene chloride. (b) O/W-dispersion method, solvent-ethyl acetate. (c) O/W-co-solvent method, solvent-methanol/methylene chloride. (d) W/O/W-multiple emulsion method. (e) Non-aqueous O/O-method.

chloride, regardless of the type of co-solvent and the preparation method (Table 2).

Another important variable was the amount of organic solvent used, with the other parameters being constant (Fig. 4). The encapsulation efficiencies increased with decreasing amount of organic solvent to values close to 90%. This was probably caused by the increased viscosity of the organic drug/polymer solution at smaller amounts of organic solvent, which slowed down the diffusion of the peptide into the external aqueous phase. Less solvent could not be used, the increasing viscosity made it difficult to disperse the gel-like solution into the external aqueous phase. Microparticles prepared with the co-solvent method (standard preparation, Table 1) have a non-porous, wrinkled surface structure, and are hollow (Fig. 2c, cross-section not shown).

Alternatively, the microspheres were formed by a multiple W/O/W-emulsion technique. The influence of the most important process and formulation parameters of this method has been described in previous papers [19,20]. These microspheres had a comparable encapsulation efficiency of 84.4% (Table 3; all parameters constant, except drug emulsified in the polymer solution). This technique produced spherical particles with a smooth surface structure (Fig. 2d). As shown, the type and polarity of the organic solvent, among several other factors [22] such as the solvent removal

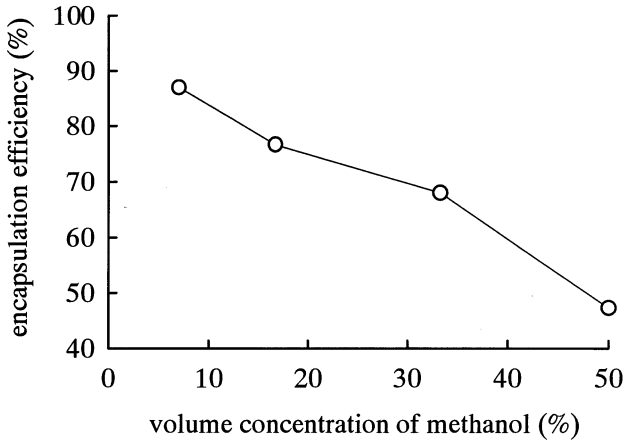

Fig. 3. Effect of the volume concentration of the co-solvent methanol on the encapsulation efficiency of somatostatin acetate. (O/W-co-solvent method, polymer-PLGA 85/15).

technique [20] and the polymer type [21], have a significant influence on the morphology of microspheres.

When the microspheres were prepared from a D,L-PLA polymer with a smaller molecular mass of 6.000 D compared with 87.000 D for the PLGA, very similar encapsulation efficiencies were achieved, except with the O/W-dispersion method (Table 3). Depending on the preparation technique, it seems possible to select a polymer according to the desired release and degradation properties, and to achieve high encapsulation efficiencies.

Although solvent evaporation methods using an aqueous external phase are favorable, the O/O-method may be useful when the use of water is prohibited due to stability problems or because of the high water solubility of the drug, which could lead to low drug loadings. Therefore, somatostatin was also encapsulated with a non-aqueous O/O-solvent evaporation technique. The drug was dissolved in methanol, and

Table 3
Effect of polymer type and preparation method on the encapsulation efficiency of somatostatin acetate

Preparation method	Encapsulation efficiency (%)	
	PLGA 85/15 (87 000 D)	D,L-PLA (6000D)
O/W-dispersion	82.5	64.1
O/W-co-solvent	76.6	74.6
W/O/W-multiple emulsion	84.4	83.5

this solution was added to a polymer solution in acetonitrile. As seen before with the methanol/ethyl acetate solvent system, the peptide did not stay in solution but precipitated to form a fine dispersion. Interestingly, in this method the volume of the internal organic phase was not an important factor with regard to the encapsulation efficiency. A larger internal volume slightly improved the encapsulation efficiency (Fig. 5). This could be explained by the fact that the peptide is essentially insoluble in the external oil phase and partitioning into the oil will be very limited. An almost constant fraction (approx. 30%) of the drug was not encapsulated. These microspheres were much smaller than the microparticles prepared with the aqueous solvent evaporation methods (Fig. 2e). This can be explained by the lower interfacial tension between the internal organic phase (acetonitrile) and the external oil phase (cottonseed oil) leading to smaller droplets and smaller microspheres after the solvent has been removed. A clear disadvantage of this method is the use of acetonitrile and hexane (used in the washing step to remove the oil from the surface of the microspheres), which are both 'Class 2 solvents' according to the ICH document [16].

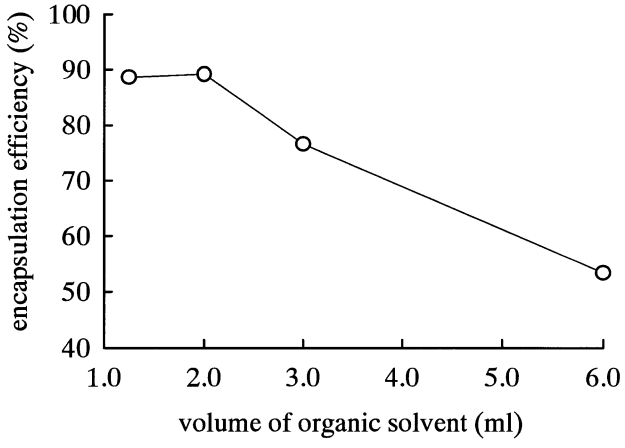

Fig. 4. Effect of the total volume of organic solvent on the encapsulation efficiency of somatostatin acetate. (O/W-co-solvent method, polymer-PLGA 85/15).

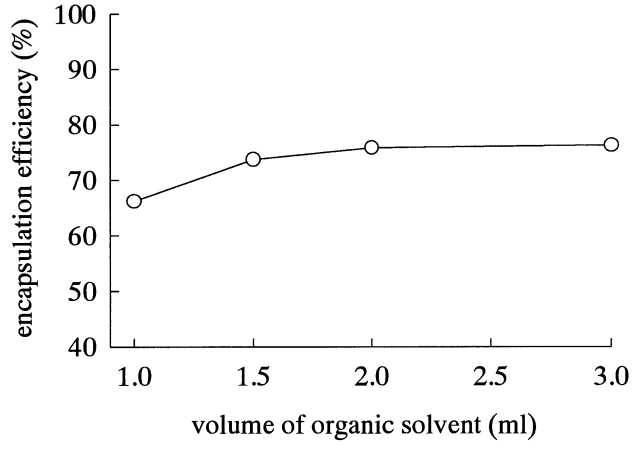

Fig. 5. Effect of the total volume of organic solvent on the encapsulation efficiency of somatostatin acetate. (O/O-method, polymer-PLGA 85/15).

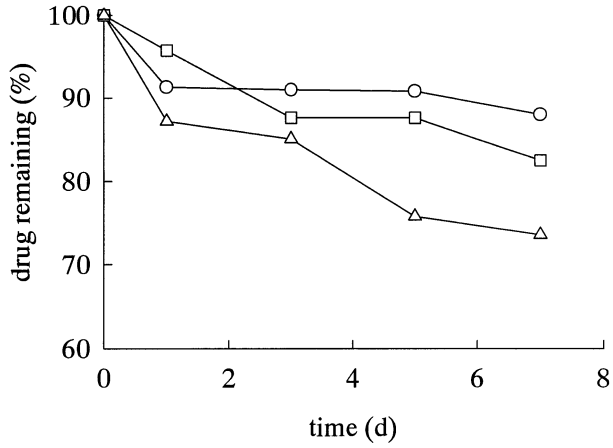

Fig. 6. In-vitro drug release from D,L-PLA 6000 microspheres. Effect of preparation method. (○), O/W-cosolvent method, drug loading = 1.4%; (□), W/O/W-multiple emulsion method, drug loading = 1.4%; (△), O/W-dispersion method, drug loading = 1.2%.

The preparation method will determine the microstructure of the microparticles and how the peptide is dispersed in the polymer matrix, (e.g. as drug particles or molecularly dispersed), which should affect the release properties of the microspheres. The initial phase of drug release (up to 7 days) was followed with preparations made from the low molecular weight D,L-PLA ($M_w = 6.000$ D). The microspheres showed an in vitro-release profile frequently seen with these systems, a small initial release of 5–15% was followed by a slower phase (Fig. 6). The microspheres prepared with the dispersion method showed the fastest release. This may be due to the porous surface structure of the particles not seen with the other preparations.

In conclusion, biodegradable microspheres containing somatostatin could be successfully prepared with high encapsulation efficiencies with various aqueous and non-aqueous solvent evaporation methods. By choosing and carefully evaluating the correct microencapsulation technique, it should be possible to prepare microspheres with the desired properties, regardless of the solubility and stability of the peptide drug to be encapsulated.

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