ELSEVIER

International Journal of Pharmaceutics 131 (1996) 171–181

international journal of pharmaceutics

Influence of the preparation method on residual solvents in biodegradable microspheres

Claudia Bitz, Eric Doelker*

School of Pharmacy, Université de Genève, Sciences II, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland

Received 3 July 1995; accepted 6 September 1995

Abstract

Spray-drying, the (w/o)w solvent evaporation method and the aerosol solvent extraction system (ASES) were used to study the influence of the preparation method on residual solvents in microspheres. Poly(L-lactic acid) and poly(DL-lactic-*co*-glycolic acid) (75:25) were selected as polymers and methanol, methylene chloride and chloroform were used as solvents to prepare drug-free and tetracosactide (model peptide) loaded microspheres. The residual solvent contents of the microparticles were determined by multiple headspace extraction from the solid samples. In all batches, residual methanol was well below the limit of 1000 ppm (Rabiant, 1991), whereas residual methylene chloride was only well below the USP XXIII limit of 500 ppm in batches prepared by spray drying at an inlet temperature of 60°C. The lowering of the inlet temperature to 50°C led to increased residual methylene chloride concentrations ranging from 300 to 700 ppm. In case of ASES even higher residual methylene chloride contents were obtained. However, when dried under mild conditions (3 days, room temperature, 35 kPa) all batches satisfied the requirements, with the exception of batches containing chloroform (prepared by spray drying at an inlet temperature of 60°C), which could not be efficiently removed. Additionally, morphology, particle size distribution, drug loading and encapsulation efficiencies of the batches prepared by the different methods were studied comparatively.

Keywords: Biodegradable microspheres; Poly(L-lactic acid); Poly(DL-lactic-*co*-glycolic acid); Residual solvents; Preparation method; Multiple headspace extraction; Headspace chromatography

1. Introduction

Various methods are described in the literature (Aftabrouchad and Doelker, 1992) for preparing biodegradable microparticles of poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA) for controlled release of water-soluble drugs. Although moderate conditions are used, appropriate for encapsulating sensitive drugs such as proteins or peptides, organic, potentially toxic solvents are indispensable to dissolve these lipophilic polymers. Only a few methods, foregoing organic solvents, such as the melting method (Wichert and Rohdewald, 1990) or the microparticle production after extrusion (Anderson et al., 1976; Smith and Hunneyball, 1986; Orsolini, 1993) are described. However, these methods require temperatures ex-

* Corresponding author. Tel.: (+41-22) 702 6148; fax: (+41-22) 702 6567.

SSDI 0378-5173(95)04320-A

ceeding 100°C and therefore applicable only to heat-resistant drugs.

The objective of this work was thus to study the influences of the preparation method, the drug content and the polymer type on residual solvents in microparticles, loaded with the model peptide tetracosactide, and to check in particular if the USP XXIII requirements (i.e. 50 ppm for chloroform and 500 ppm for methylene chloride) and the limit proposed by Rabiant (1991) for methanol (1000 ppm) are met. Therefore, spray drying the (w/o)w solvent evaporation method and the aerosol solvent extraction system (ASES) were selected as preparation methods. These methods lead to the formation of microspheres of the same size range and with a high drug entrapment efficiency, while using the same solvents (Pavanetto et al., 1993; Bleich et al., 1994; Prieto et al., 1994). L-PLA was selected, according to the results presented by Bleich et al. (1993), because it was the only polymer of the PLA/PLGA group leading to microparticles when using ASES as the preparation method. L-PLA, to our knowledge, is only soluble in such halogenated solvents as dichlormethane, chloroform and hexafluoroisopropanol (at room temperature), forcing the use of the same to get a solution. Accordingly, dichlormethane and chloroform were used in the preparation processes.

Multiple headspace extraction was an appropriate gas chromatography method for the determination of organic volatile impurities in a solid sample, not requiring their dissolution (Kolb et al., 1984). Multiple headspace extraction is based on a stepwise gas extraction procedure. The sample is placed in a tightly closed vial and heated above the boiling point of the residual solvents for a time sufficient to reach equilibrium in the gas phase. The vial is then put under pressure and vented after the injection of the gas phase, which is then analyzed. By repeating this procedure several times, the amount of solvent in the gas phase decreases exponentially from one injection to the following. Accordingly, only a few extraction steps are necessary to estimate the total peak area corresponding to the sum of the areas of an infinite number of injections as well as to the total solvent amount in the sample. To calculate this total peak area ΣA, the ln(peak area)'s of a solvent are plotted versus the injection numbers to obtain the slope k, which is dependent on the partition coefficient of the solvent and on instrumental parameters. This constant k is then entered into the following equation, where A_1 represents the peak area of the first injection:

$$\Sigma A = A_1/(1 - e^{-k}).$$

Tetracosactide, a peptide composed of the first 24 amino acids of ACTH, stimulates the surrenal cortex and is used to treat various diseases responding to corticotherapy, such as rheumatismal arthritis, dermatosis, colitis ulcerosa and is used diagnostically to investigate adrenocortical insufficiency (Morant and Ruppanner, 1994).

2. Materials and methods

2.1. Materials

The following substances and solvents were used in this work: tetracosactide (Synacthen®, Ciba Geigy, Basle, Switzerland), L-PLA (Medisorb® 100L, poly(L-lactic acid), Medisorb Technologies International, Cincinnati, OH, i.v. 1.0 dl/g), D,L-PLGA (Resomer® RG 755, poly(D,L-lactic-*co*-glycolic) 75:25, Boehringer, Ingelheim, Germany, i.v. 0.6 dl/g), poly(vinyl alcohol) (PVA) (Mowiol® 4-88, mol. wt. 26 000, Hoechst, Frankfurt, Germany), cupric sulphate pentahydrate puriss. p.a. (Fluka, Buchs, Switzerland), sodium hydroxide puriss p.a. (Fluka, Buchs, Switzerland), Tween-80 (Fluka, Buchs, Switzerland). The solvents were all of HPLC grade.

2.2. Preparation of microparticles

2.2.1. Spray drying

The hydrophilic drug was dissolved in 8 parts (v/v) of methanol and the polymer in 92 parts (v/v) of a mixture (1:1) of chloroform and methylene chloride (method I) or pure methylene chloride (method II). Then both solutions were mixed and spray dried using a Büchi® 190 laboratory spray dryer (Büchi, Flawil, Switzerland) to obtain the

microspheres. Details of spray drying conditions are presented in Table 1. Drug-free microspheres were prepared in the same way. A part of the microspheres was analyzed as such and an aliquot was dried in a desiccator for 3 days under vacuum (35 kPa) at room temperature.

2.2.2. (w/o)w Solvent evaporation method

Firstly, 0.005 g of tetracosactide (calculated on the active part) were dissolved in 0.4 ml of water, then added to 8 ml of the polymer solution, containing 0.495 g L-PLA or DL-PLGA dissolved in methylene chloride, and mixed for 2 min at 20 000 rev./min with an Ultra-Turrax® (Junke and Kunkel KG, Staufen i. Breisgau, Germany). The resulting primary emulsion was immediately poured into 300 ml of a PVA (4% (m/v)) solution and agitated for 10 min at 3000 rev./min with a Polytron® PT 6000 (Kinematica, Littau, Switzerland) leading to the (w/o)w emulsion system. The solvent was evaporated at 34°C under 20 kPa for 1 h using a Rotavapor® R-114 (Büchi, Flawil, Switzerland), connected to a Büchi 165 (Büchi, Flawil, Switzerland) vacuum controller. The microparticles were collected by filtration, washed three times with 100 ml of distilled water and dried for 3 days in a desiccator under vacuum, at room temperature.

Table 1
Parameter setting in spray drying method I (with methylene chloride and chloroform) and in method II (with methylene chloride only)

Parameter	Method I	Method II
Solution composition	8% (v/v) MeOH	8% (v/v) MeOH
	46% (v/v) CH_2Cl_2 46% (v/v) $CHCl_3$	92% (v/v) CH_2Cl_2
Solution concentration	3% (m/v)	3% (m/v)
Inlet temperature	60°C	50°C
Outlet temperature	50°	42–43°C
Air flow	700 Nl/h	700 Nl/h
Pump flow	2.5 ml/min	2.5 ml/min
Aspirator	15	15

2.2.3. Aerosol solvent extraction system (ASES)

To prepare the microspheres using the ASES, the apparatus described by Bleich et al. (1993) was used. The solution composition and concentration were exactly the same as those used for the spray drying method II (see above) and most of the apparatus parameters were those chosen by Bleich et al. (1994), i.e. column pressure 140 kPa, column temperature 34°C, pump lift 6 kg/h. The solution was sprayed into a continuous current of supercritical carbon dioxide phase and the microparticles formed were kept in the apparatus for 4 h to extract the solvents. A part of the collected microparticles was additionally dried for 3 days in a desiccator under vacuum at room temperature.

2.3. Microparticle morphology

A 20-nm thick gold layer was applied to the microspheres fixed to a slab, and the microspheres were examined by scanning electron microscopy, using a JSM 6400 scanning microscope (Jeol, Tokyo, Japan).

2.4. Particle size analysis

Microspheres were blended with a few drops of polysorbate 80, dispersed in water and sonicated for 2 min before the determination of particle size distribution by laser diffraction (Mastersizer® X, Malvern Instruments Ltd., Malvern, UK).

2.5. Determination of drug content

To quantify the encapsulated tetracosactide, set at a theoretical drug loading of 1% (m/m) of the polymer weight in the microspheres, a modification of the biuret reaction described by Itzhaki and Gill (1964) was used.

After addition of 5 ml chloroform to about 25 mg of microspheres (triplicata were performed), the tetracosactide was extracted with 5 ml of water by shaking for half an hour. To separate the two phases, the mixture was centrifuged for 20 min at 4000 rev./min. Two millilitres of the aqueous phase were then mixed with 2 ml of an aqueous cupric sulfate solution (0.25 mg/ml) containing 20% (m/v) sodium hydroxide, to form a

biuret complex. The absorption of the complex was measured 60 min later at 270 nm with a Hewlett Packard 8452A diode array spectrophotometer.

2.6. Multiple headspace extraction of residual solvents

In the methods described in USP XXIII and European Pharmacopoeia (1996) for analyzing residual solvents, the material has to be dissolved in water or the solvent mentioned in the corresponding monograph. But no such monograph exists for PLA/PLGA or microparticles and additionally, L-PLA is only soluble in few halogenated solvents, as already mentioned in the introduction, justifying the direct analysis of the solid sample by multiple headspace extraction. However, the gas chromatography column used in this study corresponds to the one described in System A of the project monograph of the European Pharmacopoeia and its stationary phase was the same as G43 of the USP XXIII.

To determine the amount of the residual solvents, 50–60 mg of microspheres were weighed into 10-ml vials, closed airtight and analyzed by performing four extraction steps (injections) per sample. Each batch was analyzed twice. The method was adapted from Dennis et al. (1992), by changing the following parameters for the Headspace Sampler HP 7694 (Hewlett Packard): oven temperature 110°C, loop temperature 120°C, transfer line temperature 120°C. The parameters of the gas chromatograph 5890 Series II (Hewlett Packard) were set as proposed, with the exception of the oven temperature program: 40°C (6 min), 25°C/min to 225°C (2 min). The column was a DB-624, 30 m × 0.32 mm (i.d.), fused silica, 1.8-μm film (J and W Scientific, Fisons, Folsom, CA), and the detector a Trio-2 VG Masslab Automated Mass spectrometry (Fisons Instruments, Folsom, CA), a quadrupole mass spectrometer, working with an electron ionization technique, by scanning a mass range of 25–250 twice per second. To treat the data, a VG Biotech Labbase was used.

3. Results and discussion

3.1. Microparticle morphology

When L-PLA microspheres were prepared by spray drying method I, spherical particles with a generally smooth surface resulted if drug was added. Only a few of them showed a rough surface (Fig. 1a). In the case of drug free microspheres (Fig. 1b), the surfaces were very smooth, but some of the particles were erythrocyte-shaped and isolated polymer filaments could be seen. The best result for spray drying was attained by using the method II with DL-PLGA. The microspheres were of a spherical shape and had a smooth surface (Fig. 1c), whether they were drug loaded or not. However, when prepared with L-PLA, some of the bigger microspheres were very porous and a few had even a honeycomb like surface (Fig. 1d).

The (w/o)w solvent evaporation method led to particles of spherical shape with a porous surface (Fig. 1e–g) for both polymers. A few of the bigger particles were split in half and, as can be seen in Fig. 1f, hollow inside.

Microspheres with a smooth to slightly rough surface resulted when ASES was used as the preparation method, regardless of drug loading. The shape of these microspheres was rather oval than spherical and a few particles were fused with one or two other ones. According to Bleich et al. (1993), the use of DL-PLGA resulted in a polymer film covering the inside of the high pressure column and the collector of the apparatus, but no microspheres could be obtained.

3.2. Size distribution of microspheres

The typical size distributions of the microparticle batches prepared by spray drying, (w/o)w solvent evaporation method and ASES are presented in Fig. 2a,b and the corresponding values in Table 2. Spray drying led to the smallest microparticles (mean diameter about 3 μm) whereas (w/o)w solvent evaporation led to the biggest ones (mean

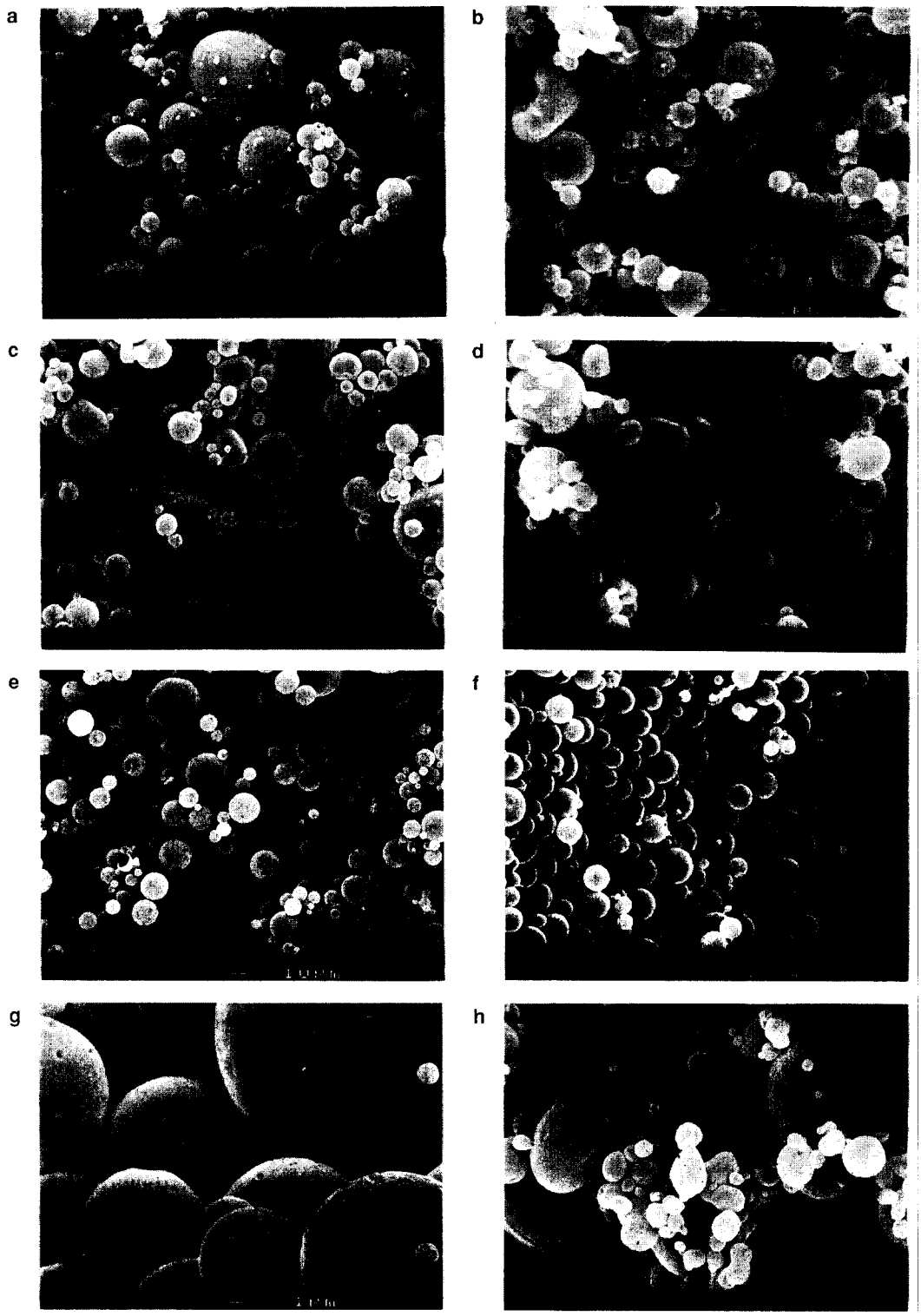

Fig. 1. Scanning electron micrographs of microparticles prepared by spray drying method I (a, L-PLA with tetracosactide; b, L-PLA, drug-free), spray drying method II (c, DL-PLGA with tetracosactide; d, L-PLA with tetracosactide), (w/o)w solvent evaporation (e, L-PLA with tetracosactide; f,g, DL-PLGA with tetracosactide) and by ASES (h, L-PLA, drug-free).

diameter about 15 μm), but also having the narrowest size distribution, when ignoring the nanoparticle fraction. Only in the batches prepared by (w/o)w solvent evaporation method was the nanoparticle population well separated from the microparticle population and additionally, for the same method, in the case of DL-PLGA, the nanoparticle population was bigger (d (0.1) = 0.3 μm vs. 7.3 μm for L-PLA).

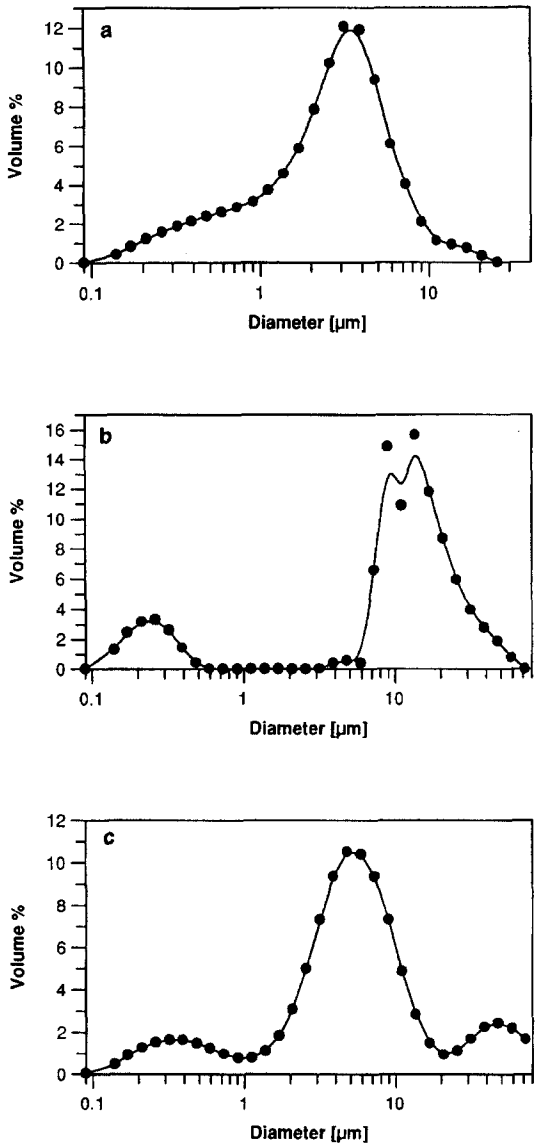

Fig. 2. Typical particle size distributions for microspheres prepared by (a) spray drying (method II, DL-PLGA with tetracosactide), (b) (w/o)w solvent evaporation method (DL-PLGA with tetracosactide) and (c) ASES (L-PLA with tetracosactide). The lines are drawn as a guide for the eye.

The slightly increased sizes of microspheres prepared by spray drying method I, compared to method II, may result from the lower vapor pressure of chloroform, leading to bigger droplets during spraying, even when working 10°C above the inlet temperature of method II.

In spite of the varying diameters of microspheres prepared by spray drying and ASES, the similarity of the particle size distributions (Fig. 2a,c) is remarkable. In fact, when analyzing the microspheres by image analysis (about 600 particles), the biggest particle prepared by ASES had a diameter of 14 μm only. This phenomenon can firstly be explained by the air fraction, which could not be removed for the calculations in the case of ASES batches (and (w/o)w solvent evaporation batches), as the bubble population overlapped the microsphere population, but could be substracted in case of spray drying, because of better separation. Secondly the existence of big agglomerates, formed of fused microspheres, cannot be excluded completely. Even when in small number only, they would strongly affect the result of the mean diameter, as the frequencies are calculated in volume percent.

3.3. Drug encapsulation efficiency and yield of microspheres

In case of an aqueous tetracosactide solution, progressive degradation leads to increasing UV absorption at the maximum of 276 nm (Aftabrouchad, 1994). Consequently, when using this quantification method, if a degradation of the drug occured during the preparation of the microspheres, this would cause erroneous results for the drug encapsulation efficiency. Since the Bio-Rad assay was also found to be unsuitable for quantification in the desired concentration range (results not presented) the micro-biuret method was tested. Therefore, tetracosactide solutions of equal concentrations were stored for 9 days in an oven at 60°C and in a freezer at −18°C and then analyzed before and after biuret complexation. Whereas the untreated solutions, stored at 60°C,

Table 2
Size distribution of drug loaded (T) and drug-free microspheres prepared by spray drying methods I (SDI) and II (SDII), by the (w/o)w solvent evaporation method and by ASES

Batch	Preparation method	Mean diameter	Diameter (0.1)	Diameter (0.5)	Diameter (0.9)
L-PLA/T	SDI	4.6	0.5	3.6	12.7
L-PLA	SDI	4.2	0.5	3.5	9.8
L-PLA/T	SDII	3.0	0.5	2.4	5.6
L-PLA	SDII	3.4	0.4	2.8	6.6
DL-PLGA/T	SDII	3.3	0.5	2.8	6.3
DL-PLGA	SDII	4.0	0.5	3.3	7.9
L-PLA/T	(w/o)w	16.6	7.3	14.2	30.7
DL-PLGA/T	(w/o)w	14.1	0.3	12.5	27.4
L-PLA/T	ASES	10.1	0.6	5.2	29.6
L-PLA	ASES	12.3	0.6	5.4	41.7

Mean volume diameter (μm) and the volume diameters (μm) undersize of 10%, 50% and 90% of the particles are presented.

showed an increased absorption at 276 nm (+ 2.2%), when compared to the solutions stored at −18°C, no significant difference in absorption resulted for the solutions after complexation (Student's t-test). Accordingly, the micro-biuret method described above was considered as an appropriate method to quantify the encapsulated tetracosactide, as the preparation methods were not expected to induce higher degradation than storing at 60°C for 9 days.

The results of the drug encapsulation efficiencies, as well as the yields of the microspheres, prepared by the various methods, are presented in Table 3. Whereas spray drying led to encapsulation efficiencies of about 90% (of the theoretical drug content of 1% (m/m)) for both polymers, (w/o)w solvent evaporation resulted in a high encapsulation efficiency only for DL-PLGA (82%), but conversly in the worst one (33%) if L-PLA was used. Schugens et al. (1994) reported a similar phenomenon in the encapsulation of inidigocarmine, a water-soluble substance, using BSA as primary emulsion stabilizer. They explained it by the rejection of the hydrophilic drug from the L-PLA matrix as it crystallizes upon hardening, in contrast to the amorphous DL-PLGA. ASES led to a drug encapsulation efficiency of 66%.

High microsphere yields were obtained for the two batches prepared by (w/o)w solvent evaporation method and the one prepared by ASES: 77.5% and 83.2% for the first ones and over 80% for the latter one. In agreement with the studies of Pavanetto et al. (1993); Conte et al. (1994), yields of only 35–55% were achieved when using spray drying as the preparation method. A considerable part of the microspheres was always found to leave the apparatus with the air flow and another part to adhere on other apparatus elements than the collector. On the other hand, microspheres were difficult to collect because of electrostatic charges. One possibility to increase the yield would be to increase the concentration of the spray drying solution, but as the solution concentration also strongly influences the shape of the microspheres, modifications of this parameter are restricted within very narrow limits.

3.4. Residual solvents in the microspheres

As presented in Table 4 and Fig. 3a, the use of chloroform to prepare microspheres by spray drying led to much too high residual contents of this solvent and even after drying, the limit of 50 ppm was exceeded by several orders of magnitude. In contrast, the concentration of methanol and methylene chloride was of a few ppm's only before and after drying. However, when preparing the microspheres by the spray drying method II (Fig. 3b; Table 4) the methylene chloride content increased to the range of the 500 ppm limit (281–705 ppm), whereas residual methanol was still much below the limit of 1000 ppm (6–40 ppm). Once the microspheres were dried for 3 days at

Table 3
Yield and drug entrapment efficiency of the microspheres, prepared by spray drying method I (SDI) and II (SDII), (w/o)w solvent evaporation method and ASES

Batch	Preparation method	Yield (%)	Drug entrapment efficiency (%)
L-PLA/T	SDI	36	88.9 ± 1.1
L-PLA	SDI	37	–
L-PLA/T	SDII	55	95.6 ± 1.9
L-PLA	SDII	43	–
DL-PLGA/T	SDII	38	87.0 ± 1.3
DL-PLGA	SDII	42	–
L-PLA/T	(w/o)w	76	33.2 ± 0.4
DL-PLGA/T	(w/o)w	83	81.8 ± 2.1
L-PLA/T	ASES	86	66.1 ± 2.9
L-PLA	ASES	83	–
DL-PLGA/T	ASES	*	*

*No microspheres resulted.
The theoretical drug loading was set at 1% (m/m) calculated on polymer weight.

room temperature at 35 kPa, the residual methylene chloride decreased to low values (2 ppm for the L-PLA microspheres and 9–17 ppm for the DL-PLGA microspheres).

The (w/o)w solvent evaporation method (drying included) led also to low residual methylene chloride contents in microspheres (4 and 14 ppm) (Fig. 3c; Table 4). In accordance with the results of Thoma and Schlütermann (1991), the use of DL-PLGA in microsphere preparation led to increased residual solvent contents, when compared to those prepared with the semi-crystalline L-PLA (both for the solvent evaporation method and the spray drying method II).

In the microspheres prepared by ASES (Fig. 3c), the methylene chloride concentrations were above the residual solvent limit before drying, indicating that the selected extraction time of 4 h was not sufficient, but in any case, the residual content was well below the limit after drying (2 and 6 ppm). As the drug loading of about 1% (m/m) did not seem to influence the residual solvent content of L-PLA microspheres prepared by the (w/o)w solvent evaporation method or spray drying, the difference of residual methylene chloride content in microspheres prepared by ASES was considered as a consequence of variations of the separator setting, which was not very easy to control, during solvent extraction. Ruchatz et al. (1995) found residual methylene chloride concentrations in microspheres in the vicinity of the limit or even lower, when extracted for 4 h, but prepared under slightly different ASES conditions.

The very low ppm values of Table 4 are to be considered as good estimates rather than exact values, as in this cases the peak of the last injection of the four-injection series of multiple headspace analysis was below the range of linearity ($< 5–10$ ppm) or even in the range of the detection limit (1.5 ppm for methanol and 0.5 ppm for methylene chloride).

4. Conclusions

The preparation of microparticles by spray drying and ASES led to comparable particle size distributions, whereas the (w/o)w solvent evaporation method resulted in particles of slightly higher diameters. The highest encapsulation efficiencies, but the worst yields, were obtained for batches prepared by spray drying. Good yields resulted with the ASES and the (w/o)w solvent evaporation method, but the encapsulation efficiencies were lower and even very low, when microcapsules were prepared with L-PLA using the (w/o)w solvent evaporation method. No microspheres could be formed with DL-PLGA by ASES.

Of the three solvents used in this study to prepare the microparticles, methanol was the most problem free, since it always led to residual solvent concentrations much below the limit of

Table 4
Residual solvents in microspheres (ppm) prepared by spray drying method I and II, (w/o)w solvent evaporation method and ASES

Batch	Preparation method	MeOH (ppm)	CH_2Cl_2 (ppm)	$CHCl_3$ (ppm)	Conforming
L-PLA/T	SDI	6.9 ± 0.4	2.7 ± 0.2	5998 ± 82	No
L-PLA/T	SDI	5.9 ± 0.6	<0.5	1534 ± 51	No
L-PLA	SDI	4.8 ± 0.7	3.5 ± 0.0	5885 ± 332	No
L-PLA	SDI	2.9 ± 0.6	2.3 ± 0.1	934 ± 78	No
L-PLA/T	SDII	39.9 ± 1.8	295.0 ± 11.4	Not used	Yes
L-PLA/T	SDII	17.4 ± 1.4	2.3 ± 0.1	Not used	Yes
L-PLA	SDII	13.2 ± 0.6	280.8 ± 6.6	Not used	Yes
L-PLA	SDII	6.3 ± 0.6	2.3 ± 0.1	Not used	Yes
DL-PLGA/T	SDII	24.8 ± 1.1	705.1 ± 25.0	Not used	No
DL-PLGA/T	SDII	18.5 ± 0.6	16.5 ± 1.0	Not used	Yes
DL-PLGA	SDII	6.0 ± 0.1	463.1 ± 20.8	Not used	Yes
DL-PLGA	SDII	5.6 ± 0.8	8.8 ± 1.2	Not used	Yes
L-PLA/T	(w/o)w	Not used	4.2 ± 1.0	Not used	Yes
DL-PLGA/T	(w/o)w	Not used	14.4 ± 2.3	Not used	Yes
L-PLA/T	ASES	3.7 ± 0.9	758.3 ± 64.3	Not used	No
L-PLA/T	ASES	2.5 ± 0.5	2.0 ± 0.6	Not used	Yes
L-PLA	ASES	4.9 ± 0.4	5283 ± 187	Not used	No
L-PLA	ASES	3.2 ± 0.3	5.9 ± 0.3	Not used	Yes

The results of the microspheres dried for 3 days under vacuum are presented in italic. The conforming limits are 1000 ppm for methanol, 500 ppm for methylene chloride and 50 ppm for chloroform.

1000 ppm, whereas methylene chloride led to residual concentrations well below the limit of 500 ppm only in the batches prepared by spray drying method I (inlet temperature 60°C) and in the case of L-PLA when using spray drying method II (inlet temperature 50°C). If the microspheres were prepared with DL-PLGA by using spray drying method II or with L-PLA by using ASES, the methylene chloride concentrations were at the limit or even above. Chloroform, only used in the spray drying method with an inlet temperature of 60°C (method I) exceeded the residual solvent limit of 50 ppm by several orders of magnitude even after drying (3 days, room temperature, 35 kPa). In contrast, methylene chloride was very easy to remove by drying, leading in all batches to residual solvent concentrations lesser than the limits, owing to the rather porous structure of the microparticles. Compared to L-PLA, DL-PLGA had a higher affinity to methylene chloride in microparticles prepared under the same conditions. However, a tetracosactide loading of 1% did not influence the residual solvents in microspheres to any large extent. In contrast to this, Spenlehauer et al. (1986) observed a decrease from 3.8 to 0.2% of residual solvents by increasing the cisplatin loading up to 44%, for microcapsules prepared by a (w/o) solvent evaporation method. The residual solvent contents of the DL-PLGA microcapsules prepared by Thoma and Schlütermann (1992), also using a (w/o) solvent evaporation method, led to residual solvent concentrations (2.1%) comparable to those found by Spenlehauer et al. (1986). The work of Thoma and Schlütermann (1992) emphasizes additionally the importance of the inlet temperature in spray drying. They used an inlet temperature of only 23°C, resulting in a residual methylene chloride content of 0.3%. Takada et al. (1994) prepared microparticles by spray drying at an inlet temperature of 100–125°C, using a water-acetonitril mixture to dissolve the thyrotropin releasing hormone and the DL-PLGA polymer, leading to residual solvent concentration of 860–4100 ppm and < 20 ppm after vacuum drying at 50°C. In the microparticles they prepared by a (w/o)w solvent evaporation method, the residual methylene chloride content was of 0.1–0.3% and < 20 ppm

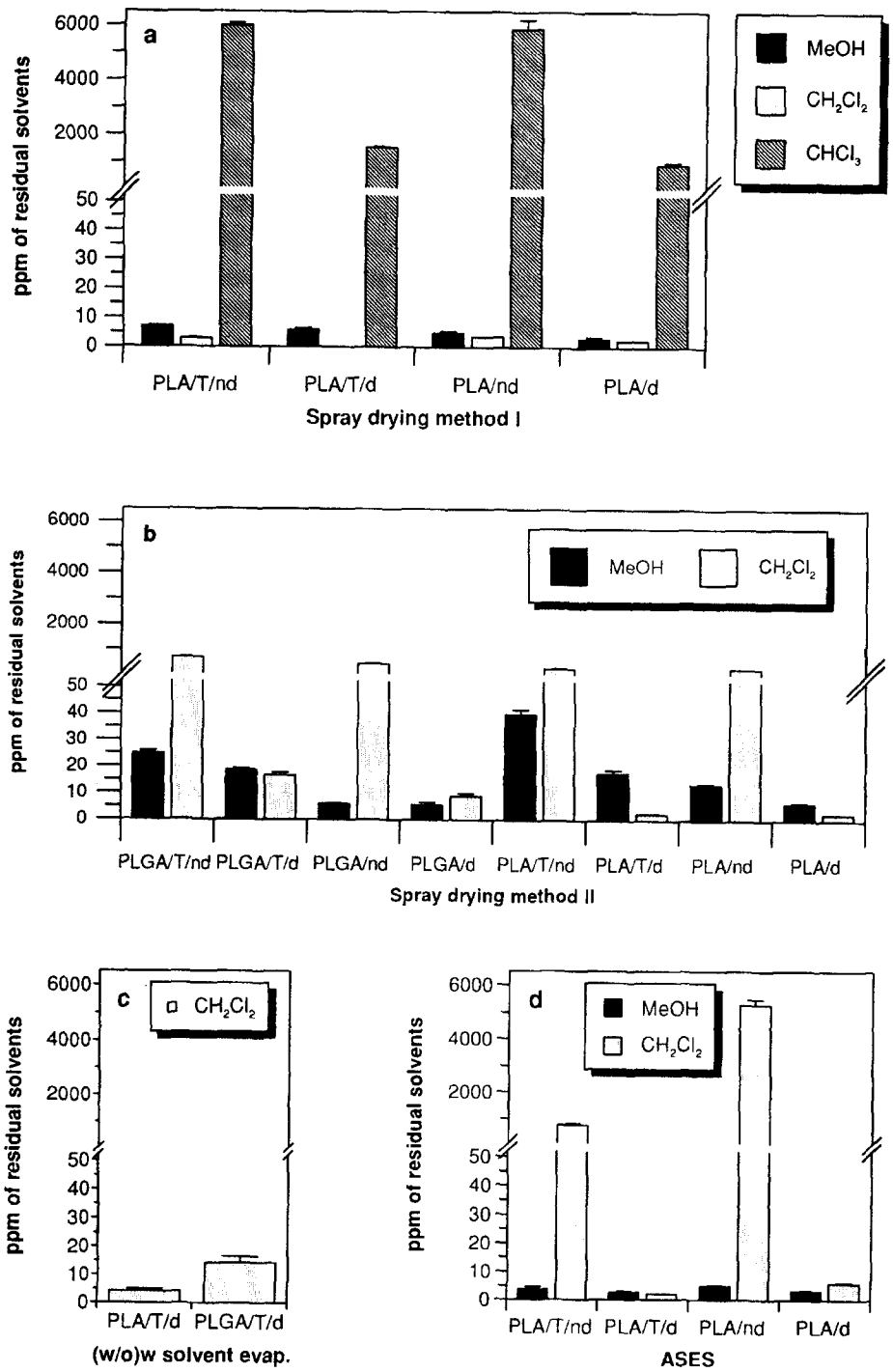

Fig. 3. Residual solvents in the microparticles prepared by (a) spray drying method I, (b) spray drying method II and (c) ASES and (w/o)w solvent evaporation method (PLA, L-PLA; PLGA, DL-PLGA; T, tetracosactide; nd, not dried; d, dried).

after drying. However, the concentration of their organic polymer solution was much higher than the one used in our preparation method.

Acknowledgements

Tetracosactide was a generous gift of Ciba-Geigy, Basle, Switzerland. We thank Professor B. W. Müller, Christian Albrechts Universität zu Kiel, Germany, for allowing us to use the ASES to prepare microspheres and Mr A. Engwicht of the same university for his technical assistance, Mr W. Klöti for his technical assistance in multiple headspace extraction and Mrs D. Massuelle of our laboratory for the preparation of scanning electron micrographs.

References

Aftabrouchad, S., *Préparation de Microparticules Biodégradables Chargées en Principes Actifs Hydrosolubles par Évaporation du Solvant, Séparation de Phases et Nébulisation*. Ph.D. Thesis, Université de Geneva, Geneva, 1994, No 2626.

Aftabrouchad, C. and Doelker, E., Méthodes de préparation des microparticules biodégradables chargées en principes actifs hydrosolubles. *S.T.P. Pharma Sci.*, 2 (1992) 365–380.

Anderson, L.C., Wise, D.L. and Howes, J.F., An injectable sustained release fertility control system. *Contraception*, 13 (1976) 375–384.

Bleich, J., Kleinebudde, P. and Müller, B.W., Influence of gas density and pressure on microparticles produced with the ASES process. *Int. J. Pharm.*, 106 (1994) 77–84.

Bleich, J., Müller, B.W. and Waßmuss, W., Aerosol solvent extraction system — a new microparticle production technique. *Int. J. Pharm.*, 97 (1993) 111–117.

Conte, U., Conti, B., Giunchedi, P. and Maggi, L., Spray dried polylactide microsphere preparation: influence of the technological parameters. *Drug Dev. Ind. Pharm.*, 20 (1994) 235–258.

Dennis, K.J., Josephs, P.A. and Dokladalova, J., Proposed automated headspace method for organic volatile impurities ⟨467⟩ and other residual solvents. *Pharmacopeial Forum*, 18 (1992) 2964–2972.

Itzhaki, R.F. and Gill, D.M., A micro-biuret method for examinating proteins. *Anal. Biochem.*, 9 (1964) 401–410.

Kolb, B., Pospisil, P. and Auer, M., Quantitative headspace analysis of solid samples: a classification of various sample types. *Chromatographia*, 19 (1984) 113–122.

Morant, J. and Ruppanner, H., *Compendium Suisse des Médicaments 1995*, 16th Edn., Documed SA, Basle, 1994, pp. 1832–1833.

Orsolini, P., Pharmaceutical composition in the form of microparticles. *US Patent 5 225 205*, 1993.

Pavanetto, F., Genta, I., Giunchedi, P. and Conti, B., Evaluation of spray drying as a method for polylactide and polylactide-co-glycolide microsphere preparation. *J. Microencapsulation*, 10 (1993) 487–497.

Prieto, M.J.B., Delie, F., Fattal, E., Tartar, A., Puisieux, F., Gulik, A. and Couvreur, P., Characterization of V3 BRU peptide-loaded small PLGA microspheres prepared by a (w1/o)w2 emulsion solvent evaporation method. *Int. J. Pharm.*, 111 (1994) 137–145.

Rabiant, J., La limitation des solvants résiduels: aspect réglementaire. *S.T.P. Pharm. Prat.*, 1 (1991) 278–283.

Ruchatz, F., Thies, J. and Müller, B.W., *Investigation of residual methylene chloride in microparticles produced by means of the ASES process*. Proceedings of the First World Meeting APGI/APV, Budapest, 1995, pp. 447–448.

Schugens, C., Laruelle, N., Nihant, N., Grandfils, C., Jérome, R. and Teyssié, P., Effect of the emulsion stability on the morphology and porosity of semicrystalline poly L-lactide microparticles prepared by w/o/w double emulsion-evaporation. *J. Control. Rel.*, 32 (1994) 161–176.

Smith, A. and Hunneyball, I.M., Evaluation of poly(lactic acid) as a biodegradable drug delivery system for parenteral administration. *Int. J. Pharm.*, 30 (1986), 215–220.

Spenlehauer, G., Veillard, M. and Benoît, J.-P., Formation and characterization of cisplatin loaded poly(D,L-lactide) microspheres for chemoembolization. *J. Pharm. Sci.*, 75 (1986) 750–755.

Takada, S., Yoshiaki, U., Toguchi, H. and Ogawa, Y., Preparation and characterization of copoly(DL-lactic/glycolic acid) microparticles for sustained release of thyrotropin releasing hormone by double nozzle spray drying method. *J. Control. Rel.*, 32 (1994) 79–85.

Thoma, K. and Schlütermann, B., Biodegradierbare Polymere als Retardierungsprinzip für Arzneistoffe. *Pharmazie*, 46 (1991) 821–830.

Thoma, K. and Schlütermann, B., Beziehungen zwischen Herstellungsparametern und pharmazeutisch-technologischen Anforderungen an biodegradierbare Mikropartikeln. *Pharmazie*, 47 (1992) 115–119.

Wichert, B. and Rohdewald P., A new method for the preparation of drug containing polylactic acid microparticles without using organic solvents. *J. Control. Rel.*, 14 (1990) 269–283.