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Preparation of microspheres by the solvent evaporation technique

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Abstract

The microencapsulation process in which the removal of the hydrophobic polymer solvent is achieved by evaporation has been widely reported in recent years for the preparation of microspheres and microcapsules based on biodegradable polymers and copolymers of hydroxy acids. The properties of biodegradable microspheres of poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) have been extensively investigated. The encapsulation of highly water soluble compounds including proteins and peptides presents formidable challenges to the researcher. The successful encapsulation of such entities requires high drug loading in the microspheres, prevention of protein degradation by the encapsulation method, and predictable release of the drug compound from the microspheres. To achieve these goals, multiple emulsion techniques and other innovative modifications have been made to the conventional solvent evaporation process. © 1997 Elsevier Science B.V.

Keywords: Microspheres; Solvent evaporation; Water soluble compounds; Peptides; Proteins; Multiple emulsion

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1. Introduction

The solvent evaporation method to produce microspheres of poly(lactic acid) (PLA), and its co-polymer poly(lactic-co-glycolic acid) (PLGA) has been studied extensively due to the biocompatibility of these polymers [1–4]. In the solvent evaporation process, the polymer is dissolved in a suitable water immiscible solvent, and the medicament is dispersed

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or dissolved in this polymeric solution. The resultant solution or dispersion is then emulsified in an aqueous continuous phase to form discrete droplets. In order for the microspheres to form, the organic solvent must first diffuse into the aqueous phase and then evaporate at the water/air interface. As solvent evaporation occurs, the microspheres harden and free flowing microspheres can be obtained after suitable filtration and drying.

The solvent evaporation method has been used extensively to prepare PLA and PLGA microspheres containing many different drugs [5–7]. Several variables have been identified which can influence the properties of the microspheres, including drug solubility, internal morphology, solvent type, diffusion rate, temperature, polymer composition and viscosity, and drug loading [8–12]. The effectiveness of the solvent evaporation method to produce microspheres depends on the successful entrapment of the active agent within the particles, and thus, this process is most successful with drugs which are either insoluble or poorly soluble in the aqueous medium which comprises the continuous phase [13]. Many types of drugs with different physical and chemical properties have been formulated into polymeric systems, including anti-cancer drugs [14,15], narcotic agents [16,17], local anesthetics [18], steroids [19,20], and fertility control agents [21,22].

Protein release from a bioerodible polymeric matrix can occur by diffusion through a tortuous water filled path through the polymeric matrix or through matrix erosion [23,24]. Recent advances in the solvent evaporation technique have allowed successful entrapment of highly water soluble drugs [25,26], reactive compounds such as amine based drugs [27], proteins [28,29], peptides [30,31], and vaccines [32,33]. This review will summarize the advances in the solvent evaporation technique and the characteristics and related properties of biodegradable microspheres produced by this method.

2. Preparation of microspheres by solvent evaporation

2.1. Conventional O/W encapsulation

The microencapsulation of pharmaceutical compounds with PLA or PLGA by the solvent evaporation method has been investigated extensively during the past 25 years. The premise for this method is the emulsification of a polymeric solution in an aqueous continuous phase. A schematic diagram of the method is shown in Fig. 1. The O/W emulsion is produced by the agitation of two immiscible liquids. The drug substance is either dispersed or in solution in the polymer/solvent system or is captured in the dispersed phase of the emulsion. Agitation of the system is continued until the solvent partitions into the aqueous phase and is removed by evaporation. This process results in hardened microspheres which contain the active moiety.

Several methods have been utilized to achieve dispersion of the oil phase in the continuous phase. The most common method is the use of a propeller style blade attached to a variable speed motor. As the speed of the motor is increased, the size of the dispersed droplets decreases as a result of the high shear induced by the propeller.

Homogenization is also used to produce an emulsion. With this type of dispersion system a homogenizer equipped with a rotor and stator type blade is attached to a variable high speed motor. Since high shear is used to produce the emulsion, the resultant product has a much smaller particle size than the emulsion produced by conventional agitation. Other methods include the use of a microfluidizer [34] to produce micro-emulsions, sonication [35], and potentiometric dispersion [36].

2.2. Influence of process parameters on the physicochemical properties of microspheres containing poorly water soluble compounds

The O/W emulsion solvent evaporation system has been successfully employed to encapsulate poorly water soluble drugs including chlorpromazine [6], prednisolone [2], and hydrocortisone [37]. The influence of surfactant ratios, rate of solvent evaporation, solvent type, and polymer molecular weight on the physicochemical characteristics, encapsulation efficiency, and release of insoluble drugs from biodegradable microspheres has been reported [38–40]. Sansdrap and Moës investigated the influence of several process parameters, including agitation rate, surfactant concentrations, organic phase volume and drug loading on microspheres of nifedipine, a poorly water soluble calcium-channel blocking agent [41]. The influence of these parameters on microsphere

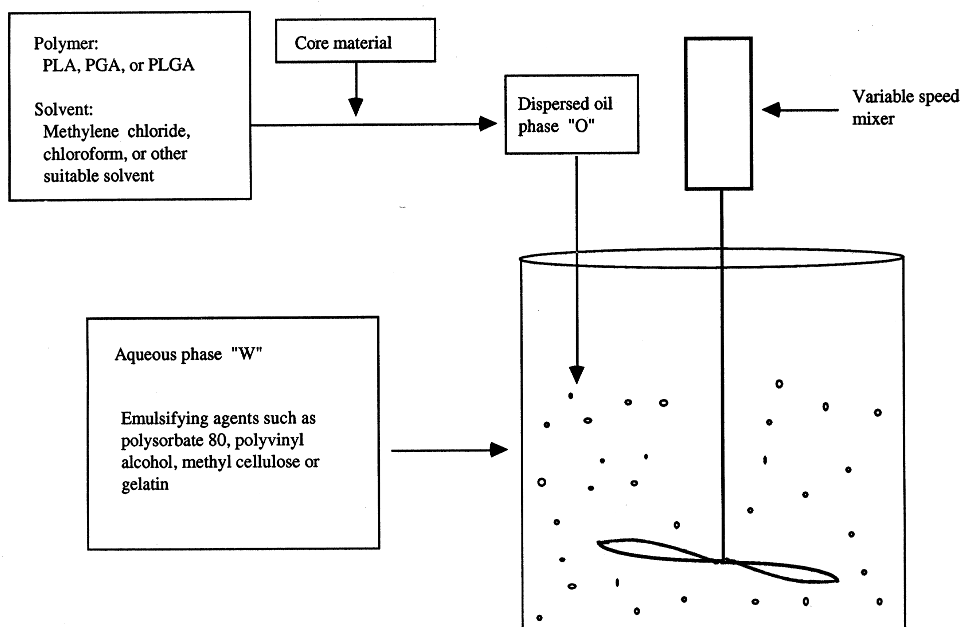

Fig. 1. Schematic diagram of O/W emulsion solvent evaporation method.

size distribution, drug content, and nifedipine release was reported. The amount of the dispersing agent hydroxypropyl methyl cellulose in the aqueous phase was varied from 0.4% to 2.4%, and it was found that the mean particle diameter decreased from 28.5 μm to 12.9 μm respectively. Similarly, it was found that when the stirring rate was increased, the microparticles became smaller and the size distribution decreased. The researchers concluded that for high levels of HPMC and stirring rates, energetic conditions were favorable for the maximum division of the organic phase. The decrease in the mean diameter as a function of the increase in organic phase volume is shown in Fig. 2. As the volume of solvent increased, the mean diameter of the microsphere decreased. These results can be explained on the basis of the viscosity of the internal phase of the emulsion increasing with decreasing volume, as the polymer weight was kept constant. The effect of particle size on the release profiles of microspheres loaded with 14% nifedipine is seen in Fig. 3. The microspheres with mean particle sizes of 12 and 18 μm exhibited an initial release of about 10% of the drug load within the initial 5 h. The larger microspheres exhibited a lag time of approximately 150 h, followed by linear release of 80% of the drug load after 400 h. The initial release of the 12 and 18 μm microspheres was attributed to the small microsphere size and resultant increase in surface area [41].

Successful entrapment of drugs, together with other properties of microspheres depend to a large extent on the selection of the organic solvent used to dissolve the polymer. Bodmeier and McGinity found

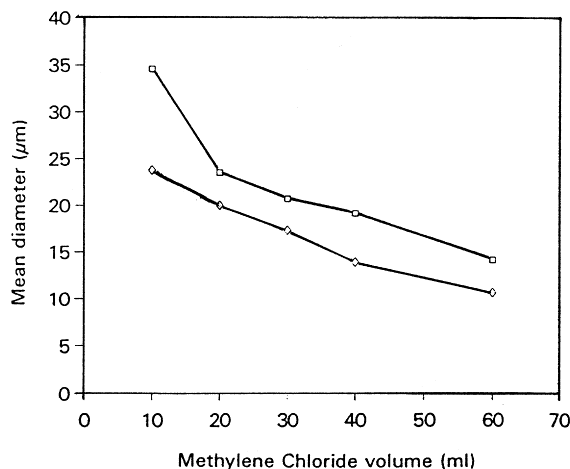

Fig. 2. Influence of organic phase volume on the mean diameter. The experiment was performed at two different HPMC concentrations ((□) 0.8% and (◇) 1.6% (w/v)), the stirring rate being 800 rpm and the aqueous phase volume 250 ml. (Reproduced with permission from [41]).

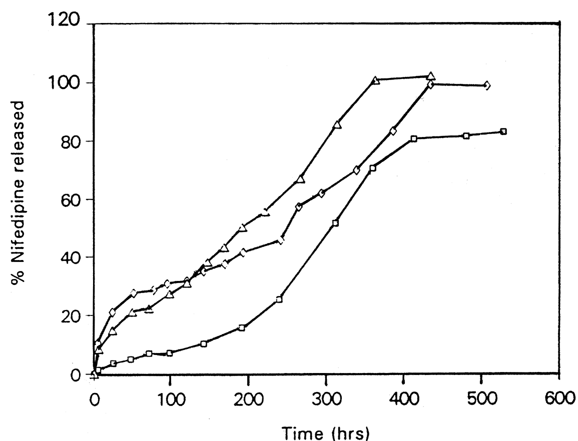

Fig. 3. Release profiles of microspheres loaded with 14% nifedipine as a function of particle size: (△) 11.7 μm, (◇) 17.5 μm and (□) 83 μm. (Reproduced with permission from [41]).

that the rate of polymer precipitation from the organic solvent phase was strongly affected by the rate of diffusion of the organic solvent into the aqueous phase [9]. Organic solvents of low water solubility resulted in slow polymer precipitation which facilitated complete partitioning of the drug into the aqueous phase. The drug loading of the microspheres was found to be enhanced by the addition of water miscible organic solvents to the organic phase of the system.

Li and coworkers established a mathematical model representing mass transfer during microsphere formation including both intrinsic variables, such as solvent polymer interaction parameters, and extrinsic variables such as dispersed phase/continuous phase ratios, dispersed phase composition and temperature [42]. This model considers the dispersed phase and the continuous phase separately, as seen in Fig. 4. The physicochemical properties of the system were classified into either transport parameters which include the diffusion coefficients of the solvent–nonsolvent system and the solvent–polymer system, or interaction parameters which include those of the polymer–solvent, solvent–nonsolvent, and nonsolvent–polymer phases. The model was tested using a salmon calcitonin (sCT) loaded PLGA microsphere system with methylene chloride as the solvent, methanol as the cosolvent, and sodium oleate solution as the non-solvent. The results showed good agreement between predicted and experimental data [42].

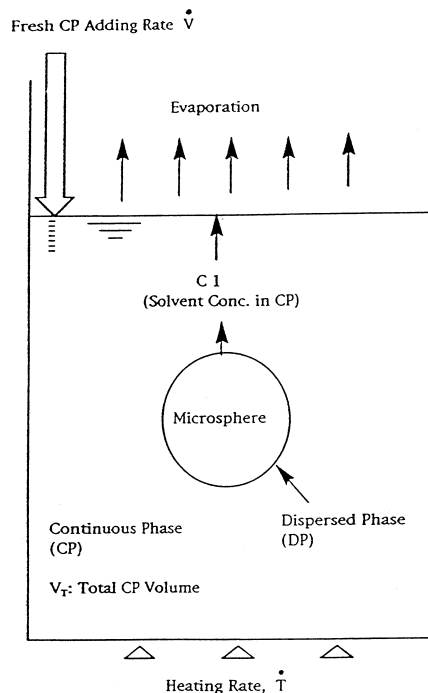

Fig. 4. Schematic diagram of microsphere formation. (Reproduced with permission from [42]).

Recently Conti et al. evaluated three process parameters in the preparation of indomethacin loaded PLGA microspheres by solvent evaporation [43]. The influence of polyvinyl alcohol concentration in the continuous phase, the emulsification stirring speed and the dispersed phase to continuous phase ratio were investigated. It was found that the encapsulation efficiency was highly dependent upon the emulsification stirring speed when low (0.5%) amounts of PVA were used in the system, with drug loading varying from 17.5% to 90%.

The rate of solvent removal from microspheres prepared by the solvent evaporation method impacts the physicochemical properties of the microspheres. Izumikawa et al. [44] found significant differences in physical characteristics and drug release profiles between progesterone loaded PLA microspheres prepared by either reduced pressure solvent evaporation or a solvent evaporation method under atmospheric conditions. Product yield and encapsulation efficiency were greater for microspheres prepared by the reduced pressure solvent extraction method

(RSE) than for those prepared by atmosphere solvent evaporation (ASE). The surface morphology of these microspheres examined by scanning electron microscopy indicated a porous and rough surface for the ASE microspheres. Conversely, the microspheres produced by the RSE method had an apparent smooth surface. The X-ray diffraction scans of the RSE and ASE microspheres are seen in Fig. 5. At 30% drug loading, the ASE microspheres exhibited peaks due to crystalline progesterone in addition to peaks due to crystalline poly(l-lactide). The RSE microspheres, on the other hand, displayed no such peak due to crystalline PLA, which suggests that the PLA was present only in the amorphous state. Additionally, the RSE microspheres exhibited no peaks which corresponded to crystalline progesterone, which indicated that the drug was dispersed in an amorphous polymer network. It was assumed that the solvent removal under reduced pressure occurred too rapidly for the polymer to crystallize.

Drug release from the microspheres was found to be significantly influenced by the crystallinity of the polymer matrices, as seen in Fig. 6. The drug release rate increased with the drug loading for both types of microspheres. For the ASE microspheres, there was a rapid release in the initial stage, and the release rate was much greater than that of the RSE microspheres [44].

Jalil and Nixon correlated the release kinetics of phenobarbitone in relation to the molecular weight of dl-PLA polymers [45]. Microcapsules containing phenobarbitone (PB) were prepared with PLA using three different molecular weights and four different core-to-polymer ratios. The microspheres showed an initial burst phase release followed by a lag phase. The lag time was affected by the polymer molecular weight in that as the molecular weight of the polymer increased the lag time also increased. The release of phenobarbitone from these microspheres was determined to be primarily diffusion controlled,

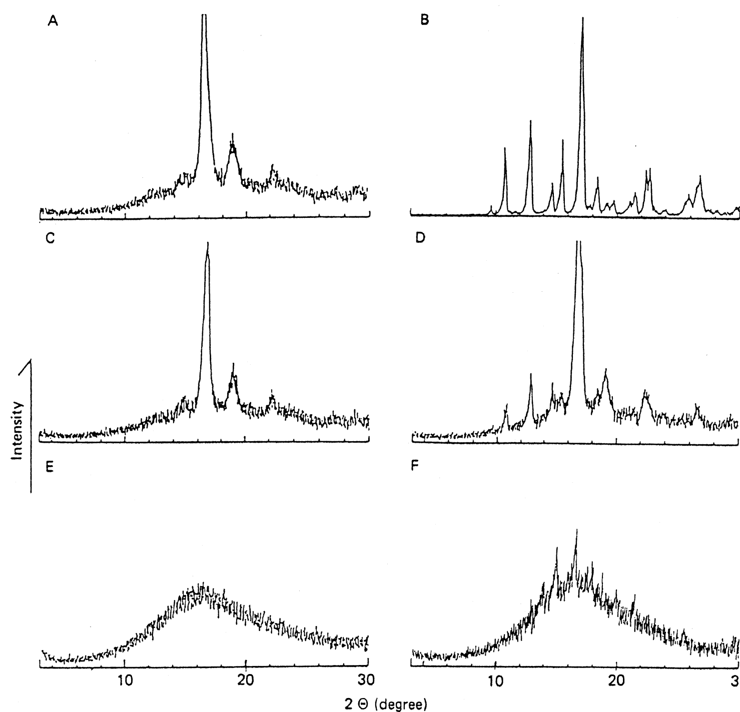

Fig. 5. X-ray powder diffraction scan of progesterone-loaded poly(l-lactide) microspheres: (A) PLA powder; (B) crystalline progesterone; (C) 5% progesterone-loaded microspheres prepared by atmosphere-solvent evaporation method; (D) 30% progesterone microspheres prepared by atmosphere-solvent evaporation method; (E) 30% progesterone loaded microspheres prepared by reduced pressure-solvent evaporation method; (F) 40% progesterone loaded microspheres prepared by reduced pressure-solvent evaporation method. (Reproduced with permission from [44]).

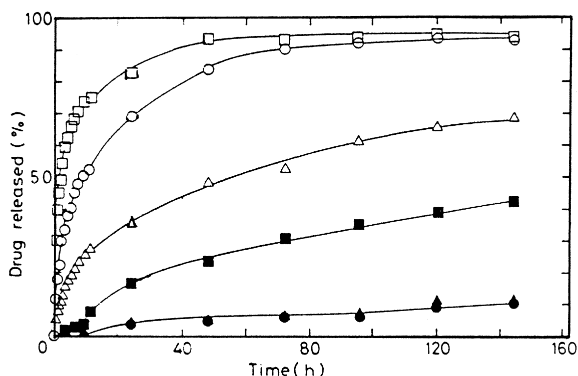

Fig. 6. Progesterone release from microspheres prepared by reduced pressure-solvent evaporation method (▲, ●, ■) and microspheres prepared by atmosphere-solvent evaporation method (△, ○, □). Progesterone loading: (▲, △) 5%; (●, ○) 10%; (■, □) 20%. (Reproduced with permission from [44]).

but swelling and erosion also contributed to the release process.

2.3. Residual solvent

Residual solvents in pharmaceutical preparations, including microspheres, are a growing concern due to the toxicological risks associated with such residuals. Solvents commonly used in microencapsulation by the emulsion solvent evaporation method, such as methylene chloride or chloroform, may be retained in the microsphere as a residual organic volatile impurity. Currently, the USP XXIII has outlined limits for residual solvents. For methylene chloride the limit is 500 ppm, and for chloroform the limit is 50 ppm [46]. To determine the level of residual solvent in microsphere preparation, Bitz and Doelker [47] used multiple headspace GC to determine the total amount of residual solvents in microspheres prepared by either emulsion solvent evaporation or by spray drying. A rotary evaporator was used (34°C, 20 kPa) to remove the methylene chloride from the microspheres, and the microspheres were stored under vacuum for three days. The researchers found that the level of residual methylene chloride was below the recommended 500 ppm for all the samples tested, but microspheres prepared with chloroform exceeded the recommended level for solvent residuals. Gas chromatographic headspace analysis of residual solvents has been used to determine residual solvent in microspheres by other researchers [48].

3. Microencapsulation of water soluble compounds, proteins and peptides

3.1. Anhydrous systems

The encapsulation of water soluble drugs by the conventional O/W solvent evaporation method will generally result in rapid partitioning of the drug from the organic phase and into the aqueous phase, resulting in microspheres with little or no drug loading. Innovative modifications to the conventional O/W solvent evaporation method have been reported to circumvent this problem.

Anhydrous systems, which are comprised of an organic polymer phase emulsified in an immiscible oil, have been used to produce microspheres of the O/O type. The elimination of water significantly reduces the tendency of the drug to partition into the continuous phase, provided that the drug is insoluble in the external oil. Sturesson and coworkers [49] used an oil-in-oil system to produce PLGA microspheres of timolol maleate. Acetonitrile was the solvent for the drug and polymer and sesame oil was used as the continuous phase. Span 80 was incorporated as an emulsion stabilizer. The PLGA/drug solution was added dropwise to the sesame oil, and the system was vigorously agitated. To further decrease the particle size, the system was sonicated. Agitation continued until the acetonitrile evaporated, at which point microspheres were collected. The microspheres were rinsed with hexane to remove the residual oil. Drug release from the microspheres was reported to be triphasic as shown in Fig. 7. Initially, a burst was observed due to release of drug located near the microsphere surface. This was followed by a period of slow release which was attributed to degradation of the microsphere and diffusion of the drug out of the microsphere. The third phase was described as a secondary burst, and was attributed to the increased solubilization and erosion of the polymeric matrix. The initial release of the drug decreased as the polymer concentration in the formulation increased. Similar release profiles of this type have been reported by other researchers [50,51].

Wang and coworkers [52] investigated the in-

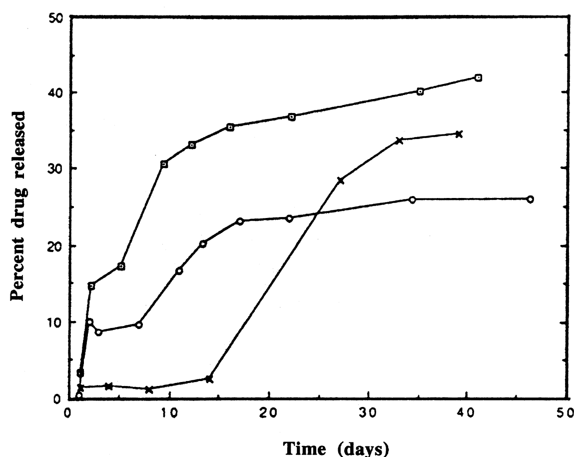

Fig. 7. Timolol released from PLGA particles showing triphasic behavior and the efect of PLGA concentration in the preparation: (□) 5% PLGA, (○) 10% PLGA, (×) 20% PLGA. (Reproduced with permission from [49]).

fluence of formulation methods on the controlled release of bovine serum albumin from PLGA microspheres. Poly(DL-lactide/glycolide) microspheres containing bovine albumin were prepared with and without carbomer 951 (Carbopol 951) by either an oil/oil, oil/water, or (water/oil)/water emulsion technique and albumin release was studied in vitro. The particle size of the microspheres was approximately 500 μm, 25–100 μm, or 10–20 μm for O/O, O/W, or (W/O)/W emulsion methods, respectively. The greatest burst of albumin release was seen with vacuum-dried microspheres formulated by the (W/O)/W method. This burst effect was eliminated by lyophilizing the microspheres after preparation. A higher initial release rate was seen with microspheres prepared by the o/w emulsion method with Carbopol 951 than from those without Carbopol 951. Albumin could be sustained for 54, 36, or 34 days in microspheres prepared by O/O, O/W, or (W/O)/W methods, respectively. The investigators concluded that albumin release from microspheres can be controlled by the method of preparation.

PLGA microspheres containing glycine and its homopeptides (diglycine, triglycine, tetraglycine, and pentaglycine) were prepared by an anhydrous O/O technique [53]. The inner phase consisted of micronized glycine homopeptides suspended in a solution of PLA and acetone. The external phase was comprised of mineral oil containing 0.3% (v/v) sorbitan sesquioleate which functioned as an emulsifier. Emulsification was carried out by adding the internal phase to the pre-cooled (5°C) external phase. The temperature was then elevated to 35°C for a sufficient period of time to allow evaporation of the solvent. The microsphere suspension was then poured into an excess of *n*-hexane and stirred for one hour, after which time the hardened microspheres were collected. Formulation parameters investigated included PLA concentration in the dispersed phase, emulsifier concentration and emulsification time, with results as shown in Table 1. Analysis of release profiles of glycine and diglycine microspheres indicated that they were released predominantly by a matrix-controlled diffusional process, where tortuosity and porosity of the diffusional path controlled the release rate. The release of tetra- or pentaglycine suggested a dissolution controlled release which is consistent with compounds of known poor aqueous solubility.

3.2. Multiple emulsion systems

3.2.1. Water–oil–water multiple emulsion systems

The preparation and characterization of the drug loading, encapsulation efficiency, and morphology of biodegradable polyester microspheres containing somatostatin acetate and poly(D,L-lactide), poly(D,L-lactide/glycolide), or poly(L-lactide) prepared by a modified solvent evaporation method based on the formation of multiple W/O/W emulsions were reported by Herrmann and Bodmeier [54]. An increase in the volume fraction of the internal aqueous phase in the primary W/O emulsion resulted in lower encapsulation efficiencies. Replacement of the methylene chloride as an organic solvent with ethyl acetate reduced the encapsulation efficiency, as seen in Table 2, and increased the porous nature of the microspheres. Except for microspheres prepared with very low molecular weight polymers, the encapsulation efficiency was not affected by the polymer type and molecular weight. The preparation conditions substantially affected the morphology and porosity of the microspheres.

Stability of the primary emulsion is a prerequisite for the successful encapsulation of multiple emulsions. The influence of emulsion stability on the morphology and porosity of microparticles prepared by the W/O/W double emulsion-evaporation technique using two semicrystalline L-polylactides of

Table 1
Formulation study of DL-PLA microspheres containing glycine: effect of DL-PLA concentration, emulsification time and solvent evaporation time on particle size distribution and entrapment

	Variable	Level		Particle size distribution (%)				Entrapment (%)
				0–38 μ	38–125 μ	125–250 μ	Total	38–125 μ
1.	DL-PLa concentration in dispersed phase (% w/w)	2.8		27.3	33.0	11.8	72.0	15.7
		5.4		34.8	27.5	4.7	67.0	60.3
		10.3		24.0	43.0	11.3	78.3	73.8
		18.7		-	-	-	-	-
2.	Emulsifier concentration (Arlacel-83) (% v/v)	0.0		-	-	-	-	-
		0.1		29.5	32.5	11.0	73.0	74.2
		0.3		24.0	43.0	11.3	78.3	73.8
		0.5		31.5	30.5	7.5	69.5	76.3
		0.7		28.0	28.5	6.5	63.5	76.8
		2.0		9.5	12.0	24.0	45.5	71.1
3.	Emulsification time (ET) and solvent evaporation time (SET) (min)	ET	SET					
		15	15	19.5	36.0	9.5	65.0	64.3
		15	1	27.8	41.8	10.8	80.3	64.3
		1	15	23.0	41.5	10.8	75.3	74.2
		1	2	24.0	43.0	11.3	78.3	73.8

Reproduced with permission from [53].

different molecular weights were studied by Schugens et al. [55]. A substantial increase in the molecular weight of L-polylactide required a dilution of the polymer solution to prevent an exceedingly high viscosity, and led to less stable primary emulsions and more porous solid microspheres. A semicrystalline polylactide was found to be poorly suited for the preparation of sustained release microparticles. It was concluded that the crystallinity of L-polylactides affected the stability of the primary emulsion by exclusion of the internal aqueous droplets from the PLA matrix. This exclusion adversely impacted the encapsulation efficiency, morphology, and porosity of microparticles prepared from a double emulsion.

Table 2
Effect of partilce size fraction and organic solvent type on the encapsulation efficiency

Particle size fraction (μm)	Encapsulation efficiency (%)	
	Methylene chloride	Ethyl acetate
45–75	89.5	58.1
75–106	85.1	63.0
106–150	85.5	63.9
150–180	86.0	65.7
180–250	84.8	63.0

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To investigate the effects of buffers or salts added to the internal aqueous and/or external aqueous phase on the properties of somatostatin acetate-containing polylactide microspheres, Herrmann and Bodmeier [30] prepared microspheres using a W/O/W multiple emulsion solvent evaporation method and characterized the resultant microspheres for encapsulation efficiency, drug release, and morphological properties. Addition of buffers or salts to the internal aqueous phase resulted in porous microspheres, as shown in Fig. 8(a). However, the addition of salts to the external aqueous medium resulted in the formation of a dense and homogenous polymer matrix (Fig. 8(b)). Drug release profiles consisted of a rapid drug release phase followed by a slow release phase. This release pattern is consistent with many matrix-type drug delivery systems in that the drug release profile can be divided into two phases; a rapid release phase which represents drug release by diffusion through fluid filled pores, and a slow release phase representing the release of the peptide by diffusion through the polymer matrix. Lower encapsulation efficiencies were obtained with the more porous microspheres. Addition of buffer salts to the internal aqueous phase promoted an influx of

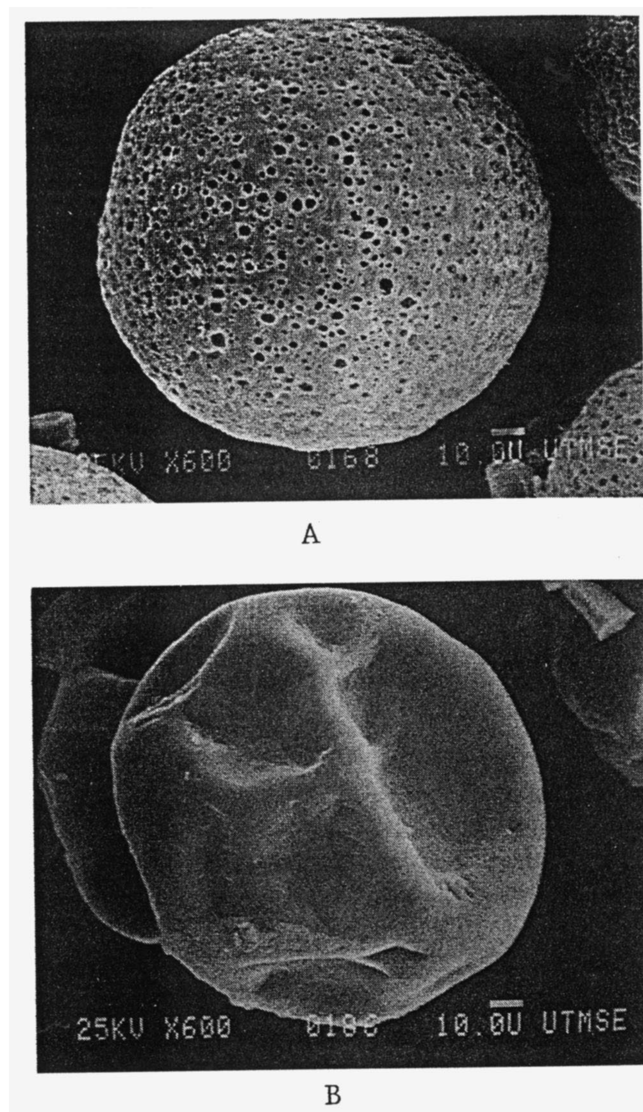

Fig. 8. Scanning electron micrographs of somatostatin containing PLA microspheres prepared with $CaCl_2$ in the internal and/or external aqueous phase: (A) 0.38 mol/l $CaCl_2$ internal aqueous phase; (B) 0.38 mol/l $CaCl_2$, internal and external phase. (Reproduced with permission from [30]).

water from the external phase due to a difference in osmotic pressure. This resulted in a more porous microsphere structure, faster drug release, and lower encapsulation efficiencies.

The impact of surfactants in the primary emulsion of the double emulsion solvent evaporation technique on the characteristics and in vitro release profile of BSA from PLA and PLGA microspheres was investigated by Soriano and coworkers [56]. The primary emulsion containing BSA was prepared using either a high pressure homogenizer or a sonicator. The emulsion was then poured into a 1% PVA solution and stirred at 8000 rpm at 5°C for one minute to produce the W/O/W emulsion. The solvent was allowed to evaporate for 2 h as stirring at 250 rpm was continued at room temperature. The microspheres manufactured with a high pressure homogenizer had a lower bovine albumin trapping

efficiency and higher burst effect than those made with the sonicator, which showed a very slow release rate. Microspheres prepared with and without surfactant agents and using the sonicator were compared. The BSA release from the PLGA microspheres was continuous, but batches that contained surfactants at HLB 6 presented an initial release of 55% of the incorporated BSA while batches with HLB 7 showed less of a burst effect and a slower release rate as seen in Fig. 9. The PLA microspheres released a percentage of incorporated drug at the beginning of the in vitro assay and reached a plateau area in which no further release of the drug was observed. The authors suggested that the BSA was bound to the polymeric matrix.

Incomplete release of encapsulated protein was also observed by Park and coworkers [57]. Carbonic anhydrase was incorporated into poly(DL-lactic-co-glycolic acid) microspheres, and protein release and stability were studied over a 2-month period. Carbonic anhydrase release was initially fast, and then followed by slow and incomplete release of the active. The slow release kinetics observed were attributed to protein aggregation and nonspecific adsorption in the microspheres. It was determined that the protein was significantly denatured and aggregated during the double emulsion formulation step. Several excipients such as dithiothreitol and sodium dodecyl sulfate improved release kinetics

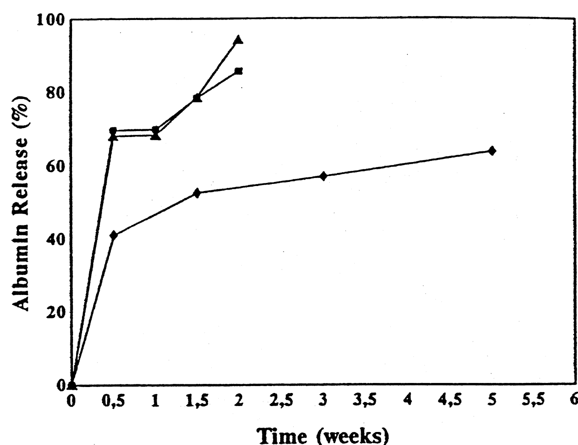

Fig. 9. Release profile of BSA from DL-PLGA (70/30) copolymer: Effects of HLB in BSA release; (▲) without surfactants, (■) HLB = 6, (◇) HLB = 7. (Reproduced with permission from [56]).

partly due to increased protein stability. Protein released from fast degrading microspheres was severely hydrolyzed and lost catalytic activity due to the accumulation of degradation products in the medium. It was concluded that carbonic anhydrase release from aliphatic polyester microspheres was a function of polymer composition.

The use of poly(lactic/glycolic acid) polymers for long term delivery of high molecular weight, water soluble proteins was investigated by Cohen et al. [58]. PLGA microspheres containing fluorescein isothiocyanate labeled bovine serum albumin and horseradish peroxidase were prepared by a modified solvent evaporation method using a double emulsion of the W/O/W type. The microspheres were spherical with diameters ranging form 55 to 95 μm and encapsulation efficiencies of more than 90% were reported. Unencapsulated horseradish peroxidase lost 80% of its activity in solution at 37°C in a few days, whereas the encapsulated enzyme retained more that 55% of its activity after 21 days incubation at 37°C. Stability studies showed that the encapsulation of the enzyme inside the PLGA microspheres can protect the enzyme from activity loss. In vitro release studies revealed that different release profiles and release rates can be achieved by simply modifying factors in the preparation procedure such as mixing rate and volume of inner water and organic phases. The cumulative release of BSA as a function of organic phase volume is shown in Fig. 10. Degradation studies by scanning electron microscopy and gel permeation chromatography suggested that the mechanism responsible for protein release was mainly through matrix erosion.

To investigate the effects of volume of the inner water phase on the internal and external structures of PLGA microspheres, five batches of bovine serum albumin in PLGA microspheres were prepared using 0 to 22.7% inner water volume and were evaluated for microsphere morphology and albumin release during solvent removal [59]. Hollow microspheres possessing dense, nonporous polymer shell layers were prepared when an initial inner aqueous phase volume fraction of 5.6% was used. However, an initial volume fraction of 22.7% resulted in hollow microspheres with porous surface structures. More than 90% of the albumin remained encapsulated after 3 h in the formulation, with a dense nonporous polymer shell layer.

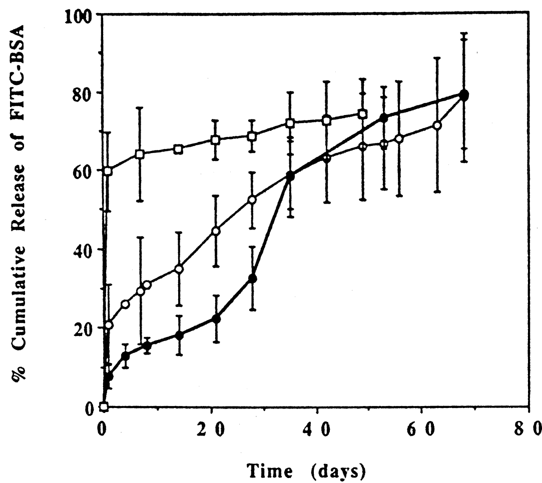

Fig. 10. In Vitro, cumulative FITC-BSA release from PLGA (75:25 L/G) microspheres as a function of organic phase volume: (□) 2 ml, (○) 1 ml, and (●) 0.7 ml methylene chloride. The results are the average of two to five experiments. (Reproduced with permission from [58]).

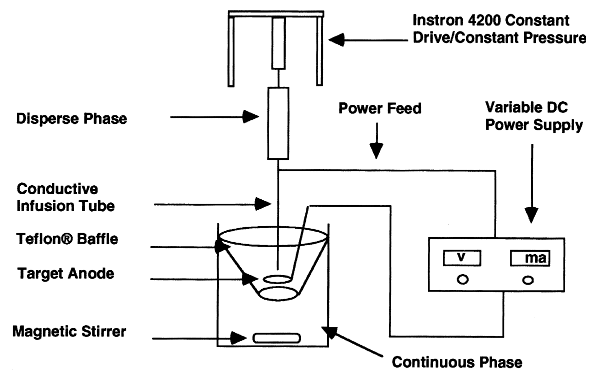

Fig. 11. Schematic diagram for production of microspheres by potentiometric dispersion. (Reproduced with permission from [36]).

3.2.2. *Multiple emulsions of the W/O/O or W/O/O/O type*

Iwata and McGinity developed a multiple emulsion of the W/O/O/O type [34]. Multiphase microspheres of either PLA or PLGA containing water-in oil (W/O) emulsions were prepared by a multiple emulsion solvent evaporation technique. Acetonitrile was used as the solvent for the polymer, and light mineral oil comprised the continuous phase for the encapsulation procedure. Drug loading efficiencies of model water soluble compounds ranged from 80 to 100% of theoretical, based on specific preparative conditions. Scanning electron microscopy of transverse cross sections of the multiphase microspheres revealed cavities in which the W/O emulsion resided. This suggested that the multi-phase microspheres of the W/O/O/O type belonged to the class of reservoir type drug delivery devices. Utilization of this type of multiple emulsion system allows the encapsulation of a primary water-in-oil emulsion within a polymeric microsphere. The oil in the primary emulsion prevents contact between the internalized protein and the polymer/solvent systems. The isolation of the protein from the polymer/solvent system prevents possible denaturization of the protein by the polymer or the solvent. Likewise, the possibility of polymeric degradation due to reactive proteins or drug compounds is also limited.

O'Donnell and coworkers [36] prepared multiphase microspheres of poly(DL-lactic-co-glycolic acid) by a multiple emulsion potentiometric dispersion technique. Water soluble compounds were dissolved in the aqueous phase (W) and emulsified in soybean oil (O) to form a stable emulsion. This primary emulsion was dispersed in a solution of PLGA and acetonitrile (**O**) to form a W/O/**O** emulsion. The W/O/**O** emulsion was then dispersed in a hardening solution of light mineral oil (O) using a potentiometric dispersion technique as illustrated in Fig. 11 to produce microspheres of the W/O/**O**/O type with a very narrow and selective size distribution. The size of the microspheres was controlled by varying the internal diameter of the conductive infusion tube or by the variation of voltage applied to the conductive tube. Particle size analysis revealed a narrow particle size distribution with 80% of the microspheres made by this method in the 20 to 40 μm range as compared to a wide distribution of 50 to 500 μm for microspheres made by conventional agitation methods. Chlorpheniramine maleate was encapsulated with a loading efficiency of 88.9% with the potentiometric method as compared to a loading efficiency of 74.3% for the agitation method [36].

3.3. *Release of proteins from microspheres*

The release of bovine serum albumin from biodegradable microcapsule formulations prepared from various polymer compositions was studied by Sah et al. [60]. Polymer composition and the ratio of

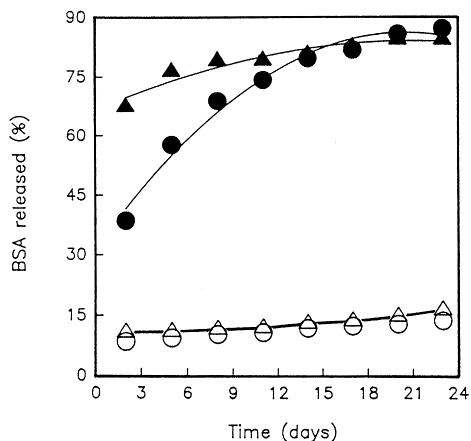

Fig. 12. The influence of polymer compositions on release profiles. BSA (15 mg) was loaded into the microcapsules prepared from 0.3 g PLGA 75:25 (Intrinsic viscosity = 0.48 dl/g) and 0.1 g DL-PLA ($M_w = 2000$) (▲); 0.4 g PLGA and 0.1 g PLA (●); 0.4 g PLGA and 0.2 g PLA (△); 0.5 g PLGA and 0.1 g PLA (○). (Reproduced with permission from [60]).

polymer to protein were found to influence the albumin release profiles as illustrated in Fig. 12. An increase in the amount of polymer used appeared to give rise to microcapsules with a dense, less porous polymeric phase, thereby inhibiting the burst effect. Porosity of the microspheres was also related to the initial burst effect and release patterns of the microspheres. Depending on the microcapsule formulation, controllable and predictable release profiles of albumin that could be described by either zero- or first-order kinetics were observed. It was concluded that zero- or first-order release kinetics of bovine albumin can be achieved using biodegradable microcapsules.

Controlled release microparticles containing ovalbumin were prepared with two poly(lactide-co-glycolide) polymers having different degradation rates, and the antibody response was studied in mice following oral administration [61]. Both polymers showed enhanced serum IgG and salivary IgA antibody responses in comparison to a group of mice immunized with soluble ovalbumin. The level of response was found to be polymer dependent. The more rapidly degrading polymer was more effective for the induction of high levels of salivary IgA antibodies, while the more slowly degrading polymer was more effective for the induction of serum IgG antibodies. It was concluded that microparticles were capable of inducing enhanced secretory and systemic antibody responses to entrapped ovalbumin after oral immunization.

To prepare microcapsules that release proteins as vaccine adjuvants, five microcapsule formulations were prepared by a multiple emulsion W/O/W method from blends of PLA and PLGA and three model proteins. The microcapsules were evaluated in vitro for water uptake, hydrolysis, and release and in mice for release and immune response. The microcapsules demonstrated continuous release of proteins. The degree of water retention of all microcapsule formulations was similar to one another, as water uptake was enhanced with increasing the amount of poly-DL-lactic acid or poly-DL-lactic acid-co-glycolic acid [62].

A microencapsulated HIV vaccine was evaluated in vitro for particle size, size distribution, microparticle surface structure, antigen loading level, efficiency of entrapment, moisture content, release, and stability [63]. The microspheres were then evaluated in guinea pigs after oral immunization alone, and combined oral and subcutaneous immunization for toxicity and efficacy. The microparticles were safe, pyrogen free, and induced high levels of both serum IgG and neutralizing antibodies against HIV.

Changes in surface characteristics of polylactide microspheres after encapsulation or adsorption of model protein antigens were studied using hydrophobic interaction chromatography and zeta potential analysis, and immune response was examined after intranasal immunization of guinea pigs. Protein adsorption followed the classical Langmuirian model and was probably influenced by polar interactions. Protein adsorption elevated the surface hydrophobicity of the particles, with the degree depending on the protein. Uncoated and protein-coated polylactide microspheres were far less hydrophobic than were latex controls. Hydrophobicity was also affected by the surfactant used in microsphere preparation. Strongly hydrophobic preparations resulted in a stronger and lasting immune response compared to those of lower hydrophobicity. It was concluded that increasing the hydrophobicity of antigen-associated microspheres may improve the immune response [64].

4. Polymeric degradation induced by macromolecules or reactive compounds

4.1. Polymeric degradation induced by proteins, reactive compounds, or processing conditions

The influence of plasma proteins on the degradation of poly(L-lactide) microcapsules in relation to the potential distribution across a microcapsule/adsorbed protein layer interface, and the increased solubility of PLA caused by the presence of proteins, was reported by Makino et al. [65]. The degradation rate of PLA microcapsules in an aqueous medium was accelerated by the addition of albumin, γ-globulins, and fibrinogen. As previously reported by these researchers, PLA molecules of intermediate molecular weights were considered to be degraded into lactic acid [66]. The amount of lactic acid released into the bulk solution as a function of the degradation period in various protein solutions is presented in Fig. 13 a–c. For all proteins, as the concentration increased, the released amount of lactic acid produced via degradation of PLA also increased. The presence of plasma proteins in the buffer solution accelerated the cleavage of ester bonds in the PLA molecules. These workers concluded that the presence of proteins can increase the solubility of PLA causing the PLA molecules to exist in an expanded form which would in turn accelerate the degradation of PLA microcapsules.

Maulding and coworkers [27] found that the degradation rate of PLA was accelerated in the presence of the tertiary compound, thioridazine. The PLA component of microcapsules containing up to 50% thioridazine free base showed a decrease in molecular weight during microcapsule fabrication and in the course of the dissolution studies. Polymer hydrolysis did not occur in placebo microspheres or when the amino group of thioridazine was protonated in the form of the pamoate salt. The enhancement of the degradation rate was attributed to amine-influenced hydrolysis of poly(dl-lactide).

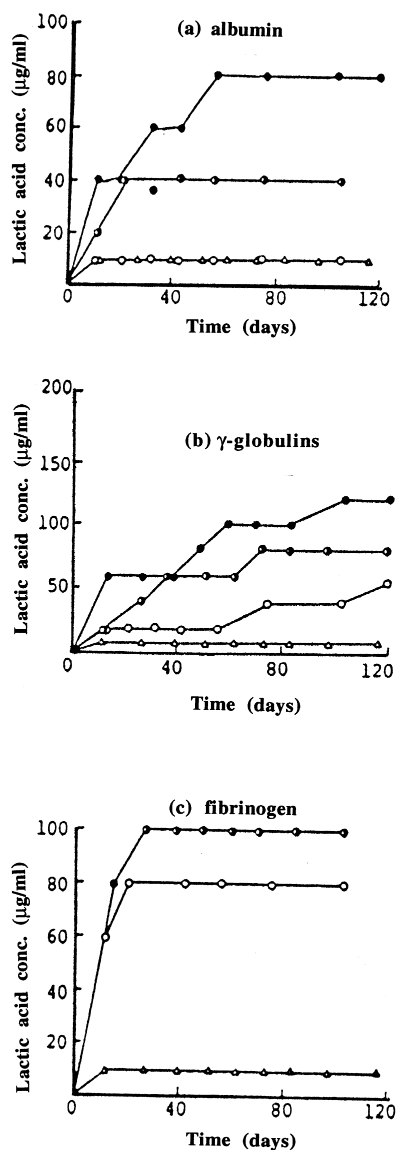

Fig. 13. Amounts of lactic acid generated from PLA microcapsules at different plasma protein concentrations of (●) 30 mg/ml; (◑) 5 mg/ml; (○) 0.1 mg/ml; (△) 0 mg/ml. (Reproduced with permission from [65]).

Agitation and sonication methods have been reported as techniques to produce microspheres, and the resultant microspheres often exhibit a wide particle size distribution and low drug loading. Sonication, either by probe or flow-through cell, can produce small regions of intense heat which may prematurely degrade the polymer [67]. The potentiometric dispersion method to produce biodegradable microspheres was previously reported by the authors [36]. In this method, an electrically charged infusion tube was positioned above a target anode. As the polymer solution was emitted from the

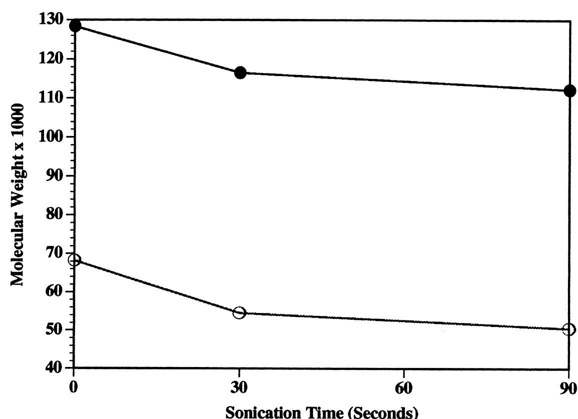

Fig. 14. Change in molecular weight of PLA in multiphase microspheres produced by sonication at 64W. (Reproduced with permission from [68]).

infusion tube, the solution dispersed due to the potentiometric force between the cathode and anode. The effect of processing techniques on the molecular weight of biodegradable microspheres of (PLA) and (PLGA) was investigated [68]. Multiphase microspheres were produced by conventional agitation, potentiometric dispersion, or sonication. Gel permeation chromatography was used to determine the molecular weight of the polymer before and after processing. Multiphase microspheres of PLA produced by sonication experienced a decrease in molecular weight, as seen in Fig. 14. The amount of degradation was found to be dependant on the type of polymer and the sonication time. PLA with an initial molecular weight of 128,000 exhibited a 10% reduction in molecular weight after 90 s of sonication. PLA with an initial molecular weight of 68,000 experienced a 21% decrease in molecular weight after 90 s of sonication. These results are in agreement with sonication induced degradation reported by other researchers [69,70]. PLGA, on the other hand, did not experience a decrease in molecular weight due to the sonication process under the experimental conditions.

4.2. Degradation of encapsulated proteins

A controlled release microreservoir-type biodegradable microcapsule containing bovine serum albumin (BSA) was prepared and characterized in vitro [71]. BSA was incorporated into microcapsules with high efficiency of 96.1%. The encapsulation did not change the molecular weight or conformation of BSA, as indicated by biochemical analyses. The composition and preparation method of microcapsules were found to be closely related to BSA release and microcapsule degradation. Depending on the formulation, the release profile was monophasic or biphasic. It was possible to control the delay before the initial release of BSA and also the total duration of its delivery. It was concluded that microreservoir-type microcapsules can act as a depot system for the controlled release of BSA by precise control of the microcapsule degradation rate which gradually increased drug permeability and porosity of the microspheres.

The preparation of multiphase microspheres involves the preparation of emulsions with vigorous agitation, which may denature certain susceptible proteins. Hayashi et al. [72] examined the usefulness of reversed micelle solvent evaporation as an alternative to agitation for preparing W/O emulsions. Reverse micellar solubilization of the proteins, super oxide dismutase (SOD), human tumor necrosis factor (TNF), and tilactase (beta-galactosidase) in organic solvents, and their encapsulation in poly(L-lactide) microspheres were studied. The activity of the proteins was decreased to varying extents during solubilization and/or extraction depending on the solvent used. The enzyme activity of SOD and tilactase recovered from the micellar chloroform solutions was greater than 90% for all surfactants studied. In contrast, TNF activity decreased significantly during solubilization with chloroform. For SOD and tilactase, the activity entrapped in the microspheres depended on the surfactant used during solubilization. It was concluded that SOD, tilactase, and TNF can be successfully encapsulated in L-polylactide microspheres in their active form by the reversed micelle solvent evaporation technique using sucrose esters of fatty acids as surfactants.

Certain compounds, such as bovine insulin, are not stable in acidic conditions [73]. These drugs, when encapsulated and in contact with PLGA, may thus be degraded by the acidic microenvironment which exists inside the microsphere. Uchida and coworkers [74] prepared PLGA microspheres containing bovine insulin as a model drug using an O/O emulsion solvent evaporation process. Encapsulation efficiency of the process was almost 100% and the average

diameter of the microspheres was between 100 μm to 200 μm. A very low release of insulin (1%) was demonstrated after 7 days. The degradation of bovine insulin in PLGA microspheres was confirmed by high-performance liquid chromatography as shown in Fig. 15. This phenonemon suggests that PLGA microspheres formed an acidic condition inside the microspheres, and accelerated the degradation of bovine insulin.

Carbonic anhydrase and bovine serum albumin were encapsulated in poly-DL-lactic-co-glycolic acid microspheres, and the effects of experimental conditions on protein release and stability were investigated by Park et al. [75]. Degradation of microspheres incubated in a polypropylene tube caused a pH decrease from pH 7.4 to below pH 3 after one month. This caused severe hydrolysis of the released protein in the medium and unreleased protein in the microspheres. Released and unreleased protein exhibited less severe degradation when microspheres were incubated in a dialysis bag which permitted constant pH. Microspheres in the tube showed a significant decrease in polymer molecular weight and morphological change, compared to those in the dialysis bag. There was no apparent effect of encapsulated protein on polymer stability. It was concluded that the stability of released and unreleased proteins in microspheres, release kinetics, and polymer degradation were dependent on experimental conditions.

Johnson et al. [76] investigated the suitability of PLGA microspheres for the controlled release of rat atriopeptin III (APIII), a 24 amino acid peptide fragment of atrial naturiuretic factor. APIII was encapsulated in PLGA microspheres at loading levels of 2%, 6%, and 10% by weight, and the APIII in these microspheres was found to be stable for several months when stored at −20°C in a desiccated chamber. However when stored at 40°C and 95% relative humidity and then extracted, the APIII was found to be 20% degraded after 8 days. Release studies were performed and chromatographic analysis revealed that much of the APIII had not been released into solution, but was degraded while inside of the PLGA microspheres. The data indicated that PLGA catalyzed the decomposition of APIII.

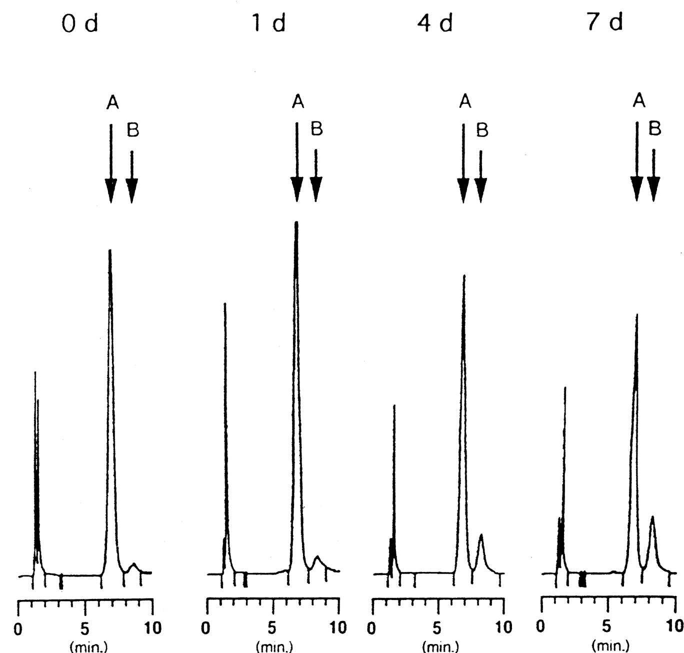

Fig. 15. Chromatograms of bovine insulin and the degradation products extracted from PLGA microspheres. A and B represent the peaks corresponding to the bovine insulin and the degradation product, respectively. (Reproduced with permission from [74]).

5. Conclusions

In conclusion, the emulsion solvent evaporation method for production of PLA and PLGA microspheres has been used extensively for the encapsulation of a variety of pharmaceutical compounds. The efficacy of this microencapsulation process is dependant on many factors, including drug solubility, partition coefficient, polymer composition and molecular weight, and method of manufacture. These variables must be considered in order to develop a successful controlled release PLGA microsphere containing drugs. Additionally, factors including polymer to core ratio, viscosity, rate of solvent evaporation and polymer crystallinity will all impact microsphere properties and drug release characteristics.

Proteins and peptides offer a unique challenge to the researcher. Proteins and peptides are susceptible to denaturization, degradation, and conformational changes which may render them inactive. These conditions can be produced by solvent interactions, mechanical processing or an acidic environment that may be encountered during microsphere production or storage. It has been shown that certain proteins may prematurely degrade the polymer used in the microencapsulation process. In response to these concerns, innovative methods of microsphere production, such as multiple emulsion systems have been investigated.

References

[1] S. Pandey, U.V. Singh, N. Udupa, Implantable flurbiprofen for treating inflammation associated with arthritis, Ind. Drugs 31 (1994) 254–257.
[2] A. Smith, I.M. Hunneyball, Evaluation of poly(lactic acid) as a biodegradable drug delivery system for parenteral administration, Int. J. Pharm. 30 (1986) 215–220.
[3] Y.W. Chien, The use of biocompatible polymers in rate-controlled drug delivery systems. Pharm. Tech. May, (1985) 50–66.
[4] C.C. Chu, Degradation phenonema of two linear aliphatic polyester fibre used in medicine and surgery, Polymerization 26 (1985) 591–594.
[5] D.H. Lewis, T.R. Tice, G.I. Zatuchni, Polymeric considerations in the design of microencapsulation of contraceptive steroids. Long Acting Contracept. Delivery Syst. (1984) 77–95.
[6] K. Suzuki, J.C. Price, Microencapsulation and dissolution properties of a neuroleptic in a biodegradable polymer, poly(dl-lactide), J. Pharm. Sci. 74 (1985) 21–24.
[7] R.U. Jalil, Biodegradable poly (lactic acid) and poly (lactide-co-glycolide) polymers in sustained drug delivery, Drug Dev. Ind. Pharm. 16 (1990) 2353–2367.
[8] J.P. Benoit, T. Painbeni, M.C. Venier-Julienne, Internal morphology of biodegradable BCNU-loaded microspheres, Proc. Int. Symp. Controlled Release Bioact. Mater. 23rd (1996) 379–380.
[9] R. Bodmeier, J.W. McGinity, Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by the solvent evaporation method, Int. J. Pharm. 43 (1988) 179–186.
[10] R. Bodmeier, J.W. McGinity, Polylactic acid microspheres containing quinidine base and quinidine sulfate prepared by the solvent evaporation method. III. Morphology of the microspheres during dissolution studies, J. Microencapsulation 5 (1988) 325–330.
[11] R. Bodmeier, H. Wang, J. Herrmann, Microencapsulation of chlorpheniramine maleate, a drug with intermediate solubility properties, by a non-aqueous solvent evaporation technique, STP Pharma. Sci. 4 (1994) 275–281.
[12] R. Jalil, J.R. Nixon, Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties, J. Microencapsulation 7 (1990) 297–325.
[13] R. Bodmeier, J.W. McGinity, The preparation and evaluation of drug-containing poly(dl-lactide) microspheres formed by the solvent evaporation method, Pharm. Res. 4 (1987) 465–471.
[14] M. Boisdron-Celle, P. Menei, J.P. Benoit, Preparation and characterization of 5-fluorouracil-loaded microparticles as a biodegradable anticancer drug carriers, J. Pharm. Pharmacol. 47 (1995) 108–114.
[15] R. Verrijk, I.J.H. Smolders, N. Bosnie, A.C. Begg, Reduction of systemic exposure and toxicity of cisplatin by encapsulation in poly(lactide-co-glycolide), Cancer Res. 52 (1992) 6653–6656.
[16] N. Mason, C. Thies, T.J. Cicero, In vivo and in vitro evaluation of a microencapsulated narcotic antagonist, J. Pharm. Sci. 65 (1976) 847–850.
[17] S. Yolles, T.D. Leafe, J.H.R. Woodland, F.J. Meyer, Long acting delivery systems for narcotic antagonists. 2. Release rates of naltrexone from poly(lactic acid) composites, J. Pharm. Sci. 64 (1975) 348–349.
[18] J.K. Lalla, K. Sapna, Biodegradable microspheres of poly-(DL-lactic acid) containing piroxicam as a model drug for controlled release via the parenteral route, J. Microencapsulation 10 (1993) 449–460.
[19] D.R. Cowsar, T.R. Tice, R.M. Gilley, J.P. English, Poly(lactide-co-glycolide) microcapsules for controlled release of steroids, Methods Enzymol. 112 (1985) 101–116.
[20] P. Giunchedi, A. Benvenga, H.O. Alpar, U. Conte, PDLLA microspheres containing steroids: Spray-drying and w/o/w emulsification as preparation methods, World Meet. Pharm. Biopharm. Pharm. Technol. 1st (1995) 389–390.
[21] L.R. Beck, R.A. Ramos, C.E. Flowers, G.Z. Lopez, D.H. Lewis, Clinical evaluation of injectable biodegradable con-

traceptive system, Am. J. Obstet. Gynecol. 140 (1981) 799–806.

[22] P.A. O'Hern, E. Goldberg, T.J. Roseman, N.A. Peppas, H.L. Gabelnick, Development of a contraceptive peptide vaccine for use with PGAL microspheres, Proc. Int. Symp. Controlled Release Bioact. Mater. 20th (1993) 394–395.

[23] R. Langer, J. Folkman, Polymers for the sustained release of proteins and other macromolecules, Nature 263 (1976) 797–800.

[24] R. Langer, New methods of drug delivery, Science 249 (1990) 1527–1533.

[25] C. Aftabrouchad, E. Doelker, Preparation methods for biodegradable microparticles loaded with water-soluble drugs, STP Pharma. Sci. 2 (1992) 365–380.

[26] P. O'Donnell, B. Schrader, M. Haase, M. Iwata, J.W. McGinity, Multiphase Microspheres of Poly (D,L-Lactic-co-Glycolic Acid) Containing Water-Soluble Compounds, Prepared by a Multiple-Emulsion Solvent Evaporation Technique, in: H.S. Kas, A.A. Hincal (Eds.), Minutes of the 9th Int. Sym. Microencapsulation, Ankara, 1993, pp. 68–75.

[27] H.V. Maulding, T.R. Tice, D.R. Cowsar, J.W. Fong, J.E. Pearson, J.P. Nazareno, Biodegradable microspheres: Acceleration of polymeric excipient hydrolytic rate by incorporation of a basic medicament, J. Control. Release 3 (1986) 103–117.

[28] B.R. Conway, H.O. Alpar, Double emulsion microencapsulation of proteins as model antigens using polylactide polymers. Effect of emulsifier on the microsphere characteristics and release kinetics, Eur. J. Pharm. Biopharm. 42 (1996) 42–48.

[29] H. Jeffery, S.S. Davis, D.T. O'Hagan, Preparation and characterization of poly(lactide-co-glycolide) microparticles. Part 2. Entrapment of a model protein using a (water-in-oil)-in-water emulsion solvent evaporation technique, Pharm. Res. 10 (1993) 362–368.

[30] J. Herrmann, R. Bodmeier, Effect of particle microstructure on the somatostatin release from poly(lactide) microspheres prepared by a W/O/W solvent evaporation method, J. Control. Release 36 (1995) 63–71.

[31] R.C. Mehta, B.C. Thanoo, P.P. DeLuca, Peptide containing microspheres from low molecular weight and hydrophilic poly(d,l-lactide-co-glycolide), J. Control. Release 41 (1996) 249–257.

[32] C. Sturesson, P. Artursson, L. Svensson, J. Carlfors, Preparation and characterization of hydrophobic PLG microspheres suitable for oral vaccination with rotavirus, Proc. Int. Symp. Controlled Release Bioact. Mater. 23rd (1996) 515–516.

[33] R. Koneberg, T. Kissel, Microencapsulation of tetanus toxoid using poly(lactide-co-glycolide)polymers and novel ABA-triblock-copolymers for single-shot vaccines, World Meet. Pharm. Biopharm. Pharm. Technol. 1st (1995) 399–400.

[34] M. Iwata, J.W. McGinity, Preparation of multi-phase microspheres of poly(d,l-lactic acid) and poly(d,l-lactic-co-glycolic acid) containing a W/O emulsion by a multiple emulsion solvent evaporation technique, J. Microencapsulation 9 (1992) 201–214.

[35] Y. Tabata, S. Gutta, R. Langer, Controlled delivery systems for proteins using polyanhydride microspheres, Pharm. Res. 10 (1993) 487–496.

[36] P.B. O'Donnell, M. Iwata, J.W. McGinity, Properties of Multiphase Microspheres of Poly(D,L-lactic-co-glycolic acid) Prepared by a Potentiometric Dispersion Technique, J. Microencapsulation 12 (1995) 155–163.

[37] M. Cavalier, J.P. Benoit, C. Thies, The formation and characterization of hydrocortisone-loaded poly((. + − .)-lactide) microspheres, J. Pharm. Pharmacol. 38 (1986) 249–253.

[38] T. Uchida, K. Yoshida, A. Ninomiya, S. Goto, Optimization of preparative conditions for polylactide (PLA) microspheres containing ovalbumin, Chem. Pharm. Bull. 43 (1995) 1569–1573.

[39] R. Bodmeier, K.H. Oh, H. Chen, Effect of the addition of low molecular weight poly(dl-lactide) on drug release from biodegradable poly(dl-lactide) drug delivery systems, Int. J. Pharm. 51 (1989) 1–8.

[40] T. Sato, M. Kanke, H.G. Schroeder, P.P. DeLuca, Porous biodegradable microspheres for controlled drug delivery. Part 1. Assessment of processing conditions and solvent removal techniques, Pharm. Res. 5 (1988) 21–30.

[41] P. Sansdrap, A.J. Moes, Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly(DL-lactide-co-glycolide) microspheres, Int. J. Pharm. 98 (1993) 157–164.

[42] W.-I. Li, K.W. Anderson, P.P. DeLuca, Kinetic and thermodynamic modeling of the formation of polymeric microspheres using solvent extraction/evaporation method, J. Control. Release 37 (1995) 187–198.

[43] B. Conti, I. Genta, T. Modena, F. Pavanetto, Investigation on process parameters involved in polylactide-co-glycolide microspheres preparation, Drug Dev. Ind. Pharm. 21 (1995) 615–622.

[44] S. Izumikawa, S. Yoshioka, Y. Aso, Y. Takeda, Preparation of poly(l-lactide) microspheres of different crystalline morphology and effect of crystalline morphology on drug release rate, J. Control. Release 15 (1991) 133–140.

[45] R. Jalil, J.R. Nixon, Microencapsulation using poly(DL-lactic acid) III: Effect of polymer molecular weight on the release kinetics, J. Microencapsulation 7 (1990) 357–374.

[46] The United States Pharmacopoeia, 23 revision, (1995) Organic Volitile Impurities. Rockville, USA, pp. 1747.

[47] C. Bitz, E. Doelker, Influence of the preparation method on residual solvents in biodegradable microspheres, World Meet. Pharm. Biopharm. Pharm. Technol. 1st (1995) 409–410.

[48] N. Gangrade, J.C. Price, Simple gas chromatographic headspace analysis of residual organic solvent in microspheres, J. Pharm. Sci. 81 (1992) 201–202.

[49] C. Sturesson, J. Carlfors, K. Edsman, M. Andersson, Preparation of biodegradable poly(lactic-co-glycolic) acid microspheres and their in vitro release of timolol maleate, Int. J. Pharm. 89 (1993) 235–244.

[50] R. Bodmeier, J.W. McGinity, Polylactic acid microspheres containing quinidine base and quinidine sulphate prepared by the solvent evaporation technique. II. Some process parameters influencing the preparation and properties of microspheres, J. Microencapsulation 4 (1987) 289–297.

[51] G. Spenlehauer, M. Vert, J.P. Benoit, F. Chabot, M. Veillard, Biodegradable cisplatin microspheres prepared by the solvent

evaporation method: morphology and release characteristics, J Controlled Release 7 (1988) 217–229.
[52] H.T. Wang, E. Schmitt, D.R. Flanagan, R.J. Linhardt, Influence of formulation methods on the in vitro controlled release of protein from poly(ester) microspheres, J. Control. Release 17 (1991) 23–31.
[53] R.S. Pradhan, R.C. Vasavada, Formulation and in vitro release study on poly(DL-lactide) microspheres containing hydrophilic compounds: glycine homopeptides, J. Control. Release 30 (1994) 143–154.
[54] J. Herrmann, R. Bodmeier, Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on w/o/w-multiple emulsions, Int. J. Pharm. 126 (1995) 129–138.
[55] C. Schugens, N. Laruelle, N. Nihant, R. Jerome, P. Teyssie et al., 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. Release 32 (1994) 161–176.
[56] I. Soriano, A. Delgado, R.V. Diaz, C. Evora, Use of surfactants in polylactic acid protein microspheres, Drug Dev. Ind. Pharm. 21 (1995) 549–558.
[57] W. Lu, T.G. Park, Protein release from poly(lactic-co-glycolic acid) microspheres: protein stability problems, PDA J. Pharm. Sci. Technol. 49 (1995) 13–19.
[58] S. Cohen, T. Yoshioka, M. Lucarelli, L.H. Hwang, R. Langer, Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres, Pharm. Res. 8 (1991) 713–720.
[59] G. Crotts, T.G. Park, Preparation of porous and nonporous biodegradable polymeric hollow microspheres, J. Control. Release 35 (1995) 91–105.
[60] H.K. Sah, R. Toddywala, Y.W. Chien, Influence of biodegradable microcapsule formulations on the controlled release of a protein, J. Control. Release 30 (1994) 201–211.
[61] D.T. O'Hagan, D. Rahman, H. Jeffery, S. Sharif, S.J. Challacombe, Controlled release microparticles for oral immunization, Int J Pharm 108 (1994) 133–139.
[62] H. Sah, R. Toddywala, Y.W. Chien, Continuous release of proteins from biodegradable microcapsules and in vivo evaluation of their potential as a vaccine adjuvant, J. Control. Release 35 (1995) 137–144.
[63] D.T. O'Hagen, J.P. McGee, R. Boyle, D. Gumaer, W.C. Koff et al., Preparation, characterization and pre-clinical evaluation of an orally administered HIV-1 vaccine, consisting of a branched peptide immunogen entrapped in controlled release microparticles, J. Control. Release 36 (1995) 75–84.
[64] H.O. Alpar, A.J. Almeida, Identification of some of the physico-chemical characteristics of microspheres which influence the induction of the immune response following mucosal delivery, Eur. J. Pharm. and Biopharm. 40 (1994) 198–202.
[65] K. Makino, H. Ohshima, T. Kondo, Effects of plasma proteins on degradation properties of poly(L-lactide) microcapsules, Pharm Res 4 (1987) 62–65.
[66] K. Makino, H. Ohshima, T. Kondo, Mechanism of hydrolytic degradation of poly(L-lactide) microcapsules: effects of pH, ionic strength and buffer concentration, J. Microencapsulation 3 (1986) 203–212.
[67] S.E. Frisbee, J.W. McGinity, Influence of nonionic surfactants on the physical and chemical properties of a biodegradable pseudolatex, Eur. J. Pharm. and Biopharm. 40 (1994) 355–363.
[68] P.B. O'Donnell, J.W. McGinity, Properties of Multiphase microspheres of poly(dl-lactic acid) or poly(dl-lactic-co-glycolic acid) produced by mechanical agitation, sonication, or potentiometric dispersion, J. Microencapsulation 13 (1996) 667–677.
[69] L.S. Liu, A. D'Emanuele, R. Langer, Experimental approach to elucidate the mechanism of ultrasound enhanced polymer erosion and release of incorporated substances, Macromolecules 25 (1992) 123–128.
[70] G. Reich, Ultrasound induced degradation of aliphatic polyesters during microsphere processing: influence of formulation variables, in: Proceedings of 1st World Meeting on Pharm. Biopharm. and Pharm. Tech., Budapest, 1995, pp. 9–11.
[71] H.K. Sah, Y.W. Chien, Evaluation of a microreservoir-type biodegradable microcapsule for controlled release of proteins, Drug Dev. Ind. Pharm. 19 (1993) 1243–1263.
[72] Y. Hayashi, S. Yoshioka, Y. Aso, A. Li Wan Po, T. Terao, Entrapment of proteins in poly(L-lactide) microspheres using reversed micelle solvent evaporation, Pharm. Res. 11 (1994) 337–340.
[73] J. Brange, L. Langkjaer, S. Havelund, A. Volund, Chemical Stability of Insulin. 1. Hydrolytic Degradation During Storage of Pharmaceutical Preparations, Pharm. Res. 9 (1992) 715–726.
[74] T. Uchida, A. Yagi, Y. Oda, Y. Nakada, S. Goto, Instability of bovine insulin in poly(lactide-co-glycolide) (PLGA) microspheres, Chem. Pharm. Bull. 44 (1996) 235–236.
[75] T.G. Park, W. Lu, G. Crotts, Importance of in vitro experimental conditions on protein release kinetics, stability and polymer degradation in protein encapsulated poly(DL-lactic acid-co-glycolic acid) microspheres, J. Control. Release 33 (1995) 211–222.
[76] R.E. Johnson, L.A. Lanaski, V. Gupta, M.J. Griffin, H.T. Gaud, T.E. Needham, H. Zia, Stability of atriopeptin III in poly(DL-lactide-co-glycolide) microspheres, J. Control. Release 17 (1991) 61–67.