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Report

Influence of Average Molecular Weights of Poly(DL-Lactic Acid-Co-Glycolic Acid) Copolymers 50/50 on Phase Separation and *in Vitro* Drug Release from Microspheres

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The phase separation of fractionated poly(DL-lactic acid-co-glycolic acid) copolymers 50/50 was determined by silicone oil addition. Polymer fractionation by preparative size exclusion chromatography afforded five different microsphere batches. Average molecular weight determined the existence, width, and displacement of the "stability window" inside the phase diagrams, and also microsphere characteristics such as core loading and amount released over 6 hr. Further, the gyration and hydrodynamic radii were measured by light scattering. It is concluded that the polymer–solvent affinity is largely modified by the variation of average molecular weights owing to different levels of solubility. The lower the average molecular weight is, the better methylene chloride serves as a solvent for the coating material. However, a paradoxical effect due to an increase in free carboxyl and hydroxyl groups is noticed for polymers of 18,130 and 31,030 SEC (size exclusion chromatography) $\overline{\text{Mw}}$. For microencapsulation, polymers having an intermediate molecular weight (47,250) were the most appropriate in terms of core loading and release purposes.

KEY WORDS: microencapsulation; polymer–solvent affinity; poly(DL-lactic acid-co-glycolic acid) copolymers 50/50; average molecular weight; drug release.

INTRODUCTION

Biodegradable parenteral delivery systems, such as implants or microspheres, can replace treatment that calls for frequent injections for chronic therapy (1). Parenterally applied microspheres and implants are complex delivery systems. The interactions between the drug substance, the polymer matrix, and the preparative technology determine their performance *in vitro* and *in vivo* (2).

In a previous paper (3), the formulation of microspheres was possible owing to phase diagrams. The overall average molecular weights of poly(DL-lactic acid-co-glycolic acid) copolymers was 49,000. The phase separation phenomenon was studied by adding an incompatible polymer. The stabilization of the coacervate droplets and, consequently, the formation of microspheres were obtained in a defined range of concentrations of the different ingredients. However, the presence of low molecular weight compounds in the different polymer batches was shown to affect the overall hydrophobicity of the matrix-forming polymers and the experimental conditions of the phase separation.

To optimize the microencapsulation of a LHRH analogue, i.e., triptoreline (4), we determined the influence of average molecular weights on the phase separation of the coating polymer and the *in vitro* drug release from microspheres. Poly(DL-lactic acid-co-glycolic acid) copolymer 50/50 was chosen as the wall material because its biodegradability allows the formulation of a parenteral delivery system (5).

MATERIALS AND METHODS

Materials

Poly(DL-lactic acid-co-glycolic acid) copolymer 50/50 (PLGA; batch number P3) was obtained at 180°C by ring-opening polymerization in a solution of dry, freshly purified acid dimers, DL-lactide and glycolide, with Sn Cl_2, $2\ \text{H}_2\text{O}$ as catalyst (6). Commercial lactide or glycolide (Boehringer, Le Vesinet, France) was purified by dissolution into the minimal amount of ethylacetate at 60°C and subsequent crystallization by cooling down to room temperature. This was repeated three times. The purified dimers were dried at 30°C in a vacuum oven for 72 hr prior to use. The composition of the various poly(α-hydroxy acid) chains was 55.6% DL-lactic units and 44.4% glycolic units.

The amounts of lactic (LA) and glycolic acid (GA) units were determined by ^{1}H-NMR. The nonfractionated polymer P3 had the following SEC (size exclusion chromatography) characteristics, 1% (w/v) in THF, i.e., tetrahydrofuranne.

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SEC $\overline{Mw}$, weight-average molecular weight:	49,590
SEC $\overline{Mn}$, number-average molecular weight:	18,000
P, polydispersity value:	2.75

Its inherent viscosity in hexafluoroisopropanol is 0.72 dl/g. The water content determined according to the Karl Fisher method is 0.03%. The T_g as given by DSC runs is located at 43°C.

Silicone oil of viscosity grade (350 cs) was obtained from Dow Corning (Valbonne, France). The LHRH analogue was supplied by Bachem CH (Budendorf, Switzerland) as a fine lyophilisate powder. Stabilized THF (SDS, Vitry sur Seine, France) and methylene chloride (Rectapur, Prolabo, Paris) were used as received. The dye for phase diagrams was purchased from Prolabo (Bleu Patenté Violet, Prolabo, Paris).

Methods

Preparative Size Exclusion Chromatography

Fractionation of a polymer P3 solution in THF (cleared through a sintering filter) was realized at room temperature using four columns (Styragel 2 × 1000 Å, 2 × 10,000 Å, 122 × 2.1 cm, Millipore Division Waters, St. Quentin en Yvelines, France) in order to obtain different fractions with determined SEC characteristics (Fig. 1). The samples (concentration of 1.25% in THF) were injected automatically and repeatedly in SEC columns. The flow rate of the THF mobile phase was 5 ml · min^{-1}. At the end of processing, five fractions were collected in the form of diluted solutions. The solutions were concentrated by evaporation at 40°C and polymers were precipitated by a nonsolvent, *n*-heptane. After decantation, the supernatant was eliminated by filtration. The five precipitates swollen by the nonsolvent were washed and dried *in vacuo* (<0.1 Torr) for 24 hr.

The amounts of residual solvents were determined by gas chromatography (Carlo Erba, Milano, Italy). The operating conditions were the following: flow rate, 25 ml/min; inox column type, Porapak Q; column size, 150 cm × 2 mm in diameter; temperature of injection, 210°C; column temperature, 190°C; flame ionization detector temperature, 240°C, aliquot, 2 µl; and gas, nitrogen. The residual solvents were 0.93% THF and 3.6% *n*-heptane.

Preparation of Phase Diagrams

Polymer solutions in methylene chloride at different concentrations (2.5, 5, and 10%, w/v) were poured into centrifuge tubes equipped with screw stoppers. The dye was then dispersed in the organic solution. Aliquots of 1 ml 350 cs silicone oil were progressively added. After each addition, the tubes were vigorously shaken with a vortex mixer (Genie K 550 GE model, Scientific Industries Inc, Bohemia, NY). Then a sample was observed under an optical microscope equipped with coatless instant pack film (Olympus BH$_2$, Tokyo). The phase diagrams were established using a 10-ml PLGA solution at 20°C.

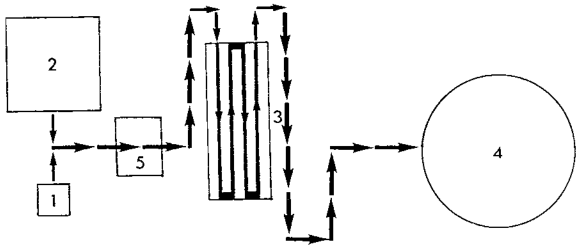

Fig. 1. Diagram of preparative size exclusion chromatography as conceived by the Laboratoire de Physico-Chimie Macromoléculaire, UA 509, Faculté des Sciences, 72017 Le Mans. (1) Sample solution in THF. (2) Solvent tank. (3) SEC columns. (4) Fraction collector. (5) Alternative pump (Gilson, model 302).

Preparation of Microsphere Batches

Preparation of microspheres was done by polymer phase separation. The LHRH analogue (2.9%, w/w) was suspended in PLGA methylene chloride solution under stirring (500 rpm). Silicone oil was added to precipitate out the five polymer obtained fractions. The suspension of semiformed microspheres was transferred to a nonsolvent solution to cause them to harden. The microspheres were then filtered (pore size, 8 µm), washed with nonsolvent solution, and dried *in vacuo*.

A typical procedure consisted of dissolving 2 g of PLGA in 81 ml of methylene chloride. After dispersion of 60 mg of LHRH analogue, 40 to 80 ml of 350 cs silicone oil was added (depending on polymer batches). The medium was then transfered to 2.5 liters *n*-heptane. The fabrication yield was based on a microsphere weight/starting material weight ratio ranging from 90 to 99%. The core loading as determined by HPLC assay ranged, for the five fractions, from 1.52 to 2.92% (w/w) after radiosterilization. Gamma radiosterilization was performed at 2.5 Mrad (Conservatome, Montluel, France).

Peptide Content

Forty milligrams of LHRH analogue-loaded microspheres was dissolved in 5 ml of methylene chloride. The peptide was extracted with 5 ml acetic acid and subjected to HPLC analysis. The HPLC operating conditions are described in Ref. 7.

Size Distribution Analysis

This was determined using a Coulter counter, TAII model (Coultronics, Margency, France).

In Vitro *Release Studies*

These were performed by incubating 50 mg microspheres in 20 ml phosphate buffer (pH 7.4) at 37°C using a stirrer (Roto-torque rotator, Cole-Parmer Instrument Company, Chicago). After 6 hr, a 100-µl aliquot of the supernatant was taken and assayed by HPLC according to the experimental conditions previously described.

Analytical Size Exclusion Chromatography

Analyses were made using a PL gel column (10 µm mixed gel, 60 × 0.7 cm, Polymer Laboratories Ltd, Church Stretton, Shropshire, UK). The flow rate of THF mobile phase was 1 ml · min^{-1}. One hundred fifty microliters of a 0.2% (w/w) polymer solution was injected in a SEC apparatus equipped with a differential refractometer (R410 model, Millipore Division Waters, St. Quentin en Yvelines, France). Calibration was made with polystyrene standards. Referring to this calibration curve, SEC number- and weight-average molecular weights (SEC $\overline{\mathrm{Mn}}$ and SEC $\overline{\mathrm{Mw}}$) were calculated by the usual method of automatic data reduction using an Apple II E microcomputer.

A special on-line viscosimetric detector (8) was connected with the refractometer. This capillary viscosimeter, fed by a 0.9-ml siphon, allowed automatic intrinsic viscosity measurements of each injected sample (9).

Light-Scattering Measurements

To prevent particle contamination, the polymer solutions were filtered on 0.22-μm PTFE membranes (Millipore, St. Quentin en Yvelines, France). A Malvern system (PCS 100 goniometer and K 7032 multi 8 bit correlator with 256 channels, Spring Lane, Malvern, Worcestershire, UK) fitted with a 4-W Spectra Physic argon laser at 488 nm was used for total intensity measurements and for photon correlation studies.

Total intensity measurements were made according to the classical Zimm plot method, using four different concentrations, and angles ranged from 30 to 150°. It was possible to determine light-scattering weight-average molecular weights, radii of gyration, and second virial coefficients.

Photon correlation measurements (10) were made on 1% (w/w) polymer solutions at a 90° observation angle. Average diffusion coefficients $\overline{D}$ were obtained from correlograms by the cumulant method (11), and according to the Einstein formula, they were converted to hydrodynamic radii (Fig. 2).

$$Rh = kT/6\pi\eta\overline{D}$$

Scanning Electron Micrographs of Peptide-Loaded Microspheres

The microspheres were dispersed in a hard resin (OCT, Miles Inc., Epernon, France) at 20°C. Cross sections in the larger diameter of particles prepared with different molecular weight polymers were made at −40°C using a congealed cutter (Jung Frigo Cut 2800 N 20-40 model, Reichert-Jung, Villepinte, France).

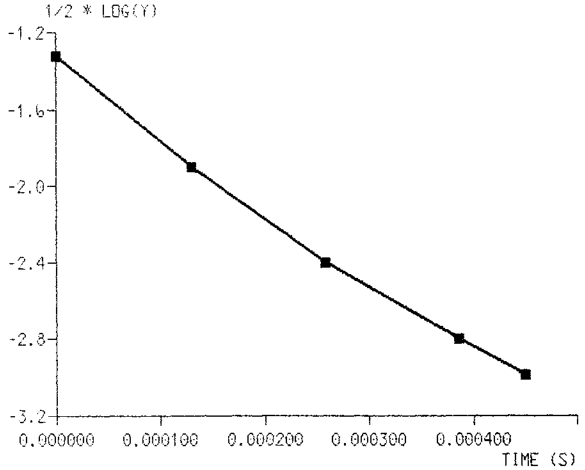

Fig. 2. Example of correlogram: average diffusion coefficient measurement. Correlation function: *Y*. Fundamental sample time: 2.0 μsec. Experiment time: 60 sec. Concentration: 1% (w/v). Sample = polymer P003; $\overline{\mathrm{Mw}}$ = 101,500; Dh = 146.4 nm; Rh = 73.2 nm.

RESULTS

Polymer Fractionation

Preparative size exclusion chromatography yields isomolecular fractions and replaces old fractionation processes such as coacervation. A commercial stabilized grade THF was selected as the mobile phase because it generally avoids interactions between solvent and gels used for SEC columns. For preparative fractionation, a 1.25% (w/v) polymer solution was prepared by stirring at room temperature (100 rpm). To avoid column damage, very high molecular weight compounds were eliminated by membrane filtration. The resulting polymer solution was limpid. Results of the fractionation are given in Table I. $[\eta]_{PS}$ represents the equivalent polystyrene intrinsic viscosity ($cm^3 \cdot g^{-1}$) and $[\eta]$ is the true intrinsic viscosity. The ratio $[\eta]_{PS}/[\eta]$ is constant near 0.8, and according to the universal calibration concept, SEC values of $\overline{\mathrm{Mn}}$ and $\overline{\mathrm{Mw}}$ could be multiplied by this factor to obtain correct calculated data (9). From these fractions, five batches of polymer were formed for subsequent studies.

Phase Diagrams

Phase diagrams were established to allow the preparation of peptide-loaded microspheres. On a phase diagram, each point corresponds to a defined weight percentage of methylene chloride, PLGA, and silicone oil. It is possible to study the sequence of events occurring with a polymer solution following the progressive addition of 350 cs-grade silicone oil. The process can be divided into four steps.

In step 1, the amount of phase inducer added to the polymer solution is low (1–5%, v/v). Silicone oil seems to form a pseudoemulsion in the organic phase.

In step 2, for a higher amount of silicone oil, the beginning of phase separation appears. The droplets of coacervate give bigger structures which merge.

In step 3, a stable dispersion of polymer coacervate droplets corresponds to the "stability window."

Step 4 is characterized by an aggregation of coacervate droplets which precipitate out.

Figures 3 and 4 show that increasing amounts of 350-cs viscosity-grade silicone oil must be added to the polymer solution to induce formation of stable coacervate droplets when weight-average molecular weights decrease. Significant differences show up in the areas of the "stability window" divided into two groups: polymers P003 with SEC $\overline{\mathrm{Mw}}$ = 18,130, SEC $\overline{\mathrm{Mw}}$ = 31,030, and SEC $\overline{\mathrm{Mw}}$ = 47,250 leading to the largest surfaces and polymers P003 with SEC $\overline{\mathrm{Mw}}$ = 70,590 and SEC $\overline{\mathrm{Mw}}$ = 101,500 exhibiting the narrowest windows.

Preparation of phase diagrams is important for determining the volume of silicone oil, *Z* (ml), inducing the formation of microspheres by polymer–polymer incompatibility (3). To define the influence of average molecular weight on the characteristics of the peptide-loaded microspheres, the ratio *Z*/*Y* was calculated, where *Y* is the volume of methyl-

Table I. SEC Data for Nonfractionated and Fractionated Polymers

Fraction	SEC $\overline{\text{Mw}}$ × 10^{-3}	SEC $\overline{\text{Mn}}$ × 10^{-3}	*P*	$[\eta]_{PS}$ (cm^3/g)	$[\eta]$ (cm^3/g)	% (w/w)[a]	Comments
Nonfractionated polymer 3, 1% (w/v) in THF	49.59	18.00	2.75	26.25	30.00	—[b]	Before fractionation
27–28	110	52.38	2.1	46.9	67	8	Before collecting
29–30	104	77.61	1.34	45.7	47.1	9.6	
31–32	70	52.24	1.34	34.5	38.8	20.2	
33–34	48.5	39.11	1.24	26.8	31.5	22.2	
35–36	32.7	24.22	1.35	20.1	22.6	18.4	
37–38	19	13.67	1.39	15.9	19.4	10.9	
39–40	11	6.18	1.78	*[c]	*	6	
41–42	7	6.36	1.1	*	*	3.3	
43–44	<4	<3	—	*	*	1.4	
27–30	101.5	72.5	1.4	45	—	17.6	After collecting
31–32	70.59	59.93	1.24	35.1	43.9	20.2	
33–34	47.25	38.4	1.23	26.5	31.3	22.2	
35–36	31.03	25.0	1.24	19.7	25.3	18.4	
37–44	18.13	9.3	1.95	13.1	23.3	21.6	

[a] Ratio (w/w) of recovered polymers.

[b] No measurement.

[c] Less than the sensibility limit.

ene chloride. For the five polymers studied, Table II shows the relationship existing between SEC $\overline{\text{Mw}}$, *Z*/*Y*, microsphere average diameter, specific surface, and core loading after radiosterilization. No significant differences are observed for microsphere average diameter and calculated specific surface, when the polymer average molecular weight increases. However, this is true only when the microspheres are isolated from the middle of the stability window. It was previously shown that, in the case of nonfractionated polymers, the microsphere average diameter increases with the added amounts of silicone oil (3). This can be correlated with the regions of the ternary diagram. When the composition of the medium evolves from step 2 to step 4 through step 3, the size of the formed microspheres progressively increases. For fractionated polymers, the results in Table II seem to contradict these findings. In fact, the increase in amounts of silicone oil allows, for each polymer, attainment of the middle of the stability window (step 3), and consequently, no significant change in microsphere diameter can be observed. The microsphere size essentially depends, for silicone oil amounts sufficient to reach the stability window, on the stirring speed during the microencapsulation process. It is a mechanical phenomenon.

The core loading increases when the average molecular weight decreases except for fraction 37–44, which is lower than fraction 35–36, certainly because of the microsphere size, which differs slightly for these two samples. With high SEC $\overline{\text{Mw}}$, especially for fraction 27–30, the entrapping is poor. The core loading is about 1.52% (w/w) after radiosterilization and is therefore very low with regard to the theoretical core loading, about 2.9% (w/w). An HPLC purity assay (7) to detect possible degradation of the peptide during irradiation indicated that peptide purity was, in any case, greater than 98%.

Figure 5 demonstrates the role of SEC $\overline{\text{Mw}}$ on amounts of peptide released over 6 hr in a phosphate buffer at 37°C. Released percentages decrease and then increase when SEC $\overline{\text{Mw}}$ increases. Peptide extraction from the microspheres in the buffer occurs more readily with low SEC $\overline{\text{Mw}}$ samples,

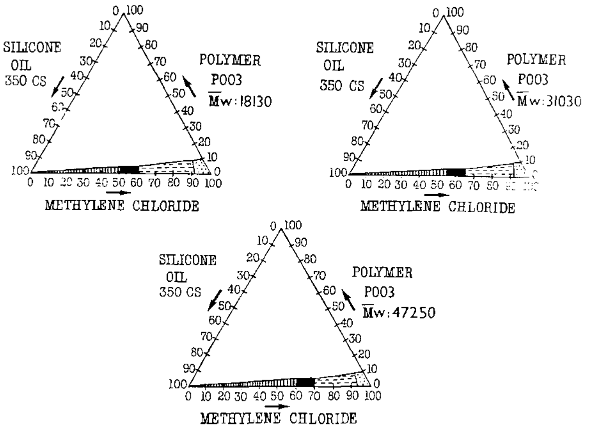

Fig. 3. Phase diagrams for the coacervation of different average molecular weight polymer batches. (⊡) Step 1; (▤) Step 2; (■) Step 3 (stability window); (▥) Step 4.

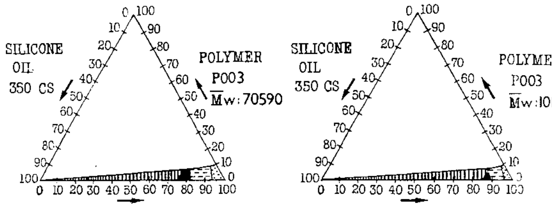

Fig. 4. Phase diagrams for the coacervation of different average molecular weight polymer batches. (⊡) Step 1; (▤) Step 2; (■) Step 3 (stability window); (▥) Step 4.

Table II. Influence of the Average Molecular Weight on the Characteristics of the Peptide-Loaded Microspheres

Fraction	SEC $\overline{\text{Mw}}$ in THF $\times 10^{-3}$	Z/Y	Average diameter (μm)	SD (μm)	Calculated specific surface $(\text{cm}^2 \cdot \text{g}^{-1})$	Core loading % (w/w) after radiosterilization	Encapsulation efficiency, % (w/w)
27–30	101.5	16.2	58.26	20.66	836	1.50	
			62.29 **60.3**	25.34	804 **832**	**1.52**	52.4
			60.37	27.37	857	1.55	
31–32	70.59	30.6	63.64	24.03	773	2.03	
			65.90 **64.8**	27.51	776 **766**	**2.00**	69.0
			64.95	22.18	748	1.97	
33–34	47.25	58.4	61.74	19.23	770	2.28	
			65.82 **64.5**	25.20	740 **744**	**2.31**	79.7
			66.06	21.81	723	2.34	
35–36	31.03	72.4	77.18	33.09	717	3.01	
			64.20 **71.0**	26.47	798 **746**	**2.92**	100.0
			71.66	30.06	724	2.84	
37–44	18.13	81.8	52.26	23.12	979	2.46	
			60.59 **60.2**	39.15	896 **889**	**2.52**	86.9
			67.66	30.55	792	2.59	

accounting for a large peptide loss. With the most important SEC $\overline{\text{Mw}}$, the microencapsulation is incomplete with peptide near the microsphere surface, resulting in a larger peptide loss than expected.

Measurement of Physicochemical Parameters: Fundamental Aspects

For this study by light scattering, two solvents were used, THF and methylene chloride. The hydrodynamic radius found in THF and polymer $\overline{\text{Mw}}$ vary in the same way. In contrast with methylene chloride, the polymer $\overline{\text{Mw}}$ appears to be more important than expected for the lowest values: the polymer seems to aggregate itself in big clusters via an association of free carboxyl and hydroxyl groups, when the SEC $\overline{\text{Mw}}$ is below 47,250 (Table III).

Methylene chloride is apolar since its polarity value according to Snyder (12) is equal to 3.4. In this work, the polarity scale goes from 0 for *n*-hexane to 9 for water. THF is more polar than methylene chloride since its polarity value is 4.2. In reality, THF solvates carboxyl and hydroxyl groups very efficiently by formation of hydrogen bonds and reduces cluster formation.

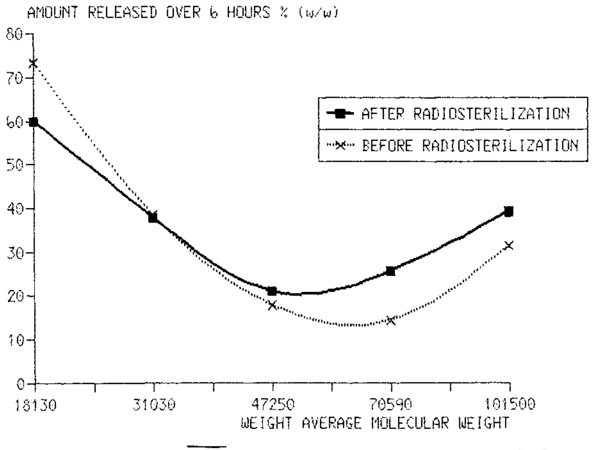

Fig. 5. Effect of SEC $\overline{\text{Mw}}$ on amounts released over 6 hr before and after radiosterilization.

The polyesters presenting a high density of hydroxyl- and carboxyl-terminal groups (low SEC $\overline{\text{Mw}}$) tend to hide these hydrophilic chemical functions in a hydrophobic environment such as methylene chloride. This behavior would be nonexistent in THF. To confirm this cluster formation, a light-scattering analysis was carried out to determine the gyration radius and the light-scattering $\overline{\text{Mw}}$ in methylene chloride. Because of the importance of aggregation effects, no reliable values of second virial coefficient were obtained. Like the hydrodynamic radius, the gyration radius and light-scattering $\overline{\text{Mw}}$ decrease and then increase when the SEC $\overline{\text{Mw}}$ decreases.

DISCUSSION

Copolymers presenting different SEC $\overline{\text{Mw}}$ values have variable affinity with solvents. We can distinguish the following.

Copolymers with a Low SEC $\overline{\text{Mw}}$ but Without Very Low Molecular Weight Compounds (<400), i.e., 31,030 and 18,130

Chains of these polymers readily solvate. Dissolution in organic solvents such as THF or methylene chloride is fast, but the density of free carboxyl and hydroxyl groups, during dissolution phenomena, induces a loss of hydrophobicity and a cluster formation. Polymer–solvent interactions are strong but less than expected. This is shown by the hydrodynamic radius, which is low in THF and abnormally high in methylene chloride.

The "stability window" in phase diagram is large but the amount of silicone oil necessary to coacervate polymer solution is less than one would expect.

Table III. Measurements of Physicochemical Parameters in THF and Methylene Chloride

Fraction	SEC $\overline{\text{Mw}}$ in THF $\times 10^{-3}$	Light-scattering $\overline{\text{Mw}}$ in CH_2Cl_2 $\times 10^{-3}$	Rg (nm) in CH_2Cl_2	Rh (nm) in CH_2Cl_2[a]	Rh (nm) in THF[a]
Nonfractionated polymer P_3	49.59	95	55.6	26.0 ± 2.7	16.0 ± 1.1
27–30	101.5	664	52.1	69.0 ± 3.1	70.0 ± 3.5
31–32	70.59	189	53.7	22.0 ± 0.7	13.0 ± 1.2
33–34	47.25	113	49.3	15.0 ± 0.6	10.5 ± 0.9
35–36	31.03	134	49.6	46.5 ± 0.8	10.0 ± 0.6
37–44	18.13	674	111.5	85.0 ± 1.8	<5[b]

[a] Average of four measurements.

[b] Less than the apparatus sensibility limit.

Figure 6 summarizes the two phenomena observed in the phase diagram. When SEC $\overline{\text{Mw}}$ decreases, solvation in methylene chloride is good, and consequently, the amount of silicone oil necessary to coacervate droplets is high. We note a displacement to the left of the stability window. But copolymers with a relatively low hydrophobic affinity owing to the presence of carboxyl and hydroxyl groups involve the use of lower amounts of silicone oil than expected. We note a displacement to the right of the stability window which counterbalance the expected effect.

Copolymers with a High SEC $\overline{\text{Mw}}$, i.e., 70,590 and 101,500

Dissolution in organic solvent is slower and often incomplete. Chains are difficult to separate. Hydrodynamic radius values are high in THF and methylene chloride as a result of weak polymer–solvent interactions. $\overline{\text{Mw}}$ given by light scattering indicates aggregate formation, and in this case the "stability window" is narrow and the amount of silicone oil to form stable droplets is low.

Copolymers with an Intermediate SEC $\overline{\text{Mw}}$, i.e., 47,250

Chains of these polymers are easy to solvate. Dissolution in organic solvent such as THF or methylene chloride is fast and complete. There is minimum aggregation in this case. Polymer–solvent interactions are strong, the hydrodynamic radius is low in THF and methylene chloride, and the light-scattering $\overline{\text{Mw}}$ is low but superior to SEC $\overline{\text{Mw}}$ data. The "stability window" in the phase diagram is wide and the amount of silicone oil necessary to stabilize coacervate droplets is large. Further, the phenomenon of polymer dissolution during processing influences the *in vitro* microsphere data.

The first type of polymer is characterized by a high core

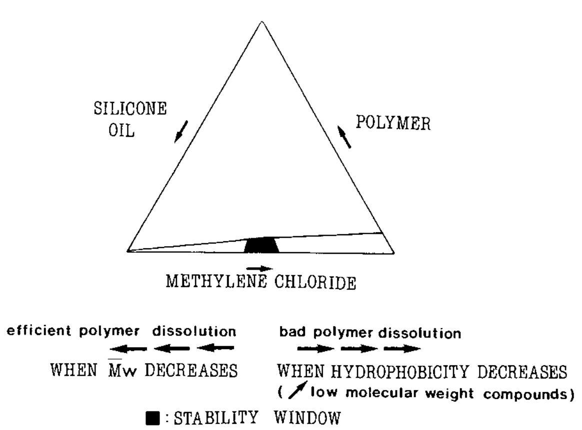

Fig. 6. Influence of polymer $\overline{\text{Mw}}$ and hydrophobicity on displacement of stability window.

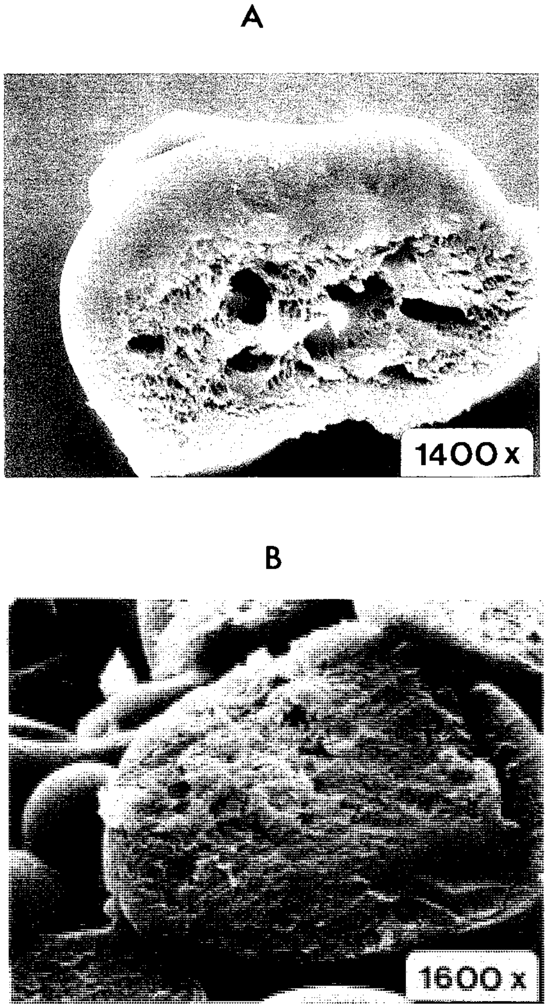

Fig. 7. (A) Microspheres prepared with polymer P003, $\overline{\text{Mw}}$ = 18,130. (B) Microspheres prepared with polymer P003, $\overline{\text{Mw}}$ = 101,500.

loading (correct encapsulation with a good incorporation of peptide in polymer coating) and an important peptide loss. The peptide extraction is facilitated in a porous microsphere by phosphate buffer at 37°C (Fig. 7A). The second type is characterized by a more compact structure (Fig. 7B). These copolymers present a peptide loss due to poor entrapping and a dramatically low core loading. For the purpose of formulation, the amount released over 6 hr and core loading can be adjusted by changing SEC $\overline{Mw}$. The third type of polymer leads to the highest microsphere loading and the smallest peptide loss.

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