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Research paper

Ultrasound-induced degradation of PLA and PLGA during microsphere processing: influence of formulation variables

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Abstract

The effect of probe sonication during microsphere processing on the stability of various aliphatic polyesters based on lactic acid (PLA) and lactic/glycolic acid (PLGA) was investigated. The weight average molecular weight (M_w) of the polymers dissolved in dichloromethane (DCM) generally decreased with an increase in duration and/or intensity of the sonication process. The extent of the M_w-reduction was more pronounced with polymers of high initial M_w and high GA content. Polydispersity indices (PD = M_w/M_n) were nearly unchanged indicating that random chain cleavage is the likely degradation mechanism. From the observation that ultrasound-induced polymer degradation slightly increased in the presence of suspended drug particles acting as cavitation nuclei, it can be concluded that the mechanical stress induced by the implosive collapse of cavitation bubbles is at least partly responsible for the observed effects in PLA/PLGA solutions. The use of ultrasound for the preparation of W/O, O/W and W/O/W emulsions exhibited different effects depending on the formulation and the type of emulsion. The preparation of W/O emulsions generally lead to M_w-changes comparable to those observed for the corresponding polymer solutions. Fatty acid free bovine serum albumin (BSA_{ff}) was found to protect PLA and PLGA against ultrasound-induced degradation in W/O-emulsions due to the formation of a semisolid interfacial film. A tremendous effect not only on the polymer M_w, but also on its PD could be observed, when ultrasound was used to emulsify an organic polymer solution or W/O-emulsion in an external aqueous phase. As this last finding was found to have rather important implications on the drug loading efficiency, the hydration, the degradation and the initial release characteristics of the resulting microspheres, it can be concluded that probe sonication can be a rather critical process step during the preparation of microspheres. © 1998 Elsevier Science B.V.

Keywords: Poly(D,L-lactide); Poly(D,L-lactide-co-glycolide); Microspheres; Ultrasound-induced polymer degradation; Formulation effects

1. Introduction

During the last two decades biodegradable microspheres based on poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) became very popular in the field of drug delivery. The most widely used methods for the preparation of polyester microspheres are solvent evaporation techniques based on O/W or W/O/W emulsions. It has been reported by several groups [1–6] that type and intensity of the dispersion method used to prepare the different emulsions are important parameters affecting particle size, morphology and thus drug loading efficiency and release characteristics of the resulting microspheres. In many cases, highly energetic homogenization techniques such as probe sonication have been used to effectively reduce the particle size below 10 μm [5], to improve drug loading efficiency [1–4] and/or to reduce the initial burst effect [1–3].

Probe sonication has, however, also been reported to impose harmful damage to the native structure of large proteins, thus reducing their biological potency [7–9]. Moreover, ultrasound has been observed to increase the

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degradation rate of biodegradable polyanhydride and polyester devices [10–14]. Interestingly, this latter aspect has only been taken into consideration as an effective means to externally control drug release rates from biodegradable implants. Its possibly negative effect during the microsphere formation process on the molecular weight (M_w) of the polymer [15] and thus on the performance of the resulting microspheres has so far not been investigated in detail.

In view of this, the present study was undertaken to systemically investigate the effect of ultrasound on the stability of various aliphatic polyesters dissolved in an organic solvent at different stages of the microsphere formation process. Special attention was paid to the effect of certain formulation variables.

2. Materials and methods

2.1. Materials

Aliphatic polyesters of various composition (PLA, PLGA 75/25 and PLGA 50/50) and weight average M_w (R 206, R 202, R 104, RG 755, RG 752, RG 505, RG 502) were purchased from Boehringer Ingelheim (Ingelheim, Germany). The following chemicals were obtained from different commercial suppliers and used without further purification: quinine (Caelo, Hilden, Germany) acetylsalicylic acid (Serva, Heidelberg, Germany), sodium chloride (Roth, Karlsruhe, Germany) bovine serum albumin (BSA_{ff}; fraction V; fatty acid free), chicken egg albumin (ovalbumin; grade V; salt-free), bovine pancreatic trypsin (Type I) (Sigma, Deisenhofen, Germany), polyvinylalcohol (PVA, Mowiol 8/88: 40 kDa, 88 % hydrolysed) (Hoechst AG, Frankfurt, Germany), dichloromethane (DCM) (Riedel de Haen, Seelze, Germany) and tetrahydrofuran (THF) (Merck, Darmstadt, Germany).

2.2. Experimental set-up

Each polymer or polymer blend was dissolved in DCM to a final concentration of 0.5 or 0.2 g/ml, respectively. In the first series of experiments, 6 ml samples of each polymer solution were probe sonicated using a Branson Model B-12P sonifier (Branson Sonic Power, Danbury, CT) equipped with a needle probe. Intensity and duration of the sonication process were varied from 20 to 60 W and from 10 to 90 s, respectively. The temperature was kept constant at 20°C.

To assess the effect of ultrasound in the presence of a dissolved and dispersed drug, quinine, acetylsalicylic acid and sodium chloride were used as model compounds. Quinine and acetylsalicylic acid were dissolved and sodium chloride (mean particle size: 10.2 μm) was dispersed in DCM solutions of RG 755, RG 755/R 104 (3/1) and RG 752, respectively. Probe sonication was carried out at 50 W for 30 s.

The same conditions and the same polymers were used for the preparation of microfine probe-sonicated W/O, O/W and W/O/W emulsions. Vortex mixing (Vortex Genie 2, Bender and Hobein, Zurich, Switzerland) for 2 min at level 8 (maximum speed) was used for comparison. In case of W/O emulsions, the inner aqueous phase was either pure distilled water or an aqueous solution of various proteins. For the preparation of the O/W emulsions, the polymer solution (4 ml) was emulsified in 20 ml of 1% PVA solution.

After the sonication or vortex mixing process the polymer solutions and dispersions including one sample of each formulation which was not probe sonicated were cast into teflon-coated dishes and dried to constant weight. The emulsions were added into 400 ml of 0.1% PVA solution and stirred for 4 h at room temperature to evaporate the solvent. The resulting microspheres were collected by centrifugation, washed with water and vacuum- or freeze-dried, respectively.

2.3. Molecular weight characterization

The molecular weight distribution (M_w, M_n, PD) of the polymer composing the microspheres and films was determined by gel permeation chromatography (GPC) using a combination of two PL gel columns (10 μm/10^3 Å; 10 μm/10^5 Å) (Polymer Laboratories, Shropshire, UK) thermostated at 35°C and a refractive index detector (Knauer, Bad Homburg, Germany). The polymer samples were dissolved in THF, filtered through a 0.5 μm teflon-filter and injected with a 100 μl sample size. The flow rate was 1 ml/min. Evaluation was carried out on a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan) using a series of polystyrene standards (Merck) for calibration.

2.4. Particle size measurements

Mean particle size and particle size distribution of the microspheres were measured by laser light scattering (Mastersizer X, Malvern, UK).

2.5. Protein entrapment and release characteristics

The amount of protein entrapped within protein-loaded microspheres was determined by the Bradford assay (BioRad, München, Germany) after digesting the microspheres with sodium hydroxide. The entrapment ratio is expressed as the ratio of actual to theoretical loading.

The in vitro release studies were carried out at 37°C in phosphate-buffered saline (PBS; 0.033 M phosphate buffer) pH 7.2.

2.6. In vitro polymer hydration and degradation

Hydration and degradation of the microspheres in the release medium were followed by differential scanning calorimetry (DSC) and GPC, respectively. DSC measure-

ments were carried out using a DSC system TA 3000 from Mettler (Greifensee, Switzerland) consisting of a TC 10 A processing unit and a DSC 30 low temperature cell. Hydrated samples (4–8 mg) in sealed aluminium pans were scanned from −100 to +100°C under a nitrogen atmosphere at a heating rate of 10 K/min and evaluated with respect to their glass transition temperature (T_{gH}).

3. Results and discussion

All polymer samples dissolved in DCM experienced a reduction in their weight average M_w when exposed to ultrasound. The extent of this M_w-reduction depended strongly on the duration and intensity of the sonication process and on the chemical composition and molecular weight distribution of the polymer. It was, however, only slightly affected by the polymer concentration in the range between 0.2 and 0.5 g/ml.

As shown in Fig. 1 for the organic solution of RG 755 at a concentration of 0.5 g/ml, the weight average M_w generally decreased with an increase in the intensity and/or the duration of the sonication process indicating polymer decomposition induced by ultrasound. From a practical point of view, it is interesting to note that at intensities of 40 W and above a significant M_w-reduction could be observed even after short exposure times of 30 or 20 s, respectively.

As shown in Fig. 2, using probe sonication for 30 s at 50 W, the sensitivity of the polymers towards ultrasound-induced degradation strongly increased with an increase in the GA content and/or the weight average M_w. Interestingly, the width of the molecular weight distribution and thus the PD remained unchanged, irrespective of the duration and/or the intensity of the sonication process (Fig. 3), the polymer composition and the initial M_w. This suggests random chain scission as the likely cleavage reaction of ultrasound-induced polyester degradation in organic solution.

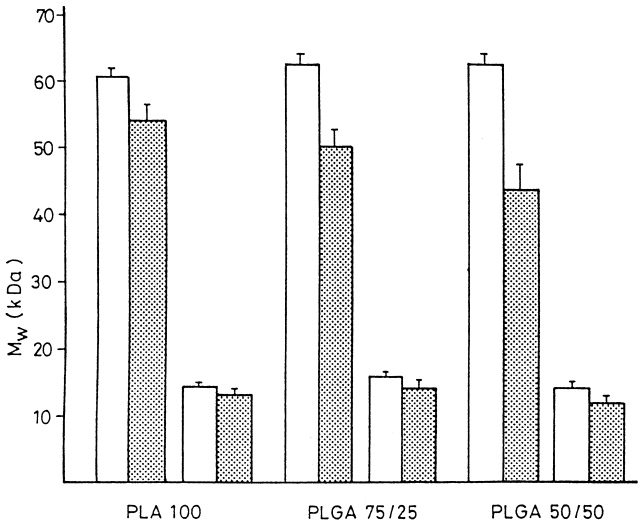

Fig. 2. Effect of polymer composition and initial weight average M_w on ultrasound-induced M_w-reduction (c_P = 0.5 g/ml; T = 20°C; organic solvent: DCM; n = 6). Unshaded area, before probe sonication; shaded area, after probe sonication (50 W/30 s).

To further elucidate the mechanism underlying the cleavage process of ultrasound-induced polyester degradation, the effect of increasing amounts of suspended particulate matter acting as cavitation nuclei was investigated. The corresponding results obtained with RG 755 solutions containing increasing amounts of sodium chloride under constant conditions with respect to intensity (50 W) and duration (30 s) of the sonication process are presented in Fig. 4. A slight, but significant increase in the M_w-reduction with increasing amounts of sodium chloride could be observed at solute concentrations above 10% m/v. PD remained constant again. As comparable results could be obtained with the other polymers, it can be concluded that the mechanical stress induced by the implosive collapse of cavitation bubbles is mainly responsible for the observed effects in PLA/PLGA solutions and drug suspensions.

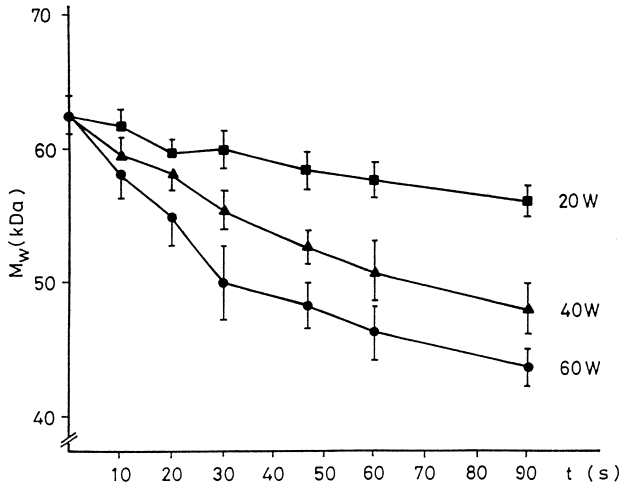

Fig. 1. Effect of sonication time and intensity on the weight average M_w of RG 755 dissolved in DCM (c_P = 0.5 g/ml; T = 20°C; n = 6).

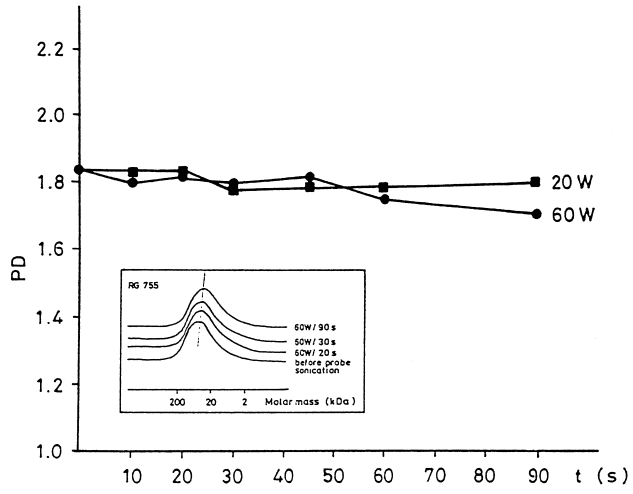

Fig. 3. Effect of sonication time and intensity on the PD = M_w/M_n of RG 755 dissolved in DCM (c_P = 0.5 g/ml; T = 20°C; n = 6).

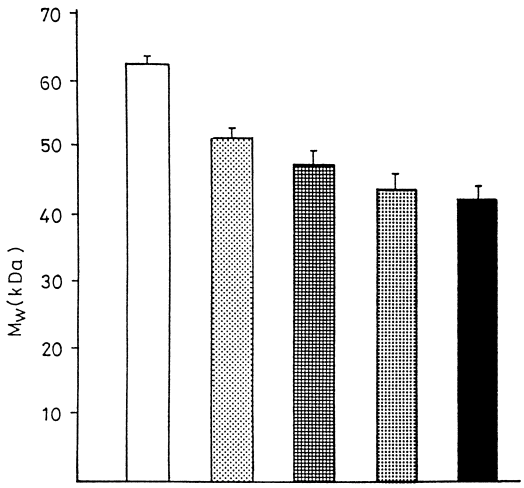

Fig. 4. Effect of dispersed sodium chloride on the ultrasound-induced M_w-reduction of RG 755 dissolved in DCM (c_P = 0.5 g/ml; T = 20°C; n = 6). Unshaded area, RG 755, before probe sonication; light shaded area, RG 755 after probe sonication (50 W/30 s); dark shaded area, RG 755 + 5% m/v sodium chloride, after probe sonication (50 W/30 s); medium shaded area, RG 755 + 10% m/v sodium chloride, after probe sonication (50 W/30 s); and black shaded area, RG 755 + 20% m/v sodium chloride, after probe sonication (50 W/30 s).

In contrast, the effect of dissolved drugs on the ultrasound-induced polyester degradation depended strongly on their chemical nature suggesting that drug/polymer interactions might be of critical importance. As shown in Fig. 5 at a concentration of 10 % m/v, no significant effect could be observed in the presence of acetylsalicylic acid. Acid-catalyzed hydrolytic chain cleavage can therefore be ruled out as a co-operative degradation mechanism. In the case of quinine, however, the concentration dependent catalytic effect of the quinuclidin-nitrogen on the ester bond cleavage reaction, which was already observed in the absence of the ultrasound, was dramatically increased upon probe sonication. This last finding suggests that in the presence of a dissolved amine drug, which acts as a nucleophilic catalyst, chemical and mechanical effects are likely to be co-operative degradation mechanisms.

The use of ultrasound for the preparation of W/O, O/W and/or W/O/W emulsions exhibited rather complex effects depending on the formulation and the type of emulsion. Probe sonication of W/O emulsions generally lead to M_w-changes comparable to those observed for the corresponding polymer solutions under otherwise identical conditions. Interestingly, only a slight, insignificant effect of the inner aqueous phase volume could be detected. The same was valid when the inner aqueous phase was composed of an aqueous protein solution containing increasing amounts of ovalbumin or trypsin (Fig. 6). An interesting feature was, however, observed in the presence of fatty acid free BSA_{ff}. As illustrated in Fig. 6, only a slight or even no reduction in the polymer weight average M_w could be detected upon increasing the amount of BSA_{ff} within the inner aqueous phase. The different effects of BSA_{ff}, ovalbumin and trypsin can neither be attributed to differences in their interfacial tensions [16] nor to differences in their intrinsic stability upon probe sonication [9], since both properties are in the same order of magnitude for BSA_{ff} and ovalbumin, but completely different for trypsin. The effect can, however, be explained by the exclusive ability of an aqueous BSA_{ff} solution to interact with the organic polymer phase in such a way as to instantaneously form a semisolid interfacial film thus obviously protecting the polymer molecules against ultrasound-induced degradation. BSA_{ff} might therefore be used as a stabilizer in the inner aqueous phase upon emulsifying a water-soluble drug in an organic PLA/PLGA solution.

As shown in Fig. 7 for RG 755 and RG 752, respectively,

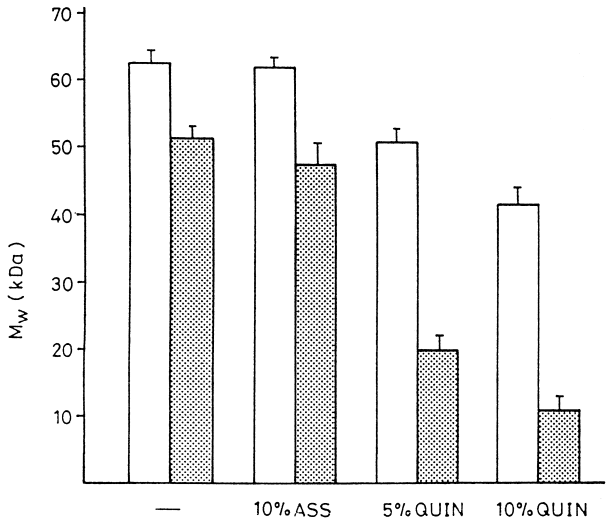

Fig. 5. Effect of dissolved drugs on the ultrasound-induced M_w-reduction of RG 755 dissolved in DCM; c_P = 0.5 g/ml; T = 20°C; n = 6; ASS, acetylsalicylic acid; and QUIN, quinine. Unshaded area, before probe sonication; and light shaded area, after probe sonication (50 W/30 s).

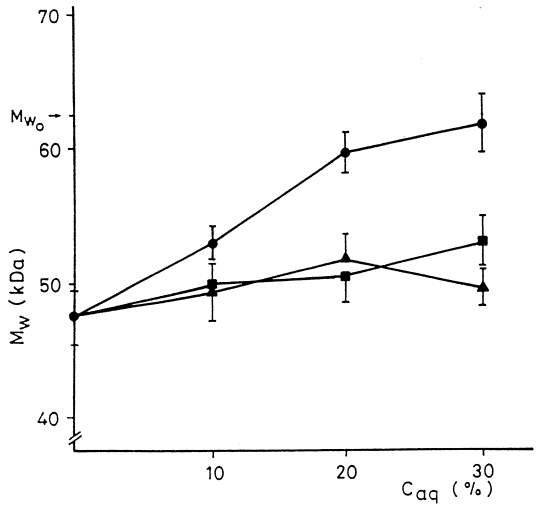

Fig. 6. Effect of inner aqueous phase composition (W_1) on the ultrasound-induced M_w-reduction of RG 755 dissolved in DCM upon W_1/O-emulsification (c_P = 0.5 g/ml; T = 20°C; W_1 = 10% v/v; 50 W/30 s; n = 6). ●, BSA_{ff}; ■, ovalbumin; and ▲, trypsin.

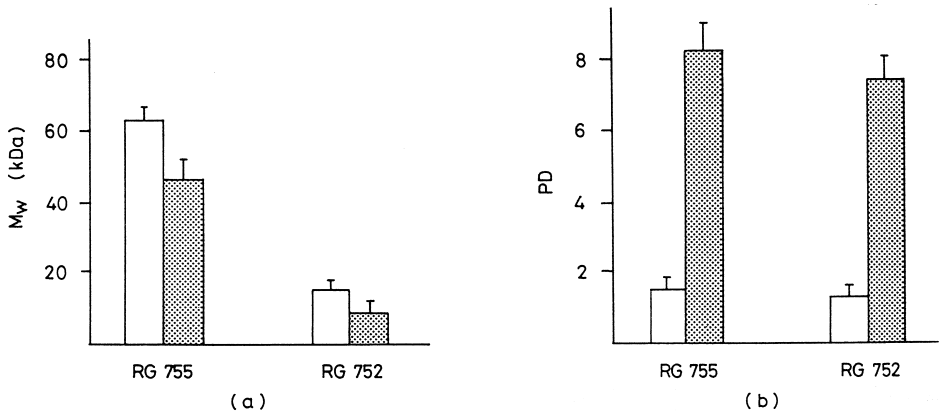

Fig. 7. Effect of probe sonication (50 W/30 s) during O/W-emulsification on the molecular weight distribution of RG 755 and RG 752 dissolved in DCM (c_P = 0.5 g/ml; T = 20°C; W = 0.1% m/v aqueous solution of PVA; n = 6). A, weight average M_w; B, PD; unshaded area, vortex mixing (level 8/2 min); and shaded area, probe sonication (50 W/30 s).

a tremendous effect not only on the polymer weight average M_w, but also on its PD and thus on the M_w distribution could be observed when probe sonication (50 W/30 s) instead of vortex mixing (level 8/2 min) was used to emulsify an organic polymer solution in an external aqueous phase containing PVA as a stabilizer. As the effect was independent of the initial weight average M_w of the polymer, it might be attributed to the additional mechanical stress that is induced on the organic polymer phase upon disruption into small droplets. Due to the formation of a large amount of low M_w molecules, rather important implications not only on the particle size distribution (see Table 1), but also on the hydration and degradation pattern of the resulting microspheres were expected. In fact, the glass transition temperature (T_{gH}) of the hydrated microspheres after 2 days in PBS pH 7.2 of 37°C was dramatically depressed from 37.8°C for the sample which was not probe sonicated to 30.5°C for the sonicated sample (Fig. 8a). The corresponding acceleration of the hydrolytic chain cleavage leading to a half-life of the M_w-reduction of 10 days compared with 40 days for the non-sonicated sample is illustrated in Fig. 8b.

As can be assumed from the aforementioned results with BSA_{ff}, the effect of ultrasound on the PD and thus on the hydration and degradation pattern of the resulting microspheres could be slightly reduced upon emulsifying a W/O emulsion containing BSA_{ff} in the inner aqueous phase. The effect was even more reduced when BSA_{ff} instead of PVA was used as a stabilizer in the outer aqueous phase. However, the PD-increase could not be fully abolished. In any case, a more or less pronounced reduction in the protein entrapment ratio (Fig. 9a) and an increase in the initial protein burst of the resulting microspheres could be detected (Fig. 9b) when probe sonication was used to prepare the second emulsion ($W_1/O/W_2$). Both effects can be attributed to the following two processes:

- Firstly, a viscosity decrease of the organic polymer phase due to the aforementioned ultrasound-induced M_w decrease and PD increase (see Fig. 7) thus providing a weaker barrier against protein spreading, i.e. protein leakage during the $W_1/O/W_2$ homogenization process.
- Secondly, a shear-induced disruption of the inner W_1/O-emulsion upon probe sonication of the $W_1/O/W_2$-emulsion thereby not only reducing mean particle size from 77.6 to 9.8 μm (see Table 1), but also increasing the amount of protein being in contact with the continuous water phase (W_2) and/or being located on the particle surface.

Table 1

Effect of W/O and W/O/W mixing method on the particle size of protein-free and protein-loaded microspheres

Batch no.	Polymer[a]	Protein[b]	Mixing method		Mean particle size (μm)
			W/O[c]	W/O/W[c,d]	
A/1	RG 755	–	V	–	55.0
A/2	RG 755	–	PS	–	2.5
B/1	RG 755	BSA_{ff}	–	V	77.6
B/2	RG 755	BSA_{ff}	–	PS	9.8

[a] Polymer concentration in DCM: c_P = 0.5 g/ml.
[b] Theoretical protein loading: 4% m/m.
[c] V, Vortex mixing; and PS, probe sonication.
[d] Probe sonication was used to prepare the primary emulsion W_1/O.

4. Conclusion

The study revealed that the use of ultrasound during the preparation of PLA and PLGA microspheres can lead to pronounced changes in the molecular weight distribution of these polymers depending on the formulation and the type of emulsion. Special attention should be paid to the use of ultrasound for the preparation of microfine W/O and W/O/W emulsions due to its dramatic effects not only on the protein loading efficiency and initial release characteristics, but also on the hydration and degradation pattern of

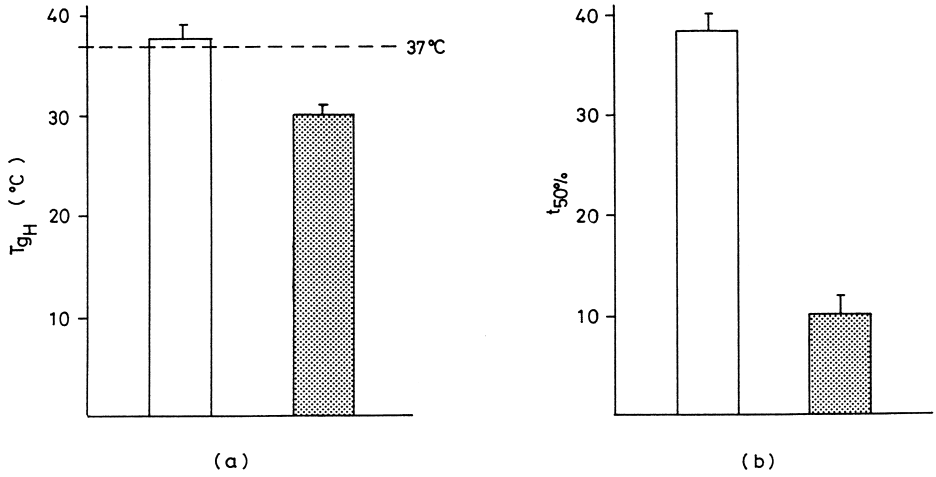

Fig. 8. Effect of probe sonication (50 W/30 s) during O/W-emulsification on (a) hydration (T_{gH}) and (b) degradation ($t_{50\%Mw}$) of the resulting microspheres (RG 755, c_P = 0.5 g/ml; W = 0.1% m/v aqueous solution of PVA; n = 4). Unshaded area, vortex mixing (level 8/2 min; batch no. A/1); and shaded area, probe sonication (50 W/30 s; batch no. A/2).

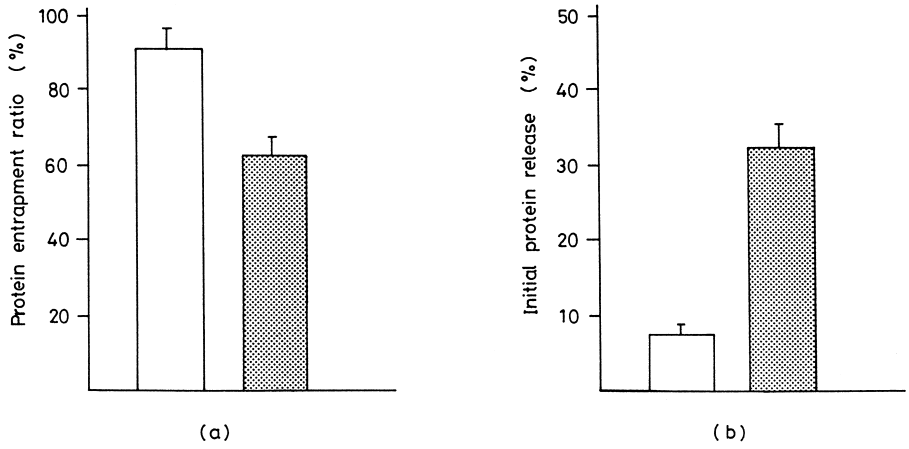

Fig. 9. Effect of probe sonication during the second emulsification step ($W_1/O/W_2$) on the properties of BSA-loaded RG 755 microspheres (c_P = 0.5 g/ml; theoretical protein loading = 4% m/m; n = 4). (a) Protein entrapment ratio; (b) initial protein release (cumulative release after 2 days); unshaded area, vortex mixing (level 8/2 min; batch no. B/1); and shaded area, probe sonication (50 W/30 s; batch no. B/2).

the resulting microspheres. The implications of the latter two aspects on the overall protein stability and release kinetics are currently under investigation and results will be presented in a forthcoming paper.

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