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Microparticle formation and its mechanism in single and double emulsion solvent evaporation

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Abstract

The emulsification is the first step of the emulsification solvent evaporation method and has been extensively investigated. On the contrary the second step, the solvent transport out from the emulsion droplets that determine the particle morphology and with great influence on the microparticles encapsulation and release behavior has been scarcely studied. This study investigates the mechanism of the solvent elimination from the emulsion droplets and its influence on the particle morphology, encapsulation and release behavior. Usually, the solvent is highly volatile that makes the solvent elimination process very fast thus difficult to observe. In order to observe in detail the microparticle formation, the initial emulsion was monitored by optical microscope under controlled solvent evaporation conditions. The results from the optical microscopic observations corroborated with laser diffractometry analysis showed that in single emulsion formulations, spherical microparticles are formed by accelerated solvent elimination due to the combined effects of high solvent volatility and polymer precipitation. The solvent expulsion accompanied by important shrinkage generates on the microparticle surface a thin layer of nanoparticles attested by scanning electron microscopy and laser diffractometry. During the intense solvent elimination, the encapsulated substance is drained, affecting the loading efficiency. Furthermore, it will concentrate towards the microparticle surface contributing to the initial burst release. In double emulsion formulations, microparticles with different morphologies are generated due to the presence of the aqueous-phase microdroplets inside the emulsion droplet. During the solvent elimination, these microdroplets generally coalesce under the pressure of the precipitating polymer. Depending mainly on the polymer concentration and emulsification energies, the final microparticles will be a mixture of honeycomb, capsule or plain structure. During the shrinkage due to the incompressibility of the inner microdroplets, the precipitating polymer wall around them may break forming holes through which the encapsulated substance is partly expulsed. Through these holes, the encapsulated substance is further partitioning with the external aqueous phase during solvent evaporation and contributes to the initial burst release during the application.

Keywords: Microparticle; Mechanism; Morphology; Emulsification solvent evaporation; Poly(lactide-co-glycolide)

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1. Introduction

The classic emulsification solvent evaporation technique (ESE) elaborated by Bodmeier and Mc Ginity [1], Ogawa et al. [2], Jeffery et al. [3], Iwata and Mc Ginity [4], and different recent variations are commonly used for encapsulation of various substances from simple pharmaceutical products to proteins and DNA [5,6].

Although the technique is well defined as methodology, there are only a few studies about the encapsulation and particle formation mechanism [7,8]. Particle formation mechanism is crucial for size distribution and morphology, which in turn determine the delivery system behavior during encapsulation and release.

It is well known that ESE is mainly a two-step process: the emulsification of a polymer solution containing the encapsulated substance, followed by particle hardening through solvent evaporation and polymer precipitation. During emulsification, the polymer solution is broken up in microdroplets by the shear stress produced either by homogenizer, sonicator or whirl mixer in the presence of a surface-active agent. This first step mainly determines the microparticles size distribution and it has been extensively investigated along with the influence of various process parameters [7–15].

This study investigates the mechanism of the second step when the initial emulsion is transformed into the final microparticles by solvent elimination and polymer precipitation. This process determines the microparticle morphology and has important influence on the microparticle encapsulation and release behavior.

2. Materials and methods

2.1. Materials

For this study, poly(DL-lactide-co-glycolide), one of the most widely used biodegradable polymer, was chosen. The copolymer, Lactel BP 0100 (PLGA) with lactide, glycolide ratio 50:50, and molecular weight 45 000–75 000 was purchased from Sigma.

Poly(vinyl alcohol), (PVA) commonly used in emulsification solvent evaporation formulations, with molecular weight 22 000, and 86–88% degree of hydrolysis, was obtained from Kanto Chemicals.

Dichloromethane (DCM) one of the most extensively used formulation solvent was purchased from Wakos.

All chemicals were used without further purification.

2.2. Equipments

High-speed emulsification was achieved by Heidolph DIAX 900 homogenizer using the 18G tool for oil in water (O/W_2), and the 6G tool for water in oil (W_1/O) emulsion. For low speed dispersion, Ika Labortechnik mixer with impeller and baffles was used.

Nikon Optiphot 114 microscope with digital imaging capabilities was used to record the microdroplet evolution into the final microparticle.

The microparticles were sized by laser diffractometry using a Shimadzu SALD 7000 laser particle analyzer.

The morphology of the microparticles was examined by scanning electron microscopy (Hitachi S-4000) after Pd-Pt coating.

2.3. Methods

The investigated single/double emulsification solvent evaporation method follows the classical method [1–4]. The values of different process parameters chosen in the present study are similar to those described in the literature.

For single emulsion formulation, a predetermined quantity of PLGA was dissolved in 5 ml DCM, forming the oil phase. Five hundred microliters of 1% w/v PVA solution in deionized water as inner aqueous phase (W_1) was used for the double emulsion formulation. High-speed homogenizer at 20 000 rpm dispersed W_1 for 2 min in the polymer solution, forming the primary emulsion (W_1/O).

The oil phase or the W_1/O emulsion was then dispersed in 30 ml of deionized water with different PVA concentrations. For high shear stress mixing, homogenizer at 8000–11 000 rpm for 2–3 min was used. For low shear stress formulations, laboratory mixer with impeller and baffles at 500–1400 rpm was

used. The oil phase or the W_1/O emulsion was added by injection through 0.2 mm needle in 15 s.

At the end of the high-speed homogenization or after 5 min in the case of low-speed formulations, the size distribution of particles was measured and one drop of emulsion was poured on a microscope slide and sealed with cover glass. The slide was mounted on the microscope and the transformation of the initial microdroplets into the final microparticles was captured by a picture sequence.

Next, the initial O/W_2 or $W_1/O/W_2$ emulsion was poured in 500 ml of 0.1% w/v PVA solution, and the solvent was let to evaporate under magnetic stirring (500 rpm) for 2 h.

The microparticles were separated by centrifugation at 3500 rpm for 5 min and washed once with 100 ml of deionized water. After separation, the size distribution of the final particles was measured and the particle morphology was examined by S.E.M.

3. Results and discussions

3.1. Single emulsion formulation (O/W_2)

In order to observe in detail the transformation of the initial emulsion into the final microparticles, one drop of the initial emulsion was poured on a microscope slide and sealed with cover glass. In this case, the solvent evaporation is restricted only through the thin emulsion–air interface located at the edges of the cover glass; thus, the whole process is slowed down allowing a proper observation of the microparticle formation.

The final microparticle is formed as the solvent is transported out from the initial droplet, transported through the aqueous phase and evaporated through the emulsion–air interface.

The solvent exits from the initial droplet only if its concentration in the aqueous phase is under the saturation limit. Right after the emulsification, the aqueous phase is practically saturated with solvent and its evaporation is possible only at the cover glass edges. The solvent evaporation at the edges of the cover glass initiates the solvent elimination from the nearby microdroplets. Once the solvent elimination initiated, the process is accelerated by the polymer precipitation and the remaining solvent is practically expulsed from the microdroplet generating the final microparticle.

Fig. 1 presents the initial and final images of the picture sequence recorded in a region near the cover glass edge. The solvent transport through the aqueous phase is controlled by diffusion. As the solvent evaporates, its concentration gradient under the cover glass propagates from the edge toward deeper regions. At the beginning of the picture sequence the aqueous

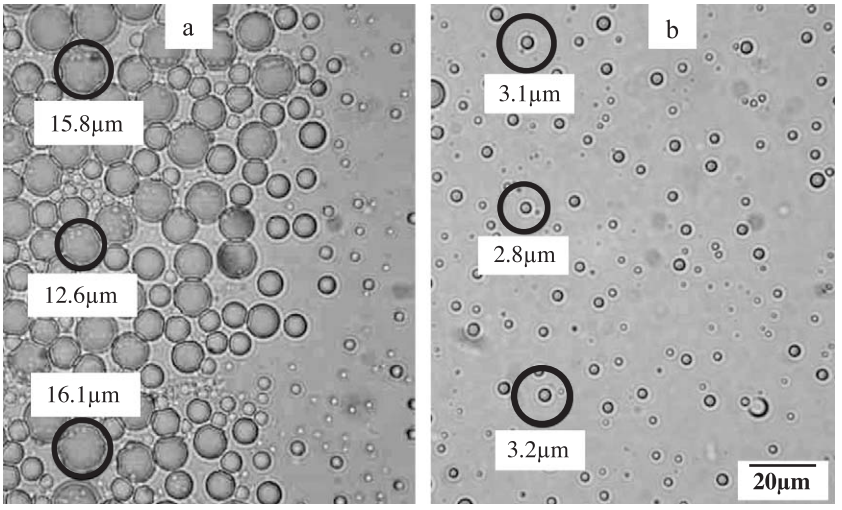

Fig. 1. The first (a) and last (b) image of the emulsion microdroplets transformation into the final microparticles. PLGA, 1% w/v; 1% w/v PVA; 8000 rpm for 3 min.

phase around the microdroplets located further from the edge (Fig. 1a) is saturated with solvent, thus their diameter remains practically unchanged until the front of low solvent concentration reaches the droplets vicinity, when they shrink very fast generating the final microparticles presented in Fig. 1b.

For usual polymer concentrations (1–10% w/v), there is a significant shrinkage during the microparticle formation, thus the final microparticle diameter is several times smaller than the corresponding microdroplet diameter (Fig. 1).

We defined the theoretical shrinkage factor (α_t) as the ratio between the diameter of the emulsion droplet and the diameter of the solvent-free microparticle. Knowing the initial polymer concentration, α_t is expressed by the following simple relation:

$$\alpha_t = \frac{d_i}{d_f} = \left(\frac{V_p + V_s}{V_p}\right)^{1/3} = \left(1 + \frac{100\rho_p}{C_p}\right)^{1/3}$$

where: d_i—diameter of the initial droplet; d_f—diameter of the final microparticle; V_p—polymer volume; V_s—solvent volume; ρ_p—polymer density (g/ml) and C_p—polymer concentration (% w/v). PGLA density measured by picnometer, and calculated using Bicerano's predictive molar volume relation [16], gave 1.31 g/ml.

Table 1 presents the theoretical (α_t) and the measured (α_m) shrinkage factors for different formulation conditions.

Considering the measuring errors of the microparticle diameter and the solvent loss during emulsification and droplet manipulation, the measured shrinkage factors are reasonably close to their corresponding theoretical values. This result sustains the fact that at the end of the fast solvent elimination, the microparticle is practically free of solvent.

Table 1
Theoretical and measured shrinkage factor of the microdroplet for different formulation conditions (1% w/v PVA)

Stirring rate/duration	C_p, % w/v	α_t	α_m
8000 rpm/3 min	1.0	5.092	4.50–5.10
1400 rpm/5 min	2	4.062	3.47–3.85
11 000 rpm/2 min	0.5	6.407	5.26–5.75
	2.5	3.766	3.75–3.85
	5.0	3.007	2.85–3.07

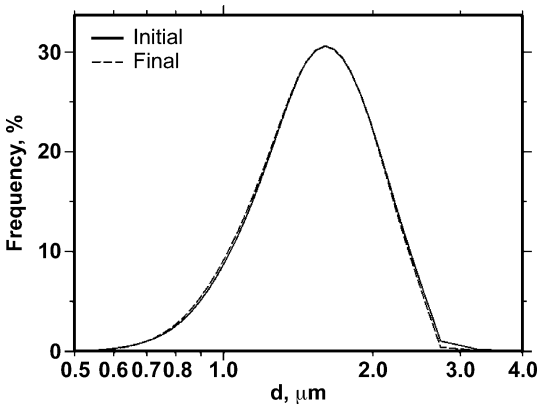

Fig. 2. Particle size distribution of the initial emulsion, and that of the final emulsion. PGLA, 1% w/v; 1% w/v PVA; 11 000 rpm, 2 min.

Fig. 2 presents the particle size distributions of the initial emulsion, right after the emulsification, and that of the final emulsion after the solvent evaporation is completed. The distributions are practically superposed sustaining the fast solvent elimination and the low residual solvent content of the final microparticles. In fact, during the size distribution analysis that takes less then 30 s, small volume of the initial saturated emulsion (1 ml) is poured in a large amount of water (250 ml) inside the analyzer where the solvent is well bellow its saturation limit, promoting fast solvent elimination.

Almost identical initial and final particle size distributions also mean that the microdroplets do not coalesce nor break up after the emulsification step, thus the particle size distribution is determined only by the emulsification step. The solvent concentration in the aqueous phase is yet another influence parameter along with all the others already investigated [7–15] that determines the particles size distribution. In order to allow the homogenizer to produce microdroplets with monomodal and narrow size distribution, the solvent concentration in the aqueous phase should be kept as close as possible to its saturation level, necessary to reach the equilibrium droplet diameter without interferences caused by the solvent evaporation.

This can be achieved by using adequate formulation and design conditions that minimize the solvent loss during emulsification. In the present study, we used an emulsification vessel with high height/diameter ratio

for smaller emulsion–air interface, and with the smallest volume possible in order to maximize the dispersion efficiency and to minimize foaming.

Taking advantage of the fast solvent elimination, the evaporation time can be significantly reduced if the initial emulsion is poured in a sufficiently large quantity of aqueous phase so that the solvent does not exceed its solubility limit.

3.1.1. Microparticle surface morphology

With a few exemptions, when the microparticle surface was found slightly rough [17–19] literature screening evidenced that microparticles are spherical and have smooth surface. Our low magnification S.E.M. micrographs showed the same spherical microparticles with smooth surface (Fig. 3a).

The high magnification S.E.M. micrographs revealed that a thin layer of nanoparticles covers the microparticle's surface (Figs. 3b, 4). Especially at high magnification, the microparticles are very sensitive to radiation. During high magnification S.E.M. observations, usually the initial shape of the particles is altered (Figs. 3b, 4), and in some cases, the crust broke-up and drift, proving the existence of the thin layer (Fig. 4b). Especially for large shrinkage extents corresponding to low polymer concentration in the initial droplet (Table 1), the nanoparticulate layer peels generating secondary particles (Fig. 4c).

For some of the formulation conditions, laser diffractometry also evidenced the nanoparticles on the microparticle surface by the presence of a secondary peak around 30 nm (Fig. 5). If the nanoparticles are assumed free, then centrifugation at low speed (3500 rpm) should eliminate them, but the secondary peak was still present after the centrifugation, proving that the nanoparticles are in fact stuck on the microparticles surface.

The nanoparticulate layer is a direct consequence of the particle formation mechanism. Once the favorable conditions for solvent elimination are set, the polymer precipitates at the water–droplet interface and tends to form a continuous crust around the emulsion droplet. But in the mean time, the particle is shrinking due to the solvent loss, thus the polymer crust around the emulsion–droplet is continuously fragmented generating the nanoparticles (Fig. 6).

3.1.2. Encapsulation and release behavior

The encapsulation efficiency is affected by the fast shrinkage because the encapsulated substance is drained during the solvent expulsion. High polymer concentration improves the encapsulation efficiency [10], mainly through smaller shrinkage and by extended entanglement of the polymer chains.

It is well known that microparticles prepared by ESE present initial burst release due to surface located encapsulated substance. Usually, single emulsion formulation is used for the encapsulation of oil soluble substances. Due its hydrophobic character, the encapsulated substance will agglomerate toward the microparticle surface during the

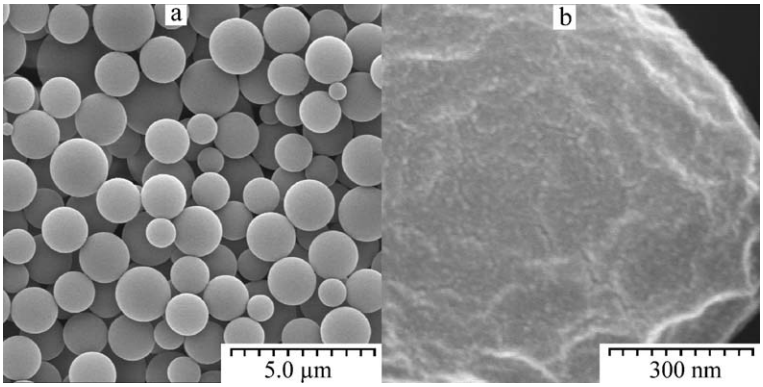

Fig. 3. S.E.M. micrographs of PLGA microparticles (a) low magnification; (b) high magnification. PLGA, 5% w/v, 5% w/v PVA 11000 rpm, 2 min.

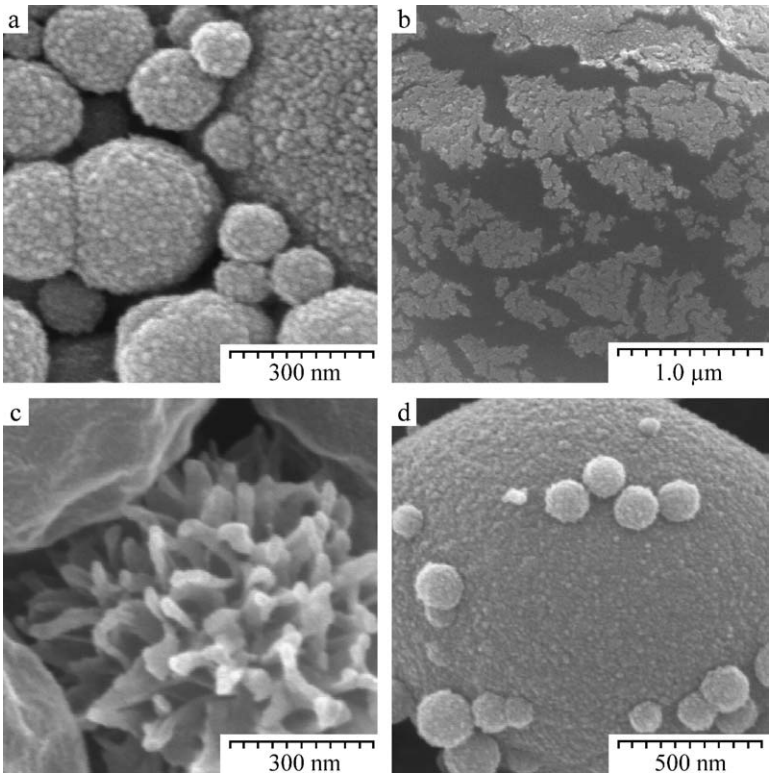

Fig. 4. High magnification S.E.M. micrographs of PLGA microparticles; (a) 1% w/v PGLA, 5% w/v PVA, 11 000 rpm, 2 min; (b) 5% w/v PGLA, 1% w/v PVA, 8000 rpm, 3 min; (c) 0.5% w/v PGLA, 1% w/v PVA, 8000 rpm; (d) 0.5% w/v PGLA, 5% w/v PVA, 11 000 rpm, 2 min.

intense solvent elimination. This enriched layer on the particle surface will contribute to the initial burst release.

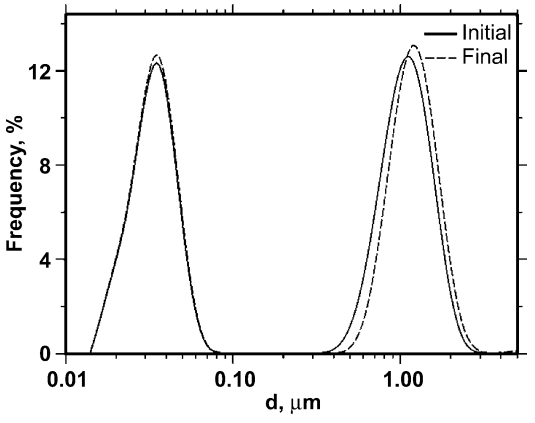

Fig. 5. Size distribution of the initial emulsion and that of the final PLGA microparticles, after centrifugation. PGLA, 1% w/v, 5% PVA, 11 000 rpm, 2 min.

3.2. Double emulsion formulation ($W_1/O/W_2$)

The evolution of $W_1/O/W_2$ droplets was recorded in a similar way to the single emulsion method. S.E.M. micrographs presented in Figs. 8b and 9b show that double emulsion formulation produces

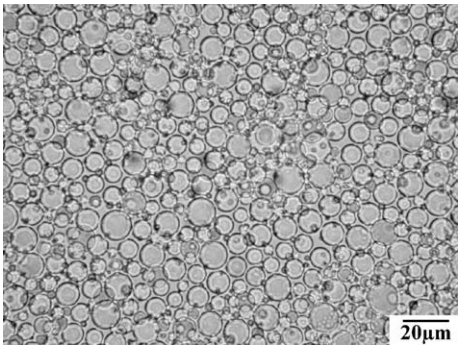

Fig. 6. Primary (W_1/O) emulsion micrograph. Five hundred microliters W_1 in 5 ml of 5% w/v PLGA; 20 000 rpm, 2 min.

microparticles with different morphologies. The origin of different microparticle morphology is the emulsion droplet with different content of inner aqueous phase. Fig. 9a presents a typical double emulsion with different droplet structures: with no entrapped inner aqueous phase (marked "1" on the figure), with one aqueous-phase microdroplet (marked "2") and with more then one microdroplet (marked "3"). These composite droplets behave differently during solvent elimination, consequently leading to different microparticle morphologies.

3.2.1. Particle morphology

The emulsion droplets without inner aqueous phase will transform in plain polymer microparticle (marked "p" on in Fig. 9b) in a similar way to the single emulsion droplets.

There are two outcomes for the droplets with one inner microdroplet: closed and open microcapsules. If the diameter of the inner microdroplet is close to that of the emulsion droplet, the polymer content is insufficient to form a resistant layer around the incompressible microdroplet, thus this layer broke during the solvent elimination forming open microcapsules with thin wall (marked "c1" on in Fig. 9b). During drying, the inner aqueous phase evaporates and the capsule collapse appearing as deflated ball on the S.E.M. micrograph. If the inner microdroplet diameter is much smaller than the emulsion droplet, closed microcapsules are generated.

The emulsion droplets with more then one inner microdroplet evolve toward capsule or honeycomb structure depending on the formulation conditions. Microcapsules are formed if the diameter of the inner microdroplet is only a few times smaller then that of the emulsion droplet and the polymer concentration is low (Fig. 7). During solvent elimination and shrinkage, the inner microdroplets gradually coalesce under the pressure of the precipitating polymer into one final microdroplet (Fig. 7, III–VI). These microcapsules are marked "c2" on the S.E.M. micrographs (Figs. 8b, 9b). If the diameter of the inner microdroplets is much smaller then that of the emulsion droplet (Fig. 8a.I) and the polymer concentration is high, microparticles with honeycomb structures are formed (Fig. 8a.IV and b). The precipitating polymer around the inner microdroplets generates this highly porous structure. As in the previous case, during solvent elimination and shrinking the inner microdroplets coalesce and the freshly formed polymer wall around them may break initiating the inner aqueous phase expulsion. The holes present on the particle surface as well as throughout the entire particle structure sustain the above mechanism (Fig. 8b).

Usually, the morphologies mentioned above are present in different proportion depending mainly on the diameter of the inner microdroplet and that of the

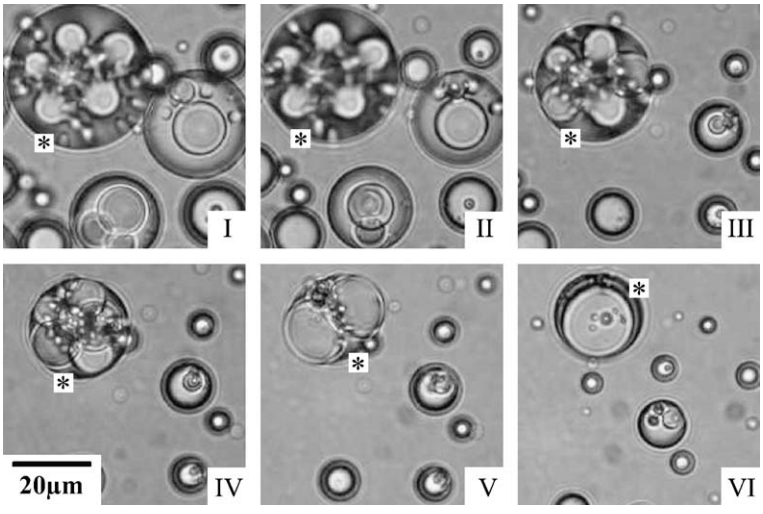

Fig. 7. Picture sequence of the secondary emulsion ($W_1/O/W_2$) transformation. PLGA, 5% w/v, 1% w/v PVA, 1400 rpm.

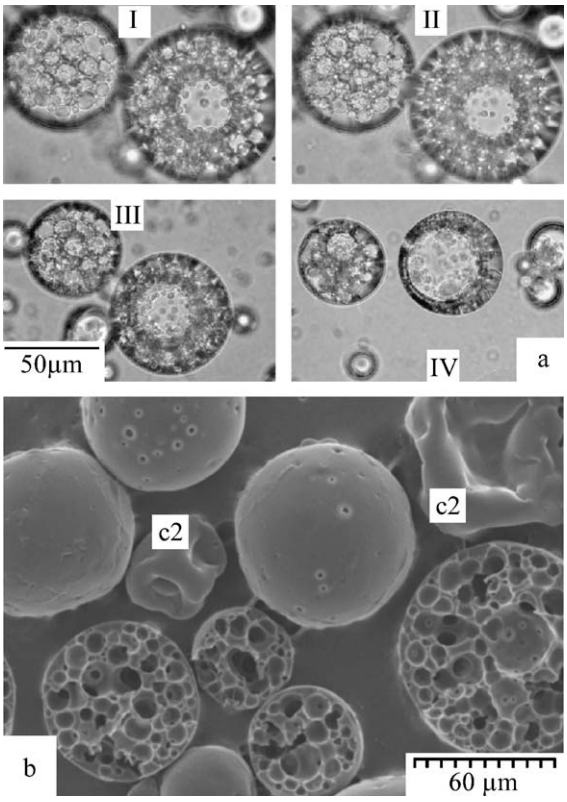

Fig. 8. Picture sequence of the initial $W_1/O/W_2$ droplet transformation (a); S.E.M. micrograph of the particle morphology (b). PLGA, 10% w/v, 1% w/v PVA, 500 rpm.

emulsion droplet. The diameter is the global parameter that encompasses all the formulation parameters: emulsification energies, polymer concentration, surface active agent concentration, phase volumes, phase viscosities, etc.

In this study, the primary emulsion was prepared always in the same conditions: 500 μl of aqueous phase in 5 ml of polymer solution was dispersed at 20 000 rpm, in order to keep its size distribution constant. Even at high stirring rates, microdroplets with diameter larger than 10 μm are still present (Fig. 6). The mean diameter of the secondary emulsion was varied by changing the stirring rate and the polymer concentration.

The honeycomb structure predominates when the diameter of the inner microdroplet is much smaller then that of the emulsion droplet (Fig. 8) [13,20,21]. In this case, the stirring rate for the secondary emulsification is much smaller (500 rpm) than that of the primary emulsification (20 000 rpm). As the stirring rate of the secondary emulsification increases, the corresponding diameter of emulsion droplets decreases and the capsule structure gradually becomes dominant (Fig. 9). Further increase of the stirring rate will produce more and more plain particles.

3.2.2. Encapsulation and release behavior

The encapsulation efficiency is directly related to the extent of W_1 phase entrapment. The honeycomb structure is the most suitable for efficient entrapment, thus for a successful encapsulation, it is necessary to choose the formulation conditions that produce W_1 microdroplets with much smaller diameter than that of the secondary emulsion [11,15].

Depending on the formulation conditions, the inner aqueous phase is partly lost during the solvent elimination and shrinkage through the holes on the surface. Comparing the initial emulsion droplet (Fig. 8a.I) to the final microparticle (Fig. 8a.IV), even in this favorable case, an important quantity of the inner aqueous phase is lost.

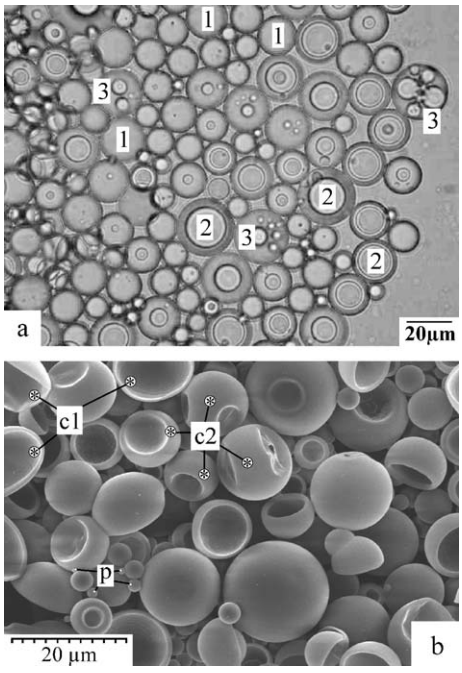

Fig. 9. Initial $W_1/O/W_2$ microdroplets (a) and final microparticle S.E.M. micrograph (b). PLGA, 5% w/v, 1% w/v PVA 8000 rpm, 3 min.

Furthermore, during the solvent evaporation, the remaining encapsulated substance is continuously partitioning with the external aqueous phase through the holes.

The holes present on the pore or capsule wall will decisively contribute to the initial burst release. For smaller particles obtained at high stirring rate, the burst release is more important [7,12,14] because the broken capsule structure is predominant in this case.

4. Conclusions

The mechanism of particle formation in single and double emulsion formulations was investigated by optical microscope under controlled solvent evaporation condition. The microparticles are generated by accelerated solvent elimination due to the combined effects of high solvent volatility and polymer precipitation. The solvent elimination accompanied by important shrinkage determines the microparticle morphology and its encapsulation and release behavior.

During the fast solvent elimination and shrinkage, the encapsulated substance is partly drained, affecting the encapsulation efficiency. Scanning electron microscopy and laser diffractometry evidenced the presence of a thin nanoparticulate layer on the microparticles surface with important role in the initial burst release. This layer is formed during the solvent elimination by the shrinkage-induced fragmentation of the precipitating polymer crust.

In double emulsion formulations, microparticles with different morphologies are generated. Depending on the inner aqueous phase content of the emulsion droplet and on the size of the inner microdroplet relative to the emulsion droplet, the outcome will be a mixture of microparticles with honeycomb, capsule and plain structure. During solvent elimination and shrinkage, the inner microdroplets gradually coalesce under the pressure of the precipitating polymer. The polymer wall around them may break forming holes on the microparticle surface, through which the inner aqueous phase is partly expulsed, affecting the loading efficiency. After particle hardening, these holes will contribute to the encapsulated substances leakage trough partitioning with the external aqueous phase and to the initial burst release.

Acknowledgements

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