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Structure and mechanical properties of polylactide copolymer microspheres and capsules

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ABSTRACT

Polylactide homopolymers, polylactide and poly(ethylene oxide) diblock and triblock copolymers are used to prepare spherical microparticles by using the single oil-in-water emulsion and solvent evaporation technique. We are able to create both bulk and hollow microspheres by altering the conditions of preparation. The experiments are carried out at two fixed temperatures of 15 and 22 °C. We show, from scanning electron microscopy data, that the microspheres produced from the homopolymers are bulk and homogeneous at both temperatures whereas they are hollow when the triblock copolymers are used. The diblock copolymers yield bulk microspheres at 15 °C and microcapsules at 22 °C. Compression experiments emphasize once more the inner morphology of the spheres. As it is expected, bulk microspheres have higher Young's modulus than the microcapsules. Nevertheless, comparative compression analysis of both morphologies shows that the microcapsules retain relatively high compressive moduli. These results have implications for the design of rigid and biodegradable microcapsules.

1. Introduction

Considerable interest exists in developing implantable and injectable biomaterials for drug delivery, tissue reconstruction and engineering [1–9]. In particular, biopolymer-based materials are the most attractive choice. This is primarily because they are easily processable, which could allow less or non-invasive therapeutic procedures. Biomaterials produced as carriers for drug delivery and scaffolds for tissue reconstruction are intended for a fixed period of use. The optimal interval corresponds to the duration of drug release or tissue growth. Hence the remaining structure might be left in place, be removed surgically, or have to degrade. Of course, degradation is preferred.

The biomaterials synthesized for drug delivery must allow fine-tuning of the device properties and to adjust the kinetic of release and the degradation time. Nevertheless the release of active molecules from drug carriers developed so far is solely controlled by erosion of the bulk material [10]. Furthermore, the use of such biodegradable materials presents some undesirable drawbacks associated with the release of toxic and low molecular weight degradation byproducts which might cause undesired in-vivo side reactions.

Among broadly studied biopolymers, polylactide (PLA) and polyglycolide (PGA) constitute a class of biocompatible polyesters that already have extensive clinical use as approved materials for implantable drug carriers and resorbable devices such as sutures and screws [10]. These biopolymers degrade by hydrolysis of ester linkages into low molecular weight molecules that are either metabolized or cleared through the renal system [11]. Moreover, these biopolymers can be readily processed into a variety of structures, while retaining high compressive moduli which make them very suitable as drug carriers, scaffolds for cell growth and bone tissue engineering applications [12–16].

Most drug carriers from PLA and PGA polymers were produced in the form of spherical particles in the micrometer size scale. To this end, single and double emulsion and evaporation techniques were used to a large extent [14,16–18]. The single emulsion preparation method involves the emulsification of the biopolymer, solubilized in an organic phase, in an aqueous phase under vigorous stirring [19]. Subsequently, the solvents are removed by evaporation. Practically, hydrophobic drug molecules are incorporated directly in the organic phase before emulsification. The double emulsion technique is a slightly improved method. Here a first aqueous or organic phase is emulsified in the biopolymer organic phase and subsequently this emulsion is re-emulsified in a large volume second aqueous phase to yield a water/oil/water emulsion.

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When the first phase is organic, the chosen solvent must be a poor solvent of the biopolymer. Among the advantages of the second method is the possibility of incorporating the desired drug molecules, either hydrophilic or hydrophobic, during the emulsification steps, in either the first aqueous phase or the organic phase respectively. It was shown that the single emulsion and solvent evaporation technique only yields bulk spheres. Advantageously the double emulsion method can produce bulk, porous and hollow spheres depending on the experimental parameters [20,21]. The characteristics of the inner aqueous phase and the solvent evaporation rate are key parameters which influence the morphology of the elaborated material.

The fabrication of hollow particles or microcapsules represents an important improvement in the area of drug carrier conception and engineering. Besides their inherent properties like light mass and large deformability, they form semipermeable membranes for the encapsulated molecules. Among other techniques utilized for the fabrication of capsules are shell-polymerization of interfacial films, assembly of polymeric particles at the oil/water interface in emulsions, and alternating layer by layer assemblies of opposite polyelectrolytes around charged colloids [22,23]. Even though these techniques have proven their effectiveness, some of them are either complex, rely on specific polymer materials, or produce shells with nanometric thickness as observed in micellar particles and vesicles [24].

Nevertheless, several strategies were investigated in order to assess which pathway is most efficient to encapsulate active species into hollow carriers [25–29]. Two primary techniques are proposed for encapsulation. The first one relies on incorporation of the corresponding molecule either prior to the synthesis of the shell or in a later stage by strained diffusion [26–28]. The second pathway relies on simultaneous administration of the specific agent with the materials that compose the capsule, which requires high stability of the agent and good chemical compatibility between all ingredients [25,29]. It is worth adding that incorporation of an active molecule prior to the fabrication of the biopolymer shell is mostly achieved by the use of the double emulsion technique mentioned above. In such case the primary emulsion phase is the drug solution in biopolymer solution. The technique has been successfully employed for the synthesis of PLA polymer capsules [26].

In the present study, we synthesized poly(L-lactide) (PLLA) and poly(DL-lactide) (PDLLA) copolymerized with poly(ethylene oxide) (PEO). We used the method of single oil-in-water emulsion technique followed by solvent evaporation to prepare polylactide microspheres. For many useful applications, the morphology of the particles and their rigidity are very important properties. For these reasons, we varied experimental parameters, such as biopolymer chemical characteristics, concentration of the biopolymer in the oil phase and temperature, in order to assess the most advantageous conditions to yield either microcapsules or bulk microspheres. The results on diblock and triblock copolymers were compared with our studies on both PLLA and PDLLA homopolymers. Furthermore, the compressibility of the obtained hollow and bulk microspheres were comparatively examined in order to evaluate the robustness of these biomaterials.

The biopolymers used herein are composed of either D or L-lactide and contain PEO blocks, whose molecular weights are large enough to be considered as amphiphilic block copolymers. Therefore, these copolymer systems, with both hydrophobic and hydrophilic blocks, represent a significant difference between the majority of PLA homopolymer systems reported in the literature [19,30,31]. Moreover, varying PEO to PLA fraction in the block copolymer provides a more complete depiction of the effect of chemical structure on the morphology of the microspheres prepared with the single emulsion technique. As discussed further below, the use of an amphiphilic block biocopolymer solubilized in a non selective solvent and the single emulsion method leads to hollow spheres with compressive moduli that are significantly high to make them suitable in various medical applications.

Investigation of encapsulation into these particular materials was not performed in this study, yet it is widely acknowledged that microcapsules have potential application as advanced functional materials for controlled release. Due to the similarities of morphology between the materials achieved here and previously reported structures, it is likely that encapsulation can be realized by two pathways. The two-step emulsion method could be used to enclose either hydrophobic or hydrophilic species into the capsules by choosing the adequate solvent on one hand [26], and the simultaneous incorporation of hydrophobic species within the shell on the other hand [29].

2. Experimental section

2.1. Materials

L and D,L-lactide dimers and homopolymers (number average molecular weight Mn = 60,000 and 75,000 g/mol for L and DL-lactide respectively), PEO (35000 g/mol), monomethylether PEO (MePEO, 2000 g/mol), polyvinyl alcohol (PVA, weight average molecular weight 31,000–50,000 g/mol, 87–89% hydrolyzed), stannous octoate, and dry organic solvents were purchased from Sigma–Aldrich (France) unless otherwise specified. L-lactide and D,L-lactide dimers were dissolved in dry ethyl acetate under reflux, left to recrystallize upon cooling, and vacuum-filtered on 0.45 μm nylon filters (Whatman) before use. Stannous octoate was diluted with dry toluene to a working concentration of approximately 0.15 g/mL. Dry dichloromethane (CH_2Cl_2), chloroform, diethyl ether and chloroform-*d* ($CDCl_3$, Cambridge Isotope) were used as received.

There are two main pathways by which PLA can be polymerized. The first involves polycondensation of aqueous lactic acid [32,33], and the second involves ring-opening polymerization of cyclic lactide dimers [34,35]. We used the second method to synthesize triblock PLA-PEO-PLA and diblock MePEO-PLA copolymers.

PLA–PEO–PLA triblock copolymers were synthesized as follows. PEO was dissolved in anhydrous toluene and dried in a bottom flask placed in an oil bath at 130 °C under nitrogen atmosphere with continuous stirring and allowed to reflux. The flask was equipped with a Dean–Stark receiver and the water-toluene azeotrope was drained from the trap before introducing the other reagents (<8 mL). Then recrystallized lactide dimers were introduced in the flask and allowed to dissolve. A second excess toluene volume (≈8 mL) was drained from the Dean–Stark trap and the system was refluxed until at least the same volume of toluene condensed in the Dean–Stark trap. Stannous octoate solution was added by syringe to initiate the reaction. The reaction was terminated after 24 h by cooling the mixture to room temperature. The product was redissolved by adding a minimum amount of chloroform under stirring. The polymer was then precipitated by slowly dribbling this solution into stirred diethyl ether. The resultant precipitate was vacuum-filtered on 0.45 μm, and the process repeated until the entire product was precipitated and isolated. The filtered polymer was dried under vacuum at room temperature for 2 days. The yield of the synthesis procedure was between 70 and 90% depending on the molecular weights.

MePEO-PLA diblock copolymers were synthesized by using monomethylether poly(ethylene oxide) to initiate the ring-opening polymerization of lactide monomers on the only free hydroxyl side

chain of the MePEO by following steps similar to those used for triblock copolymer synthesis.

The general structure of the dimer reagents, PEO homopolymers and copolymer products is schematically described in Table 1. The polymer compositions were determined by ^{1}H NMR (Bruker, 600 MHz spectrometer). The chemical characterization was carried out on the initial PEO reagent and the final product, both solubilized in $CDCl_3$, to determine the number average molecular weight of each polymer block [36].

Analysis of NMR data on the triblock copolymers was achieved by the determination of the integration ratio of resonance peak assigned to CH_2—CH_2—O groups of PEO backbone with index of polymerization n (chemical shift δ located at 3.7 ppm and integrated signal corresponding to $4 \times (n - 1)$ protons), and the two resonance peaks of CH_3 (δ 1.6 ppm, $3 \times 2y$ protons) and CH (δ 5.2 ppm, $2 \times y$ protons), both assigned to PLA blocks with index of polymerization y per block. Within this notation, we used the PEO number average molecular weight Mn, provided by the manufacturer, to determine n thereby the integrated intensity for one proton, and subsequently y.

Analysis of NMR data on the diblock copolymers, was conducted by exactly measuring the number average molecular weight of the methylated PEO (nominal Mn 2000 g/mol) thanks to the OCH_3 resonance peak located at $\delta = 3.4$ ppm. By comparing the integration ratio of this specific peak with the value integrated for CH_2—CH_2—O peak ($\delta = 3.7$ ppm; $4 \times n$ protons), the actual molecular weight was calculated to be Mn = 1880 g/mol for this specific MePEO polymer. Then we used the procedure described for the triblock polymer to evaluate the molecular weight of the lactide block with the help of integrated signals of the PLA resonance peaks corresponding to CH_3 ($3 \times y$ protons) and CH ($1 \times y$ protons). The NMR results and the number average molecular weights, Mn, of both the homopolymers and synthesized copolymers used in this study are summarized in Table 1.

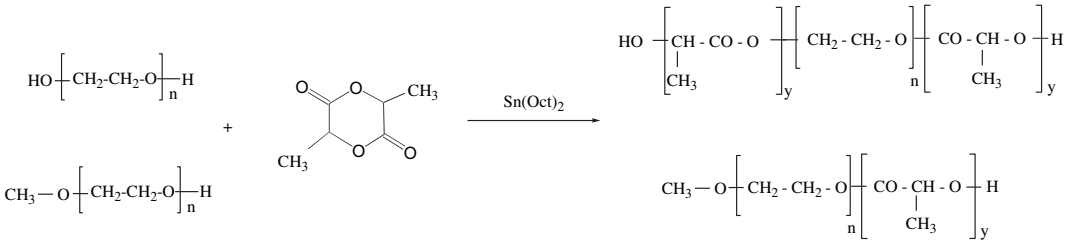
2.2. Sample preparation and instrumentation

Microspheres were prepared by the single oil-in-water emulsion and solvent evaporation method. Briefly, 0.5 ml solution of biopolymer in CH_2Cl_2, a non selective solvent towards both PEO and PLA, at a concentration of 10 wt.% was poured dropwise by the help of a syringe pump into a beaker containing 10 ml of PVA aqueous solution at a concentration of 1 wt.%. The PVA solution was mechanically stirred at 600 rpm and the temperature was fixed either at room temperature (22 °C) or 15 °C prior to pouring the organic phase. Stirring continued for 3 h to yield complete evaporation of the organic solvent and solid PLA microspheres. The spheres were collected on 80 μm filter, washed several times with de-ionized water and stored at 4 °C prior to use. We checked by optical microscopy that the filtrate does not contain visible microspheres.

Photomicrographs of the microspheres placed on cover slides were taken either on a Leitz Wetzlar (Germany) or Eclipse TE 300 Nikon (Japan) optical microscope. Some microspheres were cross-sectioned with a razor blade in order to observe the inner core. We carried out scanning electron microscopy (SEM) by using a RAITH microscope (Germany) to examine the surface and internal morphologies of the microspheres. First the samples were dried under vacuum at room temperature and few spheres were isolated and cross-sectioned under the optical microscope in order to observe their internal structure. Subsequently the whole and half spheres of each sample were mounted on silicon stubs with double sided tape and coated with aluminum to a thickness of 7 nm.

We studied the mechanical properties of the dry microspheres by the compression setup TA.XTPlus Texture Analyser (Texture Technologies, USA). To this purpose, the particles were compressed between two flat surfaces. The resulting normal force was measured as a function of the displacement applied during compression cycles at a constant speed of 10 μm/s. We used the Hertz model to analyze the compression data [37]. Hertz derived the deformation at contact for isotropic materials in the static, linear and elastic approximation. Hertz's theory gives the relationship between the deformation of a sample, enclosed between two surfaces coming into contact, and the force acting on the sample. Hertz's general expression can be simplified to the case of interest here, i.e., spherical particles compressed between two flat, rigid and parallel surfaces in the small deformation domain. It results the force F normal to the compressing surfaces acting on the sphere as a function of geometrical parameters:

Table 1
Schematic way of triblock and diblock copolymer synthesis and number average molecular weight characteristics of homopolymers, diblock and triblock copolymers.



Sample	Mn PEO	Mn PLA	Mn Total	Fraction of PLA PLA/(PLA + PEO)
PLLA	0	60,000[a]	60,000	1
PDLLA	0	75,000[a]	75,000	1
PDLLA-PEO	1880[b]	113,000[b]	114,880	0.983
	1880[b]	77,332[b]	79,212	0.976
PLLA-PEO-PLLA	35,000[a]	32,800[b]	100,600	0.652
	35,000[a]	18,750[b]	72,500	0.517
	35,000[a]	8500[b]	52,000	0.327
PDLLA-PEO-PDLLA	35,000[a]	14,800[b]	64,600	0.458
	35,000[a]	5650[b]	46,300	0.244

[a] Nominal molecular weight.
[b] Determined by ^{1}H NMR.

$$F = \frac{4}{3}\frac{E}{1-\nu^2}R_i^{1/2}\left(R_i - R_f\right)^{3/2} \quad (1)$$

where E is the material Young's modulus, ν is the Poisson ratio and R_i and R_f are the initial and final sphere radii in the force direction, respectively. The ratio $E/(1-\nu^2)$ is sometime referred to as the reduced Young's modulus of the studied material. Within this arrangement, the displacement during compression is obtained as $d = 2 \times R_i - 2 \times R_f$.

The experimental compression data were fit to the Hertz equation using least-square regression of F versus d in order to gain insight into the spheres reduced Young's moduli. To ensure reliability of Hertz theory, we restrained the fit to data in the small deformation domain, corresponding to less than 15% deformation: $d/2 \times R_i \leq 0.15$. We intentionally disregarded the morphology of the spherical particles when analyzing the data in view of the fact that Hertz model is proposed for isotropic and homogeneous spheres [38]. More detailed analysis of the mechanical compression of hollow spheres is achievable [39,40]. However, the effective compressive modulus of our samples, approximated to the reduced Young's modulus in equation (1), yields a straightforward comparison of the rigidity of the synthesized particles as a whole independently of the microscopic details as long as the size of the spheres is comparable and the applied deformation remains in the linear regime. Their actual Young's modulus is an intrinsic property of the material that does not allow direct comparison of their robustness.

3. Results and discussion

Both the preparation of the microspheres by single emulsification and their mechanical characterization by compression were repeated 3–5 times to ensure reproducibility. During the emulsification step, few samples (approximately 1 in 10) formed compact biopolymer films around the mechanical stirrer instead of a homogeneous emulsion. The failure was attributed to an incorrect setting of the stirrer in the center of the setup and these few samples were discarded. All three types of PLA polymer samples were prepared and studied under similar experimental conditions to yield the microspherical particles. All the samples listed in Table 1 yielded microspherical polymer particles after solvent evaporation, except the triblock copolymer with the highest PEO/PLA ratio which did not give stable w/o emulsion. The breakdown was attributed to the low amphiphilic character of this PDLLA-PEO-PDLLA copolymer. Indeed this copolymer has a PEO fraction of 76% which certainly triggers its swelling with water.

Optical microscopy and SEM images were taken of the microspheres prepared from each polymer, except the one with the least amphiphilicity, to reveal the particle sizes, shape and inner structure as a function of the preparation parameters. Smoothness of sphere surface appears to be a common characteristic except for the PLLA homopolymer, where rough surface spheres were obtained. Analysis of optical micrographs indicated that the sphere diameter ranged from 600 to 800 μm for diblock copolymers at 22 °C while it was smaller at 15 °C, in the range of 400 and 700 μm. No general trend was directly noticeable in the size between spheres of the same polymer material obtained at the two temperatures 22 and 15 °C for both the triblock copolymers and the homopolymers: the sphere diameter ranged from 600 to 800 μm for the triblock copolymers while it was systematically smaller for the PLLA and PDLLA homopolymers, in the range of 300 and 500 μm. Thus, it was clear that the inner morphology was the variable parameter, strongly dependent on both the chemical structure and the emulsion temperature. Moreover, the localization of the PLA microspheres after evaporation of the organic solvent was clearly associated with their respective chemical structure and temperature of preparation. PLA homopolymer spheres were located in the bottom of the aqueous solution while PLA-PEO-PLA microspheres were irremediably floating on top of the solution, for both preparation temperatures. Spheres from PLA-PEO diblock copolymers were found on top of the solution at 22 °C or at the bottom of the flask at 15 °C. Differences in sphere diameters reflected these particular localizations: small spheres settled down while larger ones floated up. It is worth adding that these assessments are appropriate because the comparison were made between microparticles obtained under identical conditions: similar concentration and volume for each phase. Further analyses were carried out to inspect the inner morphology of the spheres by examining half-cut spheres with SEM and optical microscopy.

3.1. Room temperature data

Fig. 1 (a–f images) shows representative SEM images of microparticles prepared by the single o/w emulsion and evaporation method from the homopolymer PDLLA (a–c), the PDLLA-PEO diblock copolymer (77332–1880 g/mol, d) and the triblock copolymer PLLA-PEO-PLLA (Mn = 32800-35000-32800 and 18750-35000-18750 g/mol for e and f respectively). These samples were prepared near room temperature during emulsion and evaporation process at 22 °C. All SEM patterns in Fig. 1 display well smooth external surfaces of the microspheres and confirm the particle size deduced from optical microscopy. The image a in Fig. 1 shows an intact and a cross-sectioned microsphere of PDLLA homopolymer. Images b and c show a cross-sectioned microsphere of this homopolymer at two different magnifications. The particles were perfectly spherical in shape as shown in Fig. 1-a. However, we notice that the cross-sectioned spheres were slightly compressed by the razor blade during the sectioning procedure as is evident in Fig. 1-b. We also notice that the PDLLA spheres are homogeneous and do not show any apparent porous structure at this length scale as shown in Fig. 1-c. SEM images d, e and f in Fig. 1 represent cross-sectioned microparticles of PDLLA-PEO (77332–1880 g/mol) diblock, PLLA-PEO-PLLA (32800-35000-32800 and 18750-35000-18750 g/mol) triblock copolymers respectively. They display a remarkable difference with pattern b representing the homopolymer sphere. Indeed, the emulsification of the block copolymers lead to the formation of microcapsules as it is clearly evidenced by the SEM micrographs that show that the interior of the spheres is hollow, while the homopolymer emulsion led to the formation of plain spheres. This effect is clearly evidenced in the inset of Fig. 1-e which represents a SEM magnification on the edge of the microparticle and shows that the hollow particles have a wall thickness between 8 and 14 μm. This relatively high thickness proves that these capsules do not originate from vesicles (or polymersomes) which are expected to have a thickness of the order of the polymer chain length [24,41].

At this temperature of preparation, all the SEM images reveal that the inner morphology is strongly dependent on the chemical structure of the polymers. The presence of the PEO hydrophilic moiety seems to be the main driving parameter for the formation of hollow particles or microcapsules. In order to rule out other preparation parameters associated with the method that might influence the inner morphology of the microcapsules, we carried out several experiments by varying the stirring speed from 300 to 1000 rpm. The results revealed that this parameter slightly affects the size of the resulting particles. However it does not have noticeable influence on the formation of hollow particles when amphiphilic diblock and triblock copolymers are used. The emulsification of PDLLA and PLLA homopolymers at different stirring speeds between 300 and 1000 rpm also yielded bulk microspheres

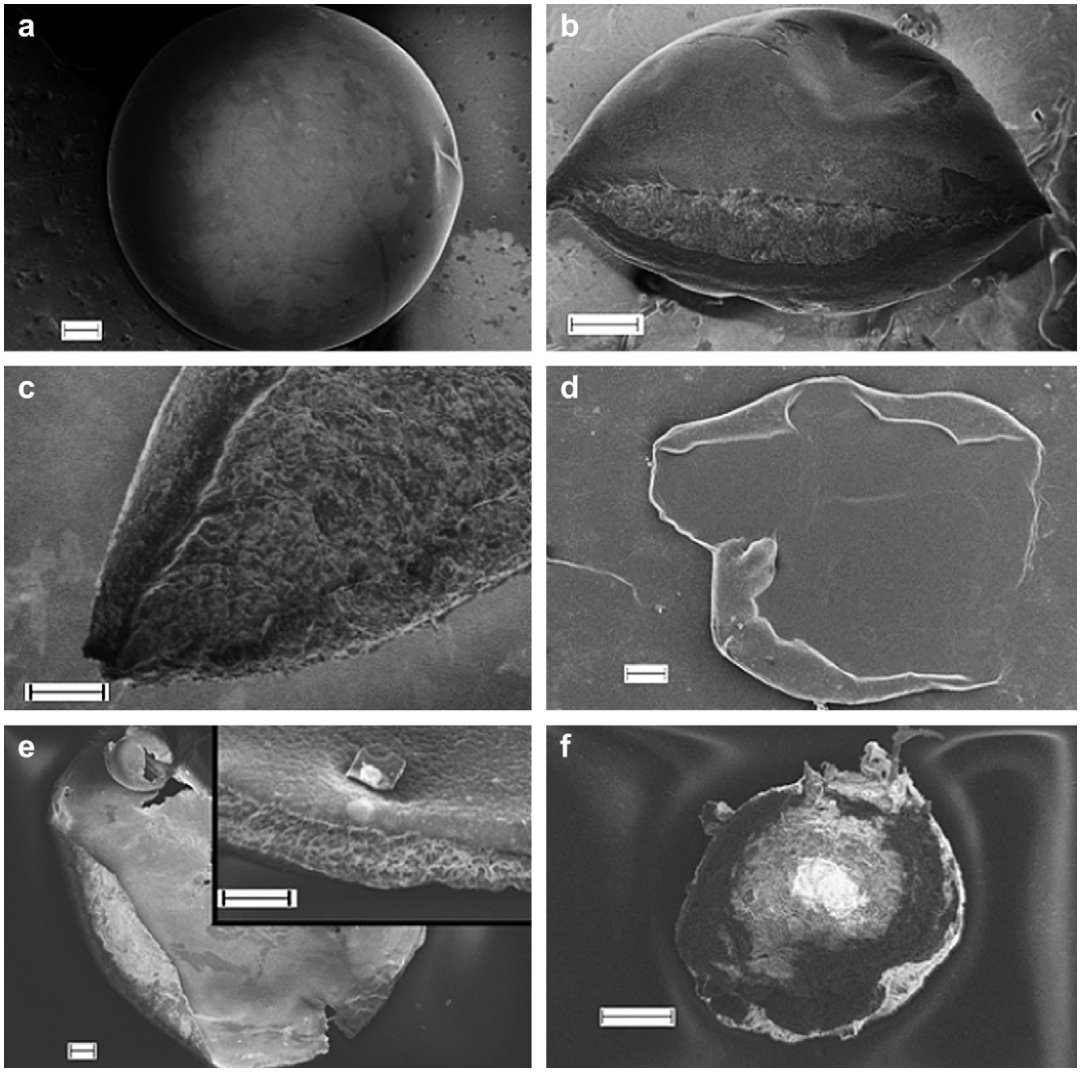


Fig. 1. Scanning electron micrographs of representative particles prepared by emulsion/solvent evaporation method at 22 °C from (a) PDLLA bulk microsphere exterior, (b and c) PDLLA half bulk microsphere interior, (d) PDLLA-PEO (77332–1880 g/mol) half microcapsule, and (e and f) PLLA-PEO-PLLA (32800-35000-32800 18750-35000-18750 g/mol) half microcapsules. Inset of image e: magnification of the microcapsule's edge, scale bare is 20 μm. The scale bar is 100 (a-b, and d-f), 40 (c) and 20 μm (inset in c).

with homogeneous inner structure at this temperature. We only noticed a decrease of the spheres diameter as a function of increasing stirring speed during the emulsification step.

3.2. Data at 15 °C

We extended the inspection of the experimental parameters effect on the inner morphology of the particles by varying the temperature during the stage of emulsification and evaporation of the organic solvent. Fig. 2 (a–f) shows representative SEM images of microparticles prepared at fixed temperature of 15 °C for same polymers as those shown in Fig. 1: homopolymer PDLLA (images a and b), diblock copolymer PDLLA-PEO (77332–1880 g/mol, images c and d) and triblock copolymer PLLA-PEO-PLLA (32800-35000-32800 and 18750-35000-18750 g/mol, images e and f respectively). The apparently small decrease of the preparation temperature from 22 to 15 °C does not affect the inner morphology of the homopolymer spheres since the resulting particles were spherically shaped (image a). Furthermore, the investigation of the inner structure of a cross-sectioned microsphere reveals that the particle core is plain, homogeneous and does not show any apparent porous structure at this length scale. This result is comparable to the data observed at 22 °C for the homopolymer and is in agreement with previous results on polylactide homopolymer microspheres from the literature [20].

The inner morphology of the cross-sectioned sphere made from PEO-PDLLA diblock copolymer contrasts with the result obtained at 22 °C. We note that as against the data in Fig. 1, the emulsification of the amphiphilic diblock copolymer at 15 °C yields bulk microspheres as it is presented in Fig. 2-c. The apparently small decrease of preparation temperature from 22 to 15 °C greatly affects the inner morphology and prompts the formation of spherically shaped bulk, homogeneous spheres, with smooth surfaces and without an apparent porous structure at this length scale. This significant change is more noticeable in Fig. 2-d which represents SEM image of where a quarter microsphere. We notice that the cross-sectioned spheres of PEO-PDLLA were more compressed by the razor blade than the PDLLA spheres, however the inner morphology and homogeneity of both materials are very close.

The preparation of microspheres from the triblock copolymers at this lower temperature did not show contrasting results as compared to data obtained at 22 °C. All the microspheres were hollow as it is evident on SEM images e and f in Fig. 2. It is worth

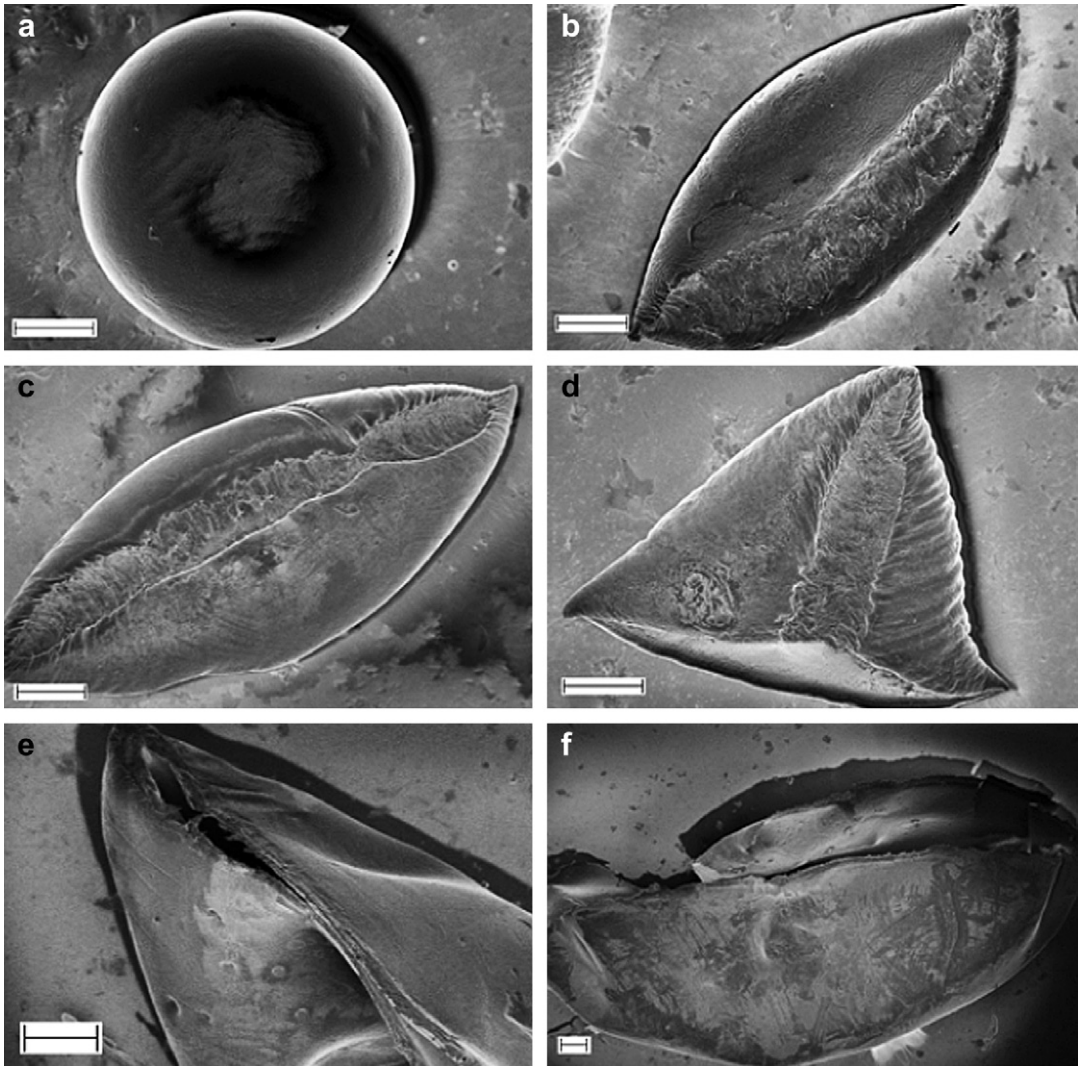


Fig. 2. Scanning electron micrographs of representative particles prepared by emulsion/solvent evaporation method at 15 °C from (a) PDLLA bulk microsphere exterior, (b) PDLLA half bulk microsphere interior, (c and d) PDLLA-PEO (77332–1880 g/mol) half and quarter bulk microsphere, and (e and f) PLLA-PEO-PLLA (32800-35000-32800 18750-35000-18750 g/mol) half microcapsule. The scale bar is 100 μm.

adding that both the SEM and optical microscopy observations were in agreement with the localization of the particles in the aqueous phase after evaporation of the organic solvent. Bulk particles, which are dense compared to water, settled down, while the hollow ones floated up.

3.3. Comparison of data

Close inspection of experimental data reveals that the temperature of preparation and the chemical nature of the polymers play key roles in the formation of bulk spheres or microcapsules. The temperature of preparation is directly related to the rate of organic solvent evaporation which prompts the condensation of the polylactide hydrophobic polymer and formation of solid particles in the aqueous phase. The chemical structure of the polymers is related to the interfacial free energy of the oil-in-water emulsion which is controllable by the amphiphilicity degree of the macromolecules used to stabilize the emulsion droplets [22,42].

Throughout the steps of the emulsion and evaporation processes carried out at 22 °C, a relatively high temperature for CH_2Cl_2, which is highly volatile, solvent evaporation is expected to be fast enough to influence the final inner morphology of the polymer spheres. Indeed, during this stage, as the solvent evaporates, remaining solvent molecules migrate to the center of the oil droplets. This mechanism might be accompanied by the migration of the polymer macromolecules towards the center of the droplets unless this inward migration is affected by any other cause. The use of polylactide homopolymer, a very hydrophobic polymer, does not influence its migration towards the center of the droplets, since their high hydrophobicity promotes small oil droplets in order to lower the interfacial free energy and stabilize the droplets. It is thus understandable that this homopolymer usually yields the formation of bulk microspheres. One would conjecture that lowering enough the interfacial free energy should promote the formation of hollow particles under the condition of fast solvent evaporation. Undeniably, the addition of small segments of the PEO hydrophilic block to polylactide stabilizes more the copolymer at the interface of the o/w droplets, with PEO blocks most likely pointing towards the water phase and polylactide blocks embedded in the oil phase. As a consequence the polymer migration to the center of the droplets slows down as compared to the situation described before and further solvent evaporation freezes up the polymer close to the

droplets interface and causes the formation of microcapsules. The addition of longer PEO hydrophilic blocks only enhances the efficiency of forming hollow microcapsules. It is worth noting that investigation of organic solutions of PEO-PLA copolymers does not show phase separation during solvent evaporation or formation of micellar structures when the solutions are inspected by dynamic light scattering. These observations provide evidence that the organic solvent used is a good solvent for the neat copolymers, and that the copolymer precipitation at the interface is driven by the w/o interface in the emulsion.

On the other hand, when the temperature is low enough to slow down the solvent evaporation, we notice that the amphiphilic diblock copolymers with very short PEO hydrophilic units allows the formation of bulk microspheres after evaporation of the solvent. This situation is most likely advantageous for the rearrangement and migration of the copolymer to the center of the droplets along with the slow migration of the solvent molecules which have lost pace due to a slower evaporation rate. The copolymer migration is favorable most likely because the interfacial free energy is not low enough to stabilize large oil droplets in water. This causes the shrinkage of the oil droplets followed by solidification of the polymer after complete solvent removal and thus formation of bulk microspheres. However, when the amphiphilic block copolymer possesses a much longer PEO hydrophilic block, there is no polymer migration to the center of the emulsion droplets during the stage of solvent evaporation and its migration to the center of the droplets. Indeed, the PEO segments used in these triblock copolymers are large enough to substantially lower the interfacial free energy and favor hollow particle structures where the interface towards the aqueous phase is efficiently stabilized by the PEO. The effect of these two parameters on the fabrication schemes is comparable to the one exploited for the realization of PDLLA anisotropic morphologies by tuning the viscosity of the emulsion [16].

3.4. Compression studies

The fabrication of hollow microspheres from polylactide with the help of the hydrophilic PEO can be useful in the area of encapsulation. In addition to their inherent properties like light mass and large deformability, their morphology is suitable for the encapsulation of specific molecules and in large amounts. However, in order to be useful in the field of drug delivery, the microcapsule rigidity should be robust enough to withstand shear stress and compression in the site of implantation [43]. This particular point requires further investigation of the mechanical properties of the solid microspheres and comparison of their relative rigidity with the data from bulk polylactide particles which were proven to be mechanically resistant for use as drug carriers. To this end the compressibility measurements were performed on dried microparticles and data analyzed to extract the apparent reduced Young's modulus by using equation (1).

Figs. 3 and 4 display the variation of the applied normal force as a function of the induced displacement, which corresponds to the variation in diameter, for particles prepared at 22 and 15 °C respectively. We checked that successive compression experiments taken for increasing maximum deformations yield overlapping force-displacement curves when the maximum deformation reached upon loading is between 0 and 10%. This proves that the linear domain was not crossed when the maximum deformation stayed below 10%. This is also a strong indication that the rigidity of the microcapsules is preserved and is as high as that of the bulk materials. The data shown on these two figures were carried out on the PDLLA and PLLA homopolymers, PDLLA diblock copolymers and PLLA triblock copolymers (see Table 1). The microparticles were

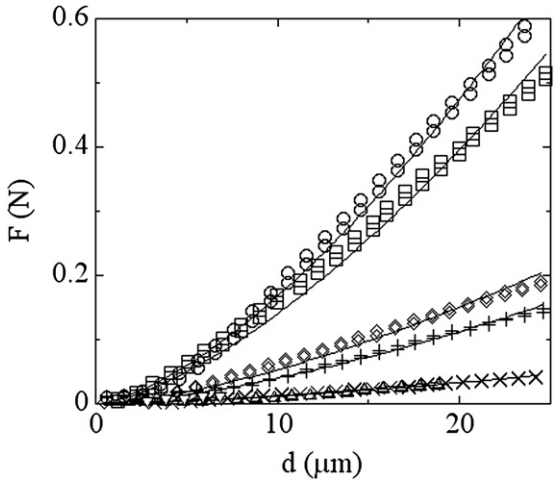


Fig. 3. Evolution of the normal force as a function of displacement during compression up to 5% deformation for the microparticles fabricated at 22 °C from: (◯ and □) PLLA and PDLLA homopolymers, (◇ and ×) PDLLA-PEO of 113000–1880 and 77332–1880 g/mol, (+, △ and ▽) PLLA-PEO-PLLA of 32800-35000-32800, 18750-35000-18750 and 8500-35000-8500 g/mol respectively. Solid lines are fits to Hertz theory.

either bulk or hollow spheres depending on the temperature of preparation and chemical structure of the material. As it is expected, compressibility data exhibit a clear distinction between the bulk and the hollow spheres. The force applied on bulk microspheres evolves rapidly with the displacement as is evident in Fig. 3 for PLLA and PDLLA homopolymer particles, and in Fig. 4 for PLLA and PDLLA homopolymer and PDLLA-PEO diblock copolymer particles even though we notice a weak normal force variation with displacement for the PDLLA-PEO copolymer with molecular weight of 77332–1880 g/mol.

As mentioned earlier, we used the Hertz model for a more detailed analysis of the compressibility curves although this model is not adequate for hollow particles since they lack homogeneity [39]. However, the evolution of the force as a function of displacement should give a clear indication on the mechanical rigidity of the bulk and the hollow spheres equally submitted to comparable compressive forces. The solid lines shown in Figs. 3 and

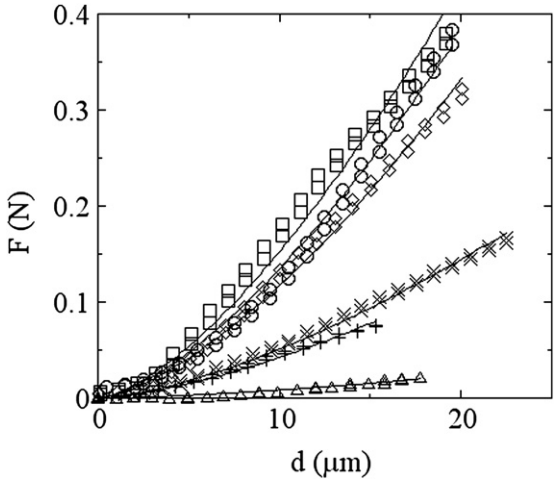


Fig. 4. Evolution of the normal force as a function of displacement during compression up to 5% deformation for the microparticles fabricated at 15 °C from: (◯ and □) PLLA and PDLLA homopolymers, (◇ and ×) PDLLA-PEO of 113000–1880 and 77332–1880 g/mol (+ and △) PLLA-PEO-PLLA of 32800-35000-32800 and 18750-35000-18750 g/mol respectively. Solid lines are fits to Hertz theory.

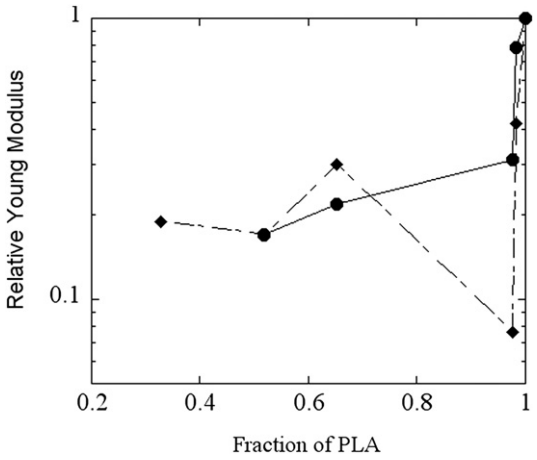


Fig. 5. Ratio of the reduced Young's modulus of the samples to the reduced Young's modulus of pure polylactide as a function of fraction of polylactide in the polymer for samples prepared at (◆) 22 and (●) 15 °C.

4 are fits to equation (1) for maximum deformation of 5% to ensure that the analysis was performed in the elastic domain. The Hertz model provides good fits to the data for all the samples studied as shown in Figs. 3 and 4. The results of the fits lead to straightforward determination of the apparent reduced Young's moduli of the spheres which constitute apt ground for comparison between the rigidity of the samples. Young's modulus can be extracted from the reduced Young's modulus if the Poisson ratio is known. The calculated reduced Young's modulus values explicitly show that the rigidity of the microcapsules fabricated from diblock and triblock amphiphilic copolymers, even though smaller than those of PLLA and PDLLA, stay substantially high. These data remarkably emphasize the considerable mechanical robustness of the microcapsules. We summarized these measurements in Fig. 5 and Table 2 for both temperatures of fabrication. We choose to represent the compressibility results in Fig. 5 in the form of the apparent relative reduced Young's modulus that is the ratio of sample apparent reduced Young's modulus/PLA homopolymer reduced Young's modulus as a function of the fraction of PLA in the polymer. We notice that there is a straightforward relationship between the value of the apparent Young's modulus and the fraction of PLA in the polymer. However, the most significant decrease of the reduced Young's modulus is triggered by the change in the inner morphology from bulk to hollow microsphere. Nevertheless, the decrease in the reduced Young's modulus does not affect considerably the compressibility of microcapsules fabricated from amphiphilic copolymers as compared to pure PLA microspheres. These results highlight the significance of the method we used by varying the chemical structure of the polymers to elaborate microcapsules in a very simple way and preserve their rigidity.

Table 2
Compressibility data.

Sample	Mn (g/mol)	Apparent reduced Young's modulus (MPa)	
		15 °C	22 °C
PLLA	60,000	425	502
PDLLA	75,000	622	494
PDLLA-PEO	113,000-1880	366	221
	77,332-1880	180	38
PLLA-PEO-PLLA	32,800-35,000-32,800	140	136
	18,750-35,000-18,750	42	46
	8500-35,000-8500		97

4. Conclusions

In summary, we have reported for the first time, a complete study of microspheres fabricated by the emulsion and solvent evaporation method from polylactide homopolymers and polylactide- poly (ethylene oxide) diblock and triblock copolymers. We have shown that varying the temperature of preparation of the emulsion and the amphiphilicity degree of the block copolymers offers a means of controlling the inner morphology of the microspheres by producing either bulk microspheres or microcapsules. These particulate systems are primarily suitable for particular drug delivery applications. The good agreement between the model fit to measured normal force as a function of lateral displacement achieved by compressibility measurements in the linear regime for bulk spheres validate Hertz theory. Furthermore, comparison of data shows that the apparent reduced Young's modulus stays high enough for the microcapsules so that their rigidity is not weakened by the inclusion of the hydrophilic poly(ethylene oxide) polymer to polylactide. The reduced Young's modulus and expansion of the linear elastic domain of the microcapsules are in the same range as several microspherical system carriers designed as drug delivery carriers.

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