Hollow Polylactide Microcapsules with Controlled Morphology and Thermal and Mechanical Properties

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Hollow polylactide microcapsules were prepared by multistage premix membrane emulsification of polylactide/dichloromethane/oil solutions in water (nonsolvent). The effects of the different oils on the morphology, thermal, and mechanical properties of the hollow microcapsules were investigated. All oils resulted in hollow microcapsules with controlled shell thickness of ∼60 nm except for eugenol, in which irregular, massive capsules were obtained. The properties of the microcapsules were strongly dependent on the oil used, for example the thermal transition temperatures found for hollow capsules were lower than for solid particles prepared without any oil. The crystallinity and transition temperatures of the capsules prepared with linear alkanes were higher than for cyclic alkanes; terpenes gave the lowest transition temperatures. The shell stiffness, measured with atomic force microscopy, was highly dependent on the oil used. Capsules prepared with dodecane showed higher stiffness (3.3 N m^{-1}) than with limonene (2 N m^{-1}) or cyclohexane (1.4 N m^{-1}). © 2009 American Institute of Chemical Engineers *AIChE J*, 55: 2827–2834, 2009

Keywords: hollow polylactide microcapsules, thermal and mechanical properties, nonsolvent, crystallinity, morphology

Introduction

Biopolymer polylactide (PLA) is used for biomedical materials because it is nontoxic, biocompatible, and biodegradable in the human body and has a high-mechanical strength.[1,2] Various types of biomaterials have been prepared from PLA, for example scaffolds for tissue engineering, implants, sutures, and films.[3–5] PLA is also used in drug delivery systems.[6–8] Encapsulation of drugs and other bioactive compounds within microcapsules may control the release of the encapsulated compounds or may protect them from fast degradation in the body.[7] Hollow PLA microcapsules can serve as ultrasound contrast agents (UCAs).[9–11] During imaging of the body with ultrasound, the gas core of these hollow capsules allows them to resonate in the acoustic field, which reflects the ultrasound and enhances the image contrast.[10] Moreover, hybrid UCAs that also encapsulate drugs have been developed. In this case, the ultrasound can be used for imaging and to trigger the release of the encapsulated drug.[9]

PLA microcapsules or UCAs are usually prepared through emulsification.[11,12] The polymer is dissolved in a proper solvent (e.g., dichloromethane) plus a poor solvent (often called oil). This solution is then emulsified in a continuous phase which consists of a nonsolvent (i.e., water) and a proper stabilizer. After emulsification, the solvent diffuses through the nonsolvent bath and evaporates at the surface of the bath. The droplets slowly become more and more concentrated in both polymer and oil, and at a certain moment these two become incompatible, and phase separation inside the droplet takes place. An internal droplet of mainly oil is created, surrounded by a polymeric solution, which solidifies and forms a skin around the oil droplet. To obtain hollow microcapsules, the oil is removed by freeze-drying.[13]

The properties of the polymer shells of microcapsules and UCAs are important for the final application. Factors like

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crystallinity, glass transition temperature (T_g), morphology, porosity, mechanical strength, and elasticity of the polymer shell will influence the drug release properties and acoustic activity of the microcapsules.[14–18] Some studies have reported that the release rate of a drug can be fine-tuned by controlling the T_g and crystallinity degree of particles[14]; drug release increases with decreasing T_g and degree of crystallinity.[14] Moreover, the biodegradation behavior and biocompatibility of the microcapsules in the human body are influenced by the thermomechanical state of the particles. Crystalline regions within the polymer matrix degrade at a slower rate compared with amorphous ones.[19] Good control may therefore improve and broaden the field of application of hollow PLA capsules.

In a previous work, we have shown that the thermal and mechanical properties of PLA films prepared through film casting can be optimized by addition of different oils to PLA casting solutions.[20] The objective of this study is to investigate these effects for PLA microcapsules. For this purpose, semicrystalline poly(L-lactide) (PLLA) was used to produce microcapsules by premix membrane emulsification. Different types of oils were used to prepare the microcapsules and the morphology and thermal properties of the capsules were determined and compared. In addition, the mechanical properties of the capsules were probed with atomic force microscopy (AFM) technique and compared with those obtained for films.

Materials and Methods

Materials

PLLA with an intrinsic viscosity of 121 cm^3 g^{-1} was obtained from PURAC (Biochem B.V., Gorinchem, The Netherlands). Dichloromethane (DCM) (HPLC, gradient grade) was purchased from Merck and used as solvent. The oils used for preparation of the capsules were decane (95%), cyclodecane (95%), eugenol, and limonene ($\geq$96%) from Fluka, hexadecane (>99%), and cyclohexane ($\geq$99.5%) from Merck, hexane (HPLC, gradient grade ($\geq$99.9%)) from Aldrich and dodecane ($\geq$99%) from Sigma-Aldrich. As nonsolvent, Milli-Q water was used with poly(vinylalcohol) (PVA 23/88) from Ter Hell (Hamburg, Germany) as a stabilizer.

Methods

Preparation of PLLA Microcapsules. The nonsolvent used consisted of 3 g of a 1% *w/w* PVA aqueous solution and 8 g of pure water. The polymer solution was 2% *w/w* PLLA in DCM. To 11 g of nonsolvent, 0.5 g of polymer solution, 1 g DCM, and 0.15 g oil were added. The premix emulsion was prepared by mixing these solutions in a closed vessel for 1 min at 900 rpm using a magnetic stirrer. The formed emulsion was then homogenized by pushing the emulsion manually through a 1 μm glass-fiber syringe membrane (Acrodisc GF syringe filter, Pall). To evaporate the solvent, the homogenized emulsion was then stirred in an open vessel for 1 h by a magnetic stirrer. Because of out-diffusion and evaporation of the solvent, the polymer solidified around the oil droplets and oil-filled PLLA microcapsules were formed. The microcapsules were then collected by centrifugation, washed with pure water, and subsequently centrifuged again to remove the PVA. After centrifugation, the microcapsules were freeze-dried with Christ Epsilon 2-6D freeze-dryer (Osterode, Germany) to remove the oil core and form hollow capsules. The freeze-drying step was conducted using the same program as was earlier applied for the films (Sawalha et al., submitted).[13]

Differential Scanning Calorimetry. The thermal characteristics of the freeze-dried microcapsules including glass transition temperature (T_g), cold crystallization temperature (T_c), melting temperature (T_m), and enthalpies of cold crystallization (ΔH_c), premelt crystallization (ΔH_{c2}) and melting (ΔH_m) were measured using differential scanning calorimetry (DSC). A specific amount of capsules (2–7 g) was placed in stainless steel pans and then scanned using PerkinElmer Diamond DSC (PerkinElmer Co., Norwalk, CT) from −60 to 200°C at a heating rate of 10°C min^{-1}.

Mostly, no peaks for the oils were found, but in some preparations, the microcapsules contained traces of oil.

The degree of crystallinity of the capsules was calculated as the ratio between the heat of fusion of the capsules and the heat of fusion of 100% crystalline PLLA as described in the following equations.

$$X_c = \frac{\Delta H_f}{\Delta H_f^0} * 100\% \tag{1a}$$

with

$$\Delta H_f = \Delta H_m - \Delta H_c - \Delta H_{c2} \tag{1b}$$

where X_c is the degree of crystallinity, ΔH_f is the heat of fusion of capsules, and ΔH_f^0 is the heat of fusion of 100% crystalline PLLA, which was given in the literature as 93 J g^{-1},[20] and ΔH_c ΔH_{c2}, and ΔH_m are enthalpies of cold crystallization, premelt crystallization, and melting, respectively.

Scanning Electron Microscope. The morphology of the microcapsules was visualized using scanning electron microscope (SEM; JEOL, JSM-5600 LV). A droplet of freeze-dried microcapsules, resuspended in water was dried on a glass plate and then coated with 10 nm platinum in a dedicated preparation chamber (CT 1500 HF, Oxford Instruments, Oxford, UK) before viewing with SEM. To view the inner core of the capsules and to estimate the shell thickness, some of the capsules were fractured before observation with SEM. To break microcapsules, the powder was put on the sticky side of transparent "household" single side sticky tape. The capsules were pushed firmly on the sticky layer with pressed airflow. The transparent tape with the microcapsules was then put onto double-sided sticky carbon tape (EMS, WA). The transparent tape was peeled off from the carbon tape, to fracture the capsules.

Atomic Force Microscopy. Force-distance curves of PLLA microcapsules were obtained using a NanoScope IIIa multimode scanning probe microscope (SPM) with a PicoForce extension (Veeco Instruments, Plainview, NY) equipped with piezoelectric scanner "E" (x, y range 12.5 μm × 12.5 μm). The force measurements were carried out using standard V-shaped contact mode cantilever (Veeco Instruments, Plainview, NY) with a nominal spring constant of 0.57 N m^{-1} and tip radius of ~10 nm. The deflection sensitivity of the photodetector of the AFM was calibrated using a hard substrate (silica chip, which was cleaned in plasma oven).

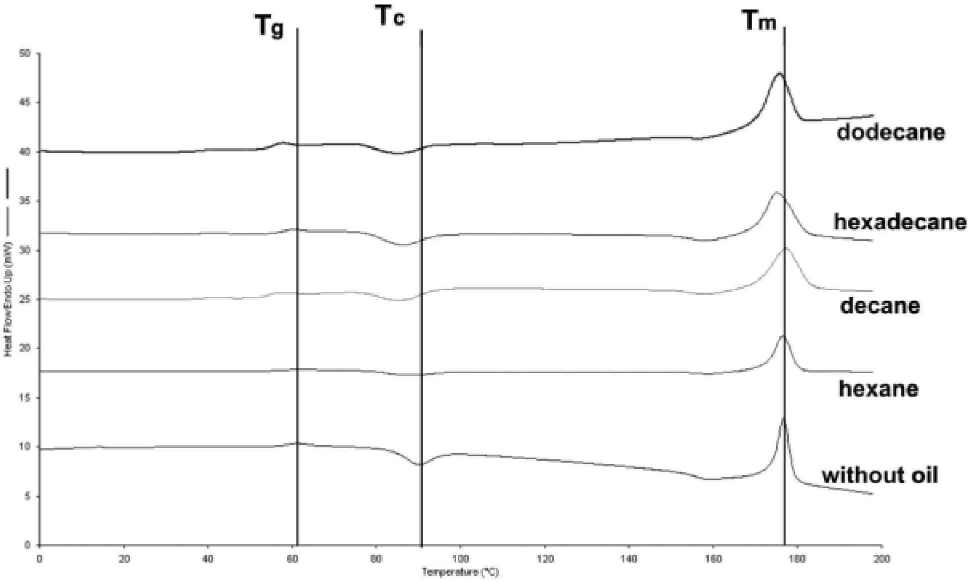

Figure 1. DSC curves of PLLA microcapsules prepared with different oils.

The measurements were conducted at a heating rate of 10°C min^{-1}.

To immobilize the microcapsules, capsules after centrifugation were suspended in pure water and a droplet of this suspension was dripped onto a silica chip after which the sample was frozen at −80°C for 1 h. The frozen silica chip with microcapsules was subsequently freeze-dried to firmly immobilize the hollow capsules on the chip. To measure the force curves, the chip with microcapsules was located onto the piezoelectric scanner. The sample was then placed under the AFM-tip and the immobilized capsule was compressed between the AFM-tip and silica chip. The vertical position of the tip and the deflection of the cantilever were recorded by instrument software and converted later into force-distance curve. All measurements were performed under ambient conditions (~20°C and 40% relative humidity) on at least five randomly selected microcapsules per sample. Topographic images of the microcapsules before and after measurements were taken by AFM.

Results and Discussion

Effects of oil on thermal properties

Figure 1 shows the DSC thermographs of PLLA microcapsules prepared with different oils. The thermal transition temperatures (T_g, T_c, and T_m) and enthalpies (ΔH_m, ΔH_c, and ΔH_{c2}) are presented in Table 1. The oils have a big effect on the thermal characteristics of the capsules. Neat PLLA particles (solid particles prepared without oil) showed T_g at 58°C, T_c at 90°C, and T_m at 177°C. The thermal transition temperatures of the hollow capsules prepared with oils were lower than those of solid particles made out of neat PLLA. Table 1 illustrates that depending on the type of oil used, the T_g of the capsules could be reduced up to 30°C. The same trend was found for T_c and T_m, in which a maximum reduction of about 20 and 15°C was observed, respectively. The T_g, T_c, and T_m of the capsules prepared with cyclic alkanes were lower than those prepared with normal alkanes (see Table 1). The terpenes showed the biggest effect on the thermal behavior of the capsules in which eugenol gave the lowest thermal transition temperatures ($T_g = 30°\mathrm{C}$, $T_c = 71°\mathrm{C}$, and $T_m = 163°\mathrm{C}$).

The type of oil did not only influence the thermal transition temperatures, but also the crystallinity of the capsules.

As can be seen in Table 1, the neat PLLA particles had an X_c of ~20%. The X_c of the capsules prepared with oils varied with the type of oil used from around 0 to 45%. The minimum X_c of 0% was obtained with cyclodecane, whereas limonene gave the maximum X_c of 44%. Cyclic alkanes produced capsules with lower crystallinity when compared with the normal alkanes (see Table 1).

Table 1. Thermal Properties of PLLA Microcapsules Prepared with Different Oils

Oil	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_c (J g^{-1})	ΔH_{c2} (J g^{-1})	ΔH_m (J g^{-1})	X_c (%)
Neat PLLA	58	90	177	17	8	43	19
Hexadecane	56	86	175	15	4	35	18
Dodecane	53	85	176	11	3	35	23
Decane	53	86	177	10	4	33	20
Hexane	55	87	177	11	3	45	33
Cyclodecane	45	78	165	15	21	24	~0
Cyclohexane	46	80	173	15	3	41	25
Eugenol	30	71	163	9	0	34	27
Limonene	41	–	174	0	0	41	44

The effects of the oils on the thermal properties of the capsules are related to the effects of the oils on the solidification process of the polymer during the formation of the capsules (Sawalha et al., submitted).[21,22] Removal of the solvent from the emulsion droplets after emulsification increases the concentrations of the polymer and the oil in the droplets. After some time, the polymer and oil in the droplet reach saturation, and phase separation will take place after which the polymer will solidify around the oil droplets. The solidification process of the polymer greatly determines the eventual properties of the resulting capsules. The low thermal transition temperatures of the capsules prepared with eugenol suggests that PLLA has better interaction with eugenol than with other oils, which makes the polymer chains more mobile during capsule formation. Correspondingly, the high crystallinity of the capsules prepared with limonene indicates strong interaction of PLLA with limonene which slows down the phase separation and gives mobility and time for crystallization to occur before complete solidification of the polymer. The polymer seems to be less compatible with alkanes when compared with the other oils, and consequently, the phase separation takes place more quickly while the mobility of the polymer is reduced faster. Therefore, the thermal transition temperatures with alkanes are higher than for other oils.

When comparing the thermal behavior of the capsules with that of air-cast films, one can see that most of oils give similar trends in which the thermal transition temperatures were the highest with normal alkanes and the lowest with terpenes, although it should be noted that the preparation method of films is different (Sawalha et al., submitted). The films were prepared by casting and evaporation to the air, whereas capsules were submersed in a nonsolvent. This indicates that the interactions between the polymer and most of the oils are rather important for the solidification of the polymer. Besides this, solvent interactions are expected to play a role, since the crystallinity in the films was different than in capsules. For example, the crystallinity of the films prepared with limonene was lowest (~15%) (Sawalha et al., submitted), whereas it was highest for the capsules with limonene (~45%). This may be ascribed to the slower removal rate of the DCM from the capsules into the nonsolvent (DCM and water are poorly miscible) which gives more time for crystallization to take place.

The results obtained so far clearly demonstrate that the oils can be effectively used to influence the thermal properties of the capsules. The next section focuses on the effects of the oils on the morphology of the capsules.

Effects of oil on morphology

Figures 2 and 3 show SEM micrographs of hollow PLLA microcapsules prepared with different oils. The majority of the capsules are spherical and have average sizes between

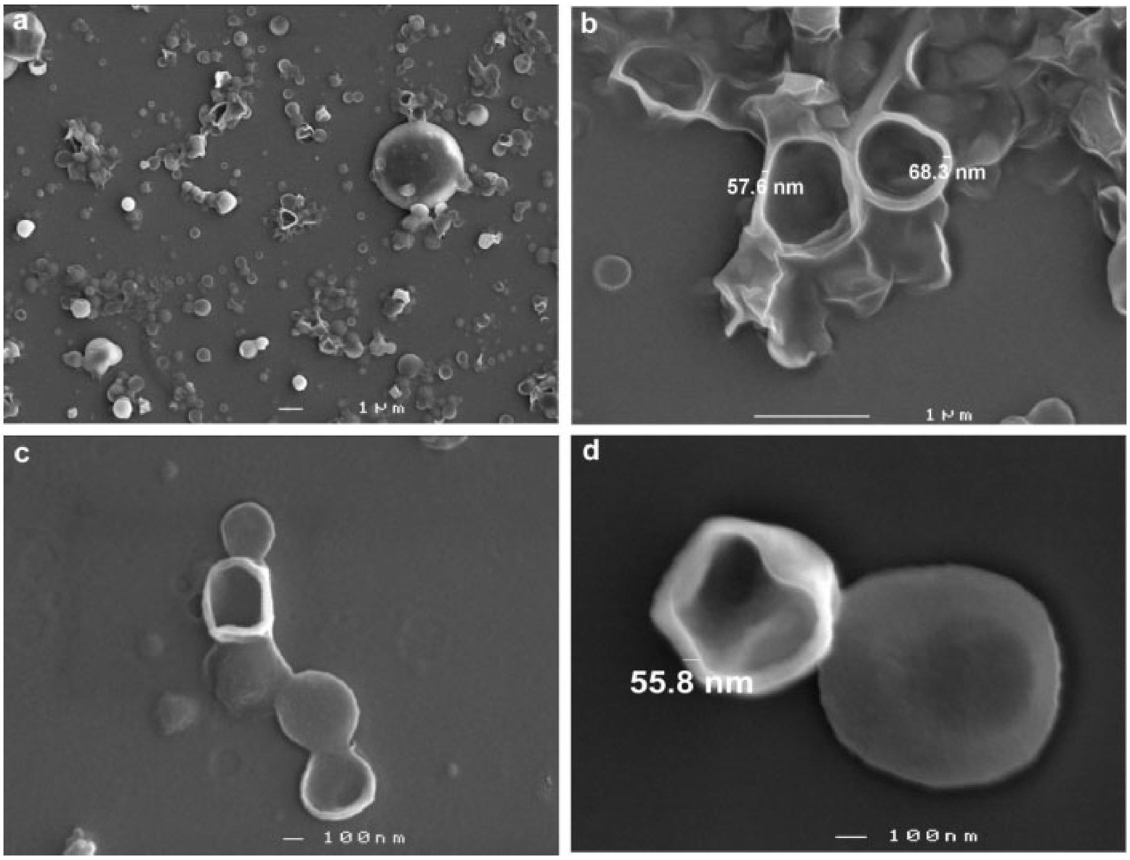

Figure 2. SEM micrographs of PLLA microcapsules prepared with different alkanes: (a) and (b) hexadecane, (c) decane, and (d) hexane.

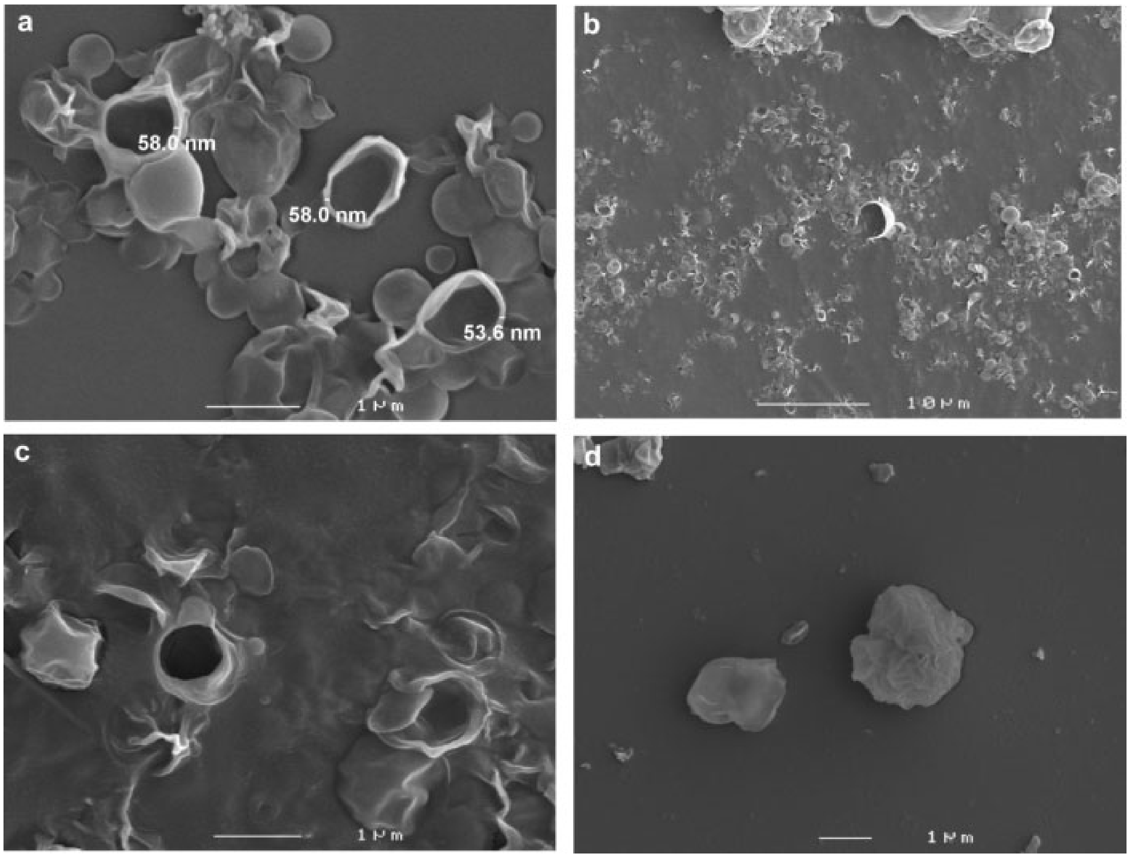

Figure 3. SEM micrographs of PLLA microcapsules prepared with different oils: (a) cyclodecane, (b) and (c) limonene, and (d) eugenol.

1 and 2 μm. With all oils hollow capsules could be produced except for eugenol, in which irregular, massive particles were formed (see Figure 3d). The formation of hollow capsules is thus influenced by the physical properties of the oil (i.e., mutual solubility and interaction with polymer, solubility in the nonsolvent, and boiling point). In Table 2, a summary of the characteristics of the oils and the resulting properties of the capsules is given. The oil must be insoluble in the polymer and nonsolvent (otherwise it will not form a separate droplet inside the droplet of polymer solution), and its boiling point should be high enough to stay inside the polymer shell during out-diffusion and evaporation of the solvent. For instance, when the capsules were prepared with volatile oils (i.e., hexane and cyclohexane), some of the oil evaporated during capsules formation which yielded solid particles. The fact that with eugenol no hollow particles were formed may be ascribed to the good interaction of this oil with PLLA, which implies that the oil did not create a single droplet of oil and leaves the polymer highly swollen with the oil. The solidification of the polymer slows down, therewith allowing enough time for the oil to diffuse out to the external nonsolvent phase (the solubility in water is higher than for other oils).

The effects of the oils on the morphology of the air-cast films and capsules prepared in this study are comparable. The oils that produced hollow capsules were the same ones that produced porous films. With eugenol, a dense and nonporous film was obtained which supports the conclusion of

Table 2. Summary of the Characteristics of the Oils and Their Effects on the Resulting Properties of PLLA Microcapsules

	Properties of Oil		Properties of Microcapsules		
Oil	Volatile	Solubility with Polymer	Hollow Capsules (Yes/No)	Yield of Hollow Capsules	Crystallinity
Hexadecane	–	–	Yes	Good	–
Dodecane	–	–	Yes	Good	–
Decane	–	–	Yes	Good	–
Hexane	+	–	Yes	Low	+
Cyclodecane	–	–	Yes	Good	–
Cyclohexane	+	–	Yes	Low	–
Eugenol	–	+	No	–	–
Limonene	–	+	Yes	Good	++

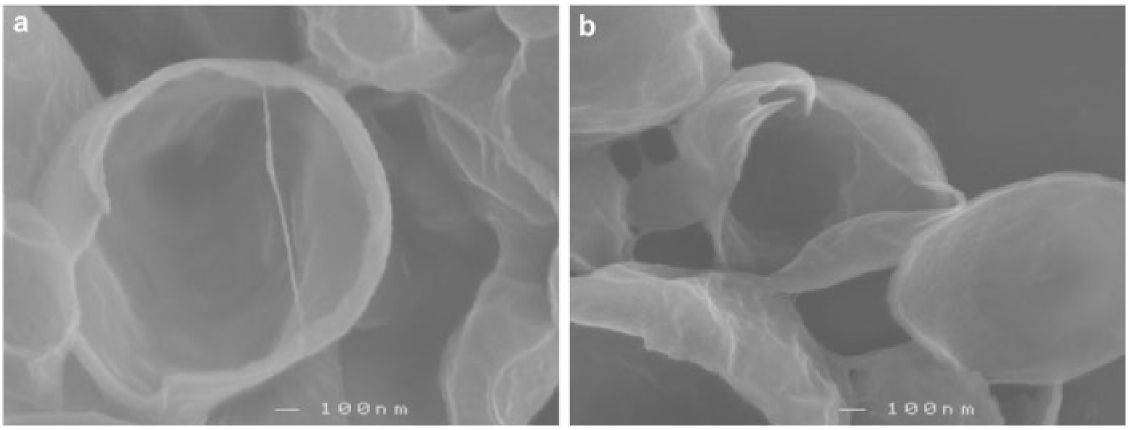

Figure 4. SEM micrographs of PLLA microcapsules prepared with different dodecane concentrations: (a) 9% *w/w* and (b) 24% *w/w*. The polymer concentration was 10% *w/w* PLLA/DCM.

strong interaction of the oil with the polymer such that phase separation was avoided and the structure collapsed into a dense film (Sawalha et al., submitted).

The shell thickness is another important property of the capsules. Figures 2 and 3 show that most of the oils yielded capsules with relatively homogeneous and controlled shell thicknesses of ~50–70 nm. The concentration of the oil is one of the factors that is expected to influence the thickness and morphology of the shell. Figure 4 shows SEM pictures of PLLA capsules prepared from a 10% *w/w* polymer stock solution and with two different dodecane concentrations. The pictures showed that with increasing the oil concentration from 9% (normal recipe) (Figure 4a) up to 24% *w/w* (Figure 4b), the shell thickness was reduced and more defects appeared in the shell. In addition, the capsules buckled and crumbled when more oil was used; and this indicates that the shell became much weaker, and could not withstand the generated capillary forces during removal of dodecane. This illustrates that the polymer/oil ratio is an important parameter that can be used to fine-tune the shell thickness and consequently the strength of the capsules.

To investigate the mechanical shell properties in detail, AFM was used, as is described in the next section.

Effects of oil on mechanical properties of capsules

The force-distance curves of hollow PLLA microcapsules prepared with different oils were obtained through AFM. The AFM images of all measured capsules showed that the sizes of these capsules were comparable (between 1 and 2 μm). In addition, no changes in the topography of capsules before and after experiments were observed, which indicates that the applied load did not cause any irreversible deformation in the capsules, and that the measurements were performed in the elastic regime. Figure 5 shows the typical cantilever deflection (d) vs. the piezo displacement (z) curve together with a schematic description of the experiment. The solid curve in the figure corresponds to the approach phase of the tip toward the surface of the capsule. This curve can be divided into three stages. In Stage I, the tip is at a large distance from the capsule and no force is noted. Once the capsule contacts the tip, due to the attractive forces (van der Waals or electrostatic) at Stage II, the cantilever deflects and upon increasing the applied load, the cantilever continues deflecting until the maximum load is reached (Stage III). After that, the retraction phase starts in which the piezo movement is reversed and the capsule starts to retract from the tip (dashed curve in Figure 5). Because of adhesion forces, the capsule will stay in contact with the tip, even though no force is applied, until the maximum adhesion force is overcome at point IV, after which the tip and the capsule separate and the cantilever returns to its starting point at Stage I. To convert the deflection-distance curve shown in Figure 5 into a force-distance curve, the applied force (F) (nN) can be estimated from the deflection [nm] using Hooke's law as shown in Eq. 2:

$$F = -k_c \cdot d \tag{2}$$

where k_c is the cantilever spring constant (nN nm^{-1} or N m^{-1}), and d is the deflection (nm).

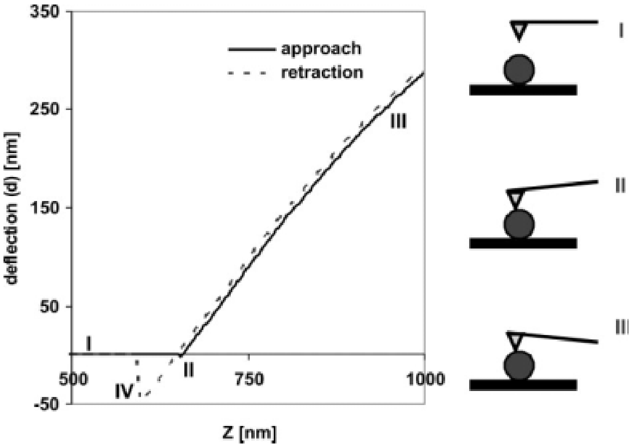

Figure 5. Typical cantilever deflection (*d*) vs. piezo displacement (*z*) curve measured by AFM (left).

On the right, a schematic representation of the positions of the tip and the surface of the sample at different stages during the measurements as indicated by the roman numerals. The actual curves were measured for microparticle prepared with dodecane.

Figure 6 shows typical force-distance curves (approach) measurements performed on clean silica chip and hollow PLLA microcapsules prepared with dodecane, limonene, and cyclohexane. The stiffness of the shell of the capsules can be calculated from the slope of the force-distance curve as described by Sboros et al.[23] According to Sboros et al., the deflection of a cantilever applied on elastically deformable material like a microcapsule combines two springs in series as shown in Eq. 3.

$$\frac{1}{k_{\text{total}}} = \frac{1}{k_c} + \frac{1}{k_s} \tag{3}$$

where k_s is the stiffness of the shell of the capsule (effective spring constant of the shell) (N m^{-1}), k_c is the cantilever spring constant (N m^{-1}), and k_{total} is the overall effective spring constant which is equivalent to the slope of the force-distance curve of the capsule shown in Figure 6.

The slope of the force-distance curve measured for a clean silica chip was equivalent to the spring constant of the cantilever (~0.57 N m^{-1}). The curves for the microcapsules showed lower average slopes than the hard silica surface (0.48 ± 0.02 N m^{-1} for dodecane, 0.44 ± 0.01 N m^{-1} for limonene, and 0.41 ± 0.01 N m^{-1} for cyclodecane). In Figure 7, the calculated average shell stiffness of these microcapsules is shown. Capsules prepared with dodecane showed significantly higher stiffness (3.3 N m^{-1}) than with limonene (2 N m^{-1}) and cyclohexane (1.4 N m^{-1}). The high stiffness of the capsules prepared with dodecane can be related to the high-thermal transition temperatures of these capsules compared with those prepared with limonene and cyclohexane (see Table 1). The shells of the capsules prepared with limonene and cyclohexane were more flexible, which correlates with strong interaction between oil and polymer and higher chain mobility of the shell as was deduced from the DSC results.

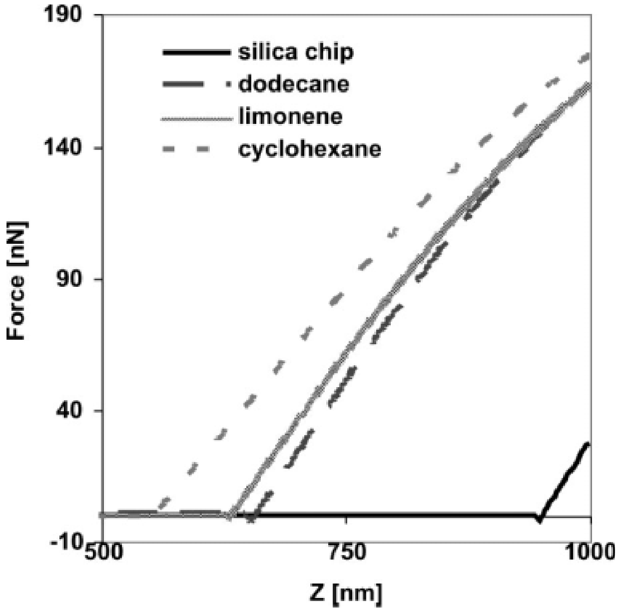

Figure 6. Force-distance curves (approach) measured for clean silica chip and PLLA microcapsules prepared with dodecane, limonene, and cyclohexane as oils.

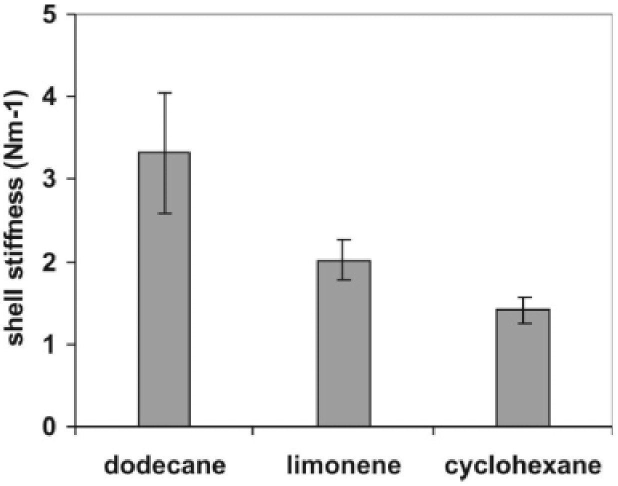

Figure 7. Average shell stiffness of PLLA microcapsules prepared with dodecane, limonene, and cyclohexane as oils.

The average stiffness shown in the figure was measured from five particles of each sample. Three force-distance curves of each particle were analyzed and the slopes were very much similar.

Comparison of the stiffness of the capsules and air-cast films shows major differences. The films prepared with cyclohexane were stiffer than those prepared with dodecane and limonene and the films prepared with limonene were the most flexible among other films. This could be ascribed to the difference in the structure of the films and capsules. The films prepared with limonene and dodecane have much higher porosity compared with those prepared with cyclohexane, which delayed the final rupture of the films. From this, it is clear that mechanical properties of films are not good indicators for microcapsule properties.

Conclusions

Hollow PLLA microcapsules were prepared using a solution of PLLA in a mixture of good and a poor solvent (oil), emulsified into water, being nonsolvent for the polymer that is immiscible with the two solvents.

The thermal and mechanical properties of hollow PLLA microcapsules depend on the type of oil used. The presence of the oil during formation of the capsules enhanced the mobility during the formation of the shell and reduced the glass transition temperature; allowing crystallization to take place to a greater extent. The crystallinity degree of the capsules could be varied from 0 to 45% by using different oils. Hollow capsules with well-defined shell thicknesses (~60 nm) were obtained with all oils, except for eugenol. The stiffness of the shell of the capsules, measured with AFM, was not related to the stiffness of the PLLA films, but was highly dependent on the type of oil. Capsules prepared with dodecane were stiffer than those prepared with limonene and cyclohexane.

Acknowledgments

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