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Preparation of polylactide microcapsules at a high throughput with a packed-bed premix emulsification system

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ABSTRACT: Core–shell polymer microcapsules are well known for their biomedical applications as drug carriers when they are filled with drugs and gas-filled microcapsules that can be used as ultrasound contrast agents. The properties of microcapsules are strongly dependent on their size (distribution); therefore, equipment that allows the preparation of small and well-defined microcapsules is of great practical relevance. In this study, we made polylactide microcapsules with a packed-bed premix emulsification system that previously gave good results for regular emulsions. Here, we tested it for applicability to a system in which droplets shrank and solidified to obtain capsules. The packed-bed column was loaded with glass beads of different sizes (30–90 μm) at various bed heights (2–20 mm), and coarse emulsions consisting of the polymer, a solvent, and a nonsolvent were pushed repeatedly through this system at selected applied pressures (1–4 bar). The obtained transmembrane fluxes (100–1000 $m^3\ m^{-2}\ h^{-1}$) were much higher than those recorded for other membrane emulsification techniques. The average size of the obtained microcapsules ranged between 2 and 8 μm, with an average span of about 1; interestingly, the capsules were 2–10 times smaller than the interstitial voids of the beds. The droplets were larger when we used thicker beds and larger glass beads, and these effect correlated with the pore Reynolds number (Re_p). Two breakup mechanisms were identified: spontaneous droplet snap-off dominated the system at low Re_ps, and localized shear forces dominated the system at higher Re_p. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43536.

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INTRODUCTION

Polylactide is a biodegradable and biocompatible polymer that is used extensively for different biomedical applications, including implants, tissue engineering applications, sutures, and microcapsules. Biodegradable microcapsules made of polylactide have been widely used in recent years for the controlled and targeted delivery of drugs and bioactive molecules.[1–3] Their good *in vivo* stability makes them superior drug carriers because they offer controlled and sustained drug release. In addition, hollow polylactide microcapsules can be used in medical imaging as ultrasound contrast agents. The gas core of the microcapsules allows oscillation in the acoustic field; this increases the backscatter signal of the ultrasound and results in a better image quality.[1,3]

The *in vivo* performance of the microcapsules either as drug-delivery systems or ultrasound contrast agents is significantly influenced by their mechanical, thermal, and morphological properties and their size and size distribution. The drug-release properties and biocompatibility of the microcapsules, for instance, are strongly dependent on the molecular weight, crystallinity, size, and size distribution of the microcapsules.[4] Microcapsules with a uniform size distribution were reported to have less inflammatory and immune responses of the tissues and cells than polydisperse ones.[5]

The preparation of polymer microcapsules involves the dissolution of the polymer (i.e., polylactide) in a proper solvent [i.e., dichloromethane (DCM)] and the addition of a poor solvent to the polymer (i.e., high alkane) followed by the emulsification of

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the polymer solution into a continuous phase, which consists of a nonsolvent for the polymer (i.e., water) and an appropriate surfactant [i.e., poly(vinyl alcohol)]. The emulsification process results in liquid polymer droplets (that contain the alkane) that are dispersed in the nonsolvent phase. The polymer droplets are then solidified into polymer microcapsules through the extraction of the solvent from the droplets into the nonsolvent, which is subsequently removed by evaporation to the air, and the alkane remains captured in the polymeric capsule. The morphology and thermal and mechanical properties of the microcapsules can be fine-tuned through the optimization of the ingredient formulation that determines the solidification of the polymer during the fabrication process.[2]

The emulsification methods, including high-pressure homogenizers, colloid mills, and sonication, used for the preparation of polymer microcapsules are the same as those reported for the preparation of emulsions. However, these traditional methods are high in energy consumption, and this may damage the fragile capsules and result in poor control over the size and size distribution. This affects the performance of the microcapsules. Therefore, milder preparation techniques are of great relevance to the field.[6]

Membrane emulsification was successfully used to prepare monodisperse microcapsules at low energy consumption; this also resulted in better control over the size and size distribution.[6,7] Membrane emulsification can be conducted in different manners, including cross-flow, microchannel, and premix membrane emulsification.[6–8] In cross-flow and microchannel emulsification, the dispersed phase is pushed through the fine pores of the membrane, and the continuous phase shears them off at the opening of the pores to form relatively uniform and small droplets.[6–8] Although these droplets are highly monodisperse, the production yield is rather low.

In premix emulsification, a coarse emulsion is forced through the pores of the membrane; this can be done at high flux.[9] Upon passage of the membrane, the large droplets are broken up into smaller ones, and repeating this process several times may result in relatively uniform and small droplets. Different types of microporous membranes, including glass, polymeric, metallic, and ceramic membranes, have been reported in the literature[6–8,10] for the preparation of emulsions and microcapsules, but there have also been issues related to the fouling and blockage of membranes due to interactions with emulsion ingredients.

Recently, Nazir *et al.*[11,12] and van der Zwan *et al.*[13] proposed a promising premix emulsification system, which consisted of packed beds of glass beads supported by a metal sieve. The new system has many advantages, including a robust and simple design, easy cleanup, low cost, allowance of higher transmembrane fluxes (*J*s), and fewer fouling issues, compared to the previously mentioned membranes. The system was tested for single and double emulsions[11,14] but not for the complex mixtures needed for polymer microcapsules, which are expected to interact very differently with the system. During the preparation of the microcapsules, the solvent is removed; this leads to solidification, which makes droplets break up less easily, and it is expected that also, because of this, the emulsion ingredients will interact differently with the device. Both aspects need to be carefully controlled to allow successful application of the premix system.

The objective of this study was to investigate for which process conditions polylactide microcapsules could be prepared successfully with the packed-bed premix emulsification technique. The glass bead size, bed height, *J*, and number of emulsification cycles through the bed were varied and their effect on the size and size distribution of the microcapsules were noted, with the ratio between the droplet and pore size and the droplet pore Reynolds number (Re_p) as convenient scaling parameters.

EXPERIMENTAL

Materials

Poly(L-lactide) (PLLA), with an intrinsic viscosity of 1.21 dL/g, was purchased from PURAC Biochem B.V. (Gorinchem, The Netherlands). As a solvent for PLLA, DCM (high performance liquid chromatography (HPLC), gradient grade) was purchased from Merck (Amsterdam, The Netherlands). The poor solvent, decane (≥99%), was obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). The nonsolvent phase consisted of Milli-Q water and methanol (HPLC, gradient grade, ≥99.9%) from Aldrich (Zwijndrecht, The Netherlands). Poly(vinyl alcohol) (23/88) from Ter Hell (Hamburg, Germany) was used as an emulsion stabilizer. All chemicals were used as received.

Preparation of the Microcapsules and Experimental Setup

A polymer stock solution with a concentration of 2% w/w was prepared by the dissolution of PLLA in DCM. The course premix emulsion was prepared by the mixture of the polymer phase, which consisted of 11.9 g of the stock PLLA solution, 23.8 g of DCM, and 3.57 g of decane, with the nonsolvent phase, which consisted of 70 g of a 1% w/w aqueous poly(vinyl alcohol) solution, 140 g of water, and 23.8 g of methanol. This mixture was magnetically stirred at 1000 rpm for 1 min. Methanol, which constituted 20% w/w of the total nonsolvent solution, was later added to the mixture to prevent immediate solidification of the polymer. The premix emulsion was then poured into a stainless steel emulsion vessel, which was connected to the packed-bed column (Plexiglas column) through pipes and valves, as shown in Figure 1.

Hydrophilic glass beads (100 HFL, Pneumix SMG-AF) with different average sizes (30, 55, 65, 75, and 90 μm) were predeposited on a supporting nickel sieve (Stork, Eerbeek, The Netherlands), which was mounted at the bottom of the column. The sieve had straight-through pores with an average size of 11.6 × 331 μm and a thickness of 350 μm (scanning electron microscopy images of the sieves can be found elsewhere[11]). The amount of glass beads was varied to achieve bed heights of 2, 5, 10, and 20 mm. To ensure a good settling of the beads above the sieve, the beads were wetted with a small amount of water, and the column was turned upside down several times and then positioned vertically.

The emulsification procedure was performed by the pressurization of the emulsion vessel to transport the premix emulsion through the packed-bed column (see Figure 1). The outlet

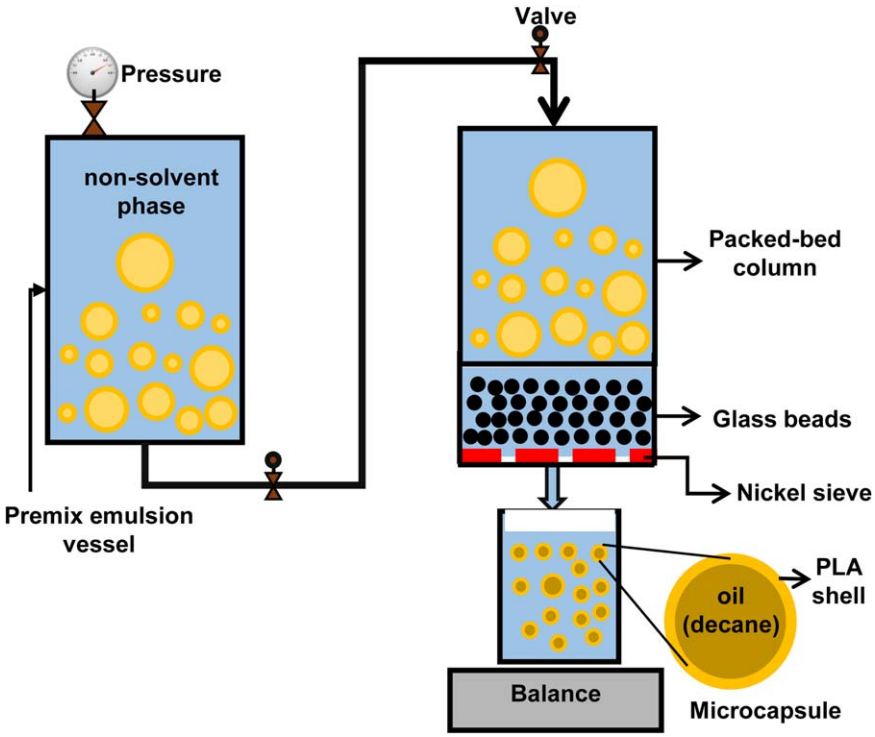

Figure 1. Schematic representation of the premix packed-bed emulsification process. PLA = polylactide. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

emulsion was collected in a beaker, which was placed on an electronic balance to measure the mass flux in time. The emulsion was then poured again into the emulsion vessel, and the emulsification procedure was repeated in the same way up to five times. After that, the emulsion was stirred for 1 h at 1000 rpm with a magnetic stirrer to remove the DCM from the polymer droplets; this consequently solidified the polymer around the decane droplets and resulted in the formation of oil-filled microcapsules.

PLLA Microcapsule Characterization

The Sauter mean diameters (d_{32}s) of the microcapsules right after emulsification and after the evaporation of the solvent were measured with laser diffraction (Malvern Mastersizer 2000, Malvern Instruments, Ltd., Worcestershire, United Kingdom). The size distribution of the samples was represented with the span, which was defined as follows:

$$\text{Span} = (d_{90} - d_{10})/d_{50}$$

where d_x is the droplet diameter corresponding to an x volume percentage in the size distribution curve.

The morphology of the solid polylactide particles was visualized by field emission scanning electron microscopy (Magellan 400, FEI, Eindhoven, The Netherlands).

Calculation of the Packed-Bed Parameters

The packed-bed structural parameters, such as the pore diameter (d_p; m) and bed tortuosity (ξ), were determined with the Comiti and Renaud capillary model for fixed beds.[15] According to the proposed model, d_p can be calculated as follows:

$$d_p = \frac{4\varepsilon}{A_{vd}(1\text{-}\varepsilon)} \quad (1)$$

where ε is the porosity of the bed and is defined as follows:

$$\varepsilon = 1\text{-}\frac{\rho_b}{\rho_p} \quad (2)$$

where ρ_b and ρ_p are the bulk and particle densities of the beads and A_{vd} is the specific area of the beads (m^2/m^3). This was related to the bead diameter (d_b) according to the following relation:

$$A_{vd} = \frac{6}{d_b} \quad (3)$$

ξ of the bed was determined as follows:

$$\xi = 1 + q\ln(1/\varepsilon) \quad (4)$$

For tightly packed spherical beads, q is a constant worth 0.41. The flow within the pores of the packed bed was characterized with Re_p, which is given as follows:

$$Re_p = \frac{\rho_e v_p d_p}{\mu_e} \quad (5)$$

where ρ_e and μ_e are the density and viscosity of the emulsions, respectively. These were calculated with the same correlations used by Nazir *et al.*[12] with the assumption that the continuous phase mainly consisted of water and the dispersed phase of DCM. Also, v_p is the superficial velocity across the bed; this was equivalent to J, which was calculated as follows:

$$J = \frac{\varphi_m}{\rho_e A} = \frac{\Delta P}{\mu_e R_{pb}} \quad (6)$$

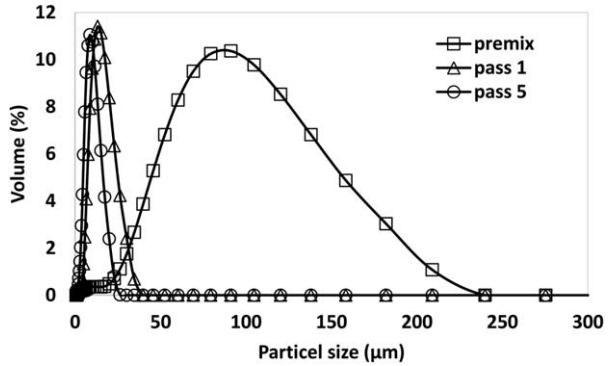

Figure 2. Particle size distribution of polylactide microcapsules prepared with 78-µm beads and a bed height of 2 mm at an applied pressure of 1 bar.

where φ_m is the mass flux through the bed (kg/s), A is the effective surface area of the bed (m^2), ΔP is the applied pressure, and R_{pb} is the resistance of the packed bed.

RESULTS AND DISCUSSION

Before describing the effect of various factors, first, we discuss our general impression of the effects created by the premix emulsification of the packed-bed system. In Figure 2, the size distributions of the premix are shown together with those after repeated passage through the system with 78-µm beads at bed height of 2 mm and an applied pressure of 1 bar. The results in Figure 2 nicely illustrate the efficacy of the emulsification; one can clearly observe that the size distribution became sharper after passage through the system and was more uniform than that of the premix sample, which appeared broad and polydisperse.

Effect of the Applied Pressure and Packed-Bed Structure

The effect of the applied pressure and packed-bed structure, that is, the bed height and glass bead size, on the flux through the packed bed was investigated first, and the results are shown in Figure 3. The flux generally increased with increasing applied pressure and bead size, whereas it decreased with increasing bed height, as expected from eq. (6). The resistance of the packed bed was higher for smaller glass beads and also for higher beds, whereas the flux scaled linearly with the applied pressure difference at a certain bead size and bed height.

Furthermore, the flux slightly decreased with increasing number of passes [see Figure 3(a)]. This could have been due to either an increase in the viscosity [see eq. (6)] or the interaction of the components with the bed, which increased the flow resistance. Unfortunately, the emulsion destabilized so fast that the viscosity could not be measured, but the actual flux values that were found were such that they compared well with those reported for hexadecane in water emulsions, which are known not to cause fouling. This was, of course, not proof that fouling did not occur; it was just an indication that it was not occurring so fast that it had a great negative effect on the process. The flux values obtained with this technique (100–1000 m^3 m^{-2} h^{-1}) were higher than those reported for any other membrane emulsification technique; this indicated the effectiveness of the packed-bed emulsification system.

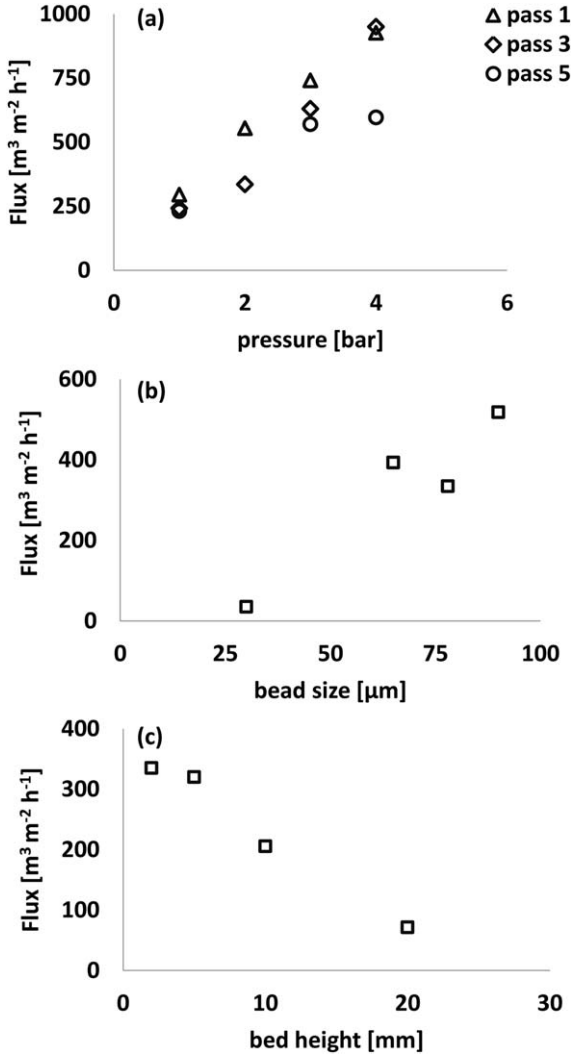

Figure 3. J as a function of the (a) applied pressure, (b) bead size, and (c) packed-bed height. The flux values shown in panels b and c are for the third pass.

To compare the various conditions, we used the dimensionless diameter (d_{32}/d_p) and the span, which was indicative of the droplet size distribution. The arrangement of the beads within the bed resulted in interstitial voids, which formed irregular paths through the bed similar to the pores of conventional membranes.[11] d_p within the bed was related to d_b and was estimated for different beads sizes with eq. (1), as shown in Table I.

Table I. Average d_b and Corresponding d_p Values of the Bed Calculated with Equation (1)

d_b (µm)	d_p (µm)
30	13
65	29
78	33
90	40

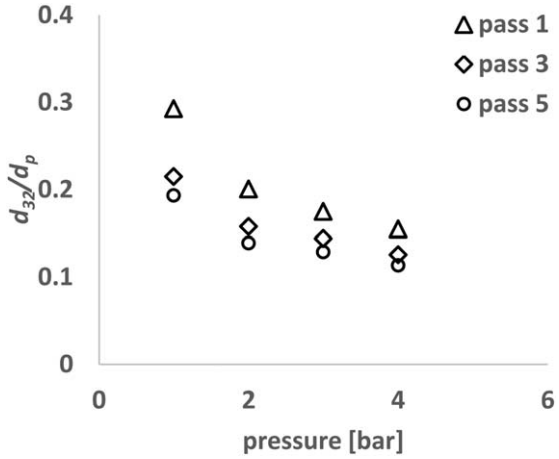

Figure 4. Effect of the applied pressure on d_{32}/d_p of the polylactide microcapsules. The experiments shown here were conducted with 78-μm beads and a bed height of 2 mm.

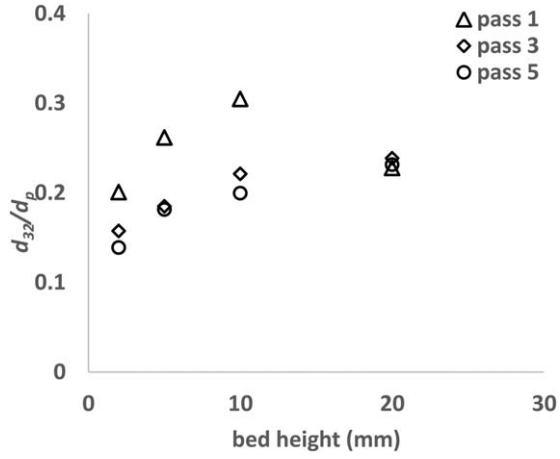

Figure 6. Effect of the bed height on d_{32}/d_p of the polylactide microcapsules. The experiments shown here were conducted at a constant pressure of 2 bar with 78-μm beads.

The bead sizes that were used were 30, 65, 78, and 90 μm, and the bed height and transmembrane pressure (ΔP; Pa) were kept constant at 2 mm and 2 bar, respectively. The size of the microcapsules was expressed as the dimensionless ratio of the microcapsule size to the pore size (d_{32}/d_p); these values are shown in Figure 4 for passes 1, 3, and 5. Generally, the d_{32}/d_p ratio decreased with increasing applied pressure for most of the passes through the bed (see Figure 4). The increase in the applied pressure increased the flux (see Figure 1) and, consequently, increased the shear forces applied on the droplets while they were breaking up; this reduced the droplet size.

With respect to the pore size, which was varied at the same applied pressure, its effect on the droplet size was twofold (Figure 5). The flux scaled linearly with pressure but not linearly with bead size; because the net effect of flux was larger, the overall effect was still a decrease in the droplet size for larger pores. In addition, the size of the microcapsules was much

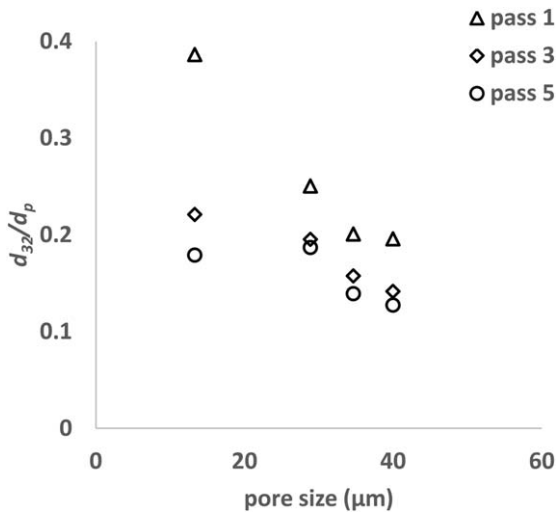

Figure 5. Effect of the pore size on d_{32}/d_p of the polylactide microcapsules. The experiments shown here were conducted at a constant pressure of 2 bar and a bed height of 2 mm.

smaller than that of the pore, as shown from the d_{32}/d_p ratios, which ranged between 0.1 and 0.4. The obtained d_{32}/d_p values were actually the smallest compared to those of the other membrane emulsification process. For instance, a ratio of around 1 was recorded for premix membrane emulsification,[16] whereas for cross-flow membrane emulsification, the ratio was much higher. It started around 2 and went up as high as 10.[11]

The effect of the bed height on the size of the microcapsules was investigated with 78-μm beads at different heights (2, 5, 10, and 20 mm; Figure 6). The results show that d_{32}/d_p increased with increasing bed height (see Figure 6). With bed height, the resistance of the bed increased; this decreased the emulsion velocity and resulted in lower shear forces during emulsification, and consequently, larger microcapsules were obtained. What could also have played a role was the time that the droplets spent together in the bed, which increased with increasing bed height; this provided more of a chance for coalescence to occur, and this would also result in bigger droplets. Looking at Figure 6, one can clearly see that at low bed height, the size was reduced steadily, whereas at a high bed height, the final size was immediately obtained, and this could indicate that in the higher beds, more recoalescence took place and that the emulsification efficiency was higher for the most shallow beds.

Although the average droplet size did not evolve much after three passes (Figures 4–6), that was not the case for the droplet size distribution. The span seemed to increase slightly with increasing pore size [see Figure 7(a,b)], whereas it decreased with increasing bed height up to 10 mm, after which the span values became generally independent of the height, as shown in Figure 7(b). The decrease in the span was ascribed to the fact that with higher beds, the droplets had more of a chance to break up inside the porous media. This resulted in more uniform droplets. However, if the bed was too high, coalescence of the droplets may have occurred. This would cancel out the breakup effects and lead to more or less constant span values.

The morphology of the microcapsules was visualized with scanning electron microscopy; the particles were spherical and had a

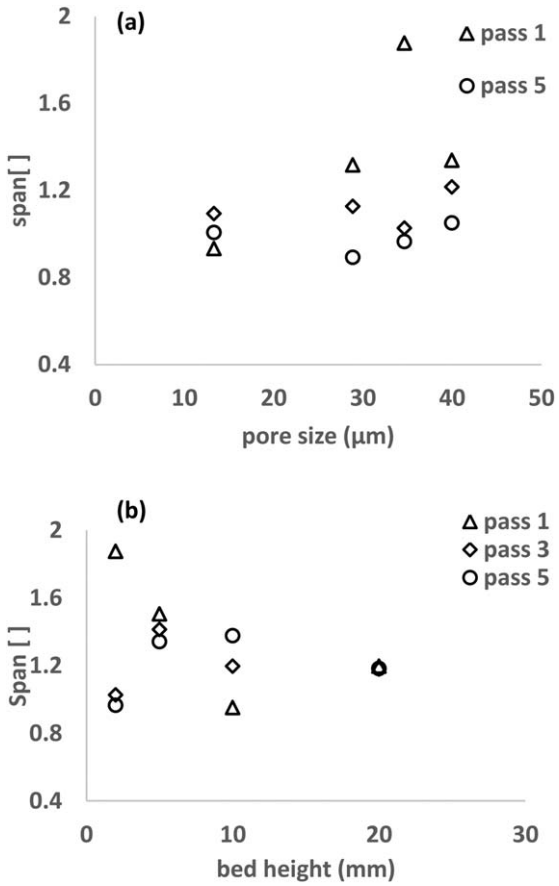

Figure 7. Effect of the (a) d_p and (b) bed height on the span of polylactide microcapsules. The experiments shown in panel a were performed at a constant pressure of 2 bar and a bed height of 2 mm, and the experiments shown in panel b were performed at the same pressure with 78-μm beads.

smooth surface (see Figure 8). Although we did not perform this step, the oil could be removed by freeze drying; this results in hollow-core microcapsules. Interested readers are referred to the study of Sawalha *et al.*[17] for images of hollow microcapsules prepared from similar recipes.

Droplet Breakup Mechanism

To allow the comparison of all of the tested process conditions and to use that to identify the droplet breakup mechanisms in our system, we used the droplet Re_p [eq. (2)]. In this number, the effects of the bed height, bead size, and ΔP were incorporated, and we plotted the dimensionless size and span of the obtained microcapsules for the fifth pass [see Figure 9(a,b)].

Figure 9(a) shows that at low Re_p (i.e., $Re_p<10$), the dimensionless size of the microcapsules decreased with increasing Re_p, whereas at high Re_p (>10), the decrease in the size was much less. This could hint at a change in the droplet formation mechanism from one that was affected by shear to one that was virtually shear independent. The span of the obtained emulsions were mostly around 1, with the exception of some higher values at low Re_p, which corresponded to low shear forces and high residence time; this might have led to coalescence. What was clear was that Re_p values greater than 10 seemed to correspond to a more uniform droplet size [see Figure 9(b)].

From Figure 9(a,b), we concluded that there were two regions, a low Re_p region ($Re_p < 10$) and a high Re_p region ($Re_p > 10$) in which the behavior of the size and the span of the obtained microcapsules varied. At low Re_p corresponding to smaller beads (and higher beds), the droplets were relatively more constricted than their counterparts, which passed through beds with larger beads. In addition, the flow velocity was relatively low, and that implied that the droplets were more susceptible to spontaneous droplet snap-off, as was reported for some microstructured systems.[18] On the other hand, at higher Re_p (i.e., when a larger bead size and/or lower bed height was used), the flow velocity was higher, and consequently, the applied shear stress within the porous media was greater. In this case, the droplet breakup process mainly occurred because of local shear forces applied on the droplets.

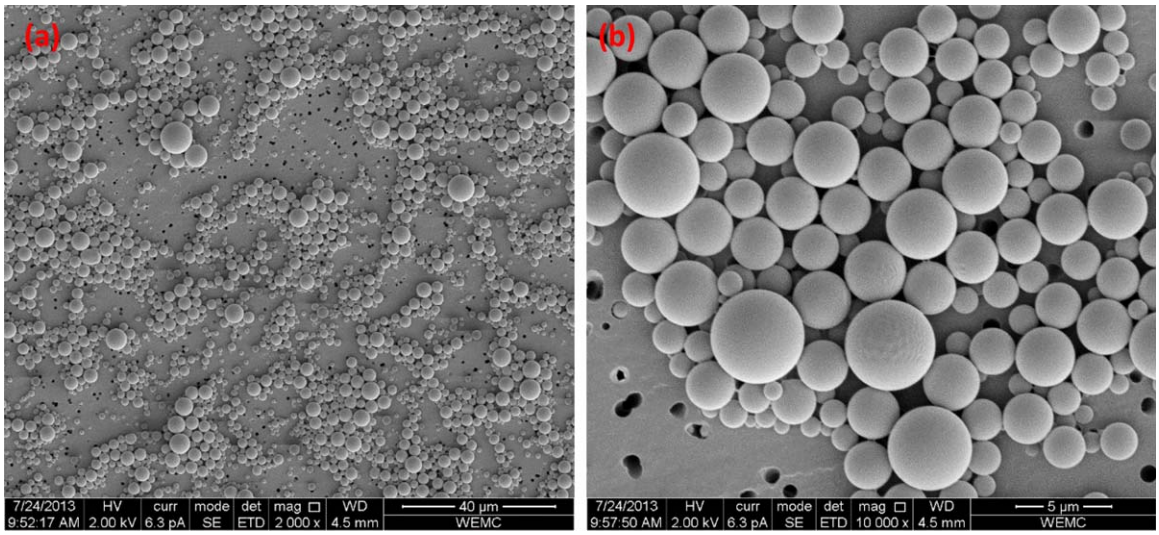

Figure 8. Scanning electron micrograph of the (a) solid polylactide microcapsules prepared without the addition of the poor solvent (decane) to the premix emulsion and (b) same microcapsules at a higher magnification. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

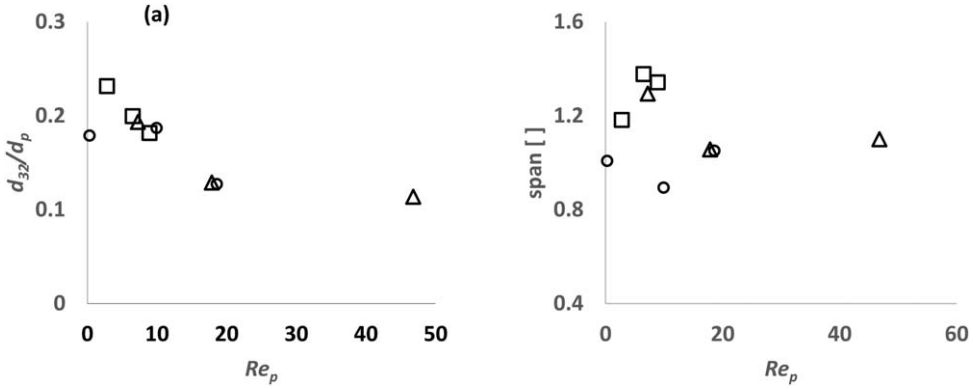

Figure 9. (a) d_{32}/d_p and (b) span of polylactide microcapsules obtained after the fifth pass as a function of Re_p. The data shown here were obtained through variations of the (Δ) applied pressure, (○) d_p, and (□) bed height. The pressure and d_p decrease and the bed height increases from right to left in both panels.

When comparing our results with those of Nazir *et al.*[11] for single oil-in-water emulsions and those of Sahin *et al.*[14] for water-in-oil-in-water double emulsions with the same emulsification system, we observed that the emulsification behavior of the polymer microcapsules resembled single emulsions more than double emulsions. This may be have been due to the fact that at the time of formation, the polymer droplets behaved as a single phase, as was the case with single emulsions, and solidification did not influence the process. In double emulsions, a second phase was present with different properties, and that may have led to different droplet formation behavior.

CONCLUSIONS

Polylactide microcapsules were successfully prepared with the packed-bed premix emulsification technique. The achieved *J* and, consequently, the yield of the microcapsules was the highest among the membrane emulsification techniques, and the obtained microcapsules were rather uniform in size.

The droplet breakup mechanism seemed to be similar to that of a single emulsion and was dependent on the droplet Re_p. At low Re_p, the spontaneous droplet snap-off mechanism seemed to dominate, whereas localized shear forces dominated at higher Re_p.

In this study, we demonstrated that the premix technology could also be used successfully in systems in which solidification took place. The timescales for solidification and processing were such that the droplets were still liquid and could be postprocessed to obtain polymer microcapsules.

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REFERENCES

1. Kooiman, K.; Vos, H. J.; Versluis, M.; de Jong, N. *Adv. Drug Delivery Rev.* **2014**, *72*, 28.
2. Sawalha, H.; Schroën, K.; Boom, R. *Chem. Eng. J.* **2011**, *169*, 1.
3. Böhmer, M. R.; Klibanov, A. L.; Tiemann, K.; Hall, C. S.; Gruell, H.; Steinbach, O. C. *Eur. J. Radiol.* **2009**, *70*, 242.
4. Anderson, J. M.; Shive, M. S. *Adv. Drug Delivery Rev.* **2012**, *64*, 72.
5. Sawalha, H.; Fan, Y.; Schroën, K.; Boom, R. *J. Membr. Sci.* **2008**, *325*, 665.
6. Vladisavljević, G. T.; Kobayashi, I.; Nakajima, M. *Microfluidics Nanofluidics* **2012**, *13*, 151.
7. Charcosset, C.; Limayem, I.; Fessi, H. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 209.
8. Schroën, K.; Bliznyuk, O.; Muijlwijk, K.; Sahin, S.; Berton-Carabin, C. C. *Curr. Opin. Food Sci.* **2015**, *3*, 33.
9. Nazir, A.; Schroën, K.; Boom, R. *J. Membr. Sci.* **2010**, *362*, 1.
10. Spyropoulos, F.; Lloyd, D. M.; Hancocks, R. D.; Pawlik, A. K. *J. Sci. Food Agric.* **2014**, *94*, 613.
11. Nazir, A.; Boom, R. M.; Schroën, K. *Chem. Eng. Sci.* **2013**, *92*, 190.
12. Nazir, A.; Boom, R. M.; Schroën, K. *Chem. Eng. Sci.* **2014**, *116*, 547.
13. van der Zwan, E. A.; Schroën, C. G. P. H.; Boom, R. M. *AIChE J.* **2008**, *54*, 2190.
14. Sahin, S.; Sawalha, H.; Schroën, K. *Food Res. Int.* **2014**, *66*, 78.
15. Comiti, J.; Renaud, M. *Chem. Eng. Sci.* **1989**, *44*, 1539.
16. Sawalha, H.; Purwanti, N.; Rinzema, A.; Schroën, K.; Boom, R. *J. Membr. Sci.* **2008**, *310*, 484.
17. Sawalha, H.; Schroën, K.; Boom, R. *AIChE J.* **2009**, *55*, 2827.
18. Kobayashi, I.; Nakajima, M.; Nabetani, H.; Kikuchi, Y.; Shohno, A.; Satoh, K. *J. Am. Oil Chem. Soc.* **2001**, *78*, 797.