Synthesis and Characterization of Novel, Highly Crystalline Poly(vinyl alcohol) Microspheres for Chemoembolization Therapy

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ABSTRACT: Biodegradable polymeric microspheres can be used to deliver drugs through controlled rate and targeted processes. The drug is released from the particles by drug leaching or degradation of the polymeric matrix. Crystallinity can play a very important role in the degradation of polymeric matrixes; it can affect the drug-release rate, especially in chemoembolization. Most commercial embolic agents have a low degree of crystallinity, and the correlation between the drug-delivery rate and the degree of crystallinity is not fully understood. This study presents the appropriated synthesis conditions for the preparation of highly crystalline poly(vinyl alcohol) and poly(vinyl alcohol)/poly(vinyl acetate) microspheres and physicochemical characterizations by scanning electron microscopy, X-ray diffraction, differential scanning calorimetry, and cross-polarization/magic angle spinning nuclear magnetic resonance. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 1417–1423, 2011

Key words: biomaterials; NMR; synthesis; X-ray

INTRODUCTION

The decodification of the human genome and the rapid technological advancement in nanotechnology has created the ability to design patient-specific drugs that can alter the cellular machinery at the genetic level in a way that controls or treats a specific disease. Designing nucleic acid drugs and engineering novel delivery vehicles that encapsulate and effectively transport genetic materials into cells provides an opportunity to enhance the understanding of disease mechanisms and may help treat or cure these diseases.[1] There has been considerable research effort into the search for novel families of nonviral vectors that can be assembled with entities, such as small molecules of drugs, peptides, proteins, and nucleic acids, to be used as a new class of therapeutic entities for targeted cancer treatment.[1] Polymeric particles with a controlled size and morphology associated with appropriate chemical properties, such as hydrophilicity, sorbability, bioaffinity, and biodegradability, have been widely used in several biomedical applications,[2,3] especially in embolization and chemoembolization.[4–7]

Embolizations are defined as therapeutic vascular occlusions that are used to prevent or treat pathological conditions *in situ.* The intravascular interventional procedures that produce artificial embolizations can be useful in mammals for controlling internal bleeding, blocking the blood supply to tumors, and relieving pressure in vessel walls near aneurysms. Chemoembolization advantageously combines arterial embolization of the vascular supply of a neoplasm with the controlled intra-arterial infusion of chemotherapeutic drugs.[8–14] Currently, some of the most commonly used embolic agents are particles made of poly(vinyl alcohol) (PVA), and their use is directly related to their biocompatibility, good elasticity, high compressibility, and effective chemical resistance to acids, bases, and detergents.[6]

PVA particles are divided into two classes of particles: nonspherical PVA foams or sponges and

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spherical particles. Traditional nonspherical PVA foams or sponges are irregularly shaped and generally contain a range of pore sizes that are produced during the manufacturing process by the whipping of air into the PVA solution before crosslinking. The disadvantages of these particles include their imprecise size and the open edges on the particles, which can cause them to clump together and subsequently plug up delivery catheters or become occluded at a site next to the target site. Usually, the undesirable characteristics related to nonspherical PVA particles can be minimized by the use of spherical particles. Spherical particles can penetrate more deeply into the vasculature than traditional particles because of their uniform shape, and they are rarely reported to occlude delivery catheters.

Calibrated particles have drastically changed the conditions of embolization because the radiologist may adapt the size of the vessels to be occluded so that accurate targeting can be achieved. Trisacryl gelatin microspheres (Embosphere Microspheres, Biosphere Medical, Rockland, MA) were the first commercially approved in Europe (approval by CE (European Community)) in 1997 and in the United States (approval by the U.S. Food and Drug Administration) for general embolization in 2000 and, specifically, for uterine fibroid embolization in 2002. Thereafter, two PVA-based microspheres, named *Contour PVA Embolization Particles* (Boston Scientific, Natick, MA) and *Bead Block* (Biocompatibles, Farnham, United Kingdom), have been developed; both were approved in 2007 in Europe and in the United States.[15]

PVA cannot be prepared by directed polymerization because of the tautomerism of the vinyl alcohol monomer, and it is usually obtained by the saponification of poly(vinyl esters), such as poly(vinyl acetate) (PVAc) and poly(vinyl pivalate), although PVAc is the most common precursor of PVA. Commonly used polymerization methods for the monomeric precursors of PVA are bulked, solution, emulsion, and suspension polymerizations. Nevertheless, the suspension polymerization method is the most used method because it has several advantages, including the easy removal of the heat produced by the strong exothermic reaction, a high rate of conversion, and the formation of polymer particles with diameters of 30–1200 μm.[16,17] Several authors have shown that particles with a regular spherical morphology and size could be obtained with the suspension polymerization technique instead of particles with irregular shapes and sizes,[5,18] as are usually found in many commercial embolization products. However, a careful and critical analysis of the polymeric crystalline structures of the synthesized particles showed that there was no improvement in the crystallinity of the synthesized microspheres.

In general, a typical X-ray diffraction (XRD) pattern of the PVA microspheres synthesized by the suspension method is characterized by a broad diffraction peak. For chemoembolization with PVA microspheres, it is important to consider not only the size distribution of the polymeric particles and their well defined morphology but also their crystalline structures. Several authors have investigated drug–polymer interactions using different polymeric matrixes carried or loaded with different drugs.[2,3] The authors concluded that the crystallinity of the polymeric matrix could affect the release rate of the drugs through the degradation rate of the chain. It was observed that degradation first occurred in the amorphous region of the polymeric matrix followed by a slower degradation in the crystalline regions. Therefore, the drugs located or loaded in the amorphous region were released more quickly than those located or loaded in the higher crystalline region.[2,3] In this article, we present our preliminary results by reporting an experimental methodology capable of producing PVA/PVAc microparticles with a controlled size and spherical morphology and also with a higher crystallinity molecular structure compared to commercially available PVA particles.

EXPERIMENTAL

Materials

All chemicals were used without purification. Vinyl acetate (minimum purity = 99.9%), benzoyl peroxide (minimum purity = 99%), and PVA (average molecular weight = 78,000 Da, degree of hydrolysis = 85%) were supplied by Vetec Química Fina (Rio de Janeiro, Brazil). Sodium hydroxide (NaOH; minimum purity = 99%) was supplied by Aldrich–Fluka (Steinheim, Germany). Distilled water was used as the suspending medium.

Synthesis of the PVA/PVAc microspheres

PVA/PVAc microspheres were synthesized from modified procedures reported in the literature.[5,6] In a typical reaction, the suspending agent, PVA (0,07 g), was dissolved in water (100 mL) under a nitrogen atmosphere and with constant stirring in a 250-mL homemade reactor fitted with a condenser. After degassing, 25 mL of the solution of the vinyl acetate monomers and 1 g of the initiator, benzoyl peroxide, were added all at once at a fixed polymerization temperature of 70°C. The system was kept under isothermal conditions with an agitation speed of 700 rpm. The reactions were interrupted after 5 h, and the particles were filtered, washed with distilled water, and then submitted to the saponification procedure.

For the saponification reaction, a flask equipped with a reflux condenser, a thermocouple, a dropped

TABLE I
Saponification Conditions and Degree of Crystallinity of the PVA Microspheres

Sample	NaOH concentration (*M*)	Degree of crystallinity (%)
1	25	87.5 ± 2.1
2	20	78.4 ± 11.5
3	16	65.8 ± 7
4	12	56 ± 3.6
5	8	19.6 ± 0.2
6	4	14.3 ± 4.6

funnel, and a stirring device and an alkali solution of NaOH at different concentrations (see Table I) were used. The PVAc microspheres were slowly added to the flask with stirring, and the time for saponification reaction varied from 2 to 6 h at 35°C. After the saponification reaction, the particles were poured into cold distilled water and kept there for approximately 12 h to separate and sink the PVA/PVAc microspheres. The final products were dried *in vacuo* at 37°C for 1 day before characterization by XRD, solid-state cross-polarization/magic angle spinning nuclear magnetic resonance (CP–MAS NMR), differential scanning calorimetry (DSC), and scanning electronic microscopy (SEM).

PVA/PVAc microsphere characterization

The XRD patterns of the synthesized polymeric particles were collected with a Rigaku RotaFlex RU200B (Tokyo, Japan) on a rotating anode source with a flat-plate Bragg–Brentano geometry, operating with Cu Kα radiation (wavelength = 1.5418 Å) at 50 kV and 100 mA, and equipped with a graphite monochromator. The powder diffraction patterns were recorded in the range $2\theta = 5$–$80°$ with a scan step of 0.02° and at a rate of 10 s/step. NMR measurements were performed with a Varian Inova spectrometer (Palo Alto, California) operating at 400 and 100.5 MHz for ^{1}H and ^{13}C, respectively. A 5-mm magic angle spinning probe (Jackobsen design) was used in the measurement. The CP–MAS method with a linear amplitude variation of the ^{1}H pulse was used.[19–21] The strength of the ^{1}H decoupling field applied during the signal acquisition was 60 KHz. A contact time of 1 ms, a spinning frequency of 10 KHz, and recycle delays of 3 s were used. SEM images were recorded with an XL30 FEG instrument, and before the analysis, a thin coating of gold was deposited onto the samples. Small amounts of dried polymeric particles (2.2 mg) were characterized by DSC (DSC 404C controlled by TASC 424/3A, Netzsch 404C controlled by TASC 424/3A, Selb, Germany) between 25 and 300°C with a platinum crucible. The equipment was calibrated against an indium standard (melting temperature = 158.5°C). A constant heating rate of 10°C/min and a flux of 0.50 cm^3/min of nitrogen were used.

RESULTS AND DISCUSSION

The XRD data shown in Figure 1 provide strong evidence that the crystalline structures of the synthesized polymeric particles changed with the increasing NaOH concentration used during the saponification process. It is clear from Figure 1 that the saponification reactions performed with NaOH concentration of 4 and 8*M* resulted in polymeric particles whose XRD patterns were characterized by very broad Bragg diffraction peaks in the region $2\theta = 7$–$30°$. The absence of well-defined and intensive Bragg diffraction peaks for these samples revealed their amorphous nature. On the other hand, when the NaOH concentration was increased from 12 to 25*M*, the XRD patterns of the resulting polymeric particles showed a completely different spectrum. It was evident from the XRD patterns that the new diffraction peaks were an indication that novel crystallographic arrangements were formed with the addition of high-concentration NaOH solutions. The

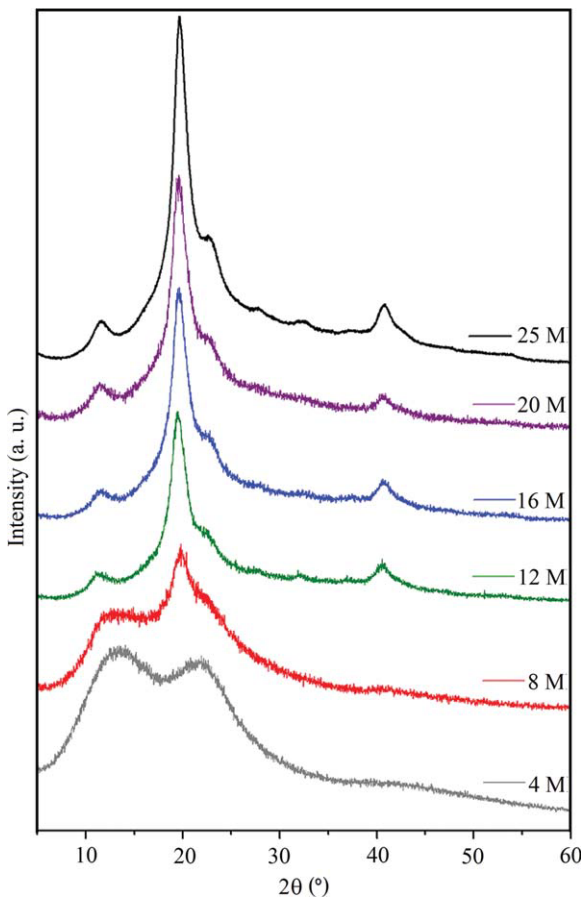

Figure 1 XRD patterns of the laboratory-made polymeric particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

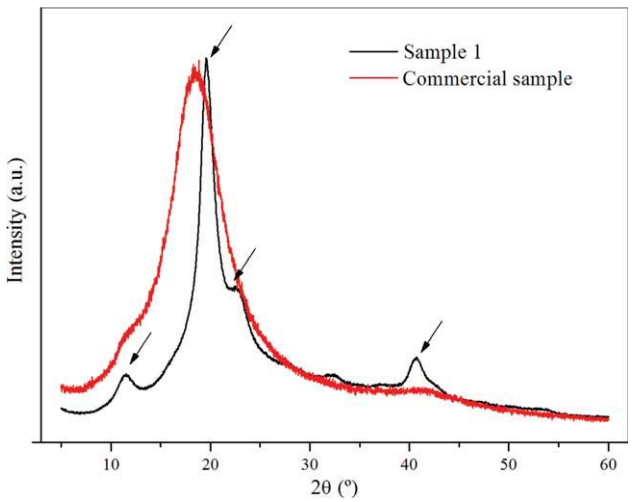

Figure 2 XRD patterns of the commercial and highly crystalline polymeric particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

most striking case occurred for the sample prepared with a 25*M* NaOH solution (sample 1) because its XRD pattern showed four distinct Bragg diffraction peaks at 2θ values of 11.5, 19.5, 23.0, and 40.5°. The breadth and peak shape of this sample in comparison with the other ones clearly indicated that sample 1 was more crystalline than the other samples prepared in this study and the other PVA/PVAc polymeric particles previously reported in the literature.[5,6,16]

Figure 2 shows a detailed XRD pattern of sample 1 compared with the XRD pattern of the commercial PVA sample available on the market (Boston Scientific). The well-defined and strong Bragg diffraction peaks of sample 1 compared to the broad Bragg diffraction peak of the commercial one indicated that sample 1 was more crystalline than its counterpart. Most of the XRD patterns of the PVA microspheres reported in the literature were similar to the XRD pattern of the commercial sample,[5,6,16] and to the best of our knowledge, this was the first XRD pattern of a highly crystalline PVA microsphere reported up to this point. PVA is a semicrystalline synthetic polymer whose structure is characterized by chains in transplanar conformation packed in a monoclinic unit cell with $a = 7.81$ Å, $b = 2.52$ Å (chain axis), $c = 5.51$ Å, and $\beta = 91.42°$ and containing single monomer units of two chains (a, b and c are the unit cell parameters and β is the angle of the monoclinic unit cell parameters). The strongest PVA crystalline reflections occurred at $2\theta = 19.4$ and 20.1°; these reflections correspond to the superposition of the equatorial $(10\bar{1})$ and (101) reflections, respectively.[22–24]

On the basis of the unit cell proposed by Bunn[22] and other more recent crystallographic studies on the structures of PVA hydrogels,[25,26] PVA microfibrils,[27,28] and PVA films,[29] it was possible to assign the diffraction peaks of sample 1 observed at 2θ values of 11.5, 19.5, 23.0, and 40.5° to the (100), $(10\bar{1})$, (200), and (201) reflections, respectively.

Figure 3 shows the degree of crystallinity of the PVA particles calculated from the DSC data with the following correlation:

$$\text{Crystallinity } (\%) = \frac{\Delta H}{\Delta H^0} \times 100$$

where ΔH is the change in the heat of fusion and ΔH^0 is the change in the heat of fusion of 100% crystalline PVA (this can be obtained directly from DSC plots and is usually considered to be 150 J/g).[27,30–32] The calculated degrees of crystallinity are listed in Table I. It was evident from the data that the degree of crystallinity of the samples increased with the concentration of the NaOH solution (see Fig. 3). Sample 1 presents the highest degree of crystallinity (87.5 ± 2.1%) compared with the other samples, and the commercial sample (PVA Foam Embolization Particles, Cook Medical (Bloomington, Indiana)) presented the lowest degree of crystallinity, which was calculated to be around 5.7 ± 0.3%.

Although the syntheses conditions described in Table I affected the degree of crystallinity of the PVA particles, in terms of morphology, we did not notice any significant difference. All of the samples prepared according to the conditions described in Table I presented a spherical morphology, unlike the commercial samples (Fig. 4), and the main differences among them, besides the degree of crystallinity, were related to their color and stickiness. Sample 1 (Fig. 5) was reddish, and the particles were free-flowing spheres with a very small amount of debris. Samples 2–5 were yellowish, sample 6 (Fig. 6) was white, and the particles were sticky spheres that easily could form aggregates.

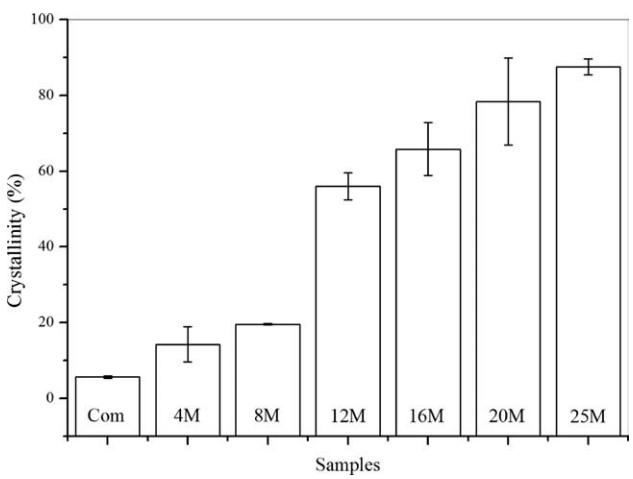

Figure 3 Comparison of the degree of crystallinity (obtained by DSC) for the commercial (Com) and laboratory-made polymeric particles.

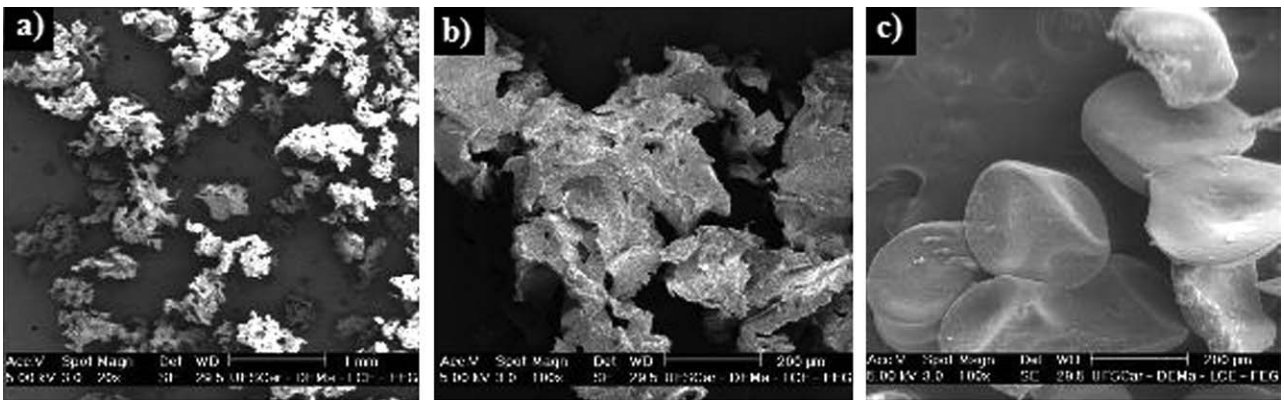

Figure 4 SEM images of the commercial PVA particles: (a,b) Cook Medical and (c) Boston Scientific.

To gain a better insight into the structural differences among the samples prepared in this study, solid-state CP–MAS NMR experiments were performed. CP–MAS ^{13}C-NMR is a powerful tool for studying the miscibility and phase structure of polymer blends and for detecting specific intermolecular interactions.[33–35] Figure 7 shows the CP–MAS ^{13}C-NMR spectra of the commercial sample (Boston Scientific) and samples 1 and 6. The spectra of samples 2–4 were very similar to the spectrum of sample 1, and the spectrum of sample 5 was very similar to the spectrum of sample 6. Therefore, only the spectra of samples 1 and 6 are shown.

The CP–MAS ^{13}C-NMR spectrum of sample 6 had some features that were very interesting: there were two sharp peaks to the far left and to the right with chemical shift values of 171 and 21 ppm, respectively, and these two peaks were assigned to PVAc, according to the literature.[34–36] The CP–MAS ^{13}C-NMR spectrum of PVAc in the solid state presented four distinct and well-characterized chemical shifts: 40, 67, 171, and 21 ppm, whereas that of PVA in the solid state showed three distinct chemical shift values of 77, 71, and 65 ppm.[34,37] Therefore, on the basis of these values reported in the literature, we concluded that the two broad peaks in the region 30–80 ppm were a peak overlap of the chemical shifts of PVAc and PVA and indicated a blend of the two polymers.

The CP–MAS ^{13}C-NMR spectrum of the highly crystalline sample 1, on the other hand, showed splits for the chemical shift peak in the regions 65 and 71 ppm. It was proposed in the literature that the chemical shifts at 77, 71, and 65 ppm observed for solid PVA originated from the intramolecular and intermolecular hydrogen bonding of the CH carbon to the hydroxyl groups.[36–38] The chemical shift at 77 ppm was assigned to the CH carbon with two intramolecular hydrogen bonds with the hydroxyl groups. The chemical shift at 71 ppm was assigned to the CH carbon with one intermolecular hydrogen bond with the hydroxyl group, whereas the chemical shift at 65 ppm was not assigned or related to any hydrogen bond with the hydroxyl groups in the crosslinked region. A theoretical model based in the literature for these assignments is shown in Figure 8.[37]

A comparison between the CP–MAS ^{13}C-NMR spectra of the commercial sample and the highly crystalline sample 1 showed that, qualitatively, the commercial sample was greatly enriched with

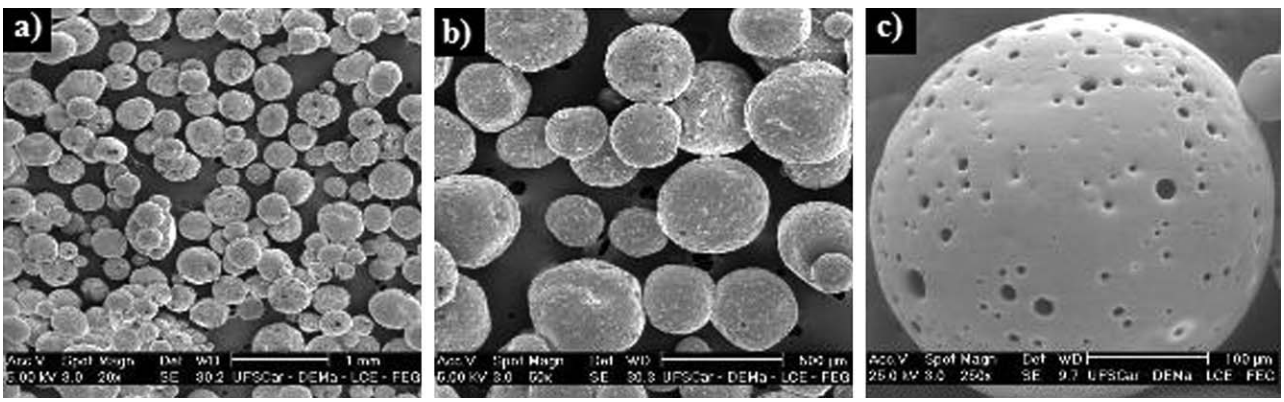

Figure 5 SEM images of the PVA/PVAc microspheres prepared by polymerization and saponification (sample 1).

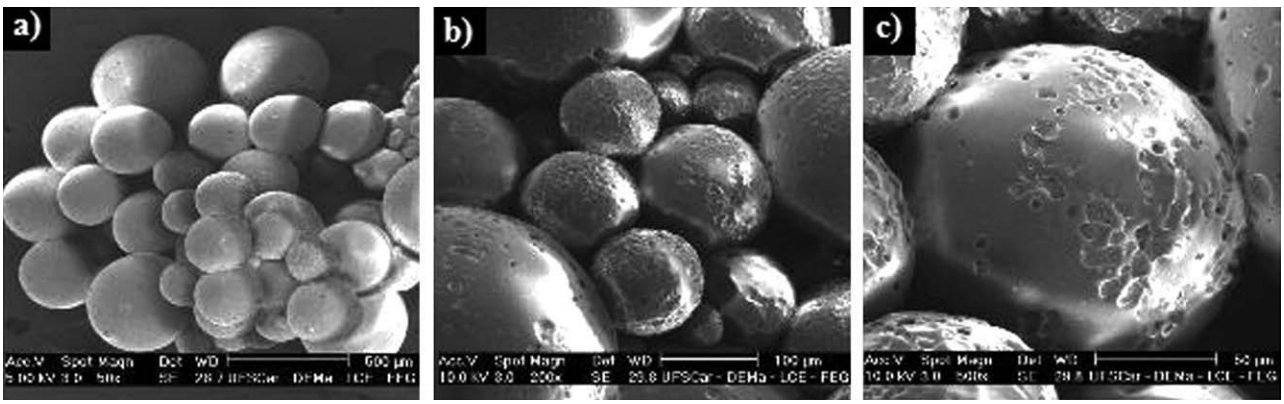

Figure 6 SEM images of the PVA/PVAc microspheres prepared by polymerization and saponification (sample 6).

intermolecular hydrogen bonds because it presented a very intense peak at 71 ppm. However, the chemical shift characteristic of intramolecular hydrogen bonds (77 ppm) was not present, and the chemical shift at 65 ppm was present only as a small shoulder. On the other hand, for sample 1, the situation was quite different because this sample presented two relatively intense chemical shift peaks (71 and 65 ppm) and a small peak at 77 ppm. The CP–MAS ^{13}C-NMR spectrum of the highly crystalline sample 1 was very similar to the spectrum of PVA in the solid state, with a predominant syndiotactic configuration, as reported in the literature.[37]

On the basis of both the CP–MAS ^{13}C-NMR and XRD data, one possible explanation or reason for the formation of highly crystalline PVA microspheres is the excess of strong base (25*M* NaOH) used in the saponification step of the reaction. The excess of strong base induced the formation of intermolecular hydrogen bonds, which were formed because of the chemical bonding of the CH carbon of the polymer interchains with the hydroxyl groups in the crosslinked region; this resulted in highly crystalline samples and also increased the intensity of the peak at 65 ppm, which is a chemical shift characteristic of a syndiotactic configuration (see Fig. 8).

At this time, chemoembolization studies with these new highly crystalline microspheres are being performed to examine the drug–polymer interactions and release rate of the loaded drugs *in vitro* and *in vivo* with the highly crystalline polymeric matrix. The results of this ongoing research will be reported in future publications.

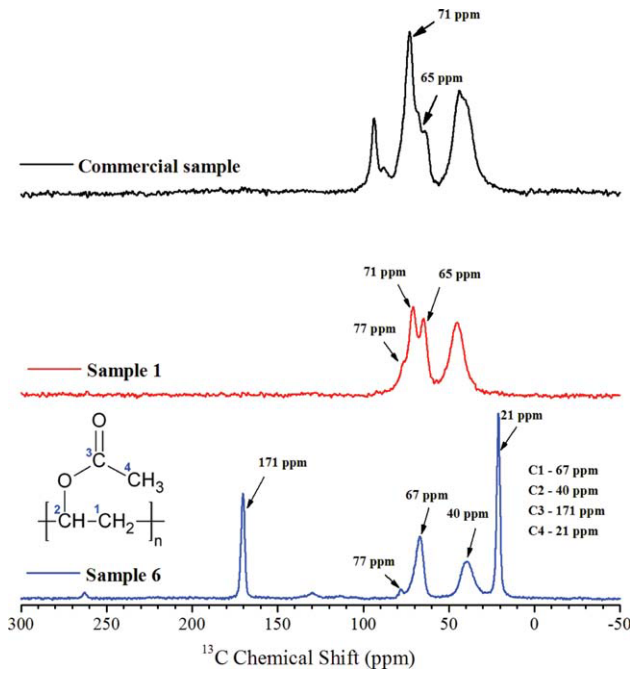

Figure 7 ^{13}C CP–MAS NMR spectra of the commercial sample, sample 1, and sample 6. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

CONCLUSIONS

Highly crystalline and spherical PVA particles were prepared through a controlled polymerization reaction followed by a saponification process. Several syntheses were made. However, the best results were obtained when NaOH solutions at high concentrations were used during the saponification process.

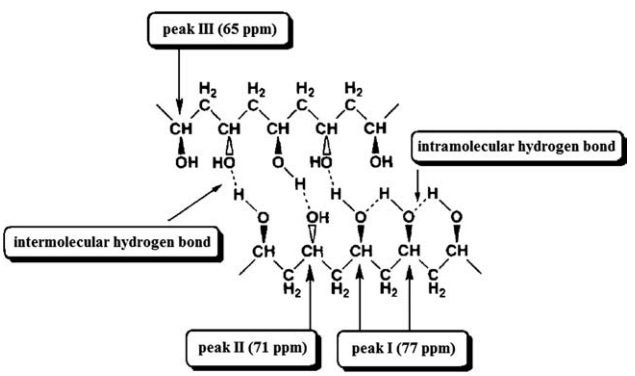

Figure 8 Theoretical model of the hydrogen bonds in PVA.

The crystallinity of the PVA microspheres obtained through this procedure was superior to commercially available ones. Correlations between the XRD and CP–MAS ^{13}C-NMR data indicated that the high crystallinity of the samples was due to the presence of the intermolecular hydrogen bonds, which were formed by the chemical bonding of the CH carbon of the polymer interchains with the hydroxyl groups in the crosslinked region.

References

1. Reineke, T. M.; Grinstaff, M. W. MRS Bull 2005, 30, 635.
2. Edlund, U.; Albertsson, A.-C. J Polym Sci Part A: Polym Chem 2000, 38, 786.
3. Edlund, U.; Albertsson, A.-C. Adv Polym Sci 2002, 157, 67.
4. Nitta, N.; Ohta, S.; Tanaka, T.; Takazakura, R.; Toyama, T.; Sonoda, A.; Seko, A.; Furukawa, A.; Takahashi, M.; Murata, K.; Kurumi, Y.; Tani, T.; Sakamoto, T.; Tabata, Y. Eur J Radiol 2009, 71, 519.
5. Pinto, J. C.; Lopez, G. B.; Niemeyer, M. A. L.; Silva, F. M.; Melo, P. A.; Nele, M. W.O. Pat. 050591A2 (2006).
6. Peixoto, L. S.; Silva, F. M.; Niemeyer, M. A. L.; Espinosa, G.; Melo, P. A.; Nele, M.; Pinto, J. C. Macromol Symp 2006, 243, 190.
7. Liu, X.; Heng, W. S.; Paul, A.; Li, Q.; Chan, L. W. J Controlled Release 2006, 116, 35.
8. Raoul, J. L.; Heresbach, D.; Bretagne, J. F.; Ferrer, D. B.; Duvauferrier, R.; Bourguet, P.; Messner, M.; Gosselin, M. Cancer 1992, 70, 585.
9. Roos, G.; Christensson, P. I.; El Hag, I. A.; Jakobsson, B.; Teder, H.; Stenram, U. Anticancer Res 1993, 13, 635.
10. Kyotani, S.; Nishioka, Y.; Okamura, M.; Tanaka, T.; Miyazaki, M.; Ohnishi, S.; Yamamoto, Y.; Ito, K.; Ogiso, T.; Tanada, S.; Terao, M. Chem Pharm Bull 1992, 40, 2814.
11. Teder, H.; Johansson, C. J. Anticancer Res 1993, 13, 2161.
12. Bechtel, W.; Wright, K. C.; Wallace, S.; Mosier, B.; Mosier, D.; Mir, S.; Kudo, S. Radiology 1986, 161, 601.
13. Burton, M. A.; Chen, Y.; Atkinson, H.; Codde, J. P.; Jones, S. K.; Gray, B. N. Int J Hyperther 1992, 8, 485.
14. Chiannilkulchai, N.; Ammoury, N.; Caillou, B.; Devissaguet, J. P.; Couvreur, P. Cancer Chemother Pharm 1990, 26, 122.
15. Laurent, A. Tech Vasc Intervent Radiol 2007, 10, 248.
16. Lee, S. G.; Kim, J. P.; Lyoo, W. S.; Kwak, J. W.; Noh, S. K.; Park, C. S.; Kim, J. H. J Appl Polym Sci 2005, 95, 1539.
17. Lyoo, W. S.; Lee, H. W. Colloid Polym Sci 2002, 280, 835.
18. Lee, S. G.; Lyoo, W. S. J Appl Polym Sci 2008, 107, 1701.
19. Peersen, O. B.; Wu, X. L.; Kustanovich, I.; Smith, S. O. J Magn Reson Ser A 1993, 104, 334.
20. Cook, R. L.; Langford, C. H.; Yamdagni, R.; Preston, C. M. Anal Chem 1996, 68, 3979.
21. Dria, K. J.; Sachleben, J. R.; Hatcher, P. G. J Environ Qual 2002, 31, 393.
22. Bunn, C. W. Nature 1948, 161, 929.
23. Sakurada, I.; Fuchino, K.; Okada, N. Bull Inst Chem Res Kyoto Univ 1950, 23, 78.
24. Bunn, C. W.; Peiser, H. S. Nature 1947, 159, 161.
25. Cho, J. D.; Lyoo, W. S.; Chvalun, S. N.; Blackwell, J. Macromolecules 1999, 32, 6236.
26. Lyoo, W. S.; Chvalun, S. N.; Ghim, D. H.; Kim, J. P.; Blackwell, J. Macromolecules 2001, 34, 2615.
27. Ricciardi, R.; Auriemma, F.; Gaillet, C.; De Rosa, C.; Lauprêtre, F. Macromolecules 2004, 37, 9510.
28. Ricciardi, R.; Auriemma, F.; De Rosa, C.; Lauprêtre, F. Macromolecules 2004, 37, 1921.
29. Strawhecker, K. E.; Manias, E. Macromolecules 2001, 34, 8475.
30. Hassan, C. M.; Peppas, N. A. Macromolecules 2000, 33, 2472.
31. Zhang, W.; Zhang, Z.; Wang, X. J Colloid Interface Sci 2009, 333, 346.
32. Mandal, S.; Pangarkar, V. G. J Appl Polym Sci 2002, 86, 2194.
33. Wu, C.; McGinity, J. W. Eur J Pharm Biopharm 2003, 56, 95.
34. Lubach, J. W.; Padden, B. E.; Winslow, S. L.; Salsbury, J. S.; Masters, D. B.; Topp, E. M.; Munson, E. J. Anal Bioanal Chem 2004, 378, 1504.
35. Cheung, M. K.; Wang, J.; Zheng, S.; Mi, Y. Polymer 2000, 41, 1469.
36. Terao, T.; Maeda, S.; Saika, A. Macromolecules 1983, 16, 1535.
37. Kobayashi, M.; Ando, I.; Ishii, T.; Amiya, S. J Mol Struct 1998, 440, 155.
38. Kobayashi, M.; Ando, I.; Ishii, T.; Amiya, S. Macromolecules 1995, 28, 6677.