The Preparation and Forming Mechanism of the Red Blood Cell-Shaped Microspheres via Electrospraying

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ABSTRACT: This article successfully prepared the red blood cell-shaped microspheres via electrospraying. The polymer solution was comprised of polyether sulfone (PES) and dimethyl sulfoxide (DMSO), and the hydrophilic polyethylene glycol (PEG) was added to regulate and control the structure of the microspheres. When the solution concentration was 7.6 wt % and PES/PEG proportion was 10 : 5 while preparing microspheres, the shape and diameter of the microspheres was the most similar to that of erythrocyte. The results suggested that the forming mechanism of the red blood cell-shaped microspheres be related to two aspects. One is that the microspheres had relatively weak mechanical strength because of the sponge pores internal nanostructure. The other is that the existence of residual solvents and water within the microspheres would dissolve the gel phase PES and then further weaken mechanical strength of the outer layer of the microspheres, which caused the structural changes on the top layer of the spheres, that is, the top layer collapsed, and the microspheres finally turned into the red blood cell-shaped microspheres. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 2552–2556, 2011

Key words: polyether sulfone; microspheres; hydrophilic polymer; nanostructure; electrospraying

INTRODUCTION

Polymer microspheres with diameter in the range from several micrometers to hundreds of nanometers possess many special functions and are widely applied in many important areas, such as in microstorage, microreactor, microseparator, microstructural unit,[1–5] etc.

The morphology of polymer microspheres is varied, including solid, hollow, porous, onions-shaped, dumbbell-shaped, red blood cell-shaped,[6,7] etc. Due to the flat shape of the red blood cell-shaped microspheres, they can possess a lower viscosity than the sphere-shaped microspheres under the high-speed shear force and contribute to better processability. So the red blood cell-shaped microspheres show better performance in paper making.[8] In addition, the red blood cell-shaped microspheres have great potential in bio-medical field because of the similarity shape to erythrocyte.[9,10] Therefore, the study of the preparation of the red blood cell-shaped microspheres has become a hotspot. It has been known from the existing literature that the red blood cell-shaped microspheres can be produced by two methods. One is the two-step seeding polymerization method introduced by Hoshino et al.,[11] etc; the other is the method that electrosprayed polyether sulfone as raw material invented by our research group.[12,13]

Electrospraying is a method based on electrohydrodynamic technology to prepare microspheres with polymer as raw materials.[14] Compared with other methods for preparing polymer microspheres, this method has several advantages: relative ease of setup, open atmosphere operation without a sophisticated chamber and well-dispersed microspheres due to self-repellence resulting from the electric charges on the microspheres.[15,16] Consequently, using the method to prepare the red-blood cell-shaped microspheres has a vast prospect.[17] At present, there is rarely report about the forming mechanism of electrosprayed red blood cell-shaped microspheres. However, the forming mechanism is the premises of accurately controlling microspheres structure. We successfully prepared red blood cell-shaped microspheres and proposed those forming mechanism, which allow it possible to accurately control the structure and size of microspheres.

EXPERIMENTAL

Materials

Polyether sulfone (PES) pellets (GAFONE 3400) with an intrinsic viscosity of 0.34 dL/g in dimethyl

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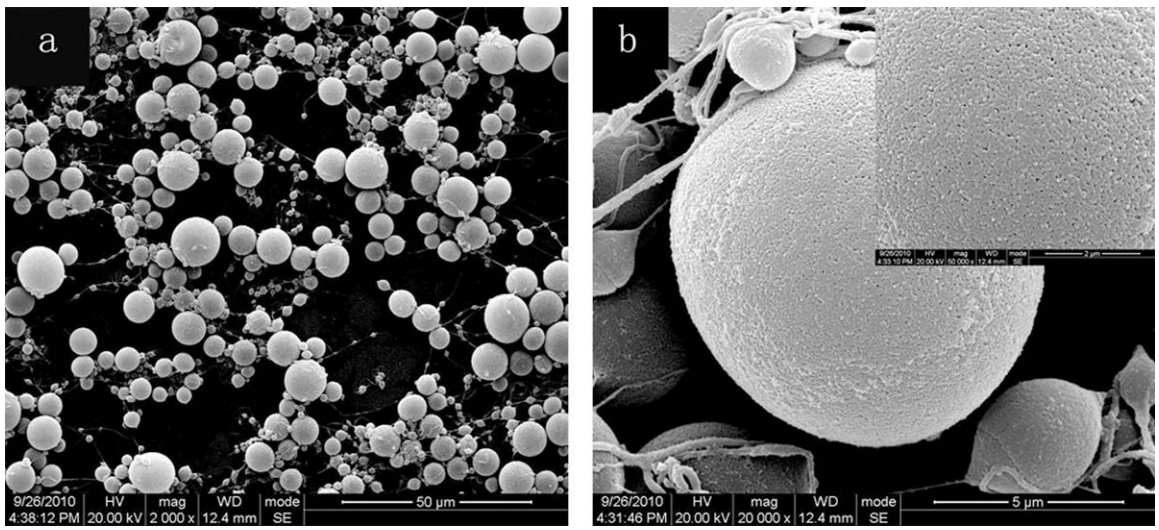

Figure 1 The Low (a)- and high (b)-magnification SEM images of polymer microsphere prepared by 12 wt % concentration solutions of PES and DMSO.

sulfoxide (DMSO) at 303.2 K, obtained from Gharda Co., India, polyethylene glycol (PEG, Mw = 10,000 g/mol) imported from Japan, and DMSO (analytical grade, 99+%, acquired from Kelong Chemical Co., China) were used as raw materials without further purification.

Preparation of microspheres

The polymer (PES) was dissolved in DMSO at 70°C for 4 h until it became a homogeneous solution. While preparing microspheres, the solution was placed in a 50-mL syringe, to which a capillary tip of 0.5 mm inner diameter was attached. The positive electrode of high-voltage power supply was connected to the capillary tip. Direct current high voltage-generator (ZGF Chuan Gao electro-tech Inc, China) was used to provide voltage of 10 kV. The grounded electrode was connected to a metallic collector immersed in a water bath. The distance between the tip and the collector was maintained at 6 cm and the flow rate was constant 1 mL/h. The spaying is stream broken up after moving a certain distance, and then microspheres are collected in the water bath.

Characterization

The morphology of microspheres was assessed using a scanning electron microscope (SEM and EDS Inspector F, FEI Company, all operation at an accelerating voltage of 20 kV). The samples were coated by an E-1045 ion sputter coater with Au/Pd to reduce charging. To observe the internal structure expediently, we cut the microspheres.[13] The microspheres were dispersed in distilled water by ultrasonic vibration (power: 500 W, frequency: 40 KHz), and then were frozen into hard ice with liquid nitrogen, the hard ice was broken into powder rapidly; the powder was naturally unfrozen and dried, the cross sections of the microspheres with morphologies maintained well were obtained.

RESULTS AND DISCUSSION

Preparation of circle microsphere via electrospraying

As shown in Figure 1(a), the particle size distribution of PES microspheres is relatively narrow and its roundness is relatively well. Moreover, the surface of microspheres is porous [Fig. 1(b)]. The average grain diameter of microspheres (9 μm) is close to that of erythrocyte (6–8 μm) in the organism; however, they haven't the flat structure as possessed by erythrocyte. So, to make the red blood cell-shaped microspheres and better simulate the structure and performance of erythrocyte, more work has been carried out.

Preparation of red blood cell-shaped microsphere

Inspired by others work that reported, the hydrophilic polymer PEG can be used to regulate and control the PES membrane structure.[18–20] When PEG which has a strong affinity was added to coagulation bath, the exchange rate of solvent/nonsolvent would increase. At the same time, when the hydrophilic polymer additive PEG was added to the solution system and precipitation emerged in the water bath, the PEG will voluntarily transfer to the interface of the membrane and the coagulation bath, thus can change the structure of microspheres.[12] Consequently, we add PEG into the PES/DMSO system to

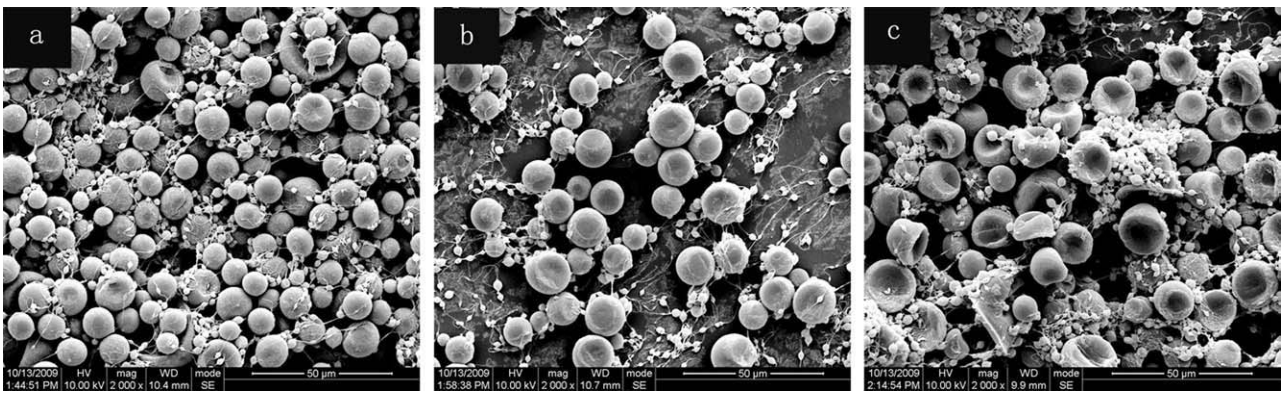

Figure 2 SEM images of polymer microsphere prepared by 12 wt % concentration solution of PES and DMSO but with different PES/PEG proportions, in which, a is 10 : 1, b is 10 : 3, c is 10 : 5.

adjust the speed of liquid–liquid phase separation, and regulate the microspheres structure, with the expecting that the flat top structure on the microspheres (i.e., the red blood cell shaped) is achievable.

Fixed PES but changing the contents of PEG

With the addition of PEG to the system which was used to perpetrate microspheres, some changes occurred in the structure of microspheres via electrospraying: their structure changed from perfect roundness to appreciably flats, [shown in Fig. 2(a)]. As gradual increase of PEG content (from 10 : 1 to 10 : 5), the flat structure of microspheres became more and more obvious and finally similar to that of erythrocyte [in Fig. 2(b,c)]. This shows that the red blood-shaped microspheres can be prepared by adding PEG. The diameter of microspheres shown in Figure 2(c) is obviously larger than 10 μm of circle microspheres [as shown in Fig. 1(a)]. Therefore, the red blood-shaped microspheres' diameter is larger than erythrocyte's (6–8 μm). The diameter of microspheres would greatly influence their application in the biological field so that we would ensure the diameter of PES microspheres is much closer to that of erythrocyte. To achieve this goal, the PES-PEG/DMSO solution concentration was gradually reduced by referring other's work.[21,22]

Fixed PEG but changing the contents of PES

PES microspheres were prepared via electrospraying by maintaining the same proportion of PES and PEG but reducing the solution concentration step by step. The influence solution concentration morphology of microspheres was shown in Figure 3. With the decrease of solution concentration, the diameter obviously reduced. However, all the microspheres prepared still showed the red blood cell-shape. When the solution concentration was 7.6 wt %, the average diameter of microspheres was 10 μm (shown in Fig. 4), which was the closest to the size of erythrocyte (6–8 μm). The test result indicated that with mass fraction of 7.6 and proportion of 10 : 5, the microspheres, whose diameter and structure agreed well with that of erythrocyte, can be successfully prepared.

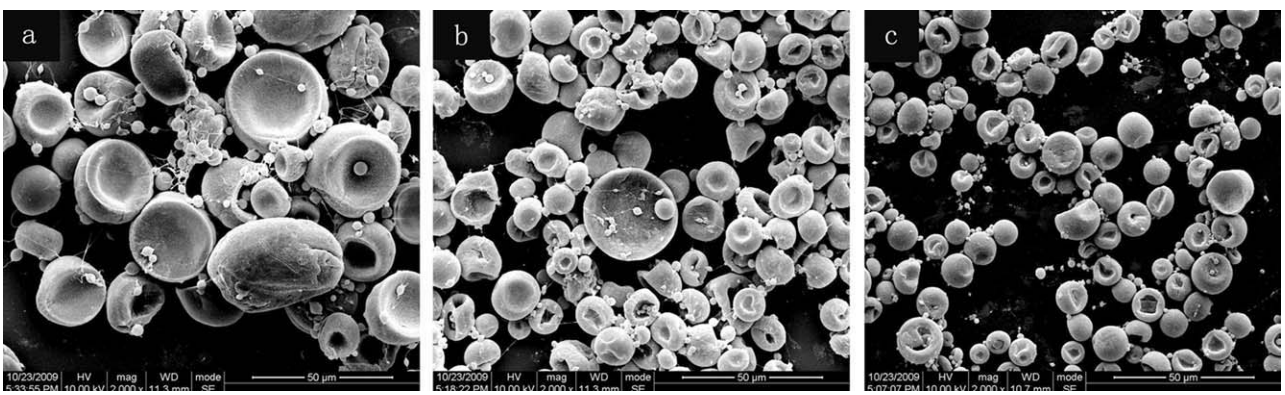

Figure 3 SEM images of polymer microsphere prepared by different concentration solution of PES and DMSO but with the same PES/PEG proportions 10 : 5, in which, a is 10.6 wt %, b is 9.1 wt %, c is 7.6 wt %.

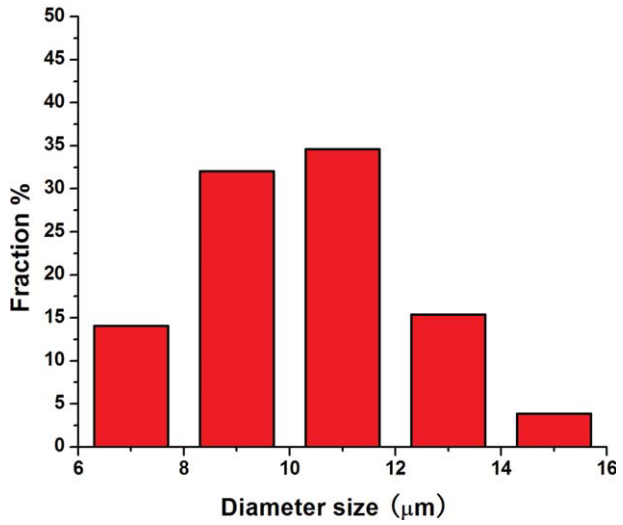

Figure 4 The diameter size distribution of PES microspheres. The concentration of PES/PEG solution is 7.6 wt % and the PES/PEG proportions 10 : 5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The forming mechanism of red blood cell-shaped microsphere

To better understand and control the forming process of red blood cell-shaped microspheres, the study of its forming mechanism was especially required. Taking the experimental condition and microspheres structure into consideration, there were two factors most likely result in the flat shape of microspheres. One was the diffusion of residual solvent within the microspheres; the other was the internal structure changes of microspheres.

The formation process of microspheres can be divided into two steps. The first step was electrospraying which was accompanied with rapid evaporation of solvent; while the second step was the figuration of microspheres with the quickly exchange between solvent and no-solvent. According to the forming mechanism of microspheres,[12] the microspheres interior should be porous structure. To easily observe the internal structure, the microspheres were cut. Figure 5(a) shows the images of the cross section of PES circle microspheres, the macrovoids throughout the cross section of the microsphere which display a uniform cellular structure, can be seen. By adding hydrophilic PEG, the red blooded cell-shaped microspheres with the sponge pores internal nanostructure were prepared, and, there is an elliptical cavity formed in the innermost [as shown in Fig. 5(b)]. The cupped side of the red blood cell-shaped microspheres is a relatively thinner loose layer, while the other side is a thicker loose layer. As far as the mechanical strength was concerned, the red blood cell-shaped microspheres with sponge pores and hollow structure were weaker than the microspheres with cellular structure.

There were residual solvent and water at the inner microspheres preliminary formed. The unshaped PES inside the microspheres was in the dynamic equilibrium between dissolving and precipitating out, with the coeffect of DMSO and water. The post-treatment of the samples was accompanied with the evaporation of DMSO and water from the inner microspheres. While the evaporation of residual solvent was slow at room temperature due to its high boiling point (189°C). The evaporation of residual solvents would dissolve PES which was gel phase inside the microspheres. What is more, the smaller the porous structure size was, the bigger the contact area between solvent and PES would be. Thus, the better the solubility property for PES can be achieved. As for the microspheres prepared from

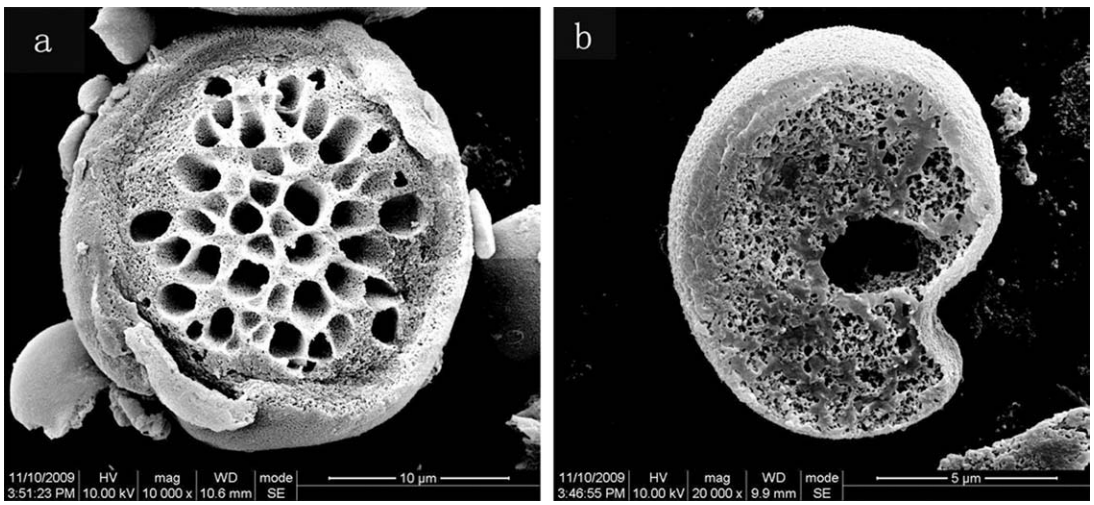

Figure 5 SEM images of the cross section of the porous microsphere prepared by different concentration solution of PES and DMSO, in which, a is 12% but without PEG, while b is 10.6 wt % (PES/PEG, 10 : 5, w/w).

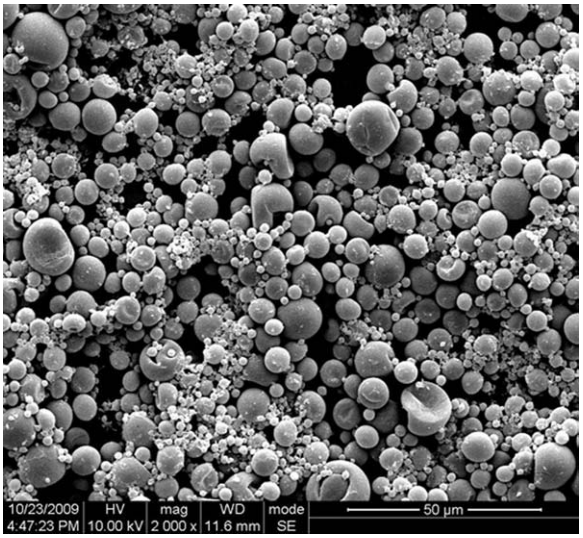

Figure 6 SEM images of polymer microsphere prepared by 7.6 wt % concentration solution of PES and DMSO (PES/PEG, 10 : 5, w/w), in which, the microsphere was boiled.

PES/DMSO system, the quantity of PES dissolved by DMSO is limited. On the other hand, the mechanical strength of the cellular structure microspheres was higher. Therefore, the microspheres would not be collapsed but remain good roundness. However, for the microspheres containing hydrophilic polymer PEG, the existence of PEG made DMSO dissolve PES more[23] and promoted, PEG which originally tangled with PES, to be dissolved into DMSO at the same time. Therefore, the volume of microspheres dissolved by the residual solvent and water was larger. Meanwhile, the sponge pores internal nanostructure had a weak mechanical strength, so the dissolution of PES and PEG would further reduce the mechanical strength on the top of microspheres. The squeezing action caused collapse in the top of microspheres, and then the structure changed; the microspheres finally had red blood cell shape.

To prove the forming mechanism, the microspheres which were prepared from7.6 wt % concentration solution of PES and DMSO (PES/PEGwas10/5, w/w) were boiled. As shown in Figure 6, the microspheres, which had been boiled, almost haven't collapsed structure and have better roundness. First, because of boiling, the temperature would rise. The residual solvent will accelerate the speed of diffusion, which broke the dynamic equilibrium between dissolving and precipitating out of PES, and PES would be quickly precipitated out. Second, while boiling, the gel microspheres finally molded in water, and the boiling water would provide anchorage force which enhanced the microspheres the mechanical strength at the inner microspheres to prevent the microspheres from being collapsed. Once the structure of microspheres was identified, their mechanical strength was strong and the microspheres would not collapse anymore, so the microspheres roundness was perfect. Therefore, through the boiling treatment, we can demonstrate that the formation of red blood cell-shape microspheres was caused by the combined action between the poor mechanical strength of the internal nanostructure and the residual solvent at the inner microspheres.

CONCLUSIONS

The red blood cell-shaped microspheres were successfully prepared via electrospraying through adding hydrophilic polymer PEG to the polymer solution. When the system was that the concentration solution was 7.6 wt % and the ratio between PES and PEG was10/5, the size and structure of microspheres was the closest to erythrocytes. The formation of red blood cell-shaped microspheres was caused by the combined action between the sponge pores internal nanostructure and the residual solvent and water in the microspheres.

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