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Preparation of Micron-sized Crosslinked Poly (Vinyl Alcohol) Microspheres via Inverse Suspension-chemical Crosslinking Method

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Abstract: Poly (vinyl alcohol) microspheres used for biomedical filed have attracted much attention for they contained lots of hydroxyl groups and can be modified with multipurpose. While the reaction steps of traditional preparation methods were tedious and uncontrollable, so the new method called inverse suspension-chemical crosslinking method was adopt to prepare poly (vinyl alcohol) microspheres by using Polyvinyl Alcohol (PVA) as monomer, Glutaraldehyde (GA) as crosslinker and Span-60 as dispersant. Effects of various factors such as amount of dispersant, stirring rate, volume ratio of oil to water phase, amount of crosslinker and hydrochloric acid, on the sphericity and size of microspheres were examined. The experimental results showed that the crosslinked microspheres with smaller size and more uniform size distribution, excellent sphericity and controllable diameter can be successfully prepared. As the amount of dispersant is less than 0.16 g, the stirring rate is lower than 250 r min^{-1}, the ratio of oil to water is less than 1:1 and amount of crosslinker is more than 2 mL, the microspheres can not be formed in the system. Via controlling the reaction conditions, the crosslinked microspheres with excellent sphericity and controllable particle diameter in the range of 100~200 μm can be gained.

Key words: Polyvinyl alcohol, glutaraldehyde, chemical crosslinking, crosslinked microspheres

INTRODUCTION

Microsphere materials especially polymer microsphere materials have been widely used in many fields of science and technology (Rasuli *et al*., 2008; Oster *et al*., 2001; Liu *et al*., 2006; Bicak *et al*., 2001; Yang *et al*., 2005), because they have many advantages such as larger specific surface area but not easy to reunite, good mechanical properties, solvent resistance and reuse performance compared with microspheres with nanometer and larger particles such as ion exchange resin microspheres (Bayramoglu *et al*., 2007; Yavuz *et al*., 2006; Bayramoglu and Arica, 2005). Functional polymer microspheres with excellent biocompatibility used for the immobilization of biological macromolecules and enzymes (Xu *et al*., 2006; Perez *et al*., 2006), drug controlled release (Krizova *et al*., 2005), the separation of biological macromolecules and DNA (Rittich *et al*., 2006) gradually become research hotspot.

Especially, the polyvinyl alcohol microspheres used for biomedical filed have aroused great concern for it contained lots of hydroxyl groups and can be modified chemically with multipurpose. Compared with the traditional preparation methods such as gelation method (Arica *et al*., 2004; Chu and Hashim, 2007), macromolecular crosslinking method and crosslinked copolymerization-alcohol solution method, for inverse suspension-chemical crosslinking method, the reaction step was simple, the reaction condition was easy to control and can prepare better sphericity and more uniform size distribution of polyvinyl alcohol microspheres.

In this study, the polyvinyl alcohol crosslinked microspheres CPVA were prepared via the Polyvinyl Alcohol (PVA) and Glutaraldehyde (GA) chemically crosslinked in the inverse suspension system and polycondensation. The influence and principle of various factors on the particle size and performance of microspheres were mainly examined, providing valuable reference for preparing polyvinyl alcohol crosslinked microsphere with good sphericity and controllable particle size. The research results have certain reference value for the fields such as biomedicine, biochemical engineer, biochemical analysis and related research of enzyme catalysis.

EXPERIMENTAL

Materials and Instruments: Polyvinyl alcohol (PVA, polymerization degree of 2200, reagent grade) was purchased from Shanxi Three-dimensional Chemical Factory. Glutaraldehyde (50%, analytical pure) was

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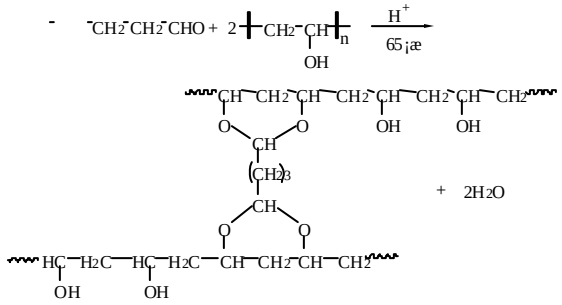

Fig. 1: Preparation reaction of CPVA microspheres

purchased from Tianjin Baishi Chemical Company. Liquid paraffin (chemical pure) was purchased from Tianjin BASF Chemical Company. Sorbitan monostearate (span60, chemical pur) was purchased from Dongli District of Tianjin chemical Reagent Factory.

The instruments used in this study were as follows: 438VP scanning electron microscope (SEM, LEO Company of UK). XSZ-4 Binocular biological microscope with micrometer (Taiyuan Optical Instrument Factory). 1700 Fourier transform infrared spectroscopy (FTIR, Perkin-Elmer Company of United States).

Preparation of CPVA microspheres: 0.16 g dispersant span-60 was dissolved in 20 mL liquid paraffin as continuous oil phase under stirring, added in the 4-neck flask equipped with stirrer and condenser. 8.0 mL 5% PVA solution and 1.0 mL 50% glutaraldehyde solution were immiscibled as aqueous phase, added 1 mL hydrochloric acid of 1.0 mol L^{-1} as catalyst to the aqueous phase. And then the aqueous phase was added to oil phase and stirred sufficiently to reverse two-phase dispersion. The temperature of system was raised to 65°C and started polymerization reaction for 7 h, thus obtaining translucent crosslinked microspheres. Microspheres were separated by filtration, washed with hot distilled water and then were washed several times with distilled water at room temperature and diethyl ether and dried in vacuum drying oven to constant weight. The preparation reaction of CPVA microspheres was shown in Fig. 1.

Characterization of CPVA microspheres: The chemical structure of CPVA microspheres was characterized using infrared absorption spectra. The morphology, particle size and size distribution were observed using Scanning Electron Microscope (SEM). The average diameter of microspheres D was calculated as the following formula using microscope with an optical micrometer:

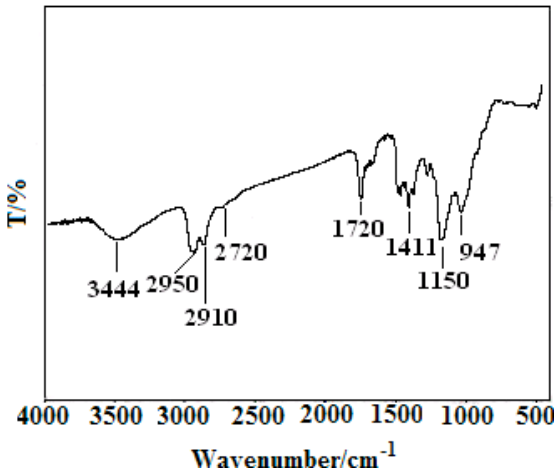

Fig. 2: FTIR spectra of CPVA mircospheres

$$D = \frac{\sum_{i=1}^{n} D_i}{n}$$

RESULTS AND DISCUSSION

FTIR spectra of the CPVA microspheres: The FTIR spectra of CPVA microspheres was shown in Fig. 2. As can be seen from the Fig. 2, the absorption peak of unit chain of PVA and ether bond appeared, the methyl bending vibration absorption appeared at 947 cm^{-1}, the ether bond absorption peak appeared at 1150 cm^{-1}. The methylene-CH_2-of main chain bending vibration absorption peak appeared at 1411 cm^{-1} and the absorption peak of-C = O at the end of main chain appeared at 1720 cm^{-1}. The hydroxyl association absorption peak appeared at 2720 cm^{-1}. The absorption peak of side chain methyl and main chain methylene were at 2910 and 2950 cm^{-1}, respectively. The characteristic absorption peak of free hydroxyl groups was at 3444 cm^{-1}. All absorption peaks indicated successful preparation of polymeric microspheres CPVA reacted from PVA and glutaraldehyde.

Morphology of CPVA microspheres: The SEM photograph of CPVA microspheres was shown in Fig.3. As can be seen from the figure, the microspheres showed good sphericity and uniform particle size. Microspheres with different particle size can be obtained by adjusting the reaction conditions and the particle size in the study was about 150 μm.

Effects of various factors on particle size and properties of CPVA microspheres

Effect of dispersant amount: Select oil-soluble span-60 as a dispersant for the reaction was water-in-oil (O/W)

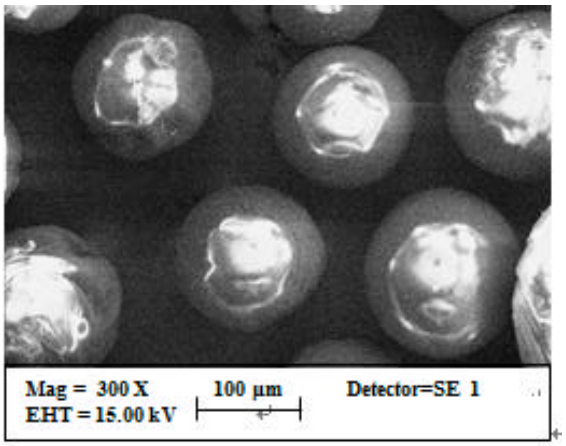

Fig. 3: SEM image of CPVA microspheres

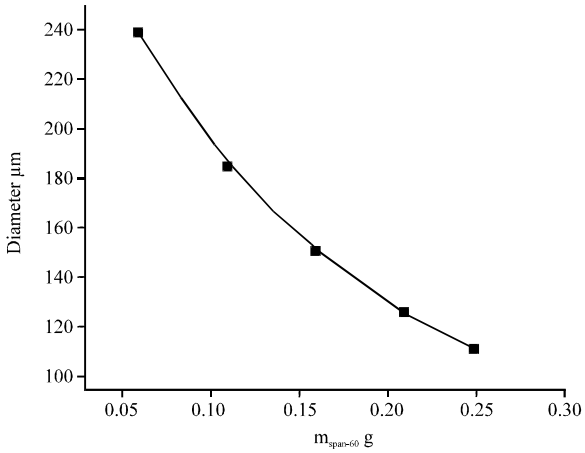

Fig. 4: Effect of dispersant amount on microsphere diameter

suspension system. The effect of dispersant amount span-60 on the particle size and properties of CPVA microspheres was investigated. It was found that when the dispersant amount used was less than 0.06 g, the microspheres can not be obtained. Dispersant span-60 can effectively reduce the oil-water interfacial tension, making the water phase layer dispersed into droplets under stirring. Span-60 molecules in the surface of water phase droplets can form steric hindrance to prevent water droplets collide with each other and occur coalescence, playing a protective role in the dispersed phase and the microspheres were successfully generated.

It was shown the average particle size of microspheres changed curve with the dispersant amount in Fig. 4. As can be seen from the figure, the particle size of microspheres was gradually decreased with the

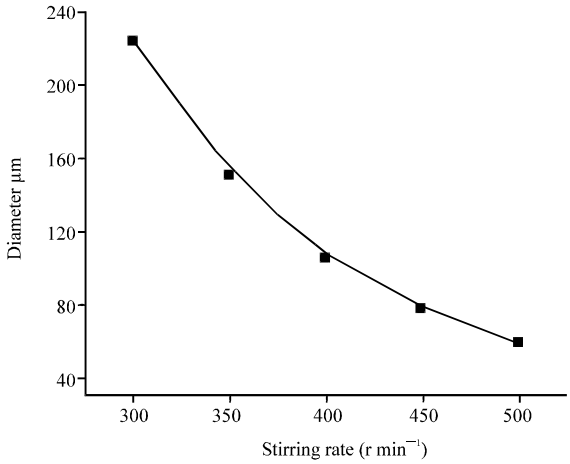

Fig. 5: Effect of stirring rate on microsphere diameter

increase of dispersant span-60 amount. This is because the oil / water interfacial tension reduced with the increase of span-60 amount, favor to dispersion of the aqueous phase droplets. More importantly, the span-60 protective film around the water droplets was thicken, decreasing the coalescence effect of dispersed phase droplets, making the microsphere size increased with the reduced amount of dispersant. As also can be seen, the size distribution of microspheres becomes more uniform. According to the needs of latter study, when the added dispersant amount was 0.16 g, the diameter of prepared microspheres was about 150 µm.

Effect of stirring speed: The effect of stirring speed on the particle size and properties of CPVA microspheres was investigated. It was found that when the stirring rate was lower (<250 rpm), the microspheres can not be obtained, the product was irregular block. Further accelerated the stirring speed, the microspheres can be obtained. It was shown the average particle size of microspheres changed curve with stirring speed in Fig. 5.

It can be seen, as the stirring rate increased, the size of microspheres decreased. The aim was to produce shear to overcome the interfacial tension, making the liquid layer dispersed into droplets. When the stirring rate is lower, the shear is smaller and not enough to overcome the interfacial tension and the liquid layer can not be dispersed into the droplets, so the microsphere can not be obtained. When the stirring rate increased, the shearing action of aqueous phase was larger and dispersed into small droplets, leading to the formation of microspheres. Besides, if the stirring rate was higher, the more fine droplet dispersed, the average particle size of microspheres was smaller. When the stirring rate was too high (>750 rpm), although the size of microspheres was

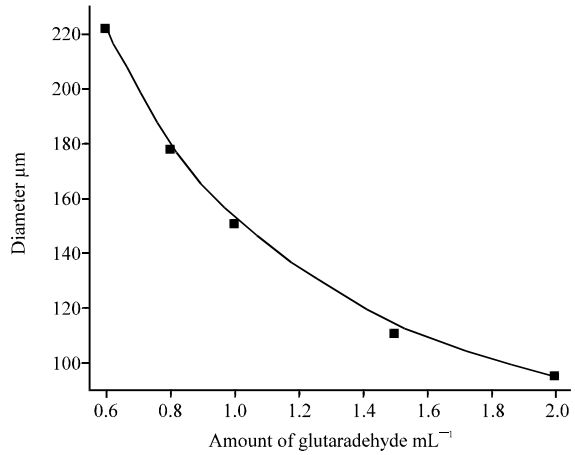

Fig. 6: Effect of crosslinker amount on microsphere diameter

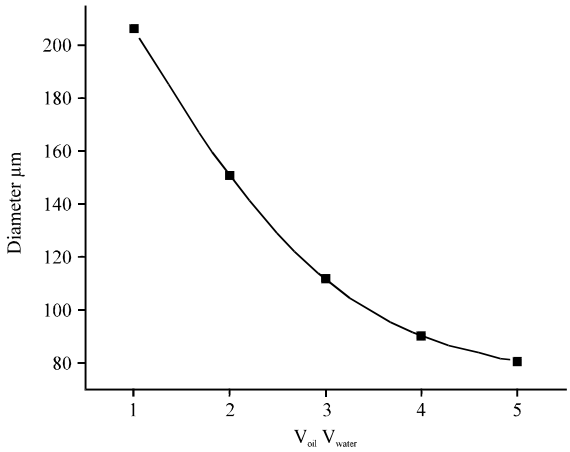

Fig.7: Effect of two phases ratio on microsphere diameter

small but prone to adhesion. According to the data, select 350 rpm as stirring rate, the particle size of about 150 μm was obtained.

Effect of crosslinker amount: The effect of crosslinker amount on the particle size and properties of CPVA microspheres was investigated. It was found the microspheres can not be obtained when the amount of crosslinker was greater than 2 mL. It was shown the average particle size changed curve with crosslinker amount in Fig. 6.

It can be seen from the figure, the average particle size of microspheres gradually decreased with the crosslinker amount increased. When a small amount of glutaraldehyde molecules added to the system, two aldehyde groups were fought by a large number of hydroxyl groups, PVA was intermolecular crosslinked firstly and with the increase amount of glutaraldehyde, only one aldehyde group may participate in aldehyde acetal reaction. The hydroxyl groups of PVA molecule were more adjacent to react with an aldehyde, occurring intramolecular crosslinking, making further contraction of PVA long chain and leading to smaller size. According to Fig. 6, the particle diameter of 150 μm was obtained when selecting the crosslinker amount as 1 mL.

Effect of volume ratio of oil to water phase: The effect of volume ratio of oil to water phase on the particle size and properties of CPVA microspheres was investigated. It was shown the average particle size of microspheres changed curve with volume ratio of oil to water phase in Fig. 7.

It can be seen from the figure, the microspheres can not be obtained when Voil/Vwater<1:1. This is because when the ratio of oil to water phase was relatively small, the number of water droplets in the dispersed phase distributed in continuous phase was larger and frequent collision occurred between each other, leading to coalescence of water droplets, so the microspheres can not be obtained. Further increased the proportion of oil and water phases, the microspheres can be obtained. As can be seen from Fig. 7, the average particle diameter of microspheres decreased gradually with the ratio of oil to water phase increased. It is because with the ratio decreased, the continuous oil phase increased and the dispersed phase droplets distributed continuous phase decreased and the chance of collision reduced, reducing the chance of droplet coalescence, so the average particle diameter of microspheres decreased. According to Fig. 7, the particle diameter of 150 μm was obtained when selecting the ratio as 2:1.

Effect of hydrochloric acid amount: The effect of hydrochloric acid amount on the particle size and properties of microspheres CPVA was investigated. It was shown the average particle size of microspheres changed curve with hydrochloric acid amount in Fig. 8.

It can be found that microspheres can not be obtained when the hydrochloric acid amount was less than 0.6 mL. As can be seen from Fig.8, the average diameter increased with the hydrochloric acid amount increased. This is because the aldehyde acetal reaction occurred, carbonyl group of glutaraldehyde was highly polar group and the carbon showed strong electropositive, while molecular oxygen on the hydroxyl of PVA contained lone pair electrons and had strong nucleophilicity, thus oxygen attacked carbonyl carbon to form hemiacetal using lone pair electrons. The reaction required an acid as catalyst. So when the hydrochloric acid amount increased, more carbonyl group of

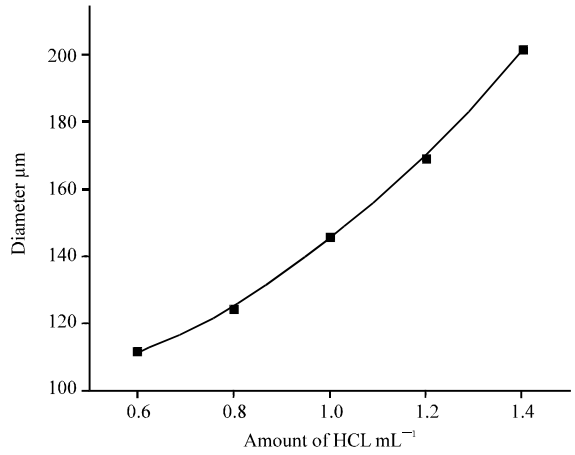

Fig. 8: Effect of hydrochloric acid amount on microsphere diameter

glutaraldehyde formed hemiacetal, thereby increasing the reaction active sites, making more polyvinyl alcohol molecules reacted with glutaraldehyde, leading to increased diameter. According to Fig. 8, the particle diameter of 150 μm was obtained when selecting hydrochloric acid amount as 1 mL.

CONCLUSION

Polyvinyl alcohol microspheres CPVA with good sphericity, controllable particle size and size distribution were successfully prepared via inverse suspension-chemical crosslinking method. The properties of microspheres obtained were affected by the main factors such as the dispersant amount, stirring rate, ratio of oil to water phase, the crosslinker amount and the catalyst hydrochloric acid amount. For the preparation of crosslinked polyvinylalcohol microspheres CPVA with good sphericity and particle size of about 150 μm, the suitable conditions were as follows, the dispersant amount was 0.16 g, stirring speed was 350 rpm, the ratio of oil-water phase was 2:1, the crosslinker amount was 1 mL and the hydrochloric acid amount was 1 mL.

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