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Synthesis of two thermo-sensitive copolymers forming aqueous two-phase systems

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ABSTRACT

Separation of bioproducts plays a key role in bio-industry. Aqueous two-phase systems are suitable for separation and purification of bioproducts like proteins, antibiotics, amino acids and organic acids. However, a key problem is the recovery difficulty of the copolymers forming aqueous two-phase systems. In the study, novel recyclable aqueous two-phase systems have been developed by using two new thermo-sensitive copolymers, P_{NBAa} and P_{NDB}. Copolymer P_{NBAa} was copolymerized by using N-isopropylacrylamide (NIPA), n-butyl methacrylate (BMA) and allyl alcohol (Aa) as monomers. Copolymer P_{NDB} was synthesized by using N-isopropylacrylamide (NIPA), 2-(dimethylamino) ethyl methacrylate (DMAEMA) and n-butyl methacrylate (BMA) as monomers. The lower critical solution temperatures (LCST) of these two polymers are 25.5 °C and 31.3 °C, respectively. P_{NBAa} and P_{NDB} can form aqueous two-phase systems with each other. Both of the two polymers can be recycled by adjusting temperature, with recoveries of over 97%. Demeclocycline was partitioned in the aqueous two-phase systems, with optimized partition coefficient of 2.61. It is believed that the recycling aqueous two-phase systems have potential applications in bioseparation engineering and biocatalysis engineering areas.

1. Introduction

The aqueous two-phase systems (ATPS) were firstly reported in 1950s [1]. The systems have low interfacial tension, high water content in two phases and mild aqueous environment, which are suitable for separation and purification of proteins, antibiotics, amino acids, organic acids and natural products. However, a big obstacle in the industrial application [2] is the recovery difficulty of the polymers forming aqueous two-phase systems, which results in high cost and environmental pollution. In order to solve the problem, researchers have focused on development of intelligent polymers forming ATPS. Recovery of phase-forming polymers could be achieved by adjusting temperature, pH, ionic strength, and electric potential.

Thermo-sensitive ethylene oxide–propylene oxide copolymers (EO–PO) were firstly reported in early 1990s and it could form ATPS with Dextran [3]. Persson et al. [4] modified random copolymer of EO and PO with aliphatic $C_{14}H_{29}$-groups and obtained an EOPO with hydrophobic groups (HM-EOPO). The modified HM-EOPO could form ATPS with mentioned EOPO polymer. In the systems, 73% EOPO and 97.5% HM-EOPO could be recycled. Other type of thermo-sensitive polymers and pH-sensitive polymers were reported as well [5–7].

In our lab, a bunch of novel polymers with high recovery were synthesized to construct different ATPS. Light–light recyclable ATPS composed of two novel light-response reversible copolymers P_{NBAC} and P_{NDBC} [8] were synthesized by using N-isopropylacrylamide, Butyl methacrylate, 2-(dimethylamino)-ethylmethacrylate, acrylic acid and chlorophyllin sodium copper salt as monomers. Polymers could be precipitated by laser irradiation in 488 nm with the least light density of 1.70×10^5 W/m^2, and their recoveries could reach up to 96.6% and 97.4%, respectively. A pH-thermo recyclable ATPS composed of one pH-response polymer P_{ADB} and one thermo-response polymer P_{NB} were also prepared by our lab. Over 98% of P_{NB} could be recovered by changing temperature to 32 °C. The P_{ADB} could be recovered by adjusting isoelectric point (pI) to 4, and its recovery could reach 95%. Several model bioproducts were partitioned in this ATPS [9]. Light-pH recyclable ATPS composed of one light-sensitive copolymer P_{NNC} and one pH-sensitive copolymer P_{ADB} [10], pH-thermo recyclable ATPS composed of one pH-response polymer P_{MDB} and one thermo-response polymer P_{NB} [11], and pH–pH recyclable ATPS composed of two pH-response copolymers P_{ADB} and P_{ADBA} [12] were also prepared by Cao and co-workers.

In this study, we have prepared two new polymers to form recycling ATPS. Both of the two polymers are thermo-sensitive, and they can be recycled by adjusting temperature to 10 °C above LCST. Their recoveries can reach 97%. Furthermore, demeclocycline used as an important antibiotic [13] was partitioned in the ATPS to

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investigate their performance. The new ATPS show great potential application in industry.

2. Experiments

2.1. Materials

N-isopropylacrylamide (NIPA) was synthesized by our laboratory [9]; n-butyl methacrylate(BMA), Allyl alcohol (Aa), 2,2′-azobis-isobutyronitrile (AIBN) was from Ling Feng Co., Ltd (Shanghai, China); Acryloyl chloride was from Bang Cheng Chemical Co., Ltd. (Shanghai, China); 2-(dimethylamino)-ethylmethacrylate (DMAEMA), n-hexane, tetrahydrofuran(THF), benzene, hydroquinone and isopropylamine were from Fei Da Co., Ltd. (Shanghai, China).

2.2. Methods

2.2.1. Synthesis of copolymer P_{NBAa} and P_{NDB}

After being tried a number of monomers with different ratios at different conditions, the final ATPS in the paper were developed by copolymer P_{NBAa} and copolymer P_{NDB}. P_{NBAa} was synthesized as following steps: 3.0 g of N-Isopropyl acrylamide (NIPA, 26.5 mmol), 0.12 ml Butyl methacrylate (BMA, 0.76 mmol) and 0.05 ml of Allyl alcohol (Aa, 0.74 mmol) were slowly added into a 100 ml conical flask containing 50 ml of benzene. Then 0.08 g of 2,2′-azobis-isobutyronitrile (AIBN) was added into the flask as initiator. The copolymerization was carried out in a shaker at the speed of 180 rpm for 24 h (60 °C) under the protection of N_2. After the reaction finished, solvent was evaporated, and then the solid in the flask was dissolved in acetone and recrystallized by using n-hexane in order to remove the initiators and unreacted monomers. At last, polymers were dried in a vacuum at room temperature.

The synthesis of copolymer P_{NDB} was similar to P_{NBAa}. The difference was that the solvent was 50 ml tetrahydrofuran (THF) and the initiator was 0.1 g AIBN. Besides, 3.0 g NIPA (26.5 mmol), 0.6 ml 2-(dimethylamino)-ethylmethacrylate (DMAEMA, 3.56 mmol) and 0.4 ml BMA (2.26 mmol) were added.

2.2.2. Characterizations of copolymers P_{NBAa} and P_{NDB}

P_{NBAa} and P_{NDB} were characterized by Bruker spectrospin DRX500 NMR equipment from Bruker (Switzerland) and Nicolet MagnaIR550 infrared equipment (Nicolet Company, USA) to determine their structures. The solvent was D_2O at room temperature for NMR. In addition, the molecular weights of copolymers were measured by Ubbelodhe viscosimeter measurement.

2.2.3. Lower Critical Solution Temperature (LCST) measurements of P_{NBAa} and P_{NDB}

LCST can be determined by the minimum in the cloud-point curve [14]. Cloud point measurements were carried out by immersing test tube containing the aqueous P_{NBAa} solutions in a water bath heated at a temperature rate of 0.5 °C/min. A 1 cm sample cell was used and the temperature was set between 22 °C and 35 °C. Concentration of solutions was at the range of 0.1% wt–10% wt. These solutions were analyzed by a Shimadzu UVmini-1240 UV–VIS spectrophotometer. The water-jacketed sample and cell holders were coupled with a THS-10(Ningbo Tianheng instrument factory) programmable circulating bath adjusted at a heating rate of 0.5 °C/min. Cloud points were defined as the temperature corresponding to a 10% reduction in the original transmittance of the solution [15]. A similar procedure was followed for the cloud point measurements of P_{NDB}. According to the lowest point in the curve of cloud points, LCST was determined.

2.2.4. Phase-forming test

Here, different concentrations of PEG1000, PEG20000, Dextran20000, polypropylene glycol (PPG), $(NH_4)_2SO_4$, P_{NBC}, P_{NNC}, P_{ABC} and P_{ADB} were used to test possibility of forming ATPS with the two copolymers. The copolymers P_{NBC}, P_{NNC}, P_{ABC} and P_{ADB} were synthesized in our laboratory [8–12]. These copolymers were dissolved in Na_2HPO_4/NaH_2PO_4 buffer (pH 7.0) or distilled water. The initial concentration of copolymer was chosen to be 10% (w/w).

2.2.5. The phase diagram

Phase composition in ternary P_{NBAa} and P_{NDB}-water systems was measured by High-Performance Liquid Chromatography (SHIMADZU, Japan) and Cloud Point Method. A reversed-phase column C18 (150 mm × 4.6 mm, 5 μm) from Phenomenex was used with the mobile phase consisted of methanol (A) and phosphate buffer (B) (V_A: V_B = 1:1), at flow rate of 1.0 ml/min. Chromatograms were monitored by UV absorbance detection at 210 nm. The sample was diluted in 1.0 ml of mobile phase and then filtered through a 0.45 μm PVDF syringe filter and 20 μl samples was injected into the HPLC system.

Series of mixtures were settled at room temperature for 3–4 h till the clear phase was observed. Take out the samples from top and bottom phases, and determine the concentrations of polymer P_{NDB} and P_{NBAa} to draw the calibration curve between peak area and concentration of polymer. Then, different concentrations of P_{NBAa}/P_{NDB} aqueous two-phase systems were prepared. After forming clear phase, concentrations of samples taken from top and bottom phases were tested by HPLC. Finally, the phase diagram was drawn by concentrations of polymer at top and bottom phases.

Cloud Point Method was used to obtain the binodal curve on the basis of the turbidity change between single aqueous phase and two-phase. Samples after phase separation were measured as following method. Two samples were prepared by taking certain amount of solution from top phase and bottom phase respectively. The absorbance of samples measured at 217 nm by ultraviolet visible spectrophotometry (SHIMADZU, Japan) was the summation of P_{NDB} and P_{NBAa}. The concentration of P_{NBAa} was determined at 420 nm after reacted with 2% (w/w) potassium permanganate for 8 h. Then P_{NBAa} concentration was used to calculate the absorbance of P_{NBAa} at 217 nm with the standard curve. The absorbance of P_{NDB} was calculated by subtracting the absorbance of P_{NBAa} from the summation. Then P_{NDB} concentration was calculated by the standard curve of P_{NDB} at 217 nm.

2.2.6. Recycle of copolymers

At nearly 10 °C above the LCST, the two copolymers could be recycled almost completely. The precipitate was separated from solution by centrifugation at 8000 rpm for 30 min and dried in a vacuum oven to constant mass. The recoveries of two polymers were measured. The two copolymers were recycled five times to estimate their recovery.

2.2.7. Effects of pH and salts on recovery of polymers and recycle of recovered polymers

The recovery of the two copolymers was investigated at different pH and salt types. 40 mM NaCl (Na_2SO_4, Na_2HPO_4, NaH_2PO_4, NH_4Cl and $(NH_4)_2SO_4$) was added to the copolymer solution to investigate the LCST and recovery of the polymer. The behavior of P_{NBAa}–P_{NDB} aqueous two-phase systems was also investigated in the presence of inorganic salts. Aqueous two-phase systems were prepared by mixing different ratios of known stock solutions of P_{NBAa} and P_{NDB}. After phase separation happened at room temperature, the two phases were separated by syringe, and the phase-forming polymers were recovered by temperature-inducing. Then these two recovered copolymers were dissolved again, and formed the same ATPS to repeat the previous operation.