



Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: [www.elsevier.com/locate/chroma](http://www.elsevier.com/locate/chroma)



# Synthesis of two thermo-responsive copolymers forming recyclable aqueous two-phase systems and its application in cefprozil partition

Daisong Hou, Xuejun Cao\*

State Key Laboratory of Bioreactor Engineering, Department of Bioengineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

## ARTICLE INFO

### Article history:

Received 6 January 2014  
Received in revised form 1 April 2014  
Accepted 18 April 2014  
Available online xxx

### Keywords:

Aqueous two-phase systems  
Thermo-responsive copolymer  
Cefprozil partition  
Correlation

## ABSTRACT

Aqueous two-phase systems are efficient purification methods for bioproducts. However, recovery difficulty of the copolymers forming aqueous two-phase systems becomes obstacle in scale-up application. In the study, novel recyclable aqueous two-phase systems have been developed by using two thermo-responsive copolymers,  $P_{NE}$  and  $P_{VAm}$ . Copolymer  $P_{NE}$  was copolymerized by using N-isopropylacrylamide and ethyl methacrylate as monomers, and  $P_{VAm}$  was synthesized by using N-vinylcaprolactam and acrylamide as monomers. The lower critical solution temperatures of  $P_{NE}$  and  $P_{VAm}$  are 28.7 °C and 35.6 °C, respectively. The recoveries of both polymers can reach over 95%. The minimal and maximal partition coefficient of cefprozil was 0.33 at 50 mM  $NH_4F$  and 6.55 at 70 mM  $LiCl$ . Two empirical equations were used to correlate with experimental binodal curve. Partition coefficients of cefprozil examined in the ATPS were correlated with tie-line length and calculated partition coefficient.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

Aqueous two-phase systems (ATPS) have been widely exploited for separation and purification of biological products since it was firstly reported in 1950s [1]. ATPS have several advantages in comparison with conventional methods for separation and purification such as low interfacial tension, fast mass transfer, high water content in two phases and mild aqueous environment. However, large-scale application of ATPS has not been realized due to the recovery difficulty of the polymers. In order to overcome the problem, researchers have focused on development of intelligent polymers forming ATPS.

The most well-known thermo-responsive monomer is poly (N-isopropylacrylamide) (NIPA) because its lower critical solution temperature (LCST) in water is close to body temperature [2]. ATPS containing thermo-responsive ethylene oxide–propylene oxide copolymers (EO–PO) were firstly studied in early 1990s [3]. Show et al. [4] purified lipase by using ATPS composed of thermo-responsive ethylene oxide–propylene oxide (EPO) copolymer and ammonium sulfate. Boyko et al. [5] synthesized and characterized a thermo-responsive polymer, poly-N-vinylcaprolactam. Caykara

et al. [6] also synthesized a new thermo-responsive polymer by using monomer N-isopropylacrylamide and acryl amide. Other types of ATPS containing thermo-responsive polymers were also reported [7–9].

In our laboratory, a lot of novel polymers forming ATPS were synthesized. ATPS consisted of thermo-responsive  $P_{NDB}$  and  $P_{NBAA}$  [10] was developed to distribute antibiotic. Their recoveries reached over 97%. Miao et al. [11] synthesized a pH-thermo recyclable ATPS composed of one pH-response polymer  $P_{ADB}$  and one thermo-response polymer  $P_{NB}$ . Over 98% of  $P_{NB}$  was recovered by changing temperature to 32 °C. ATPS composed of two novel light-response reversible copolymers  $P_{NBAC}$  and  $P_{NDBC}$  [12] were synthesized and their recoveries could reach up to 96.6% and 97.4%, respectively. Other recyclable ATPS containing light-responsive copolymer, pH-responsive copolymer and thermo-response polymer were also prepared by Cao and co-workers [13–15].

In this study, two novel thermo-response copolymers  $P_{NE}$  and  $P_{VAm}$  were prepared to form recycling ATPS. Difference of structures between monomer N-vinylcaprolactam with ring and monomer N-isopropylacrylamide is beneficial to form ATPS and partition. Both of them can be recycled easily by adjusting temperature. Their recoveries can reach over 95%. Furthermore, cefprozil used as an important antibiotic [16] was partitioned in the ATPS to investigate their performance and the relationship between partition coefficient and tie-line length was also correlated. The

\* Corresponding author. Tel.: +86 21 64252695; fax: +86 21 64252695.  
E-mail address: [caoxj@ecust.edu.cn](mailto:caoxj@ecust.edu.cn) (X. Cao).

thermo-responsive aqueous two-phase systems show great potential application in biotechnology industry.

## 2. Experiments

### 2.1. Materials

N-isopropylacrylamide (NIPA) was purchased from Aladdin; N-vinylcaprolactam (NVCL) was purchased from Aote Fine Chemical Factory (Shandong, China), 2,2'-azo-bis-isobutyronitrile (AIBN) was from Ling Feng Co., Ltd. (Shanghai, China); ethyl methacrylate was from Bang Cheng Chemical Co., Ltd. (Shanghai, China); acrylamide, methanol, ethanol and n-hexane were from Ling Feng Co., Ltd. (Shanghai, China).

### 2.2. Methods

#### 2.2.1. Synthesis of copolymer $P_{NE}$ and $P_{VAm}$

The final ATPS in the study were formed by copolymer  $P_{NE}$  and copolymer  $P_{VAm}$ . For  $P_{NE}$ , 3.0 g of N-Isopropyl acrylamide (NIPA, 26.5 mmol) and 0.05 ml of ethyl methacrylate (EMA, 0.44 mmol) were slowly added into a 100 ml conical flask containing 40 ml of deionized water. Then 0.1023 g ammonium persulfate and 0.1023 g sodium bisulfite was added into the flask as initiator. The copolymerization was carried out in a shaker at the speed of 200 rpm for 24 h (60 °C) under the protection of  $N_2$ . After the reaction, solution was crystallized by adding 100 ml ethanol in order to remove the initiators and unreacted monomers. Finally, polymers were dried in a vacuum at room temperature.

The synthesis of copolymer  $P_{VAm}$  was similar to  $P_{NE}$ . The difference was that the solvent using in reaction was 30 ml methanol, and solvent using in crystallization was 100 ml n-hexane and the initiator was 0.05 g 2, 2'-azo-bis-isobutyronitrile (AIBN). Besides, 3.0 g VCL (21.5 mmol) and 0.4 g Am (5.63 mmol) were added.

#### 2.2.2. Characterizations of copolymers $P_{NE}$ and $P_{VAm}$

$P_{NE}$  and  $P_{VAm}$  were characterized by Bruker spectroscope DRX500 NMR equipment from Bruker (Switzerland) and Nicolet MagnaIR550 infrared equipment (Nicolet Company, USA) to determine their structures. Particle sizes of copolymer were measured by Dynamic Laser Scattering (Wyatt Technology Corporation, USA). In addition, the molecular weights of copolymers were measured by Gel Permeation Chromatography (Wyatt Technology Corporation, USA) and viscosities of copolymers were characterized by Ubbelohde viscosimeter measurement.

#### 2.2.3. Lower critical solution temperature (LCST) measurements of $P_{NE}$ and $P_{VAm}$

LCST was determined by the minimum in the cloud-point curve [17]. A 1.0 cm sample cell was used and the temperature was set from 22 °C to 38 °C. Concentration of solutions was in the range 0.1–10% (w/w). 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 [18]. According to the lowest point in the curve of cloud points, LCST was determined.

#### 2.2.4. The phase diagram

The phase diagram was drawn by the Cloud Point Method [19]. Without centrifugation, two clear phases can be formed after standing for 18 h at 293.15 K in the water bath when polymers are completely dissolved. Centrifugation at low speed can shorten the

phase separation time [15]. A mixture with known total composition was agitated for 6 h to be completely dissolved. The systems were separated by centrifuging at 4000 rpm for 30 min and standing in graduated centrifuge tube at least for 3 h. In order to achieve complete phase separation, the mixture was settled for 12 h at 293.15 K in the water bath. The composition of each phase was determined in triplicate when the solutions in equilibrium were separated. Samples were taken out from top and bottom phases, and concentrations of polymer  $P_{NE}$  and  $P_{VAm}$  was determined. 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 0.7 ml/min. Chromatograms were recorded by UV absorbance detection at 220 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. Finally, the phase diagram was drawn by concentrations of polymer at top and bottom phases.

#### 2.2.5. Effects of salts on recovery of polymers

Copolymers are thermo-response, so they can be precipitated by adjusting temperature. The precipitate was separated from solution by centrifugation at 8000 rpm for 15 min and dried in a vacuum oven to constant mass. The recoveries of two polymers were measured and each recovery had three trials.

The recovery of the two copolymers was investigated at different salt types. 40 mM NaCl (KCl,  $Na_2HPO_4$ ,  $NaH_2PO_4$ , LiCl and  $(NH_4)_2SO_4$ ) was added to the copolymer solution to investigate recovery of the polymer. Each recovery had three trials.

#### 2.2.6. Partition of cefprozil in $P_{NE}$ - $P_{VAm}$ ATPS

The tie-line length (TLL) reflects the property of ATPS. TLL has close relationship with composition of ATPS. Partition coefficient in the investigated systems at different compositions was measured by the following methods.

The equilibrium time of biomolecule partitioning in aqueous two-phase systems (ATPS) is very short because low interfacial tension of ATPS makes mass transfer rate quicker. A mixture of polymers was prepared by dispensing appropriate amounts of  $P_{NE}$  and  $P_{VAm}$  solutions into a 5.0 ml tube. The total volume of final system was 2.0 ml. Solution of cefprozil was prepared at concentrations of 5 mg/ml. 200 μl cefprozil solution was added to a set of mixtures. Phase separation was achieved by centrifuging at 4000 rpm for 30 min and standing in graduated centrifuge tube at least for 3 h. In order to reach two clear phases, the mixture was settled for 12 h at 293.15 K in the water bath. After aqueous two-phase systems reached equilibrium, the upper phase in each system was removed after 100 μl samples in top phase were taken out. After that, 100 μl samples in bottom phase were also taken out. Aliquots of 100 μl from the upper and lower phases were withdrawn in duplicate for analysis. All samples were diluted to 5.0 ml solutions for measurement.

Determination by an automatic UV spectrometer, the maximum absorbance wavelength  $\lambda_m$  of cefprozil,  $P_{NE}$  and  $P_{VAm}$  were found to be 280 nm, 215 nm and 220 nm, respectively, so we can examine the concentration of cefprozil at 280 nm without the interference of polymers. Here, NaSCN, KSCN, NaCl, KCl, LiCl,  $NH_4Cl$  and  $NH_4F$  were used to investigate effect of salt species and the concentration on cefprozil partition in  $P_{NE}$ - $P_{VAm}$  ATPS. The effect of salts on partition coefficient was studied in the concentration range 20–100 mM.

## 3. Results and discussion

### 3.1. Characterizations of copolymers $P_{NE}$ and $P_{VAm}$

Figs. 1 and 2 show schematic representation of copolymerization for  $P_{NE}$  and  $P_{VAm}$ .  $P_{NE}$  was synthesized by using NIPA and

Download English Version:

<https://daneshyari.com/en/article/7612984>

Download Persian Version:

<https://daneshyari.com/article/7612984>

[Daneshyari.com](https://daneshyari.com)