Synthesis and Functionality of Poly(*N*-vinylalkylamide). X. A Novel Aqueous Two-Phase System Based on Thermosensitive Polymers and Dextran

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ABSTRACT: The use of thermosensitive polymers in an aqueous two-phase system was studied. Poly(*N*-isopropylacrylamide) (PNIPAAm) and poly(*N*-vinylisobutyramide) (PNVIBA) were used as thermosensitive polymers. Both polymers could form aqueous two-phase with dextran, respectively. The phase diagrams of each system were successfully obtained. Using myoglobin as a model protein, a preliminary separation study was performed. The separation ability of both polymers was higher than that from the poly(ethylene glycol)-dextran system. Protein separation ability appeared to be related to the hydrophilic/hydrophobic balance of the polymers. Both PNVIBA and PNIPAAm rich phases maintained their thermosensitivity after two-phase formation. PNVIBA and PNIPAAm are useful as polymers for a functional aqueous two-phase system. J Appl Polym Sci 73: 2545–2548, 1999

Key words: aqueous two-phase system; thermosensitive polymer; poly(*N*-isopropylacrylamide); poly(*N*-vinylisobutylamide); protein separation

INTRODUCTION

Aqueous two-phase systems have received a great deal of attention from researchers in recent years[1–9] (for part IX of this series, see ref. 31). In general, two-phase systems are formed in solutions with two incompatible polymers. The most commonly used polymer systems are based on poly(ethylene glycol)(PEG) and dextran. Most of the recent research on two-phase systems that are based on polymer/salt or polymer/polymer have focused on their practical industrial use. One main goal of researching the two-phase system is the development of a low-cost two-phase system, and another is to design polymers that can be easily recovered or that have a high level of separation performance. Several studies have been done on phase-separation phenomena with the above two goals.[10–15]

Thermosensitive polymers are currently being studied for use as novel functional polymers. The most well-known thermosensitive polymer is poly(*N*-isopropylacrylamide) (PNIPAAm). The aqueous solution of PNIPAAm shows a lower critical solution temperature (LCST) at 32°C,[16–18] and many studies of thermosensitive behavior have been done on PNIPAAm and its derivatives.[19–25] To develop new water-soluble functional polymers, the authors have been studying poly(*N*-vinylacetamide) (PNVA) and its related compounds.[9,26–31] One of the derivatives of NVA, *N*-vinylisobutyramide (NVIBA), is structurally quite similar to *N*-isopropylacrylamide (NIPAAm) (Fig. 1) and exhibits thermosensitivity in a polymeric form. In previous studies, the authors reported that the aqueous solution of poly(NVIBA) (PNVIBA) exhibited LCST at 39°C.[28,29]

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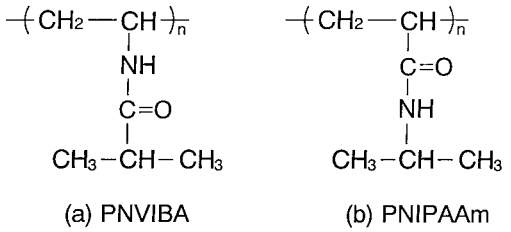

Figure 1 Chemical structures of PNVIBA and PNIPAAm.

The thermosensitive nature of polymers is believed to due to their hydrophobic–hydrophilic balance. Every thermosensitive polymer has both hydrophobic and hydrophilic moiety in its molecular structure; this bifunctional characteristic is also essential for the polymers used in aqueous two-phase systems. Therefore, thermosensitive polymers are expected to form aqueous two-phase systems. The use of thermosensitive polymers in aqueous two-phase separation is of great interest in industries such as recycling polymers, the high recovery rate of separated substances, and the formation of the two-phase system at a lower concentration of polymers. However, there have been only a few studies that have used thermosensitive polymers in aqueous two-phase systems.[32–35] In this study, as a preliminary step in developing novel aqueous two-phase systems based on thermosensitive polymers, PNVIBA–dextran and PNIPAAm–dextran systems were investigated. A protein (myoglobin)-separation study, as well as a phase-separation study, was done on both systems.

EXPERIMENT

Materials

NIPAAm was donated by Kojin Co. Ltd.(Tokyo, Japan). NVIBA was synthesized as described in a previous study.[29] PEGs (with a molecular weight of 7300–9000, 20,000, and 70,000) and dextran (with a molecular weight of 170,000–200,000) were purchased from Nacalai Tesque (Kyoto, Japan). Myoglobin (from equine skeletal muscle, isoelectric pH = 7.0) was purchased from Sigma (St. Louis, MO). All other chemicals used were of analytical grade.

The PNVIBA and PNIPAAm were prepared by the solution polymerization in methanol and in ethanol, respectively, at 60°C using 2,2′-azobisisobutyronitrile(AIBN) as an initiator.

The molecular weights of the polymers obtained were determined by gel permeation chromatography. This was performed on a Shimadzu LC-6A that was equipped with a RI detector (Shodex SE-51) with a Shodex column. LCST was measured by transmittance plots at 500 nm on a JASCO Model V-550 spectrophotometer, which included a peltier-type thermostatic cell holder for 0.2 wt % solutions of each polymer.

Two-Phase Systems

Aqueous two-phase systems (PNVIBA–dextran, PNIPAAm–dextran, and PEG–dextran) were prepared from the solutions of the polymers. The polymer solutions were mixed at room temperature, and the composition of the phases in the mixtures were determined by the following procedure. The systems were mixed and left to separate overnight at 4°C. The phases were collected separately using a syringe, and were diluted appropriately with distilled water for concentration determination. The dextran concentration was determined by polarimetry (optical rotation) against calibration curves. Polarimetric measurements were performed at room temperature using a digital polarimeter (DIP-370, JASCO, Tokyo, Japan). The concentration of other polymers (PNVIB, PNIPAAm, and PEG) in the different phases was determined by gravimetry after the solutions were freeze dried.

Partition of Myoglobin in Two-Phase Systems

The partitioning of myoglobin was described by partition coefficient K, which is defined as $K = C_T/C_B$ where C_T and C_B are the equilibrium concentration of the partitioned substance in the top (upper) and bottom (lower) phases, respectively. The protein yields were determined by ultraviolet spectroscopy at 280 nm absorption. All of the results were averaged after the partition of protein in two equal systems.

RESULTS AND DISCUSSION

PEG–dextran systems, which are well known, are used as controls in experiments to compare separation behavior. Both PNVIBA–dextran and PNIPAAm–dextran systems could be formed into aqueous two-phase systems. The top phases were synthetic polymer (PNVIBA or PNIPAAm) rich and the bottom phases were dextran rich. The same phenomenon has been observed in PEG–

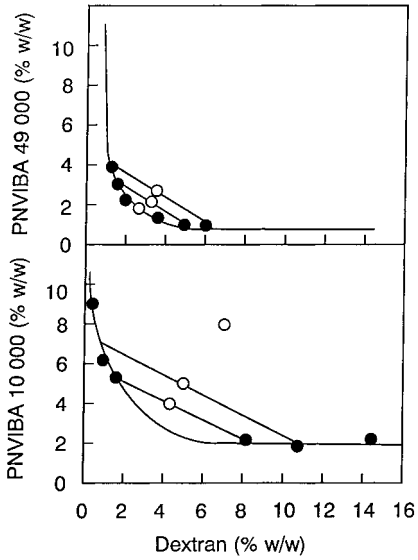

Figure 2 Phase diagram of the PNVIBA–dextran system with various molecular weights of PNVIBA.

dextran and PNVA–dextran systems.[9] Figures 2 and 3 show the phase diagrams of PNVIBA–dextran and PNIPAAm–dextran two-phase systems with synthetic polymers of various molecular weights, respectively. In both systems, the critical concentration of phase separation was affected by the molecular weight of the polymers. When polymers with a higher molecular weight were used, a two-phase system was formed at the lower concentration. This was due to the enlargement of the repulsion force between the synthetic polymer and the dextran with increasing molecular weight. There was no apparent difference in the phase diagrams between the PNVIBA– and PNIPAAm–dextran systems. This means that the hydrophilic/hydrophobic balance of the two polymers are almost equivalent.

Figure 4 shows the partition coefficient of the myoglobin in three kinds of two-phase systems. The partition coefficients (K) were less than 1 for all systems. Therefore, myoglobin tends to be distributed in the bottom (dextran-rich) phase. This is caused by the hydrophilic nature of myoglobin, i.e., as myoglobin is a hydrophilic protein, it is distributed in the hydrophilic dextran phase. The degree of distribution of myoglobin in the dextran phase increased with an increase in the concentration of polymers. A comparison of the partition coefficient between PNVIBA–dextran, PNIPAAm–dextran, and PEG–dextran shows that both the partition coefficients of the PNVIBA–dextran and PNIPAAm–dextran systems are smaller than that of the PEG–dextran system. Myoglobin,

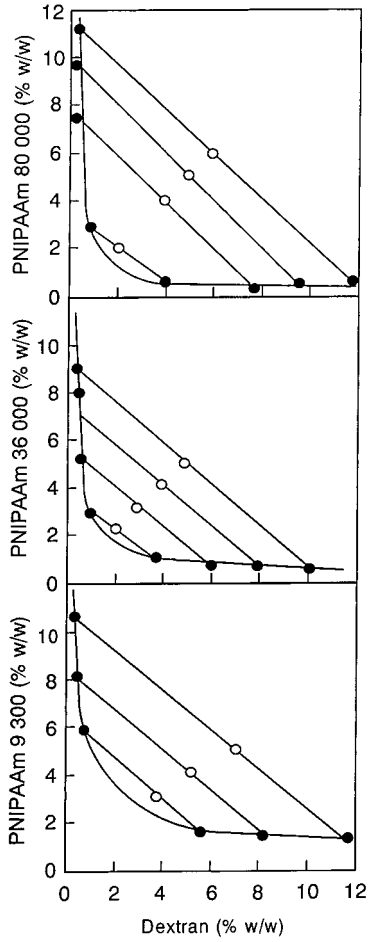

Figure 3 Phase diagram of the PNIPAAm–dextran system with various molecular weights of PNIPAAm.

therefore, is partitioned more in the bottom phase in both systems, and this indicate that the PNVIBA–dextran and PNIPAAm–dextran systems are su-

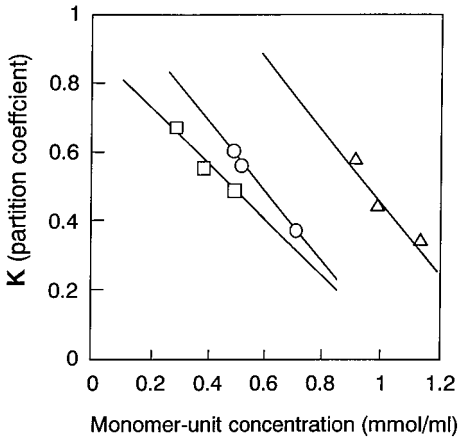

Figure 4 Partition coefficient of myoglobin separation: (○) PNVIBA–dextran system; (□) PNIPAAm–dextran system; (△) PEG–dextran system.

perior to PEG–dextran systems in regard to partitioning myoglobin. This is caused by the higher hydrophobicity of PNVIBA and PNIPAAm (compared to PEG), i.e., both polymers have a strong repulsion to myoglobin, which is a hydrophilic protein.

As the LCST of PNVIBA (39°C) was higher than that of PNIPAAm (32°C), PNVIBA seems to have higher hydrophilicity than PNIPAAm. By comparing the partition coefficients at the same concentration, it is clear that myoglobin (hydrophilic protein) partitioning was more effective in the PNIPAAm–dextran system than in the PNVIBA–dextran system. This result shows that it may be possible to determine the hydrophilicity series of water-soluble polymers using aqueous two-phase separation.

The above results show that PNVIBA and PNIPAAm are useful as polymers for use in aqueous two-phase systems. For a two-phase system with thermosensitive polymers, Kataoka et al. found that the copolymer of *N*-hydroxymethylacrylamide and *N*-phenylacrylamide formed a two-phase system by coacervation.[35] They noted that partition coefficient (*K*) drastically changed across the LCST. Although their system is slightly different from that in this study, it was expected that PNVIBA– and/or PNIPAAm–dextran systems have the thermosensitivity in regard to protein separation. In fact, when a PNVIBA- or a PNIPAAm-rich phase was heated to above its LCST, insoluble aggregates of thermosensitive polymers were observed (data not shown). Further research (including recovering and recycling of the PNVIBA and PNIPAAm) is now in progress.

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