Synthesis and Functionalities of Poly(*N*-vinylalkylamide). VII. A Novel Aqueous Two-Phase Systems Based on Poly(*N*-vinylacetamide) and Dextran

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ABSTRACT: It was found that a poly(*N*-vinylacetamide) (polyNVA)–dextran system formed two phases. The top was polyNVA-rich and the bottom phase was dextran-rich, which is the same as the poly(ethylene glycol) (PEG)–dextran system, and the phase separation occurred at a lower concentration than the PEG–dextran system. The protein separation in the polyNVA–dextran system was studied using myoglobin. Myoglobin was partitioned more in the bottom (dextran-rich) phase in a polyNVA–dextran system. The partition coefficient of the polyNVA–dextran system was smaller that of the PEG–dextran system, which suggests that the polyNVA–dextran system is superior to the PEG–dextran system for myoglobin separation. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci **67:** 255–258, 1998

Key words: poly(*N*-vinylacetamide); aqueous two-phase system; dextran; poly(ethylene glycol); myoglobin

INTRODUCTION

Aqueous two-phase systems, which were introduced as a separation method for biomolecules in the mid-1950s by Albertsson,[1,2] have been extensively studied recently.[3–8] The most commonly used polymer systems are based on poly(ethylene glycol) (PEG) and dextran. In some applications, the polymers have been modified with covalent bound groups in order to introduce hydrophobic ligands[9] and biospecific ligands for the affinity partitioning of biomolecules.[10–14] It is easy to modify dextran because it has many hydroxyl groups; however, as PEG has few functional groups, it is very difficult to attach affinity ligands to it. It would be very useful to introduce a novel polymer which can form two-phase system and has many functional groups; this type of polymer would be useful for modifications such as the introduction of affinity ligands.

Poly(*N*-vinylacetamide) (polyNVA) (Scheme 1) is an amphiphilic, neutral, stable, and cheap polymer which we have been studying recently.[15–18] PolyNVA's chemical structure can easily modified by hydrolysis in order to give poly(vinyl amine). The subsequent amidation of an amino group with carboxylic acids is also possible.[17] In an experiment, a reaction of hydrolyzed polyNVA with isobutyric acid gave poly(*N*-vinylisobutyramide) (polyNVIBA).[17] PolyNVIBA has higher hydrophobicity than polyNVA and it shows thermosensitivity like poly(*N*-isopropylacrylamide).[19–21] In this study, as a preliminary study for the use of polyNVA in an aqueous two-phase system, the formation of the two-phase systems of polyNVA–dextran system was studied. The protein separation in a polyNVA–dextran system was also accomplished by the use of myoglobin as a model protein, as compared with that in the PEG–dextran system.

For part VI of this series, see ref. 21.

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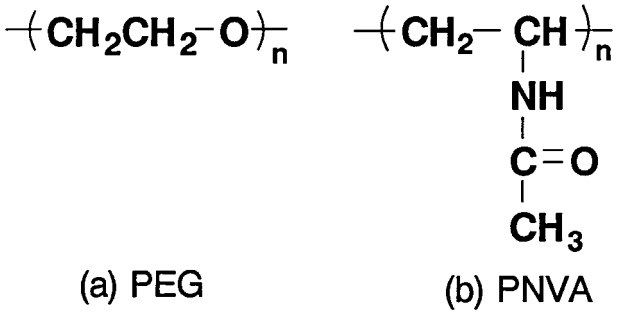

Scheme 1 Chemical composition of the (a) PEG and (b) PNVA.

EXPERIMENTAL

Materials

NVA was kindly donated by Showa Denko Co. Ltd. (Tokyo, Japan). PolyNVA was obtained by the polymerization of NVA using 2,2′-azobis-isobutyronitrile as an initiator.[15] The polyNVA that was obtained was repeatedly purified by re-precipitation, and various molecular weights of polyNVA were obtained. PEGs (molecular weights are 7300–9000, 20,000, and 70,000) and dextran (molecular weight is 170,000–200,000), were purchased from Nacalai Tesque (Kyoto, Japan). Myoglobin (from horse skeletal muscle, isoelectric pH = 7.0) was bought from Sigma (USA). All of the other chemicals that we used were of analytical grade.

Two-Phase Systems

Aqueous two-phase systems were prepared from polymer solutions (polyNVA–dextran and PEG–dextran). The compositions 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 diluted as was appropriate for the concentration determination. The dextran concentration were determined by polarimetry (optical rotation) against calibration curves. The polarimetric measurements were performed at room temperature using a digital polarimeter (DIP-370, JASCO, Tokyo, Japan). The concentration of polymers in the different phases was determined by gravimetry after the freeze-drying of the solutions.

Partitioning of Myoglobin in Two-Phase Systems

The partitioning of a substance is described by the 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 results are averaged values after the partition of a protein into two equal systems with two repeated measurements of the protein content.

RESULTS AND DISCUSSION

As a well-known system, PEG–dextran systems were used as a control for a comparison of a separation behavior. A polyNVA–dextran system formed an aqueous two-phase system. The top phase was polyNVA-rich and the bottom phase was a dextran-rich phase, which is the same as the PEG–dextran system. Figure 1 shows the

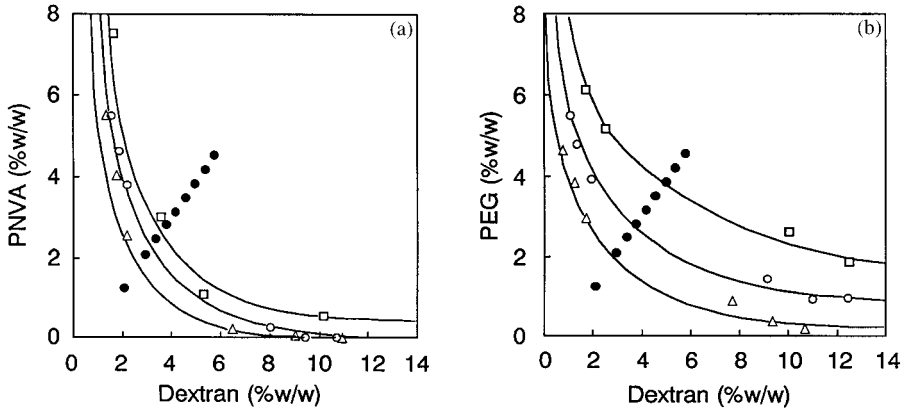

Figure 1 Phase diagrams of aqueous two-phase systems of different degree of polymerization (DP). (a) PolyNVA–dextran system. DP of polyNVA: (□) 240; (○) 590; (△) 1410. (b) PEG–dextran system. DP of PEG: (□) 140; (○) 450; (△) 1600.

Table I Composition of PolyNVA/Dextran/Water System

Total Composition (mmol/mL)		Top Phase (wt %)		Bottom Phase (wt %)	
PolyNVA	Dextran	PolyNVA	Dextran	PolyNVA	Dextran
3.5	1.2	3.0	3.5	1.1	5.3
6.0	1.7	7.5	1.7	0.5	10.2
8.1	2.0	13.7	1.4	0.0	12.7
10.5	3.3	13.7	1.1	0.0	18.5

phase-diagrams of polyNVA–dextran and PEG–dextran two-phase systems for different degrees of polymerization, respectively. It is clear that phase separation is obtained at lower concentrations as compared to the PEG–dextran system. It is possible that the phase separation in a low concentration could be useful from an industrial point of view.

The molecular weights of the monomer units were different for PEG (molecular weight monomer unit = 44) and polyNVA (molecular weight of monomer unit = 85). If they are used at the same concentration, the total number for the polyNVA monomer unit will be approximately twice that of PEG. In order to compare the repulsion of the monomer–monomer interactions of polyNVA–dextran and PEG–dextran systems, the phase separation concentration was compared in the monomer unit concentration. Tables I and II show the calculation results. Phase separation was observed at 3.5 mmol/mL for a polyNVA–dextran system, whereas a PEG–dextran system needs a higher concentration (10 mmol/mL). The repulsion between the monomer units of polyNVA and that in dextran seemed to be stronger than that of PEG and dextran. This is due to the higher hydrophobicity of polyNVA as compard to that of PEG.

The molecular weight effects of phase separation were also shown in Figure 1. Three pairs of polyNVAs and PEGs, which had approximately the same degree of polymerization, were used. In the PEG–dextran system, the critical concentration in the phase separation was strongly affected by the degree of polymerization, whereas that of polyNVA–dextran was not. One of the reasons for this is the differences of the structure in the water between polyNVA and PEG. PEG was observed; it forms a helixlike structure in water. So, when the degree of polymerization of PEG is high, the length and content of the helixlike structures increase. It affects the viscosity, the motility, and the hydration state; then, a change in the degree of polymerization affects phase separation.

Figure 2 shows the partition coefficient for myoglobin. The partition coefficients (K) were smaller than 1 for all concentrations. This means that myoglobin was distributed to the bottom (dextran-rich) phase. As myoglobin is a hydrophilic protein, it seems to be distributed in the hydrophilic dextran phase. The degree of distribution of myoglobin to the dextran phase increased with an increase in the polymer concentration. By using a comparison of the partition coefficient between polyNVA–dextran and PEG–dextran,

Table II Composition of PEG/Dextran/Water System

Total Composition (mmol/mL)		Top Phase (wt %)		Bottom Phase (wt %)	
PEG	Dextran	PEG	Dextran	PEG	Dextran
3.5	1.2	N.F.[a]	N.F.	N.F.	N.F.
6.0	1.7	N.F.	N.F.	N.F.	N.F.
8.1	2.0	N.F.	N.F.	N.F.	N.F.
10.5	3.3	7.8	0.8	0.5	17.0

[a] N.F., not formed.

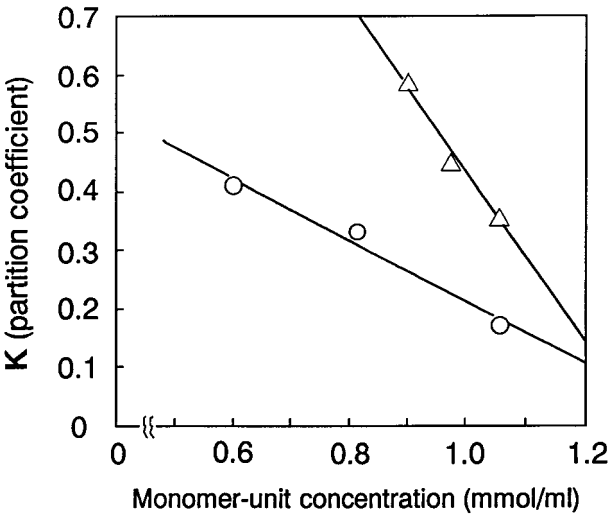

Figure 2 Partition coefficient of myoglobin separation: (○) polyNVA–dextran system; (△) PEG–dextran system.

one can see that the partition coefficient of the polyNVA–dextran system is smaller than that of the PEG–dextran system. This means that myoglobin is partitioned more in the bottom phase in a polyNVA–dextran system and that the polyNVA–dextran system is superior to the PEG–dextran system in regard to the partitioning of myoglobin. This is due to the higher hydrophobicity of PNVA than that of PEG; polyNVA has strong repulsive force in regard to myoglobin, which is a hydrophilic protein.

From these results, we have concluded that polyNVA is useful as a polymer in an aqueous two-phase system. For a two-phase system with low polymer concentrations, the ethyl hydroxyetyl cellulose (EHEC)–dextran system was reported.[22] The special character of polyNVA which might be superior to EHEC is the functionality. The thermosensitivity of polyNVIBA, which is a derivative of polyNVA, is one example. The thermosensitivity is expected to be useful for easy recovering and recycling of biomolecules.[23–25] Research about other types of functionality, such as the incorporation of affinity ligands via hydrolysis and condensation reactions between polyNVA and ligands, is now in progress.

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