

BRIEF
COMMUNICATIONS

Extraction and Preconcentration of Anthocyan Dye from Aqueous Solutions with Water-Soluble Poly-*N*-Vinylamides

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Abstract—Extraction of an anthocyan dye with poly-*N*-vinylpyrrolidone (PVP) and poly-*N*-vinylcaprolactam (PVC) under the conditions of separation of aqueous solutions of this polymer into two phases in the presence of salting-out agents (sodium chloride and sodium or ammonium sulfate) was studied.

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Heterogeneous systems containing water-soluble polymers poly-*N*-vinylpyrrolidone (PVP) and poly-*N*-vinylcaprolactam (PVC) are used in biotechnology for separation of proteins, cells, and biologically active substances, including amino acids and vitamins [1, 2]. The main advantages of application of PVP and PVC as extractants are their nontoxicity, high hydrophilicity, and ability to form complexes with many low- and high-molecular-weight organic compounds [3].

The majority of natural red pigments are derived from anthocyanins, phenolic compounds, whose structure is similar to that of plant glycosides. These compounds are readily soluble in water and form anthocyanides upon hydrolysis [4]. These dyes are used in food industry [5].

The common methods for recovery and preconcentration of anthocyan dyes from plant raw materials and aqueous solutions are based on their repeated extraction with organic solvents [5]. We believe that liquid extraction of anthocyan dyes can be improved by using two-phase aqueous systems based on water-soluble polymers containing no toxic organic solvents. Such extraction systems based on PVP and PVC have not been studied previously.

In this study, we examined the extractive preconcentration of an anthocyan dye from aqueous solutions with PVP and PVC.

EXPERIMENTAL

Anthocyan dye was extracted from black-currant berry with ethanol at 50–55°C by the procedure reported in [6]. The PVP polymer of medical grade with a molecular weight $M_{\eta} = 1 \times 10^4$ was used. Poly-*N*-vinylcaprolactam was prepared by radical polymerization of its monomer in isopropanol at 70°C in the presence of azobis(isobutyronitrile) initiator at various monomer concentrations. The polymer was precipitated by adding hexane to the reaction mixture and then vacuum-dried at 55–60°C. The molecular weights of the polymers were calculated from the intrinsic viscosity $[\eta]$ of their solutions by the formula $[\eta] = 1.5 \times 10^{-4} M_{\eta}^{0.68}$ [3]. PVC samples with the molecular weights of 1×10^4 , 6×10^4 , and 16×10^4 were prepared.

NaCl, $(\text{NH}_4)_2\text{SO}_4$, and Na_2SO_4 (chemically pure grade) were used as salting-out agents providing separation of aqueous solutions of the polymer into two phases within a wide range of polymer and salt concentrations.

A solution of anthocyan dye (10 ml), containing a salting-out agent, was placed in a vial with a ground-glass stopper, and then 1 ml of an aqueous solution of the polymer (concentration 0.001–2 wt %) was added to obtain a two-phase extraction system. The extraction was carried out on a vibra-

tion mixer until the phase equilibrium was attained (3–5 min).

The efficiency of the extraction recovery of the dye was characterized by the degree of its recovery R (%):

$$R = [(A_0 - A)/A_0] \times 100,$$

where A_0 and A are the optical densities of aqueous anthocyan dye solutions before and after extraction (KFK-2MP spectrophotometer, $\lambda = 490$ nm, 1-cm optical cell).

The content of the red pigment in the anthocyan dye was determined with a reference solution of $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ by the standard procedure [5].

As a rule, two-phase aqueous systems based on water-soluble polymers are prepared by their salting-out from homogeneous aqueous solutions [7]. The conditions of formation of heterogeneous systems by salting-out of PVP and PVC from their homogeneous aqueous solutions were elucidated from their phase diagrams (Fig. 1) obtained by titration of these polymers with standard salt solutions to the cloud point [8]. We found that, in all the systems under consideration, except PVP–sodium chloride–water, stable two-phase systems are formed. In phase diagrams, the fields of two-phase systems lie above binodal curves of approximately exponential shape. Irrespective of a particular poly-*N*-vinylamide, the strongest salting-out effect is reached in the presence of ammonium sulfate. It should be noted that the molecular volume and electronic structure of ammonium cations are close to those of water molecules, and ammonium cations form hydrogen bonds with surrounding water molecules. These factors favor stronger hydration of ammonium cations. To obtain the maximal amount of the aqueous phase enriched in the polymer, the salting-out is carried out at the salt concentration close to saturation.

Figure 2 shows that the extraction of anthocyan dyes is dependent on both the polymer concentration and particular dye. In salting-out with ammonium sulfate, the dye recovery is more complete. In single-step extraction, the degrees of recovery of dyes in the presence of ammonium sulfate and sodium sulfate are 95 and 79%, respectively.

In extraction of anthocyan dye with PVC, the more efficient recovery of the dye is reached with sodium chloride compared with ammonium and sodium sulfates. We believe that this difference is primarily caused by the difference in the effect of salts on the phase separation temperature T_{ps} of aqueous PVC.

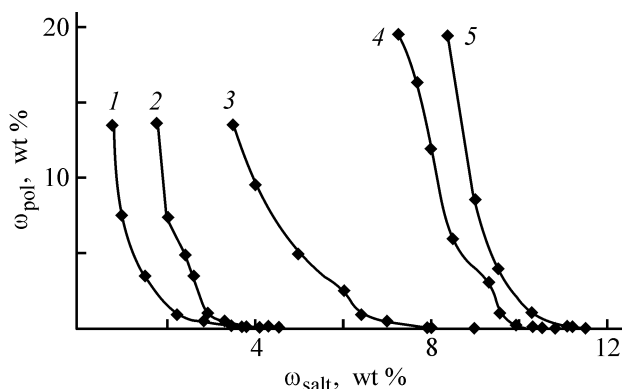


Fig. 1. Phase diagrams of the systems (1) PVC–ammonium sulfate, (2) PVC–sodium sulfate, (3) PVC–sodium chloride, (4) PVP–ammonium sulfate, and (5) PVP–sodium sulfate. (ω_{pol}) Weight fraction of polymer and (ω_{salt}) weight fraction of salt.

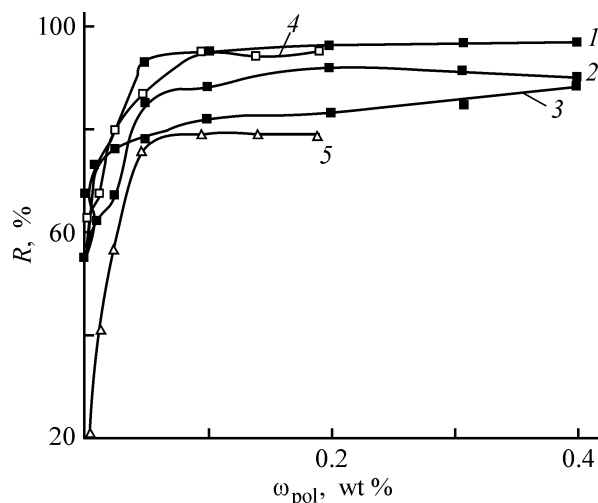


Fig. 2. Degree of extraction of anthocyan dye R from aqueous-salt solutions vs. polymer concentration ω_{pol} . Polymer: (1–3) PVC and (4, 5) PVP. Salting-out agent: (1) sodium chloride, (2, 5) sodium sulfate, and (3, 4) ammonium sulfate.

It is known [9] that, in the absence of salt, the T_{ps} of this polymer is close to the temperature of warm-blooded living bodies. At the same time, on adding NaCl, the phase-separation temperature decreases to room temperature. Therefore, we suggest that the increase in efficiency of extraction of the anthocyan dye in the system poly-*N*-vinylcaprolactam–NaCl– H_2O is caused not only by salting-out of the dye and polymer but also by changes in the structure of the polymer– H_2O complex.

Figure 3 shows how the PVC molecular weight and pH affect the degree of recovery of anthocyan dye from saturated aqueous NaCl. It is seen that, irrespective of PVC concentration and its molecular weight,

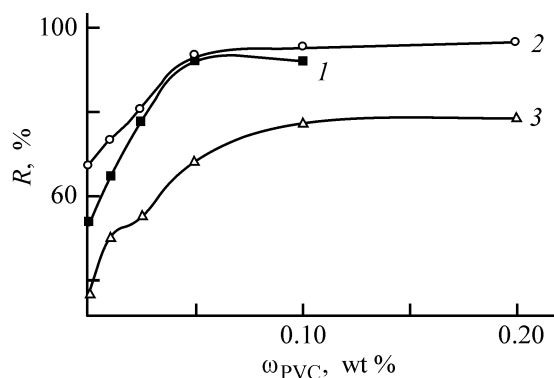


Fig. 3. Degree R of extraction of anthocyan dye from saturated aqueous NaCl vs. PVC concentration ω_{PVC} . M_n : (1) 1×10^4 (pH 3.5), (2) 6×10^4 (pH 3.5), and (3) 6×10^4 (pH 1).

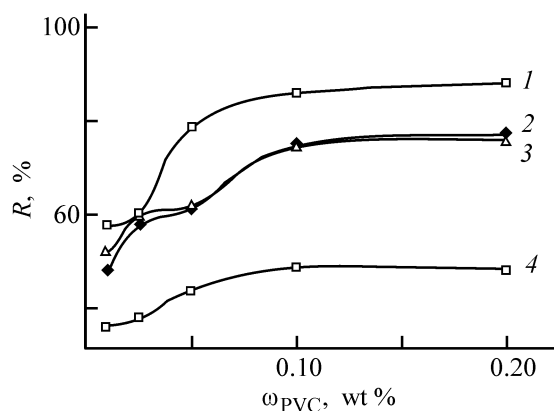


Fig. 4. Degree R of extractive recovery of anthocyan dye with aqueous PVC at phase separation induced by heating vs. the polymer concentration ω_{PVC} . M_n : (1) 16×10^4 (pH 3.5), (2) 6×10^4 (pH 3.5), (3) 1×10^4 (pH 3.5), and (4) 1×10^4 (pH 1).

the degree of recovery of the dye considerably decreases with decreasing pH. At a PVC concentration of 6×10^4 M, raising pH from 1 to 3.5 increases the degree of recovery of the anthocyan dye from 77 to 92–95%. The extraction of dyes at pH > 7 in the systems under consideration was not studied because of the chemical degradation of anthocyan dye in alkaline media [10].

Variation of the molecular weight of the polymer from 1×10^4 to 6×10^4 at pH 3.5 in the extraction systems based on PVC does not noticeably affect the degree of recovery of the anthocyan dye (Fig. 3, curves 1 and 2). A similar behavior is exhibited by PVC in its complexation with fluorescent dyes [3].

We found that, in the extraction systems based on PVP, all factors being the same (M_n , pH, salting-out

agent), the degree of recovery of the anthocyan dye was higher than in the systems based on PVC (Fig. 2, curves 3 and 4). This difference is apparently caused by the stronger complexation of PVP with the dye. This polymer is more sterically accessible and less hydrophobic because of the smaller length of hydrocarbon chains in *N*-vinylpyrrolidone, compared to *N*-vinylcaprolactam.

The high efficiency of the extraction systems based on PVP and PVC is caused by complexation of PVP and PVC with dye molecules. This complexation is manifested in the UV absorption spectra as the absorption band at 274 nm, absent in the spectra of the aqueous solutions of particular polymers. The complexation between the polymers and anthocyan dye occurs by the mechanism of hydrogen bonding between the carbonyl groups of polymer and the hydroxy groups of the dye. Upon acidification, the complexation between the polymer and dye weakens because of the protonation of the C=O groups [11] and, as a result, the degree of recovery of the dye decreases.

In contrast to aqueous PVP, aqueous poly-*N*-vinylcaprolactams have a lower critical solution point at 32–37°C [3]. Therefore, PVC allows extraction of anthocyan dye (up to 86%) from aqueous solutions without salting-out agents at temperature exceeding the lower critical solution point (33.5°C). As can be seen from Fig. 4 (curve 4), the extraction of the dye considerably decreases upon acidification of the extraction system based PVC to pH 1 before phase separation by heating.

CONCLUSIONS

(1) Two-phase systems based on water-soluble poly-*N*-vinylpyrrolidone and poly-*N*-vinylcaprolactam are suitable for efficient extractive recovery and pre-concentration of anthocyan dyes from aqueous solutions. The highest extraction efficiency is exhibited by the two-phase extraction systems ammonium sulfate–poly-*N*-vinylpyrrolidone–water and sodium chloride–poly-*N*-vinylcaprolactam–water at pH 3.5.

(2) The anthocyan dye concentrates prepared by extraction with poly-*N*-vinylpyrrolidone and poly-*N*-vinylcaprolactam can be used for coloration of food products.

REFERENCES

1. Shlyakhina, Yu.V., Mokshina, N.Ya., Khokhlov, V.Yu., et al., *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.*, 2006, vol. 49, no. 6, pp. 20–22.
2. Mokshina, N.Ya., *Ekstraktsiya aminokislot i vitaminov*

- (Extraction of Amino Acids and Vitamins), Voronezh: Voronezh. Gos. Tekhnol. Akad., 2007.
3. Kirsh, Yu.Ya., *Poli-N-vinilpirrolidon i drugie poli-N-vinilamidy* (Poly-N-vinylpyrrolidone and Other Poly-N-vinylamides), Moscow: Nauka, 1998.
 4. Tanchev, S.S., *1Antotsiany v plodakh i ovoshchakh* (Anthocyanins in Fruits and Vegetables), Moscow: Pishch. Prom-st., 1980.
 5. Kharlamova, O.A. and Kafka, B.V., *Natural'nye pishchevye krasiteli* (Natural Food Dyes), Moscow: Pishch. Prom-st., 1979.
 6. RF patent 2228344.
 7. Nifant'eva, T.I., Matousova, V., Adamcova, Z., and Shkinev, V.M., *Vysokomol. Soedin., Ser. A*, 1989, vol. 31, no. 8, pp. 2131–2135.
 8. Molochnikova, N.P., Shkinev, V.M., and Myasoe-dov, B.F., *Radiokhimiya*, 1995, vol. 37, no. 5, pp. 385–397.
 9. Kirsh, Yu.E., Yanul', N.A., Popkov, Yu.M., and Timashev, S.F., *Zh. Fiz. Khim.*, 1999, vol. 73, no. 2, pp. 313–319.
 10. Goto, T., *Prog. Chem. Org. Nat. Prod.*, 1987, vol. 52, pp. 113–158.
 11. Kirsh, Yu.E., Semina, N.V., Yanul', N.A., and Shatalov, G.V., *Zh. Fiz. Khim.*, 1994, vol. 68, no. 9, pp. 1584–1586.