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SELECTIVITY PROPERTIES OF POLY-N-VINYL PYRROLIDONE IN COLUMN CHROMATOGRAPHY OF NUCLEOTIDES, THEIR DERIVATIVES, AND RELATED COMPOUNDS: A PRELIMINARY REPORT*

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SUMMARY

Insoluble poly-N-vinyl pyrrolidone (PVP) has been found to possess selectivity properties toward various nucleotide derivatives and can be used for column chromatography employing water as eluent. Compounds emerge from the columns in this order: nucleotides, pyrimidines, purines. Total elution volumes and times for the series of compounds investigated are 45 ml (5 h) with use of a 0.9 × 22.3 cm column and 20 ml (65 min) with use of a 0.9 × 10.8 cm column.

INTRODUCTION

Poly-N-vinyl pyrrolidone can be used effectively as a complexing agent in removing polyphenols from plant enzyme preparations[1–3]. Recently it has been applied in its insoluble form by QUARMBY[1] for effecting thin-layer chromatographic separations of phenolic acids and flavonoids. He found PVP to bind phenolic compounds tenaciously and very polar solvents were necessary to move them from the plate origin. GUSTAVSON[4,5] discovered that PVP-vegetable tannin complexes can be disrupted by treatment with 6–8 *M* urea and that hydrogen bonding to hydroxyl groups of tannins is the main interaction in complex formation. Recently, ANDERSEN AND SOWERS[3] investigated conditions for PVP–plant phenol complex formation and noted binding to increase in the series, scopoletin, caffeic acid, and quercetin, *i.e.*, in the order of increasing number of free hydroxyl groups. They also reported rutin to bond least when its phenolic hydroxyls are dissociated, *e.g.*, in alkaline solution. Likewise the glycoside of scopoletin, scopolin, does not bind the polymer[3]. PVP has also been incorporated into Gas-Chrom P** (a support for use in gas chromatography) where it has been found to increase column affinity toward alcohols and selectivity for

* The following abbreviations will be used: AMP = adenosine monophosphate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; UMP = uridine monophosphate; XMP = xanthosine monophosphate.

** Mention of trade or company names does not imply endorsement by the Department over others not named.

closely related plant sterols[6]. Thus the retention times of dihydroxy alkaloids, such as morphine and reticuline, on a treated support are more than doubled[6]. PVP was also noted to be selective for aromatic moieties as evidenced by the increased retention times of papaverine and cinchona alkaloids, which contain the quinoline function[6]. Another application has been the separation of lipoproteins by flotation in solutions containing sodium chloride and PVP[7].

It is the purpose of this study to offer a preliminary report on the applicability of PVP in separations of certain nucleotide derivatives and related compounds.

METHODS

A commercial preparation of PVP, obtained from the General Aniline and Film Corporation (New York), was used in these studies. The polymer is sold under the trade name of Polyclar AT Powder, which, according to the manufacturer, is a high molecular weight, cross-linked form of PVP and is insoluble in water, organic solvents, acid, and alkali[8,9]. Polyclar AT particles range in size from greater than 60 B. S. mesh (250 μ) to less than 300 B. S. mesh (53 μ) and are known to swell slightly in water[1]. In preparing chromatographic columns, the powder was mixed with distilled water and allowed to settle with repeated decantation of the fines. The slurry was poured into two small columns of identical dimensions, designated A and B (0.9 × 10.8 cm), and into a longer column C (0.9 × 22.3 cm). In all experiments, distilled water (pH 6) was used as eluent, and this was delivered to the columns under conditions of room temperature (25°) and atmospheric pressure. Flow rates averaged 0.3 ml/min for columns A and B, and 0.15 ml/min for column C. All compounds were obtained from Nutritional Biochemical Co., except ADP and ATP, which were purchased from Sigma Chemical Co., and were used without further purification. They were applied singly or in mixtures to the columns, in amounts between 0.1 mg and 0.5 mg, and in 0.2 ml distilled water. Eluate was collected in 0.3 or 0.4 ml fractions and monitored at 260 mμ in a Gilford 220 spectrophotometer.

RESULTS AND DISCUSSION

In our initial investigations with PVP, short columns were made to study the selectivity properties of this polymer toward various nucleotides, their derivatives, and related compounds. Table I gives a list of these substances with their respective elution volumes. Band widths are given to illustrate peak spreading. Vitamin B_{12} appeared first in the elution series; this may be due to exclusion from the PVP matrix because of its large size. If this is the case, the amount of its hydrogen and hydrophobic bonding to PVP would be small, because such interactions would be limited to the particle surface. The early elutions of AMP, UMP, and 2'-AMP, despite their possession of bonding groups, may be indicative of a repulsive, electrostatic interaction between the negatively charged phosphate group in the nucleotides and the negatively polarized PVP carbonyl group. No adequate explanation can be given for the elution of riboflavin phosphate at an elution volume greater than that of the nucleotides or riboflavin. In Table I it is seen that the retention volumes of the pyrimidines range from about 9 ml to 10.5 ml. These compounds, as will be discussed below, bond weakly to the PVP matrix. On the other hand, the purines (and adenosine) bond

TABLE I

ELUTION VOLUMES OF NUCLEOTIDES, THEIR DERIVATIVES, AND RELATED COMPOUNDS ON A 0.9 × 10.8 cm COLUMN

Compound	*Elution volume*[a,b] *(ml)*	*Band width*[c] *(ml)*
Vitamin B_{12}	6.0	
AMP	7.4	0.9
UMP	7.9	0.9
2′-AMP	8.0	
Riboflavin	8.1	
5-Aminouracil	8.7, 9.3	0.9
Thymine	9.3, 9.3, 9.7	
Cytosine	9.9	
6-Methyluracil	10.4[d]	
5-Nitrouracil	10.5, 10.5	
Riboflavin phosphate	10.7[d]	
Xanthine	12.6, 12.6	1.6
Adenosine	13.8	1.3
Adenine	18.9, 19.5	1.9

[a] Volume of eluate to maximum concentration.
[b] Each figure is the value obtained in a single experiment.
[c] Peak width at half-maximum concentration.
[d] Indicates elution on column B; other values are from column A.

more strongly to the matrix and therefore are removed last from the column. Thus it is observed that short columns of PVP can be used with water as eluent to effect class separations of certain nucleotides from pyrimidines as well as pyrimidines from purines (and adenosine) in a small volume of eluate. This is done rapidly (in 65 min) under conditions of room temperature and atmospheric pressure.

Selectivity properties of PVP were examined further using a longer column, C, and distilled water again as eluent. Fig. 1 indicates that no separation of the mono-, di-, and triphosphates of adenosine from each other could be effected. Similarly, XMP was eluted with a retention volume of 14.1 ml. This was judged not to be significantly different from the volume obtained for AMP, ADP, and ATP. These nucleotides precede several pyrimidines that are also not separable. Regarding the elutions of two pyrimidines, thymine and uracil, it is assumed that each can form two hydrogen bonds with the pyrrolidone carbonyl groups of PVP. In the keto form, this interaction could involve a proton on the secondary nitrogen atoms. It is also of interest that the methyl group in thymine does not appear to influence retention of this compound. The elution behavior of 5-aminouracil seems to suggest that only two

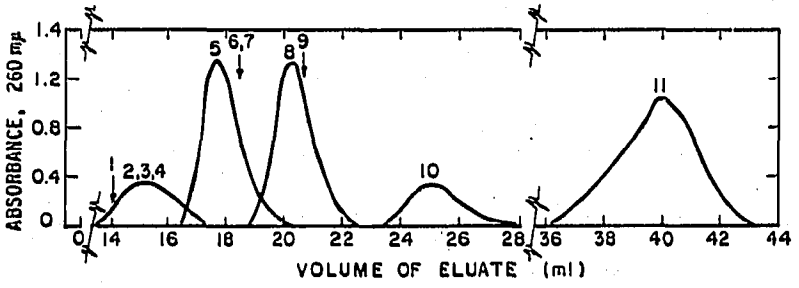

Fig. 1. Chromatogram of nucleotides and bases on column C (0.9 × 22.3 cm). 1 = XMP; 2 = AMP; 3 = ADP; 4 = ATP; 5 = uracil; 6 = thymine; 7 = 5-aminouracil; 8 = hypoxanthine; 9 = xanthosine; 10 = xanthine; 11 = adenine.

hydrogen bonds are formed between this substance and PVP. Thus, it can be postulated that the primary amino group in 5-aminouracil participates in an internal hydrogen bond with its carbonyl thereby forming a five-membered ring.

Although hypoxanthine, like the pyrimidines mentioned, only has two protons available for hydrogen bonding to the column, it has a greater retention volume. Perhaps the reason for this is its larger ring system which provides for a greater degree of nonpolar interactions with the pyrrolidone matrix. COHN[10] has observed that purine bases such as xanthine and hypoxanthine are retained to a small degree even on cation exchange resins, and this he attributes to nonpolar attractions. The influence of hydrogen bonding on elution volume is seen in the case of xanthine which has three available protons and hence a greater retention than hypoxanthine. Also, the strong retention of adenine on column C as well as on Sephadex G-10, as reported by SWEETMAN AND NYHAN[11], can be partly explained by its ability to form hydrophobic bonds to column backbones. Furthermore, while only two protons in this compound can hydrogen bond to PVP, it is thought that the presence of sp^3 orbitals in the primary amino group increases the hydrogen-donating effect by enhancing proton availability as compared with the hydrogen-donating properties of the shorter sp^2 bonds of secondary nitrogen groups. Since the primary amino group of adenine has a pK of 4.1[10], hydrogen bonding at pH 6 involves about 1 % of the charged and about 99 % of the uncharged species.

The elution of xanthosine in a retention volume of 20.7 ml (Fig. 1) indicates the effect of the ribofuranosyl moiety in reducing elution volume as compared to the free base (see data for adenine and adenosine in Table I).

Table II gives reproducibility and recovery characteristics of a PVP column. Application of a mixture of three bases to column C was repeated three times on different days. The data show that the column can be used repeatedly with only small variability in retention volume for a given compound. Similarly recovery values were found to be high. The lack of tailing (Fig. 1) is also indicative of reversible adsorption of solute. The elution profiles obtained on this column are generally symmetrical,

TABLE II

REPRODUCIBILITY AND RECOVERY CHARACTERISTICS OF CHROMATOGRAPHY ON PVP

Reproducibility experiment: application of a mixture of uracil, xanthine, and adenine to column C; eluent, distilled water. Recovery experiment: application of xanthosine, ATP, and hypoxanthine singly to column C; eluent, distilled water.

Compound	*Retention volume (ml)*		
	Run 1	*Run 2*	*Run 3*
Uracil	17.6	17.6	17.7
Xanthine	25.4	25.1	24.9
Adenine	41.3	41.9	40.0
	Amount applied (mg)		*% recovered*
Xanthosine	0.13		93
ATP	0.13, 0.50		100, 100
Hypoxanthine	0.29		88

except for adenine, which tends to show some peak "heading", and the separation of classes is good.

Some advantages are evident in the use of water; it is a mild eluent and allows sample recovery uncontaminated with salts. In reference to solute sorption effects, hydrogen bonding in a distilled water system undoubtedly is more effective than in eluents containing electrolytes. In the latter case, salt can disrupt polymer–solute interactions.

The use of small columns, although limiting sample size, permits collection in a minimal volume of eluate. With the longer column (C) it is possible to separate certain purines, nucleotides from pyrimidines, and pyrimidines from purines (and xanthosine) in a volume of water less than 45 ml (in about 5 h). This system should have applicability for rapid preliminary separation of these classes of compounds.

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