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# Functional Monomers and Polymers. LXXIX.* Application of Nucleic Acid Base Containing Polystyrene Resin to High Performance Chromatography

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## ABSTRACT

Polystyrene resins having pendant nucleic acid bases were prepared. They were found suitable for high performance liquid chromatography to separate N-substituted nucleic acid bases effectively. From the measurements of retention times and peak resolution values, it became evident that the specific base-base interaction between the solutes and the resin plays an important role in the separation purpose.

## INTRODUCTION

Much attention has been directed recently to the practical uses of synthetic polymers having pendant nucleic acid bases, particularly in the medicinal and technochemical fields [1]. One of the promising applications appears to lie in chromatography since the specific interaction realized between nucleic acid bases can be utilized for separating nucleosides, nucleotides, and their purine and pyrimidine

*For Part LXXVIII of this series, see K. Kondo, N. Mifune, S. Morita, and K. Takemoto, J. Polym. Sci., Polym. Lett. Ed., 18, 553 (1980).

bases, in a column packed with a resin containing complementary nucleic acid bases [2-6].

On the other hand, by using high performance liquid chromatography, separation of nucleosides, nucleotides, and their purine and pyrimidine bases has generally been done by a column packed with ionic exchange resins.

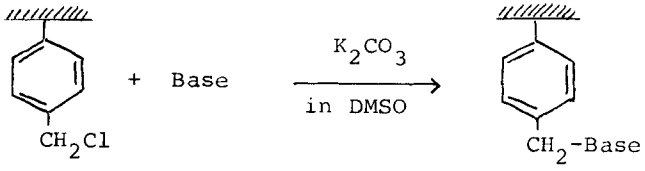
We have been searching for different types of synthetic resins applicable to high performance liquid chromatography, which would enable separation of purine and pyrimidine bases by using the specific interaction character between complementary bases. It is known that the strength of such an interaction in chloroform solution is in the order purine-pyrimidine > purine-purine > pyrimidine-pyrimidine, since the hydrogen bonding between complementary bases is rather favored, while a hydrophobic interaction predominates in water which changes the order to purine-purine > purine-pyrimidine > pyrimidine-pyrimidine [7]. If such an interaction character can be applied to the chromatographic techniques, a resin having pendant pyrimidine bases would elute out pyrimidine bases more rapidly than purine bases in chloroform, while a resin having pendant purine bases would elute out purine bases more rapidly than pyrimidine bases in chloroform, and vice versa in water.

The present paper deals with polystyrene resins having pendant nucleic acid bases which were used for high performance chromatography to separate N-substituted nucleic acid bases.



## RESULTS AND DISCUSSION

The resins for chromatography were prepared from the reaction of p-chloromethylated polystyrene with the nucleic acid bases (Scheme 1). Thus we prepared the polystyrene resins having pendant




Base: adenine (Ad), uracil (Ur)

SCHEME 1.

nucleic acid bases with different degrees of substitution (Table 1). The resins obtained were packed in a column, which was then used

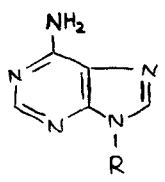
TABLE 1. Polystyrene Resins for High Performance Chromatography

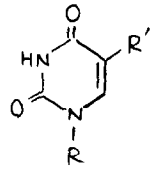
| Base | Reaction time (h) | Degree of substitution (%)[a] | Resin no. |
|---|---|---|---|
| Ur | 15 | 6 | Ⓟ-U6 |
| | 21 | 9 | Ⓟ-U9 |
| | 168 | 18 | Ⓟ-U18 |
| Ad | 24 | 10 | Ⓟ-A10 |
| | 168 | 20 | Ⓟ-A20 |

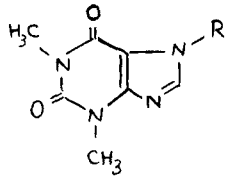
[a]Obtained from elementary analysis.



for high performance chromatographic analysis. The purine and pyrimidine derivatives shown in Scheme 2 were tested for separation.







Derivatives of

Adenine Thymine ( $R'=CH_3$ ) Theophylline
Uracil ( $R'=H$ )

R; $CH_3CH_2CH_2$- ( Pr ) and $CH_2=CH$- ( V )

SCHEME 2.

When the mixture of adenine and thymine derivatives was chromatographed by using the resin Ⓟ-A10 in chloroform, the adenine derivatives were found to be more easily eluted than the thymine derivatives. Figure 1 shows the chromatogram after separation of the base mixture of 9-propyladenine (Pr-Ad) and 1-propylthymine (Pr-Thy) by using the resin Ⓟ-A10. The result suggests that the separation was caused by complementary hydrogen bonding between the solute and the resin, since the adenine moiety on the resin is assumed to attract Pr-Thy rather than Pr-Ad. This seems also to

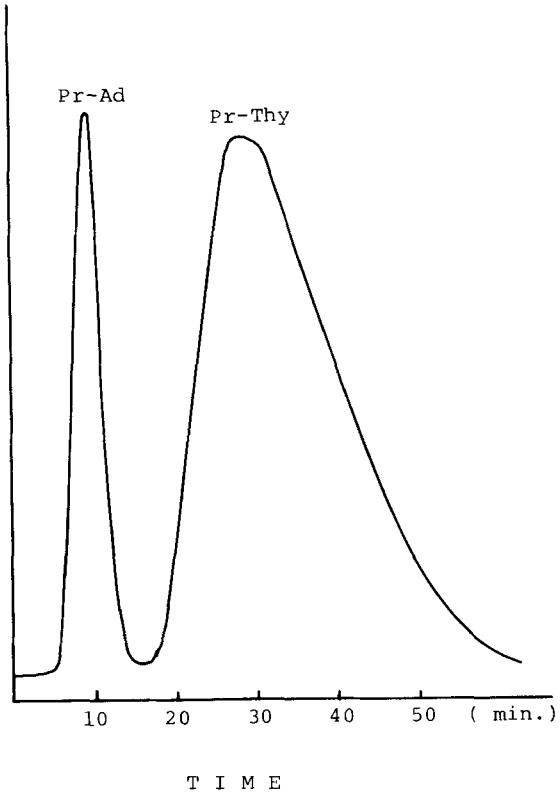



FIG. 1. Separation of Pr-Ad and Pr-Thy on Ⓟ-A10 Resin. Eluent: $CHCl_3$. Flow rate: 20 mL/h.

be supported by the fact that 7-propyltheophylline (Pr-The) was eluted much more rapidly than other bases, because such a specific base-base interaction cannot be expected in the case of Pr-The.

For practical purposes, the columns are evaluated from such parameters as the peak resolution (Rs) and the separation factor ($\alpha$), as given by

$$Rs = 2\frac{t_2 - t_1}{w_2 + w_1} \tag{1}$$

$$\alpha = \frac{t_2 - t_1}{t_1 - t_0} \tag{2}$$

where t and w denote the retention time and the peak width, respectively. Rs is also a parameter related to peak overlapping. A value of peak overlapping of 0.5% corresponds nearly to an Rs value of 0.8, and that of 0.2% corresponds nearly to an Rs value of 1.25. The column having an Rs value over 1.0 is believed to be suitable for practical use. The $\alpha$ value is correlated to the thermodynamic difference in the free energy:

$$\Delta(\Delta G^\circ) = -RT \ln \alpha \quad (3)$$

The value of Rs obtained from the chromatogram in Fig. 1 was 0.8. Taking into account the fact that no interaction is expected for Pr-The (= $t_0$), the difference in the free energy can be estimated from the $\alpha$ value to be 1.01 kcal/mol for the system Pr-Ad and Pr-Thy, which corresponds qualitatively to the energy difference in hydrogen bonding between Ad-Thy and Ad-Ad.

As compared with Ⓟ-A type resins, Ⓟ-U type resins were rather suitable for separating the mixture of adenine and thymine derivatives in chloroform (Fig. 2). In particular, Ⓟ-U18 resin showed an Rs value of 1.80 for vinyl and 2.20 for propyl derivatives, which had improved resolution and peak sharpness. The high resolution by Ⓟ-U18 resin appears to be interpreted in terms of controlled diffusion. In addition, chromatograms for VAd-VThy and VAd-VUr were easily distinguished on Ⓟ-U18, so that they are useful for evaluating the interaction between pyrimidine bases (Fig. 3).

Differences in the free energy of 0.95 kcal/mol for VAd-VThy and of 0.76 kcal/mol for VAd-VUr suggest that interaction by hydrogen bonding should be larger between Ⓟ-U resin and Ur than between Ⓟ-U resin and Thy.

Thus effective separation was attained by the hydrogen bonding character on Ⓟ-U resin in chloroform. Such an interaction was also verified for 6-dimethylamino-9-vinylpurine (DMAP), 6-methylamino-9-vinylpurine (MAP), and 9-vinyladenine (V-Ad). As can be seen from Fig. 4, DMAP was more rapidly eluted than MAP and V-Ad. This fact indicates that the amino group substituted at Position 6 of the purine ring might effectively take part in forming hydrogen bonding with the Ⓟ-U resin.

From all the results mentioned here, it is evident that the specific interaction between the solute and the resin can effectively be realized in chloroform. In protic eluents such as methanol and water, however, no separation was attained and, as a result, the peaks were completely overlapped. Moreover, as polystyrene itself has no separation ability in chloroform, it is essential that the resin be effectively substituted for by the nucleic acid bases to facilitate separation.

Examples of the high performance liquid chromatography are shown in Table 2. For the separation of N-substituted purine and pyrimidine bases which are soluble in chloroform, the Ⓟ-U type of

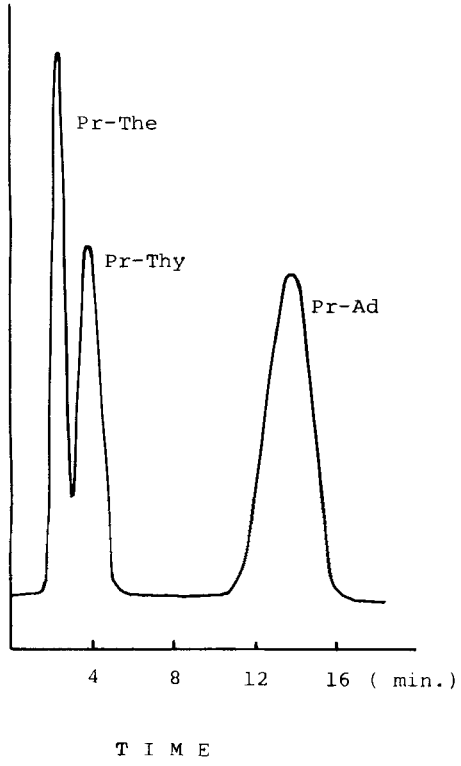



FIG. 2. Separation of Pr-The, Pr-Ad, and Pr-Thy on Ⓟ-U18 Resin. Eluent: $CHCl_3$. Flow rate: 20 mL/h.

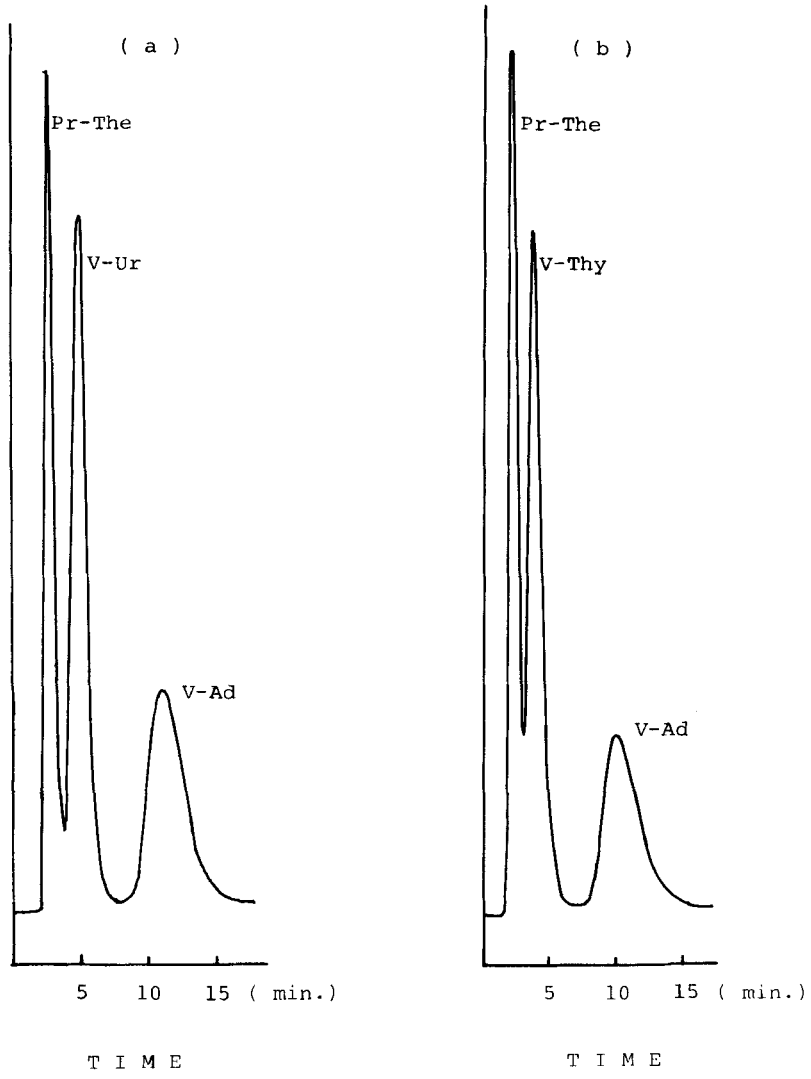



FIG. 3. (a) Separation of Pr-The, V-Ad, and V-Ur on Ⓟ-U18 Resin. (b) Separation of Pr-The, V-Ad, and V-Thy on O -U18 Resin. Eluent: $CHCl_3$. Flow rate: 20 mL/h.

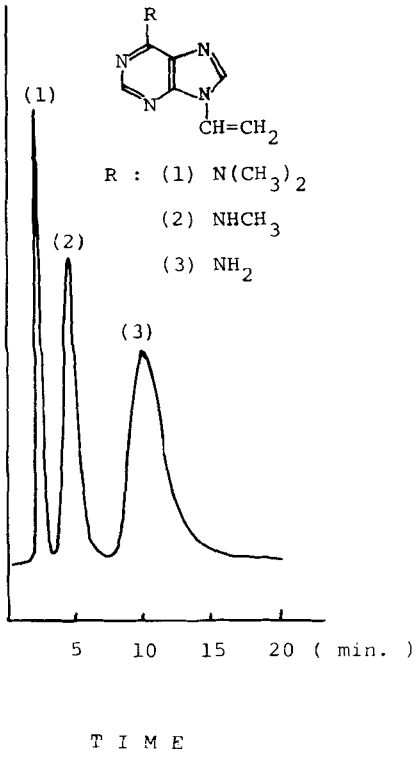



FIG. 4. Separation of DAMP (1), MAP (2), and V-Ad (3) on Ⓟ-U18 Resin. Eluent: $CHCl_3$. Flow rate: 20 mL/h.

resins are superior to the Ⓟ-A type because they have larger Rs values and smaller retention times. The former resins, however, were not able to separate water-soluble solutes, such as nucleic acid bases, effectively since the resins used here were hydrophobic in nature. Therefore, it is important to make the resins hydrophilic for practical use as observed in the case of the hydrophilic microporous polymer gels having thymine moieties [6]. Further work is now in progress on this point, and the results will be published in the future.

TABLE 2. High Performance Liquid Chromatographic Analysis for a mixture of 9-Propyladenine, 1-Propylthymine, and 7-Propyltheophylline

| Resin | Eluent | retention time (min:s) Pr-The | Pr-Thy | Pr-Ad | Rs |
|---|---|---|---|---|---|
| Polystyrene | $CH_3CN$[a] | 3:12 | 3:12 | 3:36 | - |
| Ⓟ-A10 | $CHCl_3$ | | 28:24 | 8:48 | 0.89 |
| Ⓟ-U6 | $CHCl_3$ | 2:24 | 3:24 | 8:58 | 0.95 |
| Ⓟ-U9 | $CHCl_3$ | 2:24 | 3:36 | 9:10 | 1.04 |
| | $CH_3OH$ | | 3:22 | 3:36 | - |
| Ⓟ-U18 | $CHCl_3$ | 2:20 | 3:50 | 12:58 | 2.20 |
| | $CH_3CN$ | 3:48 | 4:10 | 13:31 | 1.06 |
| | $H_2O$ | 2:29 | 2:48 | 2:48 | - |

[a]In this case, $CHCl_3$ was not used in order to avoid overpressure.



## EXPERIMENTAL

### Preparation of P-A and P-U Resins

One gram of 24% chloromethylated polystyrene (2% cross-linked with divinylbenzene, 100-200 mesh) was stirred with 270 mg of adenine or 240 mg of uracil in dimethylsulfoxide in the presence of potassium carbonate (140 mg) at 25-30°C at definite times. The precipitates were filtered, washed with aqueous sodium carbonate solution, followed by water and methanol, and dried in a vacuum oven.

### Solutes

The synthesis of propyl [8] and vinyl derivatives [9, 10] of purine and pyrimidine bases followed descriptions in the literatures.

### Column Packing

Resins made in slurry form in acetonitrile were packed into a column of 50 cm in length and 2 mm in inner diameter under a constant flow of acetonitrile.

## Chromatographic Measurements

The column packed with Ⓟ-U or Ⓟ-A resin was attached to a Varian Aerograph LC 4200, 3 to 25 $\mu$L of the solute solution ($5 \times 10^{-4}$ to $10^{-5}$ mol/L) was injected, and the chromatograms were recorded by using the absorbance at 260 nm.

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