Journal of Chromatography, 260 (1983) 161–163
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,631

Note

Volatile buffer system for high-performance anion-exchange chromatography of nucleotides

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(Received November 3rd, 1982)

In most analytical procedures for high-performance liquid chromatography (HPLC) of nucleotides, alkali-metal or ammonium phosphates were used as non-volatile components[1–4]. However, the salt content may cause interference if the nucleotides are to be recovered chemically pure. Recently, reversed-phase columns and elution with a gradient of increasing methanol concentration or ion-pair chromatography[5,7], ion-exchange chromatography with HCl[8] or trifluoroacetic acid (TFA)[6] as eluents were utilized for purification of nucleotides. While reversed-phase procedures are not very suitable for separation of nucleoside di- en triphosphates[7], elution with strong acidic compounds is not recommended for isolation of acid labile nucleotides nor is it very suitable for the materials of the chromatographic system.

The method presented here is based on Aminex A-25 resin and ammonium carbonate as eluent.

EXPERIMENTAL

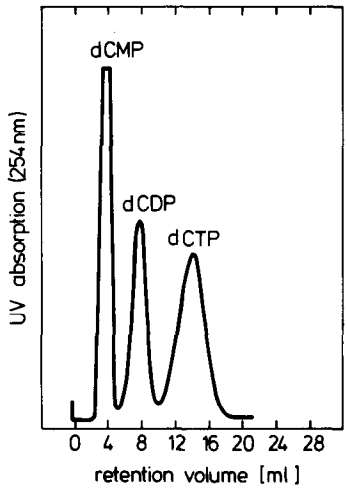
Chemicals

Aminex A-25 anion-exchange resin was obtained from Bio-Rad Labs. (Munich, G.F.R.). The deoxynucleotides were purchased from Boehringer (Mannheim, G.F.R.). [U-^{14}C]dCTP (specific activity 340 mCi/mmol) from the Radiochemical Centre (Amersham, Great Britain). All other reagents were from E. Merck (Darmstadt, G.F.R.). Buffers were prepared as 1 *M* stock solutions with triply distilled water and filtered through 0.2-μm membrane filters (Schleicher & Schüll). The solutions were diluted in triply distilled water before use and degassed by sonication or helium purging.

Apparatus

For chromatography an HPLC apparatus was used, equipped with a Latek injection valve and an UV-absorbance detector from Waters Assoc. (Model 440). A 200 × 4 mm stainless-steel column packed with Aminex A-25 anion-exchange resin (17.5 μm) was used throughout this study. Routinely, the column was run at a pressure of 140 bar at room temperature. A flow-rate of 1.2 ml/min was maintained under such conditions.

0021-9673/83/$03.00



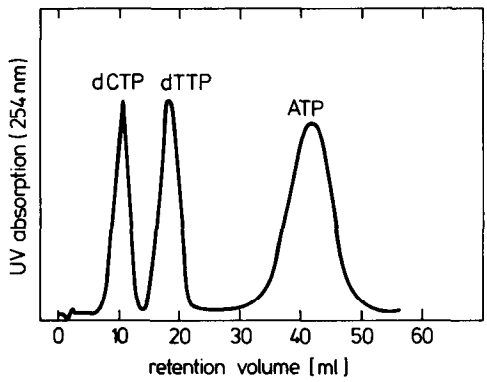


Fig. 1. Elution profile of a mixture of deoxycytidine mono(dCMP)-, di(dCDP)- and triphosphates (dCTP) with 0.4 *M* ammonium carbonate solution. For other conditions see text.

Fig. 2. Isocratic separation of dCTP, dTTP and ATP with 0.5 *M* ammonium carbonate.

RESULTS AND DISCUSSION

Initial attempts were made to purify small amounts of commercial ^{14}C-labelled dCTP from unknown impurities. Gel filtration and reversed-phase chromatography with water as eluent were not efficient enough. The corrosive nature of HCl and the fact that nucleoside triphosphates hydrolyse rapidly in acidic solvents were the main reasons to evaluate another volatile solvent system as eluent.

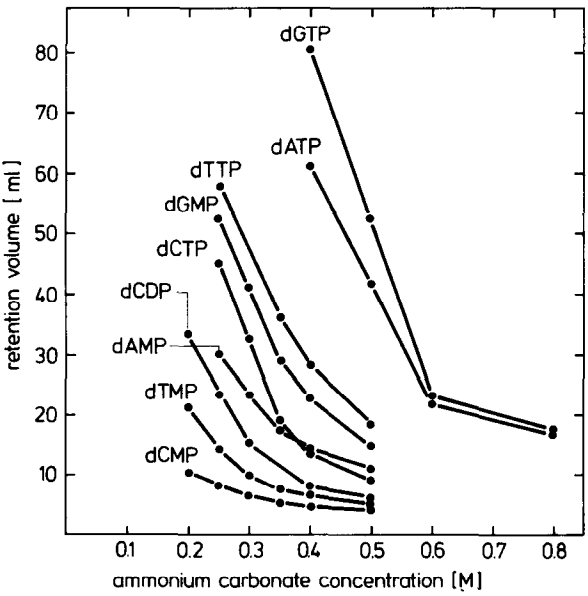


Fig. 3. Variation of retention volume with concentration of eluent for a variety of nucleotides.

Fig. 1 shows a typical isocratic elution profile of a mixture of deoxycytidine mono-, di- and triphosphates using 0.4 *M* ammonium carbonate. It is recommended to start with a low concentration run and to increase the ammonium carbonate concentration in one or two steps. Linear gradients have not proved very useful, as they cause peak broadening and lead to poorer resolution.

The method was used for separation of purine and pyrimidine nucleotides. It was generally found that pyrimidine mono-, di- and triphosphates were less strongly retained on the A-25 resin than purines under similar conditions. This result may be very useful for the purification of enzymatically synthesized pyrimidine triphosphates[9]. In these reactions ATP serves as pyrophosphate donor and is usually added in large excess. Ion-exchange procedures were not suitable to separate the product triphosphate from ATP[10], and ATP had to be removed by periodate oxidation in a further reaction step[11,12]. Fig. 2 demonstrates that, with the system described here, dCTP and dTTP are eluted from the column under conditions where ATP is still strongly retained. To illustrate quantitatively the chromatographic capabilities of the system, ^{14}C-labelled dCTP was used as test sample. Usually, 92% of the radioactivity injected could be recovered.

The dependence of the retation volume on concentration of the elution mixture for several nucleotides is shown in Fig. 3. These curves are helpful in choosing the concentration of the eluent so as to optimize the separation of a given mixture of nucleotides. Purine triphosphates were eluted in a reasonable time only if the ammonium carbonate concentration was $\geqslant 0.5$ *M*; even then elution proceeded slowly, resulting in broad peaks. Except for this weakness, the advantages of the method presented are as follows. It is a rapid procedure for separation and purification of small amounts of nucleotides in volatile solution which can easily be removed by evaporation under reduced pressure to yield chemically pure nucleotides. As nucleotides are generally more susceptible to acid hydrolysis, the alkaline ammonium carbonate can slow down or even prevent hydrolysis of the material during handling. For purification of tritiated nucleotides with acid labile protons, this method may be a great help. Finally, the separation of enzymatically synthesized triphosphates from excess of ATP can be performed easily, making additional purification steps superfluous.

REFERENCES

1 R. A. Hartwick and Ph. R. Brown, *J. Chromatogr.*, 112 (1975) 651.
2 A. Wakizaka, K. Kurosaka and E. Okuhara, *J. Chromatogr.*, 162 (1979) 319.
3 M. Ehrlich and K. Ehrlich, *J. Chromatogr. Sci.*, 17 (1979) 531.
4 C. Garrett and D. V. Santi, *Anal. Biochem.*, 99 (1979) 268.
5 R. W. Tyson and G. Wickstrom, *J. Chromatogr.*, 192 (1980) 485.
6 J. T. Axelson, J. W. Bodley and T. F. Walseth, *Anal. Biochem.*, 116 (1981) 357.
7 T. F. Walseth, G. Graff, M. C. Moos and N. D. Goldberg, *Anal. Biochem.*, 107 (1980) 240.
8 D. S. Hsu and S. S. Chen, *J. Chromatogr.*, 192 (1980) 193.
9 R. B. Hurlbert, *Methods Enzymol.*, 3 (1951) 785–805.
10 R. H. Symons, *Methods Enzymol.*, 29 (1974) 102–115.
11 R. Wu, *J. Mol. Biol.*, 51 (1970) 501.
12 P. T. Englund, *J. Mol. Biol.*, 66 (1972) 209.