Journal of Chromatography, 253 (1982) 133–137
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,277

Note

Preparative separation of nucleosides and nucleotides on a non-ionic gel column

SHOHEI AOYAGI*, KIMIKO HIRAYANAGI, TAKAO YOSHIMURA and TOSHIO ISHIKAWA
Institute of Natural Color Technology, Faculty of Engineering, Chiba University, 1-33 Yayoi-cho, Chiba 260 (Japan)
(Received August 9th, 1982)

We have been working on the synthesis of nucleotide derivatives as substrates for nucleases[1,2] and are interested in simple and effective preparative separations of nucleosides and nucleotides.

Excellent separations of these compounds by high-performance liquid chromatography (HPLC) have been reported[3–13]. However, there are some problems with preparative separations since the adsorbents are generally very expensive for packing large columns, and the desalting of separated fractions is difficult. In this report we describe a simple separation of nucleosides and nucleotides by adsorption chromatography on a column of Toyopearl HW-40. Good separations are often obtained by isocratic elutions with volatile buffer systems. The simplicity is an advantage over standard ion-exchange chromatography with gradient elutions, and there is no problem in packing large columns with this rigid, non-ionic gel.

EXPERIMENTAL

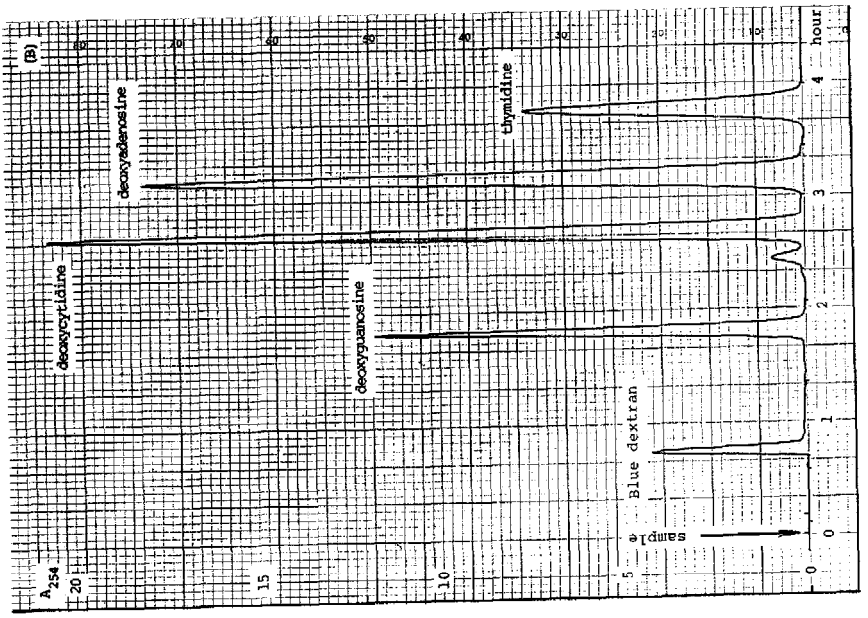
Materials

TSK-gel Toyopearl HW-40S (particle size: 20–40 μm) was obtained from Toyo Soda (Tokyo, Japan). This material is the same as Fractogel TSK HW-40, 0.025–0.037 mm, available from E. Merck (Darmstadt, G.F.R.). Blue Dextran 2000 was from Pharmacia (Uppsala, Sweden). Nucleosides and nucleotides were obtained from Yamasa Shoyu (Tokyo, Japan), Wako (Osaka, Japan), Worthington (Freehold, NJ, U.S.A.) and P-L Biochemicals (Milwaukee, WI, U.S.A.). All other chemicals were of analytical reagent grade and purchased from Wako or Kanto Chemicals (Tokyo, Japan).

Methods

The gel slurry was packed in jacketed K-26 (Pharmacia) or IEC (1.0 cm I.D.; Whatman, Clifton, NJ, U.S.A.) columns by a similar method to that described in ref. 14. Stock 1 *M* solutions of ammonium bicarbonate and ammonium acetate were prepared by dissolving appropriate amounts of the salts in distilled water. Eluents were prepared by diluting 1 *M* stock buffers in distilled water, adjusting the pH (pH meter Model HM-7A; Toa Electronics, Tokyo, Japan) with concentrated ammonium

0021-9673/82/0000–0000/$02.75


(B)
A_{254}
deoxycytidine
deoxyadenosine
deoxyguanosine
thymidine
Blue dextran
sample
hour

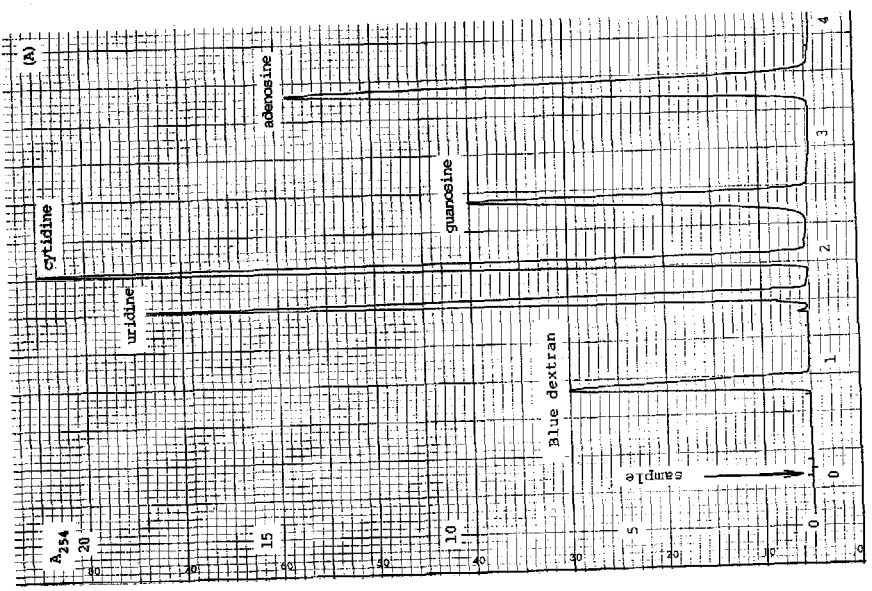

(A)
A_{254}
cytidine
uridine
adenosine
guanosine
Blue dextran
sample

Fig. 1.

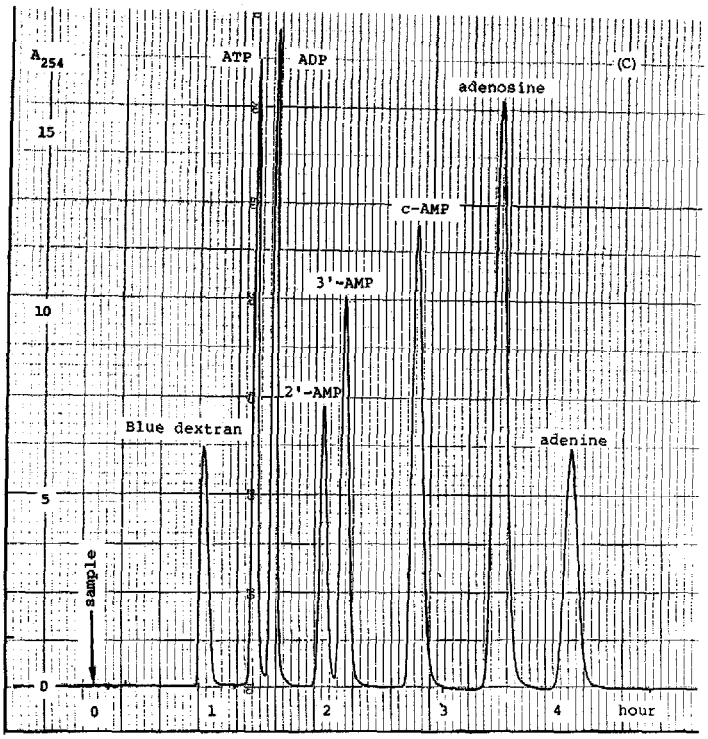


Fig. 1. (A) Separation of the four ribonucleosides. Column: Toyopearl HW-40S (2.6 × 29 cm). Eluent: 0.01 *M* ammonium bicarbonate (pH 9.2); flow-rate, 80 ml/h. Temperature: 20°C. Detection: 254 nm, 25.6 a.u.f.s. (0.05-cm cell). Sample: Blue Dextran 2000 (14.9 mg), and the ribonucleosides indicated (2–5 mg each) in 2 ml of 0.05 *M* ammonium bicarbonate (pH 9.2). (B) Separation of the four deoxyribonucleosides. The chromatographic conditions were as in (A) except that 0.01 *M* ammonium acetate (pH 4.5) was the eluent. Sample: Blue Dextran 2000 (10 mg) and the deoxyribonucleosides indicated (*ca.* 4 mg each) in 2 ml of 0.05 *M* ammonium acetate (pH 4.5). (C) Separation of adenine nucleotides, adenosine and adenine. The chromatographic conditions were as in (A) except that 0.1 *M* ammonium acetate (pH 4.6) was the eluent. Sample: Blue Dextran 2000 (*ca.* 10 mg) and the components indicated (3–5 mg each) in 2 ml of the eluent.

hydroxide or acetic acid and finally adjusting the volume to give the desired concentration. The column temperature was maintained at 20°C by use of a water-bath (Type-FN; Haake, Karlsruhe, G.F.R.). The eluents were delivered to the columns by a peristaltic pump (RP-V; Furue Science, Tokyo, Japan) at a flow-rate of *ca.* 15 ml/h · cm^2. Effluents were monitored by UV absorbance at 254 nm with a Model 230 UV-detector (Chromatronix) equipped with a 0.05-cm flow cell. Elution volumes were determined from the retention times and by use of calibrated cylinders.

RESULTS AND DISCUSSION

For preparative purposes, the following requirements are considered in selecting elution conditions: (1) isocratic elution; (2) volatile buffers (as dilute as possible); (3) avoiding extreme pH values. Typical chromatograms are shown in Fig. 1. The separations are satisfactory despite the above limitations and the results are also quite reproducible. The elution volumes, V_e, and capacity factors, k', of the peaks observed

TABLE I

ELUTION VOLUMES, V_e, AND CAPACITY FACTORS, k', OF NUCLEOSIDES AND NUCLEOTIDES

Column: HW-40S (2.6 × 29 cm).

Compounds	*Eluent*	V_e *(ml)*	k'
(A) Ribonucleosides	0.01 M NH_4HCO_3, pH 9.2		
Uridine		122.2	1.13
Cytidine		149.9	1.61
Guanosine		196.8	2.42
Adenosine		277.9	3.83
(B) Deoxyribonucleosides	0.01 M CH_3COONH_4, pH 4.5		
Deoxyguanosine		153.8	1.80
Deoxycytidine		231.2	3.21
Deoxyadenosine		276	4.03
Thymidine		331.8	5.04
(C) Adenine nucleotides	0.1 M CH_3COONH_4, pH 4.6		
ATP		86.9	0.59
ADP		98.9	0.81
2′-AMP		130.2	1.38
3′-AMP		142.1	1.60
c-AMP		187.2	2.42
Adenosine		242.2	3.43
Adenine		290.4	4.31

are summarized in Table I. The capacity factors were calculated by assuming that Blue Dextran 2000 was eluted at the void volume. This assumption may not be strictly valid, however, V_e of Blue Dextran is quite constant under various elution conditions* which cause a wide change in V_e of each nucleoside and nucleotide.

The elution orders of the solutes are roughly consistent with their hydrophobicities modulated by the ionization of the base moieties. The order observed in Fig. 1B is somewhat unexpected*. In fact, deoxyguanosine at pH 4.5 is much more soluble than expected from the solubility of guanosine at pH 9.2. Also, thymidine is much more insoluble (hydrophobic) than anticipated from the behaviour of uridine.

The capacity of the column was examined on a smaller column to avoid wastage of materials. From Fig. 2 it can be seen that appreciable loadings were possible without serious loss of resolution.

The resolution is not sensitive to the temperature. The shrinkage and swelling of the column bed with changes of ionic strength and flow-rate are very small and the column can be run repeatedly. The method has often been quite effective in medium scale purifications of nucleoside derivatives[15].

* The purpose of this note is to communicate the preparative applicability of the column. The adsorption characteristics of the chromatographic system will be discussed in detail elsewhere.

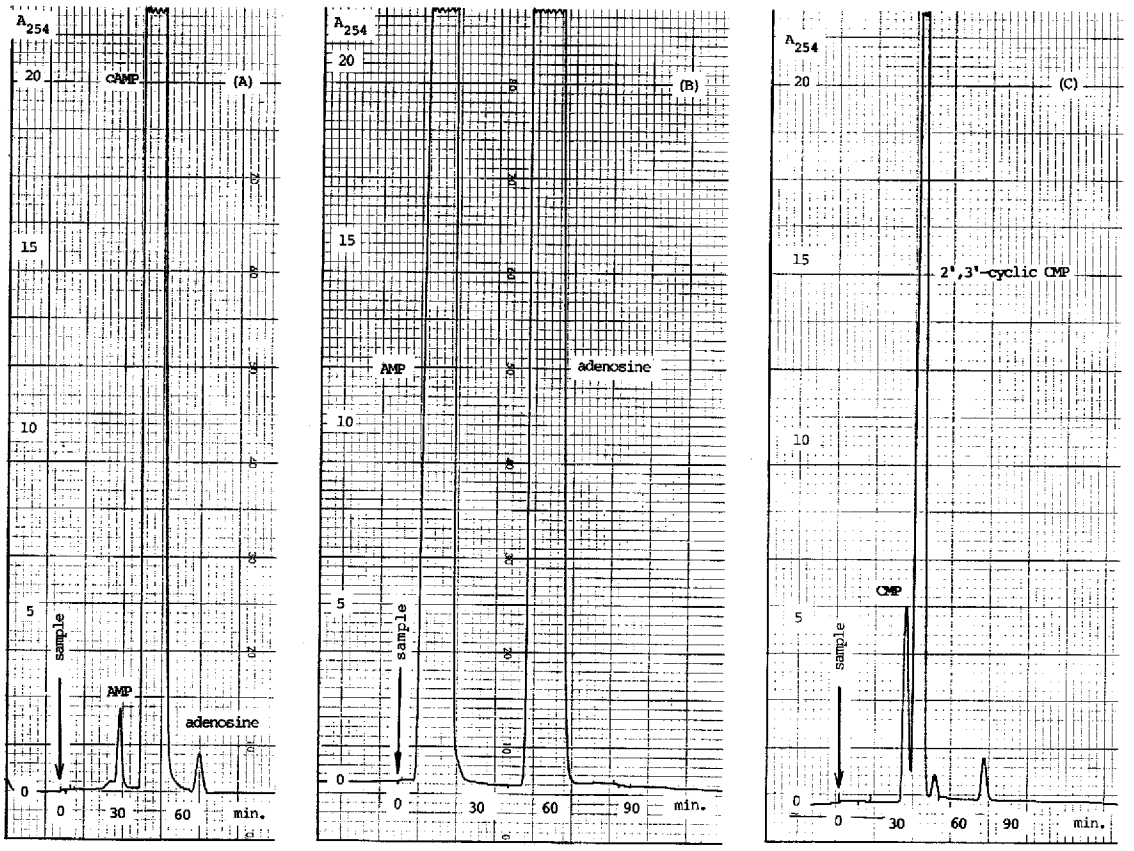


Fig. 2. (A) Removal of AMP from a sample of 3′,5′-c-AMP. Column: Toyopearl HW-40S (21 × 1 cm). Eluent: 0.1 *M* ammonium bicarbonate (pH 8.6); flow-rate, *ca.* 23 ml/h. Temperature: 20°C. Detection: 254 nm, 25.6 a.u.f.s. (0.05-cm cell). Sample: 3′,5′-c-AMP recovered from a reaction mixture, *ca.* 14 mg in 0.2 ml of the eluent. (B) Separation of 2′,3′-AMP and adenosine. The chromatographic conditions were as in (A) except that water was the eluent and flow-rate was *ca.* 25 ml/h. Sample: 2′,3′-AMP · 2Na (*ca.* 15 mg) and adenosine (*ca.* 13.5 mg) in 2.5 ml of water. (C) Removal of 2′,3′-CMP from a sample of 2′,3′-cyclic CMP. Conditions as in (A) except that 0.05 *M* ammonium bicarbonate was the eluent. Sample: 2′,3′-cyclic CMP recovered from a reaction mixture, *ca.* 23 mg in 0.7 ml of 0.14 *M* ammonium bicarbonate.

REFERENCES

1 K. C. Tsou, S. Aoyagi and E. E. Miller, *J. Med. Chem.*, 13 (1970) 765.
2 K. W. Lo, S. Aoyagi and K. C. Tsou, *Anal. Biochem.*, 117 (1981) 24.
3 M. Kratovich and B. A. Roe, *J. Chromatogr.*, 155 (1978) 407.
4 E. H. Edelson, J. G. Lawless, C. T. Wehr and S. R. Abbott, *J. Chromatogr.*, 174 (1979) 409.
5 J. C. Kraak, C. X. Ahn and J. Fraanje, *J. Chromatogr.*, 209 (1981) 369.
6 D. P. Wittmer, N. O. Nuessle and W. G. Haney, Jr., *Anal. Chem.*, 47 (1975) 1422.
7 J. H. Knox and J. Jurand, *J. Chromatogr.*, 203 (1981) 85.
8 R. A. Hartwick, S. P. Assenza and P. B. Brown, *J. Chromatogr.*, 186 (1979) 647.
9 C. W. Gehrke, K. C. Kuo and R. W. Zumwalt, *J. Chromatogr.*, 188 (1980) 129.
10 R. A. Jones, H. J. Fritz and H. G. Khorana, *Biochemistry*, 17 (1978) 1268.
11 J. E. Evans, H. Tieckelman, E. W. Naylor and R. Guthrie, *J. Chromatogr.*, 163 (1979) 29.
12 M. Ryba and J. Beránek, *J. Chromatogr.*, 211 (1981) 337.
13 W. Muller, H. J. Schuetz, C. G. Takada, P. E. Cole and R. Potts, *Nucleic Acids Res.*, 7 (1979) 2483.
14 Y. Kato, K. Komiya, T. Iwaeda, H. Sasaki and T. Hashimoto, *J. Chromatogr.*, 205 (1981) 185.
15 S. Aoyagi and K. Hirayanagi, unpublished results.