CHROM. 6035

The separation of purine and pyrimidine bases and nucleosides by thin-layer chromatography on Sephadex–cellulose and Sephadex–silica gel layers

The use of column chromatography with polymerized gel for the separation of purine, pyrimidine, nucleoside and nucleotide bases has been widespread since 1960[1], whereas that of thin-layer chromatography (TLC), which was introduced in 1962[2,3], has largely been restricted to studies on the chemical composition of proteins and amino acids[4–6], although it has found its application in the purification and separation of nucleotides[7,8], mucopolysaccharides[9] and antibiotics[10].

TLC on Sephadex has hitherto made exclusive use of the descending continuous-flow technique described by JOHANSONN AND RYMO[3,11]. The technique utilizes a layer of wet Sephadex. Transfer of the eluent from the cuvette in which it is contained to the layer of gel is achieved by means of filter paper, and migration assured by tilting the plate from 10° to 45°.

It has also been suggested that wet layers of Sephadex–agar might be used[12,13], but although they are more resilient they cannot be regenerated for further runs.

An ascending technique using a dry layer of powdered dextran had been described by DETERMANN[2]; commercial Sephadex cannot be used as the dextran does not stick to the glass plate very easily and thus causes the substances to be separated to migrate at the same time as the advancing solvent. For these reasons, all subsequent methods are modelled to a greater or lesser extent[5,14,15] on that described by JOHANSONN AND RYMO[3].

This paper describes an ascending chromatography of nucleic acid derivatives on mixed layers of Sephadex–cellulose and Sephadex–silica gel. The advantages such layers have over Sephadex alone are that they are more resilient, make for uniform migration of the eluent and require less development time (4 h as opposed to 20 h).

Experimental

Preparation of the mixed layers of Sephadex G-10. 16 g of fine Sephadex G-10 (Pharmacia, Uppsala) and 4 g of Silica Gel GF_{254} (E. Merck A.G., Darmstadt) or microcrystalline cellulose (E. Merck AG) were repeatedly washed with distilled water, equilibrated with 45 ml phosphate buffer (0.05 M, pH 7.0), and each spread on a glass plate 20 × 40 cm to a depth of 600 μ by an automatic spreading apparatus, "Stratomat" (Chemetron, Milan)[16]. The prepared plates were left for 1 h in a horizontal position and then dried in a stream of hot air until they were almost dry.

Chromatography. Aqueous solutions of adenine, thymine, uracil, xanthine, hypoxanthine, cytosine and of their respective nucleosides (Fluka AG, Buchs, Switzerland) in concentrations of 20 μg equal to 10 μl, were applied in the form of bands about 1 cm in length.

The chromatographic plates were developed with phosphate buffer (0.05 M, pH 7.0) in accordance with the usual technique for ascending chromatography. When development was complete, which, at room temperature, took about 4 h for the Sephadex–silica gel layer and 6 h for the Sephadex–cellulose layer, the plates were dried in a current of warm air and then examined under a UV lamp at a wavelength of 254 nm.

The compounds in question appeared as violet spots on the white or fluorescent yellow-green, background of the chromatographic plates.

Regeneration of the gel. The mixtures of Sephadex–cellulose and Sephadex–silica gel were scraped off the glass plate and regenerated by washing repeatedly with distilled water. When the gel has been equilibrated with phosphate buffer (0.05 *M*, pH 7.0) it may be re-used with reproducible results.

Results and discussion

Fig. 1 shows a chromatogram of the type which affords good separation of the compounds in question.

Table I shows the comparative $R_{F(\mathrm{uridine})}$ values obtained using ascending chromatography on mixed layers and on layers of silica gel or cellulose by themselves, and those obtained using descending chromatography on Sephadex G-10 alone.

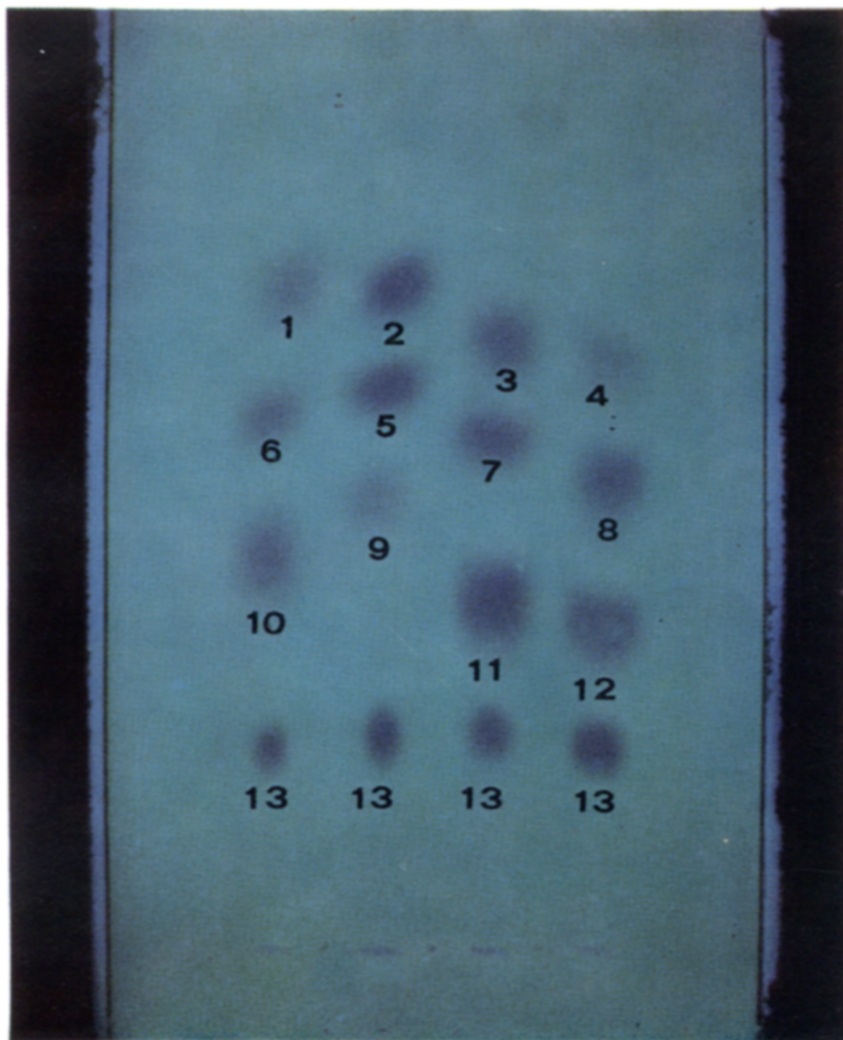

Fig. 1. Thin-layer gel chromatography of nucleic acid derivatives by ascending development. Stationary phase, Sephadex G-10–Silica Gel GF_{254} (4:1); mobile phase, phosphate buffer, 0.05 *M*, pH 7.0; detection, UV 254 nm. Numbers refer to Table I.

TABLE I

COMPARISON OF $R_{F(\text{uridine})} \times 100$ VALUES

Stationary phases: Sephadex G-10 (*a*), Sephadex–cellulose (*b*), Sephadex–silica gel (*c*), cellulose (*d*) and silica gel (*e*). Mobile phase: Phosphate buffer 0.05 *M*, pH 7.0.

No.	*Compound*	*Descending technique*	*Ascending technique*			
		a	*b*	*c*	*d*	*e*
1	Uridine	100	100	100	100	100
2	Cytidine	96	95	96	100	88
3	Thymidine	89	90	91	100	84
4	Inosine	83	83	86	85	92
5	Cytosine	80	81	83	93	74
6	Uracil	76	77	77	85	92
7	Thymine	70	67	71	84	77
8	Xanthosine	60	59	60	70	90
9	Hypoxanthine	58	56	58	61	80
10	Guanosine	53	49	52	65	93
11	Adenosine	43	41	46	80	80
12	Xanthine	42	39	—	64	45
13	Adenine	26	26	26	70	56

The $R_{F(\text{uridine})}$ values obtained on the mixed layers were the same as those obtained on Sephadex G-10 alone but different from those obtained on silica gel or cellulose alone. This might be due to the hydrated gel engulfing the individual absorbent particles, thus blocking their active centres and rendering them practically inert.

The method described offers certain advantages with regard to Sephadex-column chromatography; apart from being more practical it does not require the use of complex and costly apparatus.

In our view, the most interesting feature of the experiments regards the behaviour of the mixed layers used.

They share the chromatographic separation characteristics of Sephadex while affording the mechanical properties of cellulose or silica gel. As such they overcome the limitations of thin-layer gel chromatography that have hitherto prevented the technique from being fully exploited.

The authors wish to thank Prof. P. BIANCHINI for his valuable suggestions and M. CRISTOFORI and M. MAZZA for their skilled technical assistance.

ALFA Farmaceutici S.p.A., Chromatography Laboratory, Analytical Department, Bologna (Italy)

G. TORTOLANI
M. E. COLOSI

1 B. GELOTTE, *J. Chromatogr.*, 3 (1960) 330.
2 H. DETERMANN, *Experientia*, 18 (1962) 430.
3 G. B. JOHANSONN AND L. RYMO, *Acta Chem. Scand.*, 16 (1962) 2067.
4 H. DETERMANN AND W. MICHEL, *Z. Anal. Chem.*, 212 (1965) 211.
5 P. FASELLA, A. GIARTOSIO AND C. TURANO, in G. B. MARINI-BETTÒLO (Editor), *Thin-Layer Chromatography, Proceedings of the Symposium held at the Istituto Superiore di Sanità, Rome, 2–3 May, 1963*, Elsevier, Amsterdam, 1964, p. 205–211.
6 Z. STRÁNSKÝ, AND M. SRCH, *J. Chromatogr.*, 28 (1967) 146.
7 R. G. STICKLAND, *Anal. Biochem.*, 10 (1965) 108.

8 M. GROSS, B. SKOCZYLAS AND W. TURSKI, *Anal. Biochem.*, 11 (1965) 10.
9 G. P. ROBERTS, *J. Chromatogr.*, 22 (1966) 90.
10 M. H. J. ZUIDWEG, J. G. DOSTENDORP AND C. J. K. BOS, *J. Chromatogr.*, 42 (1969) 552
11 G. B. JOHANSONN AND L. RYMO, *Acta Chem. Scand.*, 18 (1964) 217.
12 Y. POMERANZ, *Chemist-Analyst*, 54 (1965) 57.
13 R. VENDRELY, Y. COIRAULT AND A. VANDERPLANCKE, *Compt. Rend.*, 258 (1964) 6399.
14 C. J. O. R. MORRIS, *J. Chromatogr.*, 16 (1964) 167.
15 D. JAWOREK, *Chromatographia*, 2, (1969) 289.
16 J. G. KIRCHNER, *Thin-Layer Chromatography*, Interscience, New York, 1967, p. 68.

Received January 20th, 1972

J. Chromatogr., 70 (1972) 182–186