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Note

Thin-layer chromatography of some nucleobases and nucleosides on cellulose layers

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While the bulk of the paper chromatographic work deals with partition systems, usually with water or formamide as the stationary phase, it was recognized from the beginning that good chromatograms could also be obtained with aqueous solutions as developing solvents. For example, aqueous acetic acid or hydrochloric acid was extensively used for the separation of phenols[1], flavonoids[2], anthocyanins[3], acridine dyes and other heterocyclics[4], inorganic complexes[5] and anions[6]. Such chromatographic separations were in general faster than those obtained in the usual partition systems but often yielded elongated spots owing to this fast development.

In looking for a simple system for the separation of iodotyrosines, we noted that the elongated spots, which were perhaps the main disadvantage of such systems, did not occur on suitable thin layers. It was possible to obtain good, round spots with a development time of only 10 min (distance: 55 mm) and with 5000 "theoretical plates" for most spots[7].

Clearly, this performance is comparable to that of good separations obtained by high-performance liquid chromatography. There was the additional advantage that virtually no apparatus was required: small reagent jars were used as chambers. These need not be hermetically closed as there is hardly any evaporation at room temperature from an aqueous solvent in 10 min.

We therefore felt that such systems would be of further interest and wanted to explore whether adsorption could be altered readily by suitably modifying the aqueous solvent. For this purpose, we selected the nucleobases and nucleosides, which had already been chromatographed on both paper and cellulose layers with water as the eluent[8,9].

EXPERIMENTAL

Nucleosides and bases (original sources: Sigma, St. Louis, MO, U.S.A., and E. Merck, Darmstadt, F.R.G.) were kindly donated by Dr. J. C. Kraak (Laboratory of Analytical Chemistry, University of Amsterdam).

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Plastic sheets coated with a 0.1-mm layer of cellulose were obtained from E. Merck (Art. No. 5577). For chromatography the sheets were cut into pieces of approximately 8 × 4 cm. Aqueous solutions of the nucleosides and bases were spotted on to the cellulose layers with a small piece of filter-paper, so as to form spots *ca.* 3 mm in diameter.

Chromatography was carried out in small glass jars, covered with a watch-glass, at room temperature. The plates were developed to a distance of *ca.* 6 cm; the mobile phases consisted of distilled water or aqueous solutions of inorganic salts (see Tables I–III). The spots were made visible by UV irradiation at 254 nm.

RESULTS AND DISCUSSION

Table I shows that the effect of ammonium sulphate solutions is an initial increase in R_F values at low concentrations and then an almost linear decrease at higher concentrations (Fig. 1). The initial increase, we presume, is due to desorption from the carboxyl groups of the cellulose. The decrease at higher concentrations seems to be a typical salting-out phenomenon, and it seems that the adsorption is a hydrophobic interaction.

Table II shows the effect of some other salts. Manganese and zinc sulphate give a smaller salting-out effect as they are present essentially as ion pairs and are hence less ionized than ammonium sulphate.

TABLE I

R_F VALUES OF NUCLEOBASES AND NUCLEOSIDES ON CELLULOSE THIN LAYERS AT VARIOUS CONCENTRATIONS OF AMMONIUM SULPHATE

Compound	R_F					
	Distilled water	*Ammonium sulphate*				
		0.2 M	*1 M*	*2 M*	*3 M*	*Saturated (5.3 M)*
Nucleobases						
Adenine	0.26	0.50	0.45	0.33	0.21	0.10
Hypoxanthine	0.48	0.51	0.47	0.38	0.25	0.14
Cytosine	0.63	0.85	0.83	0.80	0.80	0.60
5-Methylcytosine	0.66	0.87	0.87	0.79	0.69	0.55
Thymine	0.70	0.71	0.65	0.50	0.36	0.28
Uracil	0.73	0.72	0.58	0.57	0.47	0.40
Nucleosides						
Adenosine	0.47	0.55	0.49	0.33	0.21	0.10
Inosine	0.75	0.74	0.66	0.56	0.43	0.26
Guanosine	0.54	0.56	0.53	0.41	0.26	0.15
Xanthosine	0.81	0.55	0.50	0.35	0.21	0.15
Cytidine	0.78	0.90	0.87	0.86	0.79	0.69
Thymidine	0.82	0.83	0.73	0.56	0.41	0.27
Uridine	0.86	0.82	0.80	0.72	0.64	0.48

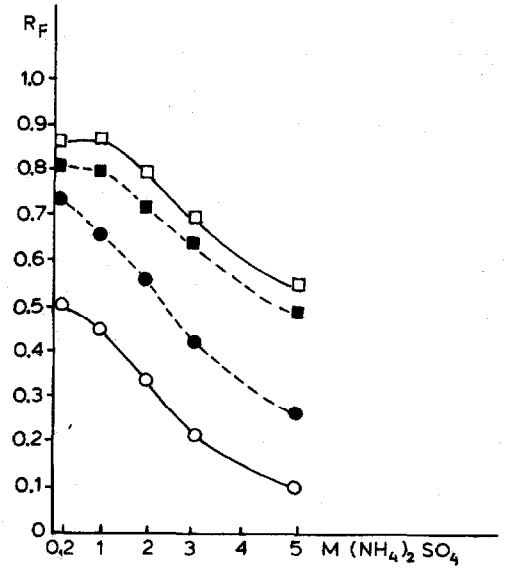

Fig. 1. Effect of ammonium sulphate concentration on R_F values of some nucleosides and nucleobases. □, Methylcytosine; ○, adenine; ●, inosine; ■, uridine.

TABLE II

EFFECT OF SOME INORGANIC SALTS ON THE R_F VALUES OF BASES AND NUCLEOSIDES ON CELLULOSE

Compound	*$MgSO_4$*		*$MnSO_4$*		*$ZnSO_4$*		*Ammonium citrate*		*1 M NaCl*	*0.5 M $MgCl_2$*
	1 M	*2 M*	*1 M*	*2 M*	*1 M*	*2 M*	*1 M*	*2 M*		
Nucleobases										
Adenine	0.44	0.35	0.64	0.62	0.77	0.77	0.44	0.45	0.42	0.37
Hypoxanthine	0.44	0.39	0.64	0.59	0.77	0.78	0.50	0.52	0.50	0.48
Cytosine	0.75	0.74	0.88	0.80	0.82	0.79	0.84	0.81	0.73	0.72
Thymine	0.58	0.42	0.62	0.46	0.60	0.45	0.69	0.66	0.71	0.69
Uracil	0.62	0.51	0.65	0.56	0.63	0.54	0.68	0.65	0.73	0.70
Nucleosides										
Adenosine	0.45	0.33	0.57	0.43		0.58	0.47	0.48	0.53	0.50
Inosine	0.65	0.51	0.75	0.63	0.77	0.74	0.82	0.81	0.73	0.73
Guanosine	0.51	0.39	0.67	0.58	0.76	0.71	0.60	0.61	0.53	0.52
Xanthosine	0.43	0.29	0.51	0.41		0.56	0.65	0.59	0.59	0.54
Cytidine	0.85	0.78	0.82	0.78	0.90	0.80	0.86	0.84	0.83	0.82
Thymidine	0.65	0.45	0.68	0.53	0.66	0.47	0.76	0.73	0.83	0.83
Uridine	0.73	0.57	0.77	0.60	0.74	0.61	0.82	0.81	0.85	0.84

TABLE III

EFFECT OF pH ON THE R_F VALUES OF BASES AND NUCLEOSIDES ON CELLULOSE

Compound	*1% borax*	*1% acetic acid*	*0.5% HCl*	*1% ammonia solution*
Nucleobases				
Adenine	0.33	0.53	0.74	0.56
Hypoxanthine	0.63	0.52	0.77	0.79
Cytosine	0.74	0.71	0.86	0.72
Thymine	0.74	0.74	0.75	0.88
Uracil	0.71	0.73	0.71	0.92
Nucleosides				
Adenosine	0.74	0,61	0.84	0.49
Inosine	0.88	0.75	0.82	0.91
Guanosine	0.85	0.55	0.72	0.84
Xanthosine	0.94	0.54		0.93
Cytidine	0.86	0.81	0.92	0.80
Thymidine	0.87	0.85	0.86	0.94
Uridine	0.86	0.84	0.88	0.96

Table III shows that lowering the pH desorbs the bases and nucleosides and so does the addition of borax or ammonia solution.

We feel that the salting-out effect with ammonium sulphate is noteworthy because it permits in most instances a large variation of the R_F values in a very simple system and hence offers a wide range of separation possibilities. It may also induce other workers to investigate such simple and inexpensive systems for large-scale separations.

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REFERENCES

1 R. J. W. Byrde, D. F. Downing and D. Woodcock, *Biochem. J.*, 46 (1950) 271.
2 T. G. Gage, C. D. Douglas and S. H. Wender, *Anal. Chem.*, 23 (1951) 1582.
3 E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta*, 4 (1950) 427.
4 A. M. Luly and K. Sakodynsky, *J. Chromatogr.*, 19 (1965) 624.
5 M. Lederer, *J. Chromatogr.*, 7 (1962) 366.
6 M. Lederer and R. Marini-Bettólo, *J. Chromatogr.*, 35 (1968) 213.
7 M. Lederer, *J. Chromatogr.*, 194 (1980) 270.
8 K. Randerath, *Biochem. Biophys. Res. Commun.*, 6 (1961/1962) 452.
9 K. Randerath and H. Struck, *J. Chromatogr.*, 6 (1961) 365.