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PAPER CHROMATOGRAPHY OF NUCLEIC ACID DERIVATIVES

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SUMMARY

A series of solvent systems for paper-chromatography, based upon mixtures of acetonitrile with aqueous buffers, is described. The R_F values of more than thirty purine and pyrimidine bases, nucleosides and nucleotides are listed.

The current high level of interest in the chemistry of nucleic acid derivatives has generated a need for analytical methods. Paper chromatography has been the classical approach, but many of the systems described in the literature[1–3] require lengthy development times, and a variety of systems are used to span the properties of the substrates of interest.

Thin-layer chromatography[4–10] constitutes an improvement over paper chromatography in certain classes, but problems of separation and quantitation still remain. Recently, acetonitrile-based solvent systems for paper chromatography have been reported by HEDRICK and coworkers[11,12]. We have studied the application of such systems in the nucleic acid field, and found them to be very useful and versatile. Solvent migration rates attained when these systems are used in conjunction with rapid-developing papers (Whatman No. 3MM, 17, 40) give development times comparable to those of silica gel TLC. Typically, using an ascending technique, a 10 cm solvent-front migration is attained in less than 1 hour.

A wide range of compounds have useful migration rates in acetonitrile containing 20–40 % aqueous buffers. Buffers in the 0.1–0.2 *M* concentration range and in the pH range 2–11 have been used with various papers. Whatman 3MM paper was chosen for general usage because of its rapid solvent migration rate, good wet strength and relatively high solute capacity.

An additional benefit, in the form of reduced spot area, is obtained through use of these systems. Migrated spots are about 50% smaller in area than those of normal paper chromatograms. A further reduction in area may be obtained by use of a hardened paper such as Whatman No. 50, but at the cost of increased development time.

EXPERIMENTAL

Chromatography was carried out on 9 × 9 in. sheets of chromatographic grade paper in 10 × 10 × 3 in. glass TLC tanks (Brinkmann Instruments Inc., Westbury,

New York). Papers were suspended from various glass, stainless steel and wire frames which allow the paper to hang free in the tank. Spots were applied at a concentration of about 1 ODU in 1–5 λ solution. Development was carried out in the ascending direction without prior equilibration. Spots were visualized by fluorescence quenching of the paper under short-wave ultraviolet light (Mineralight, Ultra-Violet Products, San Gabriel, Calif.).

The buffers used in the systems reported in the tables were 0.1 M with a pH of 7 unless otherwise noted. Acetonitrile was taken from recently opened bottles and used without further purification. Systems were prepared just prior to use. Because equilibration was not desired, tanks were emptied and flushed with air between uses. System proportions given are by volume:

A = acetonitrile–ammonium acetate (80:20)
B = acetonitrile–ammonium acetate–28% ammonium hydroxide (70:20:10)
C = acetonitrile–ammonium acetate–28% ammonium hydroxide–isopropanol (60:20:10:10)
D = acetonitrile–ammonium acetate–28% ammonium hydroxide–*n*-butanol (60:20:10:10)
E = acetonitrile–ammonium acetate–28% ammonium hydroxide (80:10:10).
F = acetonitrile–ammonium chloride, pH 5 (60:40)
G = acetonitrile–ammonium acetate–28% ammonium hydroxide (60:30:10)
H = acetonitrile–ammonium acetate (70:30)
I = acetonitrile–ammonium chloride, pH 5 (70:30).

RESULTS

The chromatographic data are presented in Tables I–IV. In most cases the R_F values given are the average of several determinations obtained from chromatograms run at different times. The R_F values are calculated on the basis of the primary or α-front, although a β-front is visible with most systems.

TABLE I

PURINES AND PYRIMIDINES

Compound	*Solvent system*								
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
Thymine	0.60	0.54	0.71	0.64	0.35	0.73	0.67	0.75	0.70
Uracil	0.54	0.50	0.42	0.41	0.38	0.66	0.59	0.66	0.64
Cytosine	0.32	0.43	0.59	0.51	0.31	0.52	0.60	0.52	0.45
Adenine	0.32	0.24	0.42	0.34	0.14	0.48	0.37	0.47	0.44
Guanine	0.16	0.14	0.29	0.20	0.06	0.35	0.28	0.34	0.30
Hypoxanthine	0.27	0.25	0.29	0.28	0.11	0.51	0.46	0.45	0.44
Xanthine	0.14	0.20	0.16	0.19	0.08	0.41	0.35	0.31	0.35

TABLE II

DEOXYRIBOSIDES

Compound	*Solvent system*								
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
Thymidine	0.57	0.66	0.65	0.61	0.35	0.83	0.74	0.73	0.70
Deoxycytidine	0.34	0.63	0.59	0.57	0.30	0.67	0.74	0.53	0.49
Deoxyadenosine	0.37	0.40	0.38	0.38	0.13	0.67	0.59	0.48	0.48
Deoxyguanosine	0.25	0.35	0.28	0.30	0.09	0.69	0.48	0.45	0.42

TABLE III

DEOXYRIBO-5'-NUCLEOTIDES

Compound	*Solvent system*								
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
Thymidylic acid	0.08	0.07	0.07	0.055	0	0.43	0.23	0.16	0.16
Deoxycytidylic acid	0	0.05	0.05	0.035	0	0.35	0.21	0.08	0.11
Deoxyadenylic acid	0	0.06	0.06	0.035	0	0.34	0.19	0.07	0.09
Deoxyguanylic acid	0	0.03	0.01	0.028	0	0.31	0.11	0.07	0.08

TABLE IV

ACYL DERIVATIVES*

Compound	*Solvent system*			
	A	*E*	*H*	*I*
TrThymidine	0.96			
MMTrThymidine	1.0			
DMTrThymidine	1.0			
T-OAc	0.93	0.77	0.93	0.92
pT-OAc	0.08			0.50
Cyanoethyl pT		0.22	0.83	0.52
Trichloroethyl pT	0.42			0.61
Trichloroethyl pT-OAc	0.57			
TrTpT	0.62			
MMTrTpT-OAc	0.57			
d-pC^{An}-OAc	0.23			0.73
d-pC^{An}				0.43
d-pA^{Bz}-OAc	0.10		0.63	
d-pA^{Bz}			0.25	
d-pA^{Bz}-OBz			0.90	
d-pG^{Ac}-OAc				0.55
d-pGpGpG				0.18
MMTrTpC^{An}-OAc	0.67			
d-TpC^{An}-OAc	0.41			
dTpA			0.15	
DMTrTpA^{Bz}-OAc	0.75			
DMTrTpA^{Bz}	0.60			0.77
DMTrTpA^{Bz}pG^{Ac}-OAc	0.50			
MMTrTpG^{Ac}-OAc	0.56			

* The abbreviations used in this table are derived from those used by KHORANA *et al.*[13,14].

DISCUSSION

The systems described have distinct advantages over ordinary paper chromatographic methods. The rapid development, small spot area, and simplicity of preparation compare favorably with those of silica gel TLC. Quantitation of areas is possible by normal paper techniques, *i.e.*, visualization under short wave ultra-violet light, excision and mincing with clean scissors, extraction with appropriate solvents and measurements of the absorption spectrum against a comparable paper blank. With care, recoveries are quantitative.

Systems can be "tailor-made" to give useful migration rates and separations of various compounds by manipulating the buffer content and pH.

Objectionable odors are absent, and the short drying times needed allow for multiple or 2-dimensional developments with minimal delay.

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