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Some Simple Thin-Layer Chromatographic Systems for the Separation of Purines and Purine Nucleosides

Studies on nucleic acid metabolism often require the analytical separation of nucleic acid derivatives and are facilitated by chromatographic methods which are rapid and convenient. Column chromatography on ion exchange resins (1), paper chromatography (2), and paper electrophoresis (3) are among the methods used routinely for such separations. Even though these techniques have been employed widely, they require lengthy development times and specialized equipment. Thin-layer chromatography (tlc) also has been used for the separation of nucleic acid derivatives and has the advantages of simple and rapid development procedures combined with high resolution power (4–6). The separation of nucleotides on anion exchange celluloses has been particularly well developed, with polyethyleneimine-cellulose (PEI-cellulose) used extensively (7). A number of tlc techniques also have been devised for the separation of nucleic acid bases and nucleosides. Most of these employ cellulose layers although silica gel plates have been used as well (4–6). However, neither appears to be commonly used. Kolassa *et al.* (8) recently described several new silica gel systems for the chromatography of purine derivatives. These systems gave good separations but required running times of three to four hours in alcoholic solvents.

We describe in this communication some simple tlc silica gel systems which employ chloroform-containing solvents and require development times of under one hour. These have been used routinely by us for the separation of purines and purine nucleosides in metabolic studies with 9-β-D-arabinofuranosyladenine (Ara-*f*-Ade).

Methods. Prescored 20 × 20 cm tlc plates with a 250 μm layer of silica gel GF254 (Merck) were obtained from Analtech, Inc., Newark, DE. Plastic sheets, 20 × 20 cm, coated with 100 μm of PEI-cellulose (PEI-cellulose, Macherey Nagel 300) were obtained from Brinkmann Instruments, Inc., Des Plaines, IL, and were converted to the borate form as described by Schrecker *et al.* (9).

The following chloroform-containing solvents were prepared by mixing chloroform, methanol, and either 3% acetic acid or 3% ammonia

(3:2:1) and using the lower phase. The composition of the lower phase may be similar to that of a chloroform–methanol–water azeotrope, i.e., 81% chloroform–15% methanol–4% water, because the boiling point of the lower phase was 52°C (uncorrected) compared to a boiling point of 52.6°C for the azeotrope (10). Solvent system I was the lower phase of the mixture prepared with acetic acid while solvent system II consisted of 17 parts of the lower phase of the mixture prepared with ammonia plus 3 parts of absolute methanol. A third solvent system consisted of 0.1 M boric acid and was used with PEI(borate)-cellulose plates. Solvent systems I and II were equilibrated before use, usually by standing overnight, and required running times of ¾–1 hr. The boric acid solvent system was used without prior equilibration and required approximately 1½ hr for development. These times permitted the solvents to migrate 13–15 cm from the origin by ascending development. Spots were visualized under uv light (254 nm).

Results. The silica gel systems described were developed so that purines and purine nucleosides would migrate on the adsorbent while nucleotides would be retained at the origin. The separations obtained are listed in Table 1. R_f values are averages of duplicate runs except as noted. The

TABLE 1
Thin Layer Chromatography of Purine Derivatives on Silica Gel Plates

Compound	R_f Value			
	Solvent system I		Solvent system II	
Adenine	0.40[a]	0.43 ± 0.04[b]	0.56[a]	0.59 ± 0.05[b]
Hypoxanthine	0.31	0.32 ± 0.05	0.42	0.43 ± 0.07
Guanine	0.05	—	0.04	—
Adenosine	0.30	—	0.47	0.51 ± 0.06
Inosine	0.17	—	0.28	0.25 ± 0.06
Guanosine	0.14	—	0.27	—
Ara-*f*-Ade[c]	0.26	0.26 ± 0.04	0.45	—
Ara-*f*-Hyp[d]	0.14	0.16 ± 0.04	0.34	—
Deoxyadenosine	0.37	—	0.60	—
Purine nucleoside-5′mono-, di- and triphosphates	0.00	0.00	0.00	0.00

[a] Average values obtained from duplicate chromatograms. Pure stock solutions of reference compounds were used.

[b] Average values ± standard deviations obtained from 30 and 20 chromatograms developed in Systems I and II respectively. In all cases dimethylsulfoxide stock solutions of reference compounds were mixed with ethanol extracts of biological material and chromatographed directly.

[c] 9-β-D-arabinofuranosyladenine.

[d] 9-β-D-arabinofuranosylhypoxanthine.

order of migration is similar in both solvent systems with the exception of hypoxanthine. In system I, adenine chromatographs ahead of hypoxanthine followed by adenosine and Ara-*f*-Ade, whereas in system II hypoxanthine follows adenosine and Ara-*f*-Ade. This difference gives a better resolution of hypoxanthine and Ara-*f*-Ade in system I, while hypoxanthine and adenosine are better separated by system II. Thus solvent system I was utilized for routine separations of the arabinosyl purines, whereas solvent system II was used for separations of the ribosyl purines (Table 1). The variation in R_f values was not random but appeared to correlate with the length of tank equilibration time, i.e. R_f values increased slightly as equilibration time increased. In all instances baseline resolution of adenine, hypoxanthine, and the corresponding arabinosyl or ribosyl nucleosides was obtained. Resolution of hypoxanthine and Ara-*f*-Ade was not complete if 100 μm plastic-backed silica gel plates were substituted for the Analtech plates.

In both solvent systems, some resolution was observed between Ara-*f*-Ade and adenosine. This could be improved to nearly complete resolution by a second development of the plate. However, a far superior resolution of the two adenine nucleosides was obtained by utilizing the binding of ribosyl compounds to borate. The method of Schrecker *et al.* (9) was used which employs PEI(borate)-cellulose plates developed in 0.1 M boric acid. Ribosyl purines were retained at the origin while purines and related nucleosides not having a *cis*-glycol moiety migrated on the cellulose layer (Table 2). Good separations were obtained between a particular purine and the corresponding ribosyl and arabinosyl nucleosides. Thus the use of silica gel plates in solvent systems I or II combined with chromatography on PEI(borate)-cellulose layers in

TABLE 2
Thin Layer Chromatography of Purines and Nucleosides on PEI(Borate)-Cellulose Plates

Compound	R_f Value[a]
Adenine	0.40 ± 0.04
Hypoxanthine	0.39 ± 0.02
Adenosine	0.06 ± 0.01[b]
Inosine	0.07 ± 0.01[b]
Ara-*f*-Ade	0.57 ± 0.02
Ara-*f*-Hyp	0.49 ± 0.02
Deoxyadenosine	0.65 ± 0.04

[a] Average values ± standard deviation obtained from 3–10 chromatograms developed in 0.1 M boric acid.

[b] Elongated spots.

0.1 M boric acid permits the characterization of a number of purines and nucleosides.

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