Separation of sugar phosphates and sugar nucleotides by thin-layer chromatography

Several papers describing the application of thin-layer chromatography to the separation of purines, pyrimidines, nucleosides and nucleotides have appeared[1–7], but this technique has not been applied to the resolution of mixtures of sugar phosphates and sugar nucleotides. Using the procedure described below, the separation of these substances can be accomplished within two hours using ECTEOLA layers.

The plates were prepared by suspending 2 g of sieved ECTEOLA-cellulose powder (Serva-Entwicklungslabor, Heidelberg, Germany) in 18 ml of 0.004 *M* ethylenediaminetetraacetic acid pH 7.0, and shaking it vigorously for 5 min. The resulting slurry was poured onto a 20 × 20 cm glass plate and spread in a uniform layer with a glass rod. The plates were dried overnight at room temperature, giving a layer with a thickness of about 200 μ. Afterwards, they were sprayed with 0.1 *M* ammonium tetraborate[8] pH 9.0 (preparation of the ECTEOLA slurry in ammonium tetraborate did not give the same results). After spraying, the layers were dried at 50° for 30 min, and cooled to room temperature before use. No difference was found if the plates were used immediately or within three days.

Solutions of phosphoric esters and nucleotides were spotted 3 cm from the edge of the plate. The layers were placed in closed tanks and the chromatograms were developed with ethanol (95 %)–0.1 *M* ammonium tetraborate, pH 9.0, (3:2). For phosphoric esters only, a solvent prepared with a pH 10 buffer was also used. In this case, the plates had been previously sprayed with buffer of the same pH.

An improvement in the shape of the spots was observed if some cellulose was erased from the layer so as to leave a pattern similar to the one described by MATTHIAS[9] for paper chromatography.

TABLE I

R_P* VALUES OF PHOSPHORIC ESTERS AND NUCLEOTIDES

Substance	*pH of the solvent* 9.0	*Substance*	*pH of the solvent*	
			9.0	10
TDP-glucose**	1.25	N-Acetylglucosamine 1-P	1.29	1.37
UDP-acetylglucosamine	0.82	N-Acetylgalactosamine 1-P	1.12	1.16
UDP-glucose	0.70	Glucose 1-P	1.20	1.15
UDP-galactose	0.47	Mannose 1-P	0.90	0.88
ADP-glucose**	0.61	Galactose 1-P	0.77	0.80
ADP-mannose**	0.50	Mannose 6-P	0.70	0.57
ADP-galactose**	0.40	Fructose 6-P	0.68	0.68
ADP-glyceric acid**	0.43	Fructose 1-P	0.59	0.54
UTP	0.37	Glucose 6-P	0.56	0.39
UDP	0.42	Fructose 1,6-P_2	0.33	0.20
UMP	0.53	3-P-Glyceric acid	0.97	0.95
ATP	0.32	2,3-Diphosphoglyceric acid	0.78	0.80
ADP	0.36			
AMP	0.42			
TMP	1.10			

* Inorganic phosphate moves about 7.2 cm from the point of application. The results are expressed as the ratio of the distance travelled by the phosphate derivative to the distance travelled by inorganic phosphate.

** These nucleotides were generously provided by Dr. E. RECONDO.

The chromatography was stopped when the solvent front had reached the top edge (after about 2 h). After examination for ultraviolet absorbing spots with a Mineralight lamp, the chromatograms were sprayed successively with benzidine–trichloroacetic acid[10] to ascertain the position of the hexose-6-phosphates, and with the molybdate reagent of BURROWS *et al.*[11] for phosphoric esters.

The results obtained for some phosphoric esters and sugar nucleotides are shown in Table I. Satisfactory separations could be obtained also for sugar nucleotides and aldose-1-phosphates by developing the chromatograms with a different solvent mixture ethanol (95 %)–0.1 *M* ammonium tetraborate, pH 9.0, (1:1).

The limits of detection of the substance with the different reagents is as follows in mμmoles: benzidine 10, molybdate 5, and ultraviolet light 1.

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Instituto de Investigaciones Bioquímicas Fundación Campomar, and Facultad de Ciencias Exactas y Naturales[§], *Buenos Aires (Argentina)*

CARL PETER DIETRICH*
SONIA M. C. DIETRICH**
HORACIO G. PONTIS***

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* On leave of absence from Instituto Osvaldo Cruz, Rio de Janeiro, Brasil.
** On leave of absence from Instituto de Botánica, San Pablo, Brasil.
*** Career Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.
§ Obligado 2490, Buenos Aires (28), Argentina.