Sodium carbonate–sodium bicarbonate buffer for the electrophoretic separation of nucleotides and phosphoric esters

Several alkaline buffers have been used for the electrophoretic separation of nucleotides. Sodium borate (0.05 *M*) pH 9.2, and a few organic buffers have been tried with limited success. Phosphate buffer (0.05 *M*) pH 7.5 gives good separations but has the obvious disadvantage of interfering with the detection of phosphate containing compounds[1].

A buffer of sodium carbonate–sodium bicarbonate (0.25 *M*) pH 9.2 (ref. 2) has been found to give fast and clear separations as shown in Table I. Electrophoresis was

TABLE I

MOBILITY OF VARIOUS COMPOUNDS

Compound	M_{P_i}	*Compound*	M_{P_i}
AMP-morpholidate	0.27	GDP	0.67
Cyclic 3′, 5′-AMP	0.29	CDP	0.67
ADP-maltose	0.33	ADP-3-phosphoglyceric acid	0.68
Diadenosine-diphosphate	0.33	UMP	0.75
ADP-glucose	0.40	Pyrophosphate	0.82
AMP-5′	0.49	Dihydroxyacetone-phosphate	0.84
CMP	0.57	UDP	0.85
ADP	0.58	2,3-Diphosphoglyceric acid	0.92
ATP	0.58	2-Phosphoglyceric acid	0.97
ADP-glyceric acid	0.60	3-Phosphoglyceric acid	1.02
UDP-glucose	0.60	Phospho-enol pyruvic acid	1.04
Galactose-1-phosphate	0.61	2,3-Cyclic phosphoglyceric acid	1.14
Glucose-1-phosphate	0.61		
Mannose-1-phosphate	0.61		
Xylose-1-phosphate	0.64		

carried out with Whatman No. 1 paper and the apparatus of MARKHAM AND SMITH[3] for 2 h at 600 V (15 V/cm) and 30–60 mA (2–4 mA/cm).

As shown in Fig. 1, good separations were obtained only at high ionic strength. This is in contrast to the results of STRANSKÝ[4] with citrate buffer at pH 4.8.

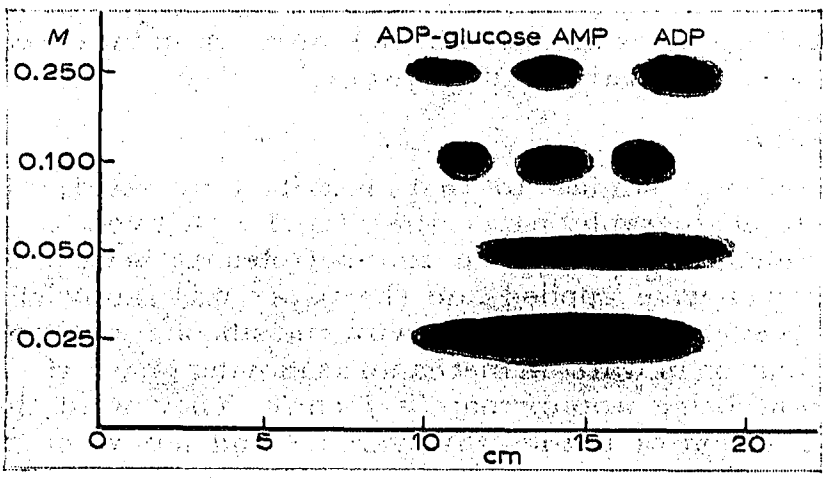

Fig. 1. Electrophoretic separation of adenine nucleotides in sodium carbonate–sodium bicarbonate buffers of different ionic strength (pH 9.2).

Compounds which are rather alkali-labile are not decomposed by the buffer. Thus UDP-glucose shows no sign of decomposition within 3–4 h. The buffer solution can be stored for months without alteration and can be recommended after considerable experience.

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Identification of dialkyl peroxides by paper chromatography

It has been shown that good separation of alkyl hydroperoxides can be obtained by paper chromatography[1] though, as yet, no satisfactory method for the separation of dialkyl peroxides has been reported. The following separation was devised in order to characterise dialkyl peroxides produced in the radiation induced oxidation of hydrocarbons but may well be of value in other work.

Experimental procedure

The apparatus used was similar to that described by CARTLIDGE AND TIPPER[1]. Whatman No. 3 chromatography paper was treated with 5 vol. % solution of silicone oil (Hopkin and Williams MS 1107) in 80–100° petroleum ether and dried in an oven at 110° for 1 h. Samples were applied and the paper was sandwiched between glass plates which had previously been treated with the silicone solution. Chromatograms were run using a solution of water in methanol as moving phase, the rate of movement of the solvent front being approximately 7 cm/h. They were then developed by spraying with a solution of ferrous thiocyanate[2] and left to stand for 20–30 min when the presence of peroxides was indicated by red spots. It was possible to detect 100 μg of peroxide.