Use of diethylamine in the paper chromatographic separation of isomeric mononitrophenols

GASPARIC[1] has separated the isomeric mononitrophenols on formamide impregnated paper with benzene–acetic acid as solvent. However, the volatile *ortho* isomer was not detected when the method was used in this laboratory. For this reason, and because formamide impregnated paper is somewhat difficult to handle, an alternative separation on conventional chromatography paper was sought. In trial runs it was found possible to separate the *meta* and *para* isomers using benzene saturated with water ascending on Whatman No. 1 paper, water saturated with benzene providing the tank atmosphere. The *meta* and *para* nitrophenols were detected, on exposure to ammonia vapour, as yellow spots with R_F values of 0.80 and 0.58, respectively, but the *ortho* isomer was still not detected. ROBINSON *et al.*[2] found it necessary to develop chromatograms at 0° to prevent volatilisation of *o*-nitrophenol from the paper.

In an effort to reduce the volatility of the *ortho* isomer, methods based on the separation of the nitrophenates with alkaline developing solvents were tried. Little or no separation was obtained on Whatman No. 1 paper with the *n*-butanol saturated with 5 *N* ammonium hydroxide system used by LEDERER[3], or with the ethanol–ammonium hydroxide system used by LONG *et al.*[4]. However, an excellent separation was achieved as follows. Benzene, diethylamine, and water (3:2:5 by volume) were shaken together in a separating funnel and the phases allowed to separate. The aqueous phase was used to provide the tank atmosphere (equilibration time 1 h) and the organic phase was used as developer (Whatman No. 1 paper, ascending technique). The nitro compounds showed up on the paper as yellow spots and the progress of the separation could be observed. The R_F values obtained by this method for the nitrophenols, naphthols, and a number of substituted phenols are given in Table I.

The results show that the isomeric mononitrophenols are separated from each other and from most of the phenols and naphthols tested. *Meta* and *para* cyanophenols are well separated from each other but *m*-cyanophenol is not separated from *m*-

TABLE I

R_F VALUES OF PHENOLS DEVELOPED WITH A BENZENE–DIETHYLAMINE–WATER SYSTEM

Phenol	$R_F \times 100$	*Method of detection*
o-Nitrophenol	44	visual
m-Nitrophenol	65	visual
p-Nitrophenol	10	visual
2,4,6-Trinitrophenol	89	visual
o-Hydroxybenzoic acid	27	U.V. light
m-Hydroxybenzoic acid	0	U.V. light
p-Hydroxybenzoic acid	0	U.V. light
α-Naphthol	100	U.V. light
β-Naphthol	100	U.V. light
o-Hydroxydiphenyl	100	U.V. light
2,2'-Dihydroxydiphenyl	77	U.V. light
m-Cresol	100	diazotised sulphanilic acid
Phenol	100	diazotised sulphanilic acid
m-Cyanophenol	61	U.V. light
p-Cyanophenol	20	U.V. light

nitrophenol. This solvent system is simple to use and would appear to have some potential for the paper chromatographic separation of other nitrophenols.

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Separation of acid polysaccharides by starch gel electrophoresis*

The acid mucopolysaccharides (APS) have differing charge densities, molecular sizes and configurations. These differing properties serve as the basis of identifying unknown APS samples with starch gel electrophoresis. This method has the advantage of simplicity, speed of operation, economy, and is sensitive to 50 μg of APS.

Materials and methods

Preparation of gel. Citric acid buffer (0.01 *M*) is adjusted to pH 3.5 with saturated sodium hydroxide. Approximately 13 g of dry starch** is added to each 100 ml of 0.01 *M* citric acid buffer in a 2-l Pyrex vacuum flask. The flask contents are heated to boiling with constant vigorous swirling. Initially, the mixture is very viscous and opaque; after 3 min of heating, it becomes less viscous and appears translucent. At this point, heating is stopped and negative pressure applied. After the mixture has boiled approximately 2 more min, it is poured into a previously prepared mold. A preparation of 200 ml is adequate for a mold 20.5 × 8 × 0.75 cm. After cooling for 1 h at 4°, the gel is ready for use[1,2].

Preparation of chamber. The electrophoretic chambers are each filled with 500 ml of 0.1 *M* citric acid buffer, pH 3.5. The prepared gel is trimmed to 7.5 cm × 20.5 cm and is placed on a glass plate of the same size on the supports of the chamber (Fig. 1). Wicks (12 cm × 8 cm) made of 2 layers of Whatman No. 3 mm paper extend from the buffer in the chamber to the top of the slab of gel. The APS sample solutions (concentrations 2 mg/ml) are placed inside wells cut in the gel. These depressions are made with a small, stainless steel spatula, are 3 mm on each side, penetrate the entire thickness of the gel, and are set a minimum of 1 cm apart.

After the samples are applied to the gel, the chamber cover is sealed. A direct current*** of 10 mA (approximately 45 V) is applied for 8 h at room temperature. After electrophoresis, the gel is placed in a tray containing a mixture of acetone

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