

CHROM 5108

**Identification of organic compounds \*****LXXIV. Paper electrophoresis of acid anthraquinone dyes**

In a previous communication of this series<sup>1</sup>, we studied the paper chromatographic behaviour of acid anthraquinone dyes and found the optimum conditions for their separation and identification. The present paper deals with the application of paper electrophoresis to the same problem. The suitability of paper electrophoresis or thin-layer electrophoresis respectively, to the separation and identification of different types of acid dyes has been studied by several authors<sup>2-8</sup>, but no special attention has been directed to a systematic study of anthraquinone dyes. It was our endeavour to find the optimum experimental conditions for the paper electrophoretic separation of these dyes and to study the relationship between the electrophoretic behaviour of these dyes and their structure which could be taken advantage of in elucidating the structure of unknown dyes.

*Experimental*

*Dyes.* All dyes under investigation were either commercial products of known structure or pure model compounds from our collection of standards. They were applied in the form of 0.5–1% solutions in aqueous pyridine.

*Apparatus.* The apparatus used was of the type with a free hanging paper strip in a moist chamber designed by the Czechoslovak Academy of Sciences.

*Paper.* Whatman No. 3 MM paper strips (27 × 35 cm) were used throughout all experiments.

*Electrolytes.* For buffer solution I (pH 9) 518 ml of 0.1 M  $\text{KH}_2\text{PO}_4$  in 50% ethanol were mixed with 482 ml of 0.1 N sodium hydroxide in 50% ethanol. For buffer solution II (pH 4), 350 ml of 0.1 M  $\text{Na}_2\text{HPO}_4$  in 50% ethanol were mixed with 750 ml of 0.1 M citric acid in 50% ethanol. The potential was 10 V/cm and the developing time was 5–6 h.

*Results and discussion*

During our preliminary experiments carried out in both alkaline and acid media, the alkaline medium was found to be more suitable. When using aqueous solutions of electrolytes, however, we observed the formation of elongated streaks. The addition of ethanol to the electrolyte solution caused a considerable decrease of the mobility of the dyes, but the spots became very sharp. The positive influence of the presence of an organic solvent in the electrolyte solution on the quality of electropherograms of acid dyes has been observed by several authors. Ethanol<sup>6</sup>, propylene glycol<sup>4</sup>, and formamide<sup>9</sup> have been used.

Optimum results have been obtained using the phosphate buffer (pH 9) containing 50% ethanol. The electrophoretic mobilities (relative to compound I) of the compounds under investigation are summarised in Table I. The migration distance of compound I was 55–60 mm within 6 h. The difference of 0.2 in the relative mobilities was sufficient for the distinct separation of two compounds. The compounds in Table I can be divided into five groups according to their structures: I–XXI,

\* Part LXXIII: Sborník VŠChT, Pardubice, in press.

TABLE I

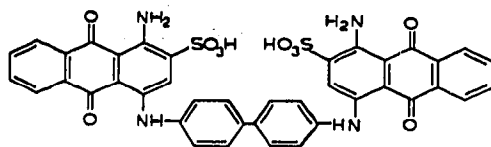
ELECTROPHORETIC MOBILITIES OF ACID ANTHRAQUINONE DYES RELATIVE TO COMPOUND I  
 Electrolyte: buffer solution, pH 9; potential, 10 V/cm; time, 6 h.

Compound	Mobility
I-XX	
I R = H	1.00
II R =	0.91
III R =	0.99
IV R =	1.04
V R =	1.04
VI R =	1.04
VII R =	1.46
VIII R =	1.60
IX R =	1.51
X R =	1.66
XI R =	1.66
XII R =	1.66
XIII R =	1.65
XIV R =	0.70
XV R =	0.82
XVI R =	0.86
XVII R =	0.82
XVIII R =	0.72
XIX R =	0.86
XX R =	1.51

(continued on p. 438)

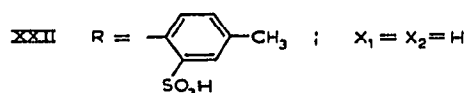
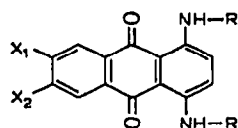
TABLE I (continued)

XXI

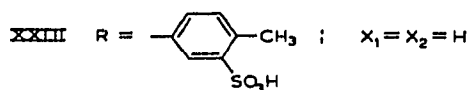


0.22

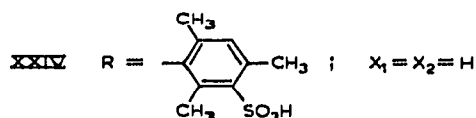
XXII-XXVI



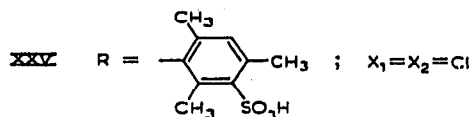
I.32



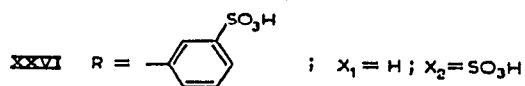
I.34



I.38

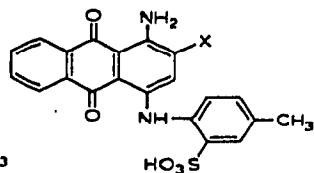


I.49



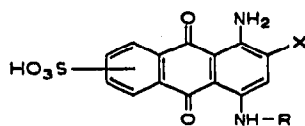
I.83

XXVII-XXVIII



0.73

XXVII X = CH<sub>3</sub>  
 XXVIII X = Br

XXIX-  
XXXI

0.75

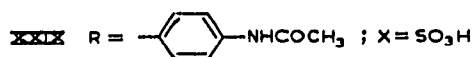
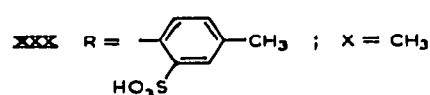
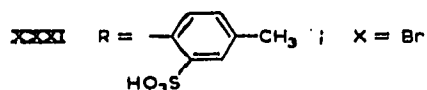
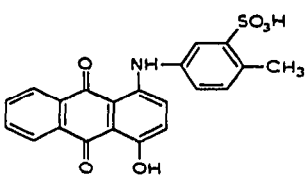
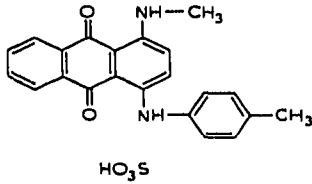
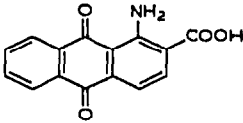
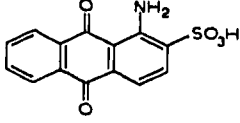
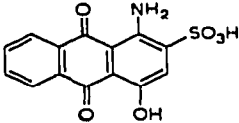
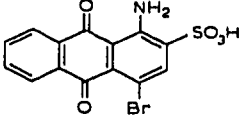
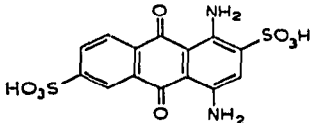
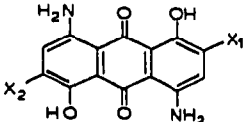
I.08  
I.22I.22  
I.42I.24  
I.44

TABLE I (continued)

XXXII		0.98
XXXIII		0.83
XXXIV		1.09
XXXV		1.22
XXXVI		1.13
XXXVII		1.17
XXXVIII		1.65
XXXIX-XLI		
	XXXIX X <sub>1</sub> = X <sub>2</sub> = H	0.00
	XL X <sub>1</sub> = SO <sub>3</sub> H ; X <sub>2</sub> = H	0.62
	XLI X <sub>1</sub> = X <sub>2</sub> = SO <sub>3</sub> H	0.86

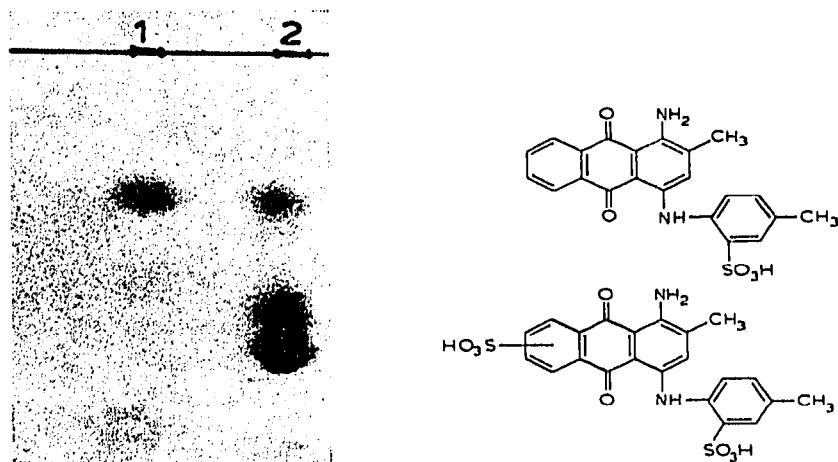


Fig. 1. Electropherogram of acid anthraquinone dyes. 1 = Kition Fast Blue CR (CI Acid Blue 47); 2 = Acilan Fast Blue REX (CI Acid Blue 49). Electrolyte, buffer solution I (pH 9); Potential, 10 V/cm; time, 6 h.

XXII–XXVI, XXVII–XXXI, XXXII–XXXVIII, and XXXIX–XLI. The mobility of the individual compounds within each group is dependent predominantly on the number of sulpho and carboxy groups, the presence of each of these groups causing a considerable increase in mobility. The substitution of the first member of each group by other functional groups is usually of only slight influence. If the buffer solution II (pH 4) is used, the carboxy groups of the dyes VII–IX containing one sulpho and one carboxy group are no more dissociated which results in a considerable decrease of the mobility of the dyes. Their mobility is close to that of the monosulfo derivatives under these conditions. Thus, the detection of the carboxy group in a dye of unknown structure is possible by comparing its electrophoretic behaviour at pH 5 and 9. The most successful result of our study has been the distinct separation of the isomeric 2,6- and 2,7-disulpho derivatives (XXIX–XXXI) demonstrated in Fig. 1. This fact is in accordance with the observation of FRANC<sup>10</sup> on the resolution of the isomeric 2,6- and 2,7-anthraquinone disulphonic acids in alkaline medium, the 2,7-isomer showing faster mobility. Using paper chromatography neither the isomeric acids<sup>11</sup> nor the isomeric dyes<sup>1</sup> could have been resolved.

The results obtained have shown that efficient separation of acid anthraquinone dyes can be achieved by paper electrophoresis in alkaline medium using buffer solutions containing ethanol, particularly in cases when mixtures of dyes after sulphonation containing derivatives of different sulphonation degree and isomeric disulpho derivatives are to be analysed. The differentiation of carboxy groups and sulpho groups is also possible.

Thanks are due to Mrs. J. ČEJKOVÁ for experimental assistance.

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Received October 28th, 1970

*J. Chromatog.*, 54 (1971) 436-441