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Note

Polyacrylamide gel electrophoresis of water-soluble coal-tar dyes

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Electrophoresis on polyacrylamide gel, since it was first developed by Ornstein and Davis[1], has been used extensively in the study of proteins and nucleic acids[2,3] and in the qualitative analysis of alginic acid[4], but not for separation of small molecules such as coal-tar dyes. Synthetic coal-tar dyes have usually been separated by solvent fractionation[5] or chromatography[6–12], but these methods are time consuming and poor reproducibility is obtained. In this paper, the application of polyacrylamide gel electrophoresis to the separation of water-soluble synthetic coal-tar dyes is described.

EXPERIMENTAL

Reagents

Fourteen synthetic water-soluble coal-tar dyes were purchased from local suppliers and 0.1% aqueous solutions were prepared. Polyacrylamide gel columns (7.5 × 0.6 cm I.D.) were prepared according to Clarke[13], using the reagent combination shown in Table I. The chamber buffer was 0.01 *M* Tris–glycine, pH 8.0, containing 0.1% of sodium chloride.

Procedure

Electrophoresis was carried out for 20–50 min at 100–130 V (10 mA per gel) in a cold chamber (*ca.* 5°). The sample size was 20–300 μl and to each sample 10–20%

TABLE I

COMBINATION OF REAGENTS FOR PREPARATION OF THE GEL

Reagents	*Concentration*	*Vol. parts combined*
Acrylamide	10%	12.5
N,N′-Methylenebisacrylamide	1%	3.0
N,N,N′,N′-Tetramethylethylenediamine	0.28%	3.0
Gel buffer (Tris–HCl, pH 6.7)	0.08 *M*	4.0
Ammonium persulphate	12%	0.1
Distilled water		2.4

of sucrose was added. The mobility (M) of each dye examined was expressed as the distance in centimetres from the top of gel to the centre of the dye band. The relative mobility (RM) of dye was expressed as the ratio of the mobility of dye being examined to the mobility of tartrazine on the same gel.

RESULTS AND DISCUSSION

Fig. 1 shows a typical electropherogram of five water-soluble coal-tar dyes on polyacrylamide gel. Because tartrazine was found to have the highest mobility, it was used as the reference compound ($RM = 1.00$) against which the RM values of other dyes were compared. Table II gives the trivial names, Colour Index numbers, colours produced and RM values of 14 synthetic coal-tar dyes. The results indicate that dyes with a difference in RM of greater than 0.05 could be separated completely under the conditions used.

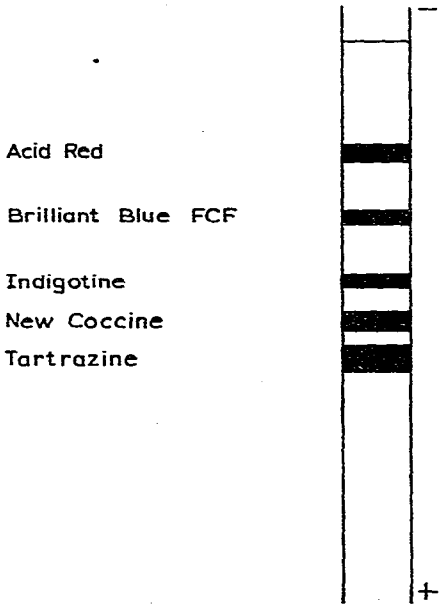

Fig. 1. Typical electropherogram of coal-tar dyes on polyacrylamide gel. Gel column size: 7.5 × 0.6 cm I.D.; gels were prepared with 5% of monomer in Tris–HCl buffer (pH 6.7). Current applied: 10 mA per gel. Time of run: 40 min. Chamber buffer: Tris–glycine buffer (0.01 M, pH 8.0, containing 0.1% of NaCl).

As the volume of the sample was increased, the mobility of the dyes decreased slightly, but the RM value of each dye remained almost constant. Increasing the monomer content (to 20%) or the bisacrylamide to acrylamide ratio had no or little effect. A chamber buffer containing 0.1–1.0% of salt (sodium chloride, ammonium chloride, etc.) was necessary in order to obtain good separations of dyes. It was found that amounts of each dye as low as 0.5–0.2 μg in a sample could be detected by this method.

From the results obtained, we conclude that polyacrylamide gel electrophoresis

is an excellent procedure for the fractionation of coal-tar dyes. The application of this technique to the detection and quantitative analysis of dyes in foods and drugs is being developed in this laboratory and will be published later.

TABLE II

RELATIVE MOBILITIES (*RM*) OF 14 COAL-TAR DYES ON POLYACRYLAMIDE GEL ELECTROPHORESIS

Trivial name	*Colour Index no.*	*Colour produced*	*RM*
Tartrazine	19140	Yellow	1.00
New Coccine	16255	Red	0.86 ± 0.01
Amaranth	16185	Red	0.81 ± 0.01
Sunset Yellow FCF	15985	Orange	0.76 ± 0.01
Indigotine	73015	Blue	0.73 ± 0.01
Fast Green FCF	42053	Green*	0.58 ± 0.02
Light Green SF Yellowish	42095	Green*	0.58 ± 0.02
Eosine	45380	Red (green)**	0.57 ± 0.02
Brilliant Blue FCF	42090	Blue*	0.56 ± 0.02
Phloxine	45410	Pink (yellowish green)**	0.49 ± 0.01
Erythrosine	45430	Red	0.49 ± 0.02
Acid Violet 6B	42640	Violet	0.44 ± 0.01
Rose Bengal	45440	Red	0.42 ± 0.01
Acid Red	45100	Purple (light yellow)**	0.36 ± 0.02

* Usually two bands were obtained on electrophoresis and the data presented here relate to the *RM* value of the main band.

** Colour of fluorescence in parentheses.

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