CHROM. 4201

Determination of the molecular weights of dyes by thin-layer gel filtration on Sephadex LH-20

There have been several recent accounts of the gel filtration of dyes on Sephadex media, *e.g.* food dyes on columns and thin layers of Sephadex G-25 (ref. 1), the histochemical reagent NBT* chloride on thin layers of Sephadex G-25 (ref. 2) and column chromatography of fluorescent dyes on Sephadex G-15 (ref. 3). Comment was usually made concerning the adsorption of dyes, by various processes, onto the gel filtration media, a topic which has also been considered more generally (ref. 4). Perhaps due to such adsorption no correlation between the molecular weights of dyes and their chromatographic mobilities has been reported.

In an effort to reduce such adsorption (see ref. 4) a number of dyes were chromatographed on thin layers of the hydroxypropylated dextran Sephadex LH-20 using dimethylformamide as solvent. The layers were prepared according to the manufacturers' instructions (ref. 5) on microscope slides and 20 × 20 cm glass plates. The latter system had no real advantages since increased resolution was masked by increased

TABLE I

DYES CHROMATOGRAPHED; CODE LETTERS FOR FIG. 1 AND DYE CLASS

Dye	*Code letter for Fig. 1*	*Class*
Picric acid	A	nitro
Azo Eosin G	B	monoazo
Crystal Violet	C	triphenylmethane
Dichlorofluorescein	D	fluoran
Acid Alizarin Blue GR	E	anthraquinone
Ponceau de Xylidine	F	bisazo
1-Hydroxy-2-(*p*-hydroxy-*o*-dianisylazo)-8-amino-3, 6-naphthalenedisulphonic acid, disodium salt	G	monoazo
Amidoblack 10B	H	bisazo
Chlorantine Fast Red	I	bisazo
Chlorophosphine	J	thiazole-azo
Chloramine Yellow	K	thiazole-azo
Chlorazol Black E	L	trisazo
Dianil Green B	M	trisazo
Sun Yellow G	N	stilbene
Trypan Red	O	bisazo
Evans Blue	P	bisazo
Eosin B	Q	fluoran
Phloxine	R	fluoran
Erythrosin	S	fluoran
Rose Bengal	T	fluoran

diffusion. The chromatograms were run with the plates horizontally, solvent flows of around 2 cm/h were maintained by adjusting the levels of eluant in the inflow and outflow reservoirs. The solvent front was marked with Indian ink. Table I lists the dyes studied, together with their code letters for Fig. 1 and their structural class. The dyes

* NBT = 3,3'-(3,3'-dimethoxy-4,4'-diphenylen)-bis-[2-(*p*-nitrophenyl)-5-phenyl-tetrazolium].

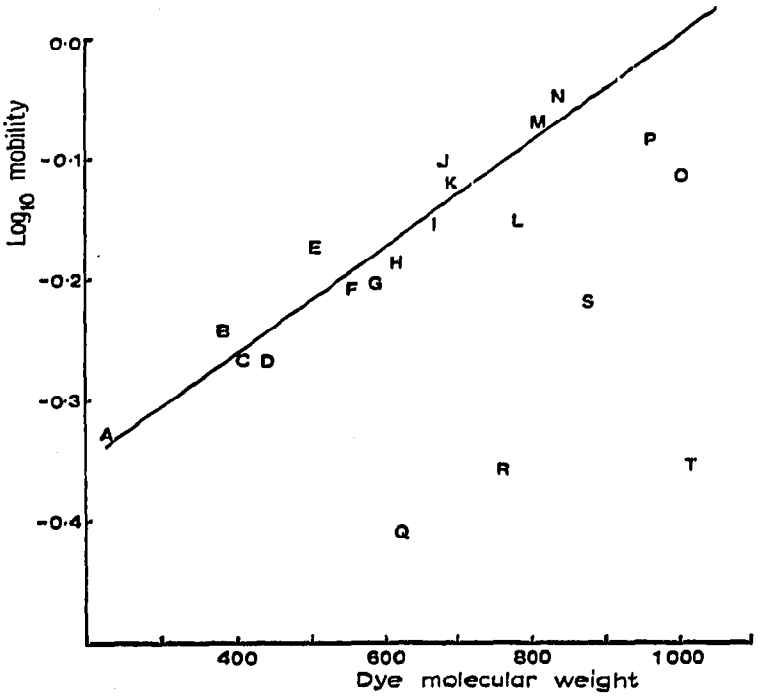

Fig. 1. A chromatographic mobility–molecular weight plot of various dyes (identities given in Table I) on thin layers of Sephadex LH-20 using dimethylformamide as developing solvent.

were spotted onto the thin layers in dimethylformamide solution. The origin line was scribed onto the back of the plate with a diamond. The chromatographic mobilities of the dyes were taken as the ratios of the distances migrated by the dyes to the distance migrated by the solvent front, *i.e.* the Indian ink spot.

In Fig. 1 the results are given in the form of a plot of $\log_{10}$ dye migration against molecular weight. The plot indicates that for dyes of a number of classes (see Table I), a chromatographic mobility–molecular weight correlation does exist. The dyes not falling in the main sequence of the plot (*i.e.* dyes Q, R, S and T) are all heavily halogenated fluorans, *e.g.* dye T (Rose Bengal) carried as substituents four chlorine and four iodine atoms. The reason for these deviations is probably that addition of, *e.g.*, an iodine atom increases the molecular weight of a dye by 127 whereas addition of, *e.g.*, the *larger* phenyl group increases the molecular weight by only 77. Other, smaller, deviations from the "main sequence" are shown by dyes L, P and O. All these dyes possess six aromatic rings and carry several groupings capable of forming hydrogen bonds, both features increasing adsorption of dye onto the Sephadex (ref. 4).

Department of Human Biology and Anatomy, R. W. HOROBIN
The University of Sheffield, Sheffield (Great Britain)

Peter Symond's School, Winchester (Great Britain) J. GARDINER

1 J. R. PARRISH, *J. Chromatog.*, 33 (1968) 542.
2 H. GROSSMANN AND H. WAGNER, *J. Chromatog.*, 35 (1968) 301.
3 J. A. DEMETRIOU, F. M. MACIAS R., M. J. MCARTHUR AND J. M. BEATTIE, *J. Chromatog.*, 34 (1968) 342.
4 M. JOUSTRA, B. SÖDERQVIST AND L. FISCHER, *J. Chromatog.*, 28 (1967) 21.
5 *Thin-layer gel filtration*, Pharmacia, Uppsala.

Received May 13th, 1969