The interaction of dyes with ion exchange resins

In analytical chromatographic systems where high resolution is required, appreciable differences in performance are often observed with different batches of resin having presumably identical particle size, cross-linkage, and composition. For example, in a recently developed method for separating purine and pyrimidine bases, nucleosides, and nucleotides, different batches of Dowex-1 X8 were found by ANDERSON, GREEN, BARBER, AND LADD[1] to give quite different results. Such results may be due to contamination of one resin type by another, by differences in the cross-linkage of different resin particles, or by small differences in the chemical composition of different batches of resin. It appeared of interest therefore to develop staining methods for determining the type of resin and if possible the cross-linkage to see if differences between individual particles could be detected.

Experimental

The possibility that a given resin preparation may be contaminated by particles having a different charge was investigated by the use of acidic and basic dyes. Methyl green (0.3 % in water) stained Dowex 50 resin a deep green while leaving Dowex 1, 2, and 3 unstained. The anion exchangers were stained a deep red with Wool Red 40-F which left Dowex 50 colorless. Carboxylic resins such as IRC-50 may be easily distinguished microscopically from the other resins used by their amorphous particle form.

Since variation in crosslinkage is also considered important in controlling the permeability of ion exchange resins, the possibility was considered that resin beads having different cross-linkages would stain differently with dyes having different molecular weights. The test this, 32 dyes, ranging in molecular weight from 240 to 1519 were prepared as 0.3 % solutions in either sodium acetate or hydroxymethylaminomethane (Tris). One ml of dye solution was added to 0.5 ml of packed Dowex-1 having cross-linkages of 2, 4, 8, or 10 % (200–400 mesh). The degree of penetration of each dye was observed usually after allowing the staining reaction to proceed for 30 min. The slurry was mounted on a slide, allowed to dry, and mounted in Permount. Very few instances of partially or non-uniformly stained beads were observed; it was likewise for beads having microscopic air bubbles in the center. The vast majority of the beads stained uniformly with a given stain, regardless of crosslinkage.

To see whether differences in the rate of penetration of the dye occurred, resin particles were wet mounted in buffer on microscope slides in such a manner as to allow test solutions to flow by. No differences in the rate of penetration could be observed between any of the dyes.

Of the 32 dyes tested, one was amphoteric, 9 basic and 22 acidic. The amphoteric dye along with two of the basic ones and three of the acid type did not stain the resins used, as shown in Table I. (Experiments in sodium acetate at pH 4.4 gave similar results and are not reported.) The remaining 26 (6 basic, 20 acidic) showed uniform staining reactions within a series (Dowex-1, X2, X4, X8 or X10) and no significant differences in the intensity of staining was observed with varying molecular weights.

To check for pH phenomena, dyes numbered 1, 3, 31 and 32 were pH adjusted to 7.5–8.0 and tested on Dow 1 (X2, X4, X8, X10), Dow 3 (X4) and Dow 50 (X8). Results are tabulated in Table II.

A corollary experiment using bovine serum albumin (BSA) stained in excess

TABLE I

STAINING REACTIONS OF DOWEX-1 SERIES RESINS

Dyes as 0.3 % solutions in 0.1 *M* Tris, pH non-adjusted.

No.	*Stain*	*Nature*	*Mol. wt.*	*Staining reaction on Dow 1 series*
1	Luxol fast blue	acid	1519	blue
2	Erie fast rubin B	acid (?)	1470	red
3	Alcian blue	amphoteric	1341	colorless
4	Solantine red	acid (?)	1268	yellow-orange
5	Rose bengal	acid	1049	red
6	Trypan red	acid	1002	orange-red
7	Chlorantine red	acid	991	red
8	Evans blue	acid	960	purple
9	Fast green	acid	808	purple
10	Ponceau S	acid	760	red
11	Aniline blue	acid	737	blue
12	Congo red	acid	696	red
13	Eosin Y	acid	691	red
14	Azocarmine B	acid	680	red
15	Bromphenol blue	acid	670	red
16	Wool red 40-F	acid	605	red
17	Cloth red B	acid	584	red
18	Azocarmine G	acid	580	red
19	Crocein scarlet MOO	acid	556	colorless
20	Janus blue	basic	505	red
21	Janus green B	basic	483	colorless
22	Rhodamine B	basic	479	yellow-orange
23	Janus red	basic	460	red
24	Methyl green	basic	458	colorless
25	Janus black B	basic	453	red
26	Orange G II	acid	452	colorless
27	Diethyl Safranin	basic (?)	350	yellow-orange
28	Methyl orange	acid	327	yellow-orange
29	Basic fuchsin	basic	323	red
30	Pyronin Y	basic	303	red
31	Methyl red	acid	291	purple
32	Alizarin	acid	240	colorless

TABLE II

STAINING REACTIONS OF VARIOUS RESINS WITH pH ADJUSTED DYES

Dyes as 0.3 % solutions in 0.1 *M* Tris, pH 7.5–8.0.

Resin	*Luxol fast blue (1)*	*Alcian blue (3)*	*Methyl red (31)*	*Alizarin (32)*
1 series	green	no staining	yellow	light pink
3(×4)	green	no staining	yellow	light pink
50(×8)	colorless	no staining	red	light yellow

with dyes numbered 1, 3, 15, 31 or 32 was performed. The stained BSA was dialyzed against Miller–Golder buffer (pH 7.5, μ 0.1)[2] for 48 h before being added to the resin beads. Though definite color still persisted in the BSA in all cases, no penetration of colored material into the beads was observed.

Discussion

It is apparent that resin beads of the polystyrene type are more permeable to molecules up to molecular weights of 1628 than might have been supposed. This suggests that separations of substances in this size range on such resins depend very little on simple permeability phenomena, and that the rationale behind choosing a resin of a given cross-linkage for a given purpose needs reconsideration.

The simple staining methods described permit the detection of accidental contamination of a cation exchanger with an anion exchanger, or *vice versa*. The experiment also indicates that particles as large as BSA cannot penetrate these beads. The uniformity of the dyed resin beads suggests that they may be useful as standards for the calibration of spectrophotometric systems.

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2 G. L. MILLER AND R. H. GOLDER, *Arch. Biochem.*, 29 (1950) 421.

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Ion-exchange separation and quantitative determination of dimethyl sulfoxide

During studies on copolymerization reactions of amino acids, peptides, proteins, and related model compounds with vinyl derivatives in dimethyl sulfoxide (DMSO) it was not possible to characterize the intermediates because DMSO could not be completely removed at reduced pressures.

To surmount this difficulty, an ion-exchange chromatographic method was developed to separate DMSO from compounds that bind to ion-exchange resins. The DMSO was quantitatively estimated by titration with an oxidizing agent. Since DMSO is currently the subject of pharmacological[1] investigations and is being widely used in chemical studies as a solvent and reactant, the proposed procedure should find extensive application.

Experimental

Glass columns, 2.5 × 50 cm, equipped with sintered-glass discs were used.