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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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.

Dowex* 50 X 8, 200–400 mesh, was treated as described by WALL[2]. The resin was washed to neutrality with water, and the sample containing about 10 g of DMSO and 1–2 g of amino acid was added to the column in about 50 ml of water. The column was then eluted with 1000 ml of water and then with sufficient hydrochloric acid to remove the amino acid[3].

A carbonate form of Dowex 1 X 8, 200–400 mesh, was prepared from the hydroxide form by passing several liters of saturated ammonium carbonate through the column. The column was then washed to neutrality, and the sample containing 10–12 g of DMSO and 1–2 g of acidic material in about 50 ml of water was added to the column. Elution was carried out with 1000 ml of water and then 1.5 *N* ammonium carbonate until the acidic compounds were removed[4].

The eluant from the columns was collected in 20-ml fractions by means of a fraction collector. Aliquots of these fractions were removed and analyzed for various components.

DMSO was determined by quantitative oxidation of the sulfoxide to the sulfone with $KMnO_4$. An 0.1-ml aliquot from each fraction, which contained 1–20 mg of DMSO, was placed in a test tube and diluted with 1 ml of 1 *N* H_2SO_4 and 5 ml of 0.1 *N* $KMnO_4$. The mixture was allowed to stand for 5 min and then was either titrated directly with 0.1 *N* $FeSO_4$ or diluted with 5 ml of 0.1 *N* $FeSO_4$ and excess ferrous ions were titrated with additional permanganate**. $Ce(NH_4)_2(NO_3)_6$ in aqueous H_2SO_4 did not oxidize DMSO.

Amino acid aliquots were evaporated to dryness under vacuum and analyzed with ninhydrin[6]. Ninhydrin-negative acids were located by spotting aliquots on Whatman No. 1 paper and spraying with bromcresol green spray[2].

Results and discussion

Dowex-50 and Dowex-1 columns were used to remove DMSO from a series of compounds that bind to ion-exchange resins. The elution pattern from the Dowex-50 column is illustrated in Fig. 1 with glutamic acid and lysine as the ninhydrin-positive

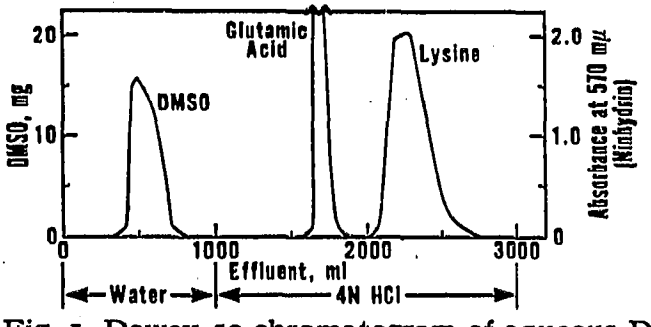

Fig. 1. Dowex-50 chromatogram of aqueous DMSO solution of glutamic acid and lysine.

components. With this system, the DMSO is retarded at around 300 ml as compared to the holdup volume, although it is completely eluted from the resin by about 800 ml of water. Since amino acids require hydrochloric acid for elution, they are therefore completely separated from the DMSO.

A Dowex-1 chromatogram which illustrates the separation of acidic compounds,

* Mention of suppliers of chemicals or specific products does not constitute preferential endorsement of their products by the U.S. Department of Agriculture.

** The use of potassium permanganate to oxidize dimethyl sulfoxide to dimethyl sulfone has been previously suggested in a footnote by T. M. DOUGLAS[5], but no details are given.

such as citric and glutamic acids, from DMSO is shown in Fig. 2. Both these acids elute in the same position, as confirmed by paper chromatographic analysis of the eluant. This column does not appear to retard DMSO since it elutes at about the holdup volume.

A quantitative procedure was developed for the estimation of DMSO. The method involves oxidation of the sulfoxide to the sulfone with potassium permanganate as described in the Experimental section. Although titration of excess ferrous ions gives a sharper endpoint, direct titration with $FeSO_4$ is more rapid and requires less solution. The stoichiometry of the oxidation ($5CH_3SOCH_3 + 2MnO_4^- + 6H^+ \rightarrow 5CH_3SO_2CH_3 + 2Mn^{2+} + 3H_2O$) was determined by titrating a series of known aqueous DMSO solutions with this oxidizing agent (Fig. 3). This technique established that DMSO was completely recovered from the ion-exchange column.

Ceric ion was also evaluated as an oxidizing agent for DMSO since this ion is a stronger oxidizing agent (by 0.1 V) than potassium permanganate and would act as

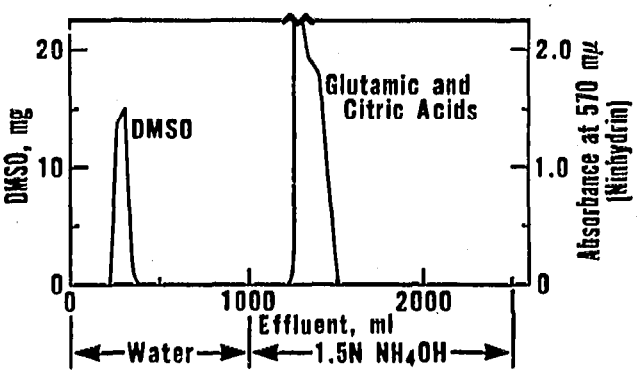

Fig. 2. Dowex-1 chromatogram of aqueous DMSO solution of citric and glutamic acids.

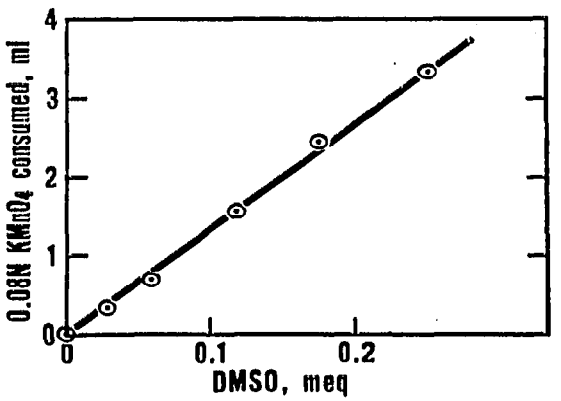

Fig. 3. Oxidative titration of aqueous DMSO solutions with 0.08 *N* $KMnO_4$.

its own indicator. Surprisingly, ceric ions did not oxidize DMSO. Apparently, one-electron oxidizing agents, such as ceric ion, are not suitable for the oxidation of sulfoxides to sulfones.

The retardation of DMSO on the Dowex-50 column is probably due to the slight basicity of this compound and to dipole–dipole interaction between a partial positive charge on sulfur of a polarized DMSO molecule and the negative charge of the sulfonic acid anion side chain of the resin.

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