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THE SEPARATION AND PURIFICATION OF IONIC AZO AND AZOMETHINE DYES BY GEL PERMEATION CHROMATOGRAPHY

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SUMMARY

Chromatography on Sephadex gels can separate water-soluble azo and azomethine dye sulfonates from colored impurities that are not removed by recrystallization. The method gives sharp bands and high resolution; as many as eleven bands have been resolved from a crude azo dye preparation. Mixtures of isomeric dyes have been separated. The azomethine dyes studied here are separated from hydrolysis and oxidation products formed during the preparation, and from other similar dyes. The separations of both classes of dyes depend upon adsorption to the gels. Variations with gel cross-linking and the nature and concentration of electrolyte in the eluent are reported.

INTRODUCTION

The preparation of highly purified samples of water-soluble dyes has often been a difficult task and one that has not benefited appreciably by advances such as gas–liquid partition chromatography (GLPC) and thin-layer chromatography (TLC). The worker is always faced with two difficulties in purifying such dyes. The first is that he is usually limited to recrystallization as a purification method. The nature of the molecules is such that they exhibit sufficient ionic behavior to be soluble only in highly polar solvents, but because such a large fraction of the molecule is hydrophobic, the dyes form aggregates in the useful recrystallization solvents and either do not crystallize well, or else do not provide adequate fractionation of similarly constituted impurities when they do crystallize. The second difficulty is that there is no satisfactory criterion of purity once a purification procedure has been adopted. The dyes do not melt at reasonable temperatures, are not volatile enough for GLPC or mass spectral analysis, and do not give good thin-layer chromatograms on the present supports. One must usually be satisfied with a good elemental analysis or an absorption curve that does not change with additional stages of purification. As we shall show in this paper, neither of these criteria is adequate for indicating the presence of small amounts of impurities.

Our need for small quantities of highly purified ionic dyes for physico-chemical studies has led to the development of a very successful method using descending column chromatography on Sephadex gels. The method gives resolution of sulfonated azo dyes comparable to that obtainable for nonionic organic substances by GLPC. It has revealed the presence of as many as six dyes in "analytical samples" that had been exhaustively recrystallized. The method is convenient for detecting the presence of colored impurities on small columns, and purifying research samples on large columns. In addition, we have achieved complete separation of isomeric azo dyes in all but one case attempted; we have also separated one pair of isomeric azomethine dyes.

EXPERIMENTAL

Azo dyes

All the series I and II dyes were prepared by coupling the diazotized anilines with the naphthol sulfonates in the usual manner. In most cases, the dyes were recrystallized repeatedly from water or ethanol–water until a good elemental analysis was obtained for C, H, N, and S, and no change in the absorption curve was obtained with additional recrystallization. The dyes were dried at 120° under waterpump vacuum and were obtained as the monohydrates in most cases. Elemental analyses for the series I dyes were within 0.3% of the theoretical for C, H, and N. The series II dyes were those used in an earlier study[1].

In a typical preparative-scale purification, 150 mg of a dye which had been recrystallized at least twice was dissolved in 15 ml of 0.05 *N* potassium hydroxide and introduced onto a 4.5 × 28 cm column of Sephadex G-25. The gel had been swollen in water, and the column had been previously washed with 0.01 *N* potassium hydroxide. The column was eluted with 0.01 *N* potassium hydroxide. Most of the colored impurity bands were held more strongly than the desired dye. The column could usually be stripped of the impurity bands with 0.01 *N* potassium hydroxide solution and used repeatedly.

The eluate containing the main dye fraction was concentrated and neutralized, and the precipitated dye was recrystallized.

In a typical separation of a synthetic mixture of isomeric azo dyes, 10-mg samples of each purified dye were dissolved in 2 ml of 0.05 *N* potassium hydroxide solution, and the solution was added by pipet to a 2.5 × 31 cm column of Sephadex G-25. The column was eluted with 0.01 *N* potassium hydroxide. Five-milliliters fractions were collected in an automatic fraction collector, and the absorption curves of the appropriately diluted fractions were recorded on a Beckman DK-2A recording spectrophotometer.

Azomethine dyes

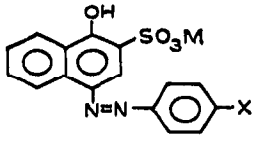
A typical preparation of series III dyes has been described[2]. The pH of the reaction mixture was lowered to about seven as soon as possible after mixing the reagents. The excess naphthol was extracted with ethyl acetate, and the dye was removed from the aqueous solution containing inorganic salts by repeated extractions with *n*-butanol. The extracts were dried with anhydrous sodium sulfate and concentrated in a rotary evaporator below room temperature until the dye separated as a

bronze precipitate. Chromatography of the product on Sephadex G-50 separated the dye from small quantities of impurities. The homogeneity of the dye band was established by collecting fractions during its elution and showing that the normalized absorption curves of fractions taken from the leading edge and tail of the band were congruent.

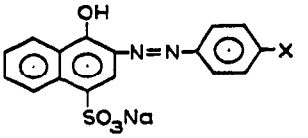
In a typical separation of synthetic mixtures of dyes previously purified by gel chromatography, 5 mg of each dye was dissolved in 5 ml of 0.01 *M* KCl and chromatographed on a 2.1 × 30 cm column of Sephadex G-50, using 0.01 *M* KCl as eluent. A flow rate of 0.8 ml/min was maintained.

CHART I

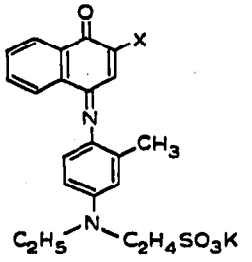
DYE STRUCTURES



I (a) X = H, M = K
(b) X = SO_3Na, M = Na
(c) X = CF_3, M = K
(d) X = OCH_3, M = K
(e) X = NO_2, M = K



II (a) X = H
(b) X = SO_3Na
(c) X = CF_3
(d) X = OCH_3



III (a) X = $CONH_2$
(b) X = $CONHC_4H_9$-*n*
(c) X = $CONHC_2H_4SO_3K$
(d) X = $CON(C_2H_5)_2$

RESULTS AND DISCUSSION

Azo dyes

The structures of the dyes used in this study are shown in Chart I. In most cases the samples had been exhaustively recrystallized prior to chromatographic analysis and were regarded as "pure" on the basis of excellent elementary analyses. Fig. 1 shows the elution curve of such a sample of Ic. The absorbances of the eluted fractions are plotted on a logarithmic scale in order to compress the absorbance of the major component of the mixture and to emphasize the minor impurities. Besides the four impurity bands shown, two additional impurities present in this mixture were separated but were not eluted. Similar elution curves were obtained with each of the "analytically pure" samples of the I and II series. The number of impurity bands ranged from one in the case of Ia up to six for Ic and Ie.

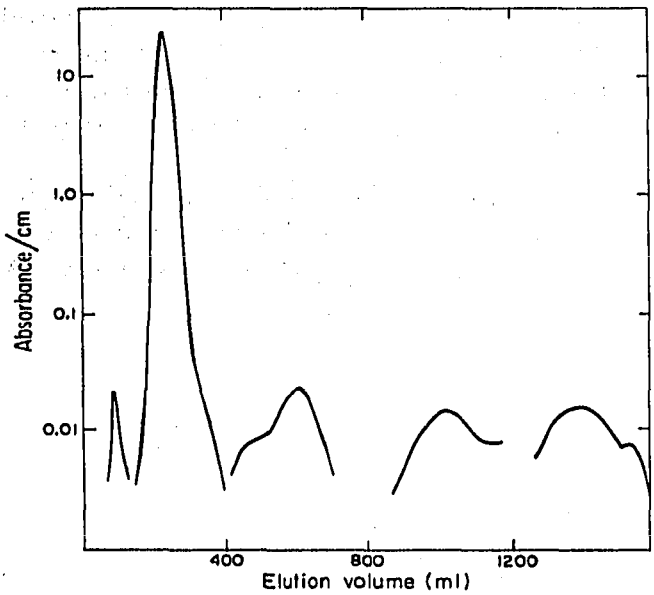


Fig. 1. Elution curve of dye Ic after repeated recrystallizations. Column, Sephadex G-25 (2.5 × 27 cm); eluent, 0.01 *M* KOH.

The excellent resolution obtained by chromatography of these dyes on Sephadex gels depends on the use of dilute alkaline solution as eluent. The naphthol OH groups of all the dyes are ionized in such a medium, and this apparently provides the highly selective adsorption and facile elution required for the resolution of complex mixtures of similar dyes. Fig. 2 shows a comparison of elution curves of a synthetic mixture of 10 mg each of the purified isomeric dyes, Id and IId, 0.01 *M* KOH and 0.01 *M* KCl being used as eluents. The same column was used for both separations and consisted of 38.6 g dry weight of Sephadex G-25 which, after swelling, gave a 2.3 × 41 cm

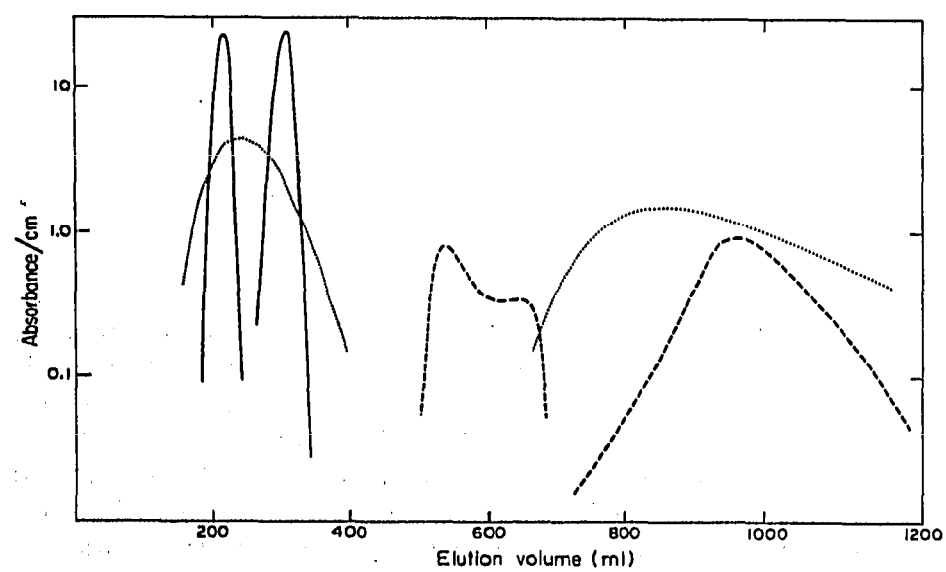


Fig. 2. Comparison of Sephadex gels and eluents for the separation of dyes Id and IId. ———, Sephadex G-25 (2.3 × 41 cm), 0.01 *M* KOH; · · · · ·, Sephadex G-10 (2.3 × 16 cm), 0.01 *M* KOH; — — —, Sephadex G-25 (2.3 × 41 cm), 0.01 *M* KCl.

column. The much greater elution volumes and broadened bands obtained with KCl show that the effectiveness of KOH as eluent is not just the result of having present a given ionic strength of electrolyte. The presence of a neutral electrolyte is beneficial, however, since attempts to separate mixtures by elution with water gave very broad bands and no resolution. The sulfonate groups are ionized in both media.

BAETZ AND DIEHL have reported the separation of complex reaction mixtures of substituted O,O'-dihydroxyazobenzenes on Sephadex G-10, using 0.001 *N* sodium hydroxide solution as eluent[3]. We have tried G-10, G-15 and G-25 with our mixtures and find little difference among them in terms of resolution. The dyes are retained more strongly by the more highly cross-linked gel (G-10) and require longer elution times, but in no case did we find that G-10 resolved mixtures that could not be resolved more rapidly on G-25. Fig. 2 shows a comparison of elution curves for a mixture of Id and IId on columns of G-10 and G-25. The same dry weight (38.6 g) of both gels was used, giving columns of swollen gel with respective dimensions of 2.3 × 16 cm and 2.3 × 41 cm. The eluent was 0.01 *M* KOH in each case. The higher elution volumes obtained on the more highly cross-linked gel show that adsorption rather than molecular weight plays the predominant role in the separations.

The Sephadex gels can resolve mixtures that are more complex than those obtained from the "purified dyes". Reaction mixtures from the dye preparations were routinely separated on large laboratory columns of Sephadex G-25 after one or two preliminary recrystallizations with complete separation of the desired dyes from other impurities in the reaction mixture. In our most severe test, we separated a crude reaction mixture of IId, which had been formed in the presence of a small amount of added naphthol, which would couple to give the isomeric dye Id. The mixture was not recrystallized and contained, in addition to the isomeric dyes, all the colored impurities found as side products from the coupling of the two isomeric naphthols. Separation on Sephadex G-15 gave resolution of the isomeric dyes along with nine other clearly resolved bands.

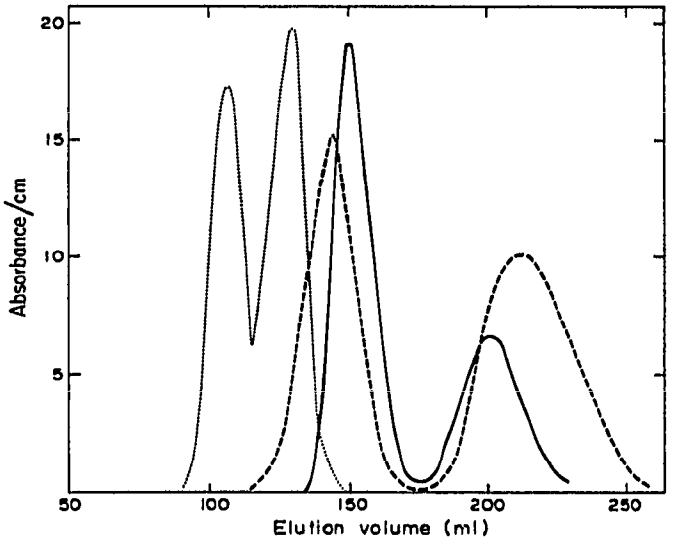


Fig. 3. Separation of isomeric dyes on Sephadex G-25. Eluent, 0.01 *M* KOH. ———, Ia and IIa; · · · · ·, Ib and IIb; — — —, Ic and IIc.

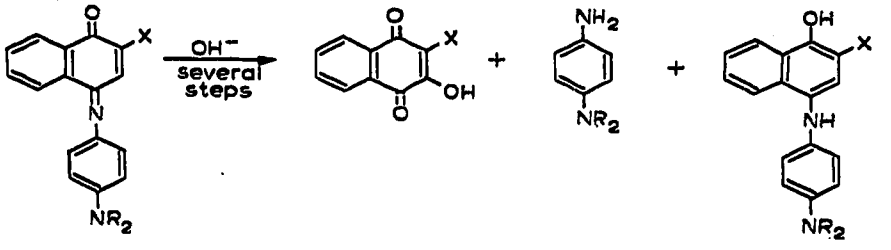
Mixtures of the isomeric pairs Ia–IIa, Ib–IIb, Ic–IIc, and Id–IId were prepared from dye samples that had been individually freed from colored impurities by chromatography on Sephadex G-25. Figs. 2 and 3 show the elution curves of the mixtures. Incomplete separation of the pair of disulfonated dyes, Ib and IIb, on G-25 is attributed to their greater water solubility. Attempts to separate this pair on G-10 and G-50 gave even poorer resolution than was obtained on G-25.

The isomeric dyes have characteristic absorption spectra which permit them to be distinguished. In every case the 2-arylazonaphthol (II) is eluted before the 4-aryl azo isomer (I). This result indicates that molecular geometry is an important factor in determining the specific adsorption to the gel.

We did not attempt to characterize the impurity dyes that were separated from the coupling reaction mixtures. An indication of the nature of the impurities was obtained from the chromatograms of the dye Ie reaction mixtures. Solutions of the nitro-substituted dyes are red-orange in neutral solution and purple in basic solution. All of the four major impurities and several minor colored impurities present in the reaction mixture were purple in basic solution, indicating that nitro dyes were present. This suggests that most of the colored impurities arise from coupling of the diazonium ions at a number of positions in the naphthol ring.

Azomethine dyes

The indoaniline dyes typified by structure III are more difficult to purify by recrystallization than are the azo dyes. These dyes probably aggregate in concentrated solutions in a way that prevents formation of nuclei. Crystalline precipitates are rarely obtained from supersaturated solutions. In addition, the azomethines are susceptible to decomposition by nucleophilic reagents and to hydrolysis catalyzed by acids and bases[2,4] (eqn. 1).



(1)

The dyes are prepared by oxidative coupling of the appropriate *p*-phenylenediamine with excess naphthol in alkaline solution[2]. The usual impurities are the dye hydrolysis products and colored materials arising from decomposition of the oxidized *p*-phenylenediamine and naphthol in the alkaline solutions. Other dyes are often present. Prior to our development of the method described here, we had to rely on countercurrent distribution for purifying small samples of these dyes[5].

Dyes IIIa–d are adsorbed to Sephadex G-25 and G-50, from which they can be eluted with an aqueous potassium chloride solution. We have used columns of G-50 routinely, eluting with 0.01 *M* KCl. With this method, the dye hydrolysis products and side products from the oxidized *p*-phenylenediamine are eluted ahead of the dye. The dye bands are not as narrow as those obtained with the azo dyes, but they are sufficiently narrow for the separation of impurities and of certain mixtures of similar dyes.

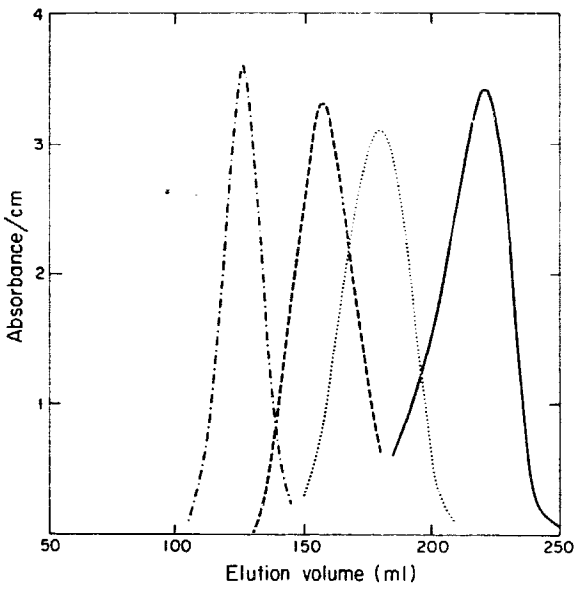


Fig. 4. Elution curves of dyes IIIa–d on Sephadex G-50 (2.1 × 30 cm). Eluent, 0.01 *M* KCl. ——, IIIa; · · · · ·, IIIb; — — — —, IIIc; —·—, IIId.

Fig. 4 shows the chromatograms of dyes IIIa–d on Sephadex G-50. The elution volumes are generally reproducible between columns. Two facts are significant: (1) The presence of additional anionic sites in the dye does not alter the degree to which the dye is adsorbed (compare IIIc with IIIb). (2) The order of increasing retention depends on the number of available protons on the amide nitrogen of the carbamoyl side chain (IIId < IIIc < IIIb < IIIa). This suggests that the adsorption of these dyes to the gel may involve hydrogen-bonding of the amide groups. Dyes IIIb and IIIc both contain secondary amide groups; mixtures of the two were not separated in spite of the difference in the number of ionic sites.

Several synthetic mixtures of dyes IIIa–d were chromatographed on Sephadex G-50 to determine how well similar dyes could be separated. We found that on a 2.1 × 30 cm column, a difference in elution volume of about 60 ml was necessary for good separation. Thus, IIIa could be separated from IIIc, but not from IIIb. A mixture of IIIb and IIIc could not be separated. All other combinations were resolved.

The azomethine dyes were more strongly adsorbed on the highly cross-linked gels. Dyes IIId and IIIb had elution volumes of 320 ml and 1050 ml, respectively, on a 2.6 × 40 cm column of Sephadex G-25, with 0.01 *M* KCl as eluent, compared to 180 ml and 220 ml for these dyes on a 2.1 × 30 cm column of Sephadex G-50. These dyes remained at the origin on a column of G-10 after elution with 200 ml of 0.01 *M* KCl. The elution volumes of the dyes were independent of the KCl concentration above 0.01 *M*. Dyes IIIa and IIId had elution volumes on G-50 of 220 ml and 125 ml, respectively, with 0.01 *M* KCl and 220 ml and 130 ml with 0.1 *M* KCl. Some KCl is necessary, however, since elution with H_2O failed to separate IIIa and IIId on Sephadex G-25.

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