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USE OF AMBERLITE XAD-2 FOR ISOLATION AND DETECTION OF WATER-SOLUBLE ACID DYES

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SUMMARY

A method is described in which Amberlite XAD-2 is used as an adsorbent for the isolation of synthetic water-soluble food dyes and for the detection of the dyes by thin-layer chromatography. The acid dyes are adsorbed on the XAD-2 resin in a presence of triethylammonium bicarbonate and then recovered in good yield by elution with methanol. In XAD-2 thin-layer chromatography, the solvent system acetone–concentrated ammonia–water (3:1:6 or 6:1:3) gave sharp resolution of the eleven dyes tested, especially of the xanthene compounds.

INTRODUCTION

Various procedures have been reported for the isolation and purification of water-soluble acid dyes from foods, including wool dyeing[1,2], solvent extraction[3], adsorption column chromatography[4–6] and ion exchange[7–9]. Of these, column chromatography using polyamide powder[5,6] gives results superior to those of solvent extraction and wool dyeing. In this paper a new method is presented in which Amberlite XAD-2 is used as an adsorbent for the isolation of water-soluble acid dyes and for the detection of the dyes by thin-layer chromatography (TLC).

Amberlite XAD-2, which is an insoluble, porous polystyrene–divinylbenzene copolymer, selectively adsorbs water-soluble organic compounds by interaction of their hydrophobic portion with the non-polar surface of the resin. XAD-2 has been widely used for the isolation and purification of many kinds of compounds from aqueous solution, *e.g.*, drugs in tissues and biological fluids[10] and organic contaminants in water[11–13]. Although XAD-2 appears to be a generally useful and powerful adsorbent for organic molecules, a limitation is encountered with polar compounds which have little or no affinity for the resin.

Scoggins and Miller[14] took advantage of the characteristics of XAD-2 to separate both aryl- and alkyl-monosulphonic acids from their disulphonic acid derivatives and sulphuric acid. The monosulphonic acids were retained on the column of XAD-2, while the others were eluted. Since the majority of the synthetic food dyes

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are sulphonic acid derivatives, *i.e.*, mono-, di- and trisulphonic acid derivatives, it seems to be difficult to isolate all of the food dyes using XAD-2. Application of XAD-2 for the separation of metal ions has been reported by Willis and Sangster[15] and by Fritz and Beuerman[16], who have achieved a selective adsorption of the ions on the resin via the formation of complexes. This indicates that the acid dyes appear to be adsorbed on the resin in a form of ionic associations with suitable cations. Recently, unique application of Amberlite XAD-4 (which is also a nonpolar synthetic adsorbent) for the adsorption and separation of polar nucleosides and nucleotides by coating the resin with triethylammonium bicarbonate (TEA) has been reported[17]. When a TEA solution containing the acid dyes is passed through a column of XAD-2, all the dyes are retained and then recovered in good yield by elution with methanol.

After isolation, the food dyes are generally detected and identified by paper chromatography and TLC, which have been studied by numerous investigators. For TLC, cellulose[18], silica gel[19] and polyamide[8,20,21] are used as adsorbents. The application of XAD-2 to TLC has not yet been reported. In this study, therefore, an attempt was made to analyze the acid dyes using an XAD-2 layer. The results indicate that the resin is a useful adsorbent in this field.

EXPERIMENTAL

Materials

The water-soluble acid dyes examined are listed in Table I and they comprise the Japanese standards of the food colours, except Fast Green FCF (reagent grade) which was obtained from Wako (Osaka, Japan). A standard solution of a dye was prepared by dissolving 10 mg of the dye in 100 ml of distilled water. A mixed dye

TABLE I

ACID DYES USED

Dye	*Colour index (C.I.) No.*	*Ionic group*
Xanthene compounds		
Erythrosine	45430	$-COONa$, $-ONa$
Phloxine	45410	$-COONa$, $-ONa$
Rose Bengal	45440	$-COONa$, $-ONa$
Acid Red	45100	$-SO_3Na$, $-SO_3^-$, $=\overset{+}{N}(C_2H_5)_2$
Azo compounds		
Amaranth	16185	$-SO_3Na$ (3)*, $-OH$
New Coccine	16255	$-SO_3Na$ (3), $-OH$
Tartrazine	19140	$-SO_3Na$ (2), $-OH$, $-COONa$
Sunset Yellow FCF	15985	$-SO_3Na$ (2), $-OH$
Ponceau 3R	16155	$-SO_3Na$ (2), $-OH$
Ponceau SX	14700	$-SO_3Na$ (2), $-OH$
Triphenylmethane compounds		
Fast Green FCF	42053	$-SO_3Na$ (2), $-SO_3^-$, $=\overset{+}{N}=$, $-OH$
Brilliant Blue FCF	42090	$-SO_3Na$ (2), $-SO_3^-$, $=\overset{+}{N}=$
Indigoid compounds		
Indigo Carmine	73015	$-SO_3Na$ (2)

* Numbers in parentheses indicate the number of substituent groups.

solution contained eleven food dyes permitted in Japan as food additives, in a concentration of 100 ng of each dye per ml.

Amberlite XAD-2 resin (20–50 mesh) was obtained from Rohm & Haas (Philadelphia, Pa., U.S.A.) and pulverized by ball-milling; different size grades were obtained by sieving through a Ro-Tap testing sieve shaker, using Japan Industrial Standard Sieves.

TEA solution (1 *M* and 0.1 *M*, pH 7.6) was prepared from triethylamine and carbon dioxide. Silica gel G (Type 60) for TLC was purchased from E. Merck (Darmstadt, G.F.R.). Organic solvents were guaranteed grade and obtained from Wako. Visible spectra were recorded on a Shimadzu Model UV-200 double-beam spectrophotometer.

Preparation of XAD-2 column and general separation procedure

The bottom of a glass column (30 × 0.5 cm) was fitted with a small glass-wool plug. The resin (0.5 ml, 100–200 mesh) suspended in methanol was poured into the column and the resin bed was washed with 30 ml of water in order to displace the methanol. Unless otherwise specified, the sample solution was mixed with an equal volume of 1 *M* TEA solution and the mixture was added to the column, followed by washing with 0.1 *M* TEA solution. The dyes retained on the column were eluted with methanol. The eluate (5 ml) was collected and applied to TLC plates and/or subjected to spectrophotometric analyses. The column was regenerated by washing with 10 ml of methanol and 30 ml of water.

Extraction of dyes from food

(1) Carbonated beverages. A sample (5 ml) was decarbonated by warming.

(2) Solid samples [jam, candy, Takuan (pickled radish), and Denbu (mushed and seasoned fish)]. A sample (2–5 g) was warmed with 5–15 ml of distilled water. After centrifugation the supernatant was filtered through glass-wool. An aqueous sample solution (5 ml) was handled as described above.

Preparation of XAD-2 layer and chromatographic procedure

To 5 g of XAD-2 (200–400 mesh) were added 15 ml of methanol and then 30 ml of water. After gentle mixing, silica gel G (10 g) was added to the mixture and the slurry thus obtained was spread on three glass plates (20 × 20 cm, thickness 0.25 mm) in the usual manner. The plates were air-dried for 1 h and dried at 80° for 1 h. A standard solution of dye was spotted on the starting line, 1.5 cm from the bottom of the layer, and the plate was developed by the ascending technique. The chamber had been equilibrated with the developing solvent for 30 min before use.

RESULTS AND DISCUSSION

Separation of dyes by XAD-2

Adsorption of dyes on XAD-2. In order to compare the adsorption ratio of dyes on the resin the procedure described in Experimental was carried out except that TEA solution was not used. One millilitre of dye solution (100 ng/ml) was passed through a column of XAD-2 (20–50 mesh), followed by washing with 25 ml of water. The filtrate was collected and checked for the eluted dye. The retained dye on the

resin was eluted with 10 ml of acetone–concentrated ammonia–water (6:1:3). As shown in Table II, both Erythrosin and Acid Red were almost quantitatively adsorbed by the resin, while Amaranth was not adsorbed to a large extent. Ponceau 3R and Ponceau SX were retained to a 35–40% extent. Amberlite XAD-2 is a nonpolar synthetic adsorbent, which adsorbs more strongly hydrophobic materials than hydrophilic materials. From these results, it is apparent that the adsorption ratio of the dye varies with the number of sulphonic acid groups in the molecule. Thus, Erythrosin, which has a carboxyl group and no sulphonic acid substituent, is adsorbed almost quantitatively on the resin, while Amaranth, a trisulphonic acid derivative, is not adsorbed on the resin. Ponceau 3R and Ponceau SX, which are disulphonic acid derivatives, are adsorbed moderately. It is generally true that organic acid materials are best adsorbed by the resin from an acid solution, where they are not ionized. However, attempts to adsorb all dyes on the resin by changing the pH of the sample solution (pH 1–7) were unsuccessful, because of the strong acidity of the sulphonic acid substituent.

TABLE II

ADSORPTION OF FOOD DYES ON AMBERLITE XAD-2 COLUMN

Sample	*In water**			*In 0.5 M triethylammonium bicarbonate***		
	Adsorbed (%)	*Unadsorbed (%)*	*Total (%)*	*Adsorbed (%)*	*Unadsorbed (%)*	*Total (%)*
Erythrosine	86.3	5.3	91.5			
Acid Red	99.2	1.0	100.2	99.5	1.2	100.7
Amaranth	3.4	91.3	94.7	91.5	3.9	95.4
New Coccine				87.8	10.4	98.2
Ponceau 3R	40.6	54.4	95.0			
Ponceau SX	35.9	57.4	93.3			

* XAD-2 (20–50 mesh) (2 ml) in the column (10 mm I.D.) and dye sample solution (100 ng/ml) were used. The washings (25 ml) obtained with water and eluates (10 ml) with acetone–concentrated ammonia–water (6:1:3) were collected.

** XAD-2 (20–50 mesh) (1 ml) in the column (10 mm I.D.) and dye sample solution (250 ng/ml) were used. The washings (10 ml) obtained with water and eluates (10 ml) with acetone–concentrated ammonia–water (6:1:3) were collected.

When the dye solution was mixed with an equal volume of 1 *M* TEA solution prior to application to the column of XAD-2 (20–50 mesh), the adsorption ratio of each dye was greatly increased (Table II). It is noteworthy that two trisulphonic acid derivatives tested, Amaranth and New Coccine, are adsorbed to the extent of *ca.* 90%. Since the ratio of the dye to resin used was five times that in the preceding experiment, it is concluded that the dye is adsorbed more strongly from the TEA solution. This result suggests that strong acidic dyes having trisulphonic acid groups are adsorbed tightly on the XAD-2 resin coated with TEA.

In one case water (10 ml) was used to wash the column after application of the dye, the coloured band on the column was slightly moved and some of the dyes, especially the trisulphonic acid derivative, were eluted to some extent. Because washing with a large volume of water seemed to cause the poorly adsorbed dye to

elute from the column, 0.1 *M* TEA solution was used for washing. The dye was retained on the column, even when large quantities of the TEA solution were passed through the column.

Elution of dyes from XAD-2. In order to choose the best eluent, the elution profile of one dye with several solvents was determined using Acid Red, which was the best adsorbed dye in Table II. The results are shown in Fig. 1. The dye was eluted more rapidly with methanol than with the ammoniacal mixture. For complete elution of the dye from the column, however, there is not much difference between the solvents. With these solvents Acid Red was eluted within 5–6 ml. Methanol was chosen as an elution solvent because Indigo Carmine, Rose Bengal and Fast Green FCF tend to decompose gradually in an alkaline medium. Acetone was a good eluent for Acid Red, but not for some other dyes, because when used to elute mixed dyes (eleven kinds of dyes) some blue colour remained on the column.

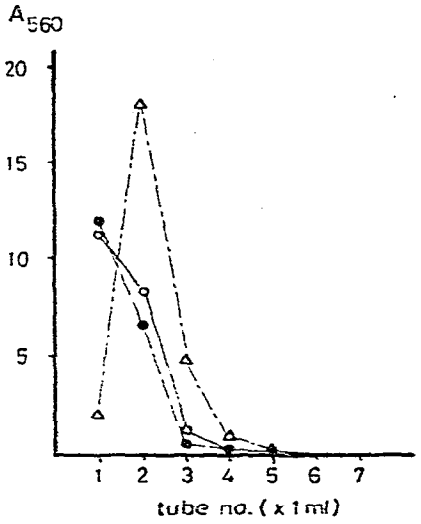

Fig. 1. Elution profile of Acid Red from XAD-2 column. A solution (5.0 ml) of Acid Red in 0.5 *M* TEA was applied to a column (5 mm I.D.) packed with 1 ml of XAD-2 (20–50 mesh). After washing with 0.1 *M* TEA, the colour was eluted with methanol (○—○), acetone (●– – –●) or acetone–ammonia–water (6:0.1:3.9) (△– · –△). The eluate was collected in 1-ml fractions. The absorption at 560 nm was measured after each fraction was diluted with phosphate buffer (pH 7.0).

Particle size and amount of XAD-2. The effect of the particle size of the resin was studied by using columns packed with different sizes of the resin (1 ml) (20–50, 100–200 and 200–400 mesh). When 10 ml of TEA solution containing Amaranth (500 ng) was passed through the column, the length of the coloured portion was determined. On the resin of 20–50 mesh, the band broadened considerably, sometimes so near the bottom of the column, depending upon the flow-rate. In other cases, using the fine sizes of the resin, the dye was tightly adsorbed at the top of the column and no difference was observed between the two sizes. The resin of 100–200 mesh was used routinely, because it gave a suitable flow-rate.

To determine the amount of the resin required, 2 ml of a TEA solution con-

taining Amaranth (100 ng) was applied to a column packed with 0.2 or 0.5 ml of the resin (100–200 mesh). No dye was detected in the filtrate and washing in both cases, indicating that 0.2 ml of the resin was enough to adsorb 100 ng of the dye. Mixed dyes, sometimes consisting of several dyes, are usually used for colouring processed foods. In some cases the content of the minor dye in a dye mixture is very low, below a few percent. To detect the minor dye, one has to isolate a large amount of the other dye(s), because the minor tends to be overlooked behind the major. For this reason, 0.5 ml of the resin was used in the following experiments. However, we found that 0.2 ml of the resin was sufficient (see below).

Recovery test of the dyes by XAD-2 isolation. A recovery test was carried out according to the procedure described in Experimental, using 1 ml of the standard solution of the dye (100 ng/ml). The recovered dye was determined spectrophotometrically after the eluate (5 ml) had been mixed with the same volume of 0.1 *M* phosphate buffer (pH 7.0). As shown in Table III, all the dyes gave 90–100% recovery.

TABLE III

RECOVERY OF FOOD DYES FROM XAD-2 COLUMN

The column (5 mm I.D.) was packed with 0.5 ml of XAD-2 (100–200 mesh). Elution solvent: 5 ml of methanol. For determination of dyes, see text.

Sample	*Amount (ng)*		*Recovery (%)*
	Added	*Found*	
Erythrosine	100	95.3	95.3
Phloxine	100	99.5	99.5
Rose Bengal	100	91.4	91.4
Acid Red	100	99.7	99.7
Amaranth	100	97.1	97.1
New Coccine	100	98.2	98.2
Tartrazine	100	99.1	99.1
Sunset Yellow FCF	100	100.1	100.1
Fast Green FCF	100	90.9	90.9
Brilliant Blue FCF	100	98.7	98.7
Indigo Carmine	100	95.8	95.8

Isolation of the dyes from foods. The XAD-2 isolation procedure was successfully applied to the analysis of synthetic dyes in foods. Samples used were processed foods, labelled "Colour additive used". The dyes in aqueous extracts were adsorbed on the top of the XAD-2 column. Methanol is a suitable solvent not only in elution processes, but also in subsequent identifications. The coloured band was so narrow that half of the resin used previously appeared to be suitable for routine analysis. Another reason for the decrease in the amount of the resin was to obtain an adequate flow-rate when viscous sample solutions prepared from sugar-rich food were applied to the column.

Thin-layer chromatography on XAD-2 layer

Attempts to separate the acid dyes by XAD-2 layer chromatography were carried out with several solvents. The best separation was achieved with solvent systems containing acetone, ammonia and water. Table IV shows R_F values of eleven

TABLE IV

THIN-LAYER CHROMATOGRAPHY OF FOOD DYES ON AN XAD-2 LAYER

Solvents: A = acetone–concentrated ammonia–water (3:1:6); B = acetone–concentrated ammonia–water (6:1:3).

Sample	*Solvent*	
	A	*B*
Erythrosine	0.13	0.34
Phloxine	0.04	0.23
Rose Bengal	0.03	0.15
Acid Red	0.28	0.53
Amaranth	0.96	0.98
New Coccine	0.62	0.82
Tartrazine	0.97	0.98
Sunset Yellow FCF	0.78	0.88
Fast Green FCF	0.47	0.77
Brilliant Blue FCF	0.30	0.58
Indigo Carmine	0.52	0.90

dyes, which are permitted in Japan. These solvent systems gave sharp resolution of the dyes, especially xanthene compounds. Other solvents tested were acetone, methanol, acetone–water, methanol–water, and methanol–ammonia–water. They did not give good separations, except for methanol–ammonia–water (6:1:3 and 7:1:2). Development with the methanolic ammonia solvent gave a fairly good separation of all the dyes, with slight tailing.

Separation of dyes by the XAD-2 layer chromatography is based on the affinity between the resin and the dye. It is clearly shown that the R_F values obtained vary with the polarity and hydrophobicity of the dyes. For example, Erythrosine, Phloxine and Rose Bengal are homologous compounds and they differ in the number of halogen substituents in their chemical structure. Thus, Erythrosine, which has four halogen substituents, migrates much faster than Phloxine and Rose Bengal, both of which have eight halogen substituents. Rose Bengal gave a lower R_F value than Phloxine. The difference in their mobilities might be attributed to the difference in halogen content: Rose Bengal is tetraiodotetrachloro derivative and Phloxine is tetrabromotetrachloro derivative. Since Acid Red is also a xanthene compound and contains a sulphonic acid group, it migrates faster than the three dyes mentioned above.

All other dyes, which contain two or more sulphonic acid groups and other ionic group(s), travelled faster than the xanthenes on the XAD-2 layer. Amaranth and Tartrazine migrated in the proximity of the solvent front. The spots near the solvent front were in narrow and sharp bands. Dyes having different colour tones could be distinguished from one another. Indigo Carmine gradually fades during the development. Therefore, a short developing time (solvent front 6–8 cm from the starting line) is recommended for the detection of Indigo Carmine.

It was possible to replace silica gel by gypsum [we used XAD-2–gypsum (1:1 and 2:1) mixtures] without any adverse effect on the separation, indicating that XAD-2 is responsible for the separation of the dyes in XAD-2–silica gel layer chromatography.

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