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Purification Procedures for Synthetic Dyes: Part 1—Dry Column Chromatography†

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SUMMARY

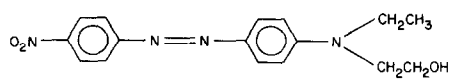
The suitability of dry column chromatography for the purification of synthetic dyestuffs has been investigated. The results of this study indicate that the utility of this method for the generation of gram quantities of pure dyestuffs is limited, from a practical standpoint, to disperse dyes. Although dry column chromatography appears to be impractical for the purification of gram quantities of acid and direct dyes, relatively simple monoazo acid dyes were successfully purified in smaller quantities by this procedure. The simplicity and low cost of this method make it an excellent way to obtain useful amounts of disperse dyes which are pure enough for use in biological assays such as Ames tests.

INTRODUCTION

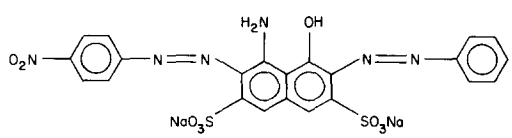
Only in relatively recent years has the presence of impurities in synthetic dyes become a matter of great concern to the textile dye chemist. This concern is largely the result of a growing need to conduct biological evaluations of dyestuffs for their potential toxicity, specifically mutagenicity and carcinogenicity. Such evaluations require the use of analytically pure dyes to avoid the ambiguities which are likely to arise when interpreting data generated from the use of impure dye samples. A search of the chemical literature for efficient and relatively inexpensive

† Abstracted in part from the MS theses of C. S. Williard and W, N. Hsu, North Carolina State University, 1985.

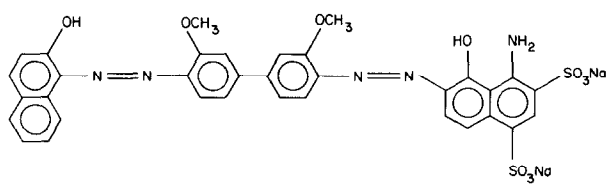
methods for purifying gram quantities of synthetic dyes affords little. This result is not too surprising when one considers that dyes are generally marketed on the basis of their ability to impart certain shades to substrates rather than their purity. In fact, many synthetic dyes are purposely adulterated to produce desired shades. The lack of an effective and inexpensive method for purifying gram quantities of dyestuffs and our desire to speed up dye synthesis work in these laboratories led to the evaluation of a number of relatively new chromatographic procedures as possible tools for preparative-scale dye purifications. Preparative high-pressure liquid chromatography[1,2] (HPLC), dry column chromatography,[3,4] flash chromatography[5] (FC), and countercurrent chromatography[6-8] (CCC) have emerged in relatively recent years as very useful procedures for purifying various organic compounds. Flash chromatography and HPLC require a good deal of solvent for each purification. FC is also limited to low-density solvents for best results, and preparative HPLC equipment is quite expensive. Dry column chromatography, on the other hand, has been found to afford separations which coincide with TLC results, requires little solvent, and is conducted at atmospheric pressure. Another virtue of dry column chromatography lies with the ability to see the separation occurring, and it was therefore selected as a procedure to evaluate the purification of dyes such as **1–3.**



1. Disperse Red 1.



2. Acid Black 1.

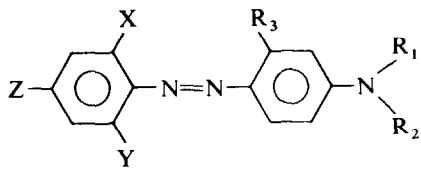


3. Direct Blue 22.

RESULTS AND DISCUSSION

Dyes were selected which are fairly popular commercial dyes and which contained 3–6 components by TLC, with varying degrees of separation. Nylon and glass chromatography columns were used in this study. The former type offered the advantage of ease of isolation of the dye components by simply slicing the column at the appropriate points. However, repeated experiments involving those columns were obviously not possible.

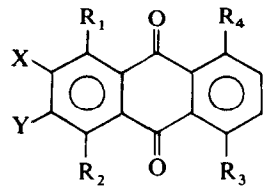
TABLE 1
Azo Disperse Dyes Purified by Dry Column Chromatography

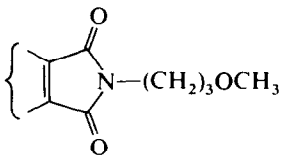


Dye	*X*	*Y*	*Z*	R_1	R_2	R_3
1 (Red 1)	H	H	NO_2	CH_2CH_3	CH_2CH_2OH	H
4 (Red 17)	H	H	NO_2	CH_2CH_2OH	CH_2CH_2OH	CH_3
5 (Orange 30)	Cl	Cl	NO_2	CH_2CH_2CN	CH_2CH_2OAc	H
6 (Orange 44)	Cl	H	NO_2	CH_2CH_2CN	CH_2CH_2CN	H
7 (Red 50)	Cl	H	NO_2	CH_2CH_3	CH_2CH_2CN	H

Dry column chromatography was successfully used to purify the disperse dyes shown in Tables 1 and 2 and the acid dyes **12** and **13**. Toluene:EtOAc and $CHCl_3$ proved to be good eluents for all of the disperse dyes. However, $CHCl_3$ attacked the plasticizer in the nylon columns. $CHCl_3$ was successfully employed only in the few instances where glass columns were employed as an alternative to the plastic films. The ratio of silica gel to the crude dyes purified was 40–60:1, depending upon the difficulty of the separation. Most of the crude dyes had components which were readily separated using a 40–45:1 ratio. It was found that the silica gel could be readily regenerated for continued use as long as it turned white again following dye removal by Soxhlet extraction. Regeneration required vacuum drying at 70°C followed by equilibration with water to give an activity of III–IV on the Brockman scale.

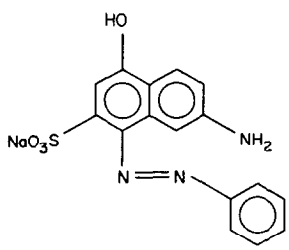
TABLE 2
Anthraquinone Disperse Dyes Purified by Dry Column Chromatography



Dye	*X*	*Y*	R_1	R_2	R_3	R_4
8 (Blue 3)	H	H	NHEt	$NH(CH_2)_2OH$	H	H
9 (Blue 60)			NH_2	NH_2	H	H
10 (Violet 28)	Cl	Cl	NH_2	NH_2	H	H
11 (Blue 118)	H	H	NHPh	OH	NH_2	OH

The pure dyes were isolated by extraction of the homogeneous column sections with $CHCl_3$, followed by concentration of the extracts with a rotary evaporator. The purity of the dyes was confirmed by ^{1}H-NMR, HPLC, and mass spectrometry. All of the dyes gave ^{1}H-NMR spectra which were consistent with the structures and afforded mass spectra which showed ^{13}C isotope peaks having relative intensities reflecting the calculated relative abundance of ^{13}C. The purity of three of the dyes (**1**, **4** and **8**) was further confirmed by combustion analyses.

The acid dyes examined required a silica gel:dye ratio of 100:1 to obtain very pure samples and, in those cases, the silica gel could not



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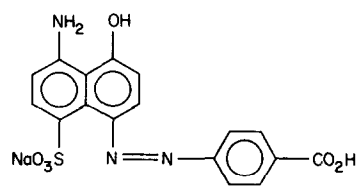

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always be reused without the possibility of sample contamination. The purifications also required the use of the complex solvent system 1-BuOH–EtOH–NH_4OH–pyridine (4:1:3:2). Dimethylformamide was employed to extract the dye from the silica gel once the chromatography was complete. Small amounts of silica gel invariably contaminated the extracted acid dyes and proved difficult to remove completely. The structures of the pure acid dyes were confirmed by ^{1}H-NMR and both were completely homogeneous by analytical HPLC.

No direct dyes were purified by this procedure. Each of the six dyes examined afforded overlapping bands on the columns. It is possible that a very high silica gel:dye ratio of 200–300:1 would effect good separation, but such ratios would also make the procedure impractical. Cationic dyes were not evaluated; however, future plans include a study of such dyes.

EXPERIMENTAL

The silica gel used in this investigation was 'silica gel for dry column chromatography' (Cat. No. 15330-4) from Bodman Chemical Co., Media, Pennsylvania. The nylon tubing employed was obtained from Ace Glass Co., Vineland, New Jersey.† The ^{1}H-NMR spectra were recorded on a Bruker 250 MHz spectrometer and a Varian EM 390 90 MHz spectrometer. The elemental analyses were performed by Atlantic Microlabs, Atlanta, Georgia. Mass spectra were recorded on a Hewlett Packard 5985B mass spectrometer. HPLC data were recorded with the aid of a Waters Series 440 absorbance detector equipped with a model 6000A solvent delivery system and a series 5000 Fisher chart recorder. Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. The dyes used were commercial samples or compounds previously prepared in these laboratories.

Purification of dye 1

Dye **1** (3 g, commercial grade) was dissolved in 50 ml of $CHCl_3$ and the resulting solution was poured over 15 g of silica gel. The dye–silica gel

† Until recently, nylon tubing for dry column chromatography was available from Ace Glass Company. We are unaware of current commercial sources and we now use custom-made columns which employ low-density polyethylene.

mixture was evaporated to dryness and the dye-coated silica gel was added to the top of a silica gel column which had been prepared by packing 30 in (762 mm) of a 1 in (25·4 mm)-diameter nylon column according to the method of Loev and Goodman.[4] The column was eluted with toluene–EtOAc (2:1) until the solvent front reached the bottom. The column was then cut into sections and each was evaluated by TLC for homogeneity. The major band was extracted with boiling $CHCl_3$ to give pure **1**, 0·8 g, m.p. 160–162°C. The compound had $R_f = 0{\cdot}68$ on silica gel with toluene–EtOAc (2:1).

^{1}H-NMR spectrum ($CDCl_3$): triplet (3H), δ 1·2; triplet (4H), δ 3·6; triplet (2H), δ 3·9; doublet (2H), δ 6·8; doublet (4H), δ 7·9; doublet (2H), δ 8·3.

Mass spectrum (70 eV), *m/e* (relative intensity): 315(18·8); 314(M^+, 100); 313(31·1); 284(9·5); 283(4·4); 282(5·3).

Analysis: Calc. for $C_{16}H_{18}N_4O_3$: C, 61·15; H, 5·73; N, 17·83. Found: C, 61·30; H, 5·78; N, 17·69%.

The procedure outlined above for dye **1** was used to purify the same quantities of the disperse dyes Red 17, Orange 30, Orange 44, Red 50, Blue 3, Blue 60, Violet 28 and Blue 118. The physical properties and spectroscopic data recorded for these compounds are described below.

Disperse dye 4

The pure dye had m.p. 158–160°C and $R_f = 0{\cdot}32$ on silica gel with toluene–EtOAc (2:1).

^{1}H-NMR spectrum ($CDCl_3$): singlet (3H), δ 2·7; triplet (4H), δ 3·7; triplet (4H), δ 3·9; doublet (1H), δ 7·9; doublet (2H) δ 8·3.

Mass spectrum (70 eV), *m/e* (relative intensity): 345(19·8); 344(M^+, 100); 343(21·8); 326(4·0); 314(5·5);

Analysis: Calc. for $C_{17}H_{20}N_4O_4$: C, 59·30; H, 5·81; N, 16·24. Found: C, 59·26; H, 5·88; N, 16·19%.

Disperse dye 5

The pure dye had m.p. 114°C and $R_f = 0{\cdot}49$ on silica gel with toluene–EtOAc (4:1).

^{1}H-NMR spectrum ($CDCl_3$): singlet (3H), δ 2·0; triplet (2H), δ 2·7; quartet (4H), δ 3·8; triplet (2H), δ 4·3; doublet (2H), δ 6·9; doublet (2H), δ 8·0; singlet (2H), δ 8·3.

IR spectrum (Nujol): 2230 cm^{-1} (—CN); 1517 cm^{-1} (—NO_2).

Disperse dye 6

The pure dye had m.p. 184–186°C and $R_f = 0{\cdot}32$ on silica gel with toluene–EtOAc (2:1).

NMR spectrum ($CDCl_3$): triplet (4H), δ7·1; doublet (1H), δ7·8; doublet (2H), δ8·0; quartet (1H), δ8·3; doublet (1H), δ8·4.

IR spectrum (Nujol): 2250 cm^{-1} (—CN); 1520 cm^{-1} (—NO_2).

Mass spectrum (70 eV), *m/e* (relative intensity): 384(17·5), 382(M^+, 51·5), 344(32·5), 342(100), 198(30·6), 158(35·9), 118(11·6), 104(18·5), 91(5·5), 77(13·9), 54(28).

Disperse dye 7

The pure dye had m.p. 128°C and $R_f = 0{\cdot}35$ on silica gel with toluene–EtOAc (2:1).

^{1}H-NMR spectrum ($CDCl_3$): multiplet (5H), δ 1·3; triplet (2H), δ2·7; multiplet (4H), δ3·7; doublet (2H), δ2·7; multiplet (4H), δ3·7; doublet (2H), δ6·8; quartet (3H), δ7·85; quartet (1H), δ8·1; doublet (1H), δ8·35.

IR spectrum (Nujol): 2240 cm^{-1} (—CN); 1517 cm^{-1} (—NO_2).

Disperse dye 8

The pure dye had m.p. 157–159°C and $R_f = 0{\cdot}50$ on silica gel with toluene–EtOAc (2:1).

^{1}H-NMR spectrum ($CDCl_3$): triplet (3H), δ 1·33; triplet (4H), δ2·93; quartet (2H), δ3·35; triplet (2H), δ3·90; doublet (2H), δ6·90; multiplet (2H), δ7·60; multiplet (2H), δ8·23.

Mass spectrum (70 eV), *m/e* (relative intensity): 311(6·9); 310(M^+, 32·7); 309(13·7); 297(19·7); 296(100; 295(44·8); 278(6·1).

Analysis: Calc. for $C_{18}H_{18}N_2O_3$: C, 69·66; H, 5·85, N, 9·03. Found: C, 69·92; H, 5·55; N, 9·00%.

Disperse dye 9

The pure compound had m.p. 186–188°C and $R_f = 0{\cdot}58$ on silica gel with toluene–EtOAc (9:1).

^{1}H-NMR spectrum ($CDCl_3$): quartet (2H), δ 1·95; singlet (3H), δ 3·35; triplet (2H), δ3·45; triplet (2H), δ3·75; multiplet (2H), δ7·75; multiplet (2H), δ8·2.

IR spectrum (Nujol): 3420 cm^{-1}, 3330 cm^{-1} (—NH_2); 1740 cm^{-1}, 1690 cm^{-1} (cyclic imide); 1560 cm^{-1} (C=O); 1140 cm^{-1} (C—O—C).

The mass spectrum had the molecular ion $m/e = 379$ as the base peak of the spectrum.

Disperse dye 10

The pure dye had m.p. 184·5–186·5°C and $R_f = 0{\cdot}8$ on silica gel with toluene–EtOAc (9:1).

^{1}H-NMR spectrum (DMSO-d_6): multiplet (2H), δ 7·6; multiplet (2H), δ 8·3.

IR spectrum (Nujol): 3400 cm^{-1} (—NH_2); 1560 cm^{-1} (C=O).

Mass spectrum (70 eV); *m/e* (relative intensity): 310(12), 309(12), 308(66), 307(22·8), 306(M^+, 100), 305(9·6), 76(6·6).

Disperse dye 11

The pure dye had m.p. 178°C and $R_f = 0{\cdot}55$ on silica gel with toluene as the eluent.

^{1}H-NMR spectrum (DMSO-d_6/D_2O): multiplet (6H), δ 7·03–7·26; multiplet (34), δ 7·27–7·43.

IR spectrum (Nujol): 3440 cm^{-1} and 3300 cm^{-1} (—NH_2).

The molecular ion $m/e = 346$ was the base peak of the mass spectrum. The spectrum also contained a ^{13}C isotope peak at $m/e = 347$ which had a relative abundance of 22·2%.

Purification of acid dyes 12 and 13

Dye **12** (0·5 g) was dissolved in MeOH and coated onto 3 g of silica gel by the procedure outlined for dye **1**. The dye-coated adsorbent was placed at the top of a 30 in (762 mm) 1/2 in (12·7 mm) diameter dry column and the column eluted with 1-butanol–EtOH–NH_4OH–pyridine (4:1:3:2). The major band was collected by extracting with hot DMF to give 0·35 g of pure **12**. TLC on silica gel showed a single component, $R_f = 0{\cdot}53$, using the chromatography solvent system as the eluent.

^{1}H-NMR spectrum (DMSO-d_6): singlet (1H), δ 6·78; doublet (1H), δ 6·80; triplet (1H), δ7·17; singlet (1H), δ 7·35; triplet (2H) δ 7·42; doublet (2H), δ 7·70; doublet (1H), δ7·98.

The same procedure was used to purify 0·5 g of dye **13**. There was obtained 0·3 g of pure dye, $R_f = 0{\cdot}53$ on silica gel.

^{1}H-NMR spectrum (DMSO-d_6): doublet (1H), δ 6·61; doublet (1H), δ 6·88; doublet (2H), δ 7·48; doublet (1H), δ 7·78; doublet (1H), δ 7·94; doublet (2H), δ 7·98.

CONCLUSIONS

It has been shown that dry column chromatography is an efficient and relatively inexpensive way to prepare gram quantities of disperse dyes. The procedure also appears to work for simple monoazo acid dyes, but the quantities of pure material produced from each experiment are much smaller. In most cases, the silica gel employed could be regenerated for use in repeat purifications. The purity of the disperse dyes generated was satisfactory for use in biological assays such as Ames tests.

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