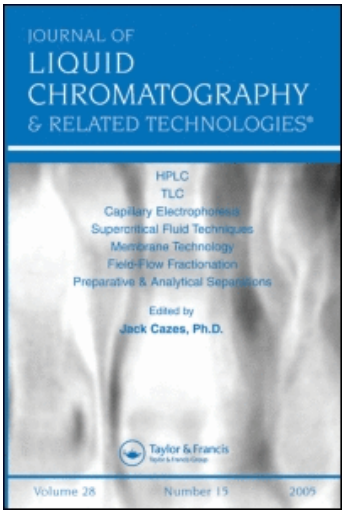


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Purification of Some Water Soluble Azo Dyes by High-Speed Countercurrent Chromatography

H. S. Freeman[a]; Z. Hao[a]; S. A. McIntosh[a]; K. P. Mills[a]
[a] Department of Textile Chemistry, Raleigh, North Carolina

To cite this Article Freeman, H. S. , Hao, Z. , McIntosh, S. A. and Mills, K. P.(1988) 'Purification of Some Water Soluble Azo Dyes by High-Speed Countercurrent Chromatography', Journal of Liquid Chromatography & Related Technologies, 11: 1, 251 — 266
To link to this Article: DOI: 10.1080/01483919808068327
URL: http://dx.doi.org/10.1080/01483919808068327

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(1), 251-266 (1988)

PURIFICATION OF SOME WATER SOLUBLE AZO DYES BY HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHY

H. S. Freeman, Z. Hao, S. A. McIntosh, and K. P. Mills

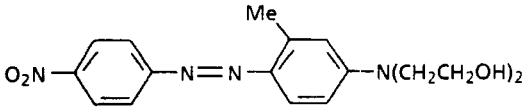
Department of Textile Chemistry
Box 8302
Raleigh, North Carolina 27695-8302

ABSTRACT

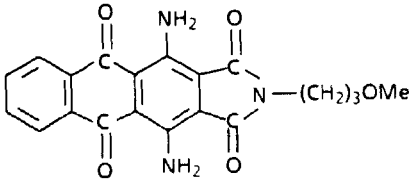
High-speed countercurrent chromatography (CCC) has been used to prepare preparative amounts of pure magenta azo dyes by a method involving the continuous extraction of an aqueous solution of the dyes by a suitable mobile phase. This slight variation in the normal way of running CCC separations permits both the removal of the impurities from the desired color component and an increase in the quantity of dye that can be purified in a single experiment.

INTRODUCTION

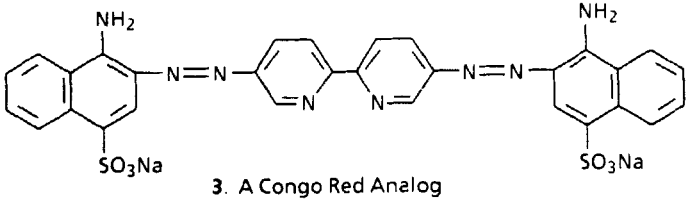
In two previous papers from these laboratories we described (1,2) the results of our evaluation of dry column chromatography (3) and countercurrent chromataography (4-8) as procedures for the purification of gram quantities of synthetic dyes. Those studies resulted from the need to have a relatively inexpensive way to produce analytically pure dyes, such as 1-4, for some toxico-



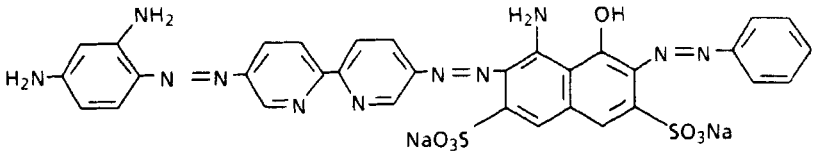
1. Disperse Red 17



2. Disperse Blue 60



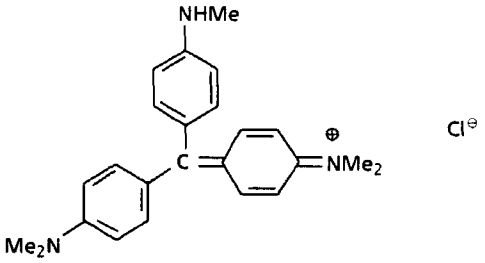
3. A Congo Red Analog



4. A Direct Black 38 Analog

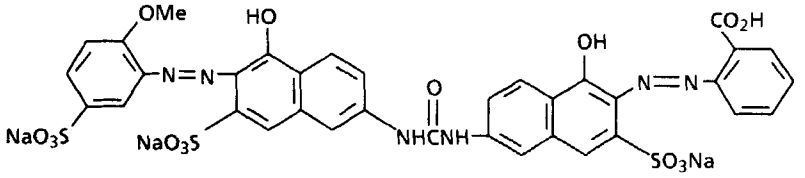
logical and structure elucidation studies of interest to us. Until that work, the literature contained very little about the use of modern techniques in dye purification. Most of what was published on this subject prior to 1985 is described in Venkataraman's book (9).

Our interest in CCC as a method for the purification of dyes was heightened by our initial experiences with the procedure (2), the paper of Fales and co-workers (10) in which the separation of the components of Methyl Violet 2B is described, and by some interesting results we were obtaining from the use of dialysis in

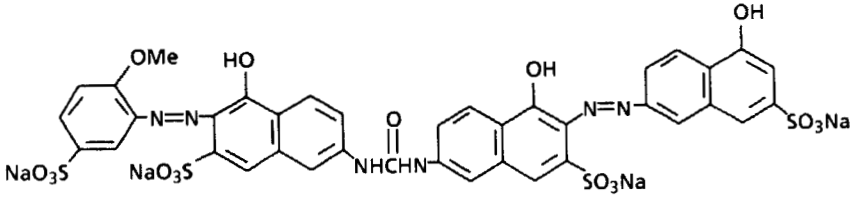


5. Methyl Violet 2B

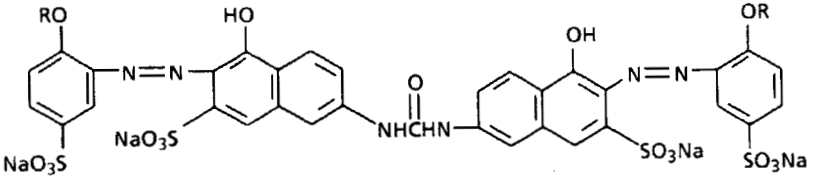
the removal of lower molecular weight impurities found in dyes **6-10**. These dyes were synthesized in our laboratories as part of a program involving the developent of some non-mutagenic azo magenta dyes for ink-jet printing. Although each dye was determined to be non-mutagenic by both the standard Ames test (11) and the Prival modification (12) even in crude form, it was necessary to get pure materials for the desired spectral data.



6

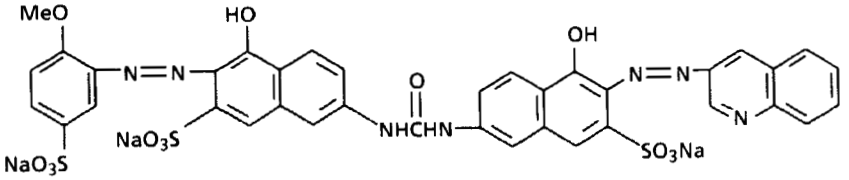


7

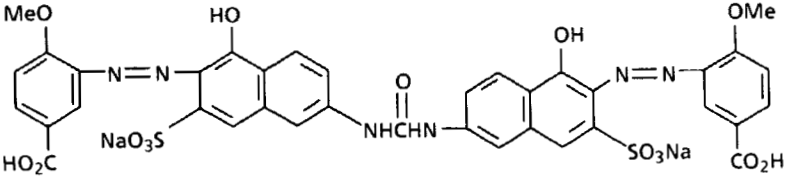


8

a. R = CH_3
b. R = H



9



10

It was clear from our initial work with CCC in the purification of some direct cotton dyes (e.g. 4) that the use of an aqueous mobile phase would always lead to the collection of dye from the column before separation of the components occurred. The aforementioned work involving the dialysis of dyes suggested, however, that dyes 6-10 might undergo purification via a continuous extraction of an aqueous stationary phase by a mobile phase containing a mixture of H_2O and organics. The pure dye would then be isolated by collecting the stationary phase and evaporating it to dryness.

A solvent system was developed that gave partition coefficients in the range of 0.1 to 10.0, and it was used to prepare pure samples of dyes 6-10. The intimate details of this study are now described.

EXPERIMENTAL

Materials

The 5 dyes used in this study were synthesized in this laboratory (13) by diazotizing the appropriate aniline derivatives followed by diazo coupling with J acid urea [N,N'-bis-(5,5'-dihydroxy-7,7'-disulfo-naphthalene-2,2'-diyl)urea] at pH 10. All of the required starting materials and solvents were obtained from Mobay Chemicals Corporation, Industrial Chemicals Division, Pittsburg, Penn. 15205, or from Aldrich Chemical Company, Milwaukee, Wis., 53233.

Countercurrent Chromatography

The partition coefficients (K) were measured with the aid of a Perkin-Elmer UV-Visible spectrophotometer Model 559A, and the procedure used was essentially the method of Conway and Ito (14). For this stage of the study, 3 solvent systems were evaluated. These were: 1) Butanol:H_2O:EtOH (1:1:0.1); 2) Butanol: H_2O (1:1); and H_2O:Butanol:Pyridine (5:3.5:1.5). The system that afforded the best K values was then freshly prepared and allowed to stand at room temperature until the two phases separated completely. (This normally required about 30 minutes for the H_2O:Butanol:Pyridine system).

The chromatography itself employed the CCC unit available from P.C. Inc., Potomac, Md. equipped with the 350 mL capacity column, 2.6 mm I.D.

A typical experiment is the following. For the purification of dye **9**, the CCC column was filled with the lower (aqueous) layer of the H_2O:Butanol:Pyridine (5.0:3.5:1.5) system at a flow rate of 6 mL/min. The column was then rotated at 800 rpm as the dye (0.2 to 0.5 g), in either 4 mL of the lower layer or 2 mL of each layer was injected into the column via a syringe, in the absence of air bubbles. The upper (organic) layer was introduced into the column at a flow rate of 4 mL/min. A fraction collector was used to collect fractions of 12 mL per tube. The organic phase was passed through the stationary phase for about 8 h. During that time period, 270 mL of stationary phase was displaced. The color of the fractions collected were first yellowish-orange, then orange,

and finally reddish-orange. The stationary phase was forced out of the column using N_2 to give the desired magenta color. Pure 9 (0.1-0.3 g) was obtained upon the removal of the solvent under reduced pressure.

Instrumental Analysis

The HPLC data were recorded using a Waters Series 400 absorbance detector equipped with a model 6000A solvent delivery system and a series 5000 Fisher chart recorder. The NMR spectra were recorded on a Bruker 250 MHz spectrometer, and the negative ion FAB mass spectra were recorded using a JEOL HX110HF double focusing mass spectrometer equipped with a DA-5000 data system.

RESULTS AND DISCUSSION

Based on the partition coefficients in Tables 1 and 2 H_2O:Butanol:Pyridine (5.0:3.5:1.5) was selected as the solvent system for purifying the dyes in this study. The data shown in the tables reflect an important point. Whereas, in an ideal (single component) system at constant temperature the partition coefficient does not vary with wavelength, in a real system, such as ours, the partition coefficient can vary with wavelength. We believe that in our case this is due to the contribution of impurities to the absorbance measurements. Interestingly, if we had considered only the wavelengths in the region of the λmax of these dyes, in determining the K values, the utility of H_2O:Butanol:Pyridine as a system for all of the dyes would not have been apparent. It was determined, however, that this system is quite satisfactory.

TABLE 1

Partition Coefficients of Dyes 6-10 Recorded in the Region Near the Wavelength of Maximum Absorbance

Solvent System	K					
	440 nm	460 nm	500 nm	520 nm	540 nm	550 nm
Dye 6						
Butanol: H_2O:EtOH (1:1:0.1)	-	0.008	0.008	0.009	0.012	-
Butanol: H_2O (1:1)	-	0.013	0.012	0.013	0.011	-
H_2O:Butanol:Pyridine (5:3.5:1.5)	-	0.025	0.026	0.026	0.032	-
Dye 7						
BuOH:H_2O:EtOH (1:1:0.1)	-	0.016	0.015	0.014	0.014	-
BuOH:H_2O (1:1)	-	0.012	0.011	0.012	0.008	-
H_2O:BuOH:Pyr. (5:3.5:1.5)	-	0.041	0.038	0.035	0.028	-
Dye 8						
BuOH:H_2O:EtOH (1:1:0.1)	0.010	0.010	-	-	0.009	0.009
BuOH:H_2O (1:1)	0.007	0.009	-	-	0.006	0.006
H_2O:BuOH:Pyr. (5:3.5:1.5)	0.027	0.026	-	-	0.022	0.021
Dye 9						
BuOH:H_2O:EtOH (1:1:0.1)	0.172	0.157	0.116	0.088	0.066	-
BuOH:H_2O (1:1)	0.138	0.130	0.094	0.071	0.045	-
H_2O:BuOH:Pyr. (5:3.5:1.5)	0.296	0.276	0.223	0.185	0.136	-
Dye 10						
BuOH:H_2O:EtOH (1:1:0.1)	-	0.053	0.034	0.024	0.025	-
BuOH:H_2O (1:1)	-	0.074	0.042	0.044	0.034	-
H_2O:BuOH:Pyr. (5:3.5:1.5)	-	0.064	0.047	0.037	0.033	-

TABLE 2

Partition Coefficients of Dyes 6-10 Recorded in the Solvent System H_2O:Butanol:Pyridine (5.0:3.5:1.5) at Wavelengths in the UV Region

Dye No.	K		
	300 nm	340 nm	380 nm
6	1.30	0.93	0.35
7	2.00	1.10	0.45
8	--	--	0.42
9	0.69	0.32	0.31
10	8.70	4.70	2.70

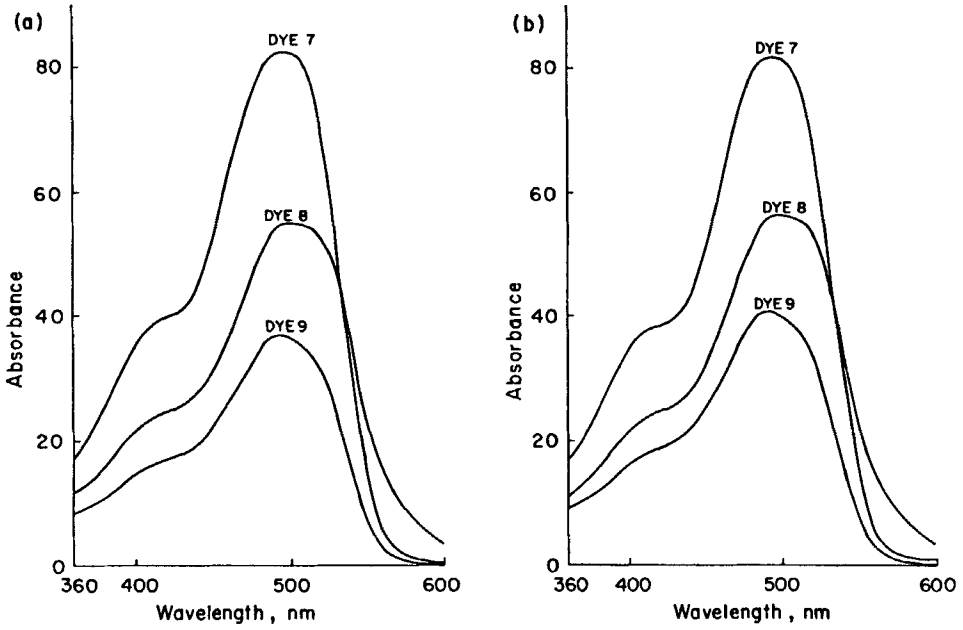


Figure 1. Absorption spectra of dyes 7-9 in (a) H_2O:Butanol:Pyridine (5.0:3.5:1.0) and (b) Butanol:H_2O(1:1)

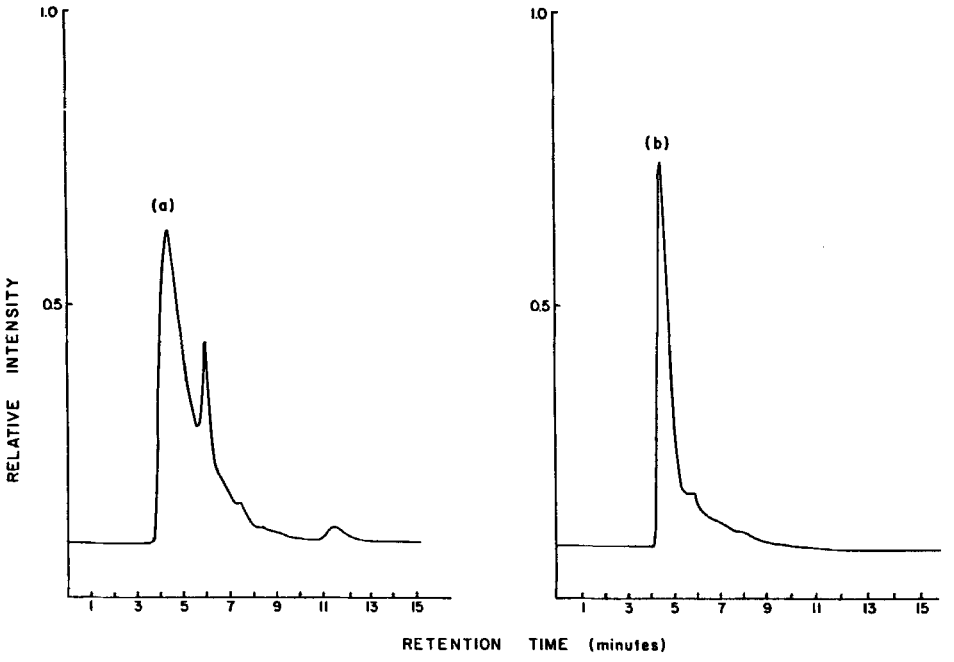


Figure 2. HPLC chromatograms of dye 9 before (a) and after (b) purification by CCC using H_2O:Butanol:Pyridine (5.0:3.5:1.5).

One of the basic assumptions in the experimental method (14) used to measure the partition coefficient K is that the effect of the solvent system on the wavelength of maximum absorption is negligible and therefore, the concentrations of the compound to be purified in the two different phases can be determined at a single wavelength. In the present study, this assumption was provided some experimental support. Figure 1 shows that the λmax of dyes **7-9** does not change by changing the solvent system.

The success of the CCC experiments was determined by HPLC, ^1H NMR, and mass spectral analysis of the resulting dyes. Figure 2

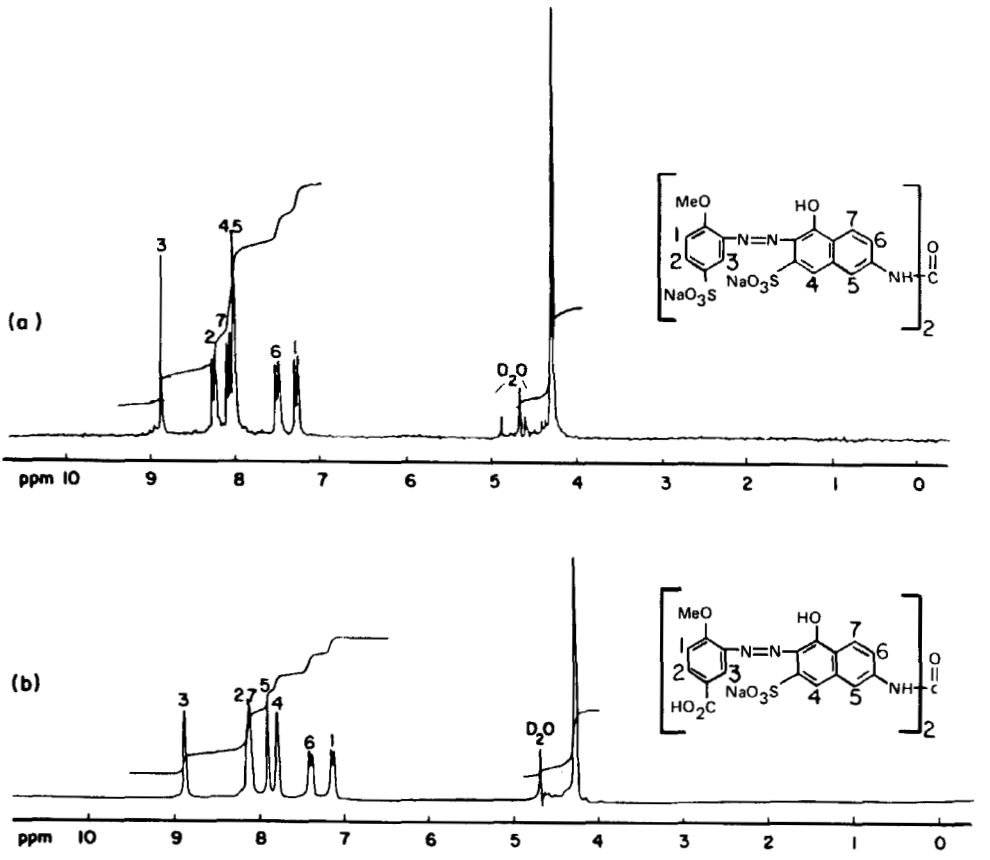


Figure 3. 250 MHz ^{1}H NMR spectra of dyes **8a** (a) and **10** (b) recorded in D_2O/DMSO-d_6.

depicts typical HPLC chromatograms recorded on one of the dyes in this study. The small impurity at retention time = 6 minutes in the chromatograph sample of **9** is readily removed by precipitating the free acid from water with the aid of excess 12 M HCl. Figure 3 and Figures 4-5 contain representative NMR and mass spectra, respectively. It is clear from the HPLC analyses that the dyes

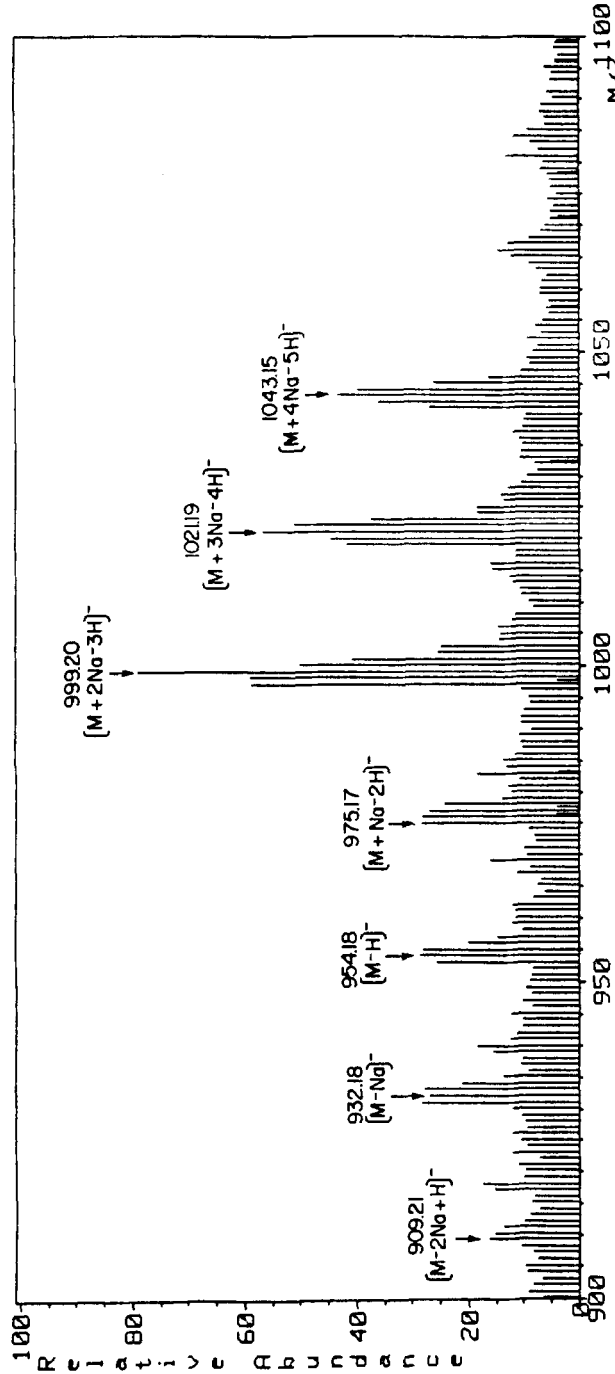


Figure 4. Mass spectrum of dye **8b** recorded using negative ion FAB and a matrix of triethanolamine/H_2O/NaCl.

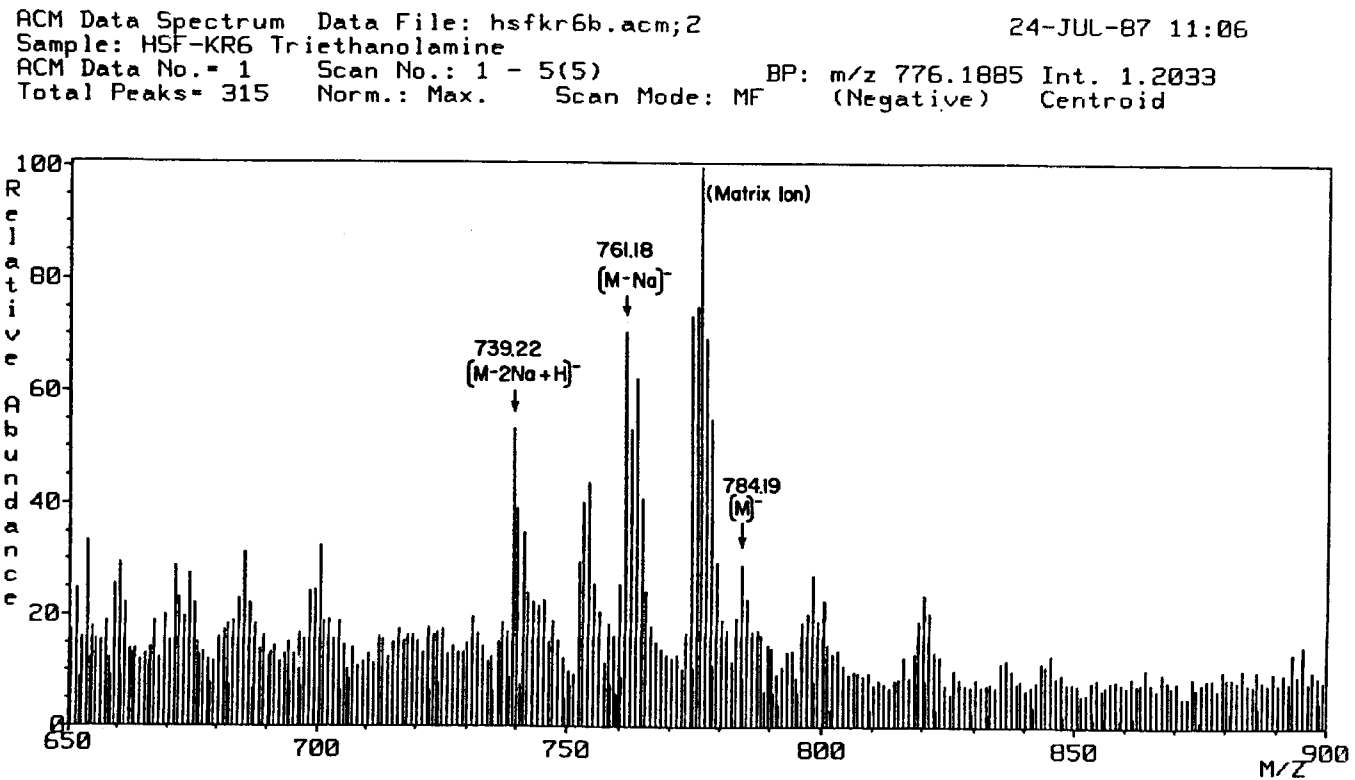
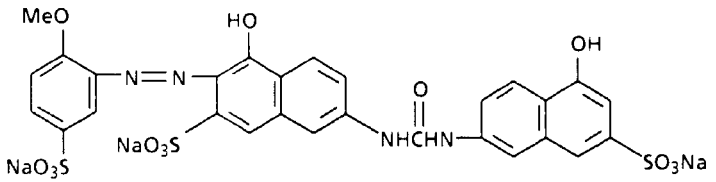


Figure 5. Mass spectrum of dye **11** recorded using negative ion FAB and a matrix of triethanolamine/H_2O/NaCl.

are pure at the end of 8-20 h of continuous extraction. The NMR and mass spectral data were entirely consistent with the proposed structures. However, it should be pointed out that not all of the NMR spectra were as well resolved as those in Figure 3. When this occurred, mass spectra were obtained, and were always satisfactory. Mass spectra analysis was also the key to determining the identity of major subsidiary colors present in these dyes. For instance the monoazo dye **11** was shown by mass spectrometry (cf. Figure 5) and ^1H NMR to be the main subsidiary color in dyes **6-9**.



11

CONCLUSIONS

The data obtained in this study show clearly that CCC was quite successful in the purification of a series of H_2O soluble magenta dyes. In addition, it was determined that the use of preparative (> 0.5 g) quantities of dye requires as long as 2 days (20 h) of continuous extraction to obtain very pure samples, and that the use of a 50/50 mixture of the upper and lower phases of the solvent system is probably not required when purifying very H_2O soluble dyes using an aqueous stationary phase.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Richard van Breemen, Head of the NCSU Biotechnology Mass Spectrometry Center for his help in recording and interpreting the mass spectra.

We also thank Mr. Mark Bowen for his artwork in the figures of this paper.

REFERENCES

1. Freeman, H.S., Williard, C.S. and Hsu, W.N., Dyes and Pigments, 7, 397 (1986).

2. Freeman, H.S. and Williard, C.S., Dyes and Pigments, 7, 407 (1986).

3. Loev, B. and Goodman, M.M., Prog. Separ. Purif., 3, 73 (1970).

4. Sandline, J.L. and Ito, Y., J. Liquid Chromatogr., 8(12), 2153 (1985).

5. Lee, Y.W. and Cook, C.E., J. Liquid Chromatogr., 8(12), 2253 (1985).

6. Putman, L.J. and Butter, L.G., J. Chromatogr., 318, 85 (1985).

7. Martin, D.G., Mizsak, S.A. and Krueger, W.C., J. Antibiot, 746 (June 1985).

8. Conway, W.D. and Ito, Y., LC, Liquid Chromatogr. HPLC Mag., 2(5), 368 (1984).

9. Papa, L.J. The Analytical Chemistry of Synthetic Dyes, Venkataraman, K., ed., Wiley-Interscience, 1977, p. 93.

10. Fales, H.M., Pannell, L.K., Sokoloski, E.A. and Carmeci, P., Anal. Chem., 57, 376 (1985).

11. Ames, B.N., McCann, J. and Yamasaki, E., Mutat. Res., 31, 347 (1975).

12. Prival, M.J. and Mitchell, V.D., Mutat. Res., 97, 103 (1982).

13. Mills, K.P. Unpublished dissertation research. The details of the synthesis work will be published elsewhere.

14. Conway, W.D. and Ito, Y., J. Liquid Chromatogr., 7(2), 275 (1984).