Dyes and Pigments 7 (1986) 407–417

Purification Procedures for Synthetic Dyes: Part 2—Countercurrent Chromatography†

H. S. Freeman and C. S. Williard

Department of Textile Chemistry, North Carolina State University, Raleigh, NC 27695, USA

(Received: 17 January, 1986)

SUMMARY

The suitability of countercurrent chromatography as a method for purifying preparative amounts of disperse dyes and direct dyes has been investigated. Solvent pairs were developed which afforded partition coefficients needed to effect the purification of dyes of these types. Although this procedure was found to give good separation of complex dye mixtures, the low solubility of the dyes in the solvent pairs prevents it from being an effective method of preparing gram quantities of analytically pure dyes.

INTRODUCTION

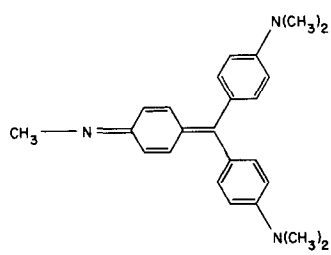
Efforts to identify a relatively inexpensive and efficient method of preparing gram quantities of synthetic dyes led to our evaluation of a number of chromatographic procedures which have not been reported previously as applicable to dyestuffs. Among such procedures are flash chromatography,[1] dry column chromatography,[2,3] preparative HPLC,[4,5] and countercurrent chromatography.[6–10] In the first paper

† Abstracted in part from the MS thesis of C. S. Williard, North Carolina State University, 1985.

Dyes and Pigments 0143-7208/86/$03·50

of this series, we reported[11] our experiences with dry column chromatography and pointed out certain disadvantages of the preparative HPLC and flash chromatography procedures. The present paper summarizes work conducted with the more recently developed chromatographic procedure, countercurrent chromatography (CCC).

Countercurrent chromatography is quite different from the more widely known droplet countercurrent chromatography[12] which utilizes a series of tubes containing the stationary phase, through which the mobile phase is pumped in the form of droplets. The engineering design and other theoretical aspects of CCC have been outlined[13–15] in papers by Ito. The intimate details of that information will not be reproduced here. It is worthwhile to point out, though, that the key aspects of CCC which make it attractive include (1) the lack of tailing of compounds being purified, (2) no sample losses, and (3) the modest amounts of solvents required to effect the purification of complex mixtures. The first two points are consequences of no solid support being used. In lieu of an absorbent, this procedure uses a pair of immiscible solvents and takes advantage of differences in the solubility of the components of a crude sample in the solvent pair. Therefore, the first step in the use of CCC is the development of a solvent pair which gives a partition coefficient in a specific range. It turns out that the range of useful values is quite wide. Values in the interval of 0·1–10 have been found to produce the desired separations.

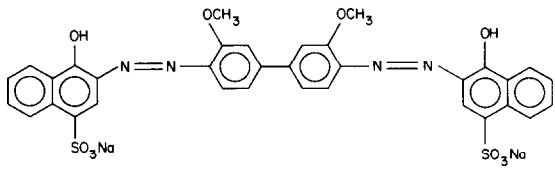


1

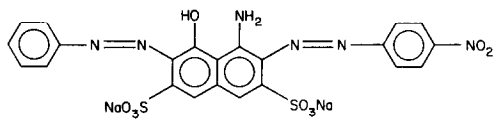
It has been suggested[16] that CCC works *best* for polar compounds. Indeed, DNP-amino acids, indole derivatives, purines, pyrimidines, and the dye Basic Violet 1 (**1**) have been purified by CCC. Consequently, this procedure was explored as a method of purifying dyes of varying polarity.

RESULTS AND DISCUSSION

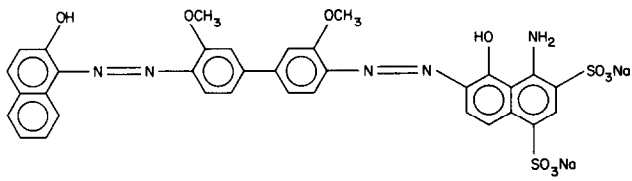
The acid/direct dyes **2–6** and disperse dyes **7–10** were selected as model compounds for evaluating the utility of CCC. The first step in the evaluation of CCC as a tool for the purification of synthetic dyestuffs involved the development of solvent systems which afforded partition coefficients in the 0·1–10 interval of values. The second and third steps involved conducting the purification process on select dyes and assessing



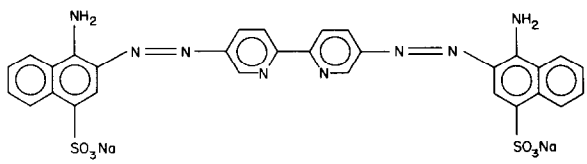
2



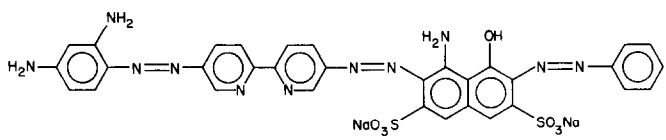
3



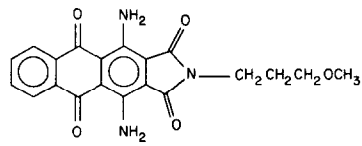
4



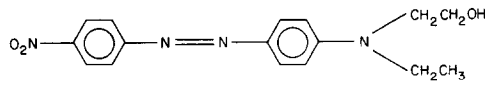
5



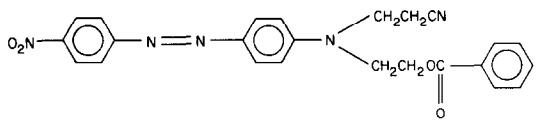
6



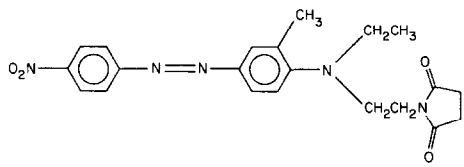
7



8



9



10

the effectiveness of each separation by TLC and HPLC analyses, respectively. Since the dye classes studied varied greatly in degree of polarity, different solvent systems needed to be developed. The starting points for the development of the solvent systems were papers by Conway and Ito.[17,18] Several of the solvent systems reported in the two papers were found to be suitable for our purposes.

The acid and direct dyes **2–6** afforded partition coefficients in the required interval when the systems 1-butanol–H_2O–EtOH and $CHCl_3$–HOAc–0·1M-HCl were employed. Table 1 shows the partition coefficients as a function of wavelength, which were recorded for each of the dyes examined. The disperse dyes evaluated (**7–10**) required the non-aqueous systems EtOAc– or Et_2O–formamide, and EtOAc– or Et_2O–ethylene glycol in order to produce the required partition coefficients. The data generated from the work with disperse dyes are shown in Table 2. The results enumerated in Tables 1 and 2 suggested that the water soluble dyes **2** and **4** and the disperse dyes **9** and **10** were the most appropriate ones to use in the CCC purification step.

TABLE 1
Partition Coefficients (K) of Certain Acid and Direct Dyes in Aqueous Solvent Systems

Solvent system	*K*								
	190 nm	*220 nm*	*280 nm*	*300 nm*	*340 nm*	*350 nm*	*360 nm*	*380 nm*	*400 nm*
Dye **2**									
Butanol–H_2O (1:1)	—	—	—	—	1·45	—	1·71	1·80	1·77
$CHCl_3$–HOAc–0·1M-HCl (12:2:1)	—	—	—	—	1·92	—	2·35	2·33	2·82
Butanol–H_2O–EtOH (1:1:0·1)	—	—	—	—	2·40	—	2·67	2·96	2·40
Butanol–H_2O–EtOH (1:1:0·2)	—	—	—	—	2·70	—	2·95	3·60	3·02
Butanol–H_2O (2:1)	2·38	4·02	—	4·44	—	5·75	—	6·64	6·36
Dye **3**									
Butanol–H_2O (1:1)	—	—	—	—	0·337	—	0·362	0·350	0·355
$CHCl_3$–HOAc–0·1M-HCl (2:2:1)	—	—	—	—	9·75	—	32·0	60·0	31·0
Butanol–H_2O–EtOH (1:1:0·1)	—	—	—	—	0·460	—	0·444	0·475	0·479
Butanol–H_2O–EtOH (1:1:0·2)	—	—	—	—	0·833	—	0·778	0·722	0·812
Dye **4**									
Butanol–H_2O (1:1)	—	—	—	—	0·327	—	0·337	0·504	0·500
$CHCl_3$–HOAc–0·1M-HCl (2:2:1)	—	—	—	—	1·09	—	0·818	0·818	0·909
Butanol–H_2O–EtOH (1:1:0·1)	—	—	—	—	0·229	—	0·246	0·300	—
Butanol–H_2O–EtOH (1:1:0·2)	—	—	—	—	0·697	—	0·715	0·882	0·929
Dye **5**									
Butanol–H_2O (1:1)	—	—	—	—	0·502	—	0·457	0·418	0·400
$CHCl_3$–HOAc (0·1M-HCl (2:2:1)	—	—	—	—	5·833	—	7·000	7·000	8·750
Butanol–H_2O–EtOH (1:1:0·1)	—	—	—	—	0·449	—	0·444	0·420	—
Butanol–H_2O–EtOH (1:1:0·2)	—	—	—	—	0·730	—	0·727	0·636	0·526
Dye **6**									
Butanol–H_2O (1:1)	—	—	—	—	0·185	—	0·224	0·240	0·214
Butanol–H_2O (2:1)	0·715	0·040	—	0·199	—	0·212	—	0·252	0·296
$CHCl_3$–HOAc–0·1M-HCl (2:2:1)	—	—	—	—	5·833	—	7·000	7·000	7·750
Butanol–H_2O–EtOH (1:1:0·1)	0·101	0·117	—	0·298	—	0·553	—	0·672	0·647
Butanol–H_2O–EtOH (1:1:0·2)	0·037	0·136	—	0·044	—	0·099	—	0·137	0·139

TABLE 2
Partition Coefficients (K) of Certain Disperse Dyes in Non-Aqueous Solvent Systems

Solvent system	*K*								
	190 nm	*220 nm*	*250 nm*	*280 nm*	*300 nm*	*320 nm*	*350 nm*	*380 nm*	*400 nm*
Dye **7**									
EtOAc–formamide (1:1)	1·140	1·870	12·690	0·714	—	—	—	—	—
EtOAc–ethylene glycol (1:1)	2·440	—	—	−0·553	0·667	0·429	0·510	0·208	0·125
Dye **8**									
EtOAc–formamide (1:1)	0·750	0·967	2·530	0·987	0·815	0·547	0·085	0·341	0·912
EtOAc–ethylene glycol (1:1)	0·223	0·392	—	0·077	0·348	0·429	—	0·125	0·714
Dye **9**									
EtOAc–formamide (1:1)	1·160	1·240	2·800	0·920	0·760	1·070	0·670	2·190	5·030
EtOAc–ethylene glycol (1:1)	1·180	1·260	0·929	0·771	0·810	1·430	4·08	5·500	6·380
Dye **10**									
EtOAc–formamide (1:1)	—	—	1·580	—	—	—	—	—	—
EtOAc–ethylene glycol (1:1)	0·540	0·581	0·517	0·269	0·426	0·271	0·278	0·764	0·893

The dye purifications required approximately 1·5 liters of solvent for each experiment. In each case, good separation of the components of the crude dyes required that the phase of the solvent pair in which the dye was more soluble be used as the stationary phase. Otherwise, the crude dye would pass quickly through the CCC column before significant separation of the components occurred. A maximum of 0·5 g of dye could be purified at one time by this procedure. This limitation is mainly the result of the relatively modest solubility of the crude dyes in the maximum allowed 5 ml of solvent mixture. The simplicity and low cost of the method, however, permit the purification of gram quantities of certain dyes by multiply repeating the process. A typical CCC purification of a dye required about 6 h. The purification of dyes with impurities possessing low solubility in the mobile phase, however, took as long as 24 h. The isolation of the purified water-soluble dyes following the CCC experiments was quite straightforward. The aqueous solution of the dye was simply evaporated to dryness with a rotary evaporator and the resulting solid was washed with $CHCl_3$ to remove residual 1-butanol. The isolation of the pure disperse dyes was possible by triturating the formamide or ethylene glycol solutions with water, extracting the resulting mixture with EtOAc, and evaporating the EtOAc solution to dryness. The purified dyes of this investigation were obtained in 0·05–0·3 g quantities, depending on their relative amounts in the crude dye mixture. The water-soluble dyes invariably remained in the stationary phase. Therefore, for such dyes, CCC purification results from the selective extraction of impurities from the main color body. The effectiveness of the CCC purification was determined with the aid of TLC and HPLC. The structures of the purified dyes were confirmed by ^{1}H-NMR.

EXPERIMENTAL

General

The partition coefficients were determined with the aid of a Perkin-Elmer UV–Visible spectrophotometer. The HPLC data were recorded with a Waters Series 400 absorbance detector equipped with a model 6000A solvent delivery system and a series 5000 Fisher chart recorder. TLC data were recorded on Bodman glass-backed silica gel plates type

K64F. The CCC purifications were conducted with the unit available from P. C. Inc., Potomac, Maryland, using the 350 ml capacity column. The solvents were purchased from Fisher Scientific Co., Springfield, New Jersey, and the dyes were obtained from commercial sources or prepared in these laboratories. The NMR spectra were recorded on a Bruker 250 MHz spectrometer and the mass spectra on a Hewlett Packard 5985B mass spectrometer.

The procedure used to measure the partition coefficients of the dyes was essentially the UV–Visible method of Conway and Ito[17] and the subsequent purifications were by standard procedures outlined in the user manual which accompanies the CCC instrument.

Partition coefficients

Approximately 0·2 ml of a methanol solution containing 1 mg/ml of dye was evaporated in a 13 mm culture tube with a N_2 stream. Exactly 1 ml of each of the two mutually saturated solvents of the solvent pair was added to the dry dye, from a pipet. The tube was closed with a Teflon-lined cap, shaken gently for 10 min, and then allowed to stand until the layers separated completely. Occasionally, warming of the tube in a water-bath was necessary to break the emulsion which formed. A pipet was then used to remove the entire *lower layer* and to transfer it to a tube which contained 5 ml of methanol. The same volume of methanol was added to the upper phase of the solvent pair. The absorbance of each of the two methanol solutions was determined at various wavelengths. Corrections for the solvent absorbance were made by subtracting absorbances of similarly diluted aliquots of the mutually saturated blank solvents. The partition coefficients were then calculated as the ratio of the net absorbance values.

Countercurrent chromatography

The solvent systems which afforded the best partition coefficients for the four dyes used at this stage of the work were prepared by shaking the solvent mixtures in a separating funnel until thoroughly mixed. The layers were allowed to separate completely by standing overnight. The CCC column was filled by pumping in the stationary phase at a rate of 6 ml min^{-1}. The CCC column was then rotated at 800 rpm as the dye, in 4–5 ml of the solvent pair (50/50 mixture), was injected into the

column from a syringe. The mobile phase was next pumped into the column at a flow rate of 4 ml min^{-1}. A fraction collector was used to collect fractions of 12 ml per test tube. The amount of stationary phase displaced by the mobile phase was recorded. It was then possible to use the total column capacity to calculate the volume of the stationary phase retained and the volume of mobile phase present throughout the chromatography. Fractions were collected from the instrument and mobile phase pumped in until very little to no color was present in the fractions being collected. The solvent pair and the relative amounts of each component dye used, ratio of stationary to mobile phase involved in the separation, and physical data recorded are described below for the individual dyes.

Purification of the direct dye 2

A mixture of 1 liter of 1-butanol, 1 liter of distilled water, and 100 ml of absolute EtOH was shaken for 2–3 min in a 4 liter separating funnel. The layers were then allowed to separate by standing overnight. The CCC column was filled with the lower layer (aqueous phase) and then rotated at 800 rpm. The dye (0·22 g) was dissolved in 4 ml of the solvent pair and the resulting solution was injected into the column. The upper layer (organic phase) of the solvent pair was then introduced at a flow rate of 4 ml min^{-1}. This introduction caused the displacement of 223 ml of the stationary phase. After 6 h of passing the mobile phase through the stationary phase, the column contents were isolated by pumping in MeOH. The green solution obtained was concentrated and the resulting dye was washed with $CHCl_3$ to remove traces of 1-butanol. There was obtained 0·13 g of pure dye, $R_f = 0{\cdot}65$ on silica gel with 1-butanol–95% EtOH–NH_4OH–pyridine (4:1:3:2).

Purification of the direct dye 4

The solvent system used consisted of 500 ml of 1-butanol, 500 ml of distilled water, and 100 ml of absolute EtOH. The dye (0·5 g) was dissolved in 5 ml of the solvent mixture. The amount of displaced stationary phase was 229 ml. The 6 h purification generated 0·28 g of pure dye **4**, $R_f = 0{\cdot}36$ on silica gel with 1-butanol–95% EtOH–NH_4OH–pyridine (4:1:3:2).

Purification of the disperse dye 9

The dye (0·52 g in 4 ml solvent) was purified using the solvent system EtOAc–ethylene glycol (1:1). The EtOAc layer (upper phase) was used as the stationary phase. The introduction of the mobile phase (ethylene glycol layer) at 800 rpm led to the displacement of 105 ml of stationary phase. After 5 h, the EtOAc phase was removed and concentrated to afford 0·120 g pure dye having $R_f = 0{\cdot}57$ on silica gel with toluene–EtOAc (4:1) and m.p. 129–131°C.

NMR spectrum ($CDCl_3$): triplet (2H), δ 2·74; multiplet (4H), δ 3·94; triplet (2H), δ 4·57; doublet (2H), δ 6·87; triplet (2H), δ 7·45; triplet (1H), δ 7·55; doublet (4H), δ 7·95; doublet (2H), δ 8·00; doublet (2H), δ 8·33.

Purification of the disperse dye 10

The dye (0·22 g) was purified as described above for the disperse dye **9**. The mobile phase displaced 100 ml of the EtOAc phase. After 6 h, the pure dye was isolated by diluting the ethylene glycol layer with water, extracting the precipitated dye into EtOAc, drying the EtOAc layer with Na_2SO_4, and evaporating the EtOAc solution to dryness. There was obtained 0·15 g pure dye, $R_f = 0{\cdot}35$ on silica gel with toluene–EtOAc (2:1), and m.p. 149–151°C.

NMR spectrum ($CDCl_3$): triplet (3H) δ 1·30; singlet (3H), δ 2·28; singlet (4H) δ 2·67; quartet (2H), δ 3·56; triplet (2H), δ 3·62; triplet (2H), δ 3·80; doublet of doublets (1H), δ 6·62; doublet (2H), δ 7·79; doublet (2H), δ 7·81; doublet (1H), δ 8·16; doublet (2H), δ 8·32.

CONCLUSION

The utility of CCC as a method of purifying acid, direct, and disperse dyes was examined. The results indicate that in a single pass, this procedure is suitable for only 0·5 g or less of such dyes. However, the low cost and relative simplicity of the procedure make it feasible to prepare larger amounts of pure dye by repeating the process with additional crude dye. The success of this procedure depends upon the development of a suitable solvent pair, reasonably good solubility of the dye in the solvent pair, and the use of solvents which permit the isolation of purified dye with relatively little difficulty.

REFERENCES

1. W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
2. B. Loev and M. M. Goodman, *Chem. Ind. (London)*, No. 48, 2026 (1967).
3. B. Loev and M. M. Goodman, *Prog. Separ. Purif.*, **3**, 73 (1970).
4. R. Rosset, *Analysis*, **5**(6), 253 (1977).
5. F. Eisenbeiss and H. Henke, *J. High Resolut. Chromatogr. Chromatogr. Commun*, **2**(12), 733 (1979).
6. J. L. Sandline and Y. Ito, *J. Liq. Chromatogr.*, **8**(12), 2153 (1985).
7. Y. W. Lee and C. E. Cook, *J. Liq. Chromatogr.*, **8**(12), 2253 (1985).
8. L. J. Putman and L. G. Butter, *J. Chromatogr.*, **318**, 85 (1985).
9. H. M. Fales, L. K. Pannell, E. A. Sokoloski and P. Carmeci, *Anal. Chem.*, **57**(1), 376 (1985).
10. D. G. Martin, S. A. Mizsak and W. C. Kreuger, *J. Antibiot.*, 746 (June 1985).
11. H. S. Freeman, C. S. Williard and W. N. Hsu, *Dyes and Pigments*, this issue, p. 397.
12. T. Tanimura, J. J. Pisano, Y. Ito and R. L. Bowman, *Science*, **169**, 54 (1970).
13. Y. Ito, M. A. Weinstein, I. Aoki, R. Harada, E. Kimura and K. Nunogaki, *Nature*, **212**, 985 (1966).
14. Y. Ito, *Anal. Biochem.*, **100**, 271 (1979).
15. Y. Ito, *J. Chromatogr.*, **188**, 33 (1980).
16. W. D. Conway and Y. Ito, *LC, Liq. Chromatogr. HPLC Mag.*, **2**(5), 368 (1984).
17. W. D. Conway and Y. Ito, *J. Liq. Chromatogr.*, **7**(2), 275 (1984).
18. W. D. Conway and Y. Ito, *J. Liq. Chromatogr.*, **7**(2), 291 (1984).