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The Selection of Noncorrelated Thin-Layer Chromatographic (TLC) Solvent Systems for the Comparison of Dyes Extracted from Transferred Fibers

ABSTRACT: The potential of using a combination of normal and reverse phase thin-layer chromatography for the comparison of disperse, acid, and reactive dyes extracted from textile fibers of forensic science origin was examined. For disperse and acid dyes, the use of a normal silica gel and a reverse phase system was shown to provide a pair of systems of low correlation. In contrast, the use of two silica gel systems provided a pair of highly correlated systems that possibly offered little more additional information than a single system. A suitable reverse phase system for the separation of reactive dyes was not found. The various pairs of silica gel systems tested for reactive dyes were all shown to be highly correlated.

KEYWORDS: criminalistics, fibers, dyes, chromatographic analysis

Thin-layer chromatography (TLC) has been applied to the separation of dyes extracted from textiles for many years [1]. It has been used more recently to compare dyes extracted from fiber monofilaments which may have been transferred during a criminal contact.[3,4]

To maximize the quantity of comparative information that can be obtained from TLC separations so as to facilitate the matching of fibers, it is desirable to develop the plate in a number of solvent systems. Resua et al. [2] suggested that the use of two systems that are correlated may offer little more information than the use of a single system. On the other hand, the use of solvent systems that are unrelated increases the significance of any data that may be obtained. In this context, solvent systems that produce unrelated R_f information, or reflect different properties of the dye molecules, have a low degree of correlation and are referred to as noncorrelated systems. If the TLC information from two systems were to be used in a court of law, it would be appropriate for the analyst to be aware of the degree of correlation between the two systems and thus be able to weigh his conclusions accordingly. Resua et al. [2] used correlation coefficients and scatter diagrams to evaluate paired solvent systems.

Another technique that has been successfully applied to the comparison of dyes is high performance liquid chromatography (HPLC). Wheals et al. [3] reported the successful use of reverse phase (C18) HPLC columns for the separation and comparison of disperse dyes. The use of reverse phase TLC methods in conjunction with conventional silica gel TLC may provide a pair of systems of lower correlation than obtained from a pair of silica gel systems.

To test this hypothesis, dyes from three dye classes were examined. These were disperse, acid, and reactive dyes. Dyes from each class were chromatographed in a variety of solvent systems in an attempt to determine the degree of correlation between the various systems. This correlation data allowed the selection of the most appropriate pair of systems for a particular dye class. The degree of correlation between the various systems and the spread of R_f values were calculated.

Experimental Procedure

The dyes were extracted by placing the fibers from manufacturers' pattern cards in glass capillary tubes with an appropriate solvent. The capillary tubes were sealed and heated in an oven at 110°C for a period of 10 min. In the case of extraction of disperse dyes from polyester fibers, the tube was heated to 130°C for 10 min.

The plates used for the tests were plastic-backed Merck DC Plastikfolien Keisgel 60F254 (20 by 20 cm) cut to produce two plates 10 by 20 cm. The reverse phase plates were glass-backed Whatman KC18F plates (10 by 10 cm). A number of plates were prepared by spotting small aliquots of each extract 2 cm from the base of the plates. The quantity of dye placed on the plate produced a dye intensity, for red and blue dyes, similar to that encountered in forensic science case work. For yellow dyes, sufficient solution was placed upon the plate to produce a visible spot.

All plates were developed by ascending TLC for a distance of 5 cm in the appropriate system in a CAMAG TLC tank. The developed plates were dried and then examined using transmitted light from a bright fluorescent light source. The R_f values of the major dye components were then determined.

TLC of Disperse Dyes

The dyes were extracted from polyester fibers with chlorobenzene. When the extract was spotted onto the plates, small spots (< 1-mm diameter) were produced. Thus, preliminary concentration of the dye into a sharp line by chromatography with methanol was not required. The plates containing disperse dyes were developed in the solvent systems shown in Table 1, and the R_f values of the major components recorded.

TLC of Acid Dyes

The dyes were extracted from the nylon fibers with water: pyridine (4:3) and the extracts spotted onto a number of TLC plates. The spots (3-mm diameter) were developed for a short distance in methanol to concentrate the dye into a sharp line. The plates were then dried, developed in the solvent systems shown in Table 2, and the R_f values of the major components recorded.

TLC of Reactive Dyes

The reactive dyes were extracted from the cotton fibers with 1.5% sodium hydroxide (NaOH) solution and immediately spotted onto a TLC plate. Preliminary tests showed that, if the extracted dye remained in contact with the 1.5% NaOH solution for extended periods of time (two to three days), decomposition of the dye occurred. The spots (3-mm diameter)

TABLE 1—*Solvent systems tested for disperse dyes. Reverse phase plates were used with System D3.*

System	Solvent Mix
D1	chloroform (single development)
D2	hexane : ethylacetate (40 : 60)
D3	methanol : water (80 : 20)
D4	hexane : tetrahydrofuran (40 : 60)
D5	chloroform : diethylamine (100 : 4)
D6	chloroform : acetic acid (100 : 4)

TABLE 2—*Solvent systems tested for acid dyes. Reverse phase plates were used with Systems A7 to A10.*

System	Solvent Mix
A1	*n*-butanol : ethanol (95%) : water (40 : 20 : 20)
A2	chloroform : methanol : acetic acid (70 : 20 : 10)
A3	chloroform : water : methanol : ammonia (25%) (11 : 1 : 7 : 1)
A4	pyridine : amyl alcohol : ammonia (4 : 3 : 3)
A5	*n*-butanol : acetic acid : water (20 : 10 : 50)
A6	*n*-butanol:ethanol (95%) : ammonia : pyridine (4 : 1 : 3 : 2)
A7	methanol : water (80 : 20)
A8	acetonitrile : water (80 : 20)
A9	acetonitrile : water (40 : 60)
A10	methanol : water : ammonia (80 : 23 : 1)

were developed a short distance in methanol to concentrate the dye into a sharp line. The plates were then dried, developed in one of the systems listed in Table 3, and the R_f values of the major components recorded.

Data Processing

Linear correlation coefficients and critical values (confidence 0.05) for the combinations of solvent systems were calculated using a Cleveland PC/XT computer and a Ecosoft Inc. Microstat statistical software package which allowed the construction of a correlation matrix. Mean and standard deviation values were calculated using a Casio FX100 calculator which was equipped with statistical functions.

Plots of the R_f values in one system against the R_f values in the second system (scatter diagrams) were constructed to assist in the detection of nonlinear correlation between two systems.

The correlation coefficients for the various paired systems were calculated to give an indication of the degree of linear correlation between the systems. High correlation is indicated for correlation coefficients close to $+$ or -1 and low correlation for correlation coefficients close to 0. Warpole [4] indicated that a system with a correlation coefficient of, for example, 0.30, is less correlated than a system with a correlation coefficient of, for example, 0.68. This mode of interpretation was adopted below. Other calculations have been made to find the critical value (two-tail test). The $+/-$ value obtained indicates the range above or below which correlation exists between the two systems (95% confidence).

An indication of the spread of R_f values was also used to assist in the interpretation of the results. The spread of any results can be calculated in terms of standard deviation (S) [4]. Thus, in the case of a hypothetical system of ten dyes evenly separated by 0.1 R_f (for R_f 0 to

TABLE 3—*Solvent systems tested for reactive dyes.*

System	Solvent Mix
R1	methanol : amyl alcohol : water (5 : 5 : 2)
R2	n-propanol : methanol : water : ammonia (8 : 6 : 2 : 1)
R4	dioxane : acetone (1 : 1)
R5	n-propanol : ethyl acetate : water (6 : 1 : 3)
R6	n-butyl acetate : pyridine : water (2 : 2 : 1)
R7	n-butyl acetate : acetic acid : water (2 : 2 : 1)

1), the calculated standard deviation is 0.3. In the case of a group of dyes, all having the same R_f in a particular system, the calculated standard deviation of R_f values is 0.0. Thus values close to zero indicate little spread, values close to 0.3 indicate a wider spread. In these calculations a normal distribution of spots is assumed.

Using the standard deviation data, linear correlation data, and scatter diagrams, the paired systems that gave the most satisfactory combination of a good spread of R_f values and low correlation were selected for each class of dyes considered.

Discussion

Case 1: Disperse Dyes

The extracted disperse dyes produced very small spots on the silica gel TLC plates, and therefore, no preliminary chromatographic development in methanol was carried out. These same dyes produced large spots on the reverse phase plates. Predevelopment in several solvents did not produce a satisfactory concentration of the dyes into a sharp line.

The solvent systems investigated were listed in Table 1. The R_f values obtained for a range of Sandoz Foron disperse dyes in the various systems are shown in Table 4.

The correlation coefficients for various combinations of the solvent systems were calculated and the scatter diagrams for the combinations of systems constructed. The correlation coefficients are shown in Table 5.

Scatter diagrams for each combination of the systems investigated indicated the absence of nonlinear correlation.

All of the systems considered gave correlation coefficients which were within the critical value limits defined by the two-tail test ($p = 0.05$), indicating a significant degree of correlation between all of the systems.

Two of the paired systems examined (Table 5) gave the lowest correlation coefficients. They were D1/D3 and D2/D3. The Systems D3/D4, D4/D5, and D3/D6, were borderline cases with correlation coefficients falling into the low to medium correlation zones. With the exception of D4/D5, each of these cases represented a combination of a conventional silica gel system and a reverse phase system. The paired systems that were used in combination with conventional silica gel plates gave, in general, medium to high correlation.

Only two of the above systems (D1 and D5) gave a good separation of the dye components examined as indicated by the standard deviation of the R_f values.

Therefore, the paired System D1/D3 that gave the minimum correlation (-0.39) and the maximum of spread of R_f values ($S = 0.26$ and 0.14) should be the optimum one for the TLC separation of disperse dyes extracted from transferred fibers.

To test the validity of this conclusion, Systems D1, D2, and D3 were tested using extracted disperse dyes from another manufacturer (Hoescht Samaron dyes). If the correlation coefficients and the standard deviation data for the Samaron dyes are similar to the data previ-

TABLE 4—R$_f$ values of the major dye components of Sandoz Foron disperse dyes extracted from polyester fibers chromatographed in selected solvent systems.

Dye Name[a]	Solvent System						
	D1	D2	D3	D4	D5	D6	D7
Yellow 202	0.28	0.52	0.18	0.62	0.70	0.50	0.64
Yellow 210	0.65	0.78	0.15	0.76	0.70	0.60	0.33
Yellow 182	0.22	0.77	0.66	0.70	...	...	...
Yellow 49	0.55	0.68	0.40	0.68	0.66	0.50	0.56
Yellow 42	0.39	0.83	0.46	0.76	0.34	0.48	0.65
Yellow 64	0.93	0.80	0.10	0.78	0.90	0.80	0.75
Yellow 23	0.18	0.80	0.15	0.72	0.05	0.40	0.65
Orange 97	0.62	0.84	0.12	0.75	0.59	0.54	0.65
Orange 96	0.52	0.62	0.24	0.58	0.70	0.48	0.56
Orange 31	0.24	0.52	0.39	0.58	0.52	0.42	0.55
Orange 25	0.61	0.68	0.29	0.66	0.76	0.54	0.62
Red 43	0.04	0.24	0.33	0.41	0.18	0.20	0.38
Red 310	0.06	0.40	0.12	0.56	0.32	0.41	0.60
Red 54	0.23	0.54	0.29	0.29	0.55	0.42	0.55
Red 210	0.22	0.56	0.19	0.60	0.62	0.42	0.62
Red 74	0.07	0.48	0.25	0.64	0.32	0.41	0.64
Red 50	0.58	0.69	0.20	0.65	0.79	0.60	0.61
Red 72	0.12	0.46	0.47	0.54	0.38	0.40	0.50
Red 312	0.95	0.90	0.04	0.84	0.98	0.90	0.78
Red 121	0.28	0.74	0.16	...	...	...	...
Red 202	0.05	0.36	0.24	0.55	0.32	0.40	0.54
Red 53	0.08	0.34	0.26	0.57	0.32	0.40	0.54
Red 60	0.81	0.86	0.13	0.83	0.90	0.80	0.74
Red 281	0.48	0.78	0.04	0.78	0.86	0.56	0.69
Red 73	0.27	0.61	0.38	0.59	0.34	0.41	0.55
Red 167	0.09	0.50	0.12	0.65	0.50	0.41	0.64
Violet 63	0.47	0.72	0.11	0.71	0.84	0.60	0.66
Violet 27	0.90	0.88	0.05	0.85	0.98	0.90	0.85
Blue 183	0.52	0.71	0.05	0.72	0.86	0.65	0.68
Blue 58	0.27	0.61	0.36	0.70	0.15	0.44	0.62
Blue 87	0.31	0.78	0.11	0.81	0.76	0.26	0.72
Blue 79	0.12	0.52	0.15	0.71	0.63	0.46	0.70
Blue 75	0.18	0.50	0.15	0.71	0.65	0.46	0.72

[a]All of the names are prefixed by C. I. Disperse.

TABLE 5—Correlation coefficients for selected paired systems and the mean and the standard deviation of the R$_f$ for a range of Sandoz Foron Disperse dyes.[a]

System	D1	D2	D3	D4	D5	D6
D1	...	0.83	−0.39	0.64	0.78	0.88
D2		...	−0.39	0.78	0.59	0.70
D3			...	−0.52	−0.58	−0.50
D4				...	0.52	0.63
D5					...	0.75
D6						...
n	31	31	31	31	31	31
CV	←——————————— ±0.35 ———————————→					
Mean R$_f$	0.37	0.63	0.22	0.66	0.58	0.51
S	0.26	0.17	0.14	0.12	0.25	0.16

[a]CV = critical value, $p = 0.05$, two-tail test.

ously obtained for the Foron dyes, it may then be concluded that the selected systems are generally applicable to disperse dyes. The R_f values obtained for these disperse dyes are shown in Table 6. Correlation coefficients and other data are shown in Table 7. These tables confirm the validity of this conclusion. Thus the selection of D1/D3 as the paired system of choice is valid for disperse dyes in general.

The results showed that the combination of the conventional silica gel TLC System D1 (chloroform) and a reverse phase TLC System D3 (methanol : water 80 : 20) provided a paired solvent system with the lowest correlation of the paired systems tested. The paired conventional silica gel systems that exhibited the lowest correlation and the highest spread of R_f values was D4 (hexane : tetrahydrofuran 40 : 60) and D5 (chloroform : diethylamine 100 : 4).

Case 2: Acid Dyes

The solvent systems used in the selection of noncorrelated paired solvent systems for acid dyes are listed in Table 3. The R_f values obtained in each of these systems is shown in Table 8. TLC Systems A3 and A8 gave R_f values of 1.00 for all of the dyes considered and were therefore not included in this table.

The R_f values for the acid dyes were treated in a similar manner to that previously de-

TABLE 6—*The* R$_f$ *values for a range of Hoescht Samaron Disperse dyes in three solvent systems.*

	Solvent System		
Dye Name[a]	D1	D2	D3
Yellow 199	0.1	0.18	0.18
Yellow 114	0.22	0.40	0.56
Yellow 103	0.02	0.16	0.75
Yellow 180	0.03	0.36	0.34
Yellow 64	0.72	0.74	0.06
Yellow 54	0.63	0.72	0.12
Yellow 203	0.12	0.18	0.16
Yellow 68	0.00	0.34	0.27
Yellow 135 ·	0.09	0.64	0.08
Orange 71	0.14	0.32	0.26
Orange 130	0.60	0.66	0.06
Orange 13	0.11	0.62	0.05
Orange 29	0.08	0.47	0.18
Orange 140	0.20	0.34	0.39
Orange 42	0.32	0.69	0.04
Red 303	0.22	0.66	0.04
Red 200	0.31	0.70	0.02
Red 185	0.15	0.50	0.09
Red 91	0.03	0.20	0.20
Red 60	0.70	0.76	0.21
Red 183	0.54	0.61	0.09
Red 65	0.57	0.52	0.12
Red 184	0.34	0.51	0.20
Red 73	0.14	0.25	0.33
Violet 26	0.68	0.76	0.12
Violet 48	0.22	0.37	0.23
Blue 165	0.37	0.35	0.12
Blue 139	0.16	0.32	0.16
Blue 290	0.16	0.18	0.24

[a]All names are prefixed C. I. Disperse.

TABLE 7—*The correlation coefficients for a selected number of paired solvent systems used for the separation of Hoescht samaron disperse dyes. The mean and standard deviation of the R_f values of each of the solvent systems examined are shown.*[a]

System	D1	D2	D3
D1	. . .	0.71	−0.39
D2		. . .	−0.44
D3			. . .
CV	←——— ±0.37 ———→		
n	29	29	29
Mean R_f	0.27	0.37	0.19
S	0.22	0.23	0.16

[a]CV = critical value, p = 0.05, two-tail test.

TABLE 8—*The R_f values obtained for the major components of various acid dyes developed in the systems shown in Table 2. The dye names in parentheses are the ICI tradenames.*

	Solvent System							
Dye Name[a]	A1	A2	A4	A5	A6	A7	A9	A10
Yellow 135	0.76	0.70	0.47	. . .	0.68	0.61	0.50	. . .
Yellow 199	0.84	0.50	0.47	0.65	0.71	0.80	0.65	. . .
(Brown A-B)	0.80	0.42	0.47	0.57	0.75	0.72	0.63	. . .
Red 396	0.78	0.30	0.40	0.55	0.63	0.86	0.72	. . .
Red 266	0.78	0.37	0.53	0.55	0.73	0.72	0.65	. . .
Red 403	0.77	0.56	0.54	0.55	0.72	0.62	0.59	. . .
Blue 62	0.64	0.14	0.48	0.59	0.54	0.70	0.66	. . .
Blue 25	0.84	0.55	0.81	0.62	0.88	0.72	0.68	. . .
Yellow 234	0.82	0.64	0.79	0.61	0.88	0.80	0.78	. . .
(Yellow B-G3)	0.82	0.40	0.39	0.58	0.57	0.84	0.71	. . .
Yellow 19	0.65	0.15	0.46	0.40	0.62	0.88	0.95	. . .
Orange 3	0.88	0.50	0.96	0.68	0.92	0.76	0.65	. . .
Red 155	0.62	0.06	0.38	0.35	0.52	0.91	0.95	. . .
Red 394	0.65	0.09	0.44	0.40	0.60	0.88	0.95	. . .
Red 57	0.78	0.35	0.61	0.56	0.75	0.84	0.71	. . .
Blue 327	0.60	0.11	0.46	0.36	0.59	0.90	0.95	. . .
Blue 41	0.77	0.42	0.76	0.56	0.84	0.74	0.72	. . .
Blue 40	0.48	0.48	0.76	0.40	0.85	0.76	0.76	. . .
(Yellow C-GD)	0.78	0.30	0.64	0.56	0.84	0.84	0.72	. . .
Yellow 64	0.72	0.48	0.49	0.57	0.66	0.70	. . .	0.94
Orange 67	0.84	0.55	0.76	0.60	0.90	0.66	. . .	0.62
Orange 116	0.79	0.57	0.68	0.58	0.80	0.50	. . .	0.43
Orange 51	0.80	0.24	0.66	0.54	0.80	0.78	. . .	0.77
(Brown C-B)	0.65	0.11	0.47	0.43	0.68	0.89	. . .	0.95
Red 111	0.65	0.20	0.45	0.43	0.64	0.78	. . .	0.78
Red 114	0.65	0.28	0.44	0.46	0.63	0.76	. . .	0.75
Red 151	0.77	0.52	0.65	0.56	0.78	0.60	. . .	0.53
Red 299	0.75	0.46	0.65	0.55	0.80	0.76	. . .	0.78
Violet 48	0.74	0.34	0.56	0.54	0.60	0.80	. . .	0.82
Blue 140	0.78	0.38	0.60	0.56	0.88	0.66	. . .	0.60
Blue 127:1	0.78	0.40	0.67	0.55	0.89	0.71	. . .	0.70
(Blue C-GN)	0.75	0.41	0.65	0.55	0.84	0.67	. . .	0.60
Blue 175	0.65	0.22	0.48	0.45	0.68	0.78	. . .	0.82
Blue 113	0.74	0.22	0.58	0.55	0.76	0.76	. . .	0.81

[a]Other dye names are prefixed C. I. acid.

scribed for the disperse dyes. Correlation coefficients and mean and standard deviation information are shown in Table 9.

Scatter diagrams constructed for each combination of the above systems indicated the absence of nonlinear correlation.

Systems A1 and A4 were found by Resua et al. [2] to have a correlation coefficient of 0.13 as compared with 0.42 in Table 9. Their calculations were based upon a combination of R_f values obtained from acid and basic dyes. The use of dyes from two different classes to calculate the degree of correlation between paired solvent systems may have caused this disparity.

Two of the systems tested exhibited correlation coefficients that were within the critical value range for a two-tail test ($p = 0.05$). They were A1/A7 and A4/A9. Both of these paired systems represent combinations of a conventional silica gel system and a reverse phase system. Other systems that exhibited low correlation included A1/A4, A4/A7, A6/A7, and A6/A9. Of the paired conventional silica gel systems under consideration, A1/A4 provided the lowest degree of correlation.

The paired System A1/A7 gave a poorer spread of components than the other systems under consideration. The paired System A4/A9 provided a significant degree of noncorrelation and a satisfactory spread of R_f values and therefore is the paired system of choice for the comparison of acid dyes.

Case 3: Reactive Dyes

The reactive dyes were extracted from the cotton fibers using 1.5% NaOH according to the method of Home and Dudley [5]. They reported good extraction of reactive dyes by this technique. This observation was confirmed during our investigations.

The gradual hydrolysis of the dye extract may present a problem if the object of the test was to identify the dye. However, in real forensic science cases, the test is comparative. Thus, care should be taken to ensure that the dyes from the transferred fiber and from the suspected source garment are extracted under similar conditions, especially with respect to time and temperature.

The R_f values obtained for reactive dyes developed in the solvent systems shown in Table 3 are listed in Table 10.

This R_f information was treated in a similar manner to that described above for disperse and acid dyes. The correlation coefficients and the means and standard deviations for the R_f data are shown in Table 11.

TABLE 9—*The statistical data obtained from* R_f *data obtained using various TLC solvent systems for the separation of acid dyes.*[a]

System	A1	A2	A4	A5	A6	A7	A9	A10
A1	...	0.61	0.42	0.87	0.51	−0.32	−0.61	−0.60
A2		...	0.64	0.72	0.68	−0.65	−0.70	−0.66
A4			...	0.52	0.88	−0.41	−0.31	−0.66
A5				...	0.54	−0.51	−0.84	−0.51
A6					...	−0.48	−0.46	−0.61
A7						...	0.80	0.87
A9							...	...
A10							...	...
CV	←			±0.34			→ 0.47	0.51
n	33	33	33	33	33	33	18	15
Mean	0.74	0.36	0.57	0.53	0.73	0.76	0.73	0.73
S	0.08	0.16	0.14	0.08	0.11	0.09	0.13	0.14

[a]CV = critical value, $p = 0.05$, two-tail test.

TABLE 10—*The R$_f$ values obtained for a range of Hoescht Reactive Dyes in the solvent systems shown in Table 3.*

	Solvent System				
Dye Name[a]	R1	R2	R4	R5	R6
Yellow 57	0.81	0.81	...	0.42	0.55
Yellow 37	0.70	0.87	...	0.25	0.50
Yellow 42	0.63	0.72	0.64	0.22	0.35
Yellow 15	0.71	0.77	0.71	0.37	0.54
Yellow 24	0.71	0.70	0.70	0.32	0.44
Brown 30	0.68	0.68	0.66	0.29	0.43
Yellow 17	0.71	0.77	0.70	0.32	0.52
Orange 74	0.63	0.73	0.62	0.20	0.34
Orange 15	0.64	0.68	0.64	0.22	0.30
Orange 82	0.64	0.68	0.64	0.20	0.28
Orange 7	0.72	0.78	0.69	0.32	0.48
Orange 16	0.72	0.79	0.69	0.32	0.48
Red 106	0.68	0.69	0.65	0.28	0.44
Red 22	0.71	0.83	0.72	0.35	0.54
Red 21	0.66	0.67	0.63	0.23	0.32
Red 180	0.52	0.66	0.60	0.16	0.15
Red 174	0.63	0.66	0.60	0.23	0.30
Red 23	0.59	0.66	0.60	0.23	0.22
Red 170	0.67	0.66	0.60	0.20	0.22
Red 49	0.72	0.77	0.71	0.32	0.48
Violet 5	0.50	0.66	0.60	0.17	0.18
Blue 158	0.44	0.66	0.55	0.12	0.10
Blue 27	0.66	0.75	0.64	0.30	0.56
(Yellow GNL 200)	0.66	0.75	0.66	0.22	0.39
(Yellow R)	0.71	0.77	0.70	0.35	0.54
(Golden Yellow RGL)	0.72	0.85	0.72	0.38	0.56
(Golden Yellow 3R)	0.67	0.74	0.66	0.26	0.44
(Brilliant Red GD)	0.66	0.76	0.65	0.22	0.38
(Red RB)	0.49	0.66	0.60	0.14	0.10
(Brilliant Red 5BB)	0.40	0.64	0.51	0.12	0.10
(Brilliant Blue BB)	0.66	0.66	0.60	0.22	0.38
(Brilliant Blue G)	0.66	0.66	0.61	0.24	0.38

[a]The names in parentheses are the Hoescht trade names. All other dye names prefixed C.I. Reactive.

TABLE 11—*Correlation coefficients and other statistical data for R$_f$ values obtained in the various solvent systems for reactive dyes.[a]*

System	R1	R2	R4	R5	R6
R1	...	0.66	0.86	0.86	0.89
R2		...	0.84	0.79	0.81
R4			...	0.91	0.87
R5				...	0.94
R6					...
CV			← ± 0.36 →		
n	30	30	30	30	30
Mean R$_f$	0.65	0.72	0.64	0.26	0.37
S	0.08	0.06	0.05	0.07	0.13

[a]CV = critical value, $p = 0.05$, two-tail test.

Scatter diagrams for each combination of the solvent systems investigated indicated the absence of noncorrelation.

None of the paired systems investigated for reactive dyes exhibited correlation coefficients that were above or below the critical value (two-tail test [$p = 0.05$]). This indicates a significant correlation between all of the systems.

Paired System R1/R2 exhibited the lowest correlation and is thus the paired solvent system that offers the maximum of comparative information.

It is possible that, in the case of reactive dyes, development in a paired system may offer little additional comparative information over that obtained from a single system. Thus if a single system is to be used, System R6 should be selected since it gave the most satisfactory spread of R_f values.

Conclusion

Reverse phase systems were found to be satisfactory for the separation of disperse and acid dyes (Cases 1 and 2). In these two cases the paired systems offering the lowest correlation were a combination of a conventional silica gel system and a reverse phase system. In contrast, a reverse phase system for the separation of the reactive dyes was not found during our investigations. In each of the reverse phase systems tested, the majority of components moved with the solvent front.

Acknowledgments

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