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Note

Thin-layer chromatography of some cyanine dyes

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In the process of evaluating indicator dyes for the study of retinal circulation, it was found necessary to check dye purity. Thin-layer chromatography (TLC) proved to be the most rapid and effective means available to fill our needs[1–3]. In our search of the literature little TLC data was found on one particular class of dyes known as cyanines[4]. This group of dyes were of high interest to us because of their wide distribution of fluorescent wavelengths over the visible spectrum. It is also well to note that this group of dyes shares a high degree of interest in dye laser work[5,6].

Various TLC systems as suggested by Stahl[7] were tested on the different subgroups of cyanine dyes, some were much better than others. This report gives the results obtained for each system tried, along with R_F values for the dyes and the impurities in these dyes.

MATERIALS AND METHODS

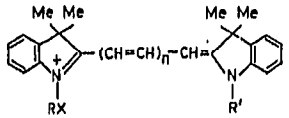
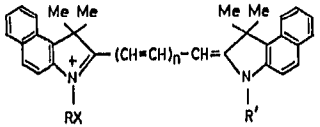
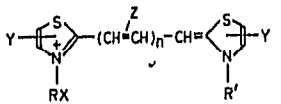
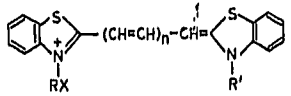
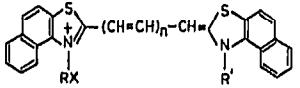
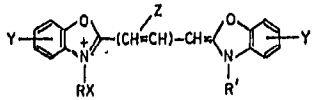
All the TLC sheets used were purchased precoated (Chromatogram sheets, 100-μm coating of silica gel, from Eastman-Kodak (Rochester, N.Y., U.S.A.) aluminum-backed 250-μm silica gel sheets from EM Labs. (Elmsford, N.Y., U.S.A.)). Solvents were mixed from high purity components obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). The dyes with NK prefixes were obtained from Nippon Kankoh-Shikiso Kenkyusho (Okayama-Shi, Japan). Indocyanine green (ICG) in high purity was donated by Hynson, Westcott and Dunning, (Baltimore, Md., U.S.A.). See Table I for a list of dyes and dye structures.

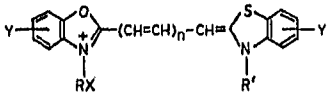
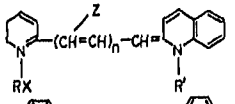
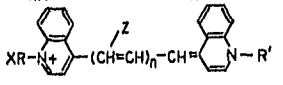
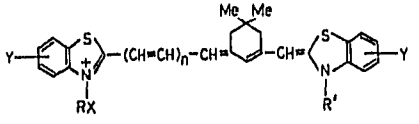
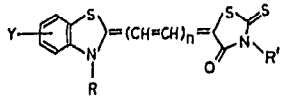
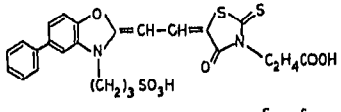
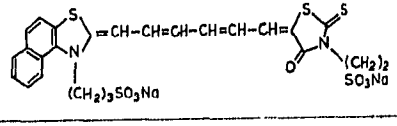
The procedure followed in running the chromatograms consisted of preparing a 0.1% (w/w) solution of each dye in methanol. These dye solutions were made up as close as possible to the spotting time, since decomposition often occurs with time in many of the cyanine family. The solvents to be used were poured in the chromatography chamber and onto a saturation pad in the chamber at least 20 min prior to a run to allow the air and solvent in the chamber to equilibrate. The TLC sheets were placed in an oven at 125° for 30 min before each run. When the sheets and chamber were ready, each solution of dye was spotted 2.5 cm from the bottom of the sheet

TABLE I

CYANINE DYE STRUCTURES

Me = CH_3; Et = C_2H_5; p-ASA = *p*-aniline sulfonic acid; p-TSA = *p*-toluene sulfonic acid; ICG = indocyanine green. In the column listing the R and R′ groups that are bound to the nitrogen atoms, there are several cases where the dye is listed as a salt in the NK catalog but the cation is not given. These dyes have been listed as possible sodium salts, *e.g.*, NK 1839 has R′ = $(CH_2)_3SO_3$(Na?). NK dye numbers listed are actual catalog numbers from the manufacturer.

Name	*Molecular structure*	*Dye No.*	*n*	*R*	*X*	*R′*	*Y*	*Z*
2,2′-Indocarbocyanine		NK 1639	1	$(CH_2)_3SO_3^-$	—	$(CH_2)_3SO_3^-$ pyridine	—	—
		NK 1405	3	CH_2COO^-	—	CH_2COOH	—	—
2,2′-Indo-4,5,4′,5′-dibenzocarbocyanine		ICG	3	$(CH_2)_4SO_3^-$	—	$(CH_2)_4SO_3Na$	—	—
2,2′-Thiacarbocyanine		NK 1839	2	$(CH_2)_3SO_3^-$	—	$(CH_2)_3SO_3$(Na?)		
		NK 156	2	Et	$EtSO_4^-$	Et	4,4′-Me	
2,2′-Thiacarbocyanine		NK 1638	1	Et	$EtSO_4^-$	Et	—	—
		NK 1407	3	C_2H_4COOH	Br^-	C_2H_4COOH	—	—
2,2′-Thia-4,5,4′,5′-dibenzocarbocyanine		NK 2075	1	$(CH_2)_3SO_3^-$	—	Et	—	—
		NK 1978	3	$(CH_2)_3SO_3^-$	—	$(CH_2)_3SO_3$(Na?)	—	—
2,2′-Oxocarbocyanine		NK 1952	1	$(CH_2)_3SO_3^-$	S	$(CH_2)_3SO_3Na$	5,5′-Diphenyl	9-Et
		NK 2073	1	$(CH_2)_3SO_3^-$	—	$(CH_2)_3SO_3H$	5-phenyl-4,5′-benzo	—

2,2′-Oxa, thiacarbocyanine		NK 1518	1	Et	—	$(CH_2)_2SO_4^-$	5,5′-Diphenyl	—
2,2′-Quinocarbocyanine		NK 179	1	Et	$EtSO_4^-$	Et	—	—
		NK 1143	2	Et	p-TSA	Et	—	11-Cl
4,4′-Quinocarbocyanine		NK 171	1	Et	$EtSO_4^-$	Et	—	—
		NK 1753	1	$C_2H_4COO^-$	—	C_2H_4COO(Na?)	—	—
		NK 1255	1	Et	p-TSA	Et	—	11-Cl
Dimethylcyclohexene substituted in bridge		NK 2237	1	$(CH_2)_3SO_3^-$	—	Et	—	—
		NK 2239	1	$(CH_2)_3SO_3^-$	—	Et	4,5,4′,5′-Dibenzo	—
Merocyanine: 2-thia derivatives		NK 1901	2	C_2H_4COOH	—	C_2H_4COOH	6-Me	—
		NK 2050	2	C_2H_4COOH	—	C_2H_4COOH	—	—
Miscellaneous merocyanines		NK 2062	—	—	—	—	—	—
		NK 2240	—	—	—	—	—	—

and allowed to air dry. The sheets were then placed in the chamber to develop. After development the sheets were removed and left in the hood until the solvent evaporated. The sheets were then observed under white light as well as short and long ultraviolet (UV) light and R_F values were recorded.

RESULTS AND DISCUSSION

Results are summarized in Tables II and III. The two solvents that seem to give the best results and most suited to the cyanine group in general are 100% methanol and the propanol–formic acid (80:20) mixture. These solvent systems were tried on many other cyanine dyes that are not reported here with approximately an 85% success rate.

TABLE II

R_F VALUES FOR CYANINE DYES

TLC on silica gel (EM Labs.). I = Methanol (100%); II = *n*-Butanol–acetic acid–water (20:10:50); III = *n*-Butanol–ethanol–water (90:10:10); IV = chloroform–methanol (80:20). Values in parentheses are spots observed under UV light (thought to be impurities). U indicates that the TLC system was unsatisfactory for the compounds. A dash (—) indicates that the compound was not tested with that TLC system.

Dye NK No.	$R_F \times 100$			
	I	*II*	*III*	*IV*
ICG	86			
156	(7)	22	—	85
171	5	24 (31)	—	86
179	5 (42)	51 (16)	—	92 (76)
1143	—	21, 46, 55, 59 (66)	—	89, 96
1255	—	19	—	85
1405	21, 54, 62, 82 (59, 68)	—	—	—
1407	51, 60 (33)	—	—	—
1518	—	38, 50, 62 (68)	—	25, 40, 57, 88, 98
1638	6	52 (17, 31)	—	87
1753	47 (54)	U	—	—
1901	70, 77	73	18, 34, 43	92
1978	66, 73, 79	30 (37)	—	U
2050	66, 74	71	22, 33, 41	92
2062	79	57	23, 44	83
2070	60	U	U	80 (90)

The two silica gel coatings tended in some cases to give different results when used with the same solvents, however, reproducibility of chromatograms on the same manufacturers' coatings was good. Several of the cyanine dyes are noted for rapid decomposition in some solvents and it was thought this might be a problem[4]. However, most dyes tested held up rather well with the solvents tried.

TLC did answer our questions concerning the purity of cyanine dyes as received from the manufacturers, in the sense that most were impure, and will supply us with some form of reference for dyes received in the future and any modification that may be done to individual dyes.

TABLE III

R_F VALUES FOR CYANINE DYES

TLC on silica gel (Eastman). V = Methanol (100%); VI = *n*-Butanol–acetic acid–water (20:10:50); VII = *n*-Butanol–ethanol–water (90:10:10); VIII = chloroform–methanol (80:20). Values in parentheses are spots observed under UV light (thought to be impurities). U indicates that the TLC system was unsatisfactory for the compound. A dash (—) indicates that the compound was not tested with that TLC system.

Dye NK No.	$R_F \times 100$			
	V	*VI*	*VII*	*VIII*
ICG	76	42	14	83
156	U	—	19	—
171	9 (34)	—	16	—
179	10 (45, 57)	—	17	—
1143	10, 35	—	—	91, 98
1255	U	—	15	—
1405	13, 44, 52, 59 64, 71 (22, 39)	50, 55, 63, 72	14, 27, 43, 50, 57, 63, 84 (21, 53)	77, 89, 96 (35, 63)
1407	46, 55	28, 36, 41	U	17, 79 (50)
1518	11, 67	—	13 (37)	—
1638	12	—	18	—
1639	74	—	—	—
1753	41	—	U	—
1839	76	—	—	—
1901	67	—	—	—
1952	73	—	—	—
1978	71	—	U	—
2050	78	—	—	—
2062	63	—	—	—
2070	28	—	—	—
2073	66	—	—	—
2237	58	—	35	95, 98
2239	26, 31	—	24	98 (92)
2240	56, 62, 64, 65 (71)	—	U	84, 94

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