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Journal of Chromatography A, 689 (1995) 269–273

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatography of cyanine dyes Multiphase separation, purification, and substitution of the counter ion

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First received 17 December 1993; revised manuscript received 30 August 1994

Abstract

Multiphase ion-pair chromatography is described for the separation of a mixture of cyanine dyes using CN and C_{18} stationary phases. Radial compression cartridges were combined to form the desired selectivity sequence and to improve chromatographic resolution. Also described is a method for the purification of 1,1'-diethyl-4,4'-cyanine iodide using lithium perchlorate as the ion-pairing agent, as well as a method for substitution of the dye's counter ion with another anion to increase solubility. The relevance of these studies to optical spectroscopy of adsorbed cyanines is also discussed.

1. Introduction

Adsorbed cyanine dyes, in the form of monomers as well as aggregates, have traditionally been used as spectral sensitizers in color photography [1,2], and in recent years have found increasing applications in optical recording devices [3,4] and as non-linear materials [5–8]. Today, the aggregation phenomenon itself is an area of active research, both in terms of understanding the structure of aggregates [9] as well as utilization of the altered spectral and dynamical properties that monomeric species experience in the aggregate environment [8].

The cyanine dyes used in the present study are those whose Raman, UV–Vis absorption and fluorescence spectra, as well as photodynamical properties on a variety of substrates (metal surfaces, vesicle, and colloidal metals) are being investigated in our laboratory, both individually and in presence of other dyes [10,11]. In general, cyanine dyes have two nitrogen heterocyclic rings joined by a conjugated chain of carbon atoms [12]. Table 1 shows the structures of the dyes used in the present study.

We have found that published methods for the HPLC of dyes generally focus on other classes of dyes. As a result, we have found it necessary to develop methods for the separation and analysis of mixtures of cyanine dyes. We have also explored the use of chromatography to improve the purity of commercially purchased cyanine dyes, and to change the counter ion (which in most commercial samples of cyanines is the iodide ion) with the aim of enhancing the dye's solubility in water. Techniques reported here for

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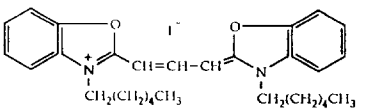
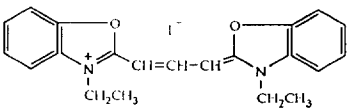
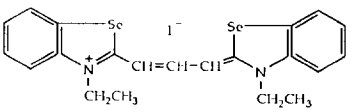
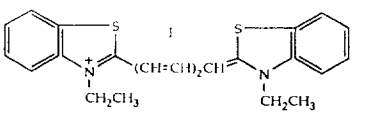
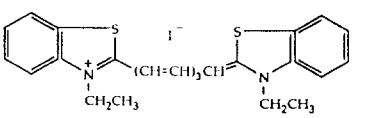
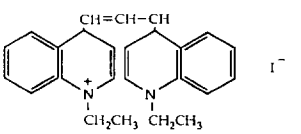
0021-9673/95/$09.50
SSDI 0021-9673(94)00849-3

Table 1
Dyes studied

Dye			Capacity factor	
No.	Name	Structure	CN	C_{18}
1	3,3′-Dihexyloxacarbocyanine iodide		3.25	2.13
2	3,3′-Diethyloxacarbocyanine iodide		3.88	1.76
3	3,3′-Diethylselenacarbocyanine iodide		4.18	1.77
4	3,3′-Diethylthiadicarbocyanine iodide		4.27	1.92
5	3,3′-Diethylthiatricarbocyanine iodide		4.30	2.12
6	1,1′-Diethyl-4,4′-cyanine iodide		4.60	2.60

replacing the iodide involve a modification of the purification procedure that is advanced as well as application of some non-chromatographic methods.

In this paper we explore the use of octadecylsilane (C_{18}) and cyano (CN) columns for the separation of mixtures of cyanine dyes. We have found, in general, that dyes which are members of the same vinylogous series are easier to separate on the C_{18} column, but remain completely unresolved on the CN column. Also, cyanine dyes in which the heteroatom is varied (e.g., O, S or Se atom), with other structural features unchanged, are found to be better resolved on the polar CN column. Thus a combination of these two stationary phases was expected to lead to better resolution of a mixture of such cyanines. Hence, we joined radial compression cartridges to form the desired selectivity capability. The wide availability of commercial

cartridges allows multiphase chromatography to be performed with minimal changes in instrument setup.

2. Experimental

2.1. Instrumentation

The chromatographic system used is manufactured by Shimadzu (Kyoto, Japan). It consists of two LC-10AD pumps, a SPDM6A photodiode array UV–Vis detector and Nova-Pak CN HP and Nova-Pak C_{18} (100 mm × 8 mm, 4 μm particle size) radial compression cartridges from Waters (Wayland, MA, USA). All experiments were conducted at room temperature (22–25°C).

2.2. Reagents

Cyanine dyes (see Table 1), methanol, methanesulfonic acid, sodium methanesulfonate, *tert.*-butyl methyl ether and methylene chloride were from Aldrich (Milwaukee, WI, USA). Lithium perchlorate was from Ventron (Beverly, MA, USA). All aqueous solutions were made using distilled deionized water.

2.3. Separation of cyanine dyes

Methanol containing 0.002 *M* sodium methanesulfonate was used as the mobile phase. The stationary phase was either CN, C_{18} or a combination of the two. The order of coupling of the cartridges was CN followed by C_{18}. Reversing the order had no effect on the chromatogram. Samples of 20 μl containing nanomolar concentration (ca. 2 nmol) of each dye in pure methanol were injected. A mobile phase flow-rate of 1.0 ml/min was used.

2.4. Purification of 1,1′-diethyl-4,4′-cyanine iodide

The CN cartridge was used in this procedure. The mobile phase was 0.01 *M* lithium perchlorate in methanol, with a flow-rate of 2.0 ml/min. Samples of 0.5 ml containing ca. 0.13 mg of 1,1′-diethyl-4,4′-cyanine iodide in pure methanol were injected. Several fractions containing the dye were combined. The solvent was evaporated under vacuum. The cyanine dye in the residue was extracted using several small portions of methylene chloride, leaving insoluble lithium perchlorate. The methylene chloride solution of the dye was filtered through a sintered glass funnel and concentrated by evaporation. *tert.*-Butyl methyl ether was added to precipitate the dye. The precipitate was centrifuged, washed with more *tert.*-butyl methyl ether and dried in a vacuum oven at 50°C for 30 min. The purity of the dye was calculated from absorbance measurements.

2.5. Substitution of the counter ion

The methylene chloride solution in the above procedure which contained the perchlorate salt of the dye was shaken with a saturated aqueous solution of potassium chloride. The methylene chloride layer was washed with distilled water, dried over anhydrous calcium chloride and filtered through sintered glass. The chloride form of the dye was precipitated by adding *tert.*-butyl methyl ether as in the above procedure.

It might be noted that in the case of 1,1′-diethyl-2,2′-cyanine iodide [also known as pseudoisocyanine (PIC); structure not shown in Table 1], switching the counter ion was easily accomplished without the use of chromatography, due to the high purity of commercial samples. This particular dye is of interest because it is the one most studied in the literature. Its structure is quite similar to that of its 4,4′-cyanine isomer, with the methine linkage occurring at the 2-positions, adjacent to the nitrogen heteroatoms.

For this latter dye, sodium perchlorate was added to the aqueous solution, leading to precipitation of the dye perchlorate. The precipitate was washed with distilled water and dissolved in methylene chloride. The methylene chloride solution was mixed with a saturated potassium chloride solution to substitute the perchlorate with chloride. The remainder of the procedure was identical to that above.

3. Results and discussion

Capacity factors determined for each dye are listed in Table 1. Some of the dyes that have essentially the same capacity factor on the CN column have different values on the C_{18} column, and vice versa. For instance dyes **4** and **5** are unlikely to separate on the CN column under the present conditions. The only difference between the two structures is the length of the polymethine chain joining the heterocyclic rings. This structural difference, however, allows their separation on the C_{18} column. Likewise, dyes **2** and **3** are expected to elute together on the C_{18} column; the oxygen and selenium atoms on the five-membered rings have little influence on retention of the dye on the C_{18} stationary phase. However, these dyes should separate easily on the CN column. Figs. 1 and 2 show chromatograms of a mixture of these on CN column and C_{18} column, respectively. Numbers on the peaks correspond to the numbering of the dyes in Table 1. The identity of the components in each peak was established by comparing the spectrum of the peak species with the spectra of individual dyes. The order of elution and overlapping of peaks in both chromatograms are well in agreement with the data in the table. Combination of the two stationary phases clearly improves separation, as shown in Fig. 3. The iodide counter ion present in all these samples is unretained and can be monitored using UV detection. The identity of this peak was established using the starch test [13].

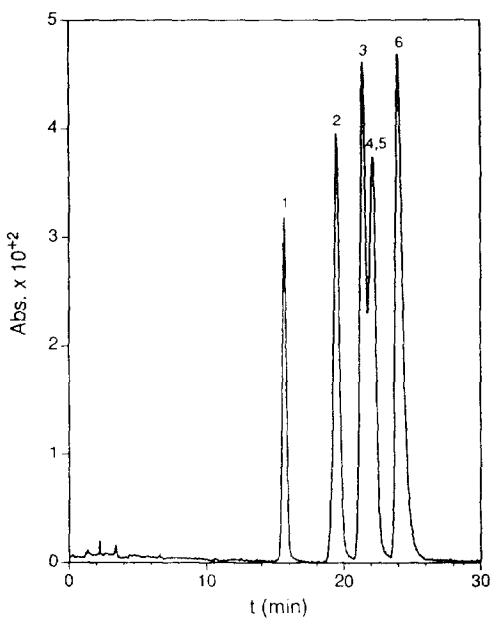


Fig. 1. Chromatogram of the mixture of cyanines using CN stationary phase. Mobile phase: methanol containing 0.002 *M* sodium methanesulfonate. Flow-rate: 1.0 ml/min. $\lambda = 350$–600 nm. Sample: 20 μl solution in methanol containing 0.14, 0.14, 0.28, 0.32, 0.46 and 0.44 μg of dyes **1**, **2**, **3**, **4**, **5** and **6**, respectively. Numbers on the peaks correspond to the numbering of dyes in Table 1.

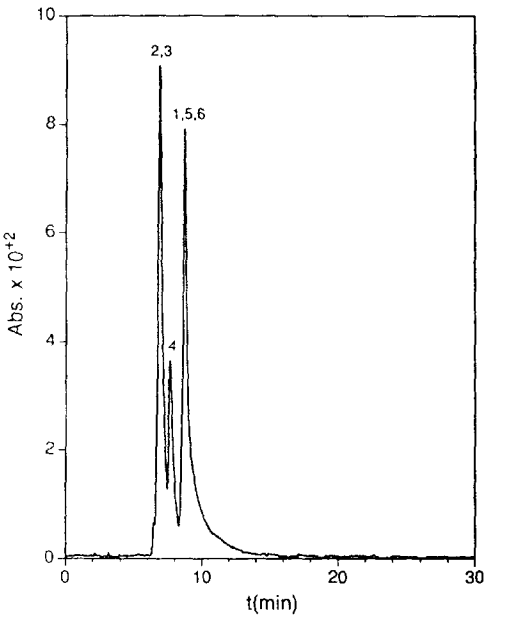


Fig. 2. Chromatogram of the mixture of cyanines using C_{18} stationary phase. Conditions and sample as in Fig. 1.

The commercial sample of 1,1′-diethyl-4,4′-cyanine contained 91.8% dye as determined using its literature reported molar extinction coefficient in methanol of value $8.8 \cdot 10^4$ M^{-1} cm^{-1} [14]. The purified product contained 96.5% dye. The yield was ca. 60%, some of the dye being lost due to adsorption on the drying agent. The perchlorate counter ion of the dye is easily changed to the chloride when the dye in methylene chloride solution is mixed with a saturated solution of potassium halide. The switching of the counter ion was confirmed by testing with

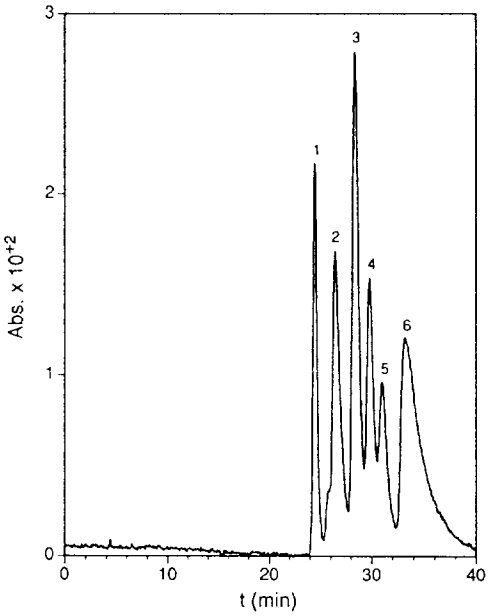


Fig. 3. Chromatogram of the mixture of cyanines on CN–C_{18} multiphase. Conditions and sample as in Fig. 1.

silver nitrate solution. Also, the product was highly soluble in water, unlike the perchlorate form of the dye.

4. Conclusions

Multiple stationary phases can take advantage of the different structural characters of cyanine dyes to accomplish the separation of mixed cyanine samples; however, if the order of elution is different in the two columns the resolution can be adversely affected. The purification procedure described here can be used to enhance dye purity of commercial samples. After chromatography, the perchlorate counter ion of the dye in methylene chloride can be exchanged if mixed with a saturated solution of potassium halide. We find that for 1,1′-diethyl-2,2′-cyanine, a dye which is the focus of many studies in our laboratory, this latter step is a quick and inexpensive way to switch the counter ion without the use of ion-exchange columns (as is often required).

Acknowledgement

Support for this research by the National Science Foundation (NSF) under Grant RII-8802964 is gratefully acknowledged.

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