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A study of *N,N′*-dicarboxyalkylthiacarbocyanines as cyanine direactive dyes covalently bound to cellulose

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Abstract

The synthesis of *N,N′*-dicarboxyalkylthiacarbocyanines and their spectroscopic properties before and fixation to microcrystalline cellulose are described. Fixation yields were determined by FTIR spectroscopy, giving an alternative to the classical indirect spectroscopic method that uses the ratio of the areas of the absorption bands for cellulose and fixed dye. Diffuse reflectance spectrometry was used to compare chemically bound dye on cellulose to physically adsorbed dye. These results, in tandem with laser induced fluorescence emission data, revealed the formation of aggregates and considerable molecular distortion, the latter resulting from a decrease in molar absorptivity compared to the case in which the dye is physically absorbed.

Keywords: Carbocyanines; Esterification; Reactive dyes; Diffuse reflectance; FTIR; Dicarboxyalkylcyanines

1. Introduction

There are various ways of linking cyanine dyes covalently to a nucleophilic substrate. One method involves the introduction of reactive groups commonly employed in textile reactive dyes [1], while another involves the use of nucleophilic groups capable of reacting with cross-linking agents [2–5].

A study directed towards obtaining cyanine-dyed macromolecules is of major interest, from an academic and a practical sense. The academic interest includes aspects such as the qualitative and quantitative nature of the dye–substrate bond, the photochemical behavior of covalently linked dye versus adsorbed dye [6–13], and the quantitative methods required to determine the fixation yields.

Studies in which aminocyanine dyes were linked to cellulose using cyanuric chloride as a cross-linking agent afforded fixation yields of 5–20%. In this case, elemental analysis (EA) and FTIR were used to characterize the dyed substrate. Although the results obtained from the two techniques were not significantly different, the latter method appeared to be more expeditious [14].

Carboxyl groups are capable of linking to macromolecules that possess nucleophilic groups. In the case of cyanine dyes, the introduction of carboxyl groups in a pendent *N*-alkyl chain can be

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accomplished via established synthetic methods [15–18].

Studies in which the groups linked to cyanine nitrogen atoms have involved those other than alkyl groups began in 1920 [16]. The results showed that λ_{max} depends on the nature of theses groups. Later, it was shown that the introduction of pendent chains containing carboxylic groups in mono-, di- and tricarbocyanines afforded strong photographic sensitization, especially in the NIR and IR regions [19].

N,N′-dicarboxyalkylcarbocyanines and certain *N,N′*-dicarboxyalkylcyanines have been used as photographic emulsion sensitizers [19,20], photopolymerization initiators [21], marking agents for substrates containing an amino group [22,23], IR photoconductor sensitizers [24], laser electrographic photoreceptors [25], supports for optical recording [26] and as agents for electrophotographic recording [27].

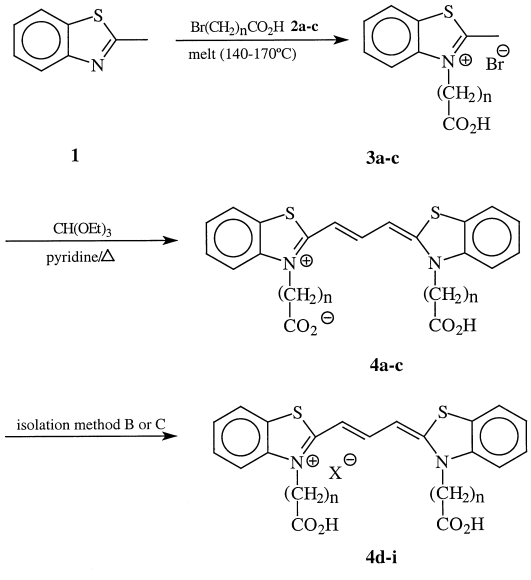
Since cellulose is a polyol, it has the potential to form esters upon condensing with carboxylic acids. While esterification of cellulosic fibers with organic acids or their derivatives at room temperature in good yield is only possible with formic acid, the reaction yields with other organic acids increase with temperature [28].

The present study pertains to the synthesis of *N,N′*-dicarboxyalkylthiacarbocyanines and the spectroscopic analyses of these dyes following fixation to microcrystalline cellulose through ester formation. The introduction of reactive functionality in the form of dicarboxyalkyl groups has several advantages: (1) the dyes can be synthesized in good yields; (2) no changes to the standard reactions are needed in order to introduce the carboxyl groups; and (3) the presence of two reactive sites increases the possibility of establishing covalent links with the substrate.

2. Results and discussion

N,N′-dicarboxyalkylthiacarbocyanines **4a–i** were synthesized, in which the carboxylic group moiety has 2, 3 and 5 carbon atoms. The two-step process involved *N*-alkylation with ω-bromoacids (**2a–c**) followed by condensation with $CH(OEt)_3$ in dry pyridine (Scheme 1) [16]. The resulting inner salts (**4a–c**) were converted to compounds **4d–f** by treatment with H_2SO_4 and to **4g–i** by sequential treatment with H_2SO_4 and 14% KI solution.

When methanol was used as a model for cellulose to examine the esterification of **4f–h**, using a 5% H_2SO_4 methanolic solution under reflux, quantitative formation of diester **5a** and monoesters **5b–c** occurred after at least 48 h (Scheme 2). Following model studies, varying amounts of cyanine **4d** were covalently linked to microcrystalline cellulose through Fischer esterification, according to the procedure outlined in Fig. 1. It was anticipated that the diesterified compound would afford the highest degree of fixation.



	X	n
4a	--	1
4b	--	2
4c	--	4*
4d	HSO_4	1
4e	HSO_4	2
4f	HSO_4	4
4g	I	1
4h	I	2
4i	I	4

* not isolated

Scheme 1. Synthesis of *N,N′*-dicarboxyalkylthiacarbocyanines **4a–i**.

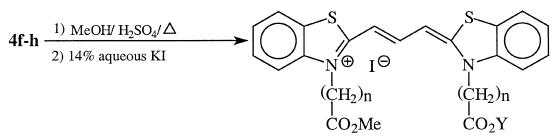
In the dyeing experiments, a control dyeing was conducted using *N,N′*-diethylthiacarbocyanines **6** (Fig. 2). In this case, practically all of the dye washed off the cellulosic substrate.

For the FTIR spectrum of each dyed cellulose sample, the ratio of the areas of the bands at 1557 cm^{-1} (aromatic C=C vibration cyanine band — Fig. 3) and 2901 cm^{-1} (cellulose C-H vibration — Fig. 3) was calculated (A_{1557}/A_{2901}). The concentration of the dye linked to cellulose was determined based on a calibration curve, showing fixation yields of 41–57% (Table 1). The calibration curve was obtained from mechanically homogenized samples containing known concentrations of the cyanine dye in microcrystalline cellulose (Fig. 4). We found a linear relationship between the concentration of dye in the dye bath and the amount of the dye covalently linked to cellulose (Fig. 5).

Although the A_{1557}/A_{2901} ratio (Fig. 4) as well as the A_{1732}/A_{2901} ratio (Fig. 6) (where 1732 cm^{-1} = C=O vibration for the $-CO_2H$ group in the cyanine dyes) exhibited linearity, with correlation coefficients of about 0.99, only the former ratio can be used to quantify covalently linked cyanine dye. This limitation is due to the inability to detect the absorption band for the carbonyl group when the cyanine dye is bound to cellulose, even at high dye concentrations (Fig. 7). However, this observation provides strong evidence for covalent bond formation between the cyanine dye and cellulose, forming ester or orthoester groups (Scheme 3). Orthoester formation can be attributed to the close proximity of the carbonyl groups to multiple hydroxyl groups.

Diffuse reflectance spectra were also recorded, in order to characterize the product from the reaction between thiacarbocyanine **4d** and powdered microcrystalline cellulose. The results (Fig. 8) can be compared with previous data for thiacarbocyanines and several other cyanines [10–13], where the dye is simply physically held by microcrystalline cellulose.

Fluorescence emission experiments using N_2 laser excitation provided evidence for appreciable quenching of the luminescence, which is associated



5a-c

	Y	n
5a	Me	1
5b	H	2
5c	H	4

Scheme 2. Esterification of *N,N′*-dicarboxyalkylthiacarbocyanine iodides **4f–h**.

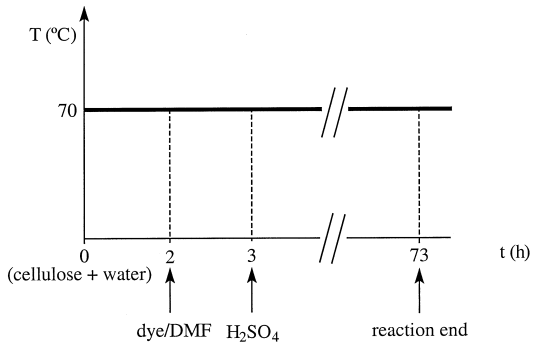
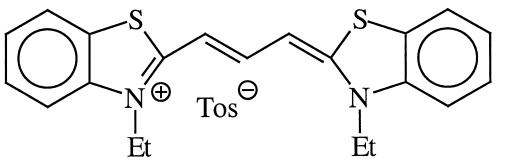


Fig. 1. Dyeing procedure used in this study.



6

Fig. 2. Structure of *N,N′*-diethylthiacarbocyanine.

Table 1
Fixation yields for cyanine **4d** in microcrystalline cellulose

Dye **4b** bath concentration (mmol dye/g cell)	A_{1557}/A_{2901}	Anchored dye (mmol dye/g cell)	Fixation yield (%)
0.0693–0.548[a]	0.0000	–	–
0.0164	0.0000	–	–
0.0287	0.0000	–	–
0.0522	0.0053	0.0225	43
0.0742	0.0189	0.0305	41
0.112	0.0664	0.0585	52
0.145	0.0667	0.0587	41
0.283	0.216	0.147	52
0.566	0.513	0.322	57

[a] Cellulose dyed with *N,N′*-diethylcarbothiacyanine **6**.

with aggregate formation and an increase in the concentration of dye covalently bound (Fig. 9). Consequently, it is clear that the dyeing method employed in the present work provided high concentrations of covalently bound dye, with concomitant significant dye–dye interaction (aggregation).

3. Experimental

3.1. General

2-Methylbenzothiazole (**1**), bromoacetic acid (**2a**), 3-bromopropionic acid (**2b**), 5-bromovaleric

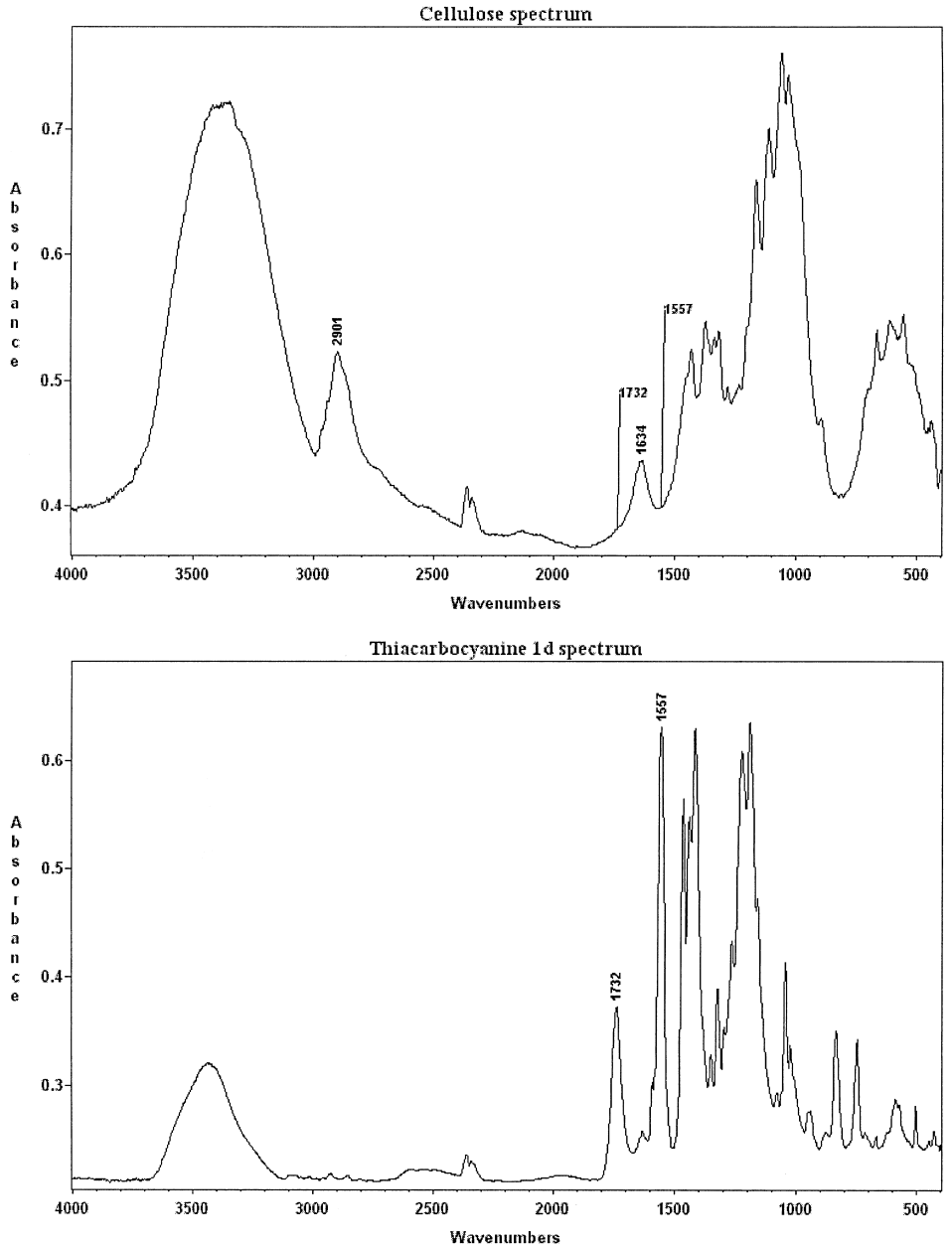


Fig. 3. FTIR spectra of cellulose (upper) and carbocyanine **4d** (lower).

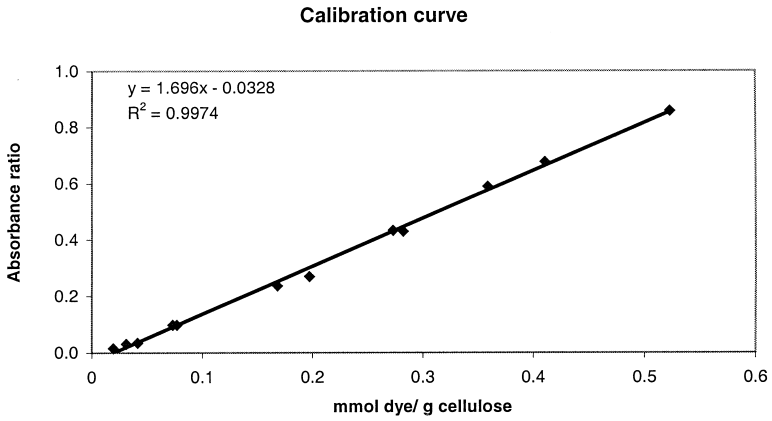


Fig. 4. Calibration curve for cyanine **4d** based on A_{1557}/A_{2901}.

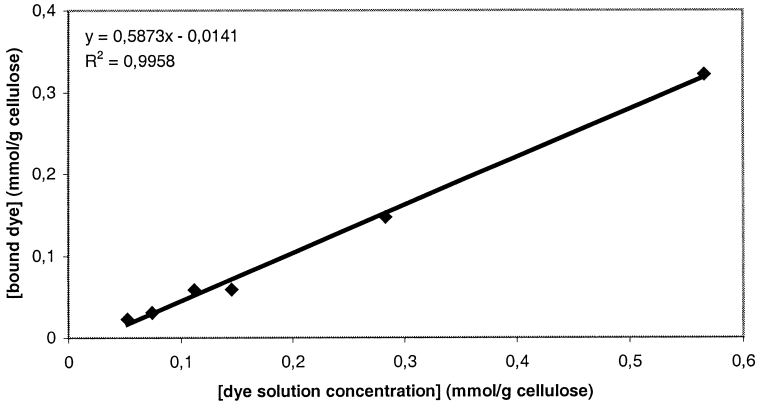


Fig. 5. Bound dye **4d** concentration as a function of dye bath concentration.

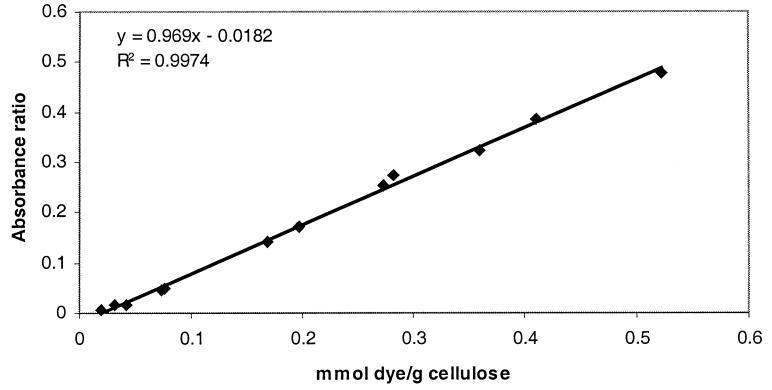


Fig. 6. Curve for cyanine **4d** based on A_{1732}/A_{2901}.

acid (**2c**), triethyl orthoformate, pyridine, KI, methanol, ethanol, DMF and H_2SO_4 of the highest purity available were purchased from Aldrich and used as received. *N,N′*-diethylthiacarbocyanine **6** was prepared by the thioalkyl method [16]. The microcrystalline cellulose was acquired commercially (Fluka DSO).

All syntheses were monitored by tlc on aluminum plates precoated with Merck silica gel 60 F_{254} (0.25 mm) using dichloromethane or dichloromethane/methanol (5–10%).

The tlc plates were observed under 254, 312 and 365 nm UV light and developed by exposure to iodine and/or Dragendorff's reagent [29].

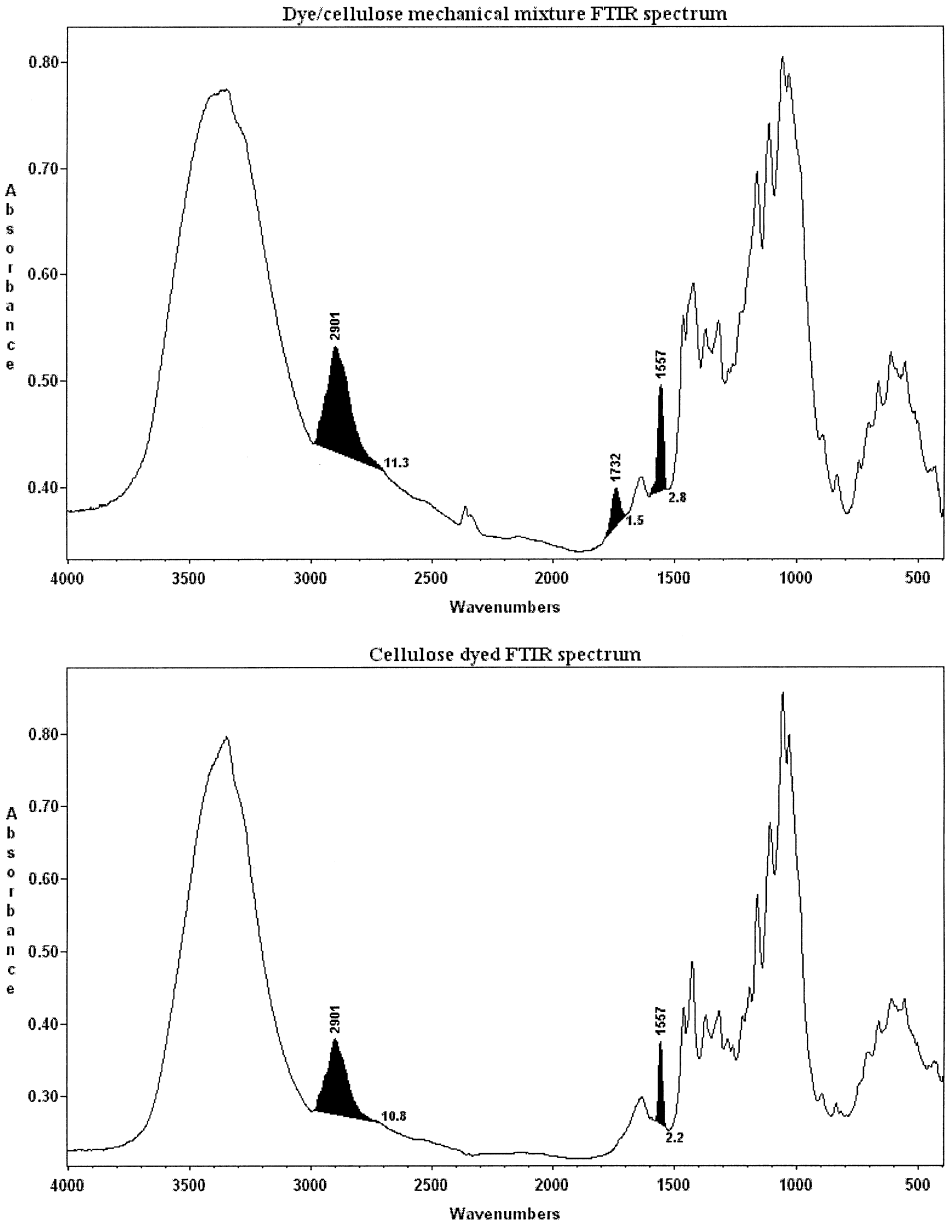
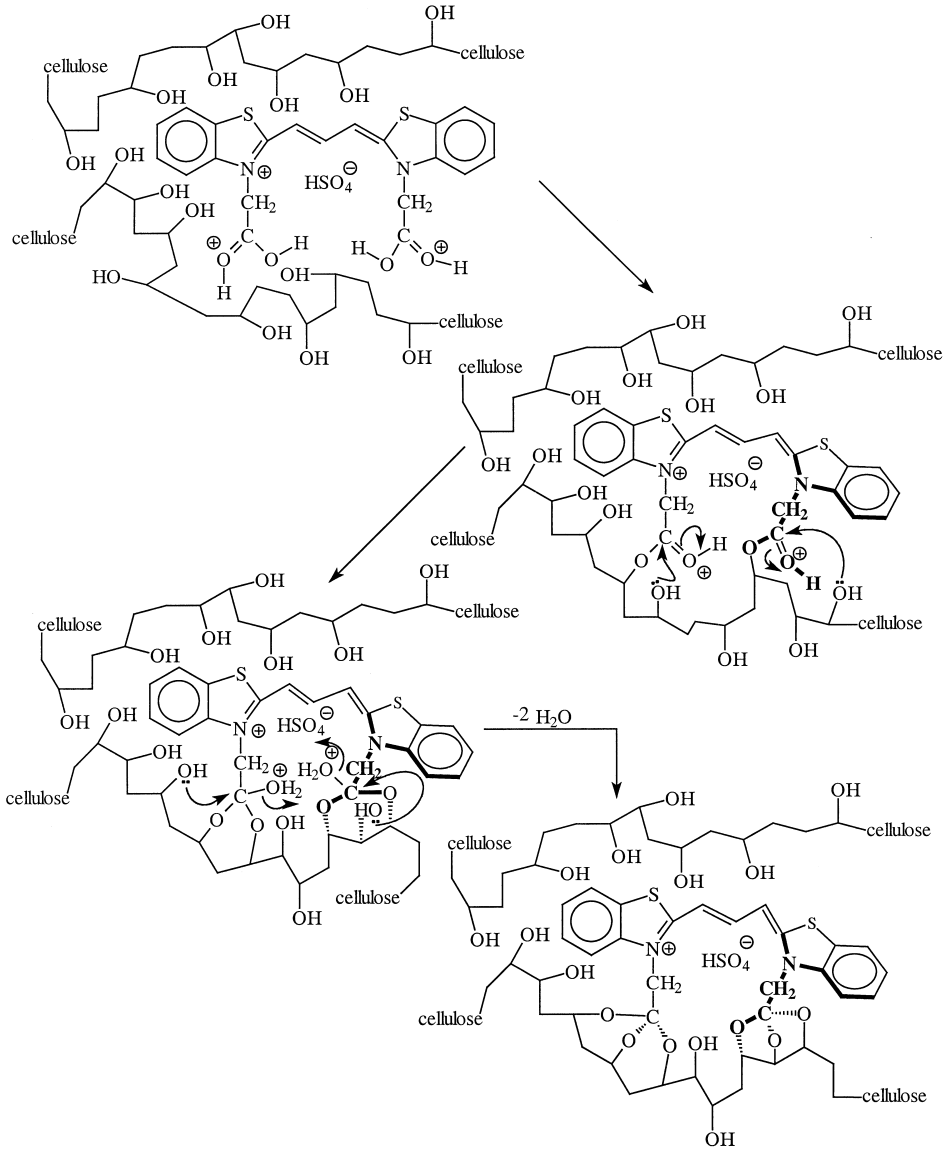


Fig. 7. FTIR spectra of cyanine **4d**/cellulose physical mixture (upper) and dyed cellulose (lower).

^{1}H- and ^{13}C-NMR spectra (300.15 and 76.48 MHz, respectively) were recorded on a General Electric QE-PLUS-300 spectrometer, at room temperature, in DMSO-d_6, DMSO-$d_6 + D_2SO_4$ or MeOD with TMS as the internal standard. COSY and HETCOR spectra were recorded on the same instrument.

Infrared spectra were performed on a Mattson 5000-FTS FTIR spectrophotometer. All samples were prepared by mixing FTIR-grade KBr (Aldrich Chemicals) with 1% (w/w) dye, and grinding the mixture to a fine powder. Spectra were recorded over the 400–4000 cm^{-1} range without baseline corrections. UV-vis spectra were recorded on a Perkin–Elmer Lambda 6 spectrophotometer using DMSO/ethanol as solvent, and melting points were determined in open capillary tubes in a Büchi 530 melting point apparatus and are uncorrected.



Scheme 3. Esther and/or orthoesther bond formation between *N,N′*-dicarboxyalkylthiacarbocyanines and cellulose.

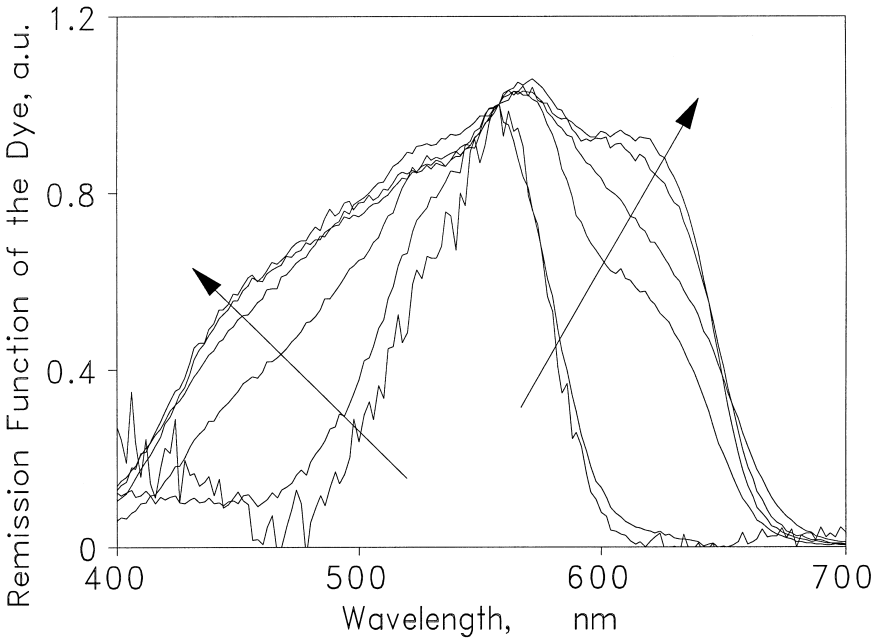


Fig. 8. Remission function of increasing concentrations (~20 to 320 μmol/g) of microcrystalline cellulose esterified with cyanine dye **4d**. The arrows show the increasing concentration.

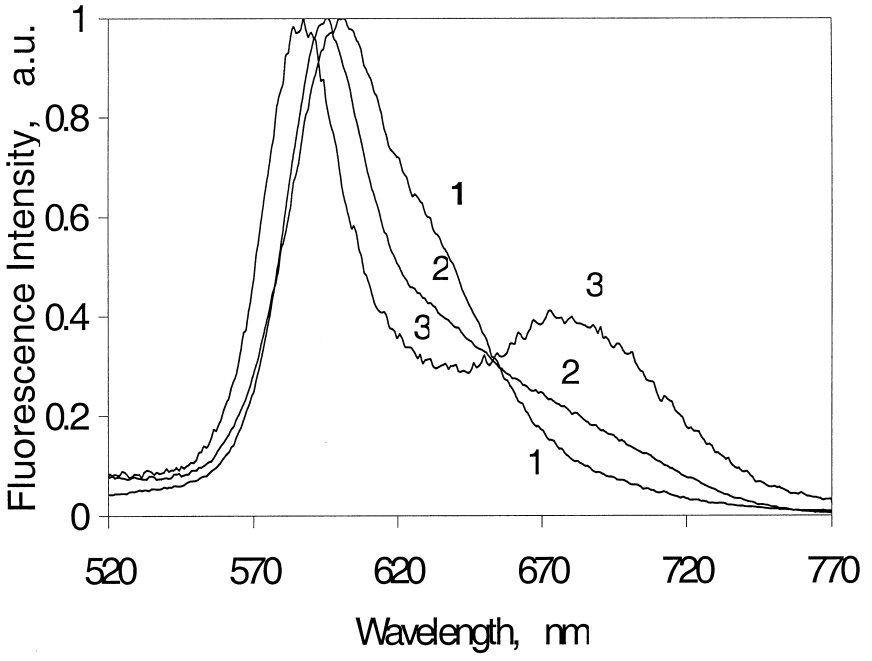


Fig. 9. Laser induced fluorescence emission of microcrystalline cellulose esterified with cyanine dye **4d**. 1, low dye concentration (~20 μmol/g); 2, intermediate dye concentration (~60 μmol/g); 3, high dye concentration (~150 μmol/g).

3.2. Synthesis

3.2.1. N,N′-dicarboxyalkylthiacarbocyanines

A mixture of 2-methylbenzothiazole (**1**; 20.0 mmol) and the ω-halogenated acid **2a**–**c** (20.2 mmol) was melted at 140–170°C. After 1–4.5 h, triethyl orthoformate (40.4 mmol) and dry pyridine (10/g of the ammonium quaternary salts **3a**–**c**) were added, producing a deeply colored solution. Heating for a further 2–3 h completed the reaction. The mixture was allowed to cool to room temperature, then diethyl ether (20 ml) was added and the solution was refrigerated. The resultant precipitate was isolated by filtration (isolation step A). In the case of the *N,N′*-dicarboxybutylcarbocyanine **4c**, a viscous oil was produced, which was isolated by decantation.

The oily or solid product produced in isolation step A was dissolved in a minimum amount of methanol and H_2SO_4 (5%, 100 ml) was added. The resultant solution was concentrated until most of the methanol was removed, yielding a solid that was collected by filtration (isolation step B).

The solid obtained from isolation step B was dissolved in a minimum amount of methanol. This solution was diluted with a five-fold volume of 14% aqueous KI and was heated until boiling began. The resultant suspension was allowed to cool to room temperature and then held at 4°C for several hours. The precipitated solid was collected by filtration (isolation step C).

All of the following compounds were obtained by recrystallizing the solids from isolation steps A, B or C using methanol.

3.2.2. 3-(2-Carboxymethyl)-2-[3-(3-carboxymethyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium inner salt (**4a**)

Bromoacetic acid (**2a**) and 2-methylbenzothiazole (**1**) were held for 2 h in the melt and then stirred at reflux for 3 h with $(EtO)_3CH$ in dry pyridine. An 81% yield of a brownish-red solid was obtained following isolation step A, m.p. 216°C; UV/vis: λ_{max} = 559 nm, λ_2 = 442 nm, ε = 1.47×10^4 cm^{-1} mol^{-1} dm^3, ε_2 = 2.35×10^3 cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.2%); IR (KBr) ν_{max} (cm^{-1}): 1729 (C=O, m), 1555 ($C{=}C_{Ar}$, s); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.29 (4H, *s*, C**H**$_2$); 6.54 (2H, *d*, *J* = 13 Hz, α-C**H**), 7.44 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.56 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.74 (2H, *d*, *J* = 8.5 Hz, **H**-4), 7.83 (1H, *t*, *J* = 12.5 Hz, β-C**H**), 8.03 (2H, *d*, *J* = 7.5 Hz, **H**-7); ^{13}C NMR (DMSO-d_6) δ (ppm): 47.7 (**C**H$_2$), 99.9 (α-**C**H), 113.8 (**C**H-4), 123.5 (**C**H-7), 125.0 (**C**H-6), 125.8 (**C**-7a), 128.5 (**C**H-5), 141.8 (**C**-3a), 147.2 (β-CH), 166.2 (**C**-2), 168.0 (**C**OOH).

3.2.3. 3-(2-Carboxymethyl)-2-[3-(3-carboxymethyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium hydrogensulfate (**4d**)

Taking the inner salt **4a** through isolation step B produced **4d** in 68% of overall yield as a dark brownish-red solid, m.p. 257–258°C (dec.); UV/vis: λ_{max} = 560 nm, ε = 3.97×10^4 cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.2%); IR (KBr) ν_{max} (cm^{-1}): 1732 (C=O, m), 1557 ($C{=}C_{Ar}$, s); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.25 (4H, s, C**H**), 6.50 (2H, *d*, *J* = 12.5 Hz, α-C**H**), 7.40 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.53 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.71 (2H, *d*, *J* = 8.5 Hz, **H**-4), 7.81 (1H, *t*, *J* = 12.5 Hz, β-CH), 7.99 (2H, *d*, *J* = 8 Hz, **H**-7); ^{13}C NMR (DMSO-d_6) δ (ppm): 47.8 (**C**H$_2$), 100.0 (α-**C**H), 113.9 (**C**H-4), 123.6 (**C**H-7), 125.1 (**C**H-6), 125.9 (**C**-7a), 128.7 (**C**H-5), 141.8 (**C**-3a), 147.7 (β-**C**H), 166.3 (**C**-2), 168.2 (**C**OOH).

3.2.4. 3-(2-Carboxymethyl)-2-[3-(3-carboxymethyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium iodide (**4g**)

Taking **4d** through isolation step C produced **4g** in 68% of overall yield as a dark brownish-red solid, m.p. 228°C; UV/vis: λ_{max} = 558 nm, ε = 1.38×10^4 cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.2%); IR (KBr) ν_{max} (cm^{-1}): 1726 (C=O, m), 1555 ($C{=}C_{Ar}$, s); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.25 (4H, s, C**H**), 6.50 (2H, *d*, *J* = 13 Hz, α-C**H**), 7.41 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.53 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.70–7.85 (3H, *m*, β-C**H** + **H**-4), 8.00 (2H, *d*, *J* = 8 Hz, **H**-7).

3.2.5. 3-(2-Carboxyethyl)-2-[3-(3-carboxyethyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium inner salt (**4b**)

3-Bromopropionic acid (**2b**) and 2-methylbenzothiazole (**1**) were held for 4.5 h in the melt and then stirred at reflux for 2 h with $(EtO)_3CH$ in dry pyridine. An 81% yield of a dark purple solid was

obtained following isolation step A, m.p. 231°C. UV/vis: λ_{max} = 450 nm, λ_2 = 562 nm, ε_{max} = 1.09×10^4 cm^{-1} mol^{-1} dm^3, ε_2 = 7.69×10^3 cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 2%); IR (KBr) ν_{max} (cm^{-1}): 1684 (C=O, w), 1562 ($C=C_{Ar}$, s); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.82 (4H, *t*, *J* = 7 Hz, C**H**$_2$COOH), 4.54 (4H, *t*, *J* = 7 Hz, C**H**$_2$), 6.66 (2H, *d*, *J* = 12.5 Hz, α-C**H**), 7.40 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.56 (2H, *t*, *J* = 8 Hz, **H**-5), 7.71–7.77 (3H, m, β-C**H** + **H**-4), 7.98 (2H, *d*, *J* = 8 Hz, **H**-7); ^{13}C NMR (DMSO-d_6 + D_2SO_4) δ (ppm): 31.8 (**C**H$_2$COOH), 42.4 (CH$_2$), 99.4 (α-**C**H), 114.0 (**C**H-4), 123.2 (**C**H-7), 125.2 (**C**H-6), 125.5 (**C**-7a), 128.3 (**C**H-5), 141.2 (**C**-3a), 146.7 (β-**C**H), 165.0 (**C**-2), 171.8 (**C**OOH).

3.2.6. 3-(2-Carboxyethyl)-2-[3-(3-carboxyethyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium hydrogensulfate (**4e**)

Taking the inner salt **4b** through isolation step B provided **4e** in 69% of overall yield as a dark purple solid, m.p. 236°C. UV/vis: λ_{max} = 562 nm, ε = 1.48×10^5 cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.2%); IR (KBr) ν_{max} (cm^{-1}): 1726 (C=O, s), 1562 ($C=C_{Ar}$, s); ^{1}H NMR (MeOD) δ (ppm): 2.88 (4H, *t*, *J* = 7 Hz, C**H**$_2$COOH), 4.61 (4H, *t*, *J* = 7 Hz, C**H**$_2$), 6.56 (2H, *d*, *J* = 12.5 Hz, α-**C**H), 7.39 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.55 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.68 (2H, *d*, *J* = 8 Hz, **H**-4), 7.81 (2H, *d*, *J* = 8 Hz, **H**-7) 7.96 (1H, *t*, *J* = 13 Hz, β-C**H**); NMR ^{1}H (DMSO-d_6) δ (ppm): 2.76 (4H, *t*, *J* = 7 Hz, C**H**$_2$COOH), 4.50 (4H, *t*, *J* = 7 Hz, C**H**$_2$), 6.61 (2H, *d*, *J* = 12.5 Hz, α-C**H**), 7.38 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.53 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.71–7.78 (3H, *m*, β-CH + **H**-4), 7.96 (2H, *d*, *J* = 8 Hz, **H**-7); ^{13}C NMR (DMSO-d_6) δ (ppm): 32.0 (**C**H$_2$COOH), 42.6 (**C**H$_2$), 99.6 (α-**C**H), 114.1 (**C**H-4), 123.4 (**C**H-7), 125.4 (**C**H-6), 125.6 (**C**-7a), 128.4 (**C**H-5), 141.5 (**C**-3a), 147.2 (β-**C**H), 165.5 (**C**-2), 171.6 (**C**OOH).

3.2.7. 3-(2-Carboxyethyl)-2-[3-(3-carboxyethyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium iodide (**4h**)

Taking **4e** through isolation step C provided **4h** in 67% of overall yield as a dark purple solid, m.p. 242–243°C. UV/vis: λ_{max} = 562 nm, ε = 1.22×10^5 cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 2%); IR (KBr) ν_{max} (cm^{-1}): 1726 (C=O, s), 1553 ($C=C_{Ar}$, s); ^{1}H NMR (MeOD) δ (ppm): 2.88 (4H, *t*, *J* = 7 Hz, C**H**$_2$COOH), 4.61 (4H, *t*, *J* = 7 Hz, C**H**$_2$), 6.57 (2H, *d*, *J* = 13 Hz, α-C**H**), 7.39 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.55 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.68 (2H, *d*, *J* = 8.5 Hz, **H**-4), 7.81 (2H, *d*, *J* = 8 Hz, **H**-7) 7.96 (1H, *t*, *J* = 13 Hz, β-C**H**); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.76 (4H, *t*, *J* = 7 Hz, C**H**$_2$COOH), 4.50 (4H, *t*, *J* = 7 Hz, C**H**$_2$), 6.61 (2H, *d*, *J* = 12.5 Hz, α-C**H**), 7.39 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.54 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.71–7.78 (3H, m, β-CH + **H**-4), 7.96 (2H, *d*, *J* = 8 Hz, **H**-7).

3.2.8. 3-(2-Carboxybutyl)-2-[3-(3-carboxybutyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium hydrogensulfate (**4f**)

5-Bromovaleric acid (**2c**) and 2-methylbenzothiazole (**1**) were heated for 1 h in the melt and then at reflux with $(EtO)_3CH$ for 2 h in dry pyridine. A 56% yield of a green solid was obtained following isolation step B, m.p. 186–189°C; UV/vis: λ_{max} = 562 nm, ε = 1.48×10^5 cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.2%); IR (KBr) ν_{max} (cm^{-1}): 1724 (C=O, s), 1561 ($C=C_{Ar}$, s); ^{1}H NMR (MeOD) δ (ppm): 1.91-2.02 (8H, *m*, $CH_2C\mathbf{H}_2$ C**H**$_2$CH$_2$COOH), 2.55 (4H, *t*, *J* = 7 Hz, C**H**$_2$COOH), 4.45 (4H, *t*, *J* = 7 Hz, *N*-C**H**$_2$), 6.66 (2H, *d*, *J* = 12.5 Hz, α-C**H**), 7.48 (2H, *t*, *J* = 6.5 Hz, **H**-6), 7.66 (2H, *t*, *J* = 7 Hz, **H**-5), 7.74 (2H, *d*, *J* = 8 Hz, **H**-4), 7.93 (2H, *d*, *J* = 8 Hz, **H**-7), 8.03 (1H, *t*, *J* = 12 Hz, β-C**H**); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.61-1.74 (8H, *m*, *N*-CH$_2$C**H**$_2$C**H**$_2$CH$_2$COOH), 2.28 (4H, *t*, *J* = 7 Hz, C**H**$_2$COOH), 4.33 (4H, *t*, *J* = 7 Hz, *N*-C**H**$_2$), 6.57 (2H, *d*, *J* = 12.5 Hz, α-C**H**), 7.38 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.54 (2H, *t*, *J* = 8 Hz, **H**-5), 7.70–7.78 (3H, *m*, *J* = 8 Hz, β-C**H** + **H**-4), 7.97 (2H, *d*, *J* = 8 Hz, **H**-7); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.1 (**C**H$_2$CH$_2$COOH), 27.2 (NCH$_2$ **C**H$_2$), 33.7 (**C**H$_2$COOH), 46.6 (*N*-**C**H$_2$), 99.2 (α-**C**H), 114.0 (**C**H-4), 123.4 (**C**H-7), 125.5 (**C**H-6), 125.7 (**C**-7a), 128.5 (**C**H-5), 141.6 (**C**-3a), 147.0 (β-**C**H), 165.3 (**C**-2), 174.2 (**C**OOH).

3.2.9. 3-(2-Carboxybutyl)-2-[3-(3-carboxybutyl-2(3H)-benzothiazolylidene)propen-1-yl]-benzothiazolium iodide (**4i**)

Taking **4f** through isolation step C gave a 54% of overall yield of **4i** as a green solid, m.p.170–171°C. UV/vis: λ_{max} = 562 nm, ε = 1.53×10^5 cm^{-1} mol^{-1}

dm^3 (DMSO/ethanol — 0.2%); IR (KBr) ν_{max} (cm^{-1}): 1726 (C=O, s), 1555 ($C=C_{Ar}$, s); ^1H NMR (DMSO-d_6) δ (ppm): 1.62–1.73 (8H, *m*, N-CH_2**C**H_2**C**H_2CH_2COOH), 2.28 (4H, *t*, *J* = 7 Hz, **C**H_2COOH), 4.32 (4H, *t*, *J* = 6.5 Hz, *N*-**C**H_2), 6.58 (2H, *d*, *J* = 12.5 Hz, α-**CH**), 7.37 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.53 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.69–7.76 (3H, *m*, β-**CH** + **H**-4), 7.96 (2H, *d*, *J* = 7 Hz, **H**-7); ^1H NMR (MeOD) δ (ppm): 1.89–2.03 (8H, *m*, N-CH_2**C**H_2**C**H_2CH_2COOH), 2.54 (4H, *t*, *J* = 7 Hz, **C**H_2COOH), 4.49 (4H, *t*, *J* = 7 Hz, N-**C**H_2), 6.69 (2H, *d*, *J* = 12.5 Hz, α-**CH**), 7.53 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.69 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.78 (2H, *d*, *J* = 8.5 Hz, **H**-4), 7.95 (2H, *d*, *J* = 8 Hz, **H**-7), 8.09 (1H, *t*, *J* = 13 Hz, β-**CH**); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.1 (**C**H_2CH_2COOH), 27.2 (NCH_2**C**H_2), 33.7 (**C**H_2COOH), 46.6 (N-**C**H_2), 99.2 (α-CH), 114.0 (**C**H-4), 123.4 (**C**H-7), 125.5 (**C**H-6), 125.7 (**C**-7a), 128.5 (**C**H-5), 141.7 (**C**-3a), 147.0 (*β*-**C**H), 165.3 (**C**-2), 174.1 (**C**OOH).

3.2.10. Esterification of N,N′-dicarboxyalkylthiacarbocyanines

A stirred solution of *N,N′*-dicarboxyalkylthiacarbocyanine hydrogensulfate **4f** or iodides **4g–h** (0.4 mmol) in methanol (20 ml) and conc. H_2SO_4 (1 ml) was heated at 50°C. The reaction was complete after 48 h and the resultant clear solution was allowed to cool to room temperature, whereupon 14% aqueous KI (20 ml) was added. Methanol was driven off at the boil, and the suspension was allowed to cool to room temperature, held at 4°C for several hours, and then filtered.

All of the following products were obtained using this method and were recrystallized from methanol.

3.2.11. 3-[2-Carboxymethyl]-2-[3-[3-[2-oxo-2-(methoxy)ethyl]-2(3H)-benzothiazolylidene]propen-1-yl]benzothiazolium iodide (**5a**)

Esterification of compound **4g** gave an 80% yield of **5a** as a brownish-red solid, m.p. 214–215°C; UV/vis: λ_{max} = 562 nm, $\varepsilon = 2.79\times10^4$ cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.4%); IR (KBr) ν_{max} (cm^{-1}): 1751 (C=O, s), 1570 ($C=C_{Ar}$, s); ^1H NMR (DMSO-d_6) δ (ppm): 3.75 (6H-6, *s*, $COOCH_3$), 5.40 (4H, *s*, N-**C**H_2), 6.49 (2H, *d*, *J* = 13 Hz, α-**CH**), 7.41 (2H, *t*, *J* = 7.5 Hz, **H**-6), 7.53 (2H, *t*, *J* = 7.5 Hz, **H**-5), 7.72 (2H, *d*, *J* = 8 Hz, **H**-4), 7.80 (1H, *t*, *J* = 13 Hz, β-**CH**), 8.02 (2H, *d*, *J* = 8 Hz, **H**-7); ^{13}C NMR (DMSO-d_6) δ (ppm): 47.5 (**C**H_2COO), 53.4 (**C**H_3), 100.0 (α-**C**H), 113.9 (**C**H-4), 123.6 (**C**H-7), 125.0 (**C**H-6), 126.0 (**C**H-7a), 128.6 (**C**H-5), 141.6 (**C**-3a), 147.7 (β-**C**H), 166.4 (**C**-2), 167.2 (**C**$OOCH_3$).

3.2.12. 3-[2-Carboxyethyl]-2-[3-[3-[2-oxo-2-(methoxy)propyl]-2(3H)-benzothiazolylidene]propen-1-yl]benzothiazolium iodide (**5b**)

Esterification of compound **4h** gave a 70% yield of **5b** as a dark purple solid, m.p. 182–184°C; UV/vis: λ_{max} = 562 nm, $\varepsilon = 1.17\times10^5$ cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.2%); IR (KBr) ν_{max} (cm^{-1}): 1732 (C=O, s), 1555 ($C=C_{Ar}$, s); ^1H NMR (DMSO-d_6) δ (ppm): 2.74–2.97 (4H, *m*, **C**H_2COOH and **C**H_2COOCH_3); 3.58 (3H, *s*, $COOCH_3$); 4.52 (4H, *t*, *J* = 7 Hz, N-**C**H_2); 6.58 (2H, *d*, *J* = 12.5 Hz, α-**CH**); 7.36 (2H, *t*, *J* = 7 Hz, **H**-6); 7.52 (2H, *t*, *J* = 6.5 Hz, **H**-5); 7.63–7.75 (2H, *m*, β-**CH** + **H**-4); 7.95 (2H, *d*, *J* = 7 Hz, **H**-7); ^{13}C NMR (DMSO-d_6) δ (ppm): 31.8 (**C**H_2COOH or **C**H_2COOCH_3), 32.1 (**C**H_2COOH or **C**H_2COOCH_3), 42.4 (N-**C**H_2CH_2COOH or N-**C**$H_2CH_2COOCH_3$), 42.7 (N-**C**H_2CH_2COOH or N-CH_2CH_2COO**C**H_3), 52.2 ($COOCH_3$), 99.6 (α-**C**H), 114.1 (**C**H-4), 123.4 (**C**H-7), 125.3 (**C**H-6), 125.7 (**C**-7a), 128.4 (**C**H-5), 141.3 (**C**-3a), 147.1 (β-**C**H), 165.3 (**C**-2), 170.8 (**C**OOH or **C**$OOCH_3$), 171.8 (**C**OOH or **C**$OOCH_3$).

3.2.13. 3-[2-Carboxybuty]-2-[3-[3-[2-oxo-2-(methoxy)pentyl]-2(3H)-benzothiazolylidene]propen-1-yl]benzothiazolium iodide (**5c**)

Esterification of compound **4f** gave a 63% yield of **5c** as a green solid, m.p. 148–149°C; UV/vis: λ_{max} = 562 nm, $\varepsilon = 1.31\times10^5$ cm^{-1} mol^{-1} dm^3 (DMSO/ethanol — 0.1/50); IR (KBr) ν_{max} (cm^{-1}): 1726 (C=O, s), 1555 ($C=C_{Ar}$, s); ^1H NMR (DMSO-d_6) δ (ppm): 1.61–1.73 (8H, *m*, N-CH_2**C**H_2**C**H_2CH_2COO); 2.27 (2H, *t*, *J* = 7 Hz, **C**H_2COOH or **C**H_2COOCH_3); 2.37 (2H, *t*, *J* = 7 Hz, **C**H_2COOH or **C**H_2COOCH_3); 3.54 (3H, *s*, $COOCH_3$); 4.32 (4H, *t*, *J* = 7 Hz, N-**C**H_2CH_2); 6.59 (2H, *d*, *J* = 13 Hz, α-**CH**); 7.38 (2H, *t*, *J* = 7.5 Hz, **H**-6); 7.54 (2H, *t*, *J* = 7.5 Hz, **H**-5); 7.70–7.79 (3H, *m*, β-**CH** + **H**-4); 7.97 (2H, *d*, *J* = 8 Hz, **H**-7); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.0

($\mathbf{C}H_2CH_2COOH$ or $\mathbf{C}H_2CH_2COOCH_3$); 22.1 ($CH_2\mathbf{C}H_2COOH$ or $CH_2\mathbf{C}H_2COOCH_3$); 27.1 ($CH_2(CH_2)_2\mathbf{C}OOH$ or $CH_2(CH_2)_2\mathbf{C}OOCH_3$); 27.2 ($CH_2(CH_2)_2\mathbf{C}OOH$ or $CH_2(CH_2)_2\mathbf{C}OOCH_3$); 33.3 ($\mathbf{C}H_2COOH$ or $\mathbf{C}H_2COOCH_3$); 33.6 ($\mathbf{C}H_2COOH$ or $\mathbf{C}H_2COOCH_3$); 46.3 (N-$\mathbf{C}H_2(CH_2)_3COOH$ or N-$\mathbf{C}H_2(CH_2)_3COOCH_3$); 46.4 (N-$\mathbf{C}H_2(CH_2)_3COOH$ or N-$\mathbf{C}H_2(CH_2)_3COOCH_3$); 51.6 (COO $\mathbf{C}H_3$); 99.2 (α-**C**H); 114.0 (**C**H-4); 123.4 (**C**H-7); 125.4 (**C**H-6); 125.6 (**C**-7a); 128.5 (**C**H-5); 141.6 (**C**-3a); 147.0 (β-**C**H); 165.1 (**C**-2); 173.4 (**C**OOH or $\mathbf{C}OOCH_3$); 174.4 (**C**OOH or $\mathbf{C}OOCH_3$).

3.3. Cellulose dyeing

3.3.1. Esterification with *N,N′*-dicarboxymethylthiacarbocyanine hydrogensulfate (**4d**)

A suspension of microcrystalline cellulose (700 mg) in distilled water (3 ml) was stirred for 2 h at 70°C as solid thiacarbocyanine **4d** (0.0164–0.5660 mmol) and DMF (16 ml) were added portionwise over a 1 h period. H_2SO_4 (18 M, 1 ml) was then added and stirring was continued for 70 h at 70°C. The dyed cellulose was collected by filtration, washed several times with ethanol, extracted with ethanol in a Soxhlet apparatus until no significant color removal was observed, and dried in vacuo over phosphorus pentoxide at 50°C for 24 h.

Control experiments were also conducted, either in the absence of dye or using the non-reactive dye *N,N′*-diethylthiacarbocyanine (**6**) at different concentrations (0.0693–0.5480 mmol dye/g cellulose).

3.4. Quantitative and qualitative studies on dyed cellulose

A visual examination of the dyed cellulose samples following Soxhlet extraction revealed the following: (1) control experiments in the absence of the dye afforded the expected uncolored cellulose, (2) dye **6** gave only a slightly pink colored cellulose (only at the highest concentrations) that had less color than the sample of cellulose dyed with the lowest concentration of reactive thiacarbocyanine **4d**, and (3) dyeing of cellulose with the reactive thiacarbocyanine gave pink to dark purple shades as dye concentration was increased.

3.5. Fixation yields

The amount of dye anchored to microcrystalline cellulose (fixation yields) was determined using FTIR on well-dried cellulose samples dyed with 0.0164–0.5660 mmol of dye **4d** and with 0.0693–0.5480 mmol of dye **6**. From the spectra obtained, the ratio of the areas of the bands for the C=C bond of **4d** (1557 cm^{-1}) and the C–H bond of the cellulose (2901 cm^{-1}) was determined. Calibration plots were obtained from known concentrations of homogeneous physical mixtures of cyanine dyes **4d** with microcrystalline cellulose using the same bands. Absorption band area ratios were also determined, using the C=O band at 1732 cm^{-1} instead of the aromatic C=C cyanine band.

3.6. Diffuse reflectance analysis

Diffuse reflectance spectra were recorded on powdered solid samples using a 90-mm diameter integrating sphere that had a standard white coating. The standard apparatus was modified to permit the use of short-wave-pass filters, which prevent the sample luminescence from reaching the detector.

Additional experimental details and a description of the system calibration used to obtain accurate reflectance (R) measurements are published elsewhere [10,11]. The remission function F(R) for a colorant adsorbed onto a powdered adsorbent and calculated with the use of the Kubelka-Munk equation for optically thick samples, after correction, is proportional to the host concentration [10–13].

Time resolved luminescence studies were performed using a nitrogen laser as the excitation source. The system included a delay unit and an intensified charge coupled device, which provided spectroscopic and kinetic information [13].

4. Conclusions

It has been shown that *N,N′*-carboxyalkylcarbocyanine dyes can be easily anchored to a cellulosic substrate using Fischer's esterification

conditions. This approach gave better fixation yields (41–57%) than those obtained from aminocarbocyanines in the presence of cyanuric chloride. We believe that the presence of two carboxyalkyl groups provides direactive carbocyanines capable of increasing dye fixation.

The generality of the title dye synthesis is another significant advantage for its use since most cyanine dye syntheses involve, as a first step, arylazole *N*-alkylation. In this approach the introduction of an *N*-carboxyalkyl group in any type of cyanine is easily achieved.

The amount of dye linked to microcrystalline cellulose varied linearly with the concentration of dye in the dye bath. Probably the main obstacles to obtaining very high fixation yields are the inherent inaccessibility of the reactive sites in microcrystalline cellulose and the low dye solubility.

The determination of the amount of dye anchored to microcrystalline cellulose by FTIR, while a simple and expeditious method, is unsuitable at dye concentrations below 0.0193 mg/g cellulose.

Diffuse reflectance studies confirm that linking the cyanine dye to cellulose destroys dye planarity and decreases the color of the dye bound to microcrystalline cellulose. These results agree with those reported from studies involving the dyeing of cellulose with aminocyanines and cyanuric chloride [14] and with Rhodamine B isothiocyanate [8]. The formation of aggregated forms of the dye bound to cellulose at high loadings is another important outcome because it demonstrates that significant dye fixation was obtained.

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References

[1] Siegel E. In: Venkataraman, K., editor. The chemistry of synthetic dyes — reactive dyes: reactive groups, vol. 6. New York: Academic Press, 1972 [chapter 1].
[2] Lewis DM. J Soc Dyes Colour 1993;109:357–64.
[3] Hensel HR, Lützel G. Angew Chem, Int Ed 1965;4(4):312–21.
[4] Lützel G. J Soc Dyes Colour 1966;82(8):293–8.
[5] Kamel MM, Kamel MM, Kamel MJ. J Soc Dyes Colour 1992;108:450–3.
[6] Vieira Ferreira LF, Freixo MR, Garcia AR, Wilkinson F. J Chem Soc Faraday Trans 1992;88:15–22.
[7] Vieira Ferreira LF, Garcia AR, Freixo MR, Costa SMB. J Chem Soc Faraday Trans 1993;89:1937–44.
[8] Vieira Ferreira LF, Cabral PV, Almeida P, Oliveira AS, Reis MJ, Botelho Rego AM. Macromolecules 1998;31:3936–44.
[9] Vieira Ferreira LF. Química 1999;72:28–46.
[10] Oliveira AS, Vieira Ferreira LF, Wilkinson F, Worrall DR. J Chem Soc Faraday Trans 1996;92:4809–14.
[11] Vieira Ferreira LF, Oliveira AS, Wilkinson F, Worrall DR. J Chem Soc Faraday Trans 1996;92:1217–25.
[12] Oliveira AS, Almeida P, Vieira Ferreira LF. Coll Czech Chem Comm 1999;64:459–73.
[13] Vieira Ferreira L.F., Oliveira A.S., Matousek P., Towrie M., Parker A.W., Worrall D.R., Wilkinson F. RAL CLF Annual Report 1998: 81-2.
[14] Pardal A.C., Ramos S.S., Santos L., Almeida P. Aminocyanines synthesis and fixation to microcrystalline cellulose, using cyanuric chloride as a crosslinking agent. J. Soc. Dyes Colour [in press].
[15] Sturmer DM, Heseltine DW. In: James TH., editor. Sensitizing and desensitizing dyes — the theory of the photographic processes. 4th ed. New York: Macmillan, 1977 [chapter 8].
[16] Hamer FM. In: Weissberger A, editor. The cyanine dyes and related compounds — the chemistry of heterocyclic compounds, vol. 18. New York: Interscience, 1971.
[17] Venkataraman K. In: Fieser LF, Fieser M, editors. The chemistry of synthetic dyes, vol. 2. New York: Academic Press 1952 [chapter 38].
[18] Ficken GE. In: Venkataraman K., editor. The chemistry of synthetic dyes, vol. 4. New York: Academic Press, 1971 [chapter 5].
[19] Brooker L, Smith L, US Patent 2231658, 1941.
[20] Sakazume K., Sakamoto E., Fujimori N. Japan. Kokai JP 50005035, 1975; Chem. Abstr. 1975; 83:36654.
[21] Ito H, Taguchi T, Imai Y, Morimitsu Y, Iino R. Jpn. Kokai Tokkyo Koho JP 03109402, 1991; Chem. Abstr. 1992;116:107009.
[22] Nakayama H, Myasaki K, Mitsumata T. Jpn. Kokai Tokkyo Koho JP 06313117, 1994; Chem. Abstr. 1995; 122:108670.
[23] Nakayama H, Myasaki K, Mitsumata T. Jpn. Kokai Tokkyo Koho JP 06313118, 1994; Chem. Abstr. 1995; 122:108669.
[24] Cavagna GA, Leifer A, Miller FN, Vermillion Jr. FJ. US Patent 3619154, 1971; Chem. Abstr. 1972;76:52971.
[25] Kamiya M, Sato H, Noguchi K. Jpn. Kokai Tokkyo Koho JP 61275760, 1986; Chem. Abstr. 1987;107: 15541.

[26] Namba K, Asami S, Aoi T, Takahashi K, Kuroiwa A. PCT Int. Appl. WO 8505078, 1985; Chem. Abstr. 1986; 105:15350.
[27] Franke W, Brahm R. Ger. Offen. DE 3404365, 1985; Chem. Abstr. 1986;104:43146.
[28] Krässig HA. Cellulose — structure, accessibility and reactivity — polymer monographs, vol. 11. Switzerland: Gordon and Breach Science Publishers, 1993.
[29] Stahl E. Thin-layer chromatography — a laboratory handbook. Berlin: Springer-Verlag, 1969.