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Expedient, accurate methods for the determination of the degree of substitution of cellulose carboxylic esters: Application of UV–vis spectroscopy (dye solvatochromism) and FTIR

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ABSTRACT

Two techniques, namely UV–vis- and FTIR spectroscopy, have been employed in order to calculate the degree of substitution (DS) of cellulose carboxylic esters, including acetates, CAs, butyrates, CBs, and hexanoates, CHs. Regarding UV–vis spectroscopy, we have employed a *novel approach*, based on measuring the dependence of λ_{max} of the intra-molecular charge-transfer bands of polarity probes adsorbed on DS of the ester films (solvatochromism). Additionally, we have revisited the use of FTIR for DS determination. Several methods have been used in order to plot Beer's law graph, namely: Absorption of KBr pellets, pre-coated with CA; reflectance (DRIFTS) of CAs-KBr solid–solid mixtures with, or without the use of 1,4-dicyanobenzene as an internal reference; reflectance of KBr powder pre-coated with CA. The methods indicated are simple, fast, and accurate, requiring much less ester than the titration method. The probe method is independent of the experimental variables examined.

1. Introduction

Cellulose and its derivatives are processed into a myriad of products, including, filters, fibers, films, membranes, food additives, etc. Cellulose possesses hydroxyl groups, linked to C-2, C-3, and C-6 of each anhydroglucose unit (AGU). The properties of cellulose derivatives, hence their applications, depend, *inter alia*, on the functional group introduced, the degree of substitution (DS), and the average degree of polymerization (DP) (Heinze & Liebert, 2001). Therefore, the search for expedient, and accurate methods for the determination of DS is always a welcomed development. The subject of the present study is to present attractive alternatives to the "classic" methods of DS determination of cellulose carboxylic esters (hereafter called "esters"), namely titration, and ^{1}H NMR spectroscopy (ASTM D 871-96, 2004; Fidale, Possidonio, & El Seoud, 2009; Heinze, Liebert, & Koschella, 2006; Marson & El Seoud, 1999).

Solvatochromic dyes (hereafter designated as "probes") are substances whose UV–vis spectra, absorption or emission, are particularly sensitive to the medium where they are present; solvent, solvent mixtures, solid surfaces, etc. (El Seoud, 2007, 2009; Reichardt, 2004, 2008). Fig. 1 shows the molecular structures of the probes employed in the present study (2,6-dichloro-4-(2,4,6-triphenylpyridinium-1-yl) phenolate, WB; 2,6-dibromo-4-[(E)-2-(1-methyl pyridinium-4-yl)ethenyl] phenolate, $MePMBr_2$). Each exhibits a medium sensitive UV–vis band, due to an intramolecular charge-transfer, from the phenolate oxygen to the positively charged nitrogen. Fig. 1 also shows their pK_a in water and log P (Martins, Lima, & El Seoud, 2006). The latter *empirical* scale is extensively employed as a measure of lipophilicity or hydrophobic character; it refers to the partition coefficient of a substance between 1-octanol and water, both mutually saturated: $\log P = \log c(\text{substance})_{1\text{-octanol}}/c(\text{substance})_{\text{water}}$ (Leo & Hansch, 1999).

The required information about the DS of the ester can be derived as follows: From the spectrum of the ester-adsorbed probe, an *empirical* medium polarity, namely, $E_T(33)$ and $E_T(MePMBr_2)$ for WB and $MePMBr_2$, respectively is calculated from Eq. (1):

$$E_T(\text{probe}),\ \text{kcal/mol} = \frac{28591.5}{\lambda_{max}(\text{nm})} \quad (1)$$

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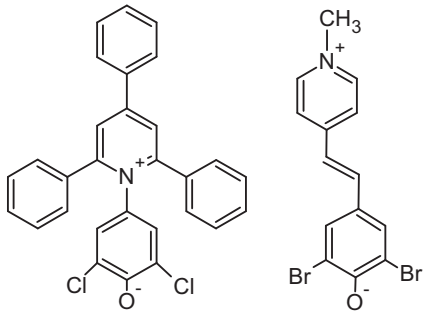
The solvatochromic probe method would constitute an attractive alternative (to titration, ^{1}H NMR spectroscopy, etc.) if E_T(probe) is *sensitively, and systematically dependent* on DS. We have evaluated this dependence not only for cellulose acetates, CAs, but also for other carboxylic esters, including cellulose butyrates, CBs, and hexanoates, CHs. Beer's law plots were constructed from the dependence of E_T(probe) on DS; these plots were r*igorously linear for all esters, and for both probes*.

FTIR spectroscopy has been employed both for qualitative and quantitative studies of CAs (Dominguez de Maria & Martinsson, 2009; Krasovskii, Polyakov, & Mnatsakanov, 1993; Samios, Dart, & Dawkins, 1997; Sereti, Stamatis, Pappas, Polissiou, & Kolisis, 2001), starch acetates (Ogawa et al., 1999), and starch maleates (Chong, Xing, Phillips, & Corke, 2001). The construction of the analytical curves, i.e., Beer's law plots between the absorbances, or the areas of the acyl-C=O group, $\tilde{\nu}_{C=O}$, and DS requires samples with different DS. These were obtained either by (laborious) synthesis, or by mixing commercial CA with an "inert" component, i.e., one that does not carry an acyl group, e.g., 2-hydroxypropylcellulose (Sereti et al., 2001). During the development of the present work, CAs samples with DS from 0.30 to 1.19 were obtained by mixing CA with pure cellulose (Dominguez de Maria & Martinsson, 2009). Alternatively, samples with known DS were obtained by controlled hydrolysis of commercial cellulose triacetate, CTA (Samios et al., 1997).

We have revisited the use of FTIR for the determination of DS. First, we show that samples of any "equivalent DS" can be conveniently obtained by mixing CTA and cellulose; the areas of the corresponding $\tilde{\nu}_{C=O}$ were found to correlate linearly with the equivalent DS. Most IR data (absorbance, or reflectance, e.g., by using DRIFTS) are recorded for solid mixtures. Homogeneous mixing of the sample with the solid matrix employed for dilution, e.g., KBr, is an essential requirement *for any quantitative determination by IR* (Culler, 1993); this is problematic for esters that cannot be easily grounded into fine powder, either manually, or with the aid of a mechanical grinder. We have introduced a procedure in order to solve this problem, and have used several approaches in order to correlate the areas of $\tilde{\nu}_{C=O}$ with DS.

The present work shows that solvatochromic probes can be fruitfully employed for the determination of DS of cellulose esters. The latter derivatives (easily grinded, or not) can be conveniently studied by IR, by recording the absorbance of KBr pellets, or reflectance from CAs plus KBr. In addition to being expedient, the methods are accurate, require simple equipment, and much less material than is needed for the saponification method.



	(WB)	(MePMBr$_2$)
pKa in water:	4.78	5.22
log P:	1.79	-0.16

Fig. 1. Molecular structures of the solvatochromic probes employed, along with the corresponding values of pK_a in water and log P.

2. Experimental

2.1. Materials

The chemicals were purchased from Acros or Merck. Microcrystalline cellulose, MCC; Avicel PH 101 was from FMC (Philadelphia; DP_v = 175; v = viscosity). Sheets of cotton (DP_v = 920), sisal (DP_v = 800), and eucalyptus (DP_v = 1049) cellulose were supplied by Lwarcel Cellulose and Paper (Lençóis Paulista, São Paulo) and Nitro Química S.A. (São Paulo), respectively. The sheets were cut into stripes and grounded in a water-cooled cutting mill (Thomas Scientific model 3383-L10, Swedesboro) against a 10-mesh stainless steel sieve. All cellulose samples were further sieved through a 100–200 mesh sieve (Fritsch Analysette 3 Spartan, Idar-Oberstein). Cellulose triacetate (CTA; CA 398-6; DS = 2.8) was supplied by Eastman. KBr was dried by heating for 2 h at 600 °C, cooled under reduced pressure, and promptly used. Ethanoic-, butanoic-, hexanoic anhydride, and DMSO were distilled under reduced pressure; 1,4-dicyanobenzene, DCB was recrystallized from ethanol and dried; spectroscopy-grade chloroform was employed as received. The solvatochromic probes WB, and $MePMBr_2$ were synthesized as given elsewhere (Kessler & Wolfbeis, 1989; Silva, Pires, Trassi, & El Seoud, 2008).

2.2. Determination of DP_v of cellulose samples

The values of DP_v were determined at 25 °C from the intrinsic viscosities of cellulose solutions in CUEN/water (1:1, v/v) according to the recommended procedure (ASTM D1795-94, 2001), by using shear-dilution Cannon-Fenske viscosimeter (Schott), inserted in Schott AVS 360 automatic viscosity determination equipment. Plots of ln(relative viscosity)/c(Cell-CUEN) versus c(Cell-CUEN) were strictly linear, whose intercepts are the intrinsic solution viscosities. The differences between DP_v calculated fom duplicate runs were ≤2%.

2.3. Synthesis of cellulose esters

Some MCC-based CAs with different DS were prepared as given elsewhere, i.e., by suspending cellulose in *N,N*-dimethylacetamide (DMAc) followed by heating; addition of LiCl; distillation of 25% of the solvent; reaction with acetic anhydride at 60 °C for 18 h, and workup of the product (Regiani, Frollini, Marson, Arantes, & El Seoud, 1999). Acylation of celluloses (MCC and fibrous) in an ionic liquid, IL, were carried out as described elsewhere (Fidale et al., 2009). Briefly, the appropriate amount of the carboxylic anhydride was added to a solution of cellulose in the IL 1-allyl-3-(1-butyl)imidazolium chloride. The acylation reaction was allowed to proceed in an oil bath kept at ca. 80 °C for 24–72 h (depending on the anhydride and the nature of the cellulose), under efficient stirring. The product was precipitated in ethanol; repeatedly suspended in hot aqueous ethanol, then filtered. The latter procedure is essential in order to remove the adsorbed acid, especially hexanoic acid. After purification and drying under reduced pressure over P_4O_{10} at 60 °C (Fisher 281A vacuum oven), *all* cellulose ester samples, including CTA, were kept in tightly stoppered bottles in a refrigerator.

2.4. Determination of DS, and of the distribution of the acetyl group in the AGU

The recommended titration method has been used (ASTM D 871-96, 2004; solution method; procedure A), with the modification that we have employed 10% of the ester sample, i.e., 0.19 g instead of 1.9 g ester. The uncertainty in DS was found to be ±0.1.

The distribution of the acetyl moiety among the carbon atoms of the AGU was calculated by integrating the (Lorentzian) $CH_3\underline{C}O$– peaks of one CA sample of each cellulose type (DS ca. 2) in DMSO-d_6 (50 mg mL^{-1}), in the presence of 20 mg mL^{-1} of Cr^{3+}-acetyl-acetonate. The ^{13}C NMR spectrum was recorded with aid of Varian Innova 300 NMR spectrometer, by using the following conditions: 80 °C; 8000 scans; pulse delay = 5 s. The inverse-gated decoupling program was employed for data acquisition (Braun, Kalinowski, & Berger, 2004); the chemical shifts employed for $CH_3\underline{C}O$– were: 168.71–168.93 ppm (C-2), 169.11–169.60 ppm (C-3), 169.83–170.04 ppm (C-6) (Kowsaka, Okajima, & Kamide, 1988). For the CAs samples tested, the relative distribution of the acetyl group among the AGU positions showed the following order: C-6 > C-2 > C-3.

2.5. Determination of DS by UV–vis spectroscopy: use of solvatochromic probes

This method is based on recording the dependence λ_{max} of the adsorbed probe on the DS of the ester film. Mixtures containing 0.025 g of the ester, and 0.25 mL of a solution of the solvatochromic probe (WB or $MePMBr_2$) in DMSO (3.7×10^{-2} mol L^{-1}) were prepared in glass vials, equipped with screw-cap covers. Care was exercised in order to minimize absorption of atmospheric moisture. The vials were closed, and the mixtures were magnetically stirred at room temperature until complete dissolution of the ester (≥30 min). CAs with low DS, obtained from fibrous celluloses did not form transparent solutions in DMSO; this did not affect the results. The probe-containing films were prepared on 2 cm × 2 cm quartz plates, with the aid of a spin-coater (model KW-4A, Chemat Technology, Northridge). The spin coating step included 3 s of slow rotation, at 1000 rpm, followed by 15 s of fast rotation, at 5000 rpm. Sample solutions in DMSO were added to the rotating plate by using a long-tip Pasteur pipette. Reproducible results (in terms of apparent film thickness) were obtained by adding one drop during the slow rotation phase, followed by 7 drops during the subsequent one. The films were dried for 30 min under reduced pressure at 40 °C; the plates were stored in a desiccator, and measured promptly. Film reflectance was recorded at room temperature by using Shimadzu UV 2550 UV–vis spectrophotometer, equipped with model IST-204A (double beam) integrating sphere reflectance attachment; $BaSO_4$ was employed as a (white) reference. We have employed Shimadzu UV-Probe, version 2.21 program for the conversion of the reflectance spectra into the corresponding absorption curves, and for calculation of the values of λ_{max} from the first derivative of the spectra. All experiments were carried out at least in duplicates. The values of E_T(probe) were calculated from Eq. (1). The uncertainties in DS were found to be ≤0.1 (CA) and ≤0.15 (CBs and CHs).

2.6. Determination of the values of $E_T(33)$ of pure ethyl alkanoates

The values of $E_T(33)$ of purified ethyl esters of ethanoic -, propanoic-, butanoic-, pentanoic-, and hexanoic acid were determined as given elsewhere (Silva et al., 2008), namely, by recording the absorption spectrum of a solution of WB in the ester (5×10^{-4} mol L^{-1}), at 25 °C, by using the above-mentioned spectrophotometer. The value of λ_{max} was calculated from the first derivative of the spectrum.

2.7. Analyses by FTIR

2.7.1. Pre-coated KBr pellets

A stock solution of CTA was prepared as follows: 250 mg of CTA (DS = 2.8) were weighted in a 25 mL volumetric flask and the sample was dissolved in chloroform. Aliquots of the latter, 1.2–2 mL, depending on the required "equivalent DS" of the pellet, were added to 200 mg dry KBr in small vials; the total chloroform volume was completed to 2 mL, by addition of pure solvent. The vials were closed, and the suspension was magnetically stirred at room temperature for 30 min, followed by solvent evaporation under reduced pressure at room temperature. The resulting (free flowing) KBr powder was then pressed into a pellet, by using a hydraulic press (Carver, model 3912, Wabash). The pellet absorbance was recorded with the aid of Bruker Vector 22 FTIR spectrophotometer; 128 scans were added at 0.5 cm^{-1} digital resolution. All $\tilde{\nu}_{C=O}$ band areas, in the range of 1860–1691 cm^{-1}, were calculated by using commercial software (Grams/32, version 5, Galactic Industries, Salem). All measurements were carried out at least in duplicates.

2.7.2. DRIFTS

These measurements were performed, at least in duplicates, with the aid of the above-mentioned FTIR spectrophotometer, equipped with a DRIFTS attachment (EasiDiff, Pike technologies, Madison). MCC-based CAs were thoroughly mixed with dry KBr at a ratio of 1/100, by wt; the resulting powder was transferred to the trough of the above-mentioned accessory. Its reflectance was recorded; 128 scans were added at 0.5 cm^{-1} digital resolution; KBr was used as background. The reflectance from the solid mixture was transformed into absorbance by using commercial software (Opus version 3.1, Bruker).

2.7.2.1. Preparation of DCB-coated KBr. KBr coated with 0.8 wt% of DCB was obtained as follows: A solution of DCB in dry acetone (0.8 g in 75 mL) was added to a round-bottom flask containing ground and dried KBr (100.0 g). The suspension was magnetically stirred for 90 min, the solvent was evaporated, and the solid was dried under reduced pressure, at room temperature.

2.7.2.2. Methods of sample preparation. We have employed two methods for sample preparation. Powdered CA samples (ca. 2 mg) were mixed with ground, dried KBr (ca. 200 mg; method (*IR-1*)), or the same amount of DCB-coated KBr; method (*IR-2*). An aliquot (0.552 g) of the solution of the CA sample in DMSO (20 mg solid plus 5.5 g solvent) was transferred into a glass vial, containing KBr (200 mg). The vial was closed, the suspension was magnetically stirred for 60 min at room temperature, and dried under reduced pressure for 4 h 30 min, at 70 °C. The resulting mixture was a free-flowing powder; method (*IR-3*).

Some CA samples showed "elastic" texture and did not give fine powder on grinding. These were treated as follows: The sample (ca. 0.2 g) was dissolved in 4.2 g DMSO, and then precipitated in 80 mL ethanol under vigorous stirring (Ultra-Turrax T18 stirrer, IKA, 6000 rpm). The product was centrifuged (IEC Centra MP4R) at 3500 × *g* for 20 min; the resulting solid was dried under reduced pressure, then pulverized into *free-flowing powder*, either by manual grinding, or by using ball milling (Puluerisette 6, Fritsch, Idar-Oberstein).

2.7.2.3. Mixtures of MCC plus CTA as a model for CA. These were prepared by weighting the appropriate amounts of dried MCC and CTA (reduced pressure at 60 °C; 2 h) in order to give equivalent DS of 1.5, 1.9, and 2.3. For example, a mixture with equivalent DS = 1.5 was obtained by grinding 0.4457 g of MCC plus 0.5543 g of commercial CTA, whose DS = 2.8.

2.7.2.4. Calculations. All band areas and, where required, band deconvolutions were calculated by using commercial software (Grams/32, version 5). The areas of the $\tilde{\nu}_{C=O}$ peak, in the range of 1820–1650 cm^{-1}, were calculated. When DCB was employed as an internal reference, the areas under the peaks at 1805–1700 cm^{-1}

($\tilde{\nu}_{C=O}$), and 2300–2200 cm^{-1} ($\tilde{\nu}_{C=O}$) were employed. Analytical curves (Beer's law plots) were constructed by plotting the areas of the $\tilde{\nu}_{C=O}$ band, or the area ratio ($\tilde{\nu}_{C=O}/\tilde{\nu}_{C\equiv N}$) as a function of *DS*. The uncertainties of *DS* of the different IR procedures were found to be ≤0.2.

2.7.3. ^1H NMR

^1H NMR was carried out with a Bruker DRX 500 (500 MHz) using DMSO-d_6 and TMS as solvent and reference, respectively. The DS was calculated by taking into consideration the area under the peaks and the corresponding number of protons from AGU (7 protons) and acetyl (3 protons) (Heinze et al., 2006).

3. Results and discussion

3.1. Use of Solvatochromic probes to determine DS of cellulose esters

As discussed above, the UV–vis spectra of solvatochromic probes are sensitive to the medium in which they are present; this applies to liquids and solids, including cellulose and its derivatives (Fischer et al., 2002; Fischer, Heinze, & Spange, 2003a; Fischer, Prause, Spange, Cichos, & Von Borczyskowski, 2003b), and inorganic solids (El Seoud, Ramadan, Sato, & Pires, 2010). For the probes employed in the present work, this sensitivity is due to the stabilization of the zwitterionic ground state, relative to the excited state. Consequently, the observed solvatochromism is negative, i.e., the value of λ_{max} decreases as a function of increasing the polarity of the medium. Thus, we have expected to find some correlation, linear or not, between E_T(probe) and DS. In order to test the generality of this assumption, we extended our investigation to CBs and CHs.

The questions that we have raised included: (i) Which probe(s) can be used? (ii) Is the relationship between E_T(probe) and DS the same for all esters? (iii) What are the effects of the experimental variables on the results? With regard to (i), we have selected two probes, WB and $MePMBr_2$, because they belong to different chemical classes, and have interesting chemical properties. Whereas protonated WB is only 2.34 times stronger acid than $MePMBr_2$, it is 89.12 more soluble in 1-octanol, i.e., it is much more lipophilic than the merocyanine probe. That is, the difference in their response to DS, if it is observed, can be traced to difference in lipophilic, rather than acidic character. The relatively low pK_a of both probes is advantageous because no solvatochromism is observed if the probe is protonated, e.g., by traces of (spurious) acidic impurities in the cellulose ester film. We have noticed that CTA stored for a long period at room temperature hydrolyze, leading to the bleaching of adsorbed 4-[(E)-2-(1-methyl pyridinium-4-yl)ethenyl] phenolate, MePM; pK_a in water = 8.37); this is the reason that we have kept all purified CAs in a refrigerator. Point (ii) above was raised because cellulose esters with DS < 3 are akin to "mixtures" of cellulose (a polyol) and CTA (polyol ester). For mixtures of protic and aprotic solvents, the dependence of E_T(probe) on mixture composition is always nonideal, i.e., nonlinear, due to the phenomenon of preferential solvation of the probe by one component of the mixture. For aqueous solvents, this deviation from ideality increases as a function of increasing the lipophilicity of the organic component of the mixture (El Seoud, 2007, 2009). In principle, the same behavior may occur with the cellulose esters, with the following order of deviation: CHs > CBs > CAs. The experimental variables investigated (point iii) included the source of cellulose (MCC or fibrous), the solvent employed in the synthesis of cellulose esters (LiCl/DMAc or IL), and the physico-chemical properties of the solvatochromic probe.

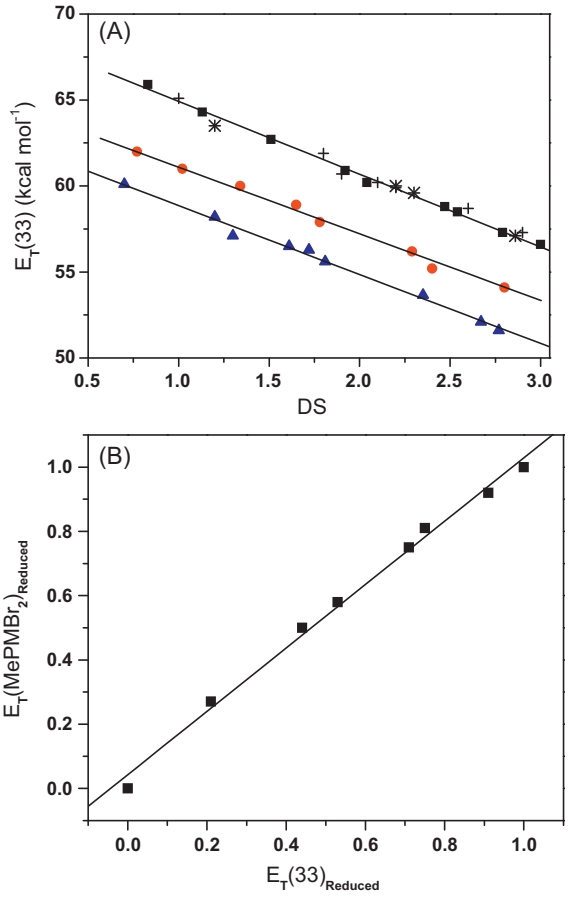


Fig. 2. (A) Dependence of $E_T(33)$ on DS of CAs. The points shown are for CAs obtained from the acetylation of MCC in LiCl/DMAc, (⁎), or in IL, (+); from the acetylation of fibrous celluloses in LiCl/DMAc, (▲); for CBs (●); for CHs (▲). The latter two esters were obtained by acylation of MCC in IL. (B) Correlation between reduced polarity scales of WB ($E_T(33)_{reduced}$) and $MePMBr_2$ ($E_T(MePMBr_2)_{reduced}$), for MCC-based CAs samples of the same DS, from 1.1 to 3.0.

Part (A) of Fig. 2 shows the dependence of $E_T(33)$ on DS of all esters studied; part (B) shows the correlation of the empirical polarities of CAs, as calculated by the two probes. For consistency, we have employed reduced values of E_T(probe) in Fig. 2B, so that the polarity scale for each probe lies between zero and unity, as shown in Eq. (2):

$$E_T(\text{probe})_{\text{Reduced}} = \frac{E_T(\text{probe})_{\text{Sample with the smallest DS}} - E_T(\text{probe})_{\text{Sample with any DS}}}{E_T(\text{probe})_{\text{Sample with the smallest DS}} - E_T(\text{probe})_{\text{Sample with the largest DS}}} \quad (2)$$

Table 1 shows the equations that describe the correlations between the parameters calculated (E_T(probe); peak area of $\tilde{\nu}_{C=O}$, or peak area ratio $\tilde{\nu}_{C=O}/\tilde{\nu}_{C\equiv N}$) as a function of *DS*.

Considering Fig. 2 and Table 1, the following is relevant:

(iv) Increasing the value of *DS* of a cellulose ester leads to substitution of the OH moiety, a highly dipolar group that acts as electron acceptor "acid" and donor "base", with an ester moiety, a less dipolar group that acts only as a base. This explains the increase in λ_{max}, or the decrease in E_T(probe) as a function of increasing DS, as shown in Fig. 2A;

(v) The films of cellulose esters were obtained by spin-coating of their solutions in DMSO, followed by evaporation of the solvent. In order to ensure the complete removal of DMSO (a *prerequisite* for obtaining correct E_T(probe) of the film) we

Table 1
Results of the application of Beer's law, by using FTIR and UV–vis spectroscopy.

Entry	Indicator(s)	Beer's law plot equations	n[a]	r[a]	ΣQ^2[a]
UV–vis					
1	WB	$E_T(33) = 68.939 - 4.107\ DS_{CA}$	20	0.995	0.267
2	WB	$E_T(33) = 65.169 - 4.0\ DS_{CB}$	8	0.997	0.249
3	WB	$E_T(33) = 62.861 + 4.006\ DS_{CH}$	9	0.996	0.270
4	$MePMBr_2$	$E_T(MePMBr_2) = 62.014 - 2.737\ DS_{CA}$	8	0.995	0.186
5	$MePMBr_2$ and WB	$E_T(MePMBr_2)_{Reduced} = 0.043 + 0.986\ E_T(33)_{Reduced}$	8	0.997	0.029
	Method				
FTIR					
6	Absorption; KBr pellets	$\tilde{\nu}_{C=O}$ area $= 24.450 + 11.180\ DS_{CA}$[b]	8	0.995	0.550
7	*IR*-1	$\tilde{\nu}_{C=O}$ area $= -6.567 + 8.770\ DS_{CA}$	8	0.996	0.574
8	*IR*-2	$\tilde{\nu}_{C=O}/\tilde{\nu}_{C\equiv N}$ area $= -0.900 + 1.065\ DS_{CA}$	7	0.984	0.119
9	*IR*-3	$\tilde{\nu}_{C=O}$ area $= -16.495 + 26.460\ DS_{CA}$	8	0.995	1.928

[a] The symbols (n), (r), and ΣQ^2 refer to the number of samples employed in order to construct Beer's law plot, the regression coefficient, and the sum of the squares of the residuals.
[b] This is an equivalent DS, obtained by adding CTA solution to KBr, vide experimental.

have carried out the following control experiments: Films of CTA in acetone or in DMSO were spin-coated, the solvent evaporated, and their $E_T(33)$ values were calculated from the corresponding UV–vis spectra. The values obtained were the same ±0.1 kcal mol^{-1}. In view of the large differences between the boiling points and $E_T(33)$ of both solvents, 56 and 189 °C, and 44.2 and 55.1 kcal mol^{-1}, for acetone and DMSO, respectively (Tada, Novaki, & El Seoud, 2000), this result shows that the drying conditions employed removes the solvent *completely* from the film. Reducing the probe concentration to half its original value resulted in a decrease of absorbance, without changing the value of λ_{max}. Thus, there is no probe "stacking" on the film, i.e., the spectrum of probe reflects a true surface property.

(vi) As shown in Fig. 2A, all plots are linear, and almost parallel, with excellent correlation coefficients, see entries 1–3 of Table 1. The intercepts decrease as a function of increasing the number of carbon atoms, N_C, of the acyl moiety, according to the polynomial equation: Intercept $= 74.14 - 2.96N_C + 0.18\ (N_C)^2$; $r^2 = 1$, where (r^2) is the non-linear regression coefficient. This agrees with our finding (Table SM-1 (Table 1 of Supplementary Material)) that the relationship between $E_T(33)$ and N_C of the acyl group of ethyl alkanoates ($N_C = 2$, 3, 4, 5, and 6; models for cellulose esters) is described by the polynomial: $E_T(33) = 48.07 - 0.93N_C + 0.06\ (N_C)^2$; $r^2 = 0.986$.

(vii) The questions raised above about the suitability of employing solvatochromic probes as an alternative, and attractive method for DS determination are all answered in Fig. 2. Thus, Fig. 2A shows that $E_T(33)$ is independent of the source of cellulose employed for obtaining the CAs (MCC, cotton, eucalyptus, sisal). Likewise, it is independent of the solvent employed in the synthesis, LiCl/DMAc or IL. Part (B) of Fig. 2, and entries 4 and 5 of Table 1 are very satisfying because they show an excellent correlation between $E_T(MePMBr_2)$ and DS. Additionally, the slope of the reduced E_T(probe) plot, 2B, is practically unity, i.e., the method is independent of the probe employed, in particular, its lipophilic character. The latter result can be explained by assuming that the probe is adsorbed almost perpendicular to the film, hydrogen bonded to the surface OH groups by its phenolate oxygen (Dawber & Williams, 1986), as indicated recently by Monte Carlo simulation of the adsorption of the same probe on the surface of TiO_2 (El Seoud et al., 2010). Therefore, the ester film-probe hydrophobic interactions appear to be negligible; probe lipophilicity does not affect its response to DS. In summary, the dependence of E_T(probe) on DS is general, i.e., it is independent of the experimental variables studied.

3.2. FTIR

Two factors can complicate the determination of DS by FTIR: The construction of Beer's law plot is laborious because of the large number of cellulose ester samples required, with regularly-spaced DS; Irreproducible results are obtained when the samples do not form homogenous mixture with the supporting matrix (KBr). In the present work, both problems have been solved, as shown in the following discussion.

(viii) Eliminating the labor involved in preparing samples with known DS for the construction of Beer's law plot:

We have prepared KBr pellets of known equivalent DS simply by adding the appropriate volume of a solution of CTA in chloroform, followed by solvent evaporation; free-flowing powders were obtained. For example, a pellet with equivalent DS = 2.1 is obtained by adding 1.5 mL of 0.062 mol L^{-1} ester solution plus 0.5 mL of chloroform to 200 mg of KBr. Using this simple procedure, a satisfactory Beer's law plot was constructed (plot not shown), as shown by the corresponding equation in entry 6 of Table 1. The DS determined by this method agreed within 0.1 with those determined by the titration method.

Unlike mixing solid CTA with cellulose, or one of its derivatives in order to obtain an equivalent DS (Dominguez de Maria & Martinsson, 2009; Sereti et al., 2001), the procedure that we have employed (dissolve CTA in solvent, add to KBr) is particularly suitable for quantitative analysis because it ensures homogeneous covering of KBr with a film of cellulose ester, as can be inferred from the excellent values of (r) and ΣQ^2, entry 6 of Table 1. This also eliminates the problem of scattering from the CA particles within the pellet (O'Connor, DuPre, & McCall, 1957). The method is suitable to CAs with high DS, i.e., is directly applicable to the commercial triacetate. As will be shown below this limitation can be eliminated by using DMSO as solvent instead of $CHCl_3$, because the former solvent dissolves CAs of still lower DS.

(ix) Determination of DS of samples that are not amenable to grinding to fine powders:

Some CA samples, with relatively low DS (<1.5) have "elastic" consistency and cannot be grinded into fine powder; the resulting mixture with KBr is relatively inhomogeneous. We have found that this leads to irreproducible absorbencies, or $\tilde{\nu}_{C=O}$ band areas, hence to large errors in DS. Note that the use of excessive grinding may degrade the sample. Dissolution of these samples in DMSO, followed by precipitation in ethanol under high solution shear, drying,

grinding or ball milling solved the problem. Although the importance of sample homogeneity has been recognized a long time ago (O'Connor et al., 1957; Fuller & Griffiths, 1978), we are unaware of any report in the literature that addresses how to obtain fine powder from any cellulose ester with elastic consistence. In summary, the two approaches introduced turn DS determination by FTIR accurate, with much less labor than that employed before.

3.2.1. DRIFTS – method (IR-1)

Fig. SM-1 (Fig. 1 of Supplementary Material) shows the DRIFTS absorbance spectra of CTA and MCC. The band attributions of both compounds are those reported elsewhere (Fengel & Ludwig, 1991; Heinze et al., 2006; Liang & Marchessault, 1959a; Liang & Marchessault, 1959b; Marchessault & Liang, 1960; Tsuboi, 1957). Because of the shoulder that appears at the lower end of the $\tilde{\nu}_{C=O}$ band, we have calculated the respective area from the interval 1820 to 1650 cm^{-1}, in order to avoid doing band deconvolution.

Fig. 3A shows the analytical curve obtained by using method (*IR*-1). A satisfactory linear correlation was obtained for the synthesized CA samples, and the mixtures of MCC plus CTA, of known equivalent DS, see entry 7 of Table 1. The use of mixtures of cellulose plus CTA as a model for CAs eliminates the labor involved in the synthesis of CAs of known DS. The fit of the mixture data with equivalent DS < 1 was, however, not satisfactory (data not shown); the reason is unclear.

3.2.2. DRIFTS – method (IR-2) using DCB as an internal reference

The use of band ratios instead of single band area should, in principle, eliminates the effects of any (equipment) operational parameters. Following literature indications, we have attempted, without success, to use the ratios between the areas of $\tilde{\nu}_{C=O}$ and the following bands of CA, in order to "normalize" the area of the former: CH_2 wagging, at 1315–1317 cm^{-1} (Yang & Wang, 1996); $\tilde{\nu}_{O-H}$ bands in the regions 3570–3450 cm^{-1} and 3400–3200 cm^{-1} (Samios et al., 1997), attributed to intra- and intermolecular hydrogen bonding of cellulose (Fengel & Ludwig, 1991), respectively. The reason is that the CH_2 wagging band appears as a shoulder; band deconvolution probably leads to larger uncertainties in DS. It is possible that the areas of the $\tilde{\nu}_{O-H}$ bands are influenced by water absorption during sample preparation, and spectra acquisition. This limits their use as internal references, at least without control of the relative humidity of the laboratory atmosphere. Therefore, we turned to the use of an extrinsic reference. DCB was selected because of its symmetrical structure; zero dipole moment (Andrews & Boxer, 2000; Sato-Toshima, Sakiyama, & Seki, 1980), and strong absorption of the two cyano groups in an IR region, where CAs do not absorb. The homogeneity and low load of the DCB-coated KBr can be corroborated by two pieces of evidence: The reproducibility of the area under the $\tilde{\nu}_{C\equiv N}$ main peak of the DCB-coated KBr; the proportionality of the areas of the same peak when the load was decreased. Six independent measurements of the $\tilde{\nu}_{C\equiv N}$ band area of 0.8% DCB-KBr gave 9.0 ± 0.4 (arbitrary units); Taking the area of 0.8% DCB-coated KBr as a reference, the ratio of areas were 0.5 ± 0.1, and 0.3 ± 0.1, for DCB load of 0.4, and 0.25%, respectively, i.e., the matrix is homogeneous and is not saturated at 0.8% DCB load.

The spectrum of a mixture of DCB with CTA and KBr, Fig. SM-2, exhibits a band with a shoulder in the 2300–2200 cm^{-1} region, assigned to the symmetric (2244 cm^{-1}) and asymmetric (2232 cm^{-1}) $\tilde{\nu}_{C\equiv N}$ stretching vibration of DCB (Andrews & Boxer, 2000). The band was deconvoluted and its main part, centered at 2233 cm^{-1}, was used in order to obtain the $\tilde{\nu}_{C=O}/\tilde{\nu}_{C\equiv N}$ area ratio. The $\tilde{\nu}_{C=O}$ peak displays two "satellite" bands at 1812 and 1691 cm^{-1} (Fig. SM-2), due to the presence of DCB in the mixture (Pavia, Lampman, & Kriz, 1996). Therefore, for calculation of the area of $\tilde{\nu}_{C=O}$, we have employed the frequency range 1805–1700 cm^{-1}.

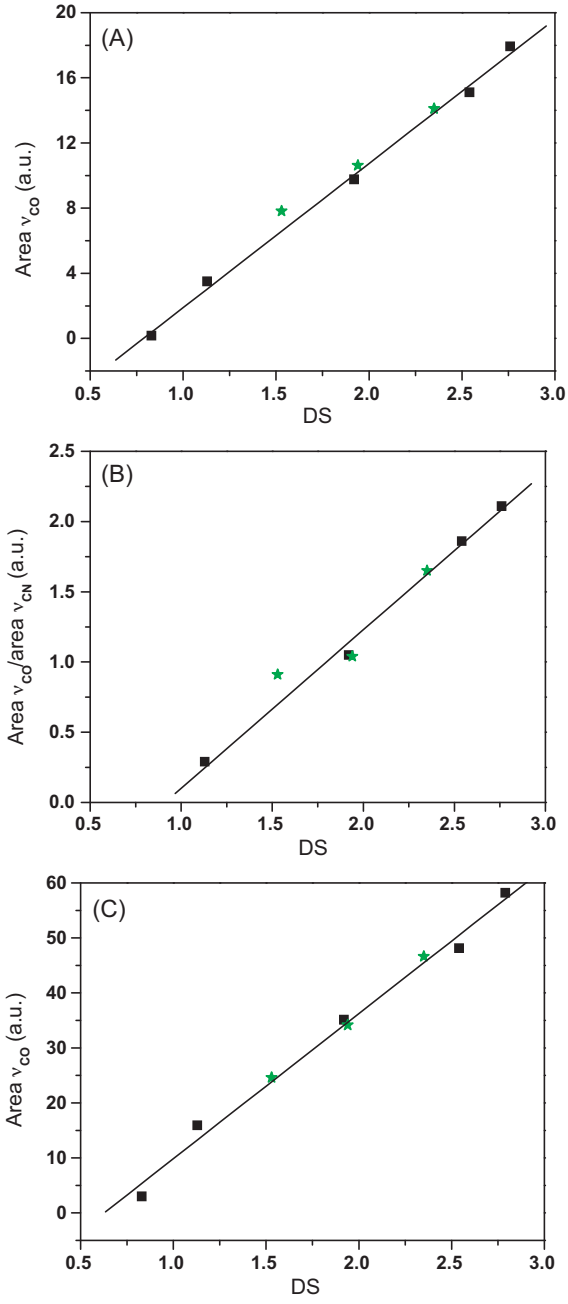


Fig. 3. Analytical curves obtained by plotting areas under $\tilde{\nu}_{C=O}$ bands or the ratios $\tilde{\nu}_{C=O}/\tilde{\nu}_{C\equiv N}$ as a function of ester DS from methods (*IR*-1), parte A; (*IR*-2), part B; (*IR*-3), part C. The symbols (■) and (★) refer to the calculated DS for CAs and mixtures of MCC plus CTA, respectively.

Fig. 3B shows the results obtained from method (*IR*-2) when DCB is used as internal reference. A satisfactory linear correlation is obtained, see entry 8 of Table 1. The lower slope observed when an internal reference is used can be accounted for because a ratio between areas was employed instead of the absolute (arbitrary) areas.

3.2.3. DRIFTS – method (IR-3)

As shown above, CA samples that did not give fine powder on grinding can be investigated after precipitation from their solution in DMSO under high shear. Alternatively, method (*IR*-3) can be satisfactorily employed, as shown in Fig. 3C. The larger slope observed for the calibration curve obtained from this method can be explained as follows: All KBr particles are covered with a CA

Table 2
A comparison of the DS of a representative sample of cellulose acetate determined by different methods.

Technique	DS
UV–vis (indicator WB)	2.0
UV–vis (indicator $MePMBr_2$)	2.0
FTIR (*IR*-1)	1.9
FTIR (*IR*-2)	1.8
FTIR (*IR*-3)	2.0
Titration	1.9
^{1}H NMR (in DMSO-d_6)	2.0

film, leading to a larger surface concentration of the ester, hence more intense $\tilde{\nu}_{C=O}$ band, in comparison with that obtained with method (*IR*-1). The goodness of fit of the results is shown in entry 9 of Table 1. All DS determination methods are compared in Table 2. As can be seen, the DS values calculated agree within ±0.1 unit.

In summary, from the results of *all runs* (UV–vis and FTIR), the uncertainty in DS is between 0.1 and 0.2, i.e., comparable to what we have experimentally determined for the titration method, ±0.1. Note that due to lack of standard, the recommended titration method makes no claims about "precision and bias".

4. Conclusions

The methods presented here are simple, fast, and of comparable accuracy to the titration method; they require apparatus (spectrophotometers) that are present in any laboratory, or are easily accessible. Much less ester is used, only 13% (solvatochromism), or 1% (DRIFTS), of the amount that is necessary for titration by the modified method, i.e., by using only 10% of the recommended weight of CA. Decreasing the amount required for DS determination is relevant because most exploratory work on acylation is carried out on 0.5–1 g cellulose. The use of solvatochromic probes proved to be a very convenient method for determining DS of esters of different acyl moieties. The method is of general applicability because it is independent of the experimental variables studied, most notably, the structure of the probe. Although we have deposited the probe-containing films on the silica plates by using spin-coating, this procedure is a convenience, not a necessity. The reason is that E_T(probe) is based on the wavelength, not the absorbance, of the CT-band; film thickness plays no role.

In the case of FTIR, the use of mixtures of CTA plus cellulose to produce mixtures with equivalent DS results in a large reduction of labor. Several approaches are indicated in order to determine DS accurately. Thus, DRIFTS can be used with ester-KBr solid–solid solution. Pre-treatments are required for ester samples that show elasticity, i.e., that cannot be conveniently pulverized. These include precipitation under vigorous stirring, followed by grinding and mixing with KBr (DRIFTS); or pre-coating of KBr with the ester, followed by measuring the absorbance (pellet), or reflectance (DRIFTS) of KBr. Provided that a UV–vis equipment with a reflectance attachment (or fiber optics reflectance accessory) is available, the solvatochromic probe method is recommended because of its accuracy and simplicity; no band area calculation or deconvolution is required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2010.09.035.

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