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A diagnostic tool for assessing the conservation condition of cellulose nitrate and acetate in heritage collections: quantifying the degree of substitution by infrared spectroscopy

Sofia Nunes¹, Francesca Ramacciotti², Artur Neves¹, Eva Marisole Angelin¹, Ana Maria Ramos¹, Élia Roldão¹, Nadja Wallaszkovits³, Alfonso Alejo Armijo¹ and Maria João Melo^{1*}

Abstract

Cellulose nitrate and acetate are materials at risk in heritage collections because it is not possible to predict the evolution of their conservation state over time. Knowing that the degree of substitution (DS) of these materials correlates with their state of conservation because the fundamental degradation mechanism is hydrolysis, in this work, DS was measured in historical objects and artworks. Infrared spectra were used to develop and optimize calibration curves for cellulose nitrate and acetate references that were next applied to calculate DS values of heritage objects. The extent of hydrolysis measured, with this tool, correlated well with the physical deterioration assessed through the sample hardness (Shore A) which was measured with a Durometer. Calibration curves were optimized in reference materials by Attenuated Total Reflectance (ATR-FTIR) and Micro Fourier Transform Infrared Spectroscopy (μ FTIR). The DS values of the AC reference materials was previously calculated by nuclear magnetic resonance spectroscopy. The calibration curves were obtained plotting DS as a function of the ratio between a reference peak (which does not suffer relevant changes during degradation) and selected peaks that monitor the degradation for cellulose acetate and nitrate polymers (avoiding the interference of plasticizers). The reference peak for both was the COC stretching mode (ν COC). The probe peak was, for cellulose nitrate, the NO_2 asymmetric stretching ($\nu_a\text{NO}_2$) and, for cellulose acetate, the OH stretching mode (ν OH). This ratio was then applied to calculate DS values of historical materials, in good and poor conservation condition; in situ by ATR, and in micro-samples collected from artworks by μ FTIR. This selection comprises cinematographic and photographic films dated from the 1890s to the 1960s, and contemporary works of art made with cellulose acetate sheets by Portuguese artist José Escada dated from the 1960s. Finally, by comparison with the original estimated DS values, we show how this tool permits to define the state of degradation of these complex polymer matrixes. Thus, establishing the quantification of the DS as a novel tool to monitor the degradation of cellulose ester plastics, contributing in this way for a sustainable preservation of an irreplaceable heritage.

Keywords: Cellulose nitrate, Cellulose acetate, Modern materials, Heritage collections, Conservation, Degradation mechanisms, Infrared spectroscopy

*Correspondence: mjm@fct.unl.pt

¹ REQUIMTE–Laboratório Associado para a Química Verde, Departamento de Química e Departamento de Conservação e Restauro, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

Full list of author information is available at the end of the article



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Introduction

A vast percentage of European cultural heritage can be found in cinematographic films and photography produced since 1899 [1–5]. Images are obtained through a complex stratigraphy that is depicted in Scheme 1 [6]. The base for these images is made of cellulose derivatives, which depending on their history of manufacture, storage and use may reveal a high degree of instability [1–5]. For this reason, more than 120 years of visual memories, based on cellulose nitrate (CN) and cellulose acetate (CA) materials, are in danger to be lost [1–5]. To overcome this risk, the European project NEMOSINE aims at improving the traditional storage solutions based on cold storage, by developing a modular package with the main goal of energy-saving and extent conservation time (<https://nemosineproject.eu>). This innovative package is based on an in-depth knowledge of the fundamental mechanisms of degradation and, on the continuous monitoring of volatiles which correlates with the conservation state of the support, Additional file 1: Scheme S1.

Knowing that hydrolysis is the fundamental degradation mechanism for both materials, the conservation condition of these supports may be correlated to their degree of substitution. For this reason, in this work, we conceive a tool to measure the degree of substitution¹ (DS) of CN and CA in historical films (photographic and cinematographic) and contemporary artworks. As it was shown that infrared spectroscopy is a powerful tool to calculate DS in cellulose acetate [7], calibration curves were based on infrared spectral data. In the field of cultural heritage, this technique may be applied in situ by Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR), or in micro-samples weighing less than 0.1 µg by Micro Fourier Transform Infrared Spectroscopy (µFTIR). For the case studies in which it is possible to predict, with some confidence, the initial (original) degree of substitution, the calculated DS allows to quantify the degradation extent, Table 1. The extent of hydrolysis measured is compared with the physical deterioration assessed through the sample hardness (Shore A) which was measured with a Durometer.

Next, the state of the art for the degradation mechanisms at play in cellulose nitrate and acetate is briefly summarized, showing how DS relates with it; a brief historical overview is also provided, in which the most common plasticizers are described in context, as they are important in the degradation mechanisms and are also detected in the infrared spectra.

Manufacture of cellulose nitrate and acetate and description of their most common plasticizers

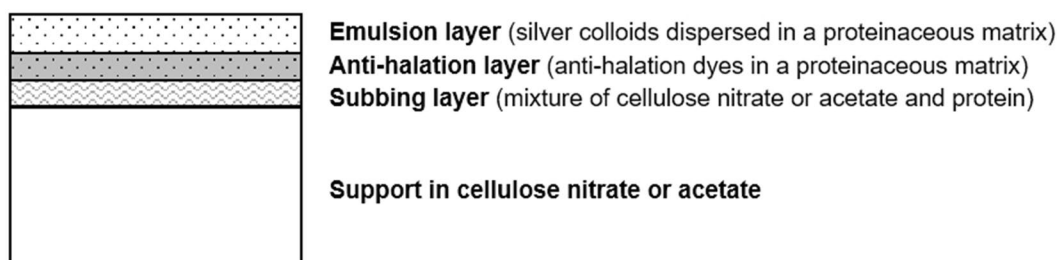
In the 1870s, John Wesley Hyatt developed the first commercially successful semisynthetic plastic—celluloid—by applying heat and pressure to a mixture of cellulose nitrate and camphor (a typical formulation could consist in the use of 30% of camphor). Celluloid's flexibility, dimensional stability, and transparency eventually led to its extensive use as a new photographic and cinematographic medium for films, as a result of the developments of Eastman Kodak and Henry Reichenbach in 1888–1889 [8]. Later camphor began to be replaced by other plasticizers, namely triphenyl and tricresyl phosphates, after 1902, and by phthalates in the 1920s [9, 10]. As support for image, cellulose nitrate was widely used between the 1890s and 1950s, but due to its inflammability, it was gradually substituted by cellulose acetate films. Its replacement started around 1904 with the commercialization of cellulose diacetate and was fully achieved in the 1950s with the large-scale manufacture of cellulose triacetate [11]. The main plasticizers used in cellulose acetate were triphenyl phosphate (TPP) and diethyl phthalate (DEP) in 20–40% by weight [12]. Recent research carried out at the Getty Conservation Institute as evidenced that plasticizer loss, in artificially aged CA samples, induced a lower ability to keep flexibility because hardness increases and, consequently, materials are more vulnerable to physical damage [10].

Degradation mechanisms for cellulose nitrate

For cellulose nitrate, experimental evidence has shown that mechanisms based on radical propagation are dominant [13–15]. Allen et al. [4, 16, 17], on their pioneer studies with artificially aged and historical CA and CN 35 mm films from the *National Film and Television Archive (United Kingdom)*, established that moisture absorption together with the accumulation of acid in the storage cans is the most important cause in film degradation. They concluded that the effect of temperature accelerates degradation in moist or acid environments and that in dry conditions degradation occurs at a very slow rate [16].

It has been proved that CN degradation occurs stepwise through side and main chain scission [13–15, 18–21]. The *first phase* is characterized by the homolytic cleavage of the RO–NO₂ bond in C2 or C3, forming an alkoxy radical (RO[•]) and releasing a NO₂[•], Scheme 2. The NO₂[•] radical can be transformed into the pair HNO₂/HNO₃ by hydrogen abstraction and reaction with H₂O [20, 22]. Over time, a complete loss of the nitrate substitution in the cellulose ring is observed, leading to a decrease of the degree of substitution (through the formation of hydroxyl groups), Scheme 2.

¹ The degree of substitution (DS) of a polymer is the (average) number of substituent groups attached per monomeric unit. The term has been used mainly in cellulose chemistry where each anhydroglucose (β-glucopyranose) unit has three reactive (hydroxyl) groups; degrees of substitution may therefore range from zero (cellulose itself) to three (fully substituted cellulose).



Scheme 1 General structure of a black and white cinematographic film. The cellulose nitrate or acetate base is the thickest layer and it is the support of the image. The image is formed by silver colloids dispersed in a proteinaceous matrix which is adhered to the support, usually, by a layer of protein-based gelatin and cellulose nitrate or acetate (subbing layer). The anti-halation layer is a proteinaceous matrix with anti-halation dyes (e.g., acid fuchsin or malachite green) that prevents light to be reflected in the emulsion causing a halo effect

Table 1 Applications of cellulose nitrate/cellulose acetate according to the nitrogen content/acetyl content and the degree of substitution (DS) [30, 34]

Polymer	Applications	Nitrogen/ acetyl content (%) ^a	Degree of substitution ^b
Cellulose nitrate	Explosives	12–13.5	2.26–2.76
	Adhesives	12	2.26
	<i>Film base</i>	12	2.26
	Lacquers, coatings	11.2–12	2.02–2.26
	Plastics	10.5–11.2	1.83–2.02
Cellulose acetate	Injection molding	52.1–54.3	2.2–2.3
	<i>Film</i>	54.3–56.4	2.3–2.4
	Lacquers	56.4–59.3	2.4–2.6
	<i>Film and fiber</i>	60.7–62.85	2.9–3.0

^a Values in italic were used for our case studies: cinematographic films and photographic negatives. In the case of the artworks from José Escada, the injection molding values were considered as the original DS

^b The degree of substitution can be calculated from the nitrogen and acetyl contents (%) by the following equations [12, 35]: $DS = \frac{3.60 \times (\% \text{ nitrogen})}{31.13 - (\% \text{ nitrogen})}$; $DS = 162 \times \left(\frac{3.86 \times (\% \text{ acetyl})}{102.4 - (\% \text{ acetyl})} \right)$

In *phase two*, main chain scission starts by the formation of hydroperoxides at C1 and their decomposition promotes a continuous cleavage of the glycosidic linkages and formation of carbonyl intermediates—the gluconolactones, identified at 1735–1740 cm^{-1} in the infrared spectrum by Berthumeyrie et al. [15]. Another possible mechanism for main chain scission is the acid-catalyzed scission of the glycosidic bonds, induced by the presence of moisture and continuous formation of HNO_2 and HNO_3 [20].

Degradation mechanisms for cellulose acetate

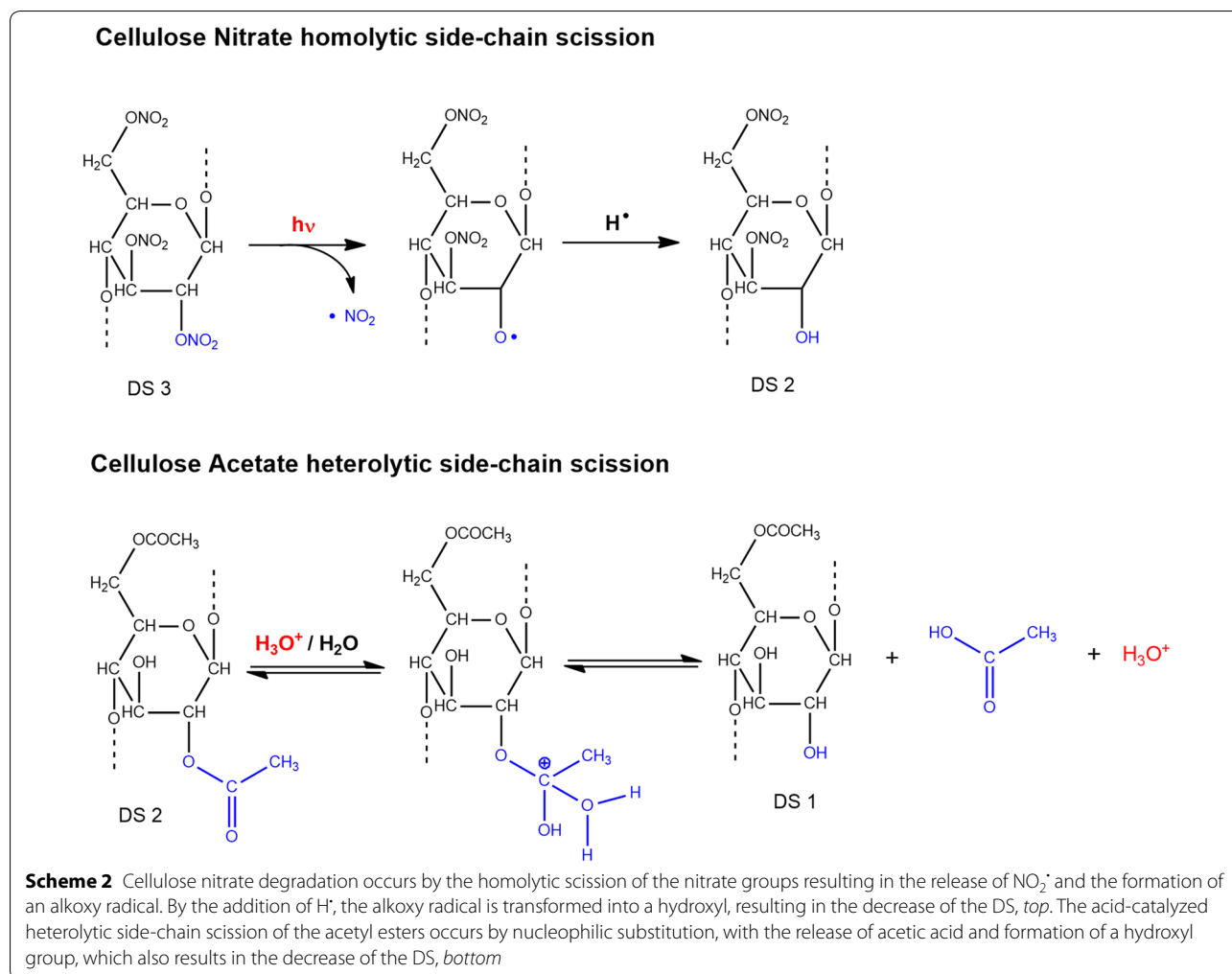
According to the literature [20, 23, 24], heterolytic bond cleavage prevails in cellulose acetate degradation mechanism catalyzed by acids; it occurs by: (i) side chain scission through ester hydrolysis, with formation of a hydroxyl group and release of acetic acid, Scheme 2, and (ii) main

chain scission by cleavage of the glycosidic bonds, Additional file 1: Scheme S2.

Although homolytic scission is not considered in archival conditions as the main degradation mechanism, photooxidation studies provide insights on radical chain reactions that can occur in the polymer matrix, as described for cellulose nitrate. In the absence of O_2 and irradiating at $\lambda \geq 235$, 280 and 340 nm, Hon [25], Merlin and Fouassier [26] demonstrated that the formation of CH_3^\bullet and H^\bullet abstraction promotes chain scission. CH_4 , CO , and CO_2 were experimentally identified as the main volatiles irradiating at $\lambda \geq 235$ and 280 (CH_3COOH was only detected for $\lambda_{\text{irr}} \geq 280$ nm) (Additional file 1: Scheme S3). Irradiating at $\lambda \geq 275$ nm cellulose acetate inside glass vials, Hosono et al. observed that photooxidation, leading to main chain scission, only occurs in the presence of photosensitizers through the formation of hydroperoxides [27].

Calibration curves to measure the degree of substitution of CA based on infrared spectra

To calculate cellulose acetate degree of substitution, Fei et al. [7] prepared calibration curves by admixing commercial powders of cellulose triacetate and microcrystalline cellulose (in KBr pellets). They observed that a 2nd degree polynomial regression of the data had higher coefficients of determination, giving the following explanation for the non-linear relationship between the degree of substitution and the chosen ratios at high degrees of acetylation ($DS > 1.8$): “With increased acetyl groups in the system of CA with a higher DS value the molar absorptivity starts to change from a linear relationship to a non-linear relationship due to the changing charge distribution at decreased average distances between particles or groups.” Before Fei et al. study, Samios et al. [28] obtained a linear calibration curve to calculate the degree of substitution for DS values below 2.5. Thus, for DS values below 2.5, it is possible to use a linear calibration fitting with a high coefficient of determination.



Aims of this research

In the heritage field, attempts made to use FTIR to measure deacetylation of CA polymers based on the hydroxyl to carbonyl peak area proved unsatisfactory [12]. On the other hand, gas chromatography/mass spectrometry provided an accurate quantification, after removal of the plasticizers by solvent extraction. A major drawback of this technique is that it requires 0.3–0.9 mg samples, which will be extremely difficult to access in heritage objects [12]. For this reason, we propose to further explore infrared spectroscopy, as it may be applied in situ or in micro-samples with weights below 0.1 micrograms.

In this work, the calculation of the degree of substitution will be first optimized on CN and AC reference samples using the approach developed by Fei et al. [7]. However, contrary to these authors, infrared spectra are collected directly in reference materials and historical artworks: (i) in situ by Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR), and (ii)

in micro samples by Micro Fourier Transform Infrared Spectroscopy (μFTIR).

Accurate DS values for the cellulose acetate reference materials were obtained by nuclear magnetic resonance spectroscopy (NMR) applying the quantitative method described by Kono et al. [29]. For cellulose nitrate reference material, DS was based on Berthumeyrie et al. quantitative elemental analysis [15]. Work is currently in progress to develop a method to calculate DS values by NMR.

The optimized calibration curves obtained for CA and CN are tested to calculate the DS in several heritage objects, as follows: (i) photographic films in good conservation condition, from the San Payo collection, studied in Roldão's Ph.D. project [30]; (ii) cinematographic films that are used in the NEMOSINE project, displaying diverse conservation conditions; (iii) cinematographic films from the National Archive of Motion Pictures (ANIM) in a severe state of degradation; (iv) three similar Portuguese artworks from José Escada with, visually, different states of degradation.

To the best of our knowledge, this will be the first time that this type of calibration curves will be employed for the quantification of cellulose nitrate degree of substitution, and for assessing the degree of degradation of CA and CN historical matrixes.

Experimental

Description of the historical samples

A preliminary analysis by μ FTIR allowed the identification of the support material and of the plasticizer class (see Additional file 1).

Cinematographic Films from NEMOSINE project

Cinematographic films from the Phonogrammarchiv of the Vienna Academy of Sciences (OEAW, Austria) and Deutsches Filminstitut & Filmmuseum (DFE, Frankfurt, Germany) were studied. Information about sample denomination, date and typology were given by the archive and are described in Additional file 1: Table S1. The thickness was measured with a micrometer in three points of each film and the average values are presented.

Cinematographic Films from the National Archive of Motion Pictures (ANIM)

35 mm cellulose nitrate cinematographic films were obtained from an aluminum can of the National Archive of Motion Pictures (ANIM, Portugal) labeled as belonging to the film *Man Are That Way* (1939), by director Arthur Maria Rabenault. Within the aluminum box, there are several reels of which the provenances are not known. The archive of the Portuguese national cinematheque was created in 1948 and in 1996 the conservation center, known as ANIM, was built. It is known from the literature that these films were widely used between the 1890s and the 1950s and that the production ceased in 1951 due to cellulose nitrate flammability. Therefore, these films can date from 1948 to 1951 or earlier. The films present inside are in an advanced stage of degradation, with tacky emulsion, loss of image and intense noxious smell. These films have already been studied in a published work by infrared and Raman spectroscopies [31].

San Payo collection

The samples analyzed belong to the Portuguese collection San Payo. Through Élia Roldão macro and molecular assessment, the samples selected are in a good state of conservation. The macro assessment consisted in the visual and olfactive identification of deterioration signs and the molecular assessment in pH, hardness (Shore A) and FTIR measurements [30].

Reliefs from José Escada

Three Portuguese artworks on cellulose acetate (CA) by the artist José Escada dated from the 1960s. They are all compositions of symmetric modules in CA sheets that have been folded and cut in different shapes creating a three-dimensional effect. These modules are supported by a sheet of the same material and a wooden grid or a panel. *Relief orange*, from 1966, currently in the Conservation and Restoration Department of FCT NOVA (Caparica, Portugal) presents warping, shrinkage, and hardening of the plastic material. *La fête*, from 1967, belongs to Galeria 111 (Lisbon, Portugal) and is currently in the Conservation and Restoration Department of FCT NOVA (Caparica, Portugal). This last artwork presents an advanced stage of degradation, showing, beyond the signs of *Relief orange*, detachment of the modules and loss of the material. *Dans la plage*, from 1968, currently in the National Museum of Contemporary Art (Lisbon, Portugal) does not show signs of degradation like the other two.

Materials and methods

Cellulose diacetate powder (Sigma-Aldrich, 39.7 wt% acetyl content), cellulose triacetate purum (Fluka), cellulose nitrate membranes (Hybond™ ECL™, GE Healthcare), microcrystalline cellulose powder (Sigma-Aldrich), racemic camphor (Fragon 33069-27, C₁₀H₁₆O), triphenyl phosphate (Sigma-Aldrich, ≥ 99%), diethyl phthalate (Alfa Aesar, 99%) were used as powders; or, dissolved in methanol (HPLC grade), dichloromethane (organic trace analysis, ≥ 99.9%) and acetone (≥ 99.9%) were used. DMSO-*d*₆ was used for NMR studies.

Cellulose acetate solutions for NMR

Cellulose acetate samples (20–25 mg) were dissolved in 500 μ L of DMSO-*d*₆. The DS of commercial cellulose acetate samples was measured by integration method of ¹H NMR and ¹³C NMR signals (see Additional file 1: Figures S1, S2, S3, and S4).

Preparation of samples with different degrees of substitution

Based on the work of Fei et al. samples with different degrees of substitution were obtained by co-grinding appropriate amounts of commercial powders of microcrystalline cellulose (MCC, DS 0) and cellulose diacetate (CDA). Commercial cellulose triacetate (CTA) was used as reference for the value DS 2.97. The relationship between the DS and the masses is described by Eq. 1:

$$DS \text{ (Degree of substitution)} = \frac{m_{CDA}/M_{CDA} \times DS_{CDA}}{m_{CDA}/M_{CDA} + m_{MCC}/M_{MCC}} \quad (1)$$

Equation 1 Relationship between DS and MCC and CDA masses. Where: m_{CDA} and m_{MCC} are the masses of CDA

and MCC in the mixture, M_{CDA} (264.48 g mol⁻¹) and M_{MCC} (162 g mol⁻¹) are the average molecular mass (g mol⁻¹) repeating units and DS_{CDA} is the degree of substitution of commercial CDA (DS 2.32).

The DS prepared by grinding MCC with CDA were 0.48; 0.95; 1.43; and 1.90. The relative mass of MCC and CDA measured for sample preparation were calculated with Eq. 2 obtained by solving Eq. 1 as follows.

$$\frac{m_{\text{CDA}}}{m_{\text{MCC}}} = \frac{264.48 \text{ g mol}^{-1} \times DS}{162 \text{ g mol}^{-1} \times 2.32 - 162 \text{ g mol}^{-1} \times DS} \quad (2)$$

Equation 2 Relationship between the DS and the mass ratio of cellulose diacetate and microcrystalline cellulose in the mixture.

The powders were ground with a mortar and pestle with the addition of droplets of water, when necessary. The mixtures obtained were put in a desiccator overnight before being analyzed.

To obtain a powder of cellulose nitrate (CN), commercial filtration membranes (GE Healthcare) were cut with scissors and scalpels. To reduce particle size, the cut membranes were sieved. The relative amounts of MCC and CN powders have been calculated by adaptation of Eq. 2 (where 261 g mol⁻¹ and 162 g mol⁻¹ are the average mass of CN and MCC, respectively and 2.2 the DS of the commercial membranes.) The standards used for the calibration curve have been prepared with DS: 0.5; 1.0; 1.5 and 2.0. The powders were ground with a mortar and pestle with the addition of droplets of water, when necessary. The mixtures obtained were kept in a desiccator overnight before being analyzed. The total mass of each mixture was approximately 6.0 mg for both CA and CN.

The making of the calibration curves

The absorbance intensities of the chosen probe and reference peaks for the ratio calculation were measured using OMNIC software. Following Fei et al. methodology, baselines were delineated using the peak height tool and the absorbance calculated from the maximum of the characteristic peak to the corresponding baseline. For μFTIR , the probe ($\nu_{\text{a}}\text{NO}_2$, $A_{1653 \text{ cm}^{-1}}$) and reference (νCOC , $A_{1067 \text{ cm}^{-1}}$) absorbances were calculated from the maximum to the baseline delineated between the points at 2000 and 1570 cm⁻¹ and at 1570 and 940 cm⁻¹. For ATR-FTIR, the probe ($\nu_{\text{a}}\text{NO}_2$, $A_{1636 \text{ cm}^{-1}}$) and reference (νCOC , $A_{1060 \text{ cm}^{-1}}$) absorbance was calculated from the maximum to the baseline delineated between the points at 1800 and 1525 cm⁻¹ and at 1190 and 930 cm⁻¹. For the DS calibration curves, for each DS, 3 spectra were used for μFTIR and 5 spectra for ATR-FTIR. The linear and polynomial (2nd degree) regressions of the data were calculated using OriginPro 8.

Equipment

Nuclear magnetic resonance spectroscopy

NMR spectra were run at 353 K on an ASCEND 500 spectrometer (500 MHz for ¹H) equipped with a Cryoprobe, ATM, Prodigy TCI 500 at 353 K.

Micro Fourier Transform Infrared Spectroscopy (μFTIR)

Infrared spectra were acquired on a Nicolet Nexus spectrophotometer equipped with a Nicolet Continuum (15× objective) microscope and a Mercury–Cadmium–Tellurium (MCT) detector cooled by liquid nitrogen. μ -samples were placed on a diamond cell and the spectra were acquired in transmission mode between the 4000–650 cm⁻¹, with a resolution of 8 cm⁻¹ and 128 scans. μ -samples from the selected case studies were collected using Ted Pella μ -tools and a Leica MZ16 stereomicroscope (between 7.1× and 115×), equipped with a Leica ICD digital camera and a Leica KI fiber optic light system 1500LCD. Spectra are shown here as acquired, without corrections or any further manipulation, except for the removal of the CO₂ absorption at approximately 2300–2400 cm⁻¹.

Attenuated Total Reflectance (ATR-FTIR)

The spectra were obtained by an Agilent Handheld Exoscan 4300 spectrophotometer equipped with a wire-wound source and DTGS detector in the 4500–650 cm⁻¹ spectral region. ATR spectra were obtained in reflectance mode with a resolution of 8 cm⁻¹ and 64 scans. The samples were analyzed in situ without prior treatments.

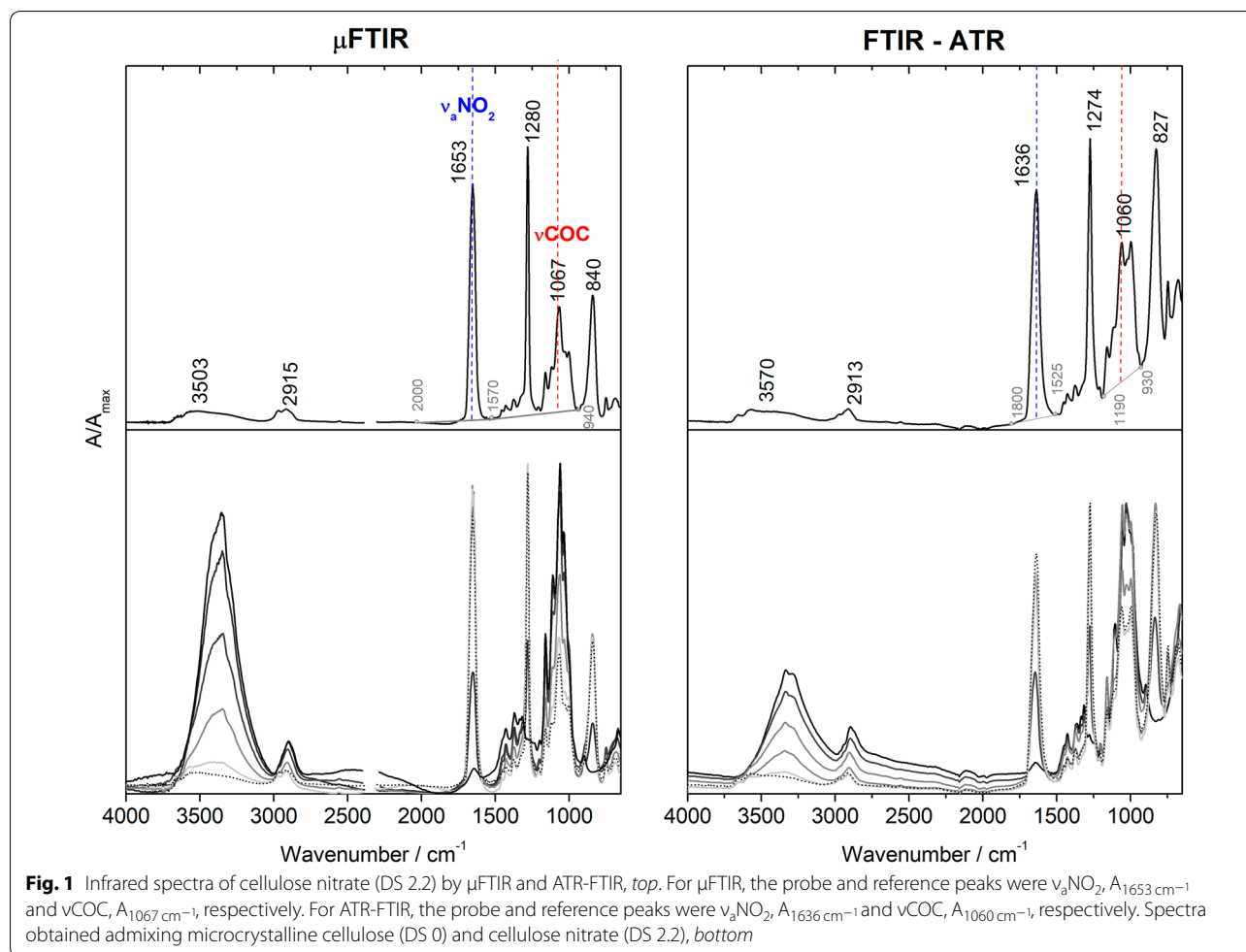
Hardness measurement

Sample hardness (Shore A) was measured with an HP Durometer (model HPSA, from Checkline Europe), with a scale from 0 to 100. For each sample, the hardness values and standard deviation were determined as the average of five independent measurements obtained in a film area of ca. 35 mm × 35 mm. The sample was placed always in the same flat surface, and the measurements were made in the middle and near the corners.

Results and discussion

DS values calculated for cellulose acetate references by NMR

The DS of commercial cellulose acetate samples was measured by integration method using ¹H NMR and quantitative ¹³C NMR (see Additional file 1). Briefly, quantification of the number of acetyl groups present in the sample could be achieved by ¹H NMR by comparison between the area of methyl group signals between 2.08 and 1.90 ppm and the area of ring proton signals between 5.06 and 3.46 ppm. The results agreed with what obtained by quantitative ¹³C NMR, by comparison between the



area of carbonyl group (around 170 ppm) or methyl group (around 20 ppm) and the area of ring carbon signals between 100 ppm and 60 ppm. The DS of commercial diacetate and triacetate cellulose samples were 2.31 and 2.97, respectively.

The DS of the CN membranes was not calculated by NMR and in this work we will use the 2.2 value measured by Berthumeyrie et al. [15] using quantitative elemental analysis (11.76% N). Work is currently in progress to synthesize CN with different degrees of substitution. In future work, these polymers will be used to prepare calibration curves that will be compared with the ones discussed here. They will be also used to optimize an NMR method for an accurate determination of DS values in CN samples.

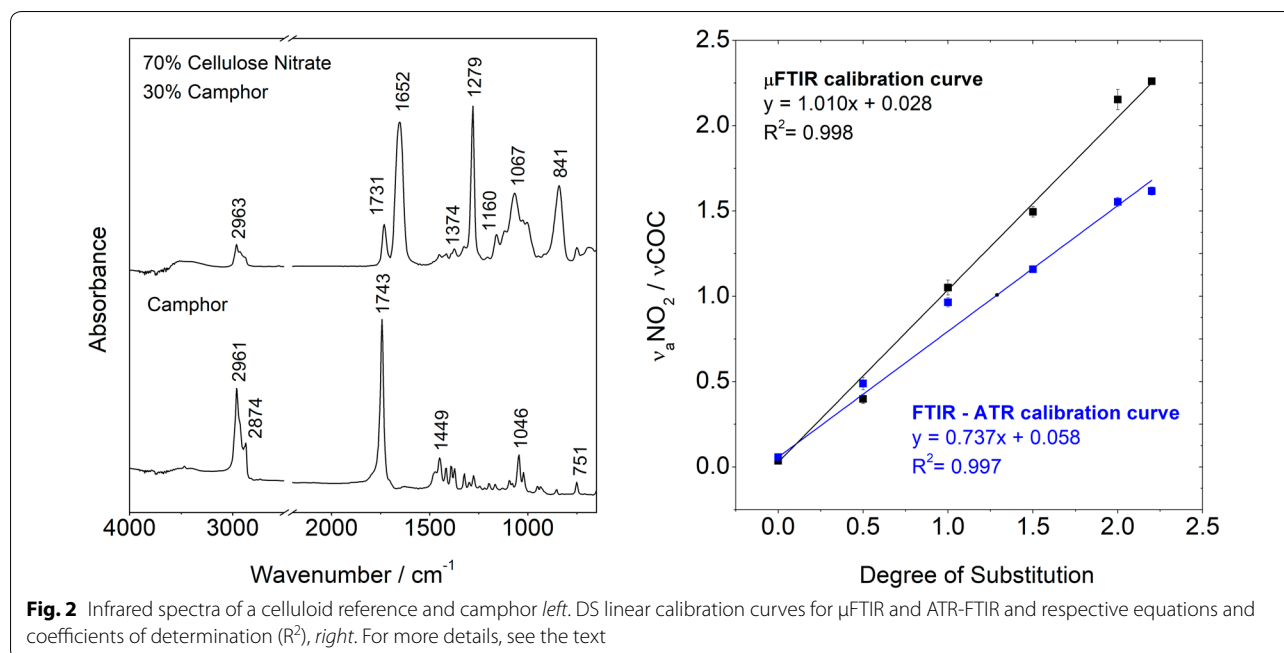
Cellulose nitrate calibration curves

The infrared spectra of cellulose nitrate obtained by μ FTIR and FTIR-ATR are plotted in Fig. 1. At time 0, both spectra are characterized by the stretching of the CH and CH_2 bonds between 2900 and 3000 cm^{-1} ; the strong nitrate vibration bands (μ FTIR: 1653, 1280 and 840 cm^{-1} ;

ATR-FTIR: 1636, 1274 and 827 cm^{-1}); the cellulosic vibrational envelope between 1200 and 900 cm^{-1} [31]. A decrease in DS leads to a decrease of the nitrate groups absorbance and an increase of the hydroxyl band (3700–3000 cm^{-1}), evolving towards the spectrum of cellulose, Fig. 1.

The main plasticizer of celluloid in heritage studies is camphor, and its influence must be assessed before the selection of the reference and probe peaks. Its infrared spectrum is characterized by the strong carbonyl stretching at 1743 cm^{-1} and low-intensity bands between 1200 and 900 cm^{-1} , Fig. 2. However, in celluloid, the carbonyl band is shifted to lower wavenumbers (1731 cm^{-1}) as a result of hydrogen bonding and does not overlap with the nitrate asymmetric stretching vibration ($\nu_a \text{NO}_2$) at 1662 cm^{-1} , Fig. 2. Furthermore, the influence of the plasticizer between 1200 and 900 cm^{-1} is residual to none.

Taking these data into account, we tested for reference peaks the COC and the C=O stretching bands. Our experimental data suggest that the best results were obtained using the first. For this reason, the COC



stretching (μ FTIR: 1070 cm^{-1} ; ATR-FTIR: 1060 cm^{-1}) was selected as the reference peak for the determination of the DS, and the hydroxyl and the nitrate groups bands can be used as probe peaks. For this work $\nu_a\text{NO}_2$ was selected and in Fig. 2, the ratio $\nu_a\text{NO}_2/\nu\text{COC}$ is plotted as a function of DS, resulting in calibration curves with high coefficients of determination (μ FTIR: $R^2=0.998$; ATR-FTIR: $R^2=0.997$).

Measuring the degree of substitution in cellulose nitrate cinematographic films and correlating it with their conservation condition

The obtained DS calibration curves were next applied to historical cinematographic films. The values calculated for NEMOSINE films ranged from 1.71 to 2.20 for μ FTIR and from 1.76 to 1.93 for FTIR-ATR, Table 2. Comparing the μ FTIR and FTIR-ATR results it is possible to observe that samples 50508 and DIF 50 500 correlate well. In the other samples (50509, S4, S5 and S6), the values calculated by ATR-FTIR are lower, with differences ranging from 0.26 to 0.33. A possible explanation is related to the fact that at the surface the polymers are more degraded than in the bulk. In that case, when using ATR-FTIR we only measure the DS at the surface, whereas with μ FTIR both bulk and surface are analyzed in a μ sample. To understand the reason for these differences, in future work, it will be necessary to make a μ sampling profiling to assess if DS changes from the surface to the bulk. Spectra and images of the cinematographic films are presented as Additional file 1: Table S2 and Figure S5.

Film S5 presents the closer match to a pristine film (DS=2.26), Table 2. The lower values obtained for the other historical cinematographic films reflect the molecular aging of the CN support. The values of DS and hardness were close for films 50509, S4, S5, which have images in good conservation conditions. In comparison, for films DIF 50500, 50508 and S6, the calculated DS values did not correlate with the image condition: DIF 50 500 presents a readable image and presented the lowest DS value (1.71). On the other hand, films 50508 and S6 presented DS values similar to films 50509, S4 and S5 but their image is in poor condition, see Table 2 and Additional file 1: Table S2. These results show that

Table 2 DS of the cellulose nitrate cinematographic films provided by the NEMOSINE project calculated using the calibration curves

Sample	DS μ FTIR	DS FTIR-ATR	Hardness (Shore A)
DIF 50 500	1.71	1.76	95 \pm 0.80
50508	1.86	1.85	96 \pm 0.63
S6	2.06	1.80	96 \pm 0.75
S4	2.15	1.89	96 \pm 1.10
50509	2.18	1.85	97 \pm 1.02
S5	2.20	1.93	97 \pm 1.17
Original DS ^a	2.26	2.26	–

For more details, see text

^a See Table 1

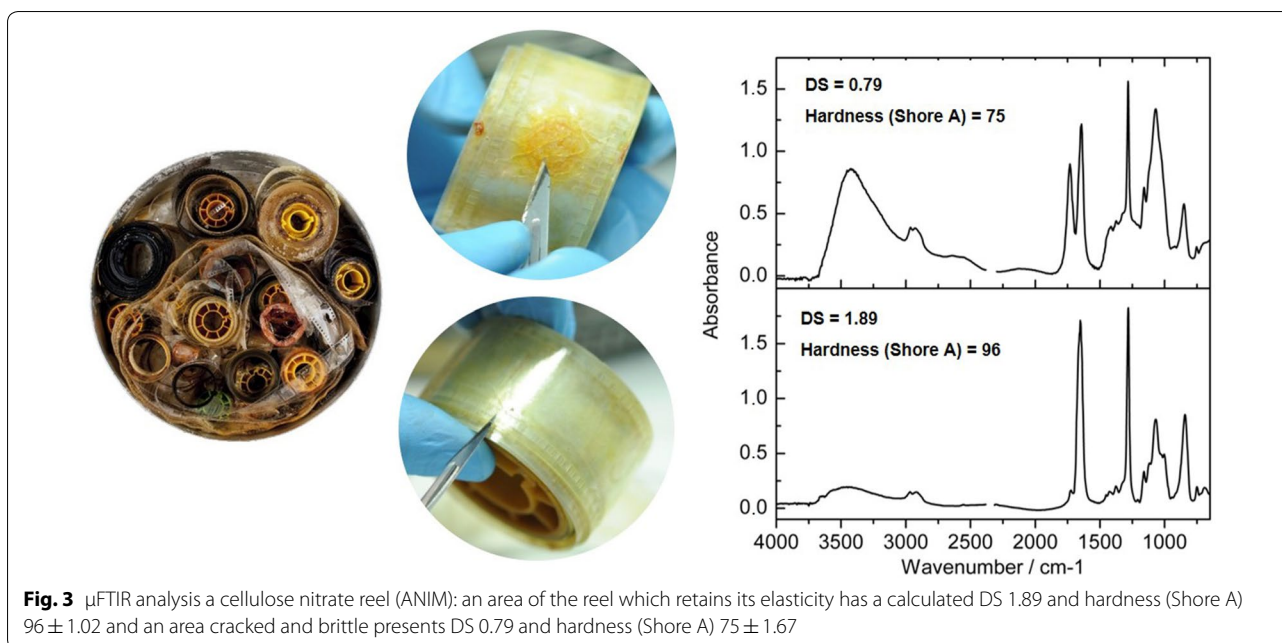


image degradation does not depend only on the degradation of the support, in this case, cellulose nitrate.

To assess the extent of degradation in a cinematographic reel from the outside to the inside, the μ FTIR calibration curve was also applied to a degraded reel from ANIM collection. The cellulose nitrate reel, kept in an aluminum box, was analyzed in two different areas: one exterior area that retains its elasticity, and an interior area extremely brittle, Fig. 3. The calculated DS 1.89 and 0.79 correlate well with the measured hardness (Shore A) 96 ± 1.02 and 75 ± 1.67 , respectively, Table 2. Thus, it was possible to correlate the molecular evolution, through the decrease in the DS values (reflecting the loss of the nitrate groups, with the formation of the pair $\text{HNO}_2/\text{HNO}_3$ that promotes main chain scission), with the observed physical alteration of cellulose nitrate: lower hardness and brittle film. The higher degradation observed in the inner film winding may be due to the accumulation of acids, not so easily off-gassing as in the outer parts.

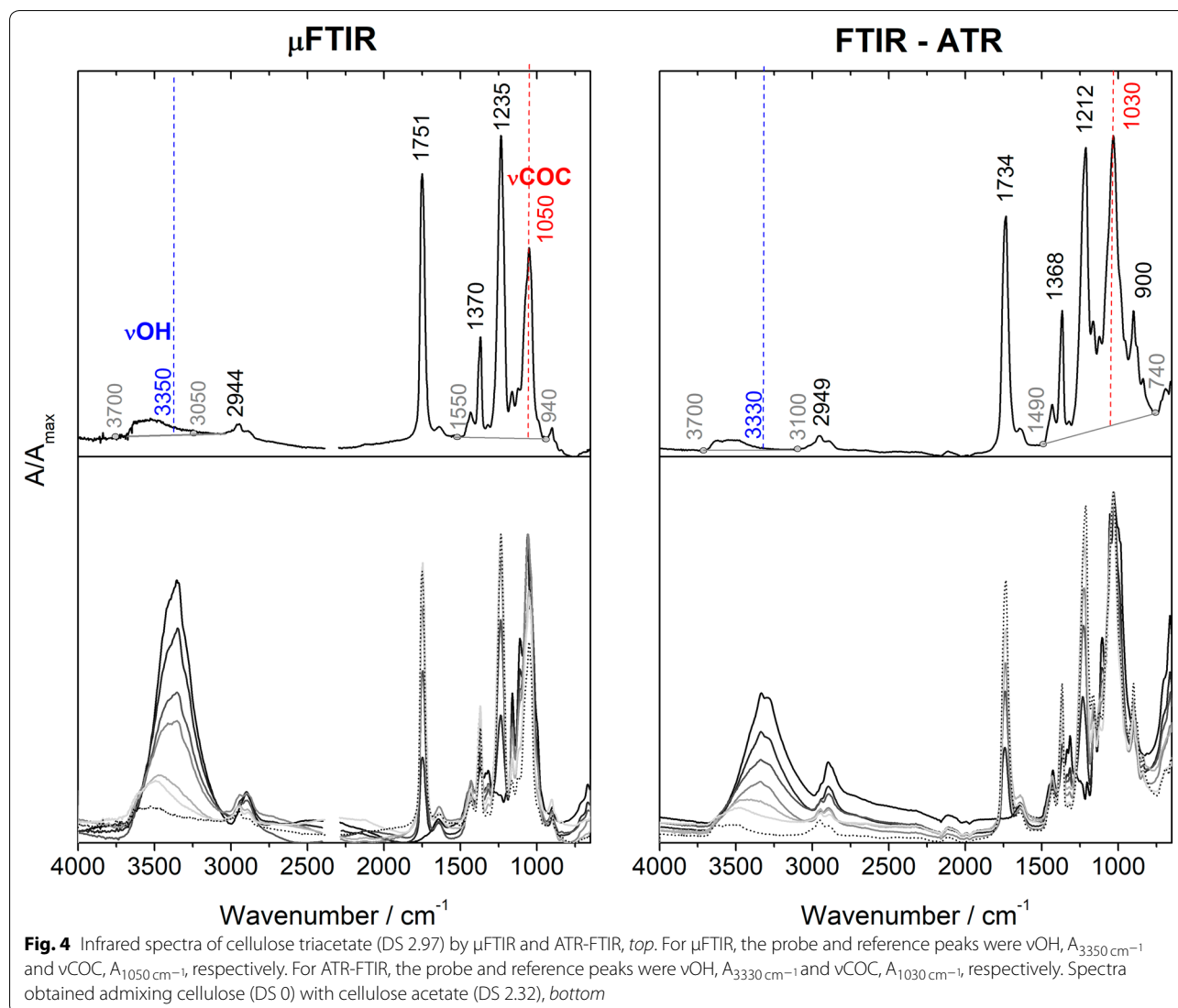
NEMOSINE cinematographic films studied presented hardness values like the less degraded zone of the ANIM reel, existing a high correlation of DS and hardness with sample 50508. The DS and hardness calculated for the interior area of the ANIM reel is in accordance with the hardness decrease observed with the decrease of the DS in the NEMOSINE films, Table 2. These results point out the potentiality of DS quantification as an early warning tool for this polymer.

Cellulose acetate calibration curves

μ FTIR and ATR-FTIR spectra of cellulose acetate, at time 0, are shown in Fig. 4. They are characterized by the carbonyl stretching (μ FTIR 1751 cm^{-1} ; ATR-FTIR 1734 cm^{-1}), the ester stretching (μ FTIR 1235 cm^{-1} ; ATR-FTIR 1212 cm^{-1}) and the methyl bending (μ FTIR 1370 cm^{-1} ; ATR-FTIR 1368 cm^{-1}); the stretching of the ether groups (μ FTIR 1050 cm^{-1} ; ATR-FTIR 1030 cm^{-1}); and the hydroxyl stretching (with maxima found at μ FTIR 3350 cm^{-1} ; ATR-FTIR 3330 cm^{-1}) [32].

The main plasticizers for cellulose acetate—diethyl phthalate (DEP) and triphenyl phosphate (TPP)—display strong absorptions due to the phthalate ester groups (1727 and 1282 cm^{-1}) that exclude the use of the acetate groups as probe or reference bands. In the historical samples selected for study, triphenyl phosphate was found as the main plasticizer, and the phosphate POC stretching (960 cm^{-1}) does not overlap the νCOC reference band, Fig. 5.

Following a similar reasoning as for cellulose nitrate and considering the experimental evidence above described, COC and C=O stretching bands were tested for reference peaks. Our experimental results show that using the first, higher linear correlation coefficients were obtained ($R^2 \approx 0.7$ versus >0.9), enabling a more accurate determination of DS (with the reference samples). Thus, cellulose acetate COC stretching was used as the reference peak for the ratio applied for the determination of the DS, Fig. 4. The probe bands to be considered are the ones influenced by hydrolysis, namely the hydroxyl and



the ester bands. For this reason, the hydroxyl stretching (μ FTIR 3350 cm^{-1} ; ATR-FTIR 3330 cm^{-1}) was selected in this work as the probe peak. For the ν COC/ ν OH ratio as a function of DS, a linear fitting was tested; however as observed by Fei et al. a quadratic polynomial fitting had higher coefficients of determination (R^2). Thus, the quadratic equations were chosen for the calculation of the DS in the case studies, Table 3.

Measuring the degree of substitution in cellulose acetate cultural heritage and correlating it with their conservation condition

Application in cinematographic and photographic films

Films from the San Payo collection, previously evaluated as in good conservation state by Roldão [30], were analyzed to assess if it is possible to propose an “original” DS

for cellulose acetate in these photographic collections. All samples presented the typical spectrum of a TPP and DEP plasticized cellulose acetate, Fig. 5 and Additional file 1: Figures S6 and S7. According to the literature, a commercialized diacetate film’s DS falls between 2.2 and 2.5 and a triacetate film between 2.9 and 3, Table 1. The DS of the San Payo samples ranged from 2.5 to 2.83, Table 4. Having been assessed as in good condition, these results suggest that samples SP 548 (DS 2.50) and SP 544 (DS 2.53) were produced as diacetate films. We propose the use of the cellulose triacetate DS interval 2.9–3 as the “original” DS for the samples that presented values ranging from DS 2.69 to 2.83. The lower DS in relation to the “original” DS is indicative of degradation.

The extent of degradation in a cinematographic reel was also studied from the outside to the inside, Table 5.

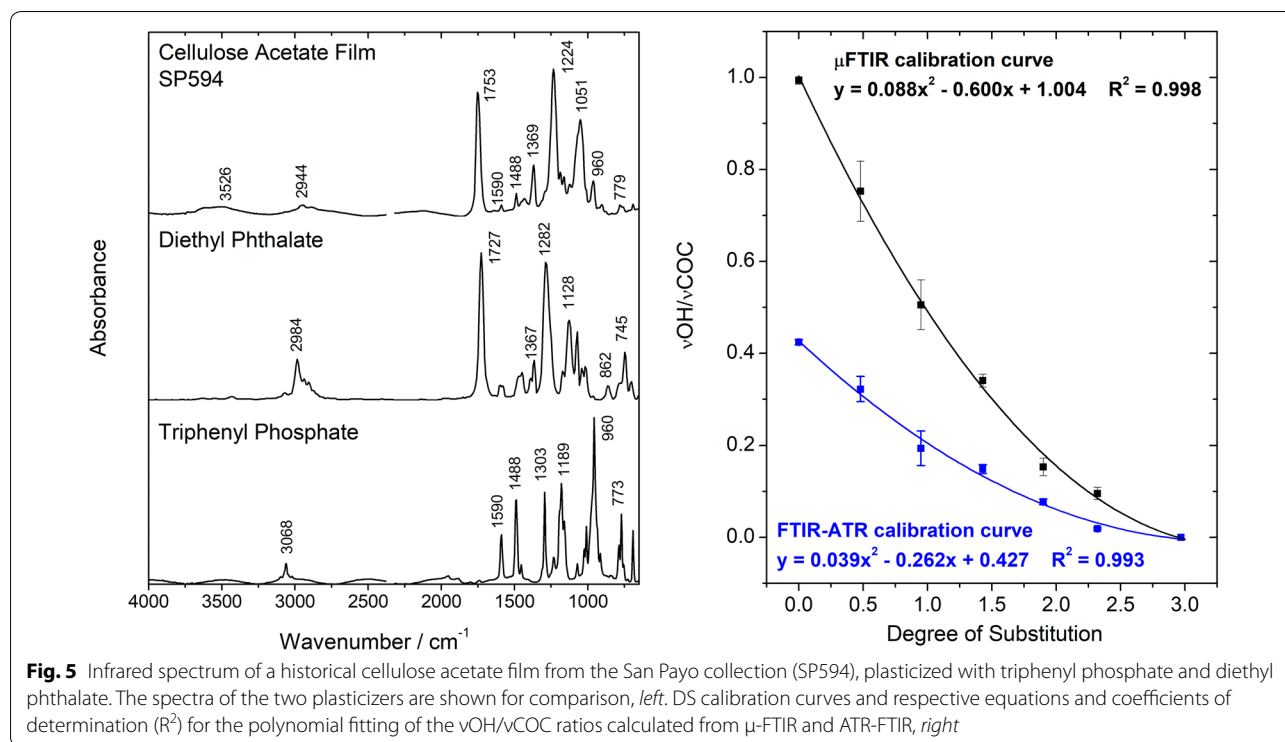


Table 3 DS calibration curves obtained by applying polynomial (2nd degree) and linear regressions

Analytical method	Polynomial fitting		Linear fitting	
	Equation	R ²	Equation	R ²
$\mu\text{FTIR } r = A_{3350\text{cm}^{-1}} / A_{1050\text{cm}^{-1}}$	$r = 0.088\text{DS}^2 - 0.600\text{DS} + 1.004$	0.998	$r = -0.341\text{DS} + 0.896$	0.948
$\text{ATR-FTIR } r = A_{3330\text{cm}^{-1}} / A_{1030\text{cm}^{-1}}$	$r = 0.039\text{DS}^2 - 0.262\text{DS} + 0.427$	0.993	$r = -0.146\text{DS} + 0.379$	0.940

Table 4 DS calculated through the calibration curve (μFTIR)


Sample	SP 548	SP 544	SP 594	SP 324	SP 543	SP 542	SP 593	SP 592	SP 600
DS (μFTIR)	2.50	2.53	2.69	2.74	2.74	2.76	2.80	2.82	2.83

The predicted pristine value for CDA and CTA are 2.2–2.5 and 2.9–3, respectively

The reel studied, SCS0057, is dated from 1950 to 1960. In this decade, the more stable cellulose triacetate was already being commercialized. The values obtained with both spectroscopy techniques ranged from 1.39 to 0.83 (μFTIR) and 1.36 to 0.76 (ATR-FTIR). These values agree with the poor conservation condition of the film, which emits an intense vinegar odor. The results show that cellulose acetate degradation is more severe in the inner parts of the reel where the cellulose acetate is covered from both sides due to film winding, as was observed for CN reels. Possibly, in these parts, the release of the degradation products is more difficult, and the accumulation

of acetic acid formed promotes a faster film’s deterioration. The μFTIR and ATR-FTIR results correlated well, the outer sample having DS values 1.39 and 1.36, respectively, and the inner samples 0.85 and 0.79, respectively. Samples taken from 0.9 and 1.8 cm, considering 0 the outer turn in a total of 8.5 cm, presented higher DS values for μFTIR in comparison with ATR-FTIR, Table 5. As proposed for historical cellulose nitrate films, this difference may be due to a higher degradation at the film surface compared to the bulk. Spectra of the samples are presented in Additional file 1: Figure S8. Considering the

Table 5 DS of cellulose acetate historical reel SCS0057, calculated on samples by μ FTIR and ATR-FTIR



Distance (cm)	0	0.9	1.8	2.7	3.5	5	5.5	5.8	6.8	8.5
DS by μ FTIR	1.39	1.01	1.02	0.87	0.83	0.88	0.85	0.85	0.83	0.84
DS by ATR	1.36	0.79	0.76	0.82	0.80	0.78	0.83	0.83	0.79	0.78

The sampling distances (cm) start from the outer turn

high level of degradation observed, it will be difficult to predict its original value.

Application in works of art made solely of cellulose acetate

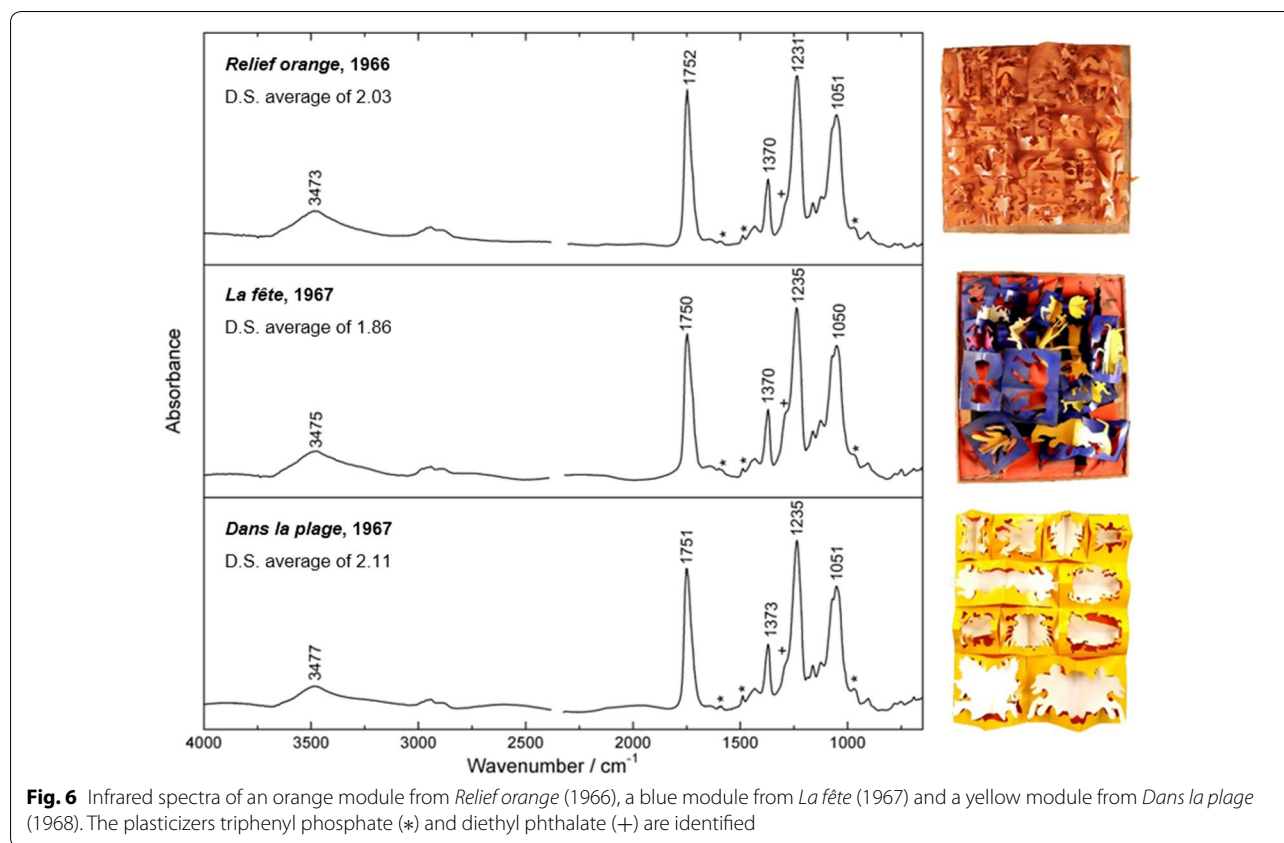
Cellulose nitrate in sheets was used in three Portuguese artworks dated from the 1960s, by the artist José Escada, Fig. 6. One of the works made by this artist is in good conservation condition (*Dans la plage*), and the two others are in an advanced stage of degradation (*La fête and Relief Orange*). The DS calculation, Fig. 6, correlated with the visual assessment of the conservation condition of the artworks: *La fête* presented the lower DS average value of 1.86 and *Dans la plage* presented the higher DS of 2.11. Considering the values presented for injection molding in Table 2, we propose the “original” DS range of the cellulose acetate sheets that compose the artworks from 2.2 to 2.3. It is important to note that the comparison between the three artworks becomes possible since they are from the same period and the plastic material was possibly purchased at the same store in Paris (*Micap*) according to the artist Lourdes Castro, which was a friend of José Escada [33].

Conclusions

In heritage collections, cellulose nitrate and acetate objects can start irreversible deterioration without any visible warning signals. Their degradation poses a threat to other materials as very reactive chemicals are released such as HNO_2 , HNO_3 , and CH_3COOH . In major museums and archives, it is possible to control the damage posed by these chemicals, but it is not possible to prevent their degradation. However, in other institutions with fewer resources, a centenary cultural heritage may be stored in inappropriate conditions, becoming

increasingly devalued, degraded and at risk of total loss. To develop innovative and sustainable preservation conditions, in a first phase, it is fundamental to correlate the molecular degradation with the physical deterioration, in order to develop early warning systems. The latter will allow professionals to make informed decisions on when it will be necessary to preserve an artwork in cold storage. This work contributes to this endeavour. For the first time, the extent of degradation was measured in historical samples through calibration curves previously optimized in reference samples. ATR-FTIR and μ FTIR calibration curves were applied in situ and in micro-samples, respectively, in photographic and cinematographic films as well as in contemporary works of art made with cellulose acetate sheets. It was possible to clearly distinguish very good and poor conservation conditions, at the molecular level, and to correlate it with a preliminary visual assessment and with hardness. In future work, the quantification of the extent of hydrolysis that was developed in this work will be integrated in multi-scale models, coupled with sensors to monitor specific volatiles, to prevent objects to reach an irreversible degradation path. This will create a breakthrough in the way collections based on cellulose derivatives are preserved, allowing the safeguard of citizens’ memories by preserving documents and photographs in centennial voluntary associations and other non-profit organizations, with innovative and affordable solutions.

Work is currently in progress, to apply this tool extensively in dated films to systematically determine original degrees of substitution in historical collections. It will be also applied to follow the accelerated ageing of highly characterized historical films to provide insight into their molecular evolution.



Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s40494-020-00373-4>.

Additional file 1. Supporting information.

Abbreviations

ANIM: National Archive of Motion Pictures; ATR-FTIR: Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy; CA: Cellulose acetate; CDA: Cellulose diacetate; CN: Cellulose nitrate; CTA: Cellulose triacetate; DEP: Diethyl phthalate; DS: Degree of substitution; DFF: Deutsches Filminstitut & Film-museum; MCC: Micro crystalline cellulose; NMR: Nuclear magnetic resonance spectroscopy; OEAW: Phonogrammarchiv of the Vienna Academy of Sciences; TPP: Triphenyl phosphite; μ FTIR: Micro Fourier Transform Infrared Spectroscopy; v: Stretching (ν_s : asymmetric stretching).

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Authors' contributions

MJM, AMR, NW and AN contributed with the conception and design of the research work; MJM, AMR, EMA, SN, FR, AN, ER, and AAA with the acquisition, analysis and interpretation of data. MJM with SN, FR, EMA and AN were responsible for the quantification of DS by infrared spectroscopy. NW and ER contributed with the selection of the historical samples. EMA and SN studied

the two artworks by José Escada (*La fête* and *Relief orange*). MJM, AR and SN prepared the first version of this paper. AAA was responsible for the NMR experiments and for the calculation of the DS in cellulose acetate samples by NMR. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its additional file).

Competing interests

The authors declare that they have no competing interests.

Author details

¹ REQUIMTE–Laboratório Associado para a Química Verde, Departamento de Química e Departamento de Conservação e Restauro, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.

² Department of Chemistry "G. Ciamician", University of Bologna, Ravenna Campus, Via Guaccimanni 42, 48123 Ravenna, Italy. ³ Phonogrammarchiv-Austrian Academy of Sciences, Liebiggasse 5, 1010 Vienna, Austria.

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