Quantification of the degree of substitution as a diagnostic tool for assessing the conservation condition of cellulose nitrate and acetate in heritage collections

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Abstract max 350

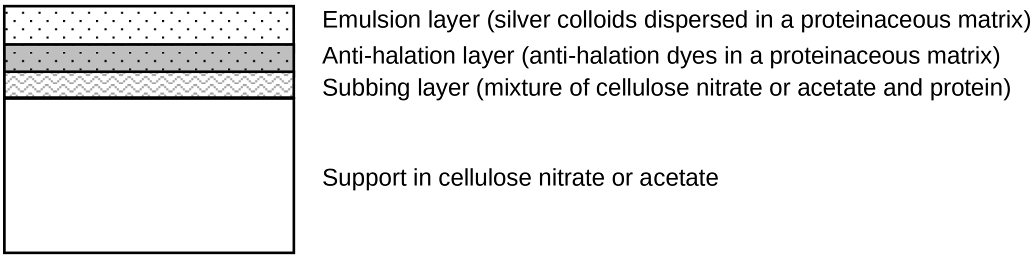
The primary goal of this work is to disclose the actual degree of substitution (DS) of cellulose derivatives in historical objects, cinematographic and photographic films or artworks, and compare them with their pristine values, establishing the quantification of the DS as a novel tool for the classification of the damage extent. Infrared spectra were used to develop and optimize calibration curves that were used to obtain DS values.

First, calibration curves were optimized in reference samples. Next, these ATR-FTIR and μFTIR curves were applied in situ and in micro-samples, respectively, in historical materials and artworks. Objects in good and poor conservation condition were selected through a preliminary visual assessment. The cinematographic and photographic films are dated from the 1890s to the 1960s, and the contemporary works of art made with cellulose acetate sheets by Portuguese artist José Escada are dated from the sixties. The calibration curves were obtained plotting DS as a function of the ratio between a reference peak (which does not change during side-chain degradation) and selected peaks that monitor the degradation for cellulose acetate and nitrate polymers. For cellulose nitrate the probe and reference peaks were ν_aNO2 and νCOC, respectively; for cellulose acetate νOH and νCOC, respectively. The extent of hydrolysis, calculated by the degree of substitution, correlate well with the physical deterioration assessed through the sample hardness (Shore A) which measured with a Durometer. Finally, we discuss how this tool will permit to explore how complex polymer matrixes degrade and how better to preserve an irreplaceable heritage.

Keywords: cellulose nitrate; cellulose acetate; modern materials; heritage collections; conservation; degradation mechanisms; infra-red

1. Introduction

A vast percentage of European cultural heritage can be found in cinematographic films and photography produced between 1895 and 1970. The support 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. The complex stratigraphy that is built on these supports is depicted in scheme 1.



Scheme 1. General structure of a black and white cinematographic film. The cellulose nitrate or acetate base is the thickest layer and its function is to provide support to 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 gelatin and cellulose nitrate or acetate (subbing layer). The anti-halation layer is a light absorbing gelatin plus anti-halation dyes (for example, acid fuchsin or malachite green) layer that prevents light to be reflected to the emulsion causing a halo effect.

For this reason, more than 75 years of visual memories, based on cellulose acetate (CA) and cellulose nitrate (CN) materials, are in danger to be lost (1–4). Innovative and sustainable solutions are badly needed for the preservation of these physical media, which have helped to preserve the cultural material that is a precious testimony of socio-cultural European evolution in the recent era. 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 (website: https://nemosineproject.eu). This innovative package will be based on an in-depth knowledge of the fundamental mechanisms of degradation and will allow for continuous monitoring of selected volatiles that will be correlated with the conservation condition of the cellulose-based support. Multi-scale modeling & sensors signals will be used to target this objective.

The degree of substitution of these materials may be correlated to their conservation condition because the fundamental degradation mechanism, in both cases, is hydrolysis, scheme S1. For this reason, in this work, we will focus on conceiving a tool that will measure the degree of substitution[1] (DS) of CA and CN in historical films (photographic and cinematographic) and contemporary artworks, by developing calibration curves based on infrared spectra. For the case studies in which it will be possible to predict, with some confidence, what was the initial (original) degree of substitution, the calculated DS will allow us to quantify the degradation extent, table 1.

Next, we will briefly summarize the state of the art for the degradation mechanisms at play in cellulose nitrate and acetate; we will also provide a brief historical overview, in which the most common plasticizers will be described in context, as they will be important in the degradation mechanisms and will be also detected in the infrared spectra.

Table 1. Applications of cellulose nitrate/cellulose acetate according to the nitrogen content/acetyl content and the degree of substitution (DS) (5,6). Films are highlighted as they were used as our case studies: cinematographic films and photographic negatives. In the case of the artworks from José Escada, the injection molding values were considered.

Polymer	Applications	Nitrogen / acetyl content (%)	Degree of substitution*
Cellulose Nitrate	Explosives	12 – 13.5	2.26 – 2.76
	Adhesives	12	2.26
	Film base	**12**	**2.26**

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[1] The degree of substitution (DS) of a polymer is the (average) number of substituent groups attached per per monomeric. 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).

	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
	Film	**54.3 – 56.4**	**2.3 – 2.4**
	Lacquers	56.4 – 59.3	2.4 – 2.6
	Film and fiber	**60.7 – 62.85**	**2.9 – 3.0**

*the degree of substitution can be calculated from the nitrogen and acetyl contents (%) by the following equations: DS = $\frac{3.60 \text{ x } (\% \text{ nitrogen })}{31.13 - (\% \, nitrogen)}$ / DS = $162 \, x \left(\frac{\% \text{ acetyl content}}{4305 - 43 \text{ x } \% \text{ acetyl content}}\right)$

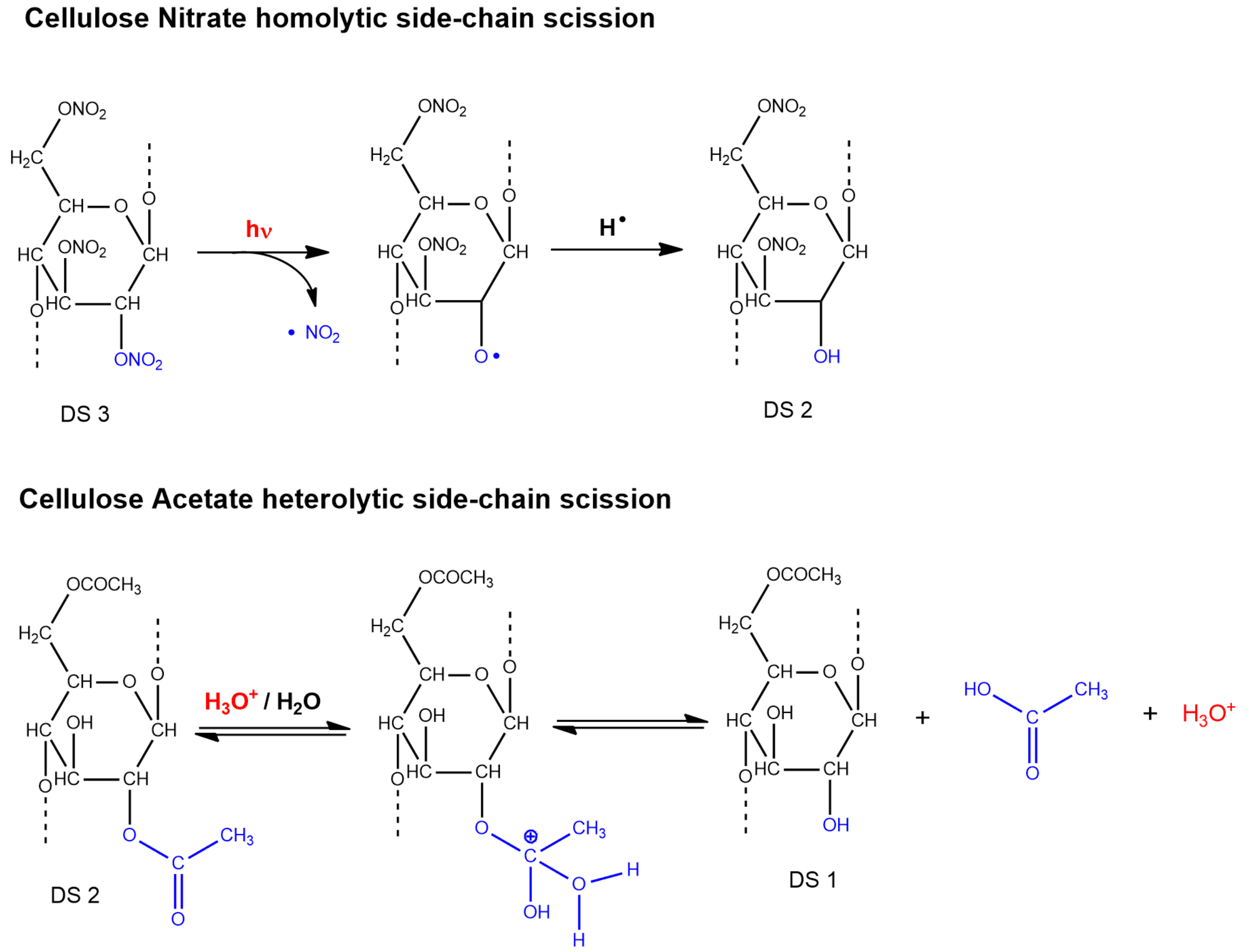
1.1 Manufacture of cellulose nitrate and acetate and description of their most common plasticizers

In the 1870’s, John Wesley Hyatt developed the first semisynthetic plastic – celluloid – by applying heat and pressure to a mixture of cellulose nitrate and camphor. Celluloid’s flexibility, dimensional stability and transparency eventually led to its extensive use as a new photographic and cinematographic medium for films, merit of the developments of Eastman Kodak and Henry Reichenbach in 1888-1889 (7). Later camphor began to be replaced by other plasticizers, namely triphenyl and tricresyl phosphates after 1902 and by phthalates in the 1920s (8). As a support for image, cellulose nitrate was widely used between 1890s and 1950s, but due to its inflammability, it was gradually replaced 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 (9). The main plasticizers used in cellulose acetate were triphenyl phosphate, diethyl phthalate and dimethyl phthalate.

1.2 Degradation Mechanisms for cellulose nitrate

For cellulose nitrate, experimental evidence has shown that mechanisms based on radical propagation are dominant (10–12). Thérias et al. demonstrated that the products and intermediates produced by light absorption (≥300 nm), i.e., are identical to what formed by thermal degradation (60ºC), however photochemical degradation displays higher rate constants (10). Allen et al.

(4,13,14), on their pioneer studies with artificially aged and historical CA and CN 35mm films from the *National Film* and Television *Archive (U.K.)*, 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.



Scheme 2*.* Cellulose nitrate degradation occurs by the homolytic scission of the nitrate groups resulting in the release of $^{\bullet}NO_2$ and the formation of an alkoxy radical. By the addition of a $H^{\bullet}$, the alkoxy radical is transformed into a hydroxyl, resulting in the decrease of the DS, *top*. The acid-catalyzed heterolytic side chain scission of the acetyl esters will occur by nucleophilic substitution, with the release of acetic acid and formation of a hydroxyl group, which also results in the decrease of the DS, bottom.

It has been proved that CN degradation occurs through side and main chain scission (10–12,15–18). The first phase of cellulose nitrate degradation is based on side chain scission and is characterized by the homolytic cleavage of the RO-NO_2 bond in C2 or C3, forming an alkoxy radical ($RO^\bullet$) and releasing a $NO_2^\bullet$ radical, scheme 2. The $NO_2^\bullet$ radical can be subsequently transformed into the pair HNO_2 / HNO_3 by hydrogen abstractions and reaction with H_2O (17,19). Thought time, a complete loss of the nitrate substitution in the cellulose ring will be observed, leading to the decrease of the degree of substitution. The release of the alkoxy radical ($RO^\bullet$), will induce the formation of hydroxyls and ketones by $H^\bullet$ abstraction or aldehyde by the cleavage of the C2-C3 bond (12,16). The main steps and intermediates formed during side chain cleavage are summarized in scheme 2.

Main chain scission will evolve through the formation of hydroperoxides at C1 and their decomposition will promote a continuous cleavage of the glycosidic linkages and formation of carbonyl intermediates - the gluconolactones, identified at 1735-40 cm^{-1} in the infrared spectrum (12). 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 (17) .

1.3 Degradation Mechanisms for cellulose acetate

According to the literature (17,20,21), cellulose acetate acid degradation mechanism occurs: i) side chain scission by the acid-catalyzed ester hydrolysis, in which heterolytic bond cleavage prevails, with formation of an hydroxyl group and release of acetic acid, scheme 2, and ii) main chain scission by the acid-catalyzed heterolytic cleavage of the glycosidic bonds, scheme S3.

Although homolytic scission is usually not considered in archival conditions as the main degradation mechanism, photooxidation studies can provide insights on radical chain reactions that can occur in the polymer matrix, as shown for cellulose nitrate. In the absence of O_2 and irradiating at $\lambda \geq 235$, 280 and 340 nm, Hon (22), Merlin and Fouassier (23) demonstrated the formation of $CH_3^\bullet$ and $H^\bullet$

abstraction promoting chain scission. CH_4, CO, and CO_2 were identified as the main volatiles irradiating at $\lambda \geq 235$, 280 (CH3COOH was only detected when $\lambda \geq 280$ nm).

Placing the samples inside glass vials and irradiating at $\lambda \geq 275$ nm, Hosono et al. observed that photooxidation of cellulose acetate only occurs in the presence of photosensitizers, proposing the formation of a radical intermediate and, subsequently, in reaction with oxygen and abstraction of a hydrogen, the formation of hydroperoxides, leading to main chain scission (24).

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

To calculate cellulose acetate degree of substitution, Fei *et al.* (25) 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." Prior to Fei *et al.* study, Samios *et al.* (26) obtained a linear calibration curve to calculate the degree of substitution, however the values used were below DS 2.5. Thus, for DS values bellow 2.5, it is possible to use a linear calibration fitting with a high coefficient of determination.

In this work, the calculation of the degree of substitution will be first optimized on reference samples using the approach developed by Fei *et al*. Contrary to Fei *et al,* infrared spectra are collected directly in the references and historical artworks based on CN and CA: *i)* in situ by ATR, and *ii)* in micro samples by microFTIR. For cellulose acetate the DS of the reference samples were calculated by NMR. The optimized calibration curves obtained for CA and CN will be tested in several historical artifacts, as follows: *i)* photographic films in good conservation condition, from the San Payo collection, studied in Roldão's Ph.D. project (6); *ii)* cinematographic films that are used in the

NEMOSINE project, displaying diverse conservation conditions; *iii)* cinematographic films from the National Archive of Motion Pictures 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 based matrixes.

3. Experimental

3.1. Materials

Cellulose diacetate powder (Sigma-Aldrich, 39,7 wt % acetyl content), cellulose triacetate purum (Fluka), cellulose nitrate membranes (Hybond™ ECL™, GE Healthcare), micro crystalline 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, ≥99.9%), dichloromethane (organic trace analysis, ≥99.9%) and acetone (≥99.9%) were used. DMSO-d_6 was used for the NMR studies.

3.2. Calculation of the degree of substitution of the cellulose acetate samples by NMR

Cellulose acetate samples (10-15 mg) were dissolved in 500 μL of DMSO-$d_{6.}$ The DS of commercial cellulose acetate samples was measured by integration method of ^{1}H NMR signals (see supporting information, figures S1 and S2). Briefly, quantification of the number of acetyl groups present in the sample could be achieved 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 DS of commercial diacetate and triacetate cellulose samples were 2.32 and 2.97 respectively.

3.3. Preparation of samples with different degrees of substitution

Based on the work of *Fei et al.*.[14] samples with different degrees of substitution were obtained by co-grinding appropriate amounts of commercial powders of micro crystalline cellulose (MCC, DS 0) and cellulose diacetate (CDA, DS 2.32). Commercial cellulose triacetate (CTA) was used as reference for a DS of 2.97 (the DS of CDA and CTA were calculated by NMR, see section 3.4.1.). The relationship between the DS and the masses is described by *Equation 1*:

$$DS\ (Degree\ of\ Substitution) = \frac{{}^{m_{CDA}}/_{M_{CDA}} \times\ DS_{CDA}}{{}^{m_{CDA}}/_{M_{CDA}} + {}^{m_{MCC}}/_{M_{MCC}}}$$

Equation 1. Relationship between DS and MCC and CDA masses. Where: $\mathrm{m_{CA}}$ and $\mathrm{m_{C}}$ are the masses of CDA and MCC in the mixture, M_{CDA} (264,48 g/mol) and M_{MCC} (162 g/mol) are the average molecular weights of repeating units and DS_{CDA} is the degree of substitution of commercial CDA (DS 2,32).

The DS prepared in addition to the pure samples (MCC (DS 0), CDA (DS 2.32) and CTA (DS 2.97) were 0,48; 0,95; 1,43; and 1,90: the relative weight of MCC and CDA measured for sample preparation were calculated with *Equation 2* obtained by deformation of *Equation 1*.

$$\frac{m_{CDA}}{m_{MCC}} = \frac{264{,}48\ g/mol \times DS}{162\ g/mol \times 2{,}32 - 162\ g/mol \times DS}$$

Equation 2. Relationship between the DS and the weight ratio of cellulose diacetate and micro crystalline cellulose in the mixture.

The powders were grinded with a mortar and pestle with seldom additions of droplets of water. The mixtures obtained were put in a desiccator over night before being analyzed.

In order to obtain a powder of cellulose nitrate (CN), commercial filtration membranes (GE Healthcare) with DS 2,2 (The DS of these CN membranes was measured by Berthumeyrie et al. (2015) [8] using quantitative elemental analysis, 11,76% N) were cut with scissors and scalpel. To reduce particle size, the cut membranes were sieved. The relative amounts of MCC and CN powders have been calculated by adaptation of equation 2 (where 261 g/mol and 162 g/mol are the average molecular weights 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 between 0 and 2,2, specifically: 0,5; 1,0; 1,5 and 2,0. The powders were grinded with a mortar and pestle with seldom additions of droplets of water. The mixtures obtained were put in a desiccator over night before being analyzed. The total weight of each mixture of was approximately of 6,0 mg for both CA and CN.

3.4. Description of the historical samples

3.4.1 Cinematographic Films from NEMOSINE project

Cinematographic films from the Phonogrammarchiv of the Vienna Academy of Sciences (OEAW, Austria) were studied. Information about sample denomination, date and typology were given by the archive and are described in table S1. The thickness was measured with a micrometer in three points of each film and the average values are presented. Also, a preliminary analysis by μFTIR allowed an identification of the support material (cellulose nitrate and cellulose acetate) and the plasticizers (camphor and triphenyl phosphate).

3.4.2. Cinematographic Films from the National Archive of Motion Pictures

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 1948-1951 or earlier. The films present inside are in an advanced stage of degradation, with tacky emulsion, loss of image and an intense noxious smell. These films have already been studied in other work by infrared and Raman spectroscopies [34].

3.4.3 San Payo Collection

The samples analyzed belong to the Portuguese collection San Payo. According to the work of 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 [16].

3.4.4 *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-UNL (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-UNL (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.

3.5. Equipment

3.5.1. Nuclear Magnetic Resonance Spectroscopy

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

3.5.2. μInfrared Spectroscopy (μFTIR)

Infrared spectra were acquired on a Nicolet Nexus spectrophotometer equipped with a Nicolet Continuμm (15x objective) microscope and a Mercury - Cadmium - Tellurium (MCT) detector cooled by liquid nitrogen. μ-samples were place 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 stereo microscope (between 7.1x and 115x), 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 CO_2 absorption at approximately 2300-2400 cm^{-1}.

3.5.3. Attenuated total reflectance (FTIR-ATR)

The spectra were obtained by an Agilent Handheld Exoscan 4300 spectrophotometer equipped with 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.

3.5.4. Hardness Measurement

Sample hardness (Shore A) was measured with a HP Durometer (model HPSA, from Checkline Europe). For each sample the hardness values were determined as the average of five independent measurements.

3.6 Construction 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 (ν_aNO2, $A_{1653\ cm^{-1}}$) and reference (νCOC, $A_{1067\ cm^{-1}}$) 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 FTIR-ATR, the probe (ν_aNO2, $A_{1636\ cm^{-1}}$) and reference (νCOC, $A_{1060\ cm^{-1}}$) absorbances were 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 FTIR-ATR. The linear and polynomial (2nd degree) regressions of the data were calculated using OriginPro 8.

4. Results and Discussion

4.1 Cellulose nitrate calibration curves

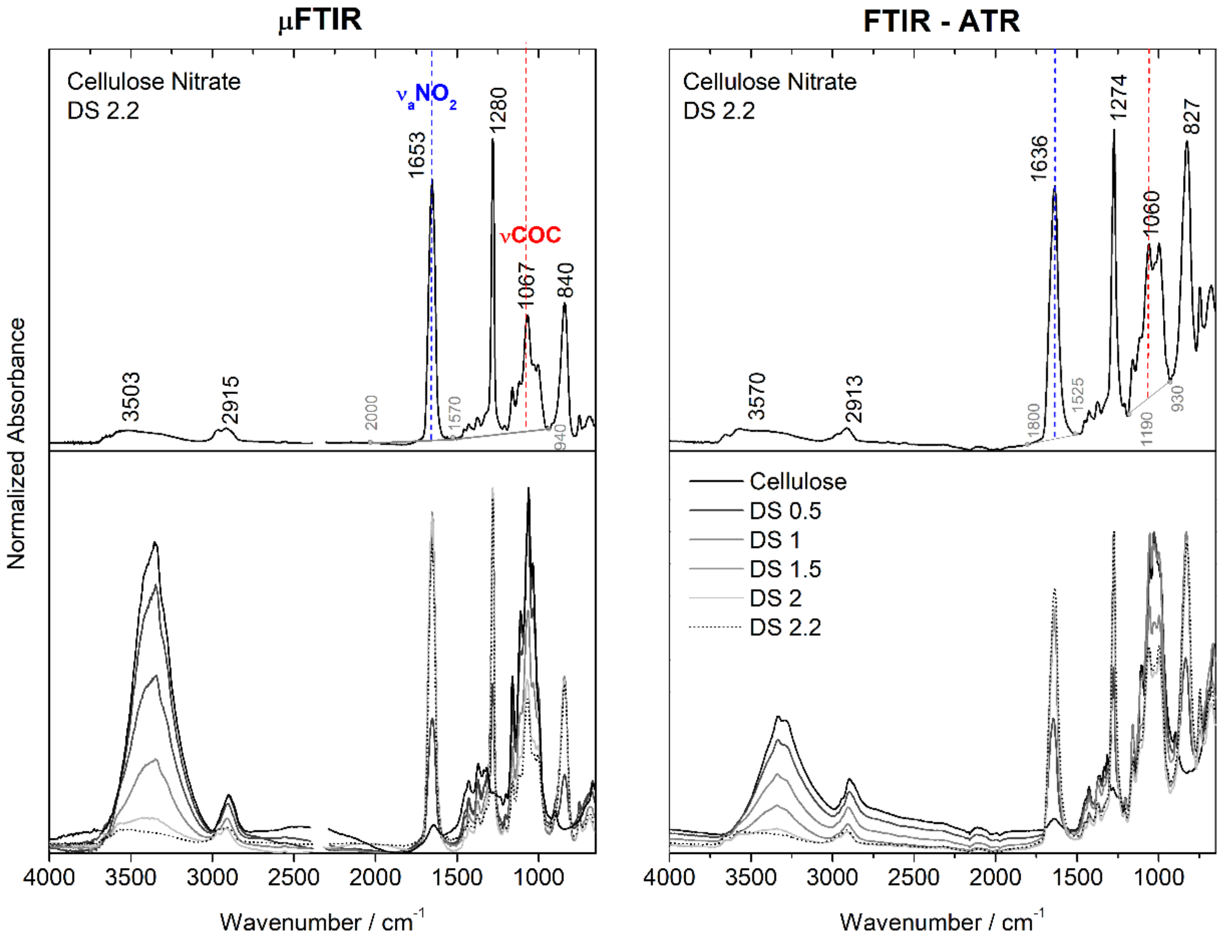


Figure 1. Infrared spectra of cellulose nitrate (DS 2.2) by µFTIR and FTIR-ATR, *top*. For µFTIR, the probe and reference peaks were ν_aNO2, $A_{1653\ cm^{-1}}$ and νCOC, $A_{1067\ cm^{-1}}$, respectively. For FTIR-ATR, the probe and reference peaks were ν_aNO2, $A_{1636\ cm^{-1}}$ and νCOC, $A_{1060\ cm^{-1}}$, respectively. Spectra obtained admixing microcrystalline cellulose (DS 0) and cellulose acetate (DS 2.4 and 3), *bottom*.

The infrared spectra of cellulose nitrate obtained by µFTIR and FTIR – ATR can be observed in figures 1. Both spectra are characterized by the stretching vibrations of the CH and CH_2 bonds between 2900 and 3000 cm-1; the strong nitrate groups vibration bands (µFTIR: 1653, 1280 and 840 cm^{-1}; FTIR-ATR: 1636, 1274 and 827 cm^{-1}); the cellulosic acetal vibrational envelope between 1200 and 900 cm^{-1} (1). The decrease of the 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, figure 1. Although the spectral profile between 2900 and 3000 cm^{-1} changes, the intensity of the acetal structure vibration remains constant. The influence of plasticizers must be assessed before the selection of the reference and probe peaks. In the infrared spectra of camphor, the main plasticizer of celluloid, the strong carbonyl stretching at 1743 cm^{-1} and low intensity bands between 1200 and 900 cm^{-1} are observed, figure 2. In celluloid, a typical formulation could consist in the use of 30% of camphor. The carbonyl band, now shifted to lower wavenumbers (1731 cm^{-1}) due to the hydrogen bond network, does not overlap with the ν_aNO_2 vibration at 1662 cm^{-1}, figure 2. Furthermore, the influence of the plasticizer between 1200 and 900 cm^{-1} is residual to none. For this reason, the COC stretching (μFTIR: 1070 cm^{-1}; FTIR-ATR: 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. In figure 2, the ratio between $\nu_aNO_2/\nu COC$ is plotted as a function of DS, resulting in calibration curves with high coefficients of determination (μFTIR: $R^2 = 0{,}998$; FTIR-ATR: $R^2 = 0{,}997$).

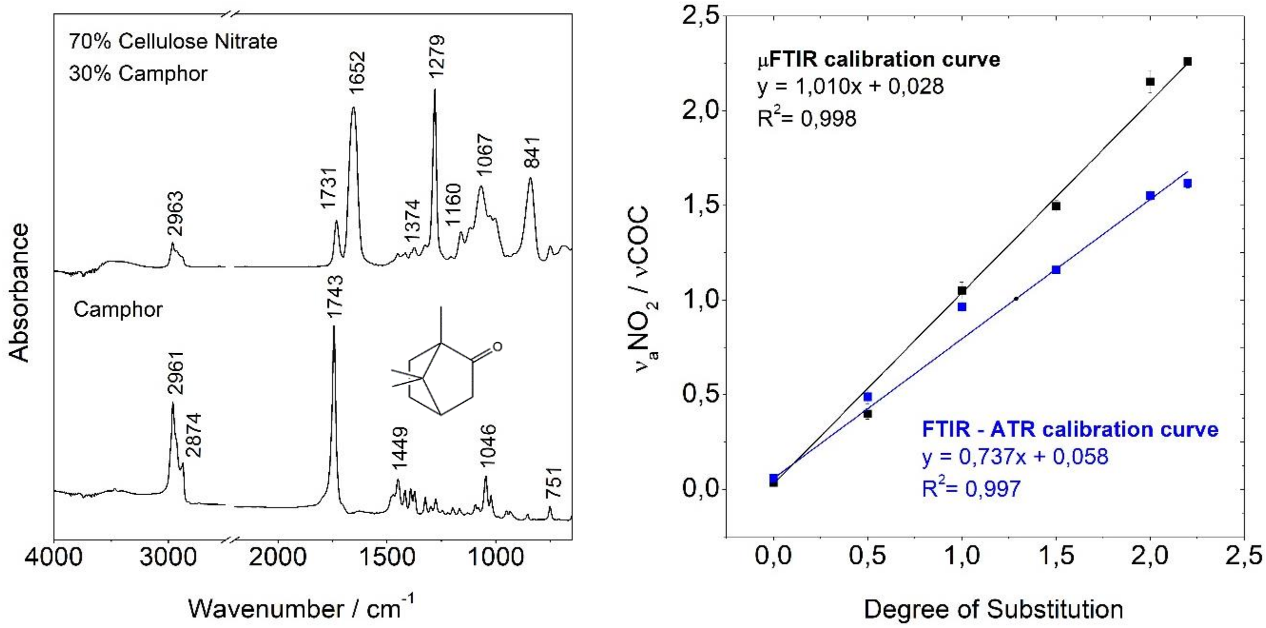


Figure 2. Infrared spectra of a celluloid reference and camphor, *left*. DS linear calibration curves for μFTIR and FTIR-ATR and respective equations and coefficients of determination (R^2), *right*. For more details, please see text.

4.2 Measuring the degree of substitution in cellulose nitrate artworks and correlating it with 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 2.26 for μFTIR and from 1.76 to 1.93 for FTIR – ATR, table 2. Comparing the μFTIR and FTIR – ATR results it is possible to observe that samples 50508 and DIF 50 500 correlate well. The other samples (50509, S4, S5 and S6) the values calculated by FTIR-ATR are lower, with differences ranging from 0.26 to 0.33. It is possible that at the surface the polymers are more degraded than in the bulk. Using FTIR-ATR we only measure the DS at the surface, whereas with μFTIR both bulk and surface are analyzed in a micro-sample. To understand the reason for these differences, in future work, it will be necessary to make a μsampling profiling to assess if DS changes from the surface to the bulk. Spectra and images of the cinematographic films are presented as supporting information, table S2 and figure S3.

Table 2. DS of the cellulose nitrate cinematographic films provided by the NEMOSINE project calculated using the calibration curves. For more details, please see the text.

Sample	DS μFTIR	DS FTIR – ATR	Hardness (Shore A)
DIF 50 500	1.71	1.76	95
50508	1.86	1.85	96
S6	2.06	1.80	96
S4	2.15	1.89	96
50509	2.18	1.85	97
S5	2.26	1.93	97

For μFTIR, film S5 is in agreement with the value reported in table 1 for a pristine film, of 2.26. 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 similar for films 50509, S4, S5, which have images in good conservation condition. In comparison, for films DIF 50 500, 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) and, in the other hand, films 50508 and S6 presented DS values similar to films 50509, S4 and S5 but their image is poor condition, see table 2 and table S2. These results show that 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 μFTIR 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, figure 3. The calculated DS of 1.89 and 0.79 correlate well with the measured hardness of shore 96A and 75A, respectively. 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 HNO_2/HNO_3 that promotes main chain scission), with the observed physical alteration of cellulose nitrate: lower hardness and brittle film.

NEMOSINE cinematographic films studied presented hardness values similar to 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 (shore 75A) 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.

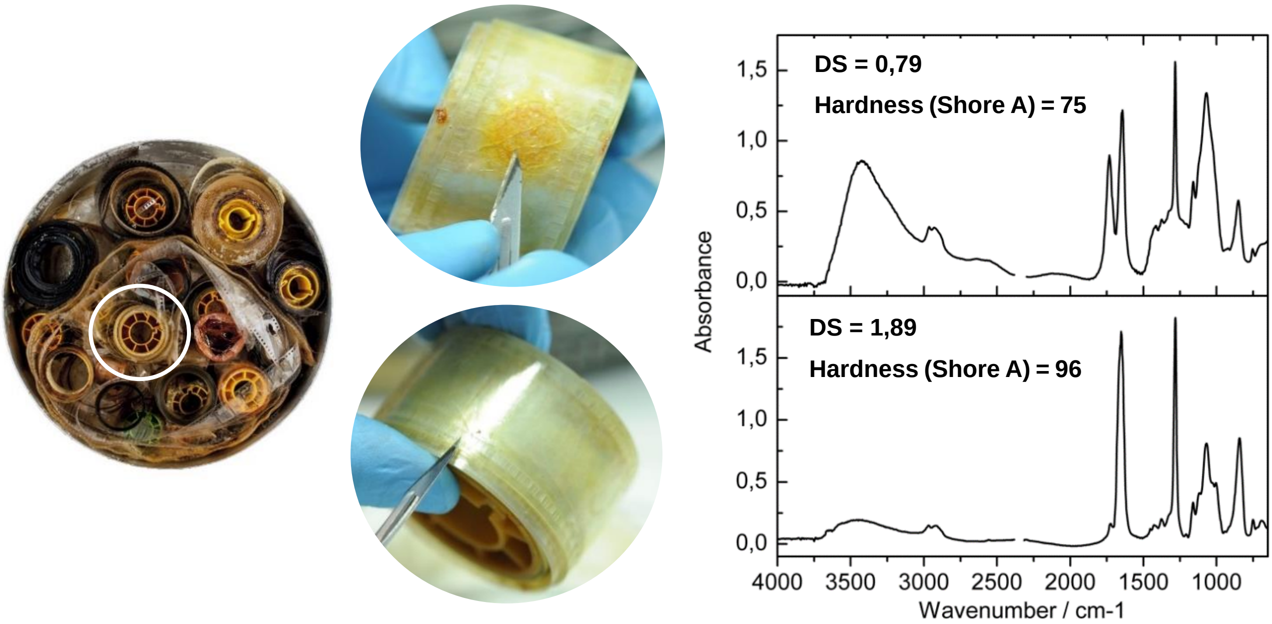


Figure 3. The μFTIR analysis a cellulose nitrate reel (ANIM) show the potentiality of the DS quantification as method for the classification of the damage extent: an area of the reel which retains its elasticity has a calculated DS of 1.89 and a shore hardness of 96A and an area cracked and brittle a DS of 0.79 and a shore hardness of 75A.

4.3 Cellulose acetate calibration curves

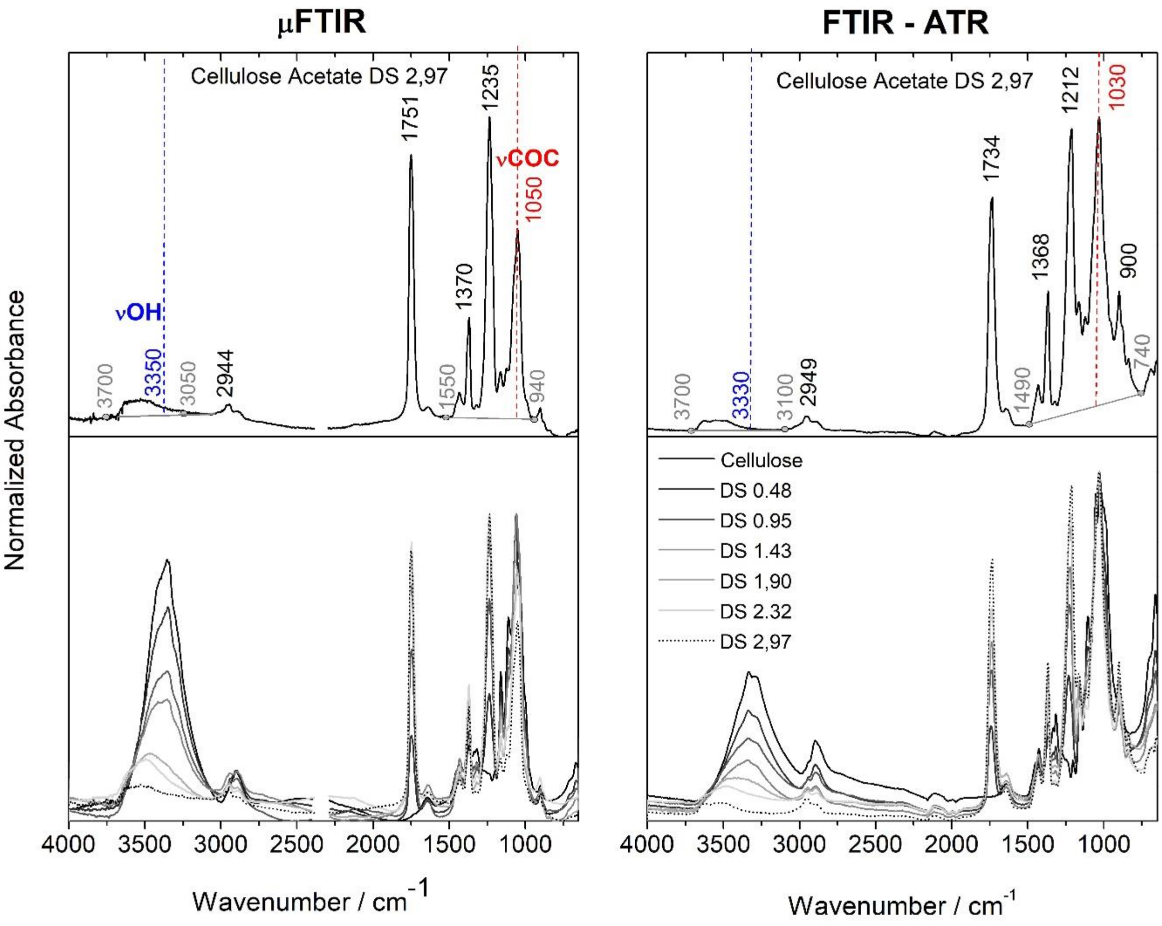


Figure 4. Infrared spectra of cellulose triacetate (DS 2,97) by μFTIR and FTIR-ATR, *top*. For μFTIR, the probe and reference peaks were νOH, $A_{3350\ cm^{-1}}$ and νCOC, $A_{1050\ cm^{-1}}$, respectively. For FTIR-ATR, the probe and reference peaks were νOH, $A_{3330\ cm^{-1}}$ and νCOC, $A_{1030\ cm^{-1}}$, respectively. Spectra obtained admixing cellulose (DS 0) with cellulose acetate (DS 2.32 and DS 2,97), *bottom*.

Both μFTIR and FTIR-ATR spectra of cellulose acetate are shown in figure 4. They are characterized by the carbonyl stretching (μFTIR 1751cm^{-1}; FTIR-ATR 1734 cm^{-1}), the carboxylate stretching (μFTIR 1235 cm^{-1}; FTIR-ATR 1212 cm^{-1}) and the methyl bending (μFTIR 1370 cm^{-1}; FTIR-ATR 1368 cm^{-1}). The stretching of the hydroxyl groups and the ether groups of the pyranose ring (μFTIR 1050 cm^{-1}; FTIR-ATR 1030 cm^{-1}), are related to the cellulosic structure (2) . Following a similar reasoning as for cellulose nitrate, cellulose acetate COC stretching was used as the reference peak for the ratio applied for the determination of the DS, figure 4.

To determine the DS, the probe bands to be considered are the ones influenced by hydrolysis, namely the hydroxyl and the ester bands. The main plasticizers for cellulose acetate - diethyl phthalate (DEP) and triphenyl phosphate (TPP) - are usually present in concentrations below 40% and the strong absorptions due to the phthalate ester groups (1727 and 1282 cm^{-1}) exclude the use of the acetate groups as probe bands. For this reason, the hydroxyl stretching (μFTIR 3350 cm^{-1}; ATR 3330 cm^{-1}) was selected in this work as the probe peak. Note that triphenyl phosphate POC stretching (960 cm^{-1}) does not overlap with the νCOC stretching reference band, figure 5.

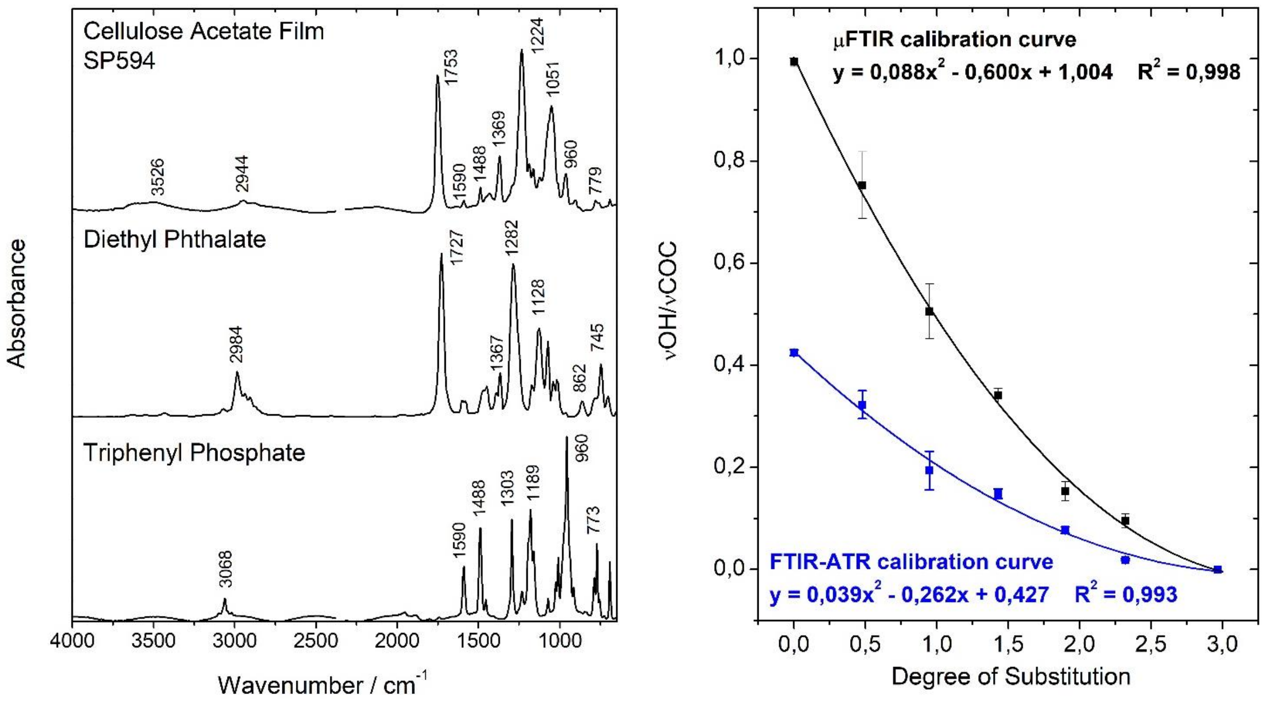


Figure 5. Infrared spectrum of an historical cellulose acetate film from the San Payo collection (SP594), plasticized with triphenyl phosphate and diethyl phthalate. The spectra of the two plasticizers are shown for comparison, *left*. DS calibration curves and respective equations and coefficients of determination (R^2) for the polynomial fitting of the νOH/νCOC ratios calculated from μ-FTIR and FTIR-ATR, *right.*

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.

Table 3. DS calibration curves obtained by applying polynomial (2nd degree) and linear regressions. The coefficients of determination (R^2) are higher using a polynomial fitting for both μFTIR and FTIR – ATR.

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

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

4.4.1. Application in cinematographic and photographic films

Films from the San Payo collection, previously evaluated as in good conservation state by Roldão (3), 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, figure 5 and figure S4 and S5. According to the literature a commercialized diacetate film 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 has in good condition, we 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 triacetate 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.

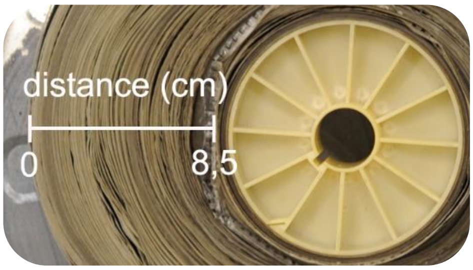
Table 4. DS of the San Payo cellulose acetate samples previously assessed by Roldão as in good conservation state. The DS of samples SP 548 and SP544 indicate that they are diacetate films. The DS ranging from 2.69 to 2.83 are indicative of triacetate film degradation.

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

The extent of degradation in a cinematographic reel was also studied from the outside to the inside, table 5. The reel studied, SCS0057, is dated from 1950-1960. 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 (FTIR-ATR). 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. The μFTIR and FTIR-ATR results correlated well, the outer sample having DS values of 1.39 and 1.36 and the inner samples a mean value of 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 FTIR-ATR, table 5. As proposed for historical cellulose nitrate films, it may be due to a higher degradation at the film surface compared to the bulk. Spectra of the samples are presented in figure S6.

Table 5. DS of cellulose acetate samples from the historical reel SCS0057, calculated by μFTIR and FTIR-ATR. The sampling distances (cm) starting from the outer turn are given. The results indicate that degradation is more severe when cellulose acetate base is covered from both sides due to the film winding.

Reel SCS0057 (cellulose acetate)



Sample distance from outer turn (cm)										
	0	0.9	1.8	2.7	3.5	5	5.5	5.8	6.8	8.5
DS Calculation										
μFTIR	1.39	1.01	1.02	0.87	0.83	0.88	0.85	0.85	0.83	0.84
ATR	1.36	0.79	0.76	0.82	0.80	0.78	0.83	0.83	0.79	0.78

4.4.2. Application in works of art made solely of cellulose nitrate

Cellulose nitrate in sheets was used in three Portuguese artworks dated from the 1960's, by the artist José Escada, figure 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, figure 6, correlated with the visual assessment of the conservation condition of the artworks: *La fête* presented a DS average value of 1.86; less degraded, *Relief orange* a DS average of 2.03; not showing signs of degradation, *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 of the cellulose acetate sheets that compose the artworks range 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 (4).

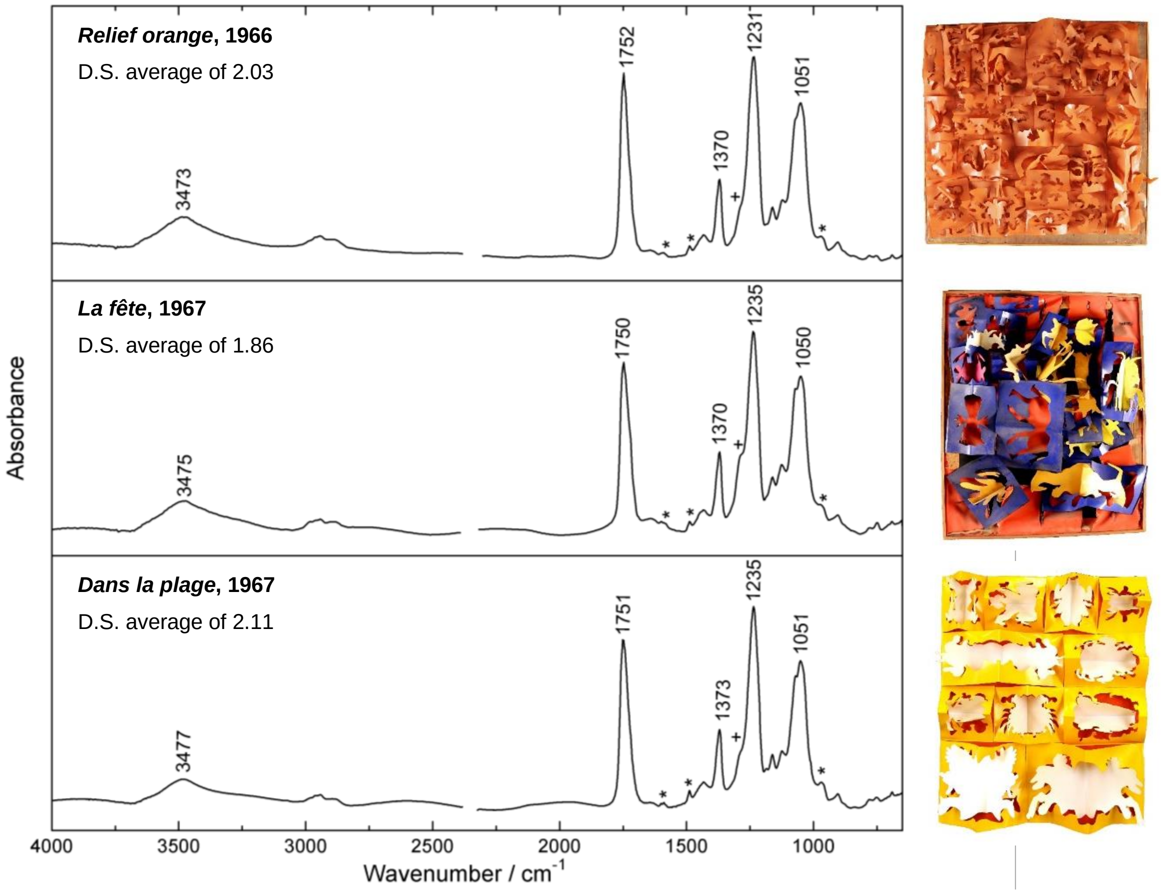


Figure 6. Infrared spectra of an orange module from *Relief orange* (1966), a blue module from *La fête* (1967) and a yellow module from *Dans la plage* (1968). The plasticizers triphenyl phosphate (*) and diethyl phthalate (+) are identified.

5. Conclusions

Cellulose nitrate and acetate are unpredictable materials in heritage collections because it is not yet possible to predict the evolution of their conservation condition over time. For this reason, 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 big museum 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 less 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 to develop early warning systems that will allow professionals to take 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 μFTIR 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 both 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, in order 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 & 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.

Declarations

Availability of data and materials

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MJM, AR, NW and AN contributed with the conception and design of the research work; MJM, AR, 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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