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DYNAMIC MECHANICAL ANALYSIS (DMA), ^{13}C SOLID STATE NMR AND MICRO-THERMOMECHANICAL STUDIES OF HISTORICAL PARCHMENT

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Abstract

DMA and solid state ^{13}C NMR techniques were used to measure historical parchment samples within the framework of the project (MAP) Micro Analysis of Parchment (EC contract No. SMT4-96-2101) in collaboration with the School of Conservation in Copenhagen. DMA was used in both thermal scan and creep modes. Thermal scans provided information on the transitions associated with the collagen polymer. Microthermal analysis was also used to obtain information on the topography and thermal conductivity of sample areas of 100 μm. Localised heating enabled measurements of softening transitions in the sample. This behaviour is influenced by the chemical composition of parchment. ^{13}C NMR provided information on the carbon atoms associated with the polypeptide chains of the collagen in parchment. The behaviour of samples immersed in water and measured in DMA creep mode was used to measure the shrinkage behaviour of the parchment samples. The different but complementary techniques provided a means for characterising the physicochemical state of parchment samples.

Keywords: ^{13}C solid state NMR spectroscopy, DMA, micro-thermomechanical analysis, parchment

Introduction

Parchment whether in the form of bookbindings or manuscripts is of considerable interest. It is produced from the dermis of animal skin. The Dead Sea Scrolls found in Qumran are dated around 250 BC to about 65 AD, and are until now the oldest known parchments. Paintings on papyrus and tomb walls indicate that parchment was manufactured in Egypt and the Middle East already in 2500 BC. The earliest description of

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European parchment production dates back to the 8th century [1]. The fibrous hide protein collagen constitutes the main part of the parchment structure. It is necessary therefore to have an understanding of the chemical composition and the physico-chemical state of historical collagen to allow for improved preservation and conservation treatment of parchment. The structure of collagen has been described in terms of three individual protein strands in the α-helix conformation [2]. These are rigidly held by strong hydrogen bonding interaction between the hydroxyl of the hydroxyproline and the amino hydrogens of adjacent glycine units. On heating collagen above the helix-coil transition temperature it causes a collapse of the rod-like three-stranded collagen unit into random coils which then constitute gelatin [3]. When collagen hide fibres are heated in water they will deform and this deformation is seen as a shrinkage of the fibres. The hydrothermal stability of the fibres decreases in proportion to the state of deterioration, and spontaneous transformation of the Dead Sea scroll parchments into gelatin has been reported [4]. The parchment samples described in this paper are based on animal hide, calf and sheep origin. Selected samples from the set studied in the MAP project (micro analysis of parchment) [1] will be discussed in this paper and their physical appearance and characteristics are described in Table 1. The remainder are discussed elsewhere together with the additional techniques which were used for their complete characterisation [1].

Table 1 Description of modern and historic parchments

Parchment	Thickness/mm	Description
NP7	0.16	Unaged calf parchment obtained from Z. H. De Groot, Rotterdam, The Netherlands. Beige, soft, thin, flexible, smooth, speckled underside.
HP16	0.43	Aged but not dated, bookbinding, back with remnants of gold print, thick, Royal Library, Denmark. Pale upper side, stiff slightly translucent.
HP22	0.36	Aged but not dated, probably bookbinding, Royal Library, Denmark, goat/calf?
HP31	0.29	Aged but not dated bookbinding board, calf, partly horned, Royal Library, Denmark. Pale yellow/brown upper side, fibrous appearance, slightly translucent.
HP28	0.24	Aged but not dated bookbinding back. Royal Library, Denmark. Yellow/brown upper side, pale underside, flexible.

The aim in this paper is to demonstrate the type of information that can be obtained from DMA and solid state NMR spectroscopy. Some preliminary assessment is also made of the potential of micro-thermomechanical analysis. The interpretation of data is based on what is generally accepted about the primary structure of collagen and gelatin. The primary structure is defined as the sequence of the amino acid residues in its polypeptide chains [5]. In terms of primary structure this sequence has been described as a block copolymer containing imino acids (proline and hydroxyproline) with glycine at

every third position i.e. $–(Gly\text{-}A\text{-}B)_x\text{-}(Gly\text{-}Pro\text{-}HyPro)_y–$. The presence and size of the imino acids residues will have some effect on the mechanical properties since they will restrict rotation and lead to a certain rigidity in blocks containing them, and so the structure can be considered in terms of soft and hard blocks depending on whether glycine or proline occurs every third position [3].

DMA measures the mechanical properties of materials under a sinusoidal mechanical stress and relates these to the internal motions of polymer molecules or their hard and soft segments. Previous measurements have been made on primed canvas [6] and leather [7] and this paper reports measurements performed on parchment samples. Materials which show both elastic (hard) and viscous (soft) and time dependent behaviour are referred to as viscoelastic. DMA characterises the viscoelastic nature of the material through the nature and intensity of the observed transitions. These in turn provide valuable markers for the physicochemical state of the material. Previous work on dehydrated gelatin using thermomechanical analysis demonstrated that two transitions were observed [3] and the interpretation of the observed thermal properties was based on known aspects of the primary structure of gelatin. The transitions are referred to as glass transitions. Below the glass transition temperature the cooperative molecular motion along the chain is frozen causing the material to respond like an elastic solid to stress. As the temperature is increased some motion commences in the flexible side chains and this causes the modulus of the sample to fall. The modulus continues to decrease as the sample approaches its glass transition temperature, T_g, where the main polymer chain is set in motion and the sample changes from the glassy to the rubbery state. Thus the transitions correspond to molecular motions of the material during its heating. Information on the viscoelastic properties of parchment samples may provide a means of determining the actual state of the collagen block copolymer in the parchment samples and give an insight into the extent of crosslinking and the degree of crystallinity in the samples.

Micro-thermal analysis (μTA®) enables thermal analysis to be performed on a micron scale and the technique has been applied to polymers and composite materials [8]. In this paper some preliminary measurements are presented for the first time on historic parchment samples. In micro-thermomechanical analysis (μTMA®) the vertical displacement of the probe is monitored as the temperature is scanned. In addition information on the sample topography can be obtained together with thermal conductivity images which provide additional information on material properties.

The effect on the mechanical properties of heating the sample while immersed in water is also explored on the same set of samples. The hydrothermal stability of collagen fibres is a particularly good measure of the strength or quality of skin, leather and parchment materials, and the degree of their deterioration. The hydrothermal stability is characterised by a shrinkage of the material when heated in water at a defined temperature. This parameter has been measured using thermomicroscopy and differential scanning calorimetry [9, 10]. X-ray diffraction studies have shown alteration in molecular packing in collagen on water removal [11]. Measurement using DMA in reverse configuration mode on selected parchments has been described in a previous paper [12]. This mode of measurement was made possible by modification of the Mark 3 Rheometric Scientific DMA in studies of wetting and drying behaviour of textiles [13].

NMR is a non-destructive technique that can yield information on the chemical environment and mobility of the nuclei in a system [14]. It has previously been used to study organic compounds in non-metallic historical seals [15]. In this project solid state ^{13}C NMR was used to study parchment samples as received to determine the state of deterioration through analysis of changes in the chemical environment of the carbon atoms of the collagen polymer.

Experimental

DMA

Thermal scans

Samples (20×10 mm) were clamped in the tensile mode and measured between –130 and 120°C at 3°C min^{-1}, frequency 1 Hz and force 4 N. A second heating of the parchment samples was subsequently performed from –130 to 220°C at the same heating rate.

Creep

Measurements were made using the DMA (Rheometric Scientific Mark 3) in reverse configuration mode as described in a previous paper [13]. Samples were mounted in tensile clamps and a small constant force (0.1 N) was applied. This allowed for any expansion or contraction of the sample during immersion and on drying to be measured.

Solid state ^{13}C NMR

Fourier transform high resolution solid state NMR spectra were obtained under conditions of magic angle spinning (MAS) with (a) cross polarization and then with (b) power dipolar decoupling. CPMAS and single-pulse MAS/Decoupling ^{13}C NMR spectra were recorded at 75.5 MHz (7.05 T) on a Bruker MSL300 spectrometer using a standard Bruker magic angle sample spinning (MAS) probe with double-bearing rotation mechanism. The samples were fitted into the cylindrical zirconia rotor (7 mm external diameter) and then spun at MAS frequency 4.5–7 kHz (with stability better than ca. ±2 Hz). All spectra were recorded at ambient probe temperature. The ^{13}C chemical shifts are given relative to tetramethylsilane.

Micro-thermal analyser µTA® 2990

The 2990 Micro-thermal analyzer has the capability to act as both a thermal microscope with µTA® capability and as a fully functional AFM. It utilizes a patented probe design consisting of a platinum wire heater/thermometer constructed from a Wollaston wire and bent into a V-shaped point. This technique combines the capability of an atomic force microscope for surface imaging with the capability of performing thermal analysis on small areas of the sample (100 µm). Preliminary measurements were made on selected samples.

Results and discussion

DMA

Figure 1 displays the typical DMA response of unaged parchment. Two curves are shown logE' (modulus or stiffness of the sample) and tanδ (ratio of inelastic to elastic moduli). The sample on heating from –130°C shows a progressive decrease in logE' and then a larger decrease between 10 and 50°C. This is followed by a small increase in modulus as the sample stiffens with loss of initially adsorbed moisture. At 200°C there is an onset of sample softening and a decrease in modulus. The tanδ curve shows two major peaks and a shoulder peak. The peak below room temperature varied in intensity and peak position. It was also observed in the tanδ curves for historic parchment samples (Fig. 2). The tanδ curve for the unaged parchment (NP7) is included for completeness. The presence of the peak below room temperature may reflect contributions from side chains of the polypeptide chain. In cellulosic samples the variation in intensity of this peak has been observed with exposure to environments of high relative humidity [6]. Therefore the peak may also reflect differences in moisture content in the samples despite the second heating of the sample. The shoulder peak at 25°C occurs also on the first heating but with increased intensity. Its intensity appears to be dependent on the residual physically adsorbed moisture. The peak at 65°C can be interpreted as the temperature at which motion of the main collagen polymer backbone occurs.

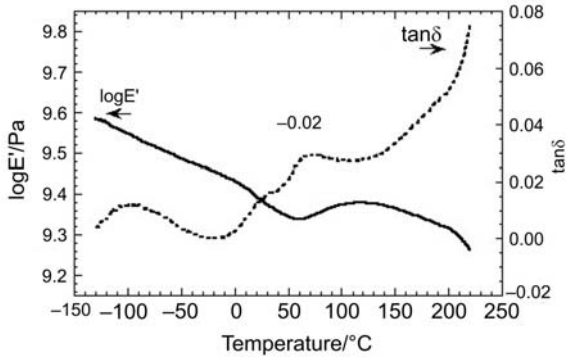

Fig. 1 DMA curves (logE' and tanδ) of unaged parchment NP7 as a function of temperature

Figure 2 displays the tanδ curves for unaged (NP7) and aged parchment samples (HP16, HP22 and HP31). The peak below room temperature is again present but differs in position and intensity in the historic parchment samples. They both exhibit two peaks at temperatures in the region of 90 and 140°C. Transitions at these temperatures have been reported in gelatin [3]. In HP16 the lower temperature peak is suppressed. The presence of these transitions will also be influenced by the degree of crystallinity and crosslinking present in the sample. So it is possible to say that some of the collagen in the aged parchments has been converted to gelatin. The presence of

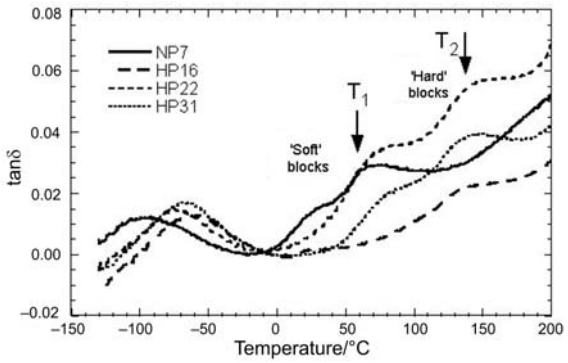

Fig. 2 DMA curves (tanδ) of unaged (NP7) and aged parchments (HP16, HP22 and HP31) as a function of temperature

the peak at higher temperatures indicates that there must be more rigid blocks containing proline and hydroxyproline along with glycine in the aged samples. Information from ^{13}C solid state NMR suggests an increase in hydroxyproline on ageing and more detailed discussion is given below.

Micro-thermomechanical analysis

Micro-TMA measurements for unaged parchment showed a softening temperature in the region of 40–50°C which is in the temperature range observed at the macroscopic level by DMA. Measurements were also made on HP31. In Fig. 3a, the left hand side, reveals the topography of the surface, and the right hand side, Fig. 3b, the corresponding thermal conductivity image (lighter regions correspond to high thermal conductivity). Numbered positions refer to locations where micro-thermomechanical measurements were made. Initially these were made on the sample as presented. Figure 4 shows the resulting μTMA® curves. The overall softening post 220°C prior to onset

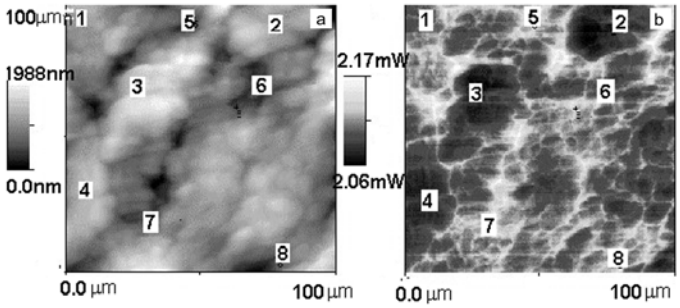

Fig. 3 HP31: a – displays the topography of the surface; b – provides the corresponding thermal conductivity image (light corresponds to high thermal conductivity). Numbered positions refer to locations where micro-thermomechanical measurements were made

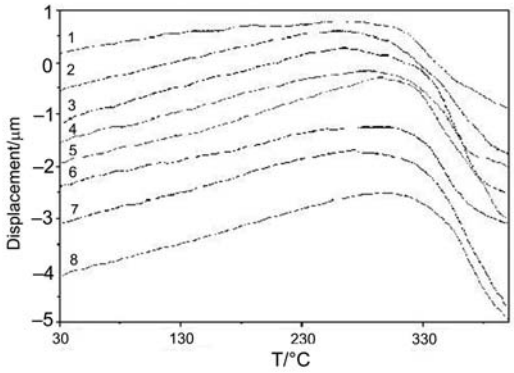

Fig. 4 HP31:μTMA curves correspond to the locations where measurements were made. 1 and 2 regions of low thermal conductivity (and therefore not visible in the right hand image) show less overall softening than area 7 region of high thermal conductivity (visible in the right hand image)

of degradation was observed. Locations 1&2 with low thermal conductivity (and therefore not visible in the right hand image) show less overall softening than location 7 with high thermal conductivity (visible in the right hand image). Micro DTA then revealed the presence of a small transition (Fig. 5) in the region of 140°C which again coincides with observations by DMA on the same sample.

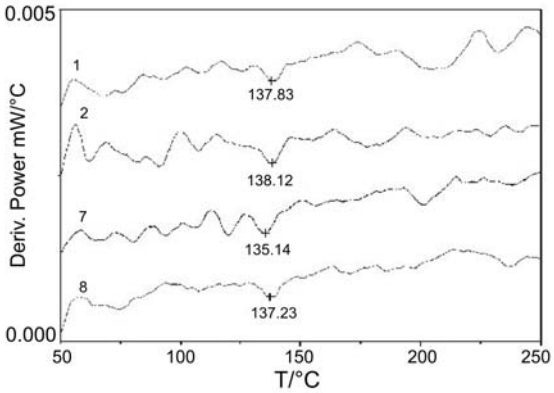

Fig. 5 HP31: The numbered μDTA curves correspond to the locations where measurements were made. Regions 1 and 2 of low thermal conductivity display transition at temperatures of 137.8 and 138.1°C, respectively, region 7 displays a transition at a slightly lower temperature 135.1°C, together with an earlier transition

In Fig. 6a the left hand side shows the surface topography of sample HP28. This sample had a similar DMA curve to HP31. The results presented here were performed on the sample prepared in thin section [16]. There is indication of improvements in image topography. Further work needs to be performed in the area of sample presentation for this type of study. Lighter areas represent the higher areas of the sample surface and the

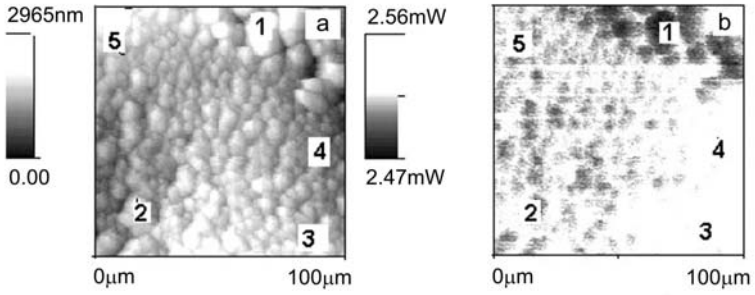

Fig. 6 HP28: a – shows the topography of the surface; b – shows the corresponding thermal conductivity image

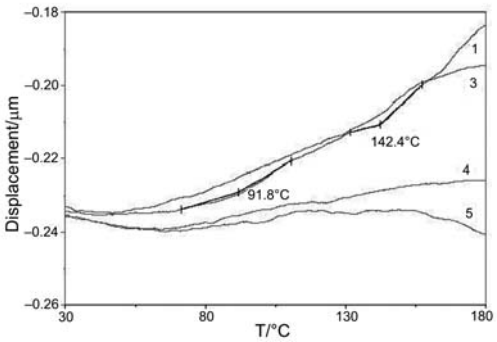

Fig. 6c Local thermal analysis experiments (μTMA®) at the locations indicated. At location 1 two transitions around 90°C and 140°C are detected with low thermal conductivity

darker areas represent the lower areas. The right hand side of Fig. 6b shows the thermal conductivity image: areas that are lighter are more conducting than those which are darker. Figure 6c provides the results of micro-thermomechanical measurements on selected marked locations of the sample area [1, 3, 4 and 5]. At location 1 two transitions around 90 and 140°C are detected with low thermal conductivity. Location 1 reveals transitions at temperatures seen in HP28 by DMA whereas location 4 (highly thermal conducting) reveals no transition. The fact that transitions at the microscale can be related to transitions at the macroscale (DMA) provides the possibility of studying archival samples where sample quantity for analysis is restricted.

DMA creep

The curve for HP31 is shown in Fig. 7. The graph is plotted as % displacement (y-axis) *vs*. time (x-axis), with the temperature profile superimposed ($y2$-axis). The stepwise increase in temperature from 30 to 85°C between 0 and 130 min can be seen in Fig. 7. On initial immersion in water the historic parchment HP31 showed a 5% expansion in length (positive displacement). Table 2 presents data from HP31 together

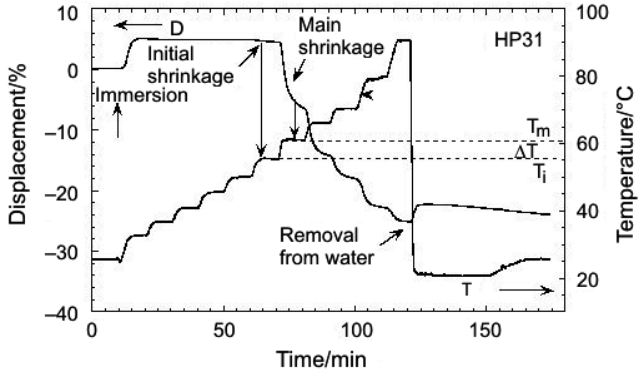

Fig. 7 Displacement (%) on heating plotted as a function of time for HP31

with the unaged sample NP7 and HP16 and HP22. The measured parameters provide a means for comparison between the samples. HP28 sample (discussed above) has the lowest shrinkage temperature. Correlation between this observation and chemical composition will be made in section below. Both HP28 and HP16 record transitions at similar temperatures to that of HP31 and HP22 but they are not as intense. The difference between these samples HP28, HP16, HP22 and HP31 can also be seen in shrinkage temperature interval. The HP type samples differ from NP7 in the measured main shrinkage (column 6 Table 2) (i.e. shrinkage occurring at the temperature of main shrinkage and the calculated shrinkage ratio (i.e. the main shrinkage divided by the total shrinkage).

Solid state ^{13}C NMR

Standard Material: Typical CPMAS spectrum of collagen

In Fig. 8 the ^{13}CPMAS spectrum of a modern parchment NP7 is presented. The features are similar to those of a rabbit skin glue film (10% by mass) that was previously tested in our laboratory and also to published work on canine Achilles tendon [17]. The spectral peaks may be assigned to functional groups of the component amino acid residues [18]. The peak at 71 ppm was allocated to C-4 of hydroxyproline. Its neighbouring peak at 60 ppm contains contributions from the C-2 of both proline and hydroxyproline, and the sharp peak at 43 ppm was attributed to the C-2 of glycine.

To enable comparison between spectra, peak fitting procedures were used to enable calculation of area ratios. This procedure is described in detail elsewhere [1]. Calculation of the area ratios of the peaks at 60 and 71 ppm *vs*. sample type shows that NP7 has the highest ratio whereas HP28 has the lowest ratio. The value for HP31 was mid-range and this was in keeping with the measured shrinkage temperatures where values for HP28 were lowest and HP31 mid-range (between unaged NP7 and HP28). In the measured ratio the numerator represents the contribution from C-2 from both proline and hydroxyproline and the denominator the contribution from C-4 of hydroxyproline. NP7 represents the intact collagen state in the non-degraded parchment. A change or decrease in this ratio indicates a change in one or other or both components i.e. decrease in proline,

Table 2 Summary of displacement (%) on immersion in water and then (%) shrinkage on heating on drying

Parchment	Change on immersion/%	Temp. of initial shrinkage, T_i/°C	Temp. of main shrinkage, T_m/°C	T_m–T_i (ΔT)/°C	Main shrinkage/%	Total shrinkage/%	Shrinkage ratio, R	Recovery on drying/%
NP7	–1.0	65	70	5	20	30	0.67	10
HP16	1.4	35	55	20	11	34	0.32	8
HP22	13.0	65	70	5	5	16	0.31	–
HP28	3.2	30	45	15	7	15*	0.47	–
HP31	5.0	55	60	5	5	30	0.17	3

*Figure approximate as sample split towards end of experiment. Actual value therefore slightly higher.

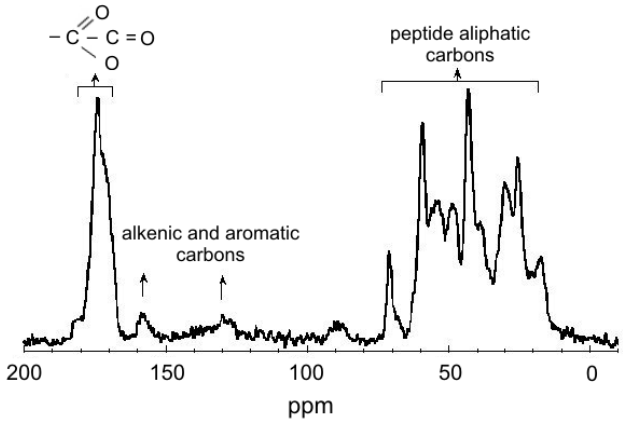

Fig. 8 Typical ^{13}C NMR CPMAS spectrum of parchment NP7

increase in hydroxyproline or both. Similarly calculation of area ratios of the peaks at 43 and 71 ppm show that NP7 has the highest ratio and HP28 the lowest. HP31 this again suggests an increase in levels of hydroxyproline which would account for the difference in mechanical behaviour of the samples.

Conclusions

Samples have been characterised according to their viscoelastic response. Some correlation between the nature of this response at the macro- and microlevel has been demonstrated and the need to optimise sample preparation procedures for micro-thermomechanical analysis of parchment demonstrated. DMA creep mode measurements enabled characterisation of shrinkage behaviour and measurement of shrinkage temperatures to be made. Lower shrinkage temperatures could be correlated with differences in amino acid residue composition, in particular proline and hydroxyproline. Data obtained by the various techniques can be linked in the following way: NMR has provided information on the amino and imino acid residues and variations in their calculated ratios have been measured in unaged and naturally aged samples. These differences correspond with differences measured in glass transition temperatures by DMA and in shrinkage behaviour, by DMA in creep mode immersed in water.

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