Structural Characterisation of Parchment at the Molecular Level by Raman Spectroscopy

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Raman spectroscopy is an excellent tool for investigating polypeptides, such as those contained in collagen and parchment. Information about bonding, vibrational modes and structural alterations resulting from damage or degradation due to exposure of parchment to various atmospheres for extended periods of time can be obtained by Raman spectroscopy. FT Raman spectroscopy has been used to study the structural properties of a large number of parchment samples (including new, historical and samples that have been "aged" in controlled laboratory conditions, the latter called hereinafter *accelerated aged samples*). Most spectra contain the basic features, which characterize a "new parchment" but some spectra contain *additional* features or *changes to existing* features.

For example, the upper spectrum in Figure 1 is obtained from a sample treated for 6 h in $SO_2/O_2/H_2O/N_2$ atmosphere. The new bands, marked by arrows, are easily observed and correlated to the presence of the gases that caused the damage.

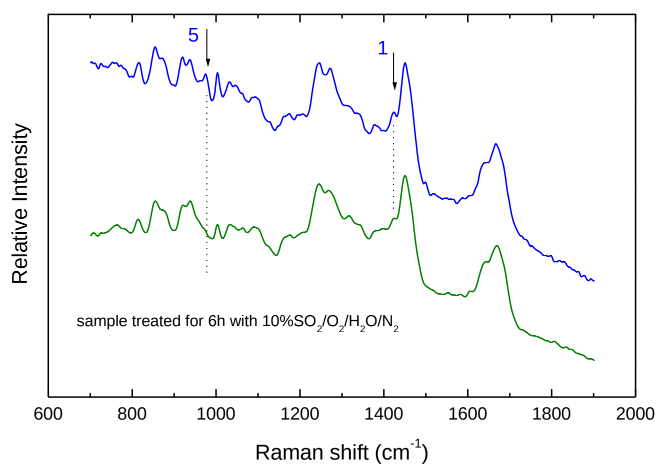

Figure 1. Raman spectra of parchment samples prior (bottom) and after (top) treatment with a 10% $SO_2/O_2/H_2O(sat)/N_2$ gas mixture. The arrows indicate new bands at 1425 and 975 cm^{-1}, marking an increase of free carboxylate ions (COO^-, band at 1425 cm^{-1}) and sulfation of parchment (band at 975 cm^{-1})

Often spectra obtained from samples that have been subjected to mild ageing conditions or even from certain historic parchment samples contain the basic spectral features, which characterize a "new parchment". Thus, to a first approach, it appears that as far as Raman spectroscopy is concerned the extent of deterioration seems to be insignificant for the applied range of ageing conditions. On a more positive note, since one of the objectives of the project is to establish an *early warning system*, a mathematical correlation (principal component analysis based on basis functions) may result in extremely useful remarks on these *seemingly* similar spectra.

The compositional and structural state of historical parchments can be assessed by identifying common *events* that among others may include: a) conversion to random coil; b) skeletal deformations; c) chemical alterations in amino acid profiles; d) hydrolysis of peptide bonds; e) denaturation; f) sulphuric acid (acid rain) attack; etc. These events, though in different extents, are reflected in the shape, position and appearance of characteristic features in the Raman spectra. As a result of the work and based on the correlations done to spectra of samples aged under known atmospheres a number of analytical markers/criteria are developed to assess the extent of damage by means of FT-Raman spectroscopy. According to these markers the above-mentioned *events* (should they have been occurred) provide *signatures* in the Raman spectra. The basic keys (analytical markers) include –but may not be restricted to–:

1. Resolution of band at ca. 1425 cm^{-1} (also spreading towards 1410 cm^{-1}), indicative of free carboxylate ions, resulting from hydrolysis of peptide bonds.
2. Alterations in relative intensities due to bands originating from different amino acid segments.
3. Disappearing or weakening/broadening features in the 890-940 cm^{-1} region (particularly at ca. 935 cm^{-1}) are indicative of skeletal (-C-C-) collapses due to transformation to random coil.
4. Broadening and shifts in position of Amide I and Amide III bands is indicative of peptide bond involvement in H-bonding and/or denaturation
5. Vanishing of the 1085 cm^{-1} band due to $CaCO_3$ is indicative of acid attack, upon which an appearance or an increase of existing feature could be identified at 975-1000 cm^{-1}(sulfate) and/or at 1145 cm^{-1} (adsorbed sulfur dioxide or $METSO_2$)
6. Emerging band at around 1045 cm^{-1}(nitrate), resulting from NO_2 pollution

The more samples are being tested the more it is possible to further validate the importance of some of the above markers. Following the recording of the spectrum from each sample we are able to classify the parchment under consideration to the four *damage categories* based on the above six analytical markers. The occurrence of the six "signatures"/criteria in the Raman spectra point to various extents of damage, although these need to be correlated with the other analytical techniques. A spectacular spectral change does not correspond necessarily to a great damage and (on the other hand) a barely visible change in the spectra may reflect a severe damage. Keeping in mind the above:

Group	Occurring "signatures"
1. No or very small changes	none
2. Minor changes	1,4
3. Medium changes	1,3,4
4. Major changes	1,3,4,5,6

Observations made in various stages of the accelerated ageing trials provide validation of markers #1, #4 and (as indicated recently) marker #3 as early warning signs resulting from exposure to atmospheres <u>not</u> containing SO_2 and/or NO_2 . Exposure to these gases is "betrayed" by markers #5 and #6.