Second Derivative and Peak Fitting in FTIR Analysis of Leather Protein: Investigation of Changes in Tanned Leather after Cleaning

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Introduction

Infra-red spectroscopy has been widely applied in materials of artworks and antiquities as it can detect the various bonds in the molecules, according to their vibrational modes. In the field of works of art, FTIR has been widely applied for characterizing both inorganic and organic materials. The peak fitting technique in FTIR spectra has been explored in the field of proteins [1,2]. When this technique is applied after deconvolution or second derivative of the amide I band, results in information on the contribution of *α*-helical, *β*-sheet or unordered structures in the material. However, little work has been done in the case of collagen-containing materials. This specific protein, abundant in bone, leather and parchment is clearly distinct from most other proteins as it contains a triple helix hyper-structure [3,4,5]. Moreover, extra difficulty arises from the fact that all collagen strands are in contact with different types of proteins, and bonded with a multitude of organic compounds included in vegetable tanning agents. The tropocollagen microfibril model has been widely accepted for the description of the collagen part of leather material [5,7,8]. A necessity arises on assessing the condition of cleaned leather with various agents and compare this to that of the original material. FTIR spectroscopy has been applied to evaluate the cleaning treatment practices on the leather material, in which, the difference spectroscopic technique has provided background evidence for changes in the various components of cleaned leather samples, including additives.

Results and Discussion

Second derivatives of FTIR spectra

The amide I band in all collagen-containing samples appears rather complex as the triple helix conformation at the Glycine, X, and Y positions (X and Y can be Proline or Hydroxyproline) of the collagen triplet region is dominated by variable hydrogen-bonding environments around the amide carbonyl groups.

In all spectra, the strongest peak-fitting contribution in the amide I band is at 1660-1663 cm^{-1}. The position of all other contributing peaks is considered relative to this basic peak. In non-quantitative terms, stronger hydrogen bonding (and generally, short-range interactions) would effectively reduce the stretching energy of the C=O group and lower frequencies of the contributing peak is observed in the infra-red spectrum. On the other hand, weaker hydrogen bonding (or long-range interactions) would result in higher frequencies of the contributing peaks.

In any FTIR spectrum of leather samples, infra red amide I band significantly tails off towards lower wavenumbers as compared to other collagen-containing materials such as animal glue and bone; this illustrates the presence of tanning agents and more significantly, the various stabilizing effects of tanning.

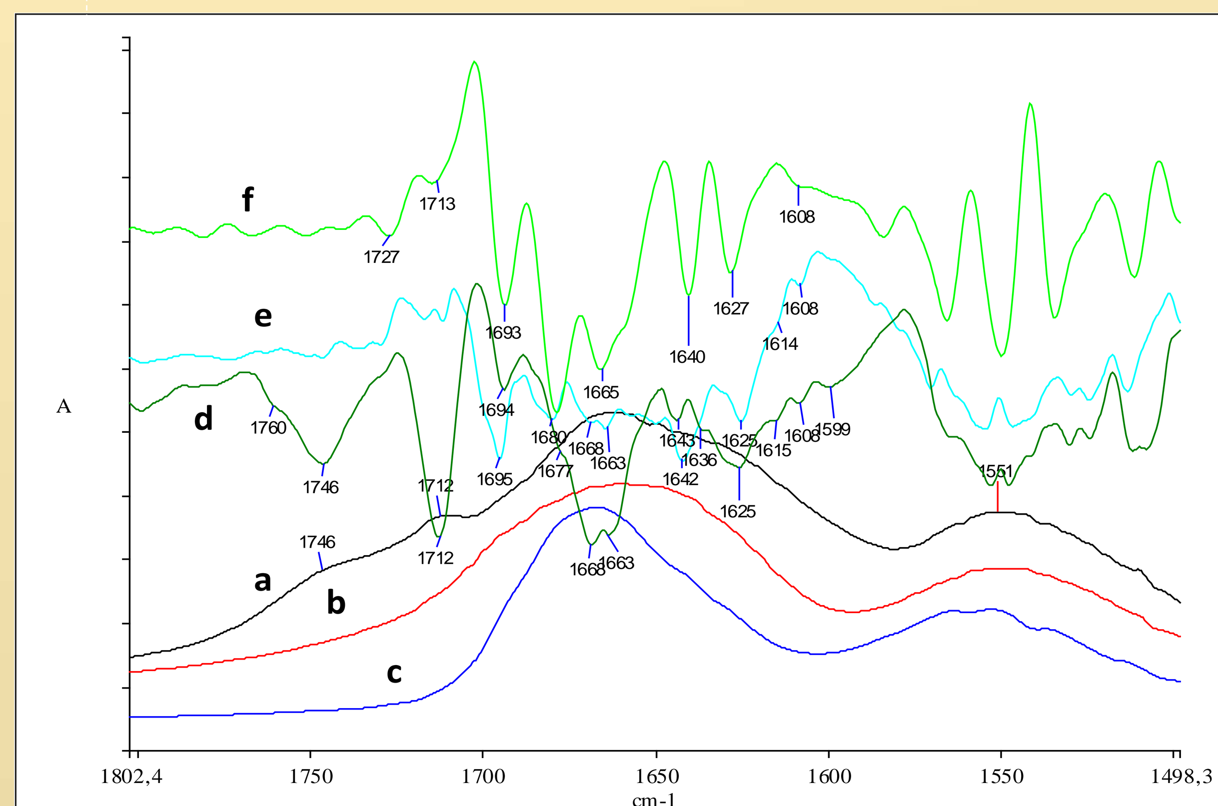

Figure 1: Amide I and II bands of two different leather coupons taken from the same oak-tanned leather sheet and other collagen-containing materials.
(a) untreated leather; **(b)** fresh ivory; **(c)** animal glue;
(d) second derivative of spectrum **a**; **(e)** second derivative of spectrum ***b*** and
(f) second derivative of spectrum ***c***.

Second derivative and peak fitting assignment

The observed second derivative peaks can be assigned/categorized into several types of amide carbonyl interactions (below ranked by significance of contribution [4,5]. Peak fitting resulted to similar overlapping bands:

i. Ordered inter-chain hydrogen bonds; these correspond to direct hydrogen bonding between proline C=O groups of one chain and the glycine NH groups of another, within the collagen triple helix (**1662** cm^{-1}).
ii. Hydrogen bonds among random-chain shift of the previous band (**1668** cm^{-1})
iii. Water-mediated hydrogen bonds due to bridges of several water molecules establishing a network that surrounds the triple helix. These water molecules significantly stabilize the collagen structure with hydrogen bonds between the hydroxyl groups of hydroxyproline and the peptide C=O and NH groups between different chains (**1640** cm^{-1}) and within each chain (**>1640** cm^{-1}).
iv. Water-mediated hydrogen bonded amide carbonyls; water molecules are incorporated between chains in places were alanine residues are located, rendering a more stabilized structure (**1625** cm^{-1}).
v. Hydrogen bonds among neighboring C=O and N-H groups within the same ordered collagen 3.3 helix; generally expected at higher wavenumbers than the α-helix of other proteins and may overlap with other peaks of collagen. The significance of this contribution is not documented. (**>1668** cm^{-1})
vi. Hydrogen-bonded amide C=O and N-H groups between neighboring triple helices (**>1680**)
vii. Water- mediated hydrogen bonding to amide carbonyls between neighboring triple helices (**>1670**)
viii. Backbone aminoacid carbonyls (around **1695** cm^{-1})
ix. Aromatic peaks from tanning agents and certain aminoacids (**1613-1600** cm^{-1}).
x. Interactions of the collagen structure with tanning agents (such as newly formed ester bonds and additional hydrogen bonds); not adequately documented in the literature.

Cleaning is an irreversible step in conservation treatments; therefore before leather cleaning, care should be taken on:

- Chemical composition of object, including additives
- The nature of depositions (impurities, dirt)
- The state of preservation of the object
- The cost of the select cleaning method

General criteria of the cleaning of leather:

- Not inducing any type of alteration to leather material
- Not forming any type of products harmful to the object
- Capable of effective cleaning, i.e. remove impurities and preserve the structure of the object
- Should be easily inspected by specialists
- Applicability of cleaning methods and safety of human been should be taken into account

Table I: Assignment of basic FTIR bands detected in a typical oak-tanned leather sample

FTIR band, cm^{-1}	Assignment
3500-3350(br,s)	vO-H
3330(br,s)	vN-H
3077(sh)	δNH_2 (overtone)
3010(w)	vC-H (aromatics in tanning agent)
2958, 1851(w)	v_{as} CH_3 (oil compounds, wax)
2929(m-s)	v_{as} CH_2 (oil compounds)
2919(sh)	v_{as} CH_2 (wax)
2853(m)	v_s CH_3
1735-1745(sh)	vC=O (ester groups in tanned material)
1712(sh)	vC=O (galloyl ester in tanning agent)
1663(s)	Amide I band of leather protein
1551(m)	Amide II band of leather protein
1457(m)	δ_s CH_2+ δ_{as} CH_3
1385(w)	$\delta_{i\text{-}p}$C-OH (ellagic acid/tannins)
1375(w)	Proline+hydroxyproline terminal aminoacid
1340(w)	δ_s CH_3
1237(m-w)	Amide III band of leather protein
1034(w)	v_{as}C-O-C=O (tanning agents and products)

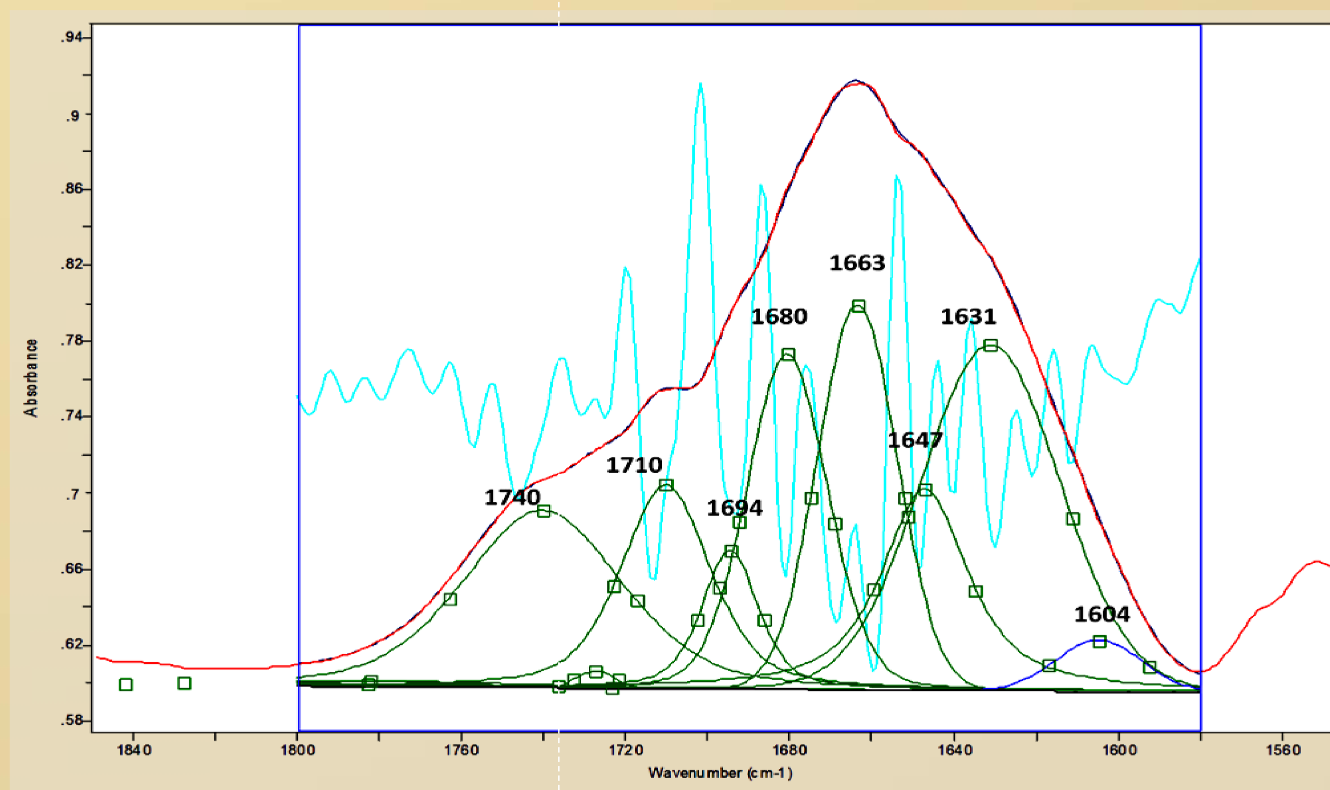

Figure 2: Peak fitting of the amide I band (KBr FTIR spectrum) of material sampled from untreated leather area; A mixed Gaussian and Laurentian (50:50) model is followed.

- Red spectral line: experimentally acquired spectrum;
- black spectral line: simulated spectrum after peak fitting;
- green curves: the contributing peaks with their wave numbers;
- light blue curve: second derivative of the experimental spectrum.

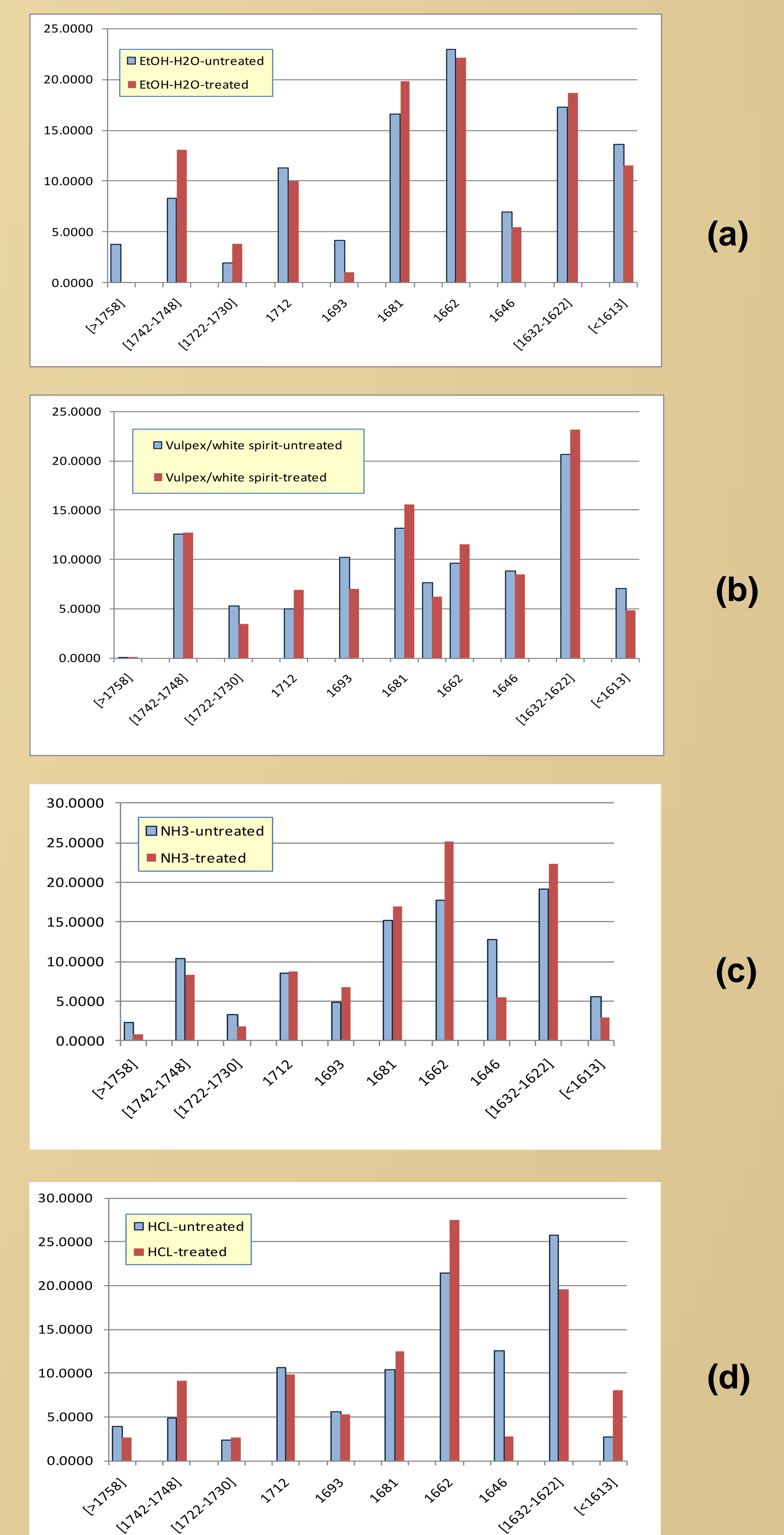

Figure 3: Histograms illustrating changes in the secondary structure of the protein material of leather.

Peak-fitting results from the amide I band of treated areas are compared with those of the corresponding neighboring untreated tanned leather areas.

Lower wavenumbers with respect to the 1662 cm^{-1} band (to the right) correspond to stabilizing short-range hydrogen bonding;

Higher wavenumbers correspond to either the destabilizing long-range hydrogen bonding, or the presence of tanning agents; the contribution at <1613 cm^{-1} corresponds to aromatics.

Treatments with: **(a)** water-ethanol; **(b)** Vulpex in white spirit; **(c)** aq. ammonia solution 1%; **(d)** hydrochloric acid (3.3%).

Conclusions

Preliminary attempts on protein secondary structure analysis through second derivative and peak fitting of FTIR spectra in the amide I region, allowed the detection of various contributing interactions of the amide bond in the protein material. Comparison between treated and untreated neighbouring areas of leather samples showed evidence that the possible structural modifications of the material can be monitored with the proposed spectroscopic methodology. Ethanol-water mixture and white spirit-based detergent (Vulpex) did not show detectable changes in the protein structure. Aqueous solution of ammonia and hydrochloric acid showed evidence for destabilization of the protein structure.

Assessing the various experimental limiting factors would lead to a reliable design for assessing the structure of tanned leather material and further, the possible induced changes after treatment with cleaning agents.

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