Microchemical Journal 114 (2014) 32–41

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Study of Egyptian mummification balms by FT-IR spectroscopy and GC–MS

M. Ménager *, C. Azémard, C. Vieillescazes

Avignon University, IMBE UMR7263/IRD237, F-84000 Avignon, France

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 22 November 2013
Accepted 29 November 2013
Available online 7 December 2013

Keywords:
GC–MS
Infrared spectroscopy
Mummification balms
Chemical markers
Resins
Lipids
Beeswax
Ancient Egypt

ABSTRACT

Fourier transform infrared spectroscopy (FT-IR) and gas chromatography coupled with mass spectrometry (GC–MS) were used in order to analyse twelve mummification balms from mummy skulls of the *Musée des Confluences* (Lyon, France).

For FT-IR analyses, a simple extraction protocol in dichloromethane and water allowed to separate the materials by their polarity. This study clearly shows that the organic fraction is the main constituent of the Egyptian balms and hides much information in the bulk analyses (made without any extraction). Infrared absorption reveals the presence of (i) several organic materials (proteins, polysaccharides), (ii) inorganic salts ($CaSO_4$, $CaCO_3$ and NH_4Cl) possibly used as natron in ancient time, and (iii) ochre used in order to dye the bandages.

GC–MS analyses were made on the organic fraction of extracted balms, previously trimethylsilylated before injection. Biomarkers and degradation products of oils, fats, resins (with oleanene, lupene, lanostane, masticadiene, and abietane compounds) and beeswax were found. These materials were often used in combination. Many identified byproducts (di and triterpenic molecules, hydroxylated fatty acids, etc) give us the opportunity to discuss the different degradation reactions taking place in such archaeological material. Furthermore, beeswax was identified in numerous samples thanks to the presence of long chain alkanes, long chain fatty acids and palmitate ester. In one balm, the co-occurrence of *brassicaceae* oil chemical markers and cholesterol (and its degradation products) shows the combine use of oil and fat. Finally, a great correlation and complementarity was observed between the two analytical techniques.

1. Introduction

From around −5000 BC to hundreds of years AD, Egyptians preserved corpses in order to insure their eternal life. Thus, in this society, mummification was of major importance. A highly elaborated process divided in numerous steps was developed and evolved throughout the ages [1,2]. Employed techniques were specific of the geographic area, time, body part, social status and maybe age and sex of the dead [3]. All along mummification, an organic balm was applied as antibacterial, water-repellent material, fragrance and maybe for religious symbolism associated with different plants [4].

Balm composition can be elucidated by various analyses [5–12]. To this point, it is known that balms can be composed of mixture of oils, fats, waxes (and especially beeswax), resins, gums, salts, bitumen and various barks and spices [13,14].

Thus, to assess the initial composition of such samples, chemists must deal with a crossover of molecules from various origins and different degrees and ways of degradation.

To overcome such difficulties, a powerful approach is to link techniques that provide general information on the sample to more selective ones. Among the possibilities, Fourier-transform infrared spectroscopy (FT-IR) and gas chromatography with mass spectrometric detection (GC–MS) are two promising and complementary techniques [15,16].

FT-IR allows a non destructive fingerprinting of the sample without any chemical transformation. On the basis of infrared absorption, it is possible to identify different materials present in a sample, as indicated in many works that performed the identification of fresh organic and inorganic materials [17–20]. In archaeological samples, materials are often in complex mixtures of components in various degradation states. For organic substances, such conditions do not allow any extensive identification by a flowchart as usually done in the case of fresh materials [17,18]. Despite that, some identifications of inorganic and organic components are still possible in mummification balms by using FT-IR [21]. Salts and gum are quite insoluble in dichloromethane but soluble in water [22], thus, they can be easily separated by extraction using the proper solvent. Such approach allows the analysis of organic and inorganic fractions and the collection of information about non extractable fraction [23]. Such kind of systematic work has never been done on an archaeological material. In Table 1, different characteristic absorption wavenumbers for some fresh material found in mummification balm are given.

* Corresponding author at: Equipe Ingénierie de la Restauration des Patrimoines Naturel et Culturel – IMBE, 33 rue Louis Pasteur, 84000 Avignon, France.
E-mail address: matthieu.menager@univ-avignon.fr (M. Ménager).

0026-265X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.microc.2013.11.018

Table 1
Characteristic infrared wavenumbers of different fresh materials possibly found in mummification balm [17,18,23,24]. All data are in cm^{-1}.

Dichloromethane soluble material Organic material, 3440–3420 (OH), 2950/2850 (νCH), 1700–1720 (C═O)	
Waxes	1460 (C═H bending), 2926 (νCH_2), 2850 (CH_2 Stretch), 1466/1462 and 730/720 (presence of doublets for semi-crystalline structures)
Oils and fats	2926 (νCH_2), 2850 (CH_2 Stretch), 1740, 3440–3420 (OH)
Resins	2926 (νCH_2), 2850 (CH_2 Stretch), 1710–20, 3440–3420 (OH)
Water soluble material	
Gums	1600 (intramolecular bound water & carboxyl group), 1455 (C═H bending), 1415 (CH deformation), shoulder at 1149, 1080 (CO), shoulder at 1035, 780, Poor C═H stretch.
Protein	3295 (bonded N═H stretching), 1650 (C═O absorption of amid I), 1554 (deformation of amide II NH_2), 1449, 1318, 1243, 1169, 1084, 1030 (unassigned bands)
Inorganic salts	cf text

In many cases, FT-IR analyses are not sufficient to allow a precise identification of aged mixtures of natural materials. Thus, GC–MS can be used as a powerful complementary analytical technique.

Thus, GC–MS has been extensively used in order to elucidate archaeological sample composition [25] and more specifically balm composition [5,6,21,26,27]. For this purpose, specific chemical markers give precious information on materials and composition of sample [28–30]. Indeed, for plant materials, the presence of different chemical markers allows to find genus or even species of the original plant [31]. Three types of chemical markers can be pointed out: (i) biomarkers that are present in the original material, (ii) natural degradation markers linked to normal alteration of initial chemical composition and (iii) anthropogenic markers that indicate a specific treatment of the sample. In Table 2, a list of different chemical markers previously found in the archaeological chemistry of Egyptian balms is indicated. However GC–MS is not suitable for inorganic materials, and cannot be used on natural polymers without specific preparation of the sample [32].

In this work, twelve balms were sampled from mummy heads of the *Musée des Confluences* (Lyon, France). These skulls were previously studied by endoscopy, radiography and archaeological data [33]. In order to obtain chemical information from such archaeological material, samples were analysed by FT-IR spectroscopy and GC–MS to allow pertinent identification of organic and inorganic materials. Thus, the aims of this work were: (i) to develop a protocol for balm analysis by FT-IR spectroscopy, (ii) to validate FT-IR results by GC–MS data, and (iii) to bring knowledge to improve the correlation between chemical and archaeological data on Egyptian mummification balms.

2. Experimental section

2.1. Sample description

The analysed samples come from the collection of the *Musée des Confluences* in Lyon [33]. The twelve mummified heads were named B1, B6, B9, B10, B13, B19, B26, B32, B33, B35 and B42. On the B19 skull, the balm was taken from two different spots: (i) from the bandage filling the left eye socket, and (ii) at the lower part of the chin. Samples were all black and amorphous pieces of resin-like material with more or less heterogeneities in the composition (white mineral inclusion, orange-brown sticky material, etc). Samples were collected on the external part of the bandage at different locations. The weight of the samples varied from 23 to 105 mg.

2.2. Materials

All reactants were of the highest purity grade available. Aqueous solutions were prepared with deionised ultrapure water which was purified with ultrapure water (18.3 MΩ·cm) from a Milli Q device (Millipore).

2.3. Microchemical analysis

The presence of sulphate was shown by the reaction of solid balms with $BaCl_2$ [42]. Specific identification of proteins requires the pyrolysis of the sample in the presence of calcium oxide according to Odegaard et al. [43]. Iron(II) detection was done by reaction with thiocyanate (KSCN, 160 $g \cdot L^{-1}$) in acidic medium according to classic protocol [42]. Iron(III) was confirmed by reaction with potassium ferricyanure ($K_3[Fe(CN)_6]$, 100 $g \cdot L^{-1}$) in acidic medium following [44]. All microchemical analyses, except protein test, were done under a binocular microscope.

2.4. FT-IR analysis

Preparation of samples was made according to the protocol described in Fig. 1. The solvents, dichloromethane and water, were chosen according to Sarmiento et al. [23]. Organic fraction was split into two in order to perform both FT-IR and GC–MS analyses.

Table 2
Characteristic chemical markers of some fresh and aged materials found in mummification balms [25,34–41].

	Botanical or animal origin	Chemical markers
Oils/fats	*Oils*: castor, balanos, safflower, horseradish, linseed, sesame, olive, almond, radish, colocynth, lettuce, poppy, cinnamon, tiger nut, rape *Fats*: beef and mutton's tallow, pig, duck and goose fat, cow, goat and sheep's milk, hen's eggs	*Biomarkers*: fatty Acid (FA), Sterol, Tri- and Di-acylglycrols (DAG & TAG). For more precise identification see [26,35]. ↙ *Oxidation of unsaturated FA*: dicarboxylic acid, ↙ *Hydroxylation of FA*: hydroxyl or dihydroxy FA, ↙ *Heating treatment*: long chain lactones, long chain ketones
Beeswax	Bees	*Biomarkers*: Palmitic acid ester (W), n-alcane (AL), FA, n-Alcool ↙ *Hydroxylation of W*: hydroxylated hexadecanoic acid esters
Mastic resins	Anacardiaceae (species *Pistacia lenticus*)	Oleanonic, Moronic, 11-hydroxyoleanolic, masticadienoic acids ↙ *Degradation products*: 28-norolean-17-ene-3-one
Olibanum resins	Burseraceae (*Boswellia*)	Boswellic, O-acetyl boswellic, 24-lupeolic, 3-O-acetyl-lupeolic, 11-ceto-β-boswellic, 3α-cetyl-11-ceto-β-boswellic acids ↙ *Heating treatment*: 24-noroleana-3,12-diene, norursa-3,12-diene, 24-norlupa-3,20(29)-diene
Conifer resins	Pinaceae (*Abies*, *Pinus*, *Cedrus*) Cupressaceae (*Cupressus*, *Juniperus*, *Tetraclinis*)	*Biomarkers*: Δ8-isopimaric, pimaric, sandaracopimaric, isopimaric, levopimaric, palustric, abietic, neoabietic acids, larixyle acetate ↙ *Natural oxidation products*: dehydroabietic (DHA), 15-DH-DHA, 7-oxo-DHA, 15-OH-7-oxo-DHA, 7,15-diOH-7-oxo-DHA ↙ *Heating treatment*: 18 and 19 nor-abietatriene, tetrahydroretene, retene, 15-OH-DHA, methyl-dehydroabietate, 7-methyl-retene, 18-nor-7-oxo abietane
Bitumen	Transformation of ancient organisms and algae	Hopanes, steranes, polycyclic aromatic hydrocarbon, AL