MaSC
Users' Group for **Mass Spectrometry and Chromatography**

Sixth MaSC Workshop on 3rd – 4th and 7th June, 2013

We will meet at the Department of Chemistry and Industrial Chemistry (Dipartimento di Chimica e Chimica Industriale), VIA RISORGIMENTO 35, Pisa, at 9:00

During the workshop participants will be divided into two main groups. Each group will be further divided into two sub-groups when working in the laboratory
The time plan of the workshop is as follows:

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<tr><td rowspan="2"></td><td colspan="2">Monday</td><td colspan="2" rowspan="2">Tuesday</td><td colspan="2" rowspan="2">Friday</td></tr>
<tr><td colspan="2">welcome, introduction</td></tr>
<tr><td>group 1</td><td>group 2</td><td>group 1</td><td>group 2</td><td>group 1</td><td>group 2</td></tr>
<tr><td>morning</td><td rowspan="3">organic pigments and dyestuff analysis</td><td>lipid material analysis</td><td>lipid material</td><td rowspan="2">organic pigments and dyestuff</td><td colspan="2">proteomics analysis</td></tr>
<tr><td>afternoon</td><td rowspan="2">proteomics</td><td>proteomics</td><td rowspan="2">discussion</td></tr>
<tr><td colspan="2">Seminar Agilent technologies</td></tr>
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1. ANALYSIS OF NATURAL ORGANIC PIGMENTS AND DYESTUFF

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Organic dyes of vegetal and animal origin have been employed through human history for colouring skin, painting, and for dyeing. Their characterisation is a challenging task for chemists, due to three main problems: the wide range of possible dye source and the vast number of chemical classes they belong to; the small amount of sample available for analysis, and the low amount of coloured compounds (chromophore-containing molecules) in them; the low stability of chromophore-containing molecules themselves and the complexity of their degradation processes (interconnected to the matrix degradation processes). The target chromophore-containing molecules are polar, water-soluble molecules; thus, the most widely used technique is reverse phase liquid chromatography HPLC with diode array detectors and/or MS detectors. The workshop will focus on the analysis of natural organic dyes by using HPLC-PDA and HPLC-MS, with particular attention to sample preparation (such as hydrolysis in acidic methanolic solution, extraction with complexing agents, or organic solvents depending on the stability of the chromophores, and the dyeing technique)

Program:

- Introduction in dyes (some chemistry, history and origin; analytical procedures; instrumental set-up)
- Introduction to HPLC-PDA. We assume that participants have basic knowledge of HPLC but not necessarily with PDA (practical experience is not strictly necessary, but nice to have). So in this block we explain about the pump, the injector, the column oven, the detector and how to treat HPLC solvents.
- Sample preparation, from reference and historical samples provided by the organizers (or by the participants in agreement with the organisers). We will discuss the practical issues and perform the analyses.
- Interpretation of data relative to reference materials which were analyzed prior to the workshop.
- Analysis of samples on HPLC-PDA, data interpretation and comparison with data obtained by HPLC-ESI-Q-ToF.

2. ANALYSIS OF LIPID MATERIALS

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Oil paint and modern oil paint. *The analysis of the glyceride fraction of a lipid paint binder can help identification the material and understanding the transformation processes undergone during curing and ageing. In addition, the analysis of the glyceride fraction of modern industrial oil paint can be extremely useful in understanding the effective composition of the commercial products. Alkyd resins were introduced as paint binders in art in the 1940s, and are an industrial evolution of the classical oil paint media. The workshop will focus on the HPLC-ESI-Q-ToF analysis of the glyceride content of commercial alkyd paints to assess the synthesis processes involved during their production, such as transesterification, heating and the addition of synthetic fatty acids.*

Archaeometry*. The identification of the source of* ***lipid materials in archaeological or historical objects*** *can yield an important contribution to the knowledge of past technologies and to the planning of conservation strategies. Oils and fats were used not only as food but also as paint media, illuminants or ingredients of cosmetics and medicines. The workshop will focus on*

the application of HPLCESI-Q-ToF analysis of overall triglycerides (TAGs) profile as a complement to the more conventional GC/MS analysis of fatty acid after hydrolysis. This represents a promising approach for the identification of the botanical or animal source of lipids in archaeological samples. (For example: APS is observed only in pork samples while MSS and MPS only in ruminant; MPP, POS and PSS are typical of animal products while plants are characterized by high levels of highly unsaturated linoleic- and linolenic-containing triglycerides; high levels of OOO are typical of olive oil; high levels of SSS are found in ovine fat.)

Program:

- Introduction about lipids, lipidomics, differences between GC-MS and HPLC-ESI-Q-ToF approach
- Sample selection under the binocular microscope (1 archaeological + 1 modern paint) – samples will be provided
- Sample treatment
- Introduction about HPLC-ESI-Q-ToF + familiarization with the hardware / software
- Analysis on HPLC-ESI-Q-ToF of reference material, archaeological and modern paint samples.
- Discussion about
    - Different columns – separation efficiency
    - Ionisation by ESI *v* sionisation by APCI
    - Different detection modes (full scan, target MS/MS, auto MS/MS)
    - Interpretation of mass spectra of lipids
    - Contamination
- Identification of lipids in the archaeological and modern paint samples and comparison with results obtained by GC/MS on the same samples– complementarities.

3. ANALYSIS OF PROTEINS

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Proteomics in Cultural Heritage. *Proteomic strategies can be adapted to overcome the requirements and difficulties presented by samples in the field of cultural heritage, allowing a rapid, reliable and often unambiguous identification of proteins in a micro-sample. As in a classical proteomic procedure, the proteinaceous materials are enzymatically digested with specific proteases, typically trypsin, which selectively cleaves the peptide bond following basic amino acids (namely lysine and arginine). From each individual protein, a specific set of peptides is released following the enzymatic digestion that constitutes a molecular "fingerprint" of that particular protein. This peptide fingerprint is extremely efficient in identifying proteins. The peptide mixture originated by enzyme digestion can be analyzed by mass spectrometry procedures leading to unambiguous identification of proteins.*

The workshop will illustrate in practice a strategy that include (1) a minimally invasive method based on the tryptic cleavage of the sample without protein extraction; (2) the analysis of the peptide mixtures by LC-MS/MS; (3) Protein sequences databases queries. Nanoliquid chromatography coupled to tandem mass spectrometry analysis (nano LC-MS/MS) is the best approach to be used with samples containing several proteins in mixture, and when the samples are degraded and small, as those from cultural heritage. In the workshop LC-MS/MS will be used on paint samples, and data will be compared to those obtained from the same samples using a nano LC-MS/MS system.

Program:

- Introduction about proteomics, analytical procedures, analysis by nanoLC-MS/MS, and databases queries
- Selection of a paint sample under the binocular microscope– samples will be provided
- Sample treatment
- Sample analysis by HPLC-ESI-Q-ToF
- Discussion about
    - Mass spectra interpretation
    - Protein sequences databases queries
    - Comparison between results obtained by LC-MS/MS and nanoLC-MS/MS on the same sample