Confocal Raman microspectrometry imaging combined with chemometric methods for environmental applications

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The atmosphere contains a large variety of particles in suspension (aerosols) that differ in chemical composition as well as in size distribution depending on the region of the atmosphere, the meteorological conditions and the anthropogenic activity. Their dimensions range from a few nanometers to tens of micrometers and their atmospheric life time can be as long as several weeks so that they can impact the global as well as the regional climates.

The knowledge of the chemical composition of aerosols is of great importance due to their potential adverse effects on the environment and human health. For instance, despite the strong decrease in industrial emission of metallic particles, lead-enriched aerosol particles are still emitted so that fallout represents the main source of lead pollution in soils. Routes of exposure for humans occur either by inhalation or through consumption of contaminated foods. The physico-chemical composition of aerosols must be known at the level of the single particle to assess impact. Due to the small size of typical aerosol particles, the challenge is physico-chemical characterization of aerosols at the micron scale.

The electron beam techniques (scanning electron microscopy coupled to energy dispersive X-ray microanalysis, SEM-EDX) are the most commonly used to characterize the internal structure and elemental composition of particles with micrometer spatial resolution.

The ion beam techniques (for instance time of flight secondary ionization mass spectrometry, ToF-SIMS) can give the composition of the very first nanometers of the surface.

However, neither SEM-EDX nor ToF-SIMS can give information from the sample in ambient conditions due to the need for operation under low pressure which may modify the aerosol particle structure.

Confocal Raman microspectrometry which combines the molecular analysis capabilities of Raman scattering and the spatial resolution of optical microscopy is well adapted for obtaining direct molecular information on individual micron size aerosol particles under ambient conditions. Currently automated Raman systems are available for acquiring two dimensional molecular images with a lateral resolution limited by light diffraction. However, the large chemical heterogeneity of aerosol particles, often compounded by a broad luminescence background can result in severe overlapping of spectral information and can limit the wide use of Raman microscopy for the study of the internal chemical structure of aerosol particles.

The combined used of automated mapping with relevant interactive self-modeling mixture analysis (Multivariate Curve Resolution) for the treatment of Raman images provides information about the molecular characteristics and distribution of molecular species within samples [1]. This procedure is especially useful for the analysis of sample mixtures where the chemistry and spectroscopy are not well known. No explicit information about the sample is required other than a set of spectral data for a mixture of several components where the composition is varying. This curve resolution technique can help to find the number of components in mixture and to extract the spectra of the individual components including fluorescence signal and corresponding semi-quantitative concentration profiles. The extracted spectra are used to identify each component by performing library searches. The reconstructed molecular images obtained from the resolved spectra and their respective contributions provide spatially resolved chemical information about the speciation and heterogeneity of environmental samples.

Characterization of particles emitted from a lead smelter.

Micron and sub-micron sized particles are emitted in the atmosphere by lead smelters; they can be transformed and transported over long distances depending upon the weather conditions. Figure 1 shows Raman images (obtained with the above procedure, using red laser excitation) of airborne particles indicating aggregation of windblown lead containing particles emitted by smelters with other atmospheric species. Particles were collected using cascade impaction. The Raman spectra for $PbSO_4$ (anglesite), $PbSO_4.PbO$ (lanarkite) and $(NH_4)_2SO_4$ were separated using the method described above, despite their spectral similarity.

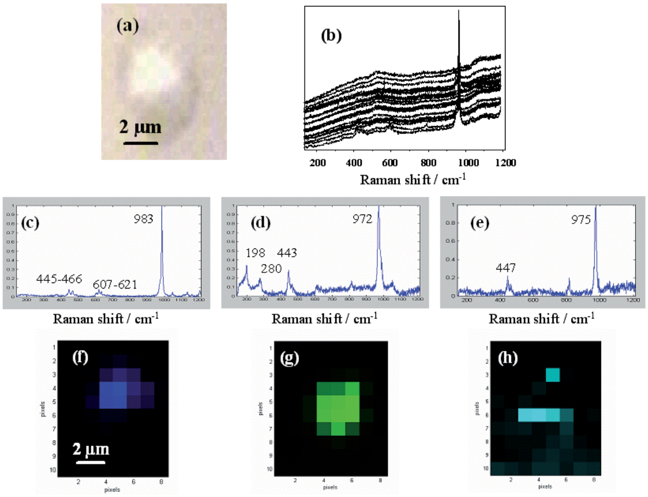

Figure 1: Raman imaging of lead containing particles emitted in the atmosphere by lead smelter facility. (a) optical image; (b) spectra of the 80 pixels of the image; Resolved Raman spectra of (c) $PbSO_4$; (d) $PbO.PbSO_4$; (e) $(NH_4)_2SO_4$; Raman images of (f) $PbSO_4$; (g) $PbO.PbSO_4$; (h) $(NH4)_2SO_4$. The smoothing procedure of the Raman imaging software was not used.

Airborne contamination of plants by lead

Due to industrial airborne pollution, Pb-containing particles can be deposited at the surface of leaves of terrestrial plants and then penetrate by crossing the leaf cuticle. The knowledge of Pb speciation inside the leaves may be of interest for risk assessment of population exposure to atmospheric metal contamination. Raman microimaging using UV laser excitation (266 nm) was applied to identify spatially-resolved speciation of lead in lettuce exposed for 43 days to atmospheric Pb smelter-originated fallouts [2]. UV excitation was necessary to probe the leaves since biological molecules such as chlorophyll exhibit strong fluorescence emission when excited with visible radiation. Thus, in these spectral regions between 400 and 800 nm, the fluorescence signal overlaps and masks any underlying Raman features. In the UV domain the Raman and the fluorescence spectra are sufficiently separated and do not interfere. We have demonstrated that lead compounds originating from primary particles (Figure 2) were located beneath the organic layer (cuticle) of the contaminated leaves and induce toxicity symptoms in the form of necrotic zones (necroses) (Figure 3).

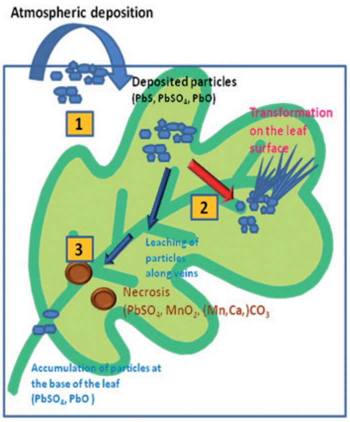

Figure 2: Tentative pathways for lead uptake after deposition of Pb-containing particles. Deposition of particles on the leaf surface (1), chemical transformation on the leaf surface leading to secondary Pb-containing phases and possibly solutes (2). Toxicity symptoms (necroses) induced by the presence of the contaminated particles on the leaf (3).

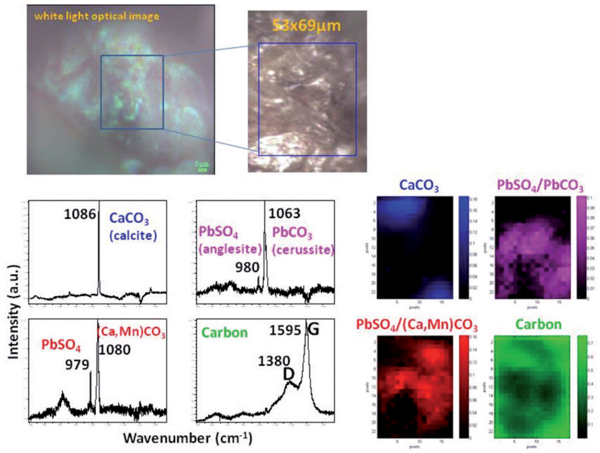

Figure 3: Optical images, Raman spectra and molecular images of the lead-rich necrotic zone on the leaf surface.

Other applications

In the field of environmental analysis high spatial resolution Raman confocal microscopy can also be used to study heterogeneous gas-aerosol particle reactions that play an important role in atmospheric chemistry. For instance, heterogeneous reaction of anthropogenic pollutant, NO_2, on NaCl(100) surfaces, taken as a surrogate of marine aerosols with or without an insoluble organic coating and as a function of humidity, can be followed by Raman microspectrometry and Atomic Force Microscopy (AFM) [3] . The chemical transformation of the NaCl surface to $NaNO_3$ by NO_2 can be demonstrated by Raman imaging while morphological transformation of the surface can be characterized by AFM images under in-situ conditions with high spatial resolution.

The «Laboratoire de Spectrochimie Infrarouge et Raman» (LASIR) manages a Raman microscopy platform equipped with a large range of HORIBA Scientific Raman microprobes covering all useful laser excitations from 1064 nm in the near infrared to 266 nm in the UV. This Raman Facility located at the University of Lille is open to both academic and industrial users.

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[2] Uzu G., Sobanska S., Sarret G., Munoz M., Dumat C., (2010) Foliar lead uptake by lettuce exposed to atmospheric fallouts. Environmental Science Technology, 44, 1036-1042.

[3] Scolaro S., Sobanska S., Barbillat J., Laureyns J., Louis F., Petitprez D., Brémard C., (2009) Confocal raman imaging and atomic force microscopy of the surface reaction of NO_2 and NaCl(100) under humidity. Journal of Raman Spectroscopy, 40, 157-163.

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