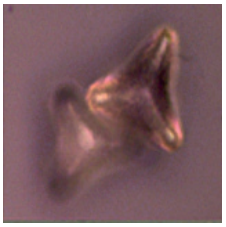
HORIBA Scientific

Raman Spectroscopy

Identification and characterization of aeroallergens based on morphological and chemical features



Application Note

Life Science RA74

Nihal Nagib, Thibault Brulé and Nicolas Buton
HORIBA FRANCE SAS, Palaiseau, France.

Abstract : The prevalence of allergies in the world is between 30 and 40%. Even though it exists medical treatments such as antihistaminic and desensitization this number is constantly increasing. Thus, in this application note, the identification and chemical characterization of aeroallergens by Raman microscopy will allow to prevent people who are affected by respiratory allergies of the presence of this type of allergens in indoor and outdoor air.

Keywords : Raman spectroscopy, Optical microscopy, Particle Size Analysis, Aeroallergens

Introduction

Aeroallergens such as pollens, dust mites, cats' and dogs' dander and mold can affect the respiratory system and these allergens can be the consequences of respiratory diseases as allergic rhinitis and asthma[1]. The identification and chemical characterization of these allergens will allow the database of Raman spectra to be developed, to more quickly and certainly determine their presence in indoor and outdoor air and to quantify them. In the past, the identification of aeroallergens has been made by Infra-Red, fluorescence and UV-visible spectroscopies[3,4,5,6,7] but also by immunobiological techniques such as ELISA or immunoblotting[8,9]. These biological identifications were supposed to determine what kind of proteins in the allergen particles were responsible for the allergy. Hence, in this work, morphological identification of different types of pollen grains from fresh flowers and commercial samples, dust mites and animals' dander by optical microscopy and particle size analysis, which gives information on the diameter of particles, will finally grant their chemical characterization by Raman spectroscopy. This spectroscopy technique should provide knowledge in their chemical composition in lipids, proteins and nucleic acids.

Instruments and methods:

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorph, crystallinity and molecular interactions. It is based upon the interaction of light with the chemical bonds within a material. Raman is a light scattering technique, whereby a molecule scatters

Figure 1: Experimental systems used for these experiments. Left: LA-960 Particle Size Analyzer. Right: LabRAM Soleil Raman micro-spectrometer

incident light from a high intensity laser light source. Most of the scattered light is at the same wavelength (or color) as the laser source and does not provide useful information – this is called Rayleigh Scatter. However, a small amount of light (typically 0.0000001%) is scattered at different wavelengths (or colors), which depend on the chemical structure of the analyte – this is called Raman Scatter. The system used is an HORIBA LabRAM Soleil. It incorporates unique and powerful functions in a reliable, high performance system, ideally suited to the research and analytical lab. It is fully confocal, not compromising image quality, spatial or depth resolution.

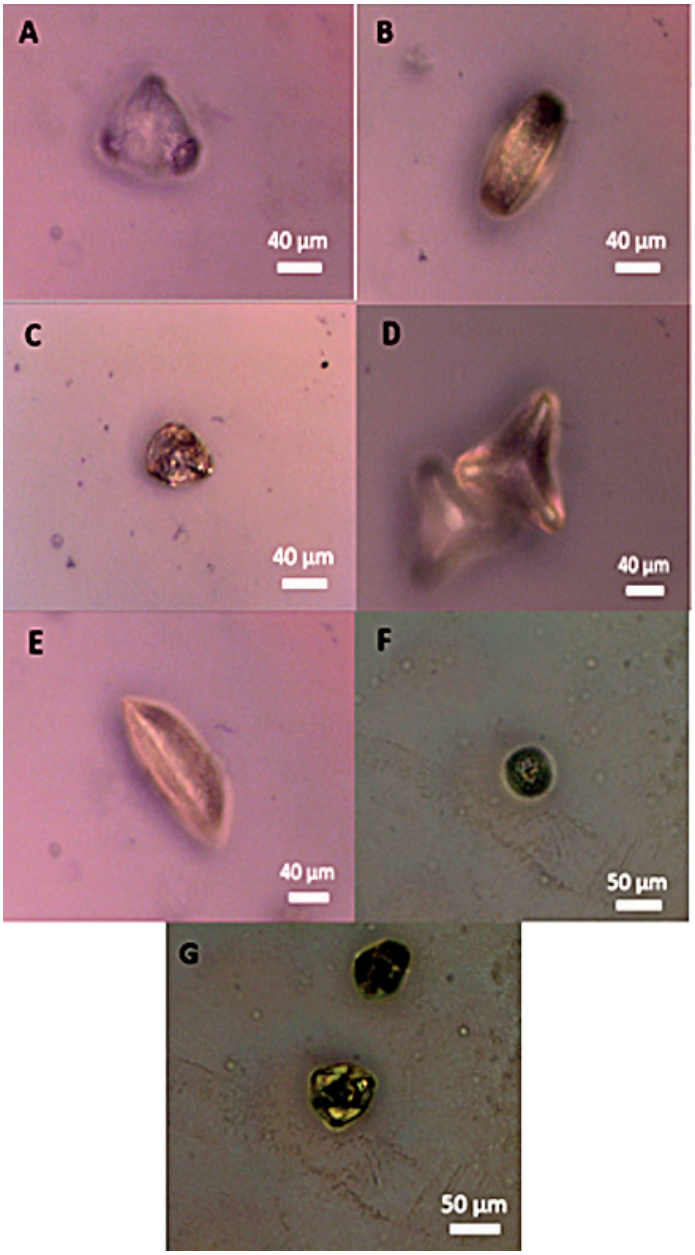


Figure 2: Optical microscopy images of pollen grains from fresh flowers. A: Camellia pollen, B: Japanese Cherry tree pollen, C: Orange Rose pollen, D: Flower thistle pollen, E: Orange Lily pollen, F: Grass pollen, G: Oak pollen.

Moreover, the ParticleFinder option in the LabSpec 6 software of the spectrometer, allows to identify in an unknown sample the particles that constitute it.

Particle Size analysis is based on laser diffraction. The central idea in laser diffraction is that a particle will scatter light at an angle determined by its particle's size. Larger particles scatter light at small angles while smaller particles scatter light at wide angles. A collection of particles produces a pattern of scattered light defined by intensity and angle that can be transformed into a particle size distribution result. The system used for this work is a HORIBA LA-960. It combines the most popular modern sizing technique with state-of-the-art refinements to measure wet and dry samples measuring from 10 nanometers to 5 millimeters.

Morphological identification

1. Pollen grains
   a) Identification by optical microscopy

Thanks to the Raman spectrometer microscope, a first observation of the pollen grains collected on fresh flowers and from commercial samples was possible. The grains being of rather large sizes, an observation with a 50x long-working distance objective is enough. Here, the differentiation between the pollen grains is made by appearance.

   b) Identification by particle size analysis

This analysis was made on pollen grains of orange Lily flowers but also from balls of pollen grains of different colors to ensure a mixture of grains. This choice of samples, especially the mixture, serves the purpose of confirming or invalidating the interest of this technique to the differentiation of the pollen grains between them. This technique being faster, it would be more effective than a microscopic observation, more laborious. The separation of pollen grains is here by size and not by appearance.
Regarding the distribution of the differents pollen grains, the

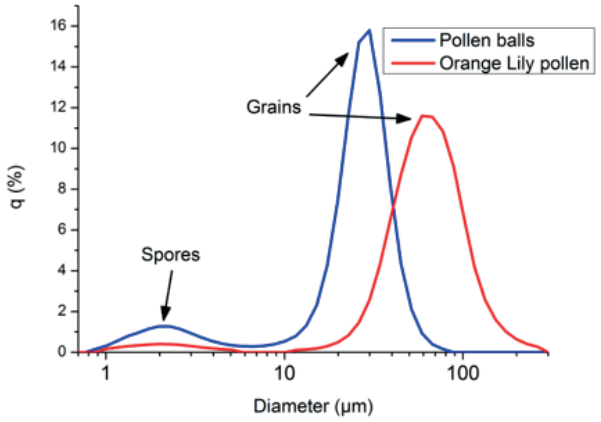


Figure 3: Particle size distributions of different pollen grains.

spore have an averange diameter of 2µm, the Lily pollen has an average diameter of 63µm, and the mixture of pollen balls have an average diameter of 24µm. The morphological identification of pollen grains by particle size analysis gave an information on the diameter of these particles but in the mixture sample this information is not sufficient because it does not allow to clearly identify each type of pollen grain as particle size measurements are not able to provide chemical information, structural identification of the pollen grain was not possible and the use of a molecular spectroscopic analysis such as Raman spectroscopy is essential to provide additional information to the previous assumption.

2. Animals' dander and dust mites

These aeroallergens were observed using the 50x long-working distance objective of the microscope which was sufficient because of their large size (Figure 4).
Optical microscopy allows to identify each pollen grain and to differentiate them from each other but also from the other allergens. Nevertheless, it is more complicated to identify dust mites and dander as their topography is not homogenous. Moreover, for an unexperimented person, it will be difficult to attribute a pollen grain to its specie. In this way, Raman spectroscopy coupled with microscopy will be a major technique to characterize these allergens and to identify them in an unknown mixture.

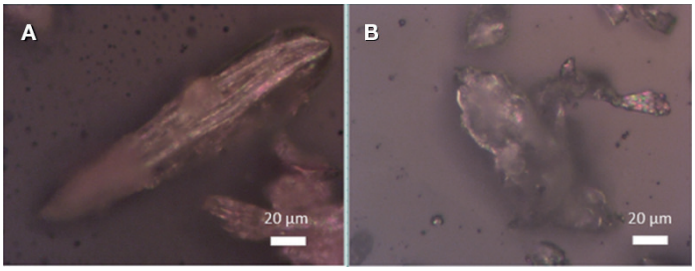


Figure 4: Optical microscopy images of commercial samples. A: Dander, B: Dust mite

Chemical characterization by Raman spectroscopy

First, aeroallergens were spectrally characterized individually resulting in Figure 5. These Raman spectra are reference spectra. In fact they were measured on single particles of allergens. Pollen grains have approximately the same spectrum, some bands are different by their intensity and Raman shift as the one at 1.170cm^{-1}, 1.600cm^{-1} and 1.650cm^{-1}. All the bands present on the spectra show the presence of proteins, lipids, sugars and nucleic acids[2,4]. The dust mites' and dander's spectra follow the same pattern as the pollen grains' spectrum. Nevertheless, the bands are quite different. These differences between all these Raman spectra will facilitate the identification of the allergens in a mixture sample.

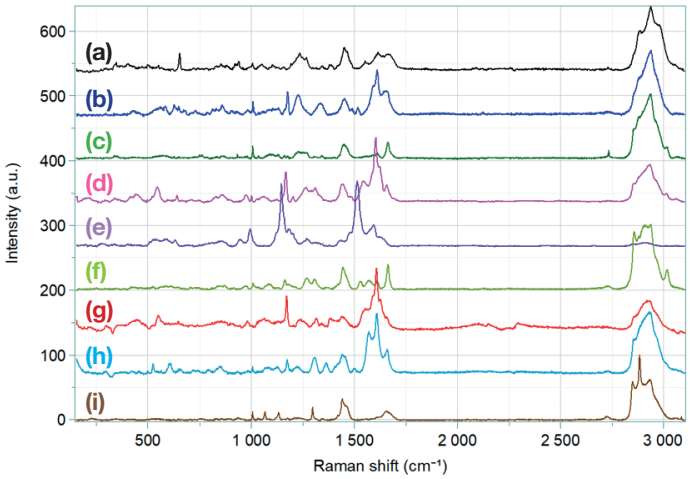


Figure 5: Reference spectra of aeroallergens. **(a)***: Dander,* **(b)***: Cherry tree pollen,* **(c)***: Camellia pollen,* **(d)***: Lily pollen,* **(e)***: Rose pollen,* **(f)***: flower of Thistle pollen,* **(g)***: Oak pollen,* **(h)***: Grass pollen,* **(i)***: Dust mite.*

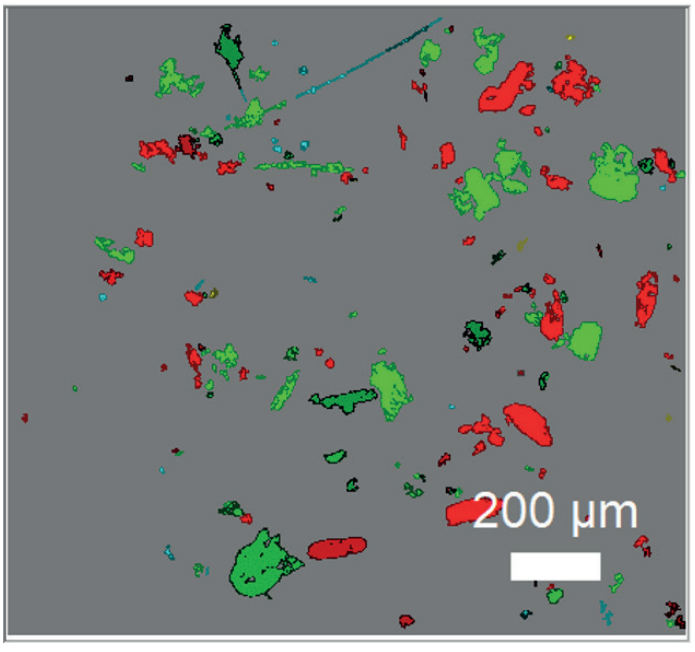


Figure 6 : ParticleFinder analysis of 174 particles of aeroallergens. Red: Dust Mite, Green: Dander, Light blue: Grass pollen, Yellow: Lily pollen.

Thus, ParticleFinder module LabSpec6 Software Suite enables to measure one spectrum by particle, locating the centers of the particles that are present in the microscopic image. Then by cross-checking the reference spectra obtained with the clustering separation, the identification of each particle in the unknown mixture is possible even if there is an important number of it. An example of such analysis is on Figure 6. So, this type of analysis allows characterizing a mixture of aeroallergens not only in terms of size of particles, but also of chemical composition, and with a high reliability on the identification as this one is based on the spectral fingerprint of the individual particles.

Conclusion

In this application note, the purpose was to identify aeroallergens in an unknown sample. Due to optical microscopy, we could observe each particle of allergen one by one which allows us to morphologically identify them and then to measure their Raman spectra in a way of chemical characterization. Considering reference spectra of these particles, we finally could find them in the mixture sample. Thus, Raman microscopy is a promising technique for the identification in real time of aeroallergens such as pollen grains, dust mites and animal's dander in the indoor and outdoor air. In fact, by taking an air sample and then filtering it, we may analyze and especially determine quickly what type of aeroallergens are present inside. This could improve the health awareness by preventing the risks of allergies for people who may be affected by it.

References

1. https://www.inserm.fr/information-en-sante/dossiers-information/allergies
2. A. Guedes, H. Ribeiro, I. Abreu et al., Talanta **2014**; 119, 473-478.
3. B.G. Pummer, H. Bauer, H. Grothe et al., J. Raman Spectrosc. **2013**; 44, 1654-1658.
4. F. Schulte, J. Lingott, U. Panne & J. Kneipp, Analytical Chemistry **2008**; 80 (24), 9551-9556.
5. B. Zimmermann, Applied Spectroscopy **2010**; 64(12), 1364–1373.
6. M; Bağcıoğlu, B. Zimmermann, A. Kohler, PLoS ONE **2015**; 10(9): e0137899.
7. B. Zimmermann, Z. Tkalčec, A. Mešić & A. Kohler, PLoS ONE **2015**; 10(4): e0124240.
8. S Spitzauer, C. Schweiger, H. Rumpold et al., Int Arch Allergy Immunol **1993**; 100:60-67.
9. Y. Cui, Q. Wang & H. Jia, Transl Allergy **2018**; 8: 14.

HORIBA Scientific

info.sci@horiba.com

www.horiba.com/scientific

USA:	+1 732 494 8660	**France:**	+33 (0)1 69 74 72 00	**Germany:**	+49 (0) 6251 8475 0
UK:	+44 (0)1604 542 500	**Italy:**	+39 06 51 59 22 1	**Japan:**	+81(75)313-8121
China:	+86 (0)21 6289 6060	**India:**	+91 (80) 4127 3637	**Singapore:**	+65 (6) 745-8300
Taiwan:	+886 3 5600606	**Brazil:**	+55 (0)11 2923 5400	**Other:**	+33 (0)1 69 74 72 00