Identification of airborne pollen by Raman spectroscopy

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Abstract
With the increase of allergic disorders over the past years, fast and reliable techniques for characterization and identification of pollen types are required.

Key words
Pollen, library search, identification, Raman microscopy

Introduction

The occurrence of allergies has increased dramatically over the past years: the prevalence of allergic diseases worldwide has doubled during the past 15-20 years.
It has been estimated that 15 to 25 % of people in western countries is affected by respiratory allergies (commonly known as pollinosis), triggered in many cases by the increase of allergenic pollen in the atmosphere, especially during the blooming season.
The allergenicity of pollen depends on the species and therefore it is important to identify, characterise and quantify the airborne pollen to have a clear picture of the allergen exposure. Raman microspectroscopy provides a rapid, selective and non destructive identification of pollen particles.

Characterisation of pollen by Raman spectroscopy

Pollen grains – varying from 10 to 200 µm diameter according to the pollen type - are the male gametophytes of seed plants being produced as part of the sexual reproduction cycle. They are considered as seasonal air pollutants, since pollen is only dispersed into the atmosphere during the flowering.
The pollen structure, morphology and the exine pattern (the outer wall of the grain) provide good taxonomic parameters. Currently these morphological parameters, obtained via optical or electron microscopy, are commonly used for identification of pollen. However, the grains present large differences in their chemical composition level: depending on the floral origin, the amount of proteins – compounds mostly responsible for the allergic disorders - may vary from 10 to 40 % of the dry weight.
These changes in the molecular composition of the sample impact the Raman spectrum, thus making Raman spectroscopy an interesting tool for the characterization and identification of pollens.

Being non-destructive, highly chemically selective, rapid, able to measure single grains, Raman spectroscopy offers an alternative to the purely optical based methods of identification or to chemical characterization approaches involving a purification step that may denature the sample.
Fig. 1 illustrates the spectral differences of various pollen species.

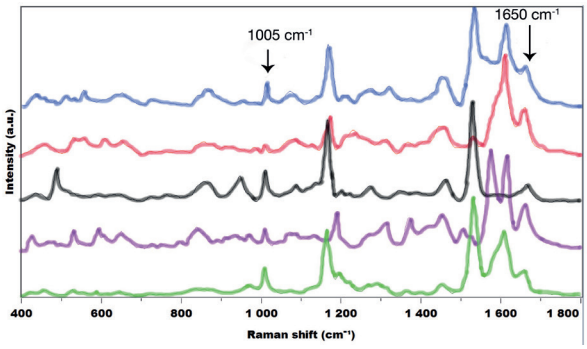

Fig. 1: Raman spectra of 5 pollen from the species: Zea Mays (Corn, in blue), Quercus coccifera (Kermes Oak, in red), Lolium perenne (Ryegrass, in black), Parietaria judaica (Spreading Pellitory, in pink) and Salix atrocinerea (Grey Willow, in green).

A more detailed examination of the spectral features indicates the presence of proteins and amino-acids with the amide I band at 1650 cm^{-1} or the typical ring breathing mode at 1005 cm^{-1} characteristic from the phenylalanine, shown in every spectrum. On the other hand, the carotenoids spectral features, revealed by the 2 intense resonance peaks at 1160 and 1530 cm^{-1}, are observed in the case of Zea mays, Lolium perenne and Salix atrocinerea – and slightly for Quercus coccifera – whereas they are absent from the spectrum of Parietaria judaica.

This illustrates the diversity in the chemical composition of the various pollen species and the ability to pick up these differences in the Raman spectra.

The differences can also be observed between different species within the same family as shown in Fig.2, where spectra of pollen types belonging to the Oleaceae family are displayed. This means that the identification can be done within a family at species level.

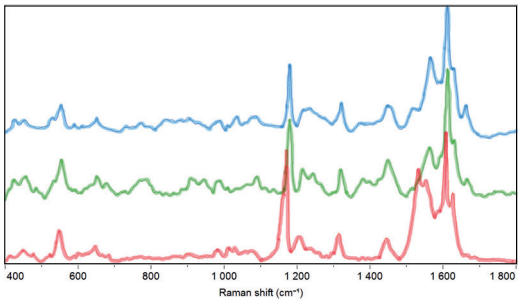

Fig. 2: Raman spectra of Fraxinus floribunda (in blue), Ligustrum lucidum (in green), Olea europaea (in red) pollen, all belonging to the Oleaceae family.

Identification of Airborne samples by library search

As the chemical and spectroscopic examination of the pollen has become increasingly important, the construction of Raman spectral libraries comprising the different pollen types found in the atmosphere should be implemented.

For that, Raman spectra of 34 pollen types were recorded and gathered in a spectral library. The list of the pollen types includes different families of trees and shrubs as well as grasses and weeds found in the atmosphere.

To check the ability of spectral identification by library search, a set of 10 airborne pollen spectra collected in spring and summer 2012 was tested against this spectral library. For all of these 10 samples, the identification to the right species is correct, as shown in Table 1, where the highest ranked reference spectrum matches the query spectrum. The higher the Hit Quality, the better is the match between the query spectrum and the library one.

Conclusion

The presented examples demonstrate that Raman microspectroscopy can be successfully applied to the analysis and the identification of airborne pollen. As it is highly chemically selective, rapid, and not requiring sample preparation, it offers an alternative to the optical microscopic methods relying only on morphological parameters. Additionnally, other spectroscopic methods (such as SPRi) provide complementary analysis for the detection of allergens. The request for such identification methods is increasing since pollinosis has become a major health issue, requiring innovative analytical characterization tools.

Further Reading

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F. Schulte, J. Lingott, U. Panne, J. Kneipp, Anal. Chem, 80, 9551-9556 (2008).

Detection of birch pollen allergen in the air, HORIBA Scientific application note.

Query spectrum	Hit	Name (Hit Quality)	Query spectrum	Hit	Name (Hit Quality)
1- Acer negundo_air (april)	1st	**Acer negundo (888.2)**	6- Acer negundo_air (may)	1st	**Acer negundo (795.4)**
	2nd	Acer negundo (888.2)		2nd	Parietaria judaica (746.9)
	3rd	Quercus coccifera (821.6)		3rd	Rumex spp. (732.3)
2- Betula pendula_air (april)	1st	**Betula pendula (876.1)**	7- Acer negundo_air (may)	1st	**Acer negundo (892.5)**
	2nd	Castanea sativa (851.1)		2nd	Quercus coccifera (827.4)
	3rd	Corylus avellana (819.8)		3rd	Parietaria judaica (793.1)
3- Platanus x acerifolia_air (april)	1st	**Platanus x acerifolia (906.2)**	8- Platanus x acerifolia_air (may)	1st	**Platanus x acerifolia (756.8)**
	2nd	Acer negundo (742.9)		2nd	Plantago lanceolata (728.5)
	3rd	Parietaria judaica (710.1)		3rd	Acer negundo (692.4)
4- Plantago lanceolata_air (april)	1st	**Plantago lanceolata (909.2)**	9- Platanus x acerifolia_air (may)	1st	**Platanus x acerifolia (726.3)**
	2nd	Platanus x acerifolia (720.9)		2nd	Parietaria judaica (718.5)
	3rd	Parietaria judaica (675.8)		3rd	Castanea sativa (957.1)
5- Acer negundo_air (may)	1st	**Acer negundo (891.7)**	10- Castanea sativa_air (august)	1st	**Castanea sativa (957.1)**
	2nd	Quercus coccifera (830.8)		2nd	Betula pendula (893.3)
	3rd	Castanea sativa (828.8)		3rd	Quercus coccifera (853.2)

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