ThermoFisher SCIENTIFIC

Webinar Q&A Report:
Infrared Spectroscopy for Biological and Biomedical Applications

Can we use peak fitting in IR to monitor protein aggregation and quantify the aggregation level? PLS, PCR model?

Yes: Peak fitting for the IR peak at 1605-1625 cm-1 can give you information on the degree of aggregation.

Does the equipment have a chemometric software to treat/analyse the results (PCA, PCR, PLS, HCA, etc.?)

Yes, OMNIC software package is equipped to do chemometric analysis like PCA, PCR, PLS etc.

Can ATR sampling be used with QCL spectrometer?

QCLs as IR source and ATR can be combined. Be aware that the QCL beam is fully polarized, other than the FT-IR beam that is unpolarized or only weakly polarized (due to reflection at mirrors). Polarization affects total reflection and thus formation of the evanescent wave.

I use IR spectroscopy a lot for chemical analysis. Normally, I use the routine KBr pellet sampling technique. Can I also use this standard technique to analyze protein samples?

In a KBr sample, a protein is fully dehydrated and experiences ionic interactions. You would not expect a native structure under these conditions.

You mentioned FT-IR spectroscopy and Quantum cascade lasers. What is the main advantage of using quantum cascade lasers?

QCLs are small, almost point sources of high power and, especially, of high brilliance (= high emission power per emitting surface area). A QCL may emit IR radiation at some mW (up to >100 mW) in contrast to the usual global source of an FT-IR (some tens or hundreds of nW for a 4 cm-1 wavelength interval): This is the key for higher SNR for spectra or for time-resolved measurements.

Samples measured in transmission or samples measure by ATR (attenuated total reflection) techniques: What are the pros and cons?

Transmission: sample preparation may be difficult because of the small cell pathlength. ATR: easy sample access/sample change.

There are relatively cheap low-end FT-IR spectrophotometers and very expensive high-end FT-IR machines. Do I need a high-end FT-IR spectrometer to analyze protein samples?

No: Most protein analyses can be performed with low-end FT-IR; their stability and SNR are sufficient. Make sure that spectral resolution of at least 4 cm-1 (better 2 cm-1) is possible.

In the lecture, you mentioned that water background absorbance in the mid-IR presents a problem for IR Biospectroscopy, and that specific sample techniques have to be developed. What is the sampling technique if I want to analyze e.g., a soluble protein?

Use a Microcon concentrator to concentrate the soluble protein to some 100s of micromolar or even mmolar concentration. This is not abiotically high - in cells, protein concentrations are higher. You will only need a few microliters to fill a demountable IR transmission cell or put a drop on an ATR unit.

You mentioned commercially available FT-IR instrumentation as well as novel Quantum Cascade Lasers (QCL). Are there IR spectrometers available based on quantum cascade lasers?

QCLs are available as (i) single wavelength emitters or (ii), using an external cavity, as tunable lasers (EC-QCL). Tuning range is around 200 -300 cm-1 for one EC-QCL. If you want to cover a wider range, you will need several EC-QCL which may be quite expensive (one 300 cm-1 EC-QCL is around USD 50.000 plus). At my knowledge, there are some manufacturers that sell spectrometers with up to 4 EC-QCL in order to cover a range of about 1000 cm-1.

Contact Information

If you have additional questions for Thermo Fisher Scientific regarding content from their webinar or wish to receive additional information about their products and services, please contact them at: Spectroscopy & Materials Analysis Inquiries

Copyright 2021 AzoNetwork and Thermo Fisher Scientific. All rights Reserved.