# FTIR analysis of Biofluids using the Gateway ATR

## Analyze large volumes of fluid using the Gateway ATR accessory

**While there are many reports on spectroscopic studies on molecules of biological significance, there are far fewer examples of spectra obtained from whole organisms.**

Many biological molecules have water as an integral part of their structure. This is problematic for infrared spectroscopy of such molecules because the water absorbs very strongly in the mid-IR, dwarfing contributions from other components in the sample.

Figure 1: The Gateway ATR

However, on the positive side there are only two main absorptions, leaving significant windows for the direct observations of absorptions from the sample.

With correct attention to factors such as the temperature of the sample it is possible to perform spectral subtractions to obtain information about the peaks that occur in the same region as the water absorptions.

The use of sampling systems employing the principle of attenuated total reflectance (ATR), is especially helpful in studying aqueous systems since the problem of high absorption by the water in minimized by the short path length characteristic of such devices.

ATR measurements rely on efficient coupling between the evanescent wave of the infrared beam and the sample.

Good coupling can be promoted by applying slight pressure to a solid sample, but difficulties arise when the spectroscopist wishes to examine particulate matter is suspension. These materials tend to sediment during scanning with the result that poor spectra are obtained.

## Experiment

The Gateway accessory is a 6-reflection ATR typically used for viscous liquids such as creams, pastes and gels. Such samples are readily applied to and cleaned from the accessible and removable crystal assembly on the ATR top plate.

In this application note we describe its use for measuring large biomolecules (yeast cells and protoplasts) in aqueous suspensions.

The samples were loaded on top of the ZnSe crystal and particulate matter precipitated out onto the crystal surface creating a good contact. The resolution of the spectrometer was set to 4 cm-1 and 1000 data scans.

### A quick look: Gateway ATR Accessory

The Gateway ATR spectrometer accessory is a versatile ATR device, capable of sampling larger fluid volumes (up to $^1/_2$ a millilitre).

- The Gateway ATR provides sensitive measurement of liquids and reactants under flow and at elevated temperatures (up to 90°C).
- The tool is ideal for oils, biofluids and the studying of liquid-lipid interactions.
- Zinc selenide, silicon and germanium crystal options are available and easy to change.
- Consistent, qualitative, averaged measurements of heterogenous solids, such as foodstuffs, are also very possible using the Gateway.
- it is an ideal accessory for applications where a single-reflection accessory is over-specified.
- The optics are purgeable and easily aligned.

**Check out 'The analysis of edible oils using ATR FTIR', for another application note on this device.**

**Visit www.specac.com and navigate to the Gateway ATR or Resources page to discover more.**

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> A quick and efficient method for analyzing particulate matter in suspension.

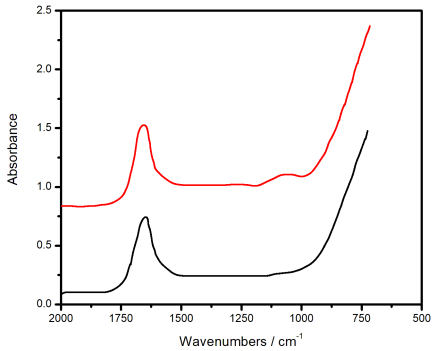


Figure 2: Upper Trace Yeast Cells in Water, Lower Trace Water

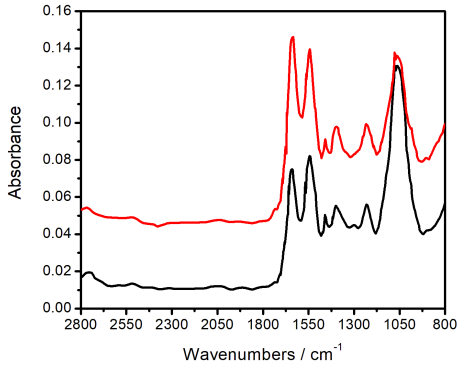


Figure 3: T.Glabrata. Upper Protoplasts, Lower Whole Cells

The spectra shown in Figure 2 have been obtained from an aqueous suspension of the yeast Torulopsis glarat (upper trace) and from distilled water (lower trace). Apart from a slight disturbance in the vicinity of 1000cm-1 these two spectra seem almost identical.

The lower trace shown in Figure 3 is the result after the digital subtraction of the water spectrum from that of the yeast suspension. It will be evident that as well as absorbances at 1050 cm-1 there are significant features at 1630, 1550, 1410 and 1240 cm-1.

The main band at 1050 cm-1 has a complex composition and represents absorptions mainly from the carbohydrates of the yeast cell wall. The peaks at 1630 and 1550 cm-1 have been shown to be indicative of the presence of amides and probably come from proteins either attached to or embedded into the cell wall.

The upper trace in Figure 3 represents the spectrum obtained from a yeast obtained from a yeast preparation treated with snail enzyme. Under the correct conditions this material can be used to break down the cell wall releasing the yeast protoplast. The production of protoplasts is an essential step In the formation of new genetic hybrids.

From the spectra it will be seen that the protoplasts and the whole cells have the same type of infrared absorbances present. The differences between the two spectra lies in the detailed band structure and in the relative peak intensities.

In the protoplast spectrum the peaks due to the carbohydrate features are significantly reduced in intensity compared with the peaks from the proteinaceous material.

The change is entirely as expected from the structures of the yeast cell and the yeast protoplast as much of the carbohydrate present is located in the cell well.

## Conclusion

The Gateway ATR provides a quick and efficient method of analysing particulate matter in suspension. This is very convenient without the disadvantage of issues associated with sedimentation in conventional transmission cells and ATR cells.

Studies such as these are capable of yielding information of value in the taxonomy of yeast hybrids produced for biotechnological reasons and can also be of use in improving methods for the production of protoplasts.

## Acknowledgements

Dr J.E Newbery, Goldsmiths College, University of London, UK.

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