

Sample Preparation for IR Spectra - Liquid Samples

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The analysis of liquid samples can be performed in a variety of ways. In this Application Note, we will discuss the techniques used in Bio-Rad's laboratories, along with potential problems associated with liquid samples.

Technique: Neat

The most common technique for analyzing liquid samples is as a neat between salt plates. This technique consists of placing a drop (or less) of the liquid sample on a suitable salt plate, followed by a second salt plate placed on top of the sample. In this way the liquid is "sandwiched" between the plates to form a thin layer of sample. Naturally, the salt plates need to be compatible with the material being analyzed. We run samples containing no water between potassium bromide crystals (32 x 5 mm). Samples containing water are placed between KRS-5 crystals. These crystal materials are used because of their spectral range (better than 4000 to 450 cm^{-1}) and durability. After running each sample, the KBr crystals are cleaned with a suitable solvent, on a tissue, followed by buffing with methanol on a felt polishing cloth.

The KBr crystals are periodically polished with polishing compound to maintain a smooth and clean surface. Due to the toxic nature of KRS-5, the KRS-5 crystals are only polished when the crystals become significantly scratched or cloudy. We recommend having KRS-5 crystals polished professionally, due to their toxicity.

Technique: ATR

For liquid analysis by ATR spectroscopy, we use a horizontal ATR equipped with a zinc selenide trough. Even though the zinc selenide has a cut-off of 650-700 cm^{-1} , its durability is much better than materials with wider spectral ranges.

After analysis, the sample is rinsed from the trough using a suitable solvent, followed by wiping with a cotton ball. This material does not usually get repolished.

Potential Problems

The most serious problem associated with running liquid samples is spectral non-linearity. Non-linearity refers to a condition where the spectral absorbance of a band does not follow the Beer-Lambert Law:

$A = abc$ where,

A = absorbance value
a = molar absorptivity
b = path length
c = concentration

The Beer-Lambert Law is typically discussed in regards to quantitative analysis. Spectral searching is a type of quantitative analysis, since most searching algorithms use band intensity as a factor. Non-linearity due to sampling typically occurs in transmission analysis and not in ATR, due to the nature of the ATR technique.

The most common cause of non-linearity is inconsistent path length across the sampling area. For our instruments at 2 cm^{-1} resolution, the sampling area is 6 mm at focus. If there are any air bubbles or unevenness in the sampling area, the path length of the sample will vary across the sample area.

These variations will cause inaccuracies in the band intensities in the spectrum. In other words, the bands will be weaker or stronger than they should be. This condition will lead to reduced spectral search quality. Figure 1 is a plot of a spectrum of 3,4-dichlorotoluene analyzed as a neat with a consistent path length. The peak intensity of the band at 808 cm^{-1} is 0.39, while the peak intensity of the adjacent band at 870 cm^{-1} is 0.24 ($A_{808 \text{ cm}^{-1}}/A_{870 \text{ cm}^{-1}} = 1.6$).

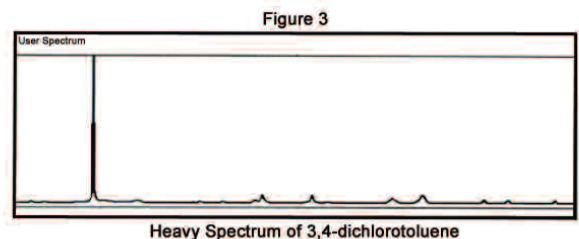
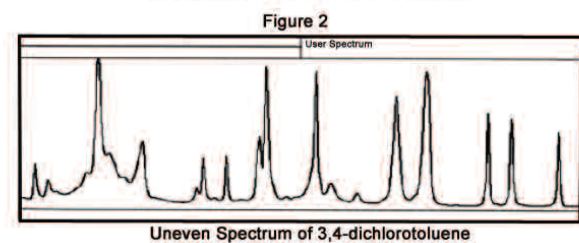
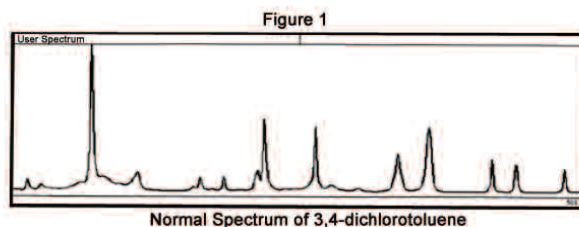
Figure 2 is the same sample analyzed with uneven coating of the sample on the crystals. As you can see, the band intensities are different. For this spectrum, the peak intensity of the band at 808 cm^{-1} is 0.76, while the peak intensity of the adjacent band at 870 cm^{-1} is 0.62 ($A_{808 \text{ cm}^{-1}}/A_{870 \text{ cm}^{-1}} = 1.2$). This represents a change of 25% from the previous spectrum. This could lead to a poor spectral search match. We check for unevenness by analyzing the sample using a 6 mm sampling area, followed by analysis with a 3 mm sampling area. The two spectra are subtracted from each other and the residual spectrum is examined.

Non-linearities due to unevenness would show up as a large residual. We also minimize unevenness by frequent polishing of the crystals.

The second cause of non-linearity in the spectrum of a liquid sample is sample thickness. A large percentage of spectra of liquid samples submitted to us for spectral search analysis have been run too thick. Most FT-IR spectrometers/detectors have a linear response up to approximately 1.2 absorbance units. Any band above 1.2 absorbance units is of questionable linearity. Non-linearity can sometimes be seen as “choppiness” on the top of the band. We only accept spectra from our lab that have a maximum absorbance value of 1.2 absorbance units. Figure 3 is a plot of the spectrum of the same sample used previously. However, the sample thickness was much larger than the previous spectrum.

The strongest band in the spectrum is over 30 absorbance units. For this spectrum, the peak intensity of the band at 808 cm^{-1} is 1.66, while the peak intensity of the adjacent band at 870 cm^{-1} is 0.94 ($A_{808 \text{ cm}^{-1}}/A_{870 \text{ cm}^{-1}} = 1.76$).

This represents a change in relative intensity of 10%, compared to the normal spectrum in Figure 1. This change in relative band intensities will have a negative effect on search accuracy.



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