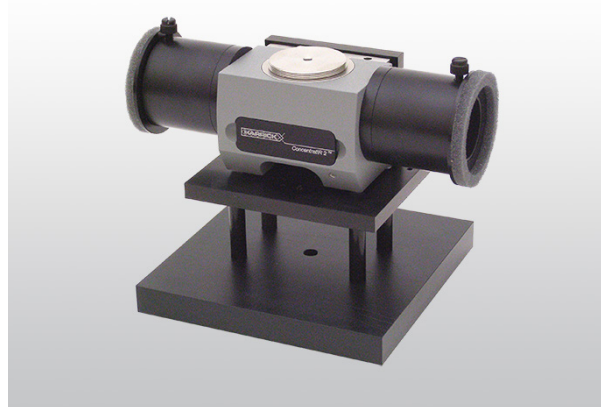


Microvolume analysis of biofluid samples using the Harrick ConcentratIR2 ATR accessory

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In this note: microliter quantities of mice urine are analysed.

Animal models, particularly mice, offer valuable insights in pre-clinical research due to their genetic and biological similarities to humans. By controlling genetics, environment, and diet, researchers can study large cohorts with reproducible conditions¹. Vibrational spectroscopy delivers molecular insights into the sample's molecular structure, composition, and dynamics without requiring any tags or dyes^{2,3}. Reports on the use of Fourier transform infrared (FTIR) spectroscopy for analyzing biofluids (such as saliva, blood, and urine), tissues, and cells are growing rapidly. In this study, we evaluate the effectiveness of the Harrick ConcentratIR2™, a multi-reflection diamond ATR accessory, in analyzing minimal volumes of wild type mice urine.



Experimental

In this study, urine samples of 6 wild types of 7-week-old C3H female mice were collected. All IR spectra were collected on a commercial FTIR spectrometer with the ConcentratIR2™ placed in the sample compartment, using a DTGS detector. Each sample was measured by collecting and averaging 128 scans with a final resolution of 4 cm⁻¹. Spectra were collected in the range 3800–700 cm⁻¹. The background interferogram is obtained using a clean diamond surface. After shaking the urine container, a 2 µL sample is pipetted onto the diamond. Due to water absorption that would mask many spectral components, the sample is dried with a gentle stream of N₂ gas before data collection to eliminate excess water.

Results and Discussion

FTIR spectra of the mice obtained with 2 µL urine with the Harrick system are shown in Figure 1. These closely resemble the spectra of healthy human urine previously reported by our group and depicted in Figure 2⁴. Mice urine spectra exhibited a consistent profile within each other, especially within the 3600–1100 cm⁻¹ region. However, variations are observed among participants in the 1100–800 cm⁻¹ range. When the human and mice urine spectra are compared, band profiles and positions are seen to be similar at many points, namely 3432, 3337, 3200, 1660, 1609, 1444, 1143, 930, 783 cm⁻¹. These bands primarily arise from urea. In the human urine spectrum,

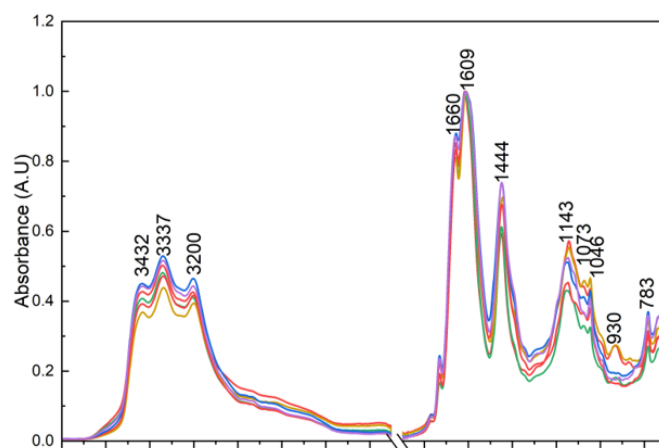


Figure 1: Urine spectra of 6 wild type female mice aged 7 weeks.

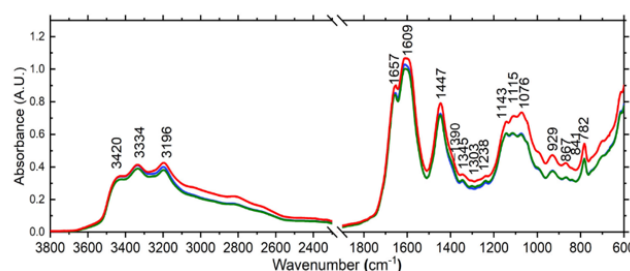


Figure 2: The average urine spectra of children (red), young adults (blue) and adults (green).⁴

the peaks at 1345 cm^{-1} , typically associated with creatinine, amino acids, and proteins (e.g., Tamm-Horsfall protein), are found at 1341 cm^{-1} in the animal urine spectrum. The differences were observed in the spectral region between 1333 cm^{-1} and 1190 cm^{-1} , as well as at 1731 , 1115 and 1046 cm^{-1} . Despite these discrepancies, the overall similarity between the two spectra is evident.

Conclusion

The Harrick ConcentratIR2 ATR accessory enables quick and simple analysis of minimal volume biofluid samples, such as mice urine. It combines the high sensitivity of multiple-reflection ATR measurements with only microvolume sample requirements. In this case just $2\text{ }\mu\text{L}$ of sample was needed to obtain good spectra. The consistency of results affirms its effectiveness in capturing comparable data across different biological models.

References

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