312 CD
DOI: 10.1017/S1431927606066220
Microsc Microanal 12(Supp 2), 2006

Quantitative Analysis of Oxalate Salts in Renal Biopsies via ATR Infrared Microspectroscopy and its Relevance to Kidney Disease.

Jennifer Anderson[†], Jennifer Haas[†], Ruta Arays[†], André Sommer[†] and Andrew Evan*

[†]Molecular Microspectroscopy Laboratory, Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056
*Department of Anatomy, Indiana University School of Medicine, Indianapolis, IN 46202

The rise in renal stone disease in developed countries around the world is cause for concern not only on the part of physicians and dieticians, but urologists and technicians as well. Due to the astonishingly high rate of renal stone component misdiagnosis using current visual techniques [1], several new methods have been suggested and attempted [2-4]. One of these new methods of diagnosis involves using reflectance infrared (IR) microspectroscopy and attenuated total internal reflectance (ATR) infrared microspectroscopy to definitively determine the components present in the sample stone or biopsied tissue [1]. Both offer direct molecular identification of the materials being studied.

Though qualitative IR reflectance work has been proven successful on tissue sections possessing mineral inclusions [5], quantitative work has yet to be applied. This paper presents preliminary quantitative results concerning the detection of calcium oxalate monohydrate (COM) and dihydrate (COD) in a simulated tissue matrix (gelatin) via ATR infrared microspectroscopy. Specific markers for these oxalates will be investigated in addition to the use of band fitting analysis. Band fitting analysis will be investigated since the strongest IR marker for tissue is the amide I band located near 1633 cm^{-1} and that for the oxalate is the asymmetric C=O stretch of the oxalate anion located near 1614 cm^{-1}. The proximity of these features may make the identification of these individual components in renal stones difficult.

In an effort to determine initial detection limits for calcium oxalate monohydrate uniformly dispersed in tissue, ATR spectra of pure gelatin and pure COM were collected. These absorbance spectra were then multiplied according to their respective concentrations by weight. The resulting spectra were then added together to yield a spectrum representative of the mixture. Figure 1 illustrates the spectra of neat gelatin (tissue matrix) and gelatin with COM added at 0.1 , 0.3 and 0.5 weight percent. Characteristic absorptions for COM include the asymmetric C=O stretch of the oxalate anion at 1614 cm^{-1}, the symmetric C=O stretch of the oxalate anion at 1315 cm^{-1}, and the O-C-O bending vibrations at 776 cm^{-1}[6]. Figure 1 clearly illustrates changes in 1315 and 776 cm^{-1} region for those spectra with added COM. No observable differences could be detected in the 1614 cm^{-1} region due to the overlap of the antisymmetric C=O stretch of the oxalate anion with the amide I band of the protein matrix located at 1633 cm^{-1}. The detection limit of COM in the protein matrix was found to be 0.3% by weight COM using the 1315 cm^{-1} band for quantitation, and that for the 776 cm^{-1} band was found to be 0.5% by weight. The detection limit represents a peak height which is 3 times larger than the peak to peak noise in a spectrum.

Preliminary curve fitting analysis of the 1633 – 1614 cm^{-1} region using GRAMs software showed that, compared to that of the pure gelatin, the absorption band of the mixed sample (1.3% COM) had a larger band width and had been shifted to lower wavenumbers, both of which are expected if the

calcium oxalate monohydrate is influencing the spectrum of the gelatin. However, in order to prove that one can quantitatively detect COM in protein using the asymmetric C=O stretch of the oxalate anion (1614 cm^{-1}), a larger sample population must be studied.

Finally, in addition to determining the minimum concentration of COM present in a homogenous mixture, our research also seeks to determine the minimum detectable size of a single oxalate particle in a protein matrix. To obtain this information, single particles of known size will be placed in the matrix and analyzed using ATR microspectroscopy. Since this method is an immersion microspectrscopic method we anticipate the ability to detect COM particles down to (ca) 7.5 micrometers in diameter. In reality we may be able to detect smaller particles since this calculation assumes that the particle diameter exactly matches the focused infrared beam diameter.

[1] J. C. Anderson, J. Dellomo, A. J. Sommer, A. P. Evan and S. Bledsoe, Urological Research 33 (2005) 213
[2] C. Paluszkiewicz, M. Galka, W. Kwiatek, A. Parczewski and S. Walas, Biospectroscopy 3 (5) (1997) 403
[3] C. G. Kontoyannis, N. C. Bouropoulos and P. G. Koutsoukos, Applied Spectroscopy 51 (8) (1997) 1205
[4] W. M. Kwiatek, J. Lekki, C. Paluszkiewicz and N. Preikschas, Nuclear Instruments & Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms B64 (1-4) (1992) 512
[5] J. Anderson, A. Sommer, P. Evan Andrew and S. Bledsoe, Applied Spectroscopy, To Be Submitted 2006
[6] R. L. White and J. Ai, Applied Spectroscopy 46 (1) (1992) 93

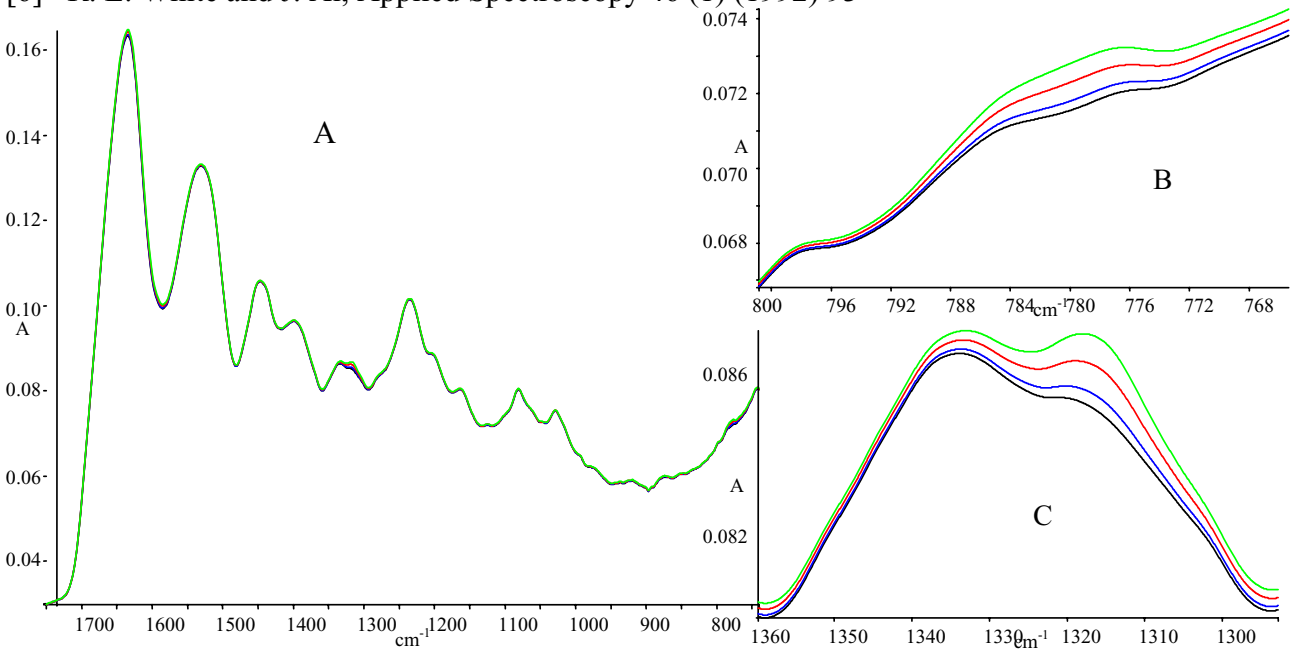

Figure 1: A) Spectra of neat gelatin (black), 0.1% COM (blue), 0.3% COM (red) and 0.5% COM (green). B) Peak centered at 776 cm^{-1}. C) Peak centered at 1315 cm^{-1}.

https://doi.org/10.1017/S1431927606066220 Published online by Cambridge University Press