BrightSpec
Molecular Rotational Resonance Spectroscopy

Isomer Specificity to Methylimidazole Powders by Direct Headspace Analysis

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Introduction

Methylimidazoles (shown in 4 different isomeric forms in Fig. 1) are common precursors and byproducts for various syntheses in industrial settings. The fundamental imidazole ring is a common substituent in nucleotide bases, which is the basis for methylimidazole importance as pharmaceutical precursor. 4(5)-methylimidazole and 2-methylimidazole have been identified as byproducts in the synthesis of certain caramel colored dyes used in the food industry. Recent toxicological studies have underscored the importance of regulating the maximum exposure levels of these two isomers and have motivated new detection protocols.[1] The California Office of Environmental Health Hazard Assessment currently regulates the exposure of 4-methylimidazole to 16µg per day.[2] Current detection methods require the use of chromatography and most proposed techniques involve derivitazation or non-organic solvent extraction.[3]

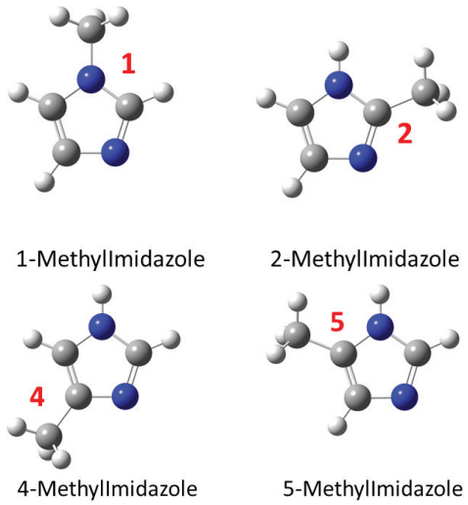

Fig 1: *Structures of the methylimidazole isomers*

Molecular spectroscopy is ideal for identifying molecules by their unique spectroscopic fingerprint. However, reliable deconvolution of a complex mixture's spectrum requires high resolution. The inherently high resolution, pure rotational spectrum is a powerful way to unambiguously identify a molecule, because it can be directly calculated based on the mass distribution (3D geometry) of the freely rotating (gas phase) molecule. The pattern of the rotational spectrum shifts appreciably with any change in mass distribution, making it possible to distinguish different molecules with the same mass without precluding the necessity of separation. Although 2-methylimidazole and 4(5) methylimidazole (a tautomeric pair, Fig. 2) exist as solids at room temperature, the vapor pressure (~ 1 mTorr) is appreciable enough for a sensitive millimeter wave rotational measurement. Typical partial pressure detection limits can be in the nanoTorr range for MRR. The purpose of this communication is to introduce BrightSpec's MRR spectroscopy as a high resolution, geometry specific detector for direct mixture analysis of vapors. The concept of measuring directly the headspace of solids also applies to routine residual solvent analysis.

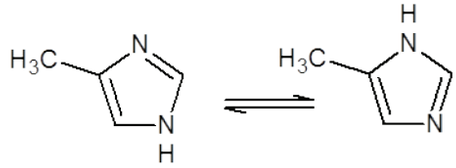

Fig 2: *4(5) Methylimidazole tautomerization. The 5-methylimidazole isomer is in approximately 30% natural abundance at room temperature vapor equililbrium.*

Experimental

The software controlled spectrometer consists of a millimeter wave light source which broadcasts an excitation pulse from 260-290 GHz, a heterodyne receiver which downcoverts the molecular free induction decay for digitization on a PCI card, and a computer. The measurement cell is a 65cm steel tube approximately 5cm in diameter and 1L in volume. A port is used to introduce gas and a separate port to evacuate gas by a turbo pump. The solids were purified by sublimation onto a glass wall in order to remove any residual solvents or reagents used in the manufacturer synthesis of 2-methylimidazole (Aldrich #M50850) and 4(5)-methylimidazole (Aldrich #199885).

Approximately 500mg of the purified crystals were placed into a sublimation chamber attached to the spectrometer measurement cell via Swagelok and the air was evacuated. Solid samples were allowed to vaporize into vacuum in the sublimation chamber for 10 to 15 minutes before being introduced to the measurement cell. The pressure of the solid's headspace was approximately 5 mTorr. 1-methylimidazole (Aldrich M50834) is a liquid at room temperature. Approximately 1mL of the liquid was transferred to a glass sample holder, frozen with liquid nitrogen, and the air was evacuated. The glass sample holder is sealed by o-ring to a kyvar glass/metal sealed tube which mates with the measurement cell by Swagelok. After warming, 6 mTorr of headspace was drawn into the measurement cell. During measurement, the cell is closed off to the sublimation chamber and the pump. The full band spectrum in high dynamic range mode was acquired (about 1.5 minutes). Typical signal level compared to the spectrometer noise level for this study is 1500:1.

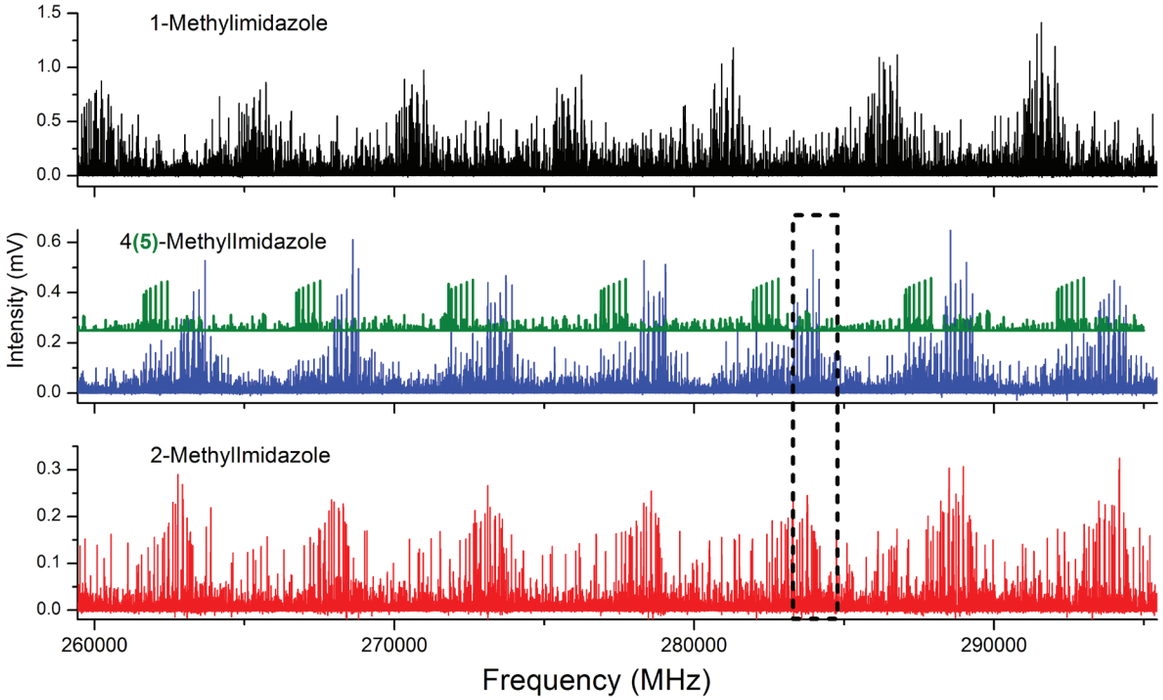

Fig 3: *The broadband spectra of the three pure methylimidazole isomers. The intense spectral patterns of the spectrum are clearly distinguishable between the isomers. A closer investigation (black box) highlights the highly resolved spectra between the 4-methylimidazole and 2-methylimidazole isomers.*

Results and Discussion

The broadband spectra of the pure samples are shown in Fig. 3. Each give a distinct, repeated spectral pattern related to their unique geometries. The green spectrum is a simulation of 5-methylimidazole calculated from the rotational constants determined from the spectrum mixture, which represent the 5-methylimidazole geometry. The high resolution nature of the measurements makes the technique amenable to measuring a mixture of all four isomers without the need for separation or complex deconvolution algorithms. Even in a place where there is expected to be a high chance of spectral overlap, the expansion of the horizontal scale in Fig. 4 shows a clear distinction and highlights the signal to noise which is not visible in the compact view. There are nearly 50,000 independent data channels in a broadband CP-FTmmW spectrum. The data set viewed on the 100x scale in Fig. 4 would carry on for nearly 300 meters.

CP-FTmmW can capture a sufficient bandwidth to take an unbiased snapshot of the mixture and analyze most polar, small (<100amu), volatile molecules all within the 1 minute measurement time. Automated detection results are based on pattern matching of the resolved spectra and abundance estimation which scales linearly with molecule number density. In the course of the study, residual imidazole precursor was detected in the powders and residual acetic acid was also identified in the spectrum before purification. The direct headspace measurement is simple and fast making it a competitive technique for residual solvent analysis of powders.

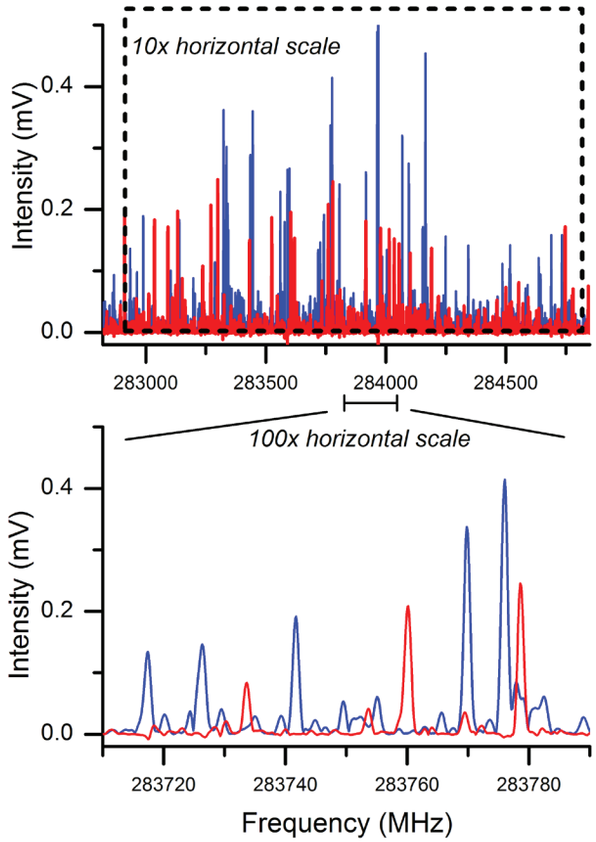

Fig 4: *An overlay of the 2-methylimidazole (red) and the 4(5)-methylimidazole (blue) spectrum. The 100x horizontal zoom highlights the high resolution of rotational spectroscopy.*

[1] National Toxicology Program Report: NTP TR-535, January 2007 [2] California Office of Environmental Health Hazard Assessment: Proposotion 65
[3] Hengel, M., Shibamoto, T., J. Ag. Food. Chem. 2013 (61), 780