SURFACE-ENHANCED RAMAN SPECTROSCOPY METHOD FOR DETERMINATION OF MALEIMIDE, A KEY MOLECULE IN MARS EARLY LIFE EXPLORATION

J. Aramendia[1,2], L. Gomez-Nubla[1], M. Tuite[1], K. H. Williford[1], J.M. Madariaga[2]*, K. Castro[2]
[1] abcLab, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr, Pasadena, CA 91109, United States, [2] Department of Analytical Chemistry, University of the Basque Country UPV/EHU, P.O. Box 644, 48080 Bilbao, Spain (juanmanuel.madariaga@ehu.eus)

Introduction: Heterocyclic compounds that contain nitrogen are fundamental components of many biologically important compounds including RNA, DNA, amino acids, and enzymes. Their presence in meteorite organic matter suggests a possible role in prebiotic chemistry and the origin of life on Earth as well as, potentially, elsewhere in the solar system. Methylated variants of maleimide (2,5-pyrroledione), a nitrogen-containing heterocycle, are products of chlorophyll diagenesis that are commonly preserved as molecular biosignatures in terrestrial sediments [1]. Maleimide, therefore, provides a good target compound for developing biologically-significant heterocycle detection methods because of its ubiquity in ancient Earth sediments and structural similarity to extraterrestrial compounds. Because maleimides in geological samples are typically analyzed via GC-MS after solvent extraction, however, the technique is inherently sample-destructive and, therefore, not compatible with analysis of small, precious extraterrestrial samples like those that might be returned to Earth by a Mars sample return mission.

Surface Enhanced Raman Spectroscopy (SERS) could serve as an appropriate non-destructive detection method for heterocyclic compounds. SERS is a surface sensitive technique used to amplify the Raman signals of low scattered compounds or to obtain much better Limits of Detection (LOD), for compounds present at minor to trace levels, through the use of metal nanoparticles [2].

The objective of this work is to develop a highly sensitive SERS method to detect and semi-quantify maleimide in terrestrial geologic specimens in anticipation of one day applying that method to Mars samples.

Materials and Methods: In order to attain the proposed objective, a 4 mg/ml maleimide solution was prepared by dissolving maleimide (99%, Sigma Aldrich) in a dichloromethane (DCM):methanol (9:1) mixture. This concentration sufficient to obtain a robust Raman spectrum that could be compared to the few maleimide spectra available in the literature.

A key step in SERS is the synthesis of the nanoparticles. In this case, Ag nanoparticles were selected following the procedure of synthesis in Leopold and Lendl [3]. This method was selected because it has been widely adopted for the detection of organic molecules in geologic samples.

The procedure starts with the preparation of stock solutions of three reagents, 10 mL of silver nitrate solution (10 mM), 90 mL solution of hydroxylamine hydrochloride (1.67 mM) and 10 mL of sodium hydroxide (3.33 mM). The sodium hydroxide and the hydroxylamine solution are mixed. The silver nitrate solution is then added drop wise (this is critical) slowly and under constant stirring. After the addition of all the silver nitrate solution, the resulting yellow/orange/grey solution must be stirred for approximately 15 minutes.

To collect the SERS spectra a hand-held Bravo Raman spectrometer from Bruker was employed. This portable device implements a new technology called DuoLaser™ to reduce fluorescence. This is based on the excitation of the sample using two lasers with wavelengths ranging from 700 to 1100 nm. The spectral resolution is ± 1 cm^{-1} in the fingerprint region and ± 2 cm^{-1} in the CH-stretching one and the overall spectral range is 300-3200 cm^{-1}. The laser output power is of <100 mW for both lasers, therefore preventing sample thermodecomposition.

Results: The first task of the work was to attain a robust Raman and SERS maleimide spectra considering the scarce bibliographic sources available. For that task maleimide standard powder was analyzed using the solid cap of the hand-held Bravo spectrometer. The Raman bands for maleimide were identified at 306 (medium, m), 331 (shoulder, sh), 404 (weak, w), 551 (w), 603 (very weak, vw), 641 (strong, s), 676 (vw), 770 (vw), 898 (s), 933 (w), 970 (w), 1067 (s), 1151 (vw), 1298 (w), 1360 (w), 1581 (s), 1607 (vw), 1708 (s), 1755 (very strong, vs), 1797 (w), 1834 (vw), 1862 (w), 1968 (w), 2866 (w), 3104 (s) and 3166 (m) cm^{-1}.

Acquisition of the maleimide SERS spectrum was performed by mixing in a vial, as the optimized conditions, 1 ml of 4 mg/ml of maleimide solution and 0.5 ml of the colloid containing the Ag nanoparticles. After mixing, the vial was set in the cap of the spectrometer and measured, obtaining the spectrum shown in Figure 1. The concentration of maleimide in solution is higher than would be expected in most natural samples, however, it was sufficient to ensure that even the weakest SERS bands were detected as it can be appreciated in Figure 1. The SERS bands for maleimide are shown at 464 (s), 669 (vs), 936 (m), 1059 (m), 1559 (sh), 1575 (s), 3083 (m) and 3142 (br) cm^{-1}.

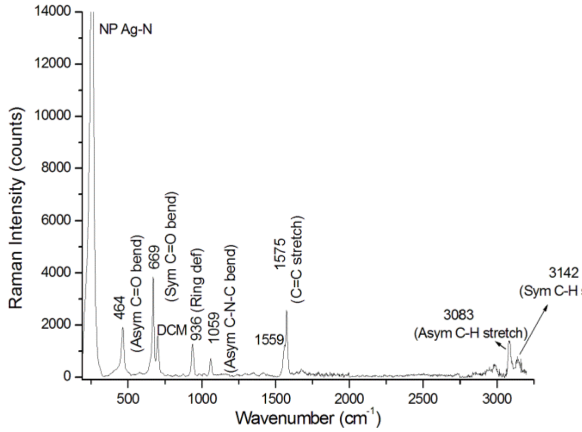

Figure 1. SERS spectra of 4 mg/ml maleimide solution in 9:1 DCM:Methanol with Ag nanoparticles.

The SERS and standard Raman spectra of maleimide differed in the relative intensity and position of the Raman bands. Most bands were shifted and others disappeared, suggesting an interaction between the maleimide molecule and the Ag nanoparticles.

The Limit of Detection (LOD) was significantly improved using the SERS method. No Raman bands were detected in the 4 mg/ml using standard Raman spectroscopy. In contrast, with the Ag colloid addition, the main SERS band of maleimide (669 cm^{-1}) was detected even in a 60 µg/ml solution, a 3 order of magnitude improvement in sensitivity.

We have observed a linear correlation between the area of the 669 cm^{-1} SERS band and the maleimide concentration in solution, in the range 60 to 120 µg/ml.

This relationship will provide an opportunity to develop an analytical method to quantify maleimide in geological samples in both the laboratory and in the field.

Conclusions: SERS has been demonstrated to be an effective method for detecting maleimide, a biologically significant heterocyclic compound. A similar method could be applied to the analysis of other organic molecules present in geological samples by using SERS on portable Raman spectrometers of high spectral resolution.

We have also demonstrated that SERS can be employed to quantify maleimides in the range between 60 to 120 µg/ml. While LOD is comparable to or slightly better than that achieved by GC-MS, SERS is much easier more rapid to perform. Moreover, GC-MS consumes the sample precluding further analysis.

Acknowledgments: J. Aramendia gratefully acknowledges financial support for her work at Jet Propulsion Laboratory, California Institute of Technology from the UPV/EHU. K. H. Williford and M. Tuite acknowledge the support of a grant from the National Aeronautics and Space Administration for work performed at the Jet Propulsion Laboratory, California Institute of Technology.

References:

[1] Summons R. E., Albrecht P., McDonald G., Moldowan J. M. (2008) Space *Sci. Rev.*, *135*, 133–159.

[2] Wilson R., Monaghan P., Bowden S. A., Parnell J., Cooper J. M. (2007) *Anal. Chem.*, *79*, 7036-7041.

[3] Leopold N., Lendl B.A. (2003) *J. Phys. Chem. B.*, *107*, 5723-5727.