Geoscience Frontiers 12 (2021) 101226

HOSTED BY

Contents lists available at ScienceDirect

Geoscience Frontiers

journal homepage: www.elsevier.com/locate/gsf

Research Paper

A new semi-quantitative Surface-Enhanced Raman Spectroscopy (SERS) method for detection of maleimide (2,5-pyrroledione) with potential application to astrobiology

J. Aramendia [a,b,*], L. Gomez-Nubla [a], M.L. Tuite [a], K.H. Williford [a], K. Castro [b], J.M. Madariaga [b]

[a] Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr, Pasadena, CA 91109, United States
[b] Department of Analytical Chemistry, University of the Basque Country UPV/EHU, P.O. Box 644, 48080 Bilbao, Spain

ARTICLE INFO

Article history:
Received 8 October 2020
Revised 3 May 2021
Accepted 8 May 2021
Available online 12 May 2021
Handling Editor: R.M. Palin

Keywords:
Maleimide
Surface-Enhanced Raman Spectroscopy
Astrobiology
Biosignature
Extraterrestrial life

ABSTRACT

Nitrogen-containing heterocyclic compounds are fundamental biochemical components of all life on Earth and, presumably, life elsewhere in our solar system. Detection and characterization of these compounds by traditional solvent extraction, chromatographic separation, and GC–MS analysis require more sample mass than will be available from samples returned to Earth from Mars. With its small sample mass requirement, Surface Enhanced Raman Spectroscopy could be an appropriate technique for analysis of returned samples. We have developed a SERS method for the detection of maleimide (2,5-pyrroledione), an N-containing heterocycle with a structure that is widespread in biochemicals. This semi-quantitative methodology accurately determines maleimide concentration in the range from 60 μg/mL to 120 μg/mL. We present a maleimide SERS standard spectrum which will be useful as a reference for future works. The present work demonstrates an easy, accurate, and effective method for the non-destructive qualitative and semi-quantitative study of maleimide as a first step toward developing a method for analysis of related compounds.

1. Introduction

Nitrogen-containing heterocyclic compounds including nucleic acids, amino acids, proteins, and enzymes are fundamental biochemical components of all life on Earth. Assuming a similar underlying biochemical framework for extraterrestrial life, this class of compounds may serve as a biosignature for life beyond the Earth. With the successful landing of the Perseverance rover on Mars in 2021, the first leg of a decade-long international sample return program, there is the very real prospect that Earth-bound laboratories will examine samples from Mars in the next decade. The limited mass of the subsamples that may be made available to investigators will necessitate methods that employ small masses and are minimally destructive.

Maleimide (2,5-pyrroledione) is an N-containing heterocycle with a structure that is widespread in fundamental biochemicals (e.g., hemoglobin and chlorophyll). Distinctive methylated forms derived from the diagenesis of chlorophylls are well preserved in ancient sediments where they serve as a record of primary production. We have selected maleimide as a useful representative compound to begin the development of a method for detection of biologically-significant heterocycles in small samples.

Maleimide and its derivatives are typically isolated from natural samples by solvent extraction, separated from other fractions by column chromatography, and then analyzed via gas chromatography/mass spectrometry (GC–MS) (Kozono et al., 2001; Shimoyama et al., 2001). This sample preparation and analytical approach is both time and resource intensive. Because of the high volatility of the maleimide, the chromatographic methodology may introduce some bias and result in low recoveries during the extraction/separation step. Although others have sought to mitigate these shortcomings (Naeher et al., 2016), sample consumption likely makes this approach untenable for returned samples.

Because of the precious nature of each returned sample, non-destructive techniques are preferred in order to preserve samples for future analyses. For this reason, Raman spectroscopy is a promising non-destructive option for characterizing organic molecular properties and has been used for(semi-)quantitative characterization of a variety of compounds employing both

* Corresponding author at: Department of Analytical Chemistry, University of the Basque Country UPV/EHU, P.O. Box 644, 48080 Bilbao, Spain.
E-mail address: julene.aramendia@ehu.eus (J. Aramendia).

https://doi.org/10.1016/j.gsf.2021.101226
1674-9871/

external calibration curves with standards (Costantini et al., 2016) and mathematical approaches (Aramendia et al., 2014).

The growing importance of Raman spectroscopy in Earth and planetary science suggests that it will play an important role in the eventual non-destructive analysis of samples returned from Mars. Significantly, Raman spectrometers are components of the scientific payload capabilities of both the Perseverance and Rosalind Franklin rovers. In order to ensure that Raman spectrometry matures in preparation for analyzing returned samples with the utmost sensitivity to a wide range of compounds, new developments that increase the sensitivity and specificity of this core analytical technique should be developed.

With that aim, several studies have been published showing characteristic Raman signatures of organic molecules relevant to Earth history and astrobiology (Edwards et al., 2011; Wei et al., 2014). These works are essential for future identification of compounds that could be detected by upcoming missions. Maleimide exhibits a good Raman response as a pure standard (Woldbæk et al., 1975). However, analysis of real geological samples containing this or other molecular biosignatures is more challenging because organic molecules are typically present in low concentrations embedded within complex mixtures with abiotic and/or inorganic phases (Dunn et al., 2007). Even in terrestrial rocks with high organic matter content, the Raman signals of compounds such as ancient pigments, proteins, or fatty acids are in such low abundance that conventional Raman spectroscopy is not able to detect them. Moreover, the signal of the rocky matrix can be so high that it overwhelms all the minute organic Raman bands. In addition, fluorescence reduces the efficiency of the Raman response, hindering the identification of the biomolecules (Chen et al., 2008). Thus, there is a compelling need for a practical, straightforward, and reproducible method for mitigating these challenges to employing Raman spectroscopy for detection of trace organics in terrestrial and extraterrestrial geological samples.

Surface Enhanced Raman spectroscopy (SERS) is a viable alternative to conventional Raman methods. SERS is a surface sensitive technique used to amplify Raman spectra and obtain much better limits of detection (LOD) through using metal nanoparticles (Wilson et al., 2007; Chen et al., 2008). The SERS effect is based on electronic and chemical interaction of the laser, the analyte, and the rough surface of metallic nanoparticles. However, reviewing the existing literature, there are few published works that apply SERS to biosignatures in geologic samples (Dunn et al., 2007; Wilson et al., 2007, 2010; Chen et al., 2008; Bowden et al., 2010). We have found only one reference in the literature to Raman analysis (not SERS) of maleimide (Aroca et al., 1991).

The objective of this manuscript is to present a highly sensitive SERS method for the detection and semi-quantification of maleimide at low abundances as a first step in the development of a method or methods to analyze related biochemicals in terrestrial and extraterrestrial geological samples, like those that may be returned to Earth from Mars. In addition, we will contribute reference spectra to Raman and SERS databases for maleimide, a common organic compound.

2. Material 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 for synthesis in Leopold and Lendl (2003). This method was selected because it has been widely adopted for the detection of organic molecules in geologic samples (Bowden et al., 2010; Wilson et al., 2010). The employed reagents were silver nitrate, sodium hydroxide and hydroxylamine hydrochloride, all of them of pro-analysis quality. The procedure is based on the reduction of the silver colloids by the hydroxylamine in a basic medium. The first step is to prepare 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 using a magnetic stirrer and a magnetic bar. After the addition of all the silver nitrate solution, the resulting yellow/orange/grey solution must be stirred for approximately 15 min. The final solution has to be wrapped with foil to avoid light degradation of the colloidal mixture.

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 nm to 1100 nm. The spectral resolution is ±1 cm^{-1} in the fingerprint and ±2 cm^{-1} in the CH-stretching region and the spectral range is 300–3200 cm^{-1}. The laser output power is <100 mW for both lasers. Prior to the analyses, the methodology was optimized to assure that there was not sample thermodecomposition. The spectrometer is provided with a liquid sample cap which facilitated the analysis of the samples. A portable Raman spectrometer was selected because it has been recently demonstrated that SERS measurements can be performed in situ using portable Raman devices with high spectral resolution, like the one used in our work (Marcaida et al., 2017).

The maleimide solutions were placed in a 2 mL chromatographic transparent glass vial (which fitted the space provided for the sample in the cap and was transparent for Raman) and the colloid solution was directly added there.

After stirring it, the vial was placed directly in the cap of the Raman spectrometer and analyzed without any further sample treatment. The automated settings were used for the spectra acquisition and different sample/colloid ratios were tested for optimization, selecting finally 1 mL of sample and 0.5 mL of the Ag nanoparticle colloids as the best ratio.

In order to obtain a reliable SERS reference spectrum of maleimide a standard solution at 4 mg/mL of maleimide was prepared. This concentration is high, if it is compared with the estimated maleimide content in real samples but in these conditions, the detection of all the SERS bands for the maleimide, even the weakest ones, was assured. Besides, a procedural blank (solvent and Ag NP) was also prepared in order to avoid misinterpretation of the maleimide signals due to the presence of the solvent and Ag colloid signals.

Moreover, in order to evaluate the solvent effect on the proposed methodology different preparations of the sample were studied. First, the maleimide was dissolved in DCM:methanol and mixed with the Ag colloid in the ratio described above. Then, in the chromatographic vial used for the measurements, the 4 mg/mL maleimide DCM:methanol solution was evaporated to dryness. After this, the nanoparticle solution was added to the solid residue left and it was analyzed with the liquid cap of the Raman spectrometer.

For the quantification works, a calibration curve was built between 30 μg/mL and 150 μg/mL. The calibration curve was constructed representing the concentration value against the area integrated from the main SERS band for maleimide (669 cm^{-1}). In order to integrate the area of the band peak some fitting tools were employed. Usually, the first step when fitting Raman bands

is to perform a baseline correction. However, taken into account the good raw spectra obtained in this work, it was considered unnecessary. Notwithstanding, if the SERS spectrum would show fluorescence, baseline correction should have been done before the peak fitting procedure. The fitting process was performed with the OPUS software (Bruker). This software integrates the area between two personally selected points (Supplementary data Fig. S1) which in our case the area between 656 cm^{-1} and 680 cm^{-1} was selected.

3. Results and discussion

First of all, the blank was measured. Then, the 4 mg/mL solution of maleimide prepared for obtaining a reliable SERS spectrum of the compound was also analyzed. By this means, the SERS bands of the solvent and NP were identified and differed from those belonging to the target compound (Fig. 1). The method proved to be successful and a good quality SERS maleimide spectrum was obtained. The detected SERS bands for maleimide were 464 (strong, s), 669 (very strong, vs), 936 (medium, m), 1059 (medium, m), 1559 (shoulder, sh), 1575 (strong, s), 3083 (medium, m) and 3142 (br) cm^{-1}. This spectrum is published for the first time, resulting a valuable contribution for the field considering the scarce bibliographic sources available.

After obtaining a valuable SERS spectrum, the solvent effect was studied by measuring the two different samples prepared as described in Material and Methods section. The result for the evaporated sample was good but comparing this spectrum with the non-evaporated one, the former resulted noisier and resolved worse the –CH region, as it can be appreciated in Fig. 2.

Raman spectrum of maleimide was also obtained in order to improve bibliographic databases. The maleimide standard powder was analyzed using the solid cap of the spectrometer. In this way, the Raman bands for maleimide were identified at 306 (m), 331 (sh), 404 (weak, w), 551 (w), 603 (very weak, vw), 641 (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 (vs), 1797 (w), 1834 (vw), 1862 (w), 1968 (w), 2866 (w), 3104 (s) and 3166 (m) cm^{-1}, as Fig. 3A shows. It is worth to point out, the high-quality spectrum obtained with the DuoLaser technology, which resulted into a low fluorescence and high signal to noise ratio spectrum. It was possible to detect multiple Raman bands with less than 30 s acquisition time and without the need of spectra treatment.

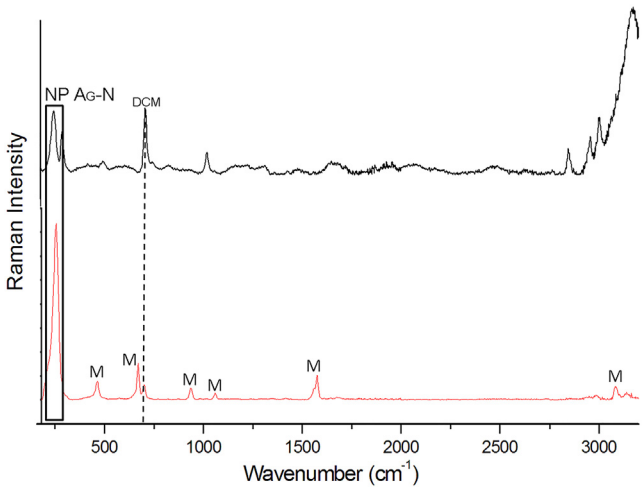

Fig. 1. SERS spectra of 4 mg/mL maleimide solution in 9:1 DCM:methanol with nanoparticles (NP) in red (M means bands belonging to maleimide) and procedural blank (DCM:methanol with NP) in black.

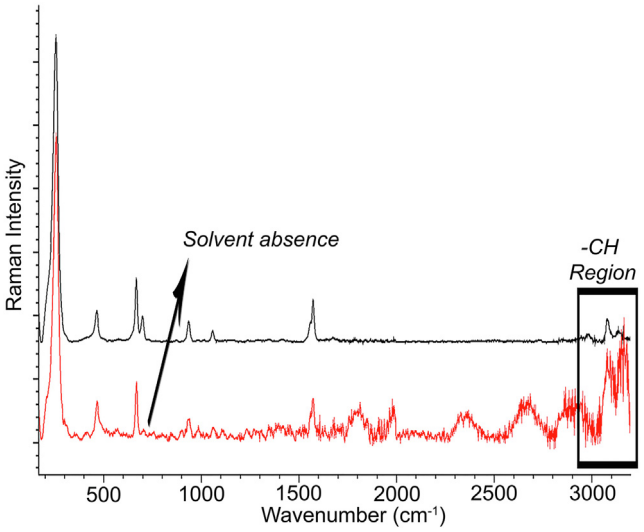

Fig. 2. SERS spectra of 4 mg/mL maleimide solution in 9:1 DCM:methanol with nanoparticles (NP) in black and SERS spectra of 4 mg/mL maleimidewith nanoparticles (NP) where the solvent was evaporated in red.

Fig. 3 shows such a comparison and the maleimide Raman bands are clearly seen. Comparing SERS and common Raman maleimide spectrum, it was seen that they differed to some extent since the relative intensity of the Raman bands varied, most were shifted and some of them disappeared (Fig. 3). This phenomenon happens for some other organic compounds such as scytonemin (Edwards et al., 2000) probably due to the formation of surface complex species between the metal of the nanoparticle and the organic molecule that has the capability of forming new metal–ligand species. This reveals the need of performing a reliable and extensive SERS spectra database for the different compounds that is still a lack in the scientific community.

In Fig. 3 there can be seen how both the symmetric (641 cm^{-1}) and the asymmetric (404 cm^{-1}) C=O bends are maintained in SERS spectrum, as well as, ring deformation (898 cm^{-1}), the asymmetric C–N–C bend (1067 cm^{-1}), the C=C stretch (1581 cm^{-1}) and the symmetric (3166 cm^{-1}) and the asymmetric (3104 cm^{-1}) C–H stretches. However, they are normally shifted and the intensities change from one another. For example, the band at 641 cm^{-1} corresponding to the symmetric C=O bend in common Raman spectroscopy shifts to 669 cm^{-1} in SERS and it becomes the main SERS band for maleimide.

In contrast, the C–H, N–H bend (1298 cm^{-1}), the Sym C–N–C bend (1360 cm^{-1}), the Asym C=O stretch (1708 cm^{-1}) and the Sym C=O stretch (1755 cm^{-1}, the main Raman maleimide band) which were detected in the common Raman spectrum, disappeared in the SERS one. Moreover, in the SERS spectrum, the main band is that belonging to symmetric C=O bend while in the common Raman spectrum it is a shoulder. Besides, the main Raman band is the one belonging to the symmetric C=O stretch and it disappears in SERS spectrum. These differences have their origin, as we mentioned above, in the formation of surface complex species between the metal of the nanoparticle and the organic molecule that has the capability of forming new metal–ligand species.

It must be noticed that all the spectra presented in Figs. 1–3 and all along the work were not processed, that means no baseline correction nor noise filtering were carried out. This underlines the good raw results extracted from the employed portable Raman spectrometer and avoids false results derived from the post-processing of spectra.

Regarding the Limit of Detection (LOD), it must be said that the Raman sensitivity increased considerably. Actually, in 4 mg/mL

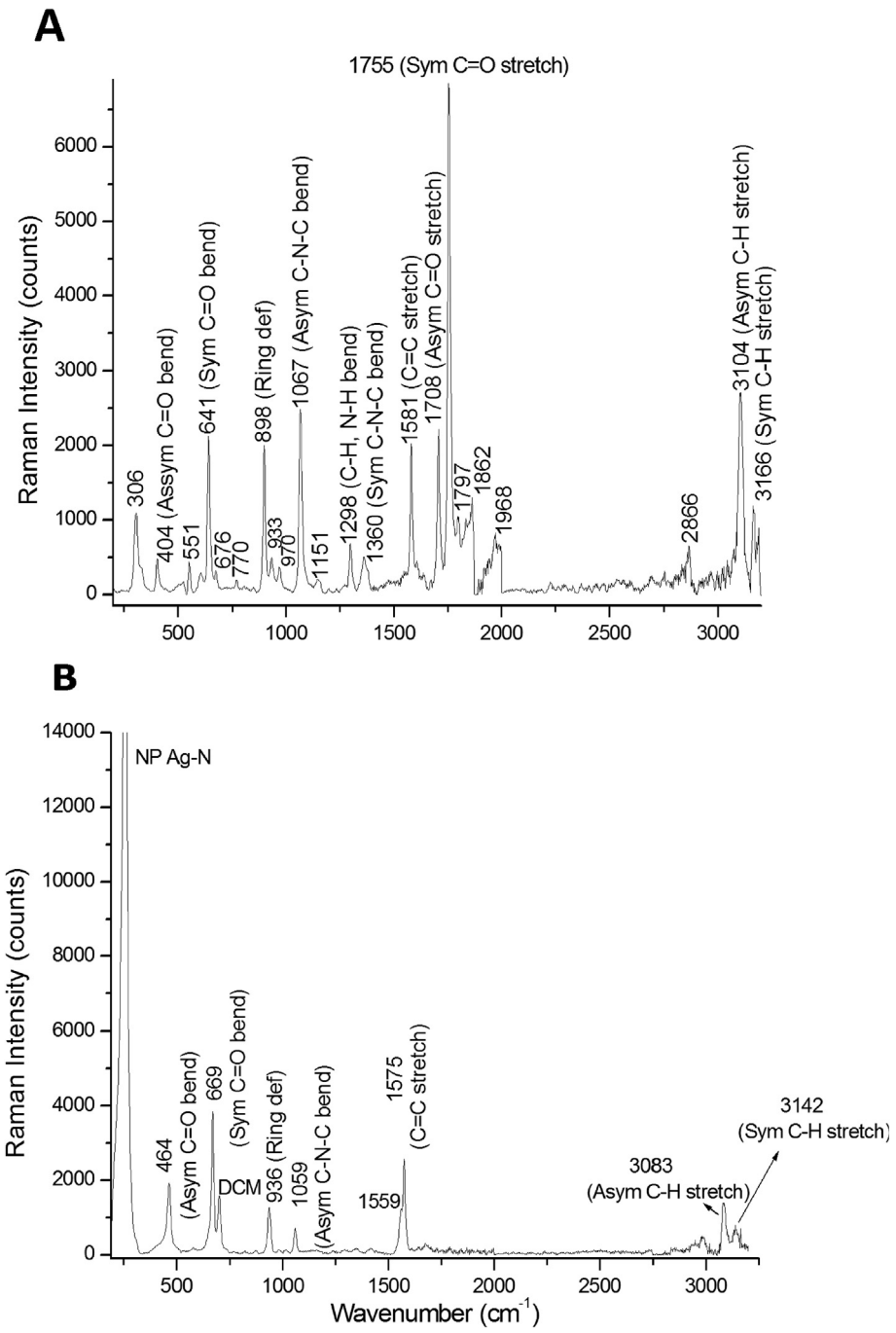

Fig. 3. (A) Maleimide standard Raman spectrum. (B) SERS spectra of 4 mg/mL maleimide solution in 9:1 DCM:methanol with nanoparticles. Differences between common Raman and SERS spectra are clear.

maleimide solutions the molecule was not detected by common Raman spectroscopy, as it can be seen in Fig. 4C where the spectrum of the 4 mg/mL maleimide solution is shown. In the mentioned figure the absence of bands different from the solvent (DCM) is clearly observed. In contrast, with the Ag colloid addition, the main SERS band of maleimide (669 cm^{-1}, which is the selected band used for the study exposed in the present work) was detected even in 60 μg/mL solutions (Fig. 4A). Therefore, the Raman sensitivity of maleimide was improved by 3 orders of magnitude with the addition of silver colloids. As no characteristic SERS bands for maleimide were observed in 50 μg/mL solutions (Fig. 4B), the LOD for this methodology can be set between 50 μg/mL and 60 μg/mL. The obtained LOD could be considered excellent if we compared it with those calculated for other techniques such as GC–MS (around 8 μg/mL (Naeher et al., 2016) as GC–MS is a more costly technique in terms of time, effort and expense.

Moreover, we observed that there was a relationship between the concentration of the solution and the area of the 669 cm^{-1} maleimide SERS main band (Fig. 5 and Table 1). Actually, in that figure, the band at 669 cm^{-1} increases as the concentration of maleimide

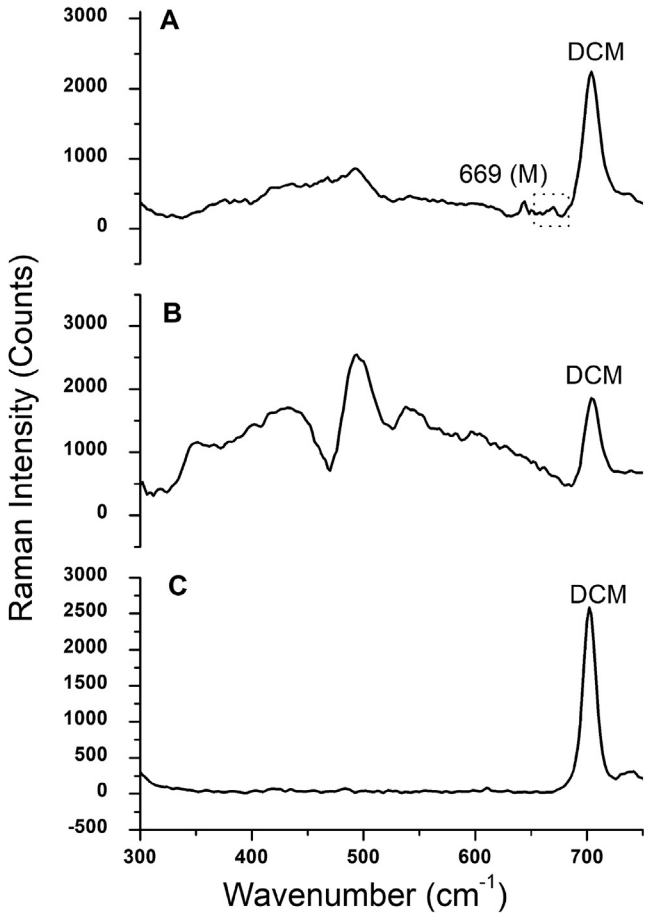

Fig. 4. SERS spectra of (A) 60 μg/mL maleimide solution and (B) 50 μg/mL maleimide solution. (C) Standard Raman spectrum of maleimide 4 mg/mL solution.

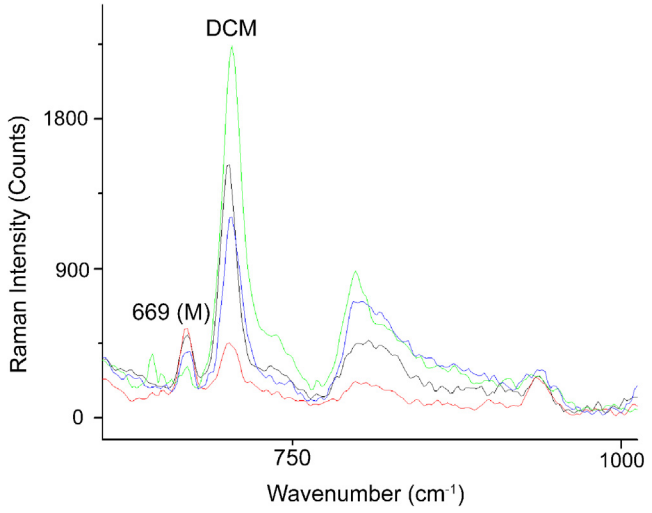

Fig. 5. Different maleimide concentration (75 μg/mL in green, 90 μg/mL in blue, 100 μg/mL in grey and 120 μg/mL in red) SERS spectra where the main band at 669 cm^{-1} is clearly seen.

increases. At the same time, the decrease of the solvent concentration is well represented by the main band of the DCM around 700 cm^{-1}.

Considering this relationship between the concentration and the main SERS band of maleimide, a semi-quantification method was developed for maleimide by monitoring the main SERS band of maleimide located at 669 cm^{-1} wavenumber as it is the main SERS band for the compound.

Table 1
Concentration and peak area values obtained through band integration procedure without outliers.

Maleimide Concentration (μg/mL)	SERS band area (cm^{-1})
50*	0
60	284
60	520
75	1064
90	2025
90	2350
100	2935
100	2718
120	3918
120	3916
150*	3858*
150*	3965*

The values marked with * were those removed for the final equation.

To develop the method, we prepared calibration set of maleimide solutions of 50, 60, 75, 90, 100, 120 and 150 μg/mL. Then, each solution was measured three times to check the repeatability of the procedure and to assess the presence of outliers. These concentration values were selected considering that the presence of maleimide is very low in Earth samples even in those with a high organic content. Therefore, concentrations higher than 150 μg/mL of maleimide would not be representative of real cases of study and solutions with less than 60 μg/mL would probably not be detected by the proposed methodology, as Fig. 4 suggests.

The repeatability of the technique was good, although a couple of points were removed as they did not fulfill the outlier test. The quality of the regression line was tested using the coefficient of determination or R^2, trying to obtain the coefficient closest to 1, in the regression line fitting of the calibration plot.

The calibration plot was obtained representing the SERS peak area of the 669 cm^{-1} band versus the concentration of maleimide. It was observed that the calibration points with the minimum and maximum values of maleimide concentrations did not follow the linearity of the developed methodology. The solution with 50 μg/mL concentration did not show a measurable value. The solution with 150 μg/mL showed a similar response to the one promoted by the solution with 120 μg/mL. Actually, the quality of the regression line was much better without them. Therefore, it was decided not to include them for the final equation. Fig. 6 shows such plot were the uncertainties in both axes are represented.

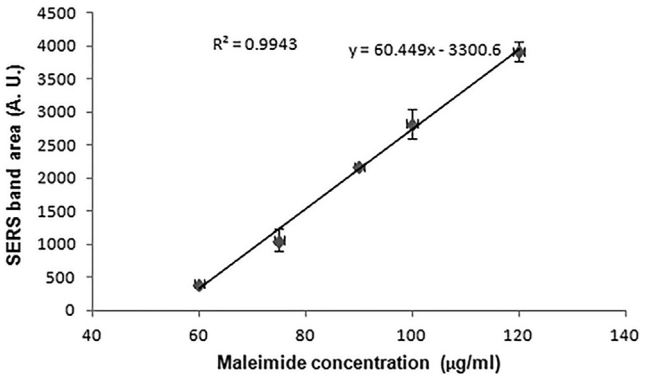

Fig. 6. Calibration plot for maleimide (band area at 669 cm^{-1}).

The equation $y = f(x)$ for this line is:

$$y = (60.4 \pm 2.6)x - (3301 \pm 242)$$

where both the intercept and the slope have associated uncertainties that fulfill the P test of significance. Moreover, the linearity index was excellent, with R^2 = 0.994. Therefore, it can be said that this method is advisable for maleimide concentrations from 60 μg/mL to 120 μg/mL because the linear response of the model is compromised for higher concentrations and the limit of detection was set of 57 ± 1 μg/mL.

4. Conclusions

This work proposes a successful SERS method for the analysis of maleimide. As the Raman spectroscopy is a non-destructive technique, this method can preserve the maleimide extracts for further analysis. This point is extremely important in the context of the Mars Sample Return, considering the incalculable value of the samples. The method development has focused on its application for geologic samples in order to study different aspects of the Earth and planetary history. A similar methodology could be developed for the analysis of other organic molecules present in geologic samples.

It must be highlighted that this is the first work presenting a semi-quantitative model for maleimide detection being accurate for the range between 60 μg/mL and 120 μg/mL. Compared with the most commonly used GC–MS (LOD around μg/mL; Naeher et al., 2016), the LOD is higher but the procedure is much easier because in less than 2 min a good signal to noise ratio SERS spectrum can be obtained. Nevertheless, the detection limit has been improved by 3 orders of magnitude with regards to Raman spectroscopy, and GC–MS only differs in 1 order of magnitude with the SERS methodology developed in this work. Moreover, the GC–MS consumes completely the sample obviating further analysis

Obviously, this methodology is thought to be applied to rock extracts. Therefore, some rock sample pre-treatment is not avoided because a given mass of the solid must be extracted with the proper solvent to obtain the extracts needed to perform the SERS analysis. The limitations of this technique (Raman spectroscopy in general and SERS in particular) for astrobiological applications are clearly exposed in this work, together with their advantages. Notwithstanding, further works will be focused on applying this method to real samples improving the LOD by testing different solvents to maximize the extractability of maleimide from the rock samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

J. Aramendia gratefully acknowledges the financial support from the UPV/EHU for her time spent at the Jet Propulsion Laboratory, California Institute of Technology. This work has been supported through the "Terrestrial and Planetary Alteration Processes" strategic project (ref. PES 18/57), funded by the University of the Basque Country (UPV/EHU).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gsf.2021.101226.

References

Aramendia, J., Gomez-Nubla, L., Bellot-Gurlet, L., Castro, K., Paris, C., Colomban, P.h., Madariaga, J.M., 2014. Protective ability index measurement through Raman quantification imaging to diagnose the conservation state of weathering steel structures. J. Raman Spectrosc. 45, 1076–1084.

Aroca, R., Scraba, M., Mink, J., 1991. SERS and normal coordinate analysis of maleimide chemisorbed on colloidal silver. Spectrochim. Acta Part A Mol. and Biomol. Spectrosc. 47 (2), 263–269.

Bowden, S.A., Wilson, R., Cooper, J.M., Parnell, J., 2010. The use of surface-enhanced Raman scattering for detecting molecular evidence of life in rocks, sediments, and sedimentary deposit. Astrobiology 10, 629–641.

Chen, B., Cabrol, N., McKay, C.P., Shi, C., Gu, C., Newhouse, R., Zhang, J., Lam, T., Pei, Q., 2008. Mix and match: enhanced Raman spectroscopy instrumentation in field applications. In: Hoover, R.B., Levin, G.V., Rozanov, A.Y., Davies, P.C.W. (Eds.). Methods, and Missions for Astrobiology 7097, 709–715. https://doi.org/10.1117/12.802772.

Costantini, I., Veneranda, M., Prieto-Taboada, N., Bellot-Gurlet, L., Castro, K., Madariaga, J.M., 2016. Comparison of semiquantification experimental methodologies using micro-Raman spectroscopy: palme software as an alternative tool for the study of salt efflorescence. J. Raman Spectrosc. 47, 1415–1421.

Dunn, D.S., Sridhar, N., Miller, M.A., Price, K.T., Pabalan, R., Abrajano, T.A., 2007. Development of a surface-enhanced raman technique for biomarker studies on mars. Appl. Spectrosc. 61 (1), 25–31.

Edwards, H.G.M., Garcia-Pichel, F., Newton, E.M., Wynn-Williams, D.D., 2000. Vibrational Raman spectroscopic study of scytonemin, the UV-protective cyanobacterial pigment. Acta Part A Mol. Biomol. Spectrosc. 56 (1), 193–200.

Edwards, H.G.M., Herschy, B., Page, K., Munshi, T., Scowen, I.J., 2011. Raman spectra of biomarkers of relevance to analytical astrobiological exploration: hopanoids, sterols and steranes. Acta Part A Mol. Biomol. Spectrosc. 78 (1), 191–195.

Kozono, M., Nomoto, S., Mita, H., Shimoyama, A., 2001. Detection of maleimides and their characteristics in Neogene sediments of the Shinjo basin, Japan. Geochem. J. 35 (4), 225–236.

Leopold, N., Lendl, B.A., 2003. New method for fast preparation of highly surface-enhanced Raman scattering (SERS) active silver colloids at room temperature by reduction of silver nitrate with hydroxylamine hydrochloride. J. Phys. Chem. B107, 5723–5727.

Marcaida, I., Maguregui, M., Morillas, H., García-Florentino, C., Pintus, V., Aguayo, T., Campos-Vallette, M., Madariaga, J.M., 2017. Optimization of sample treatment for the identification of anthraquinone dyes by surface-enhanced Raman spectroscopy. Anal. Bioanal. Chem. 409 (8), 2221–2228.

Naeher, S., Lengger, S.K., Grice, K., 2016. A new method for the rapid analysis of 1H-Pyrrole-2,5-diones (maleimides) in environmental samples by two-dimensional gas chromatography time-of-flight mass spectrometry. J. Chromatogr. A 1435, 125–135.

Shimoyama, A., Kozono, M., Mita, H., Nomoto, S., 2001. Maleimides in the Cretaceous/tertiary boundary sediments at Kawaruppu, Hokkaido, Japan. Geochem. J. 35 (5), 365–375.

Wei, J., Wang, A., Lu, Y., Connor, K., Bradley, A., Marshall, C., Steele, A., 2014. The detection of biosignatures by laser Raman spectroscopy for Mars Exploration. In: 45th Lunar and Planetary Science Conference, p. 2847.

Wilson, R., Monaghan, P., Bowden, S.A., Parnell, J., Cooper, J.M., 2007. Surface-enhanced Raman signatures of pigmentation of cyanobacteria from within geological samples in a spectroscopic-microfluidic flow cell. Anal. Chem. 79 (18), 7036–7041.

Wilson, R., Bowden, S.A., Parnell, J., Cooper, J.M., 2010. Signal enhancement of surface enhanced Raman scattering and surface enhanced resonance Raman scattering using in situ colloidal synthesis in microfluidic. Anal. Chem. 82, 2119–2123.

Woldbæk, T., Klaboe, P., Nielsen, C.J., 1975. The vibrational spectra of maleimide and N-D maleimide. J. Mol. Struct. 27 (2), 283–301.