

Evaluation of simulates for the Raman spectroscopic characterisation of biomolecular degradation products in geological matrices: PAHs under calcite and gypsum

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Abstract:

Raman spectroscopy is currently part of the instrumentation suite of the ESA Exomars mission for the remote detection of life signatures in the Martian surface and subsurface. Terrestrial analogues of Martian sites have been identified and the biogeological modifications incurred as a result of extremophilic cyanobacterial activity have been studied. In simulation of the inclusion of organic biomolecules found in crystal matrices in, for example, impact craters we have studied the Raman spectra of several PAHs and usnic acid under calcite and gypsum crystal. Spectra were obtained from anthracene, phenanthrene, naphthalene, tetracene, chrysene, pyrene and triphenylene and usnic acid crystals through calcite and gypsum surfaces. Although the resulting spectra have peaks attributed to calcite and gypsum, the PAH compounds were identified by their characteristic bands. Anthracene, phenanthrene and naphthalene were mixed together; tetracene, chrysene, pyrene and triphenylene were mixed together, and Raman spectra were collected from these mixtures under similar conditions to determine the limits of detection in admixture. Spectra of several pure compounds and in admixture could be readily obtained non-invasively from calcite and gypsum within ten minutes with little or no sample preparation. Information from this study will be useful for targeting Martian sites using a miniaturized Raman instrument where the biosignatures of relict or extant life could remain in the geological record.

Key words: Raman spectroscopy, Mars-analog, Minerals, PAHs.

تقييم المحاكاة لتوصيف رامان الطيفي لمنتجات التحلل الجزيئي الحيوي في المصفوفات الجيولوجية: PAHs تمت الكالسيت والجبس

المخلص:

يعد مطياف رامان حالياً جزءاً من مجموعة أدوات مهمة الوكالة الأوروبية للفضاء للكشف عن توقع الحياة على سطح المريخ وتحت سطحه، تم تحديد النظائر الأرضية لمواقع المريخ ودراسة التعديلات البيولوجية الجيولوجية الناتجة عن النشاط المجهرى للجراثيم.. في محاكاة إدراج الجزيئات الحيوية العضوية الموجودة في المصفوفات البلورية، قمنا بدراسة أطياف رامان للعديد من المركبات الهيدروكربونية العديدة (PAHs) وحمض عضوي تحت بلورات الكالسيت والجبس. تم الحصول على الأطياف من بلورات أنثراسين، فينانثرين، نفتالين، تتراسين، كريسين، بيرين وثلاثي فينيل وبلورات الحمض من خلال أسطح الكالسيت والجبس. على الرغم من أن الأطياف الناتجة لها قمم منسوبة إلى الكالسيت والجبس، فقد تم تحديد مركبات PAH من خلال نطاقاتها المميزة. تم خلط الأنثراسين والفينانثرين والنفتالين معاً. تم خلط التتراسين والكريسين والبيرين والثيوفينيلين معاً، وتم جمع أطياف رامان من هذه المخاليط في ظروف مماثلة لتحديد حدود الكشف في الخليط. بينت النتائج أنه يمكن الحصول على أطياف من العديد من المركبات النقية وفي الخلط بشكل غير متداخل مع الكالسيت والجبس.

الكلمات المفتاحية: مطيافية رامان، التناظرية المريخ، المعادن، PAHs.

1- Introduction:

Raman spectroscopy is currently part of the instrumentation suite of the ESA Exomars mission for the remote detection of life signatures in the Martian surface and subsurface. Terrestrial analogues of Martian sites have been identified and the biogeological modifications incurred as a result of extremophilic activity have been studied. Raman spectroscopy has been proposed as a valuable analytical technique for environmental and planetary exploration because it is sensitive to organic and is inorganic compounds and able to clearly identify key spectral markers in a mixture of biological and geological compounds; also, sample manipulation is not required and any size of sample can be studied without chemical or mechanical preparation.

Astrobiology aims at identifying the pathways through which life arose, evolved, and may have been distributed throughout the universe. With the ability to expose microorganisms to the space environment in a controlled

manner^[1] the opportunity has arisen to explore questions related to the hypothesis of interplanetary transfer of life^[2]. Possible mechanisms of interplanetary transfer include spacecraft, or natural mechanisms such as meteorites, comets or interplanetary dust particles. Interplanetary transfer of life is particularly relevant with regard to Mars and Earth because it is thought that the meteorites traveled from Mars to Earth, and possibly meteorites from Earth traveled to Mars, providing a pathway for the transport of biological material between these two planets^[3,4].

Polycyclic aromatic hydrocarbons (PAHs) have been the subject of well-documented study because of their carcinogenic effect, widespread environmental pollution. They are produced daily in great quantities by incomplete combustion of carbonaceous materials. Significant efforts have been expended to devise techniques for the identification and quantification of PAHs in water, and they are common constituents of complex mixtures such as automobile exhausts, crude oil, cigarettes, coal, and have been found at significant levels in the atmosphere, waterways and food chains^[5].

Polycyclic aromatic hydrocarbons are found to be component of organic matter in space^[6]. Their contribution is invoked in a broad spectrum of astronomical observations that range from the ultraviolet to the far-infrared and cover a wide variety of objects and environments from meteorites and interplanetary dust particles to outer Solar System bodies and to the interstellar medium in the local Milky Way.

Polycyclic aromatic hydrocarbons are believed to be the most abundant free organic molecules in space^[7,8]. Electron delocalization over their carbon skeleton makes them remarkably stable. PAH molecules are produced partly in the outer atmospheres of carbon stars or formed by shock fragmentation of carbonaceous solid material. PAHs may eventually also form in the diffuse interstellar gas by neutral-neutral atom reactions^[9] or by the energetic processing of specific ices in dense clouds^[10]. PAHs play a central role in the gas phase chemistry^[11,12]. The environmental conditions and the local ultraviolet radiation field determine their charge and hydrogenation state^[13].

The need to identify and determine these compounds has led to the establishment of methods that allow for the simultaneous determination of as many PAHs as possible.

In secondary lichen metabolites, the dibenzofuran derivative usnic acid has been without a doubt the most extensively studied. Lichens are formed through the symbiosis between fungi and algae.

Usnic acid is widely distributed in species of *Cladonia* (Cladoniaceae), *Usnea* (Usneaceae), *Lecanora* (Lecanoraceae), *Ramalina* (Ramalinaceae), *Evernia*, *Parmelia* (Parmeliaceae) and other lichen genera. *Alectoria*

(Alectoriaceae) species are often rich sources of usnic acid, and yields of up to 6% have been reported^[14]. In the literature, usnic acid has been quoted as being present in *Cetraria islandica* (Parmeliaceae), commonly known as Iceland moss or *Lichen islandicus*.

Although usnic acid has only been identified in lichens, closely related compounds have been found in fungi, e.g. the phytotoxin mycosuine and similar compounds in *Mycosphaerella*^[15] and cercosporamide and usnic acid amide in *Cercosporidium henningsii*^[16].

Raman spectroscopy is sensitive to molecular skeletal structures: characteristic wavenumber indicate bond orders, branching of chains, saturated and aromatic rings, and their substitution pattern^[17]. However, fluorescence emission is frequently observed from impurities and common by-products and often even of the main components.

The present work reports a Raman spectroscopic study of several pure fused ring organic aromatic compounds observed under crystalline calcite and gypsum, in the solid state, using confocal microspectroscopy for the detection and identification of features which are important in environmental chemistry and astrobiology. A similar study was undertaken of mixtures of relevant PAH material to assess the potential of Raman for the discrimination between specific molecular structure.

The capability of Raman spectroscopy for the detection of minerals and organic compounds in mixtures has led to adoption by the European Space Agency (ESA) as part of a life-detection instrumental suite to be sent to Mars for the ExoMars project.

2- Experimental:

2.1- Compound Samples.

Anthracene, phenanthrene, naphthalene, tetracene, chrysene, pyrene, triphenylene and usnic acid were supplied by Sigma-Aldrich (UK) and ALFA AESAR (UK). All the compounds were used as received.

Samples of crystalline calcite (calcium carbonate, CaCO_3) and crystalline gypsum (calcium sulphate, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) were kindly supplied by Prof Howell Edwards from a geological mineral collection.

2.2- Compounds Mixture:

Anthracene, phenanthrene and naphthalene were mixed together similar tetracene, chrysene, pyrene and triphenylene were mixed together. Sample mixtures were made up by mixing known amounts of the compounds, followed by grinding in an agate mortar and pestle to ensure sample homogeneity by thorough mixing of components.

2.3- Dispersive Raman microscopy.

Raman spectra were collected using a RenishawInVia Reflex dispersive Raman spectrometer (Renishawplc, Wotton-under-Edge, UK). Excitation was effected using a 785 nm near-infrared diode laser. The laser beam was focused on the sample by a 5x objective lens, resulting in a laser spot of approximately 10 μm at the surface.

Spectra were obtained at 2 cm^{-1} resolution for 10s exposure of the CCD detector in the region 100-3200 cm^{-1} using the extended scanning mode of the instrument with 100% laser power and 5 accumulations were collected for the compounds. The total acquisition time of each spectrum was therefore about ten minutes. Spectral acquisition, presentation, and analysis were performed with the Renishaw WIRE 2 (Renishawplc) and GRAM AI version 8 (Galactic Industries, Salem, NH) software.

3- Result and Discussion:

3.1- Calcite and gypsum.

The Raman spectrum of calcite presents two strong bands at 1086 cm^{-1} assigned to the $\nu(\text{CO}_3)^{2-}$ Stretch and 278 cm^{-1} (Lattice mode) and two medium sharp bands at 1747 and 708 cm^{-1} (bending mode). Also, the gypsum spectrum contains one strong peak at 1002 cm^{-1} assigned to the symmetric sulfate stretching [$\nu(\text{SO}_4)^{2-}$] and four weaker peaks at 1138 cm^{-1} which is asymmetric sulfate stretching, 615 cm^{-1} [symmetric deformation], 488 cm^{-1} [symmetric deformation] and 410 cm^{-1} as shown in figure 1 and 2.^[18,19]

3.2- PAHs under calcite and gypsum crystalline.

The spectra obtained from anthracene, phenanthrene, naphthalene, tetracene, chrysene, pyrene and triphenylene crystals under calcite and gypsum are shown in figures 3 -11. Comparison of these spectra with the reference spectra of the compounds shows that the compounds could still be easily identified using characteristic bands in their Raman spectra. Anthracene can be identified by several characteristic bands which can be used to identify it, such as one strong band at 1399 cm^{-1} due to C=C in-plane vibration and three medium bands at 1004 (C-H in-plane deformation vibration), 749 (C-H out-of-plane deformation vibration) and 391 cm^{-1} (CCC bending) as depicted in figure (3 A).

The phenanthrene spectrum contains three strong peaks at 1346, 708, 407 cm^{-1} due to C-C stretching, HCC bending, HCCC out-of-plane bending and CCC bending respectively and three medium sharp features at 1438, 1034, 543 cm^{-1} assigned to C-C stretching, HCC bending, C-C stretching, HCC bending and CCC bending, respectively, as depicted in figure (4 A).

Similarly, the naphthalene spectrum has several characteristic bands that can be used to identify the compound, for example the two strong bands 1378, 759

cm^{-1} (C=C in-plane vibration and CCC in-plane bending modes) and three medium bands at 1461, 1018 (C-H in-plane deformation vibration) and 509 (C-C ring deformation) cm^{-1} depicted in figure (5 A). Also, tetracene can be identified by its three strong Raman bands at 1540, 1381, 750 cm^{-1} due to C-C stretching, C=C in-plane vibration and CCC ring deformations, respectively, and three medium intensity Raman bands at 1194, 1177 and 1157 cm^{-1} assigned to C-H in-plane deformation vibrations as depicted in figure (6 A).

The chrysene spectrum contains one strong Raman band at 1378 cm^{-1} (C-C stretching) and four medium sharp Raman bands at 1571, 1430, 1361 and 1014 cm^{-1} assigned to C-C stretching, C-C stretching, C-C stretching and C-H in-plane bending respectively that can be used to identify the compounds as shown in figure (7 A). The pyrene spectrum has two strong Raman bands at 1403 and 589 cm^{-1} (C=C in-plane vibration, skeletal ring vibrations, CCC ring deformation) and three medium sharp Raman bands at 1238, 1063 and 403 cm^{-1} due to CH bending which can be used to identify it as depicted in figure (8 A). Similarly, the triphenylene spectrum has several characteristic bands that can be used to identify the compound, namely, one strong band at 1336 cm^{-1} (C-C stretching) and three medium-sharp features at 1455, 1058 and 695 cm^{-1} assigned to C-C stretching, C-H in-plane bending and C-H out-of-plane bending respectively shown in figure (9 A). Although Raman spectra of anthracene, phenanthrene, naphthalene, tetracene, chrysene, pyrene and triphenylene have been recorded under calcite and gypsum, the characteristic bands of each compound are still observable and are in agreement with the reference spectra of the pure compounds. Although the spectra have several bands assigned to the calcite or gypsum, these bands do not overlap with the characteristic bands of the PAHs.

3.3- PAH Mixtures.

Figure (10 A) shows the Raman spectra of a mixture of anthracene, phenanthrene, and naphthalene (Mixture 1) and figure (11 A) shows the Raman spectra of a mixture of tetracene, chrysene, pyrene and triphenylene (Mixture 2) .All the compounds in each mixture can be clearly identified by their characteristic bands with no band overlap being observed. Although the spectra of the mixtures have several bands due to the different compounds these bands do not overlap with the characteristic bands of each compound.

3.4- PAH Mixtures under Calcite and Gypsum crystalline.

Figure (10 B, C), shows the Raman spectra of Mixture 1 (anthracene, phenanthrene and naphthalene) under calcite and gypsum also Figure (11 B, C) shows the Raman spectra of Mixture 2 (tetracene, chrysene, pyrene and triphenylene) under calcite and gypsum. The characteristic bands of each

compound are still observable in the mixture under calcite and gypsum despite the presence of the calcite and gypsum bands.

3.5- Usnic acid under calcite and gypsum crystalline.

The spectra obtained from usnic acid under calcite and gypsum are shown in figure 12. Comparison of these spectra with the reference spectrum of the compound shows that usnic acid could be identified using its Raman spectrum. Usnic acid can be identified by several characteristic bands which can be used to identify it such as three bands at 1322 cm^{-1} assigned to ring stretch, 1288 cm^{-1} due to ν (COC) of the aryl alkyl ether and 539 cm^{-1} depicted in figure (11 A). The usnic acid could still be identified by its characteristic Raman bands under calcite and gypsum, although Bands due to the mineral can also be identified.

4- Conclusion.

Raman spectroscopy provides an efficient way for detection and identification of pure and mixed organic compounds through crystals of calcite and gypsum. The presence of spectral bands arising from the calcite or gypsum did not interfere with the identification of the organic compounds. The significances of which can be discriminate alone and in admixture, using confocal Raman spectroscopy.

It is clear that the observation of two or more characteristic bands for each species is necessary for the identification of the individual compound or organic mixtures.

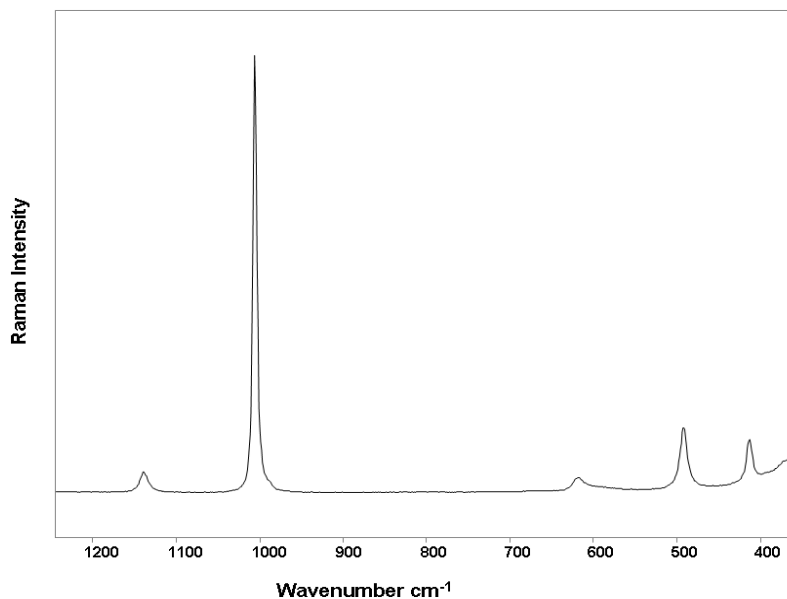


Figure 1: The Raman spectra of gypsum obtained with 785 nm excitation

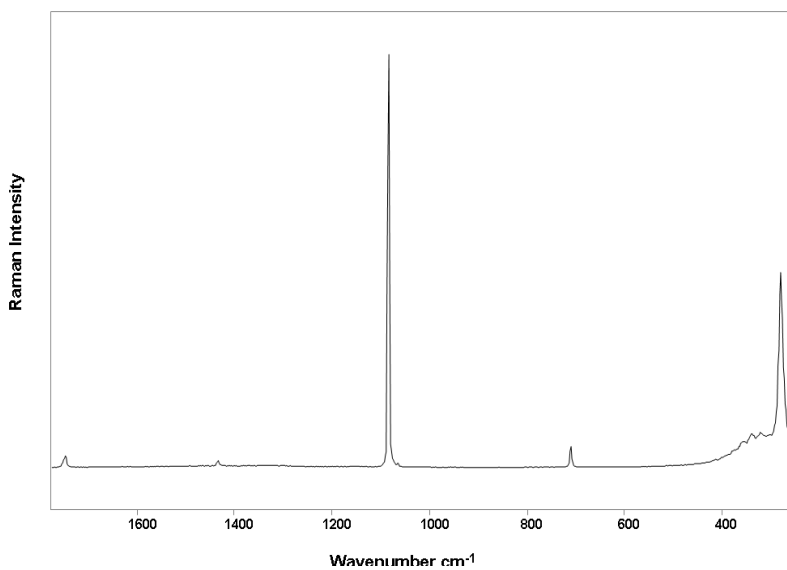


Figure 2: The Raman spectra of calcite obtained with 785 nm excitation

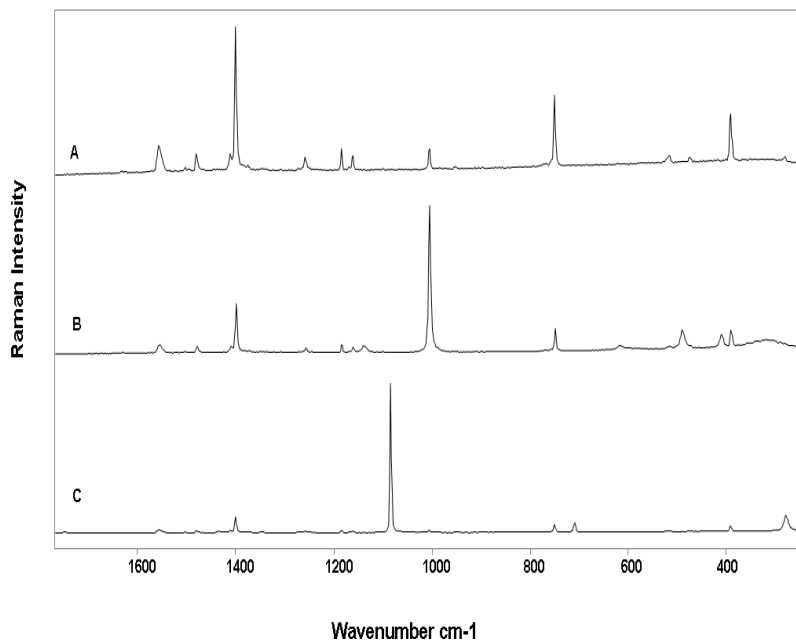


Figure 3: Raman spectra of (a) pure anthracene, (b) anthracene under gypsum and (c) anthracene under calcite

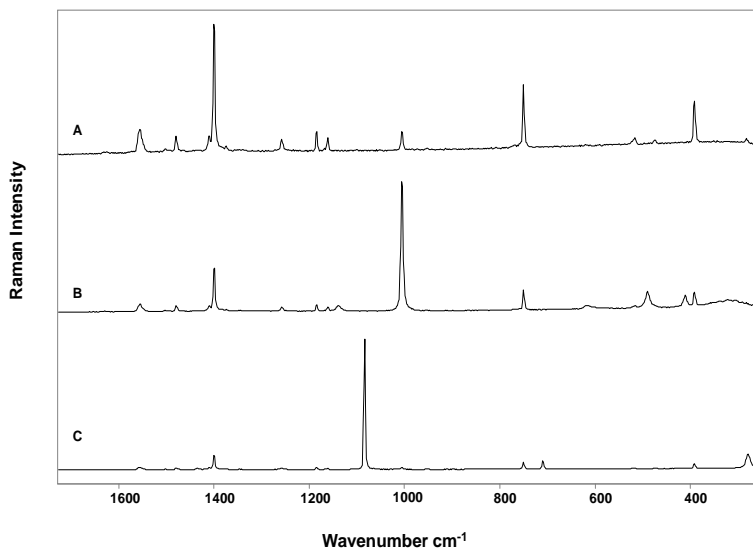


Figure 4: Raman spectra of (a) pure phenanthrene, (b) phenanthrene under gypsum and (c) phenanthrene under calcite

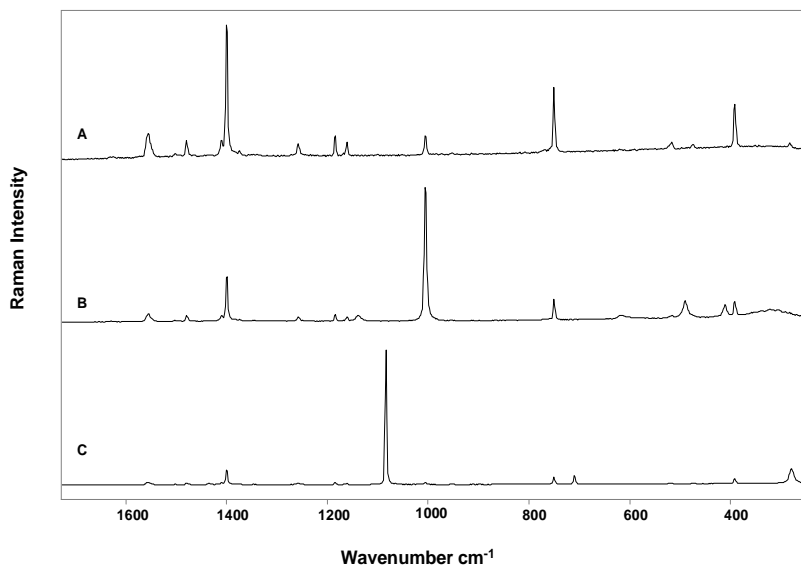


Figure 5: Raman spectra of (a) pure naphthalene, (b) naphthalene under gypsum and (c) naphthalene under calcite

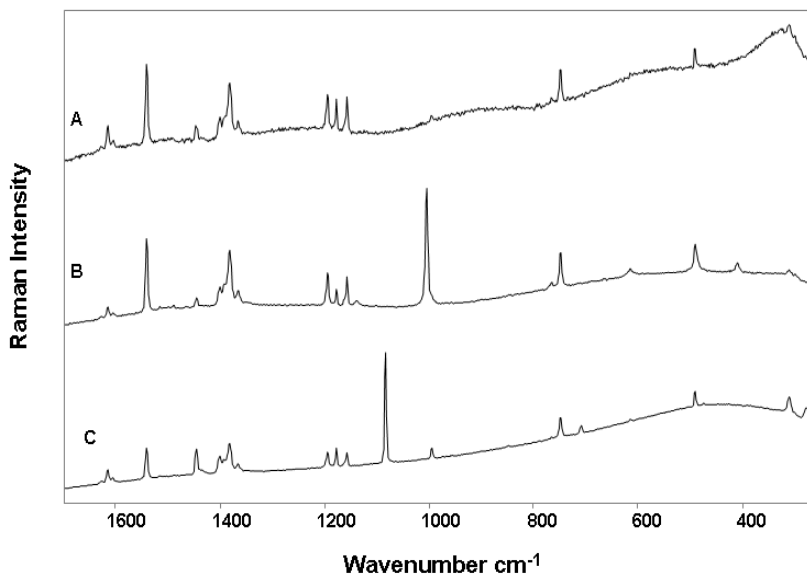


Figure 6: Raman spectra of (a) pure tetracene, (b) tetracene under gypsum and (c) tetracene under calcite

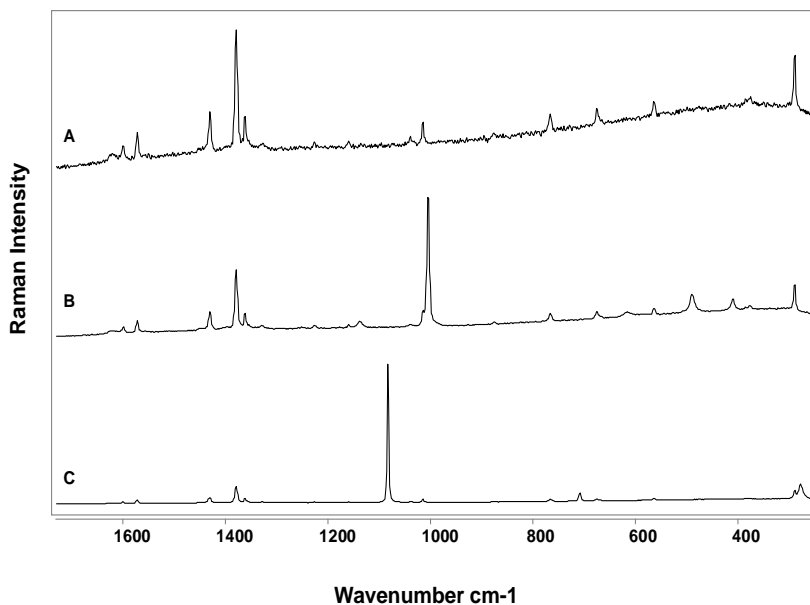


Figure 7: Raman spectra of (a) pure chrysene, (b) chrysene under gypsum and (c) chrysene under calcite

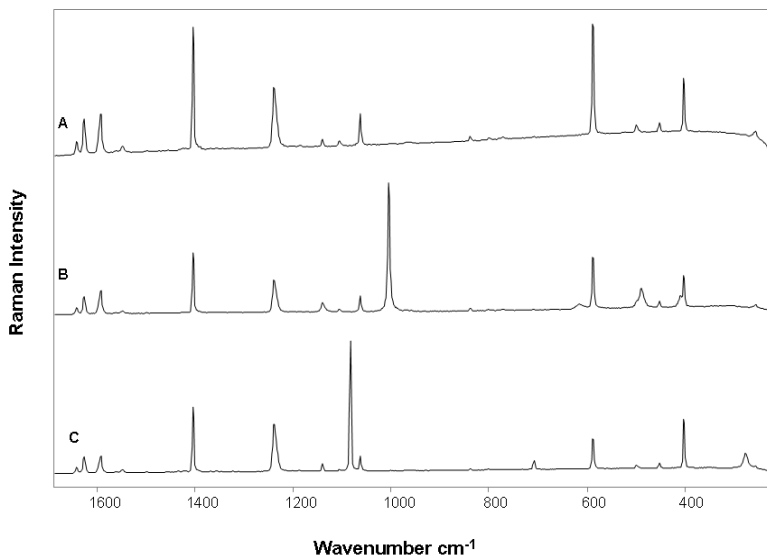


Figure 8: Raman spectra of (a) pure pyrene, (b) pyrene under gypsum and (c) pyrene under calcite

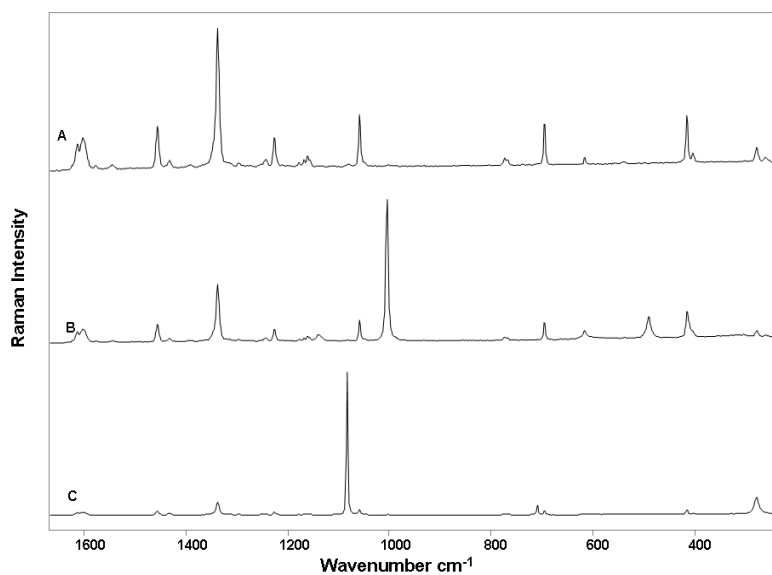


Figure 9: Raman spectra of (a) pure triphenylene, (b) triphenylene under gypsum and (c) triphenylene under calcite

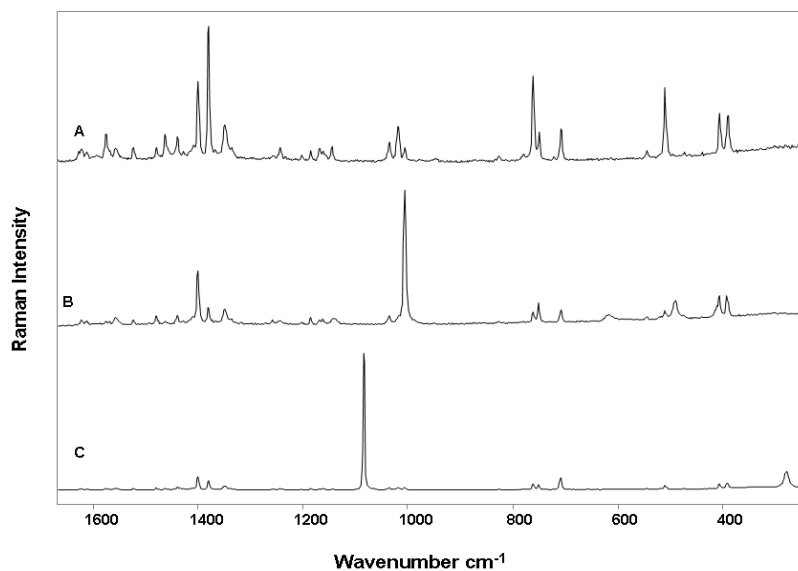


Figure 10: Raman spectra of (a) pure Mixture 1, (b) Mixture 1 under gypsum and (c) Mixture 1 under calcite

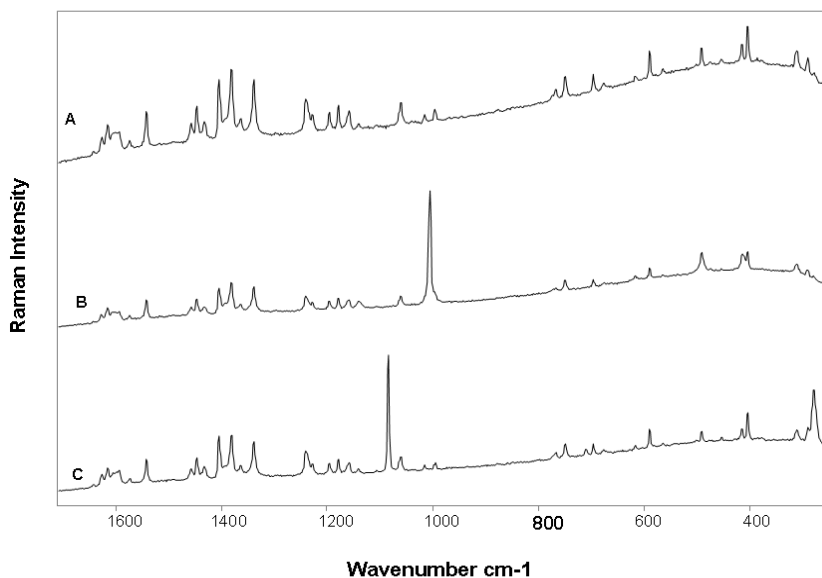


Figure 11: Raman spectra of (a) pure Mixture 2, (b) Mixture 2 under gypsum and (c) Mixture 2 under calcite

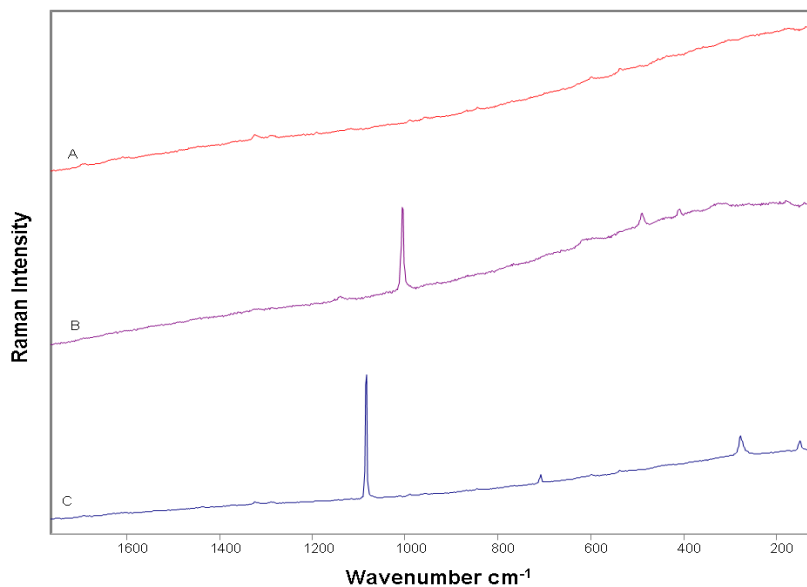


Figure 12: Raman spectra of (a) pure usnic acid, (b) usnic acid under gypsum and (c) usnic acid under calcite

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