Summary

Raman spectroscopy is a powerful tool for identification both inorganic and organic compounds including microbial biomolecules. Together with the fact, that it is considered to be the important nondestructive instrument for use on Mars within future robotic missions, it is necessary to assess its capabilities in scenarios relevant for both Martian and terrestrial conditions. In this work, the potential of Raman spectrometry was tested – including both bench-top laboratory systems as well as portable counterparts – to detect traces of life within evaporitic matrices through biomolecular identification. Due to their chemical and physical nature resulting in optical properties, pigments are important organic compounds in Raman spectroscopic analysis using visible excitation. Hence in this work we have focused on the Raman spectroscopic identification of pigments as biomarkers with relevance for investigation of life in both extreme terrestrial and potentially extraterrestrial environments.

Results of methodical work are presented in Appendices I to III, dealing particularly with β-carotene as a model carotenoid pigment. The concentration limits of this biomarker in three different evaporitic matrices (halite, gypsum and epsomite) have been determined for artificially prepared powdered mixtures alone and mixtures analysed through a single crystal of gypsum or epsomite. We detected β-carotene content even as low as 1 mg kg^{-1} in an evaporitic matrix using Raman microspectrometry equipped with 785 nm excitation wavelength (non-resonant mode) which is a universal source for biomolecular identification. Comparison of these results with resonance Raman spectroscopy using a 514.5 nm laser for excitation showed that in this case resonance enhancement of the Raman signal can improve the limits of detection by about one order of magnitude. The analysis performed through the single sulphate crystals resulted in decrease of the Raman signal; however it was still possible to register at least one carotenoid band at concentrations of 1 - 10 mg kg^{-1} of the β-carotene, depending on the excitation wavelength used.

Results obtained using a portable (hand-held) Raman instrumentation equipped with a diode laser at 758 nm showed that Raman macro analysis can be favourable for finely ground mixtures. This miniaturized instrument yielded even slightly better results when analysing a β-carotene/halite mixture than a bench-top Raman microspectrometer using the same excitation.

Real geobiological systems from Atacama Desert which is one of the driest place on Earth and is considered a close analogue to the extremely arid conditions on the surface of Mars were studied as well. Results of Raman spectroscopic analyses of natural endoevaporitic colonies from Ca-sulphate crusts in Atacama Desert (dominated by gypsum, presented in Chapter 2) exhibit systematic variations in carotenoid composition along with the presence/absence of a phycobiliprotein signal. A phycobiliprotein Raman signal is indicative of cyanobacteria and was detected typically in deeper parts of the Ca-sulphate crust where a relatively low amount of light is available. This was accompanied by two clearly distinguished ν(C=C) carotenoid bands at approx. 1516 and 1498 cm^{-1}, pointing to carotenoids of different conjugation, probably including β-carotene and the long polyene chained (13-15 conjugated double bonds) carotenoid. On the other hand, the Raman signal of algal colonies from near the rock surface exhibited a ν(C=C) carotenoid band at approx.

1525 cm^{-1} interpreted as feature of lutein or similar xantophyll compound. These spectra lack a phicobiliprotein signal. Streamline Raman mapping of the algal colonies exhibited a great potential of such type of analysis for study of endolithic communities in their original habitats. Moreover, analytical aspects of using 785 nm and 514.5 nm excitation for analysis of carotenoids are discussed.

Appendix IV describes the Raman spectroscopic identification of pigments from endolithic cyanobacterial colonies in natural halite crusts from Atacama Desert. Spectral signatures revealed the presence of UV-protective biomolecule scytonemin as well as chlorophyll, carotenoids and phycobiliproteins. The spectral features of these biomolecules differed depending on the particular microhabitat. Substantial differences in the scytonemin Raman signal have been observed and suggested to correspond to variable biosynthesis of scytonemin according to the amount of light available inside the halite crust as well as other possible parameters.

It was proved that β-carotene – a typical carotenoid – can be detected in very low content in evaporitic matrix using Raman spectroscopy. The method showed to be also a valuable tool for examination of microbial colonies in their original rock habitats on the basis of pigment composition which allowed us to insight into the phototrophic microbial life in evaporites from Atacama Desert.