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Extraction of polar and nonpolar biomarkers from the martian soil using aqueous surfactant solutions

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Abstract

The Life Marker Chip is being designed to detect the chemical evidence of life in the martian soil. It will use an aqueous surfactant solution to extract polar and nonpolar biomarkers from the martian soil and to transport them into an antibody-based detector for characterisation. Currently, a solution of 1.5 g l^{-1} polysorbate 80 in 20:80 (vol:vol) methanol:water is being considered and appears to be suitable. Here, we have investigated the ability of a range of other surfactant solutions to extract a suite of eight standards spiked on the surfaces of the martian soil simulant JSC Mars-1 and tested the compatibility of the best two surfactants with a representative antibody assay for the detection of pyrene. The results show that using 20:80 (vol:vol) methanol:water as the solvent leads to increased recoveries of standards than using water alone. The poloxamer surfactants Pluronic® F-68 and Pluronic® F-108 are not effective at extracting the standards from JSC Mars-1 at any of the concentrations tested here. The fluorosurfactant Zonyl® FS-300 is able to extract the standards, but not as efficiently as polysorbate 80 solutions. Most successful of the alternative surfactants was the polysiloxane poly[dimethylsiloxane-co-[3-(2-(2-hydroxyethoxy)ethoxy)propyl]methylsiloxane] (PDMSHEPMS) which is able to extract the standards from JSC Mars-1 with an efficiency approximately equal to that of polysorbate 80 solutions of the same concentration. Enhanced recovery of the standards using polysorbate 80 and PDMSHEPMS solutions can be achieved by increasing the concentration of surfactant, from 1.5 g l^{-1} to 10 g l^{-1} , leading to an increase in the recovery of standards of about 50%. Polysorbate 80 at concentrations of 1.5 g l^{-1} and 10 g l^{-1} and Zonyl® FS-300 and PDMSHEPMS (both at a concentration of 10 g l^{-1}) are also compatible with the representative pyrene antibody assay.

Keywords: Mars; ExoMars; Extraterrestrial life.

1. Introduction

1.1. Searching for evidence of life on Mars

The search for extraterrestrial life, past or present, is focusing on Mars, where abundant evidence indicates that habitable environments may have been commonplace in its early history. For example, channels eroded by flowing water, and minerals and sedimentary structures indicating standing water, have been detected by the NASA Mars Exploration Rovers (Squyres et al., 2004). At the present day, abundant ice has been discovered in the immediate subsurface by the NASA Phoenix lander (Smith et al., 2009) and recurring slope lineae in channels that appear to vary with the martian seasons may indicate the flow of near surface liquid brines (McEwen et al., 2011). The detection of the potential biosignature methane in the martian atmosphere (Formisano et al., 2004) that varies with seasons (Mumma et al., 2009) has led to speculation about extant life in the subsurface (Krasnopolsky et al., 2004), although the interpretations have been criticized and the issue remains controversial (Zahnle et al., 2011). The desire to search for the evidence of past or present life on Mars is the motivation behind the ESA-led ExoMars project.

The Viking landers of the 1970s searched for evidence of life, but the on-board gas chromatograph-mass spectrometer (GC-MS) detected no organic compounds in the martian soil, while the positive result of the Labelled Release experiment has generally been interpreted in terms of soil chemistry, rather than biology (Biemann, 1976). However, the Viking results are not regarded as the final word. Mars is continually being bombarded with thousands of tonnes per year of micrometeorites containing abiotic extraterrestrial organic matter, much of which survives atmospheric entry and settles on to the martian soil (Flynn, 1996). The failure of Viking to detect this meteoritic organic matter has been interpreted in terms of its destruction, via solar and cosmic irradiation (e.g., Klein, 1978; Parnell et al., 2007), or via reactions with the oxidising chemicals, such as perchlorates (Hecht

et al., 2009). As such, it is recognised that the shallow sampling depths used for the Viking samples, in unconsolidated soil, were not ideal, and that environments better suited to the preservation of organic matter exist. For example, the ability to drill 2-3 metres into bedrock would enable sampling of sediments exposed to less intense radiation, while a mobile rover could approach more attractive sites, such as the location of former hot springs. The ESA-led ExoMars mission will take advantage of our improved knowledge and seek to transform these hints of habitable environments into the discovery of past or present biology. As part of the scientific payload of the ExoMars rover, the Life Marker Chip (LMC) will attempt to extract organic matter from the martian subsurface and detect specific organic molecules within the solvent extract using antibody-based assays, following the strategy of the Specific Molecular Identification of Life Experiment (SMILE) (Sims et al., 2005).

Various mineralogically and temporally distinct areas on Mars have been detected by the OMEGA/Mars Express imaging spectrometer (Bibring et al., 2006), each of which may contain the chemical evidence of extinct or extant martian life. These orbital data have revealed that the earliest era of Mars history, the Noachian, appears to have been particularly wet, with >3.7 Ga terranes containing abundant phyllosilicates, indicating extended periods of water-rock interaction (Ehlmann et al., 2011). These oldest deposits are succeeded by younger rocks containing sulphates, generated by aqueous acidic conditions, and then by the most recent rocks characterized by iron oxides, produced in a water-free environment. Each rock type has been assigned to a particular mineralogy-based era on Mars, namely the “phyllosian”, the “theikian” and the “siderikian”, for the oldest and wettest units to the youngest and driest (Bibring et al., 2006). Each era reflects distinct environmental conditions that would have had a major control on the probability of life. It can be assumed that the earliest liquid-water rich era would represent the most habitable conditions, while the more recent dry era would be the least amenable to life. The various rock types from the three eras will present their own individual challenges for organic extraction and when, combined with the

requirements imposed by specific life detection instruments, extended tests that optimise extraction efficiency are warranted.

1.2. A solvent for the LMC

The LMC is designed to use a solvent to extract organic matter from samples of martian rocks and soil and to detect specific organic molecules present using an antibody-based assays. However, this approach meets challenges regarding the choice of solvent. Two significant issues exist. Firstly, a single solvent capable of extracting a wide range of compounds is desirable but challenging, as few solvents are capable of extracting both polar and nonpolar biomolecules, such as amino acids and hydrocarbons. Secondly, the antibodies used to detect organic species are incompatible with the organic solvents typically used to extract nonpolar species such as hydrocarbons. Instead, an aqueous solvent must be used, but the very low solubility of nonpolar hydrocarbons, such as isoprenoids, in water hinders their extraction from martian samples.

The approach chosen to circumvent both of these problems is to add a surfactant to an aqueous solvent. The aqueous solvent is compatible with the antibodies and does not denature them, while the presence of the surfactant greatly increases the ability of the aqueous solution to extract nonpolar hydrocarbons. A further advantage is the nature of surfactant solutions as wetting agents, capable of preparing microfluidic components for fluid transport. A surfactant solution consisting of 1.5 g l⁻¹ of the non-ionic surfactant polysorbate 80 (Fig. 1a) in 20:80 (vol:vol) methanol:water is intended to be used by the LMC (Court et al., 2010). The 20% methanol content is compatible with the antibody-based detector and further aids the solubility of nonpolar species. This solution has been shown to be capable of extracting nonpolar aliphatic hydrocarbons spiked on to the surface of the martian soil simulant JSC Mars-1, with extraction recoveries of up to one-third of the original mass of standard (Court et al., 2010).

For reasons of redundancy and risk reduction it is desirable to identify alternative surfactant solutions that can augment or replace polysorbate 80. Here, therefore, we have investigated the ability of solvents based on various alternative surfactants to extract organic compounds spiked on to the surfaces of the martian soil simulant JSC Mars-1. Ideally, the alternative surfactant solution should i) display high extraction efficiencies for hydrocarbons, and ii) not be confused as a target organic compound by the antibody-based detectors, and iii) be compatible with the antibody assay. Four types of surfactant have been tested – the poloxamer surfactants Pluronic® F-68 and F-108, the fluorosurfactant Zonyl® FS-300 and the polysiloxane poly[dimethylsiloxane-co-[3-(2-(2-hydroxyethoxy)ethoxy)propyl]methylsiloxane] (PDMSHEPMS) (Fig 2). Solutions of various concentrations of these surfactants, in both water and 20:80 (vol:vol) methanol:water, have been used to extract a range of organic standards spiked on to the martian soil simulant, the palagonitic tephra JSC Mars-1 (Allen et al., 1998). The organic standards were chosen to reflect the chemistry of target compounds associated with both fossil life and abiotic compounds from meteoritic infall (Parnell et al., 2007). They include aromatic hydrocarbons such as pyrene and phenanthrene typical of those found in carbonaceous meteorites, and aliphatic hydrocarbons such as squalene and phytane, along with steroids such as coprostane and stigmasterol, typically produced by biological processes on Earth. In addition, data sets for polysorbate 80 solutions have been produced to allow for direct comparisons between the abilities of different surfactant solutions to extract the nonpolar hydrocarbons, and the two best alternative surfactants have been tested for compatibility with a representative antibody assay.

2. Methodology

Briefly, samples of JSC Mars-1 were spiked with known masses of standards and extracted using a range of solvents. The extracted standards were transferred from aqueous solution to the organic solvent dichloromethane (DCM) via liquid-liquid extraction, ready for analysis by gas chromatography mass-spectrometry (GC-MS).

2.1. Samples, standards, solvents and surfactants

Surfactant solutions were used to extract organic compounds spiked on to the surface of the martian soil analogue, JSC Mars-1. This is the <1 mm size fraction of a palagonitic tephra from the Pu'u Nene cinder cone, located in the saddle between Mauna Loa and Mauna Kea volcanoes on the Island of Hawaii (Allen et al., 1998). The JSC Mars-1 was cleaned prior to spiking with standards using ultrasonic extraction, first with a 93:7 vol:vol mixture of dichloromethane (DCM) and methanol, then with deionised water, to remove any pre-existing soluble organic components that could act as contaminants. Samples of JSC Mars-1, 500 mg in mass, were spiked with 10 µg of each of eight standards – the aliphatic hydrocarbons hexadecane, phytane and squalene; the aromatic hydrocarbons anthracene and pyrene; the steroids coprostane and stigmasterol; and atrazine (Fig. 2), reflecting a range of structures and solubilities in water. Atrazine was included not as a potential biomarker, but as a control marker, representing a structure not expected to be found on Mars. Solutions of five surfactants were used to extract these standards from the JSC Mars-1. The surfactants tested were polysorbate 80, Pluronic® F-68 and Pluronic® F-108, Zonyl® FS-300 and PDMSHEPMS, present in water and in 20:80 (vol:vol) methanol:water (Table 1).

Pluronic® F-68 and Pluronic® F-108 are poloxamer surfactants, consisting of a central hydrophobic chain of polyoxypropylene, surrounded by two hydrophilic chains of polyoxyethylene. Variations in

the number of units of polyoxypropylene and polyoxyethylene units lead to differences in physical and chemical properties. This can be abbreviated to $H-[PEO]_x-[PPO]_y-[PEO]_x-OH$, where PEO is polyethylene oxide and PPO is polypropylene oxide (Fig. 1). The exact formulae of poloxamer surfactants can vary slightly but the following are good approximations: Pluronic® F-68 = $PEO_{75}PPO_{30}PEO_{75}$; Pluronic® F-108 = $PEO_{132}PPO_{50}PEO_{132}$. Dissolution of a surfactant in water produces micelles when the critical micelle concentration (CMC) of the surfactant is exceeded. However, the CMC of these poloxamers in water is not well constrained, being noted to encompass three orders of magnitude for Pluronic® F-68, ranging from 440 μM to >10 mM at room temperature (Frey and Lee, 2007), equivalent to 3.7-84 $g\ l^{-1}$. The situation for Pluronic® F-108 is little better, with values of CMC ranging from 0.32-7 $g\ l^{-1}$ (Govender et al., 2005; Kozlov et al., 2000; Lopes and Loh, 1998).

Zonyl® FS-300 is a water-soluble fluorosurfactant with the structure $F-[CF_2-CF_2]_3-[CH_2-CH_2-O]_{14}-H$, with the fluorocarbon chain being lipophilic and the ethylene oxide chain being hydrophilic. No clear information about the Zonyl® FS-300 CMC is available in the literature. PDMSHEPMS is a water-soluble polysiloxane surfactant (Fig. 1). Again, no clear information about the PDMSHEPMS CMC is available in the literature. Two solvents were used with these surfactants: water and 20:80 (vol:vol) methanol:water. It is expected that the presence of methanol will favour the dissolution and extraction of the organic standards. A wide range of concentrations of these surfactants in the solvents was tested, from 0.1-10 $g\ l^{-1}$.

2.2. Extraction procedure

Aliquots of 500 mg of JSC Mars-1, previously cleaned by extraction with 93:7 (vol:vol) DCM:methanol and reverse osmosis water, were placed in test tubes previously cleaned by baking in air at 500 °C for several hours. To each aliquot of JSC Mars-1 was added sufficient volume of a solution of the

eight standards in methanol to deposit 10 µg of each on to the JSC Mars-1. The methanol-wet JSC Mars-1 was allowed to dry overnight in a hotbox set at 35 °C. To each spiked aliquot of JSC Mars-1, 3 ml of the appropriate surfactant solution was added (Table 1). Each test tube with added solvent was sonicated for 10 minutes, using a Fisher Scientific FB-15063 sonic bath. Following sonication, the test tubes were centrifuged at 2500 rpm for five minutes to settle suspended particulates and the supernatant was pipetted away to a separate test tube. Two further cycles of addition of solvent, sonication, centrifugation and pipetting of the supernatant then followed, with the three extracts produced merged into one.

Sonication of JSC Mars-1 in these solutions has the effect of pulverising grains, producing a suspension partially resistant to centrifuging. Material remaining in suspension was therefore removed using syringe filters possessing polytetrafluoroethylene (PTFE) membranes with a 0.2 µm pore size. Previous work had used a cellulose acetate filter membrane, but preliminary work had established that this membrane material could retain aromatic hydrocarbons, producing misleadingly low values of extraction efficiency (Court et al., 2010). These aqueous extracts were unsuitable for direct analysis by GC-MS, so liquid-liquid extraction was performed to transport the dissolved standards from the aqueous phase to a suitable organic solvent, DCM. This was performed by adding about 5 ml of DCM to the aqueous solutions, followed by mixing using a Sonics & Materials, Inc. VCX-130 sonic probe. Separation of the organic and aqueous solvents was achieved using a centrifuge and a separating funnel. This liquid-liquid extraction procedure was performed three times to minimise the loss of the standards.

The volume of DCM produced by liquid-liquid extraction was reduced to 1 ml in volume under a stream of nitrogen. One µl of this was injected into the Agilent HP-5MS column on the GC-MS, comprised of an Agilent 7890N gas chromatograph and a 5975C Mass Selective Detector. The GC oven was initially held at 50 °C for 1 minute, then warmed at 4 °C min⁻¹ to 310 °C, where it was held

for 20 minutes, for a total duration of 86 minutes. The standards were identified by reference to the NIST 08 mass spectral database, and the retention times for this instrumental configuration established by previous runs of the individual standards. Further samples, consisting of 10 µg of each of the eight standards dissolved in DCM, were run as comparisons, demonstrating the chromatographic responses of 10 µg of each compound, enabling the ability of the surfactant solutions to recover the standards from the JSC Mars-1 to be calculated as a percentage. Samples of JSC Mars-1 cleaned as described above but not exposed to the set of standards were also subjected to the extraction and GC-MS analysis. No significant amounts of organic compounds were extracted from these cleaned samples, demonstrating the efficacy of the cleaning procedure.

2.3 Testing compatibility of surfactants with a representative antibody assay

The LMC instrument will include up to 25 antibody assays, for the detection of up to 25 markers of extant and extinct martian life using a multiplexed microarray format inhibition immunoassay. Within this work a single representative antibody assay, for the detection of the aromatic hydrocarbon pyrene, was used to assess antibody assay performance in the presence of alternative surfactants. Pyrene standards were prepared in 20:80 (vol:vol) methanol:water containing either 1.5 g l⁻¹ polysorbate 80, 10 g l⁻¹ polysorbate 80, 10 g l⁻¹ Zonyl® FS-300 or 10 g l⁻¹ PDMSHEPMS and analysed using an inhibition format enzyme-linked immunosorbent assay (ELISA) which has been described previously (Rix et al., 2011). IC₅₀ values (the concentration of pyrene that produced a 50% reduction in ELISA signal intensity) were calculated by fitting the data to a four parameter binding equation (Findlay and Dillard, 2007).

2.4 Comparability of laboratory procedures to the LMC

The LMC is a small instrument designed to operate on Mars, while the data described in this paper were derived from larger-scale laboratory equipment. However, many of the steps taken, e.g. sonication, will be used by the LMC, albeit with miniaturised hardware. Centrifugation will not be available but this serves only to accelerate settling and separation process thereby allowing large and statistically-significant data sets to be generated. Once laboratory scale processes are optimised, the protocols are tested for LMC compatibility using a small bench-top processing system which comprises components close to those that will make up the flight instrument. The liquid-liquid extractions and GC-MS analyses are used to assess sample extraction process efficiency and will not be necessary on Mars where the developed antibody array will represent the detector for target compounds. The data in this paper represents a complete laboratory-scale optimisation study.

3. Results

The efficiencies of recovery of each standard related to individual solvents were calculated by comparing relevant chromatographic peak areas. Representative chromatograms are illustrated in Fig. 3. The calculated recovery efficiencies for the standards are detailed in Table 1, and the relationships between extraction efficiencies are shown in Figs. 4-8, with the water-soluble atrazine omitted for clarity.

3.1. Polysorbate 80

Figs. 4 and 5 show the relationships between the recovery of standards and the concentration of polysorbate 80 in both water and methanol-water. Using water alone, without any polysorbate, results in very poor recoveries, with a maximum of 0.2% (all percentages are wt.%) for anthracene and pyrene, excluding the water-soluble atrazine. This reflects the partial solubilities of aromatic hydrocarbons in water. The aliphatic hydrocarbons and steroids used are insoluble in water. Adding methanol to water (20:80 vol:vol methanol-water), greatly increases the solubility of the aromatic hydrocarbons, anthracene and pyrene, to 4.9% and 10.8%, respectively, but recovery of the aliphatic hydrocarbons and the steroids remains at 0.0%. Addition of 1.5 g l⁻¹ polysorbate 80 to water and methanol-water enables extraction of all compounds, with the efficiency of recovery varying among the compound classes. All of the compounds are generally more soluble in polysorbate 80 with methanol-water, as opposed to polysorbate 80 with water alone, with the increase in recovery with methanol typically around 5-10 percentage units. Most soluble are the aromatic hydrocarbons, anthracene and pyrene, with recoveries around 40-70%, while the aliphatic hydrocarbons show recoveries of up to 25%, with hexadecane being easiest to extract and squalene being most difficult. Recovery of the steroids, coprostane and stigmasterol, is similar to that of squalene at around 10%.

Previous work on developing polysorbate 80 solutions for the LMC has used a concentration of 1.5 g l⁻¹ (Court et al., 2010). Fig. 5 shows how the recovery of the standards varies across a range of polysorbate 80 concentrations, from 0.1-10 g l⁻¹, with data for extraction using water and methanol-water, with and without polysorbate 80, also included. A clear trend of increasing recovery of the standards with increasing polysorbate 80 concentrations is apparent. With water as a solvent, maximum recoveries of 20-40% are measured for aliphatic hydrocarbons, while recovery of the steroids is around 20% and recovery of the aromatic hydrocarbons are around 60-80%. Addition of methanol to the solvent results in generally increased recoveries, relative to water alone, an effect most pronounced for squalene, phytane and coprostane. Maximum recoveries are attained using 10 g l⁻¹, but a solution of 10% of the strength, 1 g l⁻¹, has approximately 50% of the ability to extract the standards, demonstrating that adding further polysorbate 80 gives diminishing rewards.

3.2. Pluronic® F-68 and F-108

Fig. 5 shows the data for the poloxamers Pluronic® F-68 and Pluronic® F-108. Both surfactants show similar results – a very limited ability to extract the standards from JSC Mars-1. The recovery of aliphatic compounds or steroids did not exceed 1.0%, across a surfactant concentration range of up to 10 g l⁻¹ in both water and methanol-water. Greater success was met with the aromatic hydrocarbons, but the most efficient case, of 10 g l⁻¹ of surfactant in methanol-water, gives only a factor of 3 increase in recovery of anthracene and pyrene, relative to methanol-water without surfactant. Testing increased concentrations of these poloxamers in solution is not practical, as the concentrations of 5-10 g l⁻¹ have high viscosities that would hinder the operation of the LMC. The poor results obtained using these surfactants is surprising, given their established use as surfactants capable of encapsulating nonpolar hydrocarbons in micelles in aqueous media. It is possible that these surfactants were successfully extracting the organic standards from the JSC Mars-1, but that the standards were being retained, possibly trapped within micelles, on the PTFE filters.

Alternatively, the standards may have been retained in the aqueous phase during liquid-liquid extraction, although since subsequent evaporation of the DCM yielded abundant poloxamer surfactant precipitate, this appears unlikely.

3.3. Zonyl® FS-300

Fig. 7 shows the recovery of the standards using solutions of Zonyl® FS-300. In general, Zonyl® FS-300 gives recoveries of the standards that are superior to those of the Pluronic®, but inferior to those of polysorbate 80. Increased recovery is seen when methanol-water is used as a solvent, rather than water, matching the behaviour seen for other surfactants. Maximum recoveries are seen for 10 g l⁻¹ Zonyl® FS-300 in methanol-water, with the aliphatic hydrocarbons ranging between 10-20%, the aromatic hydrocarbons around 60% and the steroids around 10%. These recoveries are similar to those seen for the normal LMC solvent of 1.5 g l⁻¹ polysorbate 80 in 20:80 methanol:water, but are considerably lower than those seen when 10 g l⁻¹ polysorbate 80 is used.

3.4. PDMSHEPMS

Fig. 8 shows the recovery of the standards using solutions of PDMSHEPMS. In general, PDMSHEPMS was very effective at extracting the standard from JSC Mars-1, with recoveries comparable to those of polysorbate 80. Increased recovery is seen when methanol-water is used as a solvent, rather than water, matching the behaviour seen elsewhere. Maximum recoveries are for 10 g l⁻¹ PDMSHEPMS in methanol-water, with the aliphatic hydrocarbons ranging between 30-40%, the aromatic hydrocarbons around 60% and the steroids around 15-30%; however, using a solution of 5 g l⁻¹ sees only a small decrease in recoveries. These recoveries for PDMSHEPMS are similar to those seen for 10 g l⁻¹ polysorbate 80 in 20:80 methanol:water.

3.5. Compatibility of surfactants with representative antibody assay

Fig. 9 shows the immunoassay results produced with pyrene standards prepared in 20:80 (vol:vol) methanol:water with 1.5 g l⁻¹ polysorbate 80, 10 g l⁻¹ polysorbate 80, 10 g l⁻¹ Zonyl® FS-300 and 10 g l⁻¹ PDMSHEPMS. Pyrene was successfully detected in the presence of each surfactant as shown by the decreasing signal (OD at 450 nm) with increasing pyrene concentration. However, immunoassay sensitivity was affected by the surfactant under test: increasing the concentration of polysorbate 80 from 1.5 g l⁻¹ to 10 g l⁻¹ resulted in a sixfold increase in the quantity of pyrene required to reduce immunoassay signal by 50 % (IC₅₀). IC₅₀ with 10 g l⁻¹ Zonyl® FS-300 was similar to IC₅₀ with 1.5 g l⁻¹ polysorbate 80 and IC₅₀ with 10 g l⁻¹ PDMSHEPMS was approximately doubled compared to with 1.5 g l⁻¹ polysorbate 80.

4. Discussion

4.1. Comparison with previous results

Previous work, reporting the ability of 1.5 g l⁻¹ polysorbate 80 in 20:80 (vol:vol) methanol:water to extract these standards from JSC Mars-1 (Court et al. 2010) produced the data shown in Table 2, along with the data for this concentration of polysorbate solution reported here. Good agreement between the reported recoveries of hexadecane and phytane is reported. However, a significant difference can be observed for squalene, which is more efficiently extracted in the data reported here, and in particular for the aromatic hydrocarbons anthracene and pyrene, recovery of which was not reported by Court et al. (2010). The failure of Court et al. (2010) to recover the aromatic hydrocarbons was noted as surprising and unexpected, given previously reported successful extraction of aromatic hydrocarbons, such as from contaminated soil (Yeom et al., 1995). Preliminary work, between that performed for Court et al. (2010) and that performed here, suggested that the cellulose acetate filters used to removed suspended particles of JSC Mars-1 from the surfactant solutions were also causing the loss of dissolved organic matter, particularly the aromatic hydrocarbons, interpreted as a result of retention on the cellulose acetate filter membrane. Hence, this investigation used PTFE filters that had been shown to cause no significant loss of dissolved organic matter. The data showing recovery of >50% of the aromatic hydrocarbon is more in keeping with expectations, given the superior solubility of aromatic hydrocarbons in water, relative to aliphatic hydrocarbons. Consequently, the data reported here should be regarded as more reliable and more representative of the true capabilities of the surfactant solutions.

4.2. Increasing the concentration of the LMC polysorbate solution

The current surfactant solution being developed for the LMC consists of 1.5 g l^{-1} polysorbate 80 in 20:80 methanol:water. The data presented here indicates that this solution can extract the standards from JSC Mars-1, with recoveries around 20-30% for aliphatic hydrocarbons, 50-75% for aromatic hydrocarbons and around 10-15% for the steroids. The data presented here clearly indicates that recovery of the standards does improve with more concentrated polysorbate 80 solutions. Table 1 and Fig. 5 show that increasing the concentration of polysorbate 80 in methanol-water from 1.5 g l^{-1} to 5 g l^{-1} increases the recoveries of the standards by around 33-50%, with 10 g l^{-1} offering a small further increase. These findings contrast with those of Court et al. (2010) where the benefits of increased surfactant concentrations were most likely masked by the loss of standards during passage through the cellulose acetate filters used in that work.

Therefore, in terms of the extraction of these standards from JSC Mars-1, 1.5 g l^{-1} of polysorbate 80 is not the optimal concentration; better results can be achieved using a more concentrated solution of 5 or 10 g l^{-1} . Consequently, should the extraction efficiency of the current polysorbate 80 solution, of 1.5 g l^{-1} , appear inadequate to extract the low concentrations of organic matter expected to be found in the martian soil, more efficient extraction could be achieved by increasing the concentration of polysorbate 80. However, the sensitivity of the pyrene antibody assay was significantly reduced when the polysorbate 80 concentration was increased from 1.5 g l^{-1} to 10 g l^{-1} (Fig. 9). It remains to be seen whether a more concentrated solution would result in an overall improvement in instrument detection limits given the performance gain for the extraction step and the performance loss for the subsequent detection step. This is based upon only one representative antibody assay and will therefore need to be studied further.

4.3. An alternative surfactant for the LMC

We have tested the ability of four surfactants to act as an alternative to the polysorbate 80-based solvent in its role of extracting organic matter from the martian soil. Of these, the two poloxamer surfactants, Pluronic® F-68 and Pluronic® F-108, are unsuitable, as they failed to extract significant quantities of the organic standards from the aliquots of JSC Mars-1. The fluorosurfactant Zonyl® FS-300 produced better results, with a 10 g l^{-1} solution in methanol-water yielding extraction efficiencies broadly similar to those obtained by 1.5 g l^{-1} polysorbate 80 in methanol-water. However, the best results were obtained using PDMSHEPMS in methanol-water, yielding extraction efficiencies similar to those obtained using polysorbate 80 in methanol-water at identical concentrations. Both Zonyl® FS-300 and PDMSHEPMS were compatible with a representative antibody assay (Fig. 9) although some changes in assay sensitivity were observed compared to 1.5 g l^{-1} polysorbate 80. Further studies are required with a wider range of representative antibody assays to better characterise the likely interaction of these surfactants with the up to 25 antibody assays that may fly on the LMC.

Therefore, a solution of $5\text{-}10 \text{ g l}^{-1}$ PDMSHEPMS in 20:80 (vol:vol) methanol:water appears to be suitable to be considered as an alternative surfactant solution for the LMC should problems arise with the current polysorbate 80-based solution. Furthermore, the fluorosurfactant Zonyl® FS-300 can also be considered as a possible backup surfactant for further study given that for the representative antibody assay, Zonyl® FS-300 slightly improved the sensitivity whilst PDMSHEPMS slightly decreased the sensitivity when compared to polysorbate 80 at a concentration of 1.5 g l^{-1} . One additional benefit is that both the PDMSHEPMS and Zonyl® FS-300 surfactants do not include a long aliphatic hydrocarbon chain of the kind found in polysorbate 80 that, if cleaved via a process such as radiolysis, would result in the formation of long-chain fatty acids that could be misinterpreted as martian biomarkers. Little information regarding the chemical reactivity of the PDMSHEPMS and Zonyl® FS-300 surfactants is available in the literature and therefore further work

is required to assess their stability to radiation effects, storage effects, thermal processing and reactivity with expected components of martian samples such as perchlorates.

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5. Conclusions

1. The ability of a range of surfactants, in water and 20:80 (vol:vol) methanol:water, to extract nonpolar organic compounds spiked on to the surface of the martian soil simulant JSC Mars-1 has been investigated. Of the solvents tested here, the polysiloxane surfactant, PDMSHEPMS, in 20:80 (vol:vol) methanol:water has been found to offer the best extraction efficiencies, with recoveries comparable to those obtained using the current LMC solvent polysorbate 80 in 20:80 (vol:vol) methanol-water.
2. Testing revealed that the poloxamer surfactants Pluronic® F-68 and Pluronic® F-108 were not effective at extracting the standards from JSC Mars-1, with maximum recoveries of aliphatic hydrocarbons and steroids not exceeding 1 wt.%.
3. The fluorosurfactant Zonyl® FS-300 was able to extract the standards from the aliquots of JSC Mars-1, but with recoveries inferior to those obtained using similar concentrations of polysorbate 80.
4. In most cases, addition of methanol to water, to form 20:80 (vol:vol) methanol water, increased the ability of a surfactant solution to extract the suite of organic compounds from JSC Mars-1 samples. Using a surfactant in 20:80 methanol:water is therefore desirable for the LMC.
5. Increasing the concentration of polysorbate 80, or PDMSHEPMS, above the 1.5 g l^{-1} in 20:80 methanol:water currently being considered for the LMC results in significantly increased recoveries for the organic standards. This effect was not detected by a previous investigation into this surfactant solution, a result attributed here to the loss of standards on cellulose acetate filters during the previous work, avoided here by a switch to PTFE filter membranes. However, the data

indicate that although higher concentrations of surfactant favour the extraction of organic molecules from JSC Mars-1, they can also interfere with the operation of the antibody assay. The ideal surfactant concentration will also depend on interactions between mineral surfaces in the sample and the surfactant. It is expected that the determination of the ideal surfactant concentration for various martian soil chemistries will be a target of future studies.

6. Polysorbate 80, Zonyl® FS-300 and PDMSHEPMS at a concentration of up to 10 g l^{-1} in 20:80 (vol:vol) methanol-water are compatible with a representative antibody assay, although changes in assay sensitivity were observed compared to 1.5 g l^{-1} polysorbate 80 in 20:80 (vol:vol) methanol-water. Zonyl® FS-300 slightly improved the assay sensitivity whilst PDMSHEPMS slightly decreased the assay sensitivity when compared to polysorbate 80 at a concentration of 1.5 g l^{-1} .

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Table 1. Recoveries of the standards spiked on to JSC Mars-1 and extracted using various surfactant solutions, relative to the direct injection of standards into the GC-MS.

Surfactant	Solvent	Surfactant conc. (mg ml ⁻¹)	Recovery relative to direct injection into the GC-MS (%)							
			Hexa-decane	Phy-tane	Squa-lene	Anthr-acene	Pyrene	Copr-ostane	Stigm-asterol	Atra-zine
None	H ₂ O	0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	69.9
None	H ₂ O-MeOH	0.0	0.0	0.0	0.1	4.9	10.8	0.0	0.0	77.9
Poly-sorbate 80	H ₂ O	0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	69.9
		0.1	0.0	0.0	0.1	0.2	0.2	0.0	0.0	80.7
		0.2	10.4	11.4	4.7	5.9	29.3	6.9	4.0	92.7
		0.5	23.8	19.2	10.3	18.0	48.5	13.3	7.2	89.2
		1.0	24.6	15.2	9.1	35.1	64.3	9.6	4.8	89.8
		1.5	23.5	11.4	5.9	43.8	67.9	9.0	6.3	87.8
		2.0	35.5	22.2	15.8	48.7	74.8	15.4	12.7	89.3
		5.0	38.8	24.4	19.2	54.8	79.7	15.9	15.2	92.7
		10.0	42.4	26.2	22.0	56.8	82.2	19.5	16.9	93.7
		0.0	0.0	0.0	0.1	4.9	10.8	0.0	0.0	77.9
Poly-sorbate 80	H ₂ O-MeOH	0.1	5.2	6.6	3.4	6.6	20.5	5.3	0.3	94.5
		0.2	8.7	11.3	6.8	7.4	23.6	8.9	1.1	90.5
		0.5	21.3	22.2	15.3	22.9	48.0	16.0	4.7	82.2
		1.0	23.2	25.2	17.3	44.6	66.7	16.2	10.6	89.9
		1.5	28.3	24.3	16.6	49.3	74.6	17.2	7.7	92.2
		2.0	36.7	35.7	28.4	58.7	80.5	26.7	14.0	92.1
		5.0	41.1	35.4	30.7	64.3	84.3	24.3	18.0	90.5
		10.0	45.5	37.7	32.1	65.3	86.0	27.7	19.9	95.2
		0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	69.9
		1.0	0.1	0.1	0.0	0.7	0.5	0.0	0.0	103.4
Pluronic® F-68	H ₂ O	2.0	0.1	0.0	0.0	0.6	0.5	0.0	0.0	86.1
		5.0	0.1	0.0	0.1	0.9	1.4	0.0	0.0	85.0
		10.0	0.3	0.2	0.1	1.7	3.8	0.1	0.0	81.1
		0.0	0.0	0.0	0.1	4.9	10.8	0.0	0.0	77.9
		1.0	0.1	0.0	0.0	4.8	11.1	0.0	0.0	77.4
Pluronic® F-68	H ₂ O-MeOH	2.0	0.0	0.0	0.1	5.4	14.8	0.0	0.0	71.8
		5.0	0.1	0.1	0.1	6.1	19.0	0.1	0.0	67.9
		10.0	0.1	0.1	0.1	8.8	26.8	0.1	0.0	77.7
		0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	69.9
		1.0	0.4	0.7	0.6	0.7	1.0	0.4	0.0	100.2
Pluronic® F-108	H ₂ O	2.0	0.3	0.4	0.3	1.1	1.1	0.1	0.0	96.5
		5.0	0.3	0.2	0.2	1.0	3.5	0.1	0.0	75.7
		10.0	0.3	0.5	0.2	2.4	4.9	0.1	0.0	60.8
		0.0	0.0	0.0	0.1	4.9	10.8	0.0	0.0	77.9
		1.0	0.1	0.1	0.3	5.5	13.9	0.1	0.0	88.4
Pluronic® F-108	H ₂ O-MeOH	2.0	0.1	0.0	0.2	5.4	16.2	0.0	0.0	73.8
		5.0	0.2	0.2	0.2	7.5	24.3	0.1	0.0	80.7
		10.0	0.2	0.2	0.2	12.4	35.0	0.2	0.0	84.6
		0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	69.9
		1.0	0.6	2.5	1.0	8.9	26.8	0.9	1.3	87.9
Zonyl® FS-300	H ₂ O	2.0	1.4	4.1	1.4	23.2	35.0	1.5	2.8	91.5
		5.0	5.0	9.0	3.0	50.0	60.8	3.0	5.7	97.9
		10.0	10.7	9.6	3.7	54.1	67.1	4.2	4.3	97.4
		0.0	0.0	0.0	0.1	4.9	10.8	0.0	0.0	77.9
		1.0	0.2	1.4	0.5	16.3	42.9	0.7	2.0	76.7
Zonyl® FS-300	H ₂ O-MeOH	2.0	0.8	3.3	1.2	32.8	56.4	1.5	5.1	82.0
		5.0	4.5	9.6	2.8	49.1	57.6	3.7	7.2	71.7
		10.0	12.0	17.2	7.0	55.3	61.5	10.3	9.0	74.4
		0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	69.9
		1.0	14.1	9.1	5.2	14.2	26.4	5.1	3.4	87.4
PDM-SHEPMS	H ₂ O	2.0	14.5	8.3	7.3	23.3	49.8	6.1	9.2	85.3
		5.0	18.9	14.0	11.8	36.5	54.8	7.9	11.5	86.8
		10.0	16.7	12.4	9.8	42.5	67.5	6.7	10.4	76.4
		0.0	0.0	0.0	0.1	4.9	10.8	0.0	0.0	77.9
		1.0	17.4	29.0	26.5	19.1	46.0	24.6	8.4	81.4
PDM-SHEPMS	Water-methanol	2.0	23.1	29.1	25.7	34.6	55.7	21.1	8.9	75.3
		5.0	31.1	35.3	33.0	52.2	65.6	28.7	19.7	70.0
		10.0	37.2	39.1	30.4	51.3	67.0	29.4	13.6	71.8

Table 2. Recovery of standards from JSC Mars-1 using 1.5 g l⁻¹ polysorbate 80 in 20:80 (vol:vol) methanol:water, as reported by Court et al. (2010), and as reported here. See section 4.1 for discussion.

Recovery of standards from JSC Mars-1 (%)								
		Hexa-decane	Phytane	Squalene	Anthr-acene	Pyrene	Stigm-asterol	Atrazine
Court et al. (2010)	Mean	26.2	31.1	6.0	0.0	0.0	4.1	26.6
	σ	5.4	6.0	1.7	0.0	0.0	3.7	9.9
This work		28.3	24.3	16.6	49.3	74.6	7.7	92.2

Extraction of polar and nonpolar biomarkers from the martian soil using aqueous surfactant solutions

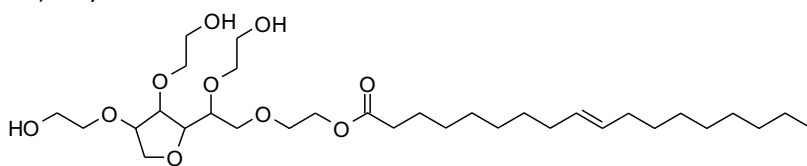
Richard W. Court^a, Catherine S. Rix^c, Mark R. Sims^b, David C. Cullen^c and Mark A. Sephton^a

Highlights

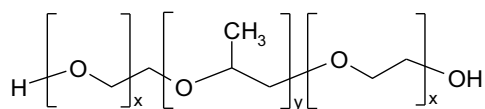
- The search for chemical evidence of life on Mars
- Testing solvents capable of extracting biomarkers from martian soil analogues
- Three surfactants suitable for the Life Marker Chip identified
- These surfactants are compatible with a representative antibody assay

Accepted manuscript

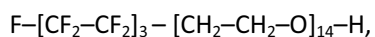
A) Polysorbate 80



B) Pluronic®



C) Zonyl® FS-300



D) PDMSHEPMS

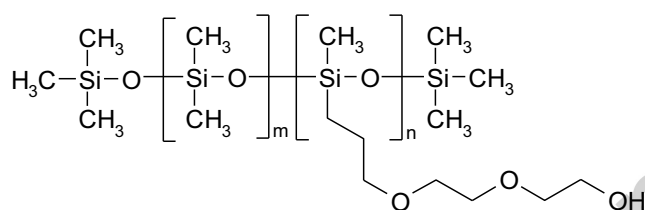


Figure 1. Structures of surfactants. For Pluronic® F-68, $x = 75$ and $y = 30$; for Pluronic® F-108, $x = 132$ and $y = 50$.

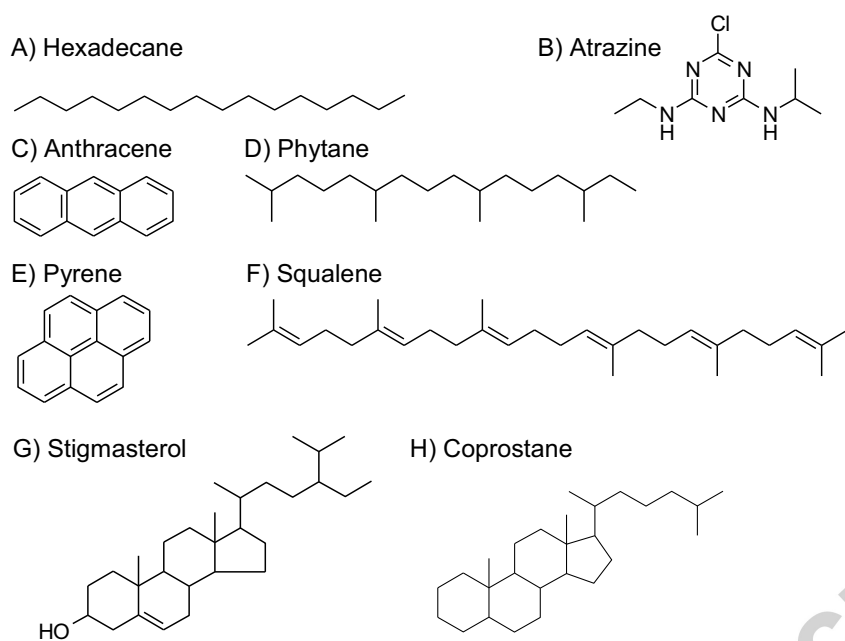


Figure 2. Structures of the organic compounds used to test the ability of the surfactant solutions to extract them from JSC Mars-1.

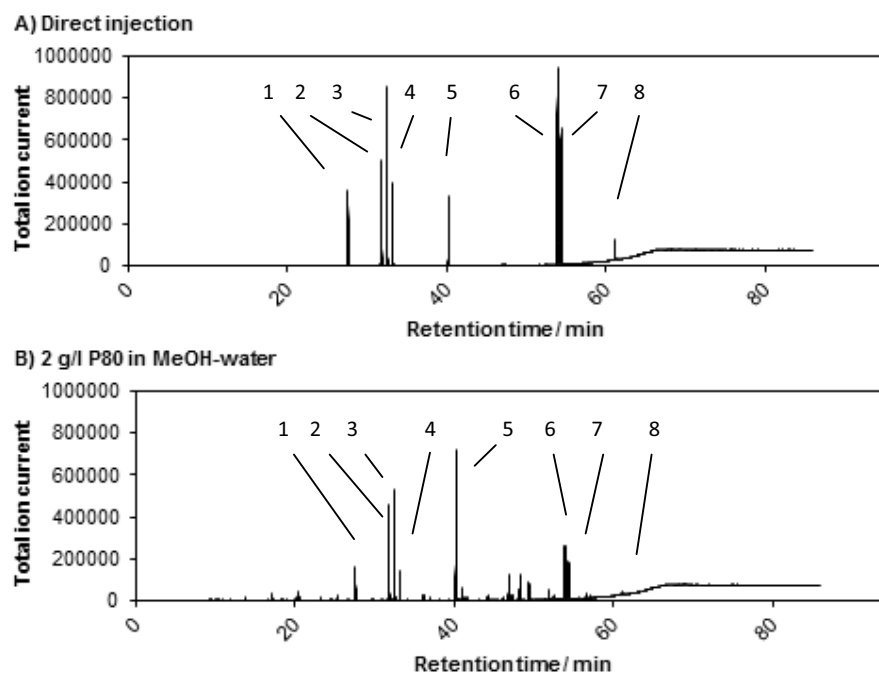


Figure 3. Chromatograms of the standards produced by direct injection into the GC-MS (A) and extracted from JSC Mars-1 using 2 g l^{-1} polysorbate 80 in 20:80 (vol:vol) methanol:water. Compounds are 1 hexadecane; 2 atrazine; 3 anthracene; 4 phytane; 5 pyrene; 6 coprostane; 7 squalene; 8 stigmasterol. Comparison of the areas of these peaks enables the efficiency of extraction of standards using the polysorbate 80 solution to be calculated.

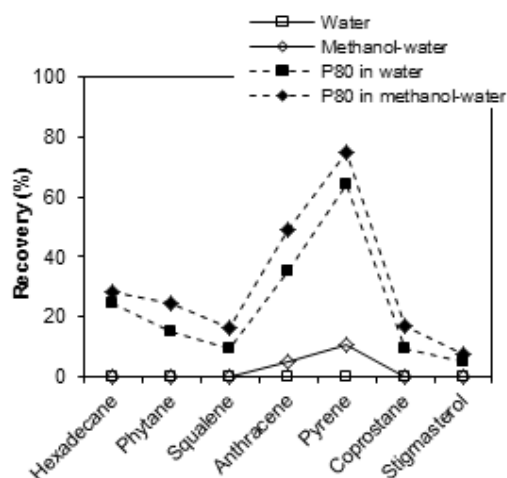


Figure 4. Recovery of 10 μg of each standard from 500 mg of JSC Mars-1 using water and 20:80 methanol:water, with and without 1.5 g l^{-1} polysorbate 80. Water alone is unable to extract the standards, and the addition of methanol helps only with the aromatic hydrocarbons. Addition of 1.5 g l^{-1} polysorbate 80, however, enables the solvent to extract all of the standards. Subsequent addition of methanol raises the percentage recoveries even further, to 20-30% for the aliphatic hydrocarbons, 50-75% for the aromatic hydrocarbons and 10-20% for the steroids.

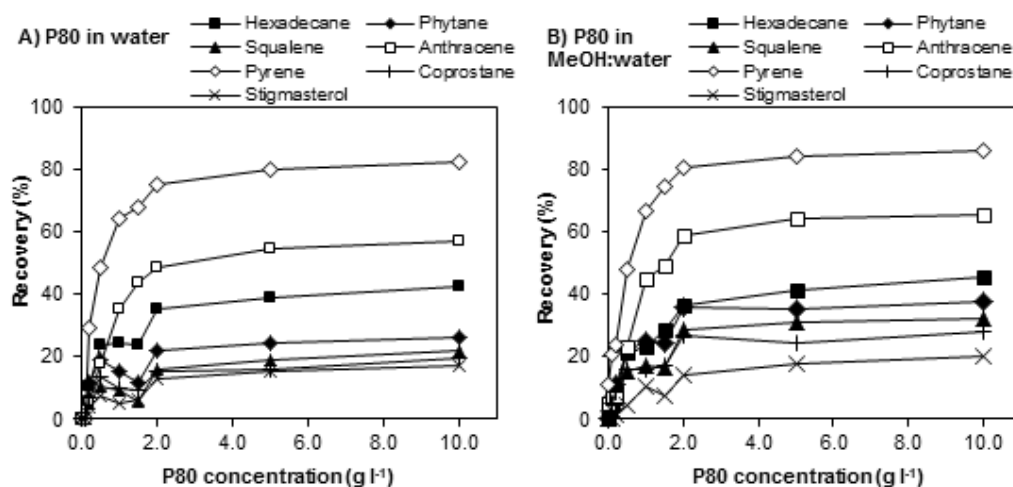


Figure 5. The recovery of standards from JSC Mars-1 using polysorbate 80 solutions in water and 20:80 (vol:vol) methanol:water. In general, the presence of methanol raises the recovery of standards, by up to around 50%. For polysorbate 80 in water, a concentration of 0.2 g l^{-1} is necessary to extract significant amounts of the standards, but the addition of methanol allows recovery at 0.1 g l^{-1} polysorbate 80. The current choice of LMC solution, 1.5 g l^{-1} polysorbate 80 in 20:80 methanol:water, gives recoveries of aliphatics, aromatics and steroids of around 20-30%, 50-75% and 10-20%, respectively. Increasing the concentration of polysorbate to 5 g l^{-1} increases those recoveries by around 50%, to 30-40% of aliphatic hydrocarbons, 65-85% of aromatic hydrocarbons and 20-25% of steroids. Hence, scope exists for more complete extraction of organic compounds, if the LMC and its antibody assays can tolerate a more concentrated polysorbate 80 solution.

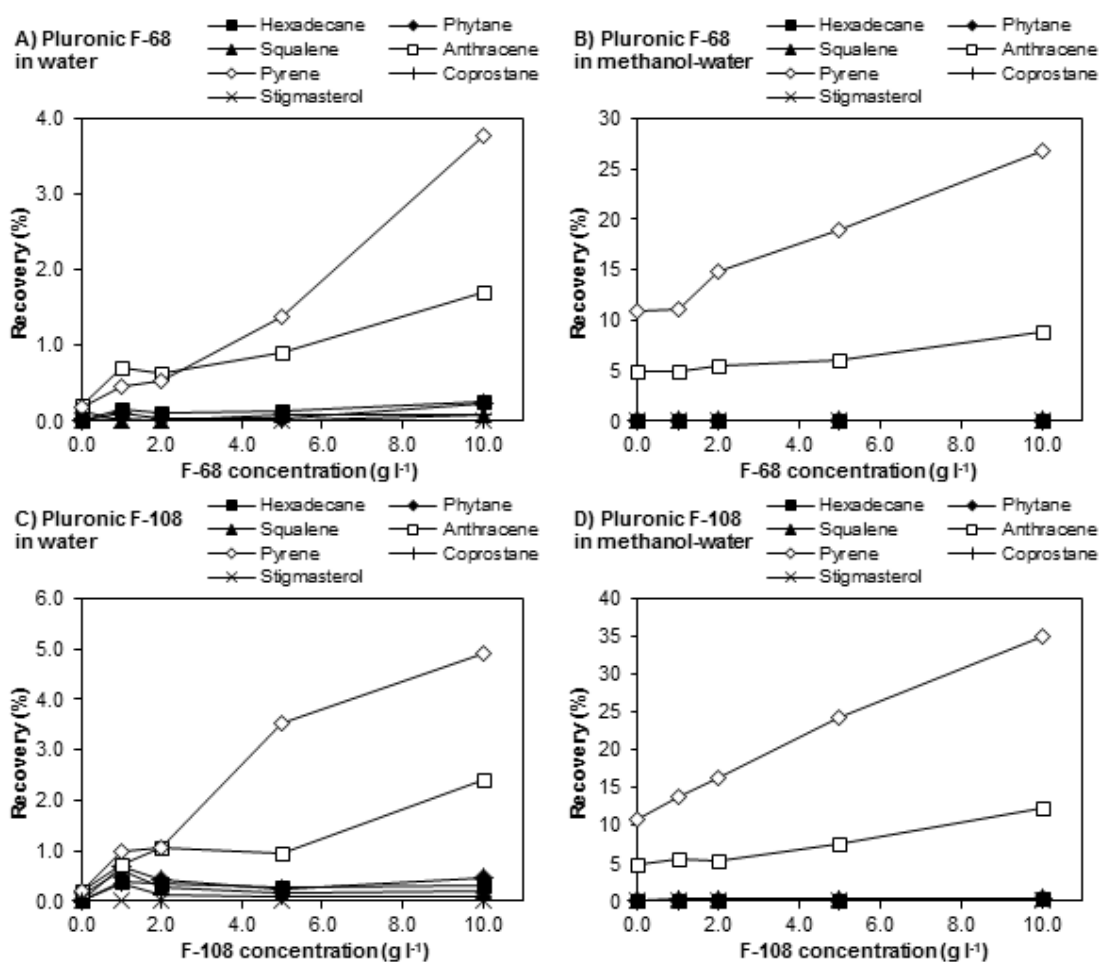


Figure 6. The recovery of standards from JSC Mars-1 using Pluronic® F-68 and Pluronic® F-108 solutions in water and 20:80 (vol:vol) methanol:water. Extraction of aliphatics and steroids by Pluronic® F-68 or Pluronic® F-108 solutions is unsuccessful, with recoveries not exceeding 1%, whether in water or methanol-water. Extraction of the aromatic species is more successful, but methanol-water is able to extract 5-10% of the aromatics without any surfactant present; addition of 10 mg ml⁻¹ Pluronic® F-68 or Pluronic® F-108 raises the recovery of these aromatics by only a factor of about three.

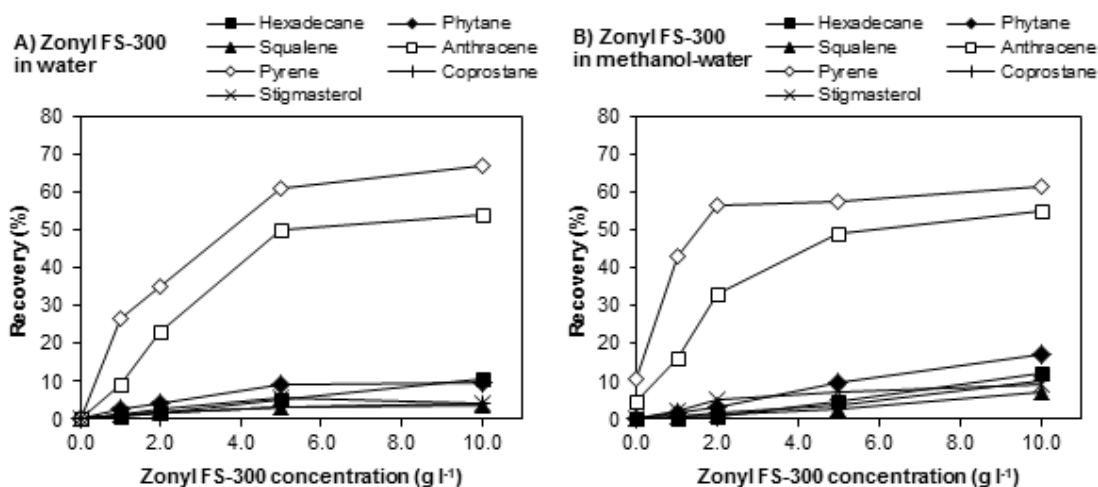


Figure 7. The recovery of standards from JSC Mars-1 using Zonyl® FS-300 solutions in water and 20:80 (vol:vol) methanol:water. Use of 20:80 methanol:water as a solvent, rather than water alone, aids the recovery of the standards. Recovery of the standards increases with increasing FS-300 concentrations, up to a maximum of around 10-20% for aliphatics, around 60% for aromatics and around 10% for steroids, in methanol-water. In general, Zonyl® FS-300 is about half as effective as polysorbate 80 at extracting aliphatics and steroids, but fairly similar in effectiveness of extracting the aromatics.

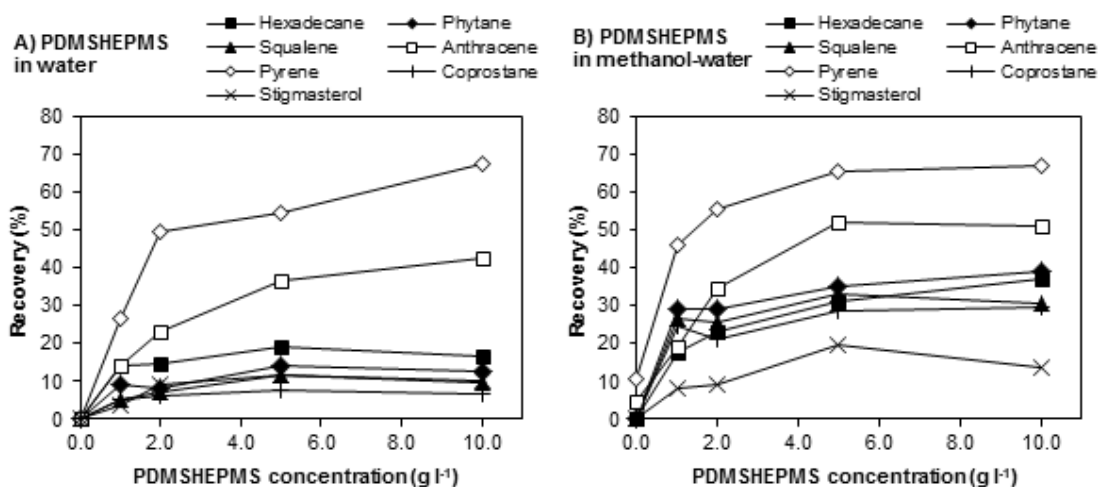


Figure 8. The recovery of standards from JSC Mars-1 using PDMSHEPMS solutions in water and 20:80 (vol:vol) methanol:water. Use of 20:80 methanol:water as a solvent, rather than water alone, greatly aids the recovery of the standards. Recovery of the standards increases with increasing PDMSHEPMS concentrations, up to a maximum of around 30-40% for aliphatics, around 50-70% for aromatic hydrocarbons and around 15-30% for steroids, in methanol-water. In general, PDMSHEPMS is about as effective as polysorbate 80 at these standards and therefore should be considered as an alternative surfactant for the LMC.

Figure 9

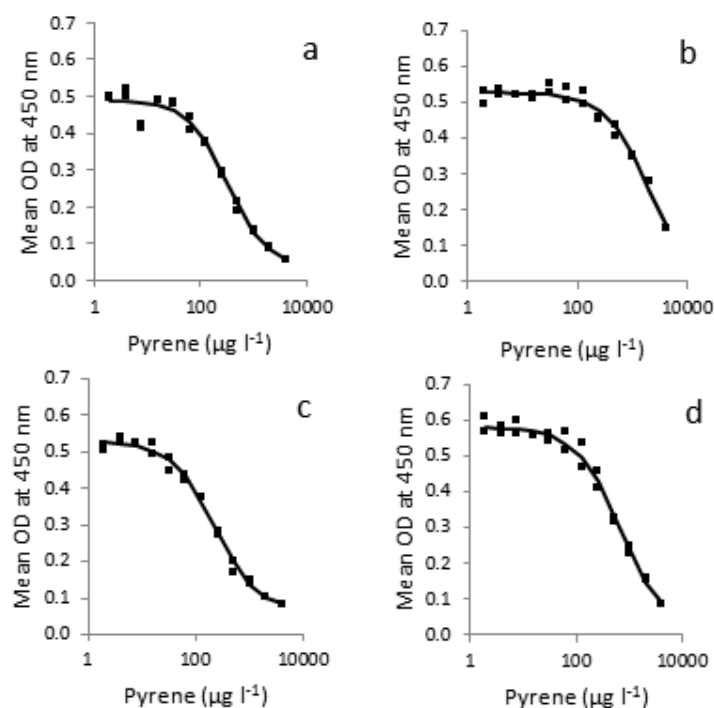


Figure 9. Inhibition ELISA data for pyrene in 20:80 methanol:water with (a) 1.5 g l^{-1} polysorbate 80 (b) 10 g l^{-1} polysorbate 80 (c) 10 g l^{-1} Zonyl® FS-300 and (d) 10 g l^{-1} PDMSHEPMS. Data points are single replicates. Plotted immunoassay curves are the fit to a four parameter binding equation. Calculated IC_{50} values were 314 $\mu\text{g l}^{-1}$ for 1.5 g l^{-1} polysorbate 80, 1890 $\mu\text{g l}^{-1}$ for 10 g l^{-1} polysorbate 80, 215 $\mu\text{g l}^{-1}$ for 10 g l^{-1} Zonyl® FS-300 and 638 $\mu\text{g l}^{-1}$ for 10 g l^{-1} PDMSHEPMS. (OD = optical density).



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Extraction of polar and nonpolar biomarkers from the martian soil using aqueous surfactant solutions

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