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Novel solvent systems for in-situ extraterrestrial sample analysis

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Abstract

The Life Marker Chip (LMC) is being designed to test for the chemical signature of life in the soil and rocks of Mars. It will use an antibody array as part of its detection and characterisation system and aims to detect both polar and non-polar molecules at the sub-ppm to tens of ppb level. It is necessary to use a solvent to transfer organic compounds from the Martian samples to the LMC itself, but organic solvents such as dichloromethane or hexane, commonly used to dissolve non-polar molecules, are incompatible with the LMC antibodies. Hence, an aqueous-based solvent capable of dissolving the biomarkers that might exist in the soil or rocks of Mars is required. Solvent extractions of a Martian soil analogue, JSC Mars-1, spiked with a range of standards show that a 20:80 (vol:vol) mixture of methanol and water is incapable of extracting compounds insoluble in water. However, addition of 1.5 mg ml^{-1} of the surfactant polysorbate 80 produces extraction efficiencies of the aliphatic standards, hexadecane and phytane, equal to 25-30% of those produced by the common organic solvent mixture 93:7 (vol:vol) dichloromethane:methanol. Extraction of squalene and stigmasterol using the polysorbate solution is less efficient but still successful, at 5-10% of the efficiency of 93:7 dichloromethane:methanol. Such aliphatic compounds with occasional functional groups represent the compound classes to which most fossil organic biomarkers belong. The polysorbate solution did not extract the aromatic compounds pyrene and anthracene with great efficiency. A solvent of 20:80 methanol:water with 1.5 mg ml^{-1} polysorbate 80 is therefore capable of selectively extracting aliphatic biomarkers from Martian samples and transferring them to the antibody sites on the Life Marker Chip.

Keywords. Mars; ExoMars; Extraterrestrial life; Astrobiology.

1. Introduction

The focus of the search for traces of past and present extraterrestrial life is on Mars, where recent discoveries indicate that the environmental conditions necessary to support life may have been commonplace at times in its history. Examples include features in sediments indicative of standing water detected by the NASA Mars Exploration Rovers (Squyres et al., 2004), the detection of the potential biosignature methane in the atmosphere of Mars by the ESA Mars Express orbiter (Formisano et al., 2004) and the confirmation of extant water ice in the subsurface near Mars' north pole by the NASA Phoenix lander (Smith et al., 2009). Forthcoming missions, such as ESA's ExoMars, seek to transform these hints of life into the discovery of past or present biology. All known life is based on organic compounds; hence carbon-based molecules are the targets for life-detection missions. To date, the only experiments designed to detect life on Mars were the biology experiments of the Viking Landers. The Viking gas chromatograph-mass spectrometer (GC-MS) detected no organic compounds in the martian soil, and the positive result of the Labelled Release experiment has generally been interpreted in terms of soil chemistry, rather than biology (Biemann, 1976; Sims et al., 2005). More recently, an alternative mechanism of detection, the Specific Molecular Identification of Life Experiment (SMILE) (Sims et al., 2005), has been proposed, in which solvents would be used to extract organic matter from the soil and introduce it into sensitive, antibody-based detection instrument (e.g., Sims et al., 2005).

However, this approach meets difficulties regarding the choice of solvent. A single solvent capable of extracting a wide range of compounds is desirable but challenging,

as target compounds include polar biomolecules such as amino acids and non-polar fossil hydrocarbons such as isoprenoids. Two approaches have emerged that can accommodate a wide range of compounds using a single solvent. One approach is to manipulate the polarity of water by varying its temperature and pressure (Aubrey et al., 2008). This method can target compounds of specific polarity by precisely matching the polarity of water to a particular class of analyte. An alternative approach is to add surfactants to aqueous solutions, forming micelles that encapsulate non-polar organic compounds within a polar solution. A surfactant approach to organic extraction on space missions has additional benefits for solutions designed for microfluidic detection equipment, as surfactants are wetting agents that prepare microfluidic component surfaces for fluid transport. Furthermore, protein-based antibodies, which represent an attractive detection mechanism, are relatively unaffected by surfactant solutions, in contrast to the deleterious effects of organic solvents (Cullen et al., personal communication).

In this paper, we report on the development of surfactant solutions as solvents for in situ life detection experiments. We have spiked a terrestrial analogue of Martian soil, JSC Mars-1 (Allen et al., 1998), with a range of organic molecules, and then used a range of solvents to gauge their extraction efficiency. The results indicate that surfactant solutions have wide applicability on space missions, especially where microfluidic systems are the final destination of any extracted organic matter.

2. Methodology

Briefly, samples of JSC Mars-1 were spiked with known masses of standards and extracted using a range of solvents. The efficiency of the solvents at extracting the standards was then determined by gas chromatography mass-spectrometry (GC-MS).

2.1. Samples, standards and solvents

The Martian soil analogue, JSC Mars-1, was chosen as the material on which the standards were applied. JSC Mars-1 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 soluble organic components.

Samples of JSC Mars-1 were spiked with seven standards – atrazine, anthracene, hexadecane, phytane, pyrene, squalene and stigmasterol (Fig 1). Standards were chosen to reflect a range of structures and solubilities in water. Four solvents were used to extract spiked samples of JSC Mars-1 – 93:7 DCM:methanol; 67:33 hexane:acetone, 20:80 methanol:water and 20:80 methanol:water with 1.5 mg ml⁻¹ of the non-ionic surfactant, polyoxyethylene (80) sorbitan monooleate, commonly known as polysorbate 80 (P80). All ratios are by volume.

2.2. Extraction procedure

Preliminary experiments indicated that P80 concentrations in the range of 1.5-2.5 mg ml⁻¹ in 20:80 methanol:water were able to extract organic compounds. Stronger concentrations of P80 did not result in significantly more efficient extraction, so a P80 concentration of 1.5 mg ml⁻¹ was chosen. Aliquots of 1000 mg of JSC Mars-1, previously cleaned by extraction with 93:7 DCM:methanol and deionised water, were placed in test tubes previously cleaned by baking in air at 500 °C for several hours. To each was added 1 ml of a solution of the seven standards in DCM, each standard being present at a concentration of 10 µg ml⁻¹. The solvent-wet JSC Mars-1 was then allowed to dry overnight in a hotbox set at 35 °C.

To each spiked aliquot of JSC Mars-1, 3 ml of the appropriate solvent was added (Table 1). Extraction using 93:7 DCM:methanol and 67:33 hexane:acetone was performed using one test tube each. Extraction using 20:80 methanol:water and P80 solution was performed in triplicate. Each test tube was sonicated for 20 minutes, using a Sonics & Materials, Inc. VCX-130 Vibra-Cell sonicator set at 40% amplitude, delivering about 2-3 W to the sonic probe head. Following sonication, the test tube was centrifuged at 1500 rpm for two minutes 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.

The accumulated supernatant was filtered using a 200 nm filter – a cellulose acetate filter for the aqueous solutions and a polytetrafluoroethylene (PTFE) filter for the organic solvents. The organic solvents were blown down under a stream of nitrogen

then redissolved in DCM, ready for injection onto a GC-MS. Evaporation of the less volatile aqueous extracts was impractical and could have caused the loss of analytes, so liquid-liquid extraction was performed to transport the dissolved standards from the aqueous phase to the DCM.

For liquid-liquid extraction, one-quarter of the volume of each aqueous extract was placed with 6 ml of DCM in test tubes and sonicated using the VCX-130 sonicator, using the same settings as described previously, for 20 minutes, to ensure good mixing between the aqueous and organic phases. The sonication of the aqueous and organic phases produced a white emulsion that was separated using 2 minutes of 1500 rpm centrifugation and standing overnight. Following separation, both phases were transferred to a separating funnel, with the test tube being rinsed three times each with DCM and the appropriate aqueous solvent, with these solvents also being added to the separating funnel, to avoid loss of analyte. Using the separating funnel the denser organic phase was separated from the aqueous solution. Two further ~3 ml volumes of DCM were then individually added to the separating funnel, with the denser organic phase being transferred to the test tube each time. Finally, the DCM solution was blown down under a stream of nitrogen to a volume of 1 ml, which was then transferred to a vial ready for GC-MS analysis, again with repeated rinsing of the test tube interior with DCM to minimise analyte loss.

2.3. Gas chromatography-mass spectrometry

The extracts of the spiked JSC Mars-1 were analysed using an Agilent 6890N gas chromatograph (GC) and a 5973 Mass Selective Detector. One μ l of solvent was

injected in to the DB-5MS column, with helium carrier gas flowing at 1.1 ml min^{-1} . The GC oven was initially held at $50 \text{ }^{\circ}\text{C}$ for 1 minute, then the temperature raised at $4 \text{ }^{\circ}\text{C min}^{-1}$ to $310 \text{ }^{\circ}\text{C}$, where it was held for 20 minutes, for a total run length of 86 minutes. The standards were identified by reference to the NIST 98 mass spectral database, and the retention times for this instrumental configuration established by previous runs of the individual standards.

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3. Results

3.1. Chromatograms of extracted JSC Mars-1

Chromatograms displaying the compounds extracted from the spiked samples are shown in Fig. 2. Fig. 2A and 2B show 93:7 DCM:methanol and 67:33 hexane:acetone extracts, showing efficient extraction of the standards added to the JSC Mars-1. Fig. 2C shows the liquid-liquid extract of the 20:80 methanol:water extract of the spiked JSC Mars-1, revealing that only the water-soluble atrazine was extracted – no trace of the other standards was detectable. However, the addition of 1.5 mg ml⁻¹ P80 to the 20:80 methanol:water enabled significant amounts of hexadecane, phytane and squalene, and some stigmasterol, to be extracted, as shown in Fig. 2D. As noted above, the aqueous extracts were each performed in triplicate and only representative chromatograms are shown in Fig. 2D.

3.2. Quantification of extraction efficiencies

The extraction efficiencies of each standard in each chromatogram were quantified by measuring the area of each peak. Comparison of these areas to the areas of these peaks in the chromatogram of the 93:7 DCM:methanol extract enables the determination of relative extraction efficiencies (Table 1). The areas of peaks in the chromatograms of the aqueous extracts were multiplied by four, to account for the division of these aqueous extracts into four aliquots. The data is also shown graphically in Fig. 3.

Extraction with 67:33 hexane:acetone is about as efficient as extraction with 93:7 DCM:methanol, except for squalene and stigmasterol, where extraction with 67:33 hexane:acetone is considerably more efficient than with 93:7 DCM:methanol. For the aqueous solvents, 20:80 methanol:water alone was capable of only extracting the partially water-soluble atrazine. However, the addition of 1.5 mg ml^{-1} P80 enabled the extraction of the aliphatic compounds hexadecane, phytane and squalene, although no pyrene or anthracene was detectable. Relative to 93:7 DCM:methanol, the P80 solution gave an extraction efficiency of up to 30% for hexadecane and phytane, and up to about 10% for squalene and stigmasterol.

4. Discussion

4.1. Solvent extraction efficiencies

A solution of 1.5 mg ml^{-1} P80 in 20:80 methanol:water allows the extraction of aliphatic standards, such as hexadecane and phytane, at an efficiency of about 30%, relative to 93:7 DCM:methanol. The extraction of squalene and stigmasterol using P80 is rather less efficient, but still achieves extraction efficiencies of ~5% of that of 93:7 DCM:methanol.

In contrast to the good extraction efficiencies for aliphatic compounds, the aromatic compounds pyrene and anthracene were not detected. This observation is unexpected as P80 has been proposed as a surfactant for solubilising polycyclic aromatic hydrocarbons from contaminated soils (Yeom et al., 1995). The result must reflect the chemistry of the P80 surfactant and/or the hydrophobicity of the low molecular weight polycyclic aromatic hydrocarbons. P80 consists of hydrophilic polyoxymethylene head and a long hydrophobic aliphatic hydrocarbon tail. Aromatic compounds, which are likely to exist prior to extraction sorbed to mineral surface or incorporated in micropores, may be less well accommodated in, or attracted to, the P80 micelles. Hence, the P80 solution is an aqueous solvent capable of selectively extracting aliphatic compounds under the conditions described.

4.2. Solvent systems for Mars

4.2.1. Extraction efficiency of P80 solution

The antibody assay system planned for the LMC will contain antibodies capable of binding to representative compounds that may be found within the Martian regolith (Parnell et al., 2007). These antibodies will denature in the presence of organic solvents such as DCM. However, surfactant-based aqueous solvents, such as the P80 solution employed here, do not denature the antibodies and are therefore suitable for the LMC. Nevertheless, it is essential that the solvent chosen is capable of efficiently extracting organic compounds from the Martian regolith. The data presented here shows a 25-30% extraction efficiency, relative to 93:7 DCM:methanol, for the P80 surfactant solution. Although the chances of successful detection of organic compounds in the Martian soil ultimately depend on the concentration of that organic matter, we consider P80 to be an acceptable compromise between extraction efficiency and antibody compatibility.

4.2.2. Spiked JSC Mars-1 as a model of the martian regolith

The standards were applied to the JSC Mars-1 by evaporating their host solvent and thereby depositing the compounds on the surfaces of mineral grains presenting easily accessible targets for extraction. However, natural organic matter in rocks can be bound to minerals and spread heterogeneously throughout a rock. Organic matter intermingled in this fashion is undoubtedly more difficult to extract than organic matter simply deposited on the surfaces of grains. However, it is worth noting that the sonication regime employed here had the effect of eroding the grains of JSC Mars-1, producing fine-grained material suspended in the solvent, demonstrating that sonication-assisted extraction, as intended to be used by the LMC, is likely to be

capable of extracting organic matter in the interiors of grains. Further work is necessary to understand the extraction of more mineralogically and organically-diverse samples.

4.2.3. Organic compounds expected in the martian regolith

The nature of the organic compounds expected in the Martian subsurface, and the alteration chemistry occurring, of relevance to the LMC was summarised by Parnell et al. (2007) and will only be discussed briefly here. Two sources of organic matter in the Martian regolith can be inferred – a definite source in meteoritic infall and a possible source in biology. These organic compounds are then processed by oxidants in the soil and by ionising radiation, resulting in a wide range of possible compounds. Meteoritic infall is estimated to deliver about 240 tonnes of organic matter annually to Mars (Flynn, 1996), much in the form of a refractory organic macromolecule, but biologically relevant amino acids (e.g., Sephton, 2002) and nucleobases (Martins et al., 2008) can also occur. The nature of organic matter produced from indigenous Martian biology is open to speculation, but the design of the LMC has assumed that it will share certain similarities with terrestrial life (Parnell et al., 2007). On Earth and presumably on Mars it is the lipid components that are most likely to persist in either recent or ancient fossil form (Sephton, 2010) and this preservation potential has ensured that the majority of terrestrial biomarker methods focus on aliphatic hydrocarbon compounds. In this context the development of an antibody compatible solvent system that efficiently and selectively extracts an information rich fraction of organic compounds can be viewed as a positive development.

5. Conclusions

1. Surfactant-based solutions are effective at extracting aliphatic standards spiked on to the Martian analogue, JSC Mars-1. Hexadecane and phytane spiked on to JSC Mars-1 are extracted by a 1.5 mg ml^{-1} solution of polysorbate 80 in 20:80 methanol:water at about 25-30% of the extraction efficiency of 93:7 DCM:methanol. Squalene and stigmasterol are extracted less efficiently, about 5-10% of the extraction efficiency of 93:7 DCM:methanol. In contrast, extraction by 20:80 methanol:water alone, without the surfactant, did not extract any of these compounds.
2. The polysorbate 80 solution did not extract the aromatic compounds spiked to JSC Mars-1, pyrene and anthracene. Hence, the polysorbate 80 solvent solution appears to selectively extract the most information rich compound class for fossil biomarker analysis, the aliphatic hydrocarbons.
3. The Life Marker Chip, being developed for the detection of the chemical signature of life in the Martian subsurface, requires a solvent to transfer biomarkers from the soil to the antibody sites on the LMC itself. This solvent cannot be organic, as it would denature the antibodies, and the aqueous solvent 20:80 methanol:water does not extract the water-insoluble organic solvents. However, addition of 1.5 mg ml^{-1} polysorbate 80 to the 20:80 methanol:water provides an effective compromise between extraction efficiency and antibody compatibility.

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Table 1. Extraction efficiencies (%) of 67:33 hexane:acetone, 20:80 methanol:water and 20:80 methanol:water with 1.5 mg ml⁻¹ polysorbate 80, relative to those determined for 93:7 DCM:methanol. Means and standard deviations of three extractions are shown.

	Extraction efficiencies (%) relative to 93:7 DCM:methanol extract				
	67:33 hx:ace	20:80 methanol:water		1.5 mg ml ⁻¹ P80 in 20:80 methanol:water	
		Mean	σ (n=3)	Mean	σ (n=3)
Hexadecane	109.1	0.0	0.0	26.2	5.4
Atrazine	109.5	36.7	12.8	26.6	9.9
Anthracene	110.2	0.0	0.0	0.0	0.0
Phytane	105.0	0.0	0.0	31.1	6.0
Pyrene	116.4	0.0	0.0	0.0	0.0
Squalene	150.8	0.0	0.0	6.0	1.7
Stigmasterol	167.7	0.0	0.0	4.1	3.7

Figure captions

Figure 1. Standards used to spike JSC Mars-1 to determine solvent extraction efficiency, and the surfactant polysorbate 80. Structures are numbered relative to elution order on a DB-5MS gas chromatograph column.

Figure 2. Chromatograms of the extracts of the spiked JSC Mars-1 aliquots, using different solvents. Peaks are: 1 hexadecane, 2 atrazine, 3 anthracene, 4 phytane, 5 pyrene, 6 squalene, 7 stigmaterol. The peak marked * is an aliphatic amide contaminant, believed to have originated from the syringes used in the process of filtering the solvent extracts. The organic solvents 93:7 DCM:methanol and 67:33 hexane:acetone (2A & 2B) show good extraction efficiencies of each standard. The aqueous solvent, 20:80 methanol:water, only extracts the water-soluble atrazine, as expected (2C). Addition of 1.5 mg ml^{-1} polysorbate 80 to the 20:80 methanol:water results in the extraction of aliphatic standards (2D), but not aromatic pyrene or anthracene. See Table 1 for quantification and extraction efficiencies.

Figure 3. Extraction efficiencies (EE) of the spiked JSC Mars-1 using 20:80 methanol:water alone (■) and 20:80 methanol:water with 1.5 mg ml^{-1} P80 added (◇). Means and standard deviations of extractions of three individual samples are shown. For methanol:water alone, only the water-soluble atrazine is extracted. Addition of the P80 enables extraction of hexadecane, phytane, squalene and stigmaterol, but not of the aromatic compounds pyrene and anthracene.

1) Hexadecane

2) Atrazine

3) Anthracene

4) Phytane

5) Pyrene

6) Squalene

7) Stigmasterol

8) Polysorbate 80

Figure 2.

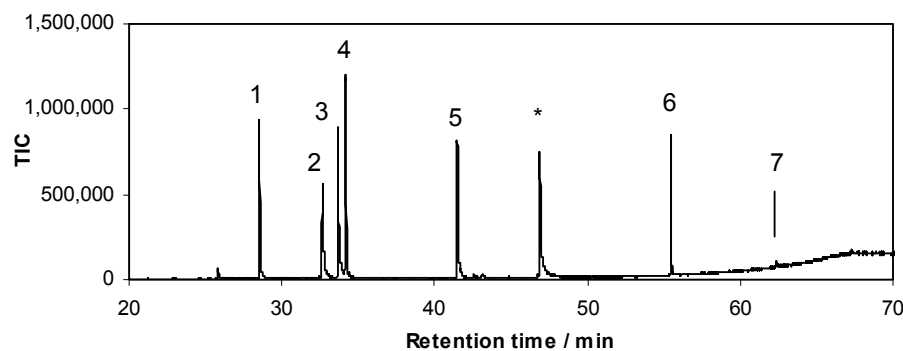
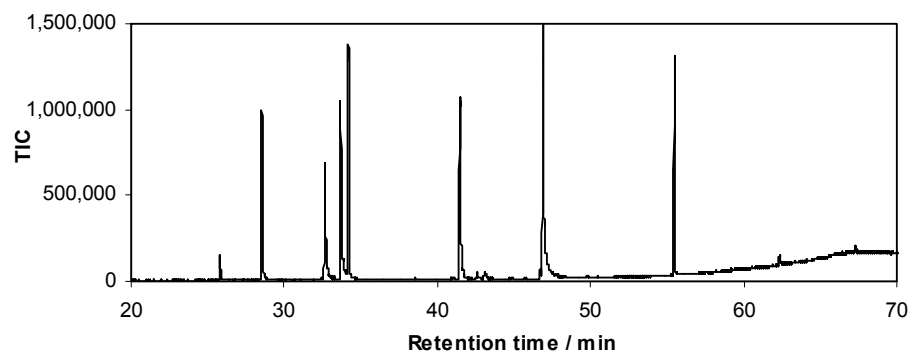
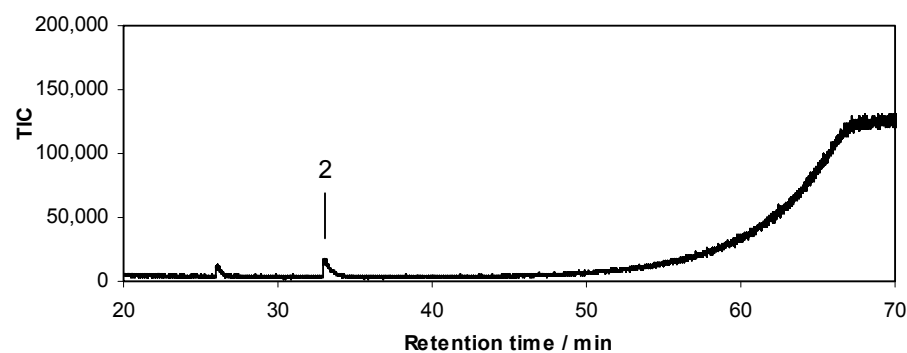
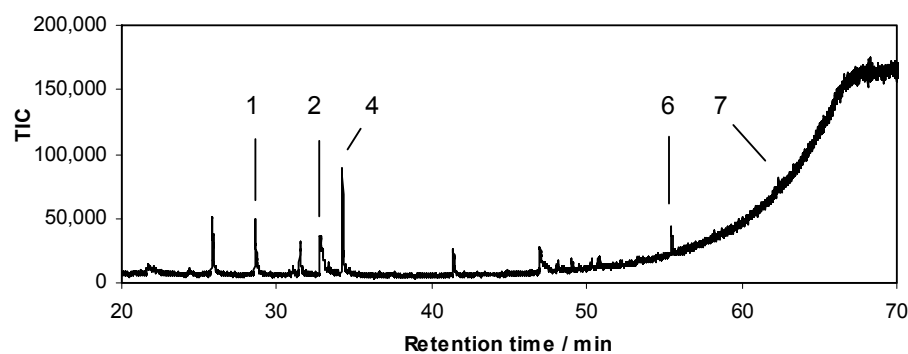
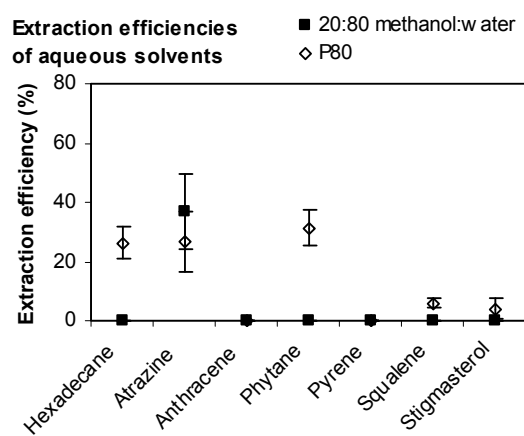
A) 93:7 DCM:Me extract**B) 67:33 Hx:Ac extract****C) 20:80 Me:H₂O extract****D) 20:80 Me:H₂O & 1.5 mg ml⁻¹ P80 extract**

Figure 3.





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