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1 Influence of pH and salts on DMF-DMA Derivatization for Future Space Applications

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14 Abstract

15 For gas chromatography - mass spectrometry (GC-MS) analyses performed *in situ*,
16 pH and salts (*e.g.*, chlorides, sulfates) may enhance or inhibit the detection of targeted
17 molecules of interest for astrobiology (*e.g.* amino acids, fatty acids, nucleobases). Obviously,
18 salts influence the ionic strength of the solutions, the pH value, and the salting effect. But the
19 presence of salts may also produce complexes or mask ions in the sample (masking effect
20 on hydroxide ion, ammonia, etc.). For future space missions, wet chemistry will be conducted
21 before GC-MS analyses to detect the full organic content of a sample. The defined organic
22 targets for space GC-MS instrument requirements are generally strongly polar or refractory
23 organic compounds, such as amino acids playing a role in the protein production and
24 metabolism regulations for life on Earth, nucleobases essential for DNA and RNA formation
25 and mutation, and fatty acids that composed most of the eukaryote and prokaryote
26 membranes on Earth and resist to environmental stress long enough to still be observed on
27 Mars or ocean worlds in geological well-preserved records. The wet-chemistry chemical
28 treatment consists of reacting an organic reagent with the sample to extract and volatilize
29 polar or refractory organic molecules (*i.e.* dimethylformamide dimethyl acetal (DMF-DMA) in
30 this study). DMF-DMA derivatizes functional groups with labile H in organics, without
31 modifying their chiral conformation. The influence of pH and salt concentration of
32 extraterrestrial materials on the DMF-DMA derivatization remains understudied. In this
33 research, we studied the influence of different salts and pHs on the derivatization of organic
34 molecules of astrobiological interest with DMF-DMA, such as amino acids, carboxylic acids,
35 and nucleobases. Results show that salts and pH influence the derivatization yield, and that
36 their effect depend on the nature of the organics and the salts studied. Second, monovalent
37 salts lead to a higher or similar organic recovery compared to divalent salts regardless of pH
38 below 8. However, a pH above 8 inhibits the DMF-DMA derivatization influencing the
39 carboxylic acid function to become an anionic group without labile H. Overall, considering the
40 negative effect of the salts on the detection of organic molecules, future space missions may
41 have to consider a desalting step prior to derivatization and GC-MS analyses.

42

43 Keywords

1 DMF-DMA derivatization, chloride, sulfate, and perchlorate salts, space applications, pH

2 **1. Introduction**

3 **1.1. Search for organic material**

4 Our understanding of the origins of life on Earth and the search for life in the solar
5 system requires the exploration of extraterrestrial materials, specifically through the search
6 for organic compounds and bioindicators – such as amino acids, carboxylic acids, and
7 nucleobases – or biomolecules – such as DNA or RNA polymers. Past (bio)geochemical
8 traces degraded on Earth because of erosion, lixiviation, and diagenesis processes, which
9 may be preserved on other planetary bodies, such as Mars or ocean worlds (e.g. Titan,
10 Europa, Enceladus). Conversely, a variety of parameters can influence organic molecule
11 preservation and detection in extraterrestrial environments, such as high temperatures,
12 cosmic/galactic rays, UV radiation, red-ox reactions, salts, acid-basic reactions, and
13 variations in pH. For example, Curiosity rover detected organic molecules in Mars surface
14 materials produced by Mars surface chemistry, infall of extraterrestrial materials, or within the
15 Sample Analysis at Mars (SAM) cups at the high temperature processes [1–4] (e.g. benzene,
16 dichlorobenzene, and C₂ to C₄ dichloroalkanes, mellitic acid) [5–7]. The *ExoMars* mission,
17 which will drill down to 2 m [8,9], might help discover more complex organics and potential
18 biosignatures on Mars within deeper geological strata (where radiation, temperature
19 variations, and surface processes such as erosion and diagenesis are limited). Another
20 significant challenge yet to be adequately studied is the variation of salt composition and
21 concentration, and sample pH, which can degrade the ability of instruments to detect organic
22 molecules in samples with *in situ* analyses.

23 **1.2. The challenging presence of salts for *in situ* analyses in extraterrestrial** 24 **environments**

25 The detection of organic molecules in a salty or mineral matrix is complicated by
26 several physicochemical processes: (i) the production of organic-salt complexes release
27 some elements (e.g. oxides) that enhance the organic degradation, (ii) organic-salt
28 adsorption on oven walls during pyrolysis or wet chemical reactions, (iii) potential
29 clogging/fouling of the organic trap(s) upstream of the GC column(s) when high
30 concentration in (organic) salts are heated over 100 °C in the space cups, (iv) degradation of
31 the GC column stationary phase [10], and (v) degradation of MS source and MS signal if
32 salts are eluted or come from thermal degradation, e.g. Cl₂ from chloride salts, which induce
33 signal suppression [11,12]. Organic molecules may also react during thermal or chemical
34 treatments in presence of salts within cups, taking organic molecules identification and
35 chromatogram interpretation more complex.

36 For almost two decades, the Mars Express, Mars Reconnaissance Orbiters (MRO),
37 Curiosity, and Perseverance rovers have been identifying different types of minerals and
38 salts on the surface of Mars. Some of these are hydrated minerals and salts, such as
39 silicates (SiO₂), hematite (FeO₃), or jarosite (K⁺Fe³⁺₃(HO⁻)₆(SO₄²⁻)₂) [13–17]. Among the
40 detected salts, Phoenix and Curiosity *in situ* probes observed Ca and Mg-perchlorates at
41 different Mars surface locations (from 0 to 3.5 wt%, and in Gale Crater from 0.8 to 1.5 wt%)
42 [18,19]. From these analyses, scientists modeled the perchlorate production at the Mars
43 surface and showed they are likely well dispersed [19–22]. Various other inorganic and

1 organic salts such as NaCl, MgSO₄, or FeSO₄ [23–26], predicted trace levels of carboxylic
 2 salts [27,28], or Ca, Mg, Fe-oxalate and acetate complexes [29] have been observed as well.

3 The presence of salts has also been demonstrated on ocean worlds and their
 4 satellites by past space missions (Pioneer 10, Voyager 1 and 2, Cassini-Huygens, and
 5 Galileo) [30–34]: (i) chloride salts are found in various forms: (a) monovalent chloride,
 6 specifically halite (NaCl), or sylvite (KCl) minerals, and (b) divalent chloride salts, such as
 7 carnallite (MgCl₂), as well as (ii) sulfates (gypsum (CaSO₄)), or (iii) other oxygen-bearing
 8 salts, such as carbonates (CaCO₃, NaHCO₃), or perchlorates (Mg/CaClO₄) [30–35] (Table 1).
 9 Despite low concentrations of some salts, salt mixtures might represent another challenge for
 10 the sample preparation (as well as for technical constraints).

11 As on Mars, ocean world surfaces (and more specifically on Europa and Enceladus
 12 satellites) present potential mineral products (observed and interpreted from Galileo imagery
 13 and spectroscopy analyses on dark yellow-brown geochemical features) such as silicates,
 14 epsomite, hexahydrate, halite, or natron [36]. These salts and minerals may encapsulate
 15 organic matter, or living cells, enhancing cellular preservation as observed on Earth
 16 [31,33,37–39]. Furthermore, multiple environments are present on the explored bodies
 17 (Mars, ocean worlds, etc.), which present acidic, neutral, or basic conditions that favor or
 18 constraint the mineralogy and the reaction between organic and salts in these soils (Table 1).

19 **Table 1.** Salt composition and concentrations expected on Mars and ocean worlds [3,13,14,16–18,23–
 20 28,30,32–34,38–53].

	Salt diversity	Concentrations	Minerals	pH value	Reference	
Mars	surface (0-10 m)	Perchlorates, organic salts, sulfates, chlorides, hydroxides, carbonates	0-3.5 wt%	Silica, Hematite, Jarosite, Olivine, Pyroxene, Halite, Gypsum, Carnallite	[4-7]	Hecht et al., 2009; Bibring et al., 2006; Carter et al., 2010; Klingelhöfer et al., 2004; Ojha et al., 2015; Navarro-González et al., 2010; Aubrey et al., 2006; François et al., 2016; Gaillard et al., 2013; Smith et al., 2014; Benner et al., 2000; Biemann, 2007; Kounaves et al., 2014
Titan	surface (0-20 km)	Sulfates, organic salts, chlorides, hydroxides, perchlorates, carbonates	few ppt	Gypsum, Silica, Olivine, Pyroxene, Halite, Carnallite	~7	Shematovich, 2018; G.M. Marion 2012; A.P. Crosta 2021; Vance et al., 2018, 2021; Fortes et al., 2007
	ocean subsurface	Sulfates, organic salts, chlorides, hydroxides, perchlorates, carbonates	10-100 ppt *		[7-12]	
Europa/ Enceladus	surface (0-10 km)	Chlorides, organic salts, sulfates, hydroxides, perchlorates, carbonates	10-100 ppt *	Halite, Gypsum, Silica, Olivine, Pyroxene, Carnallite	[2-12] Europa [9-11] Enceladus	Hand et al., 2007, 2009a, 2009b, 2016, 2022; Khurana et al., 2009; Cockell et al., 2010; Pasek and Greenberg, 2012; Zolotov, 2007; Zolotov and Shock, 2001; Kargel et al., 2000; Zolotov and Shock, 2001; Fanale et al., 2001; McKinnon and Zolensky, 2003; Kargel et al., 2000
	ocean subsurface	Chlorides, organic salts, sulfates, hydroxides, perchlorates, carbonates	0.018-1000 % *		[1-7]	

21 * Theoretical Concentrations according to models

22 1.3. DMF-DMA derivatization (sample preparation)

23 The Rosetta mission with its cometary sampling and composition GC-MS instrument
 24 (COSAC) was the first to load dimethylformamide dimethyl acetal (DMF-DMA) capsules for
 25 extraterrestrial *in situ* analysis. DMF-DMA targets organic molecules with amine and
 26 carboxylic functions, such as amino acids, fatty acids (Figure S1 in Supplementary material 1
 27 (SM1)) [54]. The efficiency of DMF-DMA to detect and keep safe the chiral conformation for
 28 these bioindicators or any labile chiral compound explains the choice to load DMF-DMA
 29 capsules in future spacecraft missions (*e.g. ExoMars and Dragonfly* missions with the MOMA
 30 and DraMS instruments, respectively). The enantiomers are then separated in the Chirasil-
 31 Dex column before the MS analysis [54,55]. The enantiomeric excess of different organics
 32 represents a bioindicator because organics are produced abiotically in both enantiomeric
 33 forms in a racemic mixture (50%-50%). However, on Earth, the presence of life privileged

1 one enantiomer for metabolic mechanisms leading to homochirality in living cells (*e.g.*
2 Levogyre form for amino acids and Dextrogyre for sugars). DMF-DMA was selected in this
3 study for its use in space applications and its stability face to a long term storage or gamma-
4 ray irradiations that could degrade the reagent during transport and use on ocean worlds
5 [56].

6 It was demonstrated that perchlorate salts present in samples collected and analyzed
7 at the Mars surface [18,19]: (i) interact with organics during the pyrolysis process [57] or (ii)
8 interact with wet chemistry reagents (N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide
9 (MTBSTFA), tetramethylammonium hydroxide (TMAH)) [58,59]. These interfering
10 interactions produce new organics considered as interfering molecules that are observed in
11 the SAM experiments, for instance, such as chlorobenzene, chloromethane, and
12 methylfluorosiloxane [2,3,15,58,60]. Thus perchlorates, and more generally salts, represent
13 (i) a technical concern on the GC-MS life duration and (ii) a chemical concern to detect
14 organic molecules due to salt interferences with the sample pre-treatment (pyrolysis,
15 MTBSTFA, and potentially with DMF-DMA). The present study will investigate and quantify
16 the salt-DMF-DMA-organic molecules interferences.

17 Understanding the kinetic and thermodynamic of reactions with salts happening
18 during the derivatization is important to mechanistically understand the influence of salts on
19 derivatization yield and find a solution to enhance the derivatization process and thereby
20 mitigate non-detection of any organics present. For the future missions to Mars (*ExoMars*),
21 Europa or Enceladus (*Europa and Enceladus Landers*), and Titan (*Dragonfly*), the issues
22 related to the presence of inorganic and organic salts have to be understood while organic
23 compounds interact with derivatizing reagents.

24 This work presents a study of the potential influence of various salts and pH during
25 the wet chemical treatment for future GC-MS analyses. We also explore the theoretical
26 processes occurring during derivatization in the presence of salts and acidic/neutral/basic
27 solution and defining how these processes can influence *in situ* analyses sample
28 preparation.

29

30 **2. Material and method**

31 **a. Standard solutions and reagents**

32 Considering the current objectives of *in situ* astrobiology explorations, we focused the
33 study on three chemical families of organic molecules known for their implication within the
34 life process, *i.e.* amino acids, carboxylic acids, and nucleobases. They represent potential
35 bioindicators to detect potential traces of life and help investigate prebiotic chemistry. For the
36 sample preparation, we studied the DMF-DMA derivatization with the objective to prevent or
37 predict the reaction yield and define interferences while organics and DMF-DMA interact with
38 various salts (studied individually in this work) in solutions with different pH. The study will
39 help determining which soil/mineral samples promote or interfere in the extraction and
40 analysis of organics using the DMF-DMA. We also tested the efficiency of DMF-DMA to
41 derivatize chiral organics without modifying their chiral conformation in presence of salts.

42 Amino acids were ordered from Fluka (glycine, D or D/L-phenylalanine, D-valine,
43 alpha-aminoisobutyric acid (or AIB), D or D/L-aspartic acid, D/L-alanine, and L or D/L-
44 cysteine at 99% purity) in racemic mix – or as pure enantiomers in order to consider the

1 potential impact on chirality during DMF-DMA derivatization [54]. The carboxylic acids
2 solution consisted of carboxylic acids detected in eukaryotes and bacteria on Earth (benzoic
3 acid, succinic acid, methyl succinic acid, and myristic acid). They were all purchased from
4 Fluka (99% purity). Finally, the uracil nucleobase (Alfa Aesar, 99%) was chosen as it is more
5 likely highly probable to be detected on an extraterrestrial surface. Uracil has been detected
6 in meteorites since it is the most easily formed nucleobase in the solar system [61–64].

7 The concentration of the standards was chosen to be representative of those found in
8 extraterrestrial samples (~0.1-10 $\mu\text{mol L}^{-1}$ (or 1-100 pmol)) [5]. In addition, the DMF-DMA
9 reagent (Sigma-Aldrich, 95% purity) is in excess compared to the organic molecules (~7.15
10 mol L^{-1} (or 143 μmol for 20 μL of DMF-DMA)). However, organic targets were considered as
11 analytes (low concentrations compared to salts). The salt concentration was chosen
12 according to the Martian range: 0.05 - 0.3 M (which corresponds to 0.8 – 17 μmol), and
13 subsurface ocean worlds: 0.3 – 5 M (which corresponds to 17 – 280 μmol) (see references
14 Table 1).

15 In this study, we conducted experiments on different salts detected on extraterrestrial
16 surfaces (Table 1): sodium chloride, potassium chloride, magnesium chloride, calcium
17 chloride, magnesium sulfate, magnesium perchlorate, and hydrogenated sodium carbonate
18 (Sigma-Aldrich, 96%).

19 Organics were diluted in HCl (0.1 M) and pure milli-Q water (TOC lower than 3 ppb),
20 except 1,3,5-benzenetricarboxylic acid diluted in ethyl acetate (Sigma-Aldrich, 99.9%). HCl
21 was used to acidify solutions (AppliChem Panreac, 99%), and NaOH to basify solutions
22 (Fluka, 99%). Methyl laurate was used as internal standard (Fluka, 97%).

23 To prevent potential biases introduced by intraday deviations of the instrumentation and
24 injected volume variations in the semi-quantitative analysis, methyl laurate (97% purity from
25 Fluka, diluted at 10^{-2} M in ethyl acetate, 99.9% purity from Sigma-Aldrich) was used to
26 normalize peak areas (1 μL in each sample). Methyl laurate is not derivatized by DMF-DMA
27 and is fully vaporized and well-eluted with the set-up and operating conditions used. To
28 quantify the organic recovery and test the reagents' efficiency to each organic, we measured
29 the peak area of each organic molecule in the chromatograms, then divided it by the peak
30 area of the internal standard (IS, or methyl laurate) using Thermo Scientific Xcalibur
31 software:

$$32 \quad A_{\text{organic}}/A_{\text{internal standard}} = \frac{\text{Area of the organic peak}}{\text{Area of the internal standard}} .$$

33 **b. Instrumentation**

34 Liquid samples were injected by a gas syringe directly in a Split/SplitLess (SSL)
35 injector of a gas chromatograph (Trace GC Ultra, Thermo Scientific) in split mode (split set to
36 50 mL min^{-1}). The injector temperature was set at 270 °C. Helium (99,9999% purity, Air
37 Liquide) was used as the carrier gas, with a constant flow rate of 1.2 mL min^{-1} . Experiments
38 were performed on a gas chromatograph (GC) coupled to a mass spectrometer (MS) ISQ LT
39 from Thermo Scientific. The MS ion source was set to 250 °C. The m/z range scanned was
40 40 to 550. The temperature of the MS transfer line was set to 300 °C. To achieve the
41 analytes separation, specifically of the enantiomers, we used the CP-Chiral Dex CB column
42 (25 m x 25 mm x 0.25 μm) from Agilent. The temperature program of the column started from
43 40 °C, and then heated up to 200 °C at a 3 °C min^{-1} rate.

1 The GC-MS detected and analyzed organic compounds were in this study fully or
2 partially derivatized forms that we sum for the quantification work (*e.g.* amino acids in Fig. S1
3 in SM1). The XCalibur 2.0 program and the National Institute of Standards and Technology
4 (NIST) chemical database were used to interpret the data. The quantification study is based
5 on statistical tests (Student or Mann-Whitney test (while the normality of the data was
6 checked using a Shapiro–Wilk test)) performed on R 3.5.1 and R Studio software (with the
7 use of six replicates by condition and a α risk of 0.05 to affirm the significant differences) to
8 present the significant differences between conditions of salts, salt concentrations, or pH
9 variations.

10 The pH of aqueous and organic phases was monitored before each experiment using
11 a Consort pH-meter and Thermo scientific Orion 9863BN pH probe.

12 13 **c. Sample preparation and gas chromatography analyses**

14 First, we prepared the three analytes mixtures rich in amino acids, carboxylic acids,
15 and a nucleobase, respectively. The amino acid concentrations were introduced in a vial at a
16 0.05 M concentration, except cysteine at 0.1 M to be detected because of the lower MS
17 sensitivity for this amino acid compared to the other. 4 μL of each amino acid solution was
18 added in a vial and evaporated under N_2 (99.9999%) flow to throw water – that can interact
19 with DMF-DMA.

20 The succinic acid concentration was at 0.04 M, methyl succinic acid at 0.05 M, and
21 1,3,5-benzenetricarboxylic acid at 0.01 M. 6 μL of each carboxylic acid solution was added in
22 the vial, and the solvent was evaporated.

23 Uracil (0.01 M) was introduced and dried out in the vial before the introduction of
24 salts, DMF-DMA reactant, and methyl laurate internal standard.

25 Salts were diluted in pure milli-Q water (TOC lower than 3 ppb) at various
26 concentrations and pH, then added over the evaporated amino and carboxylic acid (and
27 nucleobase) solution. Once the pH was set, water was evaporated under N_2 flow. Then, 30
28 μL of DMF-DMA was added – representing a molar ratio of 1:12 compared to the organic
29 molecules and 1:6 compared to the labile groups derivatized (in excess). 1 μL of methyl
30 laurate at 0.01 M solution was added as an internal standard. The solution was heated at
31 145 ± 5 °C for 3 minutes which represents the optimal conditions for the derivatization using
32 DMF-DMA [65]. Then, 1 μL of this derivatized solution was injected into the GC-MS.

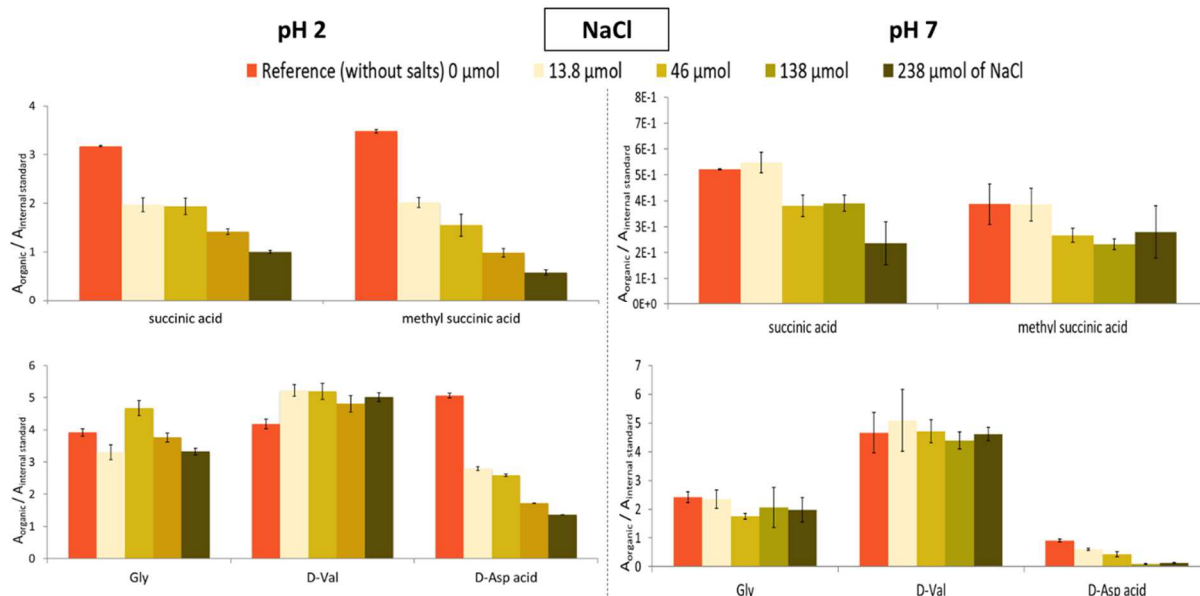
33 We varied the salt concentrations of chlorides, sulfates, perchlorates, and carbonates
34 (Table A in Supplementary Material 1 (SM1)). Concerning the influence of the pH value, it
35 has also been studied in presence of sodium chloride (one of the most abundant salts in
36 surface and subsurface environments).

37 38 **3. Results**

39 **3.1. Influence of salts**

40 **3.1.1. Effect of monovalent cations, and of their concentration, on DMF-** 41 **DMA derivatization**

1 Chloride salts, and more specifically NaCl, are expected to be present in high
 2 concentrations on extraterrestrial surfaces and oceans (e.g. Europa or Enceladus natural
 3 satellites). We first conducted derivatization experiments on amino acids, carboxylic acids,
 4 and one nucleobase in presence of a range of salt concentrations varying from 0.05 to 0.5 M
 5 (Mars concentrations) or 0.3 to 5 M (ocean world surface and subsurface concentration
 6 range). Figure 1 presents results about the influence of NaCl at pH=2 (acidic media) and
 7 pH=7 (neutral media) from 0.3 to 5 M (or 13.8 to 230 μmol of salt) on the organic recovery. In
 8 Figs. S2 and S3 (SM2), complementary results are presented using NaCl and KCl from 0.05
 9 to 0.3 (or 0.5) M (or 2 to 10 μmol) to understand the influence of salts and monovalent
 10 cations at low concentrations on the sample pre-treatment yield.



11
 12
 13 **Fig. 1.** Integrated peak area of fully derivatized amino and carboxylic acids (on amine and
 14 carboxylic functions) over the internal standard peak area (methyl laurate) in presence of
 15 different quantity of NaCl at pH=2 and pH=7. Quantity of salts from left to right for each
 16 compound: 0 μmol , 13.8 μmol , 46 μmol , 138 μmol , and 230 μmol referred to a concentration
 17 in salts of 0 M, 0.3 M, 1 M, 3 M, and 5 M. 6 replicates have been conducted to produce this
 18 quantification work and the error bars.

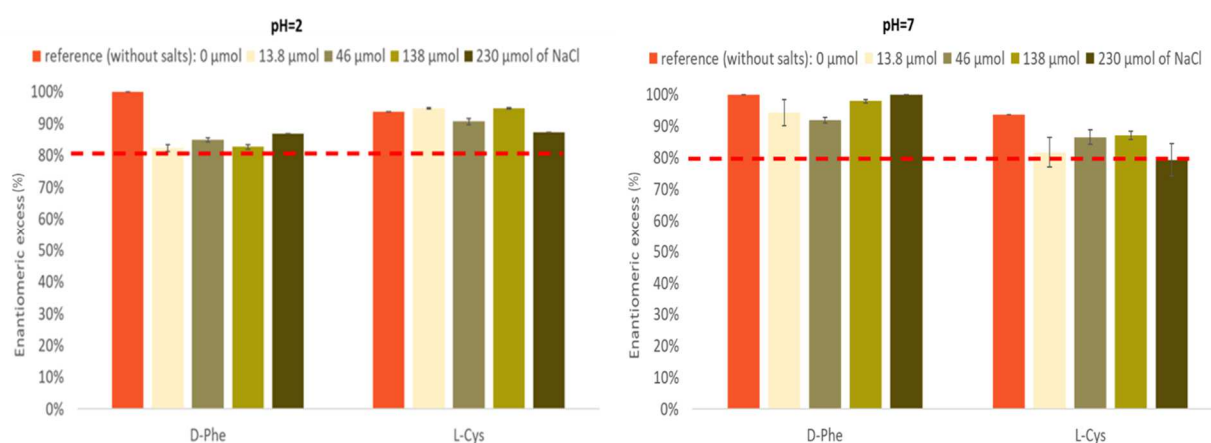
19 At pH 2, from 0.05 to 0.6 M in Fig. S2 (SM2) and 0.3 to 5 M of NaCl in Fig. 1, we
 20 observe a constant decrease in the carboxylic acids' recovery with the increase of salt
 21 concentration. Thus, monovalent cations in acidic solutions influenced the derivatization yield
 22 of the DMF-DMA reaction at 145 $^{\circ}\text{C}$ – 3 min (as observed for KCl and NaHCO_3 in SM2 and
 23 SM3). For most of the amino acids, no statistical significant differences to minimal
 24 differences (lower than a factor of two) were observed in the organic recovery in presence of
 25 NaCl and KCl (Fig. 1 and SM2: 0.05 – 5 M), and half organic recovery with the NaHCO_3 salt
 26 compared to the reference without salts (Fig. S4 in SM3 and Table B in SM8) due to the
 27 amphoteric characteristic of HCO_3^- that can interact with the labile H of COOH functions.

28 The same trend for amino acids while we increase the NaCl quantity at pH 7 is
 29 observed compared to pH 2. The same carboxylic acid trend was noted (compared with pH
 30 2), however, the organic recovery was 0.5 to 3 lower than the reference (but higher than pH
 31 2 with a loss of 2 to 5). This result was confirmed for other amino acids, carboxylic acids, and
 32 one nucleobase at the same or lower concentrations to simulate future Mars experiments

1 (SM2). Monovalent ions influenced slightly the DMF-DMA derivatization on organics while we
 2 increased the concentration, however, it represents major concerns if organics are present at
 3 trace level amounts in the extracted extraterrestrial sample (close to the LOD) or at NaCl
 4 concentrations higher than 3 M. For instance, the decrease in aspartic acid recovery (near by
 5 the GC-MS limit of detection (LOD)) compared to the absence of decreasing for other amino
 6 acid recoveries. This difference is specially explained by the MS sensitivity regarding the
 7 aspartic acid, for instance, because the quantitative approach has a low confident factor for
 8 aspartic acid [65].

9 Two hypotheses may explain the overall decreasing trend in the derivatization yield.
 10 On the one hand, the increase in salt concentration increased the ionic strength which led to
 11 numerous chemical interactions. Therefore, the collisions between ionic salts and organic
 12 molecules (or DMF-DMA) led to a decrease in the interaction of DMF-DMA and organic
 13 molecules (lower diffusion factor for organics that are highly diluted in the NaCl solution).
 14 This effect was statistically significantly measured from 138 μmol (3 M) in NaCl while DMF-
 15 DMA was at the same or in a lower amount than salts and became a chemical analyte. In
 16 parallel, organic-salt complexes were produced (discussed in part 4) (see Table B in SM8).
 17 On the other hand, the ionic strength played a second role in the derivatization process.
 18 Indeed, the permittivity of the apolar DMF-DMA is lower than water, which limits the
 19 dispersion of the solvent. To test this hypothesis, we added a polar solvent: DMF prior to the
 20 derivatization. We observed an increase (of 5-60%) up to the reference in the organic
 21 recovery compared with a condition without DMF (Fig. S5 in SM4).

22 In parallel, we looked at the possible influence of chloride salts on the organic
 23 racemization during the DMF-DMA derivatization reaction. We calculated the enantiomeric
 24 excess based on the homochiral form added in the initial organic mixture and the reverse
 25 produced enantiomeric form with the formula: $Ee (\%) = 100 \cdot \frac{AAM - AAm}{AAM + AAm}$, where AAM = the
 26 amino acid homochiral form in excess (in our case the injected one) and AAm = the
 27 enantiomeric form in minority (produced during the 145 °C – 3 min derivatization step).

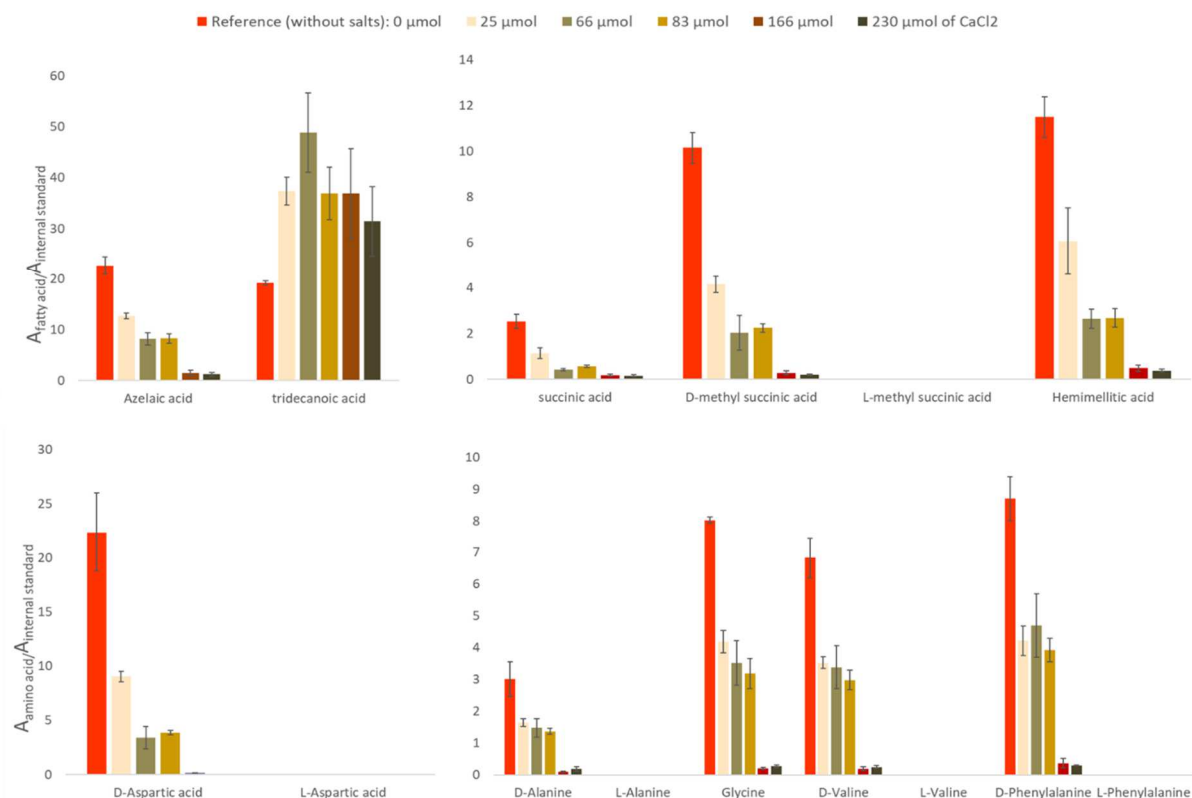


28
 29 **Fig. 2.** Enantiomeric excess of fully derivatized homochiral amino acids injected in the GC-
 30 MS at pH=2 and pH=7 varying from 0 μmol , 13.8 μmol , 46 μmol , 138 μmol to 230 μmol in
 31 NaCl (or 0.03 M, 0.06 M, 2 M, 4M, and 5 M, respectively). The red dashed line represents
 32 the lower limit for an abiotic/biotic racemization yield. 6 replicates have been conducted to
 33 produce this quantification work and the error bars.

1 In Figure 2, we observed that the homochiral form of each amino acid added for the
 2 derivatization (e.g. D-Phe and L-Cys) was the most abundant enantiomer in GC-MS
 3 analyses with an enantiomeric excess of over 80%. Thus, DMF-DMA derivatization in
 4 presence of low or high concentrations of NaCl did not influence the organic chirality during
 5 derivatization.

6 3.1.2. Effect of divalent chloride salts, and of their concentration, on 7 DMF-DMA derivatization

8 To interpret the influence of salts for *in situ* GC-MS analyses, we studied the influence
 9 of divalent cations, such as CaCl₂ (Fig. 3), on the derivatization of amino acids and carboxylic
 10 acids (or MgSO₄ and Mg(ClO₄)₂ in SM5) in a second set of experiments. The comparison of
 11 monovalent and divalent chloride salts will be discussed in part 4 (Fig. S9 in SM7).



12 **Fig. 3.** Integrated peak area of fully derivatized amino and carboxylic acids (over the internal
 13 standard peak area) in presence of different CaCl₂ quantities from left to right: 0, 25, 66, 83,
 14 166, and 230 μmol, which correspond to a salt concentration of 0, 0.54 M, 1.44 M, 1.80 M,
 15 3.60 M, and 5 M, at pH 2. 6 replicates have been conducted to produce this quantification
 16 work and the error bars.
 17

18 In Fig. 3, we observed a decrease in the organic recovery at 0.54 M with a factor of
 19 about 2 compared to the reference (no salts). The organic recovery decreased then by a
 20 factor of four to five at 1.44 M, and down to twenty to thirty at 3.60 M (different from
 21 monovalent chlorine species, which was between zero and three). We tested the influence of
 22 MgCl₂ compared to CaCl₂ and noted the same trend with an overall similar factor of
 23 difference compared with the reference on the organic recovery (Fig. S9 in SM7).

24 Fig. S6 in SM5 shows a statistical significant decrease in all organic recovery in
 25 presence of MgSO₄, except for myristic acid. Myristic acid is the only organic with a linear
 26 alkyl long chain, a single acidic group to derivatize, and therefore with a carboxylic function

1 kinetically easier to derivatize than amine groups. In contrast, $MgSO_4$ decreased the
 2 recovery of π -conjugated system, such as uracil and phenylalanine. The divalent ions and
 3 the aromatic ring might request more energy and a longer time of reaction to react totally with
 4 DMF-DMA. Despite this difference with myristic acid, namely benzoic acid, phenylalanine,
 5 alanine, and uracil, were detected way above the limit of detection of the instrument
 6 compared to succinic acid and aspartic acid, which were close to their LOD.

7 For Fig. S7 in SM5, we observed that perchlorate salts influenced the DMF-DMA
 8 derivatization, even for myristic acid (except at 0.03 M in where $Mg(ClO_4)_2$ was not in
 9 excess). Phenylalanine, or uracil, derivatized at 70-90% without salts [66] or with chloride
 10 salts in low concentrations (< 2 M), was detected at pmol instead of nmol at 0.03 M with
 11 $Mg(ClO_4)_2$.

12 In addition to the influence of perchlorate and sulfate on DMF-DMA derivatization, the
 13 chromatographic separation was also influenced by the salts (Fig. S10 in SM9), especially at
 14 the highest concentrations tested (0.3 M). Indeed, the chromatographic peaks of all the
 15 organics presented a non-gaussian form. The impact of perchlorates compared to sulfates or
 16 monovalent chloride salts was most notable on DMF-DMA derivatization with a factor of ten
 17 to two or three hundred for methyl succinic and aspartic acids (close to the LOD).

18 Other oxidized salts were tested for comparison with oxidized sulfates and
 19 perchlorates. In Fig. S4 (SM3), $NaHCO_3$ showed a similar profile as $NaCl$, but a different
 20 trend compared to sulfate or perchlorate salts. Indeed, uracil had the same recovery despite
 21 the presence of salts (< 0.1 M). A slightly higher organic recovery was observed for all
 22 compounds at higher concentrations in $NaHCO_3$ due to the amphoteric character of the salt
 23 and these monovalent ions (SM8). Despite this higher organic recovery, a decreasing factor
 24 of ~ 2 was observed at low and high $NaHCO_3$ concentrations compared to the reference –
 25 with a loss factor of about ten. The same low organic recovery was observed for methyl
 26 succinic and aspartic acid as for the other salts. We also observed in the chromatograms a
 27 poorer separation of enantiomers from the amino acid racemic mix than the reference and an
 28 S/N close to three.

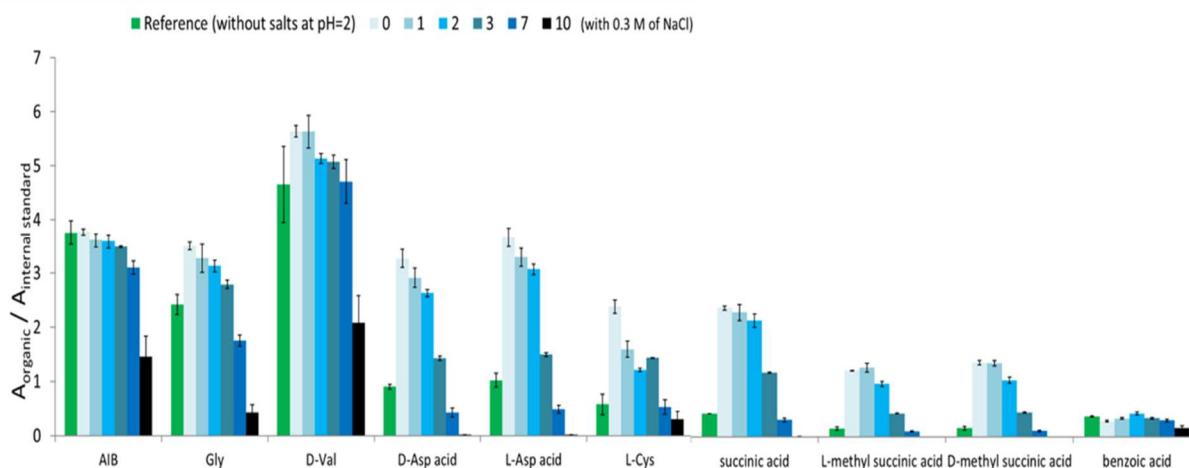
29 3.2. Influence of pH

30 To prevent the possible influence of pH variations within the original extracted
 31 samples on DMF-DMA derivatization, we conducted experiments mimicking acidic, neutral,
 32 and basic environments. These results will be helpful for future spaceflight missions to target
 33 sampled site analyses with the most favorable pH for chemical treatment with DMF-DMA.

34 **Table 2.** Measurement of pH during the DMF-DMA pre-treatment steps while we fixed pH
 35 before the introduction of DMF-DMA and internal standard (within the 0 to 10 range studied).

	pH initial	pH condition (fixed ± 0.5)	pH with DMF-DMA	pH after 145°C-3min
Organic molecules without salts	4.3	[0-10]	11.2	11.2
NaCl /KCl	3.8	[0-10]	11.2	11.2
$MgCl_2/CaCl_2$	3.3	[0-10]	11.1	11.1
pure DMF-DMA	12.10			
pure Milli-Q water	7.98			

36
 37 We observed in Table 2 that salts acidify the solutions, favoring the acidic form of
 38 organic labile H groups attacked by DMF-DMA, which is a strong base ($pK_b = 12$). The
 39 derivatization reaction did not influence the pH of the organic solution compared to the pH
 40 measured after the introduction of DMF-DMA in the organic dried solution.



1
2 **Fig. 4.** Fully derivatized amino and carboxylic acids' recovery in presence of a low amount of
3 salts (NaCl 0.3 M – Mars-like samples) at different pHs (from 0 to 10 following a blue
4 gradient). 6 replicates have been conducted to produce this quantification work and the error
5 bars.

6 In Fig. 4, we observed an increase in the organic recovery (or a similar recovery
7 compared to the reference) for a pH of 0 to 6, then no differences or a slight decrease
8 (decrease of a factor < 2) for pH 7, and finally a statistical significant decrease for pH 10. We
9 thus observed that within a wide range of pH values, the best derivatization yield was
10 obtained in acidic solutions below pH of 3-4 for carboxylic and amino acids. Up to pH 7, no
11 differences are observed for most of the amino acids (*e.g.*, AIB) and carboxylic acids (*e.g.*,
12 benzoic acid) even with 0.3 M of sodium chloride. However, basic pH seemed to decrease
13 the derivatization yield for all the organics tested (with a factor in the organic detection of ~ 2
14 to 10 lower than the reference) due to the loss of labile H on DMF-DMA targeted groups (-
15 COOH, -NH₃⁺ becomes -COO⁻ and -NH₂).

16 We also observed a slight decrease in the enantiomeric excess down to 80% of the
17 injected homochiral form, but not down to 0% as it would be if complete racemization
18 occurred during the derivatization process (SM6, Fig. S8) Thus, the pH in addition to the
19 presence of NaCl did not influence the derivatization process or change the organic chirality.
20

21 4. Discussion

22 4.1. Chemical mechanistic explanations

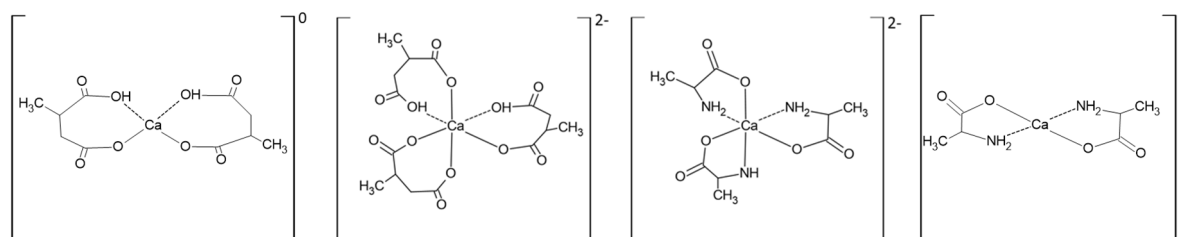
23 To evaluate Martian or ocean world organic detection interferences, we selected
24 chloride, perchlorate, sulfate, and carbonate salts from 1:1 salt:organic molar ratio to 100:1
25 (corresponding to concentrations from 0.05 M to 5 M). The results obtained with divalent
26 salts revealed three main interfering effects occurring during the reaction:

27 (i) The ionic and crystalline cage effect: Salts form either a physical barrier or
28 enhance the formation of complexes (during the 3 minutes of derivatization at 145 °C).
29 Specifically, with divalent ions, two covalent and two ionic bonds are produced, which
30 stabilize the organic-salt, effectively hiding the organic from the reagent. [67–69].

31 The cage effect hypothesis and the collision rate seemed in agreement with the
32 results at low and high concentrations in salts (Fig. 1, 3, SM2). Most of the organics tested
33 were either not detected at all (lower than the limit of detection of the GC-MS) or differed

1 from the reference with a factor of about 1.5 for NaCl or 2 for other monovalent salts, and
2 minimum 50 for CaCl₂, MgCl₂, and other divalent salts [67,68].

3 (ii) The Hard and Soft Acid-Base theory (HSAB): HSAB reveals that salt concentration
4 makes the ion more or less acid changing the electronic repartition around the nucleus and
5 avoiding the interaction and the attack of DMF-DMA on organics (while salts are in higher
6 abundance than organics). The hydration sphere of salts (made of water for ocean world
7 samples and potentially DMF in our experiments because the water was entirely removed)
8 interact at a higher rate with the organics [70]. The formation of these salt-organic complexes
9 physically blocks DMF-DMA due to their larger ionic radii. It also influences the energetic
10 barrier to get past the thermodynamic process to make the derivatization possible. Mg²⁺ and
11 Ca²⁺ impacted the derivatization more than Na⁺ or K⁺ due to chelation with organics
12 producing salt-organic complexes during the derivatization process at 145 °C, especially with
13 amino acids (4 or 6 bond-complex: 2 or 4 covalent and 2 ionic bonds between one salt and
14 two or three organics) (Fig. 5). Indeed, the cation is the most reactive when the anion is inert
15 in (i) the salt-organic complex production, (ii) DMF-DMA derivatization mechanism, and (iii)
16 DMF-DMA physical-chemical approach to labile H organic groups [71–75] (Tables 3 and 4).



18 **Fig. 5.** Salt-Organic complexes (e.g. calcium-methylsuccinic acid on the left side and
19 calcium-alanine on the right side) produced in solution prior to the DMF-DMA interaction with
20 organic molecules (e.g. methylsuccinic acid or alanine) with 4 or 6 bonds to stabilize the
21 complex.

22 The reaction between DMF-DMA and benzoic acid, phenylalanine, or uracil, led to
23 better derivatization yields compared to the other organic molecules. The explanation comes
24 both from the HSAB theory and the stabilized H acidic bond by the aromatic and/or π -
25 conjugated system. Free electrons circulate between the oxygen of the carboxylic group, the
26 nitrogen of the amine group and the aromatic group, which restrains the interaction with salts
27 and promotes the interaction with the stable and basic DMF-DMA solvent (with an organic
28 pH at 12.1). Indeed, hard and strong base, such as DMF-DMA, interacts totally with strong
29 and hard acid, such as the acidic H labile groups in our targeted organics [71–75].

30 (iii) The mask effect and hygroscopic radii: The pH involves an acid or a basic form for
31 each acid-base group targeted by the DMF-DMA reagent and salts. Thus, the pH modifies
32 the complex formation constants. The values in Table 3 were obtained at ambient
33 temperatures and acidic solutions (pH<3). For cysteine, the carboxylic acid function lost its
34 labile H at pH 3 and the thiol function at pH 9.5 [76]. Moreover, the number of moles of bases
35 in the ligand/organic per mole of salt increased with the pH [77]. Most of the time, Ca²⁺, Mg²⁺,
36 Na⁺, and K⁺ are linked to a « hard » oxygen ligand and rarely to « soft » nitrogen or sulfur
37 ligands, verified by the HSAB theory [78]. However, within the two first Mendeleïev table
38 columns, the atomic number increases within the same group of elements, and the
39 coordination distance was found to be increased from Na⁺ and K⁺ to Mg²⁺ and Ca²⁺,
40 according to the same row respectively [69]. We observed that salt complexes are easily
41 formed with divalent cations (Ca²⁺, Mg²⁺) compared to monovalent cations (K⁺, Na⁺) due to

1 thermodynamic constant with the cage effect and the hygroscopic aspects (Table 4). Kinetic
 2 aspects influence the formation constant reported in the literature to pass over an energetic
 3 barrier as well (Table 3). Table 3 reveals another interesting influence of salts, according to
 4 the form and the acidic group attacked by salts to form complexes. Indeed, the carboxylic
 5 function is highly attacked compared to sulfur and amine groups that still have a high
 6 probability of being attacked by cations. According to the organic (*i.e.* conformation, acidic
 7 functions of cysteine in Table 3), scientists noted a difference in the complex formation
 8 constants between a cysteine with and without additional groups or molecules added to an
 9 acidic function. They remarked a higher formation constant value for cystine (which is two
 10 cysteines merged at their thiol functions) compared to cysteine or N-acetyl-L-cysteine
 11 (cysteine with a branched function at the amine group) for the same cation. Comparing
 12 cations, we concluded for carboxylic, nucleobase, or other amino acids (where cysteine
 13 serves as a reference case in this study) that a salt complexation is highly probable with
 14 divalent cations compared to monovalent ones (54,62–71).

15 **Table 3.** Formation constants for complex of organics with ionic salts [69,76,77,83,84,88].

	Complex formation constant		Acid dissociation constant		
	ML		COOH	NH ₃ ⁺	SH
Cysteine*			1.88	8.38 ± 0.02	10.60 ± 0.03
Na-Cysteine**	2.39 ± 0.07				
K-Cysteine**	2.73 ± 0.01				
Ca-Cysteine*	2.99 ± 0.22				
Ca-Cystine**	2.68 ± 0.05				
Mg-Cystine**	1.93 ± 0.05				
Na-N-acetyl-Cysteine***	0.77 ± 0.06				
Ca-N-acetyl-Cysteine***	1.25 ± 0.09				
Mg-N-acetyl-Cysteine***	2.08 ± 0.13				

Values obtained at ambient temperature, where M=metal, and L=ligand

* T.D. Zucconi et al., 1978; D.A. Doornbos & J.S. Faber, 1964

** D. Aiello et al., 2021; E. Bottari et al., 2017; R. Shankar & L. Senthilkumar, 2011

*** C. Bretti et al., 2020

16

17 **Table 4.** Radii of different elements/molecules under their atomic, ionic, and hydration forms
 18 [79,80,82,85–87,89].

physical cage effect (radius in Å)	Atomic form	Ion form	Hydroscopic form	Hydration ring (H ₂ O molecules)
Na	1.86	0.97	3.60	~2.4* / 4.03**
K	2.31	1.41	3.15	~0.9* / 2.69**
Mg	1.60	0.70	3.95-5.70	5.33** / [6-18]***
Ca	1.97	1.03	3.48-6.03	3.64** / [6-18]***
Cl	1.00	1.80	2.70	1.70** / 4.0***
SO ₄ ²⁻	/	2.34	3.0-4.0	2.34** / [6-18]***
CO ₃ ²⁻	/	2.50	3.0-4.5	[6-18]***
H ₂ O	1.38	/	/	/
NaCl	3.86	3.77	5.77	~3.5*
KCl	3.31	3.21	5.21	~1.9*

From Y. Marcus, 1988; S.F. Lincoln, 2003 (with Mg and Ca-O bond of 2Å); J. Kielland, 1937

*Y. Zhong-Hua, 2014

**B.B. Tanganov, 2013 and M. Baldanov et al., 2006

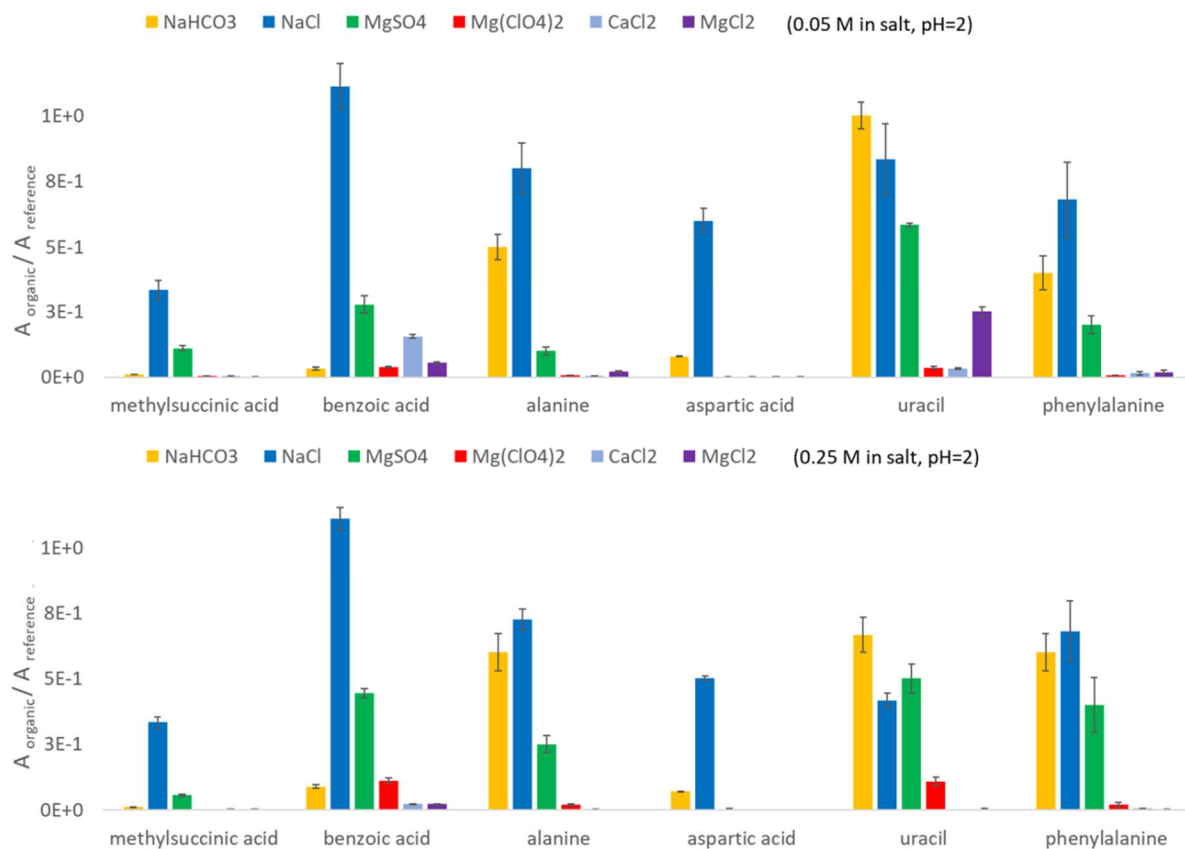
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4.2. Salts comparison on the organic recovery



4
5 **Fig. 6.** Integrated peak area of fully derivatized organic normalized by the integrated peak
6 peak area of the internal standard (methyl laurate) for each salt compared to no salts at 0.05 M or
7 0.25 M, at pH=2 (Mars concentrations and pH). 6 replicates have been conducted to produce
8 this quantification work and the error bars.

9 Fig. 6 confirms at low concentrations in salts (Mars expected concentrations) that Na⁺
10 and K⁺ cations interfere less than Mg²⁺, which interfere less than Ca²⁺ with organics.
11 Regarding the influence of the salt concentration, a negative effect on the derivatization yield
12 was observed with a drastic decrease of over 1 M in monovalent salts and 0.25 M for
13 divalent salts. Organics mixed with divalent salts had an organic recovery below 20%
14 compared to ~70% with monovalent salts.

15 To match the likely composition of the surfaces and/or subsurfaces of ocean worlds,
16 we chose a range from 0.03 M to 5 M in NaCl, according to modeling studies
17 [31,35,44,47,90]. Up to 2 M, our experiments show the same trend in the organic recovery
18 with no drastic negative influence until above 2-3 M (Fig. 1 and SM2). The loss factor in
19 organic recovery was more significant for specific compounds (student tests), such as
20 aspartic acid and 1,3,5-benzenetricarboxylic acid due to the MS sensitivity and the three
21 labile H groups to derivatize before sublimation. Removal of salt prior to wet chemistry
22 should be considered to detect the trace targeted organics.

1 Moreover, the high concentrations in salts (2-5 M) revealed supplementary
2 constraints for space and laboratory space-like works because of mandatory prerequisites: (i)
3 non-stirred *in situ* chemical treatment and (ii) the limiting volume of DMF-DMA (30-60 μ L).
4 Despite these prerequisites, we concluded with space-like experiments a 0-20 % variation
5 compared to the reference without salts on NaCl-organic sample for pH 0 to 7 (pH measured
6 on almost all planetary system surfaces). However, for future sub-surface ocean sampling
7 experiments with a pH above 7, the sample would need to be pre-processed before
8 derivatization.

9 **4.3. Consequences for space missions**

10 As introduced in Table 1, the range in salts, minerals, and pH values are widespread
11 on Mars, Titan, Europa, or Enceladus surfaces explored and analyzed in a close future by a
12 variety of instruments aboard *ExoMars*, *Dragonfly*, and *Europa or Enceladus Landers*
13 missions, respectively. The concentrations of salts (especially in chlorides) on Titan are lower
14 compared to Mars and Europa (17 μ M on the Titan surface, 0.17-1.7 mM on Europa and
15 Enceladus surfaces, and 0-1.95 mM on Mars surface of NaCl compared to 0.59 M in
16 subsurface oceans ocean worlds taking 35 grams of NaCl in 1L) [17,26,90,91]. Thus, salts
17 will represent a lesser challenge to detect organic matter of surface samples using the DMF-
18 DMA pre-treatment. Further studies have to be conducted in different pH conditions with salt
19 mixtures close in composition to what is expected on each extraterrestrial surface (Table 1).
20 These future studies will predict the results and biases in the diversity and/or amount of
21 organics detected due to the physical and chemical constraints related to the interaction of
22 the derivatization reagents (especially DMF-DMA) with salts.

23 According to our results on standard solutions, in the presence of a low amount of salt
24 (*e.g.*, <0.3 M salt solutions (<13.8 μ mol in pure NaCl or $\text{Mg}(\text{ClO}_4)_2$) corresponding to an
25 averaged desiccated 150 mg sample rich at 0.5 wt% in salt equal to 12.9 μ mol in NaCl or 3.4
26 μ mol in $\text{Mg}(\text{ClO}_4)_2$), collecting samples in extraterrestrial environments [46,92,93] with a pH <
27 3 predicted [94–96] will likely increase the derivatization yield in future *in situ* spaceflight
28 applications (Figs. S2 in SM2 and S11, S12 in SM10). The sample extraction location choice
29 may enhance the detection limit of amino and carboxylic acids at trace levels (pmol to
30 hundreds of fmol organics in these environments) despite the presence of salts (for a
31 maximum concentration of 0.3 M – maximum found on Mars). Despite the DMF-DMA
32 efficiency, some salty Mars and ocean world locations must be analyzed by other GC-MS
33 pre-treatments due to the DMF-DMA limitations observed in the study. One of the pre-
34 treatment would be a wet-chemistry process using TMAH or TMSH (derivatizing salt reagent)
35 [97] to derivatize organic molecules in presence of high concentration in salts at neutral or
36 basic pH values.

37 We expect to find in some places on Mars, Europa, or Titan at pH 7 [92,93,98–102].
38 The derivatization yield will not be as high as the reference without salts for all organics, but
39 the DMF-DMA derivatization efficiency will not be critical for a NaCl concentration lower than
40 1 M, for instance (Figs. S11 and S12 in SM10). Only basic environments, such as the water
41 oceans of Europa and Enceladus, or cryovolcanism and plume spots on Enceladus with a pH
42 over 8 would strongly decrease the recovery yield of refractory organics [102–104]. For these
43 aqueous environments, we should consider another derivatization reagent than DMF-DMA to
44 target chiral organic compounds, such as amino acids.

45 **5. Conclusions**

1 To prepare future spaceflight missions, we studied the influence of a range of pH and
2 various salts (monovalent or divalent chlorides, sulfates, perchlorates, and carbonates)
3 present on extraterrestrial surfaces during the DMF-DMA derivatization to volatilize refractory
4 or polar organics prior to the GC-MS analyses. On *in situ* spaceflight analyses, we are
5 targeting trace-level compounds (pmol-nmol) close to the limit of detections of the
6 instruments, except on Titan where organic reactions produce mmol to mol of organics.
7 Therefore, salts will statistically significantly influence the derivatization and sublimations of
8 targeted organic traces: by organic-salt reaction interferences, increase in the
9 chromatographic baseline, the physical and ionic cage effects, the strong ionic force from the
10 salts, salting/masking effect, or even a chelation of the organics.

11 This study led to two main conclusions:

12 (i) pH < 4 solutions enhance the derivatization yield while functional groups present a
13 labile H attacked by DMF-DMA solution at pH 12 (efficient base to derivatize totally
14 carboxylic acid and primary amine groups, according to HSAB theory) will increase the pH of
15 the sample solution at minimum pH 10. However, we observed a lower derivatization yield
16 (factor over 3 of difference) compared to the reference (no salts) when the pH was higher
17 than 9. From 4 to 9, the organic recovery varies from 0 to 3 compared to the reference
18 (without salts at these pH).

19 (ii) salts present in extraterrestrial surfaces in various forms (chloride, sulfate,
20 perchlorate, etc.) and state (ionic, crystalline, complexed), will decrease the derivatization
21 efficiency and the enantiomeric separation. We also observed an increase in the baseline
22 level, which may decrease the potential detection of trace elements, as well as the
23 identification of organic molecules because of interfering mass fragments. Divalent cations
24 influence detection in higher proportion the derivatization than monovalent cations (20-70 %).

25 To limit the physical cage effect, the addition of DMF to the DMF-DMA solution for
26 spaceflight applications may be a suitable solution. Additional DMF did not increase the
27 DMF-DMA derivatization without salts; however, it may allow full dissolution of the salts, and
28 limit the crystalline cage effect of NaCl over 2 M on the reaction yield. It might also enhance
29 the detection yield of the organics as it will be attacked by salts to form ionic complex with
30 divalent salts and leave organics available for derivatization with DMF-DMA. Specifically, for
31 subsurface oceans on ocean worlds, salts are expected to be found in high amount (1‰ -
32 1% instead of 1-100 ppt on extraterrestrial surfaces). In addition, an efficient universal
33 desalting routine should be performed onboard the spaceflight landers/rovers to remove salts
34 (without interfering with organics) prior to the pyrolysis or derivatization process. If salts
35 cannot be fully removed by desalting, we estimated in this study that a solution of
36 monovalent salts should not reach 2 M, or 0.03 M for divalent salts. However, tests on Mars
37 and ocean world analogues in terms of salts abundance and diversity should be conducted
38 to confirm these concentration limits since matrix or salt mix synergic effects might increase
39 or decrease these potentially required limits.
40

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Should we use DMF-DMA pre-treatment to analyze this sample in GC-MS?

What type of (bio)molecules are we looking for?

DMF-DMA derivatization is the optimal pre-treatment to use for this sample, what cross-interaction could occur?

