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In situ organic biosignature detection techniques for space applications

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The search for life in Solar System bodies such as Mars and Ocean Worlds (e.g., Europa and Enceladus) is an ongoing and high-priority endeavor in space science, even ~ five decades after the first life detection mission at Mars performed by the twin Viking landers. However, the *in situ* detection of biosignatures remains highly challenging, both scientifically and technically. New instruments are being developed for detecting extinct or extant life on Mars and Ocean Worlds due to new technology and fabrication techniques. These instruments are becoming increasingly capable of both detecting and identifying *in situ* organic biosignatures that are indicative of life and will play a pivotal role in the search for evidence of life through robotic lander missions. This review article gives an overview of techniques used for space missions (gas chromatography, mass spectrometry, and spectroscopy), the further ongoing developments of these techniques, and ion mobility spectrometry. In addition, current developments of techniques used in the next-generation instruments for organic biosignature detection are reviewed; these include capillary electrophoresis, liquid chromatography, biosensors (primarily immunoassays), and nanopore sensing; whereas microscopy, biological assays, and isotope analysis are beyond the scope of this paper and are not covered.

KEYWORDS

astrobiology, life detection, analytical chemistry, biomarker, instruments

1 Background

Is there life elsewhere in our Solar System? This inquiry, grandiose in scope but modest in words, gives rise to an array of much-debated follow-up questions: *What is life, and if it is there, how do we detect it?* There is no firm consensus regarding the definition of life (Cleland and Chyba, 2002; Tsokolov, 2009); however, NASA's current working definition is a "*self-sustaining chemical system capable of Darwinian evolution*" (Benner, 2010). In the absence of a precise classification, searching for signs of life with Earth as a reference is a pragmatic approach, such as visual (e.g., cells and fossils) or chemical (e.g., biological macromolecules, molecular evidence of metabolism, and molecular fossils) biosignatures (Domagal-Goldman et al., 2016). Organic biosignatures can be represented by complex organic molecules at concentrations requiring biotic synthesis or relative abundance of

organic molecules not found in abiotic chemical systems (Barge et al., 2021). Consequently, key objectives are to identify and quantify the abundance and distribution of organic molecules of planetary bodies such as Mars, Europa, and Enceladus, where signatures of past or present life could exist. *In situ* robotic missions accompanied by chemical analysis instruments will play a pivotal role in this endeavor (Seaton et al., 2021)—because selecting samples with the highest probability of containing biosignatures with limited prior knowledge, maintaining pristineness, and returning them to Earth is currently not feasible. However, *in situ* detection of organic biosignatures in the space environment remains a scientific and technical challenge. One of the major technical difficulties is that space-bound scientific instruments must be of small size, low mass, and low power.

Candidate biosignatures are assessed by their reliability (differentiation between abiotic and biotic), survivability (no degradation in harsh environments), and detectability (having features that could be observed or detected; National Academies of Sciences and Medicine, 2019). Biosignatures can range from patterns observed by visual inspection to chemical patterns. *The Ladder of Life Detection* is currently perhaps the most notable attempt at summarizing potential biosignatures (Neveu et al., 2018). On the basis of life as we know it, the paper provides a framework of biosignatures, how specific they are to life, and their likelihood of indicating life. However, it is important to underline that biosignatures are contextual—for example, it is essential to consider the environment in which they are evaluated.

The technical difficulties of performing reliable analysis for the identification of chemical biosignatures beyond Earth are numerous and not easily addressed. Instruments and analysis methods need to be fit for the purpose—namely, they must be within the requirements regarding detection limits, trueness, precision, reproducibility, robustness, and ruggedness (for standardized terminology, see Thompson et al., 2002; Joint Committee for Guides in Metrology, 2008; Magnusson and Örnemark, 2014). *Robustness* is defined here as the capacity to remain unaffected by instrument or method parameter (internal factors) fluctuation, and *ruggedness* refers to external factors, such as different instruments, operational environments, reagents from different batches, or sample composition variations; however, the two terms are sometimes used interchangeably in the literature.

Instruments must be able to survive and operate in extreme extra-terrestrial environments (pressure, temperature, radiation, and mechanical stress). Furthermore, they must satisfy stringent mass, volume, and power requirements. The scientific payloads typically include various instruments such as cameras, spectrometers, radiation sensors, and meteorological sensors. As a reference, a commercial benchtop gas chromatograph (GC; e.g., Agilent 7890A GC System) weighs more than 50 kg, a typical ultra-high-performance liquid chromatograph weighs 140 kg (e.g., Waters ACQUITY UPLC system), and a typical mass spectrometer (MS) weighs about 140 kg (e.g., Thermo Fisher LTQ XL). These chemical analysis systems take up entire workbenches and cannot be used for space applications without extensive reengineering.

It is challenging to develop *in situ* chemical analysis instruments with detection limits in the low-ppb (parts-per-billion, μg/kg) range, capable of quantitative analysis, and that provide sufficient chemical information for identification. Specifications are mission-dependent. However, lower limits of detection (LOD) of 1 ppb (Mahaffy et al., 2012; Pappalardo et al., 2013) or even lower (Hand et al., 2017; MacKenzie et al., 2022b) are often required.

A broad goal of chemical analysis instruments is to identify and quantify relevant analytes that can provide insight into prebiotic chemistry and analytes that are biosignatures for extant or extinct life. The strategies of agnostic life detection are discussed elsewhere (e.g., Botta et al., 2008; McKay, 2008; Summons et al., 2008; Domagal-Goldman et al., 2016; Neveu et al., 2018; Barge et al., 2021). High-priority analyte classes often include, but are not limited to, lipids (e.g., fatty acids and hopanes), amino acids, informational polymers (e.g., DNA and RNA), and biopolymers (e.g., proteins and saccharides; Neveu et al., 2018).

Previous review papers have primarily covered chemical analysis instruments previously used in spaceflight applications. Seaton et al. (2021), Seaton et al. (2022) have summarized analytical techniques and instruments used in previous and planned spaceflight missions; including those used for organic biosignature detection, inorganic analysis, and remote sensing; but also covered instruments used in space probes in addition to lander missions. Poinot and Geffroy-Rodier (2015) have provided a detailed overview of previous spaceflight-GC instruments used for organic detection. The authors also briefly discussed ongoing developments of chip-based instruments capable of detecting organic biosignatures.

We present a detailed examination of *in situ* organic biosignature detection instruments that have been developed, are under development, or are suitable for adaptation for use in upcoming *in situ* planetary applications (Figure 1). For example, a detailed and up-to-date survey is provided of recent fluorescence spectroscopy, Raman spectroscopy, and GC-MS spaceflight instruments previously used in lander missions, along with ongoing developments of the techniques. However, we also assess the *next-generation* novel approaches for the future (i.e., beyond planned missions) detection and analysis of biosignatures; as low-mass, low-power alternatives to commonly used conventional analytical techniques in the context of future astrobiology-focused space missions to those bodies. The *next-generation techniques* have not been used at planetary bodies; some are still in the method development and validation phase and have only taken some steps towards miniaturization and spaceflight compatibility.

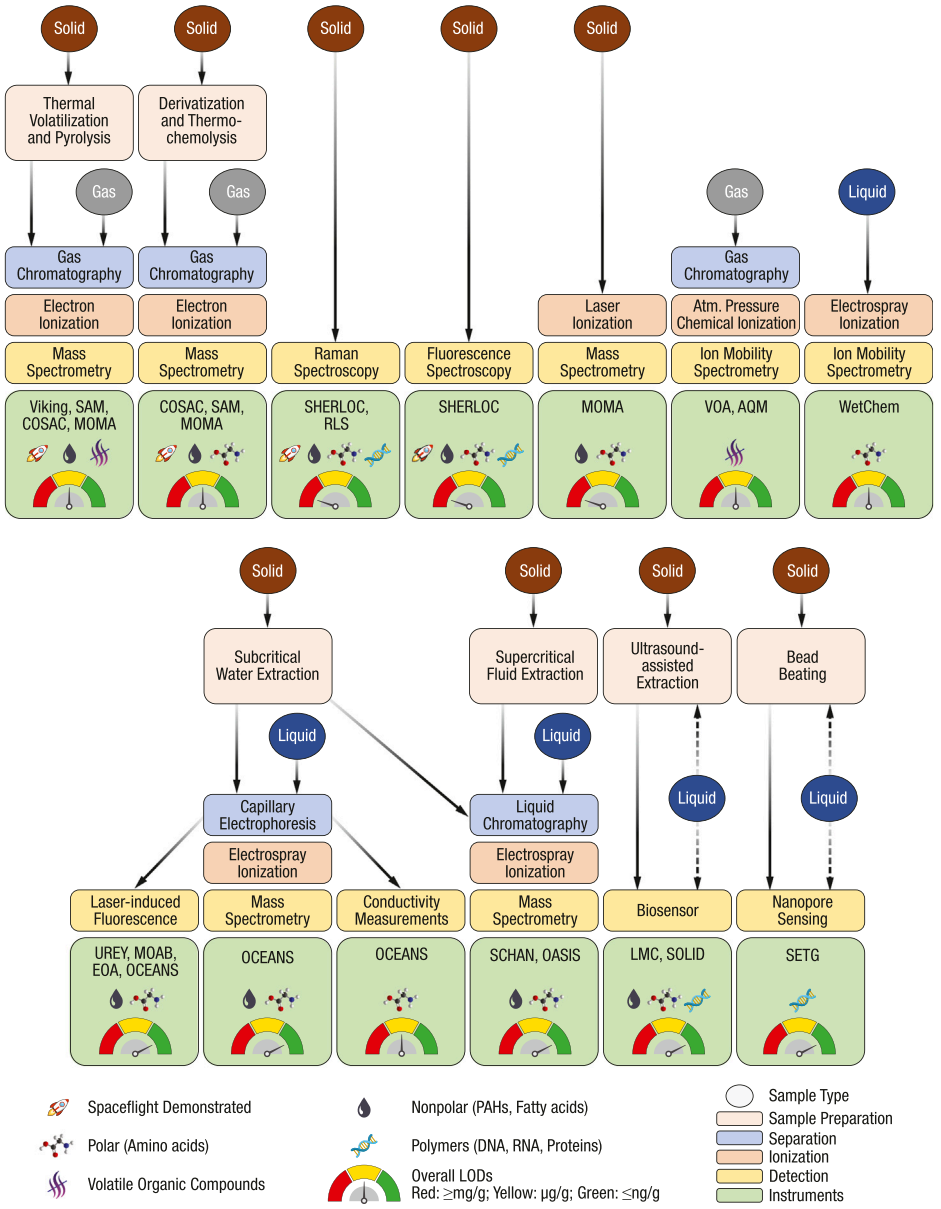

FIGURE 1
Overview of chemical analysis techniques discussed in this review. Examples of combined techniques, relevant instruments, frequently targeted analyte classes, and overall LODs are denoted with symbols. The evaluation is based on spaceflight instruments and adaptions of techniques for *in situ* space applications. The overview comes with the caveat that the target analyte classes can be extended through method development, all analytes in one class are often not detectable with a specific technique, some specific analytes may have substantially higher or lower response factors, and LODs are highly sample-dependent.

Unlike previous review papers, we focus on chemical analysis techniques for organic biosignatures rather than individual instruments that may use a combination of different techniques. More techniques are covered than previously, including the often-overlooked sample preparation step (e.g., extraction techniques). Additionally, this review assesses next-generation techniques: capillary electrophoresis, liquid chromatography, biosensors (primarily immunoassays), and nanopore sensing. We have mainly focused on chemical analysis techniques for detecting biotic organic compounds relevant to life detection in space exploration. Other avenues, although exciting but outside the scope of this review, include microscopy (e.g., Wallace et al., 2019), biological experiments (Touchette et al., 2022), and isotope analysis (Rodin et al., 2020), which provide orthogonal approaches to

life detection. This manuscript can also be seen as a stepping stone, outlining influential reviews covering each technique.

The techniques and instruments have been grouped into sections based on their generalized technology readiness level (TRL; for more details, see Smoker and Smith, 2007; Mankins, 2009). Those included in spaceflight missions are presented in Section 2. The continued developments of previously flown techniques (including ion mobility spectrometry due to long operational presence onboard the International Space Station, ISS) are presented in Section 3. Techniques without spaceflight heritage at planetary bodies are assessed in Section 4 and Section 5. Sample preparation is discussed separately to highlight its importance, which is often overlooked, because these techniques may be combined with each other and in tandem with multiple downstream analysis techniques.

All instrument developments (based on either existing or new technology) start in the laboratory environment, and some have a higher potential to evolve into spaceflight instruments than the rest. This review also reflects those intricacies by including examples of instrumentation on a clear path towards spaceflight readiness and studies demonstrating progress towards fitness for purpose, miniaturization, or spaceflight compatibility.

The path for a typical technology development from an early concept to a demonstrated spaceflight qualification is long (typically >10 years) and expensive. Costs and time can be saved by utilizing instruments and parts with spaceflight heritage. However, each newly developed technique and instrument typically start with studies in laboratory environments. Once a technique has been established as fit for purpose, it is followed by miniaturization, adaptation to the relevant environment (e.g., vacuum, low temperature, and high radiation), and then the final instrument is designed and built. The fundamental knowledge of the techniques is often fragmented; laboratory studies are essential for continuously evaluating what science can be conducted with *in situ* measurements. Consequently, many studies and reported instruments that are in development are, in fact, not flight-like—as is the case in this review. Nevertheless, they still help us understand each chemical analysis technique's performance, limitations, and opportunities.

Instruments determined as fit for purpose, miniaturized, and demonstrated in relevant environments may undergo substantial redesign after being selected for a planned mission. These redesigns are driven by, for instance, spacecraft restrictions or reduced budgets. For example, the GC-based instrument onboard the Curiosity rover was first under development with liquid extraction of solid samples prior to derivatization (Buch et al., 2003); however, a one-pot derivatization approach was finally used (Mahaffy et al., 2012; Stalport et al., 2012), due to budget constraints. Hence, it is not always possible to accurately predict the performance of the final spaceflight instrument while it is still in development (i.e., Sections 3–5 in this review). Finally, many in-development instruments have been given an acronym—this is not an indicator that it is close to spaceflight-ready, unless stated otherwise.

2 Overview of instruments used for planetary missions

Space instrumentation has advanced substantially due to research over the last few decades in engineering and manufacturing techniques. As a result, a vast and diverse number of instruments utilize a wide variety of techniques and methods that are relevant for the *in situ* detection of biosignatures in planetary missions. Comparable performance characteristics in space instrumentation with their laboratory counterparts have been progressively achieved since the 70s' in terms of high measurement sensitivity, selectivity, high precision, and resolving power while providing small instrument size, low mass, and low power required by space missions. Among these instruments, traditionally, MS-based instruments have been the most recurrent for *in situ* planetary exploration mission applications. GC, often accompanied by MS, has also been commonly used for organic detection. In this section, we will review applied techniques in planetary exploration missions (including those onboard the Rosalind Franklin rover, although they have not been flown at the time this paper was written): gas chromatography, mass spectroscopy, and optical spectroscopic techniques.

2.1 Gas chromatography

One of the most commonly used techniques for organic detection is GC (for a summary of instruments and validation data, see Poinot and Geffroy-Rodier, 2015). Table 1 summarizes GC-onboard space missions (Akapo et al., 1999; Johnson et al., 2012; Szopa et al., 2017). The first successful planetary lander mission equipped with GC involved the twin Viking landers that landed on Mars in 1976 (Rushneck et al., 1978). At the time, GC was the only chemical separation technique considered mature; alternatives such as capillary electrophoresis (Lunte and Radzik, 1996), and high-performance liquid chromatography (Snyder, 1997), did not gain mainstream popularity until the mid-80s and 90s'. Still today, GC provides unmatched peak capacity, which is the number representing how many analytes theoretically can be separated in one experiment.

The basic principle of chromatography (here we discuss GC, and liquid chromatography is discussed in Section 5.2) is based on the distribution of analytes between a mobile phase (called carrier gas in GC) and a stationary phase (an immobile material inside of a column). The sample is introduced into a stream of carrier gas that passes through the GC column; analytes that interact more with the stationary phase (e.g., a thin film coated on the walls of a GC column) are retained more strongly and exit

TABLE 1 GC onboard space missions (Akapo et al., 1999; Johnson et al., 2012; Szopa et al., 2017). The references are not a complete compilation and should be considered a starting point. The following abbreviations are used: thermal conductivity detector (TCD), neon ionization detector (NID), and electron-capture detector (ECD).

Mission/ Probe	Target body	Launch/arrival years	Sample type	Detectors	Selection of relevant references
Viking 1 and 2	Mars	1975/1976	Atmosphere Soil	TCD MS (magnetic sector, m/z ≤ 220)	Anderson et al. (1972), Biemann et al. (1976; 1977), Rushneck et al. (1978)
Pioneer	Venus	1978/1978	Atmosphere	TCD	Oyama et al. (1979; 1980)
Venera 11 and 12	Venus	1978/1978	Atmosphere	NID	Gelman et al. (1979)
Venera 13 and 14	Venus	1981/1982	Atmosphere	NID ECD	Mukhin et al. (1982)
Vega 1 and 2	Venus	1984/1985	Atmosphere[b]	TCD ECD	Mukhin et al. (1987)
Cassini-Huygens	Titan	1997/2004	Atmosphere[b]	MS (quadrupole, m/z ≤ 141)	Israël et al. (2005); Niemann et al. (2010)
Beagle 2	Mars	2003/2003*	Atmosphere Soil[a]	MS (magnetic sector, m/z range N/A)	Sims et al. (1999; 2000)
Rosetta/Philae	Comet 67P	2005/2013	Atmosphere Soil[a]	TCD MS (time-of-flight, m/z ≤ 1,500)	Rosenbauer et al. (1999); Goesmann et al. (2014)
Phobos-Grunt	Phobos	2011/ -[c]	Soil[a]	MS (monopole, m/z ≤ 44)	Sheretov et al. (2000); Zelenyi and Zakharov (2012)
Curiosity	Mars	2011/2012	Atmosphere Soil	TCD MS (quadrupole, m/z ≤ 535)	Meunier et al. (2007); Mahaffy et al. (2012); Stalport et al. (2012)
Rosalind Franklin	Mars	T.B.D.	Atmosphere Soil	TCD MS (linear ion trap, m/z ≤ 1,000)	Siljestrom et al. (2014); Goesmann et al. (2017)

[a]Did not perform analysis.
[b]Included aerosol collection and pyrolysis.
[c]Failed mission.
N/A, Not available.
T.B.D. To be determined.

(elutes) the column later than less-retained analytes. The exiting carrier gas (effluent), along with eluted analytes, are continuously transported to a detector. The retention can be shortened by increasing the temperature because it increases the analyte's vapor pressure, hence, increasing the relative time spent in the carrier gas. Analytes in a mixture, like in a natural sample, are separated based on their interaction with the stationary phase—providing different retention times that can be used for identifying unknown analytes, often together with mass spectra.

Two main requirements are imposed on analytes that are analyzed by GC. Firstly, they have to be sufficiently volatile to at least partially partition into the gas phase at the separation temperature (typically <400°C), and secondly, they need to be sufficiently thermally stable not to decompose during their migration through the capillary chromatography column. The upper temperature is limited by the stability of the stationary phase, which has improved over the decades. For example, the maximum temperature of the Viking GC was 200°C (Biemann et al., 1976). Utilizing a GC column close to its upper-temperature limit will cause so-called column bleed, where the stationary phase hydrolyses and contributes to the background signal and consequently diminishes the lower detection limits of less volatile analytes. Finally, essentially all GC methods use temperature programming by increasing the temperature over time (increases analyte vapor pressure) to allow separation of both volatile (starting at low temperatures for volatile analytes to partition into the stationary phase) and less volatile analytes (high enough temperature for them to partition into the carrier gas). Temperature-programmed GC methods require cooling down to the initial conditions before analyzing the subsequent sample, which is not trivial considering the ample thermal insulation of payload instruments.

Chemical analysis of the atmospheric composition has been rather straightforward because it only requires an atmosphere inlet port and a dust filter. Analysis of the atmosphere is also readily done with GC, as has been demonstrated by several missions: multiple Venus probes (Venera 11–14 and the multiprobe Pioneer Venus 2), Huygens (Titan), and Philae (Comet 67P). Huygens detected evaporated methane, ethane, acetylene, cyanogen, and carbon dioxide at the surface of Titan (Niemann et al., 2010). Philae detected several alcohols,

carbonyls, amines, nitriles, amides, and isocyanates in the dust and vapor stirred up by the lander (Goesmann et al., 2015).

The history of analysis of solid samples with GC has been more intriguing. Viking is the only landed mission so far that has carried dedicated life-detection instruments. The twin landers included three experiments that added substrate to the Martian regolith, which studied gas exchange (GEX), labeled release (LR), and pyrolytic release (PR; Klein et al., 1976; klein, 1978). The GEX experiment was the only analysis that involved GC.

In the GEX experiment, the gas release was determined by GC after exposure first to humidity only, followed by water, and then to immersion in a nutrient solution (Oyama, 1972; oyama et al., 1976; oyama and berdahl, 1977). The GEX experiment was primarily designed to detect heterotrophic activity in an atmosphere of mainly CO_2. The LR experiment added ^{14}C-labeled nutrients to the soil, then determined evolved radioactive gases released by potential organisms by a radiation counter (Levin, 1972; Levin and Straat, 1977). The PR experiment (also known as the carbon assimilation experiment) incubated soil with ^{14}CO and $^{14}CO_2$, followed by pyrolysis (635°C), trap and release (to separate CO and CO_2 from organic compounds), and determination of released radioactive analytes from the soil by a radiation counter (Horowitz et al., 1972; Horowitz et al., 1977).

The results and methods of these biological experiments have been put into a broader life-detection context by Neveu et al. (2018), and references therein. In summary, no conclusive results were obtained from the experiments. Surface chemistry most likely explained the positive results; however, biological activity could not be entirely rejected (Levin and Straat, 2016; Neveu et al., 2018).

Although the GC analysis of untreated soil was not classified as a life detection experiment, detected organic compounds could have served as a confirmation of life. In these experiments, the soil was stepwise heated up to 50, 200, 350, or 500°C to either volatilize analytes or produce pyrolysates of non-volatile compounds that subsequently could be separated by GC (Biemann et al., 1977).

No *native* organic compounds were detected in the soil with an overall detection limit on the order of ppb at either of the two landing sites (Biemann et al., 1977; Biemann and lavoie, 1979). The absence of detected native organic molecules by the Viking landers has been a subject of much debate (Benner et al., 2000; Glavin et al., 2001; Navarro-González et al., 2006; Biemann, 2007). Due to the rigorous validation and verification experiments performed with the Viking engineering breadboard and the detection of cleaning solvents during its flight to the red planet, it is without a doubt that the instrument performed as intended (Biemann et al., 1976; biemann et al., 1977; Biemann and lavoie, 1979). However, one of the major findings of the Phoenix lander (launched in 2007) was that the surface of Mars is rich in perchlorates (ClO_4; Hecht et al., 2009). The impact of the discovered perchlorates on the past interpretations have indeed also been a subject of great debate (Navarro-González et al., 2010; Biemann and Bada, 2011; Navarro-González and McKay, 2011). Later findings derived from data generated by the Sample Analysis at Mars (SAM, see Figure 2) instrument on the Mars Science Laboratory (MSL) of the Curiosity rover, laboratory experiments, and further re-analysis of the Viking data have provided sound evidence that Viking indirectly did detect organic molecules. The native organic molecules likely reacted during the thermal volatilization (TV)/pyrolysis (Py) process to form chlorinated organic molecules, some of which were previously and incorrectly identified as cleaning contaminants (Miller et al., 2016; Guzman et al., 2018; Szopa et al., 2020). Over the last few years, the SAM GC-MS has detected chlorobenzene (Freissinet et al., 2015), isomers of dichlorobenzene (Szopa et al., 2020), and derivatized (derivatization methods are described later) benzoic acid, ammonia, and potentially phosphoric acid and phenol (Millan et al., 2021). All chlorinated compounds detected at Mars so far have been summarized elsewhere (**Table 3** in He et al., 2021a).

The Rosetta mission to Comet 67P, carrying the lander Philae equipped with the Cometary Sampling and Composition (COSAC) system, was the second mission that attempted to analyze solid samples with GC on a planetary body. Unfortunately, the Philae lander never acquired any solid sample due to issues during the landing sequence because the lander finally settled on its side. Although no GC analysis was performed, several important developments were made that were later adopted by both SAM onboard MSL and the Mars Organic Molecule Analyzer (MOMA) onboard the European Space Agency's (ESA) Rosalind Franklin rover (previously known as the ExoMars rover).

GC is only viable for volatile and heat-stable analytes, which do not include, for example, amino acids, nucleobases, and fatty acids. These important organic biosignatures can be analyzed with GC by first performing derivatization, which converts them into volatile and more stable products; or using alternative analysis techniques described later.

All three instruments (COSAC, SAM, and MOMA) feature a so-called one-pot wet chemistry processing in addition to conventional pyrolysis, where the solid sample and the solution containing the derivatization agents are mixed without any further addition of solutions. Three derivatization reagents have been employed, namely N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), N,N-dimethylformamide dimethyl acetal (DMF-DMA), and tetramethylammonium hydroxide (TMAH). COSAC carried DMF-DMA (Goesmann et al., 2014), SAM had cups containing either MTBSTFA or TMAH (Mahaffy et al., 2012), and MOMA was designed to have the option of using any of the three derivatization reagents (Goesmann et al., 2017).

MTBSTFA is a very reactive silyl reagent, usually facilitated by DMF that acts as a proton acceptor, which enables low LODs;

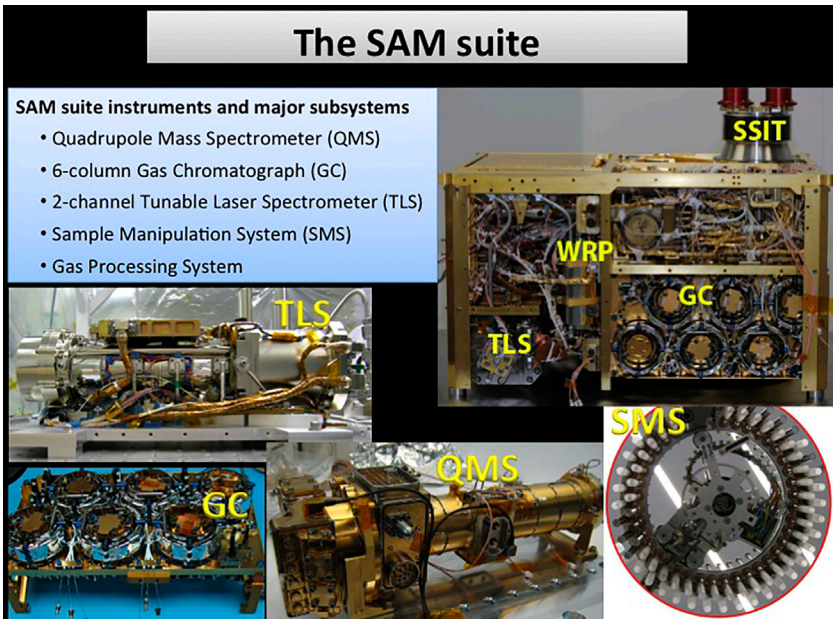

FIGURE 2
Overview of the Sample Analysis at Mars suite onboard the Curiosity rover. The suite includes a tunable laser spectrometer (TLS), a gas chromatograph (GC) with six different columns, a quadrupole mass spectrometer (QMS), a solid sample inlet tube (SSIT), sample manipulation system (SMS), and wide-range pumps (WRP; Mahaffy et al., 2012). Images courtesy of NASA/JPL-Caltech.

for example, pure amino acids can be detected between 0.3 μM and 50 μM after derivatization, followed by direct injected into a GC (Rodier et al., 2001). The derivatization reagent is suitable for carboxylic acids, amino acids, nucleobases, amines, and alcohols. However, MTBSTFA is prone to react with water, which can reduce the product yield and create a strong interfering background. The derivatization methodology is not rugged towards samples containing hydrated minerals, inorganic anions, or iron oxides; these inhibitors may render analytes completely undetectable (Stalport et al., 2012). Even so, He et al. (2021a) have extensively studied the products of MTBSTFA/DMF formed during flash pyrolysis and concluded that the derivatization reagents remained stable in the presence of calcium perchlorate during slow SAM-like temperature ramping up to 850°C. Furthermore, MTBSTFA does not allow for chiral analysis of amino acids. Derivatized benzoic acid and ammonia were detected at Mars with this methodology (Millan et al., 2021).

DMF-DMA is a methylation reagent less reactive than MTBSTFA and targets fatty acids, primary amines, and amino acids. Higher LODs are generally obtained with DMF-DMA than MTBSTFA in matrix-free samples. Most importantly, enantiomeric amino acids derivatized with DMF-DMA can be separated on chiral stationary phases, unlike MTBSTFA. It has been demonstrated that 9 out of 19 proteogenic and enantiomeric amino acids can be separated using this methodology (Freissinet et al., 2010).

TMAH is not only a methylation agent, but also facilitates hydrolysis of macromolecules at elevated temperatures, also known as thermochemolysis. The reagent is usually dissolved in methanol. TMAH is more rugged regarding sample matrix composition, and its applications have been demonstrated on a wide variety of sample types (Geffroy-Rodier et al., 2009; Williams et al., 2019). Nucleobases can also be detected with a TMAH-based methodology (He et al., 2019). The applications and reaction mechanisms of TMAH thermochemolysis have been extensively reviewed by He et al. (2020). Perhaps most importantly, TMAH appears to be better suited for samples containing perchlorates. He et al. (2021b) demonstrated that up to 5 wt% of calcium perchlorate did not significantly impact the recovery of free fatty acids at a concentration of 300 ppb. *In situ* analysis with TMAH at Mars has been performed; however, data analysis is, at this moment, ongoing (Roach et al., 2021).

Limited validation of the one-pot methodologies for COSAC, SAM, or MOMA has been published. Nonetheless, a multitude of demonstration and qualitative studies have been performed with flight-like sample chambers coupled to commercial GC-MS systems where pyrolysis, derivatization, or thermochemolysis were applied (e.g., Lewis et al., 2018; Reinhardt et al., 2020; He et al., 2021b). Whereas the one-pot derivatization methodology is versatile, it also adds risk. SAM demonstrated this, when one of the cups leaked, and the derivatization reagent formed several products, which adversely affected data

interpretation and detection limits (Glavin et al., 2013; Leshin et al., 2013).

Poinot and Geffroy-Rodier (2015) summarized publicly available validation data and pointed out that quantitative comparison of these instruments is impossible due to the lack of validation data. It has been claimed that MOMA is capable of detecting one or more amino acids with LODs of 80 ppb and 1.3 ppm with MTBSTFA or DMF-DMA, respectively (Goesmann et al., 2017). Two validation studies have been conducted using a laboratory pilot resembling SAM and MOMA instrumentation (Meunier et al., 2007; David et al., 2016). The earlier study showed an instrument mean LOD of 10 pmol of 28 organic acids derivatized with MTBSTFA, which were injected into the GC-MS system. However, the reactor's transfer yield in on-line experiments was generally low (<5.5%), and the method LODs were not determined. The second study demonstrated LODs of ~1 nmol for amino acids with off-line derivatization with MTBSTFA and where the derivatized amino acids were subsequently spiked onto 50 mg of solid sample (David et al., 2016). This corresponds to approximately 1.5–3.6 ppm LODs; however, the experiments did not consider matrix effects in the derivatization step. Comparisons between laboratory instruments and engineering test units (nearly equivalent spaceflight instruments) are rare. In the case of MOMA, the separation efficiency is substantially lower (broader peaks) due to a relatively slow heating (10–15 s) of the injection trap, compared to the conventional pre-heated injection port used on commercial instruments (Guzman et al., 2020). The same authors also extensively characterized the GC columns used in MOMA and demonstrated the vast number of analytes (e.g., noble gases, alkanes, alcohols, carboxylic acid, and amino acids) that could be separated with either of the four columns.

Despite the mentioned shortcomings of the current implementation of thermal volatilization and pyrolysis GC-MS with or without derivatization agents, the technique provides the ability to detect a wide range of relevant organic biosignatures from a variety of samples (Bishop et al., 2013; Reinhardt et al., 2020). It provides these capabilities with relatively low instrument complexity. On the basis of the successful deployments of GC-MS on Mars, it is safe to say that GC columns do not significantly degrade in the high radiation environment. Common capillary columns and consumables have been tested before and after radiation, corresponding to the levels expected for missions to the Jovian moons, without any substantial reduction in performance (Freissinet et al., 2016; Freissinet et al., 2019). Nonetheless, future missions will likely require lower LODs of biosignatures and better ruggedness regarding sample composition than what current GC-MS implementation can provide.

2.2 Mass spectroscopy

With MS-based techniques, it is possible to obtain information such as composition, atomic or molecular weight, and molecular structure of the constituents in a sample being analyzed, which is difficult to obtain by other means. An excellent general introduction to MS is given by de Hoffmann and Stroobant (2013). Mass spectrometers have a wide variety of configurations; however, they all have four essential components. These four components are the sample inlet, the ionization source, the mass analyzer, and the detector. Some instruments combine the sample inlet and the ionization source, whereas others combine the mass analyzer and the detector. The development for space applications over the last few decades has focused on minimizing power and mass while improving the mass resolution (m/Δm, Δm being at full width at half maximum, FWHM) of these instruments. These developments with emphasis on space applications have been extensively reviewed and covered by others (Palmer and Limero, 2001; Johnson et al., 2012; Snyder et al., 2016; Ren et al., 2018; Arevalo et al., 2020; Chou et al., 2021). The mass analyzer and the detector (often referred to as the back-end) function relatively independently of the front-end, which may involve a GC or a laser desorption and ionization (LDI) instrument. However, the optimum choice of ionization source depends greatly on the type of incoming sample and the environment in which it operates. The basics of various ionization techniques have been covered by Siuzdak (2005).

To date, mass spectrometry on landed robotic missions to other Solar System bodies has exclusively been used to analyze incoming gas streams such as direct atmospheric measurements, evolved gases from pyrolysis, and effluent from GC (Johnson et al., 2012). Electron ionization (EI) has therefore been the natural choice of ionization; because it provides high sensitivity, robustness, and capability of ionizing most analytes. EI is classified as a hard ionization technique that typically fragments the analyte. Hence, EI mass spectra tend to be rather complicated, but they also provide a unique opportunity for identification, as opposed to soft ionization techniques. EI provides reproducible spectra which can be matched with databases for identification, which is not always the case for other ionization techniques. Figure 3 illustrates all MS instruments utilized for spaceflight missions. Table 1 provides the mass ranges of MS instruments on the Vikings, COSAC, SAM, and MOMA.

The earliest MS instruments used for spaceflight were based on magnetic sector mass spectrometry. This was the preferred technique at the time as it was less dependent on electronic circuits (simple design) and did not require high power levels (Anderson et al., 1972); for example, for a radiofrequency generator used in the quadrupole mass analyzer. However, the magnetic sector MS is not ideal for scanning at a high m/z (mass to charge ratio) at a rate compatible with modern-day separation techniques, and its performance is greatly affected by miniaturization (Ren et al., 2018). Over the last few decades, single quadrupoles, time-of-flight (ToF), and ion traps (IT) have been the main techniques utilized. ToF and IT are the focus of

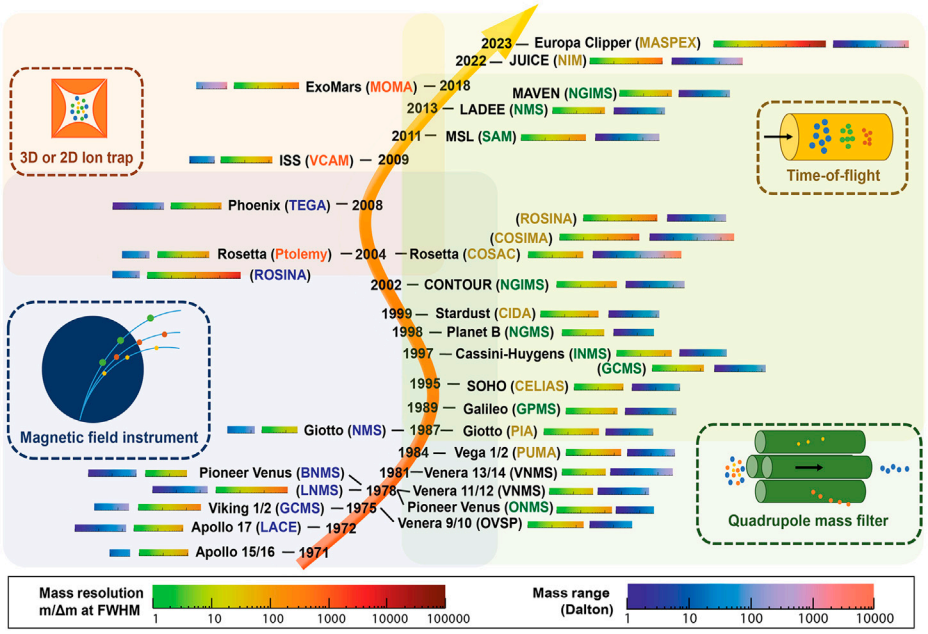

FIGURE 3
An illustrated summary of MS instruments utilized for space flight missions and their performance metrics. Reprinted by permission from Arevalo et al. (2020). Copyright 2020 Wiley.

ongoing developments, which are discussed below. The main reason for the change in focus is that the quadrupole mass analyzer suffers from reduced ion transmission and resolution when it is further miniaturized. In miniaturized versions, the assembly is critical, with positioning tolerances in the micrometer scale of the four rods that act as the ion filter (Ren et al., 2018).

The sensitivity and signal-to-noise ratio of an MS instrument cannot be determined accurately without considering the ionization and ion transmission efficiencies. Therefore, it is difficult to compare these instruments' detection limits. Validation data are, unfortunately, very scarce. Nevertheless, tremendous advancements have been made on several fronts, and development is still ongoing (see Section 3.2). The GC-MS system onboard the Viking landers weighed 25 kg and only had a scan rate of one spectrum per 10.24 s. In comparison, the MS onboard MOMA weighs 7.5 kg (11.5 kg including GC and LDI) and has a scan rate of several Hz (Goesmann et al., 2017).

2.3 Optical spectroscopic techniques

Non-invasive remote or standoff spectroscopic techniques have gained importance over the last few decades. Their role has mostly been focused on elemental composition and mineralogy analysis. Although these instruments' primary purpose was not detecting organic biosignatures, the development of the optical spectroscopic techniques has paved the way for more capable detection instruments. Standoff sensing provides unique possibilities for identifying samples or areas of interest for further chemical analysis with other techniques (e.g., GC-MS) where the total number of analyses is limited. A brief overview of existing space flight instruments is given here, and ongoing developments are discussed in detail later (see Section 3.1).

Virtually all Mars landers have employed optical spectroscopic techniques for analyzing regolith, with X-ray spectroscopy for elemental analysis being the most common (Inza and Lopez-Reyes, 2020). In fact, the most current and future planned landers have an increased number of spectroscopic instruments in their payloads, compared to past missions. The reader is referred to payload summaries of each mission: Curiosity rover (MSL; Grotzinger et al., 2012), Perseverance rover (Mars 2020; Farley et al., 2020), Rosalind Franklin rover (Vago et al., 2017), and the Tianwen-1 lander (Zou et al., 2021).

The combination of spectroscopic techniques employed by instruments onboard the Perseverance rover can be used to characterize and identify samples of interest, which has been demonstrated at a Mars analog field site in the Mojave Desert (see Figure 4; Martin et al., 2020), and in the laboratory with microbial mats from the ~3.42 Ga Buck Reef Chert in South Africa (Hickman-Lewis et al., 2022). Two remote sensing instruments, Mastcam-Z (Bell et al., 2021) and SuperCam (Wiens et al., 2021), and two microscopic proximity

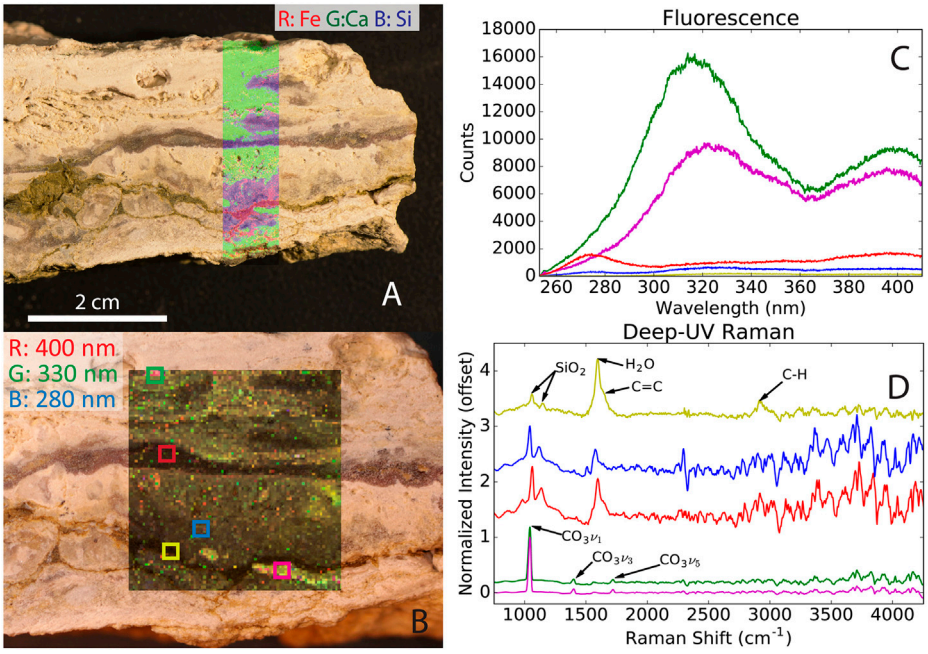

FIGURE 4
Stand-off measurements of a calcite-bearing sample at a Mars analog field site in the Mojave Desert with the techniques used by PIXL [X-ray spectroscopy, **(A)**] and SHERLOC [UV fluorescence and Raman spectroscopy, **(B–D)**]. **(A)** Overlay of the elemental abundance of Fe, Ca, and Si. **(B)** Fluorescence scan map, **(C)** averaged fluorescence spectra of the selected areas, and **(D)** averaged Raman spectra of the selected areas. The figure highlights that stand-off spectroscopy can identify interesting samples and determine mineralogy. Reprinted from Martin et al. (2020).

instruments, Scanning for Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC; Beegle et al., 2015) and Planetary Instrument for X-ray Lithochemistry (PIXL; Allwood et al., 2020), are a part of the Mars 2020 rover mission (Perseverance) instrument suite (Farley et al., 2020). The instrument suite will be used to identify samples of interest for caching for potential future return to Earth. Similarly, the Raman Laser Spectrometer (RLS, 532 nm) onboard the Rosalind Franklin rover will characterize the mineralogy of collected powdered samples, with the possibility of detecting organic compounds (Lopez-Reyes et al., 2013; Rull et al., 2017; Veneranda et al., 2020).

Fluorescence and Raman spectroscopy are the most relevant techniques for direct *in situ* measurements of organic biosignatures. In the case of fluorescence, analytes are excited (timescale of femtoseconds) by the photons from a light source (e.g., a laser) through absorption. The excited analyte then undergoes internal conversion and vibrational relaxation (picoseconds), followed by the emission of a photon (fluorescence, nanoseconds) at a lower energy (longer wavelength) than the initial excitation energy. Fluorescence competes with other processes to reach the ground state, which is molecule dependent; hence, providing different quantum yields. On the other hand, Raman spectroscopy does not rely on absorbed light—it is based on the scattering of monochromatic light. Most scattered light is at the same wavelength as the source (called Rayleigh scatter); however, a small amount (10^{-8} to 10^{-12}) of the incident light will be scattered at lower or higher energies (called Raman scattering). The transfer of energy from the photon to the analyte is called the Stokes-Raman effect, and the transfer of energy from the analyte is called the anti-Stokes effect, which is what is typically measured. The peaks in the Raman spectrum correspond to specific molecular bond vibrations, such as C-C, C=C, and O-H.

Among the spectroscopy instruments, SHERLOC is specifically geared to provide information about organic biosignatures. SHERLOC combines deep-UV (DUV, <250 nm excitation) native fluorescence (250–360 nm emission) and resonance Raman spectroscopy (810 to >3,600 cm^{-1}) with a high-resolution imager (Beegle et al., 2015). Combining the two techniques comes naturally because aromatic compounds excited by UV generate both fluorescence and resonantly enhanced Raman scattering. DUV fluorescence spectroscopy provides general LODs of sub-ppm levels for aromatic compounds and LODs of <100 ppm for aliphatic compounds with a spatial resolution of 30 µm (Farley et al., 2020). Fluorescence typically offers minimal data to facilitate the identification of organic compounds. Raman spectroscopy is better suited for deconvolution and identification; however, it

has much poorer LODs (Abbey et al., 2017). For example, 5 wt% adenosine 5′-monophosphate spiked in clay samples containing 1 wt% magnesium perchlorate was near the LOD when analyzed by Raman spectroscopy (Razzell Hollis et al., 2021).

An essential benefit of using DUV excitation for fluorescence and Raman measurements is that it reduces background autofluorescence from minerals (Bhartia et al., 2010; Abbey et al., 2017). The Raman region is also free of fluorescence from organics by DUV (Asher and Johnson, 1984). However, there is a limited capability of differentiating carbonaceous material from abiotic and biotic sources with Raman spectroscopy, particularly through the commonly used D (~1,350 cm^{-1}) and G (~1,580 cm^{-1}) band parameters (Bower et al., 2013). Even though the resolving power and the ability of analyte identification are limited, Raman and fluorescence spectroscopy (preferably combined) remain capable of identifying samples containing preserved fossilized, complex, macromolecular compounds embedded in minerals (Shkolyar et al., 2018, and references therein).

Although these spectroscopic techniques may not be ideally suited for detecting and identifying organic biosignatures, they are useful standoff sensing tools that facilitate sample selection; either for instruments limited by the number of samples they can analyze, or for potential sample return and analysis in terrestrial laboratories.

3 Current developments in organic biosignature analysis techniques

In organic-detection space missions, the main driving forces are minimizing measurement error, LODs, power, mass, volume, and risk. Lessons learned from the previous Mars missions also suggest that techniques and methods must be less affected by the sample composition. This is demonstrated by the chemical degradation of organics at elevated temperatures in the presence of perchlorates and the inhibitory matrix effects of minerals during derivatization (Navarro-González et al., 2006; Stalport et al., 2012). Furthermore, current instrumentation is not fit for the purpose in regards to targeted LODs (ca. 1 ppb) of key organic biosignatures such as fatty acids, amino acids, and informational polymers (i.e., DNA and RNA; Mahaffy et al., 2012; Pappalardo et al., 2013; Hand et al., 2017; Neveu et al., 2018). On the basis of the summary of previously used *in situ* organic detection techniques, further development is needed in order to detect a greater span of organic biosignatures at lower concentrations than what is currently possible.

Currently, no technique can universally detect all high-priority target analyte classes at desired LODs and with satisfactory ruggedness, trueness, and precision. Each mission has its scientific scope and engineering constraints; the sample type can range from dry solids to completely liquid. It is essential to underline that current developments for *in situ* organic biosignature analysis are primarily infusions of techniques utilized in larger sectors such as the biomedical industry. The main challenge is adopting these techniques for space flight applications.

3.1 Recent advances in gas chromatography

Although GC has been a part of instrument suites on several missions, the development of primarily system miniaturization is still ongoing. More recent attention has focused on scaling down the size by utilizing chip-based platforms as opposed to traditional capillary columns. The Spacecraft Atmosphere Monitor (S.A.M.) onboard the ISS is perhaps currently the most notable instrument, which consists of a very compact chip-based GC and a compact quadrupole ion trap MS (Schowalter et al., 2019). Of note here, S.A.M. should not be confused with the SAM instrument suite onboard the Curiosity rover on Mars. The instrument onboard the ISS weighs only 10 kg and is capable of measuring major air constituents and trace concentrations of VOCs. Other developments in chip-based GC instruments are in progress (Figure 5; Blase et al., 2020b; Blase et al., 2022).

So far, applications of chip-GC have mainly been restricted to the analysis of VOCs due to the limited upper-temperature range (~200°C). For example, silicon, glass, and adhesives that are frequently used for fabrication have mismatching thermal expansion coefficients, which leads to cracking and leaking after thermal cycling (Ghosh et al., 2018). GC methods for analyzing methylated fatty acids and polycyclic aromatic hydrocarbons (PAHs) almost always employ temperatures above 200°C, or even 300°C. There is a vast literature covering fundamental research to improve the upper-temperature limit of microchip GC (Ghosh et al., 2018). Among various approaches, stainless steel microchip GC columns appear particularly promising due to their high thermal conductivity and compatibility with high operating temperatures up to 350°C (Ghosh et al., 2019). Nonetheless, a chip-GC manufactured out of etched silicon substrate and bonded with anodized Pyrex glass was able to operate up to 300°C; with the ability to separate alkanes and derivatized fatty acids up to C22 (Blase et al., 2022). The authors demonstrated that the chip-GC hyphenated with an MS provided LODs between 4 µM and 43 µM (4–43 pmol), a peak capacity of 124, and retention variations of <1% RSD.

Shrinking GC from traditional capillary columns to chip-based separation also comes at the expense of chromatographic efficiency. Chip-based GC suffers from a less homogeneous stationary phase coating due to the square geometry of the flow channels as compared to the cylindrical geometry of conventional capillary columns. The excessive accumulation of stationary phase in the corners, also known as pooling, creates an

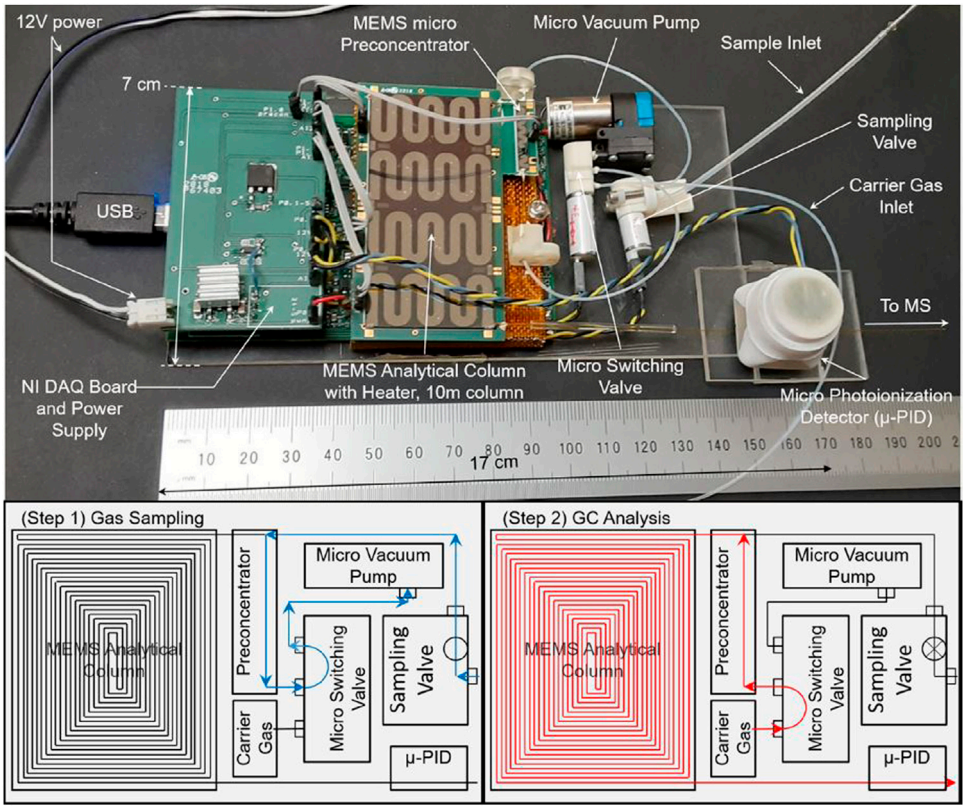

FIGURE 5
Top image shows a micro-electromechanical system (MEMS) GC and its major components. The bottom figures illustrate the operation, with first the loading step followed by the analysis step. Adapted with permission from Blase et al. (2020b). Copyright 2020 American Chemical Society.

uneven film thickness which contributes to peak broadening (Wang et al., 2014). There is currently a multitude of ongoing research efforts to improve the separation efficiency by optimizing the stationary phase coating technique, column architecture, and layout. For example, serpentine, circular-spiral, and square-spiral column layouts are the most frequently utilized designs (Regmi and Agah, 2018). The channel width and the coating thickness play an important role in the trade-off between separation efficiency and sample capacity. Narrow channels with a thin coating provide higher separation capacity but limited sample capacity. Multi-capillary columns and semi-packed columns (pillar array columns) have been developed to increase the sample capacity by increasing the relative volume of the stationary phase; yet, they still have a thin film of the stationary phase to maintain the separation efficiency, which consequently keeps the mass transfer resistance low (Zareian-Jahromi et al., 2008; Jespers et al., 2017). Furthermore, micro-columns often exhibit tailing of polar analytes compared to conventional capillary columns due to surface adsorption (Regmi et al., 2018).

Comprehensive two-dimensional microscale GC (μGC × μGC) is currently being developed for future planetary missions (Blase et al., 2020a; Blase et al., 2022). Under ideal conditions, the peak capacity of GC × GC is almost an order of magnitude higher than conventional GC for the equivalent analysis time (Klee et al., 2015). In brief, GC × GC is a multidimensional separation technique achieved by coupling two columns with different stationary phases and where the effluent of the first column is cyclically trapped, refocused, and injected onto the second column. The technique has gained immense acceptance in petroleum analysis, where the first dimension typically separates based on volatility and the second dimension primarily separates based on polarity, facilitating analysis of samples even when all analytes are not entirely resolved (Abrahamsson et al., 2017; Prebihalo et al., 2018). The interface between the first column and the second column is called modulation. Conventional modulation is typically achieved by either thermal modulation or flow modulation. Thermal modulation is accomplished by cryogenic cooling through a jet of liquid nitrogen to trap analytes in the column and then rapid heating to elute the analytes into the second column. Flow modulation typically uses sample loops and valves to sample the effluent from the first column and reintroduce the analytes to the second column.

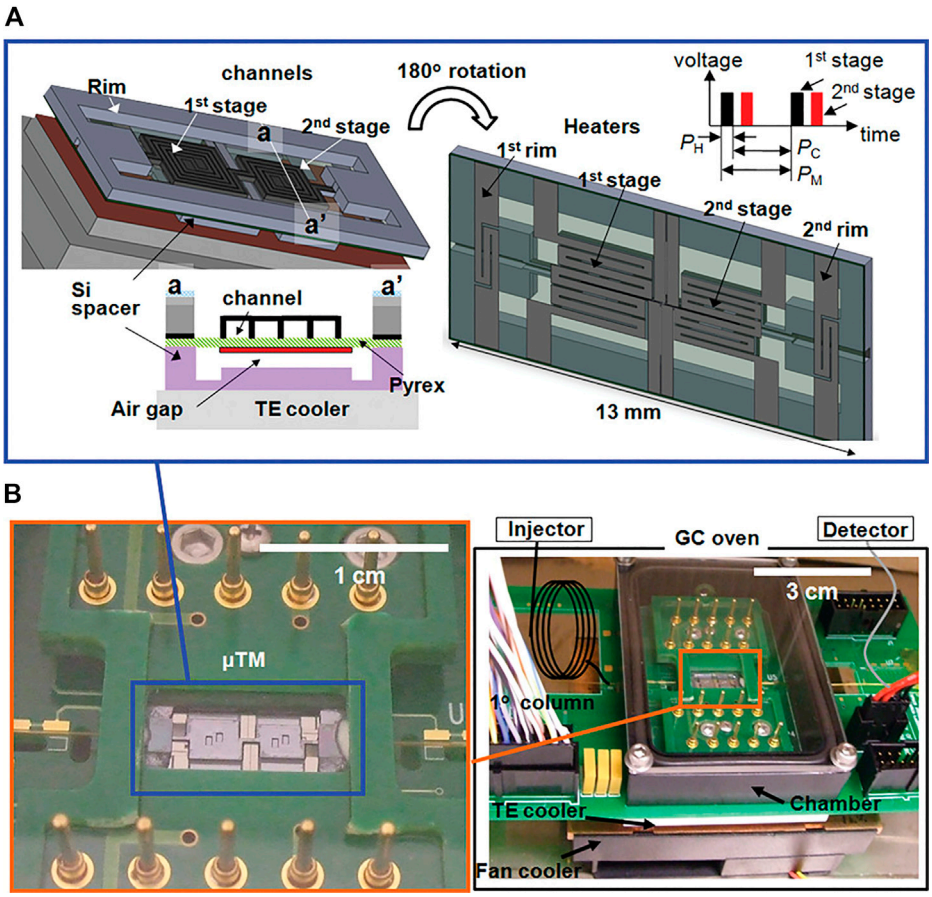

FIGURE 6
Illustration of a two-stage μTM that is both miniaturized and essential for comprehensive μGC × μGC. The two stages are cooled in order to trap analytes from the first dimension, followed by rapid heating so that the analytes are introduced in a narrow band into the second dimension. The upper figure **(A)** shows the diagram and the duration of the heating (P_H) and cooling (P_C) of one modulation period (P_M). The lower photographs **(B)** show the assembly mounted on a printed circuit board. Reprinted with permission from Kim et al. (2011). Copyright 2011 American Chemical Society.

Whereas the former approach is usually preferred for sharp peaks and lower detection limits, the latter is preferred for very volatile analytes which are not readily trapped even under cryogenic conditions. Conventional thermal modulation uses large volumes of liquid N_2 or liquid CO_2 and is unsuitable for space applications.

A microfabricated thermal modulator (μTM) has been developed for μGC × μGC, which completely retires the need for cryogenic fluids (Figure 6; Kim et al., 2010; Kim et al., 2011). The device measures 13 mm × 6 mm × 0.5 mm and is thermally controlled by a thermoelectric cooler (−35°C to −25°C at a rate as high as 168°C/s) and thin-film resistors (up to 210°C at a rate as high as 2,400°C/s; Kim et al., 2011). The cooling requires 21 W, and the heating requires 10 W. The authors reported FWHM of second dimension peaks as narrow as 90 ms. This is an impressive feat; however, it could potentially pose an interface issue with the limited scan speed of current state-of-the-art mass spectrometers.

Despite all the recent development in the miniaturization of GC, the engineering of connectors and interfaces are still lagging behind the individual components, such as, those between chip columns, sample introduction sub-systems, and detectors (Ghosh et al., 2018). These connections must be compatible with the high temperature of GC for biosignature analysis and maintain the separation's peak fidelity. Connections that introduce additional dead volume increase the dispersion in the system and consequently give rise to broader peaks and poorer separation. Currently, the performance of chip-based GC does not match conventional capillary column GC; however, rapid progress is being made. For example, Blase et al. (2022) demonstrated that capillaries between chip-GC and an MS could simply be sealed with polyimide/Hysol 1C epoxy, which provided separation efficiencies close to commercial capillary columns.

Miniaturization of GC will enable a substantial reduction of size, weight, and power compared to previous instruments applied in spaceflight missions. CE or LC cannot match the peak capacity of GC when performing conventional injections, and this may be further improved by using GC×GC at the cost of additional power requirements. However, current implementations with a trap column to interface the sample processing unit and the GC give rise to broad peaks (Guzman et al., 2020). Additionally, GC is limited to the analysis of thermally stable and volatile analytes. Most high-priority organic biosignatures are incompatible with GC and must be derivatized first. Herein lies the primary drawback of current implementations; the sample composition considerably impacts the derivatization methods. Overall LODs are often magnitudes lower than for the next-generation instruments (see Section 5), primarily due to analyte transfer efficiencies and limited sample load (~50 mg). Wet-chemistry sample preparation techniques (see Section 4) could mitigate some of these issues; however, it has not been extensively pursued. Furthermore, several techniques discussed later (e.g., LC and biosensors) do not require derivatization, which reduces risks associated with matrix effects and contamination. Finally, GC has an extensive flight heritage and may be the preferred choice for missions in the near future, with limited budgets, and when LOD requirements can be relaxed.

3.2 Recent advances in mass spectrometry

Mass spectrometry has been an essential part of space exploration, as described in Section 2.2. In the context of organic biosignature detection, in addition to mass spectrometers for landed missions, developments are ongoing for fly-by missions to attempt to capture the ejecta from the plumes of Enceladus and Europa. Recent research has studied the ionization through impact at 3–10 km/s of organic biosignatures such as amino acids and fatty acids (Klenner et al., 2020a; 2020b). The LODs achieved in these studies were generally in the low-ppm level. The upcoming Europa Clipper (Howell and Pappalardo, 2020) will carry two mass spectrometry instruments, MAss SPectrometer for Planetary Exploration (MASPEX; Brockwell et al., 2016) and SUrface Dust Analyser (SUDA; Kempf et al., 2014). Although these developments contribute to the development of MS instruments, they are beyond the scope of this review because we focus on *in situ* organic biosignature analysis primarily for landed missions.

3.2.1 Mass analyzers

The development of miniaturized mass spectrometers has experienced a surge over the last decade, and we refer the interested reader to a few excellent general review papers (Snyder et al., 2016; Mielczarek et al., 2020) and a few related to planetary exploration applications (Arevalo et al., 2020; Chou et al., 2021). A few ongoing developments are worth highlighting here, such as the quadrupole ion trap (QIT) MS onboard ISS (Darrach et al., 2015; Madzunkov et al., 2016; Schowalter et al., 2019), the linear ion trap (LIT) MS onboard the Rosalind Franklin rover (Arevalo et al., 2015; Goesmann et al., 2017), the multi-bounce time-of-flight MS developed for the Europa Clipper mission (Brockwell et al., 2016), and the adaption and miniaturization of the Orbitrap (Arevalo et al., 2018). Validation data are scarce; consequently, a quantitative performance comparison is not currently possible without such data.

Current progress toward non-spaceflight laboratory instruments mainly focuses on hybridization and combining various mass analyzers, as well as increasing the ion transmission from ambient pressure ionization sources by increased volumetric flow, which is enabled by more efficient vacuum pumps (Hager, 2004). However, an increase in complexity, mass, and power does not align with current general strategies of miniaturizing instruments (including mass and power) for spaceflight applications. Particularly, miniaturizing vacuum pumps and simpler designs of electronics are vital efforts. Heritage plays an important role in the selection and development of MS for *in situ* missions. For example, Arevalo et al. (2020) illustrated that the neutral mass spectrometer (NMS) onboard the LADEE spacecraft, with heritage components from Cassini-Huygens, took less than 3 years to deliver, as opposed to the LIT developed for MOMA, with fewer heritage components, that took more than 10 years to deliver.

Ion traps are typically easier to miniaturize because they can often operate at higher pressures (~10^{-3} Torr), and miniaturization can even improve mass resolution (Johnson et al., 2019), contrary to other types of mass analyzers. Compared to ToF-MS, the ion trap offers simpler electrical design and MS/MS analysis, but provides lower resolution (Ren et al., 2018). A handheld rectilinear ion trap MS (~10 kg, 50 W) equipped with electrospray ionization was able to detect amino acids, nucleobases, nucleosides, and peptides with LODs of approximately 1 mg/L (1 ppm) or lower (Sokol et al., 2011). The instrument was also demonstrated with multiple-stage MS up to MS^5, which greatly facilitates the identification of larger analytes. Discontinuous atmospheric pressure ionization (DAPI) was utilized to reduce the load on the vacuum pumps. The principle of the technique is that the flow path between the ionization source and the mass analyzer is only open for a short duration, allowing a short pulse of ions (Gao et al., 2008). Two newer versions of the rectilinear ion trap MS system have since then been developed, with a range up to m/z 900 and a resolution of 500 (Gao et al., 2008; Li et al., 2014).

The LIT (e.g., used in MOMA) is principally a miniaturized version of the Thermo LTQ XL (Thermo Fisher Scientific), with lower performance characteristics than its commercial counterpart due to miniaturization (Brinckerhoff et al., 2013). On one side is an EI source to interface with GC and on the

opposing side is an inlet for ions generated by the laser ionization sub-system, which utilizes the DAPI technique to reduce the requirement of the vacuum pumps. The valve is opened between 10 and 100's milliseconds while the sample is subjected to laser ionization, and the ions are pulled in by a potential applied to the inlet (Li et al., 2015a). The instrument, including GC and laser ionization sub-system, weighs approximately 12 kg and provides unit resolution up to 500 Da (Goesmann et al., 2017). Recent developments of the same MS instrument have improved the performance to an m/z range of 20–800 with an FWHM of 0.5 Da over the entire range (Brinckerhoff et al., 2022).

Although the MOMA-MS only supports positive mode MS, the LIT can operate in both negative and positive modes with minor modifications (Li et al., 2019). The instrument is being repurposed for various missions concepts, such as the Dragonfly Mass Spectrometer (DraMS) that is planned for an aerial lander mission to Titan (Lorenz et al., 2018), the EMILI instrument suite comprising capillary electrophoresis (CE) and GC that is being developed as a contender for a potential Europa lander mission (Brinckerhoff et al., 2019; Brinckerhoff et al., 2022), integration with an Orbitrap MS in the Advanced Resolution Organic Molecule Analyzer (AROMA; Arevalo et al., 2016), and the Molecular Analyzer for Complex Refractory Organic-rich Surfaces (MACROS; Getty et al., 2017).

A miniaturized Paul trap mass spectrometer, also known as a quadrupole ion trap mass spectrometer (QITMS), has been developed and is currently operating onboard the ISS for cabin atmosphere monitoring (Shortt et al., 2005; Madzunkov et al., 2016; Schowalter et al., 2019). The QITMS weighs about 7.5 kg and is hyphenated with a GC system (described in Section 3.1). The ideal operating pressure is $< 10^{-6}$ Torr, which is lower than the typical LIT (Madzunkov et al., 2016). The current configuration enables detection of m/z 30 to 300; however, it is extendable up to m/z 600, with a mass resolution of 18,000 at m/z 40, and a resolution of 600 throughout the entire mass range at a scanning frequency of 50 Hz (Simcic et al., 2021).

Several miniaturized ToF systems have been conceived; for example, a conventional but miniaturized ToF-MS has been interfaced with a miniaturized pyrolysis unit (Getty et al., 2010; Glavin et al., 2012b), or with laser-ionization (Getty et al., 2012). Recent trends and development have mostly focused on multibounce, or multiturn, ToF instruments. Multibounce ToF systems enable smaller instruments, even though they maintain a long travel distance in order to retain the high resolution. Instruments based on this technology have mainly been used with laser-ablation (Rohner et al., 2003; Wiesendanger et al., 2018, 2019; Riedo et al., 2020; Ligterink et al., 2021). The ToF instrument used in these studies resulted from developments based on the Rosetta TOF-MS (Hohl et al., 1999). It measured 160 mm in length and 60 mm in diameter and enabled two turns of the injected ions through a set of reflectrons ion optics (Riedo et al., 2017). A resolution of <900 was acquired when interfaced with laser ionization (Riedo et al., 2013).

MASPEX is another ToF instrument (Figure 7), with an adjustable number of turns or bounces, which will be a part of the Europa Clipper payload (Brockwell et al., 2016) and is also being developed for GC-MS applications as a candidate for a potential Europa lander mission (Blase et al., 2020a; Blase et al., 2020b). An average MS resolution of 380 was achieved when coupled with chip-based GC and scanning m/z 40–140 (Blase et al., 2020b). However, with slower measurement frequency, the resolution can be as high as 46,000 at the expense of mass range and sensitivity (Brockwell et al., 2016).

Orbitrap is the latest addition to MS techniques currently under investigation and development for spaceflight applications. It was introduced first in 2000 (Makarov, 2000). The Orbitrap traps the ions in an orbital motion around the inner electrode rather than in a space confined by outer electrodes like the LIT or QITMS. GC-Orbitrap, with either EI or chemical ionization, has been used to detect volatile organics from interstellar ice analogs (Javelle et al., 2021). A benchtop laser ablation (LAb)-Orbitrap has been developed (Briois et al., 2016). The MS operates at a pressure of $<10^{-6}$ Torr (typically $<10^{-10}$ Torr), which may require powerful vacuum pumps depending on the mission and the front-end instrumentation. The mass resolution increases with lower pressure. The benchtop Orbitrap instrument presents an impressive mass resolution of >90,000 up to m/z 209 even at 1 Hz scan rate (Briois et al., 2016). Multiple studies have highlighted how the benchtop instrument provides exceptional capabilities for identifying unknown organics by high-resolution MS (Arevalo et al., 2018; Selliez et al., 2019; Selliez at al., 2020). The Characterization of Ocean Residues And Life Signatures (CORALS) instrument, which is also based on LAb-Orbitrap, is estimated to weigh 8 kg and consume 41 W at peak power; and provides an m/z range of 20–600 with a mass resolution of 120,000 (at m/z 133; Willhite et al., 2021). The LIT-MS mentioned earlier, hyphenated (also known as hybridization) with a miniaturized Orbitrap, is also being investigated (Arevalo et al., 2016). The capabilities of the hybrid MS under investigation remain unknown; however, the configuration is popular in commercial systems used in analytical chemistry laboratories and provides improved LODs due to the accumulation of ions in the LIT, which are then delivered as coherent ion packs with high ion transmission to the Orbitrap (Makarov et al., 2006).

It is important to consider the pressure at which each mass analyzer typically operates when comparing them. The typical ITMS operates at $\sim10^{-3}$ Torr, the ToF at $\sim10^{-8}$ Torr, and the Orbitrap at $\sim10^{-10}$ Torr. Vacuum pumps and electronics are the main contributors to the overall mass and volume. Appropriate pumping capacity must be selected depending on the mission environment (ambient pressure) and the type of front-end selected. For example, the most common ionization sources for liquid streams operate at atmospheric pressure (760 Torr).

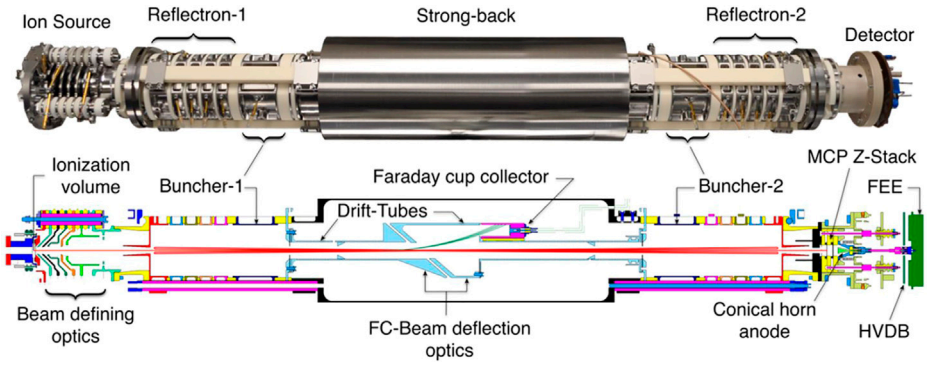

FIGURE 7
Image and illustration of the Mass Spectrometer for Organic Analysis in Space Environments (MASPEX) instrument. Multibounce is utilized to increase the flight path to achieve high-resolution measurements despite miniaturization. Adapted with permission from Blase et al. (2020b). Copyright 2020 American Chemical Society.

3.2.2 Laser-based ionization

Laser ionization MS (LIMS), including laser desorption ionization (LDI) and laser ablation (LAb), enables the detection of biosignatures through a single laser pulse (typically a few nanoseconds) that desorbs and ionizes material that is subsequently MS-detected. It can be used at various ambient pressures, including high vacuum. LIMS requires no consumables such as liquids for extraction or carrier gases and further reduces instrument size and complexity. Consequently, the number of possible analyses is not limited by consumables, and countless samples can be analyzed throughout an *in situ* mission. LIMS enables spatial resolution (micrometer scale or lower) that cannot be achieved by conventional sample acquisition techniques for downstream chemical analysis. The downside of the technique is that the ionization efficiency is relatively low, which results in low sensitivity (Feider et al., 2019).

LIMS has historically mainly been used for the elemental and inorganic analysis of solid samples in the laboratory (Azov et al., 2022). The technique has proven powerful for the elemental analysis of signatures of microbes in solid samples (Stevens et al., 2019; Riedo et al., 2020). So far, limited validation data related to planetary analogs has been reported. The MOMA engineering test unit was used for analyzing microbial-containing silica sinter and previously extracted lipids (Siljeström et al., 2021). Only chlorophyll A was detected in the untreated sample. Spiked coronene (10 ppm) and Rhodamine 6G (10 ppm) in Mars analog samples, with added ~1 wt% perchlorate salt, have been successfully analyzed with a breadboard setup of MOMA (Li et al., 2015a). Spiked tryptophan was detected at 0.1 wt% in various mineral substrates (Uckert et al., 2018). However, LIMS spectra of solid samples are often difficult to interpret and may be dominated by mineral fragments (Bishop et al., 2013). Two-stage LIMS can reduce mineral interference by tuning the desorption and ionization independently for aromatic analytes; however, the LODs remain high (Getty et al., 2012; Uckert et al., 2018).

It has been shown that most amino acids mixed with NaCl on a stainless steel surface can be detected, of which tryptophan had an LOD of 1.2 fmol mm^{-2} (Ligterink et al., 2020). Another study that used an Orbitrap adapted for spaceflight (CosmOrbitrap) showed that twelve amino acids could be detected at a concentration of 1 pmol/mm^2, which was achieved by drying 10 µL of water with 10 µM of each amino acid onto a plate of 80 mm^2 (Arevalo et al., 2018). The same authors demonstrated that pure uracil could also be detected. LIMS has also been applied to analyze isolated single cells with sub-micron resolution, of which mainly phospholipids were detected (Wang et al., 2018).

For Ocean Worlds applications, the ice or aqueous samples could be sublimated or evaporated to further increase the concentration of analytes in order to improve method LODs. However, further validation and comparison with alternative analysis techniques are still needed to assess its utility. Nonetheless, a LIMS instrument is included in the payload of the Rosalind Franklin rover (Li et al., 2017), and was included onboard the unsuccessful Phobos-Grunt mission (Managadze et al., 2010).

LIMS is a versatile technique that requires minimal or no sample preparation. However, compared to some of the more complicated techniques that also utilize MS (e.g., GC, CE, and LC), it has several orders of magnitude higher LODs. Additionally, LIMS offers similar performance to stand-off spectroscopic techniques; however, when combined with high-

resolution MS, it may offer much higher ability to differentiate analytes in a complex sample.

3.2.3 Mass spectrometer interfaces for separation techniques

Electron ionization (EI) has been used extensively for ionization in the analysis of atmospheres and the coupling of GC-MS, and has extensive spaceflight heritage. Other ionization sources that are routinely used in laboratories are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric photoionization ionization (APPI), matrix-assisted laser desorption/ionization (MALDI), fast atom/ion bombardment (FAB), and chemical ionization (CI). The choice of ionization technique depends primarily on the sample; for example, solid sample, gas from a GC, or liquid from a liquid chromatography (LC) system; and secondarily on the analytes of interest.

EI is easily implemented and generates reproducible mass spectra that can be matched with databases. However, current the usage of EI is not suitable for the emerging instruments that utilize wet chemistry for separation or direct analysis of aqueous samples on Ocean Worlds. EI requires a high vacuum ($<10^{-6}$ Torr) for ideal operation and to not burn out the filament. EI interfaces for liquid streams have been developed; however, their suitability for thermo-labile biosignatures has not yet been assessed (Termopoli et al., 2017; Termopoli et al., 2019; Rigano et al., 2019).

Conventional ionization techniques for liquid streams include ESI, APCI, and APPI, or variants of these (Covey et al., 2009; Feider et al., 2019). They all have in common that the ionization process occurs at atmospheric pressure (760 Torr). Consequently, additional instrument design considerations are added due to the lower atmospheric pressure at various target planetary bodies. In the case of atmospheric pressure ionization sources, the ions need to be transferred into the mass analyzer, which operates at vacuum $<10^{-3}$ Torr, depending on the MS technique. Among these atmospheric ionization techniques, ESI is the most commonly used. ESI, and other atmospheric pressure ionization techniques, are adversely affected by matrix effects such as salts through ion suppression, and result in diminished sensitivity (Furey et al., 2013). During the last decade, low flow (nL/min) nano-ESI, also known as nanospray ionization, has gained widespread popularity. Among the benefits mentioned are low-solvent consumption, increased sensitivity, and reduced matrix effects from components such as non-volatile salts (Juraschek et al., 1999). However, to obtain these benefits, the flow rate needs to be a few tens of nL/min or less through the emitter (Schmidt et al., 2003; Jarvas et al., 2017). The total flow rate can be increased using multiple emitters (Kelly et al., 2008; Mao et al., 2011; Jender et al., 2021).

Although ionization sources, compatible with liquid samples, are essential for many techniques in development (see Section 5), no hardware compatible with operation in a vacuum environment (while maintaining ~760 Torr in an ionization chamber) has been demonstrated. Additionally, ion optic interfaces with gradually increasing vacuum, from atmospheric pressure to vacuum levels required by MS analyzers, have just recently been interfaced with a flight-like MS (Brinckerhoff et al., 2022). Although these are standard techniques in the laboratory and portable instruments, they have not yet been fully adapted for spaceflight applications.

3.3 Ion mobility spectrometry

In this section, we discuss ion mobility spectrometry, which includes ion mobility spectrometry (IMS) and a sub-technique named differential mobility spectrometry (DMS). IMS resembles MS in many practical aspects, but the two techniques are not interchangeable. Ionized analytes in the gas phase are separated based on their mobility in a carrier buffer gas, often at atmospheric pressures. Hence, the same atmospheric pressure ionization techniques used in MS, such as ESI and APCI, are also combined with IMS. IMS has, over the last decade, established itself as a routine analysis technique hyphenated with MS in order to increase the overall resolving power by adding a dimension of separation. IMS has not been used for any planetary application; however, it has been routinely used on the ISS for air quality monitoring.

Commercial instruments are readily available and fully integrated. However, conventional IMS is not orthogonal to MS; the drift time often correlates with the m/z separation, and frequently only adds a marginal improvement in tandem instruments (Valentine et al., 2005; Shvartsburg et al., 2011). It is important to note that IMS can be used as a stand-alone technique, without MS; though some IMS techniques are only used in combination with MS to increase selectivity (Cumeras et al., 2015). In fact, for many smaller analytes, the correlation can be used to approximate the molecular weight based on their IMS drift times (Kim et al., 2005, 2009). IMS is readily miniaturized, and most IMS techniques are ideally operated at ~760 Torr, which renders coupling with atmospheric pressure ionization techniques straightforward (Cumeras et al., 2015). Consequently, the technique may be seen as a more straightforward but less powerful substitute for MS. This section focuses on describing IMS as a stand-alone analyzer, with or without chromatography, but without MS. Johnson et al. (2007) have given a brief overview of IMS and its potential in space exploration applications.

Although many variations of IMS have been reported, they all include a drift tube in which the analytes are separated under a constant or variable electric field. The various commonly used types are covered elsewhere (Cumeras et al., 2015; Dodds and Baker, 2019). The most uncomplicated design involves insulated stacked rings that act as electrodes to form a uniform electric field inside the drift tube. Ions are injected as packages using an ion gate or a shutter. As the ions move through the electric field (e.g.,

towards a Faraday plate), their mobility depends on their surface area and charge. The ions travel through a gas typically held at ambient pressures, and molecules with a larger collision cross-section (CCS) reach the detector later than smaller molecules due to their interactions with the buffer gas. The separation is carried out on the order of micro-to milliseconds, making it ideal for coupling with fast chromatographic techniques with very narrow peaks (Valentine et al., 2005; Piendl et al., 2019). Isomers that MS does not resolve can be separated using IMS because CCS influences the migration time as opposed to the molecular mass (Zhu et al., 2012).

Current state-of-the-art IMS instruments have resolving powers above 300, sufficient to discriminate analytes with <0.5% CCS differences (Dodds et al., 2017). The resolving power (R_p) is here defined as the quotient of the drift time t_d and the full width at half maximum w_{FWHM}. Some IMS techniques have also been demonstrated with on-chip separation (Costanzo et al., 2017; Deng et al., 2017).

The drift tube IMS has a simple design and operation. It consists of an ion gate, a drift region, and a shielded Faraday plate as an ion detector. The ions travel through a constant electric field that usually requires a few kV to maintain. The ions always travel through an environment of the counter-current flow of a neutral gas (mostly nitrogen, helium, argon, or carbon dioxide), called the drift gas. The separation can be optimized; for example, by the selection of buffer gas, pressure, and temperature (Matz et al., 2002; Wyttenbach and Bowers, 2003). Neutral analytes are flushed away by the buffer gas in the opposite direction of the detector. Ahrens et al. (2019) presented a simple and portable drift tube IMS (Figure 8), manufactured from PEEK and stainless-steel foils and snapped into place on a printed circuit board. The instrument provided a R_p of 63, with a drift length of 40 mm and a cross-section of 15 mm × 15 mm. Although this effort was not toward spaceflight applications, it demonstrates the ability to miniaturize IMS instruments with a simple design.

DMS has proven to be a valuable addition to mobility spectrometry applications and analyses. DMS utilizes planar electrodes, and similar high-field asymmetric waveform ion mobility (FAIMS) utilizes cylindrical electrodes (Kolakowski and Mester, 2007). An electric field, perpendicular to the analytes' direction, oscillates between low and high. Depending on the properties of the asymmetric electric field, also known as the dispersion field, stable trajectories towards the detector will be achieved for some analytes. With some similarity to a quadrupole MS, FAIMS can be used as a filter to selectively single out analytes for detection, or it can be used in a sweeping mode to produce a spectrum. Spectra are frequently plotted as intensity against the sweeping compensation voltage or compensation field, rather than a time for a drift tube IMS. Because FAIMS is not a time-based separation technique, it can accept a continuous input of ions and does, therefore, not require an ion gate (Cumeras et al., 2015). Although DMS/FAIMS can offer orthogonal separation to MS and offers more flexibility in method optimization (Shvartsburg et al., 2013), it requires more demanding electronics due to changing potentials.

In most chemical analysis laboratories, IMS is typically integrated with MS systems. However, stand-alone IMS instruments have found a niche application area where

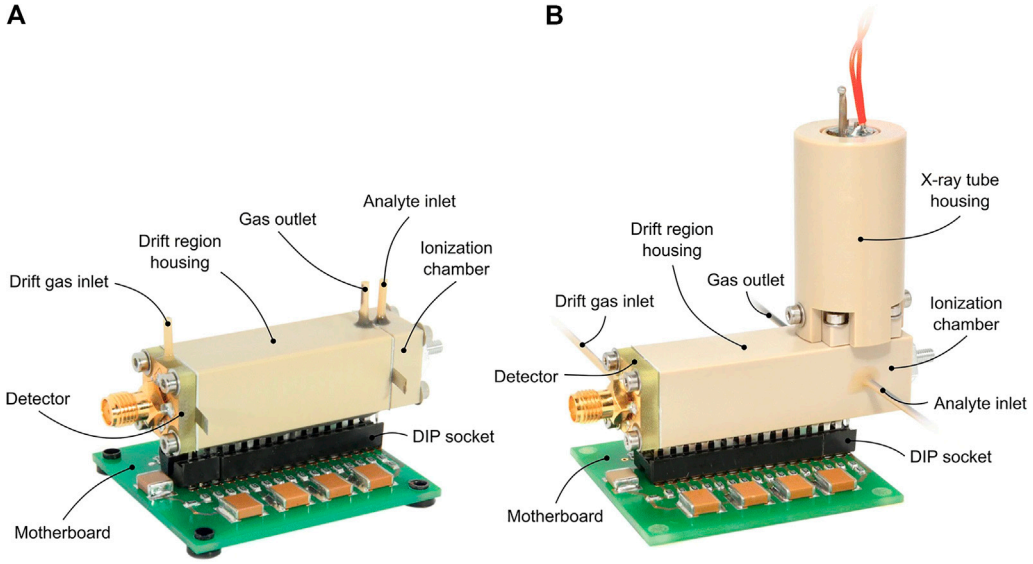

FIGURE 8
Photo of a miniaturized drift tube IMS with either a ^{3}H ionization source **(A)** or an X-ray ionization source **(B)**. This IMS is not being developed for space applications; however, it demonstrates that manufacturing a low-mass and small-volume instrument is possible. Reprinted from Ahrens et al. (2019).

simplicity, reliability, and portability are highly valued; such as, in airport security or for military purposes. Examples of such applications are: field-analysis of explosives (Eiceman et al., 2004), chemical warfare agents (Eiceman and Stone, 2004), food and air monitoring (Eiceman, 2002), and illicit drug screening (Verkouteren and Staymates, 2011). A field-portable Py/GC-IMS instrument for aerosol analysis demonstrated that 1 µg of *B. subtilis* spores could be detected (Snyder et al., 1996; Dworzanski et al., 1997). More recent developments have included tandem IMS/IMS to provide similar structural information and improved resolving power as in tandem MS/MS (Merenbloom et al., 2006; Chiluwal et al., 2019).

Although, no IMS (including DMS) instrument has been used at any planetary body, it has been extensively used for air quality monitoring onboard ISS. A GC-IMS instrument was deployed to the ISS in 2001 for air quality measurements. It was the first time a real-time trace volatile organic compounds (VOCs) air quality instrument was deployed in a spacecraft. The system used radioactive nickel-63 foil as an ionization source to detect VOCs (Limero et al., 1992, 2011). Although its operation was successful, it suffered several technical issues. This sparked the development of a smaller and modularized GC-DMS instrument, the Volatile Organic Analyzer (VOA), which was installed onboard the ISS in 2009 (Limero et al., 2008). The VOA instrument was calibrated and validated for 15 VOCs. The operation of VOA lasted for about 7 years and was replaced by two units of a new GC-DMS system named the Air Quality Monitor (AQM). The AQM was able to quantify 22 VOCs (Limero et al., 2014; Wallace et al., 2017).

The Wet Chemistry Experiment at Mars (WetChem) instrument, an early-stage project that has not been used in spaceflight, included IMS in tandem with MS to analyze samples previously characterized by electrochemical techniques (Kanik et al., 2006). In the context of astrobiology, IMS has been proven suitable for detecting and identifying of many analytes; for example, organic acids (Kim et al., 2005), amino acids (Beegle et al., 2001; Johnson et al., 2004), phosphatidylcholines (Kim et al., 2009). Although no LODs were determined, the technique was able to separate the majority of 19 tested amino acids at 10 mg/L using ESI-IMS (Beegle et al., 2001).

In summary, IMS cannot operate in a vacuum environment because atmospheric pressure (~760 Torr) is often required to achieve separation, which is based on gas-analyte interactions. An enclosure would be needed to maintain a suitable gas pressure when operating in space environments and would also require bringing additional carrier gas. However, for Mars (~5 Torr) applications, MS instruments require capable vacuum pumps (see Figure 2), making IMS instruments a potential contender. Furthermore, the electrical subsystems for MS are substantially larger than for IMS instruments. IMS could be a smaller, but less capable regarding resolving power and identification compared to MS for *in situ* organic biosignature analysis. Only a handful of studies have investigated IMS for spaceflight applications at planetary bodies; however, recent progress in the state-of-the-art has proven it to be a readily miniaturized technique.

3.4 Standoff spectroscopic techniques

Remote and standoff spectrometers (e.g., X-ray spectrometers) have been an essential part of previous space missions and will continue to play an important role in future missions. However, fluorescence and Raman spectrometers for detecting organic material were first deployed on the Perseverance rover. These two techniques are among the most convenient and suitable techniques for detecting organic content in samples. No sample collection or sample preparation is needed, and the number of analyses is only limited by the laser lifetime. Nowadays, most space instrument suites include either or both Raman spectroscopy and fluorescence spectroscopy techniques in their arsenal. The same excitation wavelengths can be used in both techniques. The miniaturization of electronics and optics enables the continuous downsizing for the suitability of these instruments for space applications. Hence, a vast literature covers field demonstrations of fluorescence and Raman spectrometers onboard various platforms, including a rock-climbing robot (Uckert et al., 2020), and an ice-sheet-penetrating platform (Eshelman et al., 2019).

3.4.1 Raman spectroscopy

Recent developments in Raman spectroscopy have improved both the spatial resolution as well as the detection limits. High spatial resolution (~1 µm) Raman imaging was used to study the hydrogen to carbon ratio in organic matter from fossilized cells (Ferralis et al., 2016). However, this study was conducted with a laboratory-based instrument, and these resolutions have not been achieved with miniaturized components. The *in situ* Spectroscopic Europa Explorer (iSEE) concept uses a digital micromirror device/photomultiplier assembly (DMD/PMT) and a microchip diode laser into a miniature Raman spectrometer which improves the signal-to-noise ratio by 3-orders of magnitude compared to conventional CCD arrays (Sobron et al., 2019). The authors also claim that the instrument has an LOD of <1 ppb for organic content. The Raman spectrometer designed for the Martian Moons eXploration (MMX) mission measures only 81 mm × 125 mm × 98 mm and weighs approximately 1.4 kg (Cho et al., 2021). There are other examples in the literature of other miniaturized standoff Raman instruments (Wang et al., 2003; Misra et al., 2016; Abedin et al., 2018; Sandford et al., 2021).

Background fluorescence, however, remains an important issue, which is often several orders of magnitude stronger than the Raman scattering signal, and inhibits the detection—for example, of cells in Martian simulant samples (Stevens et al., 2019). Two main strategies are used to reduce the background fluorescence signal: UV-range excitation wavelengths and time-

gated (TG) Raman spectroscopy (Frosch et al., 2007; Shkolyar et al., 2018; Hanke et al., 2019). Fluorescence signals from minerals have a decay lifetime of >1 ms compared to organic fluorophores that typically have decay lifetimes of ~<100 ns (Misra et al., 2016; Shkolyar et al., 2018). Blacksberg et al. (2020) demonstrated improved signal-to-noise ratios through fluorescence rejection by TG Raman spectroscopy with a 100 ps laser pulse, and a 1 ns detector gate duration with 550 ps rise time and 250 ps fall time. Consequently, the authors could detect kerogen (i.e., the insoluble sedimentary organic fraction formed through diagenesis of biological material; see Vandenbroucke, 2003 for a detailed definition) content down to 1% in various samples.

Research is still ongoing regarding how the sample composition influences the response factors of organic compounds in mineral matrices. Samples synthesized in the laboratory do not capture all matrix effects that may be present in a natural sample. In fact, most of the available literature only covers the study of pure analyte or single analyte spiking on solid samples. The latter does not consider the distribution of analytes or adsorption processes that only occur through geological diagenesis. Therefore, it is difficult to evaluate the performance of the techniques and the instruments. For example, Montagnac et al. (2021) found that 0.4 mM deoxyguanosine-5′-monophosphate (dGMP), spiked onto clay samples, could be detected; however, the response factor depended on the sample matrix.

3.4.2 Fluorescence spectroscopy

Fluorescence spectroscopy has long been a standard tool for marine sample analysis, in the laboratory or *in situ*, and is now being adapted for the Ocean Worlds applications. Of note here, the Ocean Worlds in the Solar System of particular interest, other than the Earth, are Callisto, Enceladus, Europa, Ganymede, and Titan. Many of the optical sensor technologies and marine platforms that may be applicable for Ocean Worlds have been reviewed by Aguzzi et al. (2020). The typical instrument involves absorption and fluorescence spectroscopy applied to a flow cell for the detection of organic compounds such as pigments, polycyclic aromatic hydrocarbons (PAHs), proteins (indirectly detected through tryptophan), and organic polymers such as humic acids (Wollschläger et al., 2013; Wollschläger et al., 2016). Naturally, fluorescence spectroscopy is only suitable for detecting the fluorescent fraction of the dissolved organic matter. Cells and molecules can be labeled with a fluorescent dye; however, inefficient staining, non-specific binding, and autofluorescence of mineral particles (especially those including rare-earth elements) may result in both false positives and false negatives of potential biosignatures (Gaft et al., 2001; Li et al., 2004; Shkolyar et al., 2021).

Single-wavelength excitation with full spectrum emission scanning provides limited chemical information for identification and deconvolution. Hence, development for Earth applications is moving towards using excitation and emission scanning fluorescence, or excitation-emission matrices (EEMs), which provide a 2D dataset for each analyzed sample (Bhartia et al., 2008; Ferdinand et al., 2017; Carstea et al., 2020). Such data provide more rugged deconvolution for identification and quantification through multi-way (tensorial) chemometric techniques such as parallel factor analysis (PARAFAC; Smilde et al., 2005; Toraman et al., 2018). For example, excitation and emission scanning fluorescence followed by PARAFAC enabled analysis of terrestrial humic-like species, marine humic-like species, and protein-like tryptophan in natural waters (Zielinski et al., 2018). Although no instrument is specifically being developed for space applications, EEMs have proven valuable for analyzing biological (Bhartia et al., 2008) and Ocean World (Malaska et al., 2020) analog samples, and may be a future spaceflight instrument avenue.

Ice can be directly analyzed with fluorescence spectroscopy, thereby retaining the spatial distribution. The Wireline Analysis Tool for the Subsurface Observation of Northern ice sheets (WATSON) leverages the previously developed SHERLOC instrument (currently onboard the Mars 2020 Perseverance rover) for this specific purpose (Eshelman et al., 2019). WATSON uses DUV excitation (248.6 nm) and acquires spectra between 265 nm and 440 nm. The LOD for tryptophan, tyrosine, and *E. coli* were 2 μM, 20 μM, and 5×10^6 cells/mL, respectively (Eshelman et al., 2019). The absolute cell LOD was estimated to be < 300 cells, achieved through the spatial resolution (100 μm) and low interrogation volume, where each scan covers a 75 mm by 25 mm area. The instrument demonstrated that it could identify hot spots in the Greenland ice sheet containing organic matter from the surface down to 105 m depth (Malaska et al., 2020).

TG-fluorescence spectroscopy provides valuable utility in improving detection limits and identification. Its applications and recent developments have been reviewed by Yang and Chen (2020). The detection limits are improved with TG-detection because organic materials display fluorescence decay lifetimes, which are orders of magnitude shorter than mineral (inorganic) decay lifetimes. This can allow organics to be detected as biosignatures in the presence of a mineral matrix (i.e., fossil organics in a rock; Shkolyar et al., 2018). Identification can be facilitated by time-resolved measurements, in which a sequence of TG measurements is taken with incremental gate delay. The decay profiles and spectra together provide information for analyte identification. For example, time-resolved fluorescence with a gate width of 1.5 ns was used to discriminate PAHs over the course of 20 ns following the laser pulse (Eshelman et al., 2018). Lymer et al. (2021) used UV time-resolved laser-induced fluorescence to detect and identify amino acids, intending to later be used on returned meteorite samples. Other similar instruments, such as the OrganiCam (TG-fluorescence and Raman spectroscopy) are currently being developed for

potential Mars and Ocean Worlds applications (Wiens et al., 2019, 2020; Gasda et al., 2021).

Mineralogy greatly affects response factors, and more research is needed (Laurent et al., 2019). For example, matrices rich in transition metals (e.g., Fe and Cr) may have strong UV absorbance, greatly reducing the sensitivity (Chua et al., 2001; Shkolyar et al., 2018; Carrier et al., 2019). Most studies have been conducted with pure analytes or spiked samples; therefore, they do not reflect the true LOD of the reported methods. Hence, fluorescence spectroscopy can currently be viewed as a qualitative, or possibly semi-quantitative, tool for standoff measurements that facilitate sample selection for analysis by more capable techniques.

4 Sample preparation

Most chemical analysis techniques used in the laboratory are performed with the analyte in solution. In the context of methods developed for space applications, hybrid approaches have been investigated, such as sublimation followed by derivatization and gas chromatography analysis (Glavin and Bada, 1998; Glavin et al., 2006). However, sublimation is several orders of magnitude less efficient than solvent extraction of amino acids (Skelley et al., 2005). Direct analysis of liquid samples, including liquid extracts from solids, typically provides lower LODs. Furthermore, liquid samples are easier to prepare for downstream analysis instruments; nevertheless, preconcentration or desalting may still be necessary. Extraction techniques applicable to solid samples, primarily for Mars applications, have been reported but are relatively unexplored compared to separation and detection instruments. Although sample preparation techniques, including extraction, are generally perceived as being of secondary importance to detection technologies, it is perhaps the most crucial step to achieve required LODs and measurements with acceptable errors (Traks et al., 2005).

Analytes strongly adsorbed to minerals may be more resistant to degradation (Aerts et al., 2014 and references therein), and microbes may have higher an affinity toward certain types of minerals (Röling et al., 2015 and references therein). For example, strong adsorption to solid matrices such as clay and other silicate-rich samples aids the preservation of nucleic acids, but may result in lower recoveries from the extraction process (Direito et al., 2012). Similarly, Mars-relevant minerals have proven to be difficult to extract DNA from due to adsorption (Carr et al., 2017; Mojarro et al., 2017). Competitive binders such as phosphate, pyrophosphate, ATP, random hexamers, or skim milk are often used to increase the recoveries (Takada-Hoshino and Matsumoto, 2004; Direito et al., 2012; Mojarro et al., 2017). Furthermore, although DNA may be locally well-preserved, when extracted it may degrade due to the presence of metals or extreme pH. The impact of Mars-relevant components on nucleic acid degradation has been covered by Mojarro et al. (2021). There is no universal extraction protocol that is effective for all types of soil samples, and repeatability and reproducibility varies greatly between commercial extraction kits, whose components are often trade secrets (Zielińska et al., 2017). Consequently, sample matrices such as clay-rich minerals become a double-edged sword—organic biosignatures may survive for extended periods of time, but they are at the same time challenging to extract.

The overall instrument complexity varies for sample preparation subsystems. For example, multiple solvents and reagents, that need to be stored separately, require additional pumping units and valves. A typical DNA extraction and purification protocol illustrates such an instance: the sample is desalted; the DNA is extracted with a binding buffer with or without competitive binders, and sometimes in combination with a cell lysing step; the DNA is purified on an adsorbent and washed; lastly, the DNA is eluted from the adsorbent and can be analyzed downstream (often with additional preparation steps). On the other side, sample preparation protocols may be as simple as only using water as an extraction solvent. The storage of reagents and solvents must be carefully considered. Solutions or reagents stored dry, need to be stable over several years (or even longer), potentially also in high-radiation environments. Even solvents may degrade; for example, acetonitrile degrades into cyanide when exposed to 300 krad (Creamer et al., 2019), which could be expected during a mission to Europa (Hand et al., 2017). Storage experiments of solutions and reagents are discussed later, whenever data are available, together with their accompanying technique or instrument.

Miniaturized liquid sample handling devices are currently being developed for a holistic approach to sample distribution, filtering, the addition of reagents, and concentration by evaporation, such as the Sample Processor for Life on Icy Worlds (SPLIce; Chin et al., 2017), or the similar Extractor for Chemical Analysis of Lipid Biomarkers in Regolith (ExCALiBR) for lipid analysis (Wilhelm et al., 2021).

4.1 Derivatization for gas chromatography

Pyrolysis, derivatization, and thermochemolysis have all previously been used in combination with GC for *in situ* organic analysis (see Section 2.1). Research is still ongoing to further understand the impact of oxidation agents such as perchlorates on the various methods. In the presence of perchlorates, pyrolysis forms chlorinated molecules and *in situ* derivatization with MTBSTFA or DMA/DMF is also adversely affected by perchlorates. It has been shown that fatty acids detected through thermochemolysis with TMAH remain unaffected up to 10 wt% perchlorate (Helge Mißbach et al., 2019; He et al., 2021b). However, TMAH has been found to cause isomerization of unsaturated fatty acids, resulting in

additional GC peaks (Challinor, 1991). Strategies are still being formed for selecting Mars samples that are compatible with current methods; for example, samples that do not release excessive amounts of oxygen during thermal decomposition (Lewis et al., 2018). It has been shown that the commonly used MTBSTFA, DMA-DMF, and TMAH are not adversely affected by radiation corresponding to levels expected at Europa during an *in situ* mission (<300 krad; Freissinet et al., 2019).

Alternatives to TMAH for *in situ* organic detection are also being studied, such as trimethylsulfonium hydroxide (TMSH; Kivrak et al., 2021). The processing temperatures are lower, which may reduce unwanted reactions. TMSH is commonly used for the derivatization of free fatty acids prior to GC analysis (Ishida et al., 1999; Chiu and Kuo, 2020; Gries et al., 2021). However, it has been reported that TMSH is not efficient at derivatizing some of the unsaturated fatty acids (Ostermann et al., 2014). Similarly to TMAH, TMSH can also be used to derivatize other analytes, such as nucleobases (Kivrak et al., 2021).

4.2 Subcritical water extraction

Subcritical water extraction (SCWE) and conventional liquid extraction are currently the most studied extraction techniques for extracting analytes from solid planetary analog samples. The technique is also referred to as pressurized hot water extraction, superheated water extraction, pressurized liquid extraction, or accelerated solvent extraction. At elevated temperatures (>100°C), the water is maintained in its liquid state by applying pressure (see Figure 9). Increased desorption and increased solubility are the two main benefits of SCWE. The solubility of medium-polar analytes is increased at higher temperatures due to a decreased relative static permittivity (ε, also known as the dielectric constant) of water. Liquid water at 275°C has similar solubility parameters to methanol in terms of polarity, and hydrogen-bond donating and accepting abilities; hence, can dissolve even rather non-polar analytes.

SCWE has gained particular popularity as an extraction technique for amino acids bound to their sample matrix (e.g., Amashukeli et al., 2008; Kehl et al., 2019; Brinckerhoff et al., 2022). The recovery of amino acids is influenced by desorption, hydrolysis, and degradation, which are all positively associated with increased temperatures. For example, amino acids in solid samples from the Atacama Desert could not be extracted at 30°C; however, higher recoveries were achieved at elevated temperatures up to 250°C (Amashukeli et al., 2007). The same study showed that no amino acids were detected in extracts obtained at 325°C, likely due to degradation of amino acids.

SCWE can also hydrolyze biopolymers such as proteins into a mixture of peptides and amino acids at temperatures above 200°C (Marcet et al., 2016). Complete hydrolysis is typically achieved at very acidic conditions (i.e., 6 M HCl) for approximately 24 h at ambient temperatures, or 1 h at temperatures above 160°C (Csapá et al., 1997). Noell et al. (2018) demonstrated that hydrolysis at 185°C over 20 min was improved approximately 5-fold with 10 mM of HCl (3.2% versus 15.7% recoveries of total amino acids, respectively). It is noteworthy that the recoveries of most amino acids did improve with higher temperature and longer extraction times; however, some amino acids, such as aspartic acid and glycine, degraded. SCWE is also suitable for lysing cells and spores, while simultaneously hydrolyzing proteins that can subsequently be analyzed by, for example, CE (Cieslarova et al., 2022).

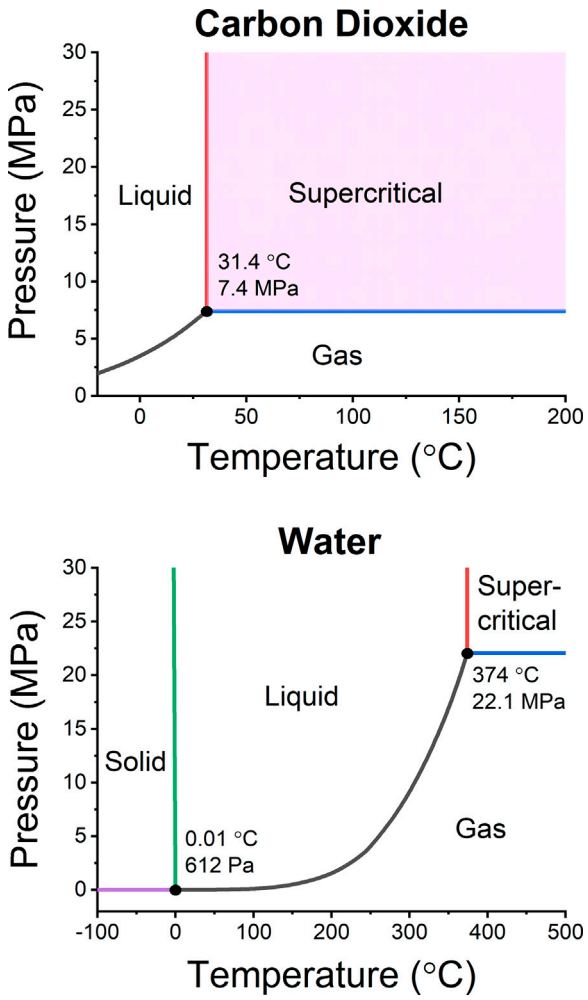

FIGURE 9
Phase diagrams of carbon dioxide (top) and water (bottom). The triple points and critical points are labeled. Extraction conditions are typically maintained at 50–150°C and 15–30 MPa for supercritical fluid extraction with CO_2 and 150–250°C and 2–10 MPa for subcritical water extraction. The pressure does not affect water as long as the liquid state is sustained; however, the density of supercritical CO_2 depends on the pressure, which positively affects analyte solubility.

Urey, an instrument based on SCWE and microchip electrophoresis (Mars Organic Analyzer, MOA, see Section 5.1), was initially under development for the Rosalind Franklin rover; however, it was descoped before the first prototype was manufactured (Amashukeli et al., 2008; Aubrey et al., 2008; Bada et al., 2008). However, a portable breadboard version of Urey was field-tested in the Atacama Desert (Skelley et al., 2007). Several follow-up studies were later published in which capillary electrophoresis—laser induced fluorescence (a separation and detection technique discussed in Section 5.1) was used to analyze amino acids extracted by SCWE with subsequent derivatization (Skelley et al., 2007; Stockton et al., 2009a; Chiesl et al., 2009). The instrument later continued developing at the Jet Propulsion Laboratory (Beegle et al., 2011; Kehl et al., 2019). The first fully automated prototype was demonstrated onboard a remotely controlled rover, as a Mars rover simulation mission, where it performed SCWE on solid samples in the Atacama Desert (Kehl et al., 2019; Mora et al., 2020). A microfluidic device with a slurry generated from a solid sample to facilitate sample transport has also been evaluated for SCWE applications (Sherrit et al., 2017). SCWE with carbonated water (5 wt% CO_2, pH ~3, 150°C) was used to extract amino acids from solid samples acquired from a geyser in Yellowstone National Park, followed by online chiral LC-MS (Abrahamsson et al., 2021).

Although (subcritical) water is efficient at extracting polar analytes, it can be used at elevated temperatures to dissolve non-polar analytes such as PAHs with limited solubility. For example, the solubility of pyrene (logP = 4.9) in water is 3 mg/L and 78 mg/L at 200°C and 300°C, respectively (Andersson et al., 2005). Consequently, large volumes of water (>8× solvent to sample ratio) are needed even for the extraction of light PAHs (Stockton et al., 2009b), and recoveries of analytes such as stigmasterol (logP = 7.0, no solubility data reported for subcritical water) are practically zero (Luong et al., 2014). It has been shown that 300°C is required to extract hydrophobic organic matter from sedimentary rocks (Luong et al., 2015). Performing extraction above 300°C is typically not desirable because of degradation issues of most analytes, particularly beyond the critical point of water (T_c = 374°C, P_c = 22 MPa). Other techniques, such as supercritical fluid extraction or liquid extraction with organic solvents, may be preferred and are discussed later.

4.3 Supercritical fluid extraction

Supercritical CO_2 ($scCO_2$) is a complementary solvent to water with similar solubility properties as n-hexane. Hence, $scCO_2$ is well-suited for wet chemistry analysis that targets fatty acids, sterols, steranes, polycyclic aromatic compounds, and other lipids. Faster extractions are possible compared to conventional liquid extraction techniques due to higher diffusivity and zero surface tension of $scCO_2$. The extraction temperature can also be ramped up (150°C) to desorb analytes bound to the solid sample matrix (Björklund et al., 1999), much like SCWE, except that $scCO_2$ maintains its polarity. The solubility is directly related to the density of the CO_2, which enables efficient preconcentration of analytes (e.g., on trap columns) at ambient pressures prior to downstream analysis techniques such as chromatography (Abrahamsson et al., 2019). Field-portable supercritical fluid extraction (SFE) instruments have previously been used to analyze polychlorinated biphenyls and PAHs in soils (Bøwadt et al., 1997).

One particular benefit is that salts are insoluble in neat $scCO_2$, rendering analysis of organic biosignatures in brines and other salt-rich samples compatible with downstream techniques, such as on-line mass spectrometry. For example, coronene was extracted from Martian regolith simulant by SFE with no measurable salts in the extract (McCaig et al., 2016). Furthermore, the Martian atmosphere contains 95% CO_2, which could potentially be harvested for SFE applications that use neat $scCO_2$. It has also been proposed that dry ice in the polar regions of Mars could be utilized as a natural extraction solvent for *in situ* SFE (Lang et al., 2002). SFE, in parallel with SCWE, followed by supercritical fluid chromatography (SFC) and LC with mass spectrometry detection, are utilized in the Supercritical CO_2 and Subcritical H_2O Analysis instrument (SCHAN; Lin et al., 2021).

4.4 Ultrasound-assisted extraction

Analysis techniques targeting biopolymers benefit or require that these targeted species remain intact. High-temperature extraction may not be feasible in these situations due to decomposition (Parro et al., 2011b). Instead, liquid extraction has been evaluated with various solvent compositions and surfactants at lower temperatures. Ultrasound-assisted extraction (UAE) has been applied to increase the extraction kinetics and to lyse cells (Fykse et al., 2003; Albero et al., 2019). The same mechanics also facilitate rupturing of solid particles to increase the accessibility of analytes. The sound waves originating from a probe form short-lived microscopic vapor bubbles that implode (cavitation process) and cause shockwaves in the sample (Albero et al., 2019). Relatively short processing times, less than a few minutes, are typically applied to avoid analyte degradation. It has been shown that UAE can be as efficient as the traditional Soxhlet extraction technique for PAHs and hopanes (Blanco-Zubiaguirre et al., 2015), and that it is more efficient than microwave-assisted extraction for multiple organic biosignatures (Timoumi et al., 2022). UAE is a part of most current biosensor instruments designed for solid samples (see Section 5.3). Although UAE improves the extraction kinetics (the extraction rate), it has been suggested that it does not improve the desorption of bound analytes. For example, nucleobases adsorbed to magnesium oxide were not extracted with higher recoveries when UAE or surfactants were applied, as opposed to traditional liquid extraction (Fornaro et al., 2013).

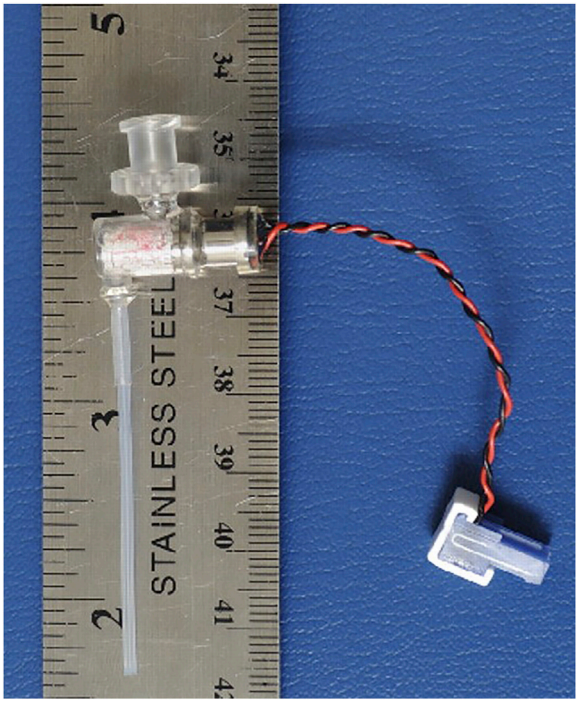

FIGURE 10
A miniaturized bead beater (OmniLyse) from Claremont BioSolutions. Some bead materials can act as an adsorbent from which the analytes can be subsequently eluted with appropriate buffer. Consequently, the bead beater can be used for lysis with or without solid-phase extraction of nucleic acids. Reprinted with permission from Vandeventer et al. (2011). Copyright 2011 American Society for Microbiology.

Non-polar organic solvents such as dichloromethane and hexane are not compatible with most affinity assays (Court et al., 2010). In order to extract hydrophobic analytes, surfactants are used in aqueous solutions with or without a small fraction of organic modifier such as methanol. Common buffers for molecular biology assays are often used, such as tris-buffered saline (TBS) and Polysorbate 20, also known as Tween 20 (Parro et al., 2011b). By adding Polysorbate 80 (1.5 g/L) to a water-methanol mixture (80:20 v/v), hydrophobic analytes such as squalene, stigmasterol, and PAHs could be extracted from pre-cleaned and spiked JSC Mars-1 sample analogs with recoveries of 17%, 8%, and >49%, respectively (Court et al., 2010, 2012). Liquid extraction with surfactants generally outperforms SCWE at ≤200°C for the very hydrophobic analytes mentioned earlier (Luong et al., 2014).

One of the main issues with surfactants is that they are not stable in solution for extended periods of time (Court et al., 2014). The same authors showed that the degradation rate greatly diminished at -20°C; however, recoveries were still lower than with fresh solutions. The degradation products can form interferents that may complicate downstream analysis. Finally, it should be noted that surfactants are generally not compatible with ESI-MS due to ion suppression and the fact that they give rise to large interfering signals.

4.5 Bead beating for cell lysis

For most chemical analysis instruments to detect metabolites inside cells or components of cell membranes, these must first be made accessible and extracted. Microorganisms, including bacteria, fungi, and algae, require lysing prior to extraction. Gram-positive bacteria and spores can be difficult to lyse due to their protective cell walls and coating structure (Harrison, 1991). Bead beating is a mechanical lysing technique often used in the laboratory and is currently investigated as a sample preparation step for DNA and RNA sequencing (see Section 5.4). It has been shown that bead beating is more efficient than UAE for extracting DNA from permafrost soil samples; however, its efficiency is sample-dependent (Raymond-Bouchard et al., 2022). A complete overview of nucleic acid sample preparation, including extraction, has been given by Emaus et al. (2020).

Miniature commercial bead beating kits with a large selection of microbeads are readily available (see Figure 10). Examples of such are the OmniLyse from ClaremontBio and SuperFastPrep from MP Biomedicals. Automated small systems that combine bead beating and extraction are also available, such as SimplePrep from ClaremontBio.

Combined pretreatment and extraction with zirconia/silica microbeads (10 μm) in a miniaturized system that lyses cells and spores, and adsorbs DNA and RNA to the beads been has shown to be a fit-for-purpose technique (Vandeventer et al., 2011). NASA's Wetlab-2 platform onboard the ISS used the microbead-beating technique without extraction buffers (OmniLyse from ClaremontBio) during the cell lysing step to extract and purify RNA (Parra et al., 2017). The Search for Extra-Terrestrial Genomes (SETG) instrument uses a modified Claremont BioSolutions solid-phase Purelyse bacterial genomic DNA extraction kit to isolate DNA and RNA from solid and liquid samples that are mixed with a binding buffer (low pH with ethylenediaminetetraacetic acid, EDTA; Mojarro et al., 2019). The adsorbed polynucleotides are then eluted with a high pH buffer containing EDTA. Extraction recoveries of 5%–14% from various Mars soil analogs spiked with 2.2×10^8 *Bacillus subtilis* spores (~1 μg DNA) per 50 mg of sample have been achieved by a method that included desalting, competitive binders (random hexamers), microbead beating, adsorption, and elution (Carr et al., 2017; Mojarro et al., 2017). Similar performance was later demonstrated with 50 mg Mars soil analogs containing 1.6×10^4 *Escherichia coli* cells or 1.6×10^4 *Bacillus subtilis* spores (~70 pg DNA) with recoveries ranging from 2%–15% and 5%–11%, respectively (Mojarro et al., 2019). The extraction recovery and the optimum methodology are sample-dependent.

4.6 Solid-phase extraction

Solid-phase extraction (SPE) is a commonly used technique for extraction from fluidic streams, mainly liquids, but also gas. It can also be used for sample clean-up by selectively adsorbing analytes of interest; for example, from a sample with high salt content or for preconcentrating dilute samples upstream from analytical instruments (Andrade-Eiroa et al., 2016b; Andrade-Eiroa et al., 2016a). In short, the SPE involves a small packed bed of material into which analytes adsorb or partition favorably. Subsequently, the captured analytes are most commonly released using a strong elution solvent.

Examples of applications for planetary science applications include desalting of samples containing amino acids on a microchip (Craft et al., 2020; Volkenburg et al., 2022), on-line preconcentration of amino acids before chromatographic analysis (Abrahamsson et al., 2021), and refocusing of extracted analytes for on-line extraction and chromatography (Abrahamsson et al., 2019).

SPE can also be used to adsorb analytes in the headspace of a sample, where small fibers are used, namely, solid-phase microextraction (SPME). The SPME-fiber can then be directly inserted into the desorption chamber of a GC-MS system. This technique has been used to identify and quantify PAHs in a Mars analog sample (Orzechowska et al., 2011).

5 Next-generation biosignature detection techniques for planetary applications

Certain trends have greatly influenced the viability of implementing various techniques, as is later shown for chip-based GC, CE, and LC. The concept of a completely integrated microfluidic system, also known as micro total analysis systems (μTAS), for sampling, sample pretreatment, separation, detection, and so on, was introduced in 1990 (Manz et al., 1990). Since then, major advances have been made in traditional manufacturing processes of microfluidic devices, such as wet etching, micromachining, micromilling, casting, hot embossing, and injection molding. Although many various materials, such as silicon, glass, polydimethylsiloxane (PDMS), poly (methyl methacrylate) (PMMA), paper, and 3D printed materials, are used for fabricating microfluidic devices, PDMS and glass remain the most commonly used (Yamamoto et al., 2021). The material must be carefully chosen depending on the application based on, for example, biocompatibility (adsorption of analytes), chemical resistance, thermal properties, physical strength, and employed detection technique. Developments are further facilitated by recent breakthroughs in 3D printing (Weisgrab et al., 2019; Balakrishnan et al., 2021). This emerging technology enables the fabrication of truly 3D devices rather than layered 2D build-up and avoids chemical bonding to form the enclosure around fluidic channels. Currently, 100 μm-sized microchannels can be manufactured by 3D printing; however, smaller sizes remain challenging (Balakrishnan et al., 2021). The recent developments and applications have been extensively reviewed (Berlanda et al., 2021; Yamamoto et al., 2021). Most of these technologies have not yet been adopted in instrument development for space flight applications but will likely do so in the upcoming decade. The advances in materials and engineering benefit many emerging technologies, such as chip-based gas chromatography, liquid chromatography, electrophoresis, nanopore sensing, and immunoassays.

The recent surge in nanotechnology has given rise to nanopore sensing, among other techniques. This has led to the development of the third-generation sequencing techniques and has enabled single-molecule detection (see Section 5.4). Advancements in electronics and specifically integrated circuits have been instrumental to nanopore sensing but also the general miniaturization of most chemical analysis instruments.

The techniques discussed in this section have never been used at any planetary body outside of Earth, and do not have spaceflight heritage in relevant environments. Consequently, the development cost of these techniques will likely be higher than for those techniques discussed earlier. However, on the basis of demonstrated performances of the next-generation techniques, these are also more capable in terms LODs and the number of organic biosignatures that can be detected. The order in which the techniques are discussed does not entail any judgment on their performance, TRL, or whether they are fit for purpose.

5.1 Capillary electrophoresis

5.1.1 Background

The first modern capillary electrophoresis (CE; inner diameters of ≤75 μm instead of ≥3 mm) instrument was developed in 1981 (Jorgenson and Lukacs, 1981), and gained tremendous popularity during the following decades (Lunte and Radzik, 1996). In essence, CE is a separation technique in which the analytes migrate in an established electric field within a channel as the primary mechanism, where the velocity depends on their electrophoretic mobility and the strength of the electric field. The secondary migration mechanism is governed by the electroosmotic flow (EOF), typically established by deprotonating the silanol groups in a fused-silica capillary, thus forming a double diffuse layer of cations that becomes the driving force of the bulk fluid within the capillary. The EOF velocity depends on the strength of the electric field and the potential between the two layers of cations (zeta potential) that is dependent on the capillary surface charge and altered by regulating the pH of the buffer.

The flat flow profile in CE reduces band broadening effects, which results in very narrow peaks, unlike in pressure-induced flow like in chromatography, where the flow profile is parabolic due to friction at the walls. CE is a well-established chemical analysis technique that is particularly useful for, but not limited to, the separation of inorganic ions, nucleic acids, amino acids, peptides, proteins, and metabolites. The interested reader is referred to the following sources regarding the fundamentals and applications of CE (Cech and Enke, 2001; Harstad et al., 2016; Voeten et al., 2018; Bernardo-Bermejo et al., 2020).

CE is a widely used separation technique in many disciplines, and its development is moving rapidly, where the most exciting topics over the last 2 years have been covered by Kristoff et al. (2020). Over the last few years, a plethora of advances have been made in preconcentration and sample introduction techniques, pseudo-stationary phases such as micellar electrokinetic chromatography (MEKC), chiral selectors and two-dimensional CE (Voeten et al., 2018). Nevertheless, CE has been criticized for its lack of repeatability, reproducibility, robustness, and ruggedness (Faller and Engelhardt, 1999; Boone et al., 2000; Schaeper and Sepaniak, 2000). However, in recent years, it has matured into a highly reliable technique. In an inter-laboratory trial, the relative migration time and peak area reproducibility were below 1.4% RSD and 30% RSD, respectively (Wenz et al., 2015). It should still be noted that the absolute migration times still vary substantially due to variations in the EOF and for *in situ* applications, and typically, an internal standard will be needed in order to establish the relative migration time. Kehl et al. (2022) have developed an alternative approach to continuously measure the flow, to normalize migration times based on the flow rate. The authors adapted a thermal flowmeter for CE and provided flow-compensated electropherograms with 25× improved RSD of migration times.

Various modes of separation are used and combined to achieve acceptable resolution of the analytes. Often capillary zone electrophoresis (CZE) and MEKC are used in combination or in a sequence of runs to analyze various analytes such as amino acids (Creamer et al., 2017). In brief, MEKC is used to separate analytes with a neutral net charge, which is performed by adding surfactants that form micelles that act as a pseudo-stationary phase. Often a strong EOF is utilized, and the micelles have an electrophoretic mobility in the opposing direction and, therefore, migrate at a reduced speed through the capillary. The migration times of analytes will be influenced by their partitioning between the micelles and the background electrolyte—similar to conventional chromatography. Furthermore, cyclodextrin is used to obtain chiral separation of enantiomers, which is further enhanced by coupling it with MEKC (Lu and Chen, 2002).

The ionic strength and the pH of samples have a major impact on the EOF in CE. To avoid or reduce the need for separate desalting steps, a high concentration (30–80 mM) of borate buffer is typically used and further improved by adding EDTA (Stockton et al., 2009a; Creamer et al., 2017). Duca et al. (2022) used a 35 mM borate buffer as the background electrolyte, which allowed for a maximum of 1 mM H_2SO_4, 10 mM Na_2CO_2, and 10 mM $MgSO_4$ (with 5 mM EDTA) in the injected sample before separation failed when analyzing amino acids. Alternatively, high concentrations of acetic acid (1–5 M) can be used to enable analysis of samples with high ionic strength (Santos et al., 2018; Mora et al., 2022). Samples containing up to 3 M NaCl or 1.5 M $MgSO_4$ were successfully analyzed using 5 M of acetic acid as BGE (Mora et al., 2022). Therefore, high concentrations of acetic acid may be a way forward; however, uncertainties remain in terms of compatibility with chiral separation and other methods that rely on a strong EOF, which is heavily suppressed by the low pH.

Miniaturized and portable CE systems have frequently been reported for purposes outside of space exploration. In brief, there are six main considerations in the design and manufacturing of an *in situ* CE system: channel dimensions, device material, the shape of the channel path, sample injection, detection technique, and placement, and generation of the electric field (Lewis et al., 2013). The systems typically fall into two categories, microchip electrophoresis (ME) systems and conventional capillary-based electrophoresis. An overview and the trade-off between the two platforms in miniaturized and portable systems are extensively discussed by Lewis et al. (2013). The benefits of CE are that capillaries are well-characterized and provide the maximum volume-to-surface ratio due to the cylindrical geometry. However, repeatable injections can be challenging with CE through the commonly used pneumatic injection technique. Alternatively, an injector valve can improve repeatability (Zamuruyev et al., 2021). ME, on the other hand, struggles with injection bias that consequently affects the measurement trueness (Duca et al., 2022). Furthermore, the channels often consist of two or more materials that may or may not be fully characterized, ultimately leading to unknown EOF characteristics. Furthermore, the fabrication process of creating the channels also influences the properties of the surface (Pugmire et al., 2002). Consequently, method development becomes more cumbersome for ME than for CE. In both cases of CE and ME, small channel dimensions are crucial to ensure low current to reduce Joule heating and lower power consumption. However, more narrow channels reduce the sensitivity because the optical pathway is diminished when optical detection techniques are used.

5.1.2 *In situ* planetary applications and state-of-the-art

CE was first proposed as a potential *in situ* instrument for Mars (Bada and McDonald, 1996; Bada et al., 1997) and the ISS (Orta et al., 1997) already in the late 90s', but has so far not been tested in a space environment. Whereas CE does not operate at high pressures like its chromatographic cousins, the lab-on-a-

TABLE 2 Summary of a selection of separated analytes and their LODs (given in parentheses as nM) using electrophoresis-based instruments in development for space applications. A slash (/) indicates co-eluting peaks without separation. Derivatization occasionally produces multiple compounds (byproducts and analyte derivatives) resulting in several peaks, which is not indicated below.

References	Analyte analyzed (LOD, nM)	Techniques and methods
Skelley et al. (2006)	**Exp. A:** Cytosine (2,100), adenine (2000), adenosine, valine, alanine/serine, glycine, 3'AMP, and 3′CMP	ME-LIF, offline labeling
	Exp. B: Urea, valine, alanine/serine, allantoin, and glycine	
	Exp. C: Isoamylamine, isopropylamine, ethylamine, methylamine, valine, aminoisobutyric acid, alanine/serine, and glycine	
	Exp D: Cadaverine, α-glucoseamine, ethylamine, methylamine, *β*-glucosamine, cadaverine, valine, AIB, alanine/serine, and glycine	
Chiesl et al. (2009)	**Method A (CZE):** Cadaverine, arginine, methyl amine, ethyl amine, propyl amine, butyl amine, citrulline, iso-leucine, valine (0.075), threonine, asparagine, alanine, glycine, glutamic acid, and aspartic acid	ME-LIF, offline labeling
	Method B (MEKC): tryptophan, arginine, phenylalanine, norleucine, leucine, AIB, methionine, norvaline, valine (0.08), citrulline, *α*-aminobutyric acid, *γ*-aminobutyric acid, histidine, taurine/*β*-aminobutyric acid, L/D-alanine, *β*-alanine, glycine, threonine, and L/D-serine	
Stockton et al. (2009b)	9,10-Diphenylanthracene (2.3), dibenzo[b,def]chrysene (0.8), anthracene (20,000), anthanthrene (1.1), benzo[a]pyrene (3.2), benzo[j]fluoranthene (50), fluoranthene (60), perylene (0.26), benzo[ghi]fluoranthene (800)	ME-LIF
Stockton et al. (2010)	Butyraldehyde/diethylketone/3-methyl-2-butanone, acetone (5), p-anisaldehyde/benzophenone (1,600), butyraldehyde/methylethylketone/ propionaldehyde, acetaldehyde (0.7), Formaldehyde (0.07)	ME-LIF, offline labeling
Benhabib et al. (2010)	Glycine (0.006), citrulline, leucine, threonine, valine, alanine, serine, glycine, glutamic acid, aspartic acid	ME-LIF, offline labeling
Mora et al. (2011)	Citrulline (0.9), valine (0.7), serine (1), alanine (1), glycine (1)	ME-LIF, on-chip labeling, LODs determined by offline labeling at higher concentrations
Stockton et al. (2011)	Heptanoic acid/octanoic acid/hexanoic acid, pentanoic acid, butyric acid, butyric acid (7), propionic acid (6), pyruvic acid, acetic acid, formic acid, malic acid (20), citric acid (230), α-ketoglutanic acid	ME-LIF, offline labeling
Kim et al. (2013)	Citrulline (16), valine (16), serine (16), alanine (16)	ME-LIF, on-chip labeling
Cable et al. (2013)	Ammonia, methylamine (2.4), ethylamine/butylamine (2.6), pentylamine (2.3), hexylamine (1.8), nonylamine (2.5), dodecylamine (1.9), hexadecylamine (1.4), octadecylamine (1.0)	ME-LIF, offline labeling
Mora et al. (2013)	2-mercaptoethanol (1.4), methyl 3-mercaptopropionate (3.1), 2-propanethiol (4.3), 1-propanethiol (4.4), 1-butanethiol (4.1), cyclohexanethiol (3.3), 3-methyl-2-butanetiol (5), 1-pentanethiol (7), butyl 3-mercaptopropionate (6), 1-hexanethiol (8), 1-heptanethiol (10), 1-heptanethiol, 1-octanethiol (10)	ME-LIF, offline labeling
Cable et al. (2014b)	Acetic acid, butyric acid, caproic acid, capric acid (1450), lauric acid (1260), myristic acid (5670), palmitic acid (85900), stearic acid (1570), arachidic acid (2110), behenic acid (1570), lignoceric acid (1110), cerotic acid (920), montanic acid (3770), melissic acid (3790)	ME-LIF, offline labeling
Creamer et al. (2017)	**Method A:** D-alanine (25), L-alanine (25), β-alanine (100), γ-aminobutyric acid (5), glycine (5), D-histidine (10), D-leucine (10), L-leucine (5), D-serine (25), L-serine (25), D-valine, L-valine (10)	CE-LIF, offline labeling
	Method B: D-aspartic acid (500), L-aspartic acid (750), D-glutamic acid, L-glutamic acid (750)	
Mora et al. (2020)	Leucine (1.3), valine (0.7), serine (3), alanine (3), glycine (2)	ME-LIF, automated fluidic management, on-chip labeling
Mora et al. (2022)	**No salt:** Glycine (1000), Alanine (1000), β-alanine (1000), serine (500), isovaline/valine (500), leucine (250), histidine (50), glutamic acid (100), aspartic acid (500), γ-aminobutyric acid (500), 2-aminoisobutyric acid (500), gly-gly (250), gly-gly-gly (50), leu-leu-leu (50), phe-val (50), adenine (100), cytosine (250), guanine (100), uracil (500), adenosine (50), cytidine (50), guanosine (100), uridine (250), thymidine (100)	CE-MS
	1 M NaCl: Glycine (2000), Alanine (2000), β-alanine (2000), serine (2000), isovaline/valine (2000), leucine (1000), histidine (1000), glutamic acid (1000), aspartic acid (2000), γ-aminobutyric acid (2000), 2-aminoisobutyric acid (1000), gly-gly (2000), gly-gly-gly (1000), leu-leu-leu (1000), phe-val (1000), adenine (2000), cytosine (2000), guanine (1000), uracil (2000), adenosine (1000), cytidine (1000), guanosine (2000), uridine (2000), thymidine (1000)	
	3 M NaCl: Glycine (20000), Alanine (20000), β-alanine (20000), serine (50000), isovaline/valine (5000), leucine (2000), histidine (5000), glutamic acid (1000), aspartic acid (2000), γ-aminobutyric acid (20000), 2-aminoisobutyric acid (20000), gly-gly (5000), gly-gly-gly (2000), leu-leu-leu (1000), phe-val (1000), adenine (10000), cytosine (10000), guanine (5000), uracil (1000), adenosine (1000), cytidine (2000), guanosine (1000), uridine (1000), thymidine (1000)	

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chip concept was easily conceived. The first chiral separation of derivatized amino acids (fluorescein isothiocyanate, FITC) with cyclodextrin on a ME system with 190 mm long × 150 μm wide × 20 μm deep channels on a 100 mm diameter glass wafer was demonstrated by Hutt et al. (1999). However, only a handful number of enantiomers were entirely resolved.

Since the first microchip electrophoresis (ME) instruments were developed, the research has focused on method development instead. ME for *in situ* analysis in space applications has almost exclusively been developed with laser-induced fluorescence (LIF) detection, and consequently, much attention has been given to optimizing labeling protocols for various target analytes. Examples of CE-LIF and ME-LIF include chiral and achiral separation of amino acids (Skelley and Mathies, 2003; Stockton et al., 2009a; Chiesl et al., 2009; Creamer et al., 2017; Fujishima et al., 2019), peptides (Fujishima et al., 2019), amines (Skelley et al., 2006; Stockton et al., 2009a; Cable et al., 2013; Cable et al., 2014a), aldehydes and ketones (Stockton et al., 2010), nucleobases (Skelley et al., 2006; Fujishima et al., 2019), carboxylic acids (Stockton et al., 2011; Cable et al., 2014b), thiols (Mora et al., 2015), and PAHs (Stockton et al., 2009b). The instrument LODs are typically in the μg/L range (see Table 2), where the most high-performance methods targeting amino acids have LODs in the range from 5 nM to 750 nM (~0.4 μg/L up to 100 μg/L; Creamer et al., 2017), except for valine that could be detected as low as 75 pM (9 ng/L; Chiesl et al., 2009).

LIF has almost exclusively been the detector of choice for detecting organic biosignatures. However, very few analytes of interest contain a natural chromophore; therefore, fluorescent labeling is necessary. Several issues arise when performing ultra-trace analysis (<1 ppm) in combination with derivatization. Derivatization reagents form byproducts during the reaction step that need to be separated from the labeled analyte, which in many applications is not completely viable due to the vast number and relatively high concentration of byproducts interfering with low-concentration analysis. An excess of reagent is, however, needed to ensure efficient labeling of analytes at very low concentrations. Derivatization efficiency also depends on the sample matrix (Fernández-Fı´gares et al., 2004); however, this has not been studied in regard to environmental samples. It is noteworthy that a substantial number of publications report LODs based on derivatizations performed at high concentrations that are subsequently diluted and hence only represent instrumental LODs rather than the relevant method LODs. In this vein, a considerable amount of work has been dedicated to developing derivatization protocols that provide efficient labeling at low concentrations with minimal byproduct formation.

Further development of the microchip platform has enabled on-chip derivatization, dilution, and addition of internal standard, as well as carrying a large number of solutions that are needed for each unique and selective CE method (Mora et al., 2011; Mora et al., 2012; Kim et al., 2013). These instruments, which use LIF detection, only weigh approximately 11 kg, are approximately 13 cm × 31 cm × 33 cm, and require only <15 W during operation (see Figure 11). Channel redundancy is readily implemented on a microchip, which reduces the overall risk of, for example, clogging by having several CE channels available for separation (Benhabib et al., 2010). On-line SCWE coupled with ME-LIF onboard a remotely controlled rover was used to detect five amino acids from soil in the Atacama Desert, Chile (Mora et al., 2020).

Capacitively coupled contactless conductivity detection (C^4D) is an electrochemical detection technique that has seen tremendous growth and quick adaption in the general field of CE over the two decades (Kubáň and Hauser, 2011, 2018). C^4D is well-suited for many analytes, particularly those that do not contain a chromophore or are not suitable for derivatization, and it is especially suitable for inorganic analysis. Two of the key benefits are that the electrodes are not in direct contact with the fluid hence avoiding dispersion, corrosion of the electrodes, and decoupling from the high voltages of the CE; and secondly, due to the simplicity, the detector is much smaller in size than optical-based detectors, allowing for further miniaturization. C^4D in tandem with LIF (Santos et al., 2018), or MS (Beutner et al., 2018), provides additional information that is useful for identifying or resolving co-migrating species. Santos et al. (2018) demonstrated how CE-C^4D could be utilized to simultaneously analyze inorganic species and amino acids. The C^4D is easily incorporated onto ME platforms (Kubáň and Hauser, 2018), and has been proposed for an integrated system based on CE-C^4D-LIF-MS (Creamer et al., 2020; Zamuruyev et al., 2021).

Over the last decade, CE hyphenated with MS (CE-MS), has gained mainstream popularity and has recently been conceptualized as an *in situ* instrument for the future exploration of the Ocean Worlds (Creamer et al., 2020; Brinckerhoff et al., 2022). A miniaturized and automated microfluidic sample delivery platform compatible with CE-MS has been developed (Figure 12; Zamuruyev et al., 2021). The multifaceted implications and considerations of the required instrumentation for CE-MS have been thoroughly described (Bonvin et al., 2012; Lindenburg et al., 2015). Applying and decoupling the voltages on the CE (>15 kV) and the ESI (<4 kV) are the primary obstacles. Typically, the outlet, which also functions as the spray tip, is grounded, and the potentials are applied at the inlet of the CE and MS, respectively. However, because capillaries are made of fused-silica (electrically insulating), the electrical connection must be made elsewhere. The connection can be achieved through a conductive liquid pumped co-axially with the CE eluent (sheath liquid), and the two liquids come in contact at the end of the CE capillary (the spray capillary tip). Alternatively, the end of the CE capillary can be made porous (e.g., by hydrofluoric acid etching), allowing contact between the two liquids (sheathless interface). In the sheathless approach, the capillary extends beyond the outer

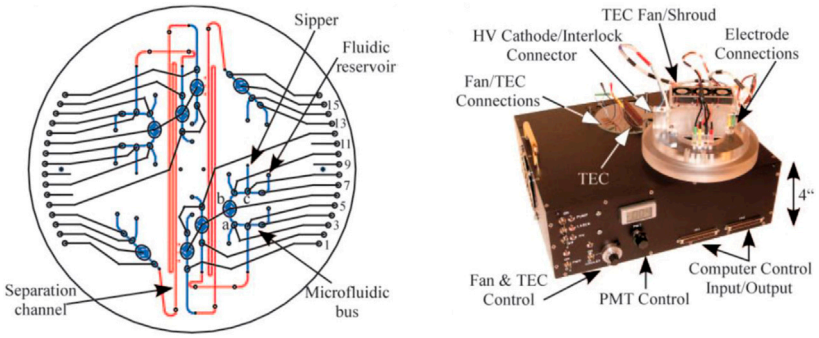

FIGURE 11
Illustration of the Mars Organic Analyzer (MOA) instrument that uses ME-LIF. In the left diagram the CE channel and other fluidic channels are highlighted. The packaged instrument is shown on the right. Reprinted from Skelley et al. (2005). Copyright 2005 National Academy of Sciences.

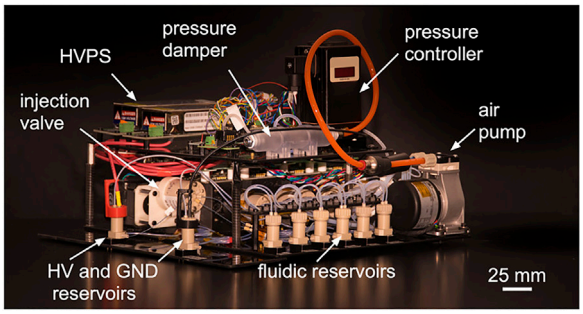

FIGURE 12
A portable and automated CE system with the main components denoted. Reprinted with permission from Zamuruyev et al. (2021). Copyright 2021 American Chemical Society.

liquid, and only the solution from the CE separation is partaking in the ESI process.

The co-axial sheath liquid interface (2–10 µL/min) is the most commonly used approach for hyphenating CE with MS (Stolz et al., 2019). However, nano-ESI benefits are only obtained at flow rates of a few tens of nL/min or lower (Schmidt et al., 2003; Jarvas et al., 2017). The commercially available sheathless CE-ESI-MS interface (CESI, Sciex) based on the work of Moini (2007) has, over the years, gained much attention. The sheathless porous interface for CE-MS can provide these flow rates so that true nano-ESI with high sensitivity (and less influence by matrix effects) is obtained (Busnel et al., 2010).

Narrow separation capillaries (≤30 µm inner diameter) and a reduced EOF are essential to maintain flow rates within the effective nano-ESI regime. However, the EOF plays a significant role in many separation methods, especially for analytes without a net charge, and it might therefore be difficult to adapt these methods for CE-MS with true nano-ESI. As mentioned earlier, methods robust against the ionic strength utilize high background electrolyte (BGE) concentrations that either require operation in the low nanoliter/min regime or substantial dilution by an added sheath liquid. Furthermore, the ionic strength of the BGE is important as excessive current may cause the electrospray voltage to deviate from its set point depending on the interface design, and potentially cause arcing in the ionization source (Flaherty et al., 2017). Even with these challenges, CE-MS is a routine analysis method in many laboratories despite not operating in the nano-ESI regime; however, there is much room for improvement. For example, sheathless CE-MS provides LODs of cationic species such as (underivatized) amino acids and biogenic amines (e.g., spermine and spermidine) in the range of 30–1,000 nM (Hirayama et al., 2018), which are approximately 100× higher LODs than for CE-LIF. Mora et al. (2022) demonstrated that CE-MS (sheathless CESI) could detect several amino acids, nucleobases, and nucleosides with LODs of 1–2 µM, in the presence of 1 M NaCl, when using 5 M acetic acid as BGE and low injection volumes (7 nL). Nevertheless, MS provides additional resolving power for co-migrating analytes and identification potential of unknown analytes.

Long-term stability tests have shown that the fluorescent derivatization reagent carboxyfluorescein succinimidyl ester (CFSE) is stable for up to 2 years if stored at 4°C (Creamer et al., 2019). If the necessary reagents and solutions for MEKC for chiral separation of amino acids are stored dry and separated, they can withstand 300 krad TID radiation (Creamer et al., 2018). A C^4D detector has been developed for high-radiation environments, and the BGE for a C^4D method was shown to be stable for 2 years and survive radiation (Ferreira Santos et al., 2022).

CE is already suited for scenarios where limited sample is available, because, CE inherently uses little sample volume (low nL range) for each analysis due to the narrow capillaries or channels used for separation. Hence, ME is being developed as an instrument with the possibility of sampling plumes and detecting

organic biosignatures; for applications such as a fly-by mission at Enceladus (Mathies et al., 2017). Research and development are also being made to enable an integrated ME system to withstand high-speed impacts (Stockton et al., 2016), which would be well-suited for potential future missions that rely on kinetic penetrators for *in situ* subsurface investigations of Ocean Worlds (Gowen et al., 2011).

Due to the early miniaturization of CE, there is a vast amount of literature regarding method development and evaluation of the fitness for purpose in space applications. Although many analyte classes can be analyzed (see Table 2), they require different derivatization and separation methods. Furthermore, the peak capacity is often limited, and relatively few analytes are separated in each run. Coupling with MS adds a mode of separation, does not require derivatization, and enables a wider range of analytes analyzed with one method. However, CE-MS is not as sensitive as ME-LIF and would be a much larger instrument when combined. Compared to the previous generation of instruments (i.e., GC-MS, fluorescence spectroscopy, and Raman spectroscopy), CE provides orders of magnitude lower LODs, especially for amino acids and other polar analytes. Engineering concerns for spaceflight applications remain rather unknown at the moment. However, several integrated and miniaturized ME-LIF systems have been reported (Kim et al., 2013; Mora et al., 2020; Golozar et al., 2022). Method demonstrations and validation have mostly been conducted on benchtop instruments with off-line sample preparation, and some caution is advised when interpreting the fitness of purpose. More research is needed with integrated, end-to-end, analysis of environmental sample analogs of Mars or Ocean Worlds.

5.2 Liquid chromatography

5.2.1 Background

It is estimated that only 20% of known organic compounds can be analyzed by GC in their native form; as in, without decomposition or derivatization to render the target analytes volatile (Snyder et al., 2011). Although GC analysis is simpler than other chromatographic and electrophoretic techniques, and offers unmatched resolution and peak capacity, LC is often the preferred method in the laboratory for less volatile analytes. Modern ultra-performance liquid chromatography (UPLC)/SFC—MS platforms offers high reproducibility; making it possible for research groups to verify, compare, and scrutinize results from *in situ* missions (Chocholoušková et al., 2021). This is currently not easily achievable with conventional TV/Py-GC-MS methods. There have been a limited number of reported developments and adaptions of LC for astrobiology applications. Consequently, this section covers said developments and the general status of miniaturized LC instruments primarily targeting point-of-care applications. The interested reader is encouraged to read the review series *Miniaturization of Liquid Chromatography Coupled to Mass Spectrometry* for a more in-depth coverage of the field (Medina et al., 2020; Mejía-Carmona et al., 2020; Vargas Medina et al., 2020).

The typical LC system consists of one or more pumps, an injector, a chromatography column, and a detector. A small volume of sample is injected into a stream of mobile phase, where the analytes are separated on the stationary phase packed inside the column based on molecular interactions. Columns packed with functionalized small particles (≤5 μm) are generally used to achieve comparable resolving power; consequently, high pressures are needed to achieve acceptable flow rates due to the flow impedance. Hence, high-performance liquid chromatography (HPLC) is often incorrectly referred to as high-pressure liquid chromatography. The most common LC technique is called reversed-phase LC, where the stationary phase is generally non-polar, and the mobile phase is mainly aqueous with an organic modifier such as methanol or acetonitrile. This mode of LC is generally used along with gradient elution—that is, when the fraction of organic solvent is increased over time to increase the elution strength. Consequently, sufficient separation of a wide range of polarities of the analytes can be achieved along with peak compression (sharper peaks).

Miniaturized separation is a relatively mature technology that mimics many of the trends seen in capillary electrophoresis. On-chip separation channels and capillary columns are readily available. However, traditional columns of 1 mm–2.1 mm inner diameter are rarely the main contributors to an LC instrument's overall mass and volume. Nonetheless, columns with narrow IDs substantially reduce the required flow rate and solvent consumption. Miniaturized detectors based on MS, spectroscopy, and electrochemistry have been covered earlier in this review. This section mainly focuses on fluid delivery technology because it remains the most challenging component to miniaturize to render LC truly portable and suitable for *in situ* biosignature detection.

5.2.2 Fluid delivery systems

Mechanical pumps in commercial benchtop instruments, such as syringe and dual piston reciprocating pumps, capable of delivering 40 MPa or above, are bulky and limited to the laboratory environment. Most commercial UPLC systems are operated at flow rates of a few hundred μL/min; however, miniaturized systems are generally designed for a few μL/min down to tens of nL/min. Achieving accurate and pulse-free flow at these low flow rates is challenging. For example, in a dual piston pump, pulses arise when one piston engages in the delivery, while the other piston is retracted to refill the chamber. In order to achieve stable flow rates, modern systems use feedback control based on flow rate measured downstream (Šesták et al., 2015). The development of commercial nanoLC has not reduced the instruments' size,

but has substantially reduced the sample volume and mobile phase consumption rate (Sharma et al., 2015b).

Multiple portable LC instruments with mechanical fluid delivery sub-systems have been demonstrated; for example, using a single stepper motor-driven syringe pump that operated up to 110 MPa and weighed 1.4 kg (Sharma et al., 2014). A second pump was added to provide the option of gradient elution, with a volume of 32.5 µL in each syringe. This fluid delivery system weighed 4 kg and consumed 29 W (Sharma et al., 2015a). Subsequent developments of the same instrument resulted in a 5.9 kg LC system that utilized larger stepper motors to increase the maximum pressure and pumping accuracy (Zhao et al., 2017). A series of studies utilized syringe pumps and small breadboard valves from LabSmith that together formed portable instruments with a pressure limit of approximately <20 MPa (Li et al., 2015b; Coates et al., 2020; Lam et al., 2020; Hemida et al., 2021a; Hemida et al., 2021b). For example, Lam et al. (2020) demonstrated a portable medium-pressure capillary LC instrument (2 kg, 245 mm × 185 mm × 160 mm) that included UV/Vis absorbance detection with LODs of <10 µg/L for a few pharmaceutical compounds. The developed instrument and method provided retention time shifts of <0.1% RSD and peak areas <0.3% RSD (Lam et al., 2020). A similar instrumental setup was packaged into a briefcase (7.2 kg, 300 mm × 450 mm × 120 mm) and tested in the field along with a portable (not miniaturized) mass spectrometer for the analysis of polyfluorinated compounds in environmental samples (Hemida et al., 2021a).

A conceptually different approach uses a high-pressure gas source to drive the mobile phase in a reservoir. This approach enabled the detection of PAHs with high flowrate stability (0.29% RSD at 148.8 µL/min, limited to isocratic elution) with a portable instrument weighing 4.2 kg, including 150 ml of mobile phase and UV/Vis absorbance detection (Chatzimichail et al., 2021). Constant-pressure fluid delivery systems operating at lower pressures have been demonstrated multiple times (Wang et al., 2009, 2010; Kiplagat et al., 2010).

Electrochemical pumps without moving parts have been under development for over 20 years (Lynch et al., 2018; Rahimi et al., 2020). They provide an impressive footprint that can fit onto a chip, and are free of moving parts. Electroosmotic pumps (EOPs) offer pulse-free flow where the direction and the flow rate are easily manipulated by varying the applied potential and its polarity. The technique has been demonstrated up to 120 MPa (Gu et al., 2012), and has generally been used to deliver a few hundreds of nL/min (Haghighi et al., 2018). Lynch et al. (2017) demonstrated a typical implementation of EOP in a miniaturized instrument of about 3 kg (excluding detector) capable of gradient elution. Despite the potential of EOPs, it has not yet reached commercial and mainstream success, partly due to the need for high voltage (typically several kV), incompatibility with high organic content mobile phases, flowrate fluctuation, and chemical breakdown within the pumping elements (Haghighi et al., 2018; Lynch et al., 2018). Electrolysis-based fluid delivery for low-pressure chip-based LC has also been demonstrated (Xie et al., 2005). Far too many on-chip fluidic delivery system concepts have been developed to be accurately covered here, and the reader is referred to the comprehensive review by Haghighi et al. (2018). However, to our knowledge, none of these are currently in development for space applications.

Open-tubular columns, as opposed to conventional packed columns, do not require as high pressures to function due to lower flow impedance. This provides an opportunity to use smaller mechanical pumps or pneumatic systems based on constant pressure (Kiplagat et al., 2010; Shelor et al., 2014). This approach has been adopted for an ion-exchange LC instrument intended for *in situ* astrobiology applications (e.g., Shelor et al., 2014).

5.2.3 Astrobiology applications and state-of-the-art

Compared to the other techniques discussed in this manuscript, there have been relatively few reported studies on the viability of LC for *in situ* organic biosignature detection; likely due to the difficulties in manufacturing small pump devices that are mechanically rugged for space application. The reported literature with an astrobiology focus can be classified into four categories: analysis of analog samples using benchtop instrumentation, on-chip reversed-phase LC and ESI, supercritical fluid chromatography, and miniaturized ion chromatography (see Table 3).

For example, benchtop LC-MS was used to detect amino acids, dipeptides, and sugars from a cometary ice analog (Eddhif et al., 2018). A similar study that also incorporated a trap-column prior to separation, also known as column-switching methodology, showed that amino acids, oligopeptides, and nucleobases could be separated and detected at approximately 1 µg/L (Ribette et al., 2019). Liu et al. (2008) used ion-pair reversed-phase LC coupled with MS to detect underivatized amino acids with instrument LODs ranging from 3 µg/L to 800 µg/L. The authors used the developed methodology to analyze amino acids from Mono Lake, Death Valley, and Haughton Crater. LC has been extensively used to characterize meteorites, including chiral amino acids, nucleobases, and other biosignature precursors (e.g., Glavin et al., 2012a; Burton et al., 2012; Ruf et al., 2019; Oba et al., 2020). Most methods require gradient elution and frequently use derivatization or ion-pairing to analyze amino acids. Abrahamsson et al. (2021) developed a column-switching method with strong cation exchange as SPE followed by enantiomeric separation on a chiral stationary phase using only carbonated water as a mobile phase in an isocratic method. LODs determined with MS detection were in the range of 0.5–500 µg/L (median LOD was 2.5 µg/L), and 12 of the 18 chiral pairs tested were separated. The same study also

TABLE 3 Summary of a selection of separated analytes and their LODs (given in parentheses as µg/L, unless stated otherwise) using liquid and supercritical-based chromatography instruments in development for space applications.

References	Analyte analyzed (LOD, µg/L, unless stated otherwise)	Techniques
Shelor et al. (2014)	Acetate (≤500 nM), formate (≤500 nM), bromate (≤500 nM), Cl^- (≤500 nM), chlorate (≤500 nM), Br^- (≤500 nM), benzoate (≤500 nM), nitrate (≤500 nM), sulfate (≤500 nM), chromate (≤500 nM)	Open tubular column ion chromatography
Shelor and Dasgupta (2017)	Tartaric acid (35), citric acid (23), malic acid (18), glycolic acid (16), formic acid (8), lactic acid (24), fumaric acid (32), α-hydroxyisobutyric acid (36), succinic acid (35), acetic acid (24), glutaric acid (92), propanoic acids (77)	Packed column ion exclusion chromatography
Sricharoen et al. (2019)	Li^+ (0.8), Na^+ (1.5), NH_4^+ (2.4), ethanolamine (10), methylamine (5), diethanolamine (16), Rb^+ (24), triethanolamine (16)	Packed column ion chromatography
Abrahamsson et al. (2019)	**Exp. A:** Naphthalene (2), anthracene (0.4), 9-phenylanthracene (0.5), fluoranthene (0.4), pyrene (0.7), cinnamic acid (3), capric acid (40), lauric acid (30), myristic acid (20), palmitic acid (20), stearic acid (15) **Exp. B:** thionaphthene (3), isoquinoline (0.02), dibenzothiophene (0.6), 7,8-benzoquinoline, acridine (0.03), indole (1)	Supercritical fluid extraction—Supercritical fluid chromatography—UV/Vis—MS
Abrahamsson et al. (2021)	Gly (5), D-Ala (2.5), L-Ala (2.5), D,L-Ser (5), D-Pro (25), L-Pro (2.5), D-Val (0.5), L-Val (0.5), D,L-Thr (10 L), D,L-Cys (500), D-Leu (0.5), L-Leu (0.025), D-Asn (5), L-Asn (5), D,L-Asp (50), D-Gln (2.5), L-Gln (2.5), D-Lys (2.5), L-Lys (5), D,L-Glu (5), D-Met (0.25), L-Met (0.25), D,L-His (0.025), D-Phe (2.5), L-Phe (2.5), D-Arg (0.5), L-Arg (0.5), D,L-Tyr (1), D-Trp (2.5), L-Trp (2.5)	Column-switching chiral liquid chromatography

demonstrated on-line SCWE applied to a spicular sinter sample from Lemon Spring in Yellowstone National Park (Abrahamsson et al., 2021).

On-chip columns with integrated ESI capabilities are readily available commercially, such as the ionKey/MS (Murphy et al., 2014), Trizaic nanoTile (Hughes et al., 2011), HPLC-Chip (Yin and Killeen, 2007), and cHiPLC-nanoflex (Hebert et al., 2011). Some of these commercial options include on-chip trapping (column switching), and on-chip flow channels that are interfaced with an external rotor to form valves; however, none incorporate on-chip injection or fluid delivery. Similar devices have also been developed for *in situ* organic analysis applications (Getty et al., 2013; Southard et al., 2014; Southard et al., 2016a; Southard et al., 2016b). As with any nano-fluidic device; clogging, detecting tiny leaks, and extra-column dispersion remain practical challenges due to miniaturization (Desmet and Eeltink, 2013; Sharma et al., 2015b; Desmet and Broeckhoven, 2019).

SFC is another avenue currently being pursued. Although it is not strictly classified as an LC technique, modern SFC with packed columns is very similar to conventional LC (Tarafder, 2016). The main difference is that CO_2, with or without co-solvents, is used as the mobile phase in its subcritical or supercritical state. Due to the non-polar nature of $scCO_2$ (similar to hexane), it is ideal for extracting and separating lipids. Abrahamsson et al. (2019) developed a novel SFE-SFC-MS method for analyzing free fatty acids and polycyclic aromatic compounds from aqueous samples. These techniques, along with conventional LC, are used in the Supercritical CO_2 and Subcritical H_2O Analysis instrument (SCHAN; Abrahamsson et al., 2022), an organic biosignature analysis instrument, which is a part of the payload on an astrobiology-focused mission concept to the Mars north polar region, Mars Astrobiology Science Exploration (MASEX; Lin et al., 2021).

Another area of research currently geared towards *in situ* chemical analysis is ion chromatography. Although it is not strictly a technique for organic biosignature detection, it does enable the analysis of small ionic organic compounds in addition to inorganic compounds. Analysis of both inorganic and small organic cations (e.g., alkali metals, amines, and alkanolamines) and anions (e.g., chloride, oxychlorides, and organic acids) are readily analyzed by ion chromatography coupled with conductivity measurements (Shelor et al., 2014; Shelor and Dasgupta, 2017). Separation is achieved with a purely aqueous mobile phase that is modified; for example, with carbon dioxide or an inorganic base (Shelor and Dasgupta, 2017; Shelor et al., 2017; Sricharoen et al., 2019). Detection limits of <100 µg/L are obtained for many small ionic analytes (Shelor et al., 2014; Shelor and Dasgupta, 2017; Sricharoen et al., 2019). Ion chromatography also allows for the analysis of perchlorates (Shelor et al., 2014), which has been of particular interest on Mars (e.g., Hecht et al., 2009; Glavin et al., 2013).

Ion chromatography differs from other LC techniques, such as reversed-phase LC, by the mode of separation and by the required hardware. In addition to a conventional HPLC system, adding a mobile phase generator and an ion suppressor is often desirable. The mobile phase generator, as the name suggests, increasingly adds the modifier to the aqueous mobile phase to increase the elution strength over

time, providing gradient elution. The ion suppressor lowers the ionic strength of the mobile phase prior to conductivity measurements by neutralizing the acid or base that was added by the mobile phase generator. Ion exchange membranes are instrumental to this end, and their role in analytical chemistry has been extensively reviewed (Dasgupta and Maleki, 2019). These components have been demonstrated at a miniaturized scale. For example, Chouhan et al. (2020) demonstrated a small hydroxide eluent generator suitable for flowrates in the nL regime and capable of gradient elution.

In the pursuit of a portable or *in situ* small ion analysis instrument, open tubular columns have been the preferred choice. The commercially available capillaries of various materials with narrow inner diameter (<50 µm) have increased tremendously over the last decade, which offers the analytical chemist more options in method development. Open tubular columns' benefits are extremely high theoretical separation efficiency, low flow impedance, and simplicity. However, the loading capacity is very limited compared to packed columns due to the much lower surface area. For example, 4 nL of injection volume is slightly too large for 25 µm inner diameter capillary columns (Chouhan et al., 2020). Lower injection volumes are currently difficult to achieve with acceptable repeatability. Naturally, the optimum injection volume also depends on the ionic strength of the sample. Due to the low flow impedance, less than 1 MPa is needed to achieve flowrates <1 µL/min using a <1 m capillary column with 25 µm inner diameter (Huang et al., 2021).

Bare open tubular capillary columns, such as those described for ion chromatography applications, circumvent many challenges relating to high-pressure fluid delivery systems. The approach is also applicable to conventional LC techniques, and other capillary column variants such as wall-coated open tubular and porous layer open tubular columns, and these may in the future offer a compromise between chromatographic efficiency, loading capacity, solvent usage, and backpressure (Xiang et al., 2021).

In summary, LC offers many of the same capabilities as CE regarding targeted analyte classes and LODs. LC achieves orders of magnitude lower LODs compared to techniques previously used in spaceflight applications. Derivatization may be used but is often not required for LC analysis, which reduces some risks and issues with sample matrix effects. However, the technique has been relatively unexplored for spaceflight applications due to issues with miniaturization in the previous decade, particularly regarding fluid delivery systems. Adaptation for spaceflight is currently ongoing, and a miniaturized integrated system was recently reported (Abrahamsson et al., 2022). More validation and demonstrations would increase the confidence in the suitability for spaceflight adaption.

5.3 Biosensors

5.3.1 Background

Bioreceptors in analytical chemistry have extremely high affinity and specificity for analytes of interest and form biosensors when combined with a detection scheme. Antibodies, nucleic acids, cellular structures, biomimetic materials, and molecular imprinted polymers are the most commonly used receptors in the laboratory. Methods and instruments in development for space applications have primarily adopted antibodies and nucleic acids, also known as aptamers, for sensitive organic biosignature detection—these are covered in this section. However, there is a vast number of alternative bioreceptors or affinity receptors that are not covered here. A few noteworthy instruments that have been developed, or are under development, are the Life Marker Chip (LMC; Sims et al., 2005; sims et al., 2012; Martins, 2011), the Signs Of Life Detector (SOLID; Parro et al., 2011b; Moreno-Paz et al., 2018), the Biochip for Organic Matter Analysis in Space (BiOMAS; Baqué et al., 2011b), and the Planetary Life Explorer with Integrated Analytical Detection and Embedded Sensors (PLEIADES) chip (Nascetti et al., 2019). The LMC mainly targeted small organic molecules (ca. <1,000 Da), and the SOLID instrument has focused on larger biomolecules (ca. >1,000 Da). The LMC was initially intended to be a part of the Rosalind Franklin rover instrument suite, but was later descoped.

Antibodies are the conventional subset of protein-based receptors. The variable region known as the complementarity-determining region (CDR), which consists of an amino acid sequence, has a high affinity towards a target antigen (analyte) through non-covalent interactions (Lipman et al., 2005). The specific site to which an antibody binds is called an epitope. An antigen may have multiple epitopes to which different antibodies bind. Furthermore, epitopes may also be very similar, causing the same antibody to bind several different antigens and consequently producing false positives, also known as cross-reactivity.

Antibodies can be obtained as monoclonal antibodies (mAbs) that are produced *in vitro* using tissue-culture techniques or as polyclonal antibodies that are easily obtained through the production in animals, typically rabbit. Monoclonal antibodies ideally only bind to one antigen and therefore have very high specificity. The process of identifying and producing monoclonal antibodies is, unfortunately, very laborious. On the other hand, polyclonal antibodies contain multiple epitope specificities. Whether to use monoclonal or polyclonal antibodies is usually decided by their commercial availability or availability from other researchers. If an antibody is not available off-the-shelf, then polyclonal antibodies are rather easily produced within a few months, contrary to monoclonal antibodies that take years and require more expertise to produce (Lipman et al., 2005).

Aptamers are an alternative to antibodies. These are typically single-stranded oligonucleotides (single-stranded DNA or RNA) that may have high specificity for a range of target molecules such as nucleic acids, proteins, metal ions, and other small molecules.

The antibodies and aptamers can be used in various configurations for detecting analytes of interest, with either mobile (in solution) or immobilized antigens or bioreceptors, with or without fluorescent labeling. The various configurations relevant for life detection have been reviewed elsewhere (Parro et al., 2008b). The following is a short overview of the two most commonly used methodologies: the sandwich and competitive assays.

Instruments that target large molecules (e.g., proteins and other biopolymers) primarily utilize a sandwich immunoassay where the immobilized bioreceptors bind to an antigen that is in solution, followed by the addition of fluorescently-labeled antibodies that also bind to the antigen to form a sandwich (Parro et al., 2008b). Sample and fluorescent probes are washed off in multiple steps. Consequently, bright spots will arise under UV excitation where an antigen has been sandwiched. The immobilized antibodies are sometimes referred to as capturing antibodies, and the fluorescently labeled antibodies are detector antibodies. This methodology requires that an antigen carry two similar epitopes (i.e., larger molecules) to generate a positive result. The assay is useful for detecting whole cells, high molecular weight complexes (organelles, membranes, cellular debris, and humic substances), biological polymers (proteins, polysaccharides, and nucleic acids), and organo-mineral complexes (Blanco et al., 2015). An example of a complete protocol for multiplex fluorescent antibody microarray that includes analysis of cross-reaction and unspecific binding has been reported by Blanco et al. (2015).

Smaller molecules with only one epitope require a fluorescent competitive inhibition assay. In the competitive assay, a conjugate of the analyte of interest and a carrier protein (e.g., bovine serum albumin) is immobilized on a solid support. The immobilized conjugates act as a capturing probe for fluorescent antibodies. When the sample is added, the analyte present will bind to the fluorescent-labeled antibodies that will be washed out in the following step; because, they no longer bind to the immobilized conjugate. Contrarily to the sandwich array, the competitive assay will produce lower fluorescent signal with higher analyte concentration as the fluorescent probes are washed off. Competitive assays do not provide as low LODs as sandwich assays (Parro et al., 2011b).

Multiplex detection (i.e., detection of multiple analytes) is performed by applying a cocktail solution to all spots simultaneously, where each spot contains affinity receptors for each analyte (see Figure 13). Such operation generally comprises injection of the sample to a flow cell containing the microarray, incubation, washing, dissolution, and addition of a fluorescent antibody cocktail from accessory chambers, incubation, washing, fluorescent excitation with a laser beam, and image capture with a CCD camera. Such methodology has, for example, been described in detail by Parro et al. (2008a). As the number of target analytes increases, the sensitivity decreases and the risk of cross-reactivity needs to be more carefully monitored (Rivas et al., 2008). Thus, it is essential to factor in that LODs deteriorates as the number of target analytes increases, and the LODs reported for a single analyte assay are not necessarily representative.

More recent developments include utilizing autonomous capillary force-driven microfluidic networks and chemiluminescence bioassays instead of fluorescent probes (Roda et al., 2018; Nascetti et al., 2019; Zangheri et al., 2019). Chemiluminescence detection, where light is generated through a chemical reaction, provides a few distinct advantages, such as not needing an excitation light source, and consequently, there is no light scattering or autofluorescence from interfering sample components (Roda et al., 2016).

5.3.2 Applications

Antibodies can be produced against specific molecules, environmental extracts, or whole cells (see Table 4). For example, Parro et al. (2008b) have focused on producing antibodies against analytes in extracts from the acidic, iron-, and sulfur-rich Rio Tinto area. This area serves as a Mars analog, and chemolithoautotrophic bacteria such as *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans* are dominant in these samples. These bacteria can subsequently rapidly be detected and identified in unknown samples (Parro et al., 2005). The multiplex sandwich array has mainly been used to pursue detecting microbial communities, which has been demonstrated multiple times with extracts of samples from extreme environments such as the Atacama Desert (Parro et al., 2011a; Blanco et al., 2013; Crits-Christoph et al., 2013; Fernández-Martínez et al., 2019), Antarctica (Lezcano et al., 2019), and other sites (Martin-Cuadrado et al., 2019). Unlike other techniques discussed in this review, immunoassays with receptors against cells do not require a cell lysing step prior to detection (Rivas et al., 2008).

The first stereoselective antibody against phenylalanine was demonstrated by Kassa et al. (2011). Later chiral discrimination of amino acids was adopted in the development plan of the LMC (Sims et al., 2012). A multiplex inhibitor immunoassay with specific antibodies towards D- or L-aromatic amino acids showed LODs of 100 μg/L and 500 μg/L, respectively (Moreno-Paz et al., 2018). The reported method did not discriminate between aromatic amino acids; specifically, it could not differentiate between phenylalanine and tryptophan. Additionally, other aromatic compounds were detected, such as finasteride and benzo [a]pyrene. The authors applied the method to extracts from Atacama Desert and kerogen-containing samples.

It was demonstrated with an early version of the SOLID instrument that some proteins (GroEL, thioredoxin, and glutathione-S-transferase) in solution could be detected at 10 μg/L

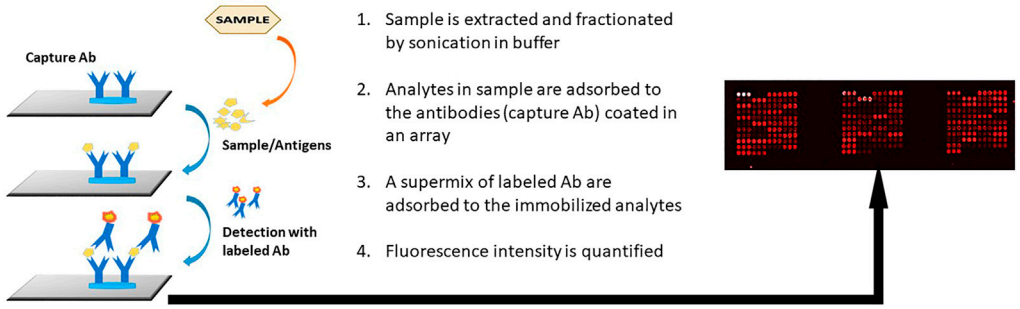

FIGURE 13
The principles of a sandwich-based immunoassay (left) and an illustration of the acquired data from a microarray where each antibody is printed in triplicate. Adapted from García-Descalzo et al. (2019).

TABLE 4 A brief selection of detected measurands and their LODs (given in parentheses) using biosensors being developed for space applications.

References	Measurand (LOD)
Fernández-Calvo et al. (2006)	2-amino-naphthalene (100–500 μg/L), 4-phenylphenol (100–500 μg/L), 4-tertbutylphenol (100–500 μg/L), terbutryn (0.2 ng/L), multiple peptides (1–10 μg/L), 10-kDa protein thioredoxin (0.5–1 μg/L), *B. subtilis* (10^4–10^5 spores/mL)
Rivas et al. (2008)	*B. subtilis* (10^3–10^4 spores/mL), *P. putida* (10^3–10^4 cells/mL), *B. fungorum* (10^4–10^5 cells/mL), *Salmonella bongorii* (10^4–10^5 cells/mL), 10-kDa protein thioredoxin (0.2 μg/L), 60-kDa protein GroEL (2 μg/L)
Parro et al. (2011b)	Multiple proteins (0.2–1 μg/L), 10^3–10^4 spores/mL
Blanco et al. (2013)	Mellitic acid (5 μg/L)
Moreno-Paz et al. (2018)	Atrazine (0.025 μg/L), sulfamethazine (10 μg/L), phthalylsulfathiazole (3 μg/L), pentachlorophenol (3 μg/L), finasteride (0.001 μg/L), L- phenylalanine (500 μg/L), D-phenylalanine (100 μg/L), peptide ModA (0.1 μg/L), benzo [a]pyrene (0.001 μg/L)
García-Descalzo et al. (2019)	Various cells (10^3–10^4 cells/mL)
Nascetti et al. (2019)	Adenosine triphosphate (60 nM)

(Parro et al., 2005). Another study using the same technique could detect 5 μg/L of mellitic acid, which was then successfully applied to extracts from Atacama Desert samples (Blanco et al., 2013). Smaller analytes such as naphthalene, 4-phenylphenol, and 4-tertbutylphenol were detected with LODs between 100 μg/L and 500 μg/L, and approximately 10^4–10^5 spores/mL with an inhibition methodology (Fernández-Calvo et al., 2006). Subsequent improvements enabled the detection of 10^3–10^4 spores/mL, approximately 1 μg/L of peptides, and 0.2–1 μg/L of thioredoxin with an instrument (SOLID3) that could perform both competitive and sandwich assays (Parro et al., 2011b).

The current state-of-the-art instrumentation designed for astrobiology applications contains <300 specific affinity receptors that include both antibodies and aptamers (Rivas et al., 2008; Parro et al., 2011b; Fairén et al., 2020). As mentioned earlier, the risks of unwanted interfering background, false-positive signals, and a reduction of LODs increase as the number of biosensors increase along with the increased complexity of the cocktail solution containing detector antibodies. This is exemplified by an order of magnitude increased LODs (from 10^3–10^4 to 10^4–10^5 spores/mL) when using a 200 antibody multiplex array compared to an assay with individual antibodies (Rivas et al., 2008). The SOLID3 instrument currently weighs 7 kg, including optics and sample preparation (ultrasonic-assisted extraction), whereas the sample analysis unit only weighs 1 kg (Parro et al., 2011b). The instrument is capable of performing both competitive and sandwich assays.

An example of a chemiluminescence bioassay includes a capillary force-driven device based on chemiluminescence that was used onboard the ISS to measure the health of an astronaut by quantifying the level of cortisol in saliva with an LOD of 0.2 μg/L (Zangheri et al., 2019). However, its potential for *in situ* space applications has remained relatively undocumented.

Compared to CE and LC, biosensors provide a very different approach to organic biosignature detection. The specificity is much higher than for separation-based chemical analysis techniques; however, the targeted analytes also have to be specified; an intrinsic difficulty unless it is clear precisely which biosignatures should be targeted. LODs are comparable with the other techniques discussed in this section; however, one tremendous benefit is that biopolymers (including those of whole cells) can easily be detected. Complete end-to-end instruments, including sample preparation, have been manufactured and demonstrated. Besides the limited number of analytes (≤300) that can be analyzed at once, the

stability of the biological receptors has presented some minor drawbacks. However, the stability of the biosensors in space environments has been scrutinized to a much larger degree than the other techniques (CE, LC, and nanopores) and is discussed separately below.

5.3.3 Environmental testing for spaceflight applications

Multiple studies have investigated the impact of radiation, thermal cycling, and long-time storage on affinity sensors (Le Postollec et al., 2009a; Le Postollec et al., 2009b; Baqué et al., 2011a; Baqué et al., 2011b; de Diego-Castilla et al., 2011; Derveni et al., 2012; Derveni et al., 2013; Vigier et al., 2013; Coussot et al., 2019b). In summary, these studies showed that antibodies and aptamers were resistant to radiation and maintained their performance. However, it was observed that grafted (immobilized) antibodies were more sensitive to general storage parameters (Baqué et al., 2011a). Antibodies are stable for extended periods of time when they are freeze-dried (Chang et al., 2005; Wang et al., 2007). Generally, the storage conditions such as temperature and moisture appear to be more critical than levels of radiation exposure.

Although ground-based radiation testing is valuable, it does not capture the reality of varying radiation fluxes, energies, and types during long-term storage and thermal variations. Two studies evaluated affinity sensors (i.e., antibodies and aptamers) that were stored for 566 days outside of the ISS (Vigier et al., 2013; Coussot et al., 2019a; Coussot et al., 2019b). Extensive validation and functionality testing were performed with a previously developed methodology (Coussot et al., 2018a; Coussot et al., 2018b; Coussot et al., 2018c). In summary, antibodies (anti-horseradish peroxidase Ab) or aptamers were evaluated either as covalently immobilized (grafted) or in the free form. The samples stored outside the ISS were exposed to 220 mGy. It was shown that both immobilized and free antibodies retained at least 40% of the binding efficiency after being exposed to freeze-drying, long-term storage, temperature fluctuations, and radiation (Coussot et al., 2019a). Similar experiments with aptamers showed that at least 50% retained their functionality after the entire study (Coussot et al., 2019b). The same study, complemented with ground-based experiments, suggested that radiation has minimal influence on assay performance; however, long-term storage with thermal cycles has a more considerable impact on the sensitivity. The chemiluminescence approach will be tested onboard a CubeSat mission, where the effect of radiation will be monitored (Brucato et al., 2021).

5.4 Nanopore sensing

5.4.1 Background

Earlier research and instrument designs have focused on detecting the presence of specific DNA or RNA by polymerase chain reaction (PCR), followed by staining and fluorescent detection (Thiel et al., 2011; Carr et al., 2013). Primarily, the well-conserved 16S and 23S ribosomal RNA (rRNA) has been of great interest. For example, within the ca. 1,500 nucleotides of the 16S rRNA, there are several 15–20-nucleotide segments that are almost identical in all known organisms on Earth (Pace et al., 1999). This fact has sparked interest in investigating the possibility that life first evolved on Mars while it was potentially habitable and organisms were transferred to Earth through meteorites (Gladman et al., 1996; Mileikowsky et al., 2000; Benner and Kim, 2015; Carr, 2022). The oldest DNA that has been recovered was over one million years old (van der Valk et al., 2021), which corresponds to the theoretical limit based on current lower detection limits of current techniques and the degradation rate of DNA in the most favorable conditions on Earth (Willerslev et al., 2004). Due to the lower temperatures on Mars and Ocean Worlds, it is possible that nucleic acids might survive even longer.

The development of third-generation sequencing (e.g., single-molecule real-time sequencing and nanopore sequencing) has dramatically improved portability and speed. The third-generation sequencing performs longer nucleotide sequence reads at a single molecule level as opposed to the second-generation, which involves fragmentation of long strands followed by amplification and synthesis from which the sequence is derived (Bleidorn, 2016). Hence, DNA and RNA can be detected and sequenced without amplification by PCR.

Nanopore sequencers, in general, use biological nanopores such as α-Hemolysin, MspA, CsgG, and Aerolysin nanopore proteins (Zhou et al., 2020). The most widely utilized and tested platform is the MinION from Oxford Nanopore Technologies (ONT), which is currently pocket-sized (105 mm × 23 mm × 33 mm). In nanopore sequencing, single-stranded DNA or RNA is driven through a small orifice (10^{-9} m in diameter), located in a membrane, by electrophoresis (Rezzonico, 2014). The electric field is held constant, and the ionic current is measured. Any polymer occupying the pore's space will partially block the flow of ions across the membrane. The ionic current depends on the nucleic acid composition that occupies the space within the nanopore from which the sequence can be derived as the DNA or RNA passes through.

Currently, ONT recommends a minimum input of 400 ng of nucleic acids without PCR and 1 ng of nucleic acids with PCR for sequencing with the MinION. However, low-input sequencing of 2 pg of DNA has been demonstrated without PCR (Mojarro et al., 2019). The long reads from single molecules are particularly useful for taxonomic identification at, or below, the species level (Benítez-Páez et al., 2016). Although nanopore sequencing at high concentrations of DNA or RNA can be achieved within minutes (Wang et al., 2017), lower amounts (on the order of pg) may require longer runs up to multiple days (e.g., Mojarro et al., 2019); depending on throughput goals. In addition, the stability of protein-based nanopores and data processing workflow have improved, almost removing any spurious signals that could cause

false-positive results; however, with adequately set noise thresholds, this risk is eliminated (Mojarro et al., 2018; Pontefract et al., 2018).

Due to favorable LODs, low power, and low mass, nanopores have quickly been adopted for instrument development that targets metagenomics (Rezzonico, 2014; Carr et al., 2016). Two key features of nanopore sequencing without PCR amplification include that it avoids PCR bias (Sabina and Leamon, 2015), and the ability to sequence non-standard bases, also known as xeno nucleic acids (XNA; Carr et al., 2017).

Nanopores can also be used to partially sequence proteins. Currently, neither nanopore nor tunneling techniques (see below) can distinguish all of the 20 different amino acids in terrestrial proteins (Restrepo-Pérez et al., 2018). However, in one study, 13 out of 20 proteinogenic amino acids have been successfully differentiated in a protein sequence of a single molecule in an aerolysin nanopore with the help of a polycationic carrier—namely, arginine heptapeptide (Ouldali et al., 2020).

Biological pores often require tight control of the feed solution, including pH, ionic strength, and temperature. Any deviation may adversely affect its performance. Solid-state nanopores, on the contrary, are more chemically, thermally, and mechanically stable (Haque et al., 2013). The holes can be manufactured with an electron beam from a transmission electron microscope (Danda and Drndić, 2019), among other methods. Currently, solid-state nanopores have limited spatial and temporal resolution, where the translation of DNA is too fast to generate more than a few data points (Yuan et al., 2020). A tradeoff exists between temporal resolution and signal-to-noise ratio, based on the bandwidth of the current measurement. At this moment, 10 MHz bandwidth (100 ns measurements) is the fastest demonstrated, with a dwell time of ~2–21 ns per nucleotide (Chien et al., 2019). Therefore, solid-state nanopores can determine the approximate diameter (based on the current) and the approximate polymer length (duration of signal) of, for example, DNA (Xia et al., 2022). Another demonstration detected ~1-nm-size charged particulates (including DNA) in pre-filtered Antarctic soil extracts using silicon nitride pores (Niedzwiecki et al., 2020).

Nanogaps are closely related to nanopore-based techniques (see Figure 14), where the tunneling current is measured between nanoelectrodes, located in nanopores or on a surface, rather than the ionic current across the membrane. The technique is used in an early-stage instrument called Electronic Life-detection Instrument for Enceladus/Europa (ELIE; Carr et al., 2020b; Lee, 2021). A thorough technical introduction and description of applications of detection by quantum tunneling and nanogaps have been given by Di Ventra and Taniguchi (2016), and a review covering the fundamentals has been given by (Lindsay et al., 2010). Whereas biological nanopores and solid-state nanopores are efficient for detecting single polymers such as DNA, RNA, or proteins (Restrepo-Pérez et al., 2018), their capabilities are

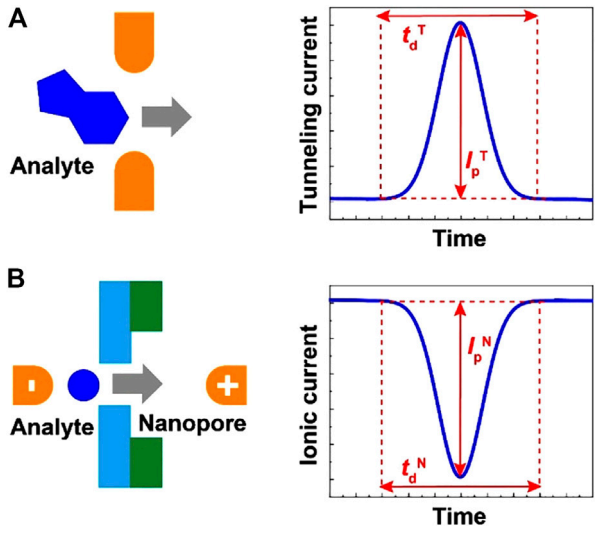

FIGURE 14
(A) The nanogap technique. A signal is acquired when a molecule passes the gap, allowing tunneling. The nanogap can be located in a nanopore or on a surface, and the analyte does not necessarily need to pass through a membrane. Analyte-nanogap interactions are on the order of milliseconds, which gives rise to a square-looking signal. **(B)** The nanopore technique. The current is decreased when an analyte occupies the pore's space. In the case of solid-state nanopores (illustrated here), the event occurs on the order of nanoseconds and the order of milliseconds for biological nanopores. Nanopores and nanogaps give chemical information about the analyte that may facilitate identification, derived from the signal's duration and intensity. Reprinted from Taniguchi (2020).

limited regarding small molecules. However, sensing over unmodified nanogaps enables the detection of single small molecules with low selectivity (Taniguchi, 2015). Additionally, the analyte interaction with a nanogap is on the order of milliseconds (equivalent to the translation speed of biological nanopores), compared to nanoseconds for typical solid-state nanopore applications, reducing the bandwidth requirement.

Even though nanogap sensing with functionalized electrodes has demonstrated that multiple amino acids can be distinguished, data complexity remains a challenge because each analyte can orient in various ways giving rise to several unique current signals (Zhao et al., 2014). Hence, chemometric techniques such as machine learning are needed to correctly classify each signal (Taniguchi, 2020). Finally, the emergence of nanodevices for single molecule detection has opened up multiple alternatives that have not yet been pursued for spaceflight applications; these have been reviewed elsewhere (Ohshiro and Taniguchi, 2022).

5.4.2 Applications

Many demonstrations with ONT's MinION have shown its capability of sequencing DNA and RNA in remote locations such as Atacama Desert (Bywaters et al., 2016),

Antarctic Dry Valleys (Johnson et al., 2017), paleochannels (Maggiori et al., 2020), and Arctic Permafrost Ice (Goordial et al., 2017). The MinION was also tested under Mars-like conditions at −60°C, 100% CO_2, and pressures between 400 and 500 Pa (Carr et al., 2019). Successful demonstrations have been performed during and after vibration exposure under microgravity (Castro-Wallace et al., 2017; Carr et al., 2020a). In its current format, it is not hampered by radiation it would experience during travel to Mars; however, additional shielding and transitioning from protein-based pores to solid-state pores is likely required for missions to Ocean Worlds (Sutton et al., 2019). Nanopore sequencing has been used onboard the ISS (Castro-Wallace et al., 2017; Burton et al., 2020). However, it is still unknown whether biological nanopores could survive the multiple years of a space mission.

The third-generation sequencing has certainly overcome previous issues for *in situ* detection of informational polymers in terms of power and mass. However, the main remaining challenges relate to the extraction and perseverance of native nucleic acids from complex and oxidative samples. In terrestrial applications, workup of environmental samples includes filtering aqueous samples to remove debris and removing enzyme inhibitors such as humic acid prior to amplification or sequencing (Tringe and Rubin, 2005). Additionally, cells and spores need to be lysed to render the DNA or RNA accessible (see Section 4.5). Purification of nucleic acid also tends to co-purify other organic material that may interfere with downstream analysis (Tringe and Rubin, 2005).

A capable *in situ* instrument will need on-line sample pretreatment, extraction, purification, library preparation, and analysis (sequencing)—end-to-end or "sample to sequence." The many steps with various solutions make developing a fully automated and on-line instrument challenging. A concept for an end-to-end system and its challenges is described by Bhattaru. (2018), Bhattaru et al. (2019). Besides desalting, extraction, and purification, the methodology must also include library preparation prior to sequencing. Library preparation is a multistep procedure that includes, for example: enzymatic DNA repair, the addition of adenine to both ends of the ssDNA, multiple cleanup steps that use magnetic beads, and the addition of a motor protein at the end of the DNA or RNA fragments (Quick, 2018). The overall fluid management system is also prone to bubble formation, which needs to be specifically addressed (Parra et al., 2017; Bhattaru, 2018; Bhattaru et al., 2019). Nucleic acids are also prone to adsorption throughout the fluid management system, where such losses impact the overall LOD of the system (Bhattaru, 2018).

So far, only manual sample handling has been demonstrated, and end-to-end analysis is still left as work for the future. Through decoupled experiments, it has been established that the LOD could be as low as 10^4 spores per 50 mg of sample without amplification (Mojarro et al., 2019).

Nanopore and nanogap sensing is the latest addition to the techniques with spaceflight potential. Biological nanopores fill a void in required capabilities that other techniques cannot provide, namely, analysis of informational biopolymers down to 2 pg (e.g., DNA and RNA). Due to the fundamental difference, nanopores cannot be directly compared to conventional spectroscopy and separation-based analysis techniques, and should be considered an orthogonal technique. Although the small biological nanopore sequencing unit has been tested in relevant environments to a limited degree, it requires extensive sample preparation, which has not yet been sufficiently demonstrated on-line. Considering that the concept is relatively young, this may be resolved in the near future. Due to extensive interest and development in other research fields, solid-state nanopores and nanogaps may take a more prominent role; however, this remains to be seen.

6 Concluding remarks

The search for signatures of extinct or extant life in Solar System bodies continues to be a challenging endeavor, even five decades after the Viking missions to Mars. Adapting instruments that fully automate rather complicated workflow, otherwise conducted in multiple steps by scientists in the laboratory, while fulfilling highly stringent size, weight, and power requirements, is not an easy task. This is an exciting new era for life detection in planetary science where detecting organic biosignatures such as informational polymers (e.g., DNA), proteins, amino acids, and various lipids at the 1 ppb level or even lower is now within our grasp, which is far beyond any capabilities that existed one or two decades ago.

Generally speaking, there has been a paradigm shift from the 70s' Viking missions where Py/GC-MS constituted the main payload, to more recent Mars lander missions where TV-GC/MS equipped with derivatization capability has played a prominent role. On the basis of current trends in the literature, we are now in the middle of a new paradigm shift towards the next-generation instruments for organic biosignatures that are liquid-based analytical chemistry techniques such as CE, LC, biosensors, and nanopores. These techniques and instruments provide lower detection limits and a more comprehensive range of biosignatures to be targeted for interrogation than their predecessors. However, the realization of this shift remains to be seen depending on payload selection for future missions to Mars, Enceladus, Europa, Ceres, Titan, and other worlds.

Standoff spectroscopy techniques (e.g., fluorescence and Raman spectroscopy) that can identify fine-scale features will likely continue to gain importance in future missions. These instruments do not currently provide required detection limits or sufficient chemical information for the identification of organic biosignatures. However, their simplicity and ability to interrogate

a vast number of samples will render them invaluable for sample selection for further analysis by instruments that are limited by the number of samples that can be analyzed.

On the basis of the reviewed literature, the next-generation biosignature detection techniques for planetary applications (CE, LC, biosensors, and nanopore sensing) are mostly fit for science requirements in mission concepts that include life detection (Pappalardo et al., 2013; Hand et al., 2017; MacKenzie et al., 2022a; MacKenzie et al., 2022b; Williams and Muirhead, 2022). Current GC-MS, fluorescence, and Raman spectroscopy instruments cannot achieve the required LODs. However, it is possible that additional sample preparation (extraction and preconcentration) and sample introduction that provides sharper peaks could remedy some of the current weaknesses (e.g., matrix effects and insufficient LODs); such efforts have not yet been reported. Nevertheless, future space missions to Ocean Worlds (e.g., Europa and Enceladus) will have different requirements than those for Mars, such as the ability to analyze aqueous samples, operate at higher vacuum, lower temperatures, high radiation environments, and with reduced data bandwidth. Within that context, the discussed techniques and instruments would require substantial development, despite having flight heritage.

We conclude that CE (including ME), LC, biosensors, and nanopore sensing have potential as payload instruments in future space missions. They have similar orders of magnitude LODs; however, this varies from one analyte to another. CE and LC are more suited for small molecules (<1,000 Da), but larger molecules can be hydrolyzed, which has been demonstrated with subcritical water. Both techniques can separate multiple enantiomeric amino acids, unlike biosensors. CE-C^4D and ion chromatography can analyze inorganic and small organic ions. CE requires multiple solutions for derivatization, rinsing, and BGE; on the other hand, only low-pressure pumping is required. LC does typically not rely on derivatization but, in most cases, requires high-pressure fluid delivery. Furthermore, LC and CE can both be coupled with MS for improved separation and identification; however, most applications have utilized ME-LIF analysis, which provides a smaller format, and has predominantly focused on amino acid analysis.

Nanopore sensing is the most suitable technique for detecting and sequencing informational biopolymers (e.g., DNA and RNA), which is impossible with CE and LC. Currently, biological nanopores are required for sequencing, but these are not as stable as solid-state nanopores. However, the sample preparation is more complicated than the other techniques and has only partially been demonstrated in an on-line setup. Biosensors are capable of detecting both small (<1,000 Da), large (>1,000 Da) molecules, and intact microbes. Most receptors have high specificity, and deciding on specific target biosignatures is problematic under the assumption that extraterrestrial life may be different from Earth. Finally, we cannot accurately assess the readiness for flight because environmental testing is not always reported in the literature. Nonetheless, CE, LC, biosensors, and nanopore sensing have been miniaturized without substantial loss in performance (e.g., separation efficiency and LODs) or even with increased performance. However, complete adaptation to spaceflight followed by extensive validation is required to determine the ultimate fitness of purpose.

It is also apparent that the next generation of life detection instruments is being scrutinized to a much higher degree than their predecessors. There is a lack of publicly available validation data for the previously flown GC-based instruments (Poinot and Geffroy-Rodier, 2015). Nonetheless, it is important to note that current instruments in development are rarely validated in an *end-to-end* fashion; namely, automated analysis from the point that the primary sample is loaded, including sample preparation. The task of validation is becoming an increasingly cumbersome effort as the targeted compounds and sample types (i.e., ice, water, liquid methane, etc.) have increased with the number of relatively recently targeted planetary bodies (e.g., Europa, Enceladus, and Titan) in the Solar System. Historically, it has become apparent that sample matrices may have unanticipated effects in the analysis of organic molecules, such as strong oxidizing effects of perchlorates on organic molecules generated during pyrolysis and thermal volatilization at Mars. Many other disciplines where analytical chemistry has a prominent role, such as pharmaceutical, food, and forensic sciences, have precise guidelines on conducting method validation to ensure reliable results (Raposo and Ibelli-Bianco, 2020). This kind of framework does not currently exist in the domain of astrobiology. We recommend that such a framework be established so instruments can be appropriately compared and avoid representing misleading performance metrics.

The emergence of high-resolution mass spectrometry and more capable separation techniques opens new possibilities for so-called non-targeted analysis (Milman and Zhurkovich, 2017). There is a wide range of potential chemical biosignatures of interest, and they may include several thousands of chemical compounds. This calls for chemical analysis techniques that are *non-targeted* that enable the detection and identification of compounds that have not been considered *a priori* (Ruf et al., 2018). A combined approach of comprehensive non-targeted analysis and suspect screening may be the most beneficial, where the latter includes a list of high-priority targets as outlined by *The Ladder of Life Detection* (Neveu et al., 2018).

No analysis technique is truly universal, and each has its strengths and weaknesses. Therefore, combining different instruments, each utilizing different but complementary detection techniques, into a single instrument suite capable of uniquely identifying biosignatures is necessary. These efforts have already been pursued in the last few years. For example, EMILI, which includes CE, GC, MS, pH, and conductivity measurements (Brinckerhoff et al., 2022), the Complex

Molecules Detector (CMOLD) that includes microscopy, Raman spectroscopy, and a biosensor array (Fairén et al., 2020), and the Mine Analogue Research (MINAR 5) project that is not an instrument but a wide array of sample acquisition instruments, spectroscopic instruments, biosensors, nanopore sequencing, and so on (Cockell et al., 2019).

Four techniques being developed for *in situ* organic biosignature detection have been identified. More may follow with possible breakthroughs in analytical chemistry in the future. However, additional development is still needed before they are spaceflight-ready. Additional end-to-end validation with miniaturized assemblies is, in many cases, still needed to prove the fitness of purpose, which is also the case for current spaceflight instruments. Nevertheless, proposed mission concepts are becoming more demanding in their measurement requirements, and the continued development of the next-generation biosignature detection techniques may be the favored path to provide an answer if there is extraterrestrial life in the Solar System.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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