



An integrated sequential injection analysis system for ammonium determination in recycled hygiene and potable water samples for future use in manned space missions



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ABSTRACT

A fully automated method for on-line ammonium determination in recycled water produced on board the International Space Station was developed and optimized. A trade off methodology is presented to evaluate and select the most appropriate analytical system, adaptable to space flight requirements. A sequential injection system meets the specifications for on-line monitoring in space conditions as it is an integrated flow manifold with small dimensions operating in a totally closed loop preventing any contact or release of the fluids/gases inside the space environment. The method is based on the reaction between ammonia and o-phthaldialdehyde in the presence of sulfite in alkaline media (pH ca. 11). The fluorescent product (isindol-1-sulfonate) is then quantified at 425 nm. The influence of chemical and flow variables as well as space adaptation characteristics which affect the performance of the system if placed on board the International Space Station have been studied, providing the appropriate conditions for the analysis of real samples. For a total analysis time of 174 s, a detection limit (3s) of 0.018 mg L⁻¹ for NH₄⁺ was obtained along with a sampling frequency of 20 h⁻¹. The working range was at values between 0.06 and 4.00 mg L⁻¹ NH₄⁺. The “intra-day precision” was 2.30% (at 0.50 mg L⁻¹), while the “inter-day precision” was 2.40% (at 0.50 mg L⁻¹). The accuracy of the proposed method was evaluated by analyzing standard reference materials as well as using the certified method (indophenol blue) for ammonium determination. The method was successfully applied for ammonium determination in water samples obtained by the Water Treatment Unit Breadboard (WTUB) located in Antarctica Concordia Research Station.

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1. Introduction

The International Space Station (ISS) is a microgravity and space environment research laboratory in low Earth orbit, suited for the testing of spacecraft systems and equipment required for long-lasting manned space missions to the Moon and Mars. Water is a basic human need and is provided to the crew of ISS from the water storage/transfer systems that are placed in there. It would be impractical, in terms of volume and cost, either to completely stock the ISS with it for long periods of time, or continuously supplying it from the Earth for long duration manned space missions, so the development of properly working water recycling systems is a critical issue for the scientists [1–3]. Water recycling systems are being developed by the European Space Agency (ESA), the Russian Federal Space Agency (ROSCOSMOS) and the National Aeronautics and Space Administration (NASA). The

recycling system developed by ESA is designed to produce hygiene and potable water either from cabin condensate or grey water (waste hygiene water) or even urine, by both physical and chemical processes in order to remove contaminants, as well as different filtration steps and temperature sterilization to ensure the quality of the water. The produced water from the recycling system demands quality control which right now is limited due to onboard technologies and crew time. Especially, in case of contamination, water monitoring is of high importance and, thus, a key challenge for the scientists. Until now, monitoring of the quality of the recycled water demands its collection and transportation to Earth, resulting in questionable measurements due to the time lapse between the sample collection and ground analysis [4]. Thus, there is a need for in-flight analytical measurements.

In the ISS, there are some requirements of the ESA (according to Statement of Work: ESA Express Procurement EXPRO On-Line Ammonium Analyzer for water recycling systems, Appendix 1 to AO/1-8029/14/NL/SFe) water quality standards [2] that should be fulfilled, in order an analyzer to be able to properly operate in space conditions. These

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requirements concern the analytical performance characteristics of the performed analytical method (limit of detection/quantification, dynamic range, selectivity, precision, accuracy) bearing in mind the fact that the recycled water will be used as potable, as well as the space adaptation characteristics (mass/volume of the analyzer, power consumption, voltage/current intensity) due to limitations in room-space and energy provision [5]. Thus, an analyzer, first of all, must be microgravity compatible, operating in a fully automated mode for a long period, even months, without any human intervention. The low consumption of sample and reagent solutions is very important to produce low wastes, considering, not only the limited room-space in the ISS or a space shuttle, but also the weight. In addition, special care should be taken into a totally closed loop system, so no leaking of fluids or release of any gas should ever happen into the orbiter due to the isolated environment of the station. Finally, the use of low toxicity reagents and minimum flammable materials is very important to ensure the safety of the crew and the whole station as well.

One of the factors that seriously affects the quality of the water is the concentration of the ammonium ion/ammonia; thus, its continuous on-line monitoring is crucial. In water, both toxic unionized ammonia (NH_3) and the, relatively, non-toxic ionized ammonium ion (NH_4^+) exist. Both forms are easily converted into each other, with the ratio of ammonia to ammonium largely depending upon pH, salinity and temperature. Ammonium is predominant when the pH is below 9.0, while ammonia exists at higher values. Generally, numerous automated analytical techniques have been developed for the quantitative determination of ammonium/ammonia species with various detectors (spectrophotometric, fluorimetric, conductimetric), but there are only few that meet the requirements for proper operation in space. A type of ammonium analyzers that is mainly used for routine analysis on Earth is the discrete batch analyzers, which are not microgravity compatible and cannot be used in space environment due to the fact that all the solutions (sample and reagents) are transported and placed into discrete open cups by a robotic arm, thus, the whole procedure is significantly affected by gravity [6]. Another type of ammonium analyzers is the flow batch analyzers which use an open reaction chamber, affected by the gravity as well, preventing their use in space [7]. A very interesting task used in routine monitoring of the water quality on ground (lakes, rivers, industry, water supplier systems) is the ion selective electrode (ISE). These systems, integrated with a closed loop system in a totally automated way, could be used for on-line monitoring of ammonia in recycled water in space, as they are easy to operate and suitable for continuous determination. However, they are characterized by low analytical sensitivity and precision [8]. Another drawback is the significant potassium interference [9]. On the other hand, there are the automated flow analyzers, which are based on the flow injection analysis (FIA) and sequential injection analysis (SIA) techniques, offering automatic on-line liquid manipulation. This kind of analyzers can be easily combined with almost all available detection systems as well as ion-selective electrodes (ISE). In addition, they use low volumes of sample and reagents solutions, resulting in minimum waste production, and they also provide a plethora of benefits regarding the operation in microgravity conditions because they work in a totally “closed loop”.

The aim of this work is to trade off the published automated flow methods for ammonium determination in terms of properly operation in space conditions and to select the top ranked one for evaluation. For this purpose, a sequential injection (SI) analyzer with a fluorimetric detector has been employed and tested. A fully automated method has been developed and optimized to meet the requirements for possible use in manned space missions. The derivatization reaction between ammonia and o-phthalaldehyde in the presence of sulfite in alkaline media (pH ca. 11) results in the formation of a fluorescent product (isindol-1-sulfonate), which is then quantified at 425 nm. All major chemical, flow and space factors affecting system operation of the system were optimized, enhancing the effectiveness of the proposed method. The accuracy and precision of the developed method were

estimated by analyzing a standard reference material (SRM) as well as using the certified method (indophenol blue). The method was applied to hygiene and potable water samples.

2. Experimental

2.1. Instrumentation

A miniSIA-1 flow analyzer (Supplementary material, SM, Fig. S1) with an acrylic Chem-on-Valve™ monolithic manifold (<https://www.globalfia.com>) was used throughout the experiments for the ammonium determination. The device is equipped with a bi-directional milliGAT™ pump coupled to a thermostated holding coil and a multi-position valve (MPV) modified in such a way in order to accept the Chem-on-Valve (COV) manifold. This configuration facilitates the fluid manipulation in a microfluidic way inside the closed system. Two fiber optic cables are used for the emission and excitation light as well as a fluorescence flow cell which is directly mounted on the COV. A monochrome white LED is used as an excitation light source (365 nm). A fluorimetric spectrometer (Ocean Optics USB-4000) is used as a detection system. The monochromator has been set at 425 nm emission wavelength. The recorded fluorescence intensity is given as arbitrary units (AU). The FloZF 5.2 software (<https://www.globalfia.com>) is used for the device control and data acquisition. Tubing is made from PEEK or PTFE. Polyethylene bottles are used as solution containers.

A Varian DMS 100S UV Visible Spectrophotometer with a 1.0 cm × 1.0 cm absorption cell is used for the ammonium determination by employing the reference method (indophenol blue). The analytical wavelength has been set at 640 nm.

A Sartorius analytical balance has been employed for mass measurements. An Orion EA940 pH-meter has been used for pH measurements.

2.2. Reagents and samples

All chemicals were of analytical reagent grade and were provided by Merck (Darmstadt, Germany, <http://www.merck.de>). All ammonium standard solutions were prepared by appropriate stepwise dilution of 1000 mg L⁻¹ NH₄⁺.

An o-phthalaldehyde (OPA, C₈H₆O₂) solution at a concentration level of 10.0 mmol L⁻¹ was prepared by dissolving 268.0 mg of solid in 50.0 mL methanol and filling the volumetric flask to 200.0 mL with double deionized water (DDW). Phosphate buffer was prepared by dissolving 26.81 g Na₂HPO₄ in 900 mL of DDW, adjusting the pH to 11.0 with 2.0 mol L⁻¹ NaOH and filling the volumetric flask to 1000 mL (0.1 mol L⁻¹). A 1000 mL solution of 3.0 mmol L⁻¹ Na₂SO₃ was prepared by dissolving 378.0 mg of solid Na₂SO₃ in phosphate buffer. The reagent solutions are light sensitive, so they were kept in dark bottles in the fridge remaining stable for at least 5 weeks.

A 20% m/v alkaline citrate solution was prepared by dissolving 50.0 g trisodium citrate and 2.5 g NaOH in 250 mL of DDW. A 5% m/v sodium hypochlorite solution was prepared by dissolving 5.0 g sodium hypochlorite (NaClO) in 100.0 mL of DDW. A 0.5% m/v sodium nitroprusside solution was prepared by dissolving 1.25 g sodium nitroprusside (Na₂Fe(CN)₅NO) in 250.0 mL of DDW by means of ultrasound. A 1% m/v sodium hypochlorite solution in 16% m/v citrates was prepared by mixing 80.0 mL of 20% m/v alkaline citrate solution and 20.0 mL of 5% m/v sodium hypochlorite solution. A 11.1 v/v phenol (C₆H₆O) solution in ethanol was prepared.

An ammonium standard reference solution from NIST (National Institute of Standard and Technology, Gaithersburg, MD, USA) NH₄Cl in DDW 1000 mg L⁻¹ NH₄⁺ CertiPUR® was analyzed for accuracy investigation.

Three different potable artificial water samples (PAW) at 0.0, 0.4 and 0.8 mg L⁻¹ concentration level of NH₄⁺ and three different hygiene artificial water samples (HAW) at 0.0, 1.0 and 5.0 mg L⁻¹ concentration level of NH₄⁺ were prepared, according to a specific chemical

composition that is similar to the available information about the levels of several compounds existed in ISS recycled water. The chemical composition of each artificial water sample is given in Table 1.

Laboratory glassware was treated with freshly prepared 10% (v/v) nitric acid solution for at least 24 h and finally rinsed with ultra-pure de-ionized water before use to avoid contamination factors as much as possible.

2.3. Analytical procedure

The manifold of the flow system is schematically presented in Fig. 1. The analysis of ammonium using the miniSIA is based on the OPA method. Three operational sequences have been used during the experiments.

At the beginning, the analyzer runs the “startup” sequence in order to prepare the device (filling of the tubes, heating of the holding coil) for the analysis. Then, the “analytical cycle” sequence begins, during which appropriate portions of sample/standard solution (40.0 μL), OPA (40.0 μL) and sodium sulfite (20.0 μL) solutions are sequentially aspirated with a flow rate of 10.0 $\mu\text{L s}^{-1}$, into the thermostated holding coil in a sandwich type format by means of the milliGAT pump in order to produce a fluorescent product (isoindol-1-sulfonate) which can be directly quantified. The chemical reaction between ammonia and o-phthalaldehyde in the presence of sulfite takes place in alkaline media (pH ca. 11). The fluorescent product, is time and temperature affected, thus, after a 2-min stop in the thermostated holding coil at 70 °C, it is delivered into the flow-cell where it is quantified at 425 nm (excitation wavelength, 365 nm). Finally, the analyzer runs the “shutdown” sequence in order to clean the whole system (rinsing of the tubing, holding coil and flow-cell).

3. Results and discussion

3.1. Trade-off methodology

There are numerous published automated flow methods for ammonium/ammonia determination in the literature. The flow systems, which are commonly used for ammonium determination, are categorized as follows: segmented flow analysis (SFA), flow injection analysis (FIA) [10–16], sequential injection analysis (SIA) [17–20], multisyringe flow injection analysis (MSFIA) [21,22], multiCommuted flow injection analysis (MCFIA) [23] and multipumping flow injection analysis

Table 1

Chemical composition of artificial potable (PAW) and hygiene (HAW) water samples. Concentrations are given in mg L^{-1} . All solutions are slightly acidic (6.0–6.5).

Parameter	PAW1	PAW2	PAW3	HAW1	HAW2	HAW3
Ammonium	–	0.4	0.8	–	1.0	5.0
Chloride	100	100	200	125	125	250
P-PO ₄	2.5	2.5	5.0	25	25	50
Nitrate	12.5	12.5	25	25	25	50
Sodium	75	75	150	140	140	280
Potassium	1.5	1.5	3.0	3.0	3.0	6.0
Magnesium	25	25	50	25	25	50
Calcium	50	50	100	50	50	100
Fluoride	0.5	0.5	1.0	5.0	5.0	10
Iron	0.15	0.15	0.30	1.5	1.5	3.0
Copper	0.5	0.5	1.0	1.5	1.5	3.0
Zinc	2.5	2.5	5.0	2.5	2.5	5.0
Cadmium	0.0025	0.0025	0.005	0.025	0.025	0.050
Nickel	0.025	0.025	0.050	0.250	0.250	0.500
Lead	0.025	0.025	0.050	0.250	0.250	0.500
Chromium	0.025	0.025	0.050	0.250	0.250	0.500
Manganese	0.025	0.025	0.050	0.250	0.250	0.500
Arsenic	0.005	0.0050	0.010	0.050	0.050	0.100
Mercury	0.001	0.001	0.002	0.010	0.010	0.020

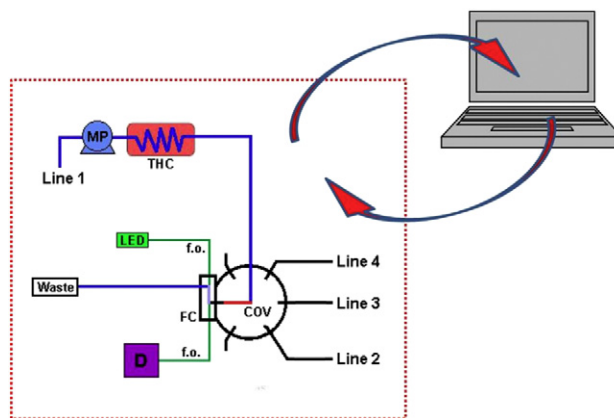


Fig. 1. Schematic diagram of the miniSIA Flow Analyzer. MP, milliGAT™ pump; D, spectrophotometric detector; THC, thermostated holding coil; FC, flow cell; COV, Chem-on-Valve device; f.o., fiber optics; LED, led excitation light source; Line 1, DDW; Line 2, Std sol. or sample; Line 3, OPA solution; Line 4, sulfate solution.

(MPFIA) [24,25] with or without a gas diffusion (GD) unit. Among them, only few meet the requirements for proper operation in space conditions.

In accordance with the ESA standard procedures, trade-off studies shall evaluate the candidate concepts for a mission candidate technique. Therefore, to evaluate and select the most appropriate of them, a trade-off methodology has been developed taking into account the detection/quantification requirements (LOD, LOQ, accuracy, repeatability, etc.) and critical items regarding the adaptation to space (microgravity applicability, volume/mass of the analyzer, solutions consumption, wastes production) as well as safety criteria (use of hazardous materials/reagents, pressure, heating, flammability). Every criterion was scored, based on defined scale/levels of each one with a specific weighting factor (WF). Each particular weighting factor has been attributed to every criterion taking into consideration the significance of the specific parameter. For instance, parameters that could affect the crews' wellbeing, such as maintenance and safety issues, were evaluated as of higher importance ($\times 10$ WF) compared with others that are subject to design adaptation and could be optimized, like volume of the analyzer ($\times 5$ WF) and power consumption ($\times 4$ WF). In addition, since the concept of the WF refers mainly to the criteria, in order to distinguish between the examined methods, a scale was required to be established for each criterion in order to provide a reliable assigned value for each one. The scale of the criteria is based on numeric features. The score of each criterion for every method was given as the result of a value (into the range of scale) \times weighting factor.

The basic requirements for the developed method are defined in the Statement of Work: ESA Express Procurement EXPRO On-Line Ammonium Analyzer for water recycling systems. The limit of detection shall be 0.05 mg L^{-1} NH_4^+ , the limit of quantification shall be 0.1 mg L^{-1} NH_4^+ and the dynamic measurement range shall be defined between 0.1 mg L^{-1} and 50 mg L^{-1} NH_4^+ . The precision shall be at a value of standard deviation of $\pm 0.05 \text{ mg L}^{-1}$, while the accuracy shall be at an error level of 0.05 mg L^{-1} . In case of the space adaptation criteria, the volume of the on-line ammonium analyzer shall be under $516 \times 440 \times 253 \text{ mm}^3$ (based on the ISS Locker), while its mass shall be under 27 kg. In addition, the consumed sample volume shall be minimal and the amount of waste generated during analysis shall be as low as possible, allowing safe disposal. Considering the power consumption and the current intensity, they shall be under 300 W and 116–126V DC, respectively. Regarding the safety criteria, the analyzer shall be automatic, tolerant to failures due to operations and maintenance and shall consist of a minimum of flammable material; wastes shall be stored for a safe disposal; no release of hazardous or toxic materials will occur.

Table 2
Trade off methodology of the examined flow methods.

Trade off criteria		Scale	Weighting factor	Published methods ^a															
				A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Analytical Performance	Limit of Detection	0–5	10	50	50	50	30	50	50	50	50	0	50	50	20	50	50	30	50
	Limit of Quantitation	0–5	9	45	45	45	27	45	45	45	45	0	45	45	0	45	45	27	45
	Working range	0–5	3	12	12	15	12	12	12	12	12	15	12	12	0	12	6	3	9
	Selectivity/interferences	0–2	5	0	0	0	0	10	10	5	10	10	10	10	10	5	0	0	0
	Precision	0–10	4	28	36	28	28	40	40	24	32	36	20	32	40	20	28	20	32
	Accuracy	0–5	6	30	18	18	30	30	30	30	18	30	24	18	30	18	18	30	30
Adaption Characteristics	Volume of analyzer	0–5	5	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	Mass of the analyzer	0–5	5	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	Sample volume	0–5	5	20	25	25	25	25	25	20	25	25	25	25	25	25	20	25	25
	Wastes Volume	0–5	5	20	20	25	25	25	25	25	20	25	25	20	25	25	20	20	25
	Power consumption	0–3	4	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Maintenance	0–5	10	0	0	0	30	30	0	30	30	0	30	30	30	0	0	0	0
	Safety Criteria	Hazardous Reagents/wastes	0–5	10	40	40	40	40	30	30	30	20	10	20	10	40	40	40	30
Heating		0–2	10	20	20	20	0	0	0	10	20	0	10	0	20	20	20	20	
Total score				327	328	328	299	359	359	303	304	263	303	324	252	322	314	277	328

^a A, FIA-GD-Conductimetric [10]; B, MPFIA-GD-Conductimetric [24]; C, MSFIA-GD-Conductimetric [21]; D, SIA-GD-Conductimetric [17]; E, FIA-OPA-SULFITE-Fluorimetric [11]; F, SIA-OPA-SULFITE-Fluorimetric [18]; G, MPFIA-OPA-SULFITE-Fluorimetric [25]; H, FIA-GD-OPA-Fluorimetric [12]; I, SIA-INDOPHENOL-UV-Vis [19]; J, FIA-INDOPHENOL-UV-Vis [13]; K, FIA-INDOPHENOL-UV-Vis [14]; L, FIA-INDOPHENOL-UV-Vis [15]; M, FIA-GD-INDICATOR-UV-Vis [16]; N, SIA-GD-INDICATOR-UV-Vis [20]; O, MSFIA-GD-INDICATOR-UV-Vis [22]; P, MCFIA-GD-INDICATOR-UV-Vis [23].

The Table 2 shows the comparison of all the examined parameters for the automated flow methods together with the score of each parameter as well as the total score of each method.

Some of the examined flow-based methods make use of the gas-diffusion (GD) technique in order to minimize the interferences arise from the matrix [17,22,24]. In this case, the sample solution is merged (or injected) with an alkaline (pH ~ 10.5) stream (typically sodium hydroxide) in the donor channel of the GD in order to convert NH_4^+ to NH_3 gas, which, then, diffuses through a gas-permeable hydrophobic membrane (e.g., PTFE) into the acceptor channel, which contains an appropriate receiving solution for quantification. The diffused ammonia is detected either by a spectrophotometric [16,20,22,23] or fluorimetric [12] or conductimetric [10,17,21,24] detector. However, the GD unit presents some inherent drawbacks. The membranes tend to clog or deteriorate during the chemical analysis making them unsuitable for long-term measurements. To avoid the clogging, the membrane has to be changed often, even every day, consuming time and complicating the work of the crew inside the ISS or a space shuttle. Another problem related with the use of GD systems is the control of the blank measurement due to possible dissolution of the ammonia from the environment into the receiving solution. This problem could be addressed by using a second gas-diffusion cell, resulting in a more complicated procedure, not compatible with the safety restrictions on board. For these reasons, the GD methods were ranked lower than the other flow methods (Table 2).

The SIA fluorimetric methods were top ranked compared to the other systems, according to the trade-off methodology. Among the commercially available SIA systems coupled with a fluorimetric detector, the miniSIA analyzer was selected to be further evaluated in terms of the ISS requirements for possible operation in space conditions. In this context, a SIA fluorimetric method was developed and optimized. The miniSIA analyzer is an automated and miniaturized integrated system with a low volume and mass, so that it could fit in a place of defined dimensions. It is an easy-to-use system which allows for an automatic handling of the solutions without any human intervention, avoiding any potential errors, regarding overpressure or overheating of the system. In addition, it uses low volumes of sample/reagents solutions resulting in low production of wastes, considering not only the restricted space inside the ISS, but also the weight. Finally, the operation of the whole procedure in a totally “closed loop” makes it microgravity applicable and prevents problems like leakage or gas elimination, which could create a negative or even fatal environment inside the limited and isolated space station or space shuttle.

3.2. Chemical and flow parameters optimization

All chemical and flow parameters of the proposed system considering the requirements for space adaptation and safety criteria, were thoroughly studied.

3.2.1. Effect of temperature and heating time

The chemical reaction which takes place in OPA method for ammonium determination is highly depended on the temperature and the heating time of the mixture inside the holding coil, affecting the formation of the fluorescent product. The effect of temperature was studied at values, ranged from 30 to 80 °C. Increasing the reaction temperature was effective in accelerating the desired reaction in the studied area, as it increased the fluorescence intensity, as it is shown in Fig. 2. However, 70 °C were selected in order to avoid any possible bubbles formation inside the closed system.

The effect of the heating time was studied from 30 to 150 s. As it was proved, the intensity was increased practically linearly by increasing the heating time up to 120 s. For a longer time of heating, the intensity was increased with a lower rate. Considering the sensitivity and the total time of analysis, a heating time of 120 s was adopted.

3.2.2. Effect of reagents and sample aspiration order

In SIA methods, the aspiration order of the reagents and sample/standard solution into the holding coil is a key factor to the formation

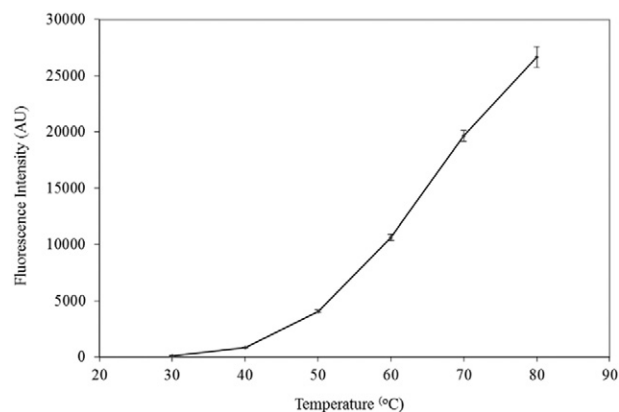


Fig. 2. Effect of temperature on the fluorescence intensity of 2.0 mg L⁻¹ NH₄⁺. Heating time: 90 s. Error bars were calculated based on standard deviation values (± 1 s).

of the fluorescent product. For this reason, six reagents/standard aspiration order combinations have been studied. The results showed that the highest sensitivity was achieved by consecutive aspiration of 20 μL of 15.0 mmol L^{-1} OPA solution, 20.0 μL of 2.0 mg L^{-1} NH_4^+ standard and 20.0 μL of 3.0 mmol L^{-1} sodium sulfite solution. Thus, the aspiration order of OPA-Standard/Sample-Sulfite was adopted for the subsequent experiments.

3.2.3. Effect of concentration and volume of OPA solution

The concentration of OPA solution and its aspirated volume inside the holding coil are of high significance to its complexation with ammonia, affecting the efficient formation of the fluorescent product. The effect of OPA concentration was studied at concentrations between 0.5 and 50.0 mmol L^{-1} , while the effect of the volume was studied at the range of 20.0–50 μL . The concentrations of NH_4^+ standard and sulfite solutions were 2.0 mg L^{-1} and 3.0 mmol L^{-1} , respectively. The fluorescent intensity increased as the concentration of the reagent increased up to 3.0 mmol L^{-1} and, then, remained practically stable. Regarding the aspirated volume of OPA solution, the experimental results showed an increase in the intensity by increasing the volume up to 40.0 μL , while at higher values a slight decrease was observed. Hence, a volume of 40.0 μL of OPA solution at a concentration level of 3.0 mmol L^{-1} was chosen as optimum.

3.2.4. Effect of concentration and volume of sodium sulfite solution

The presence of sodium sulfite as a reducing agent enhances the sensitivity and the specificity of the reaction between OPA and ammonium ion, by eliminating possible interferences of dissolved amino acids and primary amines [26,27]. Experiments using different concentrations of sodium sulfite solution showed that at 3.0 mmol L^{-1} concentration level the fluorescent reaction was maximum and, thus, it was employed for the study of the effect of its volume on the fluorescence intensity. The aspirated volume of sulfite solution was studied in the range between 5.0 and 25.0 μL . An increase of the analytical signal was observed by increasing the sulfite volume up to 20.0 μL , while then it was leveled off. Consequently, a volume of 20.0 μL was used as optimum.

3.2.5. Effect of sample volume

In on-line flow systems, sample volume plays a significant role affecting the sensitivity of the method. In order to evaluate the influence of the sample volume on the intensity, a standard solution of NH_4^+ at a 2.0 mg L^{-1} concentration level was used, varying the aspirated volume within the range 5.0–60.0 μL . The experimental results (Fig. 3) showed a positive correlation of the analytical signal with the sample volume up to 40.0 μL , leveling off at 50.0 μL , while at higher volumes, a slight decrease of the signal was observed. Therefore, a sample volume of 40.0 μL was adopted for further studies.

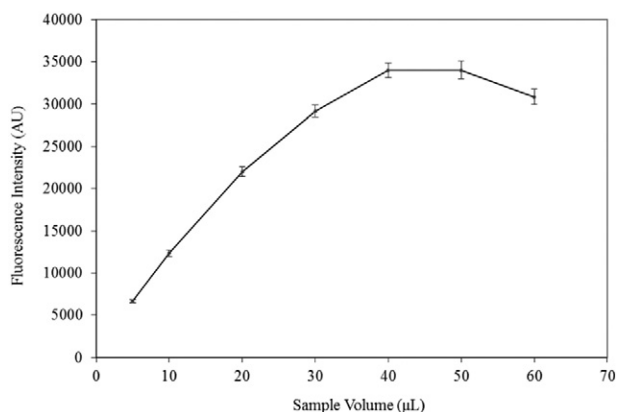


Fig. 3. Effect of sample volume on the fluorescence intensity of 2.0 mg L^{-1} NH_4^+ . Error bars were calculated based on standard deviation values (± 1 s).

Table 3
Accuracy of the miniSIA method comparing with the Certified Method.

Sample	True value (mg L^{-1})	Certified method Actual value (mg L^{-1})	miniSIA method Actual value (mg L^{-1})	Relative error (%)
PAW1	0.00	0.00	0.00	0.00
PAW2	0.40	0.33	0.32	3.03
PAW3	0.80	0.7	0.75	7.14
Overall relative error				3.39
HAW1	0.00	0.0	0.0	0.00
HAW2	1.00	1.05	1.08	2.86
HAW3	5.00 ^a	5.72	5.38	5.94
Overall relative error				2.93

^a After 1:1 dilution.

3.3. Interferences

The water system in ISS recycles cabin condensate, hygiene waters and even urine. Thus, primary amines are a significant potential interference, which has been studied at concentrations up to 0.40 mg L^{-1} CH_3NH_2 for interfering in 0.50 mg L^{-1} NH_4^+ determination using the optimized method. Higher concentrations of methylamine are not expected, according to the Reference Documents of ESA for the quality of the recycled water in ISS. Another compound that could act as interference is the silver ion which is used as a disinfectant in the ISS water system [28,29]. Experimental results revealed that CH_3NH_2 and Ag(I) can be tolerated at least up to 0.40 and 1.0 mg L^{-1} .

3.4. Analytical features

The analytical performance characteristics of the miniSIA method for ammonium determination under the optimal conditions were calculated and presented below.

With a total analysis time was 174 s, the proposed method was linear from 0.06 mg L^{-1} up to 4.00 mg L^{-1} NH_4^+ , while the sensitivity, S (slope of calibration curve) was $S = 13,218 \text{ L mg}^{-1}$. The detection limit (c_L), based on 3s criterion, was found to be 0.018 mg L^{-1} , while the quantification limit (c_Q), based on 10s criterion, was found to be 0.06 mg L^{-1} , according to IUPAC [30]. The “intra-day precision” was calculated to be 2.30% (at 0.50 mg L^{-1} , 5-subsequent times), while the “inter-day precision” was calculated to be 2.40% (at 0.50 mg L^{-1} , 5-times 5-subsequent days).

In order to validate the accuracy of the proposed method, an NTRM reference material was analyzed using the miniSIA Flow Analyzer under the proposed method. The reference material was an ammonium standard solution traceable to SRM from NIST NH_4Cl in H_2O 1000 mg L^{-1} NH_4 CertiPUR®. The relative error was calculated to be 2.52%. The accuracy of the method was also tested using the certified method (indophenol blue) for ammonium determination [31]. For this purpose, three potable artificial water samples (PAW) as well as three hygiene artificial water samples (HAW) at different concentrations of ammonium were prepared and analyzed with both the miniSIA method and the certified method. The obtained results are presented in Table 3. The overall relative errors were 3.39% and 2.33% for PAW and HAW samples respectively, showing the good performance of the proposed system.

Table 4
Volume of consumed solutions and produced wastes during the analytical cycle.

Solutions	Volumes of consumed solutions (μL)	Volumes of produced wastes (μL)
Sample/std. solution	40.0	40.0
OPA solution	40.0	40.0
Sodium sulfite solution	20.0	20.0
Carrier solution	390.0	390.0
Total volume	490.0	490.0

Table 5
Results of the analysis of WTUB water samples by CM and miniSIA.

Sample	CM (mg L ⁻¹)	miniSIA (mg L ⁻¹)	Standard error (%)
RO eff_1 (urine + shower)	3.55	3.28	-7.6
RO eff_2 (shower only)	0.34	0.32	-5.9
NF GW only	18.66	19.02	+1.9

The volume of the consumed solutions and produced wastes during the analytical cycle is also an issue to be considered in case of an analyzer for possible operation in a space shuttle. These volumes were calculated during an analytical cycle using the miniSIA ammonium analyzer and are presented in Table 4. The very small amounts of sample and reagents solutions as well as the wastes have been considered as acceptable to be used in space missions, allowing the miniSIA analyzer's operation in a green-friendly manner.

3.5. Applications

The developed method for the miniSIA analyzer was applied to the determination of ammonium in real water effluents produced by the Water Treatment Unit Breadboard (WTUB) located in the Antarctic Concordia research station. Concordia is a selected place from ESA to test the water recycling system, similar to that in ISS, as it is a realistic simulation for some aspects of human spaceflight. Three different types of water samples were provided by ESA: RO eff_1 (urine and shower water), RO eff_2 (shower water) and NF GW (grey water). These water samples are considered to have similar chemical composition as the ones of the recycled water on board the ISS. The water samples were analyzed with the miniSIA and the certified method and the results were compared (Table 5).

The results of ammonium determination in WTUB water samples were ranged between 0.3 up to 19 mg L⁻¹. Regarding the comparison of miniSIA with the CM method, good agreement was observed, and the percentage error was ranged from -7.6% up to +1.9%, which complies with the space flight requirement for accuracy.

4. Conclusions

A trade off methodology has been developed for the evaluation of the published flow methods for ammonium/ammonia determination in terms of the ISS requirements for proper operation in space conditions. The miniSIA analyzer coupled with a fluorimetric detector was selected among other flow systems and evaluated with a view to a possible use for water quality monitoring in recycled potable and hygiene water samples in a microgravity environment. The analyzer is adaptable to space flight requirements considering that its volume and mass are suitable for the limited room-space inside the ISS or a space shuttle. In addition, it allows an automatic handling of the solutions as well as the implementation of minimum sample and reagents amounts of μL , resulting in low waste generation per analytical cycle. It is an integrated system which performs the analysis principle and

measurements in a closed flow system without any crew involvement avoiding any possibility for fluid leakage or gas liberation. Thus, it conforms to the safety requirements not only for the crew, but also for the ISS environment.

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