Development of colorimetric solid phase extraction (C-SPE) for in-flight monitoring of spacecraft water supplies

by

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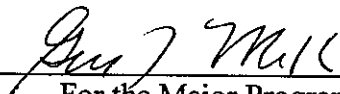
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GENERAL INTRODUCTION

Although having recently been extremely successful gathering data on the surface of Mars, robotic missions are not an effective substitute for the insight and knowledge about our solar system that can be gained through first-hand exploration. Earlier this year, President Bush presented a "new course" for the U. S. space program that shifts NASA's focus to the development of new manned space vehicles to return of humans to the moon.[1] Re-establishing the human presence on the moon will eventually lead to humans permanently living and working in space and also serve as a possible launch point for missions into deeper space.

There are several obstacles to the realization of these goals, most notably the lack of life support and environmental regeneration and monitoring hardware capable of functioning on long duration spaceflight. In the case of the latter, past experience on the International Space Station (ISS),[2] Mir,[3] and the Space Shuttle[4, 5] has strongly underscored the need to develop broad spectrum in-flight chemical sensors that:

- meet current environmental monitoring requirements on ISS as well as projected requirements for future missions, and

- enable the in-situ acquisition and analysis of analytical data in order to further define on-orbit monitoring requirements.

Additionally, systems must be designed to account for factors unique to on-orbit deployment such as crew time availability, payload restrictions, material consumption, and effective operation in microgravity.

DISSERTATION ORGANIZATION

This dissertation focuses on the development, ground testing, and microgravity flight demonstration of Colorimetric Solid Phase Extraction (C-SPE) as a candidate technology to meet the near- and long-term water quality monitoring needs of NASA. The introduction will elaborate further on the operational and design requirements for on-orbit water quality monitoring systems by discussing some of the characteristics of an "ideal" system. A description of C-SPE and how the individual components of the platform are combined to satisfy many of these requirements is then presented, along with a literature review on the applications of C-SPE and similar sorption-spectrophotometric techniques. Finally, a brief overview of diffuse reflection spectroscopy and the Kubelka-Munk function, which are used to quantify analytes via C-SPE, is presented.

Following the Introduction, four research chapters are presented as separate manuscripts. Chapter 1 reports the results from testing the C-SPE methods for the biocidal agents silver(I) and iodine on the KC-135 microgravity simulator. Chapter 2 examines the biocidal iodine platform in more detail in an attempt to determine which iodine species is responsible for the C-SPE signal. In Chapter 3, a new variation of C-SPE is introduced, the quantification of trace analytes based on the collection of an insoluble, colored precipitate. This concept is demonstrated through the determination of nickel(II) using dimethylglyoxime as a precipitating reagent. Chapter 4 combines methods for silver(I) and nickel(II) with a new method for the optical determination of sample pH to create a multiplexed C-SPE platform. The final research chapter is followed by general conclusions and a future prospectus section that concludes the dissertation.

IDEAL WATER QUALITY MONITORING SYSTEMS

There are many factors that must be considered in the evaluation of sensor systems for use in a spacecraft environment. Some of the characteristics of the "ideal" in-flight water quality monitoring system[6] are listed below.

1. <u>Accurate</u>. The analytical performance of in-flight methods should demonstrate an accuracy of 10-20% when compared to accepted ground methods.

2. <u>Dynamic.</u> The minimum dynamic range of the system should bracket the concentration ranges of target analytes established by NASA toxicologists.

3. <u>Versatile.</u> Water quality platforms must be readily adaptable in order to meet changing water quality monitoring requirements as new analytes are identified and prioritized.

4. <u>Rapid.</u> Astronaut time is extremely scarce and prioritized in great detail; analyses that can be executed in only a few minutes fit more readily into crew timelines.

5. <u>Compact.</u> Due to lack of available payload and limited storage space, platforms using small, handheld instruments requiring no ancillary hardware are more attractive.

6. <u>Battery Powered.</u> A cordless instrument eliminates possible complications associated with interfacing to ISS power.

7. <u>User Friendly.</u> Astronaut training time is a precious commodity; a smart, intuitive device not only simplifies on-orbit operation, but also minimizes the demand for crew time during training.

8. <u>Rugged and Safe.</u> Crew health and safety is paramount; the integrated platform must meet or exceed NASA flight hardware guidelines for ruggedness, noise/EMI emissions, ergonomics, and materials.

9. <u>Independent.</u> During long duration missions, the re-supply of consumable items and disposal of waste may not be feasible; techniques generating no waste and operating in a reagentless mode are therefore highly preferred.

The emphasis on the development of water monitoring techniques that embody the attributes of the "ideal" system has been greatly amplified in the wake of the Columbia accident. The temporary grounding of the Shuttle fleet has not only decreased the crew size aboard ISS, but also curtailed ISS re-supply missions. This problem is further compounded because the limited payload capacity of the Russian spacecraft and the lack of available crew time have dramatically decreased water sampling frequency. Coupled with recent occurrences of contamination, these issues make the need for in-flight water monitoring clear. Unfortunately, flight-qualified water testing methods that meet the priorities enumerated above are not currently available.

COLORIMETRIC SOLID PHASE EXTRACTION

To address this need, our research group has been pioneering the development of C-SPE, a sorption-spectrophotometric technique that combines colorimetric reagents, solid phase extraction, and diffuse reflectance spectroscopy to quantify trace analytes in water samples. In C-SPE, a syringe is used to meter a known volume of sample through a membrane impregnated with a selective colorimetric reagent along with any additives required to optimize the complexation of the reagent and analyte. As the sample is passed through the membrane, analytes are extracted and complexed, leading to a detectable change in the optical characteristics of the membrane. The analyte-reagent complex is then quantified directly on the membrane, using a hand-held diffuse reflectance spectrophotometer. This entire process is illustrated in Figure 1. Because all requisite

reagents for C-SPE methods are immobilized on solid supports and analytes are determined on-membrane, this technique operates in a solid-phase mode that markedly reduces waste generation. The role that each component of C-SPE (colorimetric reagents, solid phase extraction, and diffuse reflection spectroscopy) addresses in the effort to realize the characteristics of the ideal water quality monitoring system is discussed below.

Colorimetric Reagents

The reagents used in C-SPE facilitate the determination of analytes based on a color change that results from complexation. Incorporating the rich tradition of colorimetric methods of analysis into C-SPE makes the platform tremendously versatile. Through the use of both organic and inorganic complexing reagents, colorimetric methods have been developed to determine virtually the entire periodic table.[7, 8] Moreover, because established analysis procedures exist for many analytes, the accuracy of C-SPE can be verified through comparisons with these methods.

Although historical, solution-based procedures serve as important guides in the extension of C-SPE to new analytes, very few are directly adaptable to use in spacecraft environments. Some reagents work very well in solution, but fail to react when impregnated on an extraction membrane, which is a common observation.[9] Other reagents require labor intensive, multi-step procedures, acid digestion, heating, or do not pass toxicological review and are therefore excluded from consideration. Despite this difficulty, the judicious choice of complexing reagent and careful optimization of reaction conditions provide an invaluable starting point for the development of C-SPE methods that target most of NASA's water quality parameters.[10]

Solid Phase Extraction

By utilizing solid phase extraction (SPE) membranes as a matrix for the impregnation of colorimetric reagents, C-SPE has the ability to determine analytes at the sub-ppm level required by NASA. In SPE, a solid adsorbent is used to extract analytes from a liquid sample. Once extracted, the analytes are then eluted from the adsorbent using an appropriate solvent. The volume of solvent used for elution is typically much smaller than the sample volume, which creates a concentration factor (*CF*), defined as:[11]

$$CF = V_s / V_e \tag{1}$$

where V_s is the sample volume and V_e is the volume of eluent. Impregnation of colorimetric reagents on the adsorbent allows analytes to be concurrently complexed upon extraction. Because complexation leads to a color change, the analytes can be quantified without elution based on the change in the optical characteristics of the membrane. As such, a minimum *CF* can be calculated by equating the geometric volume of the extraction membrane with V_e. Assuming that the analytes are distributed evenly throughout the entire volume of an extraction disk, a 1.0-mL sample passed through a 13-mm diameter C-SPE membrane would provide a *CF* of ~150. The concentration of analytes on the solid phase extraction membrane enables the use of small sample volumes and is responsible for the low limits of detection achieved by C-SPE.

Diffuse Reflectance Spectrophotometer

Read-out of C-SPE membranes is accomplished using a BYK-Gardner color-guide sphere d/8° spin diffuse reflection spectrophotometer (model PCB-6830). This commercial, off-the-shelf instrument was designed for factory floor applications to monitor the quality of coatings and finishes, and as a result, is very durable. The spectrophotometer also possesses

many desirable physical characteristics for on-orbit use. It is small (8.1 x 17.8 x 9.4 cm), lightweight (0.5 kg), battery operated, and has a spectral range of 400-700 nm in 20-nm intervals, which spans the absorbance region of many common colorimetric reagents. Furthermore, the instrument has a six-month calibration cycle, can collect 10,000 spectra on a single set of batteries, employs simple, one button operation, and provides rapid data; acquisition of a single sample spectrum requires ~2 s.

LITERATURE REVIEW

Our laboratory has employed C-SPE for the determination of silver(I),[12] molecular iodine,[13] lead,[14] nickel(II),[15, 16] and copper(II), iron(III), and chromium(VI)[16] using reversed phase Empore™ SDB-XC extraction membranes. However, the combination of colorimetric reagents, solid phase extraction, and diffuse reflectance spectroscopy to determine trace analytes in water is not unique to our group. Other research groups have used variations of C-SPE for a variety of applications. The three main variations of C-SPE are defined below along with a list of example applications for each variation.

Complexation and Extraction

The first variant of C-SPE, which is also the least utilized, is complexation and extraction. In complexation and extraction, target analytes and colorimetric reagents are combined in solution and the soluble analyte-reagent complex is extracted and used for quantification. Polyamide membranes have been used as sorbents for the determinations of noble metals with azorhodanines, tyrodine, and sulfonitrophenol M[17] and vanadium in various oxidation states with sulfonitrophenol M.[18] Octadecyl silica has also been utilized to extract trace levels of nitrate in water following reaction with Shinn reagent.[19]

Extraction and Exposure

Extraction and exposure consists of extracting analytes onto adsorbents followed by exposure of the adsorbent to complexing reagent solutions. The most common adsorbents in this variant are ion exchangers, which have been employed to quantify mixtures of vanadium and molybdenum with 8-hydroxyquinoline-5-sulfonic acid and phenylfluorone,[20] rhenium with thiocyanate,[21] lead with 4-(2-pyridylazo)resorcinol,[22, 23] arsenic as a molybdoarsenic heteropoly acid,[24] mercury with dithizone,[25] as well as copper with sodium diethyldithiocarbamate, chromium with 1,5-diphenylcarazide, and nickel with dimethylglyoxime.[26] Zinc has also been determined with extraction and exposure using 1-(2-pyridylazo)-2-naphthol on cloth impregnated with vinylpyridinium groups.[27]

Impregnation and Exposure

The third form of C-SPE, utilized by our group in methods for the determination of silver(I) by complexation with 5-(p-dimethylaminobenzylidene) rhodanine,[12] molecular iodine with poly(vinylpyrrolidone),[13] and copper(II), iron(III), and chromium(VI) with dithiocarbamate, 8-hydroxyquinoline-5 sulfonic acid, and diphenylcarbazone, respectively,[16] is impregnation and exposure. Impregnation and exposure, where colorimetric reagents are loaded onto adsorbents and analytes are subsequently extracted and complexed, has been used to test for cobalt and palladium with heterocyclic azo compounds,[28] thorium with arsenazo I,[29] zinc with 1,10 phenanthroline in the presence of bromophenol blue,[30] lead and nickel with Xylenol Orange,[31] nickel with benzylglyoxime and dimethylglyoxime,[32] cationic surfactants with zincon and thiazine red ,[33] and fluoride anions with thorium complexes of arsenazo I[34] on silica gel. Iron has been measured on polyvinyl chloride membranes with bathophenanthroline[35] and cellulose supports have been used in the determination of

aluminum with eriochrome cyanine C[36] and nickel with glyoximes.[37] Beryllium has also been determined by this variant on a variety of supports using eriochrome cyanine C.[32, 38]

DIFFUSE REFLECTANCE SPECTROSCOPY

Analytes are quantified in C-SPE using diffuse reflectance spectroscopy, which utilizes radiation reflected off a rough surface to obtain compositional information about a sample. When electromagnetic radiation is reflected from a condensed phase, the reflected light can be separated into two components, a specular reflection and a diffuse reflection. Specular reflection occurs at smooth, mirror-like surfaces and diffuse reflection occurs from dull or textured surfaces. In reality, light reflected from any surface, no matter how smooth or rough, always contains both specular and diffuse components.[39-41] The difference between specular and diffuse reflection is illustrated in Figure 2.

When light impinges a textured sample, such as C-SPE membrane, a small fraction of the light is reflected specularly, but a substantial portion of the incident radiation penetrates into the sample interior. This penetrating radiation undergoes a combination of scattering (i.e., reflection, refraction, and diffraction) and wavelength-dependent absorption within the material before a portion of the light is returned to the surface. At the surface, the radiation re-emerges from the sample traveling in all directions with equal intensity, and the surface appears uniformly light at all angles of incidence. This remitted light constitutes diffuse reflection. Diffusely reflected light can be collected and used to create a reflectance spectrum which contains information about sample composition.

Because diffuse reflection arises as a result of the simultaneous scattering and absorption of light, it is typically described by two-constant theories. In these theories, one constant describes the scattering of the sample and the other constant characterizes the

sample absorbance. The most commonly employed theory used to describe and analyze diffuse reflection spectra is the Kubelka-Munk theory,[42] which enables diffuse reflectance spectra to be used for quantitative work in a manner analogous to absorbance spectra. In diffuse reflectance spectroscopy, the reflectance of a sample is defined as the ratio of the intensities of the reflected to incident light, as shown in equation 2,

$$R_\infty = J/I_0 \tag{2}$$

where R_∞ is the absolute sample reflectance, J is the intensity of the reflected light, and I_0 is the intensity of the incident light. The subscript on the sample reflectance indicates that the sample is infinitely thick. Because it is not practical to measure the absolute reflectance of a sample, the relative reflectance, R'_∞, is typically measured, as shown in equation 3.

$$R'_\infty = R_{\infty\,sample} / R_{\infty\,standard} \tag{3}$$

The Kubelka-Munk function enables relative reflectance to be linearly related to analyte concentration, similar to absorbance using Beer's law, as shown in equation 4,

$$F(R) = (1-R'_\infty)^2/2R'_\infty = 2.303\varepsilon C/s \tag{4}$$

where ε is the absorptivity, s is the scattering coefficient, and C is analyte concentration. Thorough descriptions and derivations of the Kubelka-Munk theory and the principles that govern diffuse reflection can be found in texts by Kortum[41] and Wendlandt and Hecht.[40]

DISSERTATION OVERVIEW

Using the concepts of colorimetric reagents, solid phase extraction, and diffuse refection spectroscopy presented above, this dissertation reports the progress of colorimetric solid phase extraction towards flight-qualification as a water quality monitoring platform. The data chapters that follow are presented as separate manuscripts, each describes in detail

how C-SPE addresses some of NASA's specific needs for water quality monitoring. Chapter 1 discusses the performance of the C-SPE methods for the biocidal species silver(I) and I_2 on the KC-135 microgravity simulator. The interaction of aqueous iodine solutions with PVP, which is the basis for the biocidal iodine determination, is examined in Chapter 2 to determine which form of iodine is responsible for the C-SPE signal. Chapter 3 utilizes the concept of immobilized reagents found in chapters 1 and 2 to create a new variation of C-SPE, the determination of an analyte based on the collection of an insoluble, colored precipitate. This variation is demonstrated through the determination of nickel(II), a metal leachate found in ISS water samples, with dimethylglyoxime. Chapter 4 combines the existing C-SPE methods for silver(I) and nickel(II) with a new method to measure sample pH to create a multiplexed C-SPE platform. The dissertation is concluded with a summation of the current work and a look at future directions.

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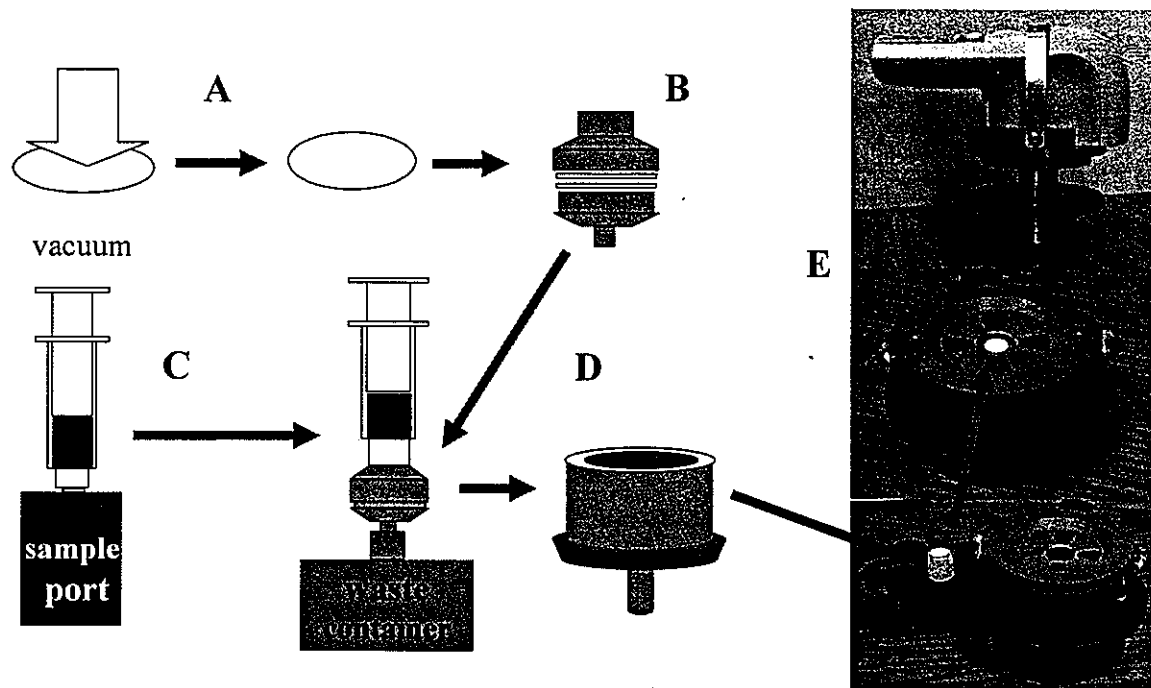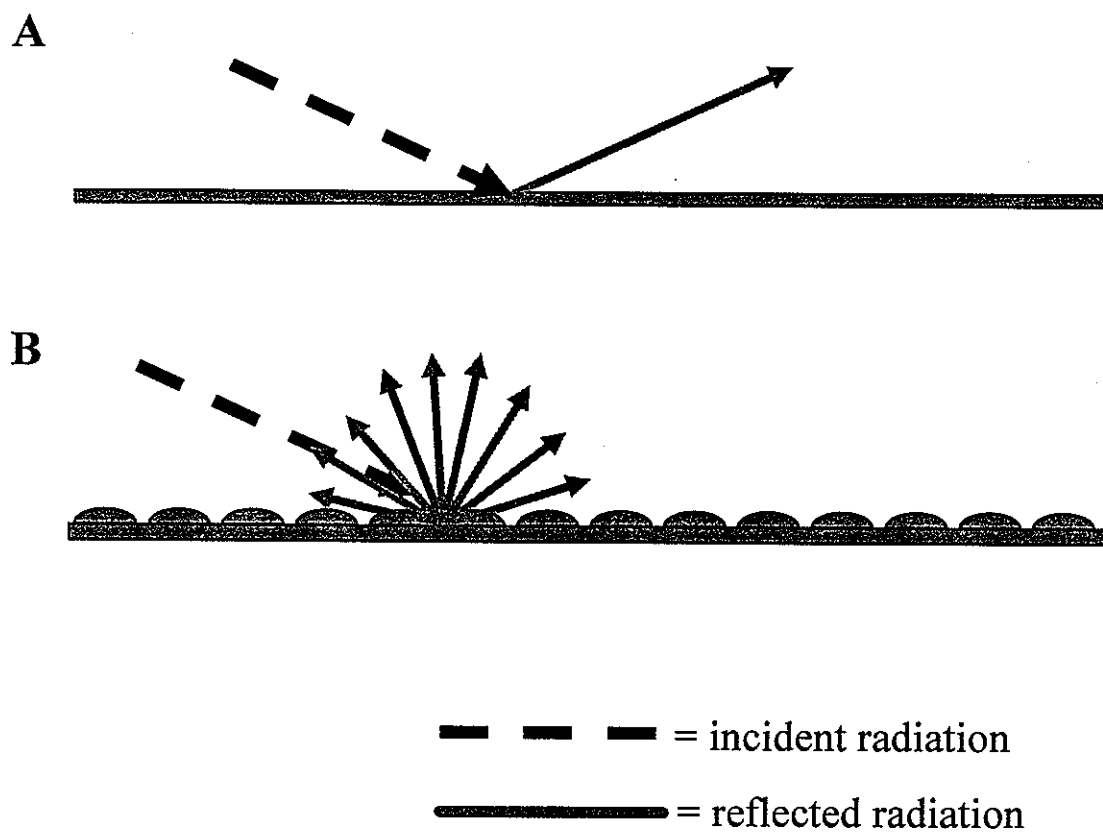
Figure 1.

A

B

= incident radiation

= reflected radiation

Figure 2.

FIGURE CAPTIONS

Figure 1. Illustration of the entire C-SPE process: (A) impregnation of membranes with colorimetric reagents using vacuum, (B) impregnated membranes are cut into 13-mm diameter C-SPE disks and loaded into plastic filter cartridges, (C) sample is collected in a plastic syringe from a Teflon sample bag, (D) sample is passed through the C-SPE disk to extract and complex analytes, and (E) filter holder is disassembled and the lower portion, with the C-SPE disk, is loaded into a sample locator to acquire a spectrum.

Figure 2. Illustration of the incident and reflected radiation is (A) specular reflection and (B) diffuse reflection.

CHAPTER 1: RAPID DETERMINATION OF BIOCIDE CONCENTRATIONS USING COLORIMETRIC SOLID PHASE EXTRACTION (C-SPE): RESULTS FROM MICROGRAVITY TESTING

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ABSTRACT

A sorption-spectrophotometric platform for the concentration and subsequent quantification of biocides in spacecraft drinking water is described. This methodology, termed Colorimetric Solid Phase Extraction (C-SPE), is based on the extraction of analytes onto a membrane impregnated with a colorimetric reagent. Quantification of the extracted analytes is accomplished by interrogating the surface of the membrane with a commercially available diffuse reflectance spectrophotometer. Ground-based experiments have shown that C-SPE is a viable means to determine biocide concentrations in the range commonly found in water samples from the Space Shuttle and the International Space Station (ISS). This paper details efforts to advance C-SPE closer to space flight qualification and ISS implementation, starting with the modification of the ground based biocide detection platform to simplify operation in a microgravity environment. The modified platform was used during KC-135 reduced gravity flights for the rapid, low level determinations of molecular iodine (I_2) and

silver ion (Ag(I)), the biocides used by NASA and the Russian Space Agency, respectively. Iodine is determined as the yellow iodine-poly(vinylpyrrolidone) (PVP) complex and Ag(I) is quantified as the pink silver-5-(*p*-dimethylaminobenzylidene) rhodanine (DMABR) complex. The results from these flight tests, along with the application of C-SPE to the determination of total iodine in a water sample by employing a pre-extraction Oxone treatment are discussed, along with issues requisite for operational improvements.

INTRODUCTION

One critical aspect of spacecraft crew health assurance is maintaining a safe, useable supply of drinking water. To ensure that water provided by the spacecraft distribution and recycling systems is potable, bacterial inhibitors are added. Aqueous iodine (I_2) and silver ion (Ag(I)) are used for this purpose by NASA and the Russian Space Agency, respectively. Ag(I) presently serves as the biocide in the water systems on the International Space Station (ISS).

During Shuttle flights, NASA adds I_2 to fuel cell derived water using the Microbial Check Valve (MCV),[1] an iodinated ion-exchange resin that imparts 2-3 mg/L of iodine and 0.5-1.5 mg/L of iodide. Shuttle water can then be transferred to the ISS using Contingency Water Containers (CWC). If the CWC contents are to be used in the Russian water systems, iodine is removed and replaced with Ag(I) during the filling process. Water supplied by the Russian Space Agency is heat pasteurized, filtered tap water to which colloidal silver is added electrolytically. On-orbit, a special mineral conditioning bed in the condensate recovery system adds low concentrations of minerals and Ag(I) to water dispensed at the galley. In both cases, only a fraction of the biocide added to the water is in its active disinfectant form.

The range of acceptable concentrations for the biocide species are listed in Table 1. The lower limits are set by the need for effective control of bacterial growth. The upper thresholds are defined to prevent negative side effects. Long term consumption of elevated silver can lead to an irreversible blue-gray discoloration of the skin known as argyria.[2] Excess iodine may curtail crew consumption because of odor and taste, and has been associated with disruption of thyroid function.[3]

At present, disinfectant levels in spacecraft water are determined on Earth either by collecting samples post flight, in the case of the Space Shuttle, or by using water samples collected on the ISS and returned by a Shuttle or Soyuz vehicle. This archival approach, however, is not ideal because of concerns over sample degradation during storage and transport. Moreover, the time lapse between sampling and analysis prohibits the detection and correction of inadequacies in biocide levels in real time. These issues can be overcome by measuring biocide concentrations in-flight.

There are a wide variety of Earth-bound analytical techniques capable of accurately determining biocide levels in water. Iodine can be determined by the leuco crystal violet (LCV) method[4, 5] and other spectrophotometric methods,[6, 7] mass spectrometry,[8] and potentiometry,[9] whereas Ag(I) can readily be quantified by ultraviolet-visible (UV-Vis) spectroscopy,[10-14] inductively coupled plasma mass spectrometry (ICP-MS),[15] and electrochemical methods.[16-19] However, the vast majority of available analytical methods do not meet the requirements for space flight implementation. Platforms for use during space flight must provide accurate information relevant to crew health in real time, operate in a reagentless mode to reduce waste and eliminate any possible contamination hazards, meet

strict storage and power guidelines, and function effectively in zero gravity. These methods must also be easy to use in order to minimize demand on crew time.

Our research team has been exploring the development of Colorimetric Solid-Phase Extraction (C-SPE) as an approach to meet the biocide monitoring needs of space exploration.[20-22] C-SPE concentrates and quantifies analytes on a solid-phase extraction membrane. By using diffuse reflection spectrophotometry as an on-membrane readout methodology, C-SPE maintains the high concentration factors of SPE (1000 or more)[23] while reducing waste by eliminating the need for an elution step.

There are three main variations of C-SPE. First, target analytes and colorimetric reagents can be complexed in solution and subsequently extracted and sampled. This approach has been used to determine noble metals on polyamide membranes,[24] nitrate on octadecyl silica,[25] and vanadium on polyamide membranes.[26] Second, analytes can be extracted onto a membrane prior to exposure of the membrane to the colorimetric reagent. This method has been employed to quantify zinc on cloth impregnated with vinylpyridinium groups,[27] mixtures of vanadium and molybdenum on ion-exchange membranes,[28] rhenium on poly(acrylonitrile) impregnated with anion exchangers,[29] lead,[30, 31] arsenic,[32] and mixtures of copper, nickel, and chromium on cation or anion exchangers.[33] Third, the colorimetric reagent can be impregnated directly on the extraction disk followed by exposure to the analyte solution. This strategy has been used to test for cobalt and palladium on functionalized silica,[34] chloride on polyurethane foams,[35] iron on poly(vinyl chloride) membranes,[36] and calcium on ion exchange polymer films.[37]

Our group has recently utilized the third approach to develop methods for the determination of sub-ppm concentrations of silver ion[20, 22] and iodine.[21] Ag(I) is detected in

~60 s as the pink complex of silver 5-(p-dimethylaminobenzylidene) rhodanine (DMABR) and iodine is determined as the yellow iodine-poly(vinylpyrrolidone) (PVP) complex in ~75 s. We have also extended our iodine test method to the determination of total iodine ($I_2 + I^- + I_3^-$) by employing an oxidative pretreatment of the sample solution with Oxone, a commercially available potassium monopersulfate salt.

The findings presented in this paper bring our work with C-SPE one step closer to implementation in space by conducting extractions and collecting sample spectra in KC-135 zero gravity situations. Water samples from ground-filled containers were collected and analyzed in-flight. Following extraction, a modified commercially available sample holder was used to simplify extraction disk positioning prior to quantification of the complexed analyte with diffuse reflection spectrophotometry. Several extractions of solutions containing either Ag(I) or I_2 were collected in zero gravity and compared with ground based-laboratory methodologies. Tests using Ag(I) and iodine samples collected from simulated ISS water dispensing ports were also conducted.

EXPERIMENTAL

Reagents and Chemicals

Iodine standard solutions. Two sets of standard solutions were used in the iodine experiments. In the ground studies (Set 1), a 20.0 ppm stock iodine solution was prepared by combining 16.4 µL of iodine volumetric standard (0.96 N Aldrich) and deionized water (Millipore) to a final volume of 100.0 mL. Standard solutions with concentrations of 0.10, 0.20, 0.50, 2.0 and 5.0 ppm were prepared by dilution of the 20.0 ppm stock.

The flight standards (Set 2) of iodine were prepared from a saturated stock solution. Standards with concentrations of 0.10, 0.20, 0.50, 2.0, and 5.0 ppm were prepared by dilution

of this stock. A 100.0 ppm iodide stock solution was prepared by dissolving potassium iodide crystals in deionized water. Iodide flight standards with the same concentrations as the iodine standards were prepared by dilution of the stock. Laboratory analysis of flight standards was performed the day of the flight using the LCV method.[5]

Ag(I) standard solutions. Two sets of standard solutions were also used in the silver experiments. The ground standards (Set 1) were prepared by diluting a 1000 ppm stock prepared by dissolving 0.157 g of silver nitrate (Fischer) in 100 mL of water (Millipore). Standards with concentrations of 0.10, 0.30, 0.60, and 1.0 ppm were prepared from the stock.

Flight standards (Set 2) were prepared from recrystallized silver fluoride and deionized water. Standard Ag(I) solutions with concentrations 0.10, 0.20, 0.60, and 1.0 ppm were prepared by dilution of a 1100 ppm stock. Laboratory analysis of the Ag(I) flight standards was performed by ICP-MS.

Oxone wool. Oxone (2.0 g, Aldrich) was dissolved in 20.0 mL of water. This solution was used to saturate a 4 inch square of glass wool (Fischer) that had been placed in a 100-mm diameter Petri dish. The wool was then heated in an oven at 110 °C for ~3 h to remove water. Prior to use, the Oxone wool was cut into 13-mm diameter disks.

C-SPE disks. 3M Empore SDB-XC (47 mm diameter) extraction membranes were used as a matrix for impregnation of the colorimetric reagents. Iodine-specific membranes were prepared by pulling a 10-mL aliquot of PVP solution through the membrane using a Millipore Glass Vacuum Filter Holder connected to a mechanical vacuum pump. A pressure difference of 250-300 torr was used for membrane preparation. The PVP solution was prepared by dissolving 3.0 g PVP (MW 10,000, Aldrich) in 100.0 mL of 1:1 methanol:water.

Residual solvent was removed from the membrane by increasing the pressure difference to 500 torr for ~10 s.

Ag(I)-sensitive membranes were prepared by pulling 10 mL of DMABR solution through the membrane with a pressure difference of 200 torr. The impregnating solution was prepared by dissolving 0.02 g of DMABR (Aldrich) in 100 mL of 80:20 methanol:dimethyl formamide (Fischer). Residual solvent was removed by increasing the pressure difference to 500 torr for 10 s. The membranes were allowed to dry on the bench top for ~30 min, and then treated with 5 mL of a 3% (w/w) aqueous Brij-30 solution by pulling the solution through the membrane at a pressure difference of 400 torr. Although the mechanism is not yet fully understood, we have previously found that treatment with Brij-30, an non-ionic surfactant, is necessary for the colorimetric reaction to proceed rapidly.[22]

Following reagent impregnation, the two sets of membranes were allowed to dry on the bench top for ~2 h. The treated membranes were then cut into 13-mm disks and stored in a lighttight and airtight container. Prior to use, the disks were positioned onto the lower portion of a plastic filter holder (Swinnex Filter Holders, Fischer part 09-753-10ASX00 0013 00) and held in place with a ring of two-sided tape (3M). The lower portion of the holder was then threaded tightly into the top portion of the holder, which contained a thin rubber gasket. The gasket has an inner diameter of 10 mm and defines the area of the C-SPE disk that is exposed to sample solution.

Spectrophotometer

A BYK-Gardner color-guide sphere d/8° spin diffuse reflection spectrophotometer (model PCB-6830) was used for spectrum collection (Figure 1). This spectrophotometer is small (8.1 x 17.8 x 9.4 cm), lightweight (0.5 kg), has a three month calibration cycle, and can

acquire 10,000 spectra on 4 AA batteries. The instrument collects spectra from 400-700 nm in 20-nm intervals. The aperture of the integrating sphere is 11 mm, which allows for sampling of the entire surface of the C-SPE disk. Acquisition of a single sample spectrum requires less than 2 s. Data transfer to a personal computer is accomplished using a standard RS-232 port and a cable provided by the instrument manufacturer.

Sample Locator

A BYK-Gardner sample holder (model PCB-6845) was modified in-house to facilitate the reproducible alignment of the surface of the extraction disk with the aperture of the integrating sphere (Figure 2). The modification was accomplished by adding a swing arm (Delrin) to the spring loaded sample platform. The arm was drilled out to hold the lower portion of the membrane holder. Once the lower portion of the holder is mounted in the arm, the arm swings into position under the spectrophotometer mount and locks the sample in place for spectrum acquisition. This modification automatically aligns the sample with the instrument aperture. A metal retaining strap is used to securely mate the spectrophotometer to the locator.

Sample Ports

Spacecraft and ISS galley water systems were emulated using two 4-L bladder tanks (Bag-in-a-Bottle P/N 17000-0040, Berghof/America, Concord, CA) mounted under the flight table, one for iodinated water (4.25 ppm total iodine) and one for Ag(I) treated water (0.261 ppm). The polyethylene shell of the tank contained a compression fitting that allowed pressure to be applied to the inner Teflon bladder. Once pressure is applied to the internal bladder, the water tanks can dispense water in a zero g environment. A fitting incorporated into the cap of the bottle dispensed the contents of the bladder. Breathing air from a K-size

bottle with a gas regulator, relief valve, and manifold system was used to pressurize the tanks. Teflon tubing and valves were used for water distribution and the dispensing ports were equipped with stainless steel male Luer-lock fittings (P/N SSA-1305, S4J Manufacturing, Cape Coral, FL), similar to the sampling interfaces used on the ISS. During the flight experiments, port samples were collected from Teflon sample bags (American Fluoroseal Corp., Gaithersburg, MD) filled from the sample port during flight.

Flight Table

A photograph of the flight table is shown in Figure 3. Preloaded C-SPE disk holders were screwed onto a sample board that was held to the experimental table with Velcro (Figure 3B). Teflon sample bags containing the standard solutions (Figure 3D) and syringes (Norm-Ject, Henke-Sass, Wolf) (Figure 3C) were held to the table with elastic straps. The modified sample locator and spectrophotometer (Figure 3A) were bolted to the top of the aluminum table. Ports used to collect water samples in zero gravity (Figure 3E) were located at the corners of the table.

Extraction Procedures

Iodine. A 10.0-mL sample was collected from one of the Teflon sample bags via a Luer-Lok port using a 20-mL plastic syringe. A preloaded disk holder was then attached to the filled syringe and the water sample was passed through the extraction disk into a waste bag. Following the extraction, the syringe was removed from the holder and the sample disk was dried by pushing ~60 mL of air through the disk into the waste container. The holder was then removed from the waste bag and the two portions of the holder were separated. The lower portion of the holder, with the colorimetric disk attached, was placed in the sample locator and a spectrum was acquired.

Iodide. The procedure for the determination of iodide paralleled the iodine procedure with one notable exception: the sample syringe was filled by pulling the aqueous sample through a membrane holder that contained a 13-mm disk of Oxone wool. After filling, the Oxone cartridge was detached from the syringe before continuing the extraction procedure described above.

Total iodine. The speciation of molecular iodine and total iodine using C-SPE requires the acquisition of two separate spectra. The first spectrum is obtained following the protocol for the determination of iodine. The second spectrum is collected after the Oxone pretreatment, and is a measure of iodine, iodide, and triiodide. By subtracting the *F(R)* value for iodine from the value for the second extraction, speciation between iodine and the sum of iodide and triiodide is possible via equation 1.

$$[I_{total}]_{ppm} = [I_2]_{ppm} + ([I^-]_{ppm} + [I_3^-]_{ppm}) \tag{1}$$

Samples utilized in the total iodine experiments were taken from bags filled from the sample ports in-flight.

Ag(I). The determination of Ag(I) followed the same procedure as iodine, but used a smaller sample volume. For Ag(I), a 2.0-mL sample was withdrawn into a 3-mL syringe.

Ag(I) port samples. The determination of Ag(I) in the port samples follow the same procedure as the Ag(I) experiments, but used samples collected from the simulated ISS dispensing ports.

KC-135 Flight Experiments

The parabolic flight path of the KC-135 simulates zero gravity when the plane changes direction at the top of each parabola. The duration of the zero gravity periods varies from 20-40 s, depending on the flight path. Extraction and collection of a spectrum for Ag(I)

takes ~60 s and iodine requires ~75 s. Since the complete procedure could not be completed during the zero g segment of a single parabola, the experiment was divided into several steps, which were carried out over the course of several parabolas. All experimental work was conducted during the zero g portions of the flight.

Readout

Post flight, the reflectance spectra were downloaded into a MS-Excel spreadsheet using BYK-Gardner QC-Link software. The data was then transferred to a Sigma Plot® notebook and a Kubelka-Munk transform was performed. The Kubelka-Munk function, $F(R)$, is defined as:

$$F(R) = (1-R)^2/2R \qquad (2)$$

where R is the percent reflectance and is measured with respect to a standard white tile from BYK-Gardner (#6841). $F(R)$ can be related to analyte concentration by

$$F(R) = 2.303\varepsilon C/s \qquad (3)$$

where ε is absorptivity, C is analyte concentration, and s is the scattering coefficient of the sample surface. The optimum detection wavelengths, 580 nm for Ag(I) and 440 nm for I_2, were previously determined from UV-Vis data collected for the reagent and the analyte-reagent complex.[21, 22] By assuming that the absorptivity and scattering coefficient of the sample disks are constant from one experiment to the next, $F(R)$ is therefore directly proportional to analyte concentration.

RESULTS

Ground-based Calibrations

Iodine and Ag(I) solutions (Set 1) were used to calibrate the C-SPE response for each analyte prior to the flight experiments. The standard concentrations were representative of

the range of iodine levels found in water collected from the Shuttle systems and Ag(I) found in samples returned from the ISS. Linear calibration plots resulted from graphing $F(R)$ as a function of concentration for each biocide standard (Figure 4). Experimental biocide concentrations were calculated using the equations from the ground based calibration plots and the $F(R)$ values obtained during the microgravity flight. Each solution was analyzed in duplicate and the average $F(R)$ was used in the calculation of the experimental concentration. Ground-based concentrations were determined by the LCV method for iodine and ICP-MS for Ag(I). Correlation plots were then constructed by plotting the experimental concentrations obtained in-flight on the y-axis and laboratory concentrations on the x-axis.

Simulated Microgravity Experiments

Iodine. The laboratory LCV results for the flight (Set 2) solutions are plotted against the experimentally determined concentrations from the KC-135 flight in Figure 5. The laboratory values are also used as flight concentrations for comparative purposes in Figure 5, yielding a straight line with a slope of unity. Ideally, the two plots in Figure 5 should be superimposable. While the flight results approximate a linear correlation with the LCV data, the flight data are consistently lower than those obtained with the ground-based LCV method.

Iodide. The results of the iodide determination in-flight are plotted in Figure 6. These data are presented in the same manner as the iodine data. Again, the experimental data displays a negative deviation from the expectations of the laboratory analysis. In this case, however, the differences are larger than those for the determination of iodine.

Total iodine. The average experimental concentrations (N=2) from the total iodine determinations are listed in Table 2 along with the LCV results (actual concentration). As

mentioned, these results were obtained using an iodinated water sample withdrawn from a water port constructed to mimic the interface currently used on the ISS. Although there is reasonable agreement between the flight and ground based determinations of total iodine (6% difference), there is considerable disagreement between the values obtained for iodine and for the sum of iodide and triiodide.

Ag(I) solutions. Because the membranes used to determine Ag(I) are initially colored by impregnation of DMABR, the flight-based C-SPE results were background corrected prior to being plotted against the ground-based laboratory values from ICP-MS. This correction was accomplished by subtracting the experimental concentration determined at 0.0 ppm from the values obtained for all other samples. Figure 7 presents the results of the flight extractions. The laboratory ICP-MS results served as the ground concentrations, and are also plotted as flight concentrations for comparative purposes. There is close agreement with the ground values at low concentration, but the plot begins to show a strong positive deviation at higher concentrations.

Ag(I) port samples. The Ag(I) sample taken from the mock-up of the ISS water dispensing ports also displayed fluctuations in the calculated concentration. Table 2 lists the ICP-MS concentration value from the silver tank samples and the average experimental Ag(I) concentration (N=5). Several of the individual Ag(I) trials yielded concentrations close (4% difference) to those obtained with ICP-MS. However, the relative standard deviation (RSD) of all the measurements is 35%.

DISCUSSION

The C-SPE methods for the determination of iodine and iodide are procedurally very similar, the only difference being the oxidation step associated with the iodide determination.

It is therefore expected that the plots in Figures 5 and 6 should be very similar. While both

plots of the flight data exhibit a negative deviation with respect to the ground-based data, the

iodide results show decreased linearity. Iodine is subject to loss by various mechanisms

including adsorption, redox reactions, and volatilization. The depletion of iodine by any of

these mechanisms would cause the solution equilibrium to shift, changing the concentrations

of all iodine species and making comparisons of the two analytical methods difficult when

the samples are not analyzed at the same time. In these trials, the flight-based determinations

were carried out several hours before the ground-based analyses. However, degradation of

the iodine samples via these mechanisms should result in a positive bias for the flight data

relative to the ground data, which is not the case.

More plausible explanations for the negative deviation in Figures 5 and 6 would be

the inability to remove trapped air while sampling in zero gravity or differences between the

LCV and C-SPE methods. Inclusion of air bubbles severely limited accurate determinations

of sample volume, generally resulting in sample volumes being lower than intended because

of the volume occupied by trapped air. This decrease is consistent with the negative

deviations in Figures 5 and 6. A systematic error of this type would be more pronounced in

the iodide and total iodine experiments because the syringes were filled by pulling a sample

through an Oxone wool cartridge. This step would likely introduce more air into the

sampling system due to the cartridge dead volume, yielding a greater deviation than that

obtained without the Oxone step. This greater, negative deviation was observed in the iodide

determinations.

There are differences between the LCV and C-SPE methods concerning which iodine

species are detected. The LCV iodine method is sensitive to I_2 and HOI, and the total iodine

LCV method measures I_2, HOI, I^-, and I_3^-. Recent experiments[38] have shown that the C-SPE iodine method measures only I_2 in solution and the total iodine method determines I_2, I^-, and I_3^-. The inability to measure HOI by C-SPE could lead to a significant negative bias vs. the LCV method, since HOI is one of the dominant iodine species in solution at neutral pH.

The positive differences in the Ag(I) data in Figure 7 cannot be explained by inaccurately determined sample volumes or differences in the species being detected by the two methods. At low concentrations, there is apparent agreement between the flight and ground results, but there is considerable positive deviation at higher concentrations. We suspect that this discrepancy arises, in large part, from sample instability. Since the KC-135 tests were performed prior to the ICP-MS experiments, it is possible that Ag(I) slowly adsorbed onto the sample container surface, lowering the analyte concentration.

CONCLUSIONS

A sorption-spectrophotometric platform, C-SPE, has been demonstrated as a potential methodology to provide real time analytical data for drinking water biocide level monitoring. Quantification of the extracted analytes was accomplished in zero gravity and compared with other laboratory methods. The modified platform produced results showing a relationship between F(R) and biocide concentration. While the results exhibited some linearity, there were differences between the C-SPE results and the LCV method for iodine and ICP-MS for Ag(I). The results from these flight tests begin to demonstrate important capabilities essential to in-flight water quality monitoring. Experiments, such as ground based trials with prototypes representing flight-like instrumentation (sample bags, tubing, etc.) to examine analyte adsorption, work to develop air removal strategies, and comparisons between C-SPE

and control methods used to determine biocide concentrations are being undertaken to minimize deviations between ground and flight results.

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Table 1. Analytical requirements for in-flight biocide analyzers.

Analyte	Concentration range (mg/L)
Ag^+	0.05-1.0
I_2	0.1-5.0

Table 2. Iodine and silver tank samples.

Analyte	Actual concentration (mg/L)	Average experimental concentration (mg/L)
I_2	0.19	1.36
Total iodine	4.25	4.45
$[I^-] + [I_3^-]$	4.06	3.09
Ag^+	0.261	0.204

Figure 1.
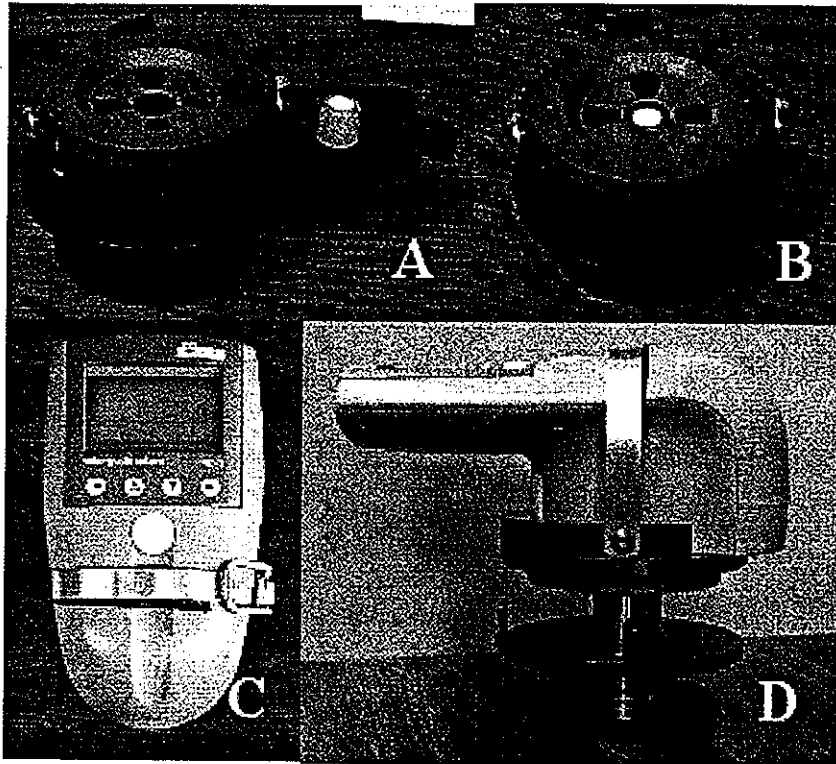
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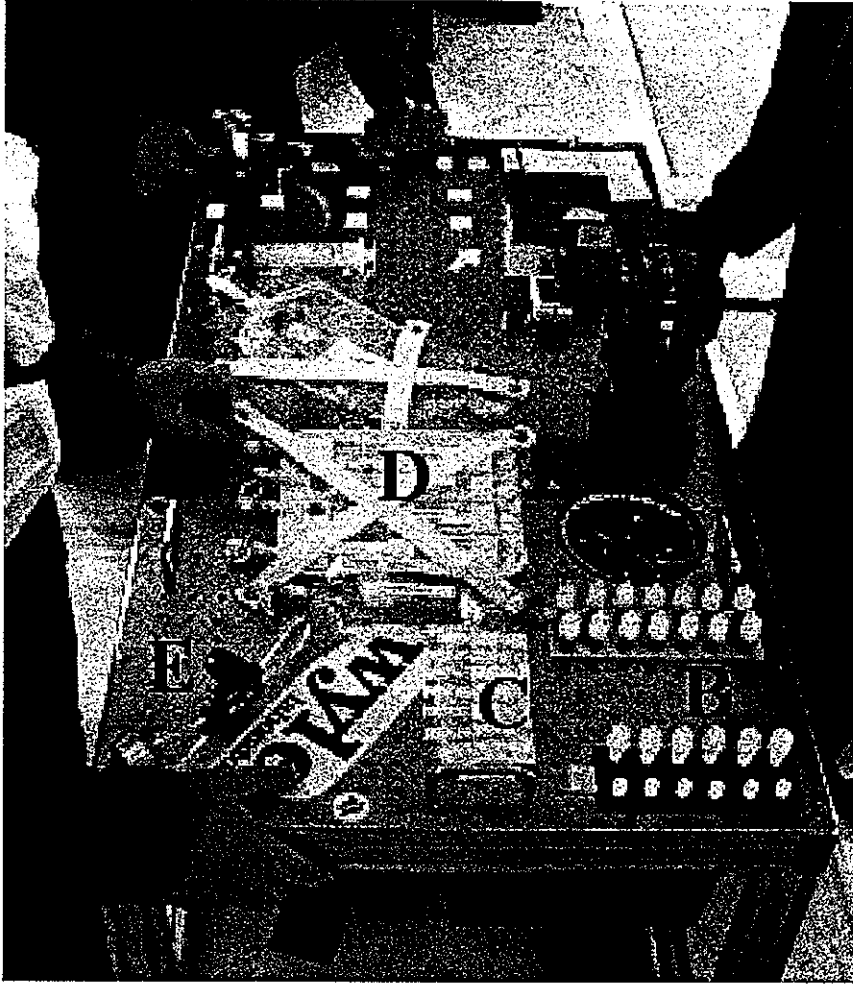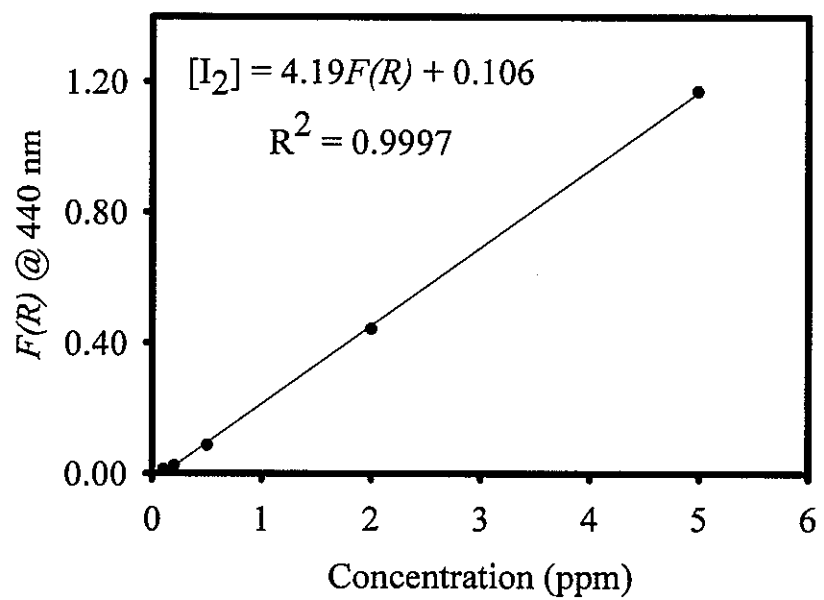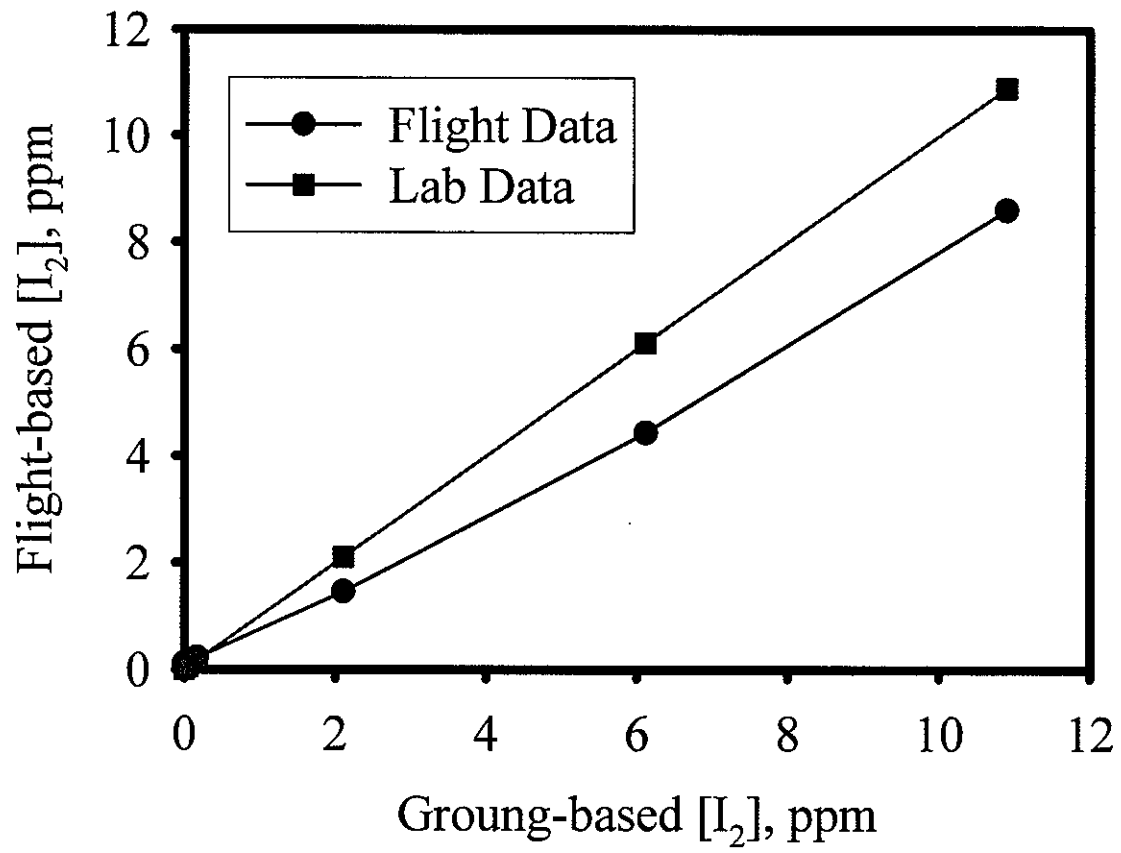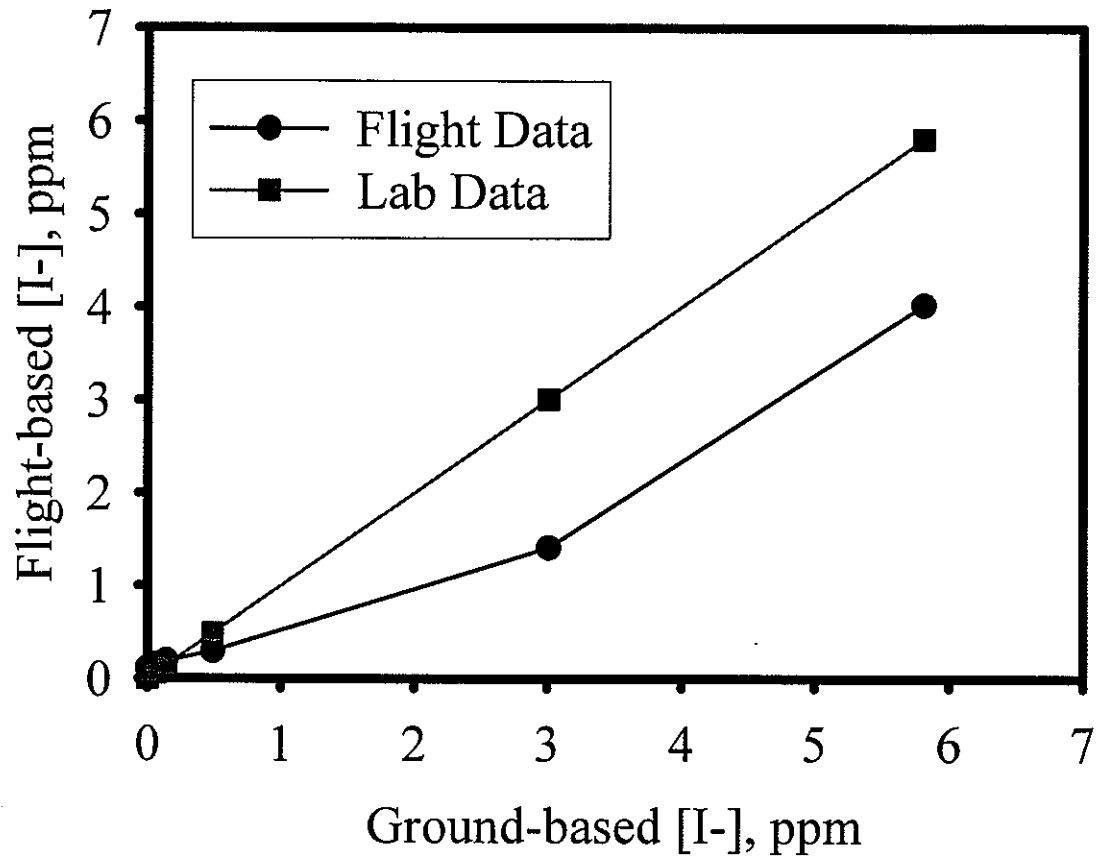
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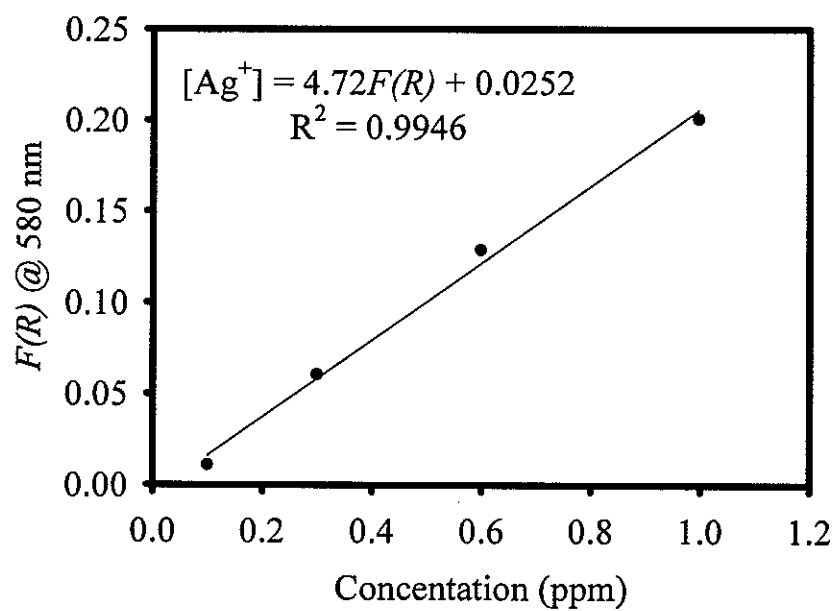
Figure 4.

Figure 5.

Figure 6.
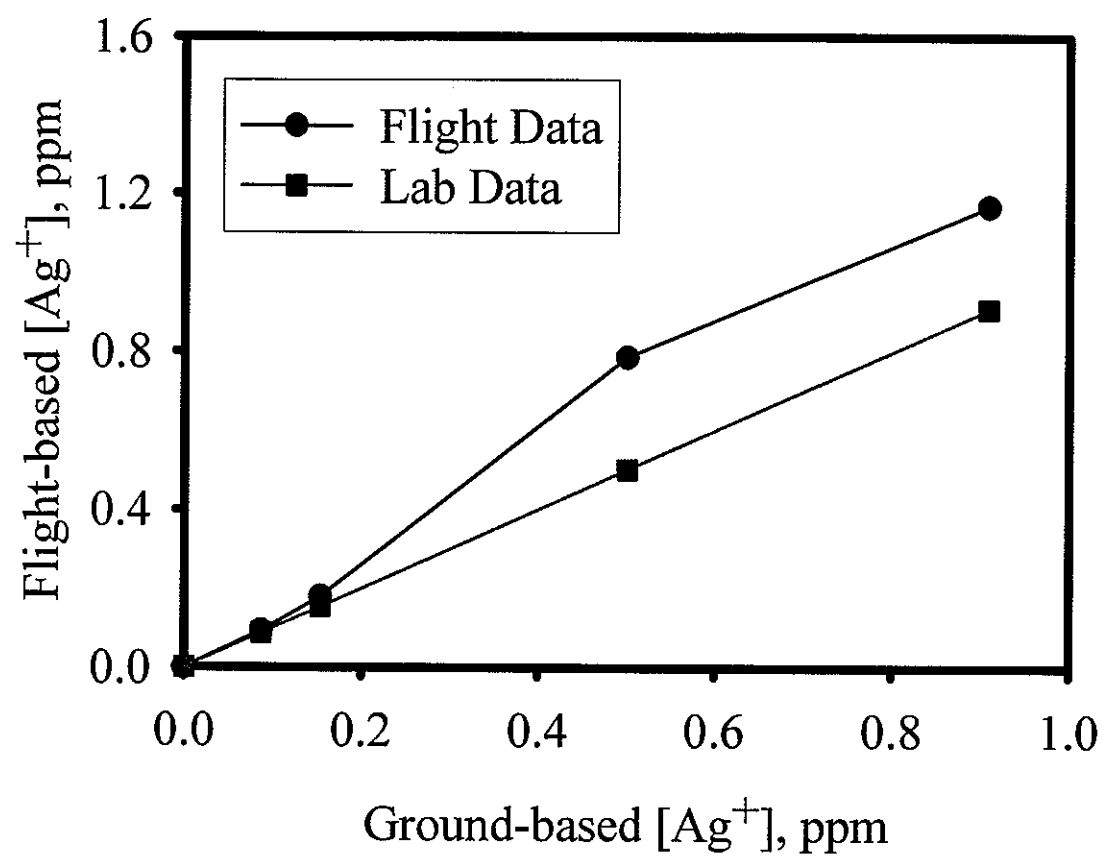
Figure 7.

FIGURE CAPTIONS

Figure 1. Hand held diffuse reflection spectrophotometer. Left photograph – top view; right photograph – side view.

Figure 2. Modified sample locator before (A,B) and after mounting the spectrometer. (A) Sample locator with extended sample arm and membrane holder. (B) Sample locator with loading arm locked into position for sample acquisition. (C) Top view of spectrometer mounted on sample locator. (D) Side view of spectrometer mounted to sample locator.

Figure 3. Flight table set-up. (A) Spectrophotometer, (B) membrane holders, (C) syringe holder, (D) sample bags, and (E) sample dispensing port.

Figure 4. Calibration Plots for iodine (top) and Ag(I) (bottom).

Figure 5. Iodine flight-and ground-based results.

Figure 6. Iodide flight- and ground-based results.

Figure 7. Ag(I) flight- and ground-based results.

CHAPTER 2: INVESTIGATION OF THE IODINE-PVP INTERACTION EMPLOYED IN THE DETERMINATION OF BIOCIDAL IODINE BY COLORIMETRIC SOLID-PHASE EXTRACTION

Daniel B. Gazda, Robert J. Lipert, James S. Fritz, and Marc D. Porter

ABSTRACT

Colorimetric solid phase extraction (C-SPE) has been previously explored as a means to monitor the iodine-based disinfectant used in the water systems on board the Space Shuttle. This same disinfectant is baselined for eventual deployment in the U.S. water recovery system planned for Node 3 of the International Space Station (ISS). With C-SPE, the I_2 concentration is determined from the diffuse reflectance spectrum (DRS) of the yellow iodine-poly(vinylpyrrolidone) (PVP) complex using the Kubelka-Munk function. However, the solution chemistry of iodine is very complex and results in a variety of inorganic species (e.g., I^-, I_2, I_3^-, HOI) that have vastly different biocidal capabilities. Thus, the nature of the interaction of iodine with PVP, and more specifically, the identity of the iodine species involved in the interaction requires more elucidation. This paper reports the findings from a series of detailed experiments conducted to elicit a more complete understanding of the iodine-PVP system employed in C-SPE. The results indicate I_2, one of the two dominant biocidal forms of iodine, is the species responsible for the analytical signal in our C-SPE platform. These findings lay the ground work for the planned development of a multiplexed

iodine determination and speciation platform for in-flight analysis of spacecraft water samples.

Keywords: colorimetry, solid phase extraction, iodine, poly(vinylpyrrolidone), diffuse reflectance spectroscopy

INTRODUCTION

NASA is currently investigating the viability of employing iodine as a biocidal agent in the water storage and dispensing systems on International Space Station (ISS). The regenerative nature of ISS water systems and the need to store water for prolonged periods necessitates the continuous addition of bacterial inhibitors to ensure a safe supply of drinking water for flight crews. Iodine solutions have been used as disinfectants for many years and NASA has employed iodine for this purpose in spacecraft water systems since the Apollo missions [1]. Water treatment has evolved considerably since that time, moving from pre-flight treated water to dynamic in-flight conditioning systems like the Microbial Check Valve (MCV). The MCV is an iodinated ion-exchange resin that releases 2-3 µg/mL of molecular iodine (I_2) and 0.5 to 1.5 µg/mL of iodide (I^-) to the water generated by the fuel cells prior to entering the Shuttle water storage tanks [1].

Presently, water samples used to determine iodine disinfectant levels are collected from the Space Shuttle water system pre- and post-flight. These samples are then transported to NASA laboratories for analysis [2]. On-ground, there are several analytical techniques capable of characterizing and quantifying iodine in water samples. These techniques include the leuco crystal violet method [3, 4], direct UV-Vis spectrophotometry [5], mass spectrometry [6], and potentiometry [7]. While these methods produce high quality analytical data, the determined iodine concentrations are representative of the sample

composition after return to Earth. Although not a major concern for short duration (10-14 day) Shuttle missions, sample integrity in ISS missions, where samples may be stored up to six months before there is a Shuttle or Soyuz flight that can return archived samples to Earth, is a major concern. To obtain accurate determinations of biocidal iodine levels in spacecraft water systems, it is therefore critical to develop a test platform capable of in-flight operation.

Our laboratory has been advancing the development of Colorimetric Solid-Phase Extraction (C-SPE) as a means to meet the design and operational requirements imposed by NASA for on-orbit biocide determinations. C-SPE concentrates and quantifies analytes on a reagent-impregnated solid-phase extraction membrane. Diffuse reflection spectrophotometry is then used as an on-membrane read out technique to quantify the analyte via the Kubelka-Munk function. Importantly, collecting the diffuse reflectance spectrum (DRS) of analyte-reagent complexes on the surface of an impregnated membrane maintains the high concentration factors of SPE (1000 or more [8]), reduces waste by eliminating analyte elution, and enables reagentless operation.

There are three main variations of C-SPE: (1) complexation and extraction, (2) extraction and exposure, and (3) immobilization and extraction. In complexation and extraction, target analytes and colorimetric reagents are combined in solution and the soluble analyte-reagent complex is extracted and sampled. Several noble metals [9] and vanadium in various oxidation states [10] have been determined in this manner. Extraction and exposure consists of analyte extraction followed by exposure to complexing reagent solutions. This variant has been employed to quantify zinc on cloth impregnated with vinylpyridinium groups [11] and mixtures of vanadium and molybdenum [12], rhenium [13], lead [14, 15], arsenic [16], and copper, chromium, and nickel [17] on ion exchangers. Immobilization and

extraction, where colorimetric reagents are immobilized and analytes are subsequently extracted and complexed, has been utilized to test for cobalt and palladium [18] and thorium [19] on silica gel, chloride on polyurethane foams [20], iron on treated polyvinyl chloride membranes [21], aluminum on cellulose [22], beryllium on a variety of supports [23, 24], and nickel on silica gel [25] and cellulose [26].

Our group has utilized immobilization and extraction in our biocide monitoring platforms for silver ion [27, 28] and molecular iodine, iodide, and triiodide [28, 29] as well as methods to determine copper(II), iron(III), and chromium(VI) [30] on polystyrene divinylbenzene. As a means of extending the applicability of C-SPE, our laboratory has also developed a fourth variation which is based on the collection of an insoluble precipitate. This variation has recently been demonstrated in methods for nickel(II) using nioxime [30] and dimethylglyoxime [31] as selective precipitating reagents.

This paper examines the interaction of aqueous iodine solutions with immobilized poly(vinylpyrrolidone) (PVP), which serves as the basis for our C-SPE biocidal platform. These studies were conducted in an effort to develop a more complete understanding of our iodine detection method and therefore validate its applicability to biocidal monitoring. The complexation of iodine with PVP is, however, somewhat controversial. Proposed structures of the colorimetric reagent during complexation include proton fixation between the carbonyl groups of two adjacent pyrrolidone rings [32] and mesomerism of the nitrogen lone pair electrons [33]. The form of iodine involved in the reaction is also unclear, with evidence for the complexation of I_2 [34], I_3^- [32-34], and possibly I_5^- [33] with PVP. Determining the identity of the interacting species is of significance because of the vastly different biocidal activities of these species [35] as well as differences in their relative abundance in solution.

Herein, experiments comparing UV-Vis absorbance spectra and C-SPE results are examined to elicit which iodine species gives rise to the C-SPE signal and to firmly set the stage for the creation of protocols for the use of this methodology as a facile and reliable means for monitoring biocide levels in spacecraft water.

EXPERIMENTAL SECTION

Reagents and Chemicals

Iodine solution. A 20.0 µg/mL stock iodine solution was prepared by combining 16.4 µL of iodine volumetric standard (0.96 N Aldrich) and deionized water (Millipore) to a final volume of 100.0 mL. The volumetric standard, as specified by the vendor, consists of 5-9% I_2 and 10-20 % potassium iodide dissolved in water and stabilized by acid (HCl). A sample solution with a concentration of 5.0 µg/mL in I_2 was prepared by dilution of the 20.0 µg/mL stock with deionized water. All solutions prepared by diluting the 20.0 µg/mL stock are referred to hereafter as volumetric iodine solutions.

Iodide solution. A 200 µg/mL stock I^- solution was prepared by dissolving 26.1 mg of potassium iodide (Fisher) in 100.0 mL of deionized water. A sample solution containing 4.0 µg/mL I^- was prepared by diluting the stock with deionized water.

C-SPE disks. 3M Empore™ SDB-XC 47-mm diameter extraction membranes were used as a matrix for impregnation of the colorimetric reagent. Membranes were prepared by pulling a 10.0-mL aliquot of PVP solution through the membrane using a Millipore Glass Vacuum Filter Holder with a mechanical vacuum pump. A pressure difference of 250-300 Torr was used for reagent impregnation. The PVP solution was prepared by dissolving 3.0 g of PVP (M_w *ca.* 10,000, Aldrich) in 100.0 mL of 1:1 (v:v) methanol:water. After treatment, residual solvent was removed from the membrane by increasing the pressure

difference to 500 Torr for ~10 s. The membranes were then allowed to dry in the ambient for ~2 h before being cut into 13-mm disks and stored in a dark, airtight container until use.

Oxone wool. A solution containing 2.0 g of Oxone (Aldrich) dissolved in 20.0 mL of water was used to load the Oxone wool. This solution was poured over a 100 x 100 mm square of glass wool (Fisher) that had been placed in a 100-mm diameter Petri dish. The dish was heated in an oven at 110 °C for ~3 h to allow evaporative removal of residual water and crystallize Oxone on the surface of the wool fibers.

Apparatus

UV-Vis spectrophotometer. All solution spectra were collected on a Hewlett-Packard model 8453 UV-Visible spectrometer using ChemStation software and a quartz cuvette with a pathlength of 1.00 cm.

Diffuse reflectance spectrophotometer. A BYK-Gardner color-guide sphere d/8° spin diffuse reflection spectrophotometer (model PCB-6830) was used to collect membrane spectra. This spectrophotometer is small (8.1 x 17.8 x 9.4 cm), lightweight (0.5 kg), battery operated (4 AA cells), and collects spectra over the range 400-700 nm in 20-nm intervals. The aperture of the integrating sphere is 11 mm, which allows for sampling of the entire surface of the C-SPE disk. Acquisition of a single sample spectrum requires ~2 s.

Sample Locator. A BYK-Gardner sample holder (model PCB-6845) was modified in-house to reproducibly align the surface of the extraction membrane with the aperture of the integrating sphere. This modification has been described previously [28].

Extraction Procedure

The 13-mm sample disks were positioned onto the lower portion of a plastic filter holder (Swinnex Filter Holders, Fischer part # 09-753-10ASX00 0013 00). The lower

portion of the holder was then threaded tightly into the top portion of the holder which contained a thin rubber gasket. The gasket has an inner diameter of 10 mm and defines the area of the C-SPE disk that is exposed to sample solution.

Water samples with a volume of 10.0 mL were collected using 10-mL plastic syringes. Preloaded sample holders were attached to the syringes, and the water samples were passed through the extraction disk. Following extraction of the analyte, the syringe was removed from the holder and the sample disk was dried by pushing 60 mL of air through the disk. At this point, the holder was separated, the lower portion of the holder, which held the C-SPE disk, was placed in the sample locator and a spectrum acquired. This entire process required ~60 s.

Oxidative pre-treatment. The procedure for treating I^- or I_2 solutions with Oxone in the C-SPE trials paralleled the extraction procedure with one notable exception; a 20-mL sample syringe was filled by pulling the water sample through a membrane holder that contained a 13-mm circle of Oxone wool. After filling, the Oxone cartridge was detached from the syringe before continuing the extraction procedure or acquiring a transmission spectrum.

Readout

After collecting a spectral data set, the hand-held spectrophotometer was interfaced to a computer using a serial cable. The reflectance spectra were downloaded into a MS-Excel spreadsheet and the Kubelka-Munk function was calculated using an in-house modified version of BYK-Gardner QC-Link software. The Kubelka-Munk function, *F(R)*, is defined as:

$$F(R) = (1-R)^2 / 2R \qquad (1)$$

where R is the percent reflectance measured with respect to a standard white tile. The value of $F(R)$ can be related to analyte concentration by [36]

$$F(R) = 2.303\varepsilon C/s \qquad (2)$$

where ε is absorbtivity, C is concentration of the complexed analyte, and s is the scattering coefficient of the sample surface. By assuming the absorbtivity and scattering coefficient of the membrane surfaces are constant at a given wavelength, $F(R)$ can be related directly to analyte concentration.

RESULTS AND DISCUSSION

Iodine Chemistry

Aqueous I_2 rapidly hydrolyzes and undergoes several other reactions that give rise to multiple iodine-containing species in solution [37]. At pH < 7, I_2, I^-, I_3^-, and HOI are the predominant species present [38]. That is, the reactions governing solution composition at pH<7 are equations 3 and 4, which have equilibrium constants of 5.44×10^{-13} [39] and 723 [40], respectively.

$$I_2 + H_2O \leftrightarrow HOI + I^- + H^+ \qquad (3)$$

$$I_2 + I^- \leftrightarrow I_3^- \qquad (4)$$

Of these four species, it is generally agreed that I_2 and HOI are responsible for the biocidal action of iodine solutions [41]. This conclusion is based on the oxidizing capability of these species and the positive correlation observed between changes in their concentrations and the rate of microbial kill [38]. Sample solutions prepared from the iodine volumetric standard, which contains I_2 stabilized by KI and HCl, typically exhibit pH values between 6.4 and 6.6. These solutions therefore meet the criteria for simplifying the number

of equilibria that govern the iodine species in solution and were used as simulated spacecraft water for the UV-Vis/C-SPE comparisons discussed in the following section.

UV-Vis/C-SPE Comparison

PVP, which serves as the complexing agent in our iodine C-SPE platform, is an iodophore commonly used in aqueous antiseptic solutions to increase the solubility of I_2. However, there is some controversy in the literature concerning the interaction of iodine with PVP. In the solid state, crystallographic and IR data argue that iodine-PVP exists not as an adduct of I_2, but rather as an HI_3 complex, where a proton is fixed via hydrogen bonding between two carbonyl groups on adjacent pyrrolidone rings and I_3^- is ionically bound to the resulting cation [32]. Experiments conducted using Raman spectroscopy show no evidence of proton fixation, but did confirm the presence of an I_3^- adduct as well as a minor I_5^- complex [33]. On the other hand, potentiometric studies performed on aqueous PVP-iodine solutions [38] and spectrophotometric investigations in organic solvents [34] suggest that I_2 and I_3^- adducts are both possible.

The interaction of iodine with PVP used in C-SPE is not necessarily analogous to either solution or solid-state experiments. In C-SPE, the colorimetric reagent is adsorbed on the surface of a hydrophobic membrane and then exposed to aqueous iodine solutions. Solutions used in this work, as well as in previous studies [28, 29], were prepared using a volumetric I_2 solution that was stabilized with acid and I^-. As a consequence, I_2, I^-, and I_3^- are all present in the water samples. HI_3, the species thought to interact with PVP by Schenk and co-workers, is regarded as a ligand-stabilized complex, existing only when complexed [32]. Because this species is absent in aqueous solution, the colorimetric signal must arise from the interaction between the polymer, I^-, I_2, and/or I_3^-.

Past work in our laboratory [29] showed that the passage of our volumetric iodine solutions through untreated polymeric extraction membranes resulted in a color change on the membrane surface. This color change was attributed to the extraction of hydrophobic I_2 molecules by the membrane. However, the volatility of I_2 precluded using this direct determination and approaches utilizing membranes pretreated with PVP were pursued. The differences in the plots of the Kubelka-Munk function for 6.0 µg/mL volumetric iodine solutions passed through untreated and PVP-treated membranes are shown in Figure 1. The differences in the spectra show that the iodine solution complexes with the PVP on the treated membrane, and is not simply adsorbed onto the hydrophobic membrane.

In order to determine whether biocidally active I_2 or biocidally inert I_3^- is responsible for the C-SPE signal, the characteristic UV-Vis absorbance wavelengths of I_2 (460 nm), I_3^- (290 and 350 nm), and I^- (226 nm) [42] were used to probe the composition of the aqueous solutions used to simulate spacecraft water. The results of these studies, with and without Oxone treatment, were then compared to data obtained by C-SPE for solutions treated in the same manner. These results are discussed in the next two subsections.

Solution studies. Figure 2 shows the absorbance spectra of an iodine solution prepared from the acid and I^- stabilized volumetric standard to have an I_2 concentration of 5.0 µg/mL and of a potassium iodide (KI) solution made to contain 4.0 µg/mL I^-. There are four distinct peaks in the volumetric iodine spectrum, which are indicative of the three different iodine species present in our simulated spacecraft water. The highest energy peak (226 nm) is from I^-, the two intermediate peaks (290 and 350 nm) are from I_3^-, and the lowest energy and weakest peak (460 nm) reveals the presence of I_2. As expected, the KI spectrum shows only a large absorbance peak for I^-.

The concentrations of I_2, I^-, and I_3^- in the volumetric iodine solution were calculated using the absorbances in Figure 2 and the Beer-Lambert Law with published molar absorbtivity values of 728 [43], 12600 [42], and 25000 (350 nm peak) L mol^{-1} cm^{-1} [5], respectively. These concentrations are listed in Table 1 as the "before Oxone" entries. Using these values, the calculated equilibrium constant for equation 4 (694) is in very good agreement with the published value (723). Moreover, leuco crystal violet (LCV) experiments revealed that the amount of HOI, the other iodine species potentially present in the sample solutions (pH 6.4-6.6), is negligible. In the LCV method, I_2 is oxidized to HOI by $HgCl_2$, with HOI in turn oxidizing leuco crystal violet to crystal violet. Any HOI present in solution prior to the oxidation of I_2 also reacts with the leuco crystal violet and the resulting absorbance of the crystal violet dye at 592 nm is representative of the amount of HOI and I_2 in solution. By removing the $HgCl_2$ from the LCV test solutions, the observed crystal violet absorbance is therefore diagnostic of any HOI initially present in solution. The plots in Figure 3 illustrate that there is no detectable HOI in the volumetric iodine solutions. These results, along with the agreement of the "before Oxone" I_2 concentration with the target solution concentration, verify the absence of HOI because the formation of HOI would decrease the concentration of I_2 in solution according to equation 3.

Figure 4 shows the spectra of the same iodine and iodide solutions following passage through a sample holder containing a circle of Oxone wool. Oxone has been used previously in the determination of I^- by C-SPE [28, 29]. The standard reduction potential of Oxone is +1.44 V (NHE), which is sufficiently positive to exhaustively drive the room temperature oxidation of the halide (I^-) to the neutral halogen (I_2) [44]. From the spectra in Figure 4 and the data in Table 1, it is clear that Oxone treatment is capable of converting not only I^-, but

also I_3^-, to I_2. This transformation is likely a result of the oxidation of all available I^- in solutions and subsequent shift in the equilibrium given in equation 4. The absence of the I^- and I_3^- peaks in Figure 4, coupled with the nearly quantitative conversion to I_2 shown in Table 1, support this conclusion.

C-SPE studies. Figure 5 plots *F(R)* values vs. wavelength obtained with the C-SPE iodine method from the volumetric iodine and the iodide solutions with and without Oxone treatment. As expected, there is no signal generated by exposure of the C-SPE disk to the I^- solution. This finding is consistent with the fact that the two species thought to interact with PVP, I_2 and I_3^-, were not detectably present in the analogous UV-Vis studies described in the last subsection. The volumetric iodine solution produces an *F(R)* value in agreement with previous studies using solutions prepared to have similar I_2 concentrations. However, because both I_2 and I_3^- are present in the volumetric iodine solution, it is not clear which species is involved in the chromogenic complexation.

The results of the UV-Vis studies indicate that exposure of I^- solutions to Oxone converts the halide ion to its neutral halogen form without detectable production of I_3^-. Those experiments also showed that I^- and I_3^- are completely removed from iodine solutions by Oxone with a concurrent increase in the absorbance of I_2. The dashed lines in Figure 5 plot the *F(R)* values gathered from the same volumetric iodine and iodide solutions following Oxone exposure. The I^- solution, which contains only I_2 following the oxidative pre-treatment, now shows a strong signal. The *F(R)* value of the volumetric iodine solution is increased as well, which is consistent with the conversion of I^- and I_3^- to I_2. The change in the *F(R)* value at 440 nm parallels the change in the I_2 absorbance observed in the solution spectra following Oxone exposure. As can be seen in Table 2, the ratio of the I_2 absorbance

and C-SPE response before and after the solution was treated with Oxone are in close agreement (<10% difference). These data argue strongly that biocidally active I_2 is the species that complexes with PVP and gives rise to the signal in our C-SPE platform.

Total Iodine

Understanding which iodine species is responsible for the observed response potentially allows C-SPE to be extended to the determination and speciation of total iodine in solution. The concentration of I_2 and the sum of the concentrations of I^- and I_3^- can be determined using the spectral approach outlined previously [28]. The first spectrum follows the protocol for the determination of I_2, while the second spectrum is collected following Oxone pretreatment and is therefore a measure of I_2, I^-, and I_3^-. Subtraction of the first $F(R)$ value from the second $F(R)$ value therefore yields the sum of the iodide and triiodide concentrations in solution.

$$[I]_{total} = [I_2] + ([I^-] + [I_3^-]) \tag{5}$$

Equation 5 is only valid, however, when the iodine test solutions contain sufficient iodide and are in the pH range (pH< 7) that suppresses the formation of HOI via equation 3. Unfortunately, water samples collected from the Space Shuttle post-flight do not always meet these criteria. As a consequence, the iodine equilibria can be more complex than those for solutions prepared from the iodine volumetric standard.

The post-flight determined pH of Shuttle water samples has ranged from 3.86 to 8.60 [45]. At low pH values, the iodine solution chemistry of Shuttle water is similar to samples prepared from the iodine volumetric standard. However, as the pH increases, the amount of HOI present in the solution increases. This conversion, i.e. the formation of HOI and loss of I_2 as pH increases above 7, is illustrated in Figure 6. Since HOI is not complexed

by PVP, both the C-SPE signal at 440 nm and the solution absorbance at 460 nm decrease with increasing pH. Importantly, the trends displayed in these plots are in close agreement with the results from both our previous work [29] and published numerical equilibrium simulations for this system [37, 38].

In order to obtain speciation information from real space water (i.e., valid measurements of I_2, biocidal iodine ($I_2 + HOI$), and total iodine ($I_2 + HOI + I_3^- + I^-$)), a third spectrum must be acquired in addition to the two mentioned above. To this end, the third spectrum should be acquired using a sample buffered at a pH that suppresses the formation of HOI (pH< 7). This additional spectrum will allow for the determination of biocidal iodine ($I_2 + HOI$). From this three-spectrum method, which could be completed in ~3 min, it should be possible to quantitate and speciate total iodine, biocidal iodine, and free I_2 in a water sample

CONCLUSIONS

The results presented in this paper clearly demonstrate that molecular iodine, I_2, is the species that interacts with PVP, producing the colorimetric signal measured in C-SPE. We have also outlined possible approaches for the determination and speciation of iodine in ground based water samples as well as samples collected post-flight from the Space Shuttle. Manipulation of solution composition through the use of immobilized reagents (redox and buffering) coupled with the data from several spectra will allow for the speciation of biocidal iodine solutions. This ability will enable C-SPE to fill a critical need in the water quality monitoring requirements of NASA. Future experiments with the C-SPE iodine platform will be geared towards gathering all necessary biocidal concentration information from a single extraction. This work will explore multiplexing the measurements of I_2, biocidal iodine, and

total iodine, potentially yielding a simple and rapid one step approach for a quantitative biocide determination.

ACKNOWLEDGEMENTS

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Table 1. Concentrations of iodine species in solution before and after Oxone treatment determined by direct UV/Vis spectroscopy

	Iodide Solution (4.0 µg/mL I^-)		Iodine Solution (5.0 µg/mL I_2)			
	µg/mL I^-	µg/mL I_2	µg/mL I^-	µg/mL I_2	µg/mL I_3^-	I_{total} µg/mL
Before Oxone	4.0	not detected	9.7	5.0	0.4	15.1
After Oxone	not detected	3.9	not detected	14.6	not detected	14.6

Table 2. Absorbance and $F(R)$ values of Iodine and Iodide solutions before and after treatment with Oxone.

Signal	Iodide Solution		Iodine Solution		Signal Ratio before/after
	I_2 before	I_2 after	I_2 before	I_2 after	
$F(R)$	not detected	1.2373	1.367	3.592	0.381
A_{460} (AU)	not detected	0.0111	0.0141	0.0411	0.343

Figure 1.

Figure 2.

Figure 3.

Figure 4.

I_2-Oxone

I_2

I^--Oxone

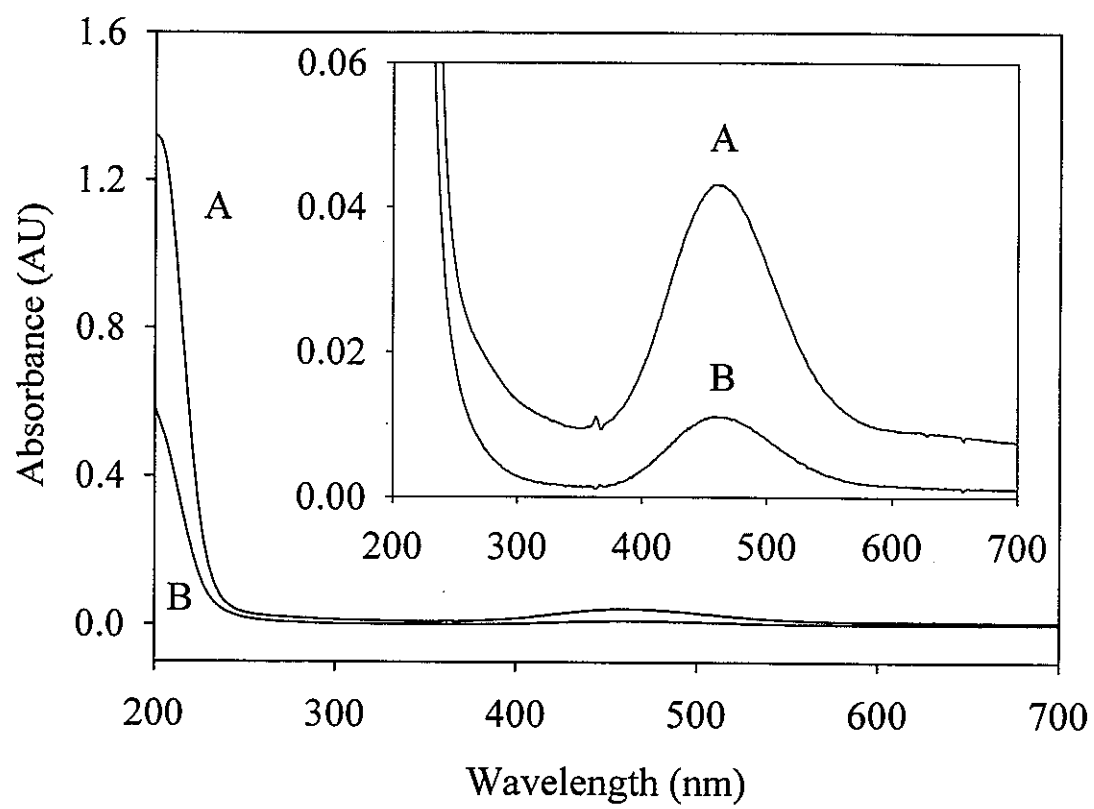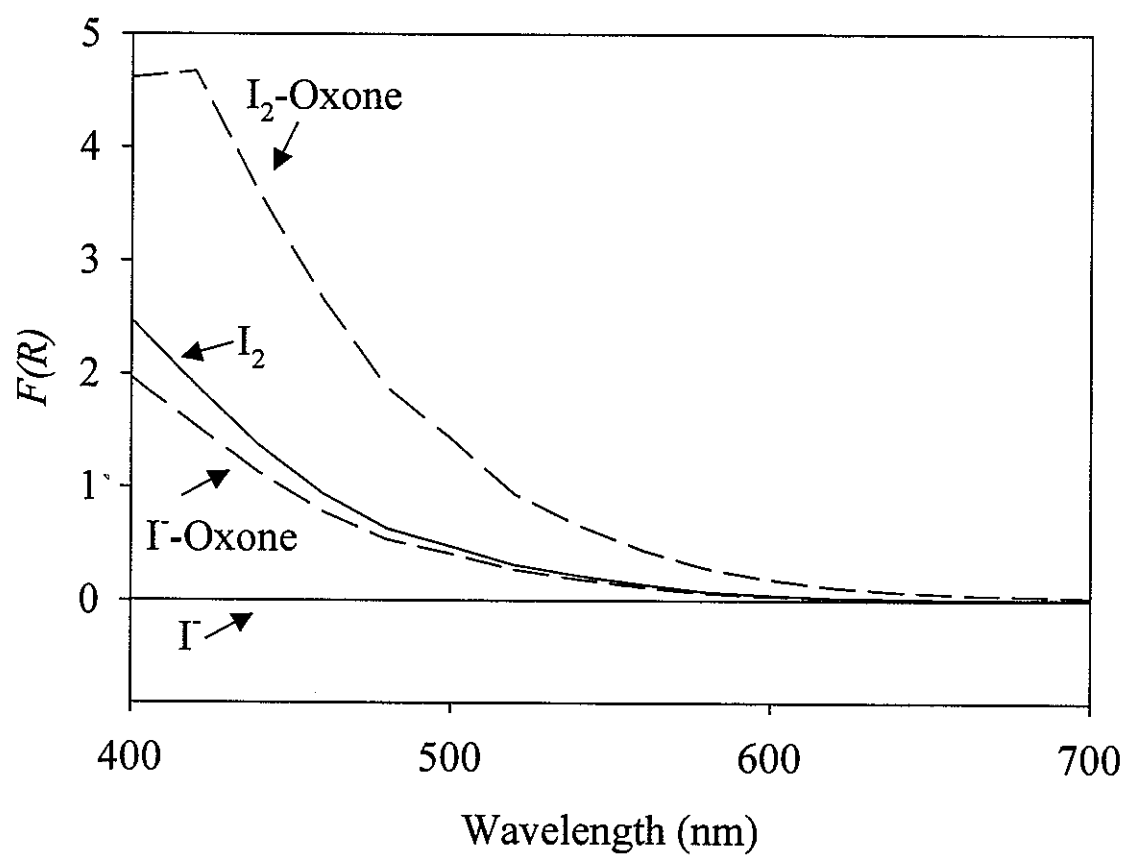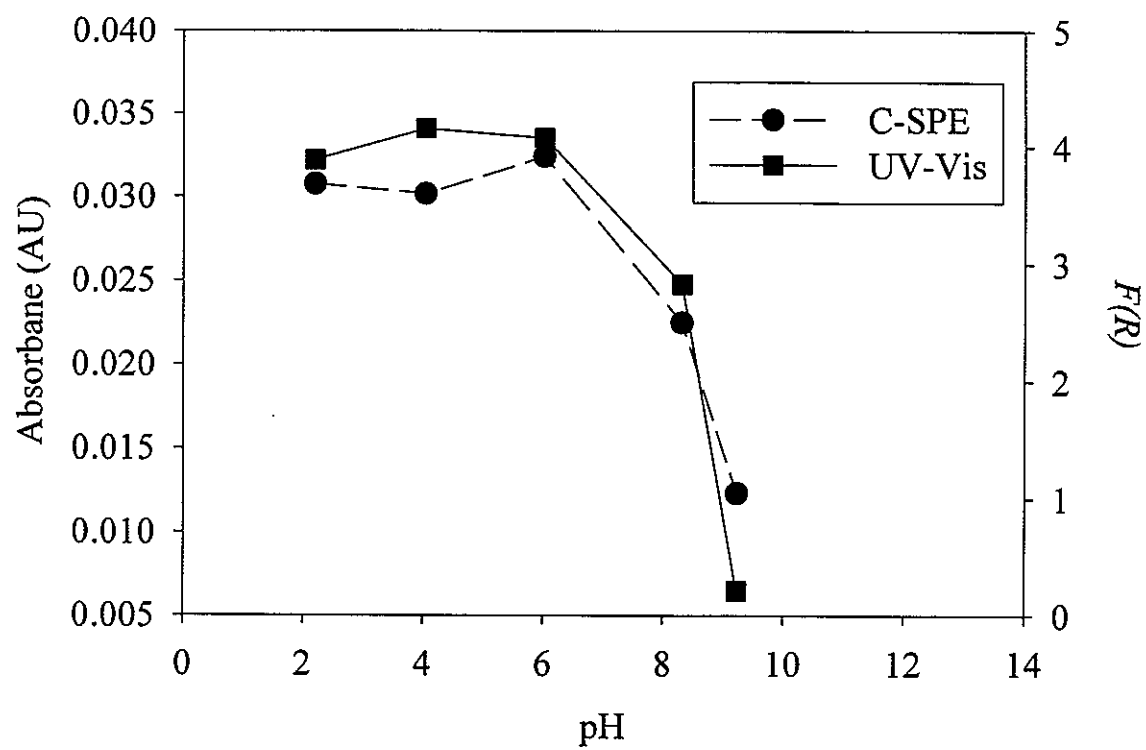I^-

$F(R)$

Wavelength (nm)
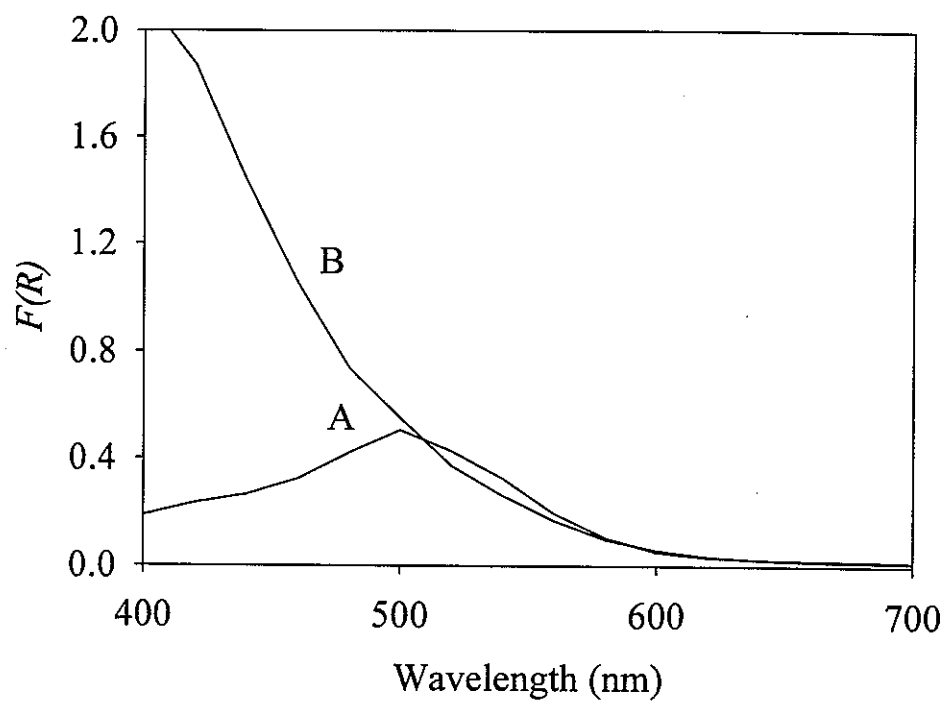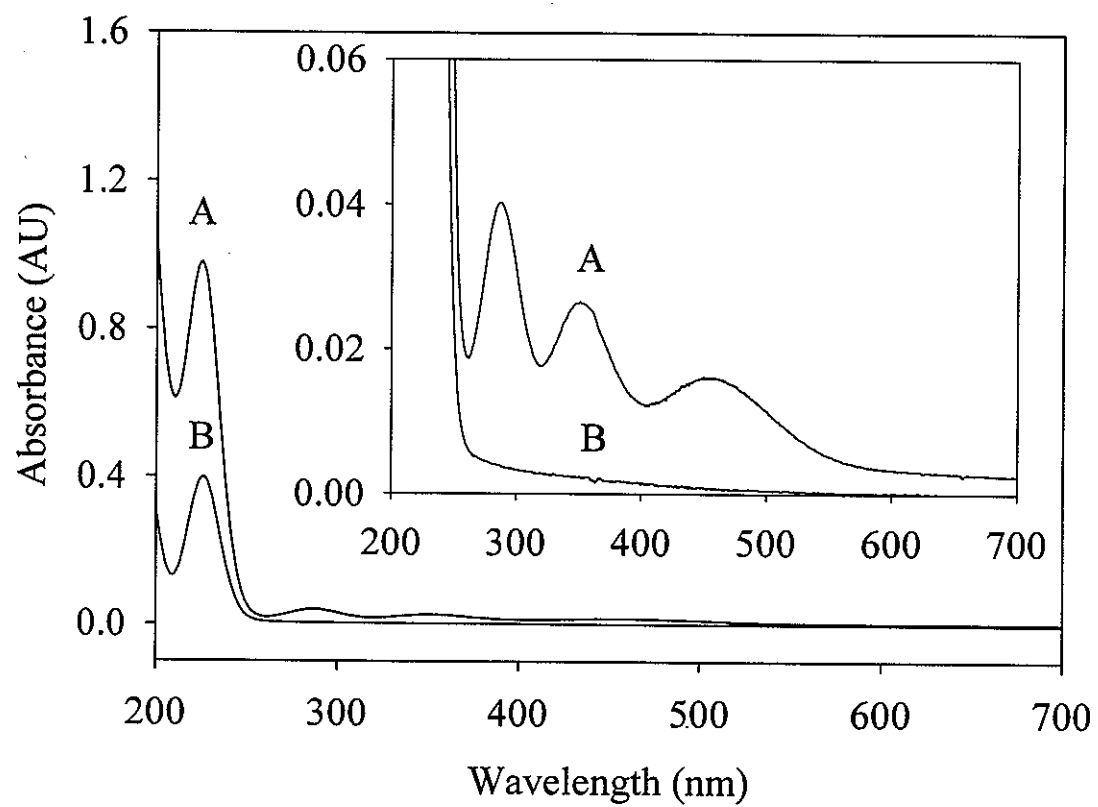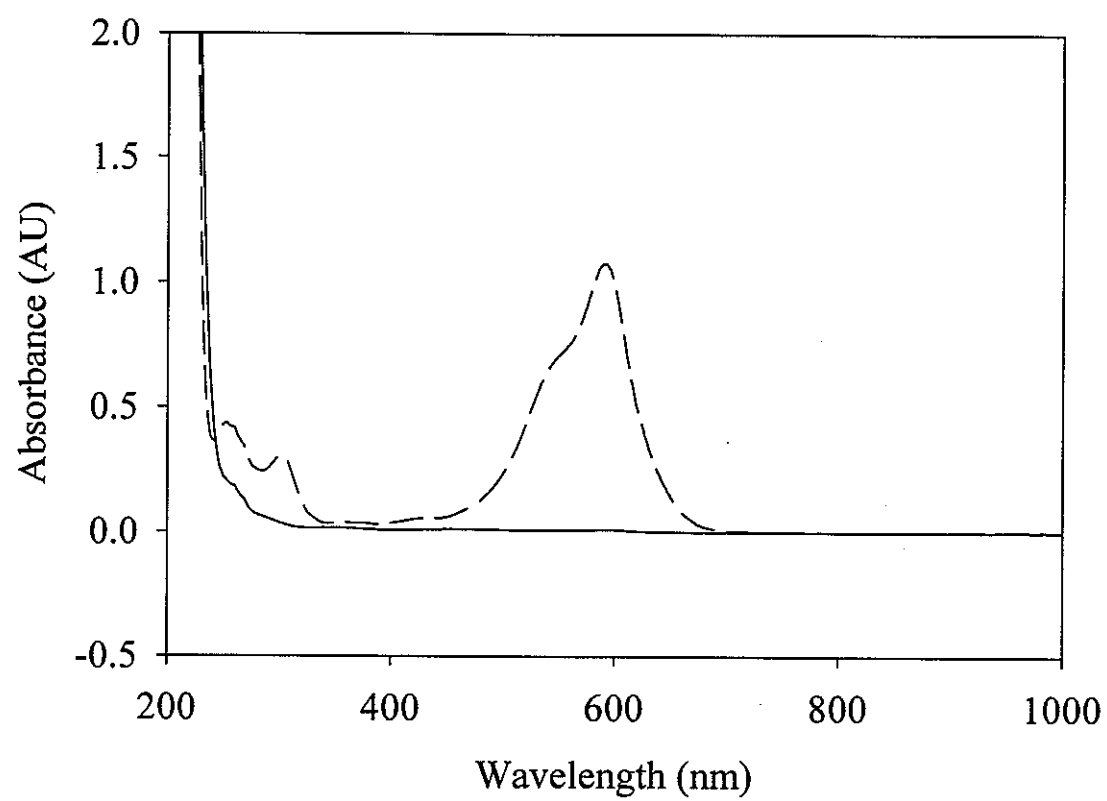
Figure 5.

Figure 6.

FIGURE CAPTIONS

Figure 1. Plots of the Kubelka-Munk function for untreated Empore disks (A) and PVP impregnated Empore disks (B) following passage of 10.0 mL of a 6.0 µg/mL volumetric iodine solution.

Figure 2. UV-Vis spectra of 5.0 µg/mL volumetric iodine (A) and 4.0 µg/mL iodide solutions (B), inset shows the same spectra with an expanded absorbance axis.

Figure 3. Results of LCV iodine determination with $HgCl_2$ (dashed line) and without oxidant (solid line) using a 2.3 µg/mL volumetric iodine solution.

Figure 4. UV-Vis spectra of 5.0 µg/mL volumetric iodine (A) and 4.0 µg/mL iodide solutions (B) following Oxone treatment, inset show the same spectra with an expanded absorbance axis.

Figure 5. C-SPE response from volumetric iodine and iodide solutions before (solid) and after (dashed) Oxone treatment.

Figure 6. C-SPE signal (440 nm) and UV-Vis signal (460 nm) from molecular 11.1 µg/mL volumetric iodine solution as a function of pH.

CHAPTER 3: DETERMINATION OF NICKEL(II) AS THE NICKEL DIMETHYLGLYOXIME COMPLEX USING COLORIMETRIC SOLID PHASE EXTRACTION (C-SPE)

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ABSTRACT

Colorimetric Solid Phase Extraction (C-SPE) is an analytical technique in which analytes in water samples are extracted onto a solid adsorbent matrix impregnated with a colorimetric reagent and then quantified directly on the adsorbent surface using diffuse reflectance spectroscopy. This paper presents a further development in C-SPE. In this case, the reagent employed to detect the analyte is not impregnated on the extraction medium. Instead, the reagent is weakly immobilized on a solid support (i.e., filter paper) and released into the sample as it flows through the support. The reagent complexes the analyte in solution, forming a highly colored precipitate that is collected on the surface of an extraction membrane. The concentration of analyte is determined using the Kubelka-Munk function calculated from the diffuse reflectance spectrum of the precipitate on the membrane surface. This precipitation-spectrophotometric platform is extensively evaluated by determining nickel(II) using dimethylglyoxime (DMG) as the precipitating reagent. The ability to optimize reaction conditions with immobilized reagents by in-line buffering is also demonstrated. Specifically, borax buffer was utilized to adjust the pH of nickel(II) samples prepared in deionized water. This combination of immobilized buffer and reagent allows C-SPE to operate in a solid-phase mode in which all the reagents requisite for optimal analyte determination are immobilized on solid supports. Using this method, nickel(II) was

determined in a single processing step over the concentration range 0.50-5.0 ppm in ~40 s with 1.0-mL sample volumes.

Keywords: colorimetric solid phase extraction, nickel, dimethylglyoxime, diffuse reflectance spectroscopy

INTRODUCTION

Contamination of spacecraft water supplies with heavy metals is a growing concern on International Space Station (ISS). Water samples collected and archived on ISS and returned to Earth for analysis have, in a few instances, contained traces of lead, cadmium, and nickel [1]. The source of these contaminants was isolated and corrected, but these observations clearly document the potential for a serious compromise in water quality as the numerous components of the ISS water system wear and degrade over time.

At present, all samples used to monitor spacecraft water quality are collected in-flight and stored until returned to Earth for chemical analysis. Bacterial count analyses are performed in-flight [2]. Following the Columbia accident, the frequency of the supply missions that retrieve water samples has drastically decreased. This decline in data collection frequency, coupled with the recent occurrences of heavy metal contamination, amplifies the need for on-board water quality monitoring. Analysis systems capable of detecting low level contaminants would therefore be valuable on three fronts: eliciting contamination sources, signaling the need to implement mitigation steps before crew safety is jeopardized, and monitoring wear and degradation of components in the water recovery and dispensing systems.

This paper describes an approach to the on-board determination of nickel(II) present in the ISS water supply. Until recently, NASA requirements for nickel(II) monitoring were

based on the maximum contamination level (MCL) set by the EPA, which was a lifetime MCL. NASA is presently establishing new, shorter term requirements for 10, 100, and 1000 day exposures, with its toxicology review subcommittee recently approving preliminary Spacecraft Water Exposure Guideline (SWEG) levels of 1.7 ppm for 1, 10, and 100 day exposures, and 0.3 ppm for a 1000 day exposure [3].

One possible solution to ISS water monitoring needs that embodies many of the necessary attributes for space flight implementation (e.g., portability, reagentless operation, versatility, ease of use, etc.) [4, 5], including the ability to function in zero gravity [6], is Colorimetric Solid Phase Extraction (C-SPE). C-SPE complexes, concentrates, and quantifies analytes on a solid-phase extraction membrane and employs diffuse reflection spectroscopy as an on-membrane read out technique. Collecting the diffuse reflectance spectrum of analyte-reagent complexes on the surface of a membrane reduces waste by eliminating analyte elution and maintains the high concentration factors of SPE (1000 or more [7]).

There are three variations of C-SPE: complexation in solution and extraction of the soluble complex, analyte extraction followed by exposure to complexing reagent solutions, and reagent immobilization with the subsequent extraction and complexation of the analyte. Noble metals [8] and vanadium in various oxidation states [9] have been determined by complexation and extraction. Extraction and exposure has been employed to quantify zinc on cloth impregnated with vinylpyridinium groups [10] and mixtures of vanadium and molybdenum [11], rhenium [12], lead [13, 14], arsenic [15], and copper, chromium, and nickel [16] on ion exchangers. Immobilization and extraction has been utilized to test for cobalt and palladium [17] and thorium [18] on silica gel, chloride on polyurethane

foams [19], iron on treated polyvinyl chloride membranes [20], aluminum on cellulose [21], beryllium on a variety of supports [22, 23], and nickel on silica gel [24] and cellulose [25]. Our group has utilized this third approach to develop biocide monitoring platforms for silver ion [5, 6] and molecular iodine, iodide, and triiodide [4, 6] and methods to determine copper(II), iron(III), nickel(II) and chromium(VI) [26] on polystyrene divinylbenzene.

This paper presents a fourth variation to C-SPE by using the formation and collection of an insoluble complex to colorimetrically determine analyte concentration. The impetus for this development lies in our desire to minimize sample volume and decrease total analysis time through the eventual development of a multiplexed C-SPE platform. This work builds on our successful employment of immobilized Oxone in an oxidative pre-treatment step in the determination of total iodine [6] and previous studies detecting nickel(II) with vic-dioximes [26]. Herein, the colorimetric nickel(II) reagent dimethylglyoxime (DMG) is immobilized on a solid support and released as the sample passes through the support. As one of the most selective nickel(II) reagents, DMG reacts to form a pink flocculent precipitate [27, 28] in solution which is collected on the surface of an extraction disk. The analyte is then quantified with a diffuse reflectance spectrophotometer. The use of DMG in this assay reflects its acceptance as a colorimetric reagent in spot tests employed to detecting the release of nickel(II) from jewelry, coins, and other metal items [29]. The flexibility and possible applications of our immobilized reagent scheme is further demonstrated by adjusting sample pH. This adjustment is accomplished by collecting sample solutions through glass wool coated with buffer salts to achieve optimum precipitation conditions in the sample. The immobilization and release of buffer salt and complexing reagent creates a rapid, reagentless platform for the determination of nickel(II) in ~40 s.

EXPERIMENTAL SECTION

Reagents and Chemicals

Nickel(II) solutions. Solutions with nickel(II) concentrations of 0.50, 1.0, 2.0, and 5.0 ppm were prepared in Nalgene® bottles by mixing the appropriate mass of a nickel atomic absorption standard (1000 ppm, Spex Certiprep, Metuchen, NJ) with either deionized water or borax buffer (pH 9.0) and bringing the solution to a final mass of 30.0 g. Buffer was prepared by dissolving 2.38 g of sodium tetraborate decahydrate (MCB, Norwood, Ohio) in 500 mL of deionized water.

Buffer solutions. Buffer solutions with pH values of 2.0, 5.0, 7.0, 8.0, 9.0, and 10.0 were prepared to determine optimum reaction conditions. The pH of these buffers was determined using an Orion model 520A pH meter. The pH 2.0 buffer consisted of 0.05 M potassium chloride adjusted to the correct pH with 0.1 M HCl. The pH 5.0 buffer was prepared from 0.05 M potassium hydrogen phthalate adjusted to pH 5.0 with 0.1 M NaOH. The pH 7.0 buffer was prepared from 0.05 M potassium dihydrogen phosphate that was brought to the correct pH with 0.1 M NaOH. The pH 8.0, 9.0, and 10.0 buffers were all made by adjusting the pH of a 0.013 M borax solution with either 0.1 M HCl or 0.1 M NaOH.

Buffer wool. A buffer slurry was prepared by adding 2.0 grams of sodium tetraborate decahydrate to 20.0 mL of deionized water. This solution was shaken to suspend any undissolved salt and poured over a 100-mm diameter disk of glass wool (Fischer) in a polystyrene Petri dish. The dish was heated in an oven at 50 °C overnight to evaporate the solvent and therefore entrap the salt within the wool.

DMG chads. A 0.022 M solution of DMG (Aldrich) was prepared by dissolving 0.250 g of DMG in 100 mL of methanol. Whatman® no.1 qualitative filter paper was cut into 10-mm chads. Each chad was then treated with 20 μL of the DMG solution and allowed to air dry.

C-SPE disks. 3M Empore™ SDB-XC 47 mm extraction membranes were used as a matrix for collection of the colorimetric complex as a precipitate. Empore™ disks are already employed in several other C-SPE methods [4-6, 26], and, as such, were a logical choice for the collection of the Ni-DMG complex. Membranes were used as received after cutting into 13-mm disks in order to fit into a polypropylene filter holder (see below).

UV-Vis Spectrophotometer

All transmission spectra were collected on a Hewlett-Packard model 8453 UV-Visible spectrometer using ChemStation software. Quartz cuvettes with a pathlength of 1.0 cm were utilized for collection of all spectra.

Diffuse Reflectance Spectrophotometer

A BYK-Gardner color-guide sphere d/8° spin diffuse reflection spectrophotometer (model PCB-6830) was used to collect membrane spectra (see Figure 1). This spectrophotometer is small (8.1 x 17.8 x 9.4 cm), lightweight (0.5 kg), battery operated, and collects spectra over the range 400-700 nm in 20 nm intervals. The aperture of the integrating sphere is 11 mm, which allows for sampling of the entire surface of the C-SPE disk. Acquisition of a single sample spectrum requires ~2 s.

Sample Locator

A BYK-Gardner sample holder (model PCB-6845) was modified in-house to reproducibly align the surface of the extraction membrane with the aperture of the integrating sphere. This modification has been described previously [6].

Sampling Procedure

The 10-mm DMG-treated chads were loaded in a plastic filter holder (Swinnex Filter Holders, Fischer part # 09-753-10ASX00 0013 00) which contained a PTFE O-ring to prevent leakage. Empore^{TM} disks (13 mm) were positioned in identical filter holders that contained a thin rubber gasket. The gasket has an inner diameter of 10 mm and defines the area of the C-SPE disk that is exposed to sample solution. The reagent cartridge and sample holders were connected in series to allow the sample solution to pass through the reagent chad and then the Empore^{TM} disk. Sample solutions buffered at optimum complexation pH as well as solutions with the pH adjusted in-line during sample collection were tested.

For buffered solutions, a 1.0-mL nickel(II) sample was collected using a 1-mL plastic syringe (Becton Dickinson) (Figure 1, step 1 A). The preloaded cartridge/holder assembly was attached to the syringe, and the water sample was passed through the unit (Figure 1, step 2). Following formation and collection of the analyte complex, the syringe is removed and the sample disk is dried by pushing 60 mL of air through the assembly. The sample holder was then separated and the lower portion was placed in the sample locator and a spectrum was acquired (Figure 1, step 3).

The procedure for the determination of nickel(II) in non-buffered samples paralleled the procedure for the buffered samples with one notable exception; a 1.0-mL sample was collected in a 5-mL syringe by pulling the aqueous sample through a filter holder that

contained a 13-mm diameter circle of buffer wool (Figure 1, step 1 B). After filling, the buffer cartridge was detached from the syringe before continuing the extraction procedure.

Readout

After collecting a spectral data set, the spectrophotometer was interfaced to a computer using a serial cable. The reflectance spectra were downloaded into a MS-Excel spreadsheet and the Kubelka-Munk function was calculated using an in-house modified version of BYK-Gardner QC-Link software. The Kubelka-Munk function, $F(R)$, is defined as:

$$F(R) = (1-R)^2 / 2R \tag{1}$$

where R is the percent reflectance measured with respect to a standard white. $F(R)$ can be related to analyte concentration by [30]

$$F(R) = 2.303\varepsilon C/s \tag{2}$$

where ε is absorbtivity, C is concentration of the complexed analyte, and s is the scattering coefficient of the sample surface. By assuming the absorbtivity and scattering coefficient of our surface are constant at a given wavelength, $F(R)$ can therefore be related directly to analyte concentration.

RESULTS AND DISCUSSION

Precipitate Formation as a Function of pH

Nickel is a metal commonly found in many naturally occurring ores. Industrially, the greatest use of nickel is in the manufacture of stainless steel and other alloys [31]. On Shuttle and ISS, the source of nickel in water samples is linked to the stainless steel components used in plumbing hardware [1]. In such systems, nickel is usually present in the +2 oxidation state which forms a 1:2 stoichiometric complex with DMG [32]. Moreover, the

complexation of nickel(II) with DMG involves the loss of a proton, so the rate and extent of complexation are pH dependent [27].

The flow through nature of our C-SPE platform necessitates the use of reagents capable of fast complexation and homogenously coating the membrane surface. Similar colorimetric approaches to nickel(II) determination report an optimum pH range for complex formation of 9 to 11 [24, 25]. To determine optimum reaction conditions in our method, 2.0 ppm solutions of nickel(II) were buffered between pH 5.0 and 10.0 and 20.0 μL of the 0.022 M DMG solution was added to a 5.0-mL aliquot of each sample. UV/Vis spectra of each sample solution were acquired every 60 s to monitor complexation. Although this is a precipitation reaction, the crystals formed by mixing the reagents stay suspended in solution for up to an hour before settling out, making it possible to monitor complex formation through the amount of scattered light measured by the absorbance in transmission spectroscopy experiments.

In these tests, the solutions formed by mixing the buffered samples with DMG exhibited different wavelengths of maximum absorbance (λ_{max}), which is attributed to the size dependent light scattering of the suspended crystals. Solutions that developed color the fastest had a λ_{max} at a lower wavelength than those that developed color more slowly. These observations, which are summarized in Figure 2 as plots of the absorbance at λ_{max} as a function of time, are consistent with the growth of larger particles when precipitates form at a slower rate. This plot shows that the most rapid precipitation of nickel(II) with DMG occurs at pH 9.0, which is the same pH that yields the strongest scattering. Because of the flow through nature of our C-SPE platform, pH 9.0 solutions were used in all further experiments

based on the fact that small crystals are indicative of fast complexation and should produce a more uniform color across the collection membrane.

DMG Release Studies

Initial experiments that utilized the reagent chad as a means of introducing DMG to the sample were conducted with 3.0-mL buffered nickel(II) solutions and demonstrated a clear trend between the $F(R)$ value at 540 nm and nickel(II) concentration. However, the trial-to-trial reproducibility was poor and the analytical signal did not scale with sample volume when experiments were performed upon changing the sample volume. As an example, a 3.0-mL sample of 0.50 ppm nickel(II) solution should yield an $F(R)$ value for the collected precipitate that is proportional to the 1.5 μg of analyte present in the sample. A 15.0-mL sample with a 0.10 ppm nickel(II) concentration should give the same result, since the total amount of nickel(II) in the two different sample volumes is the same. However, the 3.00-mL sample produced a much greater $F(R)$ value than that obtained from the 15.0-mL sample which led to the examination of the release of the colorimetric reagent from the chad and its subsequent mixing with the flowing sample solution.

To investigate this issue, five blank 5.0-mL water samples were passed through reagent chads at a constant flow rate (~5 mL/min) and the eluent was collected and analyzed by transmission spectroscopy. The results of these trials were compared to a 5.0-mL blank that was spiked with 20 μL of the DMG solution, which is the same amount of reagent used to prepare each chad. The spectra of the eluents and the spiked solution were compared by monitoring the characteristic DMG peak at 225 nm. Results of this comparison showed that essentially all of the reagent was released into the blank solutions. Additional trials showed that as flow rate was increased to greater than 10 mL/min, reagent release decreased.

However, the flow rates required to observe this phenomenon are not readily attained due to the back pressure created when the Empore^{TM} disk is used in-line for precipitate collection.

To investigate the release of DMG in more detail, the eluent from a 5.0-mL blank sample was collected in 1.0-mL aliquots and analyzed to determine the amount of reagent released as a function of sample volume. Figure 3 shows that ~60% of the colorimetric reagent is released when 1.0 mL of sample has passed through the reagent chad. Furthermore, since the dead volume of the cartridge/holder assembly is ~800 µL, the use of sample solution volumes greater than the dead volume minimizes reagent and analyte mixing. This was proven by exposing two separate membranes to a 1.0-mL and to 5.0-mL sample of a 1.0 ppm nickel(II) solution. The color difference between the two membranes is minimal ($F(R)_{1mL}$=0.15, $F(R)_{5mL}$=0.17), indicating a sample volume in excess of ~1 mL only marginally enhanced the membrane color.

Method Calibration

Buffered samples. Since volumes greater than 1 mL did not appreciably increase the analyte signal, 1.0-mL samples were used to calibrate the response. Buffered solutions with nickel(II) concentrations between 0.5 and 5.0 ppm were passed through the cartridge/holder unit. The obtained *F(R)* responses at 540 nm are plotted against sample concentration in Figure 4. The wavelength of detection is the maximum of the Kubelka-Munk function for the precipitate. The lower limit of detection for this technique, extrapolated from a regression analysis of the data and the blank response at three times its standard deviation, was 0.47 ppm. Interestingly, this limit corresponds to a mass detection limit of ~500 ng in a 1.0-mL sample and requires only ~40 s for full sample work up and analysis.

The results of the reagent release trials (shown in Figure 3) and the stoichiometry of the nickel-DMG complex were used to calculate the upper limit of the working curve for this technique. A 1.0-mL sample should release enough reagent to complex 7.4 μg of nickel(II), or a 1.0-mL sample with a nickel(II) concentration of 7.4 ppm. This claim is supported by Figure 5, which shows the response for the precipitate at 540 nm as solution concentration is increased. The data clearly begin to approach a limiting value near the predicted limit of 7.4 ppm.

Deionized water samples. Samples prepared with the same concentrations as the buffer samples in deionized water were used to examine the ability of the immobilized buffer to adjust pH and its effect on the method response. Without buffer treatment, no precipitation occurs and no color is observed on the surface of the membranes. When the buffer wool is used to adjust sample pH, the results obtained show excellent agreement with those gathered using the buffered samples. The buffer cartridge was used in two configurations: serially, as part of reagent chad/collection membrane assembly, and during sample collection as the syringe was filled. Both configurations performed well, but the dead volume of the buffer cartridge (~400 μL) increased the total dead volume of the assembly to 1.2 mL, making it difficult to reliably pass a 1.0-mL sample through the entire assembly. We are currently examining approaches to circumvent this problem.

As a result of this difficulty, the buffer wool was used in-line during syringe filling in all additional experiments, and did not appreciably increase the analysis time. The results of the extractions using buffer wool during sample collection are plotted in Figure 6, along with the slope and intercept of the linear regression. Comparison of the slope and intercept values calculated from the buffered samples (Figure 4) and the deionized water samples yields

differences of only 2.5% and 0.91%, respectively, demonstrating the ability to manipulate the solution pH with the buffer wool.

Interference Tests

There are several species that interfere with the nickel-DMG complex. Iron(III) [24, 28, 33], manganese(II) [28, 34], cobalt(II) [28, 35], and copper(II) [28, 35] have all been shown to interfere with the complexation of nickel(II) by DMG. To determine the extent of interference from each species, individual solutions containing 1.0 ppm of iron(III), manganese(II), copper(II), and cobalt(II) were prepared from the sulfate salt of that metal. Of the species tested, none produced signals that would be interpreted as in-absentia nickel(II). The iron(III) solution resulted in a yellow membrane, presumably from the precipitation of iron(III) hydroxide at pH 9.0 (K_{sp}=2.64 x 10^{-39}) [36]. Solutions containing equal concentrations (1.0 ppm) of the interfering metal and nickel(II) and a ten-fold excess of interfering metal were also tested. Manganese, copper, and cobalt showed no interference to the determination of nickel(II) when present at an equal concentration, but the presence of iron(III) caused a positive deviation in the analyte signal. When the solutions with the ten-fold excess of interferant were analyzed, all species except manganese interfered with the nickel determination. Copper(II), cobalt(II), and iron(III) all tended to yellow the surface of the membrane.

The results of these interference studies are summarized in Table 1. These results are very encouraging. Two of the four species tested, copper(II) and manganese(II), do not interfere when present at similar concentrations to nickel(II). Moreover, copper and manganese are target analytes for ISS water quality monitoring, and are likely to be present in samples used for nickel(II) quantification. Additionally, since the presence of excess

interfering species causes a yellowing of the membrane surface, it may be possible to utilize this for simultaneously monitoring these species in the presence of nickel(II) by employing multiple wavelengths.

CONCLUSIONS

The ability to optimize sample conditions and use precipitation reactions to quantify analytes represents an intriguing and valuable extension in the development of C-SPE for the analysis of ISS water. The number of possible analytes that can be determined with C-SPE is dramatically increased as are possible applications not only in space exploration, but in a wide range of Earth-bound problems as well (e.g., environmental monitoring and homeland security). Using DMG and borax buffer, 500 ng of nickel(II) was precipitated and detected in 40 s while employing only a 1.0 mL sample. In addition to meeting new NASA short term exposure concentration requirements, the minimal volume and fast analysis time are dramatic improvements over similar sorption-spectroscopic methods [24, 25, 37, 38]. These other techniques require much larger sample volumes (up to 1 L), are far more time consuming (20-40 min), and have a mass detection limit inferior to that of C-SPE. Ongoing work to lower the concentration detection limit of this method is focusing on in-line mixing chambers as a means to overcome the sample volume dependent release of the complexing reagent. An assessment of the toxicity of all components according to NASA safety requirements is also underway to expedite flight validation of this method. Whether C-SPE is used to monitor water quality or corrosion of alloys, the minimal sample volume and rapidness of the method described in this paper make it an attractive methodology for use in the facile monitoring of nickel(II) and potentially other heavy metal contaminants in spacecraft water. Further, the use of immobilized reagents to adjust sample parameters, such as pH, and controllably

release complexing reagents into a flowing sample is the first step towards isolation and determination of multiple analytes on the surface of a single membrane. Immobilized reagents are also being examined to as a means of introducing masking agents to reduce the cross reactivity of colorimetric reagents. Efforts along these lines, which include the development of a multiplexing format for simultaneous multi-analyte determination, are under way and will be reported in the near future.

ACKNOWLEDGEMENTS

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Table 1. Interference studies using 1:1 and 10:1 ratios of interferant to nickel(II).

Metal (M)	M (mg/L)	Ni^{2+} (mg/L)	*F(R)* calculated	*F(R)* obtained
Cu^{2+}	0.995	0.995	0.175	0.167
Mn^{2+}	1.00	0.994	0.175	0.165
Fe^{3+}	0.986	0.986	0.173	0.363
Co^{2+}	0.997	0.990	0.174	0.173
Cu^{2+}	10.00	0.980	0.170	0.072
Mn^{2+}	9.86	0.980	0.170	0.197
Fe^{3+}	9.63	0.944	0.160	0.010
Co^{2+}	10.10	0.979	0.170	0.012
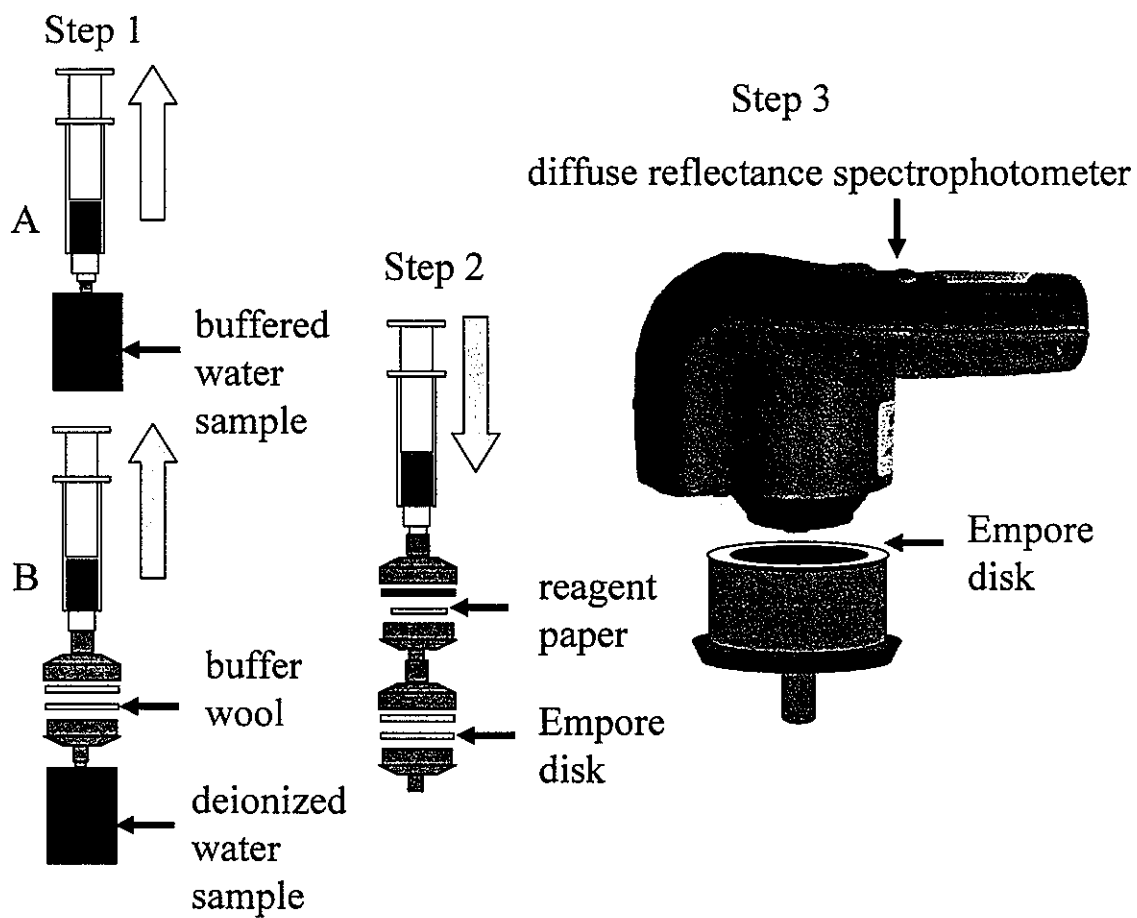
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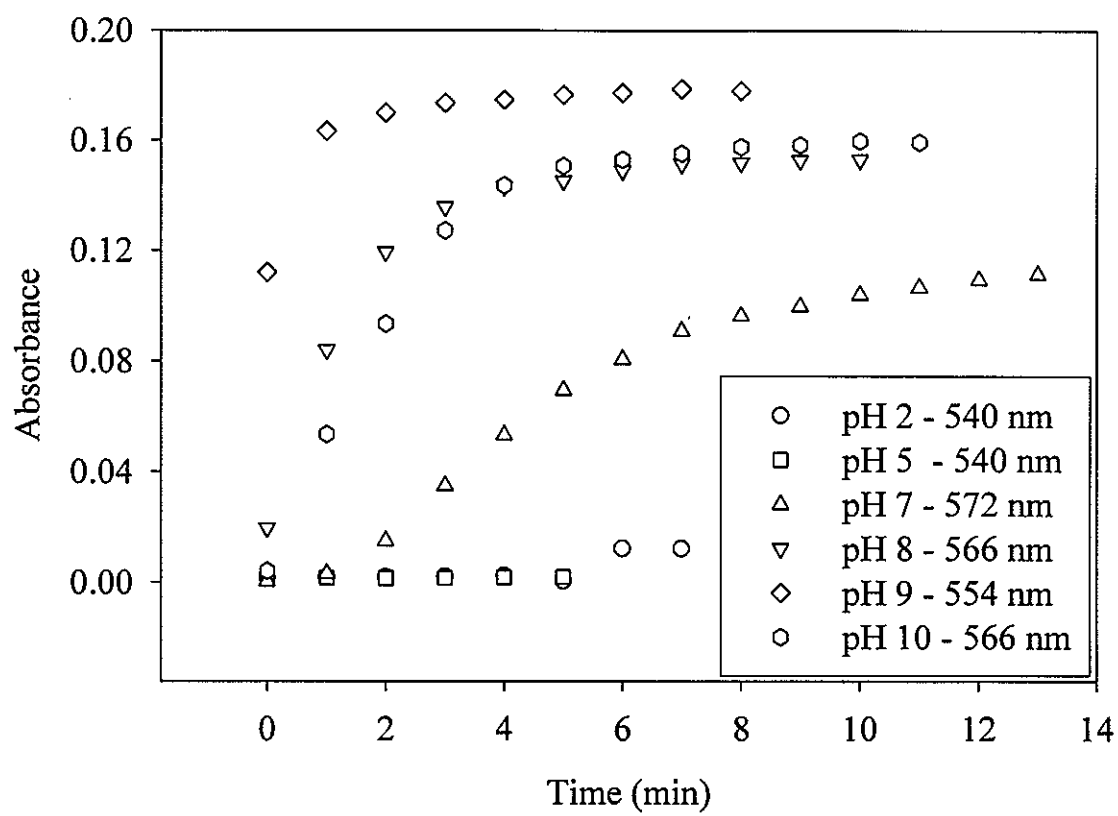
Figure 2.
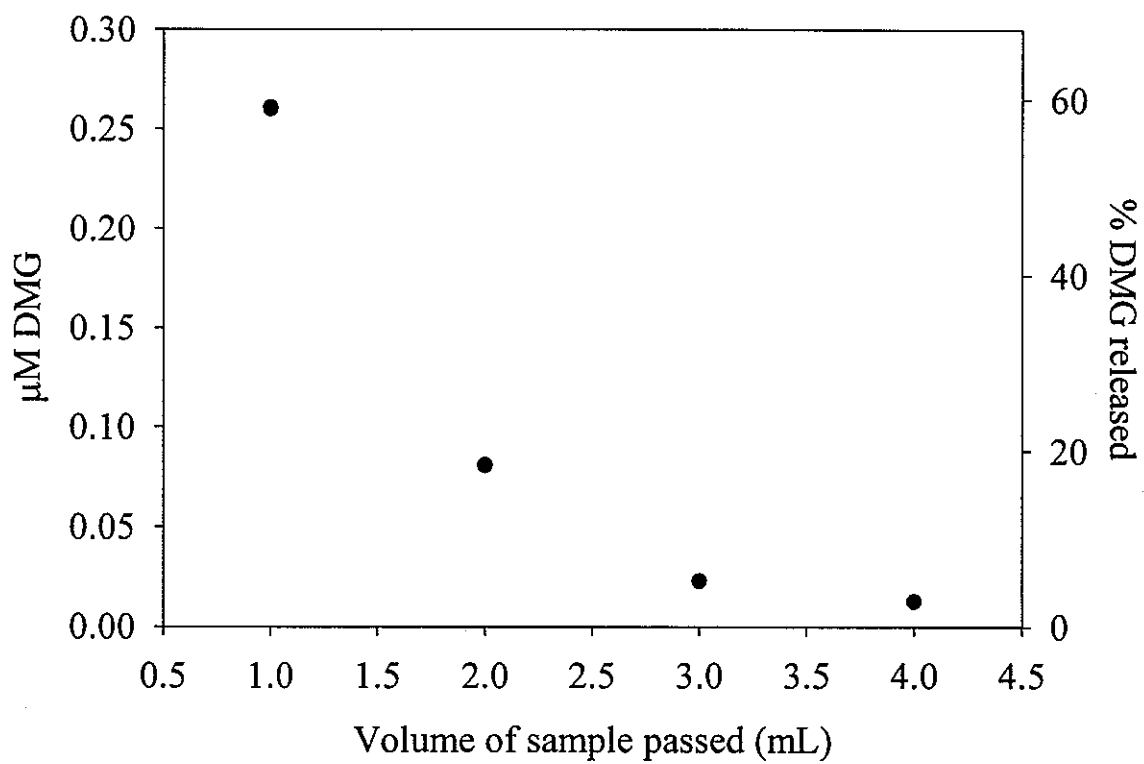
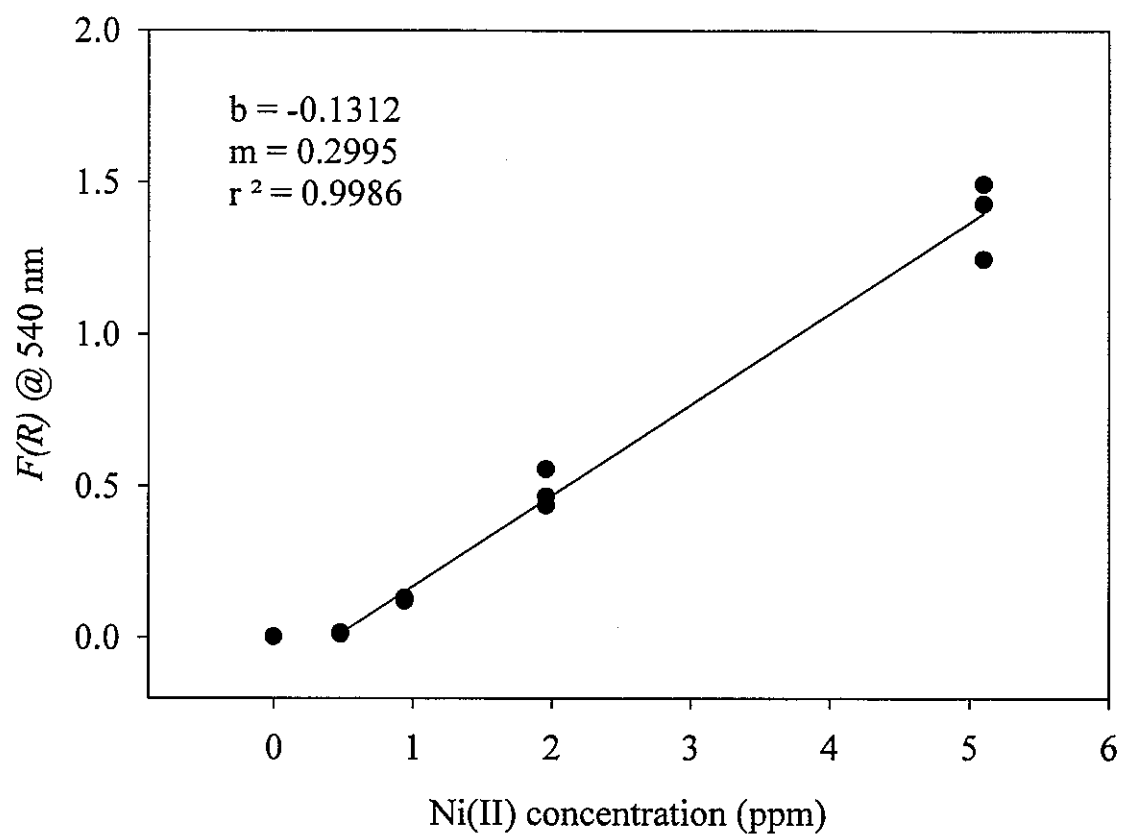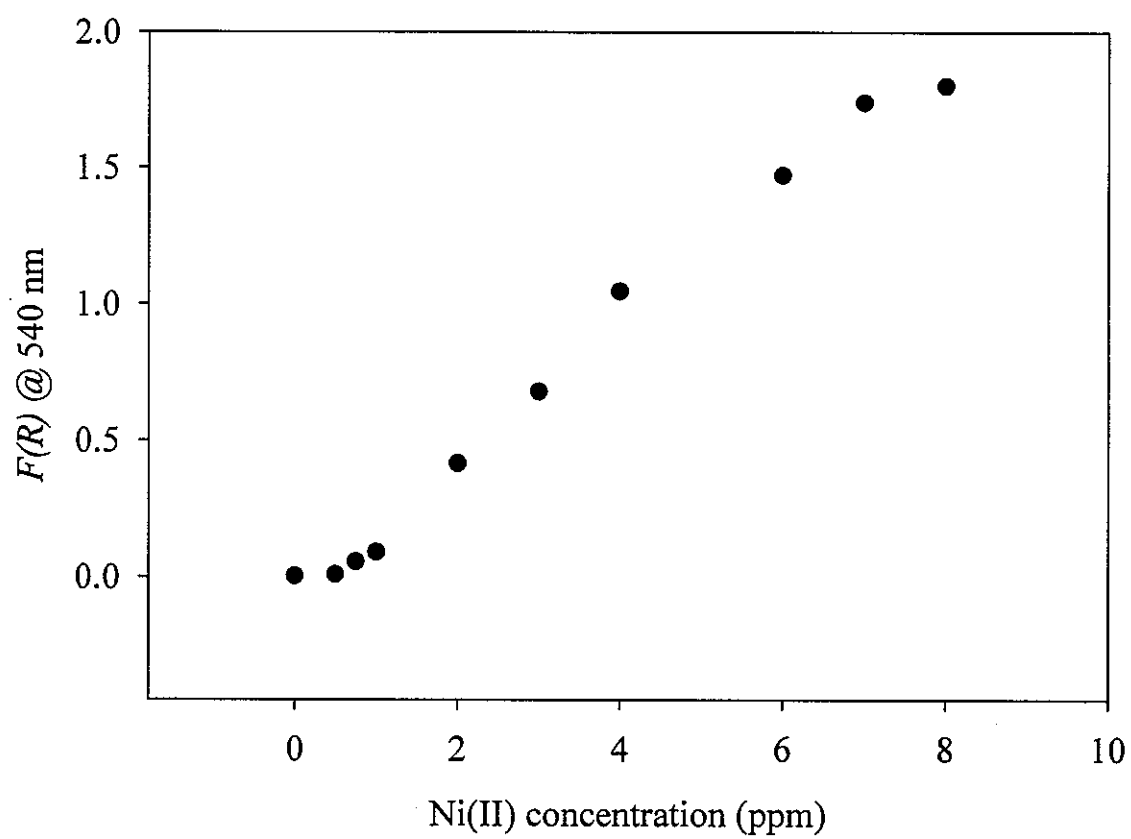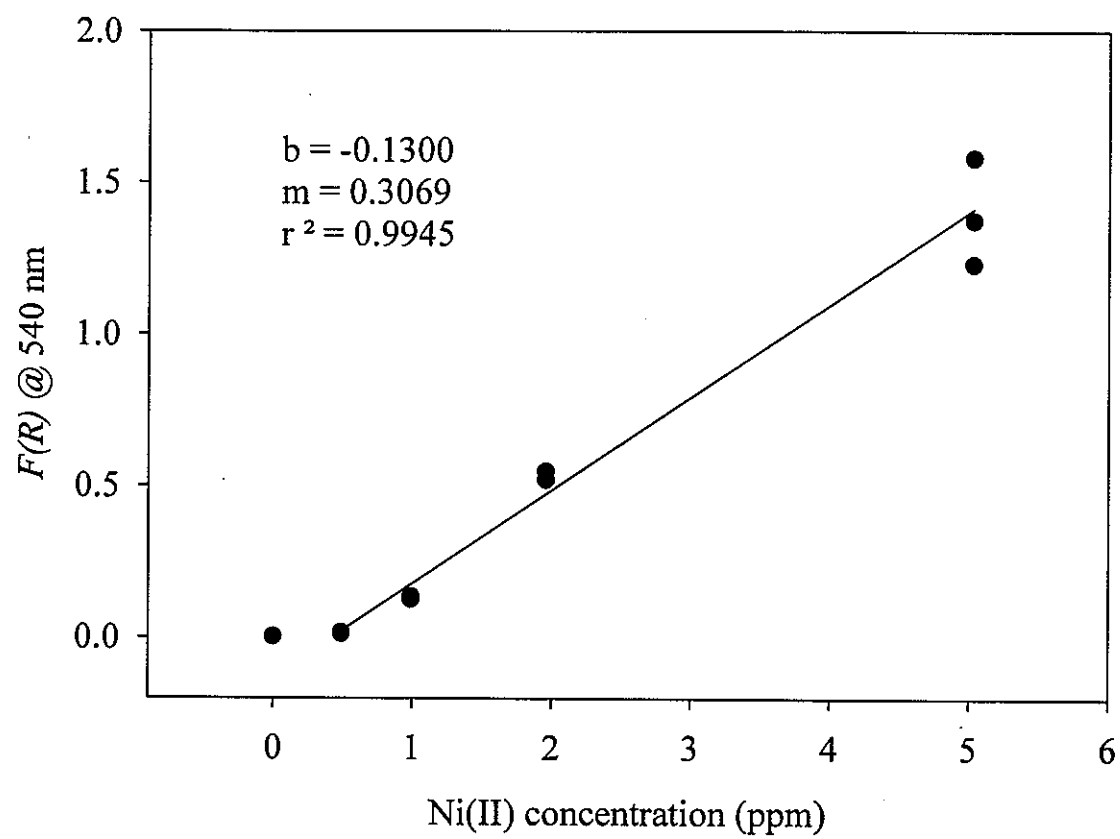**Figure 3.**

Figure 4.

Figure 5.

Figure 6.

FIGURE CAPTIONS

Figure 1. Precipitation-spectrophotometric detection scheme for buffered sample (Step 1A) and deionized water samples (Step 1B). Arrows indicate direction of fluid flow

Figure 2. Formation of nickel(II)-DMG complex at various pH values as a function of time. The noted wavelength corresponds to the λ_{max} of the solution spectrum.

Figure 3. Release of DMG from chads as a function of passed sample volume.

Figure 4. Calibration curve for nickel(II) in buffered sample solutions.

Figure 5. C-SPE response as nickel(II) concentration approaches upper limit.

Figure 6. Calibration curve for nickel(II) samples prepared in deionized water and collected through buffer wool.

CHAPTER 4: MULTIPLEXED COLORIMETRIC SOLID PHASE EXTRACTION (MC-SPE): DETERMINATION OF SILVER(I), NICKEL(II), AND SAMPLE pH

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ABSTRACT

This paper reports the design and testing of a multiplexed colorimetric solid phase extraction platform for the rapid determination of multiple water quality parameters in a simple set of operational steps. C-SPE is an analytical platform that combines impregnated colorimetric reagents on a solid phase extraction membrane and diffuse reflectance spectroscopy to quantify trace analytes in water. In extending C-SPE to a multiplexed format (MC-SPE), a filter holder that incorporates discrete analysis channels and a jig that facilitates the concurrent operation of multiple syringes have been designed, enabling the simultaneous determination of three different measures of water quality. Separate, single parameter membranes, placed in a read-out cartridge create unique, parameter-specific addresses at the exit of each channel. Following exposure to sample solutions, the diffuse reflectance spectrum of each address is collected serially and the Kubelka-Munk function is used to quantify each water quality parameter via calibration curves. Performance evaluations of the MC-SPE platform were conducted using sample pH, silver(I), and nickel(II). Determinations of silver(I) (0.05-0.5 ppm) and nickel(II) (1.8-5.0 ppm) follow established C-SPE methods on reversed phase extraction membranes using 5-(p-dimethylaminobenzylidene) rhodanine[1] and dimethylglyoxime,[2] respectively, as colorimetric reagents. Sample pH (2.5-5.0) is measured using an anion exchange membrane impregnated with fluorescein. These determinations

required ~120 s to complete using a total sample volume of 3.0 mL. The extension of MC-SPE to the determination of a greater number of analytes, and its potential application to space and earth-bound monitoring needs are briefly discussed.

Keywords: colorimetric solid phase extraction, silver, nickel, pH, diffuse reflectance spectroscopy, multiplexed analysis

INTRODUCTION

Samples used to monitor the water quality on board the International Space Station (ISS) are currently collected and archived on-orbit until returned to Earth for analysis. However, the availability of these samples for ground-based characterization has sharply declined as a result of the temporary grounding of the Shuttle fleet. Highly prioritized crew schedules and curtailed re-supply missions to ISS, coupled with the limited payload capacity of the Soyuz vehicles, have dramatically decreased sampling frequency and concurrently increased the time lapse between sample collection and analysis. These issues, along with recent incidents of heavy metal contamination,[3] illustrate the need to develop in-flight methods to measure critical quality parameters in spacecraft drinking water supplies.

Unfortunately, the vast majority of analytical techniques available for monitoring trace analytes in water do not meet the strict design, payload, and operational requirements for deployment on-orbit. Payload limitations, for example, preclude the launch of heavy, rack mounted environmental monitoring hardware, a situation exacerbated by the increased restrictions placed on flight manifests for ISS resupply missions following the Columbia accident. Water quality monitoring systems must therefore be portable, long-life instruments that function in zero gravity, require minimal consumables and operator time, and generate low levels of waste.

One technology demonstrating many of the characteristics necessary for implementation as an in-flight water quality monitoring system, including the ability to function in zero gravity,[4,5] is Colorimetric Solid Phase Extraction (C-SPE). C-SPE is a novel sorption-spectrophotometric technique that complexes and concentrates analytes on a solid phase extraction membrane, leading to a change in the color of the membrane. A hand-held diffuse reflectance spectrophotometer is then used to rapidly quantify membrane-bound analytes using the Kubelka-Munk function. Because all requisite reagents for C-SPE methods are immobilized on solid supports and analytes are determined on-membrane, this technique operates in a solid-phase mode that markedly reduces waste generation. This fact, coupled with the compactness, portability, simplicity, sensitivity, and versatility of the platform, make C-SPE an attractive candidate for deployment as an on-orbit water quality monitoring system. These same characteristics also suggest the potential application of C-SPE to numerous earth-bound needs (e.g., environmental monitoring and homeland security).

The four main variations of C-SPE, including example applications of each variation, have been recently reviewed.[2,6] To date, our group has utilized two of these variations in developing methods to meet NASA water quality monitoring requirements. Immobilization and extraction, where samples are passed through membranes impregnated with colorimetric reagents, is the basis for our approaches to monitor the biocidal agents silver(I)[1,5] and I_2[5-7] as well as the metal contaminants copper(II), iron(III), and chromium(VI).[8] Complexation and collection, where immobilized colorimetric reagents are added to samples in-line to form a precipitate that is collected on an extraction disk, was demonstrated in our methods for determinations of nickel(II) using nioxime[8] and dimethylglyoxime (DMG).[2] We have also

employed the concept of immobilized reagents to adjust pH for optimization of the complexation chemistry[2] and to oxidize an analyte to a more readily detectable product.[5]

This paper reports the design and initial performance evaluation of multiplexed C-SPE (MC-SPE), a new platform for the facile and simultaneous determination of multiple water quality parameters. The creation of MC-SPE was driven in large part by the limited astronaut time available on ISS to conduct water testing. As such, the development of a multiplexed analysis system may potentially increase the collection of water quality data with a minimal investment of on-orbit crew time.

MC-SPE uses a filter holder with discrete analysis channels and a jig that allows the simultaneous operation of multiple sample-dispensing syringes for the rapid determination of several water quality parameters. A multi-parameter read-out cartridge combines separate membranes to create unique, parameter specific addresses that are positioned at the outlet of each analysis channel. Following passage of a sample solution, the diffuse reflectance spectrum of each address is collected serially to quantify the individual sample parameters. The use of individual analysis channels and a read-out cartridge with separate membranes for each parameter allows the reaction conditions and the membrane chemistry to be optimized for each analysis while eliminating cross talk between neighboring addresses.

Three parameters were utilized in the performance evaluations of our MC-SPE platform: silver(I), a biocidal water additive on ISS, nickel(II), a metal leachate from ISS water system hardware, and pH. These analytes are Priority 1 (highest priority) water quality monitoring parameters, as classified by NASA. Determinations of silver(I) and nickel(II) follow previously established C-SPE methods using reversed phase extraction membranes and the reagents 5-(p-dimethylaminobenzylidene) rhodanine (DMABR)[1] and DMG,[2]

respectively. Sample pH is measured using anion exchange membranes impregnated with fluorescein (FL), representing the first report of C-SPE results using anion exchange membranes. These studies illustrate the versatility of the multiplexed platform by combining C-SPE methods that require different membrane disk chemistry in a single experiment. We show that in a simple set of operational steps, MC-SPE can simultaneously determine silver(I) levels ranging from 0.05 to 0.5 ppm, nickel(II) levels between 1.8 to 5.0 ppm, and sample pH between 2.5 and 5.0. Moreover, the analysis requires only ~120 s to complete and uses a total sample volume of 3.0 mL. Potential extensions to a variety of earth-bound monitoring needs are also discussed.

EXPERIMENTAL SECTION

Reagents and Chemicals

Standard solutions. Single analyte standard solutions of silver(I), with concentrations between 0.1 and 1.0 ppm, and nickel(II), with concentrations between 0.5 and 5.0 ppm, were used to calibrate the C-SPE methods in the multiplexed filter holder. These standards were prepared in Nalgene® bottles by diluting the appropriate mass of either a silver(I) or nickel(II) atomic absorption standard (Aldrich) with deionized water to a final mass of 30.0 g. The response of the pH membrane disks was calibrated using the pH of these solutions, as determined with an Orion 520A pH meter.

Sample solutions. Multi-analyte samples containing both nickel(II) and silver(I) were prepared by combining the appropriate mass of each atomic absorption standard and bringing the sample to a final mass of 30.0 g with 0.05 M sodium nitrate (Fisher) to adjust ionic strength. The pH of sample solutions was determined using the pH meter.

Buffer wool. The preparation of the buffer wool has been described previously.[2] The wool, which was cut into 10-mm diameter circles, was used to adjust the pH of the solution flowing in the nickel(II) analysis channel to 9.0.

DMG chads. The procedure used to prepare DMG has been previously reported.[2] The 10-mm diameter chads were used to introduce DMG into the solution in the nickel(II) analysis channel.

Silver(I) membranes. The preparation of silver(I) sensitive membranes followed a modification of our earlier procedure.[1, 5] A 10.0-mL aliquot of 600 ppm DMABR in 80:20 methanol:dimethyl formamide (v:v) was drawn through a 47-mm diameter 3M Empore[TM] SDB-XC extraction membrane using a Millipore Glass Vacuum Filter Holder, a mechanical vacuum pump, and a pressure difference of ~38 torr. The membranes were then treated with 5.0 mL of 3% aqueous (v:v) Brij-30, using a pressure difference of ~100 torr. Residual solvent was removed from the membranes by increasing the pressure difference to ~550 torr for 10 s.

Nickel(II) membranes. 3M Empore[TM] SDB-XC 47-mm extraction membranes were used as a collection matrix for the nickel(II)-DMG precipitate. Membranes were treated with 10.0 mL of methanol and 5.0 mL of 3% aqueous Brij-30, using a pressure difference of ~38 torr and ~100 torr, respectively. The pressure difference was then increased to ~550 torr to remove residual solvent. This treatment was necessary to reduce the back pressure of the as-received membranes.

pH membranes. 3M Empore[TM] Anion-SR 47-mm diameter extraction membranes were used for impregnation of the pH indicator FL. Membranes were pre-treated to reduce back pressure using the same procedure described for the nickel(II) membranes. Following

pre-treatment, a 5.0-mL aliquot of a solution prepared by dissolving 37.0 mg of the sodium salt of FL (Aldrich) in 100.0 mL of deionized water was passed through the membrane using a pressure difference of ~100 torr. Residual solvent was then removed from the membranes by increasing the pressure difference to ~550 torr for 10 s.

After preparation, all membranes were dried in the ambient overnight, cut into 10-mm diameter disks, placed in an airtight bag, and stored in a dark drawer until used. From here on, the term "membrane" is used to describe the 47-mm diameter extraction membranes. All smaller diameter disks cut from the membranes are referred to as "membrane disks".

Syringe Jig

The syringe jig was cut from 5.7-mm thick clear acrylic into a triangular section with 45.0-mm sides. Three holes were drilled through the triangular section so that 1.0-mL plastic syringes could be press fit into the holes and held in place. The jig, shown in Figure 1A, positions the syringes to allow all three plungers to be depressed simultaneously.

Filter Holder

The multiplexed filter holder is composed of four sections, a syringe interface, a flow chamber, a read-out cartridge, and a base. Fabrication of each section is described below and illustrated in the line drawing in Figure 2D.

Syringe interface. The upper portion of the filter holder, shown in Figure 2A, directs the sample flow from each syringe to one of the three analysis channels in the flow chamber. This piece was fabricated from 35-mm diameter white delrin. The bottom of the interface was machined to mate directly to the flow chamber, leaving no dead volume. Samples flow through the syringe interface into the flow chamber via three 1.6-mm diameter ports lined with 0.51-mm i.d. PEEK HPLC tubing. The PEEK port lining protrudes ~25 mm from the

top of the interface and is connected to nylon Luer slip fittings with Silicone tubing (Masterflex). The Luer fittings were pressed into an acrylic brace to simplify connection to the sample syringes.

Flow Chamber. The center portion of the filter holder, shown in Figure 2B, defines each analysis channel. The flow chamber was fabricated from 35-mm diameter white delrin cut to a length of 33.3 mm. Three equally spaced 7.0-mm diameter holes were drilled along the axial direction of the cylinder to create the three analysis channels. The top of each channel was bored out to accept a 9.0-mm diameter support screen. These screens rest 12.0 mm above the channel outlets and facilitate the placement of the wool and paper used for the in-line addition of immobilized reagents (nickel(II) method) in the channels. The bottom of the flow chamber was machined to fit over the base of the filter holder.

Base. The bottom portion of a 25-mm diameter Swinnex polypropylene filter holder (Millipore) was used as the filter holder base. The base of the multiplexed filter holder, which supports the read-out cartridge, is shown in Figure 2C.

Read-out cartridge. Two 25-mm diameter circles were cut from 0.05-mm thick nitrile rubber (McMaster Carr). These circles were stacked and sealed together at one point on their circumference with double-sided tape (3M). Three equally spaced 7-mm diameter holes were cut through the rubber circles to align with the analysis channels in the flow chamber and define the area of the read-out addresses. Loading individual, parameter-specific membrane disks between the two layers of nitrile rubber creates a single read-out cartridge with three separate 7.0-mm addresses, as shown in Figure 3.

Clamp. The entire multiplexed filter holder is held together with a brass screw clamp (Figure 1B). The clamp consists of two brass rings machined to fit snugly around the syringe

interface and base of the filter holder. Following assembly of the filter holder (described below), three screws are inserted through the ring at the syringe interface and threaded into the ring at the holder base. Tightening these screws secures the filter holder sections in place.

Filter Holder Construction

Three 10-mm diameter C-SPE membrane disks, one specific for each sample parameter, were loaded in the read-out cartridge. The read-out cartridge was placed on the filter holder base and the flow chamber was positioned such that the outlets of the analysis channels were aligned with the addresses on the read-out cartridge. Next, support screens were inserted in each flow channel. A DMG chad and a circle of buffer wool were added, in that order, to the support screen in the analysis channel above the nickel(II) membrane. The sample interface was positioned on top of the flow chamber ensuring that the PEEK-lined ports were oriented at the inlet of the analysis channels. Finally, the entire assembly was clamped together using the brass screw clamp, as shown in Figure 1B.

Diffuse Reflectance Spectrophotometer

A BYK-Gardner color-guide sphere d/8° spin diffuse reflection spectrophotometer (model PCB-6830) was used to collect the spectra of the read-out addresses. This spectrophotometer is small (8.1 x 17.8 x 9.4 cm), lightweight (0.5 kg), battery operated, and collects spectra over the range 400-700 nm in 20-nm intervals. The aperture of the integrating sphere is 11 mm and acquisition of a single sample spectrum requires ~2 s.

Aperture Mask

A mask was used to match the aperture size of the spectrophotometer to the diameter of the addresses on the read-out cartridge. The mask was made by cutting a 7-mm diameter

hole in a 100-μm thick polyester sheet and then painting the sheet flat black with spray paint. The mask was affixed to a BYK-Gardner sample locator using double sided tape.

Sampling Procedure

Three 1.0-mL aliquots of a water sample were collected in 1.0-mL plastic syringes. These syringes were fitted into the syringe jig and connected to the syringe interface of the filter holder. The plungers of all three syringes were depressed simultaneously to meter 1.0 mL of sample solution through each analysis channel in the flow chamber. Following passage of these solutions, the syringes were removed and the membranes were dried by passing air through the filter holder with a 60-mL syringe and an adapter mated to the analysis channels. At this point, the filter holder was disassembled and the read-out cartridge was removed. The cartridge was placed under the sample locator so that one read-out address was visible through the aperture mask. The spectrum of that address was acquired and the cartridge was repositioned to sample the other two addresses in a serial fashion. This process entire process, beginning with syringe filling, requires ~120 s.

Readout

After collecting a data set, the spectrophotometer was interfaced to a computer using a serial cable and the reflectance spectra were downloaded. An in-house modified version of BYK-Gardner QC-Link software was used to calculate the Kubelka-Munk function from the reflectance data. The Kubelka-Munk function, $F(R)$, is defined as:

$$F(R) = (1-R)^2 / 2R \qquad (1)$$

where R is the relative reflectance measured with respect to a standard white. $F(R)$ can be related to analyte concentration by:[9]

$$F(R) = 2.303\varepsilon C/s \tag{2}$$

where ε is absorbtivity, C is concentration of the complexed analyte, and s is the scattering coefficient of the sample surface. By assuming the absorptivity and scattering coefficient of the membrane surface are constant at a given wavelength, $F(R)$ can be directly related to analyte concentration.

RESULTS AND DISCUSSION

pH Method Development

The use of immobilized forms of FL to construct pH sensitive optrodes has been explored by several groups.[10-13] There are three main reasons FL was chosen to demonstrate the ability to measure pH optically in MC-SPE. First, the biodegradability[14] and low toxicity of FL make it an ideal reagent for use in spacecraft environments. Second, experiments have shown that the absorbance of aqueous FL at 490 nm changes with pH in the range 1.25 to 8.70.[15, 16] And third, most common metal ions do not interfere with the dye response.[13]

Initial attempts to impregnate Empore™ SDB-XC membranes, the membranes used in the silver(I) and nickel(II) methods, with aqueous FL were unsuccessful. Published pK_a values[17] indicate that a solution of FL dissolved in deionized water should contain a mixture of its mono- and dianion forms. Based on this, 3M Empore™ Anion-SR membranes were examined as a potential support for reagent impregnation. These membranes were selected because they are very similar to the SDB-XC membranes successfully used in other C-SPE methods. Anion-SR membranes contain polystyrene-divinylbenzene particles with a covalently linked quaternary ammonium group imbedded in a mesh of Teflon fibers. As received, the membranes appear white, but turn bright orange upon treatment with the FL solution.

The response of the FL membranes was assessed by running conventional C-SPE trials using 13-mm diameter membrane disks and 0.1 M buffer solutions with pH values between 2.17 and 6.23 as samples. The results of these experiments were compared to the published absorbance studies[15, 16] to determine if immobilization affected the reactivity of the dye. Because the diffuse reflectance spectrophotometer records relative reflectance in 20-nm intervals, the relative reflectance of the samples at 490 nm could not be recorded. Instead, the value of the Kubelka-Munk function calculated from the relative reflectance at 480 nm was used to compare C-SPE results to absorbance experiments. When 1.0-mL buffer samples were tested, the trend in the plots of the Kubelka-Munk function at 480 nm and the absorbance at 490 nm versus pH were very similar. However, because of the large absorbtivity (7.89×10^4)[15] of the FL dianion, the relative reflectance values above pH 4.5 are in the high error regime of spectrophotometric measurements.[9, 18] In order to reduce possible errors in pH measurements made by C-SPE, a detection wavelength where the pH membranes have a higher relative reflectance, and therefore a lower $F(R)$ value, was used. Based on a visual inspection of the data, the $F(R)$ value at 560 nm was selected. The $F(R)$ values at this wavelength, shown in Figure 4, follow the same trend as the data at 480 nm, enabling determinations of sample pH between 2.17 and 6.23 with reduced error.

Multi-analyte Experiments

To validate the performance of our MC-SPE system, calibration plots were constructed for silver(I), nickel(II), and pH using the three-channel filter holder. The collection of the silver(I) and nickel(II) data was accomplished by loading all three addresses in the read-out cartridge with C-SPE membrane disks specific for one analyte and running standard solutions containing only that analyte through the multiplexed holder. In the case of

nickel(II), a DMG chad and a circle of buffer wool were loaded in each analysis channel before sampling. The *F(R)* values collected in these experiments were then averaged and plotted against the analyte concentration to obtain calibration plots. Data for the pH calibration were gathered using the silver(I) and nickel(II) standard solutions and a read-out cartridge loaded with three pH sensitive membrane disks. The *F(R)* values obtained from these trials were plotted against the pH values of these standards, as measured with a pH meter.

Silver(I) calibration. The same wavelength of detection used in the conventional C-SPE experiments, 580 nm, was employed for all silver(I) measurements with MC-SPE. These determinations exhibited an upper limit of the linear response range near 0.5 ppm. Though not shown, the *F(R)* values at 580 nm begin to level off above this concentration. The lower limit of detection, calculated as the silver(I) concentration corresponding to the blank signal plus three times the standard deviation of the blank was 0.05 ppm.

The observed leveling of the response is attributed to saturation of the DMABR. Conventional C-SPE measurements, which use a 13-mm diameter membrane disk to extract analytes also exhibit saturation, but at a higher level of silver(I) (i.e., ~1.0 ppm). The addresses on the read-out cartridge are only 7 mm in diameter, representing an active area that is ~70% smaller than that used in our conventional (single analyte) C-SPE system. Since both C-SPE platforms use 1.0-mL sample volumes, the multiplexed membrane disk saturates at a lower concentration than the conventional C-SPE disk. The calibration plot is shown in Figure 5. In the linear response region, between 0.05 ppm and 0.5 ppm, all measurements showed an uncertainty of less than 15%.

Nickel(II) calibration. The same wavelength of detection used in the conventional C-SPE experiments, 540 nm, was employed for all nickel(II) measurements with the multiplexed filter holder. Unlike the silver(I) experiments, the nickel(II) studies performed with the multiplexed holder demonstrated a smaller linear response range than the single analyte method. This decrease cannot be associated with membrane disk saturation, as the upper concentration limit of the analysis has been shown to be limited by the release of DMG from the reagent chad.[2] Rather, the decreased range is a consequence of an increase in the concentration limit of detection from 0.5 ppm to 1.8 ppm.

The loss of membrane disk signal at low concentrations is ascribed to two factors: the proximity of the buffer wool to the reagent chad and the decrease in dead volume between the reagent chad and the collection disk. Both factors reduce the reaction time for the sample before passing through the membrane disk. In the conventional nickel(II) platform, the sample syringe is filled through a buffer wool cartridge. This step allows the sample to equilibrate at pH 9.0 in the syringe barrel while the buffer cartridge is detached from the syringe and the reagent chad/collection membrane assembly is attached to the syringe. In the multiplexed holder, the buffer wool sits directly on top of the reagent chad. As a consequence, solutions passing through the wool immediately encounter the reagent chad, which reduces the time available for equilibration of the buffer and sample before the sample encounters the reagent chad. Our earlier report on this method showed that if the sample does not reach the proper pH, the rate of reaction is severely diminished and the precipitate may not exhaustively form before the solution passes through the read-out address.[2] This loss of precipitate will lower the *F(R)* at all concentrations, and have a negative effect on the limit of detection.

Decreasing the dead volume between the reagent chad and the collection address should have a similar effect on the measurement. In the conventional method, once the solution passes through the reagent chad, it fills the dead volume between the chad and the collection membrane disk. This dead volume is slightly less than the total sample volume (1.0 mL), so a small portion of the sample is passed through the membrane disk immediately. The remainder of the sample sits stationary above the collection disk until it is displaced by air during membrane drying. The time lapse associated with the removal of the sample syringe and connection of the drying syringe allows more time for the analyte and reagent to complex in solution. In the multiplexed holder, this dead volume is roughly half (~0.4 mL) that in the two cartridge assembly used for conventional experiments (~0.8 mL), but the sample volume is the same. As a result, more of the sample passes through the collection address without the benefit of extra residence time. At lower concentrations, samples may not form enough precipitate to be distinguished from the background.

Despite the increased limit of detection, measurements made in the linear response region for nickel(II) in the multiplexed holder, 1.8 to 5.0 ppm, show an uncertainty similar to that of the silver(I) calibration, less than 15%. The calibration plot for nickel(II) is shown in Figure 6.

pH calibration. In initial experiments, the response from the membrane disks was somewhat erratic, failing to follow the trend observed with the buffer solutions. This behavior was attributed to the varying ionic strengths of the standards, which has a known effect on the response of pH indicators,[19, 20] including immobilized forms of FL.[13] The pH platform was tested again using silver(I) and nickel(II) standard solutions prepared in 0.05 M sodium nitrate to adjust the ionic strength of the standards. The response from these new

solutions, shown in Figure 7, was more reproducible, showing an uncertainty less than 17%, and the demonstrated relation between $F(R)$ at 560 nm and pH agreed closely with that observed using buffer samples.

The response equation listed in Figure 7 was generated by fitting an exponential function to the experimental data. The response of the pH membrane disks deviates from the sigmoidal behavior typically exhibited by pH indicators in absorbance studies. This same deviation is found in the solution absorbance studies with FL and has been attributed to the fact that all four forms of FL contribute to the solution absorbance at 490 nm.[15, 17] Because $F(R)$ at 560 nm follows the same trend with pH as absorbance studies, multiple forms of FL are clearly contributing to the C-SPE response.

Experiments were conducted to determine if the presence of 0.05 M sodium nitrate to would adversely effect the measurement of silver(I) and nickel(II). Neither Na^+ nor NO_3^- are known to interfere with either method, and comparisons with results obtained using the deionized water standards showed the addition of electrolyte had no effect. To avoid complications arising from varying ionic strength, all samples used in the multi-analyte trials were prepared in 0.05 M sodium nitrate.

Multi-analyte trials. The sample solutions used in the performance evaluation of MC-SPE were prepared to contain levels of silver(I) and nickel(II) that spanned the linear response range of the two metals as defined by the calibration studies. Each sample was run in triplicate (N=3) and the average $F(R)$ value at the wavelength of detection was used for quantification of all sample parameters. The sample pH and levels of silver(I) and nickel(II) were determined using the calibration curves constructed above and compared to the

calculated concentrations of silver(I) and nickel(II) and the measured sample pH values in Table 1.

Overall, there is strong agreement between the multiplexed determinations and the actual values of the sample parameters. All but one measurement showed a percent difference less than 14%, and all but three demonstrated a difference below 10%. The exception is the measured concentration of silver(I) in sample 5, which was ~30% higher than the calculated concentration. This difference is believed to be a result of measurement error during the preparation of the sample. This conclusion is supported by conventional C-SPE studies measurements using the same sample solution, which also yielded a measured silver(I) concentration much higher than the expected value.

Several of the results shown in Table 1 have one of the experimental determinations excluded from the calculations. These measurements, denoted with an asterisk, were discarded because of visible misalignment of the membrane disks with the addresses in the read-out cartridge. The cause of the misalignment is thought to be a shifting of the membrane disks between the layers of the read-out cartridge during assembly of the multiplexed filter holder. This created a small gap that allowed a portion of the sample to leak around the membrane in the read-out address. This issue will be addressed in future designs of the multiplexed platform by simplifying holder assembly and minimizing the need for manual alignment of platform components. Importantly, the results in Table 1 validate the performance of our MC-SPE platform for the concurrent analysis of several water quality parameters.

CONCLUSIONS

The ability of a MC-SPE to simultaneously determine three of NASA's Priority 1 water quality parameters has been demonstrated. Methods encompassing two variations of C-SPE and requiring chemically different membranes were successfully combined to measure silver(I) levels ranging from 0.05 to 0.5 ppm, nickel(II) levels between 1.8 to 5.0 ppm, and sample pH between 2.5 and 5.0. All three parameters were determined in ~120 s using a total sample volume of 3.0 mL.

Future work with MC-SPE will focus on two areas, improving the present hardware and additional applications of the multiplexed platform. Filter holder designs that provide results covering the concentration ranges required by NASA, simplify assembly, minimize alignment, and reduce the possibility of leakage at the read-out cartridge are planned. Also, a method whereby a single sample syringe can be used for sample introduction is being developed.

Extensions of MC-SPE to the speciation of aqueous iodine solutions and possible use in the extraction of trace organics from water samples are being explored. Research to enhance the range of the pH method through the co-impregnation of multiple indicators on a membrane as well as application of the multiplexed platform to meet environmental monitoring needs on Earth, such as the determination of lead and arsenic is also underway.

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Table 1. Results of the performance evaluation of MC-SPE.

Sample#	Actual			MC-SPE			% difference		
	Ag^+_{ppm}	Ni^{2+}_{ppm}	pH	Ag^+_{ppm}	Ni^{2+}_{ppm}	pH	Ag^+	Ni^{2+}	pH
1	0.0	0.0	5.0	ND	ND	4.8	N/A	N/A	4.80
2	0.336	0.0	4.3	0.321	ND	4.5	4.54	N/A	4.97
3	0.0	2.94	3.3	ND	2.86	3.2	N/A	2.87	2.99
4	0.102	4.96	3.1	0.0936	5.03*	2.8	8.18	1.49	9.17
5	0.380	2.04	3.4	0.492	2.28*	3.6*	29.48	11.55	4.67
6	0.315	2.97	3.3	0.358	3.14*	3.3	13.73	5.76	0.17

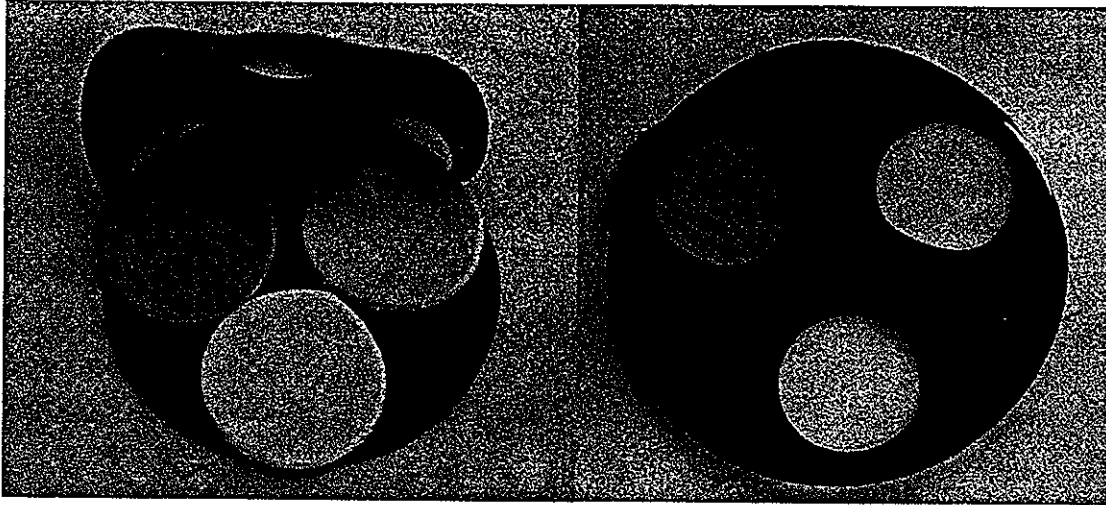N=3, except * where N=2   ND=not detected

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

$$[Ni^{2+}] = 2.34\ F(R) + 1.58$$

Figure 6.

$$pH = \frac{\ln(F(R) - 2.65e^{-2})}{1.66} + 5.54$$
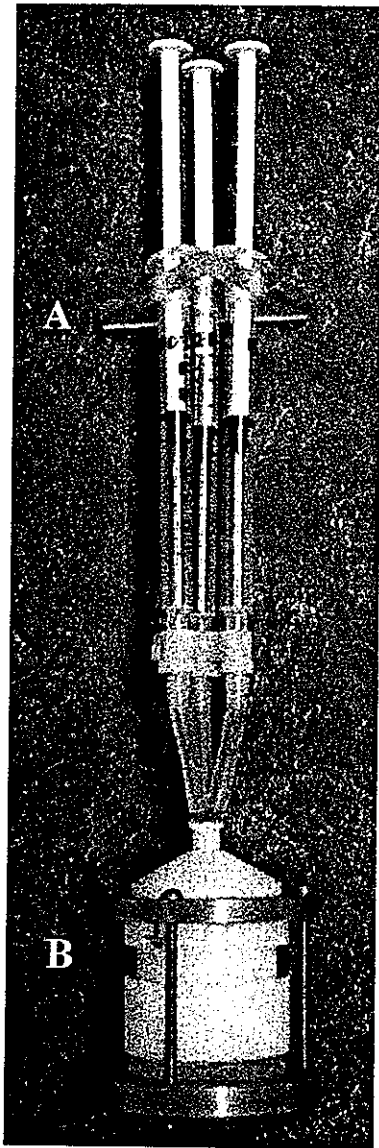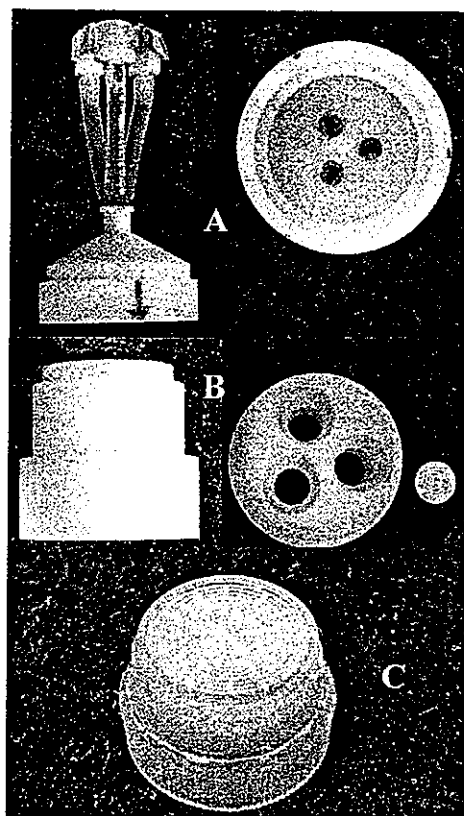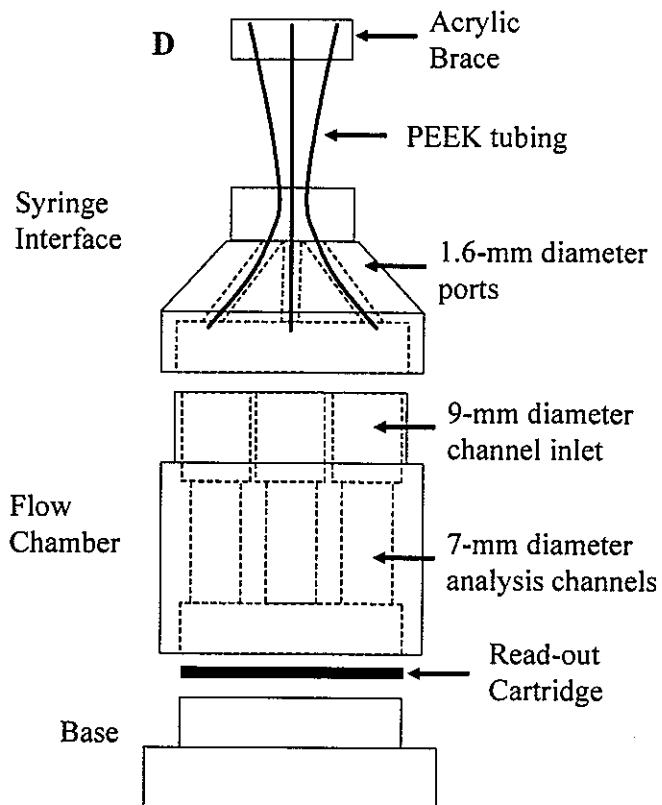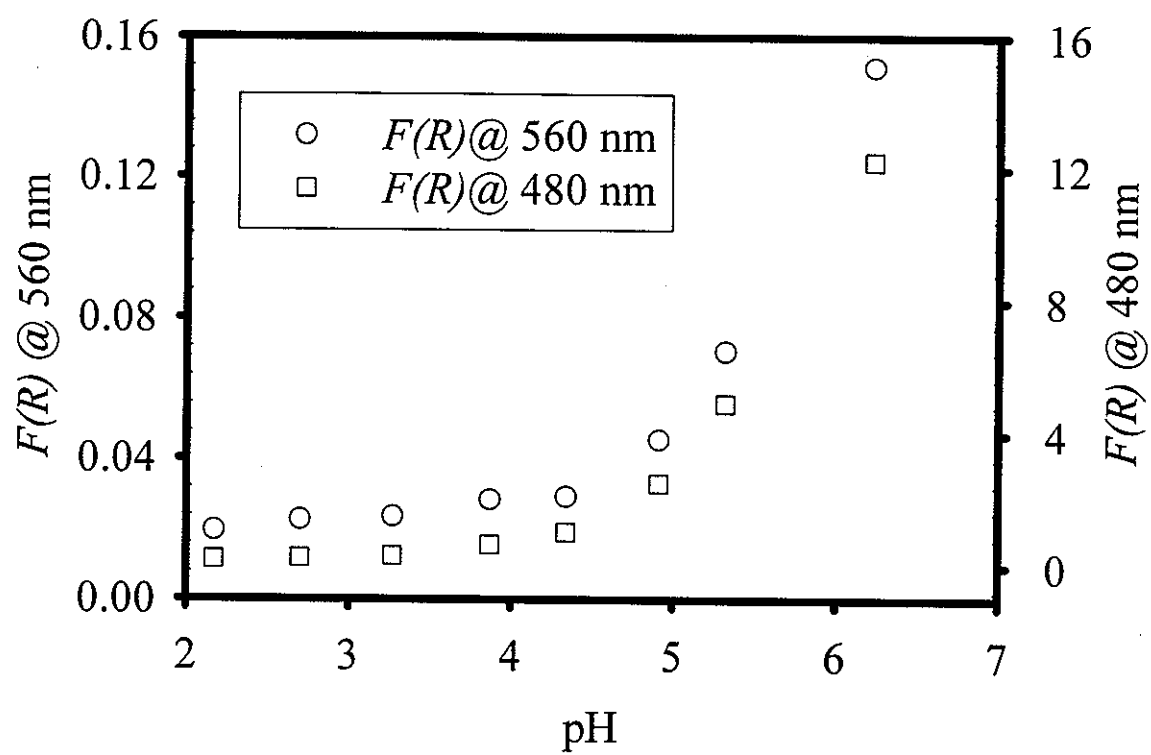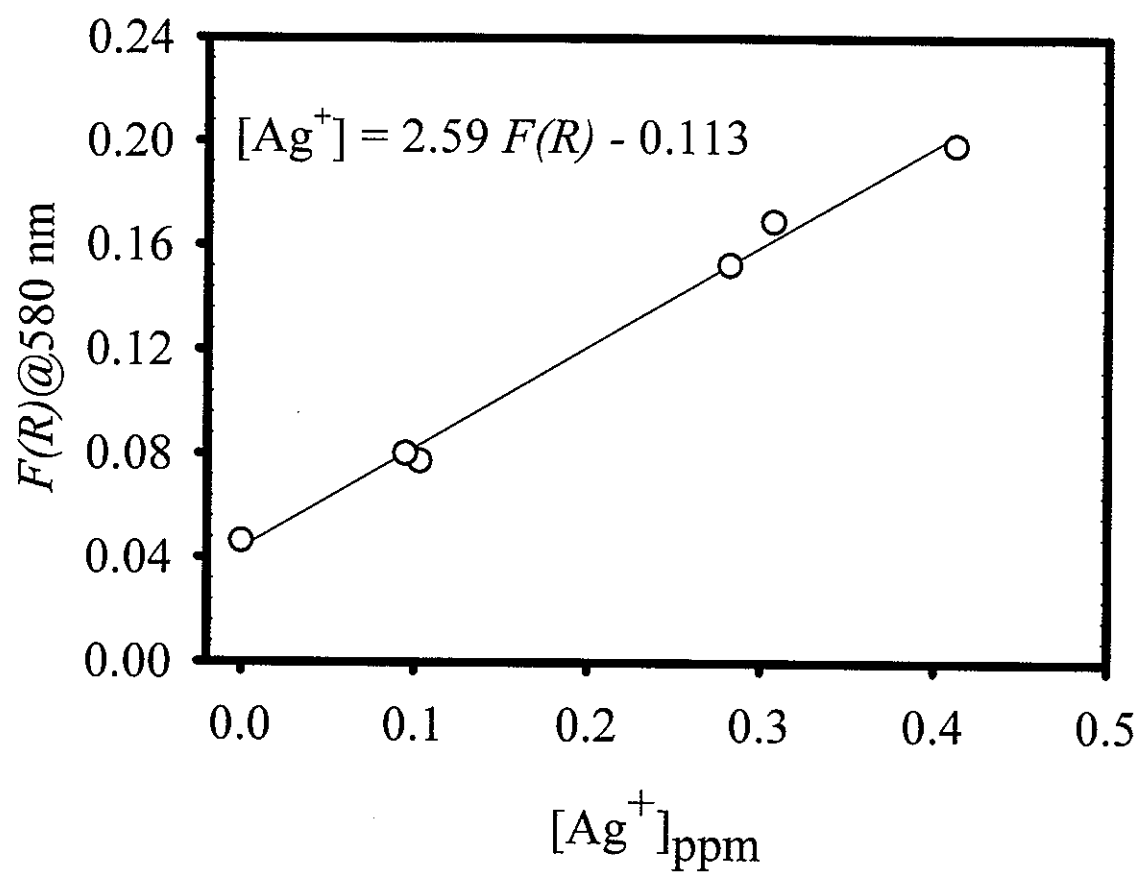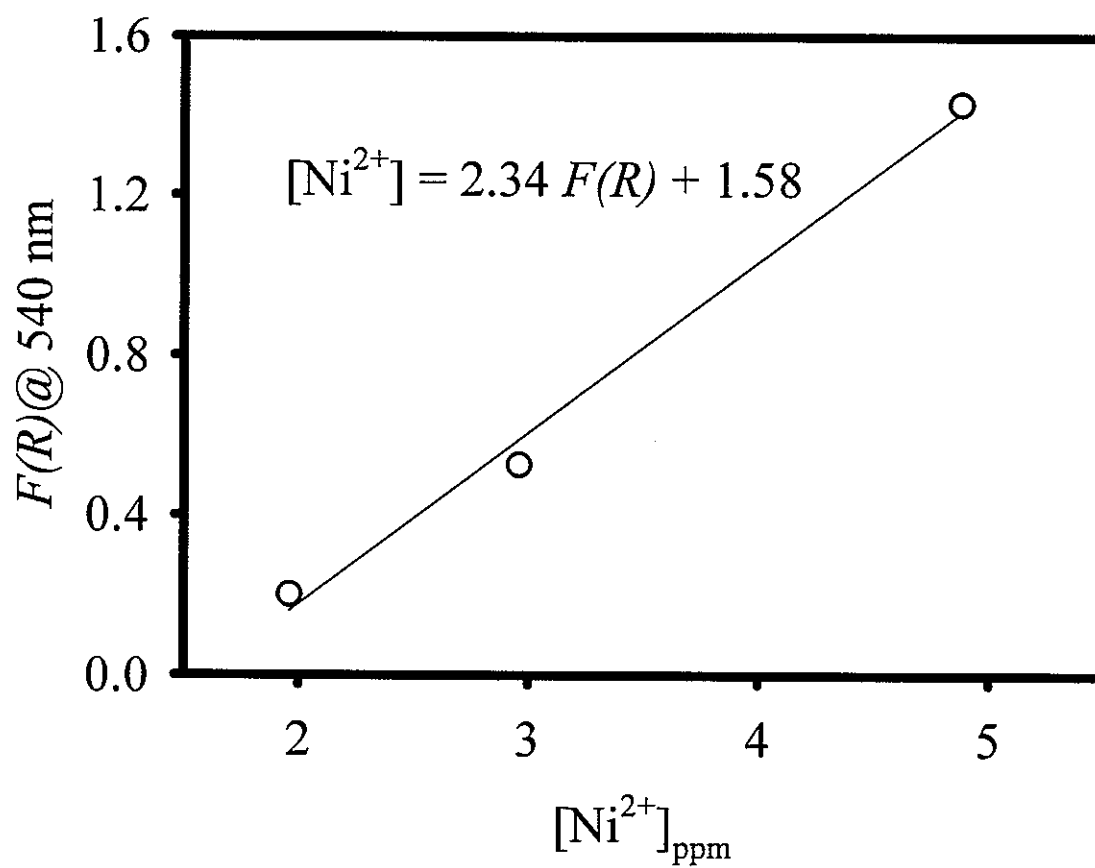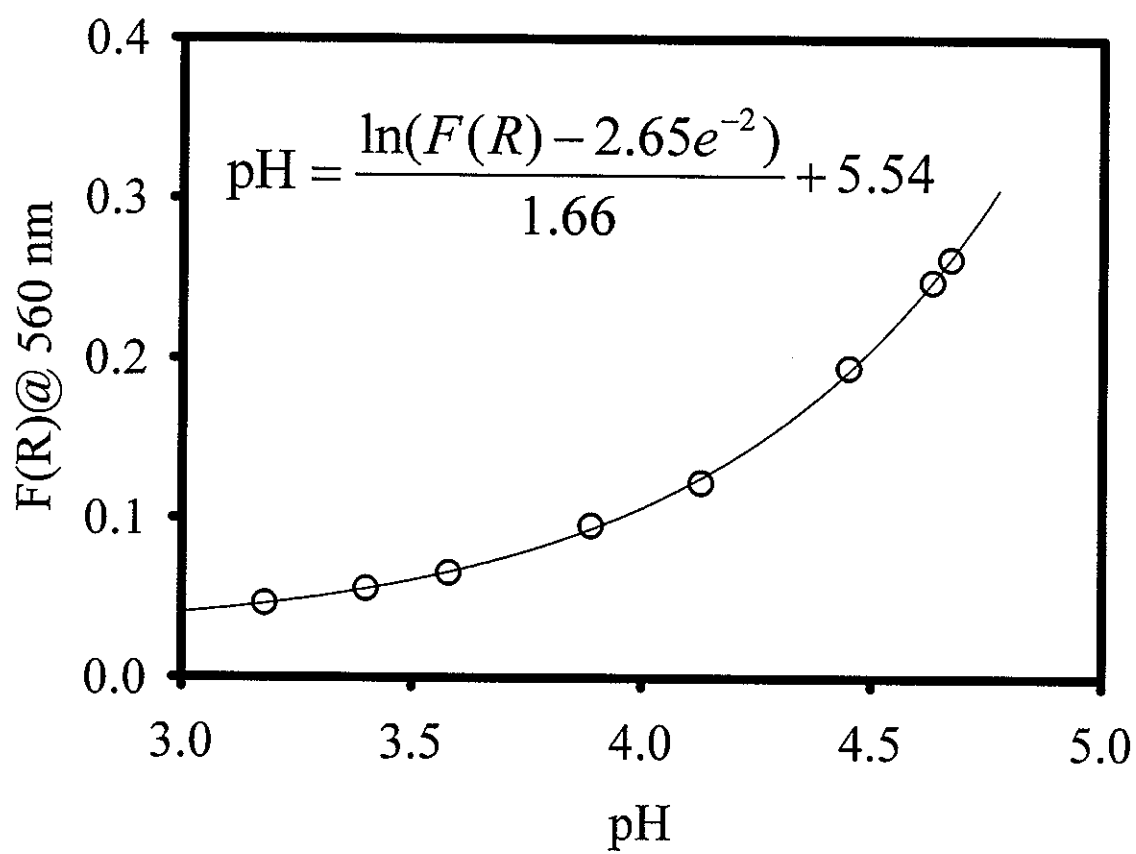
Figure 7.

FIGURE CAPTIONS

Figure 1. Assembled syringe jig and filter holder for MC-SPE. (A) Acrylic jig loaded with three 1.0 mL syringes; (B) multiplexed filter holder assembly.

Figure 2. MC-SPE filter holder components. (A) Left, side view of syringe interface; right, bottom view of syringe interface. (B) Left, side view of flow chamber; right, top view of flow chamber and support screen. (C) Base of multiplexed filter holder. (D) Line drawing of filter holder.

Figure 3. Read-out disk during membrane loading (left) and ready for use (right). Membrane disks, clockwise from the upper left, are sensitive to pH, silver(I), and nickel(II).

Figure 4. Plots of 13-mm diameter pH membrane disk response using 0.1 M buffer samples.

Figure 5. Calibration plot for silver(I) collected with multiplexed filter holder.

Figure 6. Calibration plot for nickel(II) collected with multiplexed filter holder.

Figure 7. Calibration plot for pH collected with multiplexed filter holder.

GENERAL CONCLUSIONS AND FUTURE PROSPECTUS

The pervading theme of this dissertation has been the development of C-SPE as a candidate technology for the in-flight monitoring of spacecraft water supplies. Chapter 1 demonstrates the ability of C-SPE to monitor the concentration of biocidal water additives in a microgravity environment. The performance evaluations were conducted on the KC-135 microgravity simulator using the biocidal species silver(I) and I_2. Biocide concentrations measured in microgravity with C-SPE were compared with results obtained from ground-based laboratory methods. Although the flight results demonstrated a linear relationship between $F(R)$ at the wavelength of detection and biocide concentration, there were unacceptable differences observed between the C-SPE results and the ground methods.

Chapter 2 attempted to explain the discrepancies observed between iodine concentrations measured by C-SPE and the leuco crystal violet method, which was used for comparisons in Chapter 1. Through systematic comparisons between C-SPE and UV-Visible absorbance experiments, it was determined that these techniques are sensitive to different iodine species. The signal in C-SPE was shown to be attributed to biocidally active I_2, while the leuco crystal violet method monitors not only I_2, but also HOI. This difference may account for some of the deviation observed when the techniques were compared. This Chapter also outlined a preliminary approach to the determination and speciation of total iodine in a water sample.

Chapter 3 introduced a new variation of C-SPE, the ability to determine an analyte based on the formation and collection of a colored precipitate. This approach was demonstrated using dimethylglyoxime to quantify nickel(II), a metal contaminant previously found in archived ISS water samples. This Chapter also presented the use of immobilized

reagents to adjust sample pH and introduce the complexing agents. The ability to add reagents to flowing samples and allow analytes to complex in solution enables reagents that fail to react when immobilized on a membrane surface to be utilized in C-SPE.

Chapter 4 combined the existing C-SPE methods for silver (I) and nickel(II) with a new method to optically measure pH to create a multiplexed C-SPE platform. This platform facilitates the simultaneous determination of several water quality parameters by utilizing a filter holder with discrete analysis channels and a jig that allows three syringes to be operated simultaneously. The creation of MC-SPE was motivated by the limited astronaut time available on ISS to conduct water testing. By developing a multiplexed analysis system, the amount of water quality data collected can potentially be increased without an increased investment of on-orbit crew time.

While the results presented in the data Chapters clearly demonstrate the potential of C-SPE to serve as a key element of an on-orbit water quality monitoring system, a few fundamental challenges must be overcome before C-SPE qualifies for on-orbit deployment. Most importantly, microgravity experiments that enable direct comparison of the ground and flight results are needed to ensure that biocide concentrations determined by C-SPE are accurate. Also, strategies must be devised to deal with the issue of air bubbles that become trapped in syringes during sample collection. To this end, recent KC-135 flights compared C-SPE results collected in-flight with results from laboratory C-SPE experiments conducted concurrently under similar environmental conditions (temperature, humidity, etc.). These studies eliminated uncertainty in sample volume associated with trapped air by pre-filling and de-bubbling sample syringes prior to analysis. These steps minimized differences between the flight and ground experiments, and allowed for a more direct comparison

between the results. The agreement between the data sets, detailed in a recent NASA report,[1] showed a marked improvement over results from earlier flights and provide strong evidence of the ability of C-SPE to function in microgravity.

There are several areas where the multiplexed C-SPE platform could be improved. Assembly of the present filter holder is somewhat labor intensive. New filter holders that simplify assembly and eliminate the need for manual alignment of the analysis channels with the read-out cartridge and the syringe interface would make the platform far more amenable to use in space. The technique can be further simplified by exploring modifications of the syringe interface to facilitate the use of a single sample syringe. This simplification would not only decrease the total analysis time by eliminating the loading of multiple syringes into the syringe jig, but also eliminate the need for a special adapter during membrane drying.

In addition to monitoring spacecraft water supplies, there are numerous earth-bound applications of C-SPE. As new methods are developed, C-SPE has the potential monitor analytes of interest for environmental monitoring and homeland security. Using our general platform, one can envision the assembly of field deployable test kits to measure arsenic, lead, and mercury in water and potentially even biological warfare agents. These and other avenues are currently being explored in our laboratory.

REFERENCES

(1) Porter, M. D.; James, J.; Packham, N.; Dias, N.; Gazda, D. B.; Hazen-Bosveld, A.; Lipert, R. J.; Ponton, L. M.; Kuo, M.; Rutz, J. A.; Straub, J. E.; Schultz, J. *KC-135 and Other Microgravity Simulations*; Space and Life Sciences Directorate, Human Adaptation and Countermeasures Office, Lyndon B. Johnson Space Center: Houston, TX, 2004.