Liquid Metering Centrifuge Sticks (LMCS): A Centrifugal Approach to Metering Known Sample Volumes for Colorimetric Solid Phase Extraction (C-SPE)

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ABSTRACT

Phase separation is one of the most significant obstacles encountered during the development of analytical methods for water quality monitoring in spacecraft environments. Removing air bubbles from water samples prior to analysis is a routine task on earth; however, in the absence of gravity, this routine task becomes extremely difficult. This paper details the development and initial ground testing of liquid metering centrifuge sticks (LMCS), devices designed to collect and meter a known volume of bubble-free water in microgravity. The LMCS uses centrifugal force to eliminate entrapped air and reproducibly meter liquid sample volumes for analysis with Colorimetric Solid Phase Extraction (C-SPE). C-SPE is a sorption-spectrophotometric platform that is being developed as a potential spacecraft water quality monitoring system. C-SPE utilizes solid phase extraction membranes impregnated with analyte-specific colorimetric reagents to concentrate and complex target analytes in spacecraft water samples. The mass of analyte extracted from the water sample is determined using diffuse reflectance (DR) data collected from the membrane surface and an analyte-specific calibration curve. The analyte concentration can then be calculated from the mass of extracted analyte and the volume of the sample analyzed. Previous flight experiments conducted in microgravity conditions aboard the NASA KC-135 aircraft demonstrated that the inability to collect and meter a known volume of water using a syringe was a limiting factor in the accuracy of C-SPE measurements. Herein, results obtained from ground based C-SPE experiments using ionic silver as a test analyte and either the LMCS or syringes for sample metering are compared to evaluate the performance of the LMCS. These results indicate very good agreement between the two sample metering methods and clearly illustrate the potential of utilizing centrifugal forces to achieve phase separation and metering of water samples in microgravity.

INTRODUCTION

NASA currently utilizes an archival approach to monitor the quality of the potable water supply on board the International Space Station (ISS).[1] Samples of the two main sources of potable water on ISS, recovered humidity condensate and Russian supplied Rodnik water, are collected and stored on-orbit until they can be returned to Earth for analysis. In the years following the Columbia accident, the limitations of this archival approach to water quality monitoring have become apparent. Highly prioritized crew schedules, curtailed Shuttle missions to ISS, and the limited return payload capacity of the Russian Soyuz vehicles have led to an overall decrease in sample availability and concurrent increase in the time lapse between sample collection and analysis. Since all water quality indicators are measured on the ground, this delays the implementation of corrective actions in the event that the potable water supply becomes contaminated. As NASA begins to focus on more ambitious missions, including re-establishing a human presence on the moon, the use of archival samples for water quality monitoring will no longer be feasible. In order to fulfill President Bush's bold vision of exploration,[2] it will become critical to develop in-situ methods to measure quality parameters in spacecraft drinking water supplies.

The strict design, payload, and operational requirements imposed on flight systems preclude the vast majority of environmental monitoring hardware from being deployed in space. An added challenge to hardware development is the fact that most analytical techniques capable of measuring trace analytes in water are not amenable to operation in a zero gravity environment. In the absence of gravity, phase separation becomes a serious challenge. Air bubbles become entrapped in water samples and cannot be easily displaced. The presence of such bubbles in water samples can cause significant errors in spectroscopic measurements and make most traditional electrochemical measurements impractical.

In light of these constraints, it is clear that on-orbit water quality monitoring systems must not only be portable, long-life platforms that require minimal consumables, minimal operator time, and generate low levels of benign waste; they must also be based upon technology that can function in the absence of gravity.
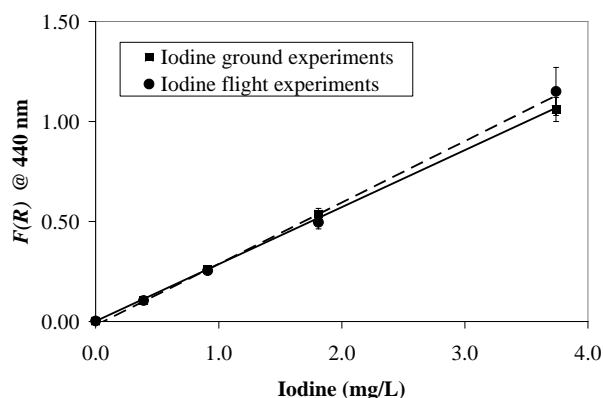
One technology being developed to meet many of the requirements for implementation as an in-flight water quality monitoring system, including the ability to function in zero gravity,[3] is Colorimetric Solid Phase Extraction (C-SPE). C-SPE is a sorption-spectrophotometric technique that complexes and concentrates analytes on a single-use 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 (KM) function. The use of solid phase extraction membranes as the matrix for impregnation of complexing reagents in C-SPE creates a concentration factor (~800) that enables low-level monitoring of target analytes using small (1-10 mL) sample volumes. Additionally, because all reagents are immobilized and analyte read-out is performed on the membrane surface, the technique operates in a solid-phase mode that markedly minimizes waste generation. These facts, coupled with the compactness, portability, simplicity, sensitivity, and versatility of the platform, make C-SPE an attractive candidate for on-orbit water quality monitoring.

The four main variations of C-SPE, including example applications, have been recently reviewed.[4] To date, two of these variations have been used in developing methods to meet several NASA water quality monitoring requirements. Immobilization and extraction, where samples are passed through membranes impregnated with colorimetric reagents, is the basis for measuring the biocidal agents silver(I)[3, 5] and I_2[3, 6, 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 methods for determinations of nickel(II) using nioxime[8] and dimethylglyoxime.[9]

Although all of the C-SPE methods developed perform very well in ground experiments, initial evaluations of the methods on microgravity flights showed significant deviations from ground results.[10] During these experiments, every step of the C-SPE method was performed in microgravity, including sample collection. Samples were collected into syringes from Teflon sample bags filled with stock solutions containing target analytes. The post flight report stated that sample collection was one of the most difficult steps in the method.[11] The samples frequently contained air bubbles which made it difficult to accurately determine the volume of the collected sample. Since C-SPE measures the mass of analyte collected on the surface

of the extraction membrane, it is necessary to accurately know the sample volume in order to determine analyte concentration.

A second set of microgravity flight experiments attempted to eliminate sample collection issues so that the effects of microgravity on the C-SPE methods could be isolated. To accomplish this, the experiments were conducted using pre-filled sample syringes. Figure 1 show plots of iodine data collected with pre-filled sample syringes during microgravity experiments and in a laboratory on the ground.[12] Both the flight and ground data provide linear responses and show excellent agreement within experimental error. This data successfully demonstrated the ability of C-SPE to function in a microgravity environment. Taken together, the experiences from the two rounds of microgravity testing underscore the need to develop better methods for sample collection and metering in microgravity.

Figure 1. C-SPE iodine response plots obtained from ground and microgravity flight experiments

As mentioned previously, perhaps the most significant obstacle encountered during the development of analytical methods for water quality monitoring in spacecraft environments is phase separation. Specifically, the elimination of entrapped air (i.e. bubbles) from water samples in microgravity. One potential solution to this problem is to utilize centrifugal force to achieve phase separation.[13] The liquid metering centrifuge stick (LMCS) is a device based on a principle previously developed for histo-chemical staining of blood smears on orbit (Centrifuge-Operated Slide Staining Technology (COSST)).[14, 15] Briefly stated, if a fluid sample containing bubbles in microgravity is placed in a *g*-field created by an on-orbit centrifuge, air/liquid phase separation will occur and any entrapped air (i.e. bubbles) will be removed from the liquid. An added benefit of this concept is that by employing a device that has physical features of specified dimensions, the centrifugal force can also be used to collect a defined volume of liquid in preparation for analysis. Further, since the fluid is under the influence of the centrifugal force, it can be directed through fluidic channels or a detection filter if the *g*-vector is applied in the correct direction with the

necessary magnitude for the appropriate period of time. This combination of fluid manipulation using centrifugal forces and defined physical features for collecting and metering known volumes of liquid has previously been used in a terrestrial setting for the development of micro-fluidic devices such as the "Lab-on-a-CD" platform.[16, 17]

This paper reports the results obtained during a ground evaluation of liquid meter centrifuge sticks with C-SPE. Ionic silver, which is the biocide used by the Russian Space Agency in potable water, was used as a test analyte for the evaluation. Ground based C-SPE experiments using the LMCS for sample metering were conducted and the data were compared to results obtained using sample syringes. Although these data indicate that minor differences exist between the two sample metering methods, they also clearly illustrate the potential of utilizing centrifugal forces to achieve phase separation and volumetric metering of liquid samples in microgravity.

EXPERIMENTAL
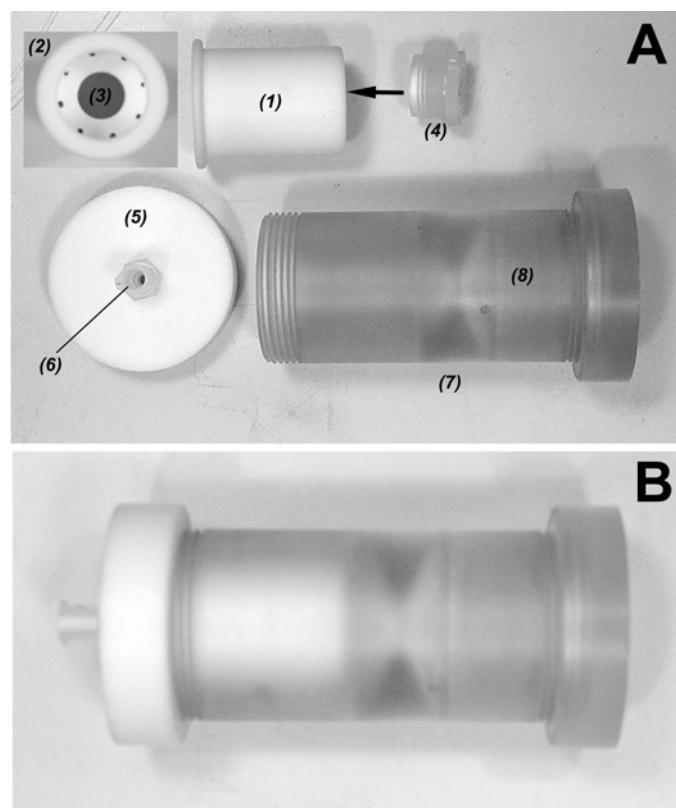
SILVER(I) SOLUTIONS.

Standard solutions of silver(I), with concentrations between 0.1 and 1.0 mg/L, were prepared in dark Teflon bottles by diluting the appropriate amount of a silver(I) atomic absorption standard (Aldrich) with deionized water. The concentration of silver in each standard was verified using inductively coupled plasma mass spectrometry (ICP-MS).

SILVER(I) MEMBRANES.

The preparation of silver(I)-sensitive membranes followed a previously reported procedure.[3] 3M Empore™ SDB-XC extraction membranes were used as a matrix for impregnation of the colorimetric reagent. A solution of 5-*p*-dimethylaminobenzylidene rhodanine (DMABR) was prepared by dissolving 150 mg of DMABR in 50 mL of dimethyl formamide and bringing the solution to a final volume of 250 mL with methanol. A 10 mL aliquot of the DMABR solution was drawn through a 47-mm diameter Empore™ 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 a 3% aqueous solution (v:v) of 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. Once prepared, the membranes were dried overnight on the benchtop in ambient conditions, cut into 13-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".

LIQUID METERING CENTRIFUGE STICK (LMCS).

LCMS devices were constructed using precision machining techniques. The metering cup was machined to dimensions that provided accurate capture of a 1 ml fluid sample. The device is capable of accepting a total sample volume of 3 mL (up to 50% air), capturing a 1 ml fluid sample, and expelling the remaining volume into the lower waste chamber. The internal metering cup portion of the LMCS device and lid were constructed from raw Teflon to avoid any potential loss of analyte by surface adsorption. The C-SPE membrane disk was secured in place directly below the 1 ml metering cup using a modified version of the lower portion of the same plastic Swinnex filter holder used in the standard C-SPE method. The LMCS also used the same PTFE O-ring to minimize leakage of the sample solution. The remaining portion of the LMCS device (the external sleeve and waste chamber) was constructed from polycarbonate in order to provide the mechanical strength needed to withstand high *g*-loads. The LMCS and all of its components are shown in Figure 2.

Figure 2. Prototype LCMS device for use with C-SPE. **(A)** Depicts the separate components of the LMCS device: *(1)* side view of the internal metering cup, *(2)* top view of internal metering cup, *(3)* area of metering cup used to collect sample, *(4)* lower portion of Swinnex filter unit used to support membrane disks which screws into the base of the metering cup *(arrow)*, *(5)* upper lid of the LMCS, *(6)* polypropylene "male" syringe connector, *(7)* polycarbonate external support sleeve , *(8)* waste chamber.**(B)** Depicts an assembled LMCS device ready for use.

CENTRIFUGE.

A Hettich Mikro 22 R centrifuge was used in the in the LMCS experiments. A custom rotor head was fabricated to accommodate the LMCS devices. The centrifuge is

microprocessor controlled and capable of spinning samples at 5000 rpm, creating a centrifugal force of approximately 3000*g*. The steady-state RPM, acceleration, and braking rates (i.e. ramp up and down) can be programmed by the operator and are controlled by the on-board microprocessor.

DIFFUSE REFLECTANCE SPECTROPHOTOMETER.

A BYK-Gardner color-guide sphere d/8° spectral included diffuse reflection spectrophotometer (model PCB-6830) was used to collect the spectra of the membrane disks. The spectrophotometer utilizes an integrating sphere to collect spectral data 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.

SAMPLING PROCEDURE.

The 13-mm DMABR membrane disks were loaded in a either a plastic Swinnex filter holder (Fischer part # 09-753-10ASX00 0013 00) or a LMCS. Both the filter holder and the LMCS contained a PTFE O-ring (10 mm i.d.) which served to prevent leakage and define the area of the C-SPE disk that is exposed to sample solution.

For the syringe experiments, a 1.0 mL sample of a silver (I) standard solution was collected using a 1-mL plastic syringe (Henke Sass Wolf, Norm-ject). One of the preloaded filter holders was attached to the syringe, and the water sample was passed through the membrane disk. Following exposure to the sample solution, the syringe is removed and the membrane disk is dried by pushing 60 mL of air through the filter holder. The sample holder was then separated and the lower portion, which contained the membrane disk, was placed in a sample locator and a spectrum was acquired.

For the LCMS experiments, 2-3 mL aliquots of the silver (I) standard solutions were collected in 3 mL syringes. The aliquots were then loaded into the LMCS, which contained the membrane disk, and the LMCS was placed into the centrifuge. The LMCS was rotated in the centrifuge to create a centrifugal force equal to 1000 *xg*. A 7 s ramp-up from 1*g* to 1000*g* was used, and the rotation at 1000 *g* was sustained for 10 s to allow complete passage of the 1 mL sample through the C-PSE membrane. After the 10 s at 1000*g*, approximately 15 s was required to stop centrifuge rotation. The LMCS was then removed from the centrifuge and disassembled so that the spectrum of the membrane disk could be acquired.

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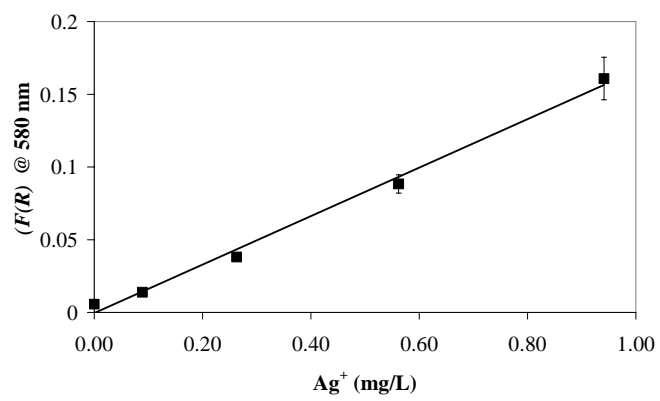
$$F(R) = 2.303\varepsilon C/s \qquad (2)$$

where ε is absorptivity, *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
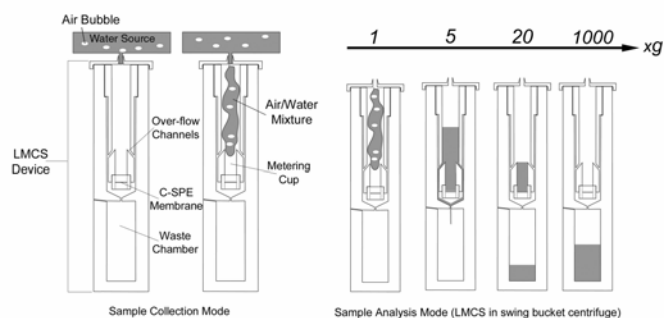
SYRINGE EXPERIMENTS.

A calibration plot for silver(I) was constructed to serve as a baseline for evaluation of the LMCS C-SPE method. To accomplish this, 1 mL samples of each silver(I) standard solution were analyzed by C-SPE using the standard filter holder assembly and a 1 mL plastic syringe for sample metering. The samples were analyzed in triplicate and the *F(R)* values at 580 nm were averaged and plotted against the analyte concentration measured by ICP-MS. Previous work with C-SPE has documented that a linear response between 0.1 mg/L and 1.0 mg/L silver(I) can be obtained using this wavelength of detection. The calibration plot for silver(I) is shown in Figure 3. The error bars on the graph represent ± 1 standard deviation. Responses from the syringe experiments showed excellent reproducibility; all measurements displayed an uncertainty of less than 13% RSD. Equation 3 is a response equation for silver(I) that was calculated from the trendline in the calibration plot. This equation was used to calculate silver(I) concentrations from the *F(R)* values obtained in the LMCS experiments and allowed for a direct comparison between the LMCS and the standard C-SPE methods.

$$[Ag^+] = 5.99F(R) + 0.0029 \qquad (3)$$

Figure 3. C-SPE response plot for silver(I) obtained from ground experiments using a syringe for sample metering.

LMCS EXPERIMENTS.

Figure 4. Graphical Representation of LMCS operation.

Adaptation of the LMCS for use with C-SPE is illustrated in Figure 4. First, the LMCS is charged with a water sample that may contain up to 50% of the sample volume as air (Figure 2 – sample collection mode). The LMCS is then loaded in a swing-out rotor of a centrifuge and exposed to a ramped g-load of 1g to 1000g (Figure 2 – sample analysis mode 1g). At low g-loads, air-fluid separation occurs and the water sample fills the metering cup in the center of the LMCS. Excess fluid over and above the volume contained in the metering cup is forced into the waste chamber located in the base of the LCMS via the over-flow channels (Figure 2 – sample analysis mode 5g-20g). As the g load increases, the sample volume in the metering cup of the LMCS is forced through the C-SPE membrane disk into the waste chamber of the LMCS (Figure 2 – sample analysis mode 1000g).   Once the centrifuge stops, the LMCS is disassembled and the C-SPE detection filter is removed and analyzed using surface reflectance spectroscopy as previously described.

FLUID PASSAGE STUDIES.

Initial experiments conducted with the LMCS revealed that the g-loads required to completely pass samples through a C-SPE membrane disk were significantly higher than anticipated. This has been attributed to the mass dependence of the centrifugal force that is created when the LMCS is rotated. Due to the hydrophobic nature of the C-SPE membrane disks, a certain minimal force must be exerted to pass a water sample through the membrane disk. In the standard C-SPE method, this force is created by depressing the plunger of the sample syringe. In this scenario, the external force applied to the syringe plunger is independent of the mass of the water sample.   In the LMCS however, the g-force created by rotating the water sample in the LMCS is directly proportional to the mass of the water sample. Once the sample begins to flow through the membrane disk in the LMCS, the g-force is reduced because the sample mass on top of the membrane disk is now smaller. When a certain amount of sample has passed through the membrane disk, the mass on top of the membrane is reduced to the point that the g-force is no longer sufficient to overcome the flow resistance of the
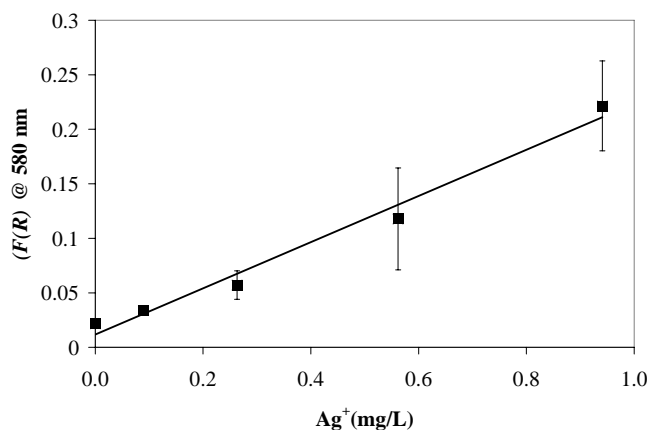
membrane disk.   If the LMCS is not rotated at a sufficient rpm, this occurs and residual sample remains in the LMCS metering cup when rotation is stopped.

A series of experiments were performed to determine the minimum g-force was required to achieve total sample passage inside the LMCS.   Samples of deionized water were injected into an LMCS that was loaded with a silver(I) membrane disk. The LMCS was then rotated to create a specific g-force and held at that rotation for 10 s. The LMCS was then disassembled and visually inspected to verify that complete sample passage had occurred. From these experiments, it was determined that a minimum force of 500g was required to affect complete sample passage in the LMCS. For the LMCS evaluation experiments, a force of 1000g (2 x the minimum g-load) was used to ensure that the entire sample volume passed through the membrane disk.

EVALUATION OF LMCS PERFORMANCE.

For the evaluation experiments, 2-3 mL samples of the silver(I) standard solutions were injected into a LMCS that contained a C-SPE membrane disk. The LMCS was then loaded into the centrifuge and rotated at 1000g for 10 s using a 7 s ramp. A blank LMCS loaded with DI water was used to balance the rotor head in the centrifuge.   The same metering cup was used in all LMCS evaluation experiments to eliminate any variability between different metering cups.   Gravimetric measurements conducted with the metering cup used in these experiments have shown the internal volume of the cup to be 0.963 mL. Previous work has shown that this is within the uncertainty of 1.0 mL sample volumes collected in a syringe. As such, no efforts were taken to account for differences in sample volume between LMCS and the standard C-SPE method.

The *F(R)* values obtained from the LMCS experiments are plotted against silver(I) concentrations measured by ICP-MS in Figure 5. The data points represent the average of three measurements and the error bars represent ±1 standard deviation. As is evident from the plot, the LMCS C-SPE data does provide a linear response for silver(I) in the same concentration range as the standard syringe method. However, the uncertainty in the measurements is significantly higher and the response from the LMCS at a given concentration is noticeably higher than the response with the syringe method. The data from both sets of experiments is summarized in Table 1.

Figure 5. C-SPE response plot for silver(I) obtained from ground experiments using the LMCS for sample metering.

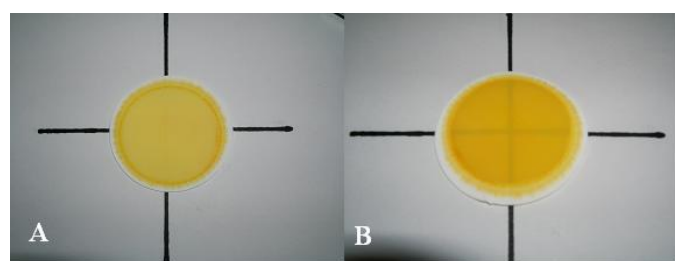**Table 1.** Silver(I) concentrations measured by standard C-SPE and LMCS C-SPE

Silver(I) (mg/L) by Standard C-SPE	Silver(I) (mg/L) by ICP-MS	Silver (I) (mg/L) by C-SPE with LMCS
0.037	0	0.133
0.087	0.089	0.204
0.231	0.263	0.345
0.532	0.562	0.708
0.966	0.941	1.33

There are several possible explanations for the increased uncertainty observed in the LMCS C-SPE experiments. One potential cause is the handling of the membrane disks between centrifugation and spectrum collection. Because the LMCS utilized a modified lower portion of a Swinnex sample holder to support the C-SPE membrane disks, the disks could not be placed directly in the standard sample locator. Instead, the membrane disks had to be removed from the modified holder with tweezers and placed on a standard holder prior to read-out. The transfer process tended to cause minor distortions of the membrane disks that could lead to variability in the collected spectra.

Another factor that likely contributed to the uncertainty was the orientation of the LMCS in the centrifuge head. Due to the limited space inside the centrifuge, the LMCS could not be loaded vertically into the centrifuge. Prior to spinning, the device was oriented at approximately a 45° angle relative to the axis of rotation. This orientation may have resulted in irregular exposure of the membrane disk to the sample solution that could create errors in the measurements.

The consistent, high response observed in the LMCS C-SPE experiments appears to be a systematic difference

between the two methods that is related to drying the membrane disks. In the standard C-SPE method, a 60 mL syringe is used to force air through the membrane disks which dries the disks before a spectrum is collected. Due to the presence of the drain holes around the metering cup in the LMCS, it was not practical to dry the membrane disks in an analogous manner. All of the drying air would pass through the drain holes and the membrane would remain wet. As a result, the spectra of the membrane disks in the LMCS experiments were collected without drying the membrane disks. Wet C-SPE membrane disks are significantly more transparent than dry disks, which reduces the amount of light scattered from the membrane disk surface. This decrease in the scattering coefficient (*s* in equation 2) manifests itself as an increase in the $F(R)$ value, which is consistent with observations from the LMCS experiments. The increased transparency of wet silver(I) membranes is shown in Figure 6.

Figure 6. (A) Dry silver(I) C-SPE membrane and (B) wet silver(I) C-SPE membrane.

CONCLUSIONS

Initial ground based evaluations of the LMCS with C-SPE have shown that the LMCS provides a linear response for silver(I) in the same concentration range as the standard C-SPE method. LMCS measurements tend to be higher than standard C-SPE measurements because the sample spectra are collected while the membrane disks are wet. The uncertainty in the current LMCS method is significantly higher than the standard method, but redesign of the centrifuge rotor and either the membrane disk support or the sample locator should minimize the variability in the measurements. Overall, the data presented indicates that while minor differences exist between the two sample metering methods, they both provide a linear response for silver(I) between 0.1 and 1.0 mg/L. Also, this study clearly illustrates the potential of utilizing centrifugal forces to achieve phase separation and volumetric metering of liquid samples in microgravity.

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