Extraction and Analysis of Lead in Sweeteners by Flow-Injection Donnan Dialysis with Flame Atomic Absorption Spectroscopy

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Flow-injection Donnan dialysis is demonstrated for the extraction of lead in sweeteners using flame atomic absorption spectroscopy (FAAS). For spiked concentrations in the low microgram per gram range, recoveries were greater than 90%, and the relative standard deviation was typically less than 10% for a 15-min dialysis procedure. The method detection limit is 350 ng/g. Donnan dialysis is shown to be successful for the extraction of lead in sucrose, corn syrup, and honey but limited in performance for molasses and artificial syrup. This paper also includes a comparison to other procedures for the determination of lead in sweeteners and presents options for realizing improved method performance with Donnan dialysis.

Keywords: *Donnan dialysis; flame atomic absorption spectroscopy; corn; honey; sweeteners; syrup*

INTRODUCTION

Natural sweeteners are classified as generally recognized as safe (GRAS) food ingredients (*1*). As common food ingredients, annual consumption is very high and since 1995 has averaged more than 150 lb/person in the United States (*2*). In terms of contamination by toxic metals, sweeteners are presently regulated for arsenic, lead, and total heavy metals (*1*). In 2000, the acceptable lead level was reduced from 500 to 100 ng/g (*3*, *4*). Regulation specifically for lead is desired because of the toxicological significance of lead and the greater bioavailability of lead to children as compared to adults (5). Lead contamination in sweeteners is possible through natural uptake from the soil or during processing (*5*). The determination of lead at these low levels in sweeteners is an analytical challenge because of the complexity of the sample matrix.

Approaches for the measurement of lead in sweeteners have involved some form of voltammetry (*6*, *7*) or atomic spectroscopy (*8*−*11*). Voltammetric measurement by simple dilution is possible, but the method of standard addition is required for quantitation with deposition times ranging from 5 to 20 min (*6*, *7*). Atomic spectrometric techniques offer a more rapid instrumentation time, and direct quantitation is possible but some form of sample digestion is required to alleviate sample matrix interferences (*8*−*11*). Therefore, alternative approaches to sample digestion are desired to extract lead from the sweetener matrix and simplify the analysis.

Donnan dialysis is one such alternative approach (*12*, *13*). Donnan dialysis involves the migration of sample ions across a membrane barrier and into a receiver or strip solution. The interaction at the membrane is based on ion-exchange, cation or anion, and the membrane serves to exclude oppositely charged ions. The receiver solution typically has a very high ionic strength value, and the diffusion of the appropriate receiver ion across the membrane must be matched in the counter direction by sample ions of like charge to ensure electroneutrality. If the receiver volume is sufficiently smaller than the sample, enrichment in addition to extraction of sample ions is possible. Enrichment is defined as the concentration measured in the dialysate relative to the initial sample concentration. Factors influencing the dialysis process include the receiver and sample ionic strength values and the temperature. In this simplistic format, Donnan dialysis does not exhibit any ion specificity beyond charge. Therefore, with a cation-exchange membrane, all divalent cations would have similar enrichment factors, but the factor would be different for monovalent and trivalent cations. Specificity is possible through membrane design and the selection of receiver solutions that promote additional processes such as complexation.

The analytical testing and development of Donnan dialysis began in the early 1970s with sheet membranes coupled to some form of electroanalytical detector (*14*, *15*). Enrichment factors were limited, approaching 10 within 30 min, but other investigations pertaining to the ideal composition of the receiver solution, sample limitations, and temperature effects were conducted. During the 1980s, tubular membranes were developed that improved enrichment factors by increasing the surface area-to-receiver volume ratio and providing for on-line interfacing with atomic spectroscopy (*16*−*18*). On-line enrichment with atomic spectrometric detection was limited because of the compromise between the low flow rate desired for the Donnan process and the higher flow rate needed for adequate signal efficiency by the atomizer (*17*, *18*). The flow rate compromise was alleviated in the late 1980s by utilizing a flow-injection analysis approach in which the tubular membrane was interfaced to the nebulizer of a flame atomic absorption spectrometer (FAAS) using a conventional six-port injection valve. The enrichment factor for copper improved to nearly 100 in 10 min (*19*). The process was also shown to be compatible with ICP-AES detection where the application to additional metal cations was demonstrated by selecting receiver solutions that were free of spectral interference (*20*).

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Analytical adaptation of Donnan dialysis as an extraction and enrichment technique has been limited despite the performance. Other efforts have centered on theoretical concerns (*21*−*23*), application of the process to the removal of contaminants from waste streams (*24*, *25*), speciation efforts where the membrane or receiver provides some type of selectivity (*26*, *27*), and anion enrichment (*28*, *29*). The objective of this work is to demonstrate the performance of flow-injection Donnan dialysis for the extraction and analysis of lead cations in sweeteners. FAAS will be used for detection so achieving regulatory limits is not expected, but the validity of the Donnan dialysis approach will be demonstrated. The performance evaluation includes various types of sweeteners, method detection limits, and spike and recovery experiments.

EXPERIMENTAL PROCEDURES

A detailed description of the dialysis cell utilized for this work has been previously published (*19*). In brief, a 1.25-m length of tubular form Nafion 811, 0.64 mm i.d. × 0.89 mm o.d. (Du Pont Polymer Products, Wilmington, DE) was coiled around a three-pronged glass holder. The membrane ends were connected to the sample ports of a standard injection valve thereby taking the place of the sample loop. The injection valve is located between a pump used for the carrier solution (1.0 M HNO_3) and the FAAS nebulizer. The volume represented by the connection to the nebulizer needs to be minimal to avoid undesirable effects from dispersion. In our case, the connection tube was 23 cm long with an internal diameter of 0.25 mm. The carrier flow rate was 4 mL/min, which was optimal for the instrument.

The dialysis procedure begins by positioning the valve to the load mode where the receiver solution (0.50 M $Sr(NO_3)_2$, 1.2 mM $Al(NO_3)_3$, and 0.10 M HNO_3) is injected into the tubular membrane while the carrier solution is diverted around the membrane and to the nebulizer. The cell is then placed in the sample solution, which was 350 mL unless otherwise noted. After allowing dialysis to occur (typically 15 min), the valve is switched to the inject mode, which causes the carrier to direct the receiver solution to the nebulizer for analysis. After analysis, the membrane is reconditioned by replacing the sample solution with distilled, deionized water and allowing the carrier solution to pass through for 5 min. The sample solution is stirred during dialysis to minimize the diffusion layer at the membrane surface.

The FAAS instrument was a Perkin-Elmer model 360, and an air−acetylene flame was used throughout. The lead line used for detection was 283.3 nm with a 0.70 nm slit width. The typical detection limit for lead achieved by conventional introduction with this instrument is 4 μg/mL for samples in deionized, distilled water. The instrument was optimized according to manufacturer protocol using a lead nitrate standard solution. A strip chart recorder was used to acquire the signals, and the peak height was used for all calculations.

As the flow-injection Donnan dialysis signal is transient in nature, it is important to ensure an adequate response time for the instrument and to minimize dead volume between the injector and the nebulizer. The minimum membrane length (volume) needed to avoid dispersion effects was determined by measuring response using stainless steel sample loops of decreasing volume. It was found that a volume as low as 250 μL produced results comparable to steady-state sample introduction, and this corresponds to a membrane length of approximately 0.80 m. The optimal time constant was established by determining detection limits using 250-μL sample injections. For this instrument, an intermediate value of 6 s was acceptable. Reproducibility computed as the percent relative standard deviation and based on triplicate injections over the calibration range averaged to be 8%. Therefore, we should anticipate similar reproducibility from the Donnan dialysis signals.

Table 1. Impact of Sample Volume and Dialysis Time on Enrichment[a]

time (min)	enrichment factor	sample vol (mL)	enrichment factor
5	35	100	90
10	64	200	113
15	79	350	110
		550	113

[a] All sample concentrations were 1.0 μg of Pb/mL. For the dialysis time study, a sample volume of 350 mL was employed, and the membrane length was 1.25 m. For the sample volume study, a 15-min dialysis time was used, and the membrane length was 0.46 m.

Samples were spiked in solution form. The desired sample mass was transferred to a volumetric flask and dissolved. Following dissolution, the spike was added, and the solution was diluted to the desired volume and mixed. Equilibration time or the time between the initial spiking of the sample and the analysis ranged from 30 min to 4 h, and no correlation was determined between recovery and time allowed for equilibration. Unspiked samples were also analyzed to provide for blank-corrected recovery calculations.

Detection limits and method limits are based on 3× the standard deviation of the signal provided by the carrier solution and represent values for the Donnan dialysis process coupled with FAAS detection. In some instances, a blank signal was noted for the unspiked sample or the calibration blank, and this signal was then subtracted from the unspiked sample signals or calibration standards, respectively, prior to final calculations. Where present, these signals are believed to be caused by trace levels of lead versus being an artifact of the measurement process. Enrichment factors were calculated as the quotient between the concentration in the dialysate over the initial sample concentration. Other trends are reported as the percent change in response for the test sample as compared to a standard prepared in distilled, deionized water.

All solutions were prepared from analytical reagent grade (or better) salts or acids dissolved in deionized, distilled water or dilute HNO_3. All glassware was thoroughly cleaned, which included a final soak in HNO_3 followed by a water rinse prior to usage.

RESULTS AND DISCUSSION

Table 1 summarizes the importance of sample volume and dialysis time on the enrichment factor for a lead standard prepared in water. From Table 1, the enrichment factor for lead is almost independent of sample volume, and this agrees with the previous report concerning flow-injection Donnan dialysis for copper with FAAS detection (*19*). The loss in enrichment for the 100-mL sample volume can be attributed to poor sample contact with the Donnan cell and could possibly be alleviated by using an alternative cell design. In our application, larger sample volumes could represent a greater initial sample concentration and therefore be one avenue to achieving lower method detection limits. A sample volume of 350 mL was employed for this paper because of the ease of configuration with the existing cell design.

The enrichment factor for lead increased with time, and this also agrees with the previous report for copper (*19*). As the increase between 5 and 10 min was 83% and this reduced to 23% between 10 and 15 min, further increases in the dialysis time would not yield substantial improvement when compared to the increase in the analysis time. Therefore, a dialysis time of 15 min was selected.

There is a discrepancy between the enrichment factor of 79 for the time study (350 mL and 15 min) and the

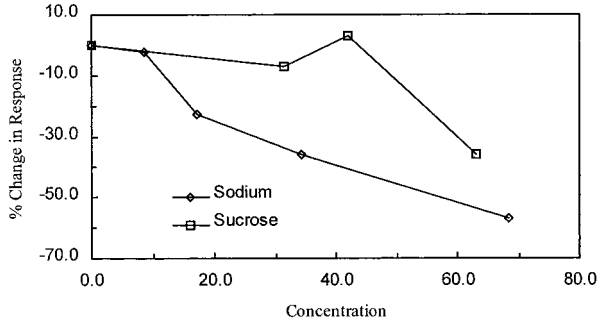

Figure 1. Impact of the concentration of sodium (mM) and sucrose (g/350 mL) on the response for lead. The solution lead concentrations were 1.0 μg/mL for the sodium study and 0.5 μg/mL for the sucrose study. The dialysis time was 15 min, the membrane length was 1.25 m, and the sample volume was 350 mL.

factor of 110 obtained for the corresponding conditions in the volume study (350 mL and 15 min). This discrepancy may be attributed to the shorter membrane (0.46 m) employed for the volume study. The shorter membrane was used to determine the minimal dialysis volume because a greater percentage of the membrane does not participate in the dialysis process (lengths between the sample surface and the injector ports) and is therefore more susceptible to sample volume effects. In either case, our observed trends for lead match the previous report for copper. The 1.25-m membrane length was employed for all additional studies to ensure that signal loss due to dispersion was not problematic.

The Donnan dialysis process is also sensitive to ionic strength effects. Previous data suggested that as the sample ionic strength exceeds 0.01, enrichment decreases (*15*). Sweeteners such as sucrose and corn syrup are of low ionic strength and therefore good sample candidates for the Donnan dialysis process. Figure 1 demonstrates the change in response for a constant lead solution concentration in the presence of increasing levels of sodium chloride (mM) and sucrose (g/350 mL). The response in both data sets was compared to a standard prepared in distilled, deionized water. As expected, as the sodium chloride concentration exceeds 10 mM (an ionic strength value of 0.01), signals decreased. In comparison, the sucrose sample concentration did not influence signals until levels approaching 40 g/350 mL or 330 mM. Rather than attributing the loss of signal at high concentrations of sucrose to ionic strength effects, it may be that the sample solution viscosity is such that the inward migration of lead cations and the outward migration of strontium cations are hindered, resulting in a lower signal. Additional studies are necessary to properly identify the cause of the signal loss, but within this application, quantitation based on external standards versus the method of standard addition is possible with initial sample sizes as large as 40 g diluted into 350 mL.

Figure 2 indicates the general utility of Donnan dialysis for the extraction and enrichment of lead from other sweeteners. For natural, simple sugars such as corn syrup and honey, enrichment of lead by Donnan dialysis is possible with trends very similar to sucrose. Complex sweeteners (artificial syrup and molasses) inhibit the dialysis process as compared to the dialysis of lead in water. It is likely that the artificial syrup and the molasses have other ingredients that effectively compete for lead or alter the sample ionic strength. Therefore, extraction and quantitation of lead in these

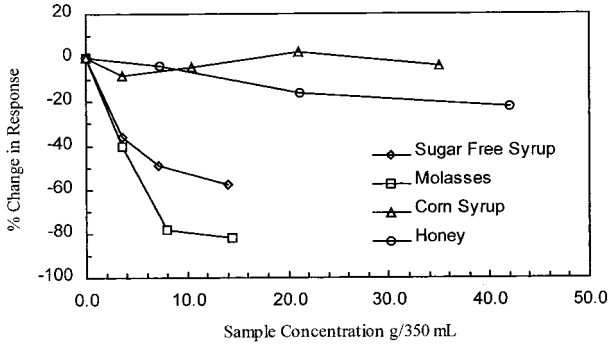

Figure 2. Impact of other sweeteners on the response for lead. The dialysis conditions were the same as those for the sucrose study (Figure 1).

Table 2. Spike and Recovery Experiments for Sucrose and Other Sweeteners[a]

sample type	spike (μg of Pb/g)	recovery %	precision (% RSD)	*N*
sucrose	1.27	99	14	3
	4.25	90	4.8	4
	8.50	93	6.8	4
corn syrup	4.25	102	5.4	5
	8.50	94	11	4
sugar	4.25	105	3.9	5
honey	4.25	89	5.2	4

[a] The dialysis conditions were the same as those identified in Figure 1 for sucrose. *N* represents the number of replicates performed.

samples would require the method of standard addition. Nonetheless, the direct Donnan dialysis approach appears effective for the extraction of lead from the simple sweeteners.

Table 2 summarizes spike and recovery experiments for various concentrations of lead in different sweeteners. Recoveries ranged from 89 to 105%, and reproducibility ranged from 3.9 to 14% RSD, which is acceptable in terms of method performance. None of the unspiked samples tested above the calibration blank. For these experiments, the typical instrument detection limit for the Donnan dialysis of the lead calibration standards was 40 ng/mL, which corresponds to a method detection limit of 350 ng/g (assumes a sample size of 40 g diluted to 350 mL). Therefore, the Donnan dialysis approach is acceptable for the extraction of lead, but detection by FAAS provides only marginal performance as compared to previous reports using the method of standard addition with voltammetric detection or sample digestion with atomic spectrometric detection (*6*−*11*).

Improvements in detection with FAAS could possibly be achieved by extending the analysis time or increasing the temperature of the dialysis cell. Alternatively, lower limits would also likely be achieved by interfacing the existing Donnan dialysis cell with ICP-AES detection. In the previous paper with ICP-AES, a detection limit of 0.8 ng/mL was reported for the Donnan dialysis of lead standards prepared in water (*20*). Extending to the proposed procedure for sweeteners (40 g of sample dissolved to 350 mL), a method detection limit approaching 10 ng/g would be possible assuming the extraction efficiency from the sweetener matrix does not deviate as the concentration drops below 350 ng of Pb/g sample. Improved performance with ICP-MS is unlikely because of the high dissolved solids content of the dialysate, which would likely cause interference at the sampling/skimmer cone interface.

This paper has demonstrated that Donnan dialysis is an acceptable procedure for the extraction and determination of lead cations in sweeteners. The benefit of Donnan dialysis is that larger sample sizes can be accommodated without a greater expense in reagents or time. The complete sample analysis time is 20 min/replicate (15-min dialysis and 5-min reconditioning of the membrane), which could be further improved by automation incorporating multiple cells. The membrane is very rugged, and replacement has not been required with an operational time in excess of 1 year. The Donnan dialysis procedure is also easy to duplicate, and undergraduate student researchers have achieved reasonable reproducibility with a minimal introduction. In comparison to other techniques, the Donnan process is not excessively costly, and the limits reported here are detector-specific. Future directions include characterization with other sample matricies, alternate cell designs to accommodate larger sample sizes, and coupling with other detectors that offer improved detection limits as compared to FAAS.

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