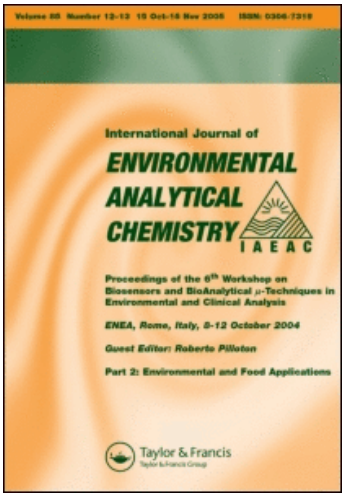

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DETERMINATION OF UREA IN ATMOSPHERIC AEROSOLS AND NATURAL WATERS – A CATION EXCHANGE METHOD

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An ion exchange chromatographic method has been developed for the determination of urea in freshwater, rainwater, and water-extracted aerosol samples. The method provides a fast and reliable way to determine the concentration of urea without the use of harmful chemicals or large amounts of time devoted to sample preparation. The method utilizes a Dionex IonPac CS12 cation exchange column and UV detection at 190 nm. A comparison between the new method described in this manuscript and a previously published method for urea determination in rainwater and water-extracted aerosol samples is also presented. This comparison shows potential interferences from other organic N compounds similar to urea when using the previously published method. Tests utilizing various pH values and the enzyme urease also confirmed the ability of the new method to determine the concentration of urea within atmospheric aerosols and natural waters.

Keywords: Organic nitrogen; Urea; Aerosol; Rainwater

INTRODUCTION

Urea is a known component of aerosols [1] and natural waters [2,3]. Urea has been stated by many authors to be a source of nitrogen (N) for marine phytoplankton [4,5], and has also been found to be preferentially adsorbed by phytoplankton even when inorganic nitrogen species such as ammonium and nitrate are in excess [6]. Our interest in urea methods stems from a lack of specificity in colorimetric and enzymatic urea analysis, and in our desire to determine accurate urea concentrations in atmospheric aerosols and rainwater. Currently the sources for urea in water-extracted atmospheric aerosol samples and rainwater, especially in samples collected in remote oceanic areas, are unknown, and possible interference from unknown atmospheric constituents makes accurate determinations difficult.

It is known that compounds such as allantoin and citrulline interfere strongly in the colorimetric analysis of urea by forming chromophores detected at the wavelength of interest in methods employing diacetylmonoxime [7,8]. Methods employing the

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enzyme urease are also questionable. Price and Harrison [7] noted that the urease method often underestimates concentrations of urea due to enzyme inhibition in seawater samples. Interference with ammonia has also been reported to be a problem associated with the urease method [9]. If these interferences exist in aerosol extracts and rainwater, they will lead to possible over-estimation of urea in cases where diacetylmonoxime is used, and where ammonia is not properly removed prior to the urease reaction. Underestimation will occur in cases where there is enzyme inhibition. In order to relieve the suspicion of inaccurate predictions concerning urea concentrations due to method failure, we explored chromatography as a means of accurately determining the concentration of urea in environmental samples.

Several HPLC methods for determination of urea are available in the literature. In a few of these methods, derivatization of urea by post-column analysis using diacetylmonoxime is undertaken [9]. Such methods are subject to the same problems as manual determinations using diacetylmonoxime unless specific peaks are identified, and co-elution of urea with allantoin and citrulline, or similar compounds, is determined to be inconsequential. Jansen *et al.* [10] introduced an HPLC method utilizing *o*-phthalaldehyde (OPA) reagent and fluorescence detection after conversion of urea into ammonia using an in-line process. This setup should not produce the interference seen with the use of diacetylmonoxime. However, the numbers of steps required for the procedure are very detailed and problems can arise while using the urease reactor and/or while using OPA. The enzymatic activity of urease within reactors is sometimes diminished by the presence of metals in the mechanics of analytical systems (e.g., pumps and tubing) and/or within the samples undergoing analysis. OPA is often used for derivitization of amino acids. Therefore, the removal of amino acids must be accomplished before urea is derivitized. The procedure of Kawase *et al.* [11] uses a strong cation exchanger. In this method a post-column reaction at an elevated temperature is used to convert urea and other compounds such as allantoin and *N*-methylurea to *N*-chloramine derivatives. The methods of Jansen *et al.* [10] and Kawase *et al.* [11] both require in-line sample preparation and special instrumentation. Therefore, we determined that an ion chromatographic method with UV detection would be ideal – a simple procedure without a great deal of sample preparation or system configuration.

Upon examination of the literature, few methods were discovered for urea determination using ion exchange chromatography and UV detection. Koebel and Elsener [12] used an anion exchange column at 190 nm to detect urea, biuret, melamine, cyanuric acid, and sodium isocyanate. Willis *et al.* [13] used an ion-moderated partition column to separate a number of amines as well as urea in marine foods using UV absorbance at 207 nm. The possibility of using a cation exchange column in the UV range for urea determinations came from examination of two papers. Yasuda *et al.* [14] described the use of a Bio Rad Aminex A-7 cation exchange gel, UV detection at 200 nm, a 0.05 M phosphate buffer at pH 3.4, and an elevated temperature of 40°C. Urea, cyanuric acid, biuret, and thymine were quantitated in this method developed for the analysis of urea in urea creams. Rey and Pohl [15] reviewed the ability of a Dionex IonPac CS 12A column, containing a combination of carboxylic and phosphonic acid cation exchangers, for separating several amines and anilines. Many of these determinations were performed using a UV detector. We tested the CS12A and its predecessor the CS 12, containing only carboxylic cation exchangers, for urea separation.

The compounds allantoin and citrulline are known to interfere with colorimetric analysis for urea. Therefore these compounds were chosen along with urea. Allantoin is also structurally similar to urea; so it is an appropriate compound to determine when evaluating a new urea method. The Dionex IonPac CS 12A proved to be incapable of separating allantoin from urea under a number of conditions, including dilution of the lowest absorbing eluent methanesulfonic acid (MSA), and a flow rate decrease. The use of other column-suitable eluents provided too high a background absorbance and were discounted as possible replacements for MSA. The Dionex IonPac CS 12 column was found to be suitable at separating all three compounds and thus a method was developed to incorporate its use. In the method presented, the pH of the eluent, ~2, allows separation of urea by absorption of the amino group (NH_2) at 190 nm.

To test the validity of the new method, a method comparison was conducted between the new ion chromatographic method introduced in this manuscript and a previously published colorimetric method for aerosol extracts and rainwater [1]. Compounds that can potentially interfere with colorimetric measurements (among them allantoin, allantoic acid, adenine, 1-citrulline, cyanuric acid, glycoxylurea, and uracil) as well as several aerosol samples (from sites described in the experimental section) were chosen for the analysis. Aerosol extracts produce very complex matrices, so their use should reflect the ability of both methods to determine accurate concentrations despite large quantities of salts or organic compounds.

EXPERIMENTAL

Chromatographic Apparatus

A Dionex DX 300 (Dionex Corporation, Sunnyvale, California) equipped with a variable wavelength detector, a gradient/isocratic pump, and AI450 software was utilized for analysis. For maximum sensitivity the UV detector was set at 190 nm. The flow rate was set at 0.5 ml/min to achieve maximum separation between the allantoin and the urea peaks. A 150 mm × 4.6 mm Dionex CS12 column and a CG12 guard column provided the means for separation. A 125 µl loop was fitted to a Rheodyne (Cotati, California, USA) model 9126 injection value with a rear loading injector. The eluent consisted of 20 mM methanesulfonic acid (Fluka, Ronkonkoma, New York, USA) delivered isocratically by the pump. This eluent is a standard eluent for the CS12 column and provided lower background absorbance than other column suitable eluents, as stated above.

Chemicals

Urea was purchased from Sigma (St. Louis, Missouri, USA) as a standard solution. Allantoin, and 1-citrulline were purchased from Fluka (Ronkonkoma, New York, USA) as solids. Adenine, allantoic acid, cyanuric acid, glycoxylurea and uracil were purchased as solids from Sigma. The urease used for the urease test and the chemicals used for the colorimetric analysis were purchased from Sigma.

Stock solutions were prepared by dissolving appropriate amounts of solution and solids in purified water (> 17.6 MΩ-cm). It should also be noted that solid urea

proved to be an impure reference for urea concentrations. Standards prepared from a previously purchased crystalline urea led to low recovery for total nitrogen determined by UV photolysis made possible by a Metrohm 705 UV digestor (Metrohm Inc., Switzerland). Therefore caution should be given to the use of "pure" solids for urea standard preparation. Crystalline urea solids can contain chemical impurities or attached water that can diminish urea concentrations. We recommend that liquid urea designated as a "standard" by chemical manufacturers be utilized to prepare working standards. In our laboratory tests these liquid standards provide ~100% recovery for N as urea following UV exposure and inorganic N ion analysis. Similar comparisons using the UV digestor were not made for allantoin and 1-citrulline because they were not found in the samples analyzed.

Sample Collection

Samples utilized in the method development were collected at a variety of sites. Aerosol samples were collected on pre-combusted glass fiber filters (450°C for 4 h in a muffle furnace) for 12 h in Bryan, Texas, in the middle of a cow pasture using a high-volume (60 L of air/min) bulk aerosol sampler. Surface fresh water samples were collected from ponds in Central Park and Gabbord Park located in College Station, Texas, and from a small yard pond in Bryan, Texas. They were stored in pre-cleaned acid washed containers at 4°C until analysis. A rain sample was collected in Bryan, Texas, using a pre-rinsed bucket left outside overnight during a rainstorm. The rain sample was also stored at 4°C until analysis.

Aerosol samples used in the method comparison were collected from a variety of sites worldwide (including biomass burning samples from Brazil, desert dust-influenced samples from Turkey, clean aerosol samples collected at a remote marine site in Australia, and cattle feedlot samples from New Mexico, USA). This suite represents highly diverse and complex samples from both anthropogenically and biogenically affected areas. Full sets of data associated with the sampling sites mentioned above are currently in preparation and will be discussed here only in the context of method comparison (aerosol sample units are therefore given only in units of concentration in liquid and not in air and are distinct from the aerosol data presented from method development samples).

Sample Pre-treatment

For aerosol filter extraction, 1/4 of a filter was placed in a 50 ml centrifuge tube and extracted with 30 ml of purified water. After a 30 min sonication the extract was filtered through a 0.4 μm Nuclepore polycarbonate filter (Whatman Inc., purchased from VWR Scientific Products, USA), and injected without further dilution. All liquid samples were also filtered through 0.4 μm Nuclepore polycarbonate filters and injected without further dilution.

RESULTS

The top panel in Fig. 1 shows typical results for an injected standard. Using a 125 μl loop, the limit of detection for urea was found to be approximately 0.01 mg/L as

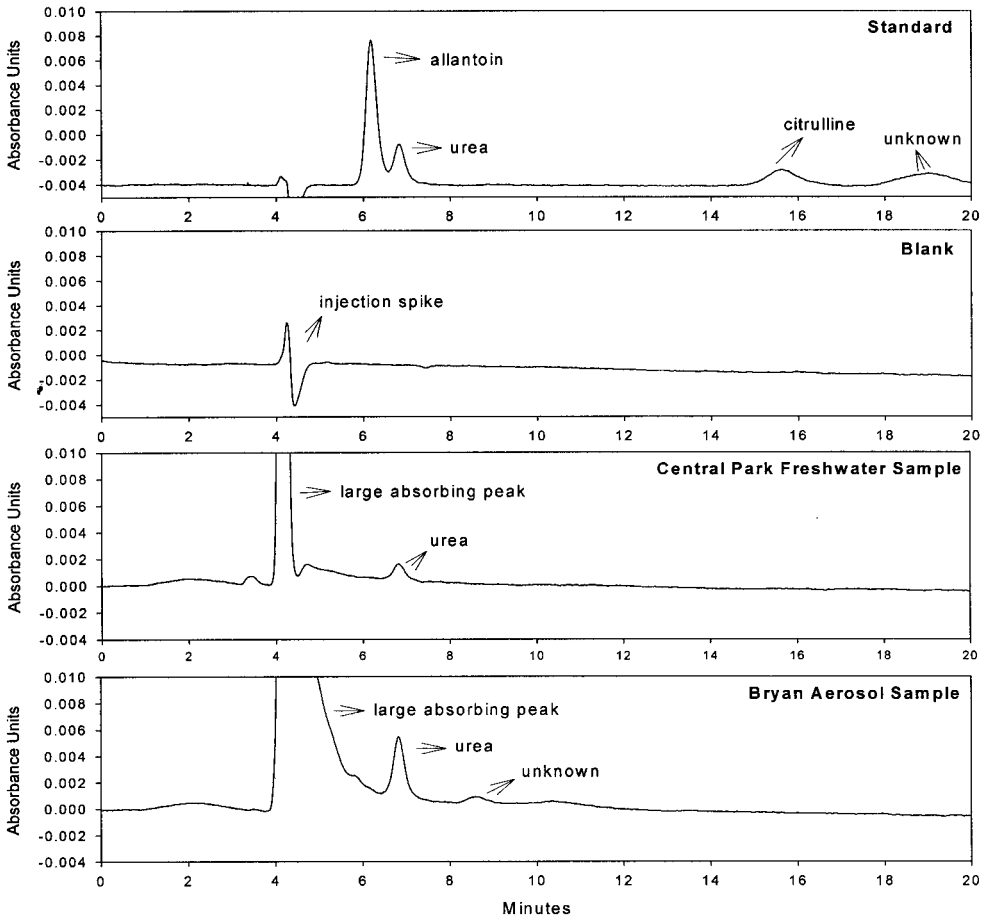

FIGURE 1 Representative chromatograms of a standard, blank, freshwater sample, and aerosol extract.

urea (or 0.3 μM N (micromoles of nitrogen per liter)). This detection limit is similar to those claimed for colorimetric methods. Urea concentrations for samples described in the lower panels of Fig. 1, as well as concentrations for samples not shown in the figures and used for the method development, are presented in Table I. In the second panel of Fig. 1 an aerosol filter blank, extracted and filtered as described in the experimental section, is shown. Chromatograms of filtered aerosol blanks sometimes show a large absorbing peak prior to 6 min, possibly attributed to salts or other compounds within the aerosol filter matrix even after pre-combustion to destroy organics, while filtered purified water samples show only an injection spike (like the one shown for this filter sample). In the lower panels of Fig. 1, an example of a freshwater pond sample and an aerosol sample injection are shown. There are strong absorbing peaks occurring in these chromatograms in the range between 4 and 6 min, yet the urea peak is clearly visible at 6.8 min. The injection of inorganic ion standards containing ammonium, nitrite, nitrate, potassium, calcium, sulfate, and chloride also produced large absorbing peaks at approximately 4 min. We attribute these large absorbance peaks to other ions and organics present in the samples.

TABLE I Concentrations of urea in analyzed samples

Test sample name	*Urea concentration*	*Urea as nitrogen (N) concentration*	*Nitrogen molar concentrations*
Aerosol sample #1 Bryan, TX	5.64 μg urea/m^3	2.63 μg N/m^3	188 nmol N/m^3
Aerosol sample #2 Bryan, TX	4.29 μg urea/m^3	2.00 μg N/m^3	143 nmol N/m^3
Aerosol sample #3 Bryan, TX	5.37 μg urea/m^3	2.50 μg N/m^3	179 nmol N/m^3
Rainwater Bryan, TX	40 μg urea/L	19 μg N/L	1.4 μmol N/L
Central Park Pond #1 College Station, TX	162 μg urea/L	75.6 μg N/L	5.40 μmol N/L
Central Park Pond #2 College Station, TX	132 μg urea/L	61.6 μg N/L	4.4 μmol N/L
Gabbord Park Pond College Station, TX	30 μg urea/L	14 μg N/L	1 μmol N/L
Yard Pond Bryan, TX	0 μg urea/L	0 μg N/L	0 μmol N/L

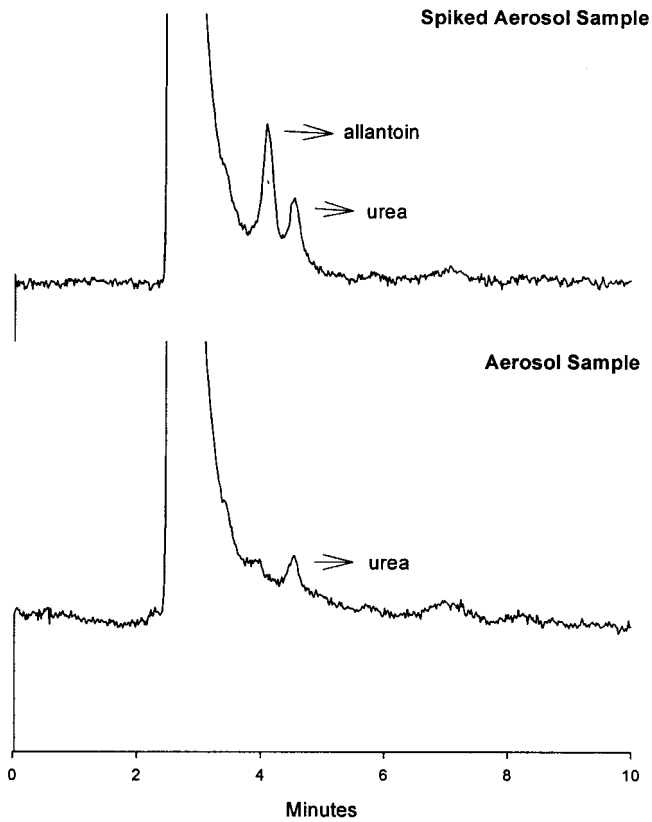

FIGURE 2 An example of a sample spiked with an allantoin and urea combined standard showing the same retention time for the urea peak.

Using internal standards in similar aerosol samples, as shown in Fig. 2, it was determined that integration should start and stop at the beginning and the ending, respectively, of the peak instead of allowing integration to return to the baseline. In Fig. 2, the peak area calculated for urea in the non-spiked sample was equivalent to the

peak area in the spiked sample minus the contribution of the urea standard. There will likely be differences in chromatography depending on the samples analyzed, and we recommend that internal standards be used at first to determine interferences from the baseline for aerosol samples. The use of internal standards has been used in the past for calibrating colorimetric urea analysis methods in complex matrices [16], and we recommend this practice. In samples containing no allantoin, standards containing only urea were used to determine concentrations based on a 9 point calibration curve (Slope = 12590, Intercept = 510.7, $R^2 = 0.999$). The percent relative uncertainty per injection, as calculated from 3 replicate injections, was determined to be 1.2%.

To determine the influence of pH on chromatographic separation, aliquots of a urea standard were adjusted with either a 1.0 M solution of hydrochloric acid or a 1.0 M solution of sodium hydroxide in a pH range from 4 to 8, filtered with a 0.45 μm Nuclepore filter, and injected onto the column without further treatment. The pH range chosen is representative for aerosol extracts, rainwater, and freshwater samples. Aerosol extracts are generally acidic due to the presence of sulfuric and nitric acid in aerosol particles [16]. Due to the dissolution of these acids in water, rainwater is generally even more acidic than the expected equilibrium pH of 5.6, the pH of water in equilibrium with atmospheric carbon dioxide [16]. Carbon dioxide buffering in water creates typical pH values in freshwater in the range of 7–8. Figure 3 shows a comparison of three pH-adjusted standards. After dilution corrections of the pH-modified standards, the comparison showed no variation in peak retention or peak area due to varying pH.

Comparison between the new ion chromatographic method and a previously published colorimetric method for aerosol extracts and rainwater [1] further showed the validity of the new method. Standards containing adenine, allantoin, allantoic acid, 1-citrulline, cyanuric acid, glycoxylurea, and uracil were tested using both methods. In Table II concentrations determined as urea N in prepared solutions (determined by each method for an identical sample) are presented. Concentrations are reported as μM N (micromoles of N as urea per liter—determined by the individual methods as urea) to emphasize the similarities between values, and because μM N is a common unit used by biogeochemists concerned with environmental budgets of N.

As seen in Table II, the colorimetric method [17] (performed as described by Cornell *et al*. [1] often estimates similar N-containing compounds as urea. Compounds such as allantoic acid, glycoxylurea, and 1-citrulline cause strong interference by their detection as urea. This is not the case for the chromatographic method. However, careful attention should be given to interpretation of peaks because two of the compounds tested, cyanuric acid and allantoic acid, produced a peak which interfered with the allantoin peak. Other compounds eluted earlier or later than the urea peak or were undetected. Therefore, urea can be detected by the chromatographic method without the interference of similar compounds. Since we are currently concerned with the presence and accurate quantitation of urea, further investigation involving elution of other compounds and incorporation into our standards for determination on a regular basis has not been undertaken.

In Table III, data from the aerosol extract method comparison are presented. As seen in Table III the colorimetric method often determines urea at higher concentrations than the chromatographic method. Higher concentrations are likely due to the absorbance of similar N species within samples, as described above. Since the individual organic N species comprising the water-soluble proportion of bulk organic N

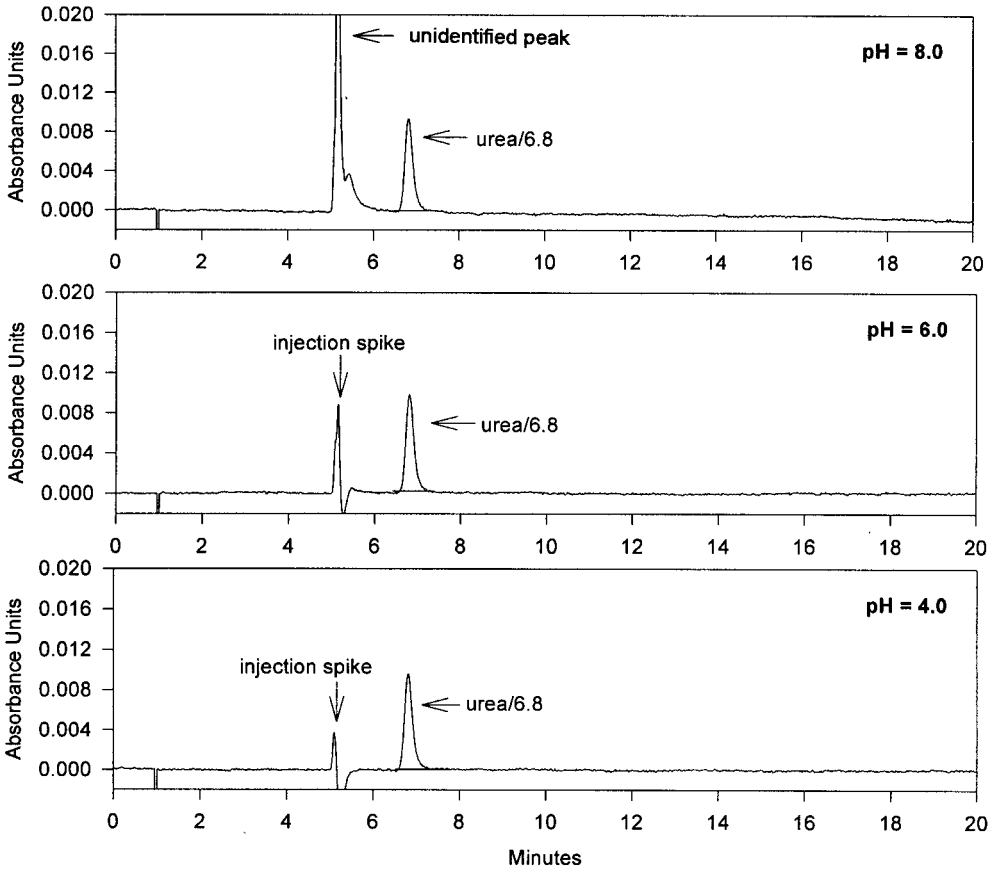

FIGURE 3 Comparison of standards showing the same retention for urea at various pH values.

within aerosols and rainwater are mostly unknown, we cannot be absolutely sure that such compounds exist within samples. However, because compounds such as allantoin and allantoic acid are a part of the biochemical degradation of purines [18], it is likely that they exist in complex matrices. A further addition of uncertainty in the colorimetric method is the analytical error associated with multiple pipeting steps (see Cornell *et al.* [1]. Also, there is uncertainty associated with the interference produced from carbon compounds such as phenols and aldehydes. Zuoguo *et al.* [19] reported that the presence of aldehydes and phenols within complex samples reduces the ability of the diacetylmonoxime colorimetric method (see Cornell *et al.* [1] to accurately determine urea due to the ability of these compounds to interrupt the chemical reaction of diacetylmonoxime (the reaction of diacetylmonoxime results in the chromophore absorbed for urea determinations). In Table III, similar reductions in absorption for the colorimetric method are seen in Brazil aerosol sample 6 that was collected under high biomass burning conditions and that contains a high proportion of poly-aromatic hydrocarbons (PAH's) and other organic compounds. When the uncertainties associated with analytical steps, the absorption of urea-like compounds, and the interferences with carbon compounds are considered, we have found that the chromatographic method is more likely to provide "actual" rather than "perceived"

TABLE II Results from the method comparison reported as μM N (micromoles of nitrogen (N) per liter) determined from colorimetric and ion chromatographic standard curves

Sample	μM *N as urea – determined by the colorimetric method*	μM *N as urea – determined by the ion chromatographic method*
0.5 μM N as adenine	0	0
5 μM N as adenine	0.8	0
20 μM N as adenine	1.2	0
0.5 μM N as allantoin	0	0
5 μM N as allantoin	0	0
20 μM N as allantoin	0	0
0.5 μM N as allantoic acid	0	0
5 μM N as allantoic acid	5.0	0
20 μM N as allantoic acid	15	0
0.5 μMN as 1-citrulline	0	0
5 μM N as 1-citrulline	1.4	0
20 μM N as 1-citrulline	4.2	0
0.5 μM N as glycoxylurea	0	0
5 μM N as glycoxylurea	3.0	0
20 μM N as glycoxylurea	15	0
0.5 μM N cyanuric acid	0	0
5 μM N cyanuric acid	0	0
20 μM N cyanuric acid	0	0
0.5 μM N uracil	0	0
5 μM N uracil	0	0
20 μM N uracil	0.2	0

TABLE III Method comparison of aerosol extracts reported as μM N (micromoles of nitrogen (N) per liter)

Sample	μM *N as urea – determined by the colorimetric method*	μM *N as urea – determined by the ion chromatographic method*
Australia aerosol 1	1.8	0
Australia aerosol 2	1.6	0
Australia aerosol 3	1.7	0
Australia aerosol 4	4.6	0
Australia aerosol 5	2.5	0
Australia aerosol 6	0.7	0
Australia aerosol 7	3.4	0
Australia aerosol 8	1.2	0
Brazil aerosol 1	0.6	0
Brazil aerosol 2	5.5	0
Brazil aerosol 3	1.5	0
Brazil aerosol 4	0.5	0.2
Brazil aerosol 5	0.4	0
Brazil aerosol 6	0.2	0.7
Brazil aerosol 7	1.9	0
Turkey aerosol 1	2.2	0
Turkey aerosol 2	8.8	0
Turkey aerosol 3	3.2	0
Feedlot aerosol 1	25.4	10.2
Feedlot aerosol 2	12.3	10.7

concentrations of urea within complex matrices than the colorimetric method using diacetylmonoxime.

To further constrain the ability of the new method to determine urea within complex matrices a test utilizing the enzyme urease that catalyzes the conversion of urea to ammonia (NH_3) was also conducted. Due to enzymatic conversion of urea to NH_3, samples treated with urease should yield samples free of urea. For this analysis, immobilized urease (purchased from Sigma) was added to filtered samples as well as to urea standards. In Fig. 4 an example of the results from this analysis are presented. As seen in Fig. 4, the urease addition produced its own absorbing peak at the outset of the injection as well as other small peaks following the urea peak (peaks due to chemicals added to the urease solution to preserve the enzyme).

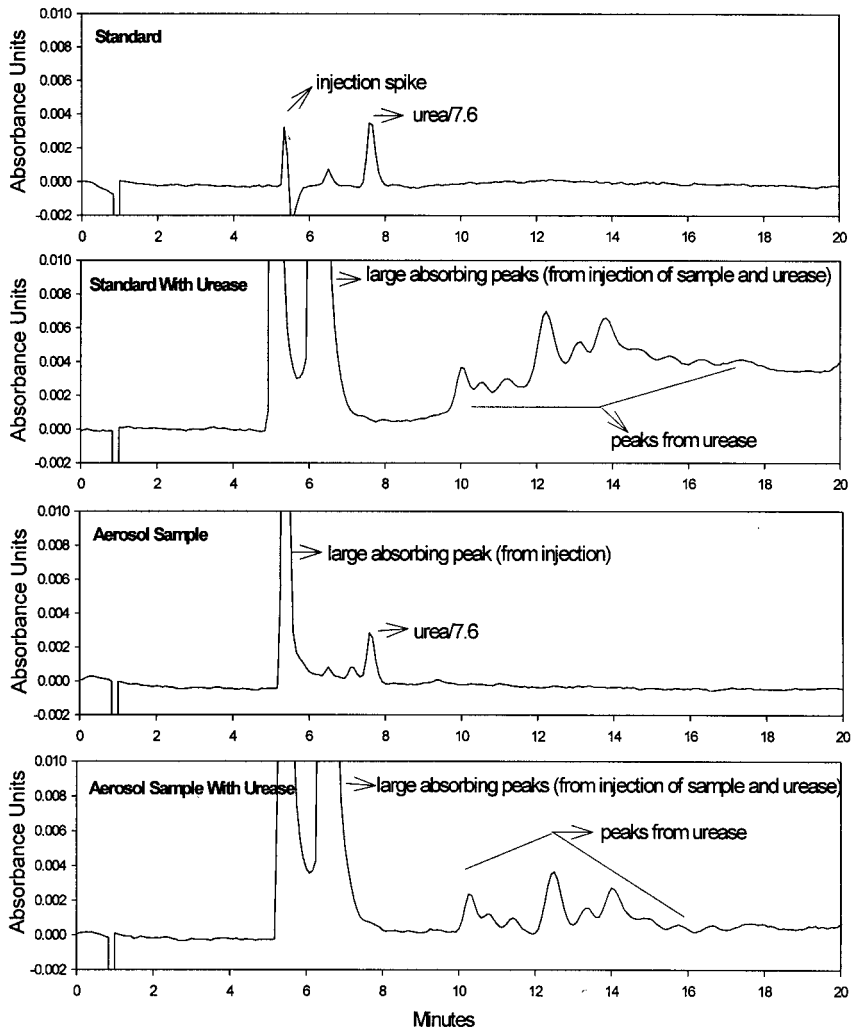

FIGURE 4 Chromatographs of a standard and an aerosol extract before urease treatment and after urease treatment.

To ensure the proper separation of urea under urease conditions, the eluent was diluted 1 : 1 with purified water and the flow was slowed to 0.3 ml/min. Figure 4 also shows the removal of urea in both standards containing urea and in an aerosol sample obtained from a cattle feedlot in New Mexico, USA, when samples are treated with urease.

CONCLUSION

Urea is a known component of natural waters [1–3], and an important nutrient for phytoplankton [4,5]. Due to the complex nature of aerobic degradation of purines, as outlined by Vogels and van der Drift [18], occurring in all animals, plants, and most microorganisms, compounds such as urea are likely to leach into the environment whether land, ocean, or air. Therefore, reliable methods for the accurate determination of urea are critical.

To combat the analytical problems described earlier, we have developed a method using a Dionex IonPac CS12 cation exchange column and UV detection at 190 nm. The described method is simple and easy to maintain. The eluent, 20 mM MSA, is a standard eluent for the column and is delivered isocratically by the pump. With a 125 µl injection volume, the limit of detection for urea is equivalent to that for manual methods employing the use of diacetylmonoxime. The IonPac CS12 column permits the detection of urea in freshwater, rainwater, and water-extracted aerosol samples without the interference of compounds similar to urea that occur when using colorimetric techniques. Tests conducted with varying pH values and with the addition of the enzyme urease show the ability of the method to determine urea within complex matrices. The method presented may also be useful to scientists interested in wastewater or other industrial processes.

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