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USE OF A POST-COLUMN REACTION AND A SPECTROPHOTOMETRIC DETECTOR FOR THE LIQUID CHROMATOGRAPHIC DETERMINATION OF WATER*

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SUMMARY

A sensitive method for the determination of water in the presence of common interferents is presented. An ion-exchange column in the Li^+ form and acetonitrile–methanol (60:40) as eluent are used to separate water from other sample components. The detection system is based on the effect of water on the equilibrium which results from the reaction of cinnamaldehyde (added to the eluent) and methanol in the eluent to form cinnamaldehyde dimethylacetal plus water. This equilibrium is shifted in the catalytic atmosphere of an H^+-form post-column reactor. The extent of the shift and the resulting change in absorbance at 310 nm are proportional to the amount of water present. The method is rapid, sensitive, relatively free from interferences and gives a linear calibration graph over approximately three orders of magnitude difference in water concentration.

INTRODUCTION

The determination of water in organic and inorganic materials is one of the most important and frequently encountered analytical problems. The Karl Fischer method has long been the most widely used method for the determination of water. However, it requires some skill to carry out and cannot be used for samples that contain oxidizing or reducing substances, or certain other chemicals. A number of gas chromatographic (GC) methods have been proposed for the determination of water[1]. The actual method chosen is dependent on the sample matrix. GC method are slow for samples containing late-eluting compounds. Decomposition of samples leading to contamination of the column can also be a problem. GC methods often cannot be used at all for samples containing non-volatile constituents.

The determination of water can be based on the reaction of phenyl isocyanate with water to produce N,N'-diphenylurea, which can then be determined by liquid chromatography[2]. However, the total reaction time prior to the chromatography is 45 min.

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* A U.S.A. Patent Application has been filed covering the work presented in this study.

Ion-exclusion chromatography is a fast and efficient way to separate and determine compounds such as carboxylic acids, carbon dioxide (as carbonic acid)[3] and neutral substances such as alcohols and sugars[4]. The determination of water by ion-exclusion chromatography should also be possible provided a suitable detection method is available. Stevens *et al.*[5] recently published a chromatographic method for water using a hydrogen-form cation-exchange column in conjunction with a methanol eluent containing a low concentration of sulfuric acid. A conductivity detector was employed, giving a decreased conductivity for the water peak. Their data indicated a response factor that varied widely with changing water concentration. The average response factor (μs per 1% water) was 240 in the 0.02–0.12% water range, 105 in the 0.22–0.42% range, 50 in the 0.42–0.80% range and 6.7 in the 1.6–3.1% range.

A method for the chromatographic determination of water is presented here that combines separation by ion-exclusion chromatography with a novel and sensitive method of detection. The eluent is a low concentration of cinnamaldehyde in methanol or in a mixture of methanol and acetonitrile. In one mode of operation, the separation column contains a cation-exchange resin in the H^+ form which causes the cinnamaldehyde and methanol to react to produce water plus an acetal that has a much lower absorbance at an appropriate wavelength than the free cinnamadehyde. In another mode of operation the chromatograpic separation of water takes place on a cation-exchange column in the Li^+ form. An H^+-form catalytic column placed just after the separation column then catalyses the reaction of cinnamaldehyde with methanol. In both modes, water in the sample partially reverses this reaction, giving an increase in absorbance for detection of the water peak. The method is fast and sensitive; it is highly selective for water and has a large, linear dynamic range.

EXPERIMENTAL

Apparatus

The instrument consisted of a Gilson Model 302 single-piston pump, a Rheodyne Model 7125 injector quipped with either a 20-μl or a 100-μl sample loop, a Scientific Systems Model LP-21 Lo-Pulse pulse damper, either a glass 10 cm × 8 mm I.D. or a stainless-steel 5 cm × 4.6 mm I.D. column packed with Bio-Rad Aminex Q-150S in the Li^+ form (separation column), a 10 cm × 2 mm I.D. stainless-steel column packed with Bio-Rad Aminex Q-150S in the H^+ form (catalyst column), a Kratos Spectroflow 783 absorbance detector and a Curken strip-chart recorder. The one-column method used either a stainless-steel 10 cm × 4.6 m I.D. or a glass 10 cm × 8 mm I.D. column packed with Aminex Q-150S in the H^+ form. The columns were packed using upward slurry packing. However, a balanced density method was not used. Owing to the large degree of shrinking and swelling that occurs in polystyrene–divinylbenzene resins when a change in solvent occurs, it was necessary to pack the column in the solvent used in the mobile phase.

Eluent and sample solution

trans-Cinnamaldehyde, 99% (Aldrich Chemical), was used without purification. Analytical-reagent grade methanol (Mallinckrodt) and HPLC-grade acetonitrile (Fisher Scientific) were dried by storing over activated 3 Å molecular sieves (Aldrich) for at least 1 week. 3-Mercaptopropionic acid (Aldrich) was 99 + % pure. All other samples were of analytical-reagent grade and used without purification.

For maximum sensitivity and reproducibility, the eluent and all samples were prepared in a nitrogen-filled glove-bag. Once prepared, the eluent was protected from atmospheric moisture by bubbling nitrogen through the solution using a drying tube filled with anhydrous calcium sulfate (Drierite). All sample solutions were placed in vials equipped with Mininert valves (Supelco) prior to removal from the glove-bag. The valve and septum of the Mininert caps allowed the removal of an aliquot without exposing the remainder of the sample to atmospheric moisture.

Titrations

Karl Fischer reagent (titer 2.8 mg/ml) was purchased from Aldrican and standardized. The buret was flushed with nitrogen prior to being filled and then blanketed with nitrogen during the titration. A large (50-ml) buret was used so that a standard and three samples could be titrated without refilling the buret. Samples were titrated in volumetric flasks blanketed with nitrogen to minimize exposure to atmospheric moisture. A visual end-point was used.

Chromatographic conditions

For the two-column method, chromatography was performed at flow-rate of either 1 ml/min (with the long separation column) or 0.8 ml/min (with the short separation column). A detection wavelength of 310 nm was used. The eluent was 0.79 m*M* *trans*-cinnamaldehyde in acetonitrile–methanol (60:40). This concentration of *trans*-cinnamaldehyde gave the best signal-to-noise ratio for water determination.

When only one column in the H^+ form was used, the eluent was 0.32 m*M* *trans*-cinnamaldehyde in methanol. With the 4.6 mm I.D. column a flow rate of 1 ml/min was used and with the 8 mm I.D. column a flow-rate of 1.2 ml/min was used. Detection was at 310 nm.

RESULTS AND DISCUSSION

Detection system

A column packed with a cation-exchange resin in the H^+ form has been shown to separate water chromatographically using dilute sulfuric acid in methanol as the eluent[5]. The separation is probably based on an ion-exclusion mechanism in which ions and most organic sample components pass rapidly through the column, but water can enter the resin beads and therefore is eluted later as a well resolved peak.

A stable and sensitive detection system is critical to the success of any chromatographic method for the separation and determination of water. Our method is based on the shift in chemical equilibrium caused by low concentrations of water. This shift in equilibrium causes an increase in absorbance that is proportional to the amount of water.

A detection system is set up by adding a low concentration (0.8 m*M*) of *trans*-cinnamaldehyde to the eluent. Cinnamaldehyde can react with methanol in the eluent to form an acetal plus water. However, spectral evidence indicates that reaction only occurs when a catalyst is present, such as when the eluent passes through a cation-exchange column in the H^+ form:

$$C_6H_5CH=CHCHO + 2\ CH_3OH \xrightarrow{H+} C_6H_5CH=CHCH(OCH_3)_2 + H_2O \quad (1)$$
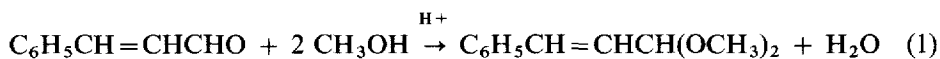
The cinnamaldehyde absorbs strongly at the detection wavelength of 310 nm, while the acetal of cinnamaldehyde shows little absorbance at this wavelength (see Fig 1). When a solution of cinnamaldehyde in methanol (with no catalyst present) was allowed to stand for 2 days, the absorbance at 310 nm remained unchanged. Although the spectrophotometric data presented in Fig. 1 indicate that a wavelength lower than 310 nm should be used for maximum sensitivity, the optimal signal-to-noise ratio was obtained at 310 nm, where the background absorbance is low.

Water in a sample injected into the chromatographic column forces the equilibrium in the reverse direction when an acid catalyst is present:

$$H_2O + C_6H_5CH=CHCH(OCH_3)_2 \xrightarrow{H+} C_6H_5CH=CHCHO + 2 CH_3OH \quad (2)$$
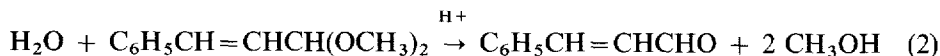
Usually the amount of water from the sample will be substantially greater than the background water. The shift in equilibrium is measured by the increased absorbance at 310 nm, which is proportional to the amount of water in the sample.

Separation–detection systems

One-column method. In this method a single column is used that contains Aminex Q-150S in the H^+ form. In the presence of the H^+-form resin in the column, water in the sample reacts with the cinnamaldehyde dimetylacetal, pushing the equilibrium in eqn. 2 further to the right. As cinnamaldehyde absorbs more strongly than the acetal at the detection wavelength used, the chromatogram shows a positive peak that is proportional to the amount of water in the sample. This peak appears a short time after the initial injection peak on the chromatogram and is well resolved from the injection peak.

The reason why the water peak has a longer retention time than the injection peak is not entirely clear. Perhaps the reaction of water with cinnamaldehyde dimethylacetal is not instantaneous in the column and water therefore moves at
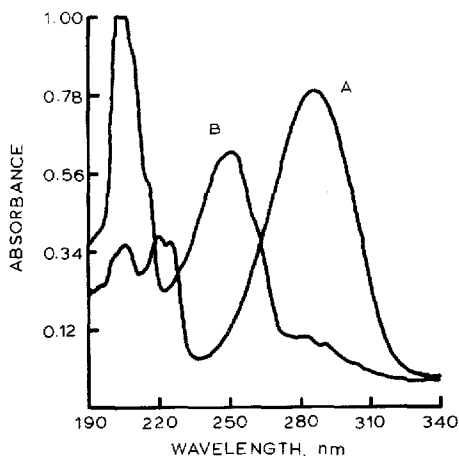

Fig. 1. Spectra of 0.0318 mM *trans*-cinnamaldehyde in methanol. (A) Spectrum immediately after the solution was prepared; (B) spectrum after the solution had been shaken with Aminex Q-150S in the H^+ form.

a slower rate through the column until reaction 2 is complete. Also, other retention experiments indicate that cinnamaldehyde moves at a slightly slower rate through the column than the acetal.

Water can be determined with excellent sensitivity using the one-column method of separation with spectrophotometric detection at 310 nm. An injection of a 20-μl sample of methanol containing only 10 ppm of water yielded an easily measured peak. A calibration graph of standard solutions of water in methanol was linear from 0.0051 to 2.40% water (correlation coefficient for linear regression 0.9995).

Many types of organic sample components either do not absorb at the detection wavelength (310 nm) or else are sufficiently well separated from water not to interfere. However, aldehydes and ketones are likely to interfere by virtue of their retention on the column (see Table I) or by reacting with methanol to produce additional water.

Two-column method. Interference from aldehydes and ketones, and possibly from other types of sample components, can be avoided by using a cation-exchange column in the Li$^+$ form for the chromatograhic separation of water, followed by a short cation-exchange column in the H$^+$ form to catalyze the reaction needed for detection. No reaction occurs in the first column (Li$^+$ form); the water itself is separated from organic and inorganic components in the sample. When the eluent enters the second, catalytic column, the reaction in eqn. 1 takes place and gives a low background absorbance. However, when the water peak enters the catalytic column, the equilibrium is shifted to the formation of more aldehyde in proportion to the amount of water injected.

The system described here is an example of a post-column reaction system which uses a solid-phase reactor. The set-up is simple and works very well. The reactants are already present in the mobile phase. The reaction simply does not occur until the catalyst column is reached. No additional reagents are mixed with the effluent stream. There is no need for the additional hardware (second pump, mixing tee or reaction chamber) commonly used in post-column reaction systems. Consequently, the problems inherent in a typical post-column reaction system are avoided. These include mixing problems, excess dead volume in the tee and reaction coil and baseline noise due to the reagent pump.

This method for the determination of water is so powerful because it combines a selective detection method with the selectivity of chromatography. Because the reaction occurs after the separation, water can be determined in the presence of substances which would interfere if the separation column were not employed, *i.e.*, aldehydes and ketone, which react with methanol in the presence of an acid catalyst to form water. This was tested by determining water in acetone (Fig. 2). Acetone would be expected to react with methanol to form water (plus a ketal) when it entered a catalytic column. However, it is already separated chromatographically from the water in the sample before it reaches the second, catalytic column and no interference is encountered.

The same reasoning holds for sample components that absorb at the detection wavelength. They do not interfere with the determination of water provided they are separated chromatographically before entering the catalytic detection system. Cinnamaldehyde works better than many aldehydes in the detection system because the wavelength of 310 nm is above the UV cut-off for many organic solvents.

Although good results were obtained with the one-column method for water
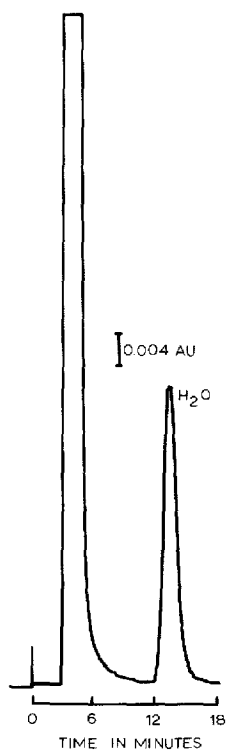
Fig. 2. Spectrum of 0.321% water in acetone. Sample loop, 20 μl; separation column, 10 cm × 8 mm I.D.; flow-rate, 1 ml/min. Other conditions are given in the text.

determination, fewer interferences were encountered with the two-column method. The remaining discussion therefore focuses on the latter method for water determination.

Column length

Many of the separations were performed on a fairly long column (10 cm × 8 mm I.D. with a 10 cm × 2 mm I.D. catalyst column) in order to obtain good resolution of the water peak in some difficult samples. The separation of water from acetone shown in Fig. 2 and the separation of water from a sample of 3-mercaptopropionic acid in Fig. 3 are examples.

In many instances a shorter column can be used and the chromatographic separation of water greatly speeded up. Using a short column (5 cm × 4.6 mm I.D. with a 10 cm × 2 mm I.D. catalyst column), good separations were obtained for 367 ppm of water in isopropyl alcohol (Fig. 4) and for 184 ppm of water in toluene (Fig. 5).

Calibration graphs

Calibration graphs were obtained with both the long and short columns using methanol containing varying amounts of water as standards. For the long column a plot of points ranging from 0.00128 to 3.40% of water had a linear regression correlation coefficient of 0.9998. Slight curvature was observed at the higher
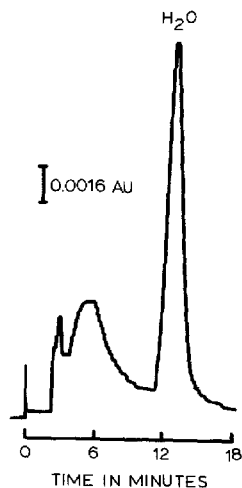
Fig. 3. Spectrum of 0.138% water in a methanolic solution of 1.15 M 3-mercaptopropionic acid. Sample loop, 20 μl; separation column, 10 cm × 8 mm I.D.; flow-rate, 1 ml/min. Other conditions are given in the text.

concentration end of the plot (above 3.0%). The lower end of the plot appeared to be strictly linear with a correlation coefficient of 0.999996 for 0.00128–0.0800% water. For the short column, similar results were obtained for calibration graphs ranging from 0.0064 to 0.50% of water.

The respons factor (RF) of the chromatographic detection system for water was measured in the following units:

$$RF = \frac{\text{signal (absorbance) at 310 nm}}{0.1\% \text{ water in sample}} \qquad (3)$$

Response factors of 0.012 and 0.071 were obtained for the long column with a 20-μl sample loop and the short column with a 100-μl sample loop, respectively. These are
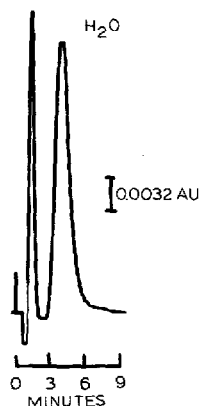

Fig. 4. Spectrum of 367 ppm water in isopropanol. Sample loop, 100 μl; separation column, 5 cm × 4.6 mm I.D.; flow-rate, 0.8 ml/min. Other conditions are given in the text.
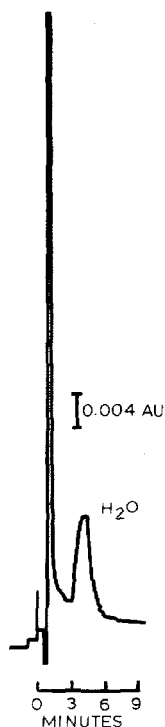
Fig. 5. Spectrum of 184 ppm water in toluene. Sample loop, 100 μl; separation column, 5 cm × 4.6 mm I.D.; flow-rate, 0.8 ml/min. Other conditions are given in the text.

fairly good, considering that the baseline noise with this system was approximately $2 \cdot 10^{-5}$ absorbance.

The limit of detection for water will depend on the response factor, the size of sample loop use and the amount of water in the eluent. The water content of the eluent can be determined by extrapolating a linear plot of peak height *vs.* water concentration in standards to zero peak height. Such an extrapolation of data from the short column gave approximately 30 ppm as the water content of the eluent. Injections of samples containing less water than the eluent give negative peaks at the retention time for water. This effect was previously noted with a different chromatographic system for water[5]. In principle, these negative peaks could be used to determine lower sample concentrations of water than those in the eluent, but we did not obtain a reasonable calibration graph for the negative peaks.

The most critical factor in obtaining extremely low detection limits for water is to prepare and use an eluent of exceptionally low water content. Because of the possibility of obtaining negative peaks when the sample contains less water than the eluent, it is not legitimate to take a positive water peak of substantial height and calculate the limits of detection by dividing the peak height (and concentration) by 2.5 times the noise[5]. By injecting 20-μl samples of decreasing water concentration on to the long column, we were able to obtain a detection limit of approximately 12.5 ppm of water (260 ng absolute) at a signal-to-noise of 3 (see Fig. 6).
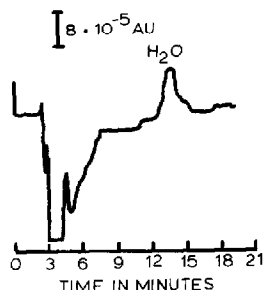
Fig. 6. Spectrum showing the limit of detection obtained with the long separation column (10 cm × 8 mm I.D.) to be 260 ng of water. Sample injected, 12.8 ppm of water in methanol; sample loop, 20 μl; flow-rate, 1 ml/min. Other conditions are given in the text.

Scope and quantitative results

The utility of this method was demonstrated by separating and determining water on the long column in each of the following samples: toluene, ethyl acetate, acetone, 3-mercaptopropionic acid, ascorbic acid (dissolved in methanol) and copper(II) chloride dihydrate (dissolved in methanol). Samples analyzed for water on the short column included isopropyl alcohol, toluene, ethyl acetate and absolute ethanol. Analysis of the mercaptan, ascorbic acid and copper(II) chloride samples by the Karl Fischer method would not be possible. Likewise, aldehydes and ketones interfere in a liquid chromatographic method that uses a different detection system[5].

Results are shown in Tables I and II. The results in Table I, for toluene, ethyl acetate and acetene, were compared with those obtained by the Karl Fischer method. Water was also determined in an ethyl acetate sample which was spiked with 0.100% water. The recovery was excellent. Table II shows results for samples for which a Karl Fischer titration would have yielded erroneous results[6]. The water content of these samples was determined by analyzing a blank, then spiking the sample and re-analyzing. With 3-mercaptopropionic acid, owing to the small amount of mercaptan available, a 10.00% solution of the mercaptan in methanol was spiked. The recoveries were again very good.

Another application of this method is the determination of water of hydration in inorganic salts. A sample of copper(II) chloride dihydrate was analyzed and found to contain 1.99 mol of water per mole of copper(II) chloride. This determination could not be effected with a Karl Fischer titration as the copper(II) would be reduced to copper(I) by the reagent[6].

TABLE I

LIQUID CHROMATOGRAPHIC DETERMINATION OF WATER IN ORGANIC SOLVENTS

Sample	Water added (%)	Water found (%)	
		This method	Karl Fischer method
Toluene	0	0.0253	0.0251
Acetone	0	0.321	0.325
Ethyl acetate	0	0.0950	—
Ethyl acetate	0.100	0.196	0.198

TABLE II

LIQUID CHROMATOGRAPHIC DETERMINATION OF WATER IN THE PRESENCE OF COMMON INTERFERENTS

Sample	Water added (%)	Water calculated (spike plus blank) (%)	Water found (%)
3-Mercaptopropionic acid	0	N.A.*	0.494
Methanolic solution of 10.00% 3-mercaptopropionic acid	0.200	0.249	0.246
Methanolic solution of 0.057 M ascorbic acid	0	N.A.	0.0080
Methanolic solution of 0.057 M ascorbic acid	0.100	0.108	0.104

* N.A. = Not available.

Interferences

Of the organic samples tested, only dimethyl sulfoxide (DMSO) was found to interfere. DMSO produced a very large peak that obscured the water peak. Inorganic metal hydroxides were also found to interfere, possibly by reaction with H^+ to produce additional water.

Samples containing large amounts of ionic materials can pose a problem by displacing more and more Li^+ from the separation column. The Li^+ removed from the separator column will exchange with the H^+ of the catalyst column, decreasing its ability to catalyze the reaction. Likewise, a large number of acidic samples will convert much of the separation column to the H^+ form and thereby cause a change in the retention time of the water peak. Periodic regeneration or the use of a replaceable Li^+-form precolumn should alleviate these difficulties.

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