CHROM. 21 990

CHROMATOGRAPHIC DETERMINATION OF WATER USING SPECTRO-PHOTOMETRIC DETECTION

JIAN CHEN and JAMES S. FRITZ*

Ames Laboratory and Department of Chemistry, Iowa State University, Ames, IA 50011 (U.S.A.)

SUMMARY

Water in various analytical samples is determined by ion-exclusion chromatography with spectrophotometric detection based on a shift in the equilibrium between cinnamaldehyde and cinnamaldehyde dimethylacetal in methanol. The shift in equilibrium is proportional to the amount of water present and occurs only in the presence of an acid catalyst. The mechanism of this unique detection system is studied in detail. The conditions for chromatographic separation and detection are optimized so that a determination of water can be completed in only 1 to 2 min. Water has been determined in a variety of samples to demonstrate the versatility of this new method.

INTRODUCTION

Although a number of approaches have been used for the determination of water in various analytical samples, the Karl Fisher titration method continues to dominate the field[1]. Even with improvements in reagents and instrumentation, this method requires a relatively large sample and is subject to a number of serious interferences. Stevens *et al.*[2] recently published a liquid chromatographic method for determination of water using a methanol eluent and a conductivity detector. Their method is fast and convenient, but the sensitivity varies widely in different ranges of water concentration. Fortier and Fritz[3] proposed a new spectrophotometric detection system for water separated by liquid chromatography. This is based on the effect of water on the equilibrium between cinnamaldehyde and cinnamaldehyde dimethylacetal in the methanol–acetonitrile eluent. Their system employed a cation-exchange column in the Li$^+$ form for separation, followed by a catalytic column containing a cation-exchange resin in the H$^+$ form.

In the present study the method of Fortier and Fritz[3] has been improved so that only a single chromatographic column is needed and a much shorter retention of water is possible. The mechanism of the detection system is now explained in detail, and the factors affecting the initial "injection" peak are elucidated.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Milton Roy Model 396/2396 mini

pump, a Model 7010 Rheodyne injector equipped with sample loops sized from 5 to 100 μl depending on the sample water content, a Model LP-21 Scientific Systems Lo-pulse pulse-dampener, a stainless-steel 15 cm $\times$ 2.1 mm column packed with Bio-Rad Aminex Q-150S resin in the H$^+$ form, a Kratos Spectroflow 783 absorbance detector, and a Curken strip-chart recorder. The Aminex Q-150S column was packed on a Shandon single-piston packing pump, using upward slurry packing method. The packing solvent was reagent-grade methanol. A Hamilton PRP-X300 ion-exclusion column (15 cm $\times$ 4.6 mm) and a Supelco LC-Diol column (25 cm $\times$ 4.6 mm) were also tested.

Reagents

trans-Cinnamaldehyde, 99% (Aldrich), was used without purification. Reagent-grade methanol and HPLC-grade acetonitrile (both from Fisher Scientific) were dried over activated 3A molecular sieves (Aldrich). The dried methanol was then refluxed over CaH$_2$ for 5 days and distilled before use. All other chemicals were reagent grade and were used without purification. The water standards (1.00 mg H$_2$O/ml and 5.00 mg H$_2$O/ml) and anhydrous methanol (Karl Fisher-grade) were purchased from Fisher Scientific.

Eluent and standard samples

Eluent was prepared simply by dissolving carefully weighed amount of cinnamaldehyde to anhydrous methanol. Standard samples were prepared by adding accurately measured amounts of water to portions of dried methanol or acetonitrile. For maximum sensitivity and reproducibility, the eluent and all standard samples were prepared under the protection of dried nitrogen. Once prepared, the eluent was protected from atmospheric moisture by bubbling the dried nitrogen through the eluent reservoir and out through a drying tube filled with anhydrous calcium sulfate (Drierite). All sample solutions were contained in vials equipped with Mininert valves (Supelco) prior to removal from the glove bag. Water-saturated organic samples were obtained by shaking the organic solvents with water for 24 h.

Chromatographic conditions

A flow-rate of 1 ml/min was employed throughout the entire experimental work. A detection wavelength of 300 nm was used. The eluent was usually 1.0 mM cinnamaldehyde in methanol. For systematic studies, however, 0.5 mM, 2.0 mM and 5.0 mM eluents were also used.

RESULTS AND DISCUSSION

Column

Experiments were performed using methanol containing 1.0 mM cinnamaldehyde as the eluent with various types of cation-exchange columns. The water peak was detected spectrophotometrically at 300 nm. A single column filled with cation-exchange resin was found to be satisfactory for most samples. It is not necessary to use the combination of Li$^+$-form and H$^+$-form cation-exchangers described previously[3]. Only a small fraction of the water in a sample is used up in shifting the cinnamaldehyde dimethylacetal–cinnamaldehyde equilibrium to the right:

$$\overset{H^+}{\text{acetal + water} \rightleftarrows \text{aldehyde}} \tag{1}$$

Most of the water remains unreacted and emerges from the column as a distinct peak with a longer retention time than the bulk of the analytical sample. The concentration of aldehyde formed in reaction 1 is proportional to the water concentration. The detection wavelength is selected to measure the aldehyde without interference from the larger amount of acetal that is present.

Several resins were tried as packing for the separation column. Aminex Q-150S (H^+) was found to work very well. It is a gel-type resin, which appears to be a desirable property for chromatographic separation of water from other substances. Hamilton PRP-X300 is said to be a good column for ion-exclusion chromatography, but it gave no separation at all for water under the conditions we used. Perhaps this is because the Hamilton resin is macroporous and not a gel. A silica-base diol column also gave no separation of water. A slight displacement of the water peak (longer retention) was due to a short Aminex Q-150S (H^+) post-column used as a catalyst.

Aminex Q-150S (H^+) columns of varying dimensions were tried. A 15 cm × 2.1 mm column, used in conjunction with a 50-μl sample loop worked the best. Columns of wider diameter gave lower detection sensitivity.

Detection system

The cinnamaldehyde added to the "dry" methanol used to prepare the eluent has the potential of reacting with the methanol to form the cinnamaldehyde dimethylacetal plus water. However, this reaction does not occur to any extent until an acid catalyst is present. This may be the H^+-form cation-exchange resin in the column, or a soluble acid can be added to the eluent. After contact with an acid catalyst, most of the cinnamaldehyde is converted to the acetal.

Equilibrium constant

The equilibrium constant for the following reaction was measured by adding varying concentrations of water to the eluent (in the presence of trace H^+) and measuring the concentrations of acetal and aldehyde spectrophotometrically:

$$\overset{H^+}{\underset{\text{(b)}}{\text{water + cinnamaldehyde acetal}} \rightleftarrows \underset{\text{(a)}}{\text{2 methanol + cinnamaldehyde}}} \tag{2}$$
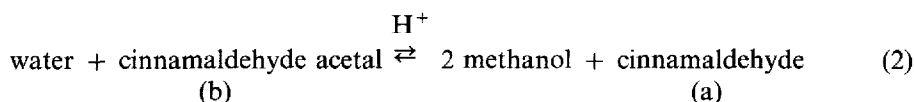
First, the absorbance of the eluent (A_a^0) was measured before addition of an acid catalyst when all of the cinnamaldehyde is present as (a). Then the absorbance (A_b^0) is measured after acid catalysis when all of the aldehyde has been converted to (b). From Beer's law

$$A_a^0 = \varepsilon_a \, l \, C^0 \tag{3}$$

$$A_b^0 = \varepsilon_b \, l \, C^0 \tag{4}$$

where ε is the extinction coefficient, l is the pass length of the detection cell, and C^0 is

the initial concentration of cinnamaldehyde in the eluent. These measurements were made at 280 nm, where both (a) and (b) absorb appreciably.

Next, varying amounts of water were added to the eluent in the presence of an acid catalyst and the total absorbance (A_{tot}) was measured at 280 nm. From Beer's law:

$$A_{tot} = A_a + A_b = \varepsilon_a l[a] + \varepsilon_b l[b] \tag{5}$$

$$= \varepsilon_a l[a] + \varepsilon_b l(C^0 - [a]) \tag{6}$$

$$= (\varepsilon_a l - \varepsilon_b l)[a] + A_b^0 \tag{7}$$

where [a], [b] and A_a and A_b are the concentrations and absorbances at equilibrium. Combining these equations

$$[a] = \frac{A_{tot} - A_b^0}{\varepsilon_a l - \varepsilon_b l} \text{ and } [b] = C^0 - [a] = \frac{A_a^0 - A_{tot}}{\varepsilon_a l - \varepsilon_b l} \tag{8}$$

The equilibrium constant for reaction 2 is:

$$K = \frac{[a]}{[b][H_2O]} = \frac{A_{tot} - A_b^0}{(A_a^0 - A_{tot})[H_2O]} \tag{9}$$

A value of $(5.3 \pm 0.4) \cdot 10^{-4} \text{ m}M^{-1}$ was found for the equilibrium constant, K.

Equation for chromatographic detection

Rearrangement of eqn. 9 gives

$$A_{tot} = \frac{A_a^0 K[H_2O] + A_b^0}{K[H_2O] + 1} \tag{10}$$

so as long as $[H_2O]$ is small, the denominator is approximately equal to 1 and eqn. 10 is essentially linear. However, the detector response (A_{det}) depends on the difference in absorbance of the eluent and sample, so eqn. 10 can be written

$$A_{det} = \Delta A_{tot} = A_a^0 K \left([H_2O]_{sample} - [H_2O]_{eluent} \right) \tag{11}$$

Introducing E as a factor of column and elution efficiency and using an eluent of low but constant water concentration, the following linear equation is obtained for detector response:

$$A_{det} = A_a^0 K E [H_2O]_{sample} - \text{constant} \tag{12}$$

Detection wavelength

An earlier paper[3] recommended 310 nm for detection of water. However, the sensitivity was found to be much better at 300 nm. A detection wavelengh of 290 nm was tried, but the background absorbance was too high.
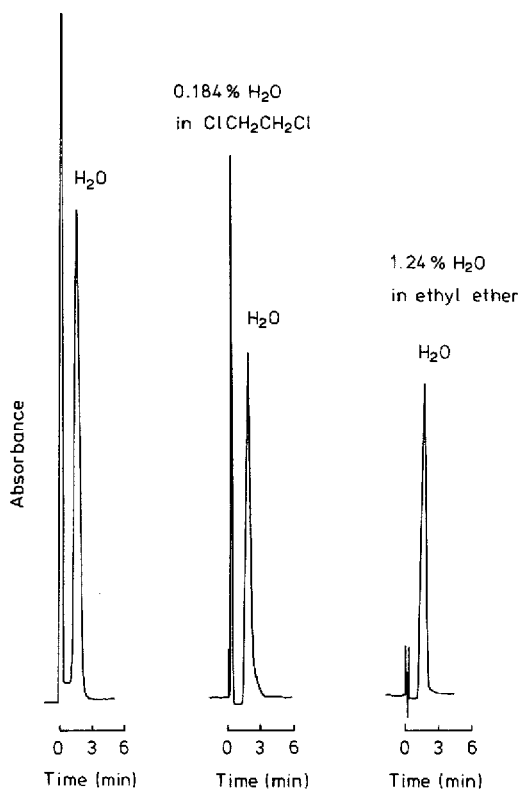
0.170 % H_2O

in Furan

Fig. 1. Determination of water in various samples. Conditions: 15 cm × 2.1 mm column; 50-μl sample loop; eluent, 1.0 mM cinnamaldehyde in methanol; flow-rate, 1.0 ml/min.

Scope

Typical chromatograms for the determinaton of small amounts of water in organic liquids are shown in Fig. 1. In each case there is an injection peak that occurs at the column dead time. This is followed by the water peak which has a retention time of 1.0 to 2.0 min, depending on the chromatographic conditions.

Successful separations of water in various organic samples were obtained. These included aromatic hydrocarbons, chlorinated compounds, alcohols, furans, esters and ethers. Aldehydes, methyl ketones and dimethylsulfoxide gave very broad injection peaks that obscured the water peak. Aldehydes and ketones can undergo an acid-catalyzed reaction with methanol to form water plus acetals and ketals, respectively. Fig. 2 shows good chromatograms for water in acetylcystine and ascorbic acid. These are reducing compounds and cannot be analyzed for water by the Karl Fisher method.

Injection peaks

The source and magnitude of injection peaks was investigated. This was done by injecting samples of four organic liquids, each containing a small amount of water, into
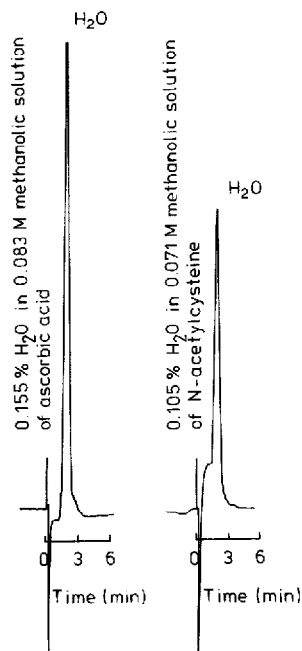
Fig. 2. Determination of water in reducing samples. Conditions as in Fig. 1.

a series of eluents containing (1) methanol only, (2) methanol plus 1 mM cinnamaldehyde, and (3) methanol containing 5 mM cinnamaldehyde. The results are summarized in Table I.

The results obtained with methanol only show that absorbance of the sample matrix can contribute to the injection peak. In this regard it should be recalled that the UV–VIS detector is very sensitive. Additional contributions to injection peaks are noted as increasing concentrations of cinnamaldehyde are added to the methanol eluent. These contributions can be explained by assuming that cinnamaldehyde can partition into the resin gel from the eluent. Then injection of a sample (which contains

TABLE I
SUMMARY OF INJECTION PEAKS

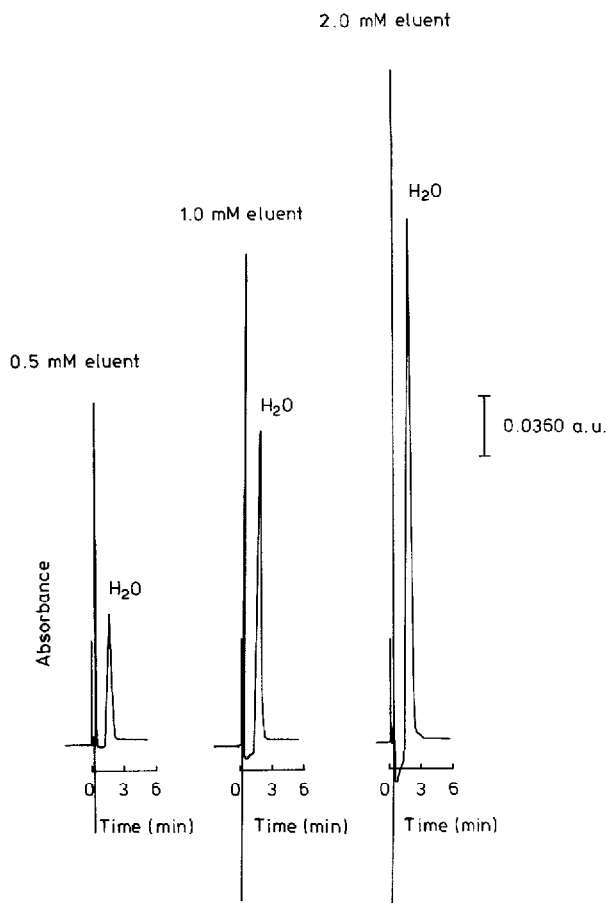| Sample	Injection peak		
	Methanol only	1 mM Aldehyde	5 mM Aldehyde
Methanol	None	Negative gap	Larger negative gap
Acetonitrile	Positive	Positive, negative gap	Larger positive, larger negative gap
Toluene	Large positive	Very large positive, small negative gap	Very large positive, negative gap
Hexane	Positive	Larger positive, small negative gap	Almost no positive large negative gap

Fig. 3. Effect of cinnamaldehyde concentration in eluent on peak height. Other conditions as in Fig. 1.

no cinnamaldehyde) causes some of the aldehyde to come from the gel back into the liquid stream and thereby contribute to the injection peak. After the sample zone has passed, some aldehyde goes back into the resin gel from the eluent, causing a negative gap in the chromatogram.

Effect of cinnamaldehyde concentration

Eqn. 11 predicts that increasing concentrations of cinnamaldehyde in the eluent should increase the detector signal for samples containing a fixed concentration of water. This is indeed the case, as is shown by the chromatograms in Fig. 3. A plot of peak height against cinnamaldehyde concentration in the eluent is linear for eluent concentration points of 0.5, 1.0, 2.0 and 5.0 mM cinnamaldehyde. The efficiency, E (eqn. 12), was calculated to be 0.18.

Quantitation

Standards were prepared by adding carefully measured amounts of water to portions of dry methanol. After chromatographic separation, linear plots of peak
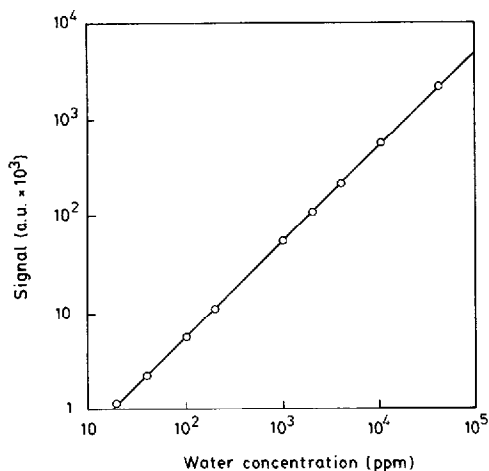
Fig. 4. Calibration curve in the low to medium range of water content. Conditions as in Fig. 1.

height against water concentration were obtained with excellent correlation coefficients for linear regression. However, such a calibration curve only shows peak height as a function of *added* water and does not account for the water already in the sample matrix and in the eluent itself.

A calibration curve of peak height *vs.* the total water in the standards was prepared with the aid of a standard (Fisher Scientific) certified to contain 1.00 ± 0.02 mg water per ml of sample. The resulting calibration plot has the same slope as that with added water, but the intercept is different. Manipulation of these two plots showed that the methanol used to prepare the standards contained 20 ppm water. The methanol eluent was calculated to contain 18 ppm water.
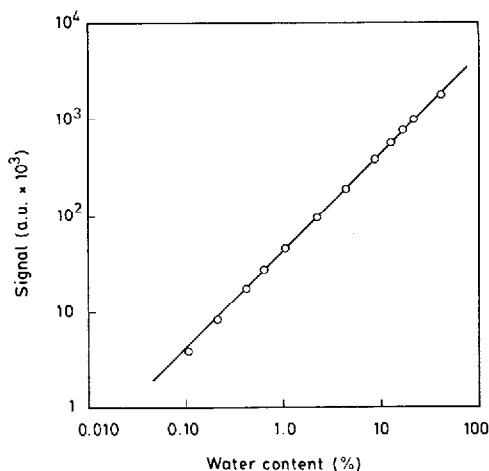

Fig. 5. Calibration curve in the high range of water content. A 5-μl sample loop was used. Other conditions as in Fig. 1.

Fig. 4 shows an excellent calibration plot for water that shows excellent linearity ($r = 0.99999$) over three orders of magnitude in water concentration. Fig. 5 shows a linear calibration curve for samples containing a high percentage of water.

Quantitative results

Samples of several organic liquids were carefully saturated with water by equilibration in a thermostat at 23°C. The water content of the organic phase was then determined by chromatographic analysis performed in triplicate. These results are summarized and compared with literature values in Table II. Some interpolation is required as the literature values are reported for slightly different temperatures than that used for the chromatographic determinations. Nevertheless, the chromatographic results are mostly in good agreement with the literature values.

TABLE II

SUMMARY OF WATER SOLUBILITY IN VARIOUS ORGANICS

Organic compound	Solubility of water (%, w/w)	
	Found (23°C)	Reported
Benzene	0.0563 ± 0.0005	0.053 (20°C)[4]
		0.066 (30°C)[4]
Furan	0.170 ± 0.001	0.141 ± 0.005 (20°C)[5]
Methylene chloride	0.154 ± 0.002	0.14 (20°C)[6]
		0.167 (25°C)[7]
Chloroform	0.088 ± 0.001	0.114 ± 0.004 (15°C)[8]
1,2-Dichloroethane	0.184 ± 0.001	0.17 (20°C)[6]
		0.187 (25°C)[7]
Diethyl ether	1.24 ± 0.01	1.2 (20°C)[9]
		1.26 ± 0.02 (RT)[8]
Carbon tetrachloride	0.022 ± 0.001	0.035 ± 0.003 (15°C)[8]
		0.0075 ± 0.0005 (20°C)[5]

Response factor

A response factor (RF) can be defined as follows

$$RF = \frac{\text{signal in absorbance units at 300 nm}}{0.1\% \ H_2O \ \text{in sample}}$$

A RF of 0.052 has been achieved with 1.0 mM cinnamaldehyde in the eluent and a 50-μl sample loop.

Detection limit

The detection limit depends on the water content of the eluent as well as the RF. The lowest detection limit achieved experimentally was 45 ppm of water.

CONCLUSIONS

Water can be determined very rapidly in a wide variety of samples by ion-exclusion chromatography using only a single separation column. Detection sensitivity is excellent over a broad concentration range using spectrophotometric detection at 300 nm with the acid-catalyzed cinnamaldehyde-acetal equilibrium system. Samples containing aldehydes or methyl ketones appear to require use of the two-column chromatographic system described earlier.

REFERENCES

1 J. Mitchell, Jr. and D. M. Smith, *Aquametry*, Wiley-Interscience, New York, 1977–1980.
2 T. S. Stevens, K. M. Chritz and H. Small, *Anal. Chem.*, 59 (1987) 1716.
3 N. E. Fortier·and J. S. Fritz, *J. Chromatogr.*, 462 (1989) 323.
4 T. I. Berkengeim, *Zavod. Lab.*, 10 (1941) 592.
5 E. P. Panteleeva, *Zavod. Lab.*, 32 (1966) 921; *Engl. Transl.*, 32 (1966) 1129.
6 W. Davies, J. B. Jagger and H. K. Walley, *J. Soc. Chem. Ind.*, 68 (1949) 26.
7 A. J. Staverman, *Recl. Trav. Chim. Pays-Bas*, 60 (1941) 836.
8 E. Eberius, *Wasserbestimmung mit Karl-Fisher-Losung*, Verlag, Weinheim, 1958.
9 M. Windholz, S. Budavari. R. F. Blumetti and E. S. Otterbein (Editors), *The Merck Index*, Merck, Rahway, NJ, 10th ed., 1983, p. 3742.