

(12) **UK Patent Application** (19) **GB** (11) **2 188 252** (13) **A**

(43) Application published 30 Sep 1987

(21) Application No **8706726**

(22) Date of filing **20 Mar 1987**

(30) Priority data

(31) **843423** (32) **24 Mar 1986** (33) **US**

(51) INT CL⁴
G01N 30/00

(52) Domestic classification (Edition I)
B1H 260 261 262 280 281 350 351 555 665

(56) Documents cited
GB 1083352 US 3935037
US 4254656
Aquametry: Part II Wiley Interscience (1984)

(71) Applicant
The Dow Chemical Company

(Incorporated in USA—Delaware),

2030 Dow Center, Abbott Road, Midland, Michigan
48640, United States of America

(72) Inventors
Timothy S. Stevens,
Hamish Small

(74) Agent and/or Address for Service
Boult, Wade & Tennant, 27 Furnival Street, London
EC4A 1PQ

(58) Field of search
B1H
Selected US specifications from IPC sub-classes B01D
G01N

(54) Determination of water by liquid chromatography

(57) The invention is a combination of liquid chromatographic separation of water from other components of an injected sample followed by electrochemical detection and quantitation of the separated water. A non-aqueous eluent is used and an electrolyte may be added to it prior to detection in order to increase the signal-to-noise ratio. Suitable apparatus is described and claimed.

GB 2 188 252 A

Fig. 1

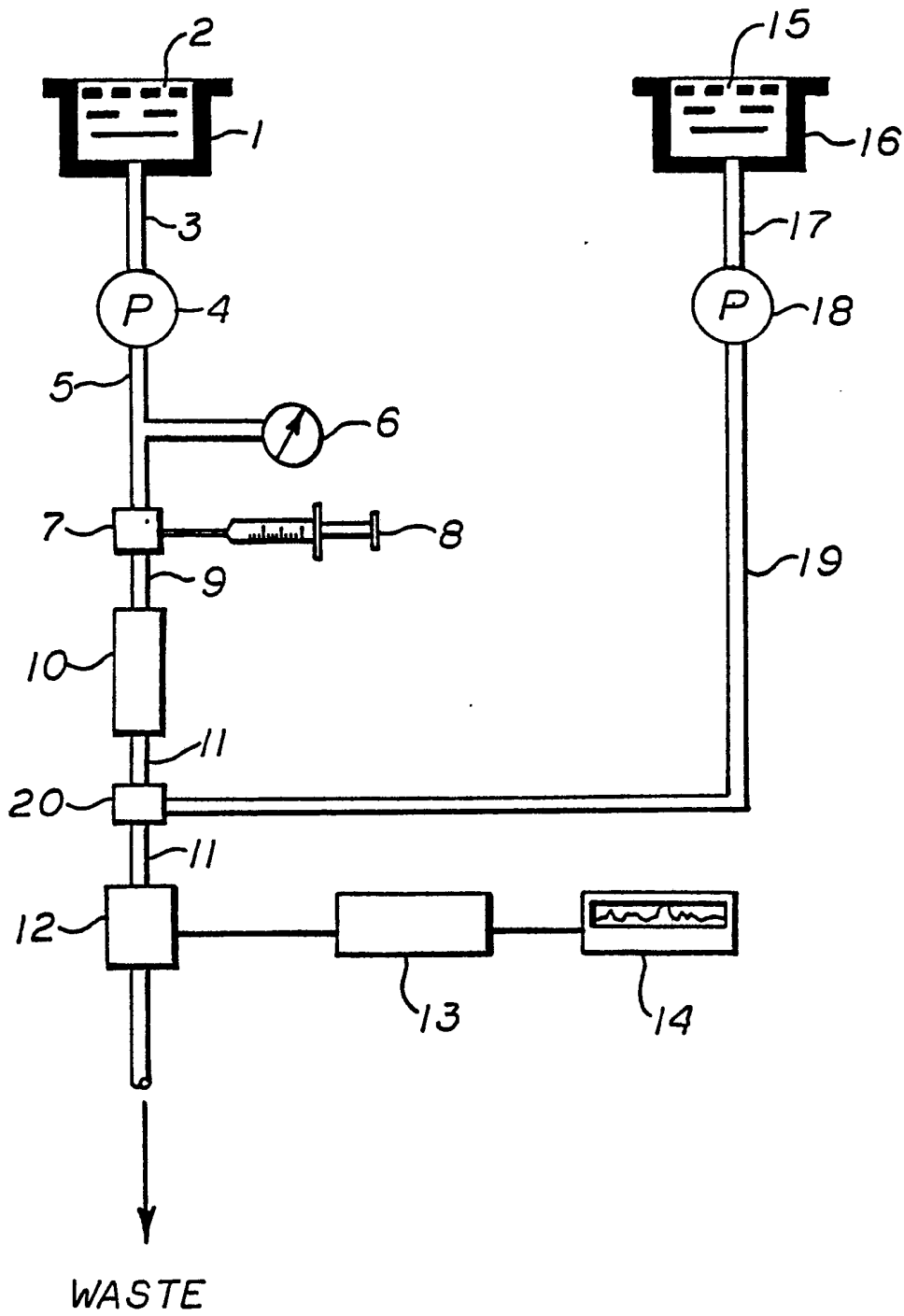


Fig. 2

HPLC DETERMINATION OF WATER
WITH NaCl IN THE ELUENT

Eluent: 0.14g NaCl in one liter of methanol at 2 mL/min.
Column: 9 x 54 mm AMINEX[®] 50WX4, Na⁺ ion form, 20-30 micron
Injection: 10 μ L loop
Detection: 7.5 μ mho cm⁻¹ per 10 mV
Recorder: 10 mV span, normal polarity

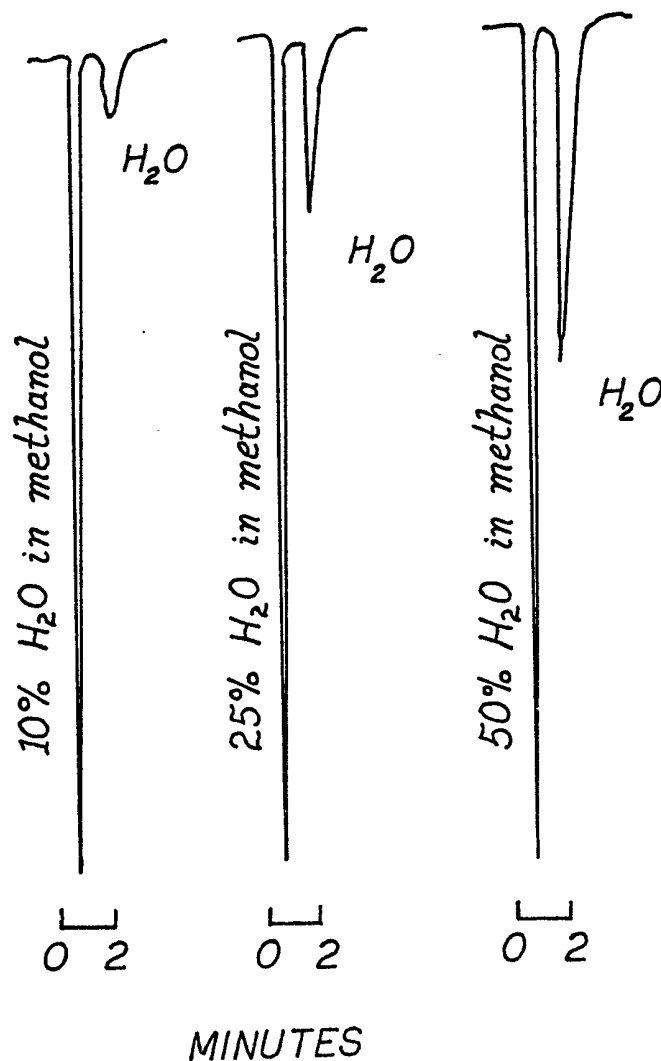


Fig. 3

HPLC DETERMINATION OF WATER USING MICROPARTICULATE
REVERSE PHASE, NORMAL PHASE AND ION-EXCHANGE
COLUMNS WITH NaCl IN THE ELUENT

Eluent: 0.14g NaCl in one liter of methanol at 1 mL/min

Column: PARTISIL[®] 10-ODS-3, ZORBAX[®]-SIL or
PARTISIL[®] 10-SCX

Injection: 10 μ L loop

Detection: 7.5 μ mho cm^{-1} per 10 mv

Recorder: 10 mv span, reversed polarity

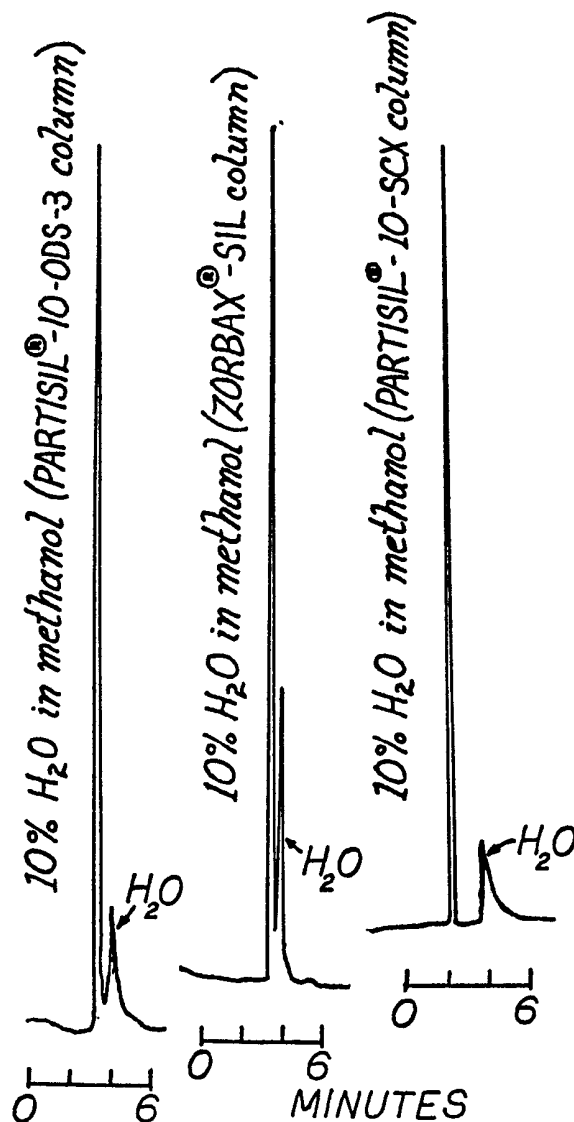


Fig. 4

HPLC DETERMINATION OF WATER WITH
VARIOUS ACIDS IN THE ELUENT

Eluent: 0.1g paratoluenesulfonic acid (PTSA) in 400 mL methanol or 0.02 mL 37% HCl in 200 mL methanol, or 0.025 mL 96% H₂SO₄ in 400 mL methanol, at 1.5 mL/min
Column: 9x21 mm, AMINEX[®] 50WX4, H⁺ ion form, 20-30 micron
Injection: 50 μL loop
Detection: 30 μ mho cm⁻¹ per 10 mv
Recorder: 10 mv span, reversed polarity

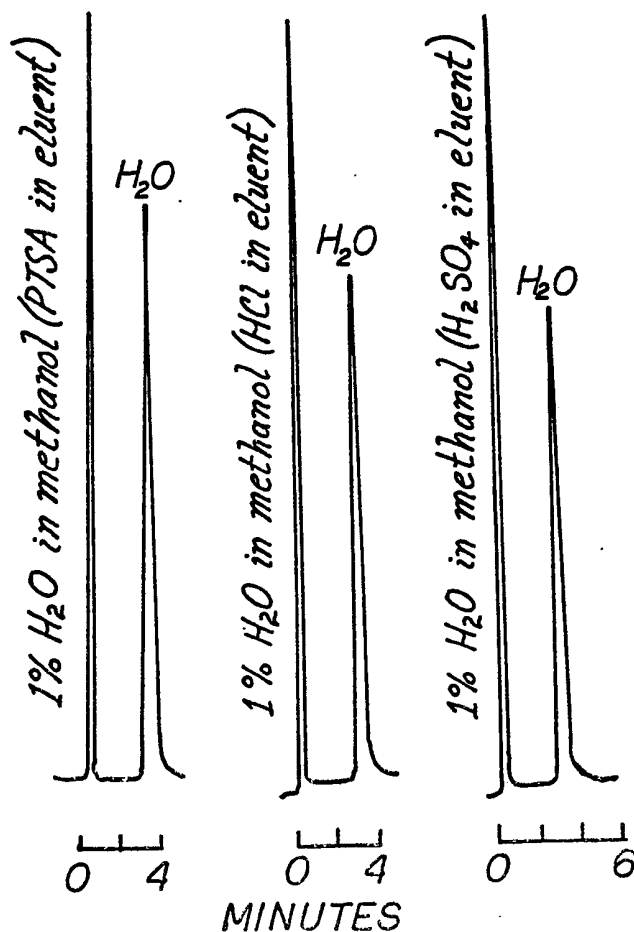


Fig. 5

HPLC DETERMINATION OF WATER
IN A FORMULATION OF DBNPA

Eluent: 0.05 mL 96% H_2SO_4 in 800 mL methanol at 1.5 mL/min
Column: 9 x 21 mm, AMINEX[®] 50WX4, H^+ ion form, 20-30 microns
Injection: 1 μ L
Detection: 60 μ mho cm^{-1} per 10 mv
Recorder: 8 mv span, reversed polarity

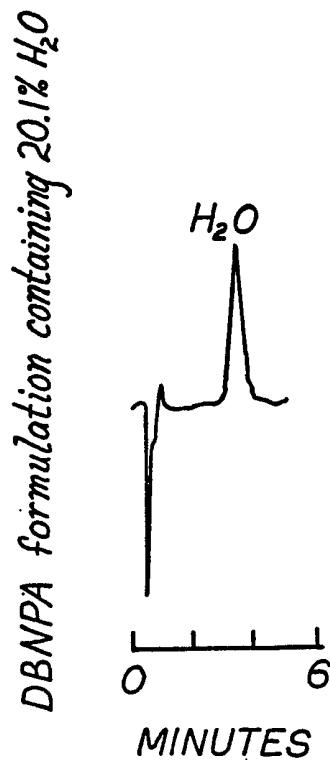


Fig. 6

HPLC DETERMINATION OF WATER
IN TELONE®-II SOIL FUMIGANT

Eluent: 0.05 mL 96% H_2SO_4 in 800 mL methanol at 1.5 mL/min
Column: 9 x 21 mm, AMINEX® 50WX4, H^+ ion form, 20-30 micron
Injection: 50 μ L loop
Detection: 7.5 μ mho cm^{-1} per 10 mV
Recorder: 10 mV span, reversed polarity

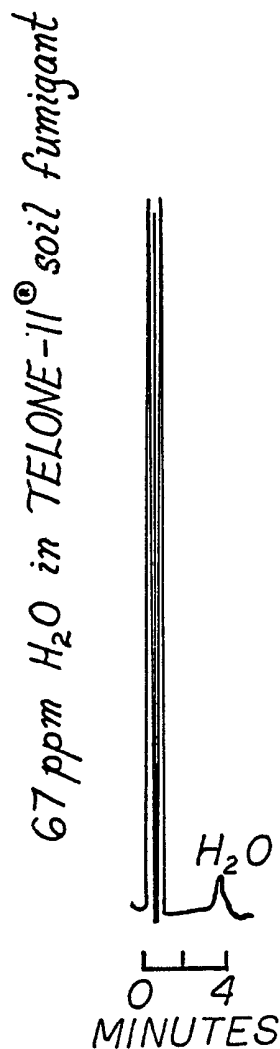


Fig. 7

HPLC DETERMINATION OF WATER USING AN
ELUENT CONTAINING ACETONITRILE

Eluent: 0.05 mL 96% H_2SO_4 in 800 mL acetonitrile at 1 mL/min
Column: 9 x 7 mm, AG[®] 1x2, SO_4^{2-} ion form, 200-400 mesh
Injection: 100 μ L loop
Detection: 15 μ mho cm^{-1} per 10 mv
Recorder: 10 mv span, normal polarity

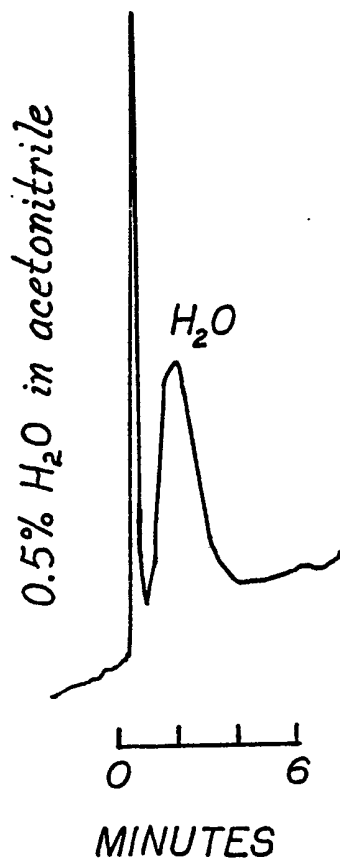
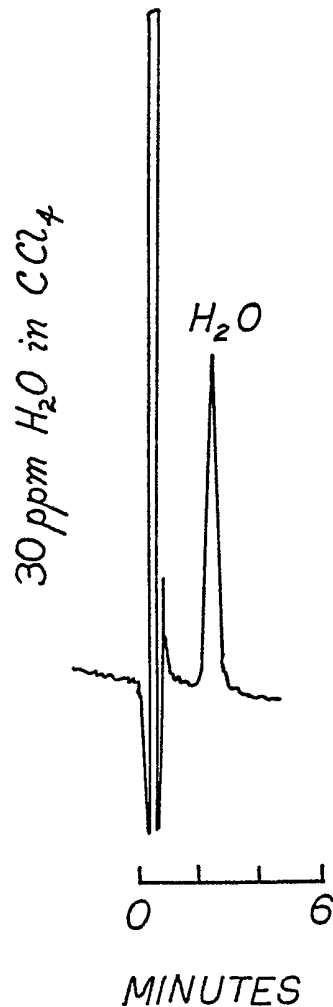


Fig. 8

HPLC DETERMINATION OF WATER
IN CARBONTETRACHLORIDE

Eluent: 0.05 mL 96% H_2SO_4 in 800 mL methanol at 1.5 mL/min
Column: 9 x 18 mm AMINEX 50WX4 H^+ ion form, 20-30 micron
Injection: 100 μ L loop
Detection: Wescan model 1CM conductivity detector, range 1, 10 mV output
Recorder: 2 mV span, reversed polarity



SPECIFICATION

Apparatus and Method for the Determination of Water by Liquid Chromatography

- 5 The invention is in the field of analytical chemistry and is directed towards a method and apparatus for the determination of water using a liquid chromatography system.
- 10 More particularly, the present invention resides in a combination of liquid chromatographic technology for effectively separating water from other components of a sample and electrochemical detection technology for effectively measuring the separated water.
- 15 Aquametry or the determination of water is an important branch of analytical chemistry. Many analytical systems have been developed to determine water in solids, liquids and gases. Most of these systems are described in 3 volumes of
- 20 Aquametry, Part I, II and III, J. Mitchell, Jr. and D. M. Smith; Wiley—Interscience; 1977, ISBN—O—471—02264—0 (Part I); 1984, ISBN—O—471—02265—9 (Part II); and 1980, ISBN—O—471—02266—7 (Part III).
- 25 Most determinations for water are easily made by a Karl Fischer titration. However, interferences are known including oxidizing agents, unsaturated compounds and thio compounds, see *Aquametry*, Part III, *supra*. Thermal conductivity detection gas chromatography (GC) is probably the second most often used method resulting in a water peak that elutes rapidly, e.g., 1 to 2 minutes, and with good sensitivity, e.g., 1 ppm, see *Aquametry*, Part I, *supra*.
- 30 However, with GC the other components of a sample can take much longer to elute than water and can even decompose on-column and interfere with the analysis.
- 35 Thus, there exists a need to determine water in commercial formulations of
- 40 dibromonitripropionamide (DBNPA), an antimicrobial product of the Dow Chemical Company, DBNPA is an oxidizing agent and reacts with iodide to yield iodine, and thus interferes with the Karl Fischer method. DBNPA is thermally labile
- 45 and decomposed on-column in a GC. The products of the decomposition (believed to include HBr) corroded and eventually severed the filaments of the GC detector.
- 50 High performance liquid chromatography (HPLC) was considered by Blasius et al. *Talanta*, 27:127, 1980. Blasius et al. determined water by HPLC using a cyclic polyether column with a refractive index detector but water and other interfering components eluted without retention.
- 55 Fehrman et al. *Z. Für Anal. Chem.*, 269(2):116, 1974 determined water by size-exclusion chromatography using a refractive index detector. Fehrman et al. used toluene as the eluent (rather than the more commonly used tetrahydrofuran)
- 60 which significantly improved separation of water from other low molecular weight interfering components. However, the DBNPA formulation was not miscible in toluene, and water itself has a limited solubility in toluene.
- 65 Bjorkquist et al., *J. Chrom.*, 178:271, 1979 reacted phenyl isocyanate with water to form N,N'-diphenylurea (NN'DPU), with a total reaction time of about 1/2 hour, and then analyzed the NN'DPU by reverse phase HPLC. However, a
- 70 simpler and faster procedure is needed.
- Roof et al. in U.S. Patent No. 3,935,097 used an anion-exchange column with a refractive index, ultraviolet absorption or differential density detector to determine water in a fluorination process stream
- 75 in about 12 minutes, but with relatively poor column efficiency, i.e., about 30 effective theoretical plates and with poorer sensitivity than desired.
- The determination of water without a prior separation by electrochemical means (for example
- 80 by electrical conductivity measurement, dielectric constant measurement [dielometry] or oxidation/reduction reactions at electrodes) is extensively discussed in the volume titled *Aquametry Part II, supra*. However, such direct measurements can be
- 85 seriously inaccurate due to variations in the sample composition unrelated to variations in water concentration.
- It is, accordingly, an objective of this invention to provide a liquid chromatographic system for the
- 90 determination of water which is generally applicable but not limited to samples containing oxidizing agents, unsaturated compounds, thio compounds and thermally labile compounds. However, the system is relatively rapid and accurate and uses an
- 95 electrochemical detector.
- The invention particularly relates to an apparatus for the determination of water by liquid chromatography comprising an eluent reservoir containing a nonaqueous eluent; said reservoir in
- 100 fluid communication with a sample injection means; said injection means in fluid communication with a chromatographic separation means; said separation means in fluid communication with an electrical conductivity detector.
- 105 The invention also resides in a method for the determination of water by liquid chromatography comprising the steps of:
- 110 adding a predetermined volume of a sample containing a water component and another component to a flowing stream of a non-aqueous eluent;
- eluting the sample through a separating medium effective to separate the water component from another component so that the separated water
- 115 component emerges from the separating medium in an effluent eluent stream from the separating medium; and
- effectively electrochemically detecting the separated water component in the effluent eluent
- 120 stream from the separating medium.
- The detector may be any one detector based on the principle of measuring dielectric constant (dielometry); electrical conductivity, or oxidation/reduction at electrodes.
- 125 Optionally, the invention includes the further step of adding an electrolyte to the eluent stream before the stream reaches the detector, the electrolyte being at least partially dissolved before passing through the detector.
- 130 The electrolyte added to the eluent is selected

from an acid such as H₂SO₄, HCl and a paratoluenesulfonic acid.

Optionally, the invention includes the further step of placing an immobilized electrolyte between the electrodes of the detector, said electrolyte being in contact with said effluent. The immobilized electrolyte comprises, for exemplary purposes, gelled electrolyte, liquid ion-exchangers and solid ion-exchangers.

10 Figure 1 is a schematic view of a typical apparatus of the invention.

Figures 2 through 8 are chromatograms showing the determination of water using the invention and are associated with Examples 1 through 10.

15 Referring to Figure 1, there is shown a liquid chromatographic system which is desirably used in practicing the invention, which comprises an eluent reservoir 1 containing an eluent 2 which is conveyed to a pump 4 by conduit 3 and then to a sample injection valve 7 by conduit 5. A pressure gauge 6 allows the monitoring of the eluent pressure in conduit 5. A sample is loaded into a sample injection valve 7 using a syringe 8. A conduit 9 conveys the eluent to a column 10. The effluent from column 10 is conveyed through a conduit 11 to flow-through detector cell 12 and then to waste. A recorder 14 visualizes the detection of the separated water peak in cell 12 by means of a detector electronic circuit 13.

20 Optionally, a reagent 15 comprising an electrolyte dissolved in a non-aqueous solvent, contained in a reagent reservoir 16, is conveyed to a pump 18 by conduit 17 and then to a mixing tee 20 by conduit 19.

Ion-exchange resins have a known affinity for water, see for example *Dowex: Ion Exchange*, published by The Dow Chemical Company, 1964, specifically page 33, and Roof, *supra*. A preferred separating medium is a chromatographic column of sulfonated styrene-divinylbenzene copolymer/acid-type ion-exchange resin such as sold by Aminex® 50WX4, 20 to 30 micron size, catalog number 147-4203, Bio-Rad Laboratories (P.O. Box 4031, Richmond, California 94804) and packed in a Cheminert® Model L9-9-MA-13 column available from The Anspec Company, P.O. Box 7730, Ann Arbor, Michigan 48107, catalog number H7224.

Also preferred are other ion-exchange mediums such as quaternized styrene-divinylbenzene copolymer base-type ion-exchange resins such as Bio-Rad Laboratories, *supra*, AG® 1×2, 200 to 400 mesh size, catalog number 745—1251. Also useful in the invention are silica based ion-exchange columns such as Whatman Corporation's Partisil® SAX anion exchange column or Partisil SCX cation exchange column, available from The Anspec Company, *supra*, catalog numbers H6303 and H6311, respectively.

Size-exclusion columns have a known ability to separate water in HPLC, for example, see Fehrman et al., *supra*. Size-exclusion columns having effective pore sizes such as the silica based Brownlee Aquapore® OH-100 column or the TSK Sphereogel® 2000 SW column available from The Anspec Company, *supra*, catalog numbers H1474 and H4548, respectively are believed useful in the invention.

Size-exclusion columns using porous polymer separation media, such as Waters Associates μ-Styrogel®, are also believed to be useful in the invention. However, as with the sulfonated or quaternized styrene divinylbenzene acid- or base-type ion-exchange resins, which swell varyingly depending on the specific eluent, many porous polymer size-exclusion media must be equilibrated with the eluent before packing the chromatographic column.

The separating media believed to be effective for the invention comprise a packed-type chromatographic column and a capillary-type chromatographic column.

80 Silica based normal phase columns such as the Du Pont Zorbax® SIL column are useful in the invention and are believed to work as size-exclusion columns.

Silica based reverse phase columns such as Whatman Partisil ODS-1 and ODS-3 columns are useful in the invention and are also believed to work as size-exclusion columns.

The essential feature of the separating medium of the invention is that it effectively

90 chromatographically separates the water of an injected sample from other components of the sample using a non-aqueous eluent. Specifically, if the electrochemical determination of water is not seriously interfered with by the other components of the sample, then there is little compulsion to use this invention. However, when one or more of the other components of a sample do interfere, then separating them from the water of the sample and presenting this water to the detector in the matrix of the eluent can be an effective means to eliminate serious interferences with detection and analysis. Therefore, the specific chromatographic column used is not critical as long as it performs the above-mentioned effective separation function in an otherwise operable system.

A preferred eluent comprises methanol or acetonitrile. Eluents believed to be effective in the invention comprise ethanol, propanol ethylene glycol, benzene, toluene, carbon tetrachloride, chloroform, cyclohexane, heptane, tetrahydrofuran and toluene. The specific eluent used is not critical as long as it effectively interacts with the chromatographic media to separate water from other components of the sample and as long as the detector used will function to effectively detect the separated water in the eluent in an otherwise operable system.

Ideally, the concentration of water in the eluent is zero. However, some water can be tolerated.

120 Preferably, the concentration of water in the eluent is no more than 100 times the concentration of water in the sample and more preferably the concentration of water in the eluent is no more than 10 times the concentration of water in the sample.

125 Most preferably, the concentration of water in the eluent is less than the concentration of water in the sample.

130 Optimally, the eluent should not react with the other components of the sample to produce significantly interfering amounts of water. For

example, ketones and aldehydes can react with methanol to form ketals and acetals with the production of water as a by-product. Some organic acids will react with methanol to form esters with the production of water as a by-product. These interferences are well known with the Karl Fischer method for the determination of water and are eliminated by replacing the methanol in the Karl Fischer reagent with another nonreactive solvent. In this invention the same can be done, for example by using an acetonitrile based eluent instead of a methanol based one.

The sample should be preferably miscible in the eluent. Thus, for many samples methanol or acetonitrile based eluents are preferred due to the excellent ability of these solvents to form homogeneous solutions with other solvents and components. However, it is not critical that the sample dissolve in the eluent. For example, the invention is used to determine water in clay based agricultural formulations by first shaking said formulation with HPLC grade methanol to extract water in the formulation and then injecting the methanol extract after the extract is filtered to remove the clay. It is also believed to be possible to determine water in a gas sample by, for example, contacting the gas with a liquid that would extract the water and then injecting the liquid.

A highly preferred detector of the invention is an electrical conductivity detector such as the Wescan Model ICM (Wescan Instrument Inc., 3018 Scott Blvd., Santa Clara, California 95050).

The test of effective electrochemical detection of the separated water of the injected sample in the effluent from the separating medium comprises the well-known signal-to-noise ratio, where the signal relates to the detectors' response to water and the noise relates to the variation in response seen at the baseline of the chromatogram. The signal-to-noise ratio must be greater than 2 for effective detection. The signal-to-noise ratio can be improved, as is well known in the art, by employing active or passive filters in the electronic circuits of HPLC systems. The signal-to-noise ratio can also be improved (made larger) by injecting a larger volume of sample or by employing a more efficient column in an otherwise operable system. However, all of these techniques are limited in their beneficial effects and thus the detection limit of the invention is also limited. On the other hand, samples containing relatively high concentrations of water, e.g., 20 percent water, may require system modifications to prevent overloading such as using a smaller injection volume.

The sensitivity of detection of water using an electrical conductivity detector is significantly increased when an electrolyte is present in the eluent. The conductivity detector responds to the electrolyte and the difference in response, between a matrix of eluent and a matrix of eluent and the separated water, is greater when an electrolyte is present in the eluent. This is an example not of direct but rather indirect measurement of water concentration. A highly preferred electrolyte is an acid such as, but not limited to, H_2SO_4 , HCl or

paratoluenesulfonic acid. However, samples containing hydroxide ion, for example, will react with acid to form water. Also preferred as an electrolyte to be present in the eluent is a salt such as, but not limited to, NaCl, KCl or LiBr. The sensitivity of detection of water using the preferred conductivity detector is not as good with a salt as with an acid, but the use of a salt does eliminate the interference from hydroxide ion and is one means of avoiding such interference in the event the interference is significant.

An electrolyte believed useful in the invention when using a conductivity detector is a base such as sodium hydroxide or tetrabutylammonium hydroxide. The specific electrolyte used, whether acid, base or salt, organic or inorganic or a mixture thereof is not critical. Optimally, the electrolyte used does not result in significantly interfering reactions with sample components and does not significantly degrade the eluent. It is believed that the most preferred acid and base electrolytes are strong acids and bases, that is acids with pK_{a1} values of less than 1 and bases with pK_{b1} values of less than 1. However, H_3PO_4 with a published pK_{a1} value of 2.12 is quite useful and to a lesser extent even acetic acid with a published pK_{a1} value of 4.73. The concentration of electrolyte added to the eluent is optimized by testing for optimum signal-to-noise ratio, supra, for a given system. However, it can be desirable to have a relatively high electrolyte concentration in the eluent when using an ion-exchange column, if the sample contains one or more electrolytes, to maintain the ion form of the column.

Where the optionally employed electrolyte is added to the eluent is not critical as long as the addition step occurs before the eluent passes to the detector and as long as the electrolyte is at least partially dissolved in the eluent. For example, it should also be possible to add the electrolyte to the eluent after it emerges from the chromatographic media and before the eluent flows to the detector.

Postcolumn reagent addition is well known to the art of HPLC. One advantage contemplated in this invention with the use of postcolumn electrolyte addition is the potential elimination of interferences. For example, when a sample contains hydroxide ion and the eluent contains an acid, the acid reacts with the hydroxide ion upon injection producing water, which is then a potential interference. On the other hand, if the chromatographic media separates water from hydroxide ion and the acid is added to the eluent following the chromatographic media, then two water peaks will be seen by the detector. One water peak will result from the water originally in the sample at the standard retention time of water. The other water peak from the water produced by the reaction of acid with hydroxide will be at a different non-standard retention time and will not interfere.

Any solvent used as a carrier with postcolumn electrolyte addition ideally has a water concentration of zero. However, some water can be tolerated. Preferably, the concentration of water in a postcolumn addition electrolyte solvent is no more

than 100 times the concentration of water in the sample and more preferably no more than 10 times the concentration of water in the sample. Most preferably, the concentration of water in a

5 postcolumn addition electrolyte solvent is less than the concentration of water in the sample.

Examples of other detectors believed to be useful in the invention comprise those measuring dielectric constant and those incorporating

10 oxidation/reduction reactions at electrodes.

It is believed to be preferred to add an electrolyte to the eluent before the eluent reaches the detector when the detector is an oxidation/reduction detector since such detectors generally require a supporting

15 electrolyte as is well known in the art. However, such detectors do not always require a supporting electrolyte. It is also believed to be preferred to add an electrolyte to the eluent before the eluent reaches the detector when the detector is a dielometry

20 detector in this invention since the dielectric constant of a solvent can be significantly altered when it contains an added electrolyte as is well known in the art.

Alternatively, the electrolyte can be added to the eluent between the sample injection means and the chromatographic separation means or at any other

25 point before the eluent reaches the detector. A highly preferred embodiment of the invention is to mix the electrolyte with the eluent in the eluent reservoir 1 of Figure 1.

The electrolyte need not be mixed with a carrier solvent and pumped into the eluent but can also diffuse into the eluent across a membrane. In other

35 words, how the electrolyte gets into the eluent before the eluent reaches the detector is not critical to the invention.

When an immobilized electrolyte is effectively employed within the detector, it should be placed between the electrodes of the detector and the

40 electrolyte should contact the effluent. When thus employed, it is believed that water, separated from other components of the sample and entering the detector, can interact with the immobilized electrolyte to enhance the detector's direct or

45 indirect response to the water. The above reference to placing the immobilized electrolyte between the electrodes should not be construed to mean directly and exactly between them as it may be preferable to effectively dispose the immobilized electrolyte away

50 from a point equidistant from the electrodes but still within or adjacent the space measured by the electrodes. The immobilized electrolyte can contact one or more of the electrodes.

The following examples further illustrate various

55 aspects of the invention.

Example 1

An HPLC system similar to Figure 1 (except that no postcolumn reagent addition system 15 to 20 is used) is assembled including an LDC

60 Constametric® III pump, a Rheodyne® 7120 sample injection valve, a 9×54 mm column of Bio-Rad Aminex 50WX4 ion-exchange resin, in the Na⁺ ion form packed in a Cheminert LC-9-MA-13 column, a Chromatronix® CMA-1 conductivity meter with

associated MCC-75 flow-through cell and a Sargent® SRG-1 strip chart recorder. The eluent is composed of HPLC grade methanol containing 0.14 g of NaCl per liter. The pump is set to deliver 2 ml of

70 eluent per minute. The injection valve is fixed with a predetermined loop size to deliver about 10 μl of sample. The detector is set to a sensitivity of 7.5 micro mho per cm for a 10 mv output. The recorder is set at a full scale response of 10 mv.

75 Three successive injections of standards of known amounts of water in methanol is then made and the chromatograms shown in Figure 2 result.

In Figure 2, two dips ("peaks") are seen in the chromatogram for each injection. The one at about

80 0.8 minutes is explained as the void volume upset. The one at about 2.1 minutes is explained as the water "peak", and its size is generally proportional to the amount of water in the injected standard.

Doubling the amount of NaCl in the eluent doubles

85 the water "peak" height and also doubles the background conductivity of the eluent from about 250 micro mho per cm to about 500 micro mho per cm. As expected, the baseline noise increases with increased background conductivity, and the limit of

90 detection observed with either eluent is about 0.1 percent water (in a standard) with a 500 μl injection. Using LiBr in the eluent instead of NaCl results in similar system performance. Reversing the recorder

95 standard operating procedure for this mode of the invention.

Example 2

The system of Example 1 is exactly reproduced

100 except that the column used in Example 1 is replaced with a Whatman Partisil® 10-ODS-3 column, or a Du Pont Zorbax® SIL column, or a Whatman Partisil® SCX column and the eluent flow rate is changed to 1 ml per minute. Figure 3 shows

105 chromatograms resulting from the injection of a standard containing 10 percent water in methanol for each column.

With the use of each column of Figure 3, a retained water peak resulted whose height is

110 generally proportional to the amount of water injected. This example demonstrates the wide range of column types useful in the invention.

Example 3

The system of Example 1 is exactly reproduced

115 except that the column is shortened to 9×21 mm and the NaCl in the eluent is replaced with paratoluenesulfonic acid (PTSA), HCl or H₂SO₄ and the flow rate of the eluent is changed to 1.5 ml per

120 minute as described in detail in Figure 4.

As shown in Figure 4, the use of HCl, H₂SO₄ or PTSA in an eluent of methanol using a 9×21 mm

125 column of H⁺ ion form Aminex® 50WX4 results in improved detection sensitivity. Using the NaCl containing eluent of Figure 2, a 50 μl injection of 1 percent H₂O in methanol results in a water peak 0.11 micro mho per cm tall. Using any of the above acids (also of a concentration sufficient to give an eluent background conductivity of about 250 micro mho

130 per cm) results in a water peak about 18 micro mho

per cm tall (see Figure 4), an increase in sensitivity of about 160 fold. This example demonstrates the improvement in sensitivity observed with the addition of acid to the eluent versus the addition of a salt to the eluent when using a conductivity detector.

Example 4

The system of Example 3 is reproduced except that the eluent was 0.05 ml of 96 percent H_2SO_4 mixed with 800 ml of HPLC grade methanol, the injection valve is changed to inject about 1 μ l of sample, the detector sensitivity is changed to 60 micro mho per cm per 10 mv output and the recorder sensitivity is changed to 8 mv full scale response. When an injection of 20 percent dibromonitriopionamide (DBNPA) in a water/glycol based formulation is made, the chromatogram shown in Figure 5 results.

Based on the water peak size for injections of known standards of water in methanol (not shown) the concentration of water in the DBNPA sample of Figure 5 is estimated to be 20.1 percent (20.1 g H_2O per 100 ml of sample). When the sample is injected 10 times, a statistical evaluation of the data indicates a standard deviation of the water concentration of 0.22 percent. This example demonstrates the utility of the invention for a sample that interferes with the Karl Fischer method and with a gas chromatographic method for the determination of water.

Example 5

The system of Example 4 is reproduced except that the sample injection volume is changed to about 50 μ l, the detector sensitivity is changed to 7.5 micro mho per cm per 10 mv output and the recorder span is changed to 10 mv. When an injection of Telone®II soil fumigant (mixed isomers of dichloropropenes, a product of The Dow Chemical Company) is made, the chromatogram shown in Figure 6 results.

Based on the water peak size for injections of known standards of water in methanol (not shown) the concentration of water in the Telone II sample of Figure 6 is estimated to be 67 parts per million (ppm) (67 mg of water per liter of sample). A GC analysis of the same sample estimates a water level of 71 ppm. In the GC analysis, the water elutes at about 2 minutes followed by dichloropropene peaks for the next 45 minutes. Thus, the HPLC procedure is faster overall. Trace levels of water can not be determined in Telone II soil fumigant by the Karl Fischer method since iodine adds across the double bonds of the dichloropropenes.

As a rule, water elutes as the last peak in the chromatogram with this embodiment of the invention, well resolved from the other components of a sample. However, dimethylsulfoxide (DMSO) elutes just before water and interferes.

Nevertheless, it is clear that the invention can be used to determine DMSO and perhaps other compounds in addition to water. A compound that responds similarly as water responds and is effectively separated from water is contemplated as

a candidate as an internal standard with the invention.

Example 6

The system of Example 5 is reproduced except that the eluent is changed to 0.05 ml of 96 percent H_2SO_4 in 800 ml of acetonitrile (at a flow rate of 1 ml per minute), the column is changed to a 9×7 mm one packed with Bio-Rad AG® 1 \times 2, SO_4^{-2} ion form, 200 to 400 mesh, ion-exchange resin, the injection volume is changed to about 100 μ l, the detector sensitivity is changed to 15 micro mho per cm per 10 mv output and the recorder polarity is reversed. When an injection of 0.5 percent water in acetonitrile is made, the chromatogram shown in Figure 7 results.

The water peak in Figure 7 is positive not negative. In addition, the baseline conductivity in Figure 7 is about 36 times lower than in Figure 6 while the response for the same amount of water injected with the system of Figure 6 or 7 (in units of micro mho per cm \times ml of the water peak width at half height) is about the same. Therefore, the use of a more efficient chromatographic column with this embodiment of the invention is expected to result in potentially increased detection sensitivity over the embodiment of Figure 6. One such column contemplated is a size-exclusion column of selected exclusion characteristics specifically designed for separation of relatively low molecular weight components such as water. Another such column contemplated is an ion-exchange column packed with partially sulfonated or partially aminated styrene-divinylbenzene copolymer beads.

Example 7

The system of Example 5 is reproduced except that the column length is changed to 18 mm, the injection volume is changed to about 100 μ l, the recorder sensitivity is changed to 2 mv full scale and the conductivity detector is changed to a Wescan Model ICM, set at range 1 and for 10 mv output. The concentration of water in the eluent is about 100 ppm. When an injection of carbontetrachloride containing 30 ppm of water is made, the chromatogram of Figure 8 results.

This example demonstrates the high sensitivity of this highly preferred embodiment of the invention.

Example 8

The system of Example 4 is reproduced except that a Kratos Post Column Reagent Addition Device, Model PCR-1 is added (as generally shown by elements 15 to 20 of Figure 1), the eluent is HPLC grade methanol containing no added H_2SO_4 at a flow rate of about 1.5 ml per minute. The post column reagent is 0.1 ml of 96 percent H_2SO_4 dissolved in 800 ml of HPLC grade methanol. The post column reagent flow rate is about 1.5 ml per minute. When an injection of DBNPA in a water/glycol based formulation is made (same sample as Example 4) a chromatogram generally similar to Figure 5 is believed to result except that the peak height is believed to be about one-half of that in Figure 5.

Example 9

The system of Example 1 is exactly reproduced except that the eluent is composed of HPLC grade methanol containing 0.14 g of sodium hydroxide per liter. When an injection of a sample containing water is made, a water response is believed to result that is generally proportional to the amount of water injected.

10 Example 10

The system of Example 4 is equivalently reproduced except that the eluent stream exiting from the detector is not directed to waste but is instead directed back to the eluent reservoir to be reused for an extensive length of time in the laboratory of a DBNPA production plant. The eluent reservoir is sealed to prevent the absorption of water from the laboratory air into the eluent. The HPLC system is used to determine water in DBNPA formulations for production control purposes and the HPLC system continues to function for more than 1 month without maintenance despite the buildup of sample (including water) in the eluent. It is contemplated that in some systems using recycled eluent that an eluent drying means could be employed to control the buildup of water in the eluent, e.g., by placing a column filled with drying agent between the detector and the eluent reservoir. A drying column can also be used between the eluent pump and the injection valve.

This example shows the economy of operation using a recycled eluent and the long term ruggedness of the system routinely used in a chemical production plant.

CLAIMS

1. A method for the determination of water by liquid chromatography comprising the steps of: adding a predetermined volume of a sample containing a water component and another component to a flowing stream of a non-aqueous eluent;

eluting the sample through a separating medium effective to separate the water component from another component so that the separated water component emerges from the separating medium in an effluent eluent stream from the separating medium; and

effectively electrochemically detecting the

50 separated water component in the effluent eluent stream from the separating medium.

2. A method as claimed in Claim 1 wherein the electrochemical detection is carried out using a dielometry detector, an electrical conductivity detector, or an oxidation/reduction detector.

3. A method as claimed in Claim 1 or Claim 2, which includes the step of adding an electrolyte to the eluent stream before the detecting step, the electrolyte being effective to increase the signal to noise ratio of the detecting step.

4. A method as claimed in Claim 3 wherein the electrolyte is an acid.

5. A method as claimed in Claim 4 wherein the acid is HCl or paratoluenesulfonic acid.

6. A method as claimed in Claim 1 wherein the detecting step is accomplished with a detector comprising electrodes, and including the step of placing an immobilized electrolyte between the electrodes of the detector, the electrolyte being in contact with the effluent.

7. A method as claimed in any one of the preceding claims wherein the eluent contains less than 5,000 parts per million water.

8. A method as claimed in Claim 7 wherein the eluent contains less than 100 parts per million water.

9. Apparatus for the determination of water by liquid chromatograph comprising: an eluent reservoir containing a non-aqueous eluent and being in fluid communication with a sample injection means; the injection means being in fluid communication with a chromatographic separation means; and the separation means being in fluid communication with a nonreactive electrochemical detector.

10. Apparatus as claimed in Claim 9 wherein the detector is a dielometry detector, an electrical conductivity detector, or an oxidation/reduction detector.

11. Apparatus as claimed in Claim 9 substantially as hereinbefore described and illustrated with reference to Figure 1 of the accompanying drawings.

12. A method as claimed in Claim 1 substantially as hereinbefore described with reference to any one of the Examples.