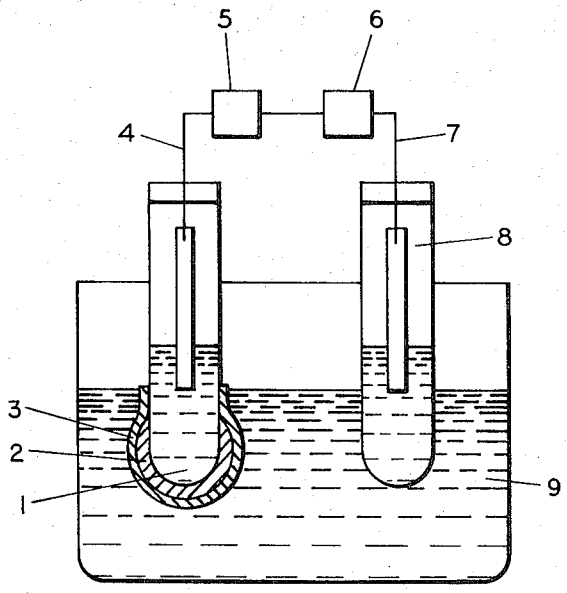
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UREA DETERMINATION AND ELECTRODE THEREFOR

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UREA DETERMINATION AND ELECTRODE THEREFOR

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ABSTRACT OF THE DISCLOSURE

The invention concerns a potentiometric method of determining the urea content of fluids by use of a cation sensitive electrode having a urease layer on its surface.

BACKGROUND OF THE INVENTION

The present invention is directed to a method of determining urea content of solutions, particularly of biological fluids such as blood. The invention is further directed to a cation-sensitive electrode having an urease coating or solution bound or confined thereto.

The invention provides a method of determining urea content of plasma or whole blood, which determinations are of value in the diagnosis of bodily ills. Procedures used for such determinations in the past have often required the isolation or removal of various blood components prior to the determination. It has previously been known that urease activity in bulk solution can be determined potentiometrically from the amount of ammonia formed by action of the enzyme upon urea in a buffered solution (Katz, Anal. Chem., vol. 36, p. 2500 (1964).

SUMMARY OF THE INVENTION

In the present invention a layer of urease solution or film is held against a cation-sensitive electrode, and this enzyme containing electrode is then placed in a solution containing urea. Ammonium ion is generated in the enzyme layer by action of the urea, and the logarithm of the ammonium ion concentration is measured versus a reference electrode. After corrections for the presence of other ions, the urea concentration is calculated. The electrod utilizes only a small quantity of enzyme and is reusable. The procedure makes it possible to determine the concentration of urea in whole blood without prior removal of other blood components, and without using excessive amounts of enzyme.

The present invention utilizes the known reaction:

$$NH_2\overset{O}{\overset{\|}{C}}NH_2 + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_3^-$$

The electrode of the present invention has the urease bound or contained close to the surface of the cation-sensitive electrode. The urease can be incorporated into a medium or film material which is held against the electrode. Such material can comprise or be coated with a dialysis medium to retard diffusion loss of the urease while permitting entry of the urea. A cation-sensitive electrode is employed as the base electrode component to which the urease is bound, such electrodes being known and avaliable. For example a glass electrode can be used, such as the common glass electrodes with an Ag/AgCl internal reference.

The figure is an illustration of the electrodes as employed in determining potentials in accordance with the invention.

The potentiometric measurements in the present invention employ a cell with a cation-sensitive electrode and a standard reference electrode, such as a saturated calomel electrode. The measurements are made on an electrometer connected across the electrodes, employing

a bucking voltage from a reference to maintain a low millivolt full scale reading on the electrometer if desired.

The figure is an illustration of the electrodes, test solution and potentiometric set up employed in the present invention. The electrode 1 has encompassing layers 2 and 3 shown in cross-section, 2 comprising urease, and 3 a covering membrane material. The lead wire 4 from electrode 1 is connected through an electrometer 5 and a precision reference voltage 6 and lead wire 7 to a saturated calomel electrode 8, and both electrode 1 and electrode 8 are in contact with test solution 9. The urease layer 2 around the electrode may be a coating of urease medium firmly adherent to the surface of the electrode, possibly comprising the urease in admixture with some resinous or gelatinous material or other film forming material, or it may be a solution or gel of the urease which is contained around the electrode by the membrane material 3. The membrane material can be adherent to the urease layer and the electrode, or it can be a physically discrete film which is mechanically affixed to the electrode to contain the urease layer. If the urease layer 2 is sufficiently adherent to the electrode surface and comprising material sufficiently resistant to attack by the test solution to substantially immobilize or prevent leaching out of the urease, the membrane layer 3 is then not essential. However it is generally preferred to employ such layer and generally that it be of some semi-permeable material which will permit migration of urea to the urease layer, but will substantially prevent migration of the urease into the body of the test solution. Materials which permit the passage of molecules of molecular weight up to say 8000 are suitable, although normally those passing molecules up to 1000 or so molecular weight will be used. Any membrane or porous diaphragm materials used in dialysis or electrolysis processes can be used.

The dimensions of the urease and membrane layers can vary greatly without impairing operability, but for good results it is desirable to employ relatively thin layers in order to have a quick potentiometric response to the test concentrations. Diffusion of the urea is apparently a rate-determining step. The urease layer is preferably of the order of ten-thousandths of an inch but is often of greater thickness; and appropriate range is about 0.0005 to about 0.005 inch. The thickness of the outer layer will depend to some extent on the material employed, particularly with the permeability and mechanical strength of such material. It may commonly be of the order of a few mills with collodion and such materials, say about 1 to 3 mils, although lesser thickness will be preferable if sufficient to prevent migration of the urease. With highly permeable materials the thickness can even be 0.01 inch or more.

The following examples are illustrative of the invention but the invention is not limited thereto.

EXAMPLE 1

An electrode was prepared by coating with a urease-gelatin film and then spraying with flexible collodion. To form the film, a solution of 50 mg. urease in 50 ml. water was added to fifty ml. aqueous solution containing 5% gelatin and 0.5% glycerol at about 40° C. The solution was coated on the electrode by alternately dipping and cooling. A flexible collodion was then sprayed on the electrode.

The electrode was then used in a cell containing bovine plasma to measure the urea content thereof. A saturated calomel electrode was used as a reference electrode. A Keithley model 610A electrometer was hooked across the electrodes to make the potentiometric measurements. An Emcee Electronics Precision Reference Voltage, Model 1118D was used as a bucking voltage to maintain a 10 mv. full scale reading on the electrometer. A Corning

Glass Co. monovalent cation glass electrode with an Ag/AgCl internal reference was the basic electrode component, such electrode being sensitive to NH_4^+ but relatively insensitive to Na^+. For the determination of the urea content, three potential measurements are necessary: the potential of the blood media (E_1), the potential of the blood media after the enzyme has converted some percentage of the urea to NH_4^+ (E_2), and the potential of the system after the addition of a known amount of urea to determine the percentage of urea converted by an electrode covered by a particular type urease film (E_3). The last measurement is unnecessary if the value has been previously established. The electrode response, E_2, in the bovine plasma was 6.6 mv. A 0.0312 molar amount of urea was added and E_3 was determined as 14.1 mv. The potential of the blood media, E_1, as determined by the same electrode minus the enzyme film was 5.2 mv. The response of the cation sensitive electrode was found to be $E=54.5 \log [NH_4^+]+83.9$ where E is millivolts vs. saturated calomel electrode at 25°. Conversion of this relationship to B.U.N. by subtraction of the background electrolyte and multiplication by a sensitivity factor yields the following equation from which the blood urea nitrogen was calculated:

$$\text{B.U.N.}=1.4\times10^3\left[\text{antilog}\left(\frac{E_2-83.9}{54.5}\right)-\text{antilog}\left(\frac{E_1-83.9}{54.5}\right)\right]\cdot K$$

where

$$K=\frac{2\ [\text{urea added}]}{\text{antilog}\left(\frac{E_3-83.9}{54.5}\right)-\text{antilog}\left(\frac{E_2-83.9}{54.5}\right)}$$

B.U.N.=12.9 mg./100 cc.

The percent conversion of the urea was 23%. The above equation can be converted into another form:

$$\text{B.U.N.}=2800\ [\text{urea added}]\ \text{antilog}\left(\frac{E_1-E_2}{54.5}\right)\left(\frac{\text{antilog}\left(\frac{E_2-E_1}{54.5}\right)-1}{\text{antilog}\left(\frac{E_3-E_2}{54.5}\right)-1}\right)$$

and when the urea added is 0.0312, this becomes

$$\text{B.U.N.}=87.36\ \text{antilog}\left(\frac{E_1-E_2}{54.5}\right)\left(\frac{\text{antilog}\left(\frac{E_2-E_1}{54.5}\right)-1}{\text{antilog}\left(\frac{E_3-E_2}{54.5}\right)-1}\right)$$

Further simplification may be possible by making mathematical approximations.

From the foregoing mathematical simplification it is evident that only voltage differences are important, and the calomel electrode standard is not significant to the determination nor required in the system. It will be recognized that the factor of 2800 in the equation is occasioned by the 2 moles of ammonium ion per mole of urea, and a factor of 1400 to convert moles of nitrogen per liter to mg./100 cc. The equation can be expressed in more fundamental form as

$$[N]=2\ [\text{urea added}]\ \text{antilog}\left(\frac{E_1-E_2}{A}\right)\left(\frac{\text{antilog}\left(\frac{E_2-E_1}{A}\right)-1}{\text{antilog}\left(\frac{E_3-E_2}{A}\right)-1}\right)$$

As reported above, the potential showed conversion of 23% of the urea in the solution. This value will vary with the time of the measurement and factors affecting the stability state achieved, but may often characteristically be in the range of 15 to 30% conversion.

EXAMPLE 2

An electrode with fibrin-urease and collodion coatings was prepared as follows. A 26.4 mg. amount of urease was added to 2.5 ml. of a solution containing 5 mg. fibrinogin per ml. water. A solution was prepared containing 5000 units thrombin per 100 ml. water buffered to pH 7.4 with a phosphate buffer, and 0.02 ml. of the solution was added to the fibrinogin solution. A fibrous mat formed within seconds and was removed from the container and placed on the tip of a glass electrode. The mat and electrode were sprayed with collodion. The electrode was then connected in electrical circuit as described in Example 1 with a saturated calomel electrode and used to measure the potential of whole blood. The potential was 2.4 millivolts. A 0.15 gram amount of urea was added (0.025 mol/liter) and the potential determined as 10.4 millivolts. The potential of 100 ml. whole blood with the untreated glass electrode was 0.6 millivolt. Utilizing the equations described herein, the B.U.N. was calculated as 13.1 mg./100 ml. The percentage of the urea converted by the urease was 25.6%.

EXAMPLE 3

A small amount of filter paper pulp was dried on a 3 cm. square piece of cellophane dialysis membrane. Five drops of water containing 14 mg. urease was dropped on and absorbed by the filter paper pulp. The piece of cellophane was then fitted to a glass electrode, being tightly stretched about the tip, with the urease-pulp between the cellophane and glass surface. The membrane was affixed to the electrode by wrapping with polytetrafluoroethylene tape at its upper edge. The electrode was used to determine the potential of a buffered solution with various amounts of added urea. The potential was found to be essentially a straight line function of the log of the cation concentration at concentrations tested in the range of 0.013 to 0.077 mole/liter.

The equations to determine nitrogen content are developed from the expression:

$$E=A \log [X]+B$$

where [X]=the concentration of cations in moles per liter.

In blood potansium and sodium ions are ordinarily present, so it is necessary to make allowances for these or other extraneous ions when determining urea nitrogen through the ammonium ion produced by urease, and this is done by measuring the potential in the absence of urease and utilizing the value in the equations as illustrated.

A series of solutions from 5×10^{-2} to 5×10^{-5} molar concentrations of $(NH_4)_2SO_4$ in distilled water were prepared and a plot of the log $[NH_4^+]$ versus the observed potential was substantially a straight line of slope 54 mv./decade and conforming to the equation

$$E=54 \log [NH_4^+]+83$$

Thus the values A and B in the previous equation in this instance are 54 and 83 respectively. As further illustrative, in the equation

$$E=A \log [X]+B$$

A can be determined by

$$A=\frac{\Delta E}{\Delta \log [X]}$$

If E′ is determined on a plasma, and E″ on a half-fold dilution thereof, the applicable equation is:

$$E'-E''=A \log \frac{[X]}{\frac{[X]}{2}}=A \log 2$$

and

$$A=\frac{E'-E''}{0.3010}$$

From values 2.4 mv. for the plasma, −14.3 mv. for half-fold dilution and −30.3 for quarter fold dilution, A was calculated as having an average value of 54.5 mv./decade. These values and further values obtained with known amounts of NH_4^+ added conformed to the equation:

$$E=54.5 \log ([K^+]+[Na^+/10]+[NH_4^+])+83.9$$

Thus the values of A and B which were employed in the calculations of B.U.N. herein were established as 54.5 and 83.9 respectively. It will be recognized that these values could be established with greater precision, although considered adequate for ordinary purposes. The value for A will vary somewhat with the concentration range, possible complexing agents in the system and other factors, and will in practice ordinarily be determined by careful calibration of the potentiometric system with the type of solution employed.

In the potentiometric determinations, the potential should be measured at some standard time interval sufficient for diffusion to come to a fairly steady state with the electrode employed. This time will vary with the thickness of the coatings on the electrode, particularly the urese coating. Ideally it will be relatively short, as a matter of seconds up to 30 seconds, but for thicker coatings can be five minutes or more, even hours, although the latter has limited practicality. Ordinarily a stable state will have been achieved within five minutes. The percent conversions herein are recognized as being a measure of how much ammonium ion reaches the electrode surface while true conversion of the urea may be much higher if only a particular portion of the ammonium ion is reaching the electrode surface.

The basic electrode structure utilized in forming the electrodes of the present invention can be any electrode capable of determining the ammonium ion potentiometrically. In general cation sensitive electrodes are suitable, with those having a strong response to the ammonium ion in the presence of other cations being preferred. Those electrodes more sensitive to monovalent cations than other cations are preferred. The pH type electrodes have some sensitivity to monovalent cations other than H^+ and can therefore be used although more sensitive electrodes are preferred. The electrodes used generally have an internal reference standard, for example silver/silver chloride, in contact with a liquid and a glass surface separating the test solution from the internal standard. The physical characteristics and composition of the glass has an influence on the selectivity of the electrode. Rather than thus using glass as a membrane, some electrodes utilize a porous membrane in conjunction with a liquid ion exchange layer between the test solution and the inner solution. Other electrodes utilize a synthetic crystal as a membrane to separate the inner liquid from the test liquid. A monovalent cation sensitive electrode manufactured by Corning Glass Works can be used. Other monovalent cation electrodes can be used, for example Beckman Instrument Co., Catalog No. 39137. Whatever the base electrode which is utilized, it must be provided with an urease layer in accord with the present invention in order to constitute the electrode of the present invention.

The present invention is useful for the determination of urea in various bodily fluids such as blood, urine, etc. It is also useful in other applications where the concentration of urea in a solution is to be determined.

What is claimed is:

1. An electrode for measuring urea concentrations comprising a cation sensitive electrode with an encompassing urease layer around and in direct contact with the electrode and confined thereto.

2. The electrode of claim **1** in which the cation sensitive electrode is a glass electrode and the urease layer is in direct contact with the glass.

3. The electrode of claim **1** in which the urease is in a gelatinous film.

4. The electrode of claim **1** in which the urease is covered with a flexible collodion film.

5. The electrode of claim **1** in which the urease is covered by a film which is substantially impermeable to urease molecules but permeable to urea molecules.

6. The electrode of claim **1** in which the urease is embedded in a coating adhering to the electrode surface.

7. The electrode of claim **1** in which the urease is confined by a separate film.

8. The method of measuring urea content of blood which comprises providing a first electrode having an encompassing urease coating, said electrode being in electric circuit with a reference electrode, placing said first electrode and said reference electrode in direct contact with a blood sample, measuring the electric potential between the electrodes, providing a corresponding electric potential determined by measuring the electric potential between the electrodes in direct contact with blood without the urease coating on the electrode, determining the difference between such electric potentials, comparing such difference with that for known urea concentrations and determining the concentration of the urea from such comparison.

9. The method of claim **8** in which the urease is confined to the electrode by a semi-permeable membrane.

10. The method of claim **8** in which the first electrode is a cation sensitive glass electrode and the blood sample is whole blood and it serves as the electrolyte between the electrodes.

11. The method of claim **8** in which 15 to 30% of the urea is converted to the ammonium ion.

12. The method of claim **8** in which the urease is in a gelatinous film.

13. The electrode of claim **1** in which the electrode is a monovalent cation sensitive electrode.

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