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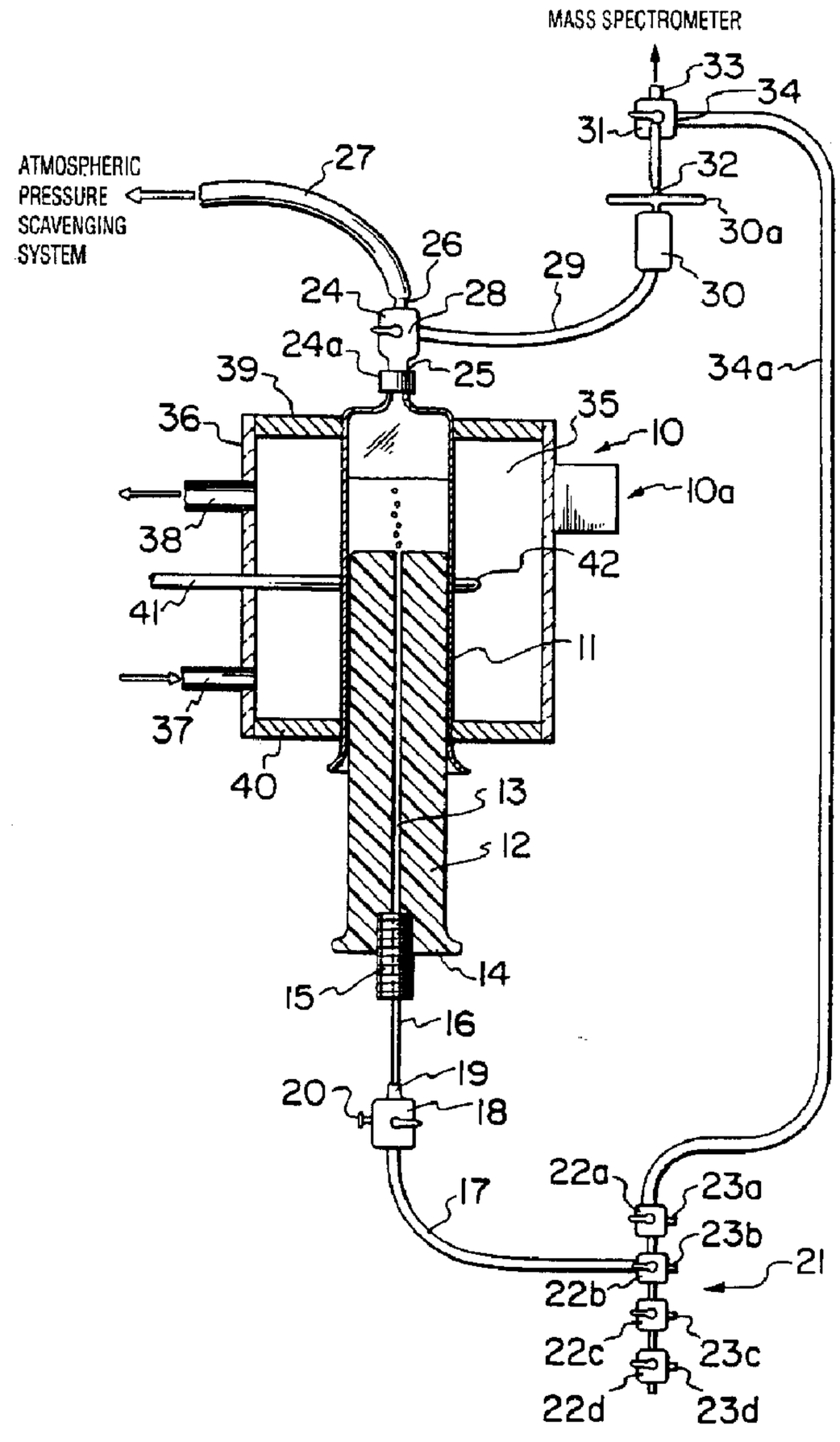
(54) **APPAREIL MESURANT LA SOLUBILITE D'UN GAZ ET LA QUANTITE DE GAZ PRESENT**

(54) **GAS AMOUNT AND SOLUBILITY INVESTIGATION APPARATUS**



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(57) A gas investigation apparatus is provided herein to determine the amount of gas in a liquid. The apparatus includes a hollow, longitudinally-extending cylindrical barrel for holding a liquid and a sample liquid with gas dissolved therewithin and with an associated gaseous headspace above. The barrel has a lower inlet and an upper outlet. At least a portion of the barrel is transparent. A plunger is slidably-fitted in a leak-proof manner within the barrel, the plunger being free to slide along the longitudinal length of the barrel, thereby forming a variable volume of air and gas in the headspace. A gas inlet tube has a lower inlet, and an upper outlet within the plunger and extends along the longitudinal axis of the plunger. A longitudinally-extending, heat transfer jacket surrounds the cylindrical barrel for the regulation of the temperature of the liquid sample. An upper valve is connected to the upper outlet of the barrel by means of a zero dead-space, butt-end connection. Selective entry means is provided through the upper valve for admitting a study liquid or tissue suspension into the barrel. A lower valve is connected to the lower inlet of the gas inlet tube by means of a zero dead-space, butt-end connection. An inlet conduit is provided for the selective introduction of test gas, a calibration gas, a carrier gas, or a flushing gas into the gas inlet tube through the lower valve. An inlet tube is provided for the selective introduction of a gas sample into the inlet conduit means through the lower valve. Finally, an exit tube is connected to the upper valve by means of a zero dead-space, butt-end connection, for leading gas exiting from the plunger-position-dependent variable volume of gaseous space at the upper portion of the barrel to a mass spectrometer.

ABSTRACT

A gas investigation apparatus is provided herein to determine the amount of gas in a liquid. The apparatus includes a hollow, longitudinally-extending cylindrical barrel for holding a liquid and a sample liquid with gas dissolved therewithin and with an associated gaseous headspace above. The barrel has a lower inlet and an upper outlet. At least a portion of the barrel is transparent. A plunger is slidably-fitted in a leak-proof manner within the barrel, the plunger being free to slide along the longitudinal length of the barrel, thereby forming a variable volume of air and gas in the headspace. A gas inlet tube has a lower inlet, and an upper outlet within the plunger and extends along the longitudinal axis of the plunger. A longitudinally-extending, heat transfer jacket surrounds the cylindrical barrel for the regulation of the temperature of the liquid sample. An upper valve is connected to the upper outlet of the barrel by means of a zero dead-space, butt-end connection. Selective entry means is provided through the upper valve for admitting a study liquid or tissue suspension into the barrel. A lower valve is connected to the lower inlet of the gas inlet tube by means of a zero dead-space, butt-end connection. An inlet conduit is provided for the selective introduction of test gas, a calibration gas, a carrier gas, or a flushing gas into the gas inlet tube through the lower valve. An inlet tube is provided for the selective introduction of a gas sample into the inlet conduit means through the lower valve. Finally, an exit tube is connected to the upper valve by means of a zero dead-space, butt-end connection, for leading gas exiting from the plunger-position-dependent variable volume of gaseous space at the upper portion of the barrel to a mass spectrometer.

A

(a) TITLE OF THE INVENTION

GAS AMOUNT AND SOLUBILITY INVESTIGATION APPARATUS

(b) TECHNICAL FIELD TO WHICH THE INVENTION RELATES

This invention relates to an apparatus which enables the study of gas content in, and interactions with, biologic and other media.

(c) BACKGROUND ART

Numerous methods exist to measure gas partial pressures and gas tensions in liquids (except inert, toxic and anaesthetic gases which are more difficult), but the measurement of gas content (i.e. the total amount of gas present) is more difficult. Content is a function of the partial pressure of the gas, its solubility in the material and any reactions with the material. Most methods calculate content from knowledge of the gas partial pressure, the temperature and the solubility of the gas in the material being studied. In many cases however, accurate solubility data is not available and this is especially true when considering inert, toxic and anaesthetic gases. In addition, biologic and other specimens are seldom of pure, known or fixed composition. Tissue specimens are always composed of a mixture of cell types. All this could invalidate assumptions about solubility coefficients.

An area of major importance is the determination of dissolved gases in blood, both those naturally-occurring, e.g., oxygen, carbon dioxide and nitrogen and those added for purposes of anaesthesia, e.g., isoflurane, halothane and nitrous oxide, or those used when diving under increased pressures (e.g., nitrogen, helium).

General anaesthesia has been accomplished by inhalation of the anaesthetic gas by the patient. The anaesthetic gases used are normally found, after application, as dissolved gas in the blood stream of the patient. It is extremely important that the level of the anaesthetic gas in the blood stream of a patient be rapidly-determined and accurately-determined particularly during any surgical operation.

The percentage of carbon dioxide or oxygen in the blood stream is a function of the adequacy of ventilation and the cardiovascular, respiratory and metabolic function of the patient who is under anaesthesia. It may also be a function of the level of anaesthetic gas in the blood stream, although the level of anaesthetic gas cannot be measured directly. For these and other reasons it is important to determine *in vivo* the level of gases dissolved in the blood stream.

Most present methods for determining the level of anaesthetic gas in the blood stream are based primarily on determining its partial pressure in gas in the lung or in the

breathing system. Many physiologic dysfunctions occur which may result in these measurements not accurately representing anaesthetic gas in the blood or brain. Other gases, including carbon dioxide and oxygen are also commonly measured in respiratory gas but in this case it is possible to take samples of blood periodically and, through electrochemical laboratory analysis, determine the partial pressure or the partial pressures or percentages of the gases under consideration in the blood stream at a remote location from the patient.

The determination of naturally-occurring blood gases is also important for clinical analysis. In particular, the determination of carbon dioxide (CO_2) and oxygen (O_2) tensions in whole blood and blood serum are among the most frequently performed analysis in a clinical laboratory. Due to the great importance of these analysis, a number of techniques have been developed and are presently being used to determine CO_2 and O_2 concentration.

A knowledge of the tension of each of the gases in the human blood stream is a valuable medical diagnostic tool. A means for continually monitoring the arterial system and analyzing the blood stream gases of one or more patients, for example, in a post-operative intensive care unit, would be an extremely valuable tool for determining the condition of patients' respiratory systems and would provide an early warning of possible malfunctioning.

At the present time, the analysis of the blood stream gases is made by withdrawing an arterial or venous blood sample and, without exposing the sample to the atmosphere, expose the blood

to oxygen and carbon dioxide electrodes which measure gas tension electrochemically. The mass spectrometer is not used for such routine measurements at the present time but has the advantage that virtually any gas can be easily identified and measured.

In one present method used to determine the <u>in vivo</u> measurement of oxygen (pO_2) in blood, an arterial needle which encloses an electrode assembly surrounded by a polyethylene membrane is inserted into a vein or artery. The dissolved oxygen in the blood diffuses through the membrane into an electrolytic solution and is reduced at a platinum cathode. The current produced is proportional to the oxygen content and is converted into a meter reading. However, this method has not found wide use because it is prone to many technical problems.

Numerous biomedical and other fields require knowledge of gas solubility and gas volumes in biological fluids or tissues. This is important in research in diving and aviation medicine, anaesthesia, toxicology and biochemistry. Although as mentioned above methods are available for measuring oxygen and carbon dioxide in blood and other fluids, it is more difficult to measure inert, poisonous, or anaesthetic gases or to measure gas production from various biological reactions. Existing methods for the determination of gas content in blood involve the use of the Van Slyke apparatus [using the method of D.D. Van Slyke which was published in the Journal of Biological Chemistry, Vol. 61, page 523 (1924)], vacuum extraction, gas chromatography, volumetric analysis or some combination of these preceding. In the basic Van Slyke method, blood serum and acid are mixed in a closed volume and the carbon dioxide in the blood is extracted

from the blood by application of vacuum. The extracted carbon dioxide is then measured volumetrically or manometrically. When the vacuum is drawn, other blood gases are released from the serum in addition to the carbon dioxide. This requires that a base, such as sodium hydroxide, be added in order to separate the carbon dioxide from the other released gases. After this, the volumetric measurement is performed by known techniques. Few advances have been made on this methodology since 1924, with the result that gas content is seldom performed except with the above method.

Another disadvantage of most prior art techniques for measuring blood gases concerns the use of a vacuum when the reagent and blood react to release the gases to be detected. Use of a vacuum means that species other than the gas to be measured (e.g., CO_2) will be released. For instance, O_2, N_2, etc. will be released from the blood and will contaminate the sample measurement where it is desired to measure CO_2. Chemical methods are required to remove unwanted gases. However, use of these methods introduces further time-consuming procedures and other possible errors including solution or adsorption of other gases by the chemicals and unknown solubility of gases in the chemicals. It would therefore be described to provide a method which can measure numerous gases simultaneously without the need to separate one from another.

Still another disadvantage of the use of vacuum relates to the possibility of leakage and lack of vacuum tightness. In vacuum systems, errors generally occur because apparatus, e.g., valves and stopcocks, develop leaks. Since the vacuum apparatus

is designed to operate reliably only when reproducibly good vacuum is provided, such techniques are critically dependent on the reliability of components which are themselves subject to numerous problems. Consequently, it is important to provide a technique which suffers only minimal interference from dissolved gases in the blood other than the species which is to be measured.

In general, such prior art methods using vacuum for measuring blood gases contain certain "non-equilibrium" features which lead to errors. The application of vacuum extracts gas from the blood which is partly reabsorbed by the blood when the vacuum is removed. Potential errors include leaks when working with high vacuums. The use of lesser vacuums can result in incomplete extraction. Leaks also occur when multiple transfers of samples between different reaction or measurement chambers are necessary. Such leaks may go undetected, especially when nitrogen or other atmospheric gases are being measured and the detection system is not gas-specific. Other problems include uncertainty that all gas has been extracted by a vacuum, inadequate control of temperature or ambient pressure and the need to make numerous assumptions or apply correction factors. In addition, many of the existing methods are highly dependent on the skill and technique of the experimenter and may suffer from inter- and intra-observer variation which is not appreciated.

In gas chromatography, the released gases are typically carried in a gas stream over a chromatographic column and then through a detector. The gas stream used as the carrier is

usually He, or some other gas having a different thermal conductivity than the gases which are to be measured. The column has different affinities for each gas in the mixture and acts to separate the different gases from one another. The detector usually comprises a hot filament wire whose resistance changes in accordance with the thermal conductivity of the gas which is in contact with the wire. Since the thermal conductivities of the gases to be measured are different from the carrier gas, and since the gases have been separated in a known order, each of the transient peaks of the detector response can be associated with one of the gases to be measured. These transient responses are usually plotted on a recorder, since the measurement is a dynamic one done in accordance with the flow of gases, rather than a stationary gas measurement. The integral under the response curve or the peak height of the response curve is then a measure of the gas content in the blood sample.

While there have been numerous publications on gas chromatography for determination of, for instance, CO_2 in blood serum, this technique has not found wide application in routine laboratory measurements. The technique is complex, requiring a significant amount of apparatus including the chromatography and column, together with recording equipment. Additionally, the method is very time consuming. Part of the time consumption is due to the burdens placed on the operator of the apparatus, who has to inject the blood sample, and then wait until the sample passes through the column and the detector. The operator then has to relate the recorder output to the signal from a calibration sample, all of which is time consuming and which can

lead to human error. The time involved means that results will seldom be available in time for them to be clinically useful. In this situation the gas chromatography cannot compete with electrochemical means of determining respiratory gases or with the non-invasive methods of pulse oximetry or analysis of exhaled gases with infrared or mass spectrometry equipment.

In addition to the disadvantages noted above, gas chromatography requires the use of a separation column which is damaged by the direct injection of blood specimens. This necessitates the use of a pre-column which can be discarded as required. Also, usually high temperatures are required in the gas chromatography resulting in vaporization of the liquid and pyrolysis of biologic and other material with the possibility of producing gases in the process perhaps including the gas it is sought to study. Gas chromatography also requires a carrier gas stream. This is a dynamic measurement rather than a static measurement, and is consequently more complex and is thought to be less reliable. With such a dynamic process, constant flow rates are required and transient responses have to be quickly recorded in order to provide accurate results. While our method using mass spectrometry also employs intermittent use of a carrier gas the flow rate merely affects the rate of washout of the gases under study and does not alter the final result. In addition it would be desirable to provide a technique which measures all gases simultaneously rather than one at a time.

In addition to the above disadvantages, using gas chromatography the carrier gas has to be a gas having a different thermal conductivity than the gas species to be detected, in

order that the measurement of the detected gas species is not altered by the presence of the carrier gas. It is for this reason that gases, e.g., He, which has a significantly different thermal conductivity than air, O_2, N_2, etc., are used.

Canadian Patent No. 1,138,226 patented December 28, 1982 by J.F. Muldoon provided an improvement with respect to electronic instrumentation associated with gas chromatography systems. The patent system included a gas processor for producing a time varying signal which was related to the constituents of the gas mixture. A converter sampled the time varying signal and converted it to digital form for providing a sampled data signal. A rate of change estimator provided a rate of change signal. The estimator include recursive digital feedback means coupled to an output of the estimator and also to the converter. In this manner, past time value signals of the estimated rate of change signal and of the sampled data signal were produced. The estimator further comprised means for combining the past time value signals with the sampled data signal for producing the estimated rate of change signal. Although this invention improved gas chromatography methods it did not improve the sample handling problems which exist when measuring gases contained in liquid.

U.S. Patent No. 2,987,912 patented June 13, 1961 by J. G. Jacobson provided an invention in the field of the determination of the amount of a gas dissolved in a liquid. The first steps in the patented method involved flushing the vessel with a neutral gas in a closed system and measuring the amount of the dissolved gas with a measuring means. The vessel was then filled

to a predetermined level with the liquid to provide a constant ratio of gas to liquid, while retaining the neutral gas in the system. The neutral gas was then circulated in the system in highly dispersed state through the liquid to extract dissolved gas from the liquid. The amount of gas dissolved in the liquid was indicated by the change in response of the measuring means after a predetermined length of time of circulation substantially shorter than needed for reaching equilibrium between the gas dissolved in the liquid and the extracted gas.

U.S. Patent No. 3,518,982 patented July 7, 1970 by R. S. Timmins et al, provided a device and method for monitoring gases in the blood stream. The patented method included the insertion of a catheter having a membrane of a material which was permeable to the gas to be measured in the blood stream. The membrane employed in the catheter had a significant rate of diffusion for at least one gaseous component of the blood stream which is to be analyzed. A gas stream of known composition and pressure, called a flush gas, was then introduced into the catheter and isolated in the chamber. Depending upon the partial pressure differences of the gaseous components on either side of the membrane wall, diffusion through the membrane occurred, which caused the normal pressure within the chamber to change with time. This pressure change was related to the concentration of the gases in the flush gas and in the blood stream to be analyzed. The pressure in the chamber at a given time was then determined, the number of pressure determinations made being at least equal to the number of gas components (n) to be analyzed in the blood stream, which components have a significant rate of

diffusion through the membrane wall of the catheter. Similar pressure determinations at a given time with additional flush gases of known composition and pressure were made to obtain a series of (n+1) pressure determinations. From these pressure determinations, the value of the actual characteristic mass transport function of the gas to be analyzed could then be determined. That value was termed the "calibration factor". This calibration factor for the patient and catheter was then employed to determine the quantitative level of the dissolved gases in the blood stream continuously or intermittently.

U.S. Patent No. 3,964,864 patented June 22, 1976 by H. Dahms provided for the determination of CO_2, or O_2 in body fluids, e.g., blood, and more particularly to an improved method and apparatus for performing such measurements. The patentee provided an improved technique for such determination of included the first step of reacting the sample and a reagent in a vessel to release CO_2 into a gas space filled with air at atmospheric pressure to produce a mixture of the released CO_2 and air. The gas space had a volume greater than the volume of sample in the vessel. At least a portion of the mixture in the gas space was transferred to a detector by adding a displacing liquid to the vessel. The concentration of the transferred gas mixture in the detector was then measured.

U.S. Patent No. 4,187,856 patented February 12, 1980 by L. G. Hall et al, provided a method for analyzing various gases in the blood stream. According to the patentee, the catheter provided with a blood-blocking membrane at its distal end was equipped with a very small tube throughout its lumen which

terminated in the area of the membrane. A "carrier" gas, e.g., helium, was introduced through the tube and against the interior surface of the membrane where it mixed with the blood gases passing through the membrane. The blood gases thus mixed with the carrier gas were under a small pressure and passed by viscous flow at a relatively-high speed through the tubing interconnecting the catheter with the sampling input leak of the mass spectrometer. Problems with this procedure, however, may include denaturation of proteins and blood components blocking the membrane, difficulties with calibration and diffusion of carrier gas into the blood stream.

In spite of these prior patents, there is a need for a rapid and effective means to calibrate and/or monitor and/or measure the level of gases in the blood stream, in tissues and in non-biologic materials. The prior art has not adequately solved the following problems, namely, certain determinations: were highly dependent on the skill of operator; used large amounts of toxic and expensive mercury, while gas interactions with mercury may not be known; were time consuming; and were prone to leaks and loss of sample during transfers of sample from one container to another.

(d) DESCRIPTION OF THE INVENTION

An object of one aspect of the present invention is to provide a method for the measurement of a specified gas carried in a liquid in a dissolved or suspended state, such method being simple, efficient, and applicable to continuous operation and control.

An object of another aspect of the present invention is to provide an apparatus which is suitable for the measurement of a specified gas carried in a liquid in a dissolved or suspended state, such apparatus being simple, efficient, and applicable to continuous operation and control.

An object of yet another aspect of this invention is to provide a method in which the determination of dissolved gas is accomplished rapidly and in a minimum of time as compared with prior procedures.

An object of yet another aspect of this invention is to provide apparatus for measuring the concentration of a gas which is dissolved in a liquid and which will have the potential to support a high degree of automation.

An object of yet another aspect of this invention is to provide a technique for low cost, reliable measurement of CO_2 and O_2 in body fluid samples, e.g., whole blood and blood serum.

An object of still another aspect of this invention is to provide an apparatus for the measurement of CO_2 and O_2 in body fluids, which apparatus can be easily cleaned, sterilized and flushed with gas after each measurement to provide increased reliability.

An object of a still further aspect of this invention is to provide a method for measuring CO_2 and O_2 in body fluids, e.g., blood, which does not require large sample volumes.

An object of another aspect of this invention is to provide a method for determining gas solubility and gas volumes in biological fluids or tissues which is not highly-dependent on the skill and technique of the experimenter and so would not suffer from inter-observer and intra-observer variations.

An object of another aspect of this invention is to provide an apparatus for determining gas solubility and gas volumes in biological fluids or tissues which is not highly-dependent on the skill and technique of the experimenter and so would not suffer from inter-observer and intra-observer variations.

An object of still another aspect of this invention is to provide a method for determining gas solubility and gas volumes in biological fluids or tissues which may be successfully-and-easily-used to measure inert, poisonous, or anaesthetic gas or to measure gas production from various biological reactions.

An object of still another aspect of this invention is to provide an apparatus for determining gas solubility and gas volumes in biological fluids or tissues which may be successfully-and-easily-used to measure inert, poisonous, or anaesthetic gas or to measure gas production from various biological reactions.

By broad aspects of the present invention, a new apparatus and a new method have been provided which are designed to overcome many of the above problems. A computer-controlled mass spectrometer is used as the primary measuring instrument. In contrast with the prior art, very small samples can be evaluated accurately and without contamination. The apparatus may be constructed so as to permit automatic cleaning

after each measurement. Further, the blood gas measured by the detector has essentially the same composition as that originally established in the vessel, thereby ensuring increased accuracy.

The new apparatus described above enables measurement of solubilities by providing a means of saturating the material with gas and includes means to remove the contained gas, thereby permitting measurement of the amount dissolved. All this is done without the need of transfers from one container to another.

Furthermore, the design of the apparatus in the form of a modified syringe enables blood or fluid specimens to be directly aspirated from the patient with no intermediate containment vessel. This prevents any gas loss and therefore reduces errors, permitting highly accurate and reliable research to be undertaken. The apparatus is easily disassembled and sterilized for repeated use with contaminated or potentially-infectious materials. The apparatus may be constructed of materials which are carefully chosen so as not to interact in any way with the gas or liquid under study.

By a first broad aspect of this invention, a gas investigation apparatus is provided to determine the amount of gas in a liquid, the apparatus, comprising: a hollow, longitudinally-extending cylindrical barrel for holding a liquid and a sample liquid with gas dissolved therewithin and with an associated gaseous headspace above, the barrel having a lower inlet and an upper outlet, at least a portion of the barrel being transparent; a plunger which is slidably-fitted in a leak-proof manner within the barrel, the barrel containing the sample liquid with the gas dissolved therewithin and with the gaseous headspace above, the plunger being free to slide along the longitudinal length of the barrel, thereby forming a variable volume of air and gas in the headspace above the liquid contained by the plunger in the barrel; a gas inlet tube having a lower inlet and an upper outlet within the plunger and extending along the longitudinal axis of the plunger; a longitudinally-extending, heat transfer jacket surrounding the hollow, longitudinally-extending cylindrical barrel for the regulation of the temperature of the liquid sample with the gas dissolved therewithin and with the gaseous headspace above, at least a longitudinally-extending portion thereof being transparent; an upper valve which is connected to the upper outlet of the barrel by means of a zero dead-space, butt-end

connection; a selective entry through the upper valve for admitting a study liquid or a tissue suspension into the barrel; a lower valve which is connected to the lower inlet of the gas inlet tube by means of a zero dead-space, butt-end connection; an inlet conduit for the selective introduction of test gas, a calibration gas, a carrier gas, or a flushing gas into the gas inlet tube through the lower valve; an inlet tube for the selective introduction of a gas sample into the inlet conduit means through the lower valve, and an exit tube which is connected to the upper valve by means of a zero dead-space, butt-end connection, for leading gas exiting from the plunger-position-dependent variable volume of gaseous space at the upper portion of the barrel to a mass spectrometer.

By one variant of this first broad aspect of this invention, the cylindrical barrel and the heat transfer jacket are entirely transparent. By one variation thereof, the cylindrical barrel and the heat transfer jacket are each made out of glass.

By a second variant of this first broad aspect of this invention and/or the above variant thereof, the plunger is formed of a rigid synthetic plastic material. By one variation thereof, the plunger is formed of an epoxy resin.

By a third variant of this first broad aspect of this invention and/or the above variants thereof, the plunger is provided with a central longitudinal bore having an inlet and an outlet, the gas inlet tube being situated within the bore.

By a fourth variant of this first broad aspect of this invention and/or the above variants thereof, the upper valve is a three-way valve or stop cock.

By a fifth variant of this first broad aspect of this invention and/or the above variants thereof, the lower valve is a three-way valve or stop cock.

By a sixth variant of this first broad aspect of this invention and/or the above variants thereof, the exit tube leading between the barrel and the spectrometer includes a further tube for the recycling of gas exiting the barrel back to the barrel.

By a seventh variant of this first broad aspect of this invention and/or the above variants thereof, the gas investigation apparatus includes a bank of interconnected valves which are connected to the further tube. By one variation thereof, one of the interconnected valves is connected to the lower valve.

By an eighth variant of this first broad aspect of this invention and/or the above variants thereof, the lower valve includes a gas injection port.

By a ninth variant of this first broad aspect of this invention and/or the above variants thereof, the transparent barrel is formed of a transparent synthetic plastic material, the plunger is formed of stainless steel, the heat transfer jacket is formed of transparent synthetic plastic material, and the inlet tube and the exit tube are each formed of polytetrafluoroethylene.

By a tenth variant of this first broad aspect of this invention and/or the above variants thereof, the syringe barrel is formed of transparent glass, the syringe plunger is formed of an epoxy resin, the heat transfer jacket is formed of a transparent acrylate resin, and the inlet tube and the exit tube are each formed of polytetrafluoroethylene.

By an eleventh variant of this first broad aspect of this invention and/or the above variants thereof, the gas investigation apparatus includes a temperature probe within the heat transfer jacket to measure the temperature of heat transfer liquid.

By a twelfth variant of this first broad aspect of this invention and/or the above variants thereof, the gas investigation apparatus includes a scavenging gas outlet tube which is connecting the upper valve of the barrel to an atmospheric pressure scavenging system by means of a zero dead-space butt-end connection.

By a thirteenth variant of this first broad aspect of this invention and/or the above variants thereof, the gas investigation apparatus includes a liquid trap and filter in the gas sampling exit tube between the exit tube from the barrel to the mass spectrometer.

By a fourteenth variant of this first broad aspect of this invention and/or the above variants thereof, the gas investigation apparatus includes an ultrasonic tissue disrupter which is operatively-associated with the heat transfer jacket and which is arranged proximate to the upper end of the barrel.

By a second broad aspect of this invention the combination is provided of (A) a gas investigation apparatus to determine the amount of gas in a liquid, the apparatus comprising: a hollow, longitudinally-extending cylindrical barrel for holding a liquid and a sample liquid with gas dissolved therewithin and with an associated gaseous headspace above, the barrel having a lower inlet and an upper outlet, at least a portion thereof being

transparent, a plunger which is slidably-fitted in a leak-proof manner within the barrel, the barrel containing the sample liquid with the gas dissolved therewithin and with the gaseous headspace above, the plunger being free to slide along the longitudinal length of the barrel, thereby forming a variable volume of air and gas in the headspace above the liquid contained by the plunger in the barrel; a gas inlet tube having a lower inlet and an upper outlet within the plunger and extending along the longitudinal axis of the plunger; a longitudinally-extending, heat transfer jacket surrounding the hollow, longitudinally-extending cylindrical barrel for the regulation of the temperature of the liquid sample with the gas dissolved therewithin and the gaseous headspace above, at least a longitudinally-extending portion thereof being transparent, an upper valve which is connected to the upper outlet of the barrel by means of a zero dead-space, butt-end connection, a selective entry through the valve for admitting a study liquid or tissue suspension into the barrel, a lower valve which is connected to the lower inlet of the gas inlet tube by means of a zero dead-space, butt-end connection; an inlet conduit for the selective introduction of test gas, a calibration gas, a carrier gas, or a flushing gas into the gas inlet tube through the lower valve, an inlet tube for the selective introduction of a gas sample into the inlet conduit means through the lower valve; and an exit tube which is connected to the upper valve by means of a zero dead-space, butt-end connection, for leading gas exiting the plunger-position-dependent variable volume of gaseous space from the upper portion of said barrel to a mass spectrometer; and (B) a mass spectrometer which is operatively-connected to the exit tube.

By many variants of this second aspect of the invention, the gas investigation apparatus is provided with any or all of the above-described variants and variations.

By a third aspect of this invention, a method is provided for determining the amount of gas in a biologic liquid, which method comprises admitting a definite volume of the biologic liquid into a sparging zone, controlling the temperature of the sparging zone to within a specified temperature range, passing a stream of carrier gas in the form of small bubbles through the sparging zone, passing a mixture of gases exiting from the sparging zone to a mass spectrometer, and determining the nature and quality of the

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individual gases in the mixture of gases by carrying out a mass spectrometer analysis thereon.

By one variant of this third method aspect of this invention, the biologic liquid is blood.

By a second variant of this third method aspect of this invention, the biologic liquid is a cellular material which is formed *in situ* by an ultrasonic nebulizer.

By a fourth aspect of this invention, a method is provided for determining the amount of gas in the blood or blood serum which comprises admitting a definite volume of the blood or blood serum into a sparging zone, controlling the temperature of the sparging zone to within a specified temperature range, passing a stream of carrier gas in the form of small bubbles through the sparging zone, passing a mixture of gases exiting from the sparging zone to a mass spectrometer, and determining the nature and quality of the individual gases in the mixture of gases by carrying out a mass spectrometer analysis thereon.

By one variant of the third and fourth method aspects of this invention, the sparging zone involves injecting a known weight of fluid corresponding to 2 ml to 4 ml volume at laboratory ambient temperature into an apparatus having a headspace gas of 20 ml to 40 ml.

By a second variant of the third and fourth method aspects of this invention, and/or the above variants thereof, the creation of the sparging zone involves opening the lower stop cock to admit carrier gas into the apparatus in order to sparge all other gases from the apparatus and the liquid, for 20 minutes, with the upper stop cock also remaining open.

By a third variant of the third and fourth method aspects of this invention, and/or the above variants thereof, the creation of the sparging zone involves opening the lower stop cock and admitting the study gas and immediately opening the upper stop cock to scavenging system, either by bubbling the study gas through liquid or passing the study gas over the surface at flow rates of 100 units/minute outflow for 20 minutes to 60 minutes.

By a fourth variant of the third and fourth method aspects of this invention, and/or the above variants thereof, the creation of the sparging zone involves opening the upper stop cock and raising the plunger to expel all gas remaining in the syringe plus a very small quantity of liquid which is collected onto a filter, and then closing the upper stop cock. By one variation thereof, the method includes the step of weighing a filter to determine the weight of fluid discarded and to calculate the weight of fluid remaining.

By a fifth variant of the third and fourth method aspects of this invention, and/or the above variants thereof, the creation of the sparging zone involves opening the lower stop cock and admitting the carrier gas and allowing the plunger to descend until 20 ml to 40 ml headspace is present, fixing the plunger and opening the upper stop cock towards the mass spectrometer, and maintaining a flow rate of 100 units/minute at the outlet.

By a sixth variant of the third and fourth method aspects of this invention, and/or the above variants thereof, the creation of the sparging zone involves continuing to sparge all test gas from the biologic fluid until the test gas signal on the mass spectrometer returns to its baseline value.

Advantages of the apparatus of aspects of this invention include the following: the provision of a syringe-type apparatus with an asymmetric placement of a fine bore inlet incorporated into plunger to enable gas to be admitted to the syringe by being passed above or by being bubbled through material in the syringe, depending on the orientation of the apparatus; plunger movement enables gas or liquid to be expelled from the apparatus while still maintaining ambient pressure; the use of ambient pressure input to the mass spectrometer; the provision of a transparent heat transfer jacket around the apparatus to maintain temperature while being able to observe the contents of the apparatus; and the allowing of equilibration of the material with gases, or the allowing of a biologic/chemical reaction to take place in the same container from which gas will be extracted by sparging with another gas.

Fundamentally the invention in its broadest aspects provides an apparatus which enables (using the methods described herein) the processing of biologic and non-biologic samples of liquids and suspensions such that the content of any gas contained therein may

be measured without errors caused by loss of gas to, or contamination by, atmosphere. This makes the apparatus particularly useful in diving and hyperbaric research when atmospheric gases are being studied. The apparatus is detector-independent and may be used with a variety of detection and measurement systems. The use of the gas amount and solubility investigation apparatus with a computer-controlled mass spectrometer will be illustrated below. Although developed for use in medical research the apparatus has numerous potential applications for industrial purposes where it is important to determine gas content in a liquid or suspension.

(e) BRIEF DESCRIPTION OF THE DRAWINGS

In the accompanying drawings,

Figure 1 is a schematic representation of the gas investigation apparatus of one embodiment of one aspect of this invention;

Figure 2 is a central longitudinal sectional view of the barrel plunge portion of another embodiment of another aspect of the gas investigation apparatus of this invention; and

Figure 3 is a flow chart for gas solubility measurement.

(f) AT LEAST ONE MODE FOR CARRYING OUT THE INVENTION

As seen in Figure 1, the gas investigation apparatus 10 includes a syringe barrel 11 which is provided with a solid plunger 12 which preferably is of solid epoxy plastics material which is slidably-fitted therein in a leak-proof manner in a manner known to those skilled in the art. A central bore 13 is formed longitudinally through the solid plunger 12. Sample liquid 45, which may be admitted before assembly of the solid plunger 12 within the syringe barrel 11, or injected afterwards, is shown near the upper part of the syringe barrel 11.

At the lower exposed end 14 of the solid plunger 12 is a hollow bolt 15 which is fitted with capillary tubing 16 which is formed, e.g., of nickel. The capillary tubing 16 is connected to tubing 17, e.g., of TEFLON$_{TM}$ (trade-mark for polytetrafluoroethylene) for the introduction of a test gas, or a carrier gas or a flushing gas, by means of a three-way, gas-tight stop cock 18, which is a zero dead-space butt-end junction 19. Such zero dead-space butt-end junction 19, as is well-known in the art, is an inert valve fitting and

adaptor which is individually-machined from an inert synthetic plastic material, e.g., KEL-F$_{TM}$, and which is specifically-designed to thread into a valve port providing a leak-tight seal at 100 psi. Such valve fitting, when so threaded, does not permit any trapped volume between the valve fitting and the valve port. An injection system 20 for gas calibration is provided on a three-way, gas-tight stop cock 18. The tubing 17, e.g., of TEFLON$_{TM}$ is connected to a bank 21 of stop cocks 22a, 22b, 22c, 22d, each being provided with a respective gas outlet 23a, 23b, 23c, 23d. By such means, a selected flushing gas, or a carrier gas or a test gas, may be introduced into the solid plunger 12.

The upper end of the syringe barrel 11 includes an opening which is fitted by means of a screw thread 24a to a three-way gas-tight stop cock 24 using a zero dead-space butt-end junction 25 (as previously-described). One outlet 26 of the three-way gas-tight stop cock 24 leads via tubing 27 to an atmospheric pressure scavenging system, which components are not shown in the drawings. The other outlet 28 of the three-way gas-tight stop cock 24 leads, via tubing 29, to a liquid trap 30, a filter 30a and then, by a connection to the three-way, gas-tight stop cock 31, using a zero dead-space butt-end junction 32, via outlet 33 of the three-way, gas-tight stop cock 31, using a zero dead-space butt-end junction 32 (as previously-described) to a mass spectrograph (not shown). The other outlet 34 of the three-way gas-tight stop cock 31 is connected via line 34a to the bank 21 of stop cocks 23a, 23b, 23c and 23d (previously-described).

A transparent water bath 35 surrounds the syringe barrel 11. The transparent water bath 35 includes a cylindrical wall 36 which is provided with water inlet line 37 and water outlet line 38. The upper and lower ends of the cylindrical wall 36 are each provided with annular walls 39,40, respectively, fitted thereto in a leak-proof fashion in a manner well-known to those skilled in the art. A temperature probe 41 and a thermometer 42 are also provided within the transparent water bath 35. An ultrasonic tissue disrupter 10a is provided in the region of the syringe barrel 11.

The description in FIG. 1 consequently shows entry means through an end of the syringe barrel for admitting a study liquid or tissue suspension. The entry means are shown to be located at the barrel end along with either the inlet tube means or the exit tube means.

As seen in Figure 2, the gas investigation apparatus 200 includes a transparent hollow cylindrical barrel 211, which is fitted with a cylindrical plunger 212, which is formed, e.g., of stainless steel. Cylindrical plunger 212 is provided with an off-centre capillary tube 213 for the inlet of gas. A gas-tight seal between the transparent hollow cylindrical barrel 211 and the cylindrical plunger 212 is provided by O-rings (not shown) which are fitted into circumferential O-ring grooves 214 in the cylindrical plunger 212.

A tubular jacket 215 is provided by means of a hollow transparent tube 216 which is disposed concentrically-around the transparent hollow cylindrical barrel 211. An annular base 217, which is formed e.g., of stainless steel, is threaded to the base end of the hollow transparent tube 216. A first inner gasket 218 is provided between the end of the hollow transparent tube 216 and the inner face of annular base 217, and a second inner gasket 218 is provided between the end of the transparent hollow cylindrical barrel 211 and the annular base 217. A hole 220 which is provided in the annular base 217 allows the cylindrical plunger 212 access into the transparent hollow cylindrical barrel 211. Annular base 217 is also provided with a water inlet 221.

A cap 222, which is formed e.g., of stainless steel, is threaded to the head-end of the hollow transparent tube 216. A third inner gasket 223 is provided between the end of the hollow transparent tube 216 and the inner face of cap 222, and a fourth inner gasket 224 is provided between the end of the transparent hollow cylindrical barrel 211 and the cap 222. Cap 222 is provided with a water outlet 225, and with an off-centre gas sampling tube 226.

As seen in Figure 3, the first step in the open solubility measurement, SET at block 310 is to set the mass spectrometer sensitivity. The next step INJECT at block 311 involves injecting a known weight of fluid at a known laboratory ambient temperature into the apparatus (e.g., 2 ml to 4 ml volume) with a headspace gas of 20 ml to 40 ml. The next step ADMIT CARRIER GAS at block 312 involves opening the lower stop cock to admit a carrier gas into the apparatus in order to sparge all other gases from the apparatus and from the liquid. This is continued for 20 minutes. The upper stop cock also remains open.

The apparatus is allowed to sit undisturbed for 30 minutes and both stop cocks are closed.

The next step ADMIT STUDY GAS at block 313 involves opening the lower stop cock and admitting the study gas, and immediately opening the upper stop cock to the scavenging system. The study gas may be bubbled through liquid or may be passed over the surface depending on the orientation of the apparatus. Gas flow rates are standardized to maintain 100 units/minute outflow. This is continued for 20 minutes to 60 minutes.

The upper and lower stop cocks are closed and the apparatus is allowed to sit undisturbed for 30 minutes. The fluid is observed with a magnifying lens to confirm the absence of bubbles.

The next step EXPEL GAS at block 314 involves opening the upper stop cock and raising the plunger to expel all gas remaining in the syringe, plus a very small quantity of liquid which is collected onto the filter. The upper stop cock is then closed.

The next step CALCULATE WEIGHT OF FLUID at block 315 involves weighing the filter to determine the weight of fluid which is discarded and to calculate the weight of fluid remaining.

The next step FLUSH TEST GAS at block 316 involves flushing all remaining test gas from lines.

The next step ADMIT CARRIER GAS at block 317 involves opening the lower stop cock and admitting the carrier gas, and allowing the plunger to descend until 20 ml to 40 ml headspace is present. Then, the plunger is fixed in place and the upper stop cock is opened towards the mass spectrometer. The flow rate of 100 units/minute at the outlet is maintained.

The next step TEST GAS SIGNAL BASELINE VALUE at block 318 involves continuing to sparge all test gas from the fluid until the test gas signal on the mass spectrometer returns to its baseline value.

The next step INJECT TEST GAS at block 319 involves injecting known volumes of test gas from the gas-tight syringe into the lower stop cock so that the test gas is flushed by the carrier gas through the liquid and towards the mass spectrometer.

The next step WAIT at block 320 involves waiting for the test gas signal to return to baseline.

The final step REPEAT at block 321 involves repeating the calibration procedures several times.

Thus, as described above, the present invention in its many aspects provides a new, more rapid and convenient method using spectrometry for the measurement of blood nitrogen (N_2) or other inert gases. The apparatus consists of a modified gas-tight syringe with stop cocks at the outlet of the barrel and also on a fine bore steel tube which traverses the plunger, creating a gas inlet.

The method of one aspect of the invention is highly-accurate and highly-reliable.

There are many variations of the method of aspects of this invention, for example the following:

A) For studies during compression/decompression, the blood is sampled directly into the apparatus or to use gas-tight syringes. Gas phase separation during decompression of the sample necessitates using the entire sample to ensure it was representative of the blood of the subject. Delicate measurements requires drawing two samples simultaneously.

B) The entire apparatus may be operated inside a hyperbaric chamber while flushing all effluent gas to a mass spectrometer outside the chamber.

C) The method of an aspect of this invention is useful in determining uptake and distribution of inert gases, including helium. It is known that carbon monoxide interferes with nitrogen measurement.

A

In general terms, one variant of the apparatus of this invention comprises a glass gas-tight syringe modified by drilling a hole in the end of the plunger and installing fine bore nickel tubing to traverse the barrel of the plunger. With this arrangement it is possible to fill the syringe with gas from either end. The syringe is surrounded by a water jacket constructed from a clear acrylate plastic (e.g., PLEXIGLASS$_{TM}$) cylinder. This arrangement enables the contents of the syringe and the volume scale on the barrel to be easily observed. Water is circulated between a thermostatically controlled water bath and the apparatus using a pump. Water in the water bath is continuously filtered using an aquarium filter to ensure that it remains clear and free of contaminants so that the interior of the apparatus is easily observed at all times. The interior temperature of the cylindrical water jacket is continuously monitored using a thermistor. The temperature of the interior of the syringe remains within 0.2°C of the temperature of the surrounding water. A three-way gas-tight stopcock is attached to each end of our apparatus using zero dead-space butt junctions. Inert TEFLON$_{TM}$ (polytetrafluoroethylene) tubing is used for all other connections. Several other stopcocks were attached to the lower stopcock on the apparatus without the need of making any connections or disconnections at all during measurements.

In another embodiment of this invention, the apparatus consists of glass syringes of any size which are modified to incorporate the following features.

The end of the plunger is cut off and a small hole is drilled in the flat top of the other end of the plunger which will be inside the syringe barrel and in contact with the liquid. Fine bore metal tubing is inserted into the plunger to join the hole. The barrel of the plunger is filled with liquid epoxy to hold the fine bore tubing in place. A hollow bolt is inserted into the epoxy at the end of the plunger. This enables other apparatus to be screwed onto the end of the plunger.

In another embodiment of this invention, the apparatus consists of special modifications to glass gas-tight syringes of any size. The modifications are similar to those described above but in this case the plunger is made of metal and the gas tight seal on the original equipment is made using gas-tight TEFLON$_{TM}$ seals. A hole is drilled into the TEFLON$_{TM}$ cap and also in the metal of the plunger base. Fine-bore tubing (about 0.01 inch bore) traverses the plunger and is silver soldered into a machined metal cap. This cap forms a seal on the TEFLON$_{TM}$ cap. The other end of the fine bore tube is held on the base of the plunger with a screw mechanism which tensions the fine bore tube ensuring that the metal cap in contact with the TEFLON$_{TM}$ is gas tight. This version of the apparatus is most satisfactory when using highly diffusible gases and non-viscous liquids or when measuring gases which have the same nominal molecular weight as atmospheric gases, leaks of which would otherwise be difficult to detect.

The barrel of the syringe is modified by incorporating a hollow bolt around a LUER$_{TM}$ lock and this is fixed in place with epoxy. This enables other apparatus to be screwed to the top of

the syringe. With this arrangement, a bracket may be installed on the plunger and the barrel of the syringe. These brackets may be connected to each other using a variety of devices, for instance a sliding micrometer for accurate measurement of movement of the plunger or a motorized screw mechanism so that the plunger may be remotely controlled.

The whole syringe arrangement is surrounded by a clear PLEXIGLASS$_{TM}$ cylinder closed at each end with rubber. This cylinder has three PLEXIGLASS$_{TM}$ tubes of smaller diameter installed through the side. Two of these are used to circulate water to and from the inside of the cylinder to a thermostatically controlled water bath. A small water pump is used for this purpose. The third port is used for inserting a thermistor for measuring the temperature. This arrangement enables precise control of the temperature of the inside of the apparatus. The contents of the syringe and the volume gradations on the syringe barrel may be easily observed through the water jacket.

Gas-tight stopcocks are installed onto the LUER$_{TM}$ lock of the syringe barrel (or preferably a zero deadspace arrangement, e.g., a butt junction) and to the fine bore tubing emerging from the other end of the plunger. This arrangement enables any gas to be introduced or removed from either end of the syringe.

In one manner of use of this apparatus, the study liquid or tissue suspension is pipetted into the syringe and allowed to equilibrate to water jacket temperature for twenty minutes (equilibration usually occurs within 10 minutes). A number of factors affect the choice of volume of liquid to be used, but

usually 2 - 4 ml is satisfactory. However, substantially smaller volumes may be used providing such volumes are measured with great precision. A carrier gas of a different nominal molecular weight to the gas under investigation is admitted into the syringe through the fine bore tubing traversing the plunger. The plunger is then withdrawn to allow adequate headspace above the surface of the liquid. The carrier gas is then bubbled through the liquid to ensure that no test gas is in solution and this may be confirmed by the absence of detectable amounts of the test gas as measured using mass spectrometry of the effluent gas. Protein containing fluids may be denatured by vigorous bubbling. This is avoided by turning the apparatus horizontally and passing the carrier gas over the surface of the liquid at a slow flow rate to avoid evaporation of water. This method requires a longer time to ensure complete removal of the trace amounts of any test gas which may already be present in the fluid. The test gas is then admitted to the syringe apparatus from below. Two basic methods have been used for saturizing the liquid with gas, namely bubbling and incubation. A third method was to combine these two.

In the bubbling mode, the test gas is bubbled through the liquid under study and this results in saturation within twenty minutes in all cases, and usually within five or ten minutes. The duration of time required varies according to a number of factors, namely: rate of gas flow/bubbling; solubility of the gas in the liquid; diffusibility of the gas; volume of liquid; volume of syringe used; and size of bubbles employed.

In addition to bubbling, the whole apparatus may be shaken by a mechanical shaker to improve mixing. Although many factors may determine the time needed to attain saturation it is easy to determine for each combination of circumstances how long is required by finding the time after which no further accumulation of gas in solution occurs as determined during the measurement stage. However, it has been found that fifteen minutes of bubbling is more than enough for even the most insoluble gas. The potential concerns with this method are that any bubbles of test gas which are not dissolved will be measured during the extraction stage and thus give a falsely high reading. In addition, it is known that the pressure inside bubbles, especially small bubbles is higher than the gas tension and hydrostatic pressure in the liquid and this leads to the possibility of supersaturation occurring inadvertently. However, by using the atmospheric pressure incubation mode described below it has been shown that the bubbling mode as described does not result in supersaturation with any gas.

In the incubation mode, the whole apparatus can be rotated so that it is horizontal, while still being clamped to a electric shaking machine. When incubation is being used for equilibration, a 50 ml syringe apparatus is employed in which the fine bore tubing is installed asymmetrically near one side of the plunger. In this way, when the apparatus is horizontal on one side test gas can flow over the top of the liquid without bubbling through it. Shaking the apparatus then causes waves on the surface of the liquid but no bubbling. Incubation requires a longer period of time for full saturation of the test liquid

but obviates concerns of supersaturation so long as atmospheric pressure is maintained within the syringe. By this means it was shown that supersaturation does not occur with bubbling methods described above because no further solution of gases occurred as compared with ambient pressure incubation modes.

All effluent gases are scavenged to a passive atmospheric system and it was shown that pressure inside the syringe never exceeded 2 mm water above atmospheric.

In the use of the present apparatus, gas in solution is removed by sparging the fluid with a carrier gas of different nominal molecular weight to the gas under study. This method has been employed for many years for de-gassing fluids but has not been little used for extracting gas for measurement. There are several mechanisms whereby this process works. Firstly, bubbles of carrier gas contain none of the study gas and therefore the latter will diffuse into the bubbles. Bubbles are buoyant and rise to the surface and burst thereby releasing study gas into the headspace above the surface of the liquid. There may also be a streaming effect whereby the study gas is carried along in the stream of gas flow, especially when using non-volatile solvents. In addition it is possible that vigorous bubbling imparts additional kinetic energy to the gas molecules enhancing diffusion. The constant stream of carrier gas also flushes the headspace of any test gas to that a diffusion gradient exists between the gas phase and the liquid phase near the surface. As gas diffuses from the surface this creates a concentration gradient within the liquid which will result in diffusion of gas

in solution towards the surface. The constant stream of bubbles also has a stirring effect.

In another method of use of this apparatus, the apparatus may be used for multiple purposes and these will be illustrated by referring to its use for measuring the solubility of a gas in a liquid.

The liquid is placed inside the syringe. The apparatus is held in a vertical position by a clamp on the water jacket, and if desired this can be attached to a laboratory stand or electric shaking equipment. A separate clamp holds the plunger in the desired position.

The test gas is introduced via the stopcock and fine bore tubing and bubbles through the liquid from the bottom. A gas head space exists above the surface of the liquid and excess gas exits through the top and the stopcock and out to an atmospheric scavenging system. When full equilibration has occurred both stopcocks are closed and the apparatus is allowed to sit undisturbed to ensure that all bubbles in the fluid have dissipated. After this the plunger of the syringe is elevated and the head space gas and a very small quantity of the test liquid is expelled through the stopcock towards the scavenging system. Then a carrier gas which is different than the test gas is introduced through the fine bore tubing to once again create a head space of gas above the surface of the liquid. The position of the stopcock on the luer-lock is changed so that all gas flowing out of the top of the syringe and the stopcock is directed towards the measuring apparatus rather than the scavenging system. The carrier gas is then bubbled through the

test liquid from below and this sparges all of the test gas out of the liquid so that the amount may be measured, for instance using a mass spectrometer or gas chromatography.

By incorporating an ultrasonic nebulizer into the new apparatus it is possible in addition to disrupt cells and tissues in a controlled gas environment or to study gas uptake into or production by tissues. Studies with blood are easily undertaken by the addition of anti-foaming agents. The apparatus may also be used to measure gases in non-biological fluids such as petrochemicals.

As described above, the present invention has provided a novel apparatus which enables all reactions, equilibrations, extractions and calibrations to be made using the same container. This reduces the risks of leaks and losing the gases under investigation during transfers. Among the advantages of this apparatus are the following: the apparatus has low/zero deadspace enabling high precision measurements for scientific research; biological or chemical reactions, gas equilibration and gas extraction all take place using the same container, so that the transfers between vessels are required, thereby reducing the risks of leaks or loss of sample; the contents of the apparatus may be easily viewed at all times; only a small sample of liquid or tissue is required, usually 5 ml or less; ambient pressure is maintained at all times, thereby avoiding leaks of atmospheric gases into the apparatus and leaks out of the apparatus; excellent temperature control and stability is maintained; the asymmetric placement of fine-bore tubing through the plunger of the syringe enables gas to either bubble through the liquid or

to flow over the surface simply by tilting the apparatus so that a combination of the bubbling and incubation methods of gas equilibration may also be used; using the multiple ion monitoring mode on the mass spectrometer with the apparatus enables leaks of atmospheric gases into the apparatus to be detected. The apparatus may be used for liquids, colloids, blood, or tissue suspensions; an ultrasonic tissue disrupter may be incorporated into the plunger of the syringe apparatus enabling homogenates to be prepared without exposure to the atmosphere; the apparatus may be used for volatile liquids by using cold water to cool the apparatus; the apparatus may be used for determining solubility or gas content in a liquid; the apparatus may be used for highly toxic gases as leaks are readily detected and effluent gas is readily scavenged; and because ambient pressure is maintained at all times, the risks of leaks out of the apparatus is minimized; direct contact of carrier gas with the liquid under study permits very efficient extraction of gas; the plunger may be moved to expel one gas or liquid from the apparatus and admit another, which enables more rapid equilibration of gas with liquid, and since the liquid never comes into contact with gas or atmosphere other than that selected, once equilibration has occurred there is no risk of losing gas from solution to another gas space prior to measurement of gas content; no liquid comes into contact with the study gas or study liquid and therefore there is no risk of losing gas into solution into another liquid; the whole process may be automated; the apparatus may be conveniently used for measuring solubility of content of inert, toxic, anaesthetic or other gases in fluids and tissue suspensions; atmospheric

pressure may be maintained without interfering with the reaction inside the apparatus; hermetic sealing is easily accomplished enabling even highly toxic gases to be studied; no vacuum extraction is required and the test liquids and gases need not be transferred from one container to another with all the inherent risks of leaks and loss of test material; for many gas-liquid combinations, the apparatus and methodology enables much faster measurements than those in current use; the temperature may be carefully controlled; the contents of the apparatus may be easily observed during any experiment; different carrier and test gases may be introduced into either end of the apparatus without the need for making any connections or disconnections, which is important for instance when working with toxic gases or with gases which are abundant in the atmosphere, since any leaks in these situations would be either dangerous or introduce significant errors into the experiment; biological reactions which produce gas may be studied, for instance by having tissue or enzyme suspensions in the syringe; measurement of virtually any gas may be used when used with a mass spectrometer, including the amount of gas that is contained in another material; it provides means to saturate a material with any gas, including atmospheric and toxic gases and determine solubility; it provides convenient, leak-free handling of gas; it may be used with toxic gases; it doesn't employ toxic mercury; it could be used in industries, e.g., petro chemicals, where gases must be measured in liquids; it could be used clinically to measure toxic gases in blood; the apparatus is most suitable for use with continuous flow gas analyzers such as a mass spectrometer and requires

modification for use with intermittent apparatus such as gas chromatographs; the plunger may be machined from stainless steel (or other materials) and fitted with double inert O-rings, or double TEFLON$_{TM}$ seals as commonly used in gas-tight syringes, to create gas-tight seal; the barrel of the apparatus and the water jacket may be made from glass, polycarbonate or other materials; the top of the barrel may have various parts machined from stainless steel and fitted to apparatus using O-ring seal.

When the nominal molecular weight of a gas under research is the same as another gas present in the system it is possible usually for research purposes and using the gas investigation apparatus here described to use a non-radioactive isotope of the gas to permit easier separation and measurement.

The apparatus and method of aspects of the present invention may be used in laboratories in the fields of medicine toxicology, biology and chemistry, whenever it is necessary to measure the solubility or content of any gas in a liquid or tissue suspension, e.g., blood. Current methods require multiple transfers of the test liquid and/or vacuum extraction of the gas. Leaks may occur at each step and it is difficult to measure the pressure inside the reaction or test vessels and thereby calculate solubility coefficients or convert to standard temperature and pressure (STP). With our apparatus the reaction or gas equilibration may take place in the same container that the gas will be extracted from while maintaining atmospheric pressure. This enables greater accuracy and sensitivity when measuring the gas using, for instance, a mass spectrometer or gas chromatography.

The apparatus is a specially constructed syringe with water jacket, zero dead-space stopcocks and fittings, with gas inlets and other apparatus incorporated into the moveable plunger. It is used to saturate liquids, cell suspensions or other materials with gases and/or extract gases from the aforementioned to enable their measurement. All this is done without contaminating the interior of the apparatus with atmosphere or vice-versa.

Leaks may exist from the syringe apparatus or its connections. To check for this possibility the following methods were used.

The whole apparatus is tested for leaks from the apparatus in the following manner. The whole apparatus is submerged so that it was covered by one centimeter of water. The apparatus is then pressurized to 300 mmHg above ambient. Ambient pressure was measured with a mercury barometer. The reservoir of the barometer was connected to one of the stopcocks of our apparatus using TEFLON$_{TM}$ tubing and the pressure inside was increased by admitting various gases (including helium) into the equipment until the pressure approximated 300 mmHg above ambient. All parts of the apparatus were carefully inspected for bubbles. A similar procedure was performed with the apparatus in air by brushing soap solution over all connections and the syringe barrel and observing for bubbles. Lastly, when pressurized with helium the air around the apparatus was sampled by the mass spectrometer.

In order to test for leaks into the apparatus in the following manner, the mass spectrometer was used in multiple ion monitoring mode and at least one atmospheric gas was monitored

during every measurement. If nitrogen was being studied then oxygen was also monitored and vice-versa. If other gases were being studied then oxygen and/or nitrogen was also monitored. Thus, leaks of atmospheric gases into the apparatus were readily apparent simply by measuring an atmospheric gas of different nominal molecular weight to the gas being studied. In addition, while conducting calibrations and experiments a flow of either carbon dioxide or helium was directed towards the base of the syringe apparatus (considered the most likely place for a leak to occur) and the presence of this gas in the syringe effluent was determined by the mass spectrometer. This procedure was repeated after reducing the pressure inside the apparatus to 300 mmHg below ambient. Finally, with the apparatus still at 300 mmHg below ambient pressure the entire assembly was submerged below water to a depth of ten centimeters and held down for ten minutes. After being removed from the water the interior of the apparatus was flushed with dry argon gas and the effluent monitored for the presence of water vapour.

Calibration for gas volume is performed under conditions which closely resembled those of each experiment. Between six and nine calibrations were performed immediately following each experimental run using known volumes of the test gas injected from a gas tight syringe using volumes which spanned the anticipated volume of test gas dissolved in the liquid. This was done using two different methods. In each, the calibration volume was injected through a septum in stopcock (i.e. below the syringe). In the first method this calibration volume was immediately flushed through the liquid and the apparatus using

an appropriate carrier gas. In the second method, the headspace of carrier gas in the syringe was reduced to approximately 1 ml and the upper stopcock was closed. The calibration volume was then injected into the septum of the lower stopcock as explained above and this was then flushed into the apparatus by allowing a few bubbles of carrier gas to enter the apparatus. The calibration volume was then allowed to dissolve in the liquid for ten minutes before being sparged in the usual way. The reason for using both these methods was that the shape of the curve obtained was different in each case and it was important to ascertain that the calculated areas and therefore the volume was the same in each case. The shape of the area curves using the second method more closely resembled those obtained on experimental runs than when using the much quicker first method.

All areas were calculated using a computer integration program. The accuracy of this integration was checked in a number of ways. Firstly, calibration injections were performed in a variety of ways, using different injection rates and different stand times for the same volumes. Also, sample volumes were injected by one author in a blinded manner while another author was responsible for gas analysis and calculation of area and therefore of injected volume. Lastly, the partial pressure-time curves were printed on a dot-matrix printer and the area calculated manually by using Simpson's rule and also by weighing the cut-out paper curves on an accurate balance. The type of calibration injection was shown to have no effect on the volumes measured.

It has been found that supersaturation does not occur providing that atmospheric pressure is maintained inside the apparatus. In addition, it is useful to allow the fluid to sit undisturbed for 10 or 20 minutes after equilibration with test gas to ensure that neither supersaturation nor persistent bubbles affect measurements.

If it is necessary only to measure the content of gas in a fluid, for example, the volume of nitrogen contained in a blood specimen, then equilibration procedures are not required. In this instance, all measurements take 15 minutes or less. This is much faster than existing methods.

CLAIMS

1. A gas investigation apparatus to determine the amount of gas in a liquid, said apparatus comprising:

(a) a hollow, longitudinally-extending cylindrical barrel for holding a liquid and a sample liquid with gas dissolved therewithin, and with an associated gaseous headspace above, said barrel having a lower inlet and an upper outlet, at least a portion thereof being transparent;

(b) a plunger which is slidably-fitted in a leak-proof manner within said barrel, said barrel containing said sample liquid with said gas dissolved therewithin, and with said gaseous headspace above, said plunger being free to slide along the longitudinal length of said barrel, thereby forming a variable volume of air and gas in said headspace above said liquid which is contained by said plunger in said barrel;

(c) a gas inlet tube having a lower inlet, and an upper outlet within said plunger and extending along the longitudinal axis of said plunger;

(d) a longitudinally-extending, heat transfer jacket surrounding said hollow, longitudinally-extending cylindrical barrel, for the regulation of the temperature of said liquid sample with said gas dissolved therewithin and with said gaseous headspace above, at least a longitudinally-extending portion thereof being transparent;

(e) an upper valve which is connected to said upper outlet of said barrel by means of a zero dead-space, butt-end connection;

(f) selective entry means through said upper valve for admitting a study liquid or a tissue suspension into said barrel;

(g) a lower valve which is connected to said lower inlet of said gas inlet tube by means of a zero dead-space, butt-end connection;

(h) inlet conduit means for the selective introduction of a test gas, a calibration gas, a carrier gas, or a flushing gas into said gas inlet tube through said lower valve;

(i) inlet tube means for the selective introduction of a gas sample into said inlet conduit means through said lower valve; and

(j) exit tube means which is connected to said upper valve by means of a zero dead-space, butt-end connection, for leading gas exiting from said plunger-position-dependent variable volume of gaseous space at the upper portion of said barrel to a mass spectrometer.

2. The gas investigation apparatus of claim 1, wherein said cylindrical barrel and said heat transfer jacket are entirely-transparent.

3. The gas investigation apparatus of claim 2, wherein said cylindrical barrel and said heat transfer jacket are each made out of glass.

4. The gas investigation apparatus of claims 1 to 3, wherein said plunger is formed of a rigid synthetic plastic material.

5. The gas investigation apparatus of claims 1 to 3, wherein said plunger is formed of an epoxy resin.

6. The gas investigation apparatus of claims 1 to 5, wherein said plunger is provided with a central longitudinal bore having an inlet and an outlet, and within said bore of which said gas inlet tube is situated.

7. The gas investigation apparatus of claims 1 to 6, wherein said upper valve is a three-way valve or stop cock.

8. The gas investigation apparatus of claims 1 to 7, wherein said lower valve is a three-way valve or stop cock.

9. The gas investigation apparatus of claims 1 to 8, wherein said exit tube leading between said barrel and said spectrometer includes a further tube for the recycling of gas exiting from said barrel back into said barrel.

10. The gas investigation apparatus of claims 1 to 9, including a bank of interconnected valves which is connected to said further tube.

11. The gas investigation apparatus of claim 10, wherein one of said interconnected valves is connected to said lower valve.

12. The gas investigation apparatus of claims 1 to 11, wherein said lower valve includes a gas injection port.

13. The gas investigation apparatus of claims 1 to 12, wherein said transparent barrel is formed of a transparent synthetic plastic material; wherein said plunger is formed of stainless steel; wherein said heat transfer jacket is formed of transparent

synthetic plastic material; and wherein said inlet tube means and said exit tube means are each formed of polytetrafluoroethylene.

14. The gas investigation apparatus of claims 1 to 12, wherein said syringe barrel is formed of transparent glass; wherein said syringe plunger is formed of an epoxy resin; wherein said heat transfer jacket is formed of a transparent acrylate resin; and wherein said inlet tube means and said exit tube means are each formed of polytetrafluoroethylene.

15. The gas investigation apparatus of claim 13, including a temperature probe within said heat transfer jacket to measure the temperature of a heat transfer liquid which is contained therein.

16. The gas investigation apparatus of claims 1 to 15, including a scavenging gas outlet tube connecting the upper valve of said barrel to an atmospheric pressure scavenging system by means of a zero dead-space butt-end connection.

17. The gas investigation apparatus of claims 1 to 16, including a liquid trap and filter in said gas sampling exit tube between exit tube means from said barrel to said mass spectrometer.

18. The gas investigation apparatus of claims 1 to 17, including an ultrasonic tissue disrupter which is operatively-associated with said cylindrical heat transfer jacket and which is arranged proximate to an upper end of said barrel.

19. In combination:

A) a gas investigation apparatus to determine the amount of gas in a liquid, said apparatus comprising:

(a) a hollow, longitudinally-extending cylindrical barrel for holding a liquid and a sample liquid with gas dissolved therewithin and with an associated gaseous headspace above, said barrel having a lower inlet and an upper outlet, at least a portion thereof being transparent;

(b) a plunger slidably-fitted in a leak-proof manner within said barrel said barrel containing said sample liquid with said gas dissolved therewithin and with said gaseous headspace above, said plunger being free to slide along the longitudinal length of said

barrel, thereby forming a variable volume of air and gas in said headspace above said liquid contained by said plunger in said barrel;

(c) a gas inlet tube having a lower inlet and an upper outlet within the plunger and extending along the longitudinal axis of said plunger;

(d) a longitudinally-extending, heat transfer jacket surrounding said hollow, longitudinally-extending cylindrical barrel for the regulation of the temperature of said liquid sample with said gas dissolved therewithin and with said gaseous headspace above, at least a longitudinally-extending portion thereof being transparent;

(e) an upper valve connected to said upper outlet of said barrel by means of a zero dead-space, butt-end connection;

(f) selective entry means through said valve for admitting a study liquid or tissue suspension into the barrel;

(g) a lower valve connected to said lower inlet of said gas inlet tube by means of a zero headspace, butt-end connection;

(h) inlet conduit means for the selective introduction of a test gas, a calibration gas, a carrier gas, or a flushing gas into said gas inlet tube through said lower valve;

(i) inlet tube means for the selective introduction of a gas sample into said inlet conduit means through said lower valve; and

(j) exit tube means connected to said upper valve by means of a zero dead-space, butt-end connection, for leading gas exiting the plunger-position-dependent variable volume of gaseous space from the upper portion of said barrel to a mass spectrometer; and

B) a mass spectrometer which is operatively-connected to said exit tube means.

20. The combination as claimed in claim 19, wherein said gas investigation apparatus is one wherein said cylindrical barrel and said heat transfer jacket are entirely-transparent.

21. The combination as claimed in claim 19, wherein said gas investigation apparatus is one wherein said cylindrical barrel and said heat transfer jacket are each made out of glass.

22. The combination as claimed in claims 19 to 21, wherein said gas investigation apparatus is one wherein said plunger is formed of a rigid synthetic plastic material.

23. The combination as claimed in claim 2, wherein said plunger is formed of an epoxy resin.

24. The combination as claimed in claims 19 to 23, wherein said gas investigation apparatus is one wherein said plunger is provided with a central longitudinal bore having an inlet and an outlet, and within said bore of which said gas inlet tube is situated.

25. The combination as claimed in claims 19 to 24, wherein said gas investigation apparatus is one wherein said upper valve is a three-way valve or stop cock.

26. The combination as claimed in claims 19 to 25, wherein said gas investigation apparatus is one wherein said lower valve is a three-way valve or stop cock.

27. The combination as claimed in claims 19 to 26, wherein said gas investigation apparatus is one wherein said exit tube leading between said barrel and said spectrometer includes a further tube for the recycling of gas exiting said barrel back to said barrel.

28. The combination as claimed in claims 19 to 27, wherein said gas investigation apparatus is one including a bank of interconnected valves connected to said further tube.

29. The combination as claimed in claim 28, wherein said gas investigation apparatus is one wherein one of said interconnected valves is connected to said lower valve.

30. The combination as claimed in claims 19 to 29, wherein said gas investigation apparatus is one wherein said lower valve includes a gas injection port.

31. The combination as claimed in claims 19 to 30, wherein said gas investigation apparatus is one wherein said transparent barrel is formed of a transparent synthetic plastic material; wherein said plunger is formed of stainless steel; wherein said

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heat transfer jacket is formed of transparent synthetic plastic material; and wherein said inlet tube means and said exit tube means are each formed of polytetrafluoroethylene.

32. The combination as claimed in claims 19 to 30, wherein said gas investigation apparatus is one wherein said syringe barrel is formed of transparent glass; wherein said syringe plunger is formed of an epoxy resin; wherein said heat transfer jacket is formed of a transparent acrylate resin; and wherein said inlet tube means and said exit tube means are each formed of polytetrafluoroethylene.

33. The combination as claimed in claim 31 or 32, wherein said gas investigation apparatus is one including a temperature probe within said heat transfer jacket to measure the temperature of a heat transfer liquid which is contained therein.

34. The combination as claimed in claims 19 to 33, wherein said gas investigation apparatus is one including a scavenging gas outlet tube connecting an upper valve of said barrel to an atmospheric pressure scavenging system by means of a zero dead-space butt-end connection.

35. The combination as claimed in claims 19 to 34, wherein said gas investigation apparatus is one including a liquid trap and filter in said gas sampling exit tube between exit tube means from said barrel to said mass spectrometer.

36. A method of determining the amount of gas in a biologic liquid which comprises:

(a) admitting a definite volume of said biologic liquid into a sparging zone;

(b) controlling the temperature of said sparging zone to within a specified temperature range;

(c) passing a stream of carrier gas in the form of small bubbles through said sparging zone;

(d) passing a mixture of gases exiting from said sparging zone to a mass spectrometer; and

(e) determining the nature and quality of the individual gases in said mixture of gases by carrying out a mass spectrometer analysis thereon.

37. The method of claim 36, wherein said biologic liquid is blood.

38. The method of claim 36, wherein said biologic liquid is a cellular material which is formed *in situ* by an ultrasonic nebulizer.

39. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone involves injecting a known weight of fluid corresponding to 2 ml to 4 ml volume at laboratory ambient temperature into an apparatus having a headspace gas of 20 ml to 40 ml.

40. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone involves opening the lower stop cock to admit a carrier gas into the apparatus in order to sparge all other gases from the apparatus and the liquid, for 20 minutes, within the upper stop cock also remaining open.

41. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone involves opening the lower stop cock and admitting the study gas and immediately opening the upper stop cock to the scavenging system, either by being bubbled through liquid or by being passed over the surface at flow rates of 100 units/minute outflow for 20 minutes to 60 minutes.

42. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone involves opening the upper stop cock and raising the plunger to expel all gas remaining in the syringe plus a very small quantity of liquid which is collected onto a filter and then closing the upper stop cock.

43. The method as claimed in claims 42, wherein said sparging zone involves weighing said filter to determine the weight of fluid discarded and to calculate the weight of fluid remaining.

44. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone involves opening the lower stop cock and admitting the carrier gas and allowing the plunger to descend until 20 ml to 40 ml headspace is present, fixing the plunger and opening the upper stop cock towards the mass spectrometer, and maintaining the flow rate of 100 units/minute at the outlet.

45. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone involves continuing to sparge all test gas from the fluid until the test gas signal on the mass spectrometer returns to its baseline value.

46. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone involves injecting a known volume of test gas from said gas-tight syringe into said lower stop cock so that said test gas is flushed by said carrier gas through said liquid and towards said mass spectrometer.

47. The method as claimed in claims 36 to 38, wherein the creation of said sparging zone includes removing all nitrogen from said apparatus by flushing with argon; injecting a blood specimen into the apparatus; removing nitrogen and other gases from the specimen by sparging with argon carrier gas; and injecting a known volume of air at said lower stop cock and sparging said known volume of air through said apparatus to calibrate the response of said mass spectrometer.

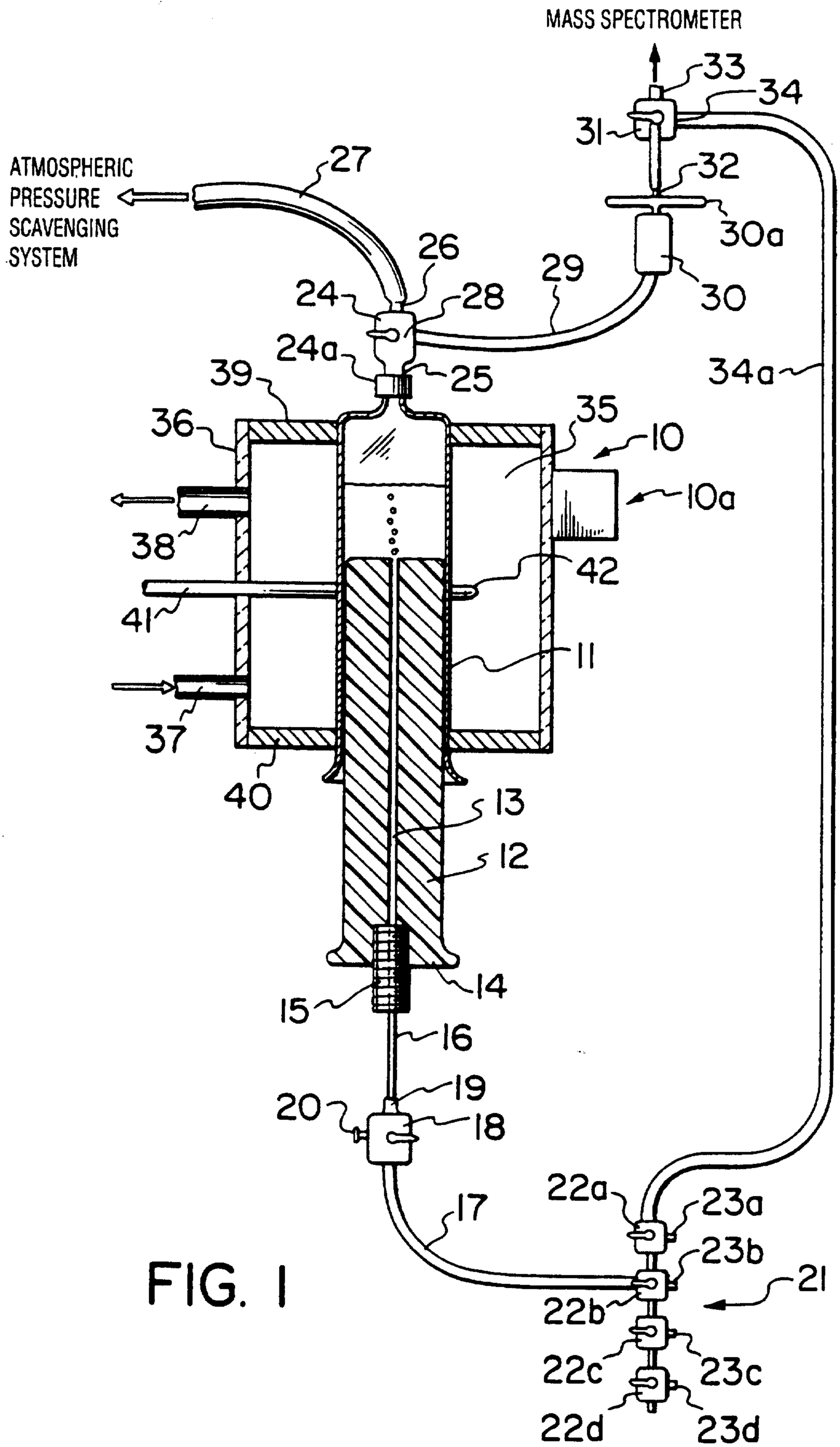
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FIG. 1

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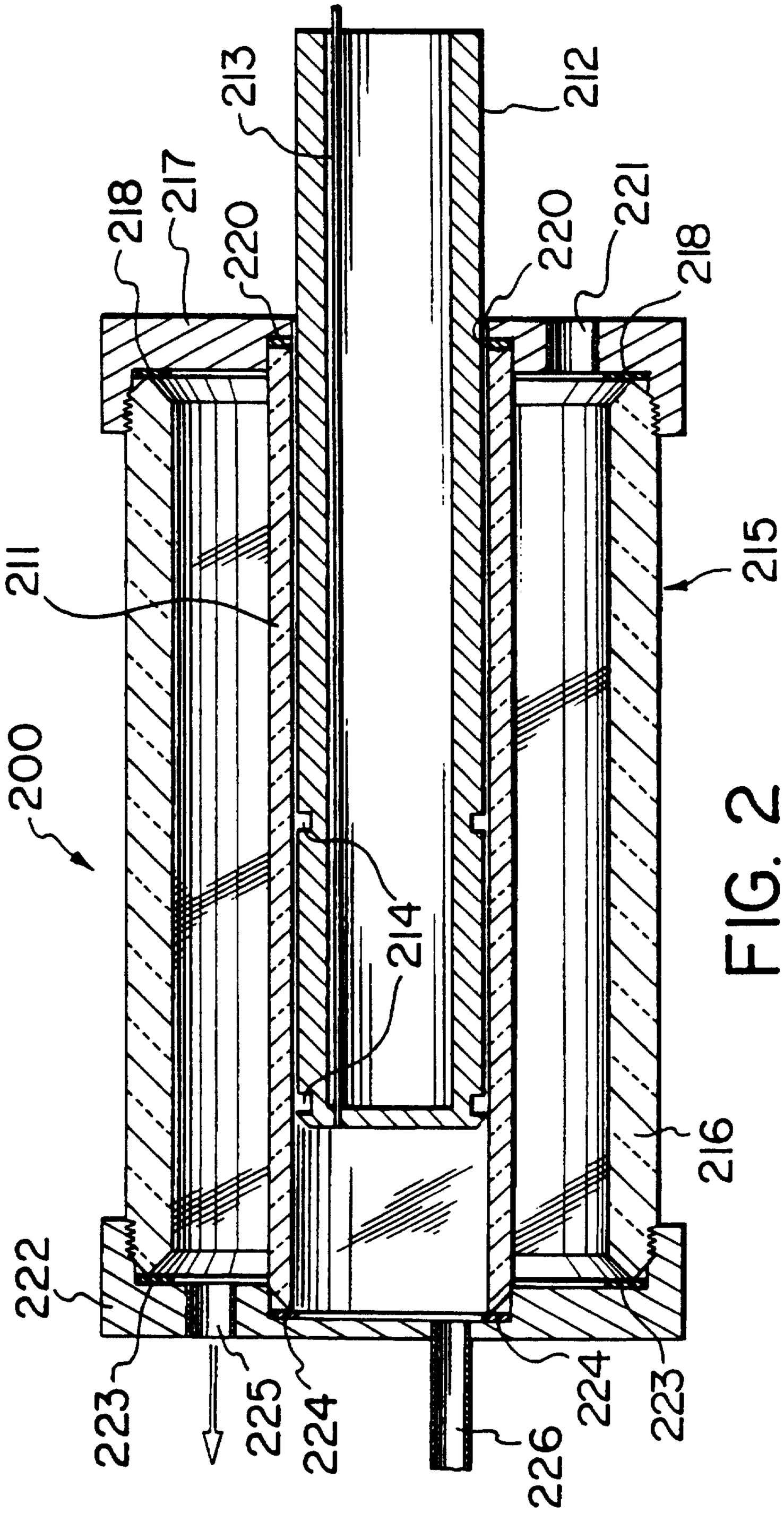
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FIG. 2

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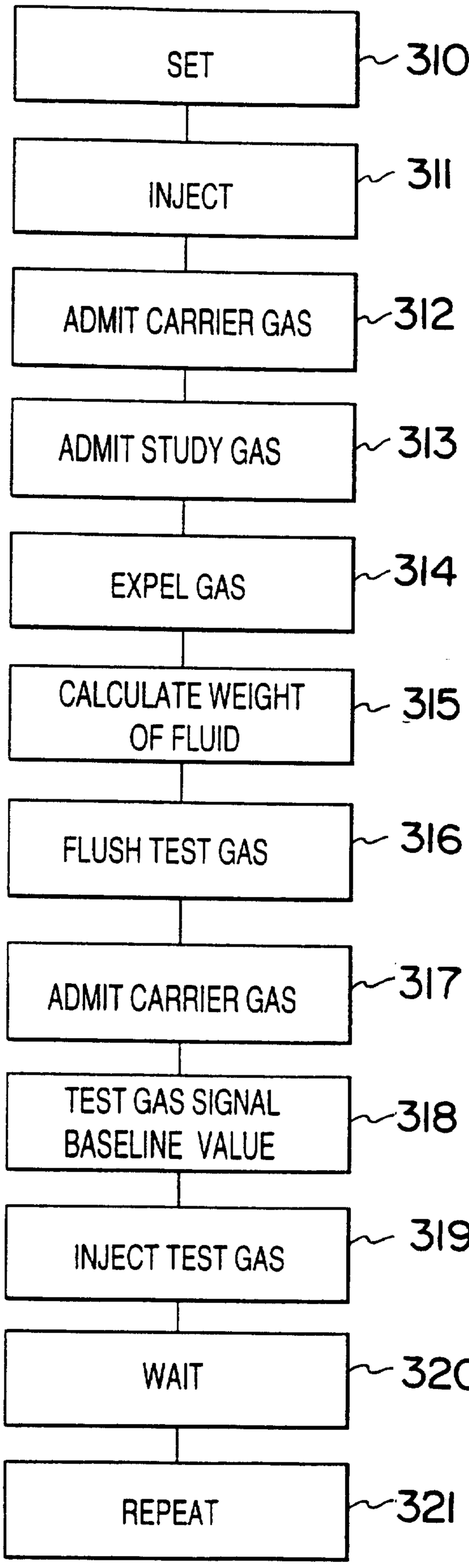
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INJECT
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ADMIT STUDY GAS
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EXPEL GAS
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CALCULATE WEIGHT OF FLUID
315
FLUSH TEST GAS
316
ADMIT CARRIER GAS
317
TEST GAS SIGNAL BASELINE VALUE
318
INJECT TEST GAS
319
WAIT
320
REPEAT
321

FIG. 3

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