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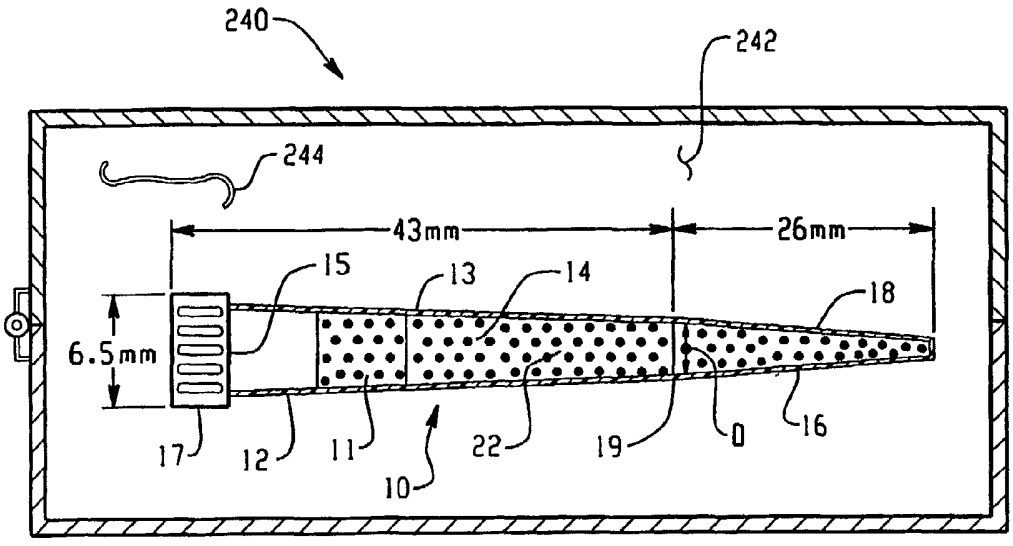
(54) Title: DEVICE FOR PRECISE CHEMICAL DELIVERY AND SOLUTION PREPARATION



(57) Abstract: A burette (10, 110, 200) suitable for delivery of a reagent into a target solution (50) employs diffusion for delivering the reagent. The reagent is in the form of a solution, which is combined with a matrix material (22), such as a gel or porous ceramic. A membrane (32) covers a delivery outlet (20) to the burette. In one embodiment, the delivery outlet comprises a plurality of fine bores (36), each one filled with or covered by a membrane (38). Stirring of the burette or target solution is achieved with a stirring means (104, 106). A heating or cooling means (80) heats a tip (16) of the burette.

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DEVICE FOR PRECISE CHEMICAL DELIVERY AND SOLUTION PREPARATION

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0001] The invention relates to a device and method for forming solutions. In particular, it relates to a diffusional burette for controlled delivery of chemicals and biochemicals into a target material, such as a liquid, and will be described with particular reference thereto.

DISCUSSION OF THE ART

[0002] Currently available methods for reagent and solution preparation involve the use of traditional tools, such as analytical balances and glassware, including beakers of different sizes, pipettes, and burettes.

[0003] Such tools are suitable for preparing reagents and solutions of relatively large volumes, in the milliliter range. However, a demand has arisen for delivery devices which are capable of delivering liquid volumes which are up to several orders of magnitude smaller, to reduce waste and associated costs, and also to accommodate the ever increasing sensitivity of the instrumentation techniques that use these reagents and solutions. To satisfy the emerging needs for solution preparation in the microliter (μL) volume range, pipettes based on liquid displacement by a precisely controlled volume of air have been introduced. For example, Eppendorf-type pipettes which use air/gas pressure and air/gas displacement for volumetric reagent transfer and delivery are now in use.

[0004] These devices deliver fixed or adjustable volumes of aqueous reagents from disposable plastic pipette tips by aspirating an appropriate volume of source solution into the tip and then delivering it into a target

solution by reversing the air flow. A piston-type arrangement, inside the pipette body, whose air volume is precisely controlled, is used to meter the liquid volume that is aspirated into the tip, and subsequently delivered in one shot into the target solution.

[0005] For continuous (as opposed to bolus type) reagent delivery, mechanized piston burettes have been introduced, where a stepping motor controls the reagent volume aspirated, as well as delivered. Such mechanized burettes cover the microliter (mL) as well as μL ranges in terms of delivered volume. For delivering sub-μL volumes, different mechanized schemes have been conceived, such as a vibrating cantilever that "shoots" nanoliter (nL) droplets into the target across air.

[0006] While such novel pipette and burette designs reach into the μL volume ranges, the mechanical working principles do not facilitate their adaptation to the handling of even smaller target liquid volumes or smaller reagent increments delivered. One constraint for the pipettes is evaporation, which becomes significant, even during short periods of time, for droplets smaller than about 1 μL. Adjusting sub-μL air volumes accurately and precisely is also difficult. Moreover, dislodging a nL-size droplet from a pipette tip is often difficult, since capillary forces become stronger relative to droplet mass as the droplet size decreases. Thus, accuracy and precision for the delivery of sub-μL volumes by a pipette based on air displacement is not readily achieved.

[0007] Furthermore, preparation of solutions of relatively low concentrations often involves multiple steps that are difficult to perform with high final accuracy and precision. The first step is, typically, weighing a very small mass of solid (crystalline or powdered) material, *i.e.*, the chemical that is to be present in the final solution, on an analytical balance. This, often tiny, amount of material may be hygroscopic (*i.e.*, it absorbs water from humidity in air). This tends to falsify the weighed amount. Additionally, a powdery material may tend to float in air, contaminating the balance and the environment. The chemical may also be hazardous, posing problems to the user. The weighed material is then transferred into a beaker. These steps, requiring utmost care, are often sources of significant errors that propagate through all subsequent steps. Typically, multiple dilutions follow, until the

desired low concentration is achieved. Further errors tend to be added in each step. In addition, such a procedure is labor intensive and prone to mistakes. Moreover, the process of serial dilution frequently uses far more of the raw material than is needed in the final solution than is needed, resulting in wastage of often expensive materials or hazardous waste production.

[0008] The mechanized burettes suffer similar drawbacks when sub-μL volumes are to be delivered in a continuous fashion. Accuracy and precision of the displaced reagent volume become worse as the delivered volume decreases, especially in the nL volume range. Parasitic diffusion between the burette tip and the target liquid may add errors that are difficult to estimate, and even more difficult to correct. On the other hand, if the burette tip is not in direct contact with the target liquid, droplets form at the tip that must be dislodged to reach the target. Thus, delivery by the burette becomes effectively discrete.

[0009] The present invention provides a new and improved reagent delivery device and method of use and fabrication which overcome the above-referenced problems and others.

SUMMARY OF THE INVENTION

[00010] In accordance with one aspect of the present invention, a delivery device for delivering a reagent into a target material is provided. The delivery device includes a body which defines an interior chamber and a delivery port fluidly connected with the chamber. The chamber holds a reagent in solution. A reagent permeable membrane is provided through which reagent passes into the target material when the delivery port is in fluid communication with the target material. Substantially no volume change occurs in the target material during delivery of the reagent to the target material.

[00011] In accordance with another aspect of the present invention, a method of forming a target solution is provided. The method includes loading a reagent solution into a chamber fluidly connected with a delivery port. The delivery port is fluidly contacted with a target liquid for a period of time such that the reagent diffuses from the chamber through a porous material and delivery port into the target liquid to form the target solution.

[00012] In accordance with another aspect of the present invention, a diffusional burette for delivery of a reagent into a target material is provided. The burette includes a body which defines an interior chamber for receiving the reagent and a delivery port fluidly connected with the chamber, the delivery port defining a plurality of holes, each of the holes having a cross sectional width of less than 500 microns. A reagent permeable matrix material is disposed within the body. A solution of the reagent is in the chamber, such that the reagent diffuses from the chamber via the holes into the target material when the delivery port is in fluid communication with the target material.

[00013] As used herein, "delivery time" means the period of time in which a diffusional burette is in fluid contact with a target material such that a reagent passes from the diffusional burette to the target material.

[00014] An advantage of at least one embodiment of the present invention is that non-volumetric (non-convective) reagent delivery is used for transfer of chemicals. As a result, substantially no volume changes are induced in the target solution.

[00015] Another advantage of at least one embodiment of the present invention is that it enables automatic delivery to be achieved using natural processes that are spontaneously occurring during delivery.

[00016] Another advantage of at least one embodiment of the present invention is that the delivered amount may be controlled by monitoring the delivery time. Delivery time can be controlled extremely precisely using readily available techniques.

[00017] Another advantage of at least one embodiment of the present invention is that ranges of delivery rates are adjustable by varying the concentration of a chemical in a burette and by varying the geometrical dimensions of the burette.

[00018] Another advantage of at least one embodiment of the present invention is that high reproducibility and precision are achieved easily and cost effectively. As a consequence, waste disposal costs and reagent costs are minimized.

[00019] Another advantage of at least one embodiment of the present invention is that very low target solution volumes and delivery rates are feasible, enabling preparation of reagents of extremely low concentrations and/or volumes.

[00020] Another advantage of at least one embodiment of the present invention is that preparation of a reagent or solution can be achieved in one single step, without the need for serial dilutions.

[00021] Another advantage of at least one embodiment of the present invention is that a target solution can be prepared with multiple reagents by introducing the reagents simultaneously or sequentially.

[00022] Another advantage of at least one embodiment of the present invention is that it enables high precision and accuracy of the resulting reagent or solution to be achieved.

[00023] Another advantage of at least one embodiment of the present invention is that natural processes of diffusion, capillary forces, and surface tension, which affect delivery rates, are extremely reproducible, where other conditions are kept the same or accounted for, such as geometry, pressure, and temperature.

[00024] Another advantage of at least one embodiment of the present invention is that operator errors are minimized.

[00025] Another advantage of at least one embodiment of the present invention is that co-delivery of several chemicals is possible in a single step, thus leading to one step production of complex mixtures.

[00026] Another advantage of at least one embodiment of the present invention is that parallel processing of several or many deliveries into several or many solutions is feasible with little human control.

[00027] Another advantage of at least one embodiment of the present invention is that the delivery methods are amenable to microfabrication and MEMS technologies for serial production.

[00028] Another advantage of at least one embodiment of the present invention is that both aqueous and non-aqueous solutions can be prepared, using both aqueous and non-aqueous reagent sources.

[00029] Another advantage of at least one embodiment of the present invention is that the provision of a suitable membrane and/or matrix material inhibits convection or flow through a delivery port, such that a reagents is transported through the port primarily by diffusion.

[00030] Another advantage of at least one embodiment of the present invention is that it enables delivery to be achieved without moving mechanical parts.

[00031] Still further advantages of the present invention will be readily apparent to those skilled in the art, upon a reading of the following disclosure and a review of the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[00032] FIGURE 1 is a schematic side sectional view of one embodiment of a delivery device in a storage holder according to the present invention;

[00033] FIGURE 2 is an enlarged side sectional view of the tip of the delivery device of FIGURE 1;

[00034] FIGURE 3 is a schematic side sectional view of a second embodiment of a delivery device according to the present invention;

[00035] FIGURE 4 is an enlarged side sectional view of the tip of a burette according to a third embodiment of a delivery device according to the present invention;

[00036] FIGURE 4A is an enlarged side sectional view of an alternative embodiment of a tip of a burette according to a fourth embodiment of a delivery device according to the present invention;

[00037] FIGURE 5 is a side view of a delivery system including the delivery device of FIGURE 1 with a dispensing device attached for delivery of a reagent into a target solution with a vibrator for mixing the target solution;

[00038] FIGURE 6 is an enlarged view of an alternative embodiment of a dispensing device adjacent a cap of a burette having a barcode thereon;

[00039] FIGURE 7 is a side view of an alternative embodiment of a delivery system including a stand for lowering a burette, an agitation means for vibrating the burette tip, and a support for a container of a target solution;

[00040] FIGURE 8 is a perspective view of a fifth alternative embodiment of a delivery device according to the present invention, supported on a stand with a tip of the delivery device positioned in a target liquid;

[00041] FIGURE 9 is a perspective view of the delivery device of FIGURE 8, supported on a stand, after removing the tip from the formed target solution;

[00042] FIGURE 10 is an enlarged sectional view of the tip of the delivery device of FIGURE 8;

[00043] FIGURE 11 is an enlarged top view of the membrane of the delivery device of FIGURE 8;

[00044] FIGURE 12 is a side sectional view of a sixth alternative embodiment of a delivery device according to the present invention, with a tip of the delivery device undergoing rotation;

[00045] FIGURE 12A is an enlarged side sectional view of the tip of the embodiment of FIGURE 12;

[00046] FIGURE 12B is an enlarged bottom view of the membrane of the embodiment of FIGURE 12;

[00047] FIGURE 13 is an enlarged sectional view of the membrane of the delivery device of FIGURE 12, illustrating the diffusion layer in a target liquid around a hole in the membrane, both with and without stirring, showing that the diffusion layer is reduced in thickness when stirring is used;

[00048] FIGURE 14 is a perspective view of a pen-type delivery device according to a seventh embodiment of the present invention;

[00049] FIGURE 15 is a perspective view of the pen-type delivery device of FIGURE 14, being manually inserted into a target liquid;

[00050] FIGURE 16 is a schematic view of a processor controlled delivery system according to another embodiment of the present invention;

[00051] FIGURE 17 is a side sectional view of an eighth alternative embodiment of a delivery device according to the present invention;

[00052] FIGURE 18 illustrates one method of preparing the delivery device of FIGURE 17;

[00053] FIGURE 19 is a side sectional view of a ninth alternative embodiment of a delivery device;

[00054] FIGURE 20 is a side view of a delivery and monitoring system for evaluating the delivery devices according to the present invention;

[00055] FIGURE 21 is a plot of cyclic voltammograms showing current in amps vs. electrode potential in volts for ferricyanide delivery times between 0 and 41.3 minutes;

[00056] FIGURES 22a, 22b, and 22c show plots of concentration of ferricyanide in a fresh target solution in mM vs. reagent delivery time in minutes for three sequential delivery procedures from the same burette;

[00057] FIGURE 23 is a plot of potassium ion concentration in mM in a target solution measured by a K^+ ion selective sensor delivered by a burette during continuous delivery;

[00058] FIGURE 24 shows the dimensions of an exemplary burette;

[00059] FIGURE 25 is a plot of potassium ion concentration in a target solution over time; and

[00060] FIGURE 26 is a schematic view of a delivery port of a diffusional burette according to the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[00061] With reference to **FIGURE 1**, a delivery device in the form of a diffusional burette **10** suitable for the delivery of a chemical, chemical species, biological, or biochemical species or molecule (hereinafter referred to generally as a "reagent") to a target material or vessel is shown.

[00062] The target material is one which, on fluid contact with the burette **10**, allows the reagent to be taken up or absorbed by the target material. Suitable target materials include liquids, such as water, aqueous solutions, and organic solvents, porous solid materials, such as medicament carriers, ceramics, and the like, absorbent fibers, semisolid materials, such as gels, and combinations thereof. While the target material will generally be referred to as being a liquid, it will be appreciated that other target materials are also contemplated.

[00063] Exemplary reagents include laboratory reagents, such as acids; bases; ions, *e.g.*, alkali metal and alkaline earth metal ions, halide ions, and the like, such as potassium ions and bromide ions; reducing and oxidizing agents and agents that can be reduced or oxidized, such as ascorbic acid and permanganate ions, ferricyanide, ferrocyanide; drugs; pharmaceuticals; antigens; antibodies; protein molecules; and the like. The reagent is preferably in the form of a solution **11**, which can be aqueous or nor aqueous. When the reagent diffuses into the target solution, it may form a target solution containing the reagent. Or, the reagent may undergo a reaction to produce a second species. Thus, the target solution need not contain the reagent or may contain less reagent than the amount actually diffused.

[00064] While the reagent is generally described in terms of a single chemical or species, it will be appreciated that a single burette **10** may deliver two or more reagents at the same time. These reagents may be in similar or different concentrations, according to the desired end use of the burette and either mixed together or separately contained within the burette.

[00065] The burette **10** of **FIGURE 1** includes a body or casing **12**, formed from glass, plastic, ceramic, or other suitable material which is preferably impermeable to and unreactive toward the reagent and its solution. The body is preferably impermeable to and unreactive toward the target material. The

body **12** includes a reagent holding portion **13**, which defines an interior chamber **14** that holds a quantity of a reagent in solution, introduced via an opening **15** at one end of the chamber **14**. The body also includes a tip portion **16**, in fluid communication with the other end of the chamber **14**, through which the reagent is delivered from the burette. The shape of the tip portion **16** affects the delivery, as will be described in greater detail below. A cap **17** or other sealing member closes off the opening **15** to the reagent holding portion **14** after filling with the reagent. Intermediate the tip portion **16** and the reagent holding portion **13** is optionally a shoulder portion **18**. The shoulder portion may be conical and have an internal diameter **D** at its widest end **19** which is wider than the diameter **d** of a delivery port **20** in the tip **16**. As shown in **FIGURE 1**, the reagent holding portion **13**, shoulder **18**, and tip **16** are integrally formed, with the shoulder being simply an extension of the tip, although other configurations are also contemplated. **FIGURE 1** provides exemplary dimensions for the burette **10**, although it will be appreciated that burettes of different sizes are readily formed. Preferably, the tip **16** has a substantially smaller interior diameter than that of the reagent holding portion.

[00066] In the embodiment of **FIGURES 1** and **2**, the tip **16** has the general shape of a bullet, which is conical with a delivery port **20** in the form of a small hole or holes at the end **21** of the tip, or near the tip end. The tip can be thus be a tapered portion of the body, which narrows between the reagent holding portion **13** and the delivery port **20.** Alternatively, the tip has a cylindrical shape or other suitable shape. The size of the port **20** can be from about 10 microns to about 10 mm in cross sectional diameter, depending, in part, on the desired delivery rate, although larger or smaller dimensions are also contemplated. In one embodiment, the port **20** is from 10 to about 500 microns in interior diameter.

[00067] In the embodiment of **FIGURE 2**, the delivery port **20** extends perpendicular to the longitudinal axis **x** of the body. It is also contemplated that the delivery port may be angled to the longitudinal axis **x** of the body, as shown in **FIG. 4**. This helps to reduce any tendency for air bubbles to become trapped in or around the delivery port.

[00068] In the embodiment of **FIGURE 1**, the reagent holding portion **13** holds a filling comprising the reagent, optionally a solvent in which the reagent is dispersed to form a reagent solution **11**, and optionally a matrix material **22**, through which the reagent and solvent are able to permeate. This ensures that there is substantially no convection inside the burette. The matrix material is a solid or "semi-solid", such as a gel or a porous material (*e.g.*, porous metals, such as inert metals e.g., nickel, titanium, and stainless steel, inert porous non-metallic materials, such as ceramic and glass, fibrous materials, such as fibrous glass, and combinations thereof) that contains the reagent solution **11**. Suitable gels include agarose, polyacrylamide, other hydrogels, hydroxyethyl methacrylate (HEMA), and the like. The matrix material is preferably electrically neutral, and of low enough density to allow the reagent to permeate therethrough.

[00069] To avoid interactions with the reagent, the matrix material is preferably chemically inert towards the reagent. In one embodiment, the matrix material comprises a gel having a permeability which can be modulated via an electric voltage, voltage gradient, or other imposed electrical property. The delivery rate can thus be varied from external to the burette by a voltage controller or the like (not shown).

[00070] The gel helps to retain the reagent solution **11** within the burette until the tip of the burette makes contact with the target liquid. The gel allows the reagent to permeate therethrough.

[00071] The reagent is preferably in the form of a solution **11**, such as an aqueous solution or non-aqueous solution. The reagent may be intimately mixed with and/or absorbed by the matrix material **22**. Alternatively, or additionally, the matrix material is disposed between the reagent solution and the delivery port, as discussed in greater detail below. In an alternative embodiment, the matrix material is omitted.

[00072] The concentration of the reagent in the solution **11** depends, in part, on the desired concentration or concentration range of solutions to be prepared with the reagent. The concentration of the reagent can also be varied, dependent on the desired delivery time. Preferably, a plurality of burettes are provided, each one having a different concentration range, such

that a user can select an appropriate burette according to the desired amount delivered, concentration, or concentration range. Alternatively, or additionally, other aspects of the burettes are modified to provide different absolute delivery rates and/or reagent, such as delivery port characteristics. For example, the target volume and desired concentration are first ascertained. From these values, the total amount to be delivered can be determined. The desired accuracy and precision of the delivered reagent and optionally the accuracy of tracking delivery time can also be taken into account in determining an optimum delivery rate. In some instances, such as in a titration or buffering in the target solution, the exact amount to be delivered is not known. In such instances, the amount delivered can be determined from the delivery time. Additionally, in some cases, the amount to be delivered cannot be determined from the volume of the target solution and the desired concentration. For example, where the reagent is undergoing a reaction as it is being delivered, such as in a titration, the desired delivered amount is dependent on the concentration of the corresponding reactant and/or the reaction end point. For example, the desired concentration of reactant in the target solution at the end point may be zero. Even though much larger amounts are actually delivered, the reactant is consumed. Similarly, where buffers are present, the amount delivered may be well in excess of what would be calculated from the final target concentration.

[00073] With reference now to **FIGURES 2** and **4**, a reagent permeable material **30** is optionally positioned in or adjacent the tip **16** of the burette, through which the reagent passes when leaving the burette. In this embodiment, the gel **22** may be omitted. As shown in **FIGURE 2**, the permeable material **30** may be in the form of a porous membrane or layer **32**, formed from cellulose, modified celluloses, such as acetyl cellulose or nitrocellulose, polyurethane, or the like. The membrane may be in the form of a single layer, as shown in **FIGURE 2**, or multiple layers, as described in greater detail below. The membrane **32** may be about 2-100 microns in thickness, more preferably, less than 50 microns, most preferably, about 5-30 microns in thickness. **FIGURE 2** shows the membrane covering the tip end **21** and thus defining the delivery port **20**, although it will be appreciated that the membrane may be positioned elsewhere in the tip **16** or shoulder **18** such

that it provides a permeable member, which acts as a conduit, through which all of the delivered reagent diffuses from the burette.

[00074] In one embodiment, the membrane **32** is formed from a gel which has an electrically controllable property, such that the rate of diffusion can be controlled electrically, by an external device, as described above for the matrix material.

[00075] In yet another embodiment, the membrane **32** is formed from a material having holes in the form of pores **36**, such as a thin layer of lipophilic or hydrophilic material, which may be 6-20 microns in thickness. The pores may be of micron or submicron diameter. For example, the pores may be from 0.01 to 100 microns in diameter. In one embodiment, the pores are at least about 0.1 microns in diameter. In one embodiment, the pores are less than about 10 microns in diameter. The pores **36** may be of uniform size or different sizes. An exemplary membrane material is a cyclopore membrane available from Whatman which has a clear and reproducible pore structure, although non-uniform pore sizes are also contemplated. Although having pores results in small semispherical volumes at the pore openings where concentrations are not uniform, the concentration at about 10-20 microns from the pores becomes substantially uniform due to the large number and small sizes of the pores. The pores thus produce a steady state delivery similar to a microelectrode array, with very little flow dependence on either side of the delivery port (flow in the target liquid or inside the burette body).

[00076] Specifically, the pores result in semispherical non-uniform volumes. This means that a local larger concentration exists at the center where the pore opening is located. A decaying concentration occurs along radial distance from the pore opening, so that local concentration depends on the distance from pore opening. If the semispherical concentration decays do not appreciably overlap, then the individual pores each deliver at steady state, which is independent of each other. If the pores are small enough, the pores produce small enough semispherical volumes that are too small for a modest flow to disturb, leading to constant delivery rate independent of small macro flows. The delivery rate is thus constant, after a negligible initial transient time during which the semispherical volumes form.

[00077] In another embodiment, shown in **FIGURE 4**, the membrane **32** is from a rigid substrate **34** of a polymeric material, ceramic or the like, in the shape of a generally cylindrical body spanning the interior of the tip **16**. The substrate **34** has a plurality of outlets **35,** which together define the delivery port **20**. Specifically, the substrate **34** is penetrated by a plurality of fine holes, such as bores **36** (**FIGURE 4**) extending through the substrate between outlets **35** and inlets **37**. The width of each of the bores **36** is dependent, to some extent, on the desired delivery rate and the method of forming the bores. For example, the bores **36** may in the general shape of hollow cylinders, although it is also contemplated that the bores may define convoluted paths or have different cross sectional shapes. The bore may be of about 0.1-300 microns in diameter. In one embodiment, the bores are about 10-200 microns in diameter. For example, the substrate **34** may contain 3, five, ten or more of such bores **36**. In one embodiment there are from about 10-1000 bores. The bores **36** can be filled with a porous material **38** similar to that used for the matrix material **22** or membrane **32** in **FIGURE 2**. As shown in **FIGURE 4**, the membrane **32** is positioned at the tip end **21,** although it is also to be appreciated that the substrate may be positioned elsewhere in the tip **16** and/or in the shoulder. It will be appreciated that the substrate **34** may be formed integrally with the tip **16** or be formed as a separate element, which is positioned in the tip.

[00078] In one embodiment, the bores **36** are all of the same width or of approximately the same width (*e.g.*, at least about 90% of the bores are within ±20% of a median width of the bores, and can be within ±10% of a median width of the bores). In another embodiment, the bores are of different widths.

[00079] In the embodiment of **FIGURE 2**, the bores are aligned with the longitudinal axis of the body. It is also contemplated that the bores **36** may be angled to the longitudinal axis, particularly where the delivery port is angled to the longitudinal axis, as illustrated in **FIGURE 4a**.

[00080] It will be appreciated that similar delivery rates can be achieved by reducing the number of bores and at the same time, increasing their diameter. For example, 10-30 bores of about 20-70 micrometers in diameter may yield a similar diffusion rate to about 3-8 bores of 100-200 micrometers in diameter.

[00081] To achieve bores **36** of suitable fineness, an Excimer laser may be used. The laser allows a single hole **36** or plurality of holes to be formed in a substrate **34** with a high degree of reproducibility-*i.e.*, the bores are virtually identical. Making techniques can be used to define the locations of the holes to be drilled. In one embodiment, the laser is used to "drill" holes in a plastic or other polymeric material, such as a polyimide, (silicone) rubber, or polytetrafluoroethylene (Teflon™) material in the form of tubing or a sheet. The tube or sheet may be cut to the appropriate size for the substrate before or after drilling the holes. Excimer lasers are capable of forming bores of as little as about 10 microns in diameter, although bores of about 50 micrometers or greater are generally satisfactory.

[00082] Another method of forming holes **36** is to use silicon microfabrication technologies to produce the holes in a silicon or similar substrate **34.** Such a substrate need not cover only the tip delivery port, it may be a large part or all of the burette body.

[00083] In one embodiment, the bores **36** are distributed in a ring, away from the center of the port. This allows for a more uniform and rapid dispersal of the reagent in the target solution, particularly when the tip is rotated, as is discussed below.

[00084] The burette **10** is intended to be disposable after use, although it is also contemplated that an empty burette may be refilled with reagent. In a preferred embodiment, large numbers of burettes are prepared by a supplier and shipped to facilities where the burettes are to be used. In one embodiment, each burette is used to prepare only a single target solution and is then disposed of.

[00085] The reagent is delivered from the burette into a target liquid by diffusion. This results in no or substantially no volume change in the target liquid during the addition of the reagent. By "substantially no volume change," it is meant that the target liquid does not increase or decrease in volume by more than 5% of the volume of the reagent solution **11** equivalent to the amount of reagent being transferred. More preferably, the volume change is no greater than 1% of the volume of the reagent solution equivalent to the amount of reagent being transferred. For example, if the reagent is in

solution at a concentration of 1 Moles/L (1M) in the burette and it is desired to deliver 1 mMoles of the reagent to the target liquid (i.e., the equivalent of 1mL of reagent solution), the target liquid increases or decreases in volume by no more than about 0.05 mL, and preferably, by no more than 0.01 mL during the delivery time.

[00086] It will be appreciated that decreases in volume of the target solution can arise due to osmotic pressure. The high relative concentration inside burette causes water to flow into the burette due to the osmotic pressure difference. In general, the extent of water flow depends on the actual osmotic pressure difference between the target liquid **50** and the reagent solution **11** as well as on the characteristics of any membrane that separates the two. In practice, the effect of osmotic pressure upon target volume is negligibly small where the delivery time is in the minutes range, or less.

[00087] In one embodiment, the water (or other solvent) flowing between the burette and the target solution accounts for less than 10%, more preferably, less than 5%, and most preferably, less than about 1% of the equivalent volumetric delivery rate. The equivalent volumetric delivery rate F can be defined by the expression

$$F = R / C_R$$

where R is the reagent delivery rate in moles per unit time and C_R is the concentration of reagent inside the burette. F depends on the delivery port characteristics and the reagent concentration inside the burette.

[00088] For example, if the reagent delivery rate is 1 micromole/second and the reagent concentration in the burette is 1 M then the equivalent volumetric delivery rate would be 1 micromole/s per 1 mmole/mL = 10^{-3} mL/s = 1 microL / s. Where delivery is achieved within a few minutes of operation, the effects of osmosis are negligible. Preferably, no more than about 10%, more preferably, less than 5%, most preferably, less than about 1% of this value will be water flow, i.e., in the example above, a volume change of less than 0.1 µL/second, more preferably, less than 0.05 µL/second, most preferably, less than 0.01 µL/second is due to water flow. In practice, the volume change due to water flow is typically in the direction from the target into the burette, and is typically far less than 5% of the equivalent volumetric

delivery rate. In some cases, there is effectively no change in volume of the target solution, since the rate of reagent delivery from the burette to the target solution is approximately balanced by the water flow from the target solution to the burette.

[00089] To form the burette **10 of FIGURE 1**, the reagent solution **11** of choice is mixed with a gel forming material, such as agarose powder or other polymer forming material which is then loaded into to the chamber **14** and the gel allowed to set. The cap **17** is then screwed or otherwise attached to the open end **15** of the burette. The reagent remains within the burette, trapped by the gel until the tip **16** is placed in contact with the desired target solution.

[00090] Where a solid matrix material such as a porous ceramic is used, the porous material is immersed in or otherwise contacted with the liquid reagent solution **11**. Vacuum infiltration techniques may alternatively be used to introduce the reagent solution to the porous material. The porous ceramic may be loaded into the chamber **14** prior to introduction of the reagent solution or loaded after introduction. Or, as described in greater detail below, the body **12** may be formed around the infiltrated porous material or gel/reagent solution mixture.

[00091] If no matrix material is used, the reagent solution **11** is introduced directly to the burette chamber. In this case, the membrane **32** is sufficiently resistant to the passage of the reagent solution therethrough that there is substantially no escape of the reagent from the body until it is able to move by diffusion into a target liquid in contact with the membrane. Where the matrix material and reagent solution are to be inserted separately, the matrix material can be added first and allowed to set before the reagent solution is introduced.

[00092] The burette **10** may be used immediately after formation or shipped and/or stored for a period of time prior to use. Accordingly, multiple burettes can be prepared and used as needed. The burette **10** holds the reagent solution until the tip is placed in fluid contact, generally in direct contact, with a target liquid **50** in which a target solution containing the reagent is to be formed **(FIGURE 5)**. Contact is maintained with the target liquid for a sufficient time (the delivery time) to form the target solution. This

operation may be carried out manually, by an operator holding the burette and contacting the target liquid with the tip, or mechanically, as described in greater detail below.

[00093] In one embodiment, the burette **10** contains sufficient reagent to form a plurality of target solutions. It will be appreciated, however, that with each additional delivery, the concentration of the reagent in the burette tends to decrease, since the volume of solution in the burette remains essentially constant. Accordingly, the delivery time for second and subsequent deliveries is generally increased for forming corresponding target solutions of the same concentration and volume. The delivery time for a particular delivery in a sequence of such deliveries can be calculated, either empirically, or by using mathematical formulae, as will described in greater detail below.

[00094] Optionally, a dispensing device is provided for the burette **10**. In one embodiment, shown in **FIGURES 3** and **5,** a dispensing device **40** selectively holds a burette in a sleeve **42** or other receptacle. A fresh burette **10** is inserted into the sleeve and a timing device, such as a clock **44** is reset to zero or to a desired delivery time, *e.g.,* 10 seconds. The resetting of the clock may be carried out automatically by the burette pressing on a reset button **46** during insertion into the sleeve.

[00095] When the burette **10** is to be used to form a target solution, the tip **16** is placed in the target liquid **50** into which the reagent is to be delivered (**FIGURE 5**) and the user simultaneously presses a button **52** on the dispensing device to start the clock **44**. The reagent flows through the membrane into the target liquid. When the clock indicates that the desired delivery time has elapsed, the user removes the burette **10** from the solution, halting the reagent flow. Alternatively, or additionally, an alarm **54**, such as a buzzer or flashing light, indicates when a desired delivery time has elapsed to alert the user to remove the burette tip **16** from the target solution. When the burette **10** is depleted, it is removed from the dispensing device **40** and the dispensing device can be reused with a fresh burette.

[00096] In one embodiment, shown in **FIGURE 6**, the user uses a keypad **60** or other input device to instruct the timer device or a control system, such as a microprocessor controller **62** associated with the timer device **44** how

much reagent is to be delivered. The control system is programmed with information concerning the delivery rate of the reagent from the burette **10** and other factors affecting delivery, such as the amount of reagent which has already been delivered from the device. The control system **62** determines how long it will take to deliver the desired amount of reagent and sets the clock **44** accordingly. In one embodiment, the control system interrogates the user by means of a screen **64** and the user inputs information which affects the delivery rate, *e.g.,* via the keypad **60**. For example, the control system **62** may ask the user for the concentration of the reagent in the burette being used, or for information regarding one or more of the ambient temperature, the desired delivery amount, the target concentration and the volume of the target solution. The control system uses this information in determining the appropriate delivery time. The control system may also ask the user for the desired final accuracy and precision. Based on the input values, the control system recommends a particular burette to be used (*i.e.*, one that has low enough delivery rate such that the total delivery time is long enough to be controlled precisely and accurately.

[00097] To accommodate the demands of a variety of consumers, it is contemplated that a series of burettes of different dimensions, sizes, shapes, and material are provided to accommodate different reagents, concentrations, delivery rates, and the like. Identifying information, such as reagent type, concentration, volume, and the like, *e.g.*, in the form of a computer recognizable indicia, such as a bar code **70,** may be provided for each burette **10**. The bar code is preferably affixed to the burette **10** or to packaging associated therewith. The bar code may also provide other information, such as the date of fabrication and filling, delivery rate, information for programming a timer device, and the like. Other recognition information may be provided, so that a user may keep track of the amount of reagent that had been delivered from a particular burette, allowing an adjustment of the calibration to be automatically made, if appropriate.

[00098] In one embodiment, shown in **FIGURES 6, 7,** and **16,** the control system **62** is programmed to recognize the bar code **70** using a bar code reader **72**. The control system optionally informs the user about the particular burette or makes calculations based on stored information about the burette which corresponds to the bar code. The bar code may be printed on an inside

surface of the burette, to protect the bar code or some other condensed digital code system from outside dirt or contamination or obscuring effects or to ensure confidentiality of the information. The control system may also display, for example, which one of the different burettes of identical type should be used for delivering a given amount with a given precision. This is because the longer the delivery time (*i.e.,* the slower the delivery rate), the higher the relative precision of the total amount delivered. The control system may include look-up tables, algorithms, or the like from which it calculates a desired delivery time, delivery rate, or the like, based on information scanned from the bar code **70** and/or or operator input information.

[00099] In place of a dispensing device **40**, with its own clock **44,** as shown in **FIGURE 3**, a stopwatch or other timing device **44** is alternatively used by the user to monitor the delivery time.

[000100] Diffusion rates depend, to some degree, on ambient temperature. Accordingly, particularly when extremely precise quantities are to be delivered, controlling the temperature and/or correcting for temperature helps to ensure accuracy. Thus, in one embodiment, a controlled temperature environment (such as is generally provided in wet chemistry laboratories) is employed for deliveries from the burette **10**. Both the burette **10** and the target liquid **50** in to which the delivery is to be made are allowed to equilibrate in the controlled temperature environment prior to delivery. Alternatively, for example, where a controlled environment is unavailable, the target liquid and/or burette are brought to a preselected temperature (*e.g.*, by heating or cooling) prior to delivering reagent from the burette. In yet another alternative embodiment, corrections are made to the delivery time depending on the detected ambient temperature. The effect of small temperature variations on the delivery characteristics is estimated by using physicochemical theory, or from prior empirical measurements.

[000101] **FIGURE 7** illustrates a dispensing device **40′**, where similar components to dispensing system **40** are labeled with a prime (′) and new elements are given new numbers. In the embodiment of **FIGURE 7**, a heating element **80**, such as a resistance heater, provides local heating of the tip **16** and optionally all or part of the reagent holding portion to heat the reagent solution therein. This allows thermostatic control of the delivered

reagent (and thus, the temperature of the delivery diffusion process) and hence a more reproducible delivery rate, particularly where ambient temperatures are prone to fluctuation. The heating element **80** may also provide different heating levels, making the delivery rate tunable. This can be used to increase or decrease the delivery rate (increasing the temperature increasing the delivery rate, decreasing the temperature decreasing the delivery rate).

[000102] In one embodiment, the heating element heats the entire delivery port and membrane and optionally also an adjacent portion of the target liquid **50**. This ensures that those elements whose temperature may affect delivery rate to some degree are at the same, reproducible temperature. For example, a partial casing or screening of a volume fraction of the target liquid closest to the delivery port is effected to ensure that the volume fraction whose temp may have an effect on the resulting effective delivery rate is effectively thermostatted. The remainder of the target liquid may then be at a slightly different temperature, without there being a significant influence on the delivery rate.

[000103] It has been found that an approximately 10°C increase in temperature may result in a doubling in the rate of a number of physicochemical processes, such as diffusion rate. A heating element **80** at or near the tip **16** of the burette **10** thus allows a constant temperature, which is higher than ambient, during the delivery by heating it above the ambient temperature. Additionally, heating enables the user to achieve different delivery rates using temperature modulation of the diffusion rate. It is also contemplated that a constant temperature may be achieved by cooling the tip. If a very small volume is to be cooled (or heated) then this can be effectively achieved using a heating or cooling element **80** employing conventional technologies (for example, for cooling, an electronic cooling system or heat pump can be used).

[000104] The delivery from the burette **10** into a target liquid **50** is continuous, with a rate which can generally be as low as practically desired. For intermittent additions, such as multiple standard additions, the burette tip **16** is removed from the target liquid and returned to the target liquid for each addition. For introducing the tip to a target liquid, a support means,

such as a stand **90** is optionally used to lower the tip **16**, either manually or by an automated operating system **92** into the solution, as illustrated in **FIGURE 7**. The automated system includes a processor **62'** analogous to processor **62**. An automatic sensing device **94,** electrically linked to the operating system **92**, optionally senses the time when the tip first contacts the solution. The automated sensor **94** may use electrical impedance for detecting contact. The timing mechanism **44'** starts counting the time from that moment. At the end of the preselected delivery period, the operating system **92** withdraws the tip from the target solution. In one embodiment, the stand **90** includes a mechanical lifting device **96**, such as a piston operated actuator, *e.g.,* operated by a motor (not shown) for removing and inserting the tip **16** into the target liquid **50** by lifting /lowering the burette **10**. The mechanical lifting device **96** can be controlled by processor **62'** to remove the tip **16** from the target liquid when a desired delivery time has elapsed.

[000105] As shown in the embodiment of **FIGURES 5** and **7**, the target liquid **50** is preferably placed in a suitable container **100**. The container is optionally formed from or lined with a containment layer **101** such as a silicone layer, for creating surface tension forces which contain the target liquid on as small area of the containment layer as an approximately hemispherical bead of liquid. Where the reagent solution is hydrophilic, containment layer **101** is preferably formed from a hydrophobic material, and vice versa. The container **100** is optionally positioned on a support **102.** Optionally, a means for stirring the target liquid **50** or reagent is provided. In the embodiment of **FIGURE 5**, the support **102**, and hence the container **100** and target liquid **50**, is vibrated by an agitator in the form of a vibrator **104,** although other suitable stirring means are also contemplated, such as fine gas jets or bubbling or integrated MEMS devices. The vibrator stirs the target liquid, helping to maintain the homogeneity of the target solution as the reagent is added.

[000106] In another embodiment, illustrated in **FIGURE 7**, the reagent in the burette is stirred. In this embodiment, the means for stirring includes an agitator, such as a vibrator or rotator **106**, which causes the tip **16** or other portion of the burette **10** to vibrate, rotate or otherwise move. The vibration/rotation or other movement of the tip **16** stirs the reagent solution in the burette **10**, maintaining its homogeneity in the burette as reagent is

dispensed. The agitator **106** also causes the tip **16** to stir the target liquid **50**, when the tip is placed in contact therewith. It will be appreciated that more than one means for stirring **104, 106** may be employed, either during or subsequent to a delivery. The stirring means **104, 106** is preferably under the control of the operating system **92**.

[000107] The rotation of the burette **10** (or other form of agitation) results in an adjustable enhancement of the delivery rate (*i.e.*, allows for reductions in the delivery time) and tends to reduce errors which may arise from unwanted spontaneous convection.

[000108] With reference now to **FIGURES 8-11**, a burette **10** similar to that shown in **FIGURES 1** and **2** is shown. For convenience, similar elements are given the same numbers and new elements are given new numbers. The burette includes a housing **107**, formed from plastic, or other suitable material which surrounds the body **12** and has an opening **108** at a lower end, through which the delivery port **20** is exposed (**FIG. 10**). The body is formed from an inert material, such as polyacetal resin, Teflon®, or the like and holds a quantity of a reagent solution **11**. A reagent permeable material **30** in the form of a membrane **32** closes the delivery port **20** and is permeable to the reagent. In one embodiment, reagent permeable material also includes a layer **109** formed from a matrix material, such as a gel, *e.g.*, an agar gel or other material allows the reagent to pass therethrough by diffusion, although it is also contemplated that the gel may be mixed with the reagent solution, as shown in **FIGURES 1-4.** The membrane layer **32** can be formed from a substrate, such as a polyimide or other plastic material or an inert metal sheet or other inert membrane material which is impermeable to the reagent, except where holes **36** are provided. The membrane may be, for example, about 20-30 microns in thickness. As shown in **FIG. 11**, the membrane **32** has a plurality of holes **36** (five in the illustrated embodiment), through which the reagent passes. The holes may be about 10-100 microns in diameter, *e.g.*, about 50 microns in diameter, or other suitable widths, as noted above, and may be a distance ***h*** of about 0.01mm to about 2 mm apart. The many holes (bores-or pores in the case of a cyclopore membrane) are ideally as close to each other as possible (to reduce overall size) without causing the resulting semispherical nonuniform concentration domains to overlap. The remainder of the polyimide film **32** is impermeable to the reagent, such that the reagent diffuses from the

burette via the holes. The holes **36** may be formed by drilling bores, *e.g.*, with a laser, as described above. Or the membrane layer **32** can be formed with pores.

[000109] In one embodiment, illustrated in **FIGURE 12**, a burette **10** similar to that of **FIGURES 8-11** includes a tip **16** which is rotatable. The burette includes a housing **107**, formed from plastic, or other suitable material, which surrounds the body **12** and has an opening **108** at a lower end, through which the delivery port **20** is exposed. The housing holds the burette body **12** and a motor **300** powered by a power source, such as a battery **310**, which in the illustrated embodiment is mounted within the housing, above the motor **300**, although other locations are also contemplated. Alternatively, a remote source of power, such as mains supply, is used to power the motor and is connected thereto by suitable electrical wiring.

[000110] The motor **300** is connected by a drive shaft **312** to the cap **17** of the burette and thus imparts rotational movement to the body **12** of the burette. A switch **314**, mounted to the housing, controls the motor. The switch may be a simple ON/OFF switch, or it may be a variable switch, which allows the speed of the motor to be varied. The motor may rotate the body at a speed of from about 50 to about 20,000 rpm. In one embodiment, the motor rotates the body at from about 1000 to about 5000 rpm.

[000111] The body **12** includes a reagent holding portion **13**, which defines an interior chamber **14** that holds a quantity of a reagent in solution **11**, which is introduced to the chamber via an opening **15** at one end of the chamber **14**. For example, the chamber may hold about 5 mL of reagent solution **11**. The body also includes a tip portion **16**, in fluid communication with the other end of the chamber **14**, through which the reagent is delivered from the burette. **FIGURE 12A** shows the tip in greater detail. Intermediate the tip portion **16** and the reagent holding portion **13** is a tubular portion **18**. The tubular portion has an internal diameter which is generally constant from the tip to the reagent holding portion, although conical or other shapes are also contemplated. The internal diameter **d** of the tip, which in this embodiment, also corresponds to the diameter of the tubular portion **18**, can be as for other embodiments of the burette. In one embodiment, the diameter **d** is about 5mm. A membrane **32** covers the delivery port. The membrane can be as

previously described, e.g., of about 10-50 microns in thickness and have a plurality of bores drilled therethrough or otherwise formed therein, similar to those illustrated in **FIGURE 4**, or be a naturally porous material. The bores/pores provide a plurality of through passages between the interior of the body and the exterior. As shown in **FIGURE 12B**, the bores **36** are located in a ring, spaced from the axial center of the membrane **32** and preferably closer to the exterior of the membrane than to the center of the membrane. In this way, the reagent is carried away from the tip **16** by the fluid flow pattern in the target liquid **50**, induced by the rotation of the tip in the target liquid. Closer to the axial center of the tip, the flow pattern tends to result in a lower flow rate.

[000112] The membrane **32** is carried by a membrane fitting **320,** which is shaped to connect with the tube **18**. Specifically, the membrane fitting is internally threaded at **322** and engages corresponding threads **324** on an exterior of the tube **18**. In this way, the membrane **32** can be removed and replaced if desired. The membrane is optionally in direct contact with a gel layer **109** as described for the embodiment of **FIGURE 11**.

[000113] As illustrated in **FIGURE 12**, ball bearings **330** or other suitable guide members are located within the housing **107**, close to the tip **16** of the burette for guiding the tube **18** during rotation of the burette.

[000114] As shown in **FIGURE 12**, the housing **117** may have a window **340**, which provides access for a non-contact tachometer (not shown) or other means of measuring the rotational speed of the burette. An optical reflector **342**, or other detectable strip is mounted to a rotating portion of the burette, such as on the cap **17**, as illustrated. The tachometer is thus able to determine the speed of rotation of the burette from the number of times the strip passes the window in a given period. The detected rotational speed can be fed to a controller which adjusts the rotational speed of the burette, for example, by increasing the power supplied to the motor, in accordance with predetermined set rotational speed values.

[000115] **FIGURE 13** shows the diffusion layer of width **w** surrounding one of the holes **36** in the membrane **32** of the device of **FIGURES 10-12** during delivery of a reagent to a target liquid **50**. The limit of the diffusion layer with

and without rotation of the tip is shown. As can be seen, rotation of the tip reduces the thickness of the diffusion layer by at least 50%, and typically by at least 70%.

[000116] It will be appreciated that the burettes shown in **FIGURES 8-12** can incorporate other features of the burettes shown elsewhere, such as heaters, control systems, timing devices, and the like.

[000117] **FIGURES 8 and 9** show the burette **10** supported on a stand **90** for raising and lowering the burette. As will be appreciated, the device of any of the FIGURES shown herein can be similarly mounted on a stand. However it will be appreciated that a burette **10** can be introduced manually to the target liquid **50**, as in the embodiment illustrated in **FIGURES 5, 14** and **15.** A pen-type burette **10** illustrated in **FIGURES 14** and **15** includes a housing **107**, which surrounds the body **12** (not shown). During delivery, the tip **16** of the body protrudes though a suitably sized aperture **108** in the housing. After delivery, the tip is retracted back into the housing. The movement of the tip into and out of the housing is controlled by a start button **52** on top of the housing, which also starts and stops delivery. For example, to start delivery the tip is pushed out to contact the target solution and to stop delivery, the tip is pulled back into the housing so that contact between delivery ports and target solution ceases. The tip **16** preferably includes a membrane **32** (not shown) similar to that illustrated in **Figs. 2, 4, 4a, 10**, or **12.**

[000118] With particular reference to **FIGURE 14**, a window **110** in the housing **107** is covered with a transparent material through which a display screen **111** is visible. The display screen **111** provides information on the volume of the target liquid (10 mL in the illustrated embodiment), and its target reagent concentration or amount delivered (100μL in the illustrated embodiment). Other information may also be displayed on the screen, as described for screen **64.** For example, a user presses a selection button **112**, mounted to the housing, for selecting the target volume and concentration, which an internal processor (not shown) in the burette **10** or associated therewith uses to calculate the delivery time. Once the delivery time is over, the tip is automatically retracted into the housing and delivery is halted.

[000119] As shown in **FIGURE 16**, a burette of the type shown in **FIGURES 8** and **9** can be readily incorporated into a computer controlled system without the need for modifications to the burette **10**. Thus a single type of burette can be used in both manual and automated systems. A support, such as a stand **90** is equipped to raise and lower the burette into and from the target liquid **50**. The stand is also equipped to rotate the burette about the longitudinal axis through the burette, which results in the tip being rotated in the target liquid. Alternatively, the stand holds the upper part of the burette still, while the lower part or tip is rotated with respect to the upper part with a motor, as described above.

[000120] The rotational movement of the burette and the lowering and lifting of the burette by the support **90** is controlled via a computer control system **62**, such as a microprocessor with a keyboard **60** and display screen **64**. Using the keyboard, the user enters the desired concentration of the reagent in the target solution, the volume of the target liquid **50**, and/or other information prompted by the computer screen, such as desired final accuracy and precision. The computer computes the delivery time and controls the delivery process using a clock **44** and by moving the stand **90** between retracted (up) and delivery (down) positions at appropriate times. During delivery, the computer instructs the stand to rotate the burette. As discussed for the embodiment of **FIGURE 7**, the computer control system **62** may also obtain information from the burette via a barcode reader **70**, detect the temperature of the target liquid and/or reagent solution, and adjust the delivery time to account for the detected temperature and/or control the temperature of the burette tip and/or target liquid using a heater (not shown) analogous to heater **80**.

[000121] Another embodiment of a burette **120** shown in **FIGURES 17** and **18** is formed by soaking or otherwise infiltrating a porous substrate **122** with the reagent in liquid form *e.g.*, a solution of the reagent in a solvent. The substrate may be, for example, a ceramic substrate or gel, which is preferably chemically inert. Examples of suitable gels include polyacrylamide and agarose. The reagent-soaked substrate is then coated (*e.g.*, by "painting", sputtering, or other means of surface coating or deposition) with a cover layer **124** formed, for example, from a hydrophobic plastic, such as Teflon™, lipophilic PVC, silicone elastomer, or the like, which, after curing, acts as the

body of the burette, analogous to body **12** of **FIGURE 1**. One or more regions of the substrate **122** are left free of the cover layer **124** to provide the holes **126** for diffusive delivery. Fabrication of such a burette **120** is relatively easy and generally results in mechanical sturdiness of the resulting burette. It also facilitates the production of extremely small (micron size, *e.g.*, 1-100 micron) delivery ports **126** by removing the coat (or leaving the substrate coat free) at such very small areas only. Microlithography or laser based methods can be used to produce such very small openings in the coating.

[000122] In one embodiment, an Excimer laser **128** or other laser is used to form the holes **126** in the cover layer **124** by applying a laser beam which selectively removes tiny areas of the cover layer **124**. In another embodiment, a mask is used to define the holes **126**. The coating is applied to the mask covered substrate and then the mask is removed.

[000123] An alternative fabrication method for the burette **120** employs a device (not shown) having one or more needles of the approximate diameter of the holes. The needle or needles are preferably heated, for example, by resistance heating, or the like, and then used to puncture the cover layer **124**.

[000124] In one embodiment, the portion of the burette tip **16** that comes into contact with the target solution **50**, or optionally the entire burette **10, 120** has a hydrophobic exterior surface **130**, provided by the material of the casing **12** or cover layer **124** or by a hydrophobic coating **132** thereon (**FIGURE 3**). This reduces the tendency for the target solution to stick to the burette and minimizes the chance of cross contamination if the burette is used in a subsequent target solution. It will be appreciated that if a hydrophobic target solution is employed, the coating **132**/surface **130** is preferably hydrophilic.

[000125] The burette **120** of **FIGURE 17** is also suited for *in vivo* applications, such as the delivery of a drug or other pharmaceutically active agent into the human or other animal body. The burette **120** may be in the form of a capsule which is ingested, or it may be implanted in the humanbody. The capsule preferably delivers a drug over an extended period at a slow rate. In one embodiment, the rate is variable. For example, a slow background delivery rate provides a continuous delivery of the drug or other reagent from

the capsule (or no delivery, if the background rate is zero). The delivery rate can be changed by including one or more piezoelectric elements **140** which selectively open or close the holes **126**. For example, the holes which define the delivery ports are opened by applying an electric voltage or current to the piezoelectric element **140** (or removing an applied electric current).

[000126] The burette **10, 120** uses diffusion for reagent delivery. Once convection is blocked (*e.g.,* by suspending the reagent in a gel), and the initial and boundary conditions of diffusion are precisely set, the delivery process occurs in the same way for each burette produced. Thus, reproducibility of such a scheme is virtually unlimited.

[000127] The lower limit of the feasible delivery rates is extremely low. For example, values of F of as low as about 10^{-12} picoliters per year (equivalent volumetric delivery rate) can be delivered with good reproducibility. For laboratory applications, delivery rates of micromoles to millimoles/hr are readily obtained. It is also contemplated that higher concentrations (in the mM range) into higher target volumes (in the mL range) be delivered by appropriately configured burettes. Practical operational ranges include: reagent or target solution concentrations from the lowest conceivable levels up to 10 mM. Target liquid volumes from femtoliters (fL) up to a few mL, or more are readily accommodated. The lower limits of delivery rates are primarily where the diffusion process occurs on such a small scale as well as rate, that continuum mechanics (thermodynamics) are no longer applicable, and statistical mechanics describe the delivery process. However, this limit is practically unlikely to be reached since delivery is ideal even on the scale of hours, days, or months for delivery and into femtoliter sized target solutions.

[000128] Upper limits of delivery rates and larger target volumes can be increased by having a burette with multiple delivery ports **20**. Parallel processing in solution preparation (multiple solutions prepared simultaneously) is also feasible with a modified device having multiple, spaced delivery ports **20**.

[000129] The delivery process is "automatic," or spontaneous in the sense that no mechanical force need be applied to the burette **10, 120**. Thermodynamic principles govern delivery. A user of the ready made burette

10, 120 can carry out a solution preparation in a one-step operation, irrespective of how low is the desired solution concentration. For example, by controlling the time of contact between the tip **16** of the burette **10** and the target liquid **50**, virtually any quantity can be delivered.

[000130] For example, as illustrated in **FIGS. 8 and 9**, a predetermined amount of a target liquid **50** is delivered to a vessel **100**, for example, with a graduated pipette, or other measuring device. The tip **16** of the diffusional burette **10** is inserted into the target liquid. After the predetermined delivery time has elapsed, the burette is removed (**FIG. 9**).

[000131] The delivery rate of the reagent and its dependence on time for the particular burette **10, 120** being used are provided to the user (or are stored in the processor for the delivery device). Solution preparation can thus be achieved with a single control variable: time. Time is a variable whose accurate and precise assessment is much easier than many other variables to control or measure. Timing devices with a high degree of accuracy and precision are available. Preferably, the delivery rate and/or concentration of the reagent are such that the burette **10** delivers quantities of reagent within the user's desired range in a readily measurable and yet convenient time, such as from about 2 seconds to about 5 minutes, more preferably, from about 10 seconds to about 1 minute. Where particularly accurate timing is possible, shorter delivery times are contemplated, such as less than a second. For the burette **120**, much longer delivery times may be appropriate, for example several hours, days, or even longer.

[000132] The desired accuracy, precision, and preparation speed are generally interrelated: longer delivery times can generally be controlled better.

[000133] Since delivery occurs via diffusion, a solution can be formed with substantially no addition of solvent volume to the target liquid **50**, *i.e.,* in a preferred embodiment, the reagent diffuses from the burette **10, 120** into the target solution **50**, leaving any solvent in which it is mixed behind in the burette. This renders the relevant calculations extremely simple since the volume of the target solution after addition can be assumed to be essentially the same as the volume prior to addition. This is particularly useful in

multiple standard addition type analytical schemes. Such schemes are frequently used in measurements involving ion selective electrodes, atomic absorption spectrometry, ICP-MS, and many other analytical techniques, especially when the samples to be analyzed involve complicated matrices (such as serum or blood) that are difficult if not impossible to approximate with any man-made calibration standards. It should be noted that although osmotic pressure differences between the target liquid and the burette reagent solution may cause some solvent movement, this is generally negligible under practical conditions (or is compensated for by an approximately equivalent rate of movement into and out of the burette).

[000134] The burette **10, 120** has applications in a variety of fields. It is particularly useful in clinical, environmental, industrial, and research laboratories, in any field that benefits from precise liquid preparations, and especially, in preparation of relatively small volumes. Industrial applications include the chemical and pharmaceutical industries, particularly whenever small batches of reagents are needed, or where the end product, being very expensive, is produced in small batches. Diagnostic applications include clinical, environmental, industrial production and quality control, as well as defense settings. Reactions performed in small scales include research, pharmaceutical, and environmental procedures, as well as where precious materials are processed or produced (such as in the noble metal related industries or in expensive and labor intensive syntheses). The burette can be used to prepare calibration samples for calibrating precise analytical equipment, due to it high accuracy and precision. Moreover, since the burette is amenable to fabrication at relatively low cost and in large numbers, it is suited to use in schools, hospitals, and other settings where large numbers of solutions are to be prepared or where precision measuring equipment or technical expertise is not readily available or is costly to use.

[000135] When preparing a solution, considerations generally include: (1) solution volume, (2) desired final concentration, and (3) desired accuracy and precision of the final solution composition. Based on this information, the characteristics of the burette that is best suited for the given task can be derived. These characteristics are used to either design a burette for the given task, or to select one from an assortment of ready-made burettes.

[000136] In one embodiment, a burette **200** is formed with a large, macroscopic, surface area **210** that is active (*i.e.*, delivering the reagent) (**FIGURE 19**). This occurs, for example, with a porous membrane **32** (*e.g.*, a Cyclopore™ mebrane) which has a large number of pores, each one delivering reagent, or a where both sides of a macroscopic diffusion membrane, such as cellophane, are effectively stirred. It is also contemplated that a single burette **10, 120** may have two or more interior reagent chambers or barrels **14,** each chamber being associated with a separate delivery port **20**. Each chamber may hold the same or a different reagent. The ports **20** may be configured to deliver the respective reagent at the same rate or at different rates, for example, by having different sized ports, different membrane materials, and or different size pores.

[000137] With reference once more to **FIGURE 1**, the burette **10, 120, 200, 220** may be stored and shipped in a holder **240**. The holder provides a closed compartment **242** in which the burette may be placed inside and in which the atmosphere is solvent (*e.g.,* water) saturated. A solvent-saturated environment can be provided by soaking a carrier **244**, such as paper tissue, cloth, or the like in a solvent, or by providing a compartment (not shown) that is porous and that contains some solvent. This creates a solvent vapor rich gaseous environment without allowing the solvent to come into contact with the burette tip **16**. The atmosphere itself can be air. If the reagent is one which is deteriorated by oxygen, an inert gas, such as nitrogen or noble gas may be used. In some circumstances, damage to the burette may result from use of water or other pure solvent because a high concentration salt, or acid, alkali, or the like is stored inside the burette, which may slowly absorb water or other solvent inside from the water saturated atmosphere in the burette holder. In such circumstances, it is preferable for the carrier **244** or porous compartment to contain the same solution as is inside the burette to saturate the air around the burette. This also reduces the risk of contaminating the burette if, for example, physical contact during storage and shipping occurs between the burette tip and the carrier solvent or solution. By using the same solution for the carrier and burette, such risks are minimized.

[000138] To ensure that the contents of the burette remain sterile, particularly when the burette is to be stored for some time prior to use, or

contains a salt solution prone to microbial growth, the solution providing the atmosphere in the holder **240** may include an antimicrobial agent.

[000139] The construction of the burette tip may vary according to the chemical to be diffused. The burette material should be compatible with the reagent which is to be diffused, *e.g.*, acids and oxidizing reagents may use different materials. For light sensitive chemicals, an opaque casing **12** or a housing **107** may be used.

[000140] While the invention has been described with particular reference to small membranes **32** covering a conical tip **16** of a burette, it is also contemplated that large, flat membranes may be used, although as discussed below, ensuring a Nernstian concentration profile within the membrane and on both sides (*e.g.*, by stirring the liquid on both sides of the membrane) may make the design internally and externally more complicated. By "Nernstian," it is meant that the profiles do not change appreciably in time, allowing for a constant delivery rate.

[000141] It will be appreciated that although the system has been described generally in terms of delivering a single reagent, multiple reagents can readily be delivered, either from a single burette (e.g., by combining both reagents in a single solution), or by using multiple chambers, each one attached to the same delivery port or to a separate delivery port, such that the reagents are simultaneously delivered when the delivery port or ports are placed in contact with the target liquid.

[000142] In another application, one of the burettes described herein is used in combination with a feedback system which monitors a change in a property of a target solution (e.g., ISE spectrophotometry, fluorescence, visible property, such as color change, pH electrochemical measurement, or the like). The feedback system determines the concentration of the reagent in the target solution from the detected change and provides feedback in the form of a signal to the burette to stop delivery when a desired concentration is reached. With such a system, calibration of the burette can be eliminated.

[000143] In another application, a multicomponent solution is prepared by having separate different delivery ports which are started and stopped at appropriate times to prepare a predetermined multicomponent target solution.

Theoretical considerations

[000144] In designing a burette the following considerations are made: (1) the initial and boundary conditions that use of the burette entails; (2) the delivery rate and its dependence on (contact) time as applicable for the specified initial and boundary conditions; and (3) the total amount of deliverable species (reagent) in the burette body **12**.

[000145] Where the shoulder region **18** is wider than the tip **16,** this "limits" the rate limiting step into the tip region. For conventional pipette tips, such as an Eppendorf type of plastic pipette tip there is no widened shoulder, only a conical tip which extends from a body. For conventional pipettes, the Cottrellian analysis of the pipette is the same as that of a microdisk electrode, except that the solid angle of the space considered is much smaller than the full sphere of a microspherical electrode or semisphere of a microdisk electrode. A steady state solution to this (spherical, semispherical, or conical) mathematical diffusion problem is possible, but it takes time to reach it. The steady state solution can be computed based on the steady state term of the microelectrode equation, as described in greater detail below.

[000146] In the present case, it is desirable to reach steady state conditions as quickly as possible, thus ensuring a constant delivery rate.

[000147] First the case is considered in which it is assumed that the inside of the burette is "semi-solid", meaning a gel **22** or a porous substance (like ceramic) is present that contains the reagent solution, so that there is no considerable convection inside the burette.

[000148] For such burettes one possible realization is very similar to a cylindrical tip, which has a very small hole at the end, or near the tip end (**FIGURE 12A**).

[000149] Therefore, a Cottrell type equation (Equation 1) describes reagent delivery where it is assumed the reagent concentration outside the burette to be negligible:

$$F(t) = \text{Cottrell transient} + \text{steady state microelectrode term} \quad (1)$$

[000150] where F(t) is reagent flux or delivery rate into the target solution, t is time with t=0 at the moment when the delivery port **20** touches the target solution. More accurately, for Equation 1 to be valid, reagent concentration inside the target solution should be always negligibly small (during the entire operation) compared to the burette bulk reagent concentration. This assumption is valid, however, for almost any type of practical application.

[000151] The delivery at time zero, t=0, will have a very high rate but only for an infinitesimally short time period. In practice, this means a very short time, negligible compared to the duration of the entire delivery operation. After the initial burst, the delivery rate decays rapidly to reach a steady state at a rate that corresponds to the stationary term of the Cottrell type "microelectrode" equation (steady state, second term microelectrode term in Equation 1).

[000152] The transient which occurs before stationary operation is reached has a duration which is roughly proportional to the diameter **d** of the delivery port opening. If the diameter **d** is in the order of 10-30 microns then we can assume that the depleted region inside the burette at steady state will be about 100 microns thick. This, for usual diffusion coefficients, means that the transient term (Cottrell transient in Equation 1) decays within about 100 - 200 milliseconds, which is a very short time, and is therefore negligible for most practical designs and operation. However, for larger openings (*e.g.*, about 100 microns to 1 mm), this transient time is larger, and may be on the order of several minutes. For such cases, calibration of the burette preferably includes an assessment of the transient. Between each delivery time period, a time period sufficient to reestablish uniform concentration in the burette is allowed to pass. Alternatively, a second or subsequent delivery is commenced immediately after the previous delivery is completed so that the steady state conditions which have been achieved in the first delivery are maintained. Alternatively, the burette tip is stored in contact with a large reservoir of buffer to allow continued delivery between two actual reagent making operations. In all these cases, identical or substantially identical initial conditions can be achieved for each delivery. This ensures reproducibility of the delivery process which is important for achieving accuracy. This makes burette calibration valid for each subsequent delivery process.

[000153] While any reproducible delivery curve can be used as long as the desired reproducibility is provided, in practice, best results are obtained when the transient at the beginning of each delivery is negligible with respect to the entire duration of the process. It is possible to design a burette for a given reagent such that term 1 (Cottrell transient) in Equation 1 is negligible with respect to term 2 (steady state term). Thus, in practice, linear (constant rate) delivery is possible within a reasonable tolerance.

[000154] Where the transient term is not negligible, there may be a short waiting period before a burette can be reused again after a delivery operation. The initial conditions to satisfy the above equations should be valid at the beginning of the next operation for reproducible deliveries. The waiting period allows reagent concentration to become homogeneous again inside the burette. For most accurate results, homogeneous conditions should be established as perfectly as possible. Therefore, this waiting period is preferably twice as long, and, more preferably, at least three times longer than the duration of the initial transient in the equations. For example, if the transient is about 50 milliseconds long then this requirement is easy to fulfill and does not make any practical difference. If it is several minutes, however, then a waiting period in the order of 10 minutes between two subsequent uses of the same burette may be appropriate. Preferably, the tip is constructed such that the waiting time is less than one minute, more preferably, less than ten seconds, most preferably, about one second or less, such that an operator is not likely to cause an error in delivery by inadvertently using the burette before the waiting time has elapsed. Alternatively, the delivery device may be programmed to provide an alarm or an ALL CLEAR signal when the waiting time is over.

[000155] Preferably, the membrane and gel (where present) are selected such that the transient term is 1 second or less, such that, after a period of contact of less than 1 second, the reagent diffuses into the target liquid at a substantially constant rate (i.e., the reagent delivery rate does not vary by more than 10%, preferably, no more than 5%) throughout the delivery period).

[000156] The above considerations apply to relatively small molecules (i.e., those having a molecular weight of about 5000, or less). For larger molecules, the diffusion coefficient may decrease such that the molecule may take several

minutes to travel a distance of a few tens of microns. In such cases, the transient time may still be significant and should be taken into consideration, particularly where a high degree of accuracy is desired. This means that a simple linear R(t) calibration may not suffice. However, a nonlinear one can be generated which achieves very good accuracy. It has been found that decreasing the diameter **d** of the tip and tube **18**, allows larger molecules to be used with greater accuracy.

[000157] Using the diffusional burette instead of a conventional method for making a solution is useful for cases when the target solution cannot be made without more than one dilution. If the target solution can be made by weighing materials on a balance, these can be dissolved in a flask, and diluted perhaps once more, then a conventional method can readily be used for its preparation. Once two or more consecutive dilutions are needed, i.e., when the target concentration is low, then the burette **10** becomes a useful tool to produce the desired (low) concentration in the target solution in a single step, precisely and accurately. This step consists of immersing the tip of the burette in the target solution for a predetermined period and then withdrawing it. In this case, i.e., when this same solution would have required two or more dilutions to make, it is unlikely that the burette itself will get depleted of the delivered substance to an extent larger than negligible.

[000158] For such applications, the equations that describe the diffusional burette operation and help designing actual burettes are as follow and are illustrated schematically in **FIGURE 26**, where: F is flux, c is concentration, subscripts b, h, l, s are as follows: **b** stands for burette bulk, **h** stands for inner or burette end of a pore **36** in the delivery membrane, **l** is lower or outer end of the pore, and s is in the bulk of the target solution. **D** is diffusion coefficient (considered here the same for burette, pore **36**, and solution, for the same chemical), **r** is pore radius, **d:** pore diameter, **A**: cross sectional area of pore, and **z** is membrane thickness = pore length. Concentration in solution bulk, c_s, is virtually zero. With these conventions:

1. Steady state operation of one pore within the burette delivery membrane is described as

$F_b = 4Dr(c_b-c_h)$	flux from burette body into pore
$F_p = (AD/z)(c_h-c_l)$	flux across pore
$F_s = 4Drc_l$	flux from pore into target solution

[000159] In steady state, all these fluxes are equal and the same. This defines two equations with two unknown variables (c_h and c_l) which can be solved:

$$c_h = [(4+\pi d/2z)/(4+\pi d/z)]c_b$$
$$c_l = [(\pi d/2z)/(4+\pi d/z)]c_b$$

[000160] From these, delivery flux by one pore is:

$$F = D\,[(\pi d^2/z)/(4+\pi d/z)]c_b$$

This gives, for a very wide pore ($d/z \rightarrow \infty$): $F = D\,d\,c_b = 2\,D\,r\,c_b$
and for a very narrow pore ($d/z \rightarrow 0$): $F = 0$

[000161] Burette delivery rate is then F times the number of pores.

2. Effect of flow in the solution on delivery rate:

[000162] Calculating the relative concentration drop within the pore compared to the entire concentration drop from burette bulk to solution bulk, i.e., $R = (c_h-c_l)/c_b$, estimates the extent to which transport within the pore is rate limiting. For example, if R = 78% then 11% of total concentration drop from burette bulk to solution bulk drops within the burette approaching the pore, 78% drops within the pore, and 11% drops within the solution adjacent to the pore. The more of the entire concentration drop is confined to within the pore the less sensitive delivery rate will be on eventual flow in the target solution. As an example, for a 10 micron thick membrane (z = 10 microns) the following can be calculated, as shown in TABLE 1:

TABLE 1

d (microns)	R (%)
100	11
10	56
1	92
0.1	99

[000163] This means that a pore **36** which is 10 microns long and 0.1 microns (= 100 nm) wide will confine 99% of the entire concentration drop to within itself. This is excellent because flow dependency is then practically eliminated.

3. Transient duration

[000164] Another characteristic to be considered is how fast the initial transients die out after immersing the burette tip into the target solution. The faster this happens, the quicker steady state delivery develops. This is characterized by the above equations and estimated flux. This flux is a constant, meaning a constant, predictable, and known delivery rate and a linear calibration. The longer the transients are, the more effect they will generally have on the ultimate amount delivered, and the less predictable the amount delivered tends to be.

[000165] From Table 1, it is clear that narrow and long pores are preferred (e.g., d/h = 0.1 or less) for avoiding flow dependency. In this case almost the entire transient will consist just of the transient within the pore and the transients in the two adjacent areas (burette and solution) will die out much faster. Therefore it is sufficient to consider transient duration within the pore. This is approximated by:

$$t_{transient} = z^2/D$$

[000166] If this value is much smaller than the total duration of reagent delivery then transient effects are negligible. For example, for a 10 microns thick membrane (10 microns long pores, or z = 10 microns) and for a small ion ($D = 10^{-5}$ cm^2/s):

$t_{transient} = 10^{-6}$ cm^2 / 10^{-5} cm^2/s = 100 ms = 0.1 s

[000167] Thus, a delivery time of as short as 10 seconds is long enough to make transients negligible (100 ms is 1 % of total delivery time).

[000168] Some bias due to excess delivery during the transient may also be incorporated in the calibration. This excess is known and can be added to an otherwise linear calibration. For example, for a 100 second delivery in the above example, the transient will only be 0.1% of total delivery and therefore any bias is negligible. For a 10 second delivery, however, it may be desirable to account for the 1% of delivery time, which is spent in the transient, in the calibration. For the example above and using a 1 μm diameter pore, the excess amount can be calculated as:

$$\text{Bias} = c_b \, z \, \pi d^2 / 3 = 10 \, \mu m \, \pi \, 1 \, \mu m^2 \, c_b = 10 \times 10^{-12} \, cm^3 \, c_b = (10^{-11} \, mL) \, c_b$$

[000169] Using a burette filled with a 1 M = 1 mmol/cm^3 = 1 mmol/mL reagent, this yields:

Bias = 10 picomol

[000170] As long as the pore is used to deliver many times more than this amount, the bias can thus be neglected. If it needs to be taken into account, however, then the following calibration can be used:

$$N(t) = (3 \, \text{Bias}/t_{transient}) \, t + \text{Bias}$$

where N(t) is the delivered amount during time period, t; t=0 at the instant of immersing the burette into the solution. For the given example, this translates to

$$N(t) = (3 \times 10^{-11}/0.1 \, s^{-1})t + 10^{-11} \text{ moles} = 3 \times 10^{-10} \, t + 10^{-11} \text{ moles}$$

$$= 300 \, t + 1 \text{ picomoles}$$

where t is the time in seconds. Thus the excess amount delivered during the initial transient is only 0.33% of the total amount when a 10 second

delivery is considered and only 0.03% for a 100 second delivery. In most cases, the bias can thus be neglected.

[000171] To determine the delivery time for achieving a selected target solution concentration from a conical tip, as shown in FIGURE 1, the following equation can be used,

$$C(t) = Cr\ (1\text{-}exp[-B.t/V]) \qquad \text{where} \qquad B \approx D\ r\ \pi\ tan\theta \qquad (5)$$

and where C(t) = the concentration of the reagent in the target solution at delivery time t

Cr = concentration of the reagent within the burette

V = volume of the target solution

B = a geometric factor (dependent on the shape of the burette tip)

D = diffusion coefficient of the reagent molecules being delivered

r = radius of hole in burette tip

θ = cone angle (see FIGURE 2)

t = time, being zero at beginning of delivery

For example, for ferriyanide, if

$D = 7.3 \times 10^{-6} cm^2/s$

$r = 0.04$ cm

$\theta = 12.5°$

then, $B = 2.03 \times 10^{-7} cm^3/s$

Since c(t) = R(t) / V

then the actually valid expression can be substituted into R(t); this for the above conical tip would make

$$B = D\ r\ \pi\ tan\theta$$

which is steady state flux in this case, so it would make:

c(t) = const flux times t / V

Since the diffusion coefficient D is dependent on temperature, the value of D for the actual delivery temperature should be used in the equation (or a correction factor for the temperature). Preferably, the burette is operated at a constant temperature such that D remains a constant. The diffusion limiting membrane is applicable to a conical design of tip and also to other embodiments.

[000172] In one embodiment, a burette **200** is formed with a large, macroscopic, surface area **210** that is active (*i.e.*, delivering the reagent) (**FIGURE 19**). With such a delivery system, larger volumes can be achieved. For example, when delivering the reagent from a larger area of which the surface is coated with a planar diffusion limiting coat (*e.g.*, "painted" on a semi-solid burette interior –see **FIGURE 17**) or any other layer similar to a limiting membrane, then macroscopic delivery rates can be achieved. To avoid the effect of a transient, depletion should not propagate far outside of this membrane **210** in either direction. Therefore, we still have the same condition fulfilled, *i.e.*, that almost all gradient will drop within a very thin layer. In this case, reproducibility as well as negligible waiting (transient) times are feasible with rates similar to mechanical delivery tools.

[000173] The transient time can be eliminated, for all practical purposes, by providing a flat section of the burette tip in which there is one, or a number of, very small hole(s) and the holes are filled with a diffusion limiting membrane (**FIGURES 4, 12**). This provides a flexible design, the number of holes being selected according to the desired delivery rate range, with exactly the same dynamic characteristics. A number of similar small holes provides an array of very small sources. The number of holes is generally proportional to the delivery rate as long as the distance between the holes is larger than the extension of the depletion (same as the semispherical region of non-uniformity, see above) around such a hole. Thus:

$$C(t) = n\, Cr\,(1-\exp[-B.t/V]) \qquad \text{where } B \approx n\, D\, r\, \pi \tan\theta \qquad (6)$$

where n is the number of holes

[000174] Depletion refers to that both inside the burette and in the target solution. The array of holes **36** can be seen as analogous to a microelectrode array that has its own particular mechanism of mass transport. Another advantage of this approach is that steady state operation is generally reached within milliseconds when the holes are very small (in the few microns range). This means a very negligible initial burst at the beginning, yet enabling high macro flux analogous to the macro current for a microelectrode array. It has been found that equations valid for such microelectrode arrays can be applied to delivery from a burette by replacing electrical current with molar flux.

[000175] In each of these design approaches, the homogeneity of the target solution **50** may be a factor, and thus for high accuracy and precision, stirring or otherwise homogenizing the target solution is optionally provided by a stirring means. Homogenizing helps to ensure that the burette is function ideally during the entire duration operation (*i.e.*, delivery is closely approximating that predicted from theory). Validity of the ideal equations (see above) is based on the assumption that the concentration in the target solution locally, adjacent to the hole or membrane of the burette, is zero (or negligible), as compared to the concentration in the body of the burette. This assumption is satisfied in many situations. For example, in the case of a conical burette tip, there is a huge solid angle of water or target solution outside the tip into which the delivered molecules rapidly diffuse away from the burette. In such cases, stirring is not generally necessary to achieve this condition. However, in the case of a large flat membrane with diffusion coefficients not much lower than in water, then stirring ensures that the reagent is dispersed at a sufficient rate to ensure the validity of the equations. Inside the bulk of the burette there may also be a need for stirring to achieve the former mentioned condition.

[000176] Stirring also has an advantage in that it is generally desirable for the target solution to be homogenous at the end of delivery. Stirring can be obtained in a variety of ways, such as with a magnetic stirring bar inside the target solution. However, when it is desired to produce very precise solutions of very small volumes and sometimes very low concentrations, other stirring methods may be appropriate as in some circumstances, as a stirring bar can cause cross contamination between different solutions, for example, the solution is in a container **100** which is stirred by an external vibrating

mechanism **104** (**FIGURE 5**). Another alternative method of producing stirring in the solution is to move the burette, *e.g.*, by rotating or vibrating the burette. As shown in **FIGURE 7** a vibrating mechanism **106** causes a small vibration or rotation of the tip **16**. **FIGURE 12** shows a rotation of the tip itself.

[000177] Movement of the burette tip **16** can be in a horizontal or vertical direction. Or, the burette **10, 110** may be moved in a circular motion, or in other directions. Preferably, the direction of the vibration is not perpendicular to the plane of the membrane **30** or delivery hole or surface, as in some cases, this may induce some (variable) pressure on the membrane leading to damage of delivery area and thus delivery is less accurate. Preferably, there is at least about 20°, more preferably, at about a 45°angle between the plane of the hole and the direction of the vibration. For example, vertical or horizontal vibration or movement is preferred if the delivery holes are in a tilted side of the burette tip (like tilted by 45° at the tip). To avoid mechanical wave resonance in the target solution resulting in amplification of the vibration, a vibration source **104, 106** which provides vibration which is generally random in nature, *i.e.*, in amplitude, frequency, and direction may be employed. Such "random" vibrations, may be produced, for example, by employing a random generator in a microprocessor **62, 62'** controlling the vibration mechanism **104, 106.**

[000178] Another option is to provide stirring of the reagent inside the burette. While this is generally not possible when the reagent is soaked and stored in a porous or gel-like substrate, where the reagent is in a liquid form, stirring may be employed. Stirring inside the burette is particularly effective where a planar diffusion membrane is used as the active surface of the burette. In this case, vibration of the burette **10, 110, 200** both stirs the interior reagent and also stirs of the surrounding target. Where stirring is used, the membrane need not be diffusion limiting to provide a high degree of accuracy and precision. The membrane may thus have a high diffusion coefficient yet function efficiently. This is because the diffusion is confined to the membrane, in this case, not by a low diffusion coefficient, but by both solutions (reagent in burette, and target solution) having agitated convection on both sides of the membrane. This may be advantageous when a high delivery rate is desired.

[000179] As discussed above, for an arrangement in which the burette tip has multiple holes **36**, it is preferable that there be little overlap, more preferably, no overlap between the depleted domains of adjacent holes inside and outside the burette. This is readily achieved for a short time with even relatively large openings or delivery ports such as, *e.g.*, 200 microns. For example, the holes may be about 0.1 to 2mm apart.

[000180] Without intending to limit the scope of the invention, the following Examples demonstrate preparation of a diffusional burette

EXAMPLES

Example 1: Preparation of a Ferricyanide Delivery Burette

[000181] Ten mL of an aqueous solution containing 3.29g of $K_3[Fe(CN)_6]$ (P-3667 Sigma) and 74.6mg of KCl (P217-500, Fisher Scientific) was prepared. To this solution, 0.5g of a gelling agent, agarose (A-6013, Type 1: Low EEO [9012-36-6], Sigma) was added. This mixture was heated up to 80 °C until the solution becomes clear. One mL of this solution was filled into a micropipette tip (101 –1000 µL, 2 13/16 inches, Eppendorf style, 21-375E, Fisher Scientific) and allowed to stand at room temperature until the filling solution became gel. The detailed dimensions of the thus prepared ferricyanide delivery burette are shown in **FIGURE 24.**

Example 2: Preparation of a Potassium Delivery Burette

[000182] Ten mL of an aqueous solution containing 74.6mg of KCl (P217-500, Fisher Scientific) 5.84mg of NaCl (S-9625, Sigma) and was prepared. To this solution, 0.5g of agarose (A-6013, Type 1: Low EEO [9012-36-6], Sigma) was added. This mixture was heated up to 80 °C until the solution becomes clear. 200 µl of this solution was filled into a micropipette tip (1 –200 µL, 1 7/8 inches, Eppendorf style, 21-375D, Fisher Scientific) and allowed to stand at room temperature until the filling solution became gel.

Example 3: Ferricyanide Delivery

[000183] A ferricyanide burette was prepared as for Example 1. **FIGURE 20** shows the experimental setup for ferricyanide (and potassium) ion delivery. 200 µL of 0.1M KCl aqueous solution was used as a sample (target) solution

50. The solution **50** was placed in a container having a silicone elastomer liquid support surface. To monitor the delivered ferricyanide concentration into this sample solution, a micro Pt electrode **250** 8μm in diameter was used in combination with a counter electrode **252** formed from stainless steel wire and a reference electrode **254** (BAS Ag/AgCl microreference electrode). The cyclic voltammograms for various delivery times from 0 to 41.3 minutes are shown in **FIGURE 21**. The current due to reduction or rather, electro-reduction of ferricyanide increases with increasing delivery time, indicating successful reagent delivery with the prepared diffusional burette **10**. **FIGURE 22** shows three sets of the ferricyanide delivery results obtained by using the same burette (labeled data 1, data 2, and data 3, respectively). Linear relationship between the delivered ferricyanide concentration in the sample solution and the delivery time was found in each set of data, as shown in plots 13a, 13b, and 13c. **Table 2** lists five sets of data for the delivered ferricyanide concentrations after 15 minutes reagent delivery by using the same burette. Very good reproducibility of the reagent delivery was obtained with 0.020mM as the standard deviation. This result demonstrates the potential of this diffusional burette for the quick and accurate preparation of standard sample solutions.

Table 2

Run	Conc. Of $[Fe(CN)_6]^{3-}$ in the Target Solution
1	0.778 mM
2	0.821 mM
3	0.793 mM
4	0.817 mM
5	0.825 mM
Average	0.807 mM
Standard deviation	0.020 mM

Example 4: Potassium delivery

[000184] A diffusional burette for delivery of potassium (and chloride) ions was prepared as for Example 2. Ten mL of 0.01M NaCl aqueous solution was used as a target solution. To monitor the delivered concentration of potassium in the sample solution, a liquid membrane-type ion-selective electrode based

on valinomycin was used in pace of the platinum electrode system of **FIGURE 20**. **FIGURE 23** shows the relationship between the delivered potassium concentration in the sample solution and the delivery time. The plot does not show actual data points but is derived from data obtained every one to two minutes and fitted to the curve shown by the least squares method ($C = 12 \times 10^{-4} t + 8.0 \times 10^{-4} t^{0.5}$). Almost perfect linear relationship was observed.

Example 5: Potassium delivery

[000185] Potassium ions were delivered from a burette of the type illustrated in **FIGURES 8-11** in which the reagent concentration in the burette C_b was 1M, the diameter **d** of each hole **36** was about 250μm, and the number **N** of holes was 5. The delivered amount, in Moles was plotted against delivery time in minutes, as shown in **FIGURE 25**. The linearity (r^2) was at least =0.999.

[000186] **FIGURE 25** can be used to calibrate a burette. Good linearity can justify using a linear calibration for R(t), but other cases are satisfactory as long as a reproducible delivery process is ensured. Knowing the desired amount of potassium ions to be delivered, the delivery time can be found from the plot.

Example 6: Comparison of a Diffusional Burette with a Conventional Pipette and Transfer Burette

[000187] A burette of the type used in Example 5 was prepared. with N=5. "Identical" deliveries were made with the burette to target solutions. The standard deviation was 0.7%, which was within the range of error of the potassium ion selective sensor used to measure the potassium ion concentration delivered (i.e., with more accurate detection techniques, the standard deviation could well have been lower). By comparison, a conventional method with a conventional burette, calibrated delivered solutions resulted in a standard deviation of 8.5% in final concentration, and a pipette tip system had a standard deviation in produced final delivery concentration of 8.8%.

[000188] The delivery time with the pre-prepared diffusional burette was generally less than ten minutes, which compares favorably with the time

taken to prepare reagent solutions and load the transfer burette or pipette tip system and deliver the reagent.

Example 7: Effect of Pore Number and Stirring Speed on Delivery

[000189] Expt 1: Diffusional burettes were prepared as for Figures 8-11, one with a single hole (pore) of diameter = 350μm, the other with five holes of diameters = 150μm. Both burettes was filled with 1M KCL and 10mM NaCl solution.

[000190] A target solution of 10mL of 10mM NaCl solution was prepared. Potassium ion in the target solution before delivery was zero (checked by ion-selective electrode that it is at least far less than 10^{-6} M).

[000191] A potassium ion selective electrode (based on Valinomycin) and a double junction type reference Ag/AgCl electrode were prepared. The output voltage was frequently calibrated by measuring the voltages on 10mM NaCl plus 0, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M KCl solutions, respectively. Outer and inner filling solution in the reference electrode was 10mM and 3M NaCl solutions, respectively. Data processing was carried out using Matlab software.

[000192] A first experiment used fast stirring and simultaneous sensing. The electrodes and a temperature sensor (which is connected a to pH meter), were put in the 10mL NaCl target solution in a 20mL size beaker. A stirring bar was placed on the bottom of the beaker and rotated very fast (about 10 rotation/s). Then, the selected burette was placed in the target solution for 15-18 min. Output voltages from the electrodes were automatically transferred to and recorded in a PC every 20 seconds, with zero time corresponding to when the burette was put in the target solution. Voltages were translated to concentration using the calibration curve. Temperature in the solution increased 2.5°C during the delivery due to simultaneous stirring.

[000193] Expt. 2: Experiments with burettes as described above in Expt. 1 were performed similar to those described above, but were carried out without continuous stirring over a 15min delivery period. 10mM of NaCl target solution was prepared in the 20mL size beaker and the burette was put in it for 15min. A stirring bar was placed on the bottom of the beaker, but it was rotated only before each concentration measurement for mixing up the

delivered reagent in the solution. After 15 min of delivery, the burette was taken out from the solution, and the solution was stirred, and then the electrodes and temperature sensor were put in the solution to read the output voltage and temperature. This experiment was repeated 3-4 times to see reproducibility for both versions of burettes.

[000194] Voltages translated to concentration, and temperatures at measurement were:

5 holes
(1st experiment) 1.0394e-004 M, 22.9°C
(2nd experiment) 1.0054e-004 M, 22.7°C
(3rd experiment) 1.0129e-004 M, 22.5°C
(4^{th} experiment) 9.6178e-005 M, 22.4°C
average: 1.0049e-004 M
standard deviation: 3.2232e-006 M (3.21%)

1 hole
(1st experiment) 6.3477e-005 M, 22.4°C
(2nd experiment) 5.4502e-005 M, 22.4°C
(3rd experiment) 5.3057e-005 M, 22.4°C
average: 5.7012e-005 M
standard deviation: 5.6454e-006 M (5.65%)

[000195] It was found that the limitation in reproducibility was largely accounted for by errors in measurement with the potassium electrode. This was checked by measuring the concentration of the same solution (0.1mM KCl, 10mM NaCl solution) 4 times with 15 min interval between measurements. Result showed ~3% of reproducibility error (std).

[000196] Expt. 3: A further experiment was carried out as for Expt. 2, but using slow stirring and sensing after every 15min delivery. The procedure was the same as for Expt. 2, except that the stirring bar was rotated slowly (about 2rotations/sec) during delivery. Results showed reproducibility which was not as good as for Expt. 2. It was concluded that for the burettes studied, stirring by stirring bar may fluctuate the depletion field and cause less than optimal reproducibility of delivered reagent amount.

[000197] Expt. 4: A further experiment was carried out as for Expt. 2, without stirring using the 5 hole burette, to check for linearity. The burette put in the target solution for 3 minutes and taken out from the solution. The solution was stirred, and concentration was measured by electrodes. The same burette was then replaced in the measured solution for a further 3 minutes and the measurement procedure was repeated. Concentrations were measured after 0, 3, 6, 9, 12, 15min deliveries. Between taking out from the solution and putting back into the solution, the burette was placed in a dummy 10mM NaCl solution. The burette was washed with distilled water and wiped carefully before put back it into the solution.

[000198] Expt. 5: A further experiment was carried out with stirring and sensing with almost zero intervals for 15min delivered solutions. Five 10mM NaCl target solutions in the 20mL size beakers were prepared. A five hole burette prepared as for Expt. 1 was put in each solution for 15min. Care was taken to ensure air pockets did not form around the burette tip, by tilting the burette.

[000199] After each 15 min delivery, each solution was stored by covering the solution by parafilm. A stirring bar was placed on the bottom of each beaker, but it was rotated only during concentration measurement. After all 15min deliveries were done, output voltages and temperatures from all 5 stored solutions were read. Voltages translated to concentration, and temperatures at measurement were:

5 Hole

(1st solution) –96.4mV >>> 8.54e-005 M, 22.4°C
(2nd solution) –96.2mV >>> 8.61e-005 M, 22.4°C
(3rd solution) –95.9mV >>> 8.71e-005 M, 22.4°C
(4^{th} solution) –96.3mV >>> 8.58e-005 M, 22.4°C
(5^{th} solution) –96.1mV >>> 8.64e-005 M, 22.4°C
average: 8.6163e-005 M
standard deviation:6.3057e-007 M (0.73%)

[000200] The reproducibility obtained was comparable to the measurement error of the potassium electrode. Thus the good delivery reproducibility of the burette is demonstrated.

[000201] Expt. 6: A further experiment was carried out as for Expt. 5, with no stirring and measurements taken after all deliveries. Nine beakers were prepared and heated to one of three temperatures. The delivered amount changed dramatically with temperature.

22.9 °C ~ 65μM
25.0 °C ~ 70μM
28.5 °C ~ 90μM

[000202] The diffusion coefficient, D, is understood to be linear with temperature, T, i.e., D=kT. However, the temperature dependence measured was not linear with T. It is proposed that convection may be responsible for the nonlinear increase of delivery rate of potassium with temperature.

[000203] Expt. 7: A further experiment was carried out to check the difference of reproducibility and delivery amount, between rotation (of the burette) and no rotation, and between stirring and no stirring using the following conditions

Rotation (5 hole) 0.59-0.63A motor current.
Stirring; slow (stirring bar rotating 5-15rps)
10min delivery to 150mL solution for each experiment.

[000204] The results for delivery amount are as follows:

1. No stirring / No rotation :	mean:	170μM
	std:	0.8%
2. No stirring / Rotation:	mean:	233μM
	std:	1.8%
3. Stirring / No rotation:	mean:	226μM
	std:	4.2%
4. Stirring / Rotation:	mean:	218Mm
	std:	5.0%

[000205] It can be seen from these results that rotation of the burette tip in the target liquid increased the delivery rate by 37%. Although rotation was not entirely in axial alignment, and the electric current of the motor fluctuated somewhat (0.59-0.63A), the reproducibilty was still 1.8%. By more careful orientation of the rotational burette to achieve more uniform rpm and closer alignment, it can be expected that the results for rotation will be better than those obtained here.

[000206] Expt. 8. Comparison of conventional glassware with the diffusional burette.

A. Conventional Method

[000207] The procedure is as following. All necessary solutions (including 10mM NaCl solution) were prepared with the following glassware: 1st 100 mL glass measuring beaker; 2nd 100 mL glass measuring beaker; 3rd 10 mL glass measuring beaker; and 1mL Transfer burette. The beakers were washed with pure water 3 times followed by washing by 10mM NaCl solution 1 time. The beakers were filled with 10mM NaCl solution. 372.8g of KCL was weighed (mw 74.56) and added to the first beaker. 1mL of the solution was transferred to the second beaker, using the transfer burette and shaken. 1mL was transferred from the second to the third beaker in the same way. This was repeated five times. The procedure, from beginning to end, was also timed. The results for time, sensor voltage, and calculated concentration were as follows:

1st) 23min05sec / 122.1mV / 49.3µM
2nd) 19min07sec / 124.0mV / 45.9µM
3rd) 17min38sec / 123.6mV / 46.6µM
4th) 15min05sec / 119.0mV / 55.5µM
5th) 14min12sec / 119.8mV / 53.8µM
mean: 50.2µM
std 8.5%

B. Diffusional Burette

[000208] Using a burette as described for Expt. 1, delivery was made into three solutions to determine a delivery rate. The delivery rate, taking the

average of 3 results was 2.08uM/min. From this, a delivery time for achieving about 50μM was calculated as 2min 24sec. 5 beakers were prepared by washing with water 3 times, and 1 time with 10mM NaCl solution. The beakers were filled with 10mL of 10mM NaCl solution. The tip of the burette was washed and wiped. The tip was contacted with each beaker solution for 2min24sec. The procedure from beginning to end was timed with a stopwatch. The results were as follows:

1st) 4min34sec /-121.8mV / 50.7uM
2nd) 4min11sec /-122.0mV / 50.3uM
3rd) 3min59sec /-122.6mV 49.2uM
4rd) 4min02sec /-122.3mV 49.7uM
5th) 3min57sec /-123.0mV 48.4uM
mean 49.7uM
std 1.9%

[000209] As the delivery time (2min 24sec) was relatively short, and due to potential errors of the potassium, sensor the standard deviation was 1.9%. However, the results achieved with the diffusional burette were far superior to those achieved by the conventional method, both in terms of time taken and reproducibility. The reproducibility can be increased using an automated control of the up/down motion of the burette and use of an automated timer. The above experiments were carried out simply by hand and with a stopwatch.

Having thus described the preferred embodiments, the invention is now claimed to be:

1. A delivery device for delivering a reagent into a target material including a body which defines an interior chamber and a delivery port fluidly connected with the chamber, the chamber holding a reagent in solution, the delivery device being characterized by:

a reagent permeable membrane in fluid communication with the chamber and the delivery port, through which reagent passes when the delivery port is in fluid communication with the target material, such that substantially no volume change occurs in the target material during delivery of the reagent to the target material.

2. The delivery device of claim 1, further characterized by:

a matrix material contained within the body, the reagent diffusing through the matrix material to the delivery port.

3. The delivery device of claim 2, further characterized by:

the matrix material being positioned intermediate the reagent solution and the membrane, such that reagent passes through the membrane and the matrix material to enter the target material.

4. The delivery device of claim 2 or 3, further characterized by:

the matrix material being located throughout the reagent solution.

5. The delivery device of claim 4, further characterized by:

the matrix material including a porous material, the reagent being carried within pores of the material.

6. The delivery device of claim 5, further characterized by:

the porous material is selected from the group consisting of nickel, titanium, stainless steel, ceramic, glass, and combinations thereof.

7. The delivery device of any one of claims 2-4, further characterized by:

the matrix material includung a gel.

8. The delivery device of claim 7, further characterized by:

the gel is selected from the group consisting of: polyacrylamide, agarose, hydroxyethyl methacrylate, and combinations thereof.

9. The delivery device of any one of claims 1-8, further characterized by:

the body narrows intermediate the delivery port and the chamber.

10. The delivery device of any one of claims 1-9, further characterized by:

the delivery port having a diameter of from about 10microns to about 10mm.

11. The delivery device any one of claims 1-10 further characterized by:

the membrane having a thickness of less than about 100 microns.

12. The delivery device of claim 11, further characterized by:

the reagent permeable membrane having a thickness of from about 2-50 microns.

13. The delivery device of any one of claims 1-12, further characterized by:

the membrane being in or adjacent to the delivery port.

14. The delivery device any one of claims 1-13, further characterized by:

the membrane including a plurality of holes which define through passages through the membrane.

15. The delivery device of claim 14, further characterized by:

the holes having a diameter of less than about 300 microns.

16. The delivery device of claim 14 or 15, further characterized by:

the holes have a diameter of at least 100 nm.

17. The delivery device of any one of claims 14-16, further characterized by:

the holes having a diameter of about 10-20 microns.

18. The delivery device of any one of claims 14-17, further characterized by:

the holes being filled with a reagent permeable material.

19. The delivery device of any one of claims 1-18, further characterized by:

an agitator which agitates at least a portion of the body.

20. The delivery device of any one of claims 1-18, further characterized by:

at least the delivery port being rotatable, relative to a stationary portion of the delivery device.

21. The delivery device of claim 20, further characterized by:

a housing which houses the body and a motor, the motor being capable of rotating the delivery port.

22. The delivery device of claim 21, further characterized by:

a power source for the motor, carried by the housing.

23. The delivery device of claim 21 or claim 22, further characterized by:

the housing defining an opening which provides access to the delivery port for the target liquid.

24. The delivery device of any one of claims 1-23, further characterized by:

a heating or cooling device which selectively heats or cools at least a portion of the body.

25. The delivery device of any one of claims 1-24, further characterized by:

a control system which calculates a delivery time for preparing a target solution of a preselected reagent concentration and volume.

26. The delivery device of any one of claims 1-25, further characterized by:

the target material comprising a liquid.

27. The delivery device of any one of claims 21-23, further characterized by:

a scanner which reads a computer recognizable indicia on the body, the indicia including information sufficient for the control system to determine a delivery rate of reagent from the burette, the control system receiving signals from the scanner and calculating the delivery time from the signals.

28. The delivery device of claim 27, further characterized by:

means for inputting information into the control system which affects the delivery rate.

29. The delivery device of any one of claims 1-28, further characterized by:

a hydrophobic coating on an exterior surface of the body.

30. The delivery device of any one of claims 1-29, further characterized by:

the reagent diffusing from the delivery device into the target liquid with substantially no volume change in the target liquid.

31. A method of forming a target solution which includes loading a reagent solution into a chamber fluidly connected with a delivery port, the method characterized by:

fluidly contacting the delivery port with a target liquid for a sufficient time such that the reagent diffuses from the chamber through a porous material in or adjacent to the delivery port into the target liquid to form the target solution.

32. The method of claim 31, further characterized by:

the delivery port comprising a plurality of holes and the step of contacting further includes the reagent passing through the holes.

33. The method of claim 32, further characterized by:

the holes being packed with a reagent permeable material, the step of passing including passing the reagent through the reagent permeable material.

34. The method of any one of claims 31-33, further characterized by:

agitating at least one of the reagent solution within the chamber and the delivery port during the step of contacting.

35. The method of claim 34, further characterized by:

the step of agitating including rotating at least the delivery port relative to the target liquid.

36. The method of any one of claims 31-33, further characterized by:

heating or cooling at least one of the reagent solution within the chamber and the delivery port during the step of contacting.

37. The method of any one of claims 31-36, further characterized by:

the reagent diffusing into the target solution at a substantially constant rate after a period of contact of less than 1 second.

38. The method of any one of claims 31-37, further characterized by:

determining a delivery time for preparing a target solution of a desired concentration from the reagent and the target liquid; and

automatically controlling the fluid contact of the delivery port with the liquid for the determined time.

39. The method of any one of claims 31-38, further characterized by:

forming the holes by drilling bores in a substrate material with a laser.

40. The method of any one of claims 31-39, further characterized by:

the burette having a conical tip and wherein the delivery time is determined from the following equation:

$$C(t) = Cr\ (1-exp[-B.t/V])$$

and, optionally, determining B using the expression

$$B = D\ r\ \pi\ tan\theta$$

where C(t) = a concentration of the reagent in the liquid at delivery time t;

Cr = a concentration of the reagent within the body;

V = volume of the liquid;

B = a constant for the body;

D = diffusion coefficient of the reagent being delivered;
r = radius of delivery port opening;
θ = cone angle of delivery port; and
t = time.

41. The method of any one of claims 31-39, further characterized by:
the delivery port including a membrane with at least one pore and the method further including:
determining the delivery rate by the following equation:

$$\text{Delivery rate} = n D [(\pi d^2/z)/(4+\pi d/z)]c_b$$

where D is the diffusion coefficient;
n is the number of pores;
d is the pore diameter;
z is the membrane thickness; and
c_b is the concentration of the reagent in the reagent solution in the chamber.

42. A method of forming a target solution of a desired concentration characterized by:
contacting a target liquid with a delivery port of a body for a period of time, the body containing a reagent in a solution, the delivery port including a plurality of holes, whereby the reagent diffuses through the holes into the liquid to form the target solution, the target solution having substantially the same volume as the target liquid.

43. The method of claim 42, further characterized by:
the change in volume of the target liquid being less than 5% of that of the volume of the reagent solution corresponding to the amount of the reagent transferred from the body to the target liquid to form the target solution.

44. The method of claim 42 or claim 43, further characterized by:
the holes having a diameter of less than about 300 microns.

45. The method of any one of claims 42-44, further characterized by:
the step of contacting including allowing the reagent to diffuse through a matrix material to the delivery port.

46. The method of any one of claims 42-45, further characterized by:
the time period being predetermined.

47. The method of any one of claims 42-46, further characterized by:
the period of time being determined by measuring a property of the target liquid which corresponds to a concentration of the reagent in the target liquid or a species derived from the reagent.

48. A diffusional burette for delivery of a reagent into a target material characterized by:
a body which defines an interior chamber for receiving the reagent and a delivery port fluidly connected with the chamber, the delivery port defining a plurality of holes, each of the holes having a cross sectional width of less than 500 microns;
a reagent permeable matrix material within the body; and
a solution of the reagent in the chamber, such that the reagent diffuses from the chamber via the holes into the target material when the delivery port is in fluid communication with the target material.

49. The diffusional burette of claim 48, further characterized by:
the reagent permeable matrix material being disposed in or adjacent to the holes, such that the reagent passes through the reagent permeable matrix material during delivery of the reagent.

50. The diffusional burette of any one of claims 48-49, further characterized by:
the reagent permeable matrix material being selected from the group consisting of cellulose, modified celluloses, polyurethane, agarose, polyacrylamide, hydrogels, cyclopore films, microporous filters, and combinations thereof.

51. The diffusional burette of any one of claims 48-50, further characterized by:
the plurality of holes including at least three holes.

52. The diffusional burette of any one of claims 48-51, further characterized by:

the solution being dispersed through the reagent permeable matrix material and wherein the reagent permeable matrix material comprises one of a porous solid and a gel.

53. The diffusional burette of any one of claims 48-52, further characterized by:

the holes being less than 100 microns in width.

54. The diffusional burette of any one of claims 48-53, further characterized by:

a housing which houses the body, the delivery port being movable between a first position, in which the delivery port extends through an opening in the housing, and a second position, in which the delivery port is retracted within the housing.

55. The diffusional burette of any one of claims 48-54, further characterized by:

a means for agitating at least one of the delivery port and the target material.

56. The diffusional burette of claim 55, further characterized by:

the means for agitating includes a motor which rotates the body.

57. A method of forming a delivery device including providing a body which defines an interior chamber and a delivery port fluidly connected with the chamber, the method characterized by:

closing the delivery port with a reagent permeable porous material;

introducing a reagent in solution to the chamber, the reagent permeable porous material being such that reagent diffuses through the reagent permeable porous material to the delivery port when the delivery port is brought into fluid contact with a target liquid and is held within the body when the delivery port is removed from fluid contact with the target liquid.

58. The method of claim 57, further characterized by:

the reagent permeable porous material including at least one of a gel and a membrane, the membrane defining a plurality of through passages.

a reagent permeable membrane in fluid communication with the chamber and the delivery port, through which reagent passes when the delivery

port is in fluid communication with the target material, such that substantially no volume change occurs in the target material during delivery of the reagent to the target material

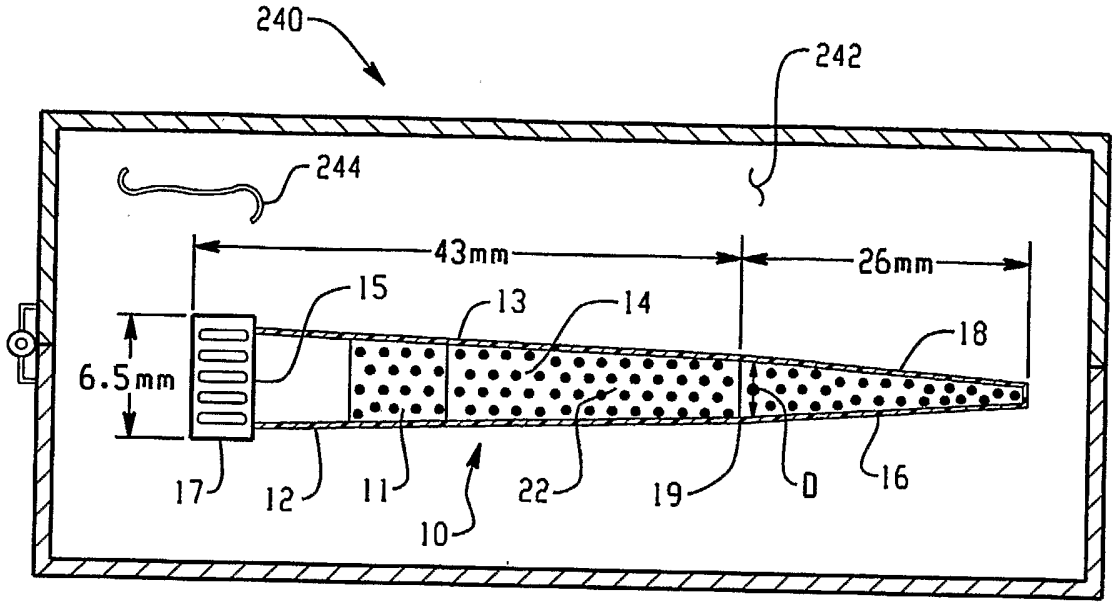
240
242
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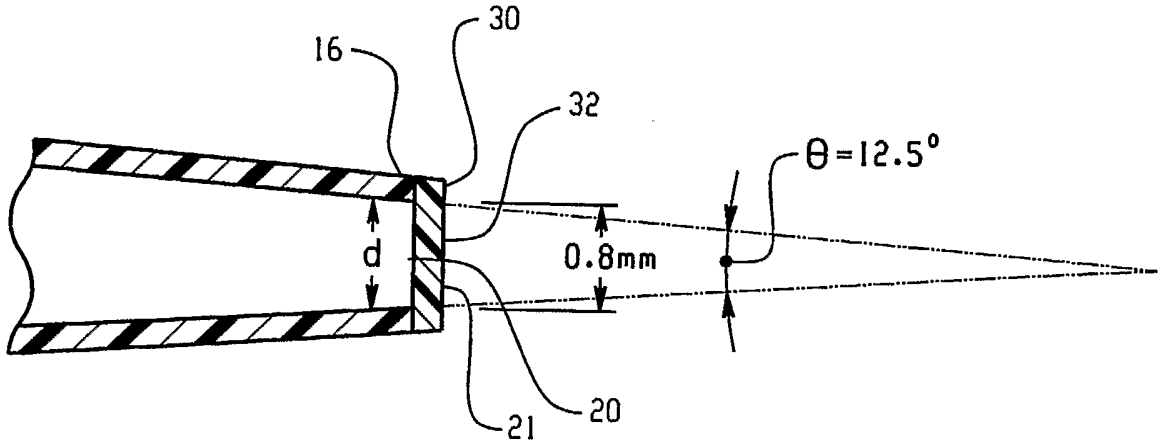
Fig. 1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32
θ=12.5°
d
0.8mm
21
20

Fig. 2

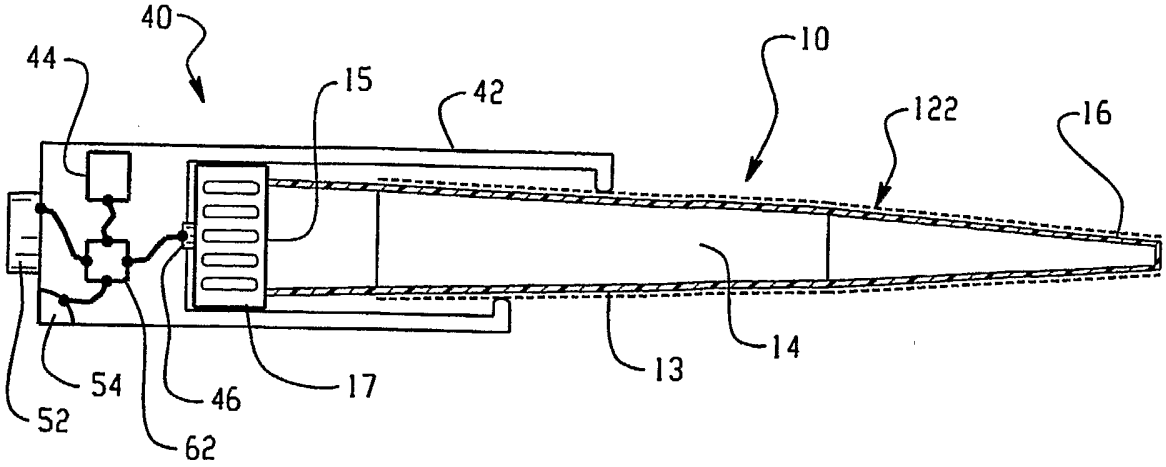


Fig. 3

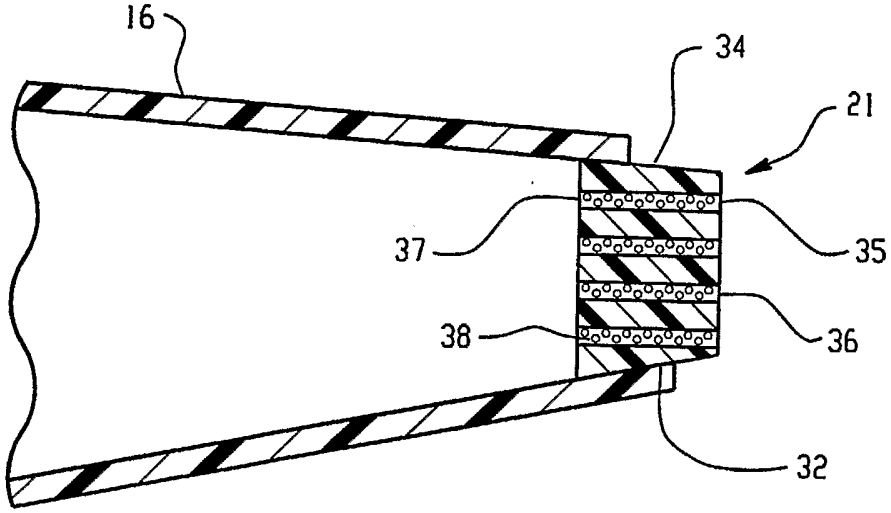


Fig. 4

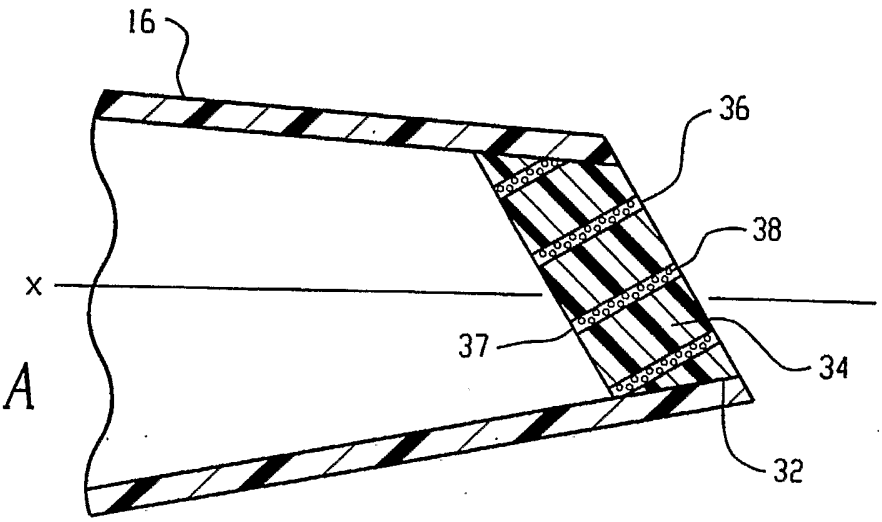
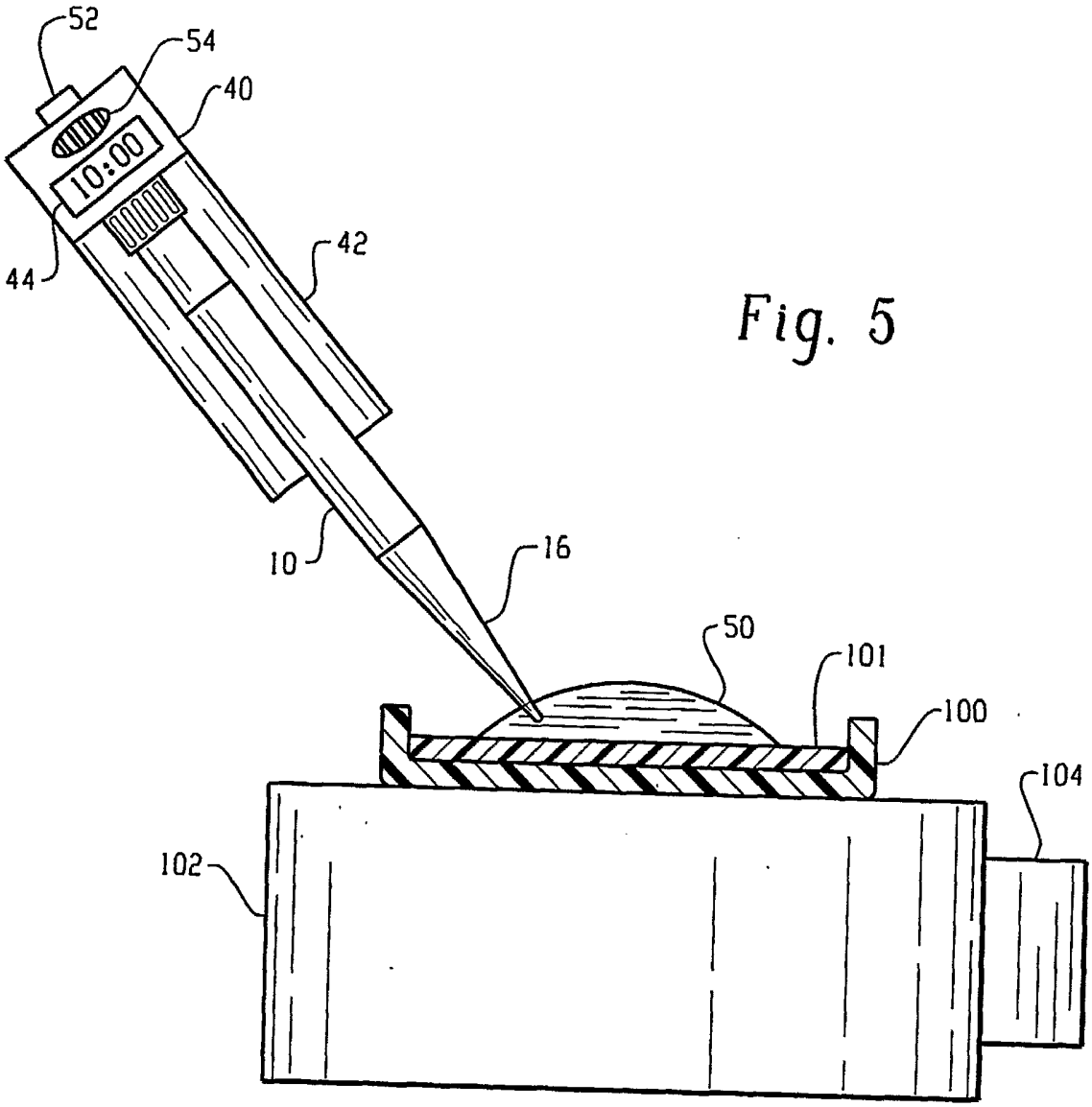


Fig. 4A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42
10
16
50
101
100
104
102

Fig. 5

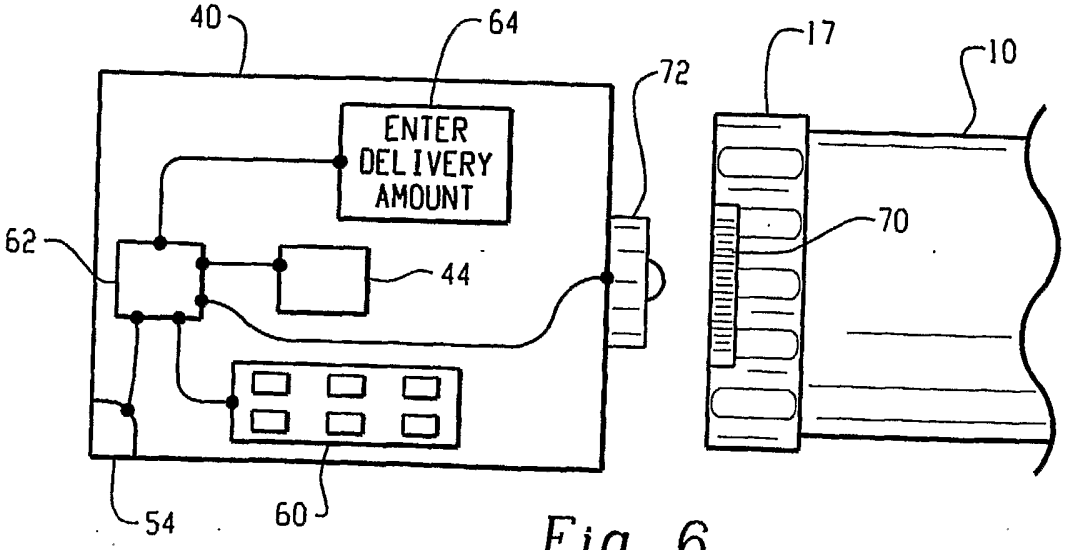

40
64
72
ENTER
DELIVERY
AMOUNT
62
44
54
60
17
10
70

Fig. 6

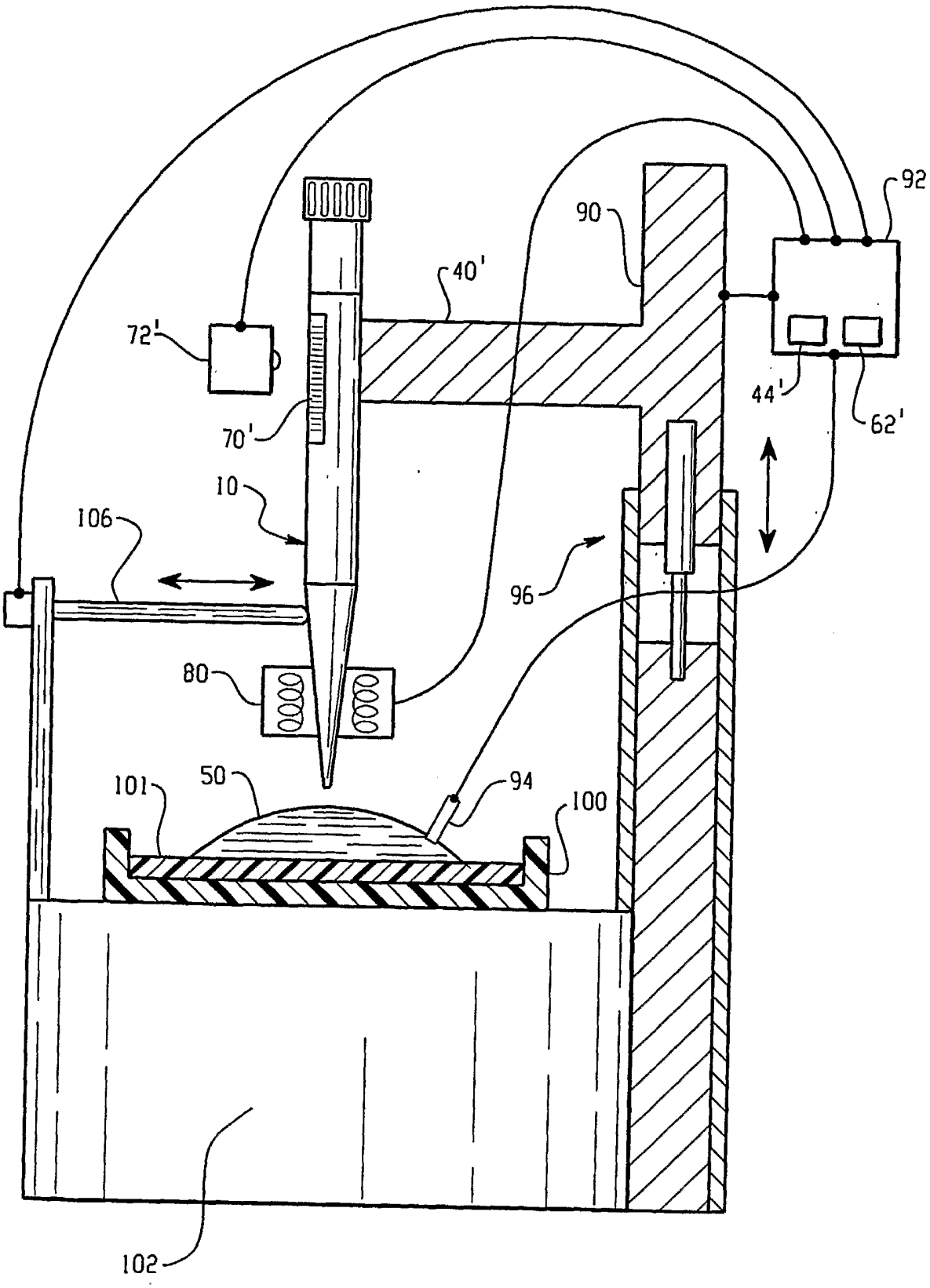


Fig. 7

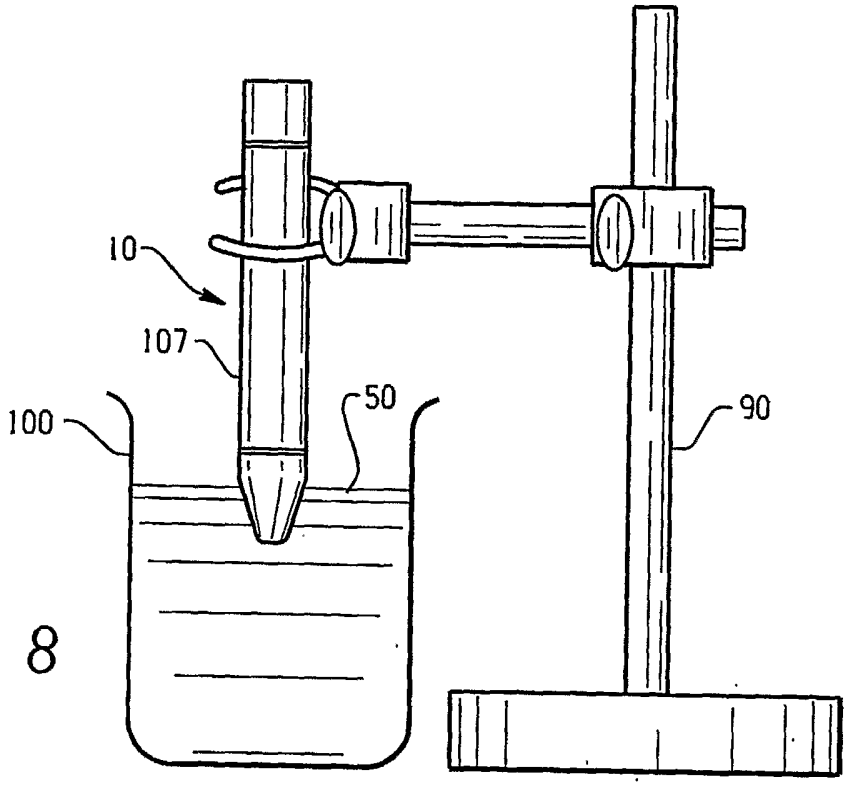


Fig. 8

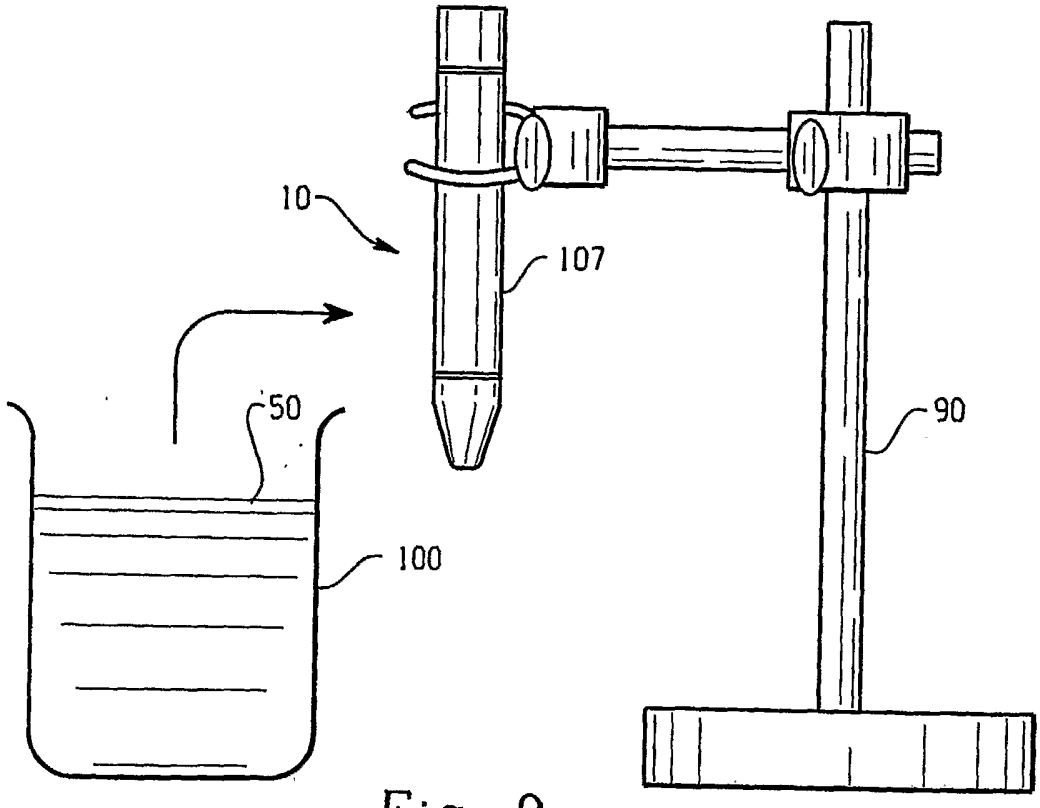


Fig. 9

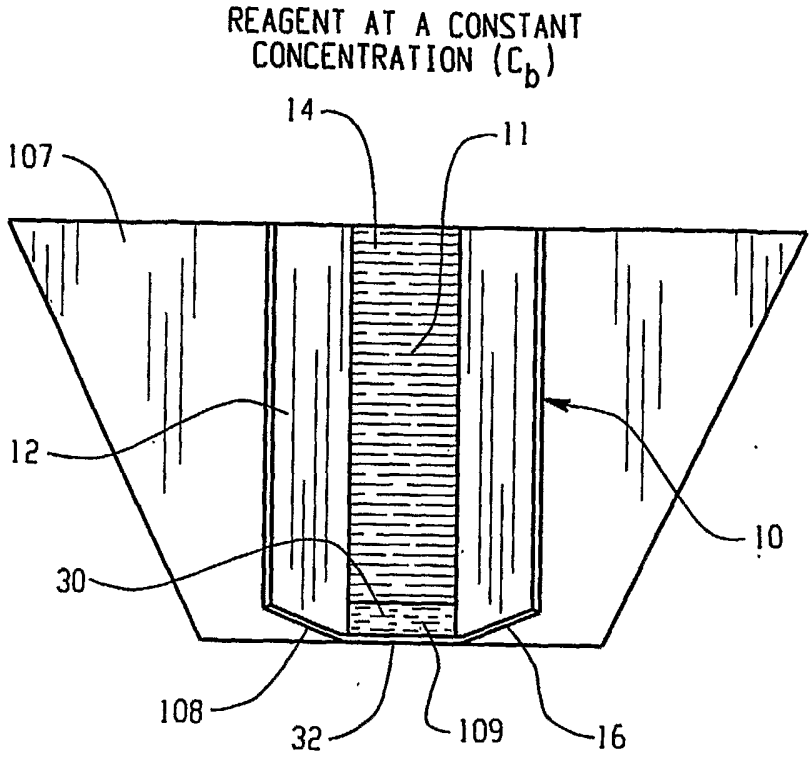

REAGENT AT A CONSTANT CONCENTRATION (C_b)
14
11
107
12
10
30
108
32
109
16

Fig. 10

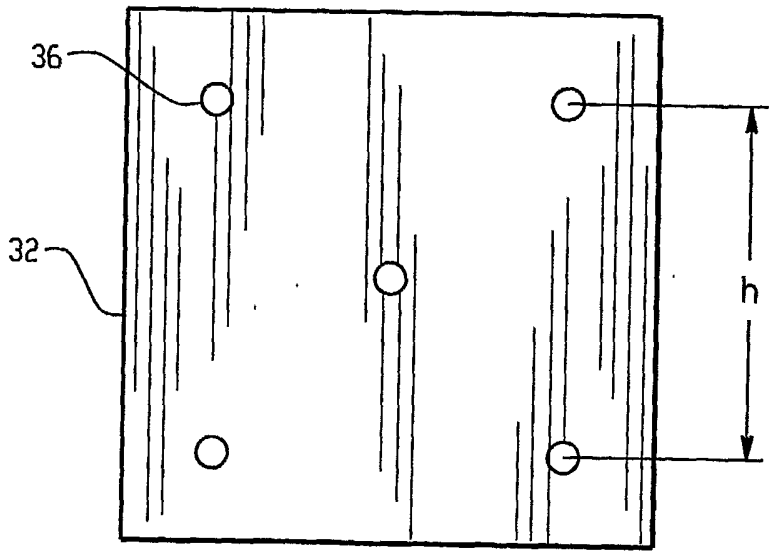

36
32
h

Fig. 11

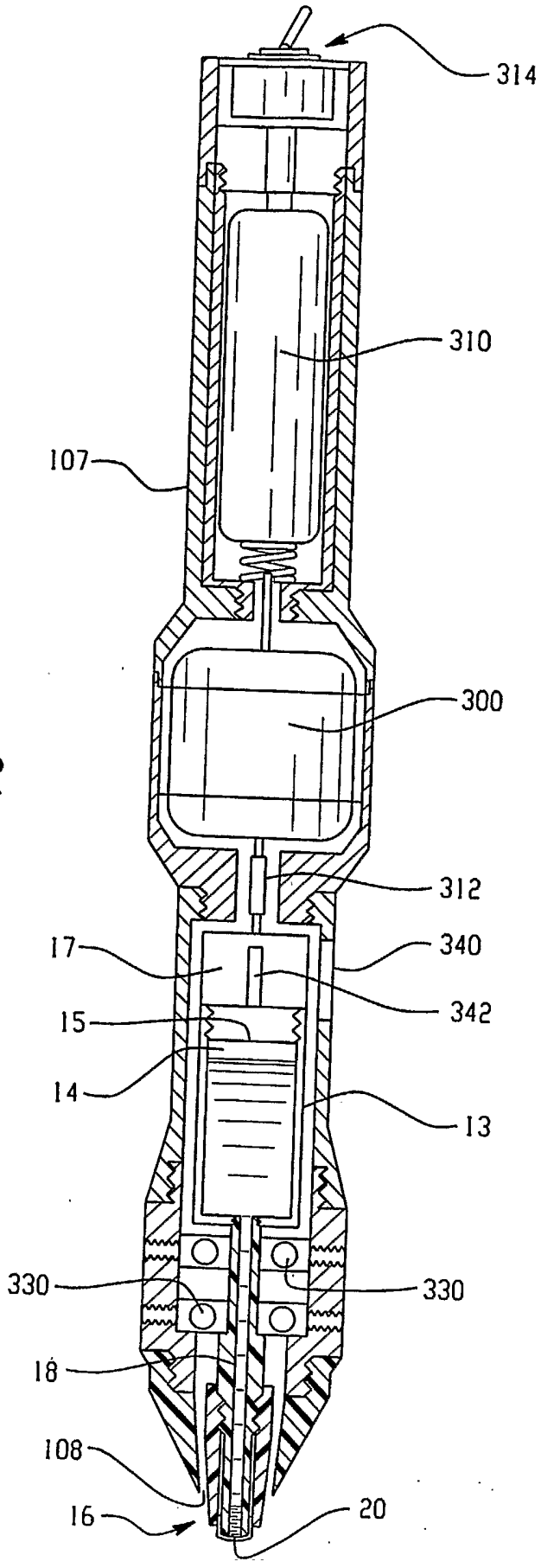


Fig. 12

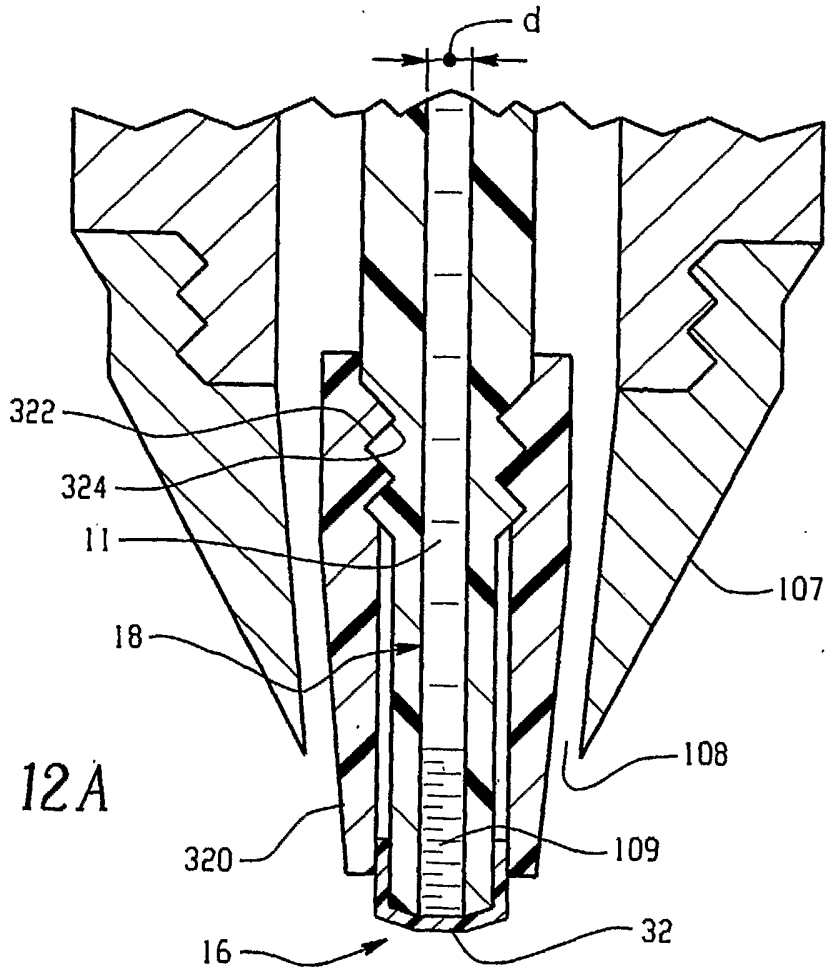


Fig. 12A

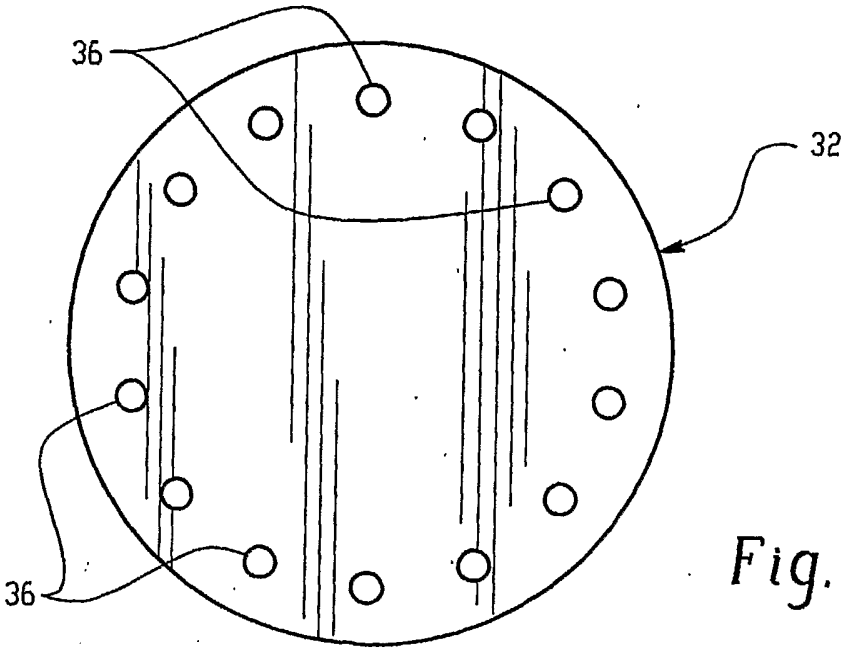


Fig. 12B

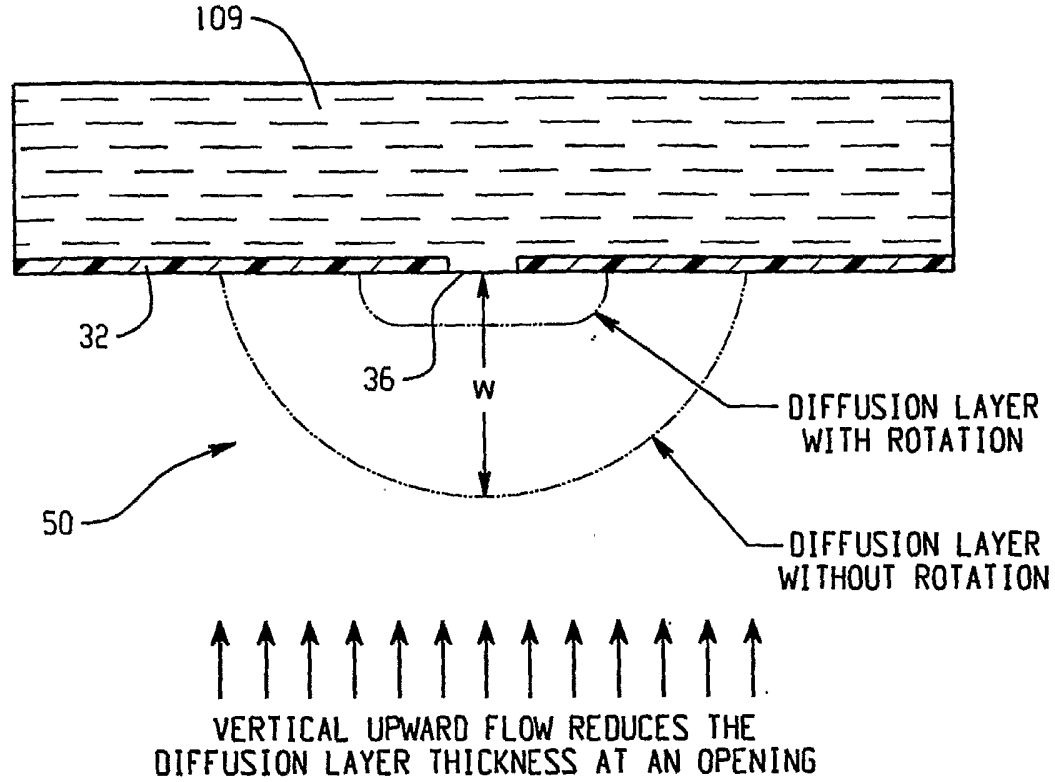

109
32
36
w
DIFFUSION LAYER WITH ROTATION
50
DIFFUSION LAYER WITHOUT ROTATION
VERTICAL UPWARD FLOW REDUCES THE DIFFUSION LAYER THICKNESS AT AN OPENING

Fig. 13

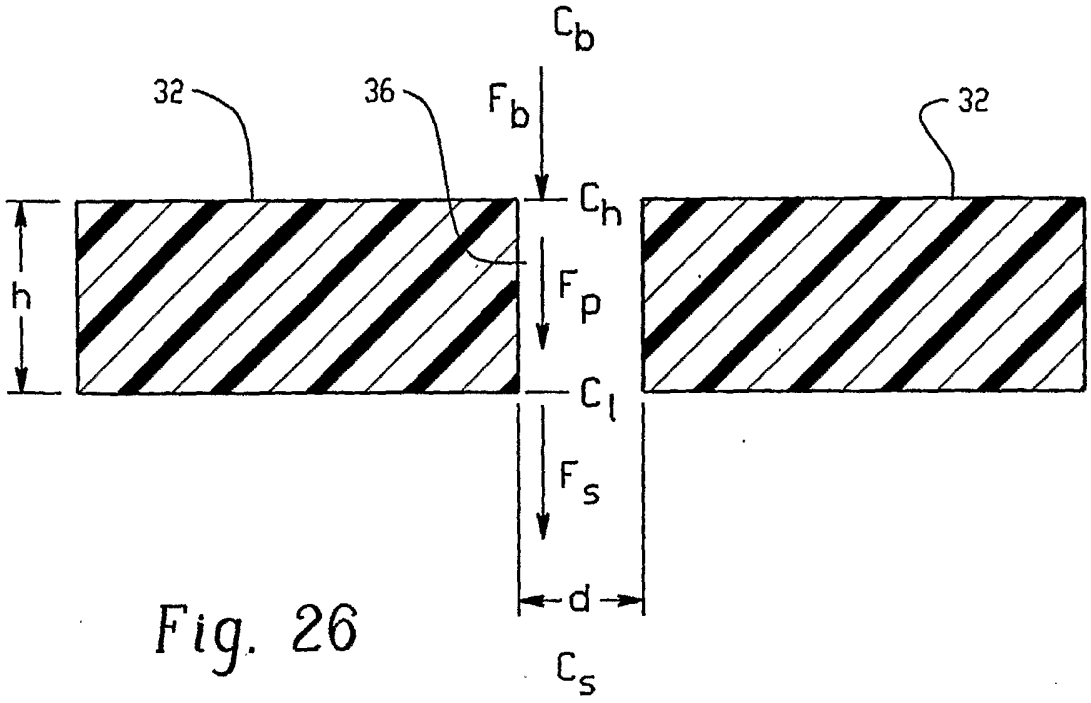

C_b
32
36
F_b
32
C_h
h
F_p
C_l
F_s
d
C_s

Fig. 26

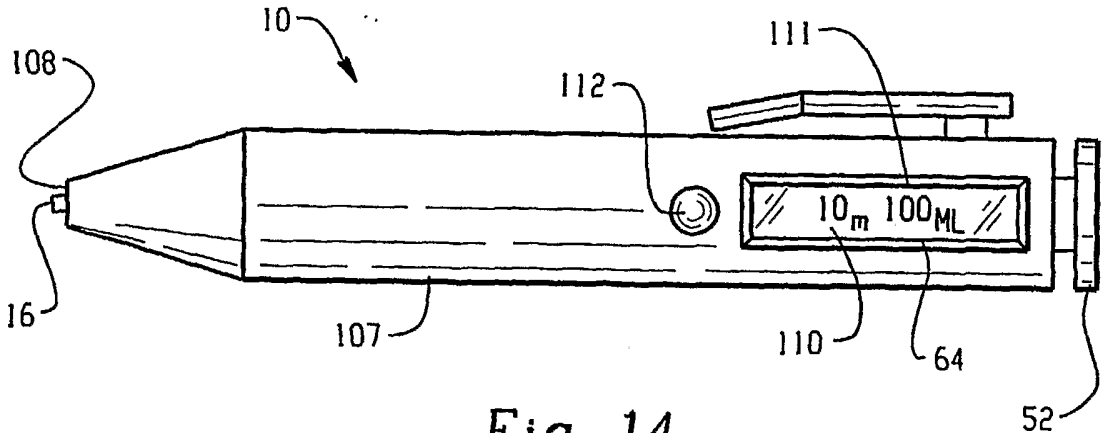


Fig. 14

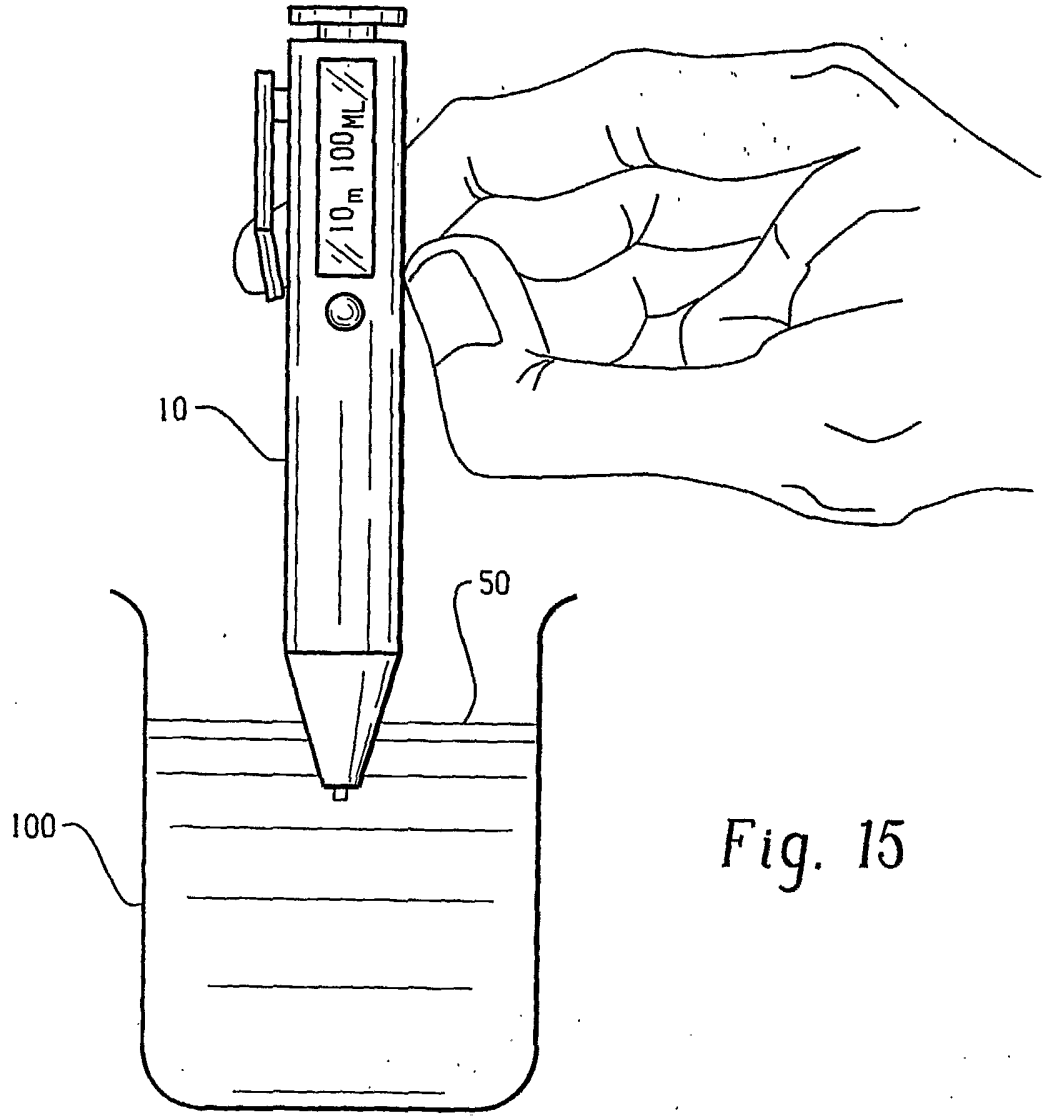


Fig. 15

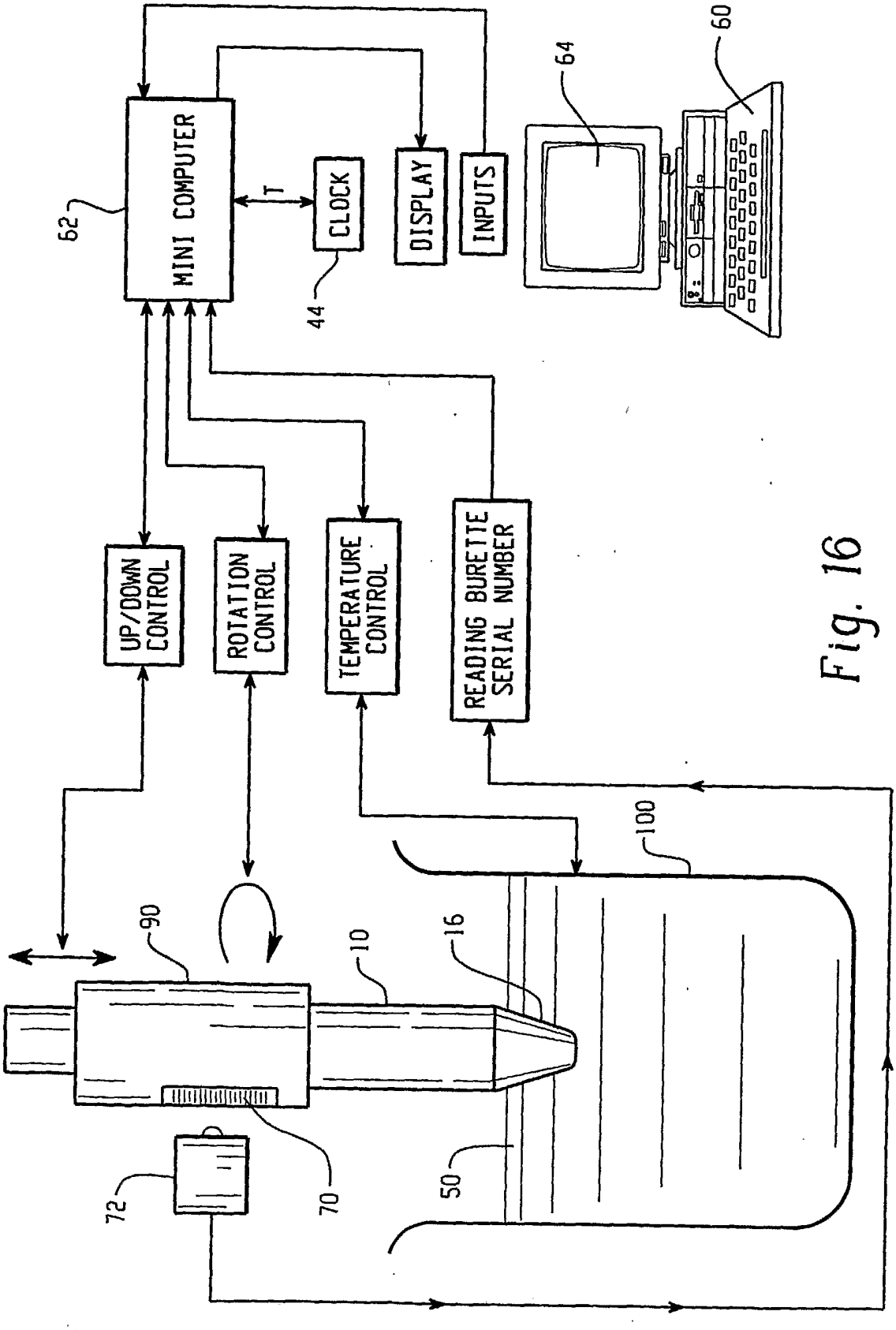

MINI COMPUTER
62
T
CLOCK
44
DISPLAY
INPUTS
64
60
UP/DOWN CONTROL
ROTATION CONTROL
TEMPERATURE CONTROL
READING BURETTE SERIAL NUMBER
100
90
10
16
70
72
50

Fig. 16

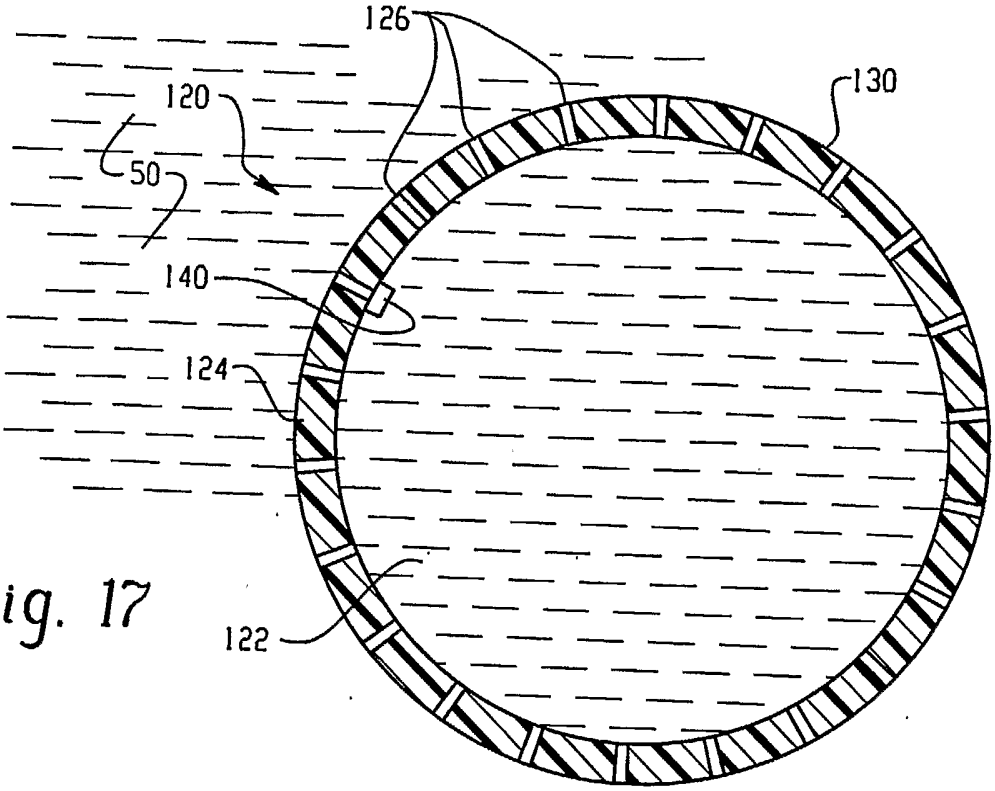


Fig. 17

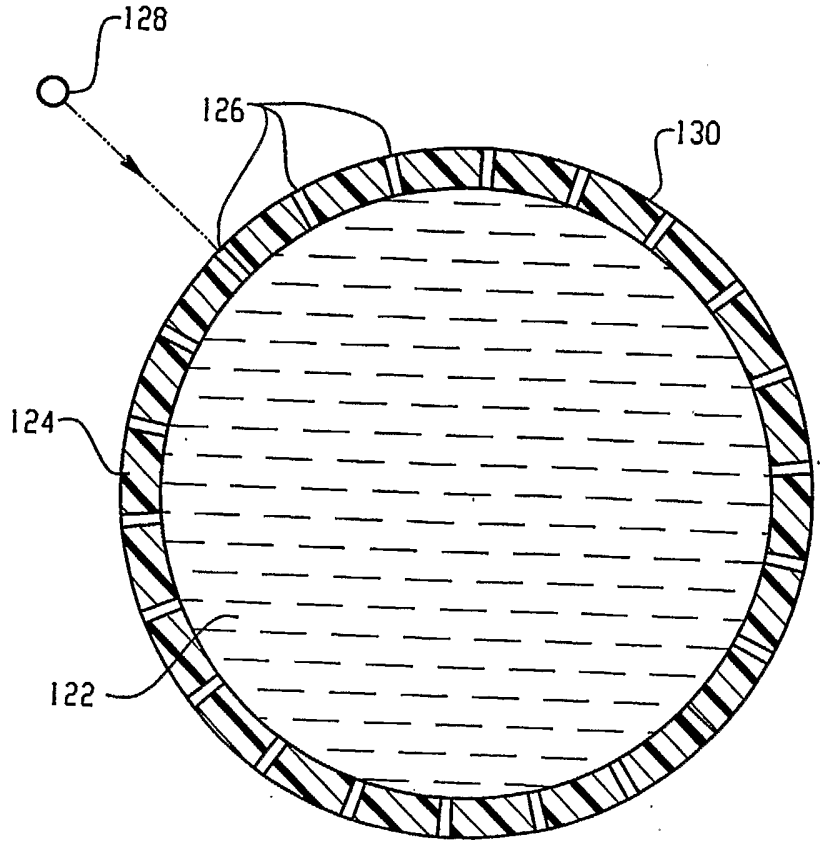


Fig. 18

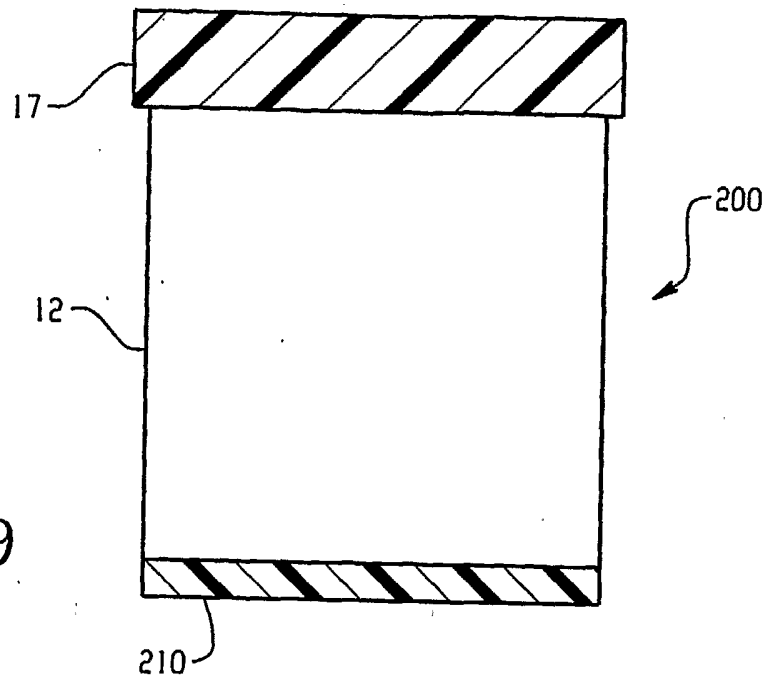


Fig. 19

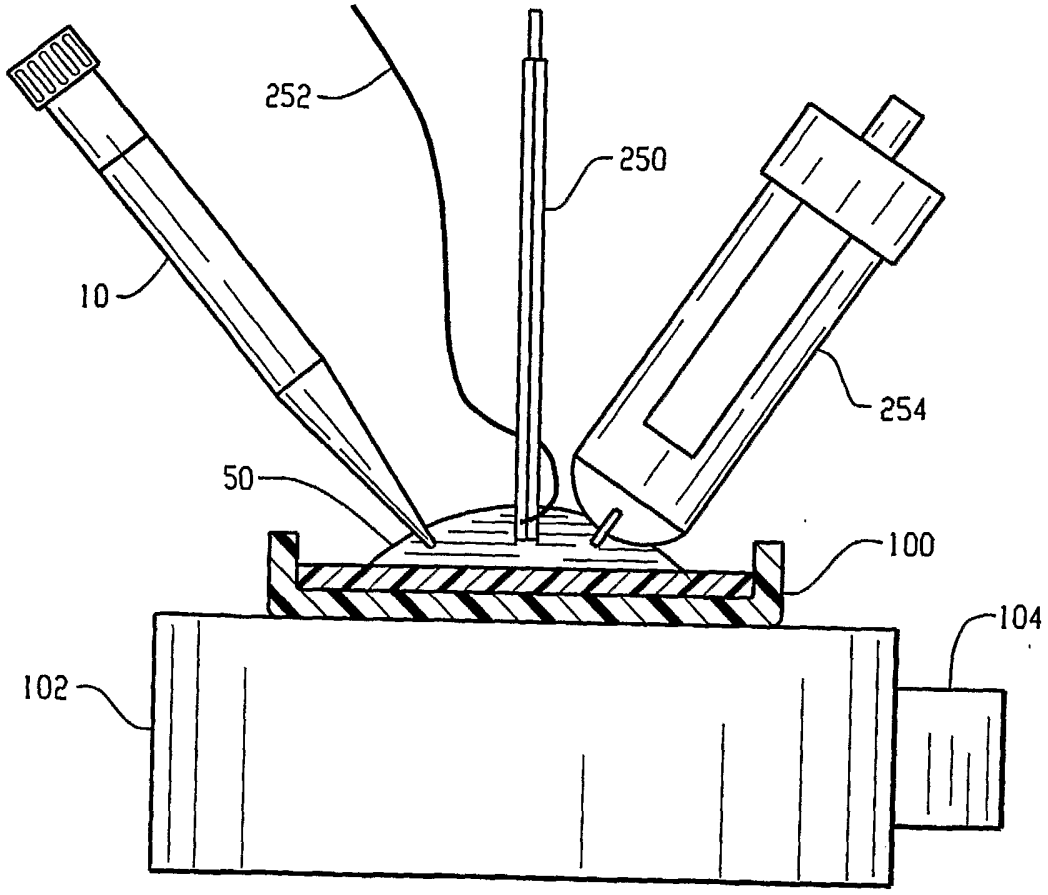


Fig. 20

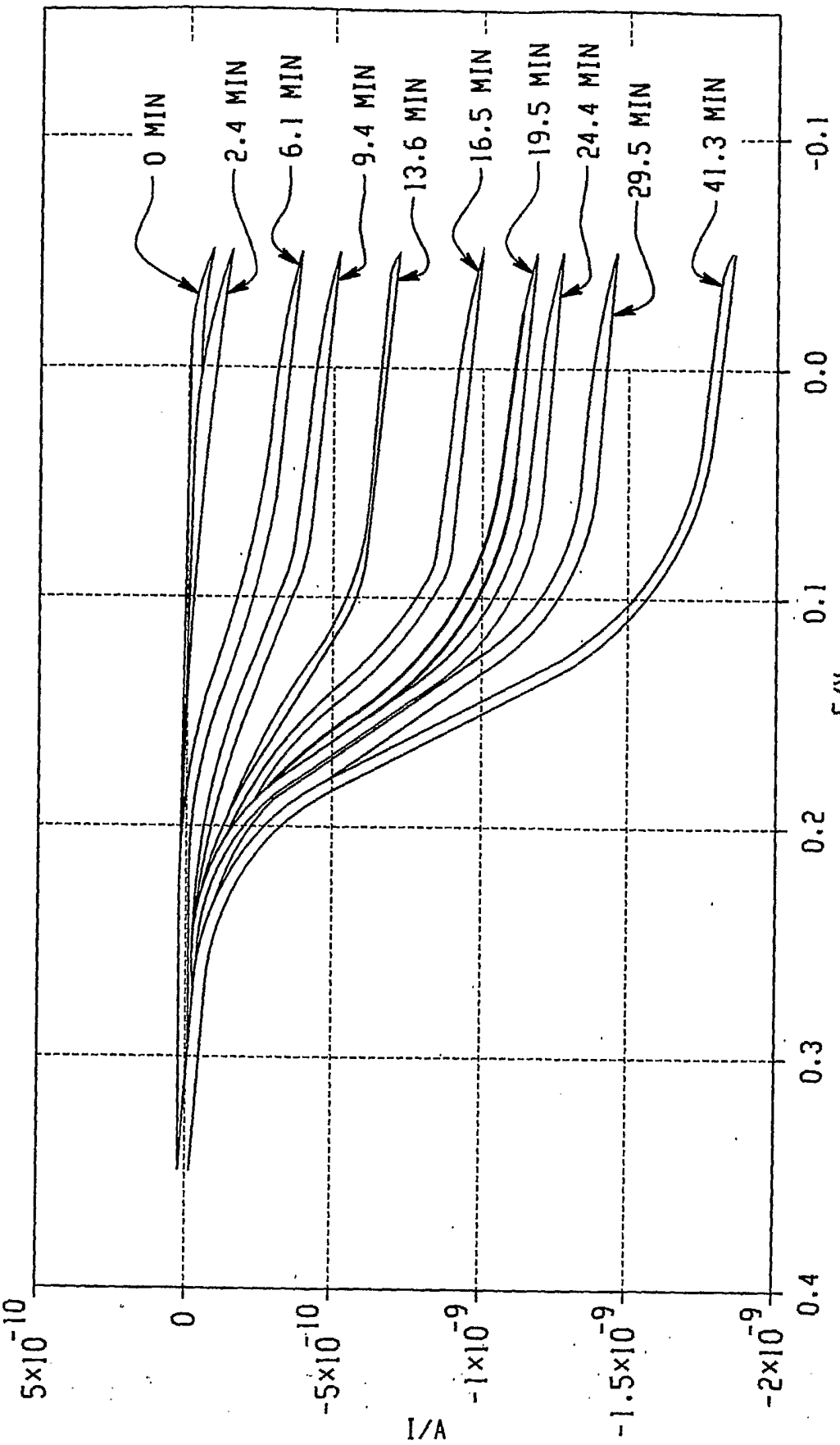

0 MIN
2.4 MIN
6.1 MIN
9.4 MIN
13.6 MIN
16.5 MIN
19.5 MIN
24.4 MIN
29.5 MIN
41.3 MIN
-0.1
0.0
0.1
0.2
0.3
0.4
E/V
5x10-10
0
-5x10-10
-1x10-9
-1.5x10-9
-2x10-9
I/A

Fig. 21

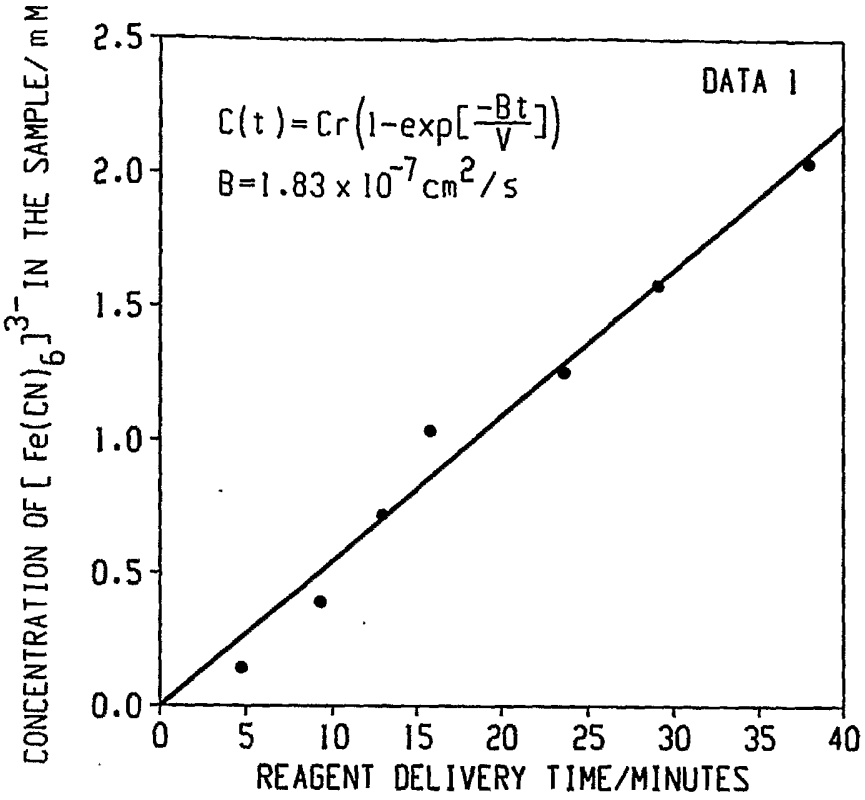


Fig. 22A

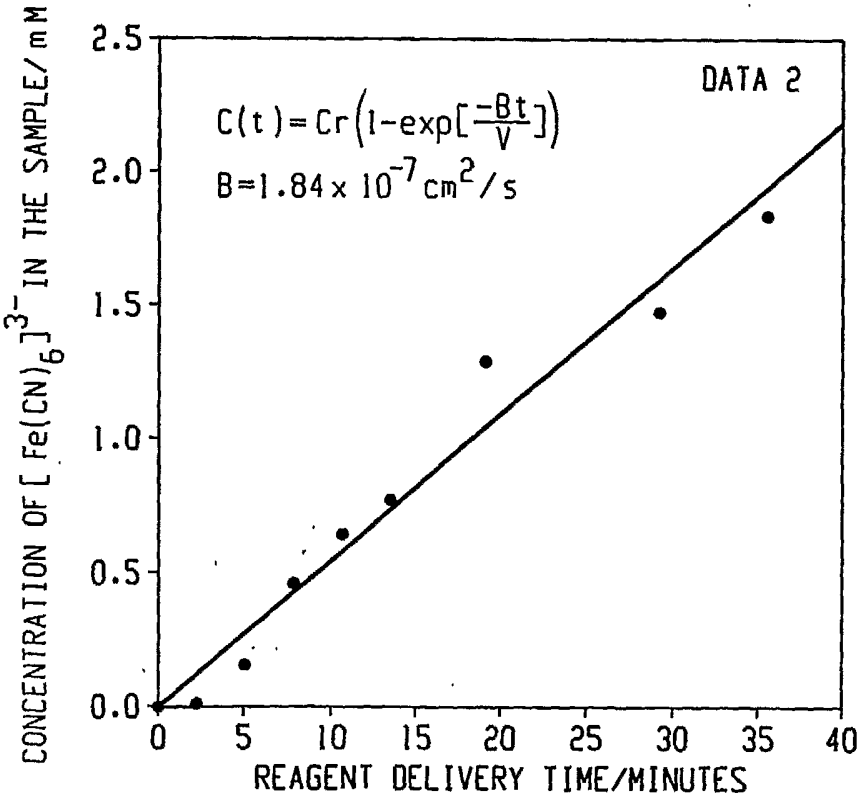


Fig. 22B

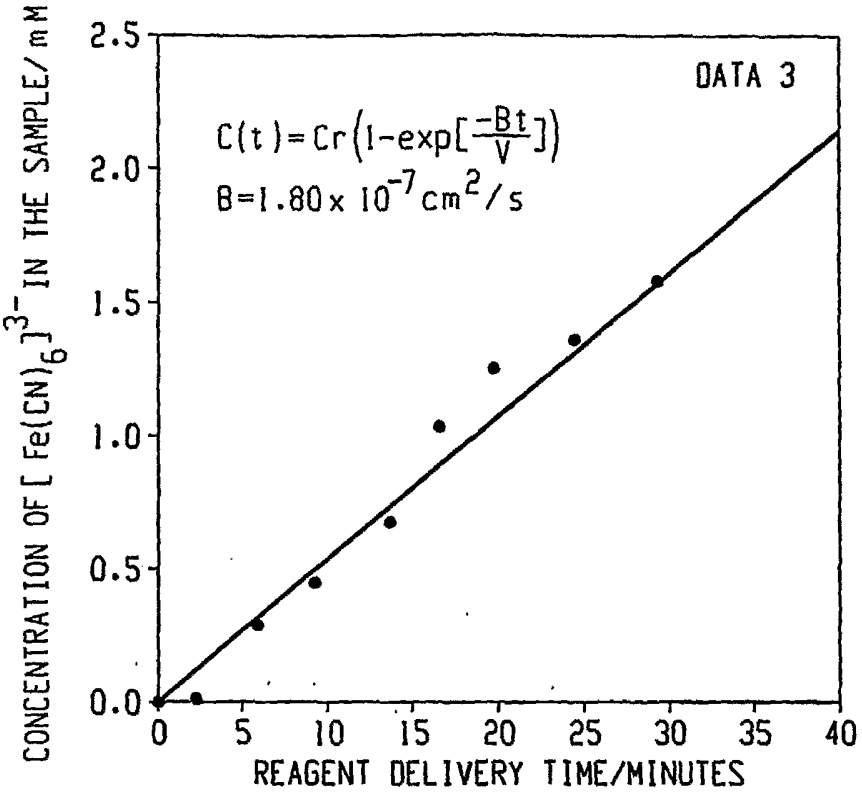


Fig. 22C

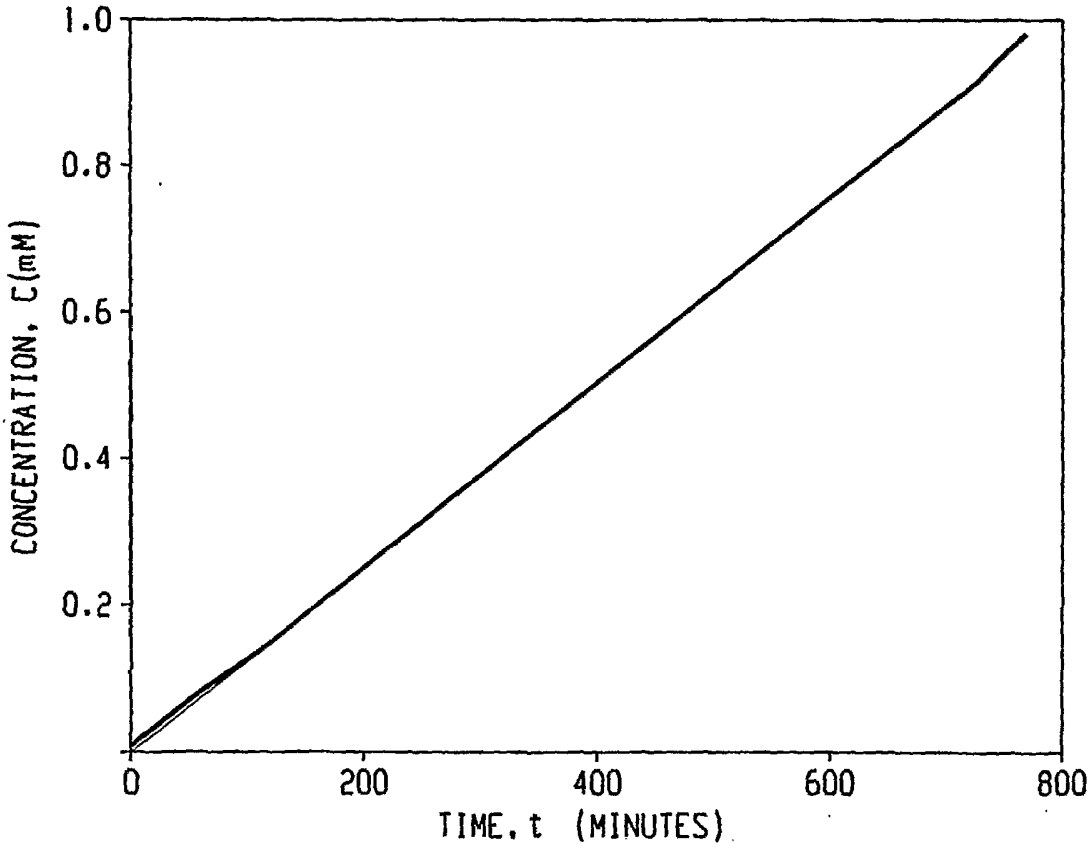
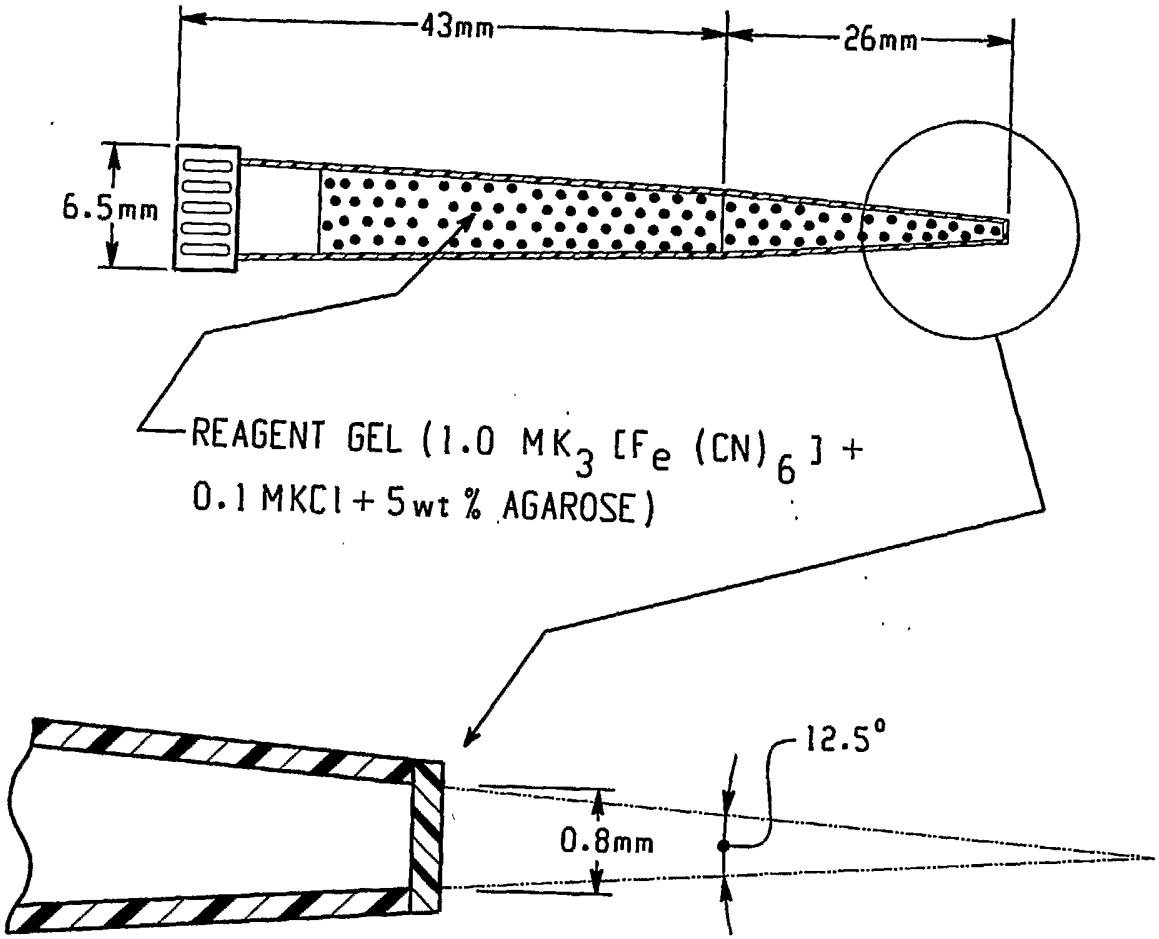


Fig. 23



$$C(t) = Cr\left(1-\exp\left[\frac{-Bt}{V}\right]\right)$$

$$B \approx Dr\,\pi \tan\theta$$

$D = 7.3 \times 10^{-6}\ cm^2/s$

$r = 0.04\ cm$

$\theta = 12.5^\circ$

$B = 2.03 \times 10^{-7}\ cm^3/s$

Fig. 24

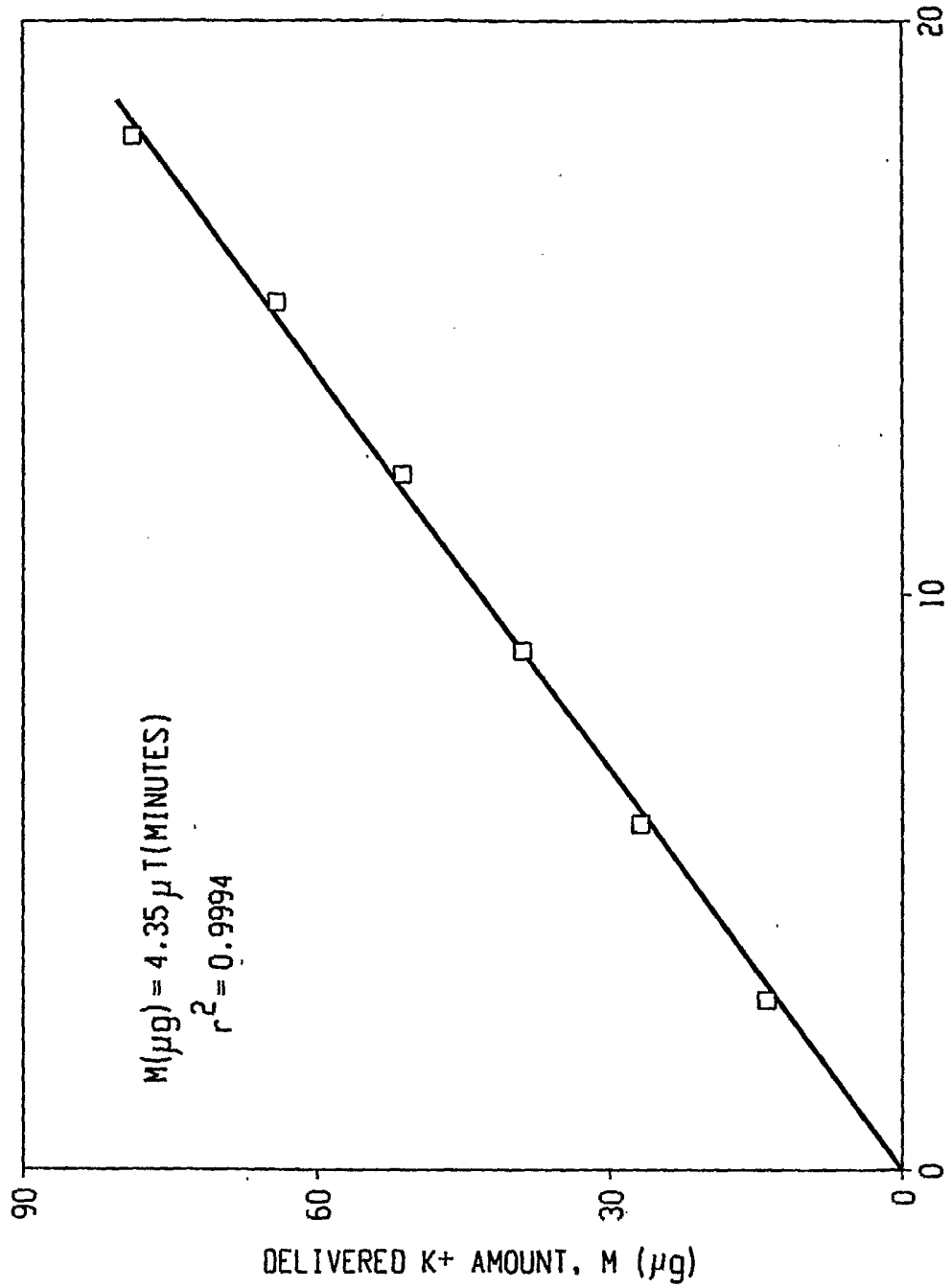

M(μg) = 4.35 μ T(MINUTES)
r² = 0.9994
DELIVERED K+ AMOUNT, M (μg)
90
60
30
0
0
10
20
DELIVERY TIME, T (MINUTES)

Fig. 25

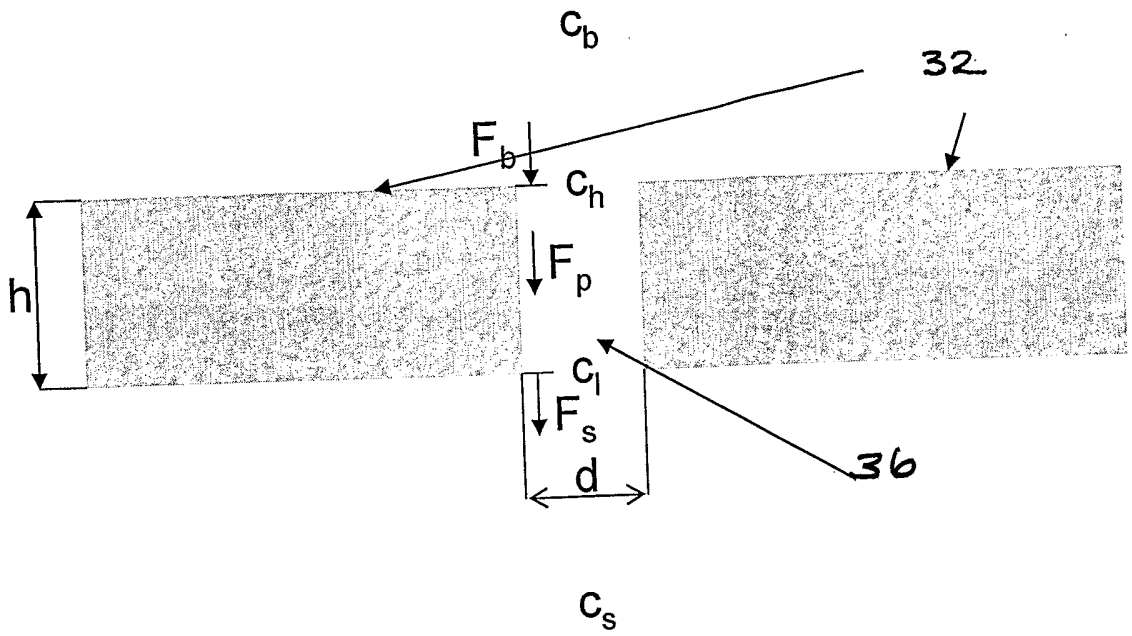


FIG. 26