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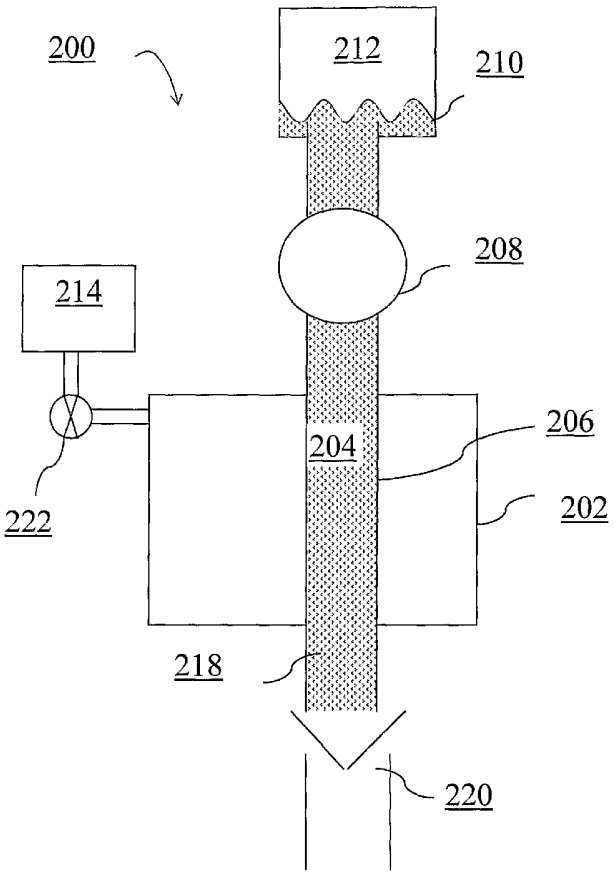

(57) Abstract: A method and apparatus are described herein for the delivery of NO into an aqueous solution, such as blood or a physiologically acceptable infusion solution. NO is provided from an NO source that is under pressure. NO flows from the NO source via a member comprising a portion being NO permeable and resistant to NO, and this portion is in contact with an aqueous solution. Thus NO is delivered into the aqueous solution. According to one aspect the member through which NO flows is a sealed catheter that is inserted to a blood vessel, thus providing NO directly to the blood. In another aspect NO is delivered into a physiologically acceptable infusion solution, which in turn may be infused to the blood vessel.

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DELIVERY OF NITRIC OXIDE TO A BLOOD VESSEL

FIELD OF THE INVENTION

This invention relates to methods for delivering nitric oxide to a blood vessel of a mammal, as well as to apparatus and composition that are useful for the practicing of the method.

BACKGROUND OF THE INVENTION

Physiologically present nitric oxide (NO) is involved in numerous processes that take place in the walls of blood vessels. NO is considered an Endothelial Delivered Relaxing Factor (EDRF), and can influence essential key processes in the development of arteriosclerosis, such as relaxation of smooth vascular muscles, dilatation of arteries, inhibition of adhesion of thrombocytes, granulocytes and monocytes to the vascular wall, inhibition of the proliferation of secretory smooth muscle cells and direct effects on endothelial metabolism and prevention of proliferation of smooth muscle cells with development of intimal hyperplasia which is the basic cause of arterial occlusion. In atherosclerotic blood vessels, secretion of endogenous NO decreases and cannot fulfill the function of preventing clotting and occlusion.

US 6,103,769 teaches bolus injection of an NO-containing solution. The solution is prepared by removing air from a 0.9% NaCl solution, saturating the solution with NO, and diluting the saturated solution upon requirement.

US 5,634,895 discloses method and apparatus for treating blood vessels for vascular thrombosis and angioplasty restenosis, by administering a conager of an endothelium-derived bioactive agent, to an extravascular treatment site at a therapeutically effective dosage rate.

SUMMARY OF THE INVENTION

The present invention teaches direct administration of molecular NO into blood vessels, which permits steady delivery of a relatively large thereof. This results not only in dilatation of the blood vessels, but also prevention of intimal hyperplasia and also better distribution of the NO throughout the body, which permits better tissue oxygenation as well as Co2 removal.

According to a first method aspect, the present invention provides a method for delivering a controlled amount of nitric oxide (NO) into a blood vessel of a mammal, the method comprising:

(a) providing an NO source under pressure;

(b) flowing NO from said NO source via a member comprising a portion being NO permeable and resistant to NO, said NO permeable portion being in contact with an aqueous solution, thereby delivering a controlled amount of NO to the aqueous solution.

The aqueous solution may be either a physiologically acceptable solution (such as a saline infusion solution) or it may be blood. Where the solution is an infusion solution it may be then administered to a blood vessel, thus administering a controlled amount on NO to a patient's blood. Where the aqueous solution is blood the NO may be provided directly into the blood within the patient's body.

According to one option, the present invention provides a method for delivering nitric oxide (NO) to a blood vessel of a mammal, the method comprising infusing at a substantially constant rate into said blood vessel a physiologically acceptable solution containing NO. NO may be infused into the blood vessel a time period ranging from about 1 hour to about 24 hours, preferably from about 2 to about 6 hours. Repetitive infusions are also possible, i.e. infusion over a time period and after a break infusing again over a similar or different time period. The physiologically acceptable solution that contains NO is

preferably infused using a drip rate of from about 0.25 to about 5cc/min, preferably about 2cc/min.

The rate of infusing NO into the blood vessel in accordance with this aspect of the invention may be for example lower than 100ng/min, and typically between 30ng/min and 80ng/min. Such rates require preparation of solutions having NO concentrations of between about 5 and about 400ng/cc or between about 15 and about 40ng/cc.

In accordance with one embodiment of the present invention, the infused solution is prepared *in situ*, namely just before it is being infused to the patient. Thus, in accordance with this embodiment, the NO may be introduced into the flow system in which the infused solution flows before being introduced into the blood, whereby the preparation site is in flow communication with the blood vessel. This would permit to adjust the NO concentration of the solution to meet the physiological parameters of the individual patient that may be measured at the time of infusion. Such adjustment may be preformed manually or automatically.

According to another option a method for continuously producing an infusion solution containing NO is provided, the method comprising contacting a flow of infusion solution with an NO permeable barrier separating between said solution and an NO-comprising pressurized gas. The NO-comprising pressurized gas may consists entirely or primarily of NO gas. In some embodiments of this aspect the NO gas may be mixed with other gases, primarily chemically inert gases that either may be administered to the recipient without causing harm or that cannot permeate the NO permeable barrier. Thus, in accordance with this embodiment the infusion solution flows through a unit having at least two compartments, one accommodating said solution and the other accommodating said pressurized gas.

The methods in accordance with above two aspects of the invention may be carried out using an apparatus, denoted below as a first apparatus aspect of the invention.

According to a first apparatus aspect of the invention, an apparatus is provided that includes a gas compartment containing an NO-comprising gas there being a barrier between the two compartments that is permeable to gaseous NO. The NO-comprising gas may consist essentially of only NO or at times NO mixed with other gases, primarily chemically inert gases that either may be administered to the recipient or that cannot permeate the NO permeable barrier of and an infusion solution compartment. Thus, gas permeates through the NO permeable barrier from the gas compartment to the infusion solution compartment. The liquid compartment has a solution inlet and a solution outlet, such that liquid that enters the liquid compartment through the solution inlet may leave through the solution outlet after absorbing NO.

According to one embodiment the gas compartment has a gas inlet and a gas outlet whereby NO can flow from an NO reservoir to the gas compartment while the spent gas can the flow out, e.g. to an NO exhaust system or circulated back to the reservoir.

In another embodiment, the gas compartment has only an inlet, and while a constant gas pressure is maintained therein by the NO-comprising gas source linked thereto. When the gas compartment is to be emptied, for instance for maintenance, it may then be vacuumed through the gas inlet aperture.

The NO concentration of the liquid that leaves the apparatus depends, *inter alia*, on the surface area and on the thickness of the barrier through which gas permeates into the liquid compartment. Therefore, it may be advantageous to design an apparatus in accordance with the invention to include a replaceable barrier, such that use of various barriers having different surface areas and/or thicknesses will result in various NO concentrations when working in given temperature and pressure. Another manner of controlling the NO concentration is through control of the NO gas pressure (or partial pressure if another gas is included as well). Another method of controlling the NO concentration in the infusion solution is to change the temperature of the apparatus. As the absorption rate of NO into a fluid increases with temperature, increasing the

temperature would lead to an increase in the concentration of NO in the infusion solution. Another method of affecting the NO concentration is to increase or decrease the active length (or area) of the permeable barrier.

The inlet and the outlet of the liquid compartment may, in accordance with an embodiment of the invention be made one and the same, such that in operation liquid enters the liquid compartment, stays in it, and after absorbing NO, leaves the compartment from the same aperture it entered.

According to one preferred embodiment, the liquid compartment is configured as a conduit extending through the gas compartment. The conduit has a wall that is NO permeable at least in a portion thereof located inside the gas compartment. This conduit is connectable to a liquid reservoir, from which liquid may enter the conduit. The liquid may be flown through the conduit by any means known in the art per se, such as by gravity, by a pump, etc.

According to another preferred embodiment, the gas compartment is a conduit extending through the liquid compartment. The conduit has a wall that is NO permeable at least in a portion thereof, said portion being located inside the liquid compartment. This conduit is connectable to a gas reservoir, from which gas may enter the conduit, while the gas flow may be controlled by a pump or by a valve system. The temperature of the gas and the infusion solution is one of the parameters that influence the NO concentration in the solution that leaves the apparatus. Accordingly, by some embodiments, either or both the gas temperature and the solution temperature is controlled, for example by controlling the temperature of the gas reservoir with a heating sleeve.

Naturally, in both embodiments, the conduit may be in any form, such as a pipe, a plurality of pipes connected to each other through a manifold at the inlet and outlet, a plurality of independent pipes connected to different liquid or gas reservoirs, and the like.

The gas compartment of the apparatus of the invention may be first purged from oxygen, then connected to an NO reservoir and filled with NO in the desired pressure Once the gas compartment is loaded with NO at the required

pressure, the apparatus may be connected to an infusion solution reservoir, such as an infusion bag, from which infusion solution is entered into the liquid compartment in a controlled rate, for instance, by an electrically controlled pump, where it absorbs NO through the barrier separating it from the gas compartment, and to an infusion needle, which transfuses the NO-containing infusion solution from the apparatus into a blood vessel.

Additionally, a kit may be composed comprising an apparatus according to the above aspect of the invention and a plurality of NO permeable barriers having same of different surface areas and/or thicknesses. These barriers may be used to replace one another either when a barrier may no longer be used (e.g. due to clogging or wear and tear) or in order to change the rate of NO perfusion.

Thus, according to another aspect of the invention there is provided a system including an apparatus according to the invention connected to an infusion solution reservoir, and to an infusion needle.

Naturally, the apparatus of the invention also includes suitable flow control means such as valves and/or pumps, which preferably may be operated automatically (which is the preferred mode for regular operation) or manually (for maintenance and the like). These control means allow filling the gas compartment with NO-comprising gas before use, controlling removal of oxygen from the gas compartment, controlling NO pressure, stopping the apparatus operation at emergency, etc. Furthermore, an apparatus in accordance with the invention preferably includes at least one manometer, to check purging of the system, i.e. removal of oxygen, NO-meter and NO_2-meter for monitoring leaks inside the apparatus and monitoring functioning the apparatus, etc. The various meters may be connected to control module that can alert when any malfunction is detected by any of these meters.

According to yet another method aspect of the invention, a method for delivering NO into a blood vessel of a mammal is provided, the method comprising inserting into said blood vessel a conduit having a sealed distal end, at least said distal end that is inserted into said blood vessel being made of a

pressure resistant material, preferably such that is chemically inert, that is NO permeable, said conduit holding gaseous NO at a pressure, such that NO permeates from said conduit into said blood vessel to create therein therapeutically effective NO concentration.

The term "therapeutically effective NO concentration" refers to any concentration that may produce a detectable desired effect in a patient, such as dilation of blood vessels, measurable increase in blood flow, etc. It may also be detectable via observation of changes in the behavior or feeling of the patient. Such concentration may be achieved for example when infusing the NO at about 10-20nmol NO/minute (such as about 16.42nmol/minute).

In accordance with an embodiment of the invention, the conduit is at an end of a tube that is connectible to a pressurized gaseous NO reservoir or source. In accordance with one preferred embodiment of this method aspect, the conduit may be made of a fluorinated hydrocarbon polymer, such as Teflon.

As NO gas may be toxic, it is preferred that said reservoir will hold the minimal amount of NO, required to retain the pressure during treatment. Thus, typically the NO gas will thus be provided in small vessels or delivered to the patients' bed site through conduits from a remote site.

According to a second apparatus aspect of the invention, there is provided a catheter that is sealed at its one end, and includes at least a portion at said end made of a substance that is permeable to NO and resistant thereto, (for example a fluorinated hydrocarbon polymer, such as Teflon). The apparatus may include an integral NO reservoir, to which the catheter is connected in a second end thereof.

In operation, the catheter is inserted into a blood vessel of the mammal, e.g. a human, to be treated, NO pressure of up to about 10 atmospheres is created in the catheter, and thus NO permeates through its NO permeable portion into the blood vessel.

In accordance with the invention, the NO permeable barrier should be made of a material that is also NO resistant, (for example a fluorinated hydrocarbon polymer, such as Teflon).

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, preferred embodiments will now be described, by way of non-limiting examples only, with reference to the accompanying drawings, in which:

Fig. 1 is an illustration of the application of the method of the invention in accordance with one embodiment thereof;

Fig. 2 is a schematic view of an apparatus in accordance with one embodiment of the invention, wherein the gas compartment is enclosed in the liquid compartment;

Fig. 3 is a schematic view of an apparatus in accordance with another embodiment of the invention, wherein the gas compartment encloses therein the liquid compartment;

Fig. 4 is a schematic view of an apparatus in accordance with still another embodiment of the invention; and

Fig. 5 is a schematic view of an example of a gas supply system for use in the apparatus of the present invention.

Fig. 6 is a schematic representation of the NO concentration in air as a function of the NO pressure in catheters manufactured according to an embodiment of the invention.

Fig. 7 is a schematic representation of the NO concentration in a solution as a function of the NO pressure in a device according to an embodiment of the invention.

DETAILED DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates in a general manner a method of the invention in accordance with one embodiment, in which an infusion solution **2** is prepared *in situ* from a solution **2'** and from NO, obtained from an NO source **3**. The infusion solution **2'** does not contain NO, or if it does contain some traces thereof, the NO concentration is not sufficient to be therapeutically effective. **Fig. 1**

schematically shows a blood vessel **4**, into which the physiologically acceptable infusion solution **2** is infused. Also shown in **Fig. 1** is that the preparation of the solution **2** takes place in a site **6** that is in flow communication with the blood vessel **4**.

In operation the apparatus may remain connected to some kind of NO source. This allows adjusting the site **6** to produce the infusion solution **2** with NO concentration in accordance with physiological parameters of the patient that may be measured during the infusion (by means not shown). Such adjustment may be preformed manually, by a healthcare specialist that controls the preparation site **6** by a manual control panel (not shown) provided therein or automatically, by a microprocessor **10** which controls the preparation site **6** and is connected to other measuring devices, not shown.

Fig. 2 shows a schematic view of an apparatus **100** in accordance with the invention. The apparatus **100** includes an NO gas compartment **102** passing through a liquid compartment **104.** A portion of the gas compartment **102** is inside the liquid compartment **104** and has a Teflon wall **106** that is NO permeable. The portions of the gas compartment **102** that are not inside the liquid compartment **104** are made of a material that is not NO permeable, such as stainless steel. In operation, the apparatus **100** is connected to an infusion bag **112**, from which a state of the art infusion solution **118** is pumped into the liquid compartment **104**, and to an infusion needle **116**, which transfuses the NO-containing infusion solution **119** from the liquid compartment into a blood vessel **120**. Before operation begins, the apparatus **100** is also connected to an NO reservoir **109**, from which NO is discharged into the gas compartment **102** through a gas control unit **108** to build a pressure in the gas compartment such that it may permeate through the Teflon walls **106** into the infusion solution **119**, to produce an NO enriched infusion solution. After the required pressure is build, the apparatus **100** may be disconnected from the NO reservoir **109**.

Fig. 3 shows a schematic view of an apparatus **200** in accordance with another embodiment of the invention. The apparatus **200** includes a gas

compartment **202** that has in it a tube portion **204** with a wall **206** that is NO permeable. In operation, the apparatus **200** is connected to an infusion bag **212**, and to an infusion needle **216**, which transfuses NO-containing infusion solution **218** from the liquid compartment **204** into a blood vessel **220**. Before operation begins, NO is discharged from its reservoir **214** into the gas compartment **202** via a gas valve **222**. The valve **222** may also be opened to allow addition of NO to the gas compartment **202** if elevation of the pressure inside the compartment is required, or it may be opened in the opposite direction, to allow lowering the gas pressure in the compartment. Such adjustments may be carried out manually or automatically. If the apparatus **200** is connected to an NO reservoir during treatment, such adjustment may also be carried out during treatment, to adjust the treatment to parameters that are being measured during the treatment, such as the patient's blood pressure.

Some non-limiting examples of possible parameters of the method and apparatus in accordance with the invention (such as the gas temperature, the temperature of the infusion solution, the dimensions of the NO permeable barrier, the NO pressure and the flow rate of infusion solution) are given below:

The gas temperature should be such that the enriched infusion solution would be approximately equal to body temperature. This typically requires the gas temperature to be between 37°C and about 70°C. For this purpose, a heating sleeve (not shown) may be added to the NO reservoir **109**, or any other appropriate location.

The NO permeable barrier may be made of Teflon and may be between about 10 and about 40cm long and between about 0.01 and about 0.5mm thick.

The NO pressure may be between about 0.5 and about 10 atmospheres. The flow rate of the infusion solution (e.g. saline) may be between about 0.5 and about 5cc/min.

Although in the two embodiments described above one of the compartments encloses therein the other compartment, this is not necessarily so, and the two compartments may be positioned in any other arrangement, as long

as NO gas may pass under pressure through a permeable barrier from the gas compartment to the liquid compartment. One other non-limiting example for such an arrangement includes the two compartments arranged side by side and separated by an NO-permeable wall (not shown).

Fig. 4 shows another apparatus **300** in accordance with the present invention. The apparatus **300** includes a catheter **303** having a sealed end **305** and an open end **307**, connected to an NO reservoir **309,** via a gas control unit **311** for controlling the transfer of gas from the reservoir **309** to the catheter **303.** The catheter **303** includes a portion **313** that is a conduit with a Teflon wall **315,** through which NO may permeate.

In operation, the catheter **303** is inserted into a blood vessel of the mammal treated (not shown), such that the portion **313** is within the blood vessel region, to which NO is to be delivered. NO is discharged from the reservoir **309** into the catheter **303** through the control unit **311** until pressure of up to about 10 atmospheres is produced in the catheter **303,** and thus NO permeates through Teflon wall **315** into the blood vessel. In cases where in operation the gas pressure in the catheter **303** varies from its required value, such that the permeation rate of NO into the blood vessel changes in a therapeutically significant degree, the control unit **311** passes gas between the reservoir **309** and the catheter **303** to compensate for this change. Similarly, NO pressure manipulations may be carried out manually during treatment, if so desired by the treating healthcare professional.

A control unit similar to unit **311** may also be used in other embodiments of the invention, such as those described with reference to Figs. 2 and 3.

The apparatus of the invention may also include an appropriate NO gas supply system, housed in a housing **350**, and **Fig. 5** illustrates an example of such a system.

Referring to **Fig. 5**, the components labeled **C1** to **C11** are quick connectors with integral valves (male or female), designed to seal upon

disconnect to prevent gas leakage; the components labeled **S1** to **S6** are solenoid valves; the components labeled **PTX1** and **PTX2** are pressure transducers or transmitters; the components labeled **PG1** and **PG2** are pressure gauges; **PR1** is a pressure regulator; **VG** is a vacuum gauge; **VP** is a vacuum pump; **FM1** is a flowmeter; and **CV** is a control valve.

Such an NO gas supply system comprises an NO gas source unit **400** including an NO gas source **402** (e.g. one or more NO gas cylinders); an NO gas delivery unit **500**; an NO gas leak and alarm unit **600** including an NO sensor **602**, an NO_2 sensor **604** and a pump **606**; and typically a gas supply control unit (e.g. a controller, typically with a display and input control - not shown).

The NO gas source **402** may be charged with NO via solenoid **S1** by an NO supply (not shown) attached at valve **C1**. Thus, source **402** may be a relatively small gas source, which after being charged may then be connected to the gas delivery unit **500**. The pressure in the gas source **402** must obviously be at least as great as that to be supplied downstream (via valve **C4**) to the gas compartment **102** or liquid compartment **104** (containing infusion solution). The gas pressure downstream of the source **402** is set by pressure regulator **PR1** and monitored by pressure gauge **PG1** and pressure transducer **PTX1**.

In preparation for operation, the NO gas supply system should be first tested for leaks. After the gas source **402** is charged with NO - say to 10bar – leaks can be tested by opening solenoids **S2**, **S3** and **S4** at which point the pressure indicated by **PG2** and **PTX2** should remain constant for an appropriate period if there are no leaks.

Next, the gas delivery unit **500** is purged several times to ensure that no more than an insignificant amount of air remains therein. It is important that there is virtually no oxygen in the system to react with the NO. To eliminate the air in the unit **500**, solenoid **S2** is shut (solenoid **S6** has not been opened) and vacuum pump **VP** is operated until a suitably low pressure is attained, as verified by vacuum gauge **VG**. The gas (NO/air mixture) may be pumped out via control valve **CV**.

Solenoid **S5** is then closed, solenoid **S2** is opened to allow the gas delivery unit **500** to be refilled with NO, and the purging/vacuuming is repeated a suitable number of times.

To check the alarm unit **600**, at the beginning of the first purge cycle, valve **CV** is closed and solenoid **S6** is opened for a few seconds. This flows NO via flowmeter **FM1** into the alarm unit **600** and past NO sensor **602** and NO_2 sensor **604**. To ensure a rigorous test (i.e. a check with a low NO/NO2 concentration), the gas supply system may include an orifice **502** to limit the NO flowing to the sensors **602** and **604** and may further include an inlet **504** via which air is provided to dilute the NO.

Now the system is ready to deliver NO (via valve **C4** to the gas compartment **102** or liquid compartment **104**) and for this purpose solenoids **S2**, **S3** and **S4** are opened and solenoid **S5** is closed.

During operation, the pump **606** operates to draw in air via inlet **504** and thus if any NO leaks out into the housing **350** it will be detected by at least one of the sensors **602** and **604**.

Example 1 - Evaluation of Permeation Rates

The permeation rate of special catheters and special infusion units constructed in accordance with some embodiments of the present invention were evaluated by measuring the concentration of NO in a flow of 250cc/min air, directing into the input of an NOX Analyzer (AP1 Chemiluminence Analyzer 0-4000ppb).

a. Catheter Permeation Rate

These evaluations were made using 3Fr diameter catheters constructed in accordance with **Fig. 4**, being 40 cm long, diameter 0.021", and the NO flow being 250 cc/min., and he results are shown in **Fig. 6**. Curves (1) and (2) depict the NO permeation at 37.4°C, whilst curve (3) depicts the NO permeation at room temperature (25°C)

As can be seen, the permeation increased with the increase of NO pressure over the range 1-8bar NO. The deviation between curves (1) and (2) (i.e. at 37.4°C) was within the measuring tolerances of the equipment used. Maximum error is 15% (±7.5%) at 7bar and 10% (±5%) at 8bar. At pressure of up to 4bar, tolerance between the catheters was negligible. The difference in permeation rates between the catheter held at 25°C (curve (3)) and 37°C (curves (1) and (2)) was within the calculated values for permeation rates at different temperatures – less then ±5%.

$$\text{Log } P1 = \text{Log } P2 - 2950(1/T1 - 1/T2)$$

Where Log P1, P2 are permeation rates at temperature T1, T2. (Gas Mixture – preparation and control by Gary O. Nelson (1992)

b. Infusion Unit Permeation Rate

The permeation rate of a device constructed according to Fig, 3was measured by connecting the infusion solution output of the infusion unit directly to the input of a NOx Analyzer (AP1 Chemiluminence 0-4000ppb) at 250cc/flow rate. The Teflon barrier was 30 cm long on a 1/4" and the experiment was done at 37.4°C.The NO permeation into the solution increased with the NO pressure in the gas chamber over the range 1-8bar NO, as can be seen in **Fig. 7**.

Example 2 – administration saline containing NO to rabbits

In the course of the experiment an automatic system for injecting NO gas into a solution was employed. This system, constructed according to **Fig. 3**, and essentially as described above, controlled the exact rate and amount of the NO gas in the solution. The gas dissolved in saline was administered under the control of a conventional drop-counter attached to the various sized cannulas employed in the animals' blood vessels (the cannula size being chosen to conform to the caliber of the subject's blood vessel).

The experiments were conducted on 28 New Zealand white rabbits (23 males and 5 females, weighing 3.5 – 4.5 Kg.) after approval was obtained from the Helsinki committee on live animal research.

Under general anesthesia, the femoral artery and vein in the groin were explored. NO containing saline was infused to one superficial femoral artery and, as a control; ordinary saline (i.e. without added NO) was infused to second superficial femoral artery, using 3Fr Teflon catheters. During operation, the pressure of NO in the system was between 2Bar – 10Bar and the rate was 1cc/min-5cc/min. The gas infusion lasted for three hours.

During the experiment, blood pressure, pulse rate and O_2 saturation were monitored using a pulse oxymeter on the leg and a laser flowmeter on the thigh with limb temperature control. Monitoring began before the administration of NO, and continued throughout the experiment (three hours) and for additional two hours after the dripping of saline was stopped. Blood was taken from the femoral artery and vein and from the auricular vein at the following times: (a) before the experiment, (b) every hour for three hours after NO administration commenced. The blood was assayed for NO (using ELISA).

Results

Blood flow, measured before the insertion of the gas was 167 Bpu (±5%) (laser flowmeter units). Within the first hour after administration of NO commenced, the blood flow increased to 242 Bpu (±5%). This high level was maintained throughout the administration of NO (three hours).

The average NO concentration in the blood before the experiment was 144.25μM. It rose during the experiment to 334.90μM and two hours after the administration of NO ceased, it still measured 303.76μM. No changes were observed in the other measured parameters (pulse rate, blood pressure or limb temperature).

Of the 28 rabbits, 18 were sacrificed immediately after measurement of the parameters ceased (i.e. two hours after the administration of NO ceased. As for the other 10 rabbits, their femoral arteries were ligated, thus limiting blood flow to the posterior limbs. The rabbits kept alive for 72 hours for observation. During this time, 5 rabbits were kept untreated. They developed paresis of the lower leg and were restless. This may be attributed to the ligation of the arteries. The other five rabbits were re-operated 24 hours after the end of the above experiment. This time NO was infused for three hours into the infra-renal aorta as described above, and the rabbits were monitored with the laser flowmeter and pulse oximeter. Bpu in the posterior limbs was very low-about 20. However, upon infusion of NO the animals started to move their posterior legs, indicating that administration of NO might have caused some improvement of peripheral blood flow in the limbs.

Example 3 –administration of NO to pigs

These experiments were conducted on 10 Landrof hybred white large female pigs (weight range: 80-100 Kg.) after approval was obtained from the Helsinki committee on live animal research.

During the experiments pigs were kept under general anesthesia. They were monitored for: O_2 saturation of the blood and pulse rate, using a pulse oximeter, limb blood flow, using laser Doppler flowmeter and ultrasonic flowmeter (transonic) and body temperature. Blood samples were obtained before administration of NO, every hour during the administration (3 hours in total) and 2 hours after NO administration was ceased, from the femoral vein and artery and/or an upper body source for determining: NO (μM) in the blood by Enzyme Linked Immunosorbent Assay (ELISA), blood sugar, urea, creatinine, sodium, potassium and complete blood count, all performed using routine blood assay procedures.

NO administration: NO was introduced percutaneously to the pigs via the femoral artery cannulation, and, if necessary, and incision was made in he groin region to gain access to the femoral artery and vein, to perform cannulation in an open fashion. Administration of NO containing saline and angiography (necessary to position the NO containing catheter, in accordance with Fig. 4) was performed via a 2-3 Fr Teflon catheter.

NO containing saline: Into one leg of the animal, saline containing NO (1500nM) was infused for a period of three hours at a rate of 1-3 cc per minute using a drop-counter (dosage of NO gas was 16.42 nmol/min). Simultaneously, under the same conditions, saline without NO was infused into the other leg. Measurements of blood flow and temperature were made by the laser flowmeter continuously during the experiment. Urine and blood samples were taken far from the incision.

Blood flow was measured in the femoral and iliac arteries via the ultrasonic flowmeter (transonic) before, during, and one hour after the gas infusion. Blood samples were taken for testing from either the artery or vein. Blood samples were again taken three hours after the experiment.

Results

In the first group of animals which, were infused with saline containing NO in their thighs, the blood flow rose in the limb which received NO within 30 minutes from the beginning of administration of NO by between 70-140%, and remained substantially constant throughout the administration of NO. NO concentration in the blood: Mean difference – 159.51 (Mean of 12 minus mean of 1). The 95% confidence interval of the difference: 157.08 to 161.95. t-181.76 with 4 degrees of freedom. The two – tailed P value is<0.0001, considered extremely significant. Two hours after the cease of administration of NO, the blood flow was still above the initial rate. NO concentration before the experiment began was 67.72μM. It increased to 87.05μM within 2 hours and two hours after the flow of NO ceased, NO was 95.15μM.

In the limbs not receiving NO directly, a smaller increase was noted – which is probably indicative of the systemic effect of the gas – this was up to 40%.

In the blood tests (gas and chemistry), increases were found only in glucose that reached approximately 30% above normal during the experiment. Repeat studies 24 hours after the experiment showed return to normal blood glucose. No other parameters showed any changes.

In the second group of animals, in whom gas was infused into their aortas or left iliac arteries (without saline), increase in blood flow in the limb which received the NO was 30-60%. In this group, the systemic influence of the gas was shown to be 15-20%. .

It must be mentioned that increase in blood flow appeared 15-30 minutes after commencement of the infusion of the NO gas and continued for about 2 hours after its cessation, regardless whether NO was introduced via a sealed catheter or with an infusion solution. In other words, the flow

remained increased and the blood vessels remained widened even after infusion was discontinued.

Experimental Conclusions

Gaseous NO which comes into contact with arterial blood rapidly affects vascular walls causing their widening and increasing the amount blood flowing through them. These changes persist for up to two hours even after infusion of the NO has ceased. This indicates that although the NO breaks down in the blood, its influence commences rapidly and continues for some time. NO can be delivered directly into blood vessels as a gas or via permeation as a liquid in physiologic saline. After infusing NO directly into arteries in the amount of 5-6 nanograms under pressure of 8-10 bar – no general effect was seen on the animal body, neither during nor after the infusion. NO, as liquid or permeation, widened blood vessels and its influence continued even after the infusion has stopped. No significant harmful effects were noted during or after the treatment.

The blood level of Nitric-Nitrate increases during the treatment and remains elevated for several hours thereafter.

Those skilled in the art will readily appreciate that various modifications and changes can be applied to the embodiments of the invention as hereinbefore exemplified without departing from its scope defined in and by the appended claims.

CLAIMS:

1. A method for delivering a controlled amount of nitric oxide (NO) into a blood vessel of a mammal, the method comprising:

(a) providing an NO source under pressure;

(b) flowing NO from said NO source via a member comprising a portion being NO permeable and resistant to NO, said NO permeable portion being in contact with an aqueous solution, thereby delivering a controlled amount of NO to the aqueous solution.

2. The method according to claim 1, wherein the NO containing aqueous solution is a physiologically acceptable infusion solution.

3. The method of Claim 2, wherein said physiologically acceptable infusion solution is infused into said blood vessel of a mammal for a period of from one hour to 24 hours.

4. The method according to the preceding claim, wherein said period of time is from 2 to 6 hours.

5. The method according to any one of the preceding claims, used repetitively.

6. The method according to any one of the preceding claims, wherein the rate of infusing NO into the blood vessel is lower than 100ng/min.

7. The method according to the preceding claim, wherein said rate is between 30ng/min and 80ng/min.

8. The method according to any one of the preceding claims, wherein the infusion solution is prepared *in situ*, such that the preparation site is in flow communication with the blood vessel.

9. The method according to the preceding claim, further comprising adjusting the NO concentration of the physiologically acceptable solution in accordance with physiological parameters of the patient in real time.

10. The method of Claim 1, wherein the aqueous solution is blood.

11. The method of Claim 10, wherein the member having a portion being permeable to NO is a conduit inserted into said blood vessel, this conduit holding NO at such a pressure, that NO may permeate from said conduit to said blood vessel to produce in the blood a therapeutically effective NO concentration.

12. The method according to the preceding claim, wherein the conduit is a portion of a catheter having first end and a second end, said catheter being closed at said first end and connectible to an NO reservoir at said second end.

13. A method according to any one of claims 11 or 12, wherein said conduit is made of a fluorinated hydrocarbon polymer, such as Teflon.

14. A method for continuously producing a physiologically acceptable solution containing NO, the method comprising contacting a flow of physiologically acceptable solution with an NO permeable barrier, which is under a pressure of NO gas.

15. The method according to any one of the preceding claims, comprising connecting the gas compartment to an NO reservoir to create a predetermined NO pressure in the gas compartment and disconnecting the gas compartment from the NO reservoir.

16. An apparatus comprising: a gas compartment and a liquid compartment, wherein the gas compartment has a first inlet and optionally also a first outlet and is in flow communication with said liquid compartment having a second inlet and a second outlet, said flow communication being through an NO permeable barrier, such that gas may permeate through the NO permeable barrier from the gas compartment to the liquid compartment and liquid that enters the liquid compartment through said second inlet may leave it through the second outlet after absorbing NO.

17. The apparatus according to claim 16, wherein said barrier is replaceable.

18. A kit comprising an apparatus according to claim 17 and a plurality of NO permeable barriers having different surface areas and/or thicknesses.

19. The apparatus according to claim 18, wherein the second inlet and the second outlet are unified in a single aperture, such that in operation liquid may

enter the liquid compartment, stay in it, and leave the compartment from the same aperture it entered.

20. The apparatus according to any of claims 16, 17, or 19, wherein the liquid compartment is a conduit going through the gas compartment and has a wall having at least a portion thereof that is NO permeable, said portion being located inside the gas compartment.

21. The apparatus according to the preceding claim, wherein the conduit is connectable to a liquid reservoir, from which liquid may enter the conduit.

22. The apparatus according to any of claims 19 or 20 having no gas outlet, such that gas may exit the gas compartment via the first inlet, which may function as outlet, or via the permeable barrier.

23. The apparatus according to any of claims 16, 17, or 19, wherein the gas compartment is a conduit going through the liquid compartment and has a wall that is NO permeable at least in a portion thereof, said portion being located inside the liquid compartment.

24. The apparatus according to the preceding claim, wherein the conduit is connectible to a gas reservoir, from which gas may enter the conduit.

25. The apparatus according to any of claims 19 or 21, further comprising a pump for pumping gas into the gas compartment from a gas reservoir.

26. The apparatus according to any one of claims 19 to 20, having means for controlling the temperature of NO.

27. The apparatus according to the preceding claim, wherein said means is a heating sleeve positioned around an NO reservoir.

28. The apparatus according to any one of claims 21 to 27, wherein the conduit is in a form of a pipe, a plurality of pipes connected to each other at the inlet and outlet, or a plurality of independent pipes connected to different liquid or gas reservoirs.

29. A system including an apparatus according to any one of claims 17 to 28 connected to an infusion solution reservoir and to an infusion needle.

30. The system according to the preceding claim, wherein said apparatus is also connected to an NO gas reservoir.

31. The method for delivering NO into a blood vessel of a mammal, comprising inserting into said blood vessel a conduit made of a material that is NO permeable and resistant and this conduit holds NO at such a pressure, that NO may permeate from said conduit to said blood vessel to produce therein a therapeutically effective NO concentration.

32. The method according to the preceding claim, wherein the conduit is a portion of a catheter having first end and a second end, said catheter being closed at said first end and connectible to an NO reservoir at said second end.

33. The method according to claim 31 or 32, wherein said conduit is made of a fluorinated hydrocarbon polymer, such as Teflon.

34. A catheter having first end and a second end, said catheter being closed in said first end and connectible to NO reservoir in said second end and has at least a portion made of material that is resistant to NO and NO-permeable.

35. A catheter according to the preceding claim, wherein said material is a fluorinated hydrocarbon polymer, such as Teflon.

36. A catheter according to any of claims 34 or 35, connected to an NO reservoir.

37. An infusion solution containing NO in a concentration that is smaller than 10ppm.

38. An infusion solution according to claim 35, wherein said concentration is from 5 to 80ng/cc.

39. An infusion solution according to claim 35, wherein said concentration is from 15 to 40ng/cc.

40. A method for delivering nitric oxide (NO) to a blood vessel of a mammal, the method comprising infusing into said blood vessel a physiologically acceptable solution containing NO.

41. The method according to claim 40, wherein NO is infused into said blood vessel for a period of from one hour to 24 hours.

42. The method according to the preceding claim, wherein said period of time is from 2 to 6 hours.

43. The method according to any of claims 40-42, used repetitively.

44. The method according to any of claims 40-43, wherein the rate of infusing NO into the blood vessel is lower than 100ng/min.

45. The method according to the preceding claim, wherein said rate is between 30ng/min and 80ng/min.

46. The method according to any of claims 40-45, wherein the infused solution is prepared *in situ*, such that the preparation site is in flow communication with the blood vessel.

47. The method according to the preceding claim, further comprising adjusting the NO concentration of the physiologically acceptable solution in accordance with physiological parameters of a patient in real time.

48. A method according to any one of claims 14, 40-47, comprising connecting the gas compartment to an NO reservoir to create a predetermined NO pressure in the gas compartment and disconnecting the gas compartment from the NO reservoir.

49. The apparatus according to any one of Claims 16-17, 20-29 for use with the method of any of Claims 1-9, or 13-16.

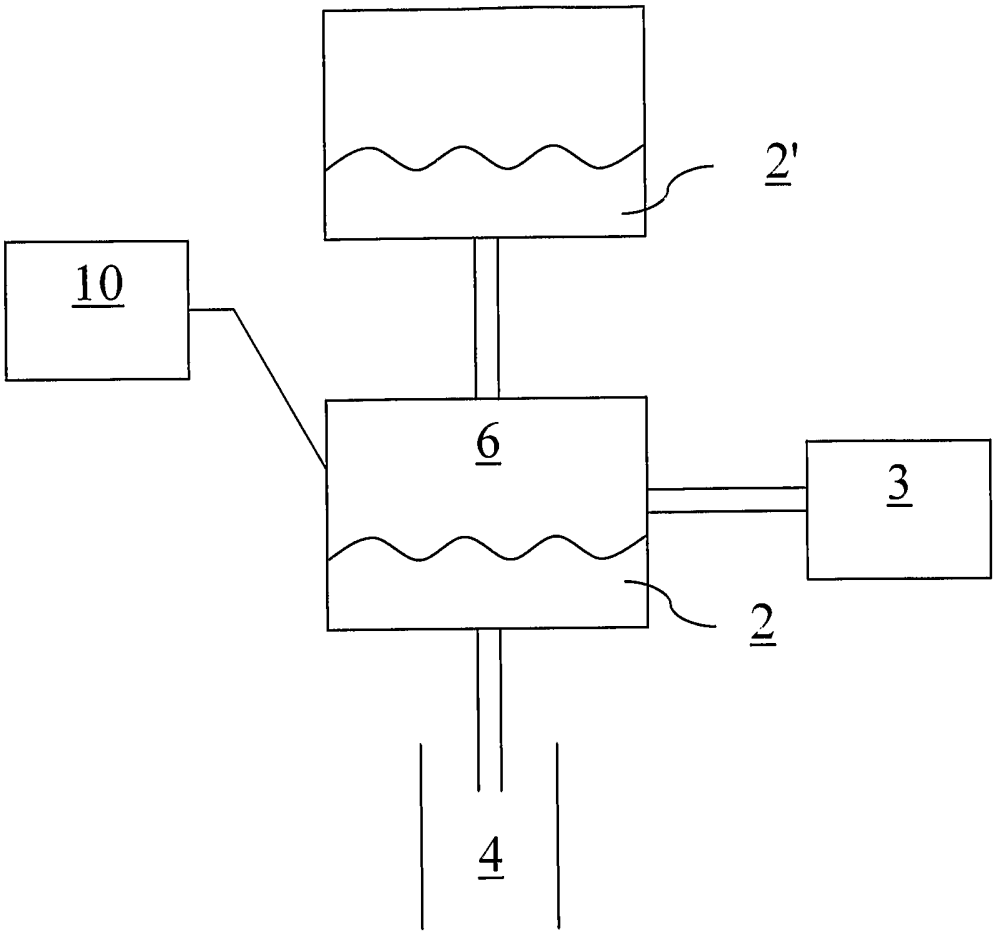


Fig. 1

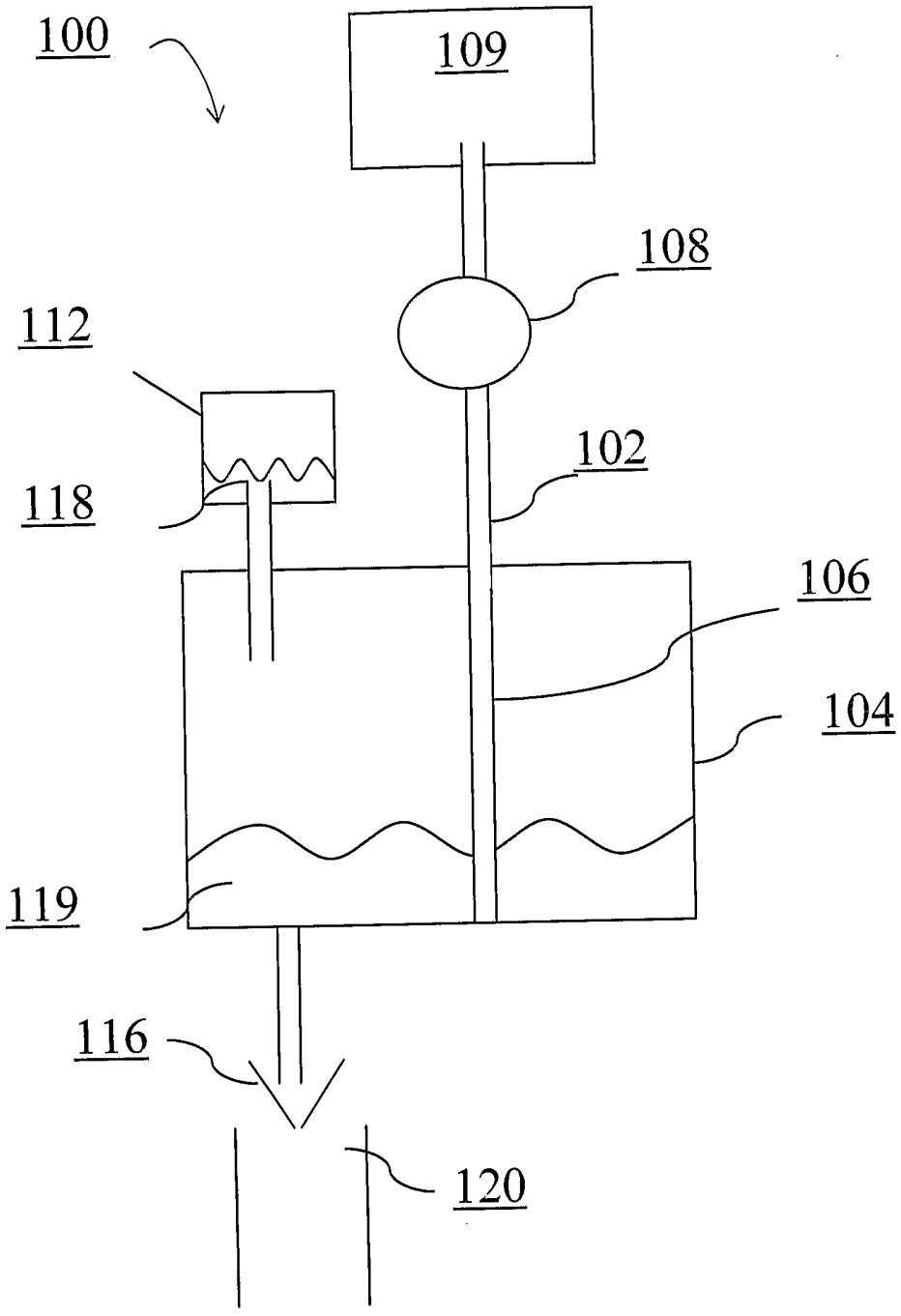


Fig. 2

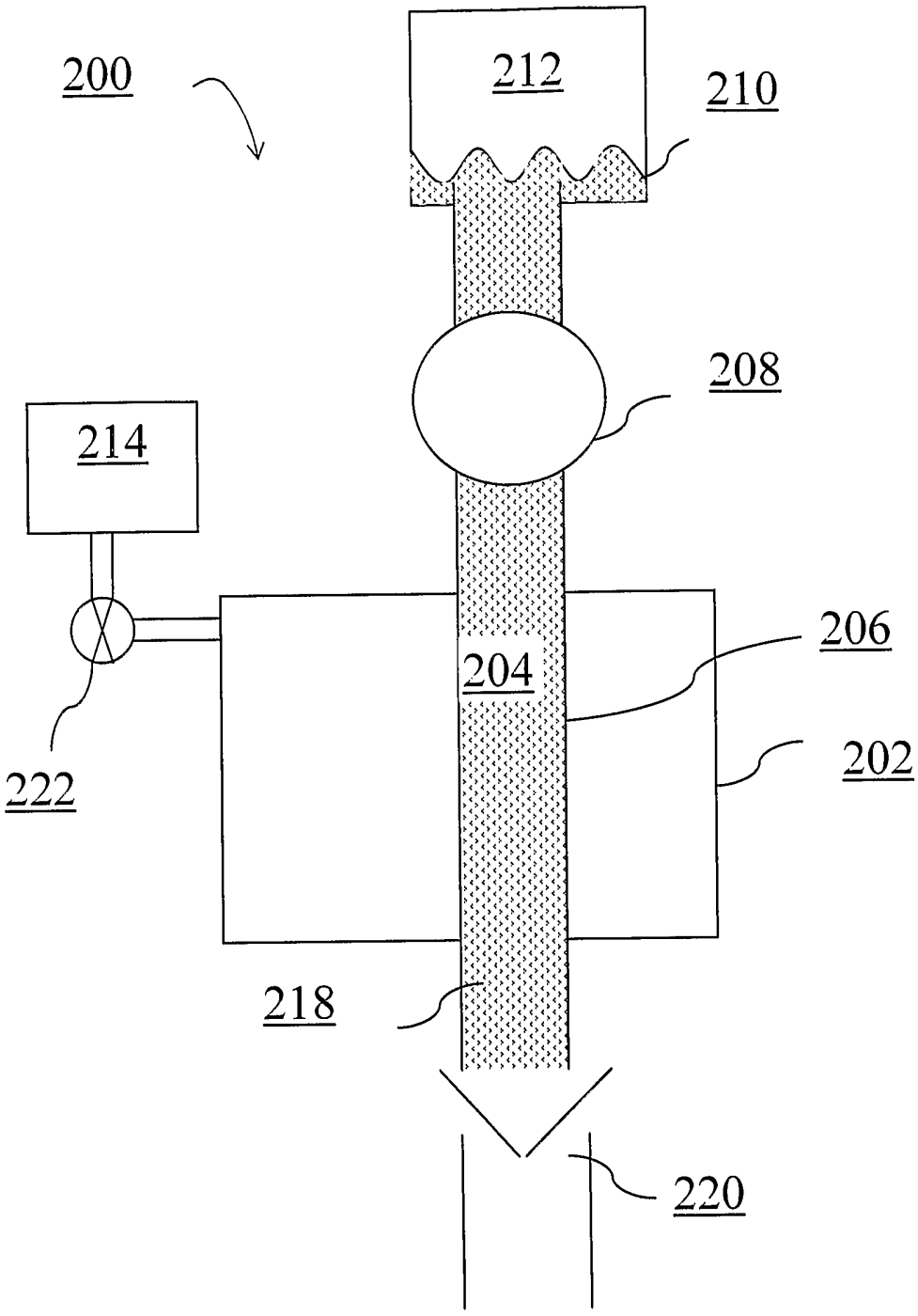


Fig. 3

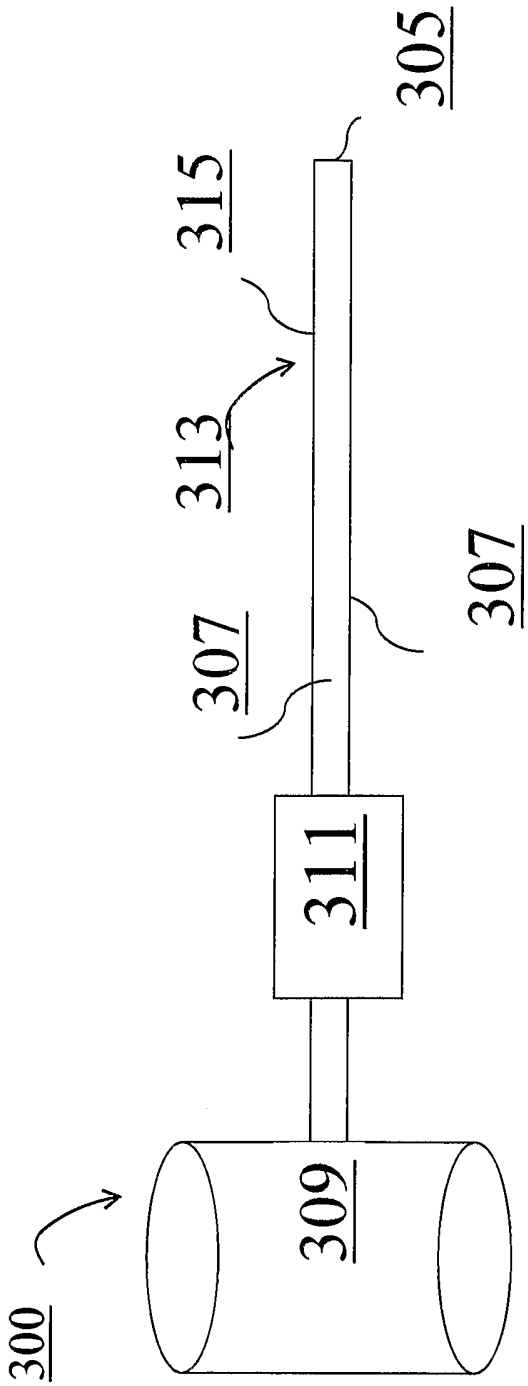
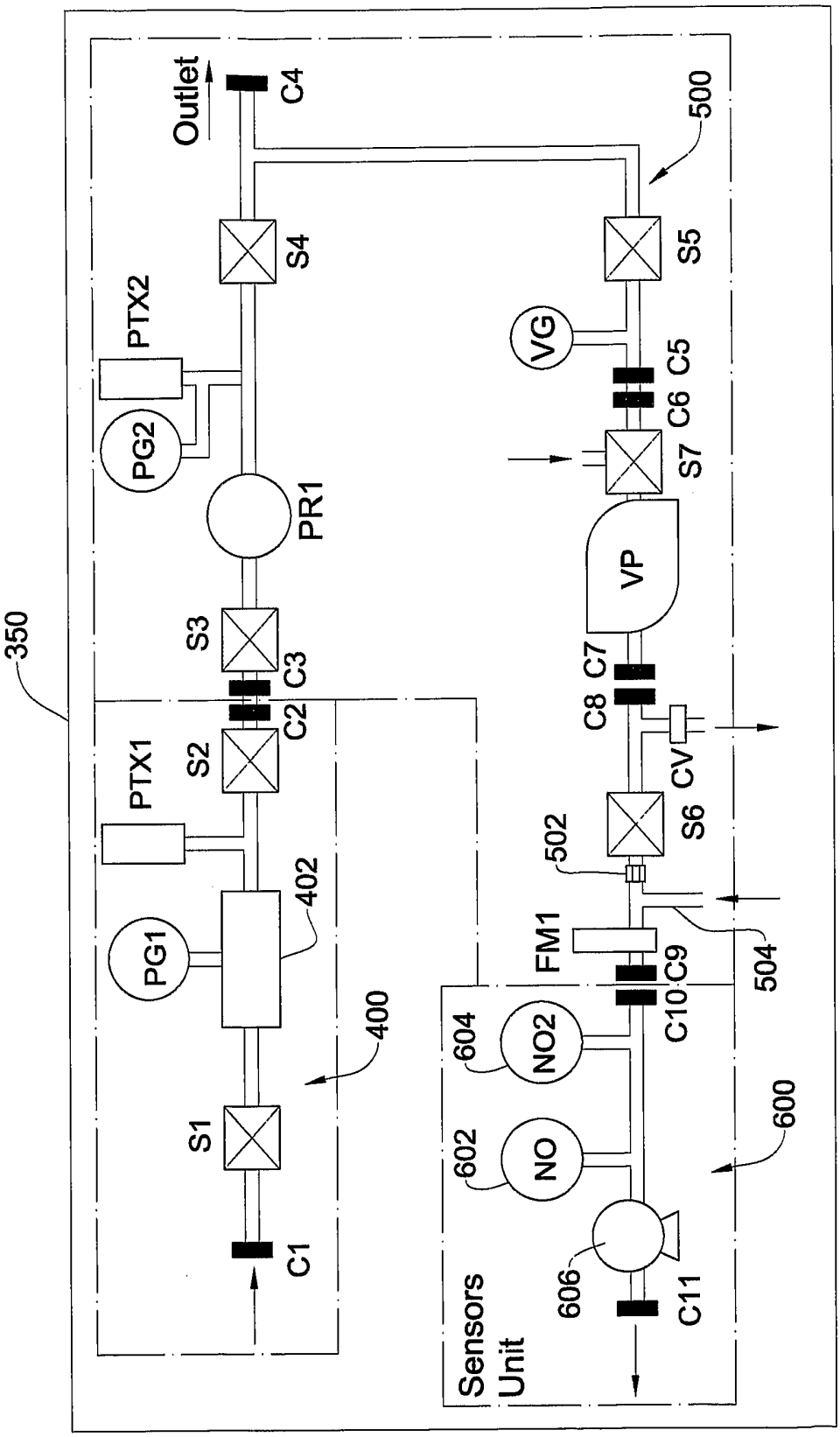


FIG.4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S5
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PG2
VG
C5
C6
S7
PR1
VP
S3
C3
C7
C8
C2
CV
PTX1
S2
S6
502
402
PG1
FM1
504
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C10
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604
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S1
602
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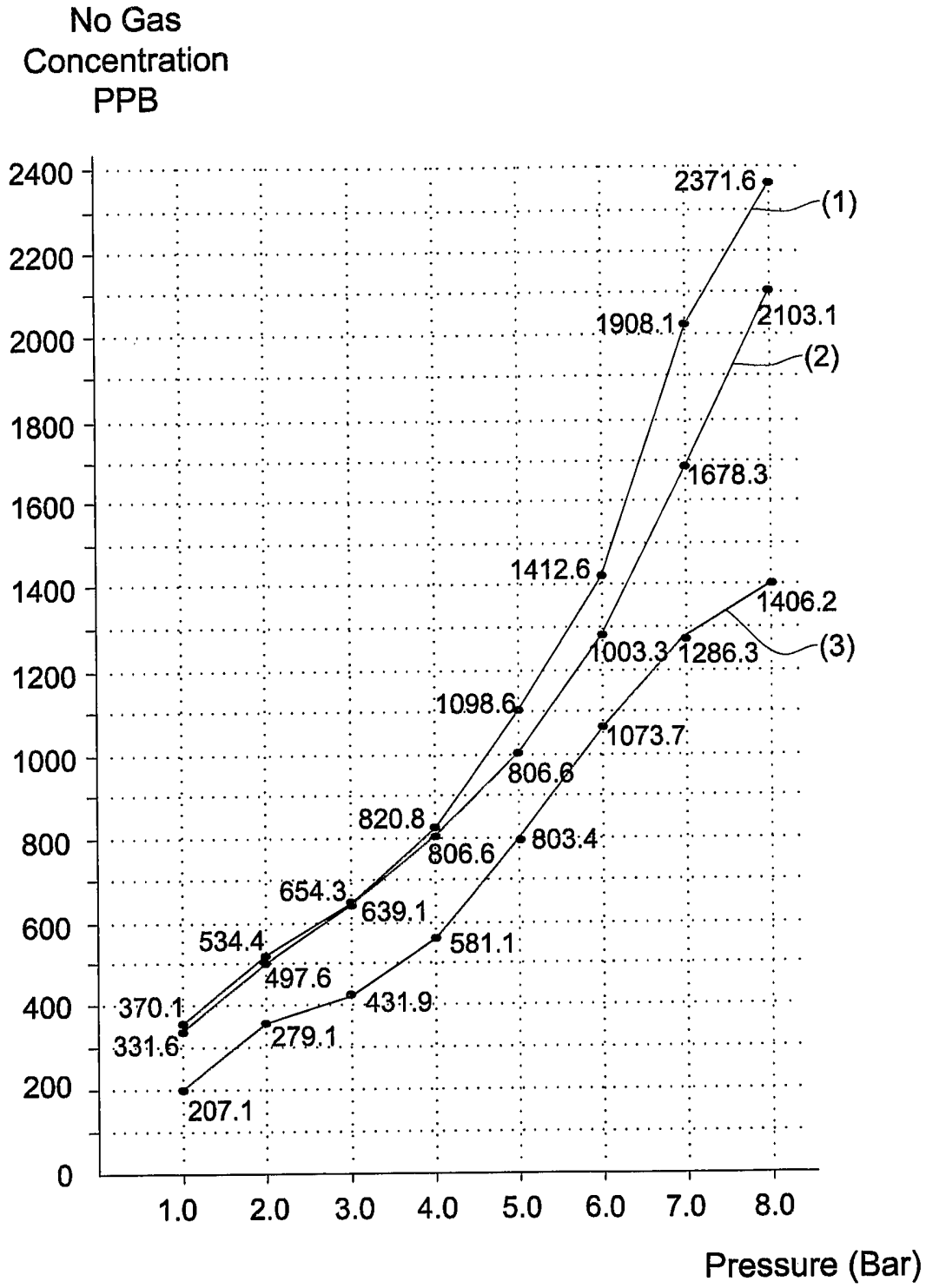
600
C1
606
C11
Sensors Unit

FIG. 5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1400
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1000
800
600
400
200
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2.0
3.0
4.0
5.0
6.0
7.0
8.0
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(1)
1908.1
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(2)
1678.3
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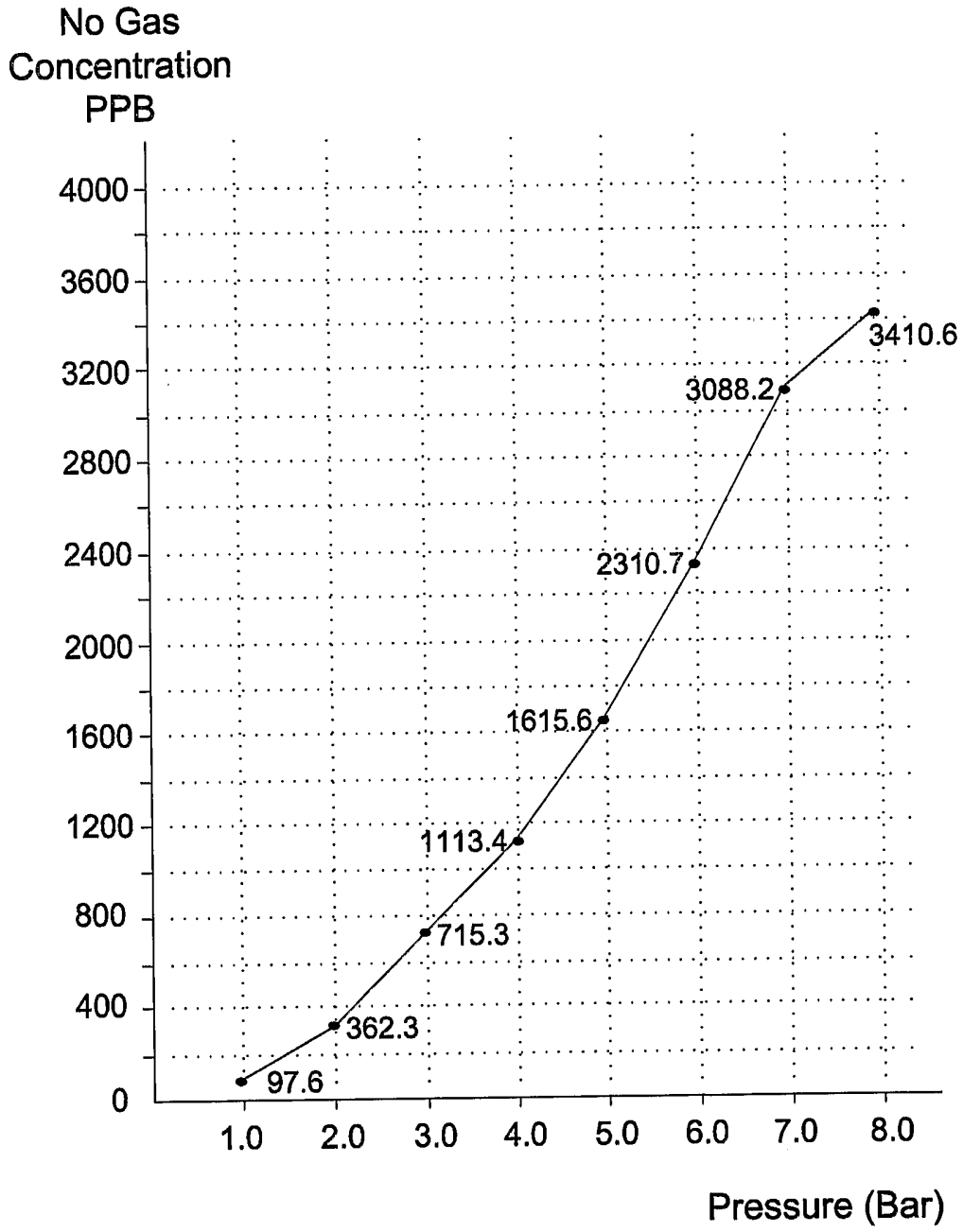
FIG. 6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1600
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800
400
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715.3
1113.4
1615.6
2310.7
3088.2
3410.6
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Pressure (Bar)

FIG. 7