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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :		(11) International Publication Number:
G01N 1/00, A61K 49/00	A1	WO 91/00507
		(43) International Publication Date: 10 January 1991 (10.01.91)

(21) International Application Number: PCT/US89/02940

(22) International Filing Date: 5 July 1989 (05.07.89)

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(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent)*, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: PERFLUOROCARBONS FOR USE AS STANDARDS IN GAS PARTIAL PRESSURE MEASUREMENTS

(57) Abstract

A method for calibrating an instrument used to determine the pO_2 and pCO_2 in the blood, comprising, introducing into the instrument a sample quantity of an emulsion comprising an aqueous phase, an oxygen-carrying fluorocarbon in an amount of 45 % to 125 % weight per volume having known stable values for pO_2 and pCO_2, and an effective amount of an emulsifying agent, the emulsion being biocompatible and maintaining the stable values through heat sterilization and storage for at least three months, and adjusting the calibration of the instrument to correspond to the known values of pO_2 and pCO_2 in the emulsion.

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PERFLUOROCARBONS FOR USE AS STANDARDS IN GAS PARTIAL PRESSURE MEASUREMENTS

Background of the Invention

The present invention relates to calibration of instruments used for the measurement of pO_2 and pCO_2 with certain biocompatible perfluorocarbon emulsions.

Instruments that measure the partial pressure of oxygen (pO_2) or carbon dioxide (pCO_2) in a liquid are in widespread use. These instruments find particular use in medicine, and in particular in the monitoring of dissolved gas levels in the blood.

One problem encountered in the use of such instruments is calibration. A reference solution, for example, is often used to calibrate instruments used in measurement of various values in liquids. The frequency of calibration varies. In some instruments used in the measurement of various values in liquids, a reference is used continuously and the measured value is determined by direct comparison against the reference value. In other circumstances, the instrument is frequently removed from service and calibrated prior to use in further measurements.

It has been proposed that fluorocarbon emulsions, which have substantial oxygen and carbon dioxide carrying capacities, could be used in the calibration of such instruments. See U.S. Patent Nos. 4,299,728 and 4,369,127. Such emulsions, however, have not been entirely suitable for this purpose. In particular, sterility and toxicity concerns have limited widespread use of these emulsions where contact with body fluids is a consideration. Moreover, these emulsions have not been entirely stable insofar as their pO_2 and pCO_2 values are concerned, particularly after long storage. Finally, the oxygen and carbon dioxide capacities of prior art emulsions used for this purpose have not been completely satisfactory.

In the past, fluorocarbon emulsions particularly formulated for oxygen carriage have been taught to have upper limits on the fluorocarbon concentration. For example, efforts directed toward perfluorocarbon emulsions with phospholipid emulsifiers have been proposed having 20% to 40% weight per volume of the fluorocarbon and 2% to 6% weight per volume of lecithin, but such emulsions have a limited stability. Moreover, it has been taught that emulsions having fluorocarbon concentrations higher than 75% weight per volume are too viscous to be used intravascularly. See for example, Sloviter, U.S. Letters Patent No. 4,423,177. Such concentrations, however, necessarily limit the capacity of the emulsion and the quantity of oxygen and of contrast enhancement which the emulsion can provide. (Note that as used herein, the term "weight per volume" or "w/v" means the amount in grams of that material in 100 ml of resulting liquid. Thus, for example, an emulsion having a "5% w/v" of an ingredient has 5 grams of that ingredient per 100 ml of the final emulsion.)

In the prior art fluorocarbon emulsions, sterilization can only take place without damage to the emulsion, at temperatures lower than 121°C, on the order of, for example, 60°C, and with repeated heatings. Many of these emulsions, further, must be stored frozen and thawed shortly before use, thus restricting handling and uses. Indeed, even in those emulsions previously taught as being sterilized at normal sterilizing temperatures, the desired emulsion is not obtained until centrifuging at 4°C at 100 times gravity for some period of time. See Sloviter, U.S. Letters Patent No. 4,423,077, mentioned above.

Where yolk lecithin, a frequently chosen emulsifying agent because of its known biocompatibility, is used, the emulsion is subject to degradation in the presence of oxygen. Oxygen attacks normally available lecithin, such as yolk lecithin, to oxidize the lecithin molecule which may result in a possible introduction of toxicity and

degradation of the emulsion. Thus, in the presence of oxygen, the pH of the emulsion decreases due to the accumulation of carbon dioxide and fatty acids, and the PO_2 pressure of the emulsion decreases. For this reason, it has been generally considered important to store such emulsions under or sparged with nitrogen which is believed to be inert with respect to the emulsion.

Yolk lecithin, as well as other lecithins have fatty acids characterized by one or more carbon-carbon double bonds. These double bonds are vulnerable to oxidation, leading to production of free fatty acids and other products. The lecithin thus changes into toxic components including fatty acids and lysolecithin which may produce adverse effects or toxicity. Over time, the oxygen dissolved in the fluorocarbon particle provides such an attack. To avoid such an attack, many such fluids are sparged with nitrogen and kept substantially oxygen-free until use. A Pluronic, such as Pluronic F.68 is an emulsifying agent normally less sensitive to oxidation, but may cause undesirable reactions in some intravascular applications.

When used for oxygen carriage or transport, fluorocarbon emulsions which cannot maintain substantially consistent partial oxygen pressure (pO_2) through sterilization, storage, processing and administration must be oxygenated immediately prior to use.

It is desired to provide a more uniform and more reliable pre-oxygenated, hence more immediately efficacious emulsion by performing the oxygenation during or shortly after the emulsion preparation before extended storage. Partial oxygen pressure (pO_2) and pH maintenance and stability during and through heat sterilization, and through extended time storage, preferably at room or ambient temperatures tend to indicate that there is no oxidation or degradation of the emulsion. It is a desired objective, therefore, to provide a biocompatible fluorocarbon emulsion which maintains pO_2 and pH during

sterilization procedures and during extended periods of storage.

It is desired further to provide fluorocarbon emulsions having a higher concentration of fluorocarbon in emulsion. It is desired yet further to provide such high fluorocarbon concentrations in emulsion with less concentrations of emulsifying agents, yet having biocompatibly satisfactory fluidity, i.e., biocompatibly low viscosity.

It is additionally desired to have methods of preparing and formulating high fluorocarbon concentrations with relatively low emulsifying agent concentrations in emulsion which do not have physical or practical commercial limitations affecting the quantity manufactured.

Finally, with these emulsions in hand, it is desired that they be used in calibration of instruments that measure oxygen and carbon dioxide tension in a liquid such as blood.

SUMMARY OF THE INVENTION

Thus, in accordance with one aspect of the present invention, there is provided a method for calibrating an instrument used to determine the po_2 and pCO_2 in the blood, comprising, introducing into the instrument a sample quantity of an emulsion comprising an aqueous phase, an oxygen-carrying fluorocarbon in an amount of 45% to 125% weight per volume having known stable values for pO_2 and pCO_2, and an effective amount of an emulsifying agent, the emulsion being biocompatible and maintaining the stable values through heat sterilization and storage for at least three months, and adjusting the calibration of the instrument to correspond to the known values of po_2 and pCO_2 in the emulsion. Preferably, the emulsifying agent is a phospholipid having saturated bonds. Although any of a number of fluorocarbons may be used in the emulsion, monobrominated fluorocarbons, such as perfluoroctylbromide are particularly advantageous. It is also preferred that the emulsifying agent having substantially saturated bonds

is saturated with hydrogen. Preferred emulsifying agents include phosphatidylcholine, synthesized lecithins, 1,2-dipalmitoyl-sn-glycero-phosphocholine, 1,2-dimyristoyl-sn-glycero-phosphocholine, lecithin derived from soy beans and then hydrogenated, and lecithin derived from egg yolk and then hydrogenated.

In one embodiment of the method, the instrument is in fluid communication with the circulatory system of an animal during the calibrating procedure. In another, the emulsion has a viscosity biologically compatible for use intravascularly of an animal.

DETAILED DESCRIPTION OF THE INVENTION

A fluorocarbon emulsion comprises from 20% weight per volume to at least 125% weight per volume of a fluorocarbon or a highly fluorinated compound (hereafter called a "fluorocarbon." The fluorocarbon could be any fluorocarbon or fluorocarbon mixture which, in emulsion, is biocompatible. Such a fluorocarbon in the emulsion may be bis(F-alkyl)ethanes such as $C_4F_9CH=CHC_4F_9$ (sometimes designated "F-44E"), i-$C_3F_7CH=CHC_6F_{13}$ ("F-i36E"), and $C_6F_{13}CH=CHC_6F_{13}$ ("F-66E"); cyclic fluorocarbons, such as C10F18 ("F-decalin," "perfluorodecalin" or "FDC"), F-adamantane ("FA"), F-methyladamantane ("FMA"), F-1,3-dimethyladamantane ("FDMA"), F-di- or F-trimethylbicyclo[3,3,1]nonane ("nonane"); perfluorinated amines, such as F-tripropylamine ("FTPA") and F-tributylamine ("FTBA"), F-4-methyloctahydroquinolizine ("FMOQ"), F-n-methyldecahydroisoquinoline ("FMIQ"), F-n-methyldecahydroquinoline ("FHQ"), F-n-cyclohexylpyrrolidine ("FCHP") and F-2-butyltetrahydrofuran ("FC-75" or "RM101"). Other stable fluorocarbons in emulsion are monobrominated perfluorocarbons, such as 1-bromoheptadecafluoroctane ($C_8F_{17}Br$, sometimes designated perfluorooctylbromide or "PFOB"), 1-bromopentadecafluoroheptane ($C_7F_{15}Br$), and 1-bromotridecafluorohexane ($C_6F_{13}Br$, sometimes known as perfluorohexylbromide or "PFHB"). Additional stable fluorocarbon emulsions that can achieve small particle

sizes and long shelf lives when made in accordance with this invention include perfluoroalkylated ethers or polyethers, such as $(CF_3)_2$ $CFO(CF_2CF_2)_2$ $OCF(CF_3)_2$, $(CF_3)_2$ $CFO(CF_2CF_2)_3$ $OCF(CF_3)$, $(CF_3)_2$ $CFO(CF_2CF_2)_2F$, $(CF_3)_2$ $CFO(CF_2CF_2)_3F$, $(C_6F_{13})_2O$, and F[CF(CF)CF O] CHFCF. Further, fluorocarbon-hydrocarbon compounds, such as, for example, $C_8F_{17}C_2H_5$ and $C_6F_{13}CH=CHC_6H_{13}$ can also be used in practicing the methods and achieving the emulsions of this invention.

Some fluorocarbons have vapor pressures too high for intravascular use. 1-bromotridecafluorohexane ($C_6F_{13}Br$) and F-2-butyltetrahydrofuran ("FC-75" or "RM-101") are two such fluorocarbons. Such fluorocarbons and their biocompatible emulsions may be used, however, in the respiratory system, gastrointestinal tract and cerebrospinal space, cavities and ventricles.

The fluorocarbon emulsion includes an emulsifying agent which must not reduce fluidity unnecessarily, and which will not permit viscosity to become so high that the emulsion will not be useful in the animal body. It has been discovered that very high fluorocarbon concentrations in emulsion, much higher than 76% weight per volume, can be achieved, including even on the order of 90%, 100% and 125% weights per volume but yet the viscosity of such emulsions remains suitable for use in the most constricted or limited body tissue, such as the vascular system, including the veins, arteries and lymphatics, and the cerebrospinal space.

In addition, these emulsions have been achieved with surprisingly low amounts of emulsifying agents. For example, with lecithin, which is an emulsifying agent of choice frequently used because of its known biocompatibility. Also, lecithin is used in fat emulsions for parenteral nutrition. Yet lecithin contributes to the increase in viscosity and is subject to attack by oxygen, the carriage of which is one of the major possible objects of fluorocarbon emulsions. It is believed that there is a

relationship between the amount of lecithin and the viscosity, and that the lecithin per given weight contributes disproportionately more than do comparable weights of fluorocarbons towards increasing viscosity in emulsions.

Fluorocarbon emulsions having fluorocarbon concentrations of 90%, 100% and 125% weights per volume have been obtained which have small particle size stability through heat sterilization and through storage for extended periods of time, on the order of months, at room or ambient temperatures using concentrations of lecithin in the emulsion of only 6%, 4.5% and 3.5% weights per volume where the mean particle sizes are in the range of approximately 100 nanometers (nm) to 300 nm in diameter. For emulsions having larger particle size means, even less lecithin is needed. For example, a 125% w/v of fluorocarbon in emulsion having a mean particle size of 600 nm has remained very stable through heat sterilization and through accelerated shelf life tests with only 3% w/v of lecithin. Such emulsion have a ratio of the fluorocarbon in emulsion to the emulsifying agent in emulsion of from 10:1, an emulsifying agent concentration which is approximately 10% of that of the fluorocarbon in emulsion, to 15:1, i.e., an emulsifying agent concentration which is approximately 6.7% of that of the fluorocarbon in emulsion, to as high as 41.7:1, i.e., an emulsifying agent concentration which is approximately 2.4% of that of the fluorocarbon in emulsion. These emulsions have been obtained by special mixing or homogenization procedures which do not require sonication and which can be formulated and manufactured more easily in large quantity.

Surprisingly, these emulsions are still very fluid, that is to say, they have a sufficiently low viscosity that is still compatible with vascular use, where the particle sizes are appropriate, and are otherwise suitable for other applications where relatively low viscosity is required.

The particles began to become larger, as shown by

larger mean particle size measurements, at lecithin concentrations of around 3.5% weight per volume or less, where fluorocarbon concentrations are around 100% weight per volume. Such larger particle sizes could be useful for use in certain applications in animal body parts where larger particle sizes, such as, for example, 600 nm mean diameter, could be tolerated or even preferred.

Fluorocarbon emulsions having relatively high concentrations, on the order of 80% w/v to 125% w/v and having a relatively higher concentration of the emulsifying agent, on the order of 7% w/v to 14% w/v have a higher viscosity than the emulsions mentioned hereinabove. Some of these higher emulsifying agent concentration emulsions have a viscosity, when stirred or mixed, sufficient for holding to the skin in topical applications where the emulsion is exposed to the air. If a high amount of oxygen is dissolved into such an emulsion, the emulsion would be a good emollient. In burns, such a malagma could suitably coat the burn area to protect the skin from dirt, drying and bacterial contamination, yet the malagma would permit diffusion of oxygen to the burned skin. Such a high fluorocarbon concentration emulsion could have mixed therein additional ingredients, such as antibiotics, nutrients, steroids, corticosteroids and other medicines which may be gainfully employed in the treatment of burns. It is an advantage of the present invention that such high fluorocarbon concentration emulsions favorably have a high oxygen concentration and diffusiveness so that by permeability, the oxygen is delivered to the burned topical areas, while providing a protective barrier against microorganisms and dehydration.

Moreover, as described in greater detail herein, these emulsions, if they employ a lecithin emulsifying agent that is fully saturated with hydrogen and they are kept in a sealed container, they will maintain in solution the oxygen in the emulsion at ambient temperatures for substantial periods of time, making such an emollient expedient and

highly useful for use by ordinary persons not necessarily trained in the medical arts.

It also has been discovered that some very highly concentrated fluorocarbon emulsions can be heavily oxygenated during and shortly after preparation of the emulsion, and remain heavily oxygenated during sterilization and through storage for extended periods of time when using an oxygen resistant surfactant as the emulsifying agent. Such a surfactant can be a lecithin which has been fully or substantially hydrogenated, that is to say where the double bonds have been saturated with hydrogen so as to make the lecithin resistant to oxygen attack. It has also been discovered that certain synthetic lecithins or lecithin analogs are resistant to oxidation, and in which the presence of sites sensitive to oxidation have been avoided. In a further possible emulsion, fluorinated surfactants which are resistant to oxidation can be made.

In particular, some of the highly concentrated fluorocarbon emulsions of the present invention, when prepared with the appropriate surfactant, have been found to maintain substantial stability of both the partial pressure of oxygen (pO_2) and the partial pressure of carbon dioxide (pCO_2) through heat sterilization and room temperature storage for extended periods of time. Such stability is useful when using the fluorocarbon emulsions of the present invention as a fluid in calibrating instruments used for measuring, for example, the pO_2 and pCO_2. It is sometimes desired when using such instruments that such a fluid be biocompatible, so that should any of the fluid used in or with such an instrument later interact or pass on to a patient, there will be no danger of toxicity or injury to the patient or instrument. Indeed, in one aspect of the present invention, the emulsion is contained in or contacted with the very catheter that is introduced into the patient for purposes of measuring the blood gas values on a constant basis. The emulsion can be

permitted to slowly enter the catheter and to contact the blood of the patient. Because of the low viscosity, the sterility, and the stable pO_2 and pCO_2 values of the emulsions described herein, excellent results are obtained.

Fluorocarbon emulsions can be oxygenated by way of several methods. One method found to be particularly useful is by placing the fluorocarbon emulsion into a pneumatically closed or closable container, and filling the space unoccupied by the emulsion with oxygen. This method takes advantage of the fact that the fluorocarbon and fluorocarbon emulsions by virtue of their low surface tension tend to form a film or layer on the inner surface of the wall of the container. Other oxygenation methods can also be used, such as the use of conventional blood oxygenators. Oxygenation should be carried out in such as manner as to insure sterility of the final emulsion.

The emulsions of the present invention may be made by a process that may be accomplished in several ways. Primarily, the preferred embodiment of the process envisages subjecting a mixture of the fluorocarbon in the vehicle, which contains the surfactant and other ingredients of the emulsion to an extremely high pressure and high flow rates in a mechanical emulsification apparatus. One method could include a cavitation procedure, which could accomplish the desired emulsion characteristics of small particle size with maximum or most efficient use of the emulsifying agent. Other methods providing sufficient turbulence or high shear conditions may also be employed.

Initially, it is contemplated that a vehicle be prepared by providing an aqueous continuous phase, optionally containing suitable buffering agents and osmotic agents in order to maintain the pH and the osmolality of the ultimate emulsion through sterilization and storage. Suitable osmotic agents include hexahydric alcohols such as, for examples, mannitol and sorbitol, certain sugars such as glucose, mannose and fructose, as well as glycerol,

sodium chloride, and osmotic agents such as hydroxyethyl starch ("HES," dextrans, gelatins and albumin. Suitable buffering agents include, for examples, imidazole, tris(hydroxymethyl)aminomethane, also known as Tham, sodium bicarbonate, monobasic potassium phosphate, dibasic potassium phosphate, monobasic sodium phosphate and dibasic sodium phosphate. Tham is also known as Trizma and is available from Sigma Chemical Company of St. Louis, Missouri. Tham and imidazole do not precipitate calcium, and thus may be a desired buffer where calcium-containing compounds are used in the emulsion or where the blood or emulsion might otherwise be exposed to calcium compounds. Imidazole may also be selected as a buffer in emulsions used to improve radiation treatments for, for example, a tumor because imidazole appears to sensitize the tumor to radiation and enhance the desired effects of the radiation to the tissue containing it. Imidazole may be used as a substitute buffer for phosphates, which appear to shield occupied tissue from the necrotic effects of radiation.

An emulsifying agent is included in the mixture. A common emulsifying agent is yolk lecithin, as it is known to be biocompatible. Lecithin, and generally those unsaturated phospholipids used as emulsifying agents, are normally subject to oxidation or attack by free oxygen as the oxygen seeks to bond with the double bonds within the lecithin molecule. The chemical changes may weaken the membranes of the emulsion particles or may form unacceptably high concentrations of fatty acids or lysolecithin or other oxidation or degradation products.

It has been found that these effects can be eliminated or reduced in the emulsions of higher fluorocarbon concentrations, that is on the order of 50% w/v or more, by having an oxygen-resistent, saturated lecithin or lecithin analog as the emulsifying agent. Such lecithins include 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and 1,2-dimyristoyl-sn-glycero-3-phosphocholine. Additional such saturated lecithins include a hydrogen-saturated soy

derived lecithin which, initially before hydrogenation, comprised 61.602% linoleic residues, 18.297% palmitic residues, 10.351% oleic residues, 5.311% linolenic residues, 4.117% stearic residues, 0.17% palmitoleaic residues and 0.153% myristic residues; and saturated hydrogenated yolk extract lecithins. Fluorocarbon emulsions having very good particle size stability, and having stable partial pressure of oxygen and partial pressure of carbon dioxide characteristics have been found without the need of an anti-oxidant and without the need of any other emulsifying agent.

It has been found advantageous to include in the emulsion a chelating agent to neutralize the effects of certain heavy metals. Certain metals, such as copper and iron, for example, catalyze oxidation and hydrolysis of lecithin. The addition of a sequestering agent, such as, for example, disodium calcium ethylenediaminetetraacetic acid (Na_2Ca EDTA), in very small quantities, can eliminate or reduce the oxidation effect of such heavy metal catalysts. Sequestering agent in the amount of as low as 0.005% w/v and as high as 0.04% w/v have been found to help in reducing the catalytic effects of the heavy metal on the oxidation of the lecithin, with the preferred amount being from approximately 0.01% w/v to 0.02% w/v.

Anti-oxidants, such as, for examples, tocopherol including alpha tocopherol acetate, mannitol or ascorbic acid optionally may be included in the mixture. Such anti-oxidants would not be necessary, or their use could be greatly reduced when using substantially fully hydrogen-saturated synthetic phospholipids as suggested herein. It is possible to hydrogenate yolk lecithin and soybean-derived lecithin, but such hydrogen-saturated lecithin tend to be less fluid than unhydrogenated lecithin.

The vehicle mixture containing the surfactant has the fluorocarbon mixed thereinto. Preferably, the fluorocarbon is mixed in an even, measured rate to obtain the most

efficient mixture. The fluorocarbon may be one of the fluorocarbons described hereinabove.

The resulting mixture is then forced at very high pressure into a flow path in an emulsification apparatus. The pressure should be sufficient to achieve high flow velocity to increase energy input to the mixture. In accordance with one aspect of the process invention, the flow is pumped at more than 10,000 pounds per square inch (psi) at a high flow rate through one or two flow paths which open into a cavity. Pressures of as low as 4,000 psi have been used with satisfactory results where the fluorocarbon concentration in the emulsion is lower, on the order of 10% to 25% w/v. In one suitable process, the flow paths are directed so that the flows of the mixture from each path impinge upon each other within the cavity. The mixture then flows to strike a surface, and is removed from the cavity. It is believed that cavitation occurs in the mixture when it is directed from the flow path into the cavity. Other equivalent methods for subjecting the mixture to the high shear, cavitation, or mechanical stress necessary to form a stable, heat sterilizable emulsion may also be used.

In accordance with another embodiment of this aspect of the process invention, a single flow path is provided. The fluorocarbon mixture is forced at 10,000 psi to 25,000 psi pressure through this flow path, which is defined by an axial vein through a cylindrical plug. The plug fits within the inside of a pipe. The fluorocarbon mixture exits the path into a cavity or chamber. At the pressures indicated, the mixture expands upon entering the cavity, and cavitation results.

Where the fluorocarbon concentration in the emulsion is lower, below 50% w/v for example, lower pressures may produce satisfactory emulsions. Pressures as low as 4,000 psi, will produce cavitation and provide some emulsification where the fluorocarbon is in the emulsion in the range of approximately 10% w/v to 25% w/v.

Some multiple runs or passes, three, four or more in accordance with the preferred embodiment of this invention, through such a procedure will increase the desired stability, decrease the particle size, and optimize the efficiency characteristics of the emulsion. Moreover, it has been found that the temperature of the emulsion rises during such procedures and methods as set forth herein. It is believed that the emulsion forms more reliably and results in an emulsion that is more stable in particle size, pH, osmolality, pO_2 and other characteristics when the temperature of the mixing and cavitation chambers or cavities are maintained cool, as with an ice or a water bath for examples. When hydrogenated lecithin or synthesized lecithins are used, the emulsion is manufactured, for example, in a cavity which is being cooled by a water bath maintained at from 15°C to 22°C.

The invention can be better understood by way of the following examples which are representative of the preferred embodiments thereof:

EXAMPLE I

A one-liter batch of emulsion made in accordance with the procedure described above, contained perfluorooctylbromide (PFOB) at 90% w/v, yolk-derived lecithin at 4.5% w/v, suitable non-calcium precipitating buffering agent, Tham at 0.05% w/v, suitable osmotic agent at 0.5% w/v, $CaCl_2$ 0.015% w/v and $MgSO_4$ at 0.003% w/v as buffering to control the pH, alpha-tocopherol acetate at 0.05% w/v and NaCl at 0.378% w/v, and a quantity sufficient of water (H_2O). In particular, the lecithin, Tham, osmotic agent, $CaCl_2$, $MgSO_4$, alpha-tocopherol acetate, NaCl and water were mixed together, forming the vehicle. The perfluorooctylbromide was mixed evenly into the vehicle. The result was forced at 14,000 psi pressure into two flow paths which were re-directed towards each other in a cavity, and the resultant was withdrawn. The passage through the two flow paths and into the cavity was repeated four times. This emulsion was oxygenated before use by placing

approximately 65 milliliters (ml) in a 500 millimeter plastic, flexible bag. Nitrogen was removed and the bag was expanded by injecting 100% oxygen to achieve a partial pressure of oxygen (PO_2) of 653 Torr in the emulsion. The bag was turned around so that the emulsion formed a relatively thin film on the inside surface of the wall of the bag, allowing the oxygen to dissolve into the fluorocarbon more readily. The oxygenated solution was storage stable, and the pO_2 and pCO_2 values remained constant over extended storage.

EXAMPLE II

A batch was prepared having 125% weight per volume of mono-brominated perfluorocarbon ($C_3F_{17}Br$), 0.03% weight per volume of Tham, a suitable buffer to maintain pH, o.4% weight per volume of mannitol, 0.2% weight per volume of NaCl, a quantity sufficient of water, with a soy lecithin as an emulsifying agent at 3.5% weight per volume. The soy lecithin was hydrogenated, that is to say, substantially all of the double bonds on the fatty acids were saturated with hydrogen.

The emulsion was equilibrated with 100% oxygen during formulation and bottled with 100% oxygen in the remaining space. The emulsion was then sterilized by autoclave at 121°C for eight (8) minutes. Fifteen (15) hours after sterilization and storage at room temperature, pCO_2 and pO_2 were measured as pCO_2 = 0.3 mm Hg, and pO_2 = 810 mm Hg, where the barometric pressure was 748 mm Hg.

The mean particle size was measured on the Nicomp submicron particle sizer manufactured by Pacific Scientific Co. of Anaheim, California. This analyzer determines relative quantities of various sized particles by a method of dynamic light scattering. Results are given digitally as shown, for example, in examples given in my co-pending application Serial No. 818,690 referenced hereinabove. Before sterilization, the mean particle size was measured at 311 nanometers (nm), with the particle size distribution showing a Gaussian curve. After sterilization by autoclave

at 121°C for eight minutes and 15 psi, the mean particle size measured 361 nm. After an additional autoclave heat sterilization performed for 60 minutes at 121°C, the mean particle size measured 358 nm.

To determine its biocompatibility, this emulsion was injected intravenously in six rats in a dose of 4 gm fluorocarbon per kg of body weight. Six additional rats were injected with a comparable amount, that is 4 ml/kg of normal saline for comparison purposes. One week after injection, the rats receiving the fluorocarbon emulsion had showed a mild, transit anemia, 0.95 hemoglobin concentration as compared with control rats injected with normal saline, but otherwise had blood characteristics comparable to those of the control rats. The fluidity or viscosity of the emulsion was, therefore, biocompatible for injection in the blood vessels in the body.

EXAMPLE III

A batch was prepared having 100% weight per volume of mono-brominated perfluorocarbon ($C_8F_{17}Br$), 0.03% weight per volume of Tham, a suitable buffer to maintain pH, 0.4% weight per volume of mannitol, 0.2% weight per volume of NaCl, a quantity sufficient of water, with a soy lecithin as an emulsifying agent at 6% weight per volume. The lecithin was not saturated with hydrogen, that is to say the carbon double bonds were not saturated with hydrogen. The emulsion was saturated with oxygen during formulation or manufacture. The oxygen attacks the non-hydrogenated double-bonds, oxidizing the lecithin. Measurements taken after twelve days revealed a decrease in the pO_2 from 359 mm Hg to approximately 4.5 mm Hg, and an increase in the pCO_2 from 1.8 mm Hg to approximately 8.5 mm Hg.

A comparison of the pO_2 and pCO_2 measurements after set time periods of the emulsion of Example II with that of Example III, shows that the hydrogenated lecithin allows for a stable oxygenation, while the emulsion with a lecithin that is not saturated with hydrogen suffers rapid oxidation in the presence of oxygen:

TABLE II

	15 hours		12 days		32 days	
	pO_2	pCO_2	pO_2	pCO_2	pO_2	pCO_2
Ex. III (H)	810	0.3			787	0.1
Ex. IV	359	1.8	4.5	8.5	0	12.2

EXAMPLE IV

A fluorocarbon emulsion was prepared having 102.5% perfluorodecalin, 4.5% weight per volume of lecithin, 0.05% w/v of an anti-oxidant, 1.2% weight per volume of mannitol to assist in osmolality and anti-oxidation, 0.036% w/v of Tham as a buffer, and water in quantity sufficient to form the emulsion. The emulsion was made by mixing at a substantially steady, even rate the fluorocarbon into the vehicle, which comprised the remaining ingredients. The resultant mixture was passed through a flow path at a high pressure, 17,000 psi, and divided into two flow paths which were directed to impinge upon each other in a cavity into which the flow paths are directed. The procedure was repeated through five passes. The emulsion had good fluidity and presented no viscosity problem.

Before sterilization, the emulsion's particle sizes were measured using the Nicomp particle sizer as described above in Example II, and the mean particle size was measured at 180 nm. Twenty-four (24) hours after heat sterilization in an autoclave at 121°C for eight minutes, the mean particle size was measured at 225 nm. The osmolality was 258. The pH varied only by 0.02 from before and after sterilization.

The emulsion was then subjected to a series of freeze and thaw cycles where the emulsion was first frozen rapidly to a temperature of approximately minus (-) 20°C. Then the frozen emulsion was thawed at ambient temperatures on the order of 17°C to 21°C. The cycle was then repeated after storage of the emulsion at ambient or room temperatures, on the order of 17 to 21°C for at least 180 minutes between

each cycle. The freeze-thaw cycle has been described, and is frequently used as a test that accelerates shelf life and other time related storage stresses for emulsions. See, for example, Advances in Clinical Nutrition at pages 228-229, I.D.A. Johnston, ed. (1982), published by MTP Press Limited of Boston, Massachusetts.

EXAMPLE V

An emulsion having substantially the same composition as set forth in Example IV above, except that the fluorocarbon is 100% w/v F-44E, was prepared using the procedure including the heat sterilization as set forth in Example IV. Mean particle size measurements were taken using the same analyzer as set forth in Example IV, and the results are set forth in Table III below. The pH decreased only by 0.10 from before sterilization until 24 hours after sterilization. The emulsion formed had good fluidity and presented no viscosity problem.

EXAMPLE VI

An emulsion having substantially the same composition as set forth in Example IV above, except that the fluorocarbon is 100% w/v F-2-butyltetrahydrofuran ("RM-101" or "FC-75") was prepared using the procedure including the heat sterilization as set forth in Example IV. Mean particle size measurements were taken using the same analyzer as set forth in Example IV, and the results are set forth in Table III below. The pH decreased only by 0.08 from before sterilization until 24 hours after sterilization. The emulsion formed had good fluidity and presented no viscosity problem.

EXAMPLE VII

An emulsion having substantially the same composition as set forth in Example IV above, except that the fluorocarbon is 100% w/v F-66E. Mean particle size measurements were taken using the same analyzer as set forth in Example IV, and the results are set forth in Table III below. The emulsion formed had good fluidity and presented no viscosity problem.

The mean particle sizes before sterilization, after sterilization and immediately after each of several freeze-thaw cycle tests for these emulsions are given in the following Table IV, where the numbers in the row entitled "PreSter" represent the mean particle size measured immediately prior to sterilization; the numbers in the row entitled "PostSter" represent the mean particle sizes after sterilization by autoclave at 121°C for eight (8) minutes, the numbers in the rows entitled "1st F-T," "2nd F-T" and "3rd F-T" represent the mean particle sizes measured after the respectively numbered freeze-thaw cycle ("F-T"):

TABLE III

	Decalin (Ex.IV)	F-44E (Ex.V)	Rm101 (Ex.VI)	F-66e (Ex.VII)
Prester	180	167	135	188
PostSter	225	225	209	189
1st F-T	299	276	266	198
2nd F-T	280	264	255	200
3rd F-T	301	287		194

EXAMPLE VIII

An emulsion having substantially the same composition as set forth in Example IV above, except that the fluorocarbon is 100% w/v $C_8F_{17}C_2H_5$ ("F-octylethyl-hydride"). Mean particle size measurements were taken using the same analyzer as set forth in Example IV. The pH decreased only by 0.12 from before sterilization until 24 hours after sterilization. The emulsion formed had good fluidity and presented no viscosity problem.

EXAMPLE IX

To ascertain particle size stability over extended periods of time in a high concentration fluorocarbon emulsion, a batch of 100% weight per volume of perflouro-octylbromide (PFOB) was prepared, using the method or procedure set forth in the application Serial No. 818,690. Specifically, an amount of yolk derived lecithin was mixed into an aqueous phase such that the amount of lecithin in

the ultimate emulsion was 6% weight per volume. Sodium phosphates were added as a buffer to maintain pH level, and sodium chlorides were added to maintain desired osmolality. An amount of alpha-tocopherol acetate was added, to limit oxygen degradation of the lecithin. Water was added in a quantity sufficient for the composition. An amount of perfluorooctylbromide was introduced into the mixture at a measured rate, and the mixture was forced through a flow path into an impingement chamber or cavity under 15,000 pounds per square inch of pressure. The flow path was of the type that divided the flow into two paths, and directed the flows at each other within impingement cavity. Four passes were made through the cavity.

The emulsion was then sterilized by autoclave at 121°C for 15 minutes. The sterilized emulsion was then stored at room temperatures which ranged during the trial from 15° to 30°C. The average particle size was measured using a Nicomp particle analyzer, as described in Example III above. The mean particle size was measured initially after sterilization as 239 nm, at one month as 262 nm, at four months as 252 nm and at ten months as 209 nm, thus indicating a very substantial particle size stability notwithstanding the high concentration of the emulsion and the low concentration of the surfactant. The fluidity, or lack of viscosity was suitable for use of the emulsion intravascularly in humans with no adverse toxicity.

EXAMPLE X

A 10-liter batch of 100% perfluorooctylbromide emulsion as described for Example IX above was kept at a different location. The emulsion was sterilized by autoclave at 121°C, but for eight minutes. The emulsion was then stored at ambient temperatures which were maintained substantially at from 15° to 30°C. Mean particle size measurements were taken at various times after sterilization, as follows: At sterilization, 265 nm; at one month, 270 nm; and, at eight months, 251 nm. Again, the mean particle size measurement appeared stable at room

temperature for substantial and extended periods of time. The emulsion was used intravascularly in humans satisfactorily using doses of 3 gm of fluorocarbon per kg of body weight.

EXAMPLE XI

Each of the emulsions prepared above is oxygenated and exposed to carbon dioxide prior to heat sterilization and is then stored in a glass bottle. After 6 months, the pO_2 levels are compared to those of the emulsions before storage. The levels of all of the emulsions except those of Example III remain substantially stable. These emulsions are then used as calibrants in blood gas measurement instruments. In one instance, the emulsion used as a calibrant is permitted to slowly trickle into a catheter inserted in the patient during the measurement, with no untoward results.

Although the present invention has been described in the context of certain examples and preferred embodiments, it will be understood that the scope of the present invention should be determined by reference to the claims that follow.

WHAT IS CLAIMED IS:

1. A method for calibrating an instrument used to determine the po_2 and pCO_2 in the blood, comprising;

introducing into said instrument a sample quantity of an emulsion comprising an aqueous phase, an oxygen-carrying fluorocarbon in an amount of 45% to 125% weight per volume having known stable values for pO_2 and pCO_2, and an effective amount of an emulsifying agent, said emulsion being biocompatible and maintaining said stable values through heat sterilization and storage for at least three months; and

adjusting the calibration of said instrument to correspond to said known values of po_2 and pCO_2 in said emulsion.

2. The method of Claim 1, wherein said emulsifying agent is a phospholipid having saturated bonds.

3. The method of Claim 1 or 2, wherein said fluorocarbon is perfluoroctylbromide.

4. The method of Claim 1 or 2, wherein said fluorocarbon comprises a mono-brominated fluorocarbon.

5. The method of Claim 2, wherein said emulsifying agent having substantially saturated bonds is saturated with hydrogen.

6. The method of Claim 2, wherein said emulsifying agent is phosphatidylcholine.

7. The method of Claim 2, wherein said emulsifying agent is a synthesized lecithin.

8. The method of Claim 2, wherein said emulsifying agent is 1,2-dipalmitoyl-sn-glycero-phosphocholine.

9. The method of Claim 2, wherein said emulsifying agent is 1,2-dimyristoyl-sn-glycero-phosphocholine.

10. The method of Claim 2, wherein said emulsifying agent is lecithin derived from soy beans and then hydrogenated.

11. The method of Claim 2, wherein said emulsifying agent is lecithin derived from egg yolk and then

hydrogenated.

12. The method of any of Claims 1-11, wherein said instrument is in fluid communication with the circulatory system of an animal during the calibrating procedure.

13. The method of any of Claims 1-12, wherein the emulsion has a viscosity biologically compatible for use intravascularly of an animal.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US89/02940

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): G01N 1/00; A61K 49/00

U.S.Cl.: 424/2; 424/9

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/2; 424/9

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,299,728 (CORMIER) 10 November 1981, see the entire document	1-13
Y	US, A 4,369,127 (CORMIER) 18 January 1983, see the entire document.	1-13

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 OCTOBER 1989	07 NOV 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	FREDERICK E. WADDELL