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[54] **MAGNETIC RESONANCE IMAGING WITH FLUOROCARBONS ENCAPSULATED IN A CROSS-LINKED POLYMERIC SHELL**

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[58] **Field of Search** 424/9; 436/173, 806; 128/653.4, 654; 514/12, 54, 57, 59

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[57] **ABSTRACT**

In accordance with the present invention, compositions comprising fluorine-containing magnetic resonance imaging agent(s) contained within polymeric shells are provided. Invention compositions are useful, for example, as contrast agents for magnetic resonance imaging (MRI). Fluorinated compounds in general are hydrophobic and as such have limited water solubility; thus the invention method permits preparation of such compounds in a biocompatible form suitable for ready delivery. The shell diameter is typically approximately 2 microns in diameter. Consequently, these materials have organ specificity due to rapid scavenging by the reticuloendothial system (RES) or the mononuclear phagocyte (MNP) system upon intravenous injection. Furthermore, fluorocarbon filled polymeric shells of the invention can be used to measure and monitor local oxygen and temperature.

16 Claims, No Drawings

MAGNETIC RESONANCE IMAGING WITH FLUOROCARBONS ENCAPSULATED IN A CROSS-LINKED POLYMERIC SHELL

The present invention relates to medical imaging, specifically to the use of contrast agents for magnetic resonance imaging (MRI). In a particular aspect, magnetic resonance agent(s) is(are) encased in a polymeric shell formulated from a biocompatible polymer.

BACKGROUND OF THE INVENTION

Contrast agents are desirable in radiological imaging because they enhance the visualization of organs (i.e., their location, size and conformation) and other cellular structures from the surrounding medium. The soft tissues, for example, have similar cell composition (i.e., they are primarily composed of water) even though they may have remarkably different biological functions (e.g., liver and pancreas).

The technique of magnetic resonance imaging (MRI) or nuclear magnetic resonance (NMR) imaging relies on the detection of certain atomic nuclei at an applied magnetic field strength using radio,frequency radiation. In some respects it is similar to X-ray computer tomography (CT), in that it can provide (in some cases) cross-sectional images of organs with potentially excellent soft tissue resolution. In its current use, the images constitute a distribution map of protons in organs and tissues. However, unlike X-ray computer tomography, MRI does not use ionizing radiation. MRI is, therefore, a safe non-invasive technique for medical imaging.

While the phenomenon of NMR was discovered in 1954, it is only recently that it has found use in medical diagnostics as a means of mapping internal structure. The technique was first developed by Lauterbur [Nature 242:190–191 (1973)].

It is well known that nuclei with the appropriate nuclear spin align in the direction of the applied magnetic field. The nuclear spin may be aligned in either of two ways: with or against the external magnetic field. Alignment with the field is more stable; while energy must be absorbed to align in the less stable state (i.e. against the applied field). In the case of protons, these nuclei precess or resonate at a frequency of 42.6 MHz in the presence of a 1 tesla (1 tesla=104 gauss) magnetic field. At this frequency, a radio-frequency (RF) pulse of radiation will excite the nuclei and change their spin orientation to be aligned against the applied magnetic field. After the RF pulse, the excited nuclei "relax" or return to equilibrium or alignment with the magnetic field. The decay of the relaxation signal can be described using two relaxation terms. T_1, the spin-lattice relaxation time or longitudinal relaxation time, is the time required by the nuclei to return to equilibrium along the direction of the externally applied magnetic field. The second, T_2, or spin-spin relaxation time, is associated with the dephasing of the initially coherent precession of individual proton spins. The relaxation times for various fluids, organs and tissues in different species of mammals is well documented.

One advantage of MRI is that different scanning planes and slice thicknesses can be selected without loss of resolution. This permits high quality transverse, coronal and sagittal images to be obtained directly. The absence of any mechanical moving parts in the MRI equipment promotes a high degree of reliability. It is generally believed that MRI has greater potential than

X-ray computer tomography (CT) for the selective examination of tissues. In CT, the X-ray attenuation coefficients alone determine the image contrast, whereas at least three separate variables (T_1, T_2, and nuclear spin density) contribute to the magnetic resonance image.

Due to subtle physio-chemical differences among organs and tissue, MRI may be capable of differentiating tissue types and in detecting diseases that may not be detected by X-ray or CT. In comparison, CT and X-ray are only sensitive to differences in electron densities in tissues and organs. The images obtainable by MRI techniques can also enable a physician to detect structures smaller than those detectable by CT, due to its better spatial resolution. Additionally, any imaging scan plane can be readily obtained using MRI techniques, including transverse, coronal and sagittal.

Currently, MRI is widely used to aid in the diagnosis of many medical disorders. Examples include joint injuries, bone marrow disorders, soft tissue tumors, mediastinal invasion, lymphadenopathy, cavernous hemangioma, hemochromatosis, cirrhosis, renal cell carcinoma, uterine leiomyoma, adenomyosis, endometriosis, breast carcinomas, stenosis, coronary artery disease, aortic dissection, lipomatous hypertrophy, atrial septum, constrictive pericarditis, and the like [see, for example, Edelman & Warach, Medical Progress 328:708–716 (1993); Edelman & Warach, New England J. of Medicine 328:785–791 (1993)].

Routinely employed magnetic resonance images are presently based on proton signals arising from the water molecules within cells. Consequently, it is often difficult to decipher the images and distinguish individual organs and cellular structures. There are two potential means to better differentiate proton signals. The first involves using a contrast agent that alters the T_1 or T_2 of the water molecules in one region compared to another. For example, gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) shortens the proton T_1 relaxation time of water molecules in near proximity thereto, thereby enhancing the obtained images.

Paramagnetic cations such as, for example, Gd, Mn, and Fe are excellent MRI contrast agents, as suggested above. Their ability to shorten the proton T_1 relaxation time of the surrounding water enables enhanced MRI images to be obtained which otherwise would be unreadable.

The second route to differentiate individual organs and cellular structures is to introduce another nucleus for imaging (i.e., an imaging agent). Using this second approach, imaging can only occur where the contrast agent has been delivered. An advantage of this method is the fact that imaging is achieved free from interference from the surrounding water. Suitable contrast agents must be bio-compatible (i.e. non-toxic, chemically stable, not reactive with tissues) and of limited lifetime before elimination from the body.

Although, hydrogen has typically been selected as the basis for MRI scanning (because of its abundance in the body), this can result in poorly imaged areas due to lack of contrast. Thus the use of other active MRI nuclei (such as fluorine) can, therefore, be advantageous. The use of certain perfluorocarbons in various diagnostic imaging technologies such as ultrasound, magnetic resonance, radiography and computer tomography has been described in an article by Mattery [see SPIE, 626, XIV/PACS IV, 18–23 (1986)]. The use of fluorine is

advantageous since fluorine is not naturally found within the body.

Prior art suggestions of fluorine-containing compounds useful for magnetic resonance imaging for medical diagnostic purposes are limited to a select group of fluorine-containing molecules that are water soluble or can form emulsions. Accordingly, prior art use of fluorocarbon emulsions of aqueous soluble fluorocarbons suffers from numerous drawbacks, for example, 1) the use of unstable emulsions, 2) the lack of organ specificity and targeting, 3) the potential for inducing allergic reactions due to the use of emulsifiers and surfactants (e.g., egg phophatides and egg yolk lecithin), 4) limited delivery capabilities, and 5) water soluble fluorocarbons are quickly diluted in blood after intravenous injection.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided compositions useful for obtaining fluorine magnetic resonance images of organs and tissues of the body. Invention compositions comprise organofluorine compounds entrapped in a biocompatible polymer shell. Also provided are methods for entrapping fluorinated compounds in a polymeric shell. Still further in accordance with the present invention, there are provided means for obtaining local oxygen and temperature data, and for obtaining fluorine magnetic resonance images of body organs and tissues.

For example, a suspension of polymeric shells of the invention can be administered intravenously, making imaging of vascularized organs (e.g., liver, spleen, and lung) and bone marrow possible. Organ target specificity is achieved as a result of uptake of the micron-sized organofluorine-containing polymeric shells by the reticuloendothelial system (RES) (also known as the mononuclear phagocyte (MNP) system). Organs such as the liver and spleen play an important role in removing foreign species (e.g., particulate matter) from the bloodstream, and hence are often referred to as the "blood filtering organs". These organs make up a major part of the RES. In addition, lymph nodes within the lymphatic circulation contain cells of the RES. Consequently, imaging of the lymphatic system is possible employing micron-sized organofluorine-containing polymeric shells of the present invention. Given orally or as a suppository, imaging of the stomach and gastrointestinal tract can be carried out. Such suspensions can also be injected into non-vascular space, such as the cerebro-spinal cavity, allowing imaging of such space as well.

As a further embodiment of the present invention, paramagnetic cations such as Gd, Mn, Fe, and the like can be bound to polyanions, such as alginate, and used as an effective MRI contrast agent.

The present invention overcomes the drawbacks of the prior art by providing 1) injectable suspensions of polymeric shells containing organofluorine-containing compounds, 2) organofluorine-containing compounds in a form having enhanced stability compared to simple emulsions, 3) organ targeting specificity (e.g., liver, spleen, lung etc.) due to uptake of the polymeric shells of the invention by the RES or MNP system, 4) emulsifier-free system, thereby avoiding agents that may potentially cause allergic reactions, and 5) the ability to inject relatively small doses and still acquire good images because the organofluorine compound-containing polymeric shells of the invention are concentrated in the targeted organ.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided compositions for obtaining in vivo fluorine magnetic resonance images, said composition comprising fluorine-containing magnetic resonance imaging agent(s) substantially completely contained within a polymeric shell.

In accordance with the present invention, it has been found that organofluorine-containing compounds, which in general are hydrophobic, water immiscible and consequently difficult to administer, can be entrapped in polymeric shells for ease of delivery. Organofluorine-containing compounds entrapped within polymeric shells are readily usable and biocompatible. The particle size of polymeric shells produced in accordance with the present invention have an average diameter of approximately 2 microns, which is ideal for medical applications, since intravenous or intraarterial injections can be accomplished without risk of small blood vessel blockage and subsequent tissue damage (e.g., caused by ischemia due to oxygen depravation). For comparison, red blood cells are approximately 8 microns in diameter (thus injectable biomaterial should be smaller than 8-10 microns in diameter to prevent blood vessel blockage).

Naturally occurring fluorine atoms (^{19}F) give a clear nuclear magnetic resonance signal and thus can function as contrast agents or "probes" in MRI. The specific advantages for the use of ^{19}F include: 1) an extremely low native concentration in the body (fluorine is not naturally found in the body), 2) a high nuclear magnetic resonance sensitivity, 3) a magnetogyric ratio close to that of ^{1}H, thus permitting ^{19}F magnetic resonance imaging to be carried out with only minor modifications of existing MRI devices, and 4) low toxicity of most organofluorine-containing compounds.

In general, fluorocarbons are non-toxic and biocompatible. Fluorocarbons are stable and unreactive, and consequently are not likely to be metabolized due to their strong carbon-fluorine bonds (approximately 130 kcal/mole). For comparison, carbon-hydrogen bonds (approximately 100 kcal/mole) are weaker and much more reactive. The FDA has approved two fluorocarbons, perfluorotripropyl amine and perfluorodecalin, for medicinal use as blood substitutes under the trade name of Fluosol DA.

A number of different fluorocarbons can be used in the practice of the present invention. For example, compounds satisfying the following generic formulae can be incorporated into polymeric shells employing the invention procedure as described herein:

(a) $C_xF_{2x+y-z}A_z$, wherein:
- x=1-30, preferably 5-15,
- y=2; or 0 or −2, when $x \geqq 2$; or −4 when $x \geqq 4$,
- z=any whole number from 0 up to (2x+y−1), and
- A is selected from H, halogens other than F, —CN, —OR, wherein R is H, alkyl, fluoroalkyl, alkenyl, fluoroalkenyl, alkynyl, fluoroalkynyl, aryl, fluoroaryl, alkanoyl, fluoroalkanoyl, alkenoyl, fluoroalkenoyl, alkynoyl, fluoroalkynoyl,

(b) $[C_xF_{2x+y'-z}A_z]_aJR_{b-a}$, wherein:
- x, z, A and R are as defined above,
- Y'=+1; or −1 or −3, when $x \geqq 2$; or −5 when $x \geqq 4$,
- J=O, S, N, P, Al, or Si,
- a=1, 2, 3, or 4, and

b=2 for a divalent J, or 3 for a trivalent J, 4 for a tetravalent J,

(c) A′—[$(CF_2)_x$—O]$_c$—A″, wherein:

x is as defined above,

A′ is selected from H, halogens, —CN, —OR, wherein R is H, alkyl, fluoroalkyl, alkenyl, fluoroalkenyl, alkynyl, fluoroalkynyl, aryl, fluoroaryl, alkanoyl, fluoroalkanoyl, alkenoyl, fluoroalkenoyl , alkynoyl , fluoroalkynoyl,

A″ is selected from H or R, wherein R is as defined above,

c=1–200, preferably 2–50, or

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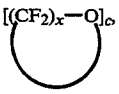


wherein:

x is as defined above, and

c′=2–20, preferably 2–8,

as well as mixtures of any two or more thereof.

Included within the above generic formulae are compounds having general formulae such as:

C_xF_{2x}, such as, for example perfluoro-1-hexene (C_6F_{12}), perfluoro-2-hexene (C_6F_{12}), perfluoro-3-hexene (C_6F_{12}), and the like,

cyclo-C_xF_{2x}, such as, for example, perfluorocyclohexane (C_6F_{12}), perfluorocyclooctane (C_8F_{16}), and the like,

C_xF_{2x-2}, such as, for example, perfluoro-1-hexyne (C_6F_{10}) , perfluoro-2-hexyne (C_6F_{10}), perfluoro-3-hexyne (C_6F_{10}), and the like,

bicyclo-C_xF_{2x-2}, such as, for example, perfluorodecalin ($C_{10}F_{18}$), and the like,

C_xF_{2x+2}, such as, for example, perfluorononane (C_9F_{20}) , perfluorodecane ($C_{10}F_{22}$), perfluorododecane ($C_{12}F_{26}$), and the like,

C_xF_{2x-4}, such as, for example, perfluoro-2,4-hexadiene, and the like,

$C_xF_{2x+1}A$, such as, for example, perfluorotripropyl amine [$(C_2F_7)_3N$], perfluorotributyl amine [$(C_4F_9)_3N$], perfluoro-tert-tributyl amine, and the like,

$C_xF_{2x-2}A_2$, such as, for example, $C_{10}F_{18}H_2$, and the like, and the like.

Besides linear, branched-chain and cyclic fluorine-containing compounds as noted above, fluorinated crown ethers (such as, for example, perfluoro 12-crown-4, perfluoro 15-crown-5, perfluoro 18-crown-6, and the like) are also contemplated for use in the practice of the present invention.

In order to obtain good magnetic resonance images with high signal to noise ratios, it is advantageous to have a high number of equivalent fluorines. As used herein, the term "equivalent fluorines" refers to those fluorine substituents of a fluorine-containing compound which exist in a substantially similar micro-environment (i.e., substantially similar magnetic environment). Equivalent fluorines will produce one imaging signal. A high number of equivalent fluorines will produce a strong signal, undiluted by competing signals of "non-equivalent" fluorines.

As used herein, the term "non-equivalent fluorines" refers to those fluorine substituents of a fluorine-containing compound which exist in a substantially dissimilar micro-environment (i.e., substantially dissimilar magnetic environment), relative to other fluorine substituents on the same fluorine-containing compound. Thus, in contrast to equivalent fluorines, non-equivalent fluorines will give multiple signals due to their different chemical shifts. Thus, while compounds with a large number of non-equivalent fluorines are satisfactory for MRI applications, such compounds are not ideal for maximum imaging.

Fluorine-containing compounds entrapped in polymeric shells according to the present invention can be used for a variety of purposes, e.g., to obtain magnetic resonance images of various organs and/or tissues, to obtain oxygen profiles in organs and/or tissues, and also to measure local temperature. Invention contrast agents are not limited to use in MRI applications, but can also be used for such applications as ultrasonography and radiology. The other isotope of fluorine, ^{18}F, can be used as a positron emission tomography (PET) contrast agent. Thus, with one fluorine-containing contrast agent, both PET and MRI diagnosis can be accomplished. Entrapment of other imaging agents, such as technetium and thallium compounds that are used in radiocontrast media, is also possible. Two examples of such contrast agents include Neurolyte and cardiolyte.

The use of invention compositions for oxygen detection is based upon the dramatic changes in NMR relaxation rate of ^{19}F in the presence of a paramagnetic species such as oxygen. Since oxygen is paramagnetic, it will interact with the fluorine nucleus, increasing the relaxation rate of ^{19}F from the excited state to the normal state. By monitoring this change in relaxation rate, it is possible to determine the oxygen concentration in a local area (by calibrating the MRI signal to a known concentration of oxygen).

The novelty of this system lies, for example, in 1) the use of MRI to obtain oxygen information, 2) the use of the oxygen paramagnetic influence on the ^{19}F MRI (NMR) signal, 3) the use of polymeric shells to provide a constant and protective environment that is also permeable to oxygen, and the like.

By using fluorine-containing compounds that are solids which undergo a phase transition over physiological temperature ranges (e.g., high molecular weight compounds, or combinations of fluorine-containing compounds), MRI can also be used to measure local temperature. Relaxation times are much longer in solids than in liquids, thus relaxation times will decrease dramatically as the transition temperature (i.e., from solid to liquid) is reached. Dramatic changes are observed in the NMR spectrum during phase transition of solid to liquid. The shape of the MRI signal for a given fluorine-containing compound can be calibrated to a known temperature. By using a high molecular weight fluorine-containing compound within the polymeric shell (i.e., a fluorine-containing compound having a melting point of $\geqq 15°$ C.), or by using a combination of fluorine-containing compound with non-fluorinated compound within the polymeric shell, the contents of the interior of the polymeric shell can be selected so as to provide a desired temperature range for phase transition to occur (typically in the range of about 22°–55° C.). The fluorocarbons within the shell will undergo a solid to liquid phase transition over the desired temperature range, altering substantially the observed relaxation rates, thus permitting in vivo temperature determination. Local temperature information would be especially useful, for example, in monitoring cancer patients

during the hyperthermia treatment of cancer or in the detection of cancer cells (cancer cells are cooler than normal cells).

The fluorine-containing composition employed will determine the temperature range of the phase transition. Thus, this technique can be used over a wide temperature range, simply by changing the makeup of the fluorine-containing composition. For example, pure perfluorododecane ($C_{12}F_{26}$) entrapped in a polymeric shell will undergo a solid to liquid phase transition at the melting point of the fluorocarbon (75° C.). However, this transition would be sharp and only a small amount of temperature information would be obtained. To obtain greater information, the melting point of the fluorine-containing composition can be spread over a wider range, for example, by simply adding another component to the pure fluorine-containing composition. It is well known in the art that a mixture will have a lower and broader melting point range than the corresponding pure components. Accordingly, for example, formulating perfluorododecane with a lower molecular weight fluorocarbon will broaden the melting point range of the encapsulated composition. Similarly, a mixture of a fluorine-containing compound (e.g., perfluorododecane) with an alkane (e.g., pentane), for example, will broaden the melting point range of the entrapped composition.

In addition, chemically modified long chain fatty acids (e.g., heptadecanoic acid [$C_{17}H_{34}O_2$], nonadecanoic acid [$C_{19}H_{38}O_2$], and the like), alcohols (e.g., nonadecanol [$C_{19}H_{40}O$], Docosanol [$C_{22}H_{46}O$], and the like) to which fluorines can chemically be added can also be used in the practice of the present invention. For example, a dehydration coupling reaction between perfluoro-tert-butanol (t-C_4F_9-OH; PCR CHEMICALS) with any of the above-described reactive oxygen-containing compounds will produce a molecule that undergoes a solid to liquid phase transition and one that has nine equivalent fluorines. Similarly, a mixture of a fluorinated fatty acid and cholesterol, for example, will broaden the melting point range compared to the pure fluorinated fatty acid, thereby allowing for local temperature measurements to be made.

The novelty of this temperature detection system lies, for example, 1) in the use of MRI to obtain spatially resolved temperature information, 2) in the use of the temperature dependence of the MRI (NMR) signal, 3) in the use of a fluorocarbon-containing composition that undergoes a solid to liquid phase transition in the desired temperature range, 4) in the use of the polymeric shell to provide a constant and protective environment for the medium, and 5) to obtain temperature information simultaneously with morphology information.

A number of biocompatible polymers may be employed in the practice of the present invention for the formation of the polymeric shell which surrounds the fluorine-containing composition. As used herein, the term "biocompatible" describes a substance that does not appreciably alter or affect in any adverse way, the biological system into which it is introduced. Essentially any polymer, natural or synthetic, bearing sulfhydryl groups or disulfide bonds within its structure may be utilized for the preparation of a disulfide crosslinked shell about particles of fluorine-containing composition, The sulfhydryl groups or disulfide linkages may be preexisting within the polymer structure or they may be introduced by suitable chemical modification. For example, natural polymers such as proteins, lipids, oligopeptides, polypeptides, polynucleic acids, polysaccharides (e.g., starch, cellulose, dextrans, alginates, chitosan, pectin, hyaluronic acid, and the like), and so on, are candidates for such modification.

As examples of suitable biocompatible polymers, naturally occurring or synthetic proteins may be employed, so long as such proteins have sufficient cysteine residues within their amino acid sequences so that crosslinking (through disulfide bond formation, for example, as a result of oxidation during ultrasonic irradiation) can occur. Examples of suitable proteins include albumin (which contains 35 cysteine residues), insulin (which contains 6 cysteines), hemoglobin (which contains 6 cysteine residues per $\alpha_2\beta_2$ unit), lysozyme (which contains 8 cysteine residues), immunoglobulins, α-2-macroglobulin, fibronectin, vitronectin, fibrinogen, and the like.

A presently preferred protein for use in the formation of a polymeric shell is albumin. Optionally, proteins such as α-2-macroglobulin, a known opsonin, could be used to enhance uptake of the shell encased particles of substantially water insoluble pharmacologically active agents by macrophage-like cells, or to enhance the uptake of the shell encased particles into the liver and spleen.

Similarly, synthetic polypeptides containing cysteine residues are also good candidates for formation of a shell about the substantially water insoluble pharmacologically active agents. In addition, polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrol idinone, and the like, are good candidates for chemical modification (to introduce sulfhydryl and/or disulfide linkages) and shell formation (by causing the crosslinking thereof).

In the preparation of invention compositions, one can optionally employ a dispersing agent to suspend or dissolve the fluorine-containing composition. Dispersing agents contemplated for use in the practice of the present invention include any nonaqueous liquid that is capable of suspending or dissolving the fluorine-containing composition, but does not chemically react with either the polymer employed to produce the shell, or the fluorine-containing composition itself. Examples include vegetable oils (e.g., soybean oil, mineral oil, corn oil, rapeseed oil, coconut oil, olive oil, safflower oil, cotton seed oil, and the like), aliphatic, cycloaliphatic, or aromatic hydrocarbons having 4–30 carbon atoms (e.g., n-dodecane, n-decane, n-hexane, cyclohexane, toluene, benzene, and the like), aliphatic or aromatic alcohols having 2–30 carbon atoms (e.g., octanol, and the like), aliphatic or aromatic esters having 2–30 carbon atoms (e.g., ethyl caprylate (octanoate), and the like), alkyl, aryl, or cyclic ethers having 2–30 carbon atoms (e.g., diethyl ether, tetrahydrofuran, and the like), alkyl or aryl halides having 1–30 carbon atoms (and optionally more than one halogen substituent, e.g., CH_3Cl, CH_2Cl_2, Ch_2Cl—CH_2Cl, and the like), ketones having 3–30 carbon atoms (e.g., acetone, methyl ethyl ketone, and the like), polyalkylene glycols (e.g., polyethylene glycol, and the like), or combinations of any two or more thereof.

Particles of fluorine-containing composition contained within a polymeric shell, prepared as described above, are delivered as a suspension in a biocompatible aqueous liquid. This liquid may be selected from water, saline, a solution containing appropriate buffers, a solution containing nutritional agents such as amino acids,

sugars, proteins, carbohydrates, vitamins or fat, and the like.

In accordance with another embodiment of the present invention, there is provided a method for the preparation of fluorine-containing compounds for in vivo delivery, said method comprising subjecting aqueous medium containing biocompatible polymer capable of being crosslinked by disulfide bonds and said fluorine-containing magnetic resonance imaging agent to high intensity ultrasound conditions for a time sufficient to promote crosslinking of said biocompatible polymer by disulfide bonds;

wherein said agent is substantially completely contained within a polymeric shell,

wherein the largest cross-sectional dimension of said shell is no greater than about 10 microns, and

wherein said polymeric shell containing agent therein is suspended in a biocompatible aqueous liquid for in vivo delivery.

Thus, in accordance with the present invention, organofluorine compounds contained within polymeric shells are synthesized using high intensity ultrasound. Two non-linear acoustic processes are involved in the formation of stable polymeric shells (i.e., acoustic emulsification and cavitation). First, acoustic emulsification disperses the organofluorine compounds into the aqueous protein solution. The dispersion formed is then chemically crosslinked and stabilized by the formation of disulfide bonds. The disulfide bonds are formed from the cysteine residues (in the case that the polymer is a protein such as albumin) that are oxidized by superoxide which is produced via acoustic cavitation.

As used herein, the term "in vivo delivery" refers to delivery of fluorine-containing compounds by such routes of administration as oral, intravenous, subcutaneous, intraperitoneal, intrathecal, intramuscular, inhalational, topical, transdermal, nasal, suppository (rectal), pessary (vaginal), and the like.

A nonobvious feature of the above-described process is in the choice of dispersing agent, specifically with respect to the polarity of the dispersing agent. The formation of a shell about the particles of fluorine-containing compounds involves unfolding and reorientation of the polymer at the interface between the aqueous and non-aqueous phases such that the hydrophilic regions within the polymer are exposed to the aqueous phase while the hydrophobic regions within the polymer are oriented towards the non-aqueous phase. In order to effect unfolding of the polymer, or change the conformation thereof, energy must be supplied to the polymer. The interfacial free energy (interfacial tension) between the two liquid phases (i.e., aqueous and non-aqueous) contributes to changes in polymer conformation at that interface. Thermal energy also contributes to the energy pool required for unfolding and/or change of polymer conformation.

Thermal energy input is a function of such variables as the acoustic power employed in the high intensity ultrasound process, the exposure time to high intensity ultrasound, the nature of the material being subjected to high intensity ultrasound, the volume of the material being subjected to high intensity ultrasound, and the like. The acoustic power of high intensity ultrasound processes can vary widely, typically falling in the range of about 1 up to 1000 watts/cm^2; with an acoustic power in the range of about 50 up to 200 watts/cm^2 being a presently preferred range. Similarly, exposure time to high intensity ultrasound can vary widely, typically falling in the range of a few seconds up to about 5 minutes. Preferably, exposure time to high intensity ultrasound will fall in the range of about 15 up to 60 seconds. Those of skill in the art recognize that the higher the acoustic power applied, the less exposure time to high intensity ultrasound is required, and vice versa.

The interfacial free energy is directly proportional to the polarity difference between the two liquids. Thus at a given operating temperature a minimum free energy at the interface between the two liquids is essential to form the desired polymer shell. Thus, if a homologous series of dispersing agents is taken with a gradual change in polarity, e.g., ethyl esters of alkanoic acids, then higher homologues are increasingly nonpolar, i.e., the interfacial tension between these dispersing agents and water increases as the number of carbon atoms in the ester increases. Thus it is found that, although ethyl acetate is water-immiscible (i.e., an ester of a 2 carbon acid), at room temperature (~20° C.), this dispersing agent alone will not give a significant yield of polymer shell-coated particles. In contrast, a higher ester such as ethyl octanoate (ester of an 8 carbon acid) gives polymer shell-coated particles in high yield. In fact, ethyl heptanoate (ester of a 7 carbon acid) gives a moderate yield while the lower esters (esters of 3, 4, 5, or 6 carbon acids) give poor yield. Thus, at a given temperature, one could set a condition of minimum aqueous-dispersing agent interfacial tension required for formation of high yields of polymer shell-coated particles.

Temperature is another variable that may be manipulated to affect the yield of polymer shell-coated particles. In general the surface tension of a liquid decreases with increasing temperature. The rate of change of surface tension with temperature is often different for different liquids. Thus, for example, the interfacial tension ($\Delta\gamma$) between two liquids may be $\Delta\gamma_1$ at temperature T_1 and $\Delta\gamma_2$ at temperature T_2. If $\Delta\gamma_1$ at T_1 is close to the minimum required to form polymeric shells of the present invention, and if $\Delta\gamma_2$ (at temp. T_2) is greater than $\Delta\gamma_1$, then a change of temperature from T_1 to T_2 will increase the yield of polymeric shells. This, in fact, is observed in the case of ethyl heptanoate, which gives a moderate yield at 20° C. but gives a high yield at 10° C.

Temperature also affects the vapor pressure of the liquids employed. The lower the temperature, the lower the total vapor pressure. The lower the total vapor pressure, the more efficient is the collapse of the cavitation bubble. A more efficient collapse of the ultrasonic irradiation bubble correlates with an increased rate of superoxide (HO_2^-) formation. Increased rate of superoxide formation leads to increased yields of polymeric shells at lower temperatures. As a countervailing consideration, however, the reaction rate for oxidation of sulfhydryl groups (i.e., to form disulfide linkages) by superoxide ions increases with increasing temperature. Thus for a given liquid subjected to ultrasonic irradiation conditions, there exists a fairly narrow range of optimum operating temperatures within which a high yield of polymeric shells is obtained.

Thus a combination of two effects, i.e., the change in surface tension with temperature (which directly affects unfolding and/or conformational changes of the polymer) and the change in reaction yield (the reaction being crosslinking of the polymer via formation of disulfide linkages) with temperature dictate the overall conversion or yield of polymer shell-coated particles.

The ultrasonic irradiation process described above may be manipulated to produce polymer shell-coated particles containing fluorine-containing compounds having a range of sizes. Presently preferred particle radii fall in the range of about 0.1 up to about 5 micron. A narrow size distribution in this range is very suitable for intravenous drug delivery. The polymer shell-coated particles are then suspended in an aqueous biocompatible liquid (as described above) prior to administration by suitable means.

Variations on the general theme of dissolved fluorine-containing compounds enclosed within a polymeric shell are possible. A suspension of fine particles of fluorine-containing compounds in a biocompatible dispersing agent could be used (in place of a biocompatible dispersing agent containing dissolved fluorine-containing compounds) to produce a polymeric shell containing dispersing agent-suspended particles of fluorine-containing compounds. In other words, the polymeric shell could contain a saturated solution of fluorine-containing compounds in dispersing agent. Another variation is a polymeric shell containing a solid core of fluorine-containing compounds produced by initially dissolving the fluorine-containing compounds in a volatile organic solvent (e.g. benzene), forming the polymeric shell and evaporating the volatile solvent under vacuum, e.g., in a rotary evaporator, or freeze-drying the entire suspension. This results in a structure having a solid core of fluorine-containing compounds surrounded by a polymer coat. This latter method is particularly advantageous for delivering high doses of fluorine-containing compounds in a relatively small volume.

Variations in the polymeric shell are also possible. For example, a small amount of PEG containing sulfhydryl groups could be included with the polymer. Upon ultrasonic irradiation, the PEG is crosslinked into the polymer and forms a component of the polymeric shell. PEG is known for its nonadhesive character and has been attached to proteins and enzymes to increase their circulation time in vivo [Abuchowski et al., J. Biol. Chem. Vol. 252:3578 (1977)]. PEG has also been attached to phospholipids forming the lipidic bilayer in liposomes to reduce their uptake and prolong lifetimes in vivo [Klibanov et al., FEBS Letters Vol. 268:235 (1990)]. Thus the incorporation of PEG into the walls of crosslinked protein shells alters their blood circulation time. This property can be exploited to maintain higher blood levels of the fluorine-containing compounds and prolonged release times thereof.

Of particular interest for application to vascular imaging are fluorocarbon-containing polymeric shells having prolonged circulation times. Currently used angiography techniques utilize X-ray contrast media and are invasive procedures. The potential of ^{1}H-MRI has been recently demonstrated for angiography applications [Edelman & Warach, New England J. of Medicine 328:785–791 (1993)]. Similarly, F-MRI is useful for angiography, with a number of advantages, such as the ability to achieve high contrast with reference to surrounding tissue (which does not contain any native fluorine). Examples of applications of such methodology include the diagnosis and identification of intracranial aneurysms, arteriovenous malformations, occlusions of the superior vena cava, inferior vena cava, portal vein, pelvic vein, renal vein, renal mesenteric artery, peripheral mesenteric artery, and the like.

One skilled in the art will recognize that several variations are possible within the scope and spirit of this invention. For example, the dispersing agent within the polymeric shell may be varied, other natural and synthetic polymers may be used in the formation of the walls of the polymeric shell, and the like.

According to the present invention, particles of fluorine-containing composition are contained within a shell having a cross-sectional diameter of no greater than about 10 microns (as used. herein, the term "micron" refers to a unit of measure of one one-thousandth of a millimeter). A cross-sectional diameter of less than 5 microns is more preferred, while a cross-sectional diameter of less than 1 micron is presently the most preferred for the intravenous route of administration.

Contrast agents of the present invention may be introduced into the body space in various ways depending on the imaging requirements. For example, aqueous liquid suspensions may be placed in the gastrointestinal tract by oral ingestion or suppository (e.g., to obtain images of the stomach and gastrointestinal tract), inserted by syringe into non-vascular spaces such as the cerebro-spinal cavity, or injected into the vascular system generally or into the vessels of a specific organ such as the coronary artery. In addition, contrast agents of the invention can also be injected into other body spaces such as the anterior and posterior eye spaces, the ear, the urinary bladder (e.g., by way of the urethra), the peritoneal cavities, ureter, urethra, renal pelvis, joint spaces of the bone, lymphatic vessels, the subarachnoid spaces, the ventricular cavities, and the like.

The polymeric shell containing solid or liquid cores of fluorine-containing composition allows for the directed delivery of high doses of the fluorine-containing composition agent in relatively small volumes. This minimizes patient discomfort at receiving large volumes of fluid.

The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLE 1

Synthesis of Dodecafluorononane (C_9F_{20}) Entrapped within a Polymeric Shell

A 20 ml glass reaction cell, titanium horn and collar were washed with alcohol and sterile saline prior to synthesis as was all equipment used. In a typical reaction, 3.5 ml of sterile 5% w/v USP (United States Pharmacopaeia) human serum albumin (Alpha Therapeutics Corporation) was added to a reaction cell and the cell attached to the ultrasonic horn (Heat Systems XL2020, 20 KHz, 400 W maximum power). The horn and cell were then submerged in a temperature control bath set to 22° C. Reactions run at 22° C. appeared to be optimum, however product can be synthesized over a wide range of temperatures (0 up to about 40° C.). Temperature control is critical to high yields of material, and the optimum temperature depends on the specific experimental configuration.

Six milliliters of dodecafluorononane (C_9F_{20}) was next added, and the ultrasonic source turned on at a power setting of 7. The amount of fluorocarbon added can be varied from less than one ml up to about 13 ml with good yield of protein polymeric shells. The reaction is complete in about 30 seconds. Yields at shorter and longer reaction times appear to be less. The homogeneous suspension produced contains the entrapped dodecafluorononane in protein polymeric shells and is

approximately 60% perfluorononane by volume. The aqueous suspension may then be stored in a sterile container at 4° C.

A typical reaction yields a solution that contains approximately 1×109 shells per mL with an average shell diameter of 2 microns with a standard deviation of 1 micron. This synthetic procedure is seen to yield high concentrations of micron-sized biomaterial with narrow size distributions.

EXAMPLE 2

Synthesis of Perfluorotributyl amine ($C_{12}F_{27}N$) or Perfluorotripropyl amine ($C_9F_{21}N$) Entrapped within Polymeric Shells

The 5% w/v USP human serum albumin (3.5 ml) and fluoroamine (6 ml) were added to a glass reaction cell and irradiated with high intensity ultrasound. The reaction conditions were a power setting of 7, a bath temperature of 22° C. and a reaction time of approximately 30 seconds. Once again high concentration of both perfluorotripropyl amine $[(C_3F_7)_3N]$ and perfluorotributyl amine $[(C_4F_9)_3N]$ entrapped in a protein polymeric shell are synthesized (1×10^9 shells/mL) with an average diameter of 2 microns.

EXAMPLE 3

Synthesis of Perfluorodecalin ($C_{10}F_{18}$) Entrapped within a Polymeric Shell

The 5% w/v USP human serum albumin (3.5 ml) and perfluorodecalin ($C_{10}F_{18}$; 6 ml) were added to a glass reaction cell and irradiated with high intensity ultrasound. The reaction conditions were a power setting of 7, a bath temperature of 22° C. and a reaction time of approximately 30 seconds.. High concentration with narrow size distributions of perfluorodecalin contained within a protein polymeric shell were synthesized. Furthermore, since perfluordecalin and perfluorotripropylamine are the major constituents of the FDA approved fluorocarbon, Fluosol DA, the medicinal use of these compounds in medical imaging should be readily accepted by regulatory authorities.

EXAMPLE 4

Synthesis of Perfluoro 15-crown-5 ($C_{10}F_{20}O_5$) Entrapped within a Polymeric Shell

The 5% w/v USP human serum albumin (3.5 ml) and the fluorocrown ether ($C_{10}F_{20}O_5$; 6 ml) were added to a glass reaction cell and irradiated with high intensity ultrasound. The reaction conditions were a power setting of 7, a bath temperature of 22° C. and a reaction time of approximately 30 seconds. As before, high concentrations of fluorocrown ether contained in a protein polymeric shell with narrow size distributions are synthesized. In fact this experimental procedure to synthesize fluorocarbon filled polymeric shells was typical for all of the fluorocarbons investigated.

EXAMPLE 5

Synthesis of Perfluoro-t-butylbutene ($C_{10}F_{18}H_2$) Entrapped within a Polymeric Shell

The 5% w/v USP human serum albumin (3.5 ml) and $C_{10}F_{18}H_2$ (6 ml) can be added to a glass reaction cell and irradiated with high intensity ultrasound. Reaction conditions comprising a power setting of 7, a bath temperature of 22° C. and a reaction time of approximately 30 seconds would typically be employed. By this procedure, protein polymeric shell having a high concentration of fluoro-t-butylbutane entrapped therein could be synthesized. EXAMPLE 6

Parameters Affecting Polymeric Shell Formation

Several variables such as protein concentration, temperature, ultrasonic irradiation time, concentration of pharmacologically active agent, and acoustic intensity were tested to optimize formation of polymeric shell. These parameters were determined for crosslinked bovine serum albumin shells containing toluene.

Polymeric shells made from solutions having protein concentrations of 1%, 2.5% 5% and 10% were counted with the particle counter to determine a change in the size and number of polymeric shells produced. The size of the polymeric shells was found not to vary with protein concentration, but the number of polymeric shells per ml of "milky suspension" formed increased with the increase in concentration of the protein up to 5%. No significant change in the number of polymeric shells was found to occur above that concentration.

Initial vessel temperatures were found to be important for optimal preparation of polymeric shells. Typically, initial vessel temperatures were maintained between 0° C. and 45° C. The aqueous-oil interfacial tension of the oils used for formation of the polymeric shell was an important parameter, which also varied as a function of temperature. The concentration of pharmacologically active agent was found not to significantly effect the yield of protein shells. It is relatively unimportant if the pharmacologically active agent is incorporated in the dissolved state, or suspended in the dispersing medium.

Ultrasonic irradiation time was an important factor determining the number of polymeric shells produced per ml. It was found that a ultrasonic irradiation time greater than three minutes produced a decrease in the overall count of polymeric shells, indicating possible destruction of polymeric shells due to excessive ultrasonic irradiation. Ultrasonic irradiation times less than three minutes were found to produce adequate numbers of polymeric shells.

According to the nomograph provided by the manufacturer of the sonicator, the acoustic power rating of the sonicator employed herein is approximately 150 watts/cm^2. Three power settings in order of increasing power were used, and it was found that the maximum number of polymeric shells were produced at the highest power setting.

EXAMPLE 7

Preparation of Crosslinked PEG-walled Polymeric Shells

As an alternative to the use of thiol (sulfhydryl) containing proteins in the formation of, or as an additive to polymeric shells of the invention, a thiol-containing PEG was prepared. By varying the concentration of PEG-SH (i.e., PEG modified to contain sulfhydryl and/or disulfide end groups) added to the 5% albumin solution, it was possible to obtain protein polymeric shells with varying stabilities in vivo. Fluorocarbon-containing protein shells having prolonged circulation times in vivo were found to have particular benefit for imaging the vascular system. These shells remained within the circulation for extended periods, relative to shells not containing PEG in the shell walls. This allowed, for example, visulation of cardiac circulation, and provided a non-invasive means of evaluating the

coronary circulation, instead of using conventional invasive techniques such as angiography. PEG-SH was prepared by techniques known in the art (such as the technique of Harris and Herati, as described in Polymer Preprints Vol. 32:154–155 (1991)).

PEG-SH of molecular weight 2000 g/mol was dissolved at a concentration of 1% (0.1 g added to 10 ml) in a 5% albumin solution. This protein/PEG solution was overlayered with oil as described in Example 2 and sonicated to produce oil-containing polymeric shells with walls comprising crosslinked protein and PEG.

Other synthetic water-soluble polymers that may be modified with thiol groups and utilized in lieu of PEG include, for example, polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrolidinone, polysaccharides (such as chitosan, alginates, hyaluronic acid, dextrans, starch, pectin, etc), and the like.

EXAMPLE 8

Toxicity of Polymeric Shells

Suspensions of soybean oil entrapped in protein (human serum albumin—HSA) polymeric shells were analyzed over time at three different temperatures to determine long-term stability. These suspensions were synthesized in a similar manner as described in Examples 1 through 4 except instead of a fluorocarbon, soybean oil was used. The solution was diluted to 20% v/v based on encapsulated soybean oil and were counted over a 27 day period using an Elzone particle counter.

Vesicle Concentration in Saline (#/ml × 10^{10})			
Day	4° C.	25° C.	38° C.
0	7.9	8.9	8.1
1	7.4	6.9	6.8
7	7.3	8.3	5.0
9	7.8	8.1	5.8
17	7.8	8.3	6.1
23	6.9	7.8	7.4
27	7.2	8.8	7.1

As seen from the above concentration data, soybean oil entrapped in polymeric shells remained fairly constant over the 27 day period at all temperatures.

EXAMPLE 9

Toxicity of Fluorocarbons Contained within Polymeric Shells

Five rats were injected through a catherized jugular vein with 5 ml of a 20% v/v fluorocarbon suspension (perfluorononane contained in an HSA protein polymeric shell) over 10 minutes. Fluorocarbons in general are nontoxic due the strong fluorine-carbon bonds; indeed, fluorocarbons have been successfully used as FDA approved artificial blood substitutes (Fluosol DA). The rats were harvested at specific times and autopsied. Besides observing the general health of the rat, the liver, spleen, lungs and kidneys were carefully examined. Rats examined at 0.5, 2, 8 and 24 hours were all healthy with no inflamed tissues or organs. The fifth rat is still alive and healthy after 90 days. For comparison, this dose of FDA approved soybean oil in a rat is the LD50 amount, further suggesting that fluorocarbons are nontoxic and safe.

EXAMPLE 10

In vivo Biodistribution as Determined by an Entrapped Fluorescent Probe

To determine the uptake and biodistribution of liquid entrapped within protein polymeric shells after intravenous injection, a fluorescent dye, rubrene (Aldrich), was entrapped within the HSA protein polymeric shell and used as a marker. Rubrene/toluene contained within polymeric shells were synthesized in a manner as described in Example 1. The resulting milky white suspension was diluted to a 20% v/v solution and 2 mL were injected into the tail vein of a rat over ten minutes. One animal was sacrificed one hour after injection and another at 24 hours. 100 micron frozen sections of lungs, liver, kidney, spleen and bone marrow were examined under the fluorescent microscope for the entrapped dye and released dye. At one hour the majority of the particles appeared to be intact and located in the lungs and liver. At 24 hours, the dye was located in the liver, lungs, spleen, and bone marrow. A general staining of the tissue was also observed indicating that the shell wall had been digested and dye liberated.

EXAMPLE 11

^{19}F Nuclear Magnetic Resonance Spectroscopy of a Neat Fluorocarbon and a Fluorocarbon Entrapped within a Polymeric Shell

NMR spectra of the fluorocarbons contained within a protein polymeric shell and neat fluorocarbons were obtained on a Bruker 500 MHz NMR instrument. The instrument was tuned for ^{19}F at its resonance frequency of 470.56 MHz. A deuterium solvent was used for locking and all spectra were externally referenced to Freon (CCl_3F) at 0 ppm. Perfluorononane and $CDCl_3$ were placed in a 5 mm NMR tube. The spectrum of pure perfluorononane was obtained with two sets of sharp peaks, one at −87 ppm, and the second set of peaks at −27, −128, and −133 ppm.

A suspension of perfluorononane entrapped within HSA protein polymeric shells was resuspended in D_2O and a similar NMR spectrum was obtained. Strong signals were obtained from the 20% v/v fluorocarbon suspension with peaks or resonances at −81, −121, −122 and −126 ppm. The entrapment of the fluorocarbon in the polymeric shell during ultrasonic irradiation resulted in no chemical or structural changes of the perfluorononane. For example, with C_9F_{20} two separate resonance were observed: one corresponding to the CF_3 at approximately −80 ppm and the second set of resonances at approximately −125 ppm, corresponding to the CF_2 group.

EXAMPLE 12

^{19}F Nuclear Magnetic Resonance Spectroscopy of Fluorocarbons to Measure Local Temperature

Variable temperature NMR spectra of fluorocarbons were obtained on a Bruker 500 MHz NMR instrument. The instrument was tuned for ^{19}F at its resonance frequency of 470.56 MHz. A deuterium solvent (d_6-dimethyl sulfoxide [d_6-DMSO]) was used for locking and all spectra were externally referenced to freon (CCl_3F) at 0 ppm. Perfluorododecane, which has a melting point of 77° C., and d_6-DMSO were placed in a 5 mm NMR tube at room temperature. Fluorine spectra were collected at different temperatures and the line-

widths were measured. Linewidth data at −81 ppm, as a function of temperature, are shown below:

Linewidth @ −81 ppm (Hz)	Temperature (°C.)
51.1	102
57.0	82
64.65	60

The broad spectrum at lower temperatures starts to sharpen as the temperature increases, resulting from the perfluorododecane undergoing its solid to liquid phase transition. The change is sharp and sudden with temperature, as expected for a pure material.

In order to broaden and lower the melting temperature, pentane was added (approximately 2% v/v) to the perfluorododecane. As was seen above, the broad spectra at lower temperatures sharpened as the perfluorododecane goes through its solid to liquid phase transition. Linewidth data as a function of temperature for the perfluorododecane/pentane mixture are shown below:

Linewidth (Hz)		
−82 ppm	−123.3 ppm	Temperature (°C.)
21.26	87.17	77
165.89	280.50	67
216.6	341.2	57
290.77	436.15	47
578.27	451.33	37
577.62	525.11	27

The resulting perfluorododecane/pentane mixture has a lower melting point that is broadened as expected. With this system, temperature measurements can be made in the range from 27° to 77° C. Thus, given a linewidth, it is possible to determine the local temperature.

An example of use of this technique to determine localized temperatures in vivo involves the injection of protein shells containing fluorocarbon mixtures (e.g., such as described above) with broad melting transitions having temperature-linewidth correlations (which can be empirically obtained). Such a formulation will localize within the liver or spleen and, in addition to serving as a ^{19}F MRI contrast agent, may simultaneously be utilized to determine locally variant temperatures within the organ (allowing the elucidation of the pathology of significant abnormalities within the tissues).

Example 13

^{19}F Magnetic Resonance Imaging of Phantoms

Two types of entrapped fluorocarbons contained in polymeric shells were used in this phantom study. Perfluorononane and perfluorotributyl amine contained within HSA protein polymeric shells were synthesized as described in Examples 1 and 2. The synthesized suspension that was 60% fluorocarbon per volume was diluted with saline and 2 milliliters placed in polystyrene tubes. The polystyrene tubes were than placed in a commercially available Siemens 2T MRI instrument (10 cm ^{19}F coil) operating at 1.5 tesla. ^{19}F magnetic resonance images of the tubes were taken over a 5 minute period with an echo time (TE) of 10 milliseconds and a time of repetition (TR) of 300 seconds (256×256 matrix).

Perflurononane Contained in Polymeric Shells		
Dilution	[conc], M	Image Clarity
1	1.8	excellent
1/2	0.9	excellent
1/4	0.45	good
1/10	0.18	good
1/50	0.09	good
1/100	0.02	marginal

Good MR phantom images were observed even at low concentrations of perfluorononane entrapped within polymeric shells. Very similar data was observed with polymeric shells that contained perfuorotributyl amine. Only at high dilution (1/100; 0.02M) was the image of poor quality and resolution.

EXAMPLE 14

^{19}F Magnetic Resonance Imaging of Liver and Spleen In Vitro

300 gram rats were injected with 2 ml of 20% v/v perfluorononane contained within an HSA protein polymeric shell suspension. At 2 hours and at 5 days, a rat was sacrificed and the liver, spleen, kidneys, and lungs were removed. The entire liver, for example, was then placed in a 4 tesla MRI instrument operating with a 10 cm ^{19}F coil. ^{19}F magnetic resonance images of the liver, spleen and kidney were obtained using a T_1 weighted sequence with a TR=1 second, a TE=20 milliseconds and a data matrix of 256×128 (i.e., 128 phase encoding steps, 16 signal averages).

^{19}F MRI images of the liver showed regions of varying intensity which correlated to varying degrees of liver uptake of the polymeric shells. For example, a dark region corresponding to the portal vein was observed where one would not expect the presence of the perfluorononane-containing polymeric shells since most of the shells are concentrated intracellularly within the RES of the liver.

The average image intensity of the liver scan at two hours after injection was approximately 20–30% higher than that of a scan recorded 5 days after injection, indicating partial dissipation of the perfluorononane, possibly through breakdown of the polymeric shells. Overall, excellent quality images showing liver morphology were obtained, demonstrating the potential of this technique in the diagnosis and localization of abnormal pathology within the liver.

EXAMPLE 15

In Vivo ^{19}F Magnetic Resonance Imaging of Liver and Spleen

A 150 gram rat was injected with 2 ml of a 20% v/v perfluorononane (C_9F_{20}) contained within HSA polymeric shells over 10 minutes. The entire rat was then placed in a 4 tesla MRI instrument operating with a 10 cm ^{19}F coil. The rat was anaesthetized with ketamine before collecting images. ^{19}F magnetic resonance images of the entire rat, as well as individual organs such as the liver, spleen and kidney, were obtained using a T_1 weighted sequence with a TR=1 second, a TE=20 milliseconds, and a data matrix of 256×128 (i.e., 128 phase encoding steps, 16 signal averages).

Rats were imaged 15 minutes, 2 hours, and 24 hours after injection of the perfluorononane-containing HSA protein polymeric shells. Overall, excellent quality images showing liver and spleen morphology were ob-

tained, demonstrating the potential of this technique in the diagnosis and localization of abnormal pathology within the liver RES containing organs.

EXAMPLE 16

Determination of Local Temperature using In Vivo ^{19}F Magnetic Resonance Imaging

A 300 gram rat is injected with 5 ml of a 20% v/v perfluorododecane/2% pentane (or perfluorononadecanoic acid and 1% cholesterol) contained within HSA polymeric shells over 10 minutes. The rat is then placed in a 15 cm coil (a Siemens 1.5 tesla MRI magnet). ATE of 10 milliseconds and TR of 300 seconds is used to collect the images (256×256 matrix). The rat is anaesthetised with ketamine before collecting data. The liver and spleen are imaged over a 15 minute period, by taking a 5 millimeter slice thickness. Data are collected at room temperature and at approximately 37° C., by wrapping the subdued rat in a heating pad.

EXAMPLE 17

In Vivo Oxygen Determination Using ^{19}F Magnetic Resonance Imaging

A 300 gram rat is injected with 5 ml of 20% v/v perfluorononane contained within HSA polymeric shells over 10 minutes. The rat is next placed in a 15 cm coil (a Siemens 1.5 tesla MRI magnet). ATE of 70 milliseconds and TR of 3 seconds is used to collect the images (256×256 matrix). The rat is placed in a restraining harness before collecting data. The rat is first put in an oxygen chamber to increase oxygen metabolism, and the linewidth and image are collected. The rat is next injected with ketamine, to reduce the consumption of oxygen, and again the linewidth and image are collected. The linewidth and the intensity of the image are observed to change, corresponding to the amount of dissolved oxygen in the rat. The largest linewidth is observed at higher oxygen concentrations. The liver and spleen are imaged over 15 minutes taking a 5 millimeter slice thickness. Two data sets are collected, one at room temperature and another at 37° C., by wrapping the anaesthetized rat in a heating pad.

EXAMPLE 18

Synthesis of Paramagnetic Cations Bound to Polyanions

Synthesis of Gd-alginates can be carried out, for example, by dispersing the alginate in a solution of $GdCl_3$. For example, small spherical particles of Gd-alginate suitable for intravascular injection may be synthesized by ultrasonic irradiation of a solution containing Gd ions (e.g., $GdCl_3$) and adding small quantities of Na-alginate solution. The alginate is dispersed into the solution of Gd ions by the ultrasonic irradiation, and crosslinked by the multivalent Gd ions, producing micron sized particles of Gd-alginate. Besides ultrasonic irradiation, low or high speed mixing can also be used.

Alternatively, a solution of Na-alginate is overlaid or layered on an immiscible organic solvent or oil (e.g., soybean oil, sunflower oil, toluene, methylene chloride, chloroform, and the like). The liquids are subjected to ultrasonic irradiation whereby the alginate-containing aqueous phase is dispersed into the organic phase, then a solution of multivalent ions (e.g., $GdCl_3$, $MnCl_3$, $FeCl_3$, etc.) is added. The Na-alginate is thereby crosslinked, producing tiny spherical particles of Gd-alginate which are suitable for use as an MRI contrast agent following intravascular injection. Essentially any synthetic technique using alginates and multivalent cations can be used to form spheres, fibers, plates, blocks and the like.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

That which is claimed is:

1. A composition for obtaining in vivo fluorine magnetic resonance images, said composition comprising fluorine-containing magnetic resonance imaging agent(s) substantially completely contained within a polymeric shell; wherein said polymeric shell comprises a biocompatible naturally occurring polymer which is substantially crosslinked by way of disulfide bonds,

wherein said biocompatible naturally occurring polymer, prior to crosslinking, has covalently attached thereto sulfhydryl groups or disulfide groups.

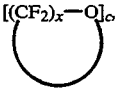
2. A composition according to claim **1** wherein said fluorine-containing magnetic resonance imaging agent is selected from:

(a) $C_xF_{2x+y-z}A_z$, wherein:
x=1-30,
y=2; or 0 or −2, when x≧2; or −4 when x≧4,
z=any whole number from 0 up to (2x+y−1), and
A is selected from H, halogens other than F, —CN, —OR, wherein R is H, alkyl, fluoroalkyl, alkenyl, fluoroalkenyl, alkynyl, fluoroalkynyl, aryl, fluoroaryl, alkanoyl, fluoroalkanoyl, alkenoyl, fluoroalkenoyl, alkynoyl, fluoroalkynoyl,

(b) $[C_xF_{2x+y'-z}A_z]_aJR_{b-a}$, wherein:
x, z, A and R are as defined above,
y'=+1; or −1 or −3, when x≧2; or −5 when x≧4,
J=O, S, N, P, Al or Si,
a=1, 2, 3, or 4, and
b=2 for a divalent J, or 3 for a trivalent J, or 4 for a tetravalent J,

(c) A'—$[(CF_2)_x$—O$]_c$—A'', wherein:
x is as defined above,
A' is selected from H, halogens, —CN, —OR, wherein R is H, alkyl, fluoroalkyl, alkenyl, fluoroalkenyl, alkynyl, fluoroalkynyl, aryl, fluoroaryl, alkanoyl, fluoroalkanoyl, alkenoyl, fluoroalkenoyl, alkynoyl, fluoroalkynoyl,
A'' is selected from H or R, wherein R is as defined above,
c=1-300, or

(d)



wherein:
x is as defined above, and
c'=2-20,

as well as mixtures of any two or more thereof.

3. A composition according to claim **1** wherein said composition is capable of undergoing a change in relaxation rate due to changes in the local oxygen concentration, and is therefore useful for the in vivo determination of local oxygen concentrations.

4. A composition according to claim 1 wherein said composition is capable of undergoing a solid to liquid phase transition in the temperature range of about 22 up to 55° C., and is therefore useful for the in vivo determination of local temperature.

5. A composition according to claim 1 wherein said biocompatible naturally occurring polymer is selected from proteins containing sulfhydryl groups and/or disulfide groups, polypeptides containing sulfhydryl groups and/or disulfide groups, polynucleic acids containing sulfhydryl groups and/or disulfide groups, or polysaccharides containing sulfhydryl groups and/or disulfide groups.

6. A composition according to claim 1 wherein the disulfide bonds on the crosslinked polymer are formed by ultrasonic irradiation.

7. A composition according to claim 1 wherein said biocompatible naturally occurring polymer is albumin.

8. A composition according to claim 1 wherein said polymeric shell containing agent therein is suspended in a biocompatible aqueous liquid.

9. A composition according to claim 8 wherein said biocompatible aqueous liquid is selected from water, buffered aqueous media, saline, buffered saline, solutions of amino acids, solutions of sugars, solutions of vitamins, solutions of carbohydrates, or combinations of any two or more thereof.

10. A composition according to claim 1 wherein said agent is contained within said shell neat.

11. A composition according to claim 1 wherein said agent within said shell is dissolved or suspended in a biocompatible dispersing agent.

12. A composition according to claim 11 wherein said biocompatible dispersing agent is selected from soybean oil, mineral oil, corn oil, rapeseed oil, coconut oil, olive oil, safflower oil, cotton seed oil, aliphatic, cycloaliphatic or aromatic hydrocarbons having 4–30 carbon atoms, aliphatic or aromatic alcohols having 2–30 carbon atoms, aliphatic or aromatic esters having 2–30 carbon atoms, alkyl, aryl, or cyclic ethers having 2–30 carbon atoms, alkyl or aryl halides having 1–30 carbon atoms, optionally having more than one halogen substituent, ketones having 3–30 carbon atoms, polyalkylene glycol, or combinations of any two or more thereof.

13. A composition for in vivo delivery of fluorine-containing magnetic resonance imaging agent,

wherein said agent is a solid or liquid, and is substantially completely contained within a polymeric shell,

wherein the largest cross-sectional dimension of said shell is no greater than about 10 microns,

wherein said polymeric shell comprises a biocompatible polymer which is substantially crosslinked by way of disulfide bonds, and

wherein said polymeric shell containing agent therein is suspended in a biocompatible aqueous liquid.

14. A method to obtain in vivo magnetic resonance images by administering to a subject a composition as described in claim 1.

15. A method for the delivery of fluorine-containing magnetic resonance imaging agents to a subject, said method comprising administering to said subject an effective amount of composition according to claim 1.

16. A composition for obtaining in vivo fluorine magnetic resonance images, said composition comprising fluorine-containing magnetic resonance imaging agent(s) substantially completely contained within a polymeric shell; wherein said polymeric shell comprises lipid(s) containing sulfhydryl groups and/or disulfide groups which is substantially crosslinked by way of disulfide bonds,

wherein said lipid, prior to crosslinking, has covalently attached thereto sulfhydryl groups or disulfide groups.

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