Basic mechanisms of gas transport and past research using perfluorocarbons

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Key words
Perfluorocarbons, gas solubility, oxygen, nitrogen, carbon dioxide, nitric oxide, solubility, review article

Abstract
Perfluorocarbon compounds have been utilized either in pure (neat) form or as emulsions suspended in aqueous fluids. These man-made chemicals possess a unique physical property allowing them to dissolve much more of the respired gases (oxygen, nitrogen and carbon dioxide) than any water-based system. Understanding the basic physical chemistry surrounding these emerging medical technologies will assure they are utilized to maximum benefit for mankind. It is clear they should not simply be viewed as ‘blood substitutes’ but rather as enhanced gas-transport pharmaceuticals.

Introduction
Carbon fluoride chemistry had its early beginnings in the 1930s−40s, during which time a unique feature of carbon halide bonds was discovered – a very high-energy ionic attachment. When a carbon chain or cyclic structure is completely substituted with halogens the ability of other carbon compounds to attack or change the parent structure is greatly limited. They become, to a very great extent, inert and unable to be changed. The carbon-fluoride bond is unique in having the highest energy of any organic bond, 120 kcal.mole-1. Solid perfluorocarbon (PFC) coatings are utilized to make non-stick pans. Liquid perfluorocarbon oils have other very useful properties.

During the Manhattan Project, it was discovered that such pure PFC oils were inert insulators. Uranium and plutonium could be stored safely in containers of PFC oils without fear of degradation and/or reaction. One would suppose unplanned reactions between uranium and plutonium could be a bad thing! A serendipitous observation occurred during such storage of radioactive material when it was noted that a tremendous amount of oxygen (O2) dissolved in the oil. That observation went uninvestigated until the late 1960s and early 1970s, when a group of physiologists suggested that perhaps such oils could be used for medical purposes. LC Clarke, the famous physiologist and inventor of electrodes for pressure and biochemical measurements, along with Geyer and Galon, began experimenting with such PFC liquids. They quickly found that tremendous amounts of dissolved O2 and other respiratory gases could be harbored in equilibrium in such PFC oils. The now classic demonstration of rodents spontaneously breathing oxygenated PFC created outcries both of animal cruelty and fascination. Goldfish could swim in the water above the PFC and, as long as the PFC was in contact with 100% O2, it seemed animals could live with liquid-PFC breathing for long periods, emerge and survive. This ‘scientific trick’ was picked up by Hollywood in the movie The Abyss. This was not just science fiction, as the producer had turned to advice from several excellent scientists such as Thomas Shaffer and Marla Wolfson, who were in liquid PFC-breathing research.5–8 The Abyss did, however, spawn conjecture that perhaps such human liquid-PFC breathing could either be used as a way to escape from a disabled submarine or work as a new technology for deep sea exploration. Today, science fiction may be yet closer than we had previously believed.

Physiology of PFC usage
This article, however, is intended to discuss the physiology and physical chemistry whereby PFCs, either as pure oils or in intravenous emulsions, can enhance and change mammalian gas exchange.9 The understanding of how these technologies work may well soon change medicine and mankind’s future; but it is only through a careful understanding of their capabilities and limitations that science, not science fiction, will move them to technological reality.

Respiratory gases are transported both by active chemical binding and by passive solubility in fluids.10 O2 and CO2 bind to haemoglobin as well as other metalloproteins throughout the body. The reactions are complex and widely reviewed elsewhere. What is important with regards to PFCs is the fact that, although erythrocytes carry vast amounts of O2 within them, it is dissolved O2 that ultimately fuels the energy production in target mitochondria. The red cells create a microenvironment of high-pressure O2 immediately outside of their cellular membrane. Within Angstroms from their surface, the dissolved O2 levels drop.11 A popular myth exists that somehow cells pull O2 from erythrocytes. It is rather that red cells, through biochemical changes in their cellular pH and 2,3 diphosphoglycerate (2,3-DPG), chloride ion, etc, release more O2 thereby increasing dissolved O2 in the local environment. The closer a red cell approaches the wall of a blood vessel the higher the local O2 concentration gradient for cellular uptake. Total
O₂-carrying capacity can be calculated from a standard equation (Table 1).\(^9\) That equation takes into account, but downplays, dissolved O₂ in plasma. Indeed, in most medical teaching the content of dissolved O₂ is disregarded, yet it is dissolved O₂ that the mitochondria actually utilize. Therefore, erythrocytes function as a bank of stored O₂ that continuously overpressurizes the aqueous plasma fluid such that the net flow of O₂ is to the mitochondria of metabolizing cells. As blood courses through tissues, it exchanges O₂ between venous and arterial blood, as well as driving it into tissues. The levels of various gases within tissues is noted in Table 2.\(^12\) The plasma not only is a conduit for gas movement but a resistor, as its capacity for gas solubility is quite limited (Figure 1).\(^13\)

Understanding that the plasma gap functions as a resistor brings to light how PFCs may well be utilized for the future.\(^13\) First, one has to understand the physics of gas solubility in PFCs. Henry’s Law states that “at a constant temperature, the amount of gas dissolved in a liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid.”\(^9\) Every fluid has an inherent solubility coefficient for every gas, dependent upon relative molecular polarity and molecular size. Water is a highly polar molecule, whereas lipids tend to be considerably less polar. Most fats, however, still have a large number of protons (hydrogen atoms) as side chains and, therefore, are relatively polar. Once a hydrocarbon molecule has all its available valences substituted with halogens (preferably fluoride), then the resultant carbon-based oil becomes highly non-polar. PFC gas solubility is noted in Table 3.\(^14\)

Pure PFC can carry large amounts of O₂ dissolved at 101.3 kPa.\(^14\) Even more soluble than O₂ is carbon dioxide (CO₂), and nitrogen (N₂) is somewhat less soluble in PFC than is O₂. However, N₂ is highly insoluble in water. Remember the O₂ solubility in plasma is 0.0031 ml 100ml⁻¹, whereas for a PFC emulsion (not pure PFC) the solubility of O₂ is 50-fold higher (Table 1). These facts can be utilized in making gas solubility and content equations (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>O₂ content equation with PFC present</th>
<th>O₂ content equation done a different way with a second-generation PFC – Perfluorobron</th>
<th>Gradient of O₂ from an erythrocyte (Hb) to the mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{aO2} = [1.36 × Hb_{conc} × Hb Sat] + (0.0031 × PaO₂))</td>
<td>(C_{aO2} = [1.36 × Hb_{conc} × Hb Sat] + (0.0031 × PaO₂) + (0.1432 × PaO₂ × \beta))</td>
<td>(V_{O₂} – DO₂ (P_{O₂} – P_{mitO₂}))</td>
</tr>
</tbody>
</table>

Where: \(\beta\): Fluorocrit (percentage of whole blood taken up by PFC particles – Oxycyte) \(Y\): relative saturation of Hb; \(O_{2\text{conc}}\): maximum O₂-carrying capacity of Hb (100% saturation; ml O₂ 100 ml⁻¹ blood) and equals 0.45 × %haematocrit; \(V_{RBC}\): fractional volume of the red blood cell; \(V_{\text{plasma}}\): fractional volume of the plasma; \(P\): total ambient pressure.

A 1 g.PFC per kg BW dose added to the blood produces a 30% increase in total O₂ in the blood (all present and available for metabolism, since it is dependent only on Henry’s law) \(V_{O₂}\): O₂ uptake; \(D_{O₂}\): O₂ diffusing capacity; \(P_{O₂}\): average capillary partial pressure O₂; \(P_{mitO₂}\): average mitochondrial partial pressure O₂.

### Table 2

<table>
<thead>
<tr>
<th>Sample site</th>
<th>(O₂)</th>
<th>(CO₂)</th>
<th>(N₂)</th>
<th>(H₂O)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspired air</td>
<td>158</td>
<td>0.3</td>
<td>596</td>
<td>5.7</td>
<td>760</td>
</tr>
<tr>
<td>Expired air</td>
<td>116</td>
<td>32</td>
<td>565</td>
<td>47</td>
<td>760</td>
</tr>
<tr>
<td>Alveolar air</td>
<td>100</td>
<td>40</td>
<td>573</td>
<td>47</td>
<td>760</td>
</tr>
<tr>
<td>Arterial air</td>
<td>100</td>
<td>40</td>
<td>573</td>
<td>47</td>
<td>760</td>
</tr>
<tr>
<td>Venous blood</td>
<td>40</td>
<td>46</td>
<td>573</td>
<td>47</td>
<td>706</td>
</tr>
<tr>
<td>Tissues</td>
<td>≤30</td>
<td>≥50</td>
<td>573</td>
<td>47</td>
<td>700</td>
</tr>
</tbody>
</table>

\(O₂\)-carrying capacity can be calculated from a standard equation (Table 1).\(^9\) That equation takes into account, but downplays, dissolved O₂ in plasma. Indeed, in most medical teaching the content of dissolved O₂ is disregarded, yet it is dissolved O₂ that the mitochondria actually utilize. Therefore, erythrocytes function as a bank of stored O₂ that continuously overpressurizes the aqueous plasma fluid such that the net flow of O₂ is to the mitochondria of metabolizing cells. As blood courses through tissues, it exchanges O₂ between venous and arterial blood, as well as driving it into tissues. The levels of various gases within tissues is noted in Table 2.\(^12\) The plasma not only is a conduit for gas movement but a resistor, as its capacity for gas solubility is quite limited (Figure 1).\(^13\)

### Figure 1

Representation of a tissue bore with a capillary running through it; the cylinders inside the capillary represent red blood cells separated by plasma gaps; various resistances to \(O₂\) movement are indicated, with corresponding \(P_{O₂}\) within the plasma, vessel wall and tissue\(^15\) (adapted with permission)
Pure PFC has been utilized to enhance O$_2$ delivery in the lungs. Small amounts have been nebulized, causing a coating of PFC in the alveoli and terminal bronchi. These experiments seemed to use the PFC as a surfactant, although the enhanced O$_2$ solubility may have enhanced gas delivery. Also, fully filling the tracheo-bronchial tree with pure PFC and creating liquid-PFC breathing has been accomplished in both animal and human studies. This works to enhance gas transport, and data from respiratory distress syndromes suggest that it may have a clinical application there in the future. Some studies have suggested that by using liquid-PFC breathing, possibly in conjunction with intravenous PFC, decompression sickness could be averted.

Most PFC utilizations in commercial development have focused upon intravenous emulsion technology. PFC, an oil, is immiscible with plasma, therefore, micro-particles of fat emulsions (micelles) have been created. At least one technology utilizes perfluorododecacontane, which has a boiling point close to body temperature. This is injected in liquid form and flashes instantly into micro-gas particles, leading to enhanced gas transport within the particles. However, the rest of this article will focus upon the biophysics and chemistry of the emulsions in preparation today. Modern-day emulsions are made from egg yolk phospholipids, quite similar to propofol and intralipid. The particles closely range around 0.2 microns in diameter, in comparison to an erythrocyte which is 5–8 microns. The micelles are considerably denser than other formed elements of the blood, and they will separate out in low-flow or standing fluids. However, with normal blood flow the PFC micelles are dispersed between red cells and also pushed to the walls of the blood vessels. The plasma gap essentially is replaced with micelles forming a gas-conduit bridge from red cells to endothelium and vice versa (Figures 1 and 2).

If one considers that the solubility of respiratory gases is up to 50 times higher than in plasma, then this bridge of microparticles allows for rapid gas transfer. Diffusion speed appears to be enhanced by PFC micelle presence, but in reality it is simply a function of enhanced solubility of gas within each micelle and the close proximity of each micelle that makes gas movement appear to speed up.

Because gas molecules in PFC micelles are not chemically bound but are held through enhanced solubility, every molecule of O$_2$ is available for metabolic utilization. In whole blood, haemoglobin has a complex interaction between each added O$_2$ molecule in the four haem moieties, as well as being modulated by pH, chloride ion, and 2,3–DPG. Therefore, under normal physiologic conditions, the maximum 21 volumes per cent of O$_2$-carrying capacity can only release from haemoglobin approximately 5–6 volumes per cent of O$_2$ (tissue demand). PFC micelles are

### Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility (mLO$_2$.100ml$^{-1}$ of compound)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorodihexyl ether</td>
<td>55.42</td>
</tr>
<tr>
<td>Perfluorodibutyl sulfur tetrafluoride</td>
<td>48.02</td>
</tr>
<tr>
<td>Perfluorotrisobutylamine</td>
<td>44.37</td>
</tr>
<tr>
<td>Perfluoro-(-N-ethylmorpholine)</td>
<td>50.10</td>
</tr>
<tr>
<td>Perfluoro-N,N-dipropylimethamine</td>
<td>52.60</td>
</tr>
<tr>
<td>Perfluorotriethylamine</td>
<td>53.86</td>
</tr>
<tr>
<td>Perfluoro-N-methylpiperidine</td>
<td>41.33</td>
</tr>
<tr>
<td>Perfluoro-N-methylmorpholine</td>
<td>37.57</td>
</tr>
<tr>
<td>Perfluoro-N,N-dimethyl-N-hexylamine</td>
<td>51.51</td>
</tr>
<tr>
<td>Perfluoro-N-butylmorpholine</td>
<td>50.59</td>
</tr>
<tr>
<td>Perfluoro-4-(N,N-dimethyl-2-aminoethyl)-morpholine</td>
<td>45.07</td>
</tr>
<tr>
<td>F-Tertbutylperfluorocyclohexane</td>
<td>43.00</td>
</tr>
</tbody>
</table>

### Figure 2

Human sickle red cells in an artificial capillary; A: normal plasma, B: PFC has been added; note the granular appearance of the PFC micelles. The dramatically increased solubility of O$_2$ in these micro particles overcomes the resistance of the plasma gap when PFC is added to whole blood (reproduced with permission)
rapidly in equilibrium with any microenvironment they inhabit. It is gas solubility according to Henry’s Law and the relative gas partial pressures in those tissue/blood or lung microenvironments that determine the content of gases dissolved within them at any one time. PFC can be effective as a third compartment of gas-carrying capacity within the blood stream. The amount of added potential gas-carrying capacity can be calculated (Table 1).

However, gas delivery from the red cells should be thought of as the most important physiologic contribution of PFC. Using normal and low haematocrit blood, adding PFC emulsions increases the mass transfer coefficient by 14% or more. Convective gas movement (forward propulsion of blood) and diffusive gas movement are considerably different. With normal whole blood, convective movement must be present, otherwise tissues become hypoxic very quickly and withdraw all available O2 from the microcirculation. In several recent studies, it has been shown that, with PFC present in the microcirculation at low or no-flow convective states, tissue O2 delivery remains present. This must be due to the massively enhanced diffusion effects.

O2 diffusion is important for normal metabolic function. The first generation PFC that garnered approval for treatment of myocardial ischaemia during balloon angioplasty was not easy to use commercially and was withdrawn from the market. Today, a third-generation compound, (Oxyxyte™, Oxygen Biotherapeutics Inc, USA) is being tested for a wide range of tissue ischaemia indications, including traumatic brain and spinal cord injury, organ preservation, carbon monoxide poisoning and cardiopulmonary resuscitation.

N2 is highly insoluble in whole blood and tissue. Rapid changes in ambient pressure can cause supersaturation leading to formation of a gas phase in blood and tissues. PFC has been shown to increase xenon (another highly insoluble gas) movement out of striated muscle by well over 100%. In multiple experiments using PFC infusions, air embolism leading to formation of a gas phase in blood and tissues. PFC may change the stress induced upon endothelial cells and withdraw all available O2 from the microcirculation. In several recent studies, it has been shown that, with PFC present in the microcirculation at low or no-flow convective states, tissue O2 delivery remains present. This must be due to the massively enhanced diffusion effects.

Conclusion

In the future, it is likely that an intravenous PFC will become approved as a treatment for tissue ischaemia. Today, a phase Ib double-blind, placebo-controlled, large (128 patients), dose-escalation trial of PFC for the treatment of civilian closed-head injury is underway. Animal studies in blast-induced traumatic brain injury are showing efficacy, and that programme is expanding quickly due to its military importance. The use of PFC infusions for sickle cell crisis and carbon monoxide poisoning is being researched as are other indications. In a second article, the use of PFCs for decompression illness will be reviewed. Suffice it to say that success in such a treatment is dependent upon understanding of the physico-chemical means by which PFC emulsions can carry both O2 and N2. The future investigation of enhanced delivery/removal of respiratory gases by PFC will almost certainly encompass a more basic understanding of the physiology of CO2 and NO fluxes when PFC is present. To look way into the future, the use of liquid-inhaled PFC may yet find a medical usage. It does offer the possibility of being used as a method to create or enhance suspended animation as well as for individual organ preservation. Work and discussions are on-going with space agencies to understand how this might be possible. PFC as a tool for medical application, temporarily changing the way that respiratory gases are transferred within the body, is very exciting.

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References

The potential role of perfluorocarbon emulsions in decompression illness
Bruce D Spiess

Key words
Perfluorocarbons, decompression sickness, decompression illness, air embolism, treatment, research, review article

Abstract
(Spiess BD. The potential role of perfluorocarbon emulsions in decompression illness. Diving and Hyperbaric Medicine. 2010;40:28-33.)

Decompression illness (DCI) is an occasional occurrence in sport, professional, and military diving as well as a potential catastrophe in high-altitude flight, space exploration, mining, and caisson bridge construction. DCI theoretically could be a success-limiting problem in escape from a disabled submarine. Perfluorocarbon emulsions (PFCs) have previously been investigated as ‘blood substitutes’ with one approved by the United States Food and Drug Administration for the treatment of myocardial ischaemia. PFCs possess enhanced (as compared to plasma) respiratory gas solubility characteristics, including oxygen, nitrogen and carbon dioxide. This review examines approximately 30 years of research regarding the utilization of PFCs in gas embolism as well as experimental DCI. To date, no humans have been treated with PFCs for DCI.

Introduction
Decompression illness (DCI) is an incompletely defined clinicopathological diagnosis in humans with a wide spectrum of presenting signs and symptoms.1,2 The disease is caused by gas bubble formation/movement in tissues and within the vascular tree or by gas forced into the circulation from pulmonary barotrauma. These gas bubbles cause either primary direct tissue destruction or secondary events from decreased blood flow (oxygen delivery), endothelial cell dysfunction, inflammation, coagulopathy/thrombosis and many other effects. The readership is familiar with many of the manifestations and difficulties with the diagnosis of DCI and is referred elsewhere for review.1-3

Mankind lives and works most often in a narrow range of ambient gas pressures. The gas column above us functions as a fluid, exerting continuous equal pressure to all parts of the body. Gases are soluble in tissues and blood, based upon Henry’s law. At 101.3 kPa (1 bar) the human body is saturated, with all respiratory gases in equilibrium with the partial pressures of each gas. Seventy per cent of the body is made up of water, therefore the relative solubility coefficients for respiratory gases in water versus fat (oils) determine the total amount of gas dissolved in aqueous media or tissues at any one time.4,5 It is through a sudden decrease in ambient pressure that tissues and blood potentially become supersaturated with gases. Supersaturation leads to bubble formation. The respiratory gases leave their dissolved state when some, as yet undefined, parameter allows for a small nidus of micro-bubble formation to occur.6 It has been suspected that micro-particles allow for the original formation of micro-bubbles.6 Once formed the micro-bubbles grow, potentially rapidly, as local, supersaturated gases move from tissue and blood into the gaseous phase of the micro-bubble.

Growth of a bubble is dependent upon gas composition, internal/external pressures and the surface tension of the bubble itself.6 Bubble dynamics is an entire study unto itself, but, suffice to say, particularly within the blood stream, bubbles are rapidly coated with proteins that themselves then have complex interactions with tissues, cells and the micro-environment.6-7 DCI is often thought to be a disease of diving, with inadequate times for gas equilibration between various faster and slower equilibrating tissue categories. However, any rapid reduction in ambient pressure may cause DCI and as man ventures into ever more unique