PERFLUOROCARBONS COMPOUNDS USED AS OXYGEN CARRIERS: FROM LIQUID VENTILATION TO BLOOD SUBSTITUTES

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Abstract
Perfluorocarbon compounds are fluorine substituted hydrocarbons. They exhibit unique properties due to the specificity of the carbon –fluorine linkage. Among these, the high gas solubility and the low surface tension are the most interesting characteristics for their use in clinic. There are several biological fields of potential applications of perfluorocarbons. Concerning the clinical applications for perfluorocarbons, they have been used as gas carriers and for liquid ventilation. Several clinical trials for commercial pharmaceutics of perfluorcarbon based on these compounds have been also already made.

Key-words
Blood substitutes, Clinical use, Perfluoroalkanes, Chemical properties

Resumo
Os compostos perfluorados são análogos aos hidrocarbonetos mas onde a ligação carbono-hidrogénio (C-H) é substituída pela ligação carbono-flúor (C-F). A ligação C-F confere propriedades aos compostos perfluorados que são diferentes das dos hidrocarbonetos que lhes são análogos. A capacidade de dissolução de elevadas quantidades de gases e a baixa tensão superficial apresentam são propriedades que têm levado ao desenvolvimento da investigação aplicada, baseada nestes compostos. Relativamente à parte clínica, alguns deles têm sido ensaiados com sucesso como "substituintes do sangue", no sentido em podem ser utilizados com veículos distribuição de gases na circulação, e como líquidos adjuvantes da ventilação pulmonar, em casos de existência de obstruções pulmonares graves.

Palavras-chave
Substituintes do sangue, uso clínico, compostos perfluorados, propriedades químicas

1. **INTRODUCTION**

Perfluorocarbons, PFC's, are linear, cyclic or polycyclic fluorine substituted hydrocarbons.

In the last 20 years fluorine chemistry and perfluorocarbon compounds have been an important field of research. The perfluorocarbons properties and applications have been investigated by researchers in several areas like biophysics, biochemistry, pharmacology, engineering and biotechnology and clinics. Clinical trials of some pharmaceuticals have already been made, some of which are already in Phase II/III trials. Due to their unique properties, pure PFC's or fluorocarbon in water emulsions have a promising wide utilization in biomedical field, from artificial liquid ventilation to oxygen carriers as blood substitutes, passing through to their uses in controlled release drug delivery (Lowe, K.C., 2006), (Krafft, M. P., 2001). The inertness of PFC's allied to the capacity of dissolving great amounts of gases (Riess, J.G., 2001)) are the main properties that make PFC's a selected group of compounds for utilization in gas delivery therapeutics.

The use of PFC's as ventilator liquids have some advantages in patients with chronic obstruction once the problem allied to surface tension air/liquid aspects are minimized, but the biggest future application could be in blood cell substitution in acute loss of blood.

Blood transfusion in developed countries is a very safe clinical procedure, but it is not a zero-risk intervention: allogenic donors can cause haemolytic reactions. Also, the need to minimise the risk of transmission of prions, the emergence of human immunodeficiency virus among others, are effective risks that are allied to this classic technique. In fact, artificial blood carriers, such as perfluoroalcanes, have been identified as a good answer for replacing acute blood loss during surgery or following trauma and for treatment of patients who refuse blood transfusions for clinical or religious reasons, (Fratantoni, J.C., 1998).

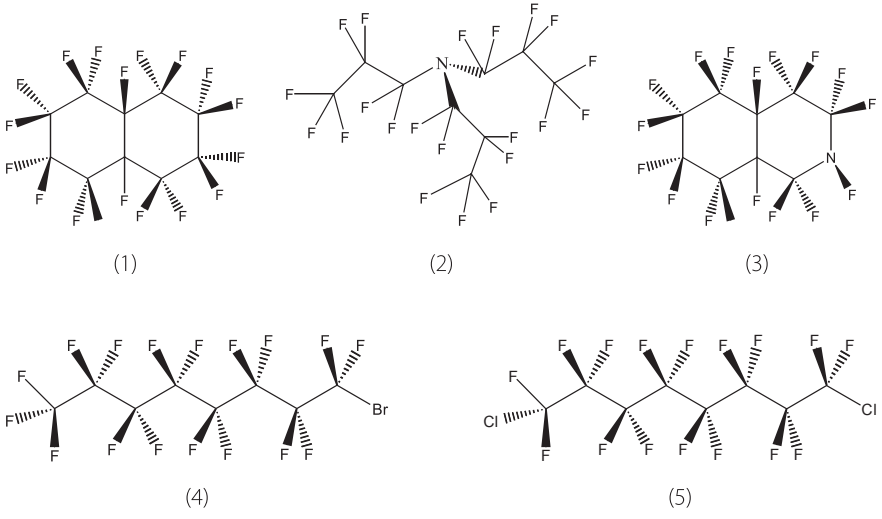

Figure 1 - Schematic representation of the most used perfluorcarbonds in clinical trials. (1) Perfluorodecalin ($C_{10}F_{18}$); (2) Triperfluoropropylamine, $N(C_3F_7)_3$; (3) C_9NF_{17}; (4) Bromoperfluoro-*n*-octane, (Perfluorobron), (5) $C_8F_{17}Br$; Dichloroperfluoro-*n*-octane, $C_8F_{16}Cl_2$.

In spite of the general inertness and the high vapour gases partial pressures, not all PFC's are of interest for biological and economical evaluation. The most interesting PFC's for biological uses are presented in Figure 1. The biological use is conditioned by the molecular weight of the compounds (from *ca* of 460 to 520 g.mol^{-1}). The most used in formulations are Perfluorodecalin and Perfluorobron (bromoperfluoro-*n*-octane) (Riess, J.G., 2001). The biocompatibility of these compounds is already assured. This means that they can be obtained with high purity level, have the ability to form stable emulsions, present rapid excretion rate and absence of side effects of the product or metabolic sub-products. Also, the cost of effective industrial production is low.

2. PHYSICAL AND CHEMICAL ASPECTS OF PFC'S

2.1. GENERAL ASPECTS

In perfluorocarbons compounds, the hydrogen atoms of a linear, cyclic or polycyclic hydrocarbon are replaced by fluorine atoms. The fluorine atom is more electronegative than the hydrogen atom and its volume size is significantly larger (Van der Waals radius is 1.20 Å for H and 1.47 Å for F). These differences allow the covalent bond between the element and carbon atom to have quite different physical chemical properties. PFC's are much more inert than their similar hydrocarbons. The thermal and chemical stability may arise from the C-F bond strength (~485 kJ mol^{-1}) as compared to the C-H one (~425 kJ.mol^{-1}). The stability of the C-F single bond maybe due to the effective overlapping of the sp^3 carbon atom orbital with the *p* fluorine atom orbital. The biological and biochemical inertness results from the repellent effect that the electronic cloud of fluorine exerts against reagent approaches.
The volume size of perfluoroalkanes is approximately one and a half times bigger when compared with homologous alkanes. The average volumes of the $–CF_2$ and $–CF_3$ groups are 38 Å^3 and 92 Å^3 respectively, while for $–CH_2$ and $–CH_3$ the same values are 27 Å^3 and 54 Å^3, respectively. The perfluoride chains assume a great stiffness, probably due to the loss of the cis/trans freedom of rotation which alkenes have. These allow the perfluorinated chains to adopt a helical conformation in order to minimize steric effects (H. Hoffmann, H & all, 1983); (Eaton, D. F. & Smart, B. E., 1990); (Gruen, D.W.R., 1985). The differences in conformation between alkenes and perfluoroalkenes are exemplified in Figure 2 with the seven carbon atoms compounds.

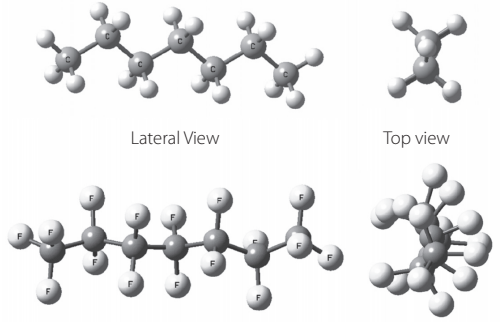

Figure 2 - Differences in conformation between hydrocarbons and perfluorocarbons. These differences are here exemplified with the 7 carbon atom molecules. Top: *n*-heptane. Button: perfluoro-*n*-heptane. The molecules were geometrically optimized using G03W at B3LYP/6311++G(d,p) level of theory (Freire, M.G. 2006).

The fluorine atom has a low polarizability so the Van der Waals interactions between their chains are weak leading to low cohesive energies in liquids. This characteristic can explain the observed properties of PFC's, like low surface tension values, high spread values, high fluidity, high vapour pressures and high gas solubility.
PFC's are efficient dissolvers for gases such CO_2, O_2 and N_2. The solubility of oxygen in PFC liquids at 37 ºC and 1 atm used in biomedical applications is of about 40 to 50% (v/v) while for CO_2 this percentage rises to about 200% (v/v). Table 1 gives the solubility values for the most used compounds in biological systems (Riess, J. G., 2001).

Table 1 - Solubility of oxygen and carbon dioxide and organ half-life values for some of perfluorocarbons used in clinic.

Molecular Formula	Solubility O_2 (% v/v at 37 ºC)	Solubility CO_2 (% v/v at 37 ºC)	Clinical Uses	Organ half-life (days)
Perfluorodecalin $C_{10}F_{18}$	42	142	Liquid ventilation Organ preservation Lung lavage Cell cultures	7
$N(C_3F_7)_3$	45	146	Organ preservation Cell cultures	65
C_9NF_{17}	42		Organ preservation Cell cultures	11
Bromoperfluoro-*n*-octane (Perfluorobron) $C_8F_{17}Br$	50	210	Oxygen carrier Cardiovascular bypass	4
Dichloroperfluoro-*n*-octane C8F16Cl2	43		Oxygen carrier Cardiovascular bypass	7

Many studies have been made in order to elucidate the site occupation of O_2 and CO_2 in perfluorocarbons and the effects of substitution of the fluoride atoms by other halogens. The substitution of a fluoride atom by a bromide or chloride causes a slight decrease in the solubility of dioxygen and a significantly increase of the solubility of carbon dioxide (Costa Gomes M. F & al., 2004), Deschamps, J. & al., 2007).

The gas content in the liquid is proportional to the gas phase pressure given by Henry's law. Relative to oxygen, that linear relation contrasts with the sigmoid relationship between oxygen content and oxygen partial pressure for haemoglobin. The dioxygen concentration in PFC pharmaceuticals can be increased by increasing the dioxygen concentration in the inspired air of the patient. Due to the weak interaction between the solvent and solute in PFC, the release of O_2 to the tissue is greatly facilitated, giving extraction rates of typically 90% that are higher than the allow by the dissociation O_2-Fe process that occurs in Haemoglobin (typically 25-30%) (Keipert, P.E., 1996).

2.2. **EMULSION PREPARATIONS**

PFC's are immiscible in water, so a preparation for injection must be oil in water emulsion or a reverse one, when the preparations are to be used for drug delivery. Concerning emulsion preparations companies had to solve two main problems: the monitoring of the target drop diameter and the emulsifier agent.

Droplet emulsion size should lie within the 0.1-0.2 μm range. The main problem arising from the control of the drop size is the "Ostwald ripening". This is due to the fact that individual fluorocarbon molecules leave the smaller droplets to join the larger ones, thus increasing the volume of the particles. The interfacial tension conditions the largest upper pressure which the smaller drops have compared to the larger ones, driving the force to the ripening. The speed of the increment of the drop size is then dependent on the diffusibility and solubility of PFC in water or vice-versa. The solubility of water in PFC seems to increase with the carbon number of the solvents. This could be because larger molecules can more easily accommodate a molecule of solute, increasing its solubility. It has also been shown that the solubility of water in the α-(ω-) substituted fluorocarbons is increased by the inclusion of Br atoms (Freire, M.G. & all, 1996).

Several substances have been tried as emulsifier agents. The most effective surfactant system, without clinical side effects, is the equimolar amounts of egg yolk phospholipids and mixed fluorocarbon/hydrocarbon amphiphile.

3. **CLINICAL APPLICATIONS AS GAS CARRIERS**

These compounds have been studied as biological gas carriers since the sixties. First, they have been studied by their possible uses in liquid ventilation. Liquid ventilation eliminates the gas-liquid interface reducing the alveolar surface tension. This improves the lung compliance and lung oxygenation. This characteristic is particularly important when there is insufficiency of surfactant.

Biomedical research has been made in liquid ventilation of premature babies with respiratory disease (Greenspan , J. S. & all , 2000). This has been tried in the treatment of the respiratory distress syndrome: for this proposes, the compound is installed in the lungs of the patient during the treatment. For temporary blood substitution the fluorocarbon must be an injectable emulsion.

Concerning the oxygen distribution in blood the effective biomedical application had to wait for the advance in biotechnology of formulations. Droplet emulsion size lies within the 0.1-0.2 μm range. This is 30-70 times smaller than blood cells. Emulsion drops can thus be much more numerous than red blood cells and can be present in the plasma gaps between erythrocytes in the microcirculation structures. This can be very valuable for O_2 supply to the cells during acute anaemia or hemodilution. The high pO_2 provides a strong driving force for dioxygen diffusion to the tissue. The same phenomenon happens with CO_2 but in the reverse way.

There are several commercial pharmaceuticals of PFC-based oxygen carriers that have been in clinical trials.

Fluosol® (Green Cross and Alpha Therapeutic) was an emulsion of two main components (perfluorodecalin and perfluorotripropylamine, approved in 1990 in the USA and some European countries. This association was used for the oxygenation of heart tissue during angioplasty and to preserve human organs prior to transplantation. The production ceased in 1994 since the maximum oxygen carrier capacity was significantly lower than that of Hb (K. C. Lowe, 2005).

Perftoran® (Russian Academy of Sciences and Perftoran company) was a two component PFC with Perfluorodecaline and Perfluoromethylcyclopiperidine. It was introduced in 1996 in Russia as an alternative anti-ischaemic agent and for the preservation of perfuse organs for transplantation. It has been used in several patients in Russia and Ukraine (Maevsky, K. E. & all, 2005).

Oxygent ™ (Alliance Pharmaceutical USA). Oxygent™ has two perfluorcarbons in the formulation: perfluorodecyl bromide and perflubron. The emulsion was stabilised with egg phospholipids (Keipert, P.E., 2005). This formulation had a higher stability than the previous ones (more than one year at 5-10 ºC and the total amount of oxygen carrier capacity was significantly higher than the previous ones, since the PFC content of the formulations was of about 60% (v/v) compared with 20% (v/v) in the others). It has been tried as oxygenation fluid in cases of high blood losses surgeries such orthopaedic ones. It was shown that the use of Oxygent™ reduces the frequency and the volume of blood use during surgery (Frumento, J. R., 2002). For some time the company suspended the phase III trial in order to evaluate the effect of the use of the pharmaceutical in the stroke that was noted in the patients that could be due to the pre-surgical acute normovolaemic heamodilution protocol (Niller, E., 2002). Anyhow, in 2005 the product was licensed again in China, Europe and Canada.

Other PFC's pharmaceuticals
Nowadays there are some new products in phase I or II of trials like for instances, Ocycyte™ for prevention of tissue hypoxia during orthopaedic and cardiac surgery.

4. **METABOLISM AND TOXICITY**

The molecules are sequestered by phagocyte cells of the monocyte /macrophage system. They diffuse back to the blood where they are carried in plasma lipids to the lungs and then exhaled as vapour (Riess, J. G., 2001, 2005).

The LD50 for Furosol® is of ~25g/kg of body (Okamoto, H., 1973) and for Oxygent ™ is about 54 g/ kg body (Flaim, S. F., 1994). Side effects that had been reported in some volunteers consisted of early effects like headache and, occasional lower backache. Delayed effects (2-12 h) consisted of fever, chills and nausea. These effects were fully reversible within 12-24 h. It has been shown that these effects were related to the normal circulations clearance process for emulsions. The phagocytation of the emulsion droplets can activate the macrophages and release products of the arachidonic acid cascade (Flaim, S. F., 1994).

5. **FINAL REMARKS**

The research concerning the perfluoroalkenes and their applications is still in a growing phase. Concerning biomedicine and bioengineering we can find that there are several working groups all over Europe, North and South America, India and Japan that are investing their resources in this field. We expect new incomings and applications in the clinical and medical area.

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