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Physical Characterization of Blood Substitutes by Carbon-Fluorine Spectroscopy

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Abstract

Blood substitutes, *aka* artificial oxygen carriers, such as perfluorocarbon emulsions, aim at improving oxygen transport and oxygen unloading to the tissue. Thereby, artificial oxygen carriers can replace allogeneic blood transfusions and improve tissue oxygenation, subsequently contributing to the function of organs with marginal oxygen supply. Carbon-Fluorine Spectroscopy (CFS™) *aka* Spectro-Fluor™ patented by Fluorotronics, Inc., is a green, disruptive, non-destructive, non-invasive and progressive analytical technology that was shown reliable and promising for various (nano-) pharmaceutical and bio-medical applications. The key feature of CFS™ is based on the capability to specifically, sensitively and rapidly detect carbon-fluorine bond(s) in the fingerprint spectral area of 550-850 cm^{-1} allowing F-imaging as well as qualitative and quantitative characterization of fluoro-organics *in vitro*, *ex-vivo* or *in-vivo*.

In this study, we show perfluorocarbons (PFCs), such as perfluorobron (PFB) and perfluorodecalin (PFD) can be easily, reliably and rapidly detected by CFS™ in various containers, especially under visible excitation (510.6 nm), opening a possible avenue for enhancing blood substitutes product security (anti-counterfeiting) or performing advanced metabolic and toxicological studies of these compounds *in vivo* (e.g. pharmacokinetics, bioavailability). Indeed, the range of specific signal wavelength related to the C-F bond in PFCs was besides confirmed by density functional theory (DFT) calculations.

Keywords: Carbon-Fluorine spectroscopy; Blood substitutes; Perfluorocarbons; Perfluorobron; Perfluorodecalin; Density functional theory calculations

Introduction

Perfluorochemicals can dissolve significant quantities of many gases including oxygen (O_2) and carbon dioxide (CO_2). Optimal use of artificial blood substitutes such as perfluorocarbon emulsions in conjunction with 100% oxygen ventilation are valuable to patients with relatively low hemoglobin levels (*e.g.* sickle cell anemia patients or patients suffering of surgical blood loss.[1-4] Indeed, they can help to enhance oxygen tissue delivery, reduce allogeneic blood transfusion requirement which consequently reduce potential infections (*e.g.* hepatitis, HIV) [1-4].

Perfluorochemicals are hydrophobic, then stable perfluorocarbon emulsion with exceptionally smaller particles (median diameter < 0.2 μm) than red blood cells (median diameter ~ 7 μm) have been generated for intravenous use [5]. Thereby, perfluorocarbon molecules such as perfluorobron (also referred to as perfluorooctyl bromide or bromoperfluoro-*n*-octane, $C_8F_{17}Br$) or perfluodecalin (*aka* octadecafluoronaphthalene, $C_{10}F_{18}$) represent the main component of commercialized artificial blood substitutes Oxygent® (Alliance Pharmaceutical Corporation, San Diego, California, USA) [5] and Fluosol® (Green Cross Corporation, Osaka, Japan) [6], respectively. In fact, perfluorobron (PFB) and perfluorodecalin (PFD) are the most used components in formulations [7].

While the metabolism of fluorocarbon molecules is still unclear in humans, it seems that after intravenous administration, the droplets of perfluorocarbon emulsions are taken up by the reticulo-endothelial system (RES) before being slowly broken down and transported by the blood to the lungs where the unaltered perfluorocarbon molecules are finally excreted *via* exhalation [8,9]. The intravascular half-life of perfluorocarbons, determined by the RES uptake and the dose used, is relatively short (< 24 hours) [10,11].

Up-to-date, Fluosol® is the only blood substitute product approved by the U.S. Food and Drug Administration (FDA) for clinical use (e.g. supplemental oxygenation of ischemic tissue in percutaneous transluminal coronary angioplasty (PTCA) because of its ability to diffuse into poorly vascularized tissue [12].

In order to characterize qualitatively and quantitatively perfluorocarbons and its derived products (e.g. metabolized ones), we used an innovative and powerful technology named carbon-fluorine spectroscopy (CFS™) [13,14]. In fact, CFS™ is a progressive, non-destructive and non-invasive laser-based technology, which based on the discovery of a characteristic signature of carbon-fluorine (C-F) bond(s) in the fingerprint spectral area of 550-850 cm^{-1}, allows fast, sensitive and specific detection as well as structural characterization, imaging, screening, tracking and quantitative determination of any fluoro-organics, regardless their physical state [13,14].

In this study, we report the by CFS™ spectral profiling of two major PFCs, PFB and PFD, obtained by under different *in vitro* experimental conditions.

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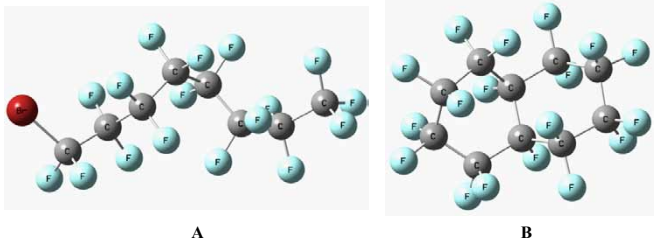
Material and Methods

Fluoro-materials

Undiluted main components of liquid blood substitutes, pure perfluorobron ($C_{18}F_{17}Br$) and pure perfluorodecalin ($C_{10}F_{18}$) were obtained from Alliance Pharmaceutical Corp. (San Diego, CA, USA) and Green Cross Corporation (Osaka, Japan), respectively. Their respective optimized structure determined by DFT calculations are presented in Figure 1.

Perfluorocarbons analysis by CFS™ technology

The detailed development, work system and features of the CFS™ technology have been previously described [13]. Fluoro-Raman spectra of those compounds were obtained, in several experimental conditions and the reliability of the data was confirmed by at least two independent experiments. Thereby, the samples have been placed either into (i) glass container (Figure 2), (ii) flow cell (Agilent) (Figure 3), (iii) high power fiber optics (Figure 4). The general set-up of CFS was performed in order to detect Raman spectra at 90 degrees with respect to the incident pulse using a pulsed copper vapor laser (CrystalTechno, Moscow, Russia) and a double monochromator (Jobin Yvon). The main set-up parameters were the following: (i) excitation at 510.6 nm (visible region) or 271.2 nm (ultraviolet (UV) region to observe resonance CFS™ spectra), (ii) repetition rate of 10 kHz, (iii) pulse duration of 17 ns and, (iv) average power of 200 mW. Globally, the high energy laser pulses irradiated the fluoro-organic sample and the consequent Raman vibrational scattering emissions were detected during a narrow time internal ("gate") approximately equal to the laser pulse duration, so the



A B

Figure 1: Optimized structure of **(A)** perfluorobron ($C_8F_{17}Br$) and **(B)** perfluorodecalin ($C_{10}F_{18}$) obtained by DFT calculations.

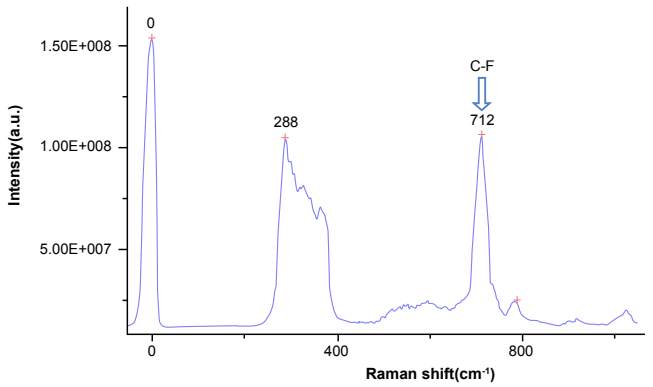


Figure 2: CFS spectrum of undiluted liquid blood substitute, perfluorobron, measured *via* glass container at 510.6 nm.

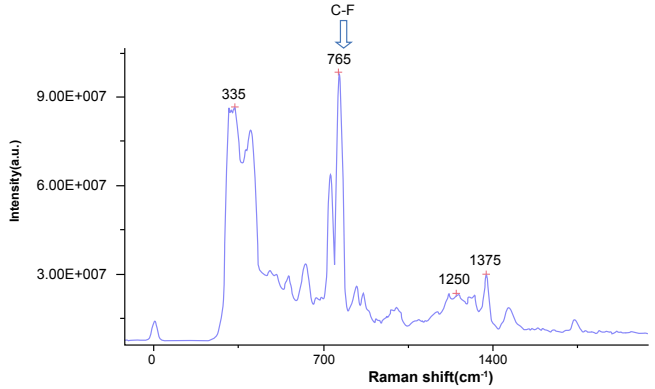


Figure 3: CFS spectrum of undiluted liquid blood substitute, perfluorobron, measured *via* fiber optics at 510.6 nm.

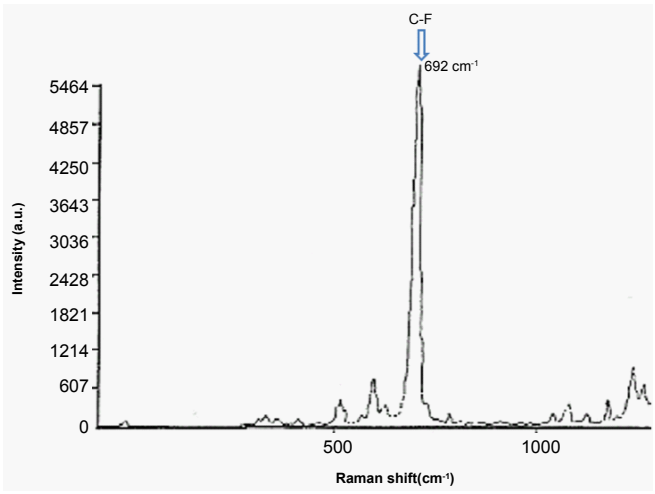


Figure 4: CFS spectrum of undiluted liquid blood substitute, perfluorobron, measured *via* flow cell at 510.6 nm.

CFS™ spectrum was not affected by fluorescence. The absorbance filter removed scattered radiation and passed the pulsed Raman radiation to the spectrometer. Eventually, signals were photo-multiplied and sent to a synchronized pulse recording system. The data were collected on computer and processed using the software-time resolved Solis(t) (Andor, Northern Ireland) and the software package Spectramax (Jobin Yvon).

DFT Calculations

All the density functional theory (DFT) calculations reported herein were performed with Gaussian 03 software package [15]. Ground state structure was obtained by carrying out a full geometry optimizations with DFT/6-31G (d) basis set using hybrid Becke's 3 Parameter exchange functional and Lee, Yang and Parr correlation functional (B3LYP). Raman spectra were calculated on the optimized geometry. Since B3LYP level overestimate the calculated vibrational frequency due to neglect of anharmonicity in real system, a scaling factor of 0.9613 [16] has been used to produce good agreement with experiment. We have not observed any imaginary frequency which suggested that the molecule was in local minimum on the potential energy surface.

Results

The respective CFS™ spectrum of the two perfluorochemicals, perfluorobron ($C_8F_{17}Br$) and perfluorodecalin ($C_{10}F_{18}$), which differed by their chemical structure (e.g. linear and cyclic, respectively) (Figure 1), have been obtained using undiluted blood liquid substitutes at determined experimental CFS™ parameters (e.g. excitation wavelength in visible or UV area), and were reproduced in different material supports for validation studies.

Thereby, using an excitation wavelength in the visible region (510.6 nm), we show that through:

1. Glass container, the C-F bond optical signature characteristic of perfluorobron (PFB) is detectable by CFS™ as a sharp and thin peak at 712 cm^{-1} (emission wavelength). The larger peak observed at 288 cm^{-1} is assigned to the glass container itself (Figure 2).
2. Fiber optics, the specific C-F assignment of PFB is detectable at 765 cm^{-1}. The larger peak observed at 335 cm^{-1} is assigned to the optical fiber itself (Figure 3).
3. Flow cell, the C-F bond-contained PFB is obtained at 735 cm^{-1}, which is represented by a sharp and thin signal. The larger peak observed at 311 cm^{-1} is assigned to the flow cell itself (Figure 4).

Taken together, we can conclude that, in our reproducible experimental conditions, the C-F assignment of PFB is observed within the minimal spectral region of 712-765 cm^{-1}. Further and interestingly, DFT calculations have predicted the observed bands assigned as CF_2 scissoring mode to occur at 667 cm^{-1}, thereby confirming and validating CFS™ measurements. Indeed, the small difference between the obtained experimental and theoretical wavenumber can be attributed to the measurement conditions. It is worth noting that fluorine, a hydrogen bond acceptor, can be subjected to such interactions owing to the different materials support which can perturb slightly the wavelength shift of many modes while the calculation has been performed on a single molecule in gas phase.

The characterization by CFS of the other liquid blood substitute, perfluorodecalin (PFD), was repeated in flow cell as a selected container. In brief, we can observe a sharp and very intense optical emission signal detectable at 692 cm^{-1}, which is assigned to a symmetrical vibration involving the difluoromethyl ($-CF_2$) group (Figure 5). Our DFT calculation predicted the C-F signal wavelength at 645 cm^{-1} which also confirms (taking in account the different experimental conditions employed) the expected C-F signal wavelength.

Besides, as any of the physical material support used did not significantly interfered with the emitted PFB C-F Raman spectrum and provided similar data for its physical characterization by CFS, we decided to finally use again the flow cell as unique container to characterize the two blood substitute products, PFB and PFD, in a UV region where both products have been shown to display the maximum ultraviolet absorption (271.2 nm). Nevertheless, we noticed that the C-F emitted peak intensity of PFB (Figure 6) and PFD (Figure 7) compounds were much weaker than those for which the measurements have been performed in visible region (510.6 nm) (Figure 4 and 5).

Eventually, we further observed that the global emission spectral signal of PFD obtained by CFS™ was globally enhanced comparatively to the PFB´s one, certainly because of the high self-absorbance of fluorinated aromatic rings that characterize the PFD structure [14].

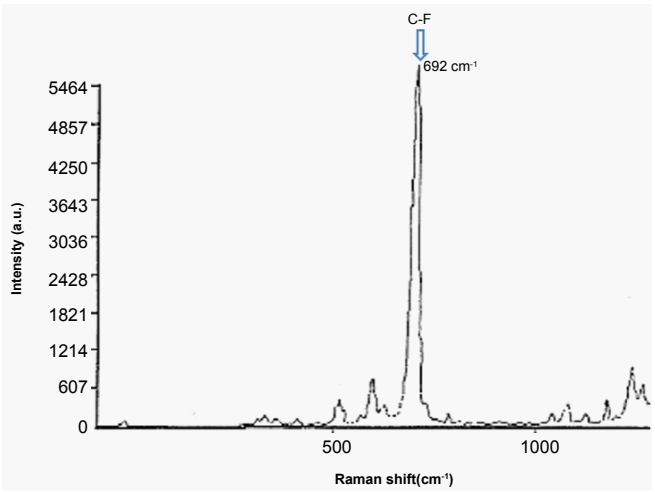


Figure 5: CFS spectrum of undiluted liquid blood substitute, perfluorodecalin, measured *via* flow cell at 510.6 nm.

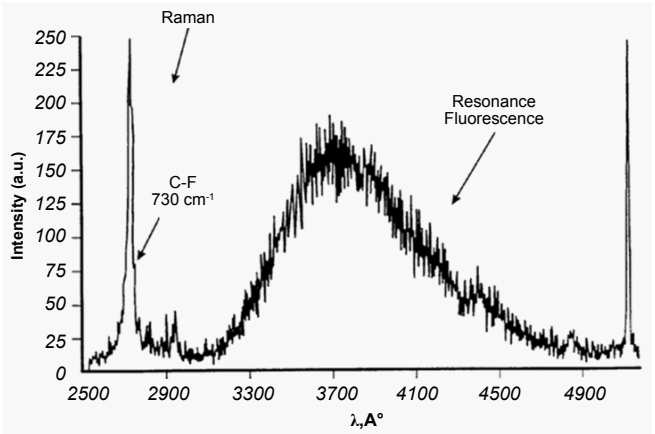


Figure 6: CFS spectrum of undiluted liquid blood substitute, perfluorobron, measured *via* flow cell at 271.2 nm.

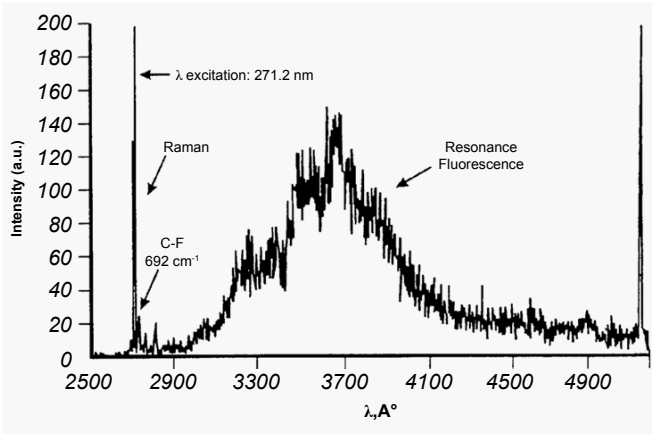


Figure 7: CFS spectrum of undiluted liquid blood substitute, perfluorodecalin, measured *via* flow cell at 271.2 nm.

Taken together, our data show that the physical characterization of perfluorocarbons by CFS™ is better performed after excitation in the visible region (i.e. at 510.6 nm) than in the ultraviolet region (i.e. at 271.2 nm), regardless of the type of container used.

Discussion

Fluid resuscitation is intended to eliminate microcirculatory disorders and restore adequate tissue oxygenation. The safety limits for a restrictive transfusion policy are given by individual tolerance of acute normovolemic anemia. Artificial oxygen carriers, based on PFCs or hemoglobin, are attractive alternatives to allogenic red blood cells. Indeed, there are many risks involved in allogenic blood transfusions (e.g. transmission of infections, delayed postoperative wound healing, immune reactions). Regardless of whether artificial oxygen carriers are available for routine clinical use, further studies are needed in order to show the safety, security and efficacy of these substances for clinical practice.

CFS™ offers a potent tool to characterize PFCs in order, for instance, to understand better their metabolism (e.g. pharmacokinetics, systemic bioavailability). Indeed, the CFS™ spectrophotometer, based on pulsed laser irradiation, is a non-destructive and non-invasive method allowing a better resolution of the signals compared to the conventional Raman spectroscopy, as already demonstrated earlier[13]. Further, the method allows rapid, sensitive and specific detection as well as measurement of C-F bonds without requiring to unravel the entire structure of the organo-fluorine product, which besides is also possible [13,14].

In this work, we showed that it is possible to apply CFS™ technology to detect reliably, quickly and cost-effectively blood liquid substitutes such as PFB and PFD thanks to the C-F bond assignment. Furthermore, our *in vitro* experimental studies underlined the importance to characterize the blood substitutes by CFS™ after photo-excitation at 510.6 nm (visible region) rather than at 271.2 nm (ultraviolet region) regardless of the analytical containers used (*e.g.* flow cell, glass, optic fiber, quartz).

Conclusions and Perspectives

CFS™ technology demonstrates excellent potential over conventional Raman spectroscopy techniques to analyze reliably PFCs *in vitro*. We further aim to apply CFS™ technology combined to confocal laser microscope, high pressure liquid-chromatography (HPLC) and high-throughput screening (HTS) systems to: (i) ensure safety and security of PFCs-based products (*e.g.* avoidance of counterfeited blood substitutes); (ii) qualitatively and quantitatively characterize PFCs and F-metabolites in biological fluids using various physical-chemical conditions (*e.g.* pH, temperature); (iii) determinate *in vivo* (*e.g.* using animal models of disease such sickle cell disease or tumor-bearing mice) the presence of PFCs, their localization and, their metabolism notably through pharmacokinetic studies; (iv) develop PFCs-based nanomaterials for early diagnosis (*e.g.* "green" enhanced imaging) and progressive/controlled-drug delivery (*e.g.* personalized targeted therapy).

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