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Design of Nanocapsules Based on Novel Fluorophilic Cyclodextrin Derivatives and Their Potential Role in Oxygen Delivery

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Abstract

The authors report an amphiphilic and fluorophilic β-cyclodextrin derivative synthesized (perfluoro-β-cyclodextrins: β-CD-C_8^F) according to a patent registered by Skiba *et al.* A new type of nanocapsule (PFC-NC) based on perfluoro-β-cyclodextrins was prepared in a single step by colloidal nanoprecipitation. PFC-NC is fluorophilic carrier that could potentially be used in various types of pharmaceutical posology. Their potential as controlled release oxygen delivery systems was evaluated. PFC-NC were spherical and homogeneous particles with mean diameter of 335 nm and zeta potential of −73 mV. They were stable for 35 days at 4 and 25 °C. PFC-NC have a prolonged delivery of oxygen with a delayed release as compared to water. The results of this study suggests that highly fluorinated-cyclodextrin could constitute a promising new component of nanocapsules. Fluorocarbon-in-water nanocapsules also represent a safe and cost-effective vehicles for *in-vivo* oxygen delivery.

Introduction

For several years, modifications of cyclodextrins have provided an interesting organic host molecules for the encapsulation, solubilisation and transport of drugs [1]. The resulting cyclodextrin derivatives are known to increase the bioavailability of drugs [2]. They have also been considered for use in more complex delivery systems. Such systems sometimes require that the cyclodextrins should have amphiphilic properties. Amphiphilic derivatives of cyclodextrins have been obtained by the introduction of lipophilic groups linked to the primary or secondary face by thio, thioxo, amino, ester or ether linkages [3–8] particularly with a wide range of long alkyl chains as hydrophobic substitutes. These molecules have been demonstrated to form self-assembling systems such as monomolecular layers, micelles, nanospheres and nanocapsules [5, 9–12].

Perfluorocarbons (PFCs) or fluorocarbons are chemicals essentially composed of carbon and fluorine instead of carbon and hydrogen as hydrocarbons.

Highly fluorinated materials have multiple properties, i.e., repellence to water and oil, unique dielectric, rheological and optical characteristics, as well as their exceptional chemical and biological inertness. Enzymatic cleavage of a fluorocarbon has to our knowledge never been reported.

This property reflects the strength of the intramolecular chemical bonds. The C-F bond is the strongest single bond with carbon in an organic molecule. Furthermore, vesicules made from perfluroalkylated amphiphiles materials have recently been reported [13–15]. These vesicules have an internal fluorinated film within the lipid bi-layer. The fluorinated chains, due to their strong hydrophobic, lipophobic and fluorophilic character, impart unique properties to the vesicles, including enhanced particle size stability [13, 14], prolonged intravascular persistence [16], and increased drug encapsulation stability [17].

We have recently reported that combinations of cyclodextrin and linear perfluorocarbon lead to a novel amphiphilic and fluorophilic β-cyclodextrin derivative which has been synthesized (Perfluoro-β-Cyclodextrins. β-CD-C_8^F) according to Skiba *et al.* [18]. A new type of nanocapsules (PFC-NC) has now been prepared from these β-CD-C_8^F by colloidal nanoprecipitation. These nanocapsules might be suitable vehicles for oxygen solubilization and delivery.

In this study, we evaluated this potential by monitoring the diffusion through the fluorocarbon phase of oxygen. In comparison, we have also studied the release of oxygen from water.

Experimental procedure

Chemicals and instruments for analysis

The β-cyclodextrin was purchased from Roquette Frères (Lestrem, France) and recrystallized before use. The synthesis of the 2,3-di-O-decafluorooctanoylcyclomaltooctaose (β-CD-C_8^F) was obtained according to a three stage synthetic route previously described by Skiba *et al.* [18]. Briefly, this synthetic route consists of the protection of primary hydroxyl groups at the O6 position of the β-cyclodextrin with t-butyldimethylsilylchloride in dry pyridine at 24 h at room

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temperature. Esterification of the secondary hydroxyl groups at the O2 and O3 positions was then performed with decafluorooctanoyl chloride in the presence of NaH as acylation catalyst in dry dichloromethane at 60 °C for 1 h. Finally, the last step consisted in the removal of silyl protecting group by the use of the $BF_3O(Et)_2$ in cold dichloromethane for 6 h to produce the Perfluoro-β-Cyclodextrins (β-CD-C_8^F) derivative. Its chemical purity (>95%) was determined using **TLC**, **FT-IR**, **DSC**, **elemental analysis** and **TOF-MS**. Fluorodecalin was obtained from SIGMA and all other substances were of analytical grade.

Preparation and characterization of the nanocapsule

Preparation of PCF nanocapsules

PFC nanocapsules were obtained according to the method of Skiba *et al.* [18]. A lipophilic phase, composed of perfluorodecalin and β-CD-C_8^F, was dissolved in acetone and added under mechanical stirring to an aqueous phase consisting of distilled water containing dissolved surfactant. The PFC nanocapsules were formed immediately. Acetone was totally removed by evaporation under vacuum, together with concentrated colloidal suspension.

Size distribution evaluation

The mean nanocapsules size and size distribution were determined by photon correlation spectroscopy using a Nanosizer instrument N4MD (Beeckman Coulter, Roissy, France) which analyses the fluctuations in scattered light intensity generated by diffusion of nanocapsules in suspension. Experimental conditions were: temperature, 25 °C; reference index, 90°; viscosity, 0.899×10^{-3} Pa; refractive index, 1.330.

Long-term stability study

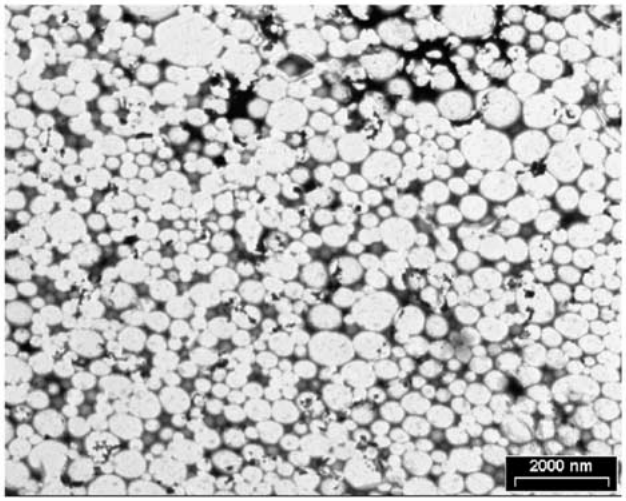
Fifteen-ml aliquots of the nanocapsules suspension prepared as mentioned above were transferred into ampules, which were sealed and kept at 4, 25, and 40 °C. Each week, an ampoule was opened and the sample was pipetted, diluted to the appropriate concentration with water and the mean diameter and size distribution were determined.

Zeta potential

The charge of PFC nanocapsules was measured with a Malvern Zetasizer 2C instrument equipped with a tubular cell of 2.6 mm. The operating principle of this instrument is based on the Doppler shift caused by the movement of nanocapsules across interference fringes which are produced by the intersection of two laser beams. The nanocapsules were suspended in KCl (10^{-3} M), and the measurement was made at 25 °C.

Transmission electron microscopy

In the various staining procedures available for imaging nanovesicles [19], the drop method was adopted here as the standard procedure [19]. In this method a single drop of freshly prepared PFC nanocapsules was placed onto a carbon-coated copper grid, left to stand for 1 min, and then



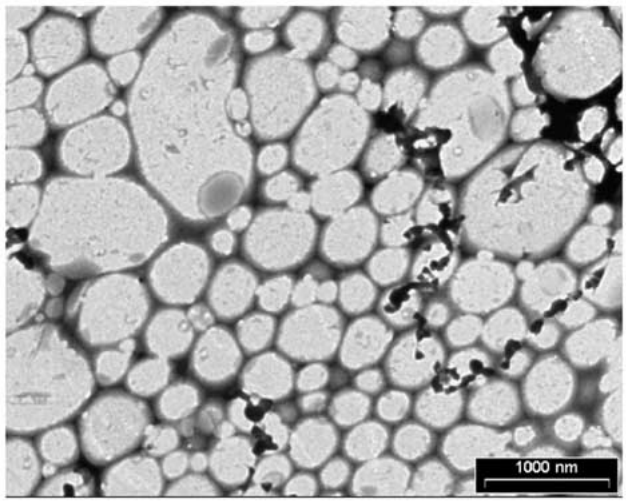


Figure 1. Electron transmission microphotography of PFC-CD nanocapsules.

dried using a wick of filter paper (Whatman No. 1). The stain, representative of an aqueous solution of 2% (w/v) phosphotungstic acid , was then placed onto the grid and left for an additionel minute. A fresh filter paper wick was used to remove excess stain from the gird. The sample was then immediately imaged with a transmission electron microscope (Philips EM 301 G) operating at an acceleration voltage of 100 kV with zero tilt in order to avoid the artefacts known to occur as a consequence of prolonged drying [20].

Oxygen delivery

We obtained the oxygenation of the PFC-NC suspension with pure oxygen at a flow of 1 ml/min for 5 min. Oxygen solubilized in water, under the same condition was used as control. We evaluated the liberation of oxygen from PFC-NC and from water control.

Table 1. Influence percent of the β-CD-C_8^F on the formulation size and distribution

β-CD-C_8^F (%) (w/w)	PFC-NC mean diameter (nm ± SD)	Polydispersity index
0	389 ± 110	0.10
10	430 ± 130	0.12
50	321 ± 100	0.09
75	335 ± 92	0.08
90	332 ± 82	0.09
100	427 ± 110	0.19

Oxygen delivery curves were measured by the automatic oxygen sensor recording system (Microprocessor Oximeter CellOx 325) interfaced to an IBM-PC computer system for on-line data acquisition, storage and analysis at a temperature of 37 °C.

Results and discussion

Preparation and characterization of PFC-NC

The nanocapsules were prepared in a single step by colloidal nanoprecipitation of Perfluoro- β-Cyclodextrins (β-CD-C_8^F) and perfluorodecalin. These nanocapsules showed an homogenous size distribution with a mean diameter of 350 ± 92 nm. The morphological examination of perfluorodecalin nanocapsules (PFC-NC) was performed by TEM and showed a spherical and homogenous system. We observed that nanocapsules having a mean diameter of 300 ± 60 nm were successfully prepared by the colloidal precipitation method (Figure 1). In order to indirectly determine the surface charge of nanocapsules, zeta potential values of PFC-NC were analysed. We obtained a zeta- potential of −73 mV. Long-term stability of PFC-NC at 4, 25 and 40 °C could be conserved for 3 months at 4 or 25 °C without any change in their diameter or poly-dispersity index. The study of the percentage of β-CD-C_8^F, of the system, showed an influence on the formulation size and distribution. Optimal results were obtained for a 75% (w/w) with a mean diameter of 335 ± 92 nm (Table 1).

Oxygen delivery

The study of the *in vitro* release of oxygen from PFC-NC showed a prolonged delivery of oxygen. They have a delayed release (1/2 equivalent concentration (Ceq): 108 min) comparatively to water (1/2 equivalent concentration (Ceq): 33 min) (Figure 2).

Injectable fluorocarbon emulsion (i.e., 'blood substitutes') are the most thoroughly investigated fluorocarbon-based products for medical applications. This application relies on the combination of two unique properties of fluorocarbon: outstanding gas dissolving capacities and extreme inertness. Fluorocarbon emulsions are thought to deliver a potent anti-ischemic drug: oxygen [21]. The first fluorocarbon emulsion developed as an oxygen carrier, FluosolR (Green Cross Corp., Osaka, Japan) was approved by the FDA in 1989 for use as an adjuvant to percutaneous tranluminal coronary angioplasty in high risk patients [22]. Other first generation fluorocarbon emulsion include Perftoran (Perftoran Co, Pushchino, Russia) and Emulsion No. II (Institute for Organic Chemistry, Shanghai, China). As a consequence of poor stability, first generation emulsions require frozen storage. Fluosol required reconstitution prior to use from a stem emulsion and two annex solutions. Perftoran has in fact now been approved in Russia as an all-purpose blood substitute and anti-hypoxic agent. In other reported studies 60% w/v concentrated perfluorooctyl bromide emulsion, Oxygent TM (Alliance Pharmaceutical Corp., San Diego, US) after successful Phase II clinical trials [23, 21], has now completed Phase III in Europe.

The drawback of currently available emulsions, which are produced from different fluorocarbons, are numerous: i.e., the problems of stability, the large size of the globules, the problem of toxicity which may occur due to the high concentration of surfactants. The maximum fluorocarbon charge in these emulsions is 60% w/v. However, above that value the fabrication of these types of emulsions are no longer possible. This type of emulsion becomes unstable as fluorocarbon density is 2. In fact, with our system a fluorocarbon charge of 100% w/v is possible by only adding approximately 5% of tenso-active stabilizer. Previous studies [24] have demonstrated that emulsions, which are produced from different fluorocarbons, permitted to obtain higher concentrations of dissolved oxygen. This could be achieved at a rate as high as 40 ml/100 ml but this would require a 20 min oxygen filling period and 40% w/v of fluorocarbon. In contrast, when producing nanocapsules with only a fluorocarbon rate of 25% and an oxygen filling rate of 5 min, a rate above 20% w/v of oxygen can be achieved (Table 2).

Table 2. The solubility of oxygen in aqueous high-fluorocarbon-content emulsions and nanocapsules

Volume percent fluorocarbon	Formulations	Calculated solubility (37 °C) of O_2 in neat fluorocarbon (ml O_2/100 ml)
25%	Nanocapsules[c] PFC-NC	20.72[a]
40%	Emulsion[d] $C_8F_{17}Br$	44[b]
40%	Emulsion[d] $C_{10}F_{21}Br$	Low[b]
43%	Emulsion[d] *n*-$C_{10}F_{22}$	30[b]
43%	Emulsion[d] FD C-47	25[b]
43%	Emulsion[d] $[(CF_3)_2CF]_2C_3F_7$-*n*	37[b]

[a] This study.
[b] Data from reference [24].
[c] Nanocapsules were then oxygenated for 15 min.
[d] Emulsion were then oxygenated for 20 min.

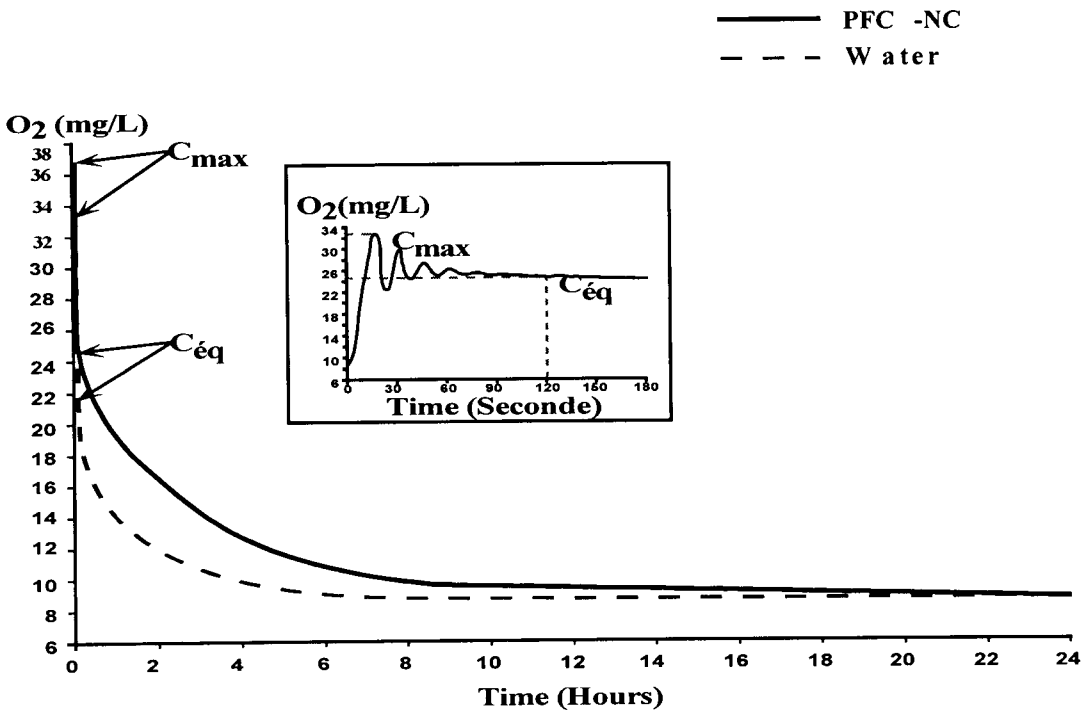


Figure 2. Oxygen delivery of PFC-NC comparatively to water.

Therefore, the number of particles in a colloid solution which is higher than in this type of emulsion will permit a greater rate of dissolved oxygen.

PFC-NC made of perfluoro-cyclodextrine (β-CD-C_8^F) was corrected most of the drawbacks of the second-generation. These new nanocapsules made from β-CD-C_8^F might have broad applicability and they could serve as novel fluorophilic carriers in various pharmaceutical dosage forms in the near future.

In conclusion, Our results suggest that highly fluorinated-cyclodextrin, could be a new promising component, which permit the preparation of nanocapsules. PFC-NC made of β-CD-C_8^F may constitute a new vehicle for *in vivo* oxygen delivery.

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References

1. J. Szejtli: in J.E.D. Davies (ed.), *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, The Netherlands (1988).
2. K.H. Fromming and J. Szejtli: *Cyclodextrins in Pharmacy*, Kluwer Academic Publishers, Dordrecht, The Netherlands (1994).
3. J. Boger, R.J. Corcoran, and J.M. Lehn: *Helvetica Chimica Acta* **61**(6), 2190 (1978).
4. S. Taneva, K. Ariga, W. Tagaki, and Y. Okahata: *J. Colloid Int. Sci.* **131**(2), 561 (1989).
5. F. Djedaïni, A.W. Coleman, and B. Perly: *Proceedings of the Vth International Symposium on Cyclodextrins*, Paris, 328 pp. (1990).
6. L. Jullien, T. Lazrak, J. Canceil, L. Lacombe, and J.M. Lehn: *J. Chem. Soc. Perkin Trans.* **2**, 1011 (1993).
7. P. Zhang, C.C. Ling, A.W. Coleman, H. Parrot-Lopez, and H. Galons: *Tetrahedron Lett.* **32**, 2769 (1991).
8. A. Mazzaglia, B.J. Ravoo, and R. Darcy: *Proceedings of the Xth International Symposium on Cyclodextrins*, Ann Arbor (2000).
9. R. Aurely-Velly, F. Djedaïni-Pilard, and B. Perly: *Langmuir* **16**, 3727 (2000).
10. B.J. Ravoo: *Proceedings of the Xth International Symposium on Cyclodextrins*, Ann Arbor (2000).
11. M. Skiba, D. Wouessidjewe, H. Fessi, J.P. Devisaguet, D. Duchene, and F. Puisieux: PCT Apllic. Fr. 93/00593 (1993).
12. M. Skiba, D. Wouessidjewe, A.W. Coleman, H. Fessi, J.P. Devisaguet, D. Duchene, and F. Puisieux: PCT Apllic. Fr. 93/00594 (1993).
13. C. Santaella, P. Verling, and J. G. Riess: *Angew. Chem. Int. Ed. Engl.* **30**, 567 (1991).
14. M.P. Krafft, F. Giulieri, and J.G. Riess: *Angew. Chem. Int. Ed. Engl.* **32**, 741 (1993).
15. L. Zarif, T. Gulik-Krzywicki, J.G. Riess, B. Pucci, C. Guedj, and A.A. Pavia: *Colloids & Surf.* **83**, 107 (1994).
16. F. Frezard, C. Santaella, P. Vierling, and J. G. Riess: *Biochim. Biophys. Acta.* **1192**, 61 (1994).
17. C. Santaella, F. Frezard, P. Vierling, and J.G. Riess: *FEBS Letters* **336**, 481 (1994).
18. M. Skiba, M. Skiba-Lahiani, R. Duclos, J.-C. Combret, and P. Arnaud: French Patent No. 99/09863 (1999).
19. S. Lukac and A. Perovic: *J. Colloid Interface Sci.* **103**, 586 (1985).
20. Y. Talmon: *J. Colloid Interface Sci.* **93**, 366 (1983).
21. M.P. Krafft: *Advanced Drug Delivery Reviews* **47**, 209 (2001).
22. K.M. Kent, M.W. Cleman, M.J. Cowley, M. Forman, C.C. Jaffe, M. Kaplan, S.B. King, M. Krucoff, T. Lassar, B. McAuley, R. Smith, C. Wisdom, and D. Wohlgelernter: *Am. J. Cardiol.* **66**, 279 (1990).
23. D.R. Spahn, R. Van Brempt, G. Theilmeier, J.P. Reibold, M. Welte, H. Heinzerling, K.M. Birck, P.E. Keipert, and K. Messmer: *Anesthesiology* **91**, 1195 (1999).
24. C.M. Sharts and H.R. Reese: *Journal of Fluorine Chemistry* **11**, 637 (1978).