This is an **Accepted Manuscript** of an article published by Taylor & Francis in: Journal of Microencapsulation 2014, 31, 738-745 on 25 June 2014, available online: https://doi.org/10.3109/02652048.2014.918668

Perfluorodecalin-soluble fluorescent dyes for the monitoring of circulating nanocapsules with intravital fluorescence microscopy

J. Laudien,[1] D. Naglav,[2] C. Groß-Heitfeld,[3] K. B. Ferenz,[1] H. de Groot,[1] C. Mayer,[3] S. Schulz,[2] A. Schnepf,[4] M. Kirsch*[1]

[1] *University Hospital Essen, Institute of Physiological Chemistry, D-45122 Essen, Germany*

[2] *University of Duisburg-Essen, Institute of Inorganic Chemistry, CENIDE, D-45141 Essen, Germany*

[3] *University of Duisburg-Essen, Institute of Physical Chemistry, CENIDE, D-45141 Essen, Germany*

[4] *Eberhard Karls University Tuebingen, Institute for Inorganic Chemistry, D-72076 Tuebingen, Germany*

*) Prof. Dr. M. Kirsch, Institute of Physiological Chemistry, University Hospital Essen, Hufelandstr. 55, 45122 Essen, Germany
Tel.: (49) 201-723 4108
Fax.: (49) 201-723 5943
E-Mail: michael.kirsch@uni-duisburg-essen.de
* to whom correspondence should be addressed

Keywords: perfluorocarbon, *in vivo* detection, artificial oxygen carriers, nanocapsules, fluorescent dye, intravital microscopy

Abstract

Perfluorodecalin (PFD) is an established artificial oxygen carrier due to its physical capability to solve the respiratory gases molecular oxygen and carbon dioxide (Bauer et al., 2010, Kirsch et al., 2012, Riess, 2001, Riess, 2006, Stephan et al., 2013 in press). For *in vivo* monitoring of PFD-filled poly(*n*-butyl-cyanoacrylate) (PACA) nanocapsules by intravital fluorescence microscopy an applicable fluorescent dye is missing. In order to develop such a dye, modifications of a BODIPY derivative and mesoscopic systems, e.g. semiconductors and gold nanoparticles with a long-chained fluorinated thiol function, as well as functionalised porphyrin derivatives were investigated. Due to the good solubility of all synthesised dyes in PFD, it should be possible to stain PFD-filled particles in general. However, only the functionalised BODIPY derivative was suitable for *in vivo* monitoring of the PFD-filled PACA nanocapsules.

1. Introduction

With regard to clinical research the development of artificial blood substitutes, such as artificial oxygen carriers, has gained importance over the past years (Lowe, 2003, Sazama, 1990, Stanworth et al., 2002, Williamson et al., 1999). In general, there are two groups of oxygen carriers: hemoglobin-based oxygen carriers and perfluorocarbon-based ones (Chang, 2003, Tappenden, 2007, Veeckman, 1997, Winslow, 2006). Liquid perfluorocarbons (PFCs) are highly effective solvents for molecular oxygen as well as carbon dioxide in their cavities, with the amount linearly depending on the partial pressure of the gas (Riess, 2001). PFCs are chemically and metabolically inert due to the high carbon-fluorine bond energy (ca. 480 kJ mol^{-1}), even in presence of reactive compounds common in the human body. PFCs are immiscible with aqueous or organic phases, but they can act as a solvent for other perfluorinated compounds. On account of their high vapour pressure, PFCs can be exhaled easily (Leland and Gollan, 1966, Lowe, 2003, Winslow, 2006) and exhibit moderately short half-lives *in vivo* of about a few days (Riess, 2001). One of the most studied PFC in biological systems is the bicyclic perfluorinated alkane perfluorodecalin (PFD - $C_{10}F_{18}$; Figure 1) (Lowe, 2003).

Geyer showed that the intravenous administration of PFC liquids in rats as bolus or as dispersion causes severe alterations in the lung, involving a disturbed respiration of the animals (Geyer, 1970). The reason for this mortal action is a spontaneous foaming of the PFC liquids within the lungs. This drawback can be smartly avoided by encapsulating the PFCs. Therefore, PFD is already discussed in literature for the use in poly(*n*-butyl-cyanoacrylate)

(PACA) nanocapsules as artificial oxygen carriers (Stephan et al., 2013 in press). For the development of such a drug with PFD and poly(*n*-butyl-cyanoacrylate) as key building materials, it is necessary to collect various information, particularly about their *in vivo* behaviour. For this purpose, the detection of PFD-filled nanocapsules by intravital fluorescence microscopy (IVM) would be a very helpful monitoring tool. Stephan *et al.* described the preparation of PFD-filled PACA nanocapsules for super-resolution fluorescence microscopy using fluorinated porphyrin derivatives to label the PFD core of the capsules (Stephan et al., 2013 in press). Those porphyrin derivatives could be excited at 405 nm. Due to the fact that a radiation with an excitation wavelength of the UV-B range would accelerate tissue damage in biological applications, those porphyrin derivatives can't be used as reporter dyes in order to monitor organs in living animals via IVM.

Unfortunately, a PFD-soluble fluorescent dye with a physiologically harmless excitation wavelength is presently unavailable, so that the IVM technique can't be used as a monitoring tool for *in vivo* circulating PFD-enriched capsule systems. In order to discard this drawback, a PFD-soluble fluorescent dye with an adequate excitation wavelength was developed. For this purpose, fluorescent systems (molecules and mesoscopic systems) were modified with molecules with high fluorine content.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich (Steinheim, Germany), if not stated otherwise.

Synthesis of CdSe/ZnS-quantum dots functionalised with $C_{10}H_5F_{17}S$ (3)

3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluor-1-decanthiol (500 µl) was added to a solution of CdSe/ZnS-quantumdots in toluene (150 µl). The reaction mixture was stirred over night at ambient temperature. The product was dried under vacuum and dissolved in PFD.

Synthesis of gold nanoparticles functionalised with $C_{10}H_5F_{17}S$ (7)

A stock solution of gold nanoparticles was prepared and modified as described by Liu *et al*. (Liu, 2008). An aqueous solution of tetrachloroauric acid $HAuCl_4$ x 3 H_2O (0.1 M, 400 µl) (ABCR, Karlsruhe, Germany) was mixed with dimethylformamide (40 ml) and stirred for 10 min. Subsequently, the reaction mixture was heated to 140 °C and stirred for 4h. After cooling down to ambient temperature, the mixture was centrifuged (500 x *g*, 45 min) and filtered.

Solvents were evaporated under vacuum, the remaining residue dissolved in methanol (5 ml) and finally kept as a stock solution. For functionalisation, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluor-1-decanthiol (175 µl) was added to 100 µl of the stock solution. The reaction mixture was stirred for 12h at ambient temperature under light exclusion. The product was dried under vacuum and dissolved in PFD.

Synthesis of tin-5,10,15,20-tetrakis(pentafluorophenyl)-porphyrin and cadmium-5,10,15,20-tetrakis(pentafluorophenyl)-porphyrin (10a/b)

$Sn[N(SiMe_3)_2]_2$ and $Cd[N(SiMe_3)_2]_2$ were prepared as described by Harris and Lappert (Harris and Lappert, 1974). To a solution of 5,10,15,20-tetrakis(pentafluorophenyl)-21H,23H-porphyrin (0.02 mmol) in carefully dried heptane (5 ml) (Carl Roth, Karlsruhe, Germany) either $Sn[N(SiMe_3)_2]_2$ or $Cd[N(SiMe_3)_2]_2$ (0.02 mmol) was added. The mixture was stirred for 45 min at ambient temperature under exclusion of air in a nitrogen atmosphere. The solvent was evaporated under vacuum and the resulting dark-red product was dissolved in PFD and separated from insoluble precipitates by filtration or centrifugation (500 x *g*, 10 min).

Synthesis of BODIPY-3-$CH_2NHCOCH_2SC_{10}H_5F_{17}$ (12)

KOH (0.89 mmol) was added to a solution of 500 µL $C_{10}H_5F_{17}S$ in methanol (50 ml). The resulting mixture was stirred for 10 min at ambient temperature. BODIPY-3-$CH_2NHCOCH_2I$ (Molecular Probes, Eugene, Oregon, USA) (0.01 mmol) dissolved in methanol (15 ml) was added dropwise within 30 min. The reaction mixture was stirred for 6 h at ambient temperature, The solvents evaporated in vacuum and the resulting product dissolved in PFD.

Animals

Two male Wistar rats (Rattus norvegicus, 446 and 450 g) were obtained from the central animal unit of the Essen University Hospital. Animals were kept under standardised temperature conditions (22±1°C), humidity (55±5 %) and 12/12-h light/dark cycles with free access to food (ssniff-Spezialdiaeten, Soest, Germany) and water. All animals received humane care according to the standards of Annex III of the directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes(Council, 2010). The experimental protocol was approved by the North Rhine-Westfalia state office for Nature, Environment and Consumer Protection (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), Germany, based on the local animal protection act.

Anesthesia, analgesia, and surgical procedure

Rats were anaesthetised with isoflurane (2.0 % in 100 % medical O_2 at 4.0 l/min for induction, 1.5-2.0 % isoflurane in 100 % medical O_2 at 1.0 l/min throughout the experiment) through face masks connected to a vaporizer (Isofluran Vet. Med. Vapor; Draeger, Luebeck, Germany) and received ketamine (50 mg/kg s.c., purchased from CEVA, Santé Animale, Libourne, France) into the right chest wall for analgesia. After local xylocain administration (5 mg/kg s.c., purchased from AstraZeneca, Wedel, Germany), a median skin-deep inguinal incision of about 2 cm was made along the right groin and a Portex catheter (0.58 mm ID, 0.96 mm OD) was placed within the right femoral artery and the right femoral vein. Each catheter was fixed with a surgical suture. The core body temperature of the rats was continuously monitored using a rectal sensor; the cooling below 37 °C was prevented by both an underlying thermostat-controlled operating table and by covering the animals with aluminum foil.

At the end of experiment, the animals were sacrificed by resection of the heart under deep isoflurane anaesthesia.

Intravital fluorescence microscopy (IVM)

The abdominal cavity was opened surgically and either gut or kidney was exteriorized and placed on a specially designed stage. A bolus of 10 ml of a suspension of labelled microcapsules in 0.9 % normal saline solution was administered intravenously via a catheter within the right femoral vein. During microscopy, the hemodynamic parameters (arterial blood pressure and heart rate) were monitored continuously via a catheter within the right femoral artery. The monitoring of circulating PFD-filled PACA nanocapsules was performed with a Leica DMLM epifluorescence microscope (Leica Microsystems, Wetzlar, Germany), equipped with an I3 filter cube (Leica Microsystems) possessing an excitation filter of 450-490 nm (BP 450-490) and a long distance working objective (HC PL Fluotar 20x/0.40, Leica Microsystems). The overall magnification was 160x. To avoid microcirculation disorders due to epi-illumination, circulating nanocapsules were monitored briefly (30–60 seconds) with a monochrome charge-coupled device camera (Zelos-285M GV, Kappa optronics, Gleichen, Germany), using KCC Zelos recording software (Kappa optronics, Gleichen, Germany). In order to check the localisation of the nanocapsules, blood vessels were stained by using the plasma dye tetramethylrhodamine isothiocyanate dextran (TRITC-dextran, TdB consultancy, Uppsala, Sweden) and visualised by using a N2.1 filter block with an excitation filter of 515-

560 nm (BP 515-560). TRITC-dextran was administered intravenously in a concentration of 0.35 µmol/kg.

Size distribution measurements

Size distribution measurements were obtained by using particle tracking with a dark field microscope. Due to the fact that submicron particles or capsules are subjected to the Brownian motion, the Stokes-Einstein equation

$$D = \frac{k_B T}{6\pi\eta r_H} \tag{1}$$

can be used, in which D is the diffusion coefficient, T the absolute temperature, η the dynamic viscosity and r_H the hydrodynamic radius. With

$$D = \frac{\Delta^2}{2t} \tag{2}$$

the equation leads to the Einstein-Smoluchowski equation. Δ^2 is the mean square displacement of a particle and t represents the corresponding reaction time.

$$D = \frac{k_B T t}{3\pi\eta r_H \Delta^2} \tag{3}$$

The dark field microscope is equipped with a CCD-camera. Using equations (2) and (3) the hydrodynamic radius of particles can be calculated with the aid of a software programme by observing their velocities ($\upsilon = \Delta/t$) (Finder et al., 2004).

$$r_H = \frac{2k_B T}{3\,\pi\eta} * \frac{t^2}{(\Delta^2)^2} \tag{4}$$

3. Results and Discussion

Fluorescent mesoscopic systems such as quantum dots and nanoparticles as well as molecular fluorescent dyes were selected as potentially appropriate PFD-reporter dyes. With regard to the mesoscopic systems, CdSe/ZnS-quantum dots and gold nanoparticles were selected as potential reporters for the PFC phase. Gold nanoparticles offer an excitation wavelength of 532 nm (Geddes, 2003). The advantage of CdSe/ZnS-quantum dots is that they can be purchased with eligible excitation wavelengths. Both mesoscopic systems offer the facility for modifying the surface, so that PFD-solubility can be achieved by functionalising those substances with molecules possessing a great number of fluorine atoms. The CdSe/ZnS-

quantum dots were modified with a long-chained fluorinated thiol, i.e. 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-1-decanthiol **2** (Figure 2; Zeichnung hat eine CF_2-Gruppe zu viel; dies gilt auch für alle anderen Zeichnungen mit diesem Molekül). During this reaction, which was performed at ambient temperature, a disulphide bond between the sulphur atom of the zinc sulphide of the quantum dot **1** and the sulphur atom of the thiol **2** was formed. In order to ensure a sufficient surface stabilisation and to avoid disulphide formation of two thiols **2**, the fluorinated thiol was added dropwise to the stock solution of quantum dots **1**. The obtained, functionalised quantum dots **3** were readily soluble in PFD and showed the expected fluorescence when excited at 495 nm. After a few hours in PFD, quantum dots **3** became unstable and formed a white precipitate that showed no fluorescence. The high oxygen content that is present in the PFD solution most likely initiated the degradation process by oxidation of CdSe/ZnS to their corresponding oxides. Due to the fact that CdSe/ZnS-quantum dots functionalised with a long-chained thiol were not stable in the presence of oxygen, they can not be suited as a reporter dye for PFD-filled nanocapsules. Nevertheless, such functionalised quantum dots may be used as reporters for other fluorinated systems since their solubility in PFD could be increased by using the long-chained fluorinated thiol **2**. Based on the fact that this thiol increased the solubility in PFD successfully, a functionalisation of gold nanoparticles (GNP) was tried additionally with this molecule. The used GNPs were obtained from tetrachloroauric acid acid **4** by reduction with dimethylformamide (DMF) **5** (Liu, 2008). Subsequently, GNPs were functionalised by attaching the fluorinated thiol **2** to their surface (Figure 3).

Geddes *et al.* observed that pure gold nanoparticles exhibit strong fluorescence when excited at 532 nm. In contrast, the functionalised GNPs **7** did not exhibit any fluorescence properties at this excitation wavelength, but in the UV-B range at 260 nm (Figure 4). Indeed, the functionalised GNPs **7** were slightly soluble in PFD, but as already mentioned above, a fluorescence dye with a low excitation wavelength can not be suited for biological applications. Furthermore, the functionalised GNPs agglomerated after a few hours so that the suspension became unstable and the formed precipitates built up sediments. This phenomenon was tentatively explained with a piling of the GNPs in the cavities within the PFD liquid, so that GNP agglomeration proceeded. Even after changing the reaction conditions, it was not possible to produce a stable suspension of the functionalised GNPs in PFD that could be excited at 532 nm. It had to be assumed that the excitation maximum of GNPs shifted to lower wavelengths after the functionalisation with the long-chained fluorinated thiol **2**. Thus, this approach to receive a PFD soluble fluorescent dye failed too. After these failures of the

mesoscopic systems, the focus of investigation moved to molecular fluorescent dyes for reporting PFD-filled nanocapsules.

Since Stephan *et al.* used 5, 10, 15, 20-tetrakis(pentafluorophenyl)-21*H*,23*H*-porphine palladium(II) (PdT), 5, 10, 15, 20-tetrakis(pentafluorophenyl)-21*H*,23*H*-porphine platinum(II) (PtT) and 5, 10, 15, 20-tetrakis(pentafluorophenyl)porphyrin (T) as fluorescent dyes for the PFD phase, the first idea was to create an improved fluorescent dye from such a porphyrin system. 5,10,15,20-tetrakis(pentafluorophenyl)-21H,23H-porphyrin **8** was selected due to its high solubility in the PFD phase. Unfortunately the excitation wavelength of this porphyrin system is λ = 380 nm. Consequently, it would cause tissue damage during *in vivo* monitoring with IVM. In order to increase both the fluorescence intensity and the excitation wavelength, a divalent metal ion was placed in the centre of the porphyrin derivative **8** (Figure 5). The divalent ions tin and cadmium were chosen due to their well-known properties to accelerate fluorescence when acting as central ions of complex compounds. Moreover, cadmium(II) represents the highest oxidation state of this element. A porphyrin system modified with a divalent cadmium ion is therefore expected to be stable to oxygen in any case. The complexes were synthesised by reaction between 5,10,15,20-tetrakis(pentafluorophenyl)-21H,23H-porphyrin **8** and **M**$[N(SiMe_3)_2]_2$ (**M** = Sn^{2+} **9a**, Cd^{2+} **9b**) under nitrogen atmosphere at ambient temperature. **10a** and **10b** (Figure 5) were highly soluble in the PFD phase and did not migrate into other phases, independently from the chosen metal ion. Both modified porphyrin systems offered fluorescent properties when excited with UV-light (Figure 6). Furthermore they offered a wide excitation range and could be excited between 380 and 500 nm. Unfortunately, the fluorescence intensity decreased by about 90 % when the excitation wavelength was increased from 380 nm up to 500 nm. For IVM application, it is imperative that the desired fluorescent dye offers good fluorescence properties when excited at physiologically suitable wavelengths. Because of the immense decrease in the fluorescence intensity within this range, the modified porphyrin systems could not be established for the monitoring of PFD-filled PACA-nanocapsules by IVM.

Another approach to develop a PFD-soluble fluorescent dye with a physiologically suitable excitation wavelength was to functionalise a so-called BODIPY derivative in order to gain PFD-solubility. BODIPY-dyes, i.e. 4,4-difluoro-4-bora-3a,4a-diaza-s-indacenes, afford diminutive stoke-shifts, sharp excitation bands and sharp emission peaks as well as excellent quantum yields (Galangau et al., 2010, Ulrich et al., 2008). The intention was to functionalise the BODIPY derivative BODIPY® FL C1-IA, *N*-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-yl)-methyl)iodoacetamid **11**, having an excitation maximum at 500 nm

with a long-chained fluorinated thiol **2** (Figure 7). Therefore, the BODIPY derivative **11** was allowed to react with 3,3,4,4,5,5,6,6,7,7,8,8,9,9,-10,10,10-heptadecafluoro-1-decanthiol ($C_{10}H_5F_{17}S$) **2** in order to replace the iodine in 3-position with the thiol **2**. The obtained product **12** was highly soluble in PFD. In the presence of an organic solvent it showed only a slight migration from PFD to the organic solvent. Most of the functionalised BODIPY derivative **12** remained in the PFD phase. The excitation maximum was found at $\lambda = 505$ nm, offering the possibility that this synthesised fluorescent dye **12** may be applicable for the monitoring of intravascular circulating PFD-filled PACA nanocapsules via IVM. To verify this thesis, a capsule preparation using BODIPY-thiol-labelled PFD was performed according to a procedure previously described by Stephan *et al.* (Stephan et al., 2013 in press). The prepared nanocapsules had a mean diameter of 528 nm, as measured by dark-field microscopy. To ensure fluorescence of the prepared nanocapsules, they were scanned by laser scanning microscopy (LSM). Easy detection of the PFD-labelled PACA nanocapsules by LSM offered the hope that they could also be detected *in vivo* using IVM. To verify this assumption a suspension of PFD-labelled nanocapsules in 0.9 % normal saline solution was administered into the right femoral vein of a living, anaesthetised male Wistar rat. Briefly after application, microvessels in gut and kidney could be monitored by IVM (Figure 8). Previous to the capsule injection, vasculature and tissue appeared completely dark, so that an unlikely autofluorescence could be safely excluded (data nor shown). In order to double-check whether the PFD-labelled nanocapsules were located intravascularly, blood plasma was stained after the application of capsules by administering the fluorescent dye TRITC-dextran to ensure labelling of blood vessels. Vasculature could be monitored by switching the filter blocks, because the synthesised BODIPY-thiol **12** offers an excitation maximum in the range of blue radiation at 505 nm, whereas the excitation maximum of TRITC-dextran is located in the range of green radiation at 550 nm (Figure 9). The injection of the plasma protein reporter TRITC-dextran clearly demonstrated that the prepared PFD-filled PACA nanocapsules were located intravascularly, because vessels monitored after the application of TRITC-dextran were consistent with the structures monitored after injection of the labelled nanocapsules (Figures 9A and 9B). The functionalised BODIPY derivative **12** did not only offer a physiological harmless excitation maximum at 505 nm, but also exhibited good fluorescence properties at this wavelength. Thus, it could be demonstrated for the first time that the monitoring of circulating PFD-filled PACA nanocapsules *in vivo* via IVM was successful by using BODIPY-thiol **12** as a reporter dye for the PFD core of the nanocapsules.

4. Conclusion

For *in vivo* monitoring of PFD-filled PACA nanocapsules by IVM, it was necessary to synthesise a PFD-soluble fluorescent dye. Apart from PFD-solubility this new fluorescent dye must offer a "**harmless**" excitation wavelength to avoid severe tissue damage during microscopy investigation under radiation. In order to develop such a fluorescent dye, modifications of a BODIPY derivative and mesoscopic systems, e.g. CdSe/ZnS-quantum dots and gold nanoparticles with a long-chained fluorinated thiol, as well as functionalised porphyrin derivatives with divalent fluorescent ions, i.e. cadmium and tin, were investigated. Since all novel synthesised dyes were soluble in PFD, they may be used for the *in vitro* staining of PFD-filled particles or for the labelling of other highly fluorinated systems. Noteworthy, only the BODIPY derivative functionalised with the thiol (compound **12**) was suitable for the *in vivo* monitoring of circulating PFD-filled PACA nanocapsules by IVM. Due to the development of this novel PFD-soluble fluorescent dye, it is now possible to monitor the circulation of PFD-filled PACA nanocapsules *in vivo*.

5. Acknowledgement

The authors thank Anna Wrobeln from the research group of Prof. Dr. Dr. H. de Groot, Institute of Physiological Chemistry of the University Hospital Essen for checking the fluorescence of the BODIPY-thiol-labelled PFD-filled PACA nanocapsules by laser scanning microscopy.

.

6. Declaration of Interest

.

The authors report no declarations of interest.

.

References

BAUER, J., ZAHRES, M., ZELLERMANN, A., KIRSCH, M., PETRAT, F., DE GROOT, H. & MAYER, C. 2010. Perfluorocarbon-filled poly(lactide-co-gylcolide) nano- and microcapsules as artificial oxygen carriers for blood substitutes: a physico-chemical assessment. *J Microencapsul,* 27**,** 122-32.

CHANG, T. M. 2003. Future generations of red blood cell substitutes. *J Intern Med,* 253**,** 527-35.

COUNCIL, E. P. A. E. 2010. European Commission (2010) Directive 2010/63/EU on protecion of animals used for scientific purposes. Ispra, Italy: Institute for Health and Consumer Protection.

FINDER, C., WOHLGEMUTH, M. & MAYER, C. 2004. Analysis of Particle Size Distribution by Particle Tracking. *Part. Part. Syst. Charact.,* 21**,** 372 – 78.

GALANGAU, O., DUMAS-VERDES, C., MEALLET-RENAULT, R. & CLAVIER, G. 2010. Rational design of visible and NIR distyryl-BODIPY dyes from a novel fluorinated platform. *Org Biomol Chem,* 8**,** 4546-53.

GEDDES, C. D. 2003. Luminescent blinking of gold nanoparticles. *Chemical Physics Letters,* 380**,** 269-72.

GEYER, R. P. 1970. Whole animals perfusion with fluorocarbon dispersions. *Fed Proc,* 29**,** 1758-63.

HARRIS, D. H. & LAPPERT, F. 1974. Monomeric, Volatile Bivalent Amides of Group IV_B Elements, $M(NR1_2)_2$ and $M(NR1R2)_2$ (M=Ge, Sn, or Pb; R1=Me_3Si, R2=Me_3C). *J.C.S. Chem. Comm.***,** 895-96.

KIRSCH, M., BRAMEY, T., WAACK, I. N., PETRAT, F., MAYER, C. & DE GROOT, H. 2012. The necessity for the coating of perfluorodecalin-filled poly(lactide-co-glycolide) microcapsules in the presence of physiological cholate concentrations: Tetronic-908 as an exemplary polymeric surfactant. *J Microencapsul,* 29**,** 30-8.

LELAND, C. C. J. & GOLLAN, F. 1966. Survival of Mammals Breathing Organic Liquids Equilibrated with Oxygen at Atmospheric Pressure. *Science,* 152**,** 1755-56.

LIU, X. 2008. Surfactant-free Synthesis and Functionalization of Highly Fluorescent Gold Quantum Dots. *J. Phys. Chem.,* 112**,** 10778-83.

LOWE, K. C. 2003. Engineering blood: synthetic substitutes from fluorinated compounds. *Tissue Eng,* 9**,** 389-99.

RIESS, J. G. 2001. Oxygen carriers ("blood substitutes")--raison d'etre, chemistry, and some physiology. *Chem Rev,* 101**,** 2797-920.

RIESS, J. G. 2006. Perfluorocarbon-based oxygen delivery. *Artif Cells Blood Substit Immobil Biotechnol,* 34**,** 567-80.

SAZAMA, K. 1990. Reports of 355 transfusion-associated deaths: 1976 through 1985. *Transfusion,* 30**,** 583-90.

STANWORTH, S. J., COCKBURN, H. A., BORALESSA, H. & CONTRERAS, M. 2002. Which groups of patients are transfused? A study of red cell usage in London and southeast England. *Vox Sang,* 83**,** 352-7.

STEPHAN, C., SCHLAWNE, C., GRASS, S., WAACK, I. N., FERENZ, K. B., BACHMANN, M., BARNERT, S., SCHUBERT, R., BASTMEYER, M., DE GROOT, H. & MAYER, C. 2013 in press. Artificial Oxygen Carriers Based on Perfluorodecalin-Filled Poly(n-butyl-cyanoacrylate) Nanocapsules. *J. Microencapsul.*

TAPPENDEN, J. 2007. Artificial blood substitutes. *J R Army Med Corps,* 153**,** 3-9.

ULRICH, G., ZIESSEL, R. & HARRIMAN, A. 2008. The chemistry of fluorescent bodipy dyes: versatility unsurpassed. *Angew Chem Int Ed Engl,* 47**,** 1184-201.

VEECKMAN, L. 1997. Artificial blood substitutes. *Current Opinions in Anaesthesiology*.

WILLIAMSON, L. M., LOWE, S., LOVE, E. M., COHEN, H., SOLDAN, K., MCCLELLAND, D. B., SKACEL, P. & BARBARA, J. A. 1999. Serious hazards of transfusion (SHOT) initiative: analysis of the first two annual reports. *Bmj,* 319**,** 16-9.

WINSLOW, R. M. 2006. Current status of oxygen carriers ('blood substitutes'): 2006. *Vox Sang,* 91**,** 102-10.

DOI: 10.3109/02652048.2014.918668
URN: urn:nbn:de:hbz:464-20201119-170644-5

This is an **Accepted Manuscript** of an article published by Taylor & Francis in:
Journal of Microencapsulation 2014, 31, 738-745 on 25 June 2014, available online:
https://doi.org/10.3109/02652048.2014.918668