The structure peculiarity of perfluorocarbon nanodispersions

I N Kuznetsova
Russian Research Institute for Haematology and Transfusiology (Rus Rsch Inst Hem/Trans), 2-d Sovietskaya Street, 16, 191024 Saint Petersburg, Russia
e-mail: kuz-ir@ inbox.ru

Abstract. Nano-dispersions of perfluorocarbon (PFC) or PFC emulsions are substitutes of erythrocytes. They perform the function of the gas transport on condition when the particle structure will be preserved. The invariable values of the size and surface properties of the spherical PFC particles characterize the stability of their structure. The stable nano-dispersion on base PFC and phospholipids (PL) as the basis of the oxygen carrier preparation have been prepared. This PFC/PL nano-dispersion conformed to the essential requirements: the heat sterilization of the final product and the structure preservation of particles: size (~100 nm) and surface properties, during one year of storage in the unfrozen state. The obtained PFC/PL nano-dispersion conformed to medicine-biology employment.

1. Introduction
Blood transfusion may cause risks of emerge of infection diseases. The creation of artificial blood substitutes with effective gas-transport function is actual task of modern medicine.

Perfluorocarbons (PFCs) contain large amounts of various gases including oxygen and carbon dioxide. There are many publications [1-4]about chemical and physico-chemical properties of PFCs that are used in biology and medicine for elaboration of different emulsions. The authors pay particular attention to the peculiarities of molecular structures of these compounds. That molecular structure brings about increased physical solubility of gases in PFC without the formation of intermolecular chemical bonds PFC - gas.

Nano-dispersions of perfluorocarbon (PFC) or PFC emulsions are basis of oxygen carrier blood substitutes. There are several elaborated PFC preparations, in which nature phospholipids as emulsifier have been used: – Oxygent, Oxyfluor, Oxycyte. Differ adverse reactions were observed during preclinical trials of the preparations according to GMP demands [5]. The reasons of these side effects are not fully understood. There is question about improving the quality of the final product – the PFC emulsion preparations [2]. The assertion have been stated that progress in studying of the quality and stability of PFC emulsions was related to the increase of notions about their structure [6].

It is more correctly to represent PFC nano-dispersions as red cell substitutes because they do not replace all components and functions of blood (coagulation factors and white cells) [5]. PFC particles are a model of erythrocyte indeed because they have the structure of two-layer sphere [7]. In the sphere centre is PFC – the nucleus of particle, where dissolves blood gases. On the surface of nucleus there is the thin layer of surfactant, the shell. Surface properties and size of two-layer sphere particles characterize stability of their structure.

PFC emulsions (nano-dispersions) are the basis of pharmacology preparations. The stability of PFC emulsions and their behavior in a vascular bed depend on a surface layer firmness of surfactant around
the particles. The study of solely a particle size of these dispersing preparations is not enough to judge of their stability and quality. It is essential to have simultaneously information about the particle size and structure changes, taking place in these media.

2. Materials and methods
The nano-dispersions were prepared by high pressure method. Fluorocarbon phase was perfluorodecalin (PFD) with additions of other known PFCs (perfluorotripropylamine and perfluorooctylbromide) in an optimal ratio. The PFC mixture was dispersed at a high pressure Gaulin-type homogenizer. Soybean (or egg yolk) phospholipids in the form of some complex aqueous dispersion was used as surfactant.

The particle size was found by turbidity spectrum. Surface properties of particles were characterized using indirect physico-chemical parameter [8] – the coefficient of PFC nanodispersion interaction with blood serum: \( K = \tau_1/\tau_2 \), where \( \tau_1 \) – turbidity of the mixture of blood serum / nanodispersion (experience), \( \tau_2 \) – turbidity of a mixture of blood serum / saline (control). Ratio of components in both mixtures "experience and control" was the same.

3. Results and discussion
PFC molecules do not form complexes with blood gases. Therefore, the preservation of the corpuscular nature of PFC particles (the structure) under the circulation in the bloodstream is the main condition to fulfill gas transport function. The decisive role to ensure the PFC emulsion stability in vitro and in vivo belongs to the surface surfactant layer around the particles. This layer became loosened as a result of molecular diffusion (Ostwald ripening or isothermal distillation), which is the main mechanism of destruction of PFC emulsions during storage [9]. Molecule PFC passage through the disperse medium from small particles to larger ones. At the same time the particle size can vary a little, but the surface properties change significantly. Into the bloodstream, the particles with the loosened surfactant shell will be more intensive to interact with the plasma macromolecules as compared to the original emulsion (Figure 1).

**Figure 1.** Schematic representation of behavior of PFC particles with strong (A) and loosen (B) surfactant layer when they are infused into the bloodstream. I – state in vitro; II – status in vivo (bloodstream). A – adsorption of plasma macromolecules is negligible. B – absorption of macromolecules is significant.
It was shown, that the turbidity of blood serum/PFC emulsion mixtures is not additive in contrast to the turbidity of blood serum/saline and saline/PFC emulsions mixtures. These differences indicate the interactions between two systems – blood serum and PFC emulsion. We proposed to evaluate the change of surface properties of PFC nano-particles using indirect physico-chemical parameter $K$ – the coefficient of PFC nano-dispersion interaction with blood serum as a model system [8, 10]. This parameter reflects the response reaction extent of nano-particles with plasma macromolecules.

Values $K$ for the emulsions of different compositions was different and the relationship between the amount of bound protein on the particle surface and $K$ parameters was established (Figure 2) [10].

![Figure 2](image.png)

**Figure 2.** Values of coefficients of interaction of PFC emulsions of different composition with blood serum.

$a$ – serum / emulsion ratio = 1 / 0.05, $b$ – serum / emulsion ratio = 1 / 0.10

The stable nano-dispersions on base of PFC and phospholipids (PL) have been prepared. These PFC/PL nano-dispersions conformed to the essential requirements for model of pharmacological preparation: the heat sterilization of the final product and the structure preservation of particles: size (~100 nm) and surface properties ($K$) during one year of storage in the unfrozen state. The relative viscosity (relative to water) prepared nano-dispersions did not exceed the critical values of plasma viscosity (1.5 – 1.75) [11].

On a large experimental material is shown that the $K$ values for different series nano-dispersions PFC/PL of the same composition fluctuate within a narrow range and did not change after one year storage in the unfrozen state – unpublished results [12] (Figure 3).

The obtained PFC/PL nano-dispersions conformed to medicine-biology employment. The constancy of surface properties and particle size of PFC nano-dispersions in vitro reduces significantly the probability of manifestation of adverse reactions in vivo.

We did not encounter such a detailed study of the physico-chemical properties of different PFC emulsion preparations on the stage of pre-clinical study. Data on the study of PFC particle surface properties for the known blood substitutes – Oxygent, Oxyfluor, Oxycyte are not described in the literature.

Obviously, it is necessary before the clinical study of such complex PFC emulsion preparations have advanced information about their physical-chemical parameters characterizing their quality: the
average diameter of particles and particle size distribution, integrity of PFC particle structure, viscosity, stability and others. The data about the structure integrity of particles and the use of indirect physico-chemical parameter $K_\tau$ can help to solve technological problems of manufacture of new effective PFC emulsions.

Figure 3. Values of coefficient of interaction with blood serum ($K_\tau$) for PFC/PL nano-dispersions. 
A – $K_\tau$ values for different series of nano-dispersions of the same composition. B – $K_\tau$ values for the same series of nano-dispersions during different periods of storage in unfrozen form.

4. Summary
Evaluation of the integrity of the structure of pharmaceutical preparations based on PFC nano-dispersions (PFC emulsions) is a prerequisite for the conclusion about their stability. The structure is characterized by size and surface properties of particles.
The indirect physico-chemical parameter $K$ – coefficient of PFC emulsions interactions with blood serum have been proposed. $K$ reflects the dynamic of change or constancy of the surface properties of PFC emulsified particles.

The nano-dispersions on base PFC and phospholipids have been prepared. These nano-dispersions conformed to the necessary requirements for a model of pharmacological preparations: heat sterilization of the final product and preservation of the particle structure integrity (size and surface properties) during to one year of storage in the unfrozen state.

Acknowledgements
I wish to express my appreciation to all my co-authors.
This work was supported in part by NPO “BioMed-Nd” Samara.

References
[1] Riess J G 2001 Oxygen carriers ("blood substitutes") - Raison d'Etre, chemistry, and some physiology Chemical Reviews 101 2797-2919
[2] Riess J G 2005 Understanding the fundamentals of perfluorocarbons and perfluorocarbon emulsions relevant to in vivo oxygen delivery Art. Cells, Blood Subs., and Immob. Biotech. 33 47-63
[3] Riess J G 2006 Perfluorocarbon-based oxygen delivery Art. Cells, Blood Subs., and Immob. Biotech. 34 (6). P. 567-80
[4] Krafft M P, Riess J G 2009 Chemistry, Physical Chemistry, and Uses of Molecular Fluorocarbon-Hydrocarbon Diblocks, Triblocks, and Related Compounds-Unique "ApbLar" Components for Self-Assembled Colloid and Interface Engineering Chemical Reviews 109 1714-92
[5] Cohn C S and Cushing M M 2009 Oxygen therapevtic: perfluorocarbon and blood substitute safety Crit. Care Clin. 25 399–414
[6] Kuznetsova I N 2003 Perfluorocarbon emulsions. Stability in vitro and in vivo.(View) Russian Chemico-Pharmacetical J. 37 20-5
[7] Kuznetsova I N, Bezrukova A G, Lopatin V N and Parshin A V 1988 On determination of refractive index and shell thickness of the perfluorocarbon particles of dispersed blood substitutes Russian Biophysic 33 126–9
[8] Kuznetsova I N, Gokhman N Sh and Lavrova Y V 1993 Interaction of perfluorocarbon emulsion particles with blood serum Russian J. Physical Chem 67 1884–8
[9] Trevino L, Sole-Violant L, Daumur P, Davallet B, Postel M and Riess J G 1993 Molecular diffusion in concentrated fluorocarbon emulsion and its effect on emulsion stability New J. Chem. 17 275–8.
[10] Kuznetsova I N and Rybakova L P 2007 Blood serum protein sorption on the surface of perfluorocarbon emulsion particles Russian Chemico-Pharmacetical J. 41 40–3
[11] Kuznetsova I N, Yurchenko V S and Kochetkova G A 2009 Physico-chemical parameters of perfluorocarbon emulsions of different osmolarity Russian Chemico-Pharmacetical J. 43 34-40
[12] Kuznetsova I N, Yurchenko V S and Kochetkova G A (in print) The stability of nano-dispersions on base of perfluorocarbon and phospholipids Russian Chemico-Pharmacetical J.