GAVIN PUBLISHERS

Archives of Natural and Medicinal Chemistry

Kuznetsova IN. Arch Nat Med Chem 4: 120.

Review Article

DOI: 10.29011/2577-0195.000020

Functional Activity and Quality of Nano-Dispersed Preparations Based on Perfluorocarbon

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Citation: I N Kuznetsova (2019) Functional Activity and Quality of Nano-Dispersed Preparations Based on Perfluorocarbon. Arch Nat Med Chem 4: 120. DOI: 10.29011/2577-0195.000020

Received Date: 05 December, 2018; **Accepted Date:** 11 February, 2019; **Published Date:** 18 February, 2019

Abstract

The central aim of PFC emulsion application under circulation in the bloodstream is to reduce hypoxia by improving oxygen delivery to tissues. The methods for comprehensive assessing of gas-transport properties of PFC emulsions have been offered. The quantitative physical-chemical and biophysical parameters that are characterized quality of disperse PFC drugs have been elaborated. Analysis of gas exchange and oxygen delivery to tissues in the living organism showed, that PFC emulsion is the correction mean of gas transporting property blood. The cause is increasing of the diffusion rate of O_2 molecules from red blood cells through plasma to tissues and CO_2 molecules in opposite direction.

Introduction

The treatment scheme improving of different hypoxic states and the creation of new infusion fluids, capable of oxygen delivering to tissues is one of the most pressing problems in biology and medicine. The ability of Perfluorocarbons (PFCs) to dissolve of large amounts of gases and chemical inertness of these compounds is the foundation for the development and creation (on their basis) of blood substitute with oxygen transport function. The development of this problem was begun in the last century (the sixties-seventies). So, we can assume that this direction is a young field of science that produces a new type of knowledge, formulation of questions, assumptions, conclusions. It should be noted the most significant from the many special matters:

- Role of particle size into PFC emulsions. Presence of large particles (greater than 0.4 microns) increases their toxicity [1].
- Importance of degree of PFC purity for biology and medicine. The presence of impurities into perfluorocarbon liquids has toxic effects on animals at the cellular level. In Russia (USSR), the comprehensive program "PFCs in biology and medicine" under the guidance of G.R. Ivanitskiy was created [2]. The technology for producing of some number of PFCs for medicine have been developed.
- Choosing of new emulsifiers - natural phospholipids, which allow sterilize finished PFC emulsions and store them in non-frozen state [3].
- Creation of PFC drugs using synthetic water-soluble or mixed emulsifiers Fluosol (Japan), Perftoran and Perfukol (Russia) and study of their properties [3-5].

In the previous review the author [6] paid attention to works of Japanese scientists [7], which have been proved:

- Thin physico-chemical mechanisms of capture and release oxygen by PFC particles;
- The saving of principles of gases physical solubility not only in PFC liquid, but in emulsion form;
- and (the most importantly) the depending on the shell thickness of the emulsifier from the nature of surfactant and PFC for various emulsions.

This circumstance must lead to some different degree of emulsion stability and the different surface properties of particles. These received results are largely based on the theoretical concepts of Physical and Colloid Chemistry. Later, the results were confirmed in animal studies, (experimental study): more rapid clearance from the bloodstream the emulsifier in comparison with PFC core [8-11], sorption of plasma macromolecules (phospholipids and proteins)

the surface particles [12,13]. These results have suggested about the need to have additional prognostic criteria of emulsion stability under storage *in vitro* that must be broader than the preservation of particle size. Japanese scientists also carried out experiments on blood exchange [7]. They were selecting the concentration of perfluorocarbon phase for Fluosol-DA. They were taking various concentrations of PFC phase from 15% up to 35% with 5% step between these values and determined that 20% concentration was as the most optimal. When rats 'blood was exchanges with emulsions with various of PFC concentrations, contents of oxygen capacity in rats' blood was almost similar. With that, rats' life span was longer, when emulsion, containing 20% of PFCs was used. This means that physiological effect is not connected with only oxygen capacity of emulsion, but depends on other properties, i.e. its quality.

Unfortunately, a comprehensive analysis of published results in the creation of other PFC drugs was replaced by a mechanistic point of view [14]. The proposition that a larger proportion of the fluorocarbon phase content into emulsion (which means larger oxygen capacity OC) will better provide oxygen delivery to tissues. This proposition was the base for new PFC preparations. Such drugs including Oxygent, Oxyfluor, Oxycyte, were established by American firms with Phospholipids (PHL) as an emulsifier. Clinical trials to prove their effectiveness in accordance with international requirements of GMP, revealed the side effects [15-17]. The causes of the phenomenon are not established. The preparations said (above) did not come out on medicine market. Thus, the development of the problem was clashed with formidable obstacle. Currently work on the creation of basic PFC drugs as oxygen carriers for medicine almost have stopped. Opinion about the decisive role of OC for drugs of this type does not include (rather exclude) general biological view on this problem and does not take into account the behavior of PFC emulsion as a blood substitute in a living organism.

At first: - Functional activity of the PFC emulsion *in vivo* is determined by the circulation duration of Nano-particles in the bloodstream together with red blood cells, i.e., no particles circulate independently from other blood cells. Therefore, gas transport properties of PFC emulsions should be considered in connection with the gas transport properties of blood.

Secondly: - The PFC particles have their own size and structure. Being introduced into the bloodstream the particles contact with blood cells. However, blood is a living system. And the particles as foreign substances (PFC emulsion) occupy their own volume. So, they can be cause unpredictable changes in complex intercellular interactions in mixed medium "blood / PFC emulsion".

Thirdly: - Dispersed PFCs are not metabolized in the body, and are derived from the organs and tissues by the exhausted air in chemically unaltered state. Thus, the PFC particles are foreign bodies in relation to the whole organism.

Obviously, at the present stage of biomedical science (and medicinal chemistry) development, we need to look for multivariate answer to the question: what should be the PFC blood substitute. It is necessary in succession to consider several complicated interconnected layers. The analyzing of which (through which) permits us to bring closer to the answer to this question. The author of this review has identified the target setting for the drugs on the basis of dispersed PFCs. The creation of the based emulsion will conform to the following requirements: to improve oxygen delivery to tissues at the joint circulation of PFC particles with erythrocytes. This target setting is the real problem of transfusiology: to improve body function by deliberate action on the blood properties by means of introducing of infusion fluids.

Comprehensive Physical-Chemical Estimation of Blood Gases Transport by PFC Emulsions

Consideration of PFC emulsions efficiency is carried out largely by one-sided. The researchers take into account mainly the amount of oxygen contained into PFC emulsions, and its contribution to the common oxygen system transport. Such an approach is obvious, but not sufficient. They do not appreciate the much solubility of carbon dioxide into PFC liquids [18]. Process of oxygen supply to tissues is closely related with excretion of carbon dioxide as a regulator of many physiological parameters. We have been proposed the comprehensive assessment of specific (gas transportation) activity of PFC emulsions [19]. In the literature, these issues are not reflected.

It is necessary to know content of PFC phase in emulsions in volume units - C_v (ml/dl). This is explained that gas solubility into liquids has the same units of measurement as Bunsen coefficient -α - ml gas/ml.

In accordance with a generalized law of Dalton Henry the content of O_2 or CO_2 to PFC liquids and PFC particles is proportional to their partial pressure and does not depend from the solubility of other gases. Therefore, the oxygen content C'_{PFC} (ml O_2/dL) and carbon dioxide C''_{PFC} (ml CO_2/dL) in the fluorocarbon phase of emulsion can be calculated according to the formulas (2.1) and (2.2):

$$C'_{PFC} = C_v \cdot \alpha'^{o2}_{PFC} \cdot pO_2/760 = pO_2 \cdot C_v \cdot q'_{PFC} \quad (2.1) \cdot$$

Where pO_2 - partial pressure of oxygen mm Hg; C_v - PFC content in the emulsion (or blood), (PFC ml / dl); α'^{o2}_{PFC} - Bunsen solubility coefficient for oxygen in the PFC at a partial pressure of 760 mm Hg; $\cdot q'_{PFC}$ - the so-called 'reduced' solubility coefficient of oxygen in the PFC (ml O_2) / (mL PFC).

$C''_{PFC} = C_v \cdot \alpha''^{co2}_{PFC} \cdot pCO_2/760 = pCO_2 \cdot C_v \cdot q''_{PFC}$ (2.2)

Where pCO_2 - partial pressure of carbon dioxide in the emulsion (or blood), mm Hg; C_v - PFC content in the emulsion/blood (PFC ml/dl); α''^{co2}_{PFC} - Bunsen solubility coefficient for carbon dioxide at a partial pressure of 760 mm Hg (ml CO_2 / ml PFC); q''_{PFC} - 'reduced' CO_2 solubility coefficient in the PFC (ml CO_2) / (mL PFC) • mm Hg.

Values of $\cdot q'_{PFC}$ and q''_{PFC} are respectively in the range (5,0÷6,5) 10^{-4} for O_2 and (1,4÷2,5) 10^{-3} for CO_2. Evaluation of the contribution of PFC emulsion in the common transport of carbon dioxide *in vivo* using the formula (2.2) is wrong. The CO_2 contribution in the blood (in contrast of O_2 to PFC and blood) is not only physically dissolved state. But also, it forms the complex buffer system: acid-base balance - pH, pCO_2, HCO_3-, BE, which is associated with the acidity of the medium. Therefore, it is necessary to take into account *in vivo* (experiment and control) other indicator - total carbon dioxide TCO_2:

TCO_2 (mmol/L) = [HCO_3-] + [H_2CO_3] + [CO_2 solubility.]

You can compare the amount of carbon dioxide carried by the blood and by the fluorocarbon phase of the emulsion *in vivo* using this parameter-TCO_2. Knowing the Avogadro's law, it is not difficult to transfer from one measurement of carbon dioxide C''_{PFC} (ml / dl) to the other units - $TCO_{2\ PFC}$s (mmol / L) [19]:

$TCO_{2\ PFC}$s (mmol / L): = C''_{PFC} 10 / 22.4 (2.3)

Where is- coefficient of 22.4 (ml) - volume of 1 Mmol of gas under normal conditions.

The proposed methodological approaches of evaluating the effectiveness of emulsion are based on an analysis of application rules of basic physical and chemical laws of gases solubility in liquids. This part of review is a fragment of the problem, the first step (the first layer) of its decision, when it is not considered (not included) gas transport mechanism *in vivo*. We used this approach to show their correctness in model conditions *in vitro* [20] and *in vivo* [21].

Quantity Physic-Chemical Criteria of Quality of Disperse Preparations on the Basis of PFC and PhL

PFC emulsions are hydrophobic meta-stable systems. A lot of energy is required for their preparation. Homogenizers of different marks are used to disperse PFC liquids. Different surfactants (or mixtures thereof) are employed to ensure the stability of emulsions formed during this process. The emulsifiers provide structural and mechanical barrier at the interface PFC/water phase of emulsions. Strength of surfactant adhesion on the PFC/H_2O interface maintains the individuality of particles, i.e. their structure. The notion about structure for PFC emulsions as medical drags has the fundamental importance.

About Structure of Perfluorocarbon Emulsions

PFCs have high solubility of oxygen and carbon dioxide without the formation of intermolecular chemical bonds "PFC - gas". 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. But the PFC emulsion introduction into the blood is stress factor for such colloid medium. The first, emulsion is under went dilution, that may weaken the bonds of surfactant molecules with the particle surface. And the second, the PFC particles interact with biological active macromolecules of plasma. Both of these factors can lead to some change in the composition of surfactant around the particles and have negative act on the stability of particle structure in the real conditions of their circulation in the bloodstream. As a result, the ability to transport blood gases by particles will be quickly neutralized.

For clearness

PFC particles are a model of erythrocyte indeed because they have the structure of two-layer sphere [22]. In the sphere center is PFC-the nucleus of particle, where dissolve blood gases. On the surface of nucleus there is the thin layer of surfactant, the shell. PFC emulsions (Nano-dispersions) are the basis of pharmacology preparations. The stability of PFC emulsions and their behavior in 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. Surface properties and size of two-layer sphere particles characterize stability of their structure. Therefore, the elaborators of PFC medical drugs must have criteria that allow them to control both the size and the structure of particles during preparing, storage or under some model situations.

About Criteria of Integrity of Particle Structure

The analysis of behavior PFC emulsions *in vivo* has shown that the quality criteria of this infusion media should include the preservation of size and surface properties of emulsified particles. The quantitative evaluation of surfactant layer cohesion is difficult because of the uncertainty of its constituent parts: the molecular structure peculiarity of the surfactants, mutual orientation and positioning of surfactant molecules in the shell, evaluation of contact strength of surfactant molecules with the particle surface, surface topography, etc. There is no universal method to obtain the complete information about the properties of adsorption layer of the shell. It is Requires independent methodological approaches to overcome this uncertainty.

It is necessary the complex Physico-Chemical parameters that would allow to judge about the structure integrity of particles

during the development of new PFC drugs. Fine-dispersed PFC emulsions are ill defined turbid media. The process of PFC dispersing using PL as surfactant is very complicated because both components did not practically dissolve in water [23-26]. Therefore, it is necessary the resulting emulsion must be characterized by the complex of Physico-Chemical parameters that can adequately reflect its quality.

The methodical approaches for assessing of PFC particle structure integrity, and their quality have been elaborated and published [6]. These criteria must include such parameters as:

Size and particle size distribution

This is a common approach. Use variety of methods and devices.

Homogeneous nature of all particles in emulsion

PFC liquids are practically insoluble in water and is a poor solvent for a large number of substances (including PL) [24-26]. As a result, two different types of particles may be present into emulsion: particles with strong attached FL to fluorocarbon core of particles, and free or liposomal FL into dispersion phase. Free or micellar PL, as more light forms on specific weight compared with PFC, emerge up under centrifugation. This technique centrifugation of obtained sample of any new emulsion can be considered as a test approach that help empirically to select the emulsion composition on the first phase of drug development, as well as modify the technological method of PFC dispersing.

The next step. Experimental and calculated values of turbidity τ must be found (τ exp. and. τ calc.) for emulsions at different periods of observations:

$$\tau \text{ exp.} = 2{,}3\ D / l;\ \tau \text{ calc.} = \sum Vi\ \tau i\ (\sum Vi = 1)$$

Where-D and l are optical density a path optical length in given medium, respectively;

τi and Vi are turbidity and volume fraction of i separated part of the emulsion after centrifugation for evaluation of its optical parameters.

Good agreement between these values (τ exp. and. τ calc.) testify to preservation of the corpuscular nature of the emulsion particles.

Integrity of Particle Structure (corpuscles), which is Determined by the Conservation Both Size and Surface Properties of Emulsified Particles

It was said, there was no universal method to obtain the complete information about the properties of adsorption layer of the shell. But indirect method has been elaborated. It was established, 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 are indicated by interactions between two systems - blood serum and PFC emulsion [27]. We proposed to evaluate the change of surface properties of PFC Nano-particles using indirect physico-chemical parameter K_τ - the coefficient of PFC Nano-dispersion interaction with blood serum as a model system: $K\tau = \tau_1/\tau_2$, where τ_1 - turbidity of the mixture of blood serum/Nano-dispersion (experience), τ_2 - turbidity of the mixture of blood serum / saline (control). Ratio of components in both mixtures "experience and control" is the same.

Description of the details and an explanation of some details of these methodological approaches using are presented in the review [6] and articles [28-29].

Solidity of Surfactant Shell (the structure) *in vitro* and Side Effect of PFC Emulsions *in vivo*

The predisposition of PFC emulsions to cause undesirable side reactions is not fully established. The manifestation of adverse reactions may be associated with an additional interaction (receptor character is most likely) of drug particles with biological fluids: blood, plasma, blood cells and other organs. The basic mechanism of PFC emulsion destruction is the process of molecular diffusion (or isothermal distillation or by Ostwald ripening) [30,31]. In this process, PFC molecules pass through the aqueous disperse medium from the small particle to larger ones. Surfactant layer around the particles became loosened. In consequence the thin changes of surface properties of particles will take place *in vitro*. Relative change (increment) of surface properties and particle size will be different.

Because difference in sizes of PFC molecules (10^{-10} m) and PFC particles (100 nm or 10^{-7} m), the surface properties will change significantly, but the particle size will vary a little. The display degree of particle interaction with the molecular structures of plasma (adsorption) may vary due to changes in their surface properties. The particles with loosened surfactant layer (due to molecular diffusion) being injected into the bloodstream will be more intensively interact with macromolecules of plasma in comparison with the original emulsion. (This process is shown schematically in Figure 2 [6-29]). Increasing of adsorption layer strength (increased strength of the structure) will slow down the process of molecular diffusion *in vitro* and reduce the likelihood of side effects *in vivo*. Thus, the term "structure" for PFC emulsions is factor that characterizes the implementation of gas transport function by PFC particles in conditions *in vivo*, i.e. quality. Preserving of the structure integrity can be considered as the next (second) step of evaluation of their quality.

About Mechanism of Gas Transport by Erythrocytes and PFC Particles

During establishment of the problem on different early

stages of its development, the efficiency of PFC emulsions was proven in model of bleeding animals and organ perfusion: exchange replacement of blood upon reduction of hematocrit (Ht) level by four or more times or perfusion of isolated organs [32]. Clearness of these experiments considerably simplified the nature of the problem and facilitated formulation of idea concerning the decisive role of absolute values of Oxygen Capacity (OC) as the factor determining the efficacy oxygen- transporting blood substitutes.

Gas transporting properties of PFC emulsion and blood is usually characterized by considering their dissociation curve: dependence of quantity oxygen contained in the test mediums (emulsion and blood) from their oxygen partial pressure. Oxygen solubility in PFC emulsions is directly proportional to the gas partial pressure, as chemical bonding "PFC↔gas" is absent. This is linear dependence. Hemoglobin binds O_2 though (by means of) a strong covalent bond to the erythrocyte iron. So, this dependence is sigmoid curve [14]).

The main! These dissociation curves represent the quantitative aspect of oxygen content in experiments *in vitro*. They do not take into account the conditions of oxygen delivery *in vivo*. According to the general biological approach, the increase of fluorocarbon phase in some PFC preparation for increasing its Oxygen Capacity (OC) meant an increase of affect (impact) of foreign medium (emulsion) on a living system (blood). Increase OC emulsion will not lead to an increase of oxygen amount that delivered to tissues, because the mechanism of oxygen transport is not considered. But the central purpose of PFC emulsions using in the real conditions of their circulation in the blood stream (co-circulation with erythrocytes) is to reduce hypoxia by improving of oxygen delivery to tissues. Therefore, the high OC values for medical PFC drug *in vitro* can't be the basis for the automatic transfer of the property on the whole organism.

Regularities of Gas Transport by Erythrocytes and PFC Particles under their Joint Circulation Effect of PFC emulsions on kinetic of gas transport by erythrocytes

The process of gas delivery by blood is determined by micro-kinetik, i.e. the diffusion rate of O_2 molecules through the plasma from red blood cells to the tissues and CO_2 molecules in the opposite direction. Schematically, the mechanism is described in various manual (handbooks) [33]. The magnitude of average particle diameter (100-200 nm) is an order (or more) smaller than the size of blood cells (erythrocytes, leukocytes, and platelets). The particles occupy certain volume on the diffusion way of O2 and CO2 molecules under co-circulating with erythrocytes. Thus, they can change the delivery conditions of the blood gases increasing or decreasing their rate of diffusion. This circumstance should effect on the conditions of oxygenation of red blood cells. Earlier Japanese scientists [34-36] have been shown that (the first) the layer of the emulsifier on the particle surface (shell) is not a barrier for passage of oxygen and (the second) the gas outlet from the particle is determined by its internal diffusion. Consequently, the PFC particles should increase the rate of oxygen diffusion in the aqueous medium (plasma). And thus, the rate of oxygenation and de-oxygenation of red blood cells must increase.

The erythrocyte oxy- and de-oxygenation kinetics and the influence of PFC emulsions on blood were studied using the devise modeling blood circulation in organism [37-38]. The hematocrit values (Ht) in the experiment (addition of the emulsion) and in the control (addition of the emulsifier) were 25-30% (hemodilution model). The content of emulsion in the experiments was~1 vol.%. The rate of oxygenation (deoxygenation) of erythrocytes dα/dt as a function of the degree of oxygenation α has been studied. It is shown that PFC emulsion of in the joint circulation with blood increases both the oxygenation rate and the rate of deoxygenation of erythrocytes (Figures 1,2). Thus, the presence of a small volume of emulsion of PFC in the blood increases the diffusion rate of O_2 molecules in the path erythrocyte-plasma-tissue. The influence of PFC emulsions in the bloodstream embolism and improve oxygen transport by blood cells *in vitro* have been noted in later works [39- 40].

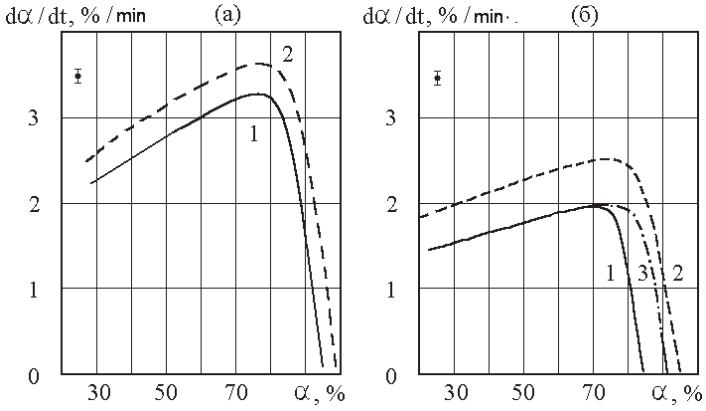

Figure 1: Oxygenation rate dα/dt vs. oxygenation degree α of blood with low ψ* potential (a) on adding physiological solution 1 (control), and PFC emulsion-2; Oxygenation rate dα/dt vs. oxygenation degree α of blood with high ψ* potential (б) on adding physiological solution 1 (control), PFC emulsion-2, emulsifier solution -3; *ψ –the erythrocyte transmembrane electrostatic potential [38].

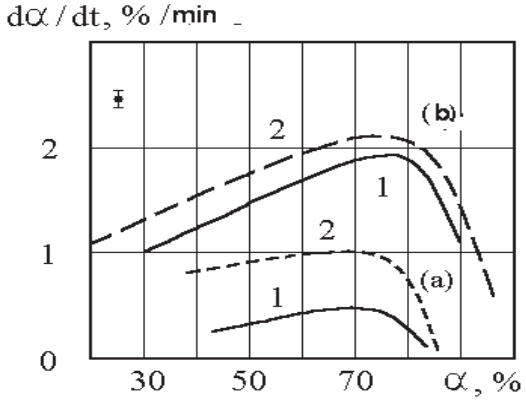

Figure 2: Deoxygenation rate dα/dt vs. oxygenation degree α of blood with high ψ* potential (a) on adding physiological solution 1 (control), and PFC emulsion-2; Deoxygenation rate dα/dt vs. oxygenation degree α of blood with low ψ* potential (b) on adding physiological solution 1 (control), and PFC emulsion-2; *ψ –the erythrocyte trans-membrane electrostatic potential [38].

About PFC emulsion influence on gas transport in organism

Diffusion of gases (oxygen) is some passive process. Its rate is depended from gradient concentrations. Analysis of transport gas in the tissues of the living organism has shown the next: it is necessary to take into account lightweight diffusion in addition to the physical diffusion. It is defined by an increased coefficient of solubility of the substance (oxygen) in a certain area of tissue or membrane [33-41]. Analyzing the gas exchange in the body (complicated object), the Physiologist and Biophysics take into consideration diffusion coefficient (D) together with coefficient of gas solubility (α), i.e. constant of diffusion on the Krogh (K_a) or constant of permeability:

$$K_a = 60 \cdot D \cdot \alpha \ [cm^3\ O_2 / (cm \cdot min \cdot atm.]$$

Where 60– coefficient of transition from seconds to minutes;

α - Bunsen solubility coefficient – (the unit volume of gas per unit volume of the liquid at partial pressure atm.);

Practically K_α - the mass transfer (mass moving) coefficient, has a dimension of mass moving coefficient (a quantitative measure of the diffusion of the unit volume of gas at a distance of 1cm for 1 minute at a partial pressure of atmosphere).

Our calculations showed that K_α magnitude for O_2 and CO_2 in PFC liquid is higher almost an order in comparison with H_2O (Table 1).

Liquid	Gas	α, cm^3 (gas) / (cm^3(l) ·atm)	$D \cdot 10^{-5}$ cm^2/c	K_a cm^3 (gas)/ (cm·min ·atm)
PFT-BA*	O_2	0,37	2,0	$4{,}4 \cdot 10^{-4}$
	CO_2	1,42	1,3	$1{,}1 \cdot 10^{-3}$
H_2O	O_2	0,023	3,0	$5{,}0 \cdot 10^{-5}$
	CO_2	0,7	1,8	$7{,}6 \cdot 10^{-4}$
*perfluorotributylamine				

Table 1: Coefficient of solubility (α), Diffusion coefficient (D), Constant of diffusion on the Krogh (K_a) [42-45].

Therefore, the presence of PFC particles in blood or other biological medium will increase the mass transfer rate of gases. From this position, the increasing of oxygenation/de-oxygenation rates of erythrocytes is a consequence of the increase of mass transfer of oxygen in mixed media: "blood / emulsion". From the point of the Physiologist there are some physical-chemical mechanisms of functional activity emulsion.

It is known that the diffusion coefficients for colloidal particles are smaller on 2-4 orders in comparison with molecules. Therefore, emulsified PFC particles in an aqueous medium can be considered as a stationary medium from the point of view the molecular diffusion process for O_2 and CO_2 molecules. The PFC particles can themselves capture and deliver any gas in accordance with the difference in its partial pressure from the arterial end of vessel to the venous part, acting as a passive carrier. However, their role is not limited by this action. Direct exchange of gases between tissue cells and red blood cells is carried out by free O_2 and CO_2 molecules diffusing through the plasma. The diffusion resistance of gases by the erythrocyte membrane is small. Since the solubility of any gas in PFC is proportional to its partial pressure and does not depend on the solubility of another gas (Henry-Dalton law). So, PFC emulsion in the blood stream can be considered as a two-channel amplifier of O_2 and CO_2 flows.

The solubility of gases in PFCs is much bigger than in H_2O. At one level of the partial gas pressure, its content in PFC particles will be an order higher than in the equivalent plasma volume. PFC particles create an additional reservoir, an additional capacity, where a certain stock of blood gases is concentrated. Therefore, PFC particles are a damper for the blood gases. They can create an additional amount for O_2 when it is consumed and maintain a

higher level of pO_2 in the arterial blood, thereby creating a greater rate of O_2 diffusion to tissues. The illustration of biophysics processes of O_2 and CO_2 delivery by blood at the joint circulation of PFC particles and red blood cells is shown on Figure 3 [46,47].

This circumstance allows us to consider the PFC emulsion as a correction means of gas transport properties of blood, increasing the reserve possibility of red blood cells for the O_2 delivery to tissues.

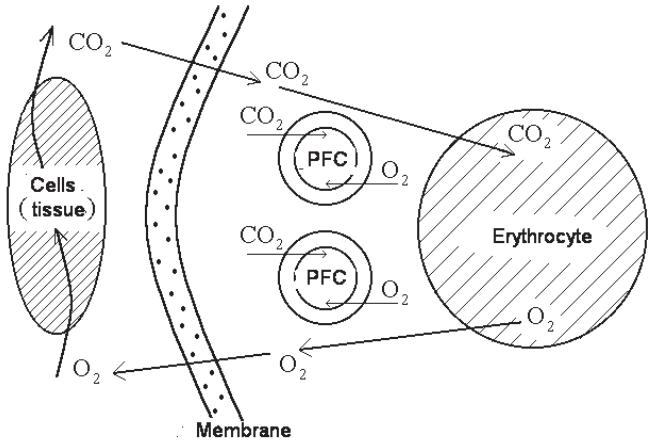

Figure 3: The illustration of biophysics processes of O_2 and CO_2 delivery by blood at joint circulation of PFC particles and red blood cells.

From the point of the Physiologist there are some physical-chemical mechanisms of functional activity emulsion. The particles carry out some next roles:

- Role of carrier of gases to proportional of partial pressure exchange from arterial to venous part of vessel;
- Role of intensifier of O_2 and CO_2 fluxes by increasing of theirs mass transfer (mass moving);
- Role of dumper{Damper} i.e. additional capacity for gases in plasma that create the support (the prop) for oxygen under its consumption.

Effects of PFC emulsions on blood rheology parameters

Therapeutic efficacy PFC emulsions depend on a favorable effect that they have on blood rheology. Japanese scientists showed 'Fluosol-DA' is non-Newtonian fluid as the blood, i.e. depend on various shear rate (τ). But the elevation of Fluosol-DA viscosity at lower shear rate is far lower than blood (Hct =46 %). Equal mixture of Fluosol and blood behaves in between the both. This fact as believe the scientists may indicate beneficial oxygen supply of blood/Fluosol-DA' mixture in peripheral vessel-capillaries. The specific task of the article [48] was to evaluate the effect of the relative concentration of PFC particles on the rheological parameters of the blood at a hematocrit (Ht or Hct) of 0, 25 (or 25 %). It turns out the in the non-Newtonian range of flow ($\tau \leq$ 0.6 Pa) asymptotic viscosity ($\tilde{\eta}$) of blood/emulsion mixtures rose nonlinearly with the volume ratio rose of PFC particles (C_v). While the $\tilde{\eta}$ of plasma emulsion mixtures varied only slightly. These data suggest that erythrocytes interacted with emulsion particles in the blood/emulsion mixtures, significantly affected on their rheological properties and about a capability to form aggregates.

The ability of erythrocytes to form stable aggregates was characterized by calculating the coefficient A according to the formula given by Merril [49].

$A=\tau_0/(Ht-Ht_0)^n$

Where τ_0 is the minimal shear stress required for initiating the blood flow at a given hematocrit;

Ht_0 is the hematocrit (0.04-0.05) at which blood flowing through a capillary behaves as a Newtonian fluid;

n is the exponent of the power function, empirically estimated at 3 in adults [49] (Table 2).

`	Medium						
	Blood Ht=0.49	Blood +emulsifier Ht=0.25	Blood+emulsifying+ emulsion Ht=0.25				
	0	0	0.86	1.72	2.86	4.3	8.6
A, Pa	0.27±0.01	0.060±0.01	0.05±0.01	0.07±0.01	0.09±0.01	0.11±0.01	0.71±0.01

Table 2: Coefficient for erythrocyte aggregation A in blood and its mixtures with emulsifying agent and with the emulsion added with various ratios C_v [47].

The erythrocyte aggregation coefficient A in blood mixtures with the relatively low emulsion content (0.86÷4.3 vol. %) was smaller than in the whole blood. At C_v = 8.6 vol. % the aggregation coefficient A of erythrocytes was significantly enhanced. We can only state that the emulsion should be used at low relative concentration to be efficient in improving the blood flow under some circulation disorders. Effect of new PFC emulsion on viscosity of plasma and blood in the presence of some blood expanders (under different Hct 40-13 %) have been studied [50]. The addition of PFC emulsion to blood hemodiluted with volume expanders significantly increased its viscosity (even at low Hct 13%). The selection of programs and treatments of various hypoxic conditions using PFC preparations depend on quality of PFC emulsions and their biocompatibility with blood expanders.

Impact of PFC emulsions on biophysical parameters of blood *in vitro* is modeling the behavior of mixed system "blood / emulsion" *in vivo*. These results reflect the qualitative and quantitative aspects of compatibility these mediums. This is the third step of quality evaluating of emulsions. This test has predictive power for the composition selecting of PFC drug, as well as some choice of optimum schemes of infusion therapy in experiment and clinic.

About Foreignness of PFC Emulsions

Nano-particles are a definite form of the product, separated from the medium by boundary. Depending on the nature of the substance, enclosed into particles-it may be an emulsion or suspension. The PFC particle dimensions are not more than 100-200 nm. The difference between the structure of the Nano-particles and the body's own molecules makes them alien. In terms of general biological lows Nano-particles are irritates of the immune system, which seek to remove them. Existing schemes of assess of immune system response to the introduction of different foreign particles was reflected in the thematic issues of the journal Current Bio nanotechnology.

The relationship between efficiency and foreignness of particles for PFC drugs has not been enough studied. The general scheme of PFC particles stay in the body is as follows: PFC particles is trapped by leukocytes, fall into various organs and output from theirs with exhaled air in a chemically unaltered state [51]. Experiments on animals have shown that neutrophils with vacuolated PFC particles preserve the ability to digest microorganisms [52]. However, the reaction of the Mononuclear Phagocyte System (MPS) on the introduction of PFC emulsions is not simple [53]. This reaction is biphasic [54,55]. Initial inhibition of macrophage phagocytic function is replaced by its activation. The duration and depth of the depression depends on the dose and composition of emulsions. Therefore, investigations that exclude the conducted steps of this process can lead to the opposite results. According to the calculations [46,47], the number of particles per unit volume of blood in standard organism is much more than the corresponding number of blood cells (by several orders of magnitude). Each cell, circulating in the bloodstream, is surrounded from one thousand to one million of PFC particles. As a result, the PFC particles will inevitably have an impact on the specific activity of blood cells circulating in the blood stream. This suggestion has been tested in a series experiments *in vitro* using the stable emulsion of PFC/PL. The surface properties and the particle size of emulsions do not change within one year of storage in the unfrozen state [56,57]. The erythrocyte morphology, function of neutrophiles and platelets, and the oxidative properties of blood were depended on emulsion dose in mix- medium "blood/emulsion" [58-61]. Discoid form of erythrocytes, bactericide and phagocyte function of neutrophiles was not changed under relations "blood/emulsion" = 100/1 ÷ 10/1. But the metabolic activity of neutrophiles (according NBT-test) was increased. The gradual increasing of echinocyte number, the NBT-test decreasing, and the non-completion of phagocytes were received when relations "blood/emulsion" was=10/2÷10/5) [57-61]. PFC emulsion was capable to increase (or decrease) initially normal (or activated) platelet aggregation [58].

These results showed that the blood cells retain their function if the entered volume of PFC emulsion in the biological mix-medium does not exceed~20-25%. Many questions of behavior of foreign particles in the living body are not resolved. Effect of PFC emulsions on morpho-functional state of white blood cells is a paramount response of the immune system on the infusion of foreign particles. This reaction can be minimized by reducing the dose. The security of medicinal product containing alien Nano-particles can't be solved when we use the standpoint of a single discipline. Analysis of PFC emulsion behavior with positions of the fundamental laws of chemistry and biology *in vivo* has been allowed us to extend the requirements to the number of controlled physical and chemical parameters *in vitro*, that are responsible for the drug quality, and thereby to reduce the likelihood of side reactions *in vivo*.

Conclusion

Based on analysis of published information, the author of this review proposes some another point of view on the evaluation of PFC emulsion functional activity. Some authors used the postulate which was based on a simple syllogism: the higher of Oxygen Capacity (OC) emulsions-the better Oxygen transport will be in organism.

In contrast, the position expressed by author is based on alternative concept: to evaluate the functional activity of PFC emulsions it is necessary to take into account the achievement of final goal-the delivering of blood gases up to cells and tissues. Only the understanding of the mechanism of action according to

biophysical and physiological laws *in vivo* will help to solve this goal. Another provision was also stated: the functional activity of PFC emulsions should be considered in connection with the gas transport properties of blood, i.e. in real conditions of PFC particle circulation in living organism. The requirement of the stability of particle structure (two-layer sphere coated by surfactant) is the cornerstone of the implementation of functional activity of emulsions *in vivo*. The elaborated methodology for assessing of PFC particle integrity is added by substantially the idea about the quality of these type medicines. In fact, these approaches are a private bioinformatics to create a particular product (PFC emulsion). The using of the set of experimental approaches allows us to work with the components of future medical PFC preparation and predict its effectiveness. The results, produced in the survey and their analysis have shown that the properties of mixed system ‘’blood / PFC emulsion’’ does not obey some simple additive rule: changes of physico-chemical and biophysical parameters of the mix system are not proportional of contents of its constituents. It was found that a relatively small amount of emulsion can change the delivery conditions of oxygen by erythrocytes, changing the following parameters:

- Increasing of diffusion rate of O_2 molecules from erythrocytes to tissues and CO_2 in the opposite direction;
- Increasing of rate of gas mass transfer (mass-moving);
- Changing (improving) of blood flow by reducing of cells aggregation (obviously).

The impact of PFC emulsions on functional activity of blood cells has a subtle nature. Her understanding is required of independent research. However, we can talk about the mutual consistency of PFC emulsion impact at different levels of organization in a living organism. Firstly, at the macro-level: changes in the physico-chemical and biophysical parameters of blood that available to our understanding at this stage of research. Secondly, at the micro level: dose dependent effect of emulsion on the functional activity of blood cells. Cells retain their function under the number reducing of particles contacting with their surface.

In both cases, the optimal emulsion dose is relatively low in comparison with the large common volume of blood in some person. This circumstance makes possible to reconcile opposing forces (counteracted connection) between the two inherent pharmacological properties of PFCs: efficiency, i.e. ability to dissolve large amounts of blood gases and foreignness, i.e. the inability to be metabolized in the body. Obviously, their unity requires to change principles of new PFC drug construction. Apparently, we can talk about the necessity to reduce the maintenance of fluorocarbon phase in some preparation, as well as to optimize the amount (dose) of infused emulsion. This final position is not exhaustive and involves the positing of special research. Success depends primarily on the technological decisions of preparing of strong stable (preserving the structure integrity) PFC emulsions.

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