ORGANO-FLUORINE COMPOUNDS AS ARTIFICIAL BLOOD SUBSTITUTE

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The rate of fluoro-carbon compounds as artificial substitute for blood has been discussed. Blood is a complicated body fluid. At first glance it seems impossible that such a complicated body fluid can be replaced by artificial synthesized fluids or any substitute for this can be synthesized. Fluoro-carbon compounds however have advanced research in this area because a number of fluoro-carbon compounds have been found to be highly inert and have high solubility for O_2/CO_2 and are able to transport O_2/CO_2 *in vivo*.

A number of fluorinating agents and processes are known for synthesis of fluoro-carbon compounds, but the following three methods are important [1,2].

Direct Fluorination with fluorine gas. A stream of fluorine gas diluted with nitrogen is reacted with the compound to be fluorinated at temperature about 130-300°C. Disadvantages of the procedure is that the reaction is highly exothermic and number of fragmented and partially fluorinated compounds are formed as by-products.

Cobalt trifluoride fluorination. In this method vapours of the compound to be fluorinated are diluted with nitrogen and reacted with cobalt trifluoride between 200-250°C. Reaction is less exothermic as compared to first one, but partially fluorinated compounds are produced as reaction by-products.

Electrofluorination method. This is the method most commonly used. It involves electrolysis of the organic compounds in anhydrous hydrofluoric acid.

Fluoro-carbon compounds are colourless, dense and apparently non-toxic liquids, having very low surface energies[3], low boiling points, etc. They have much lower surface tension than the corresponding hydrocarbons. They are very poor solvents and are insoluble in water and alcohols. They show high solubility for O_2, N_2, CO_2 and inert gases. It is the high solubility of O_2 in fluoro-carbon compound that has made *FC*-80, a fluoro-carbon compound, so useful in liquid breathing experiments.

Another unique property of organo-fluorine compounds is their chemical and thermal stability. The factors responsible are (1) *C-F* bond strength is 116 K. cal/mol. (2) shielding effect from the high electro negativity of the fluorine atoms. They are unaffected by boiling with conc. nitric and sulfuric acid and aqueous alkali. They react with H_2, Br_2 and Cl_2 only under pyrolytic conditions of 700°C or above. Certain other type of compounds having high solubility for O_2/CO_2 have also been studied for this purpose such as chelating agents of *Fe* and *CO*[3].

The term fluoro-carbon has wide meaning. Fluoro-carbon compounds used as artificial blood substitute can be designated as perfluorinated carbon compounds[4]. These are the fluoro-carbon compounds in which all the *H* atoms have been replaced by fluorine atom i.e. these have neither have *H* nor other halogen except the fluorine, although certain exceptions are there.

These liquid perfluoro-carbon-compounds cannot be used as such to substitute the blood as they cannot transport necessary water soluble salts and other metabolites i.e. they are unable to perform the function of blood plasma. They are used as substitute for erythrocytes.

Certain important properties which make these compounds useful as artificial blood erythrocytes are :

Efficient O_2/CO_2 *transport.* Excellent O_2/CO_2 solubility of perfluoro-carbon compounds has made them useful for transporting O_2/CO_2 *in vivo*. Certain perfluoro-carbon compounds have been found to

have even higher solubility for O_2 as compared to the natural blood erythrocytes for example *FC*-80 has about 10% more solubility of O_2. Experimental studies have, however, shown that capacity of artificial erythrocytes to deliver O_2 is less as compared to the natural one[5]. It is because the state in which O_2 is present in the two is different. In natural erythrocytes O_2 forms a stable but dissociable complex with haemoglobin while in parfluoro-carbon-compounds O_2 is encapsulated within the thin film of perfluoro-carbon compound. However, the solubility of O_2 is sufficient to perform the function of erythrocytes. Search of new compounds with even more solubility of O_2 may be more advantageous.

Biological inertness. Biological inertness is an essential requirement for perfluoro-carbon compounds to be used as artificial ertythrocytes. The higher the inertness of a compound the lesser will be its toxicity in the living system. Highly fluorinated and naturated compounds are found to be more inert[6]. Introduction of unsaturation [7,8] or other substitution such as methylation increases the toxicity of these compounds. Even the substitution of other halogens has adverse effect. Inertness of these compounds for blood components is an additional advantage because in number of cases these artificial substitutes are used alongwith natural blood, such as oxygenation of blood through artificial erythrocytes[9,10]. Two factors which facilitate the blood oxygenation are (*i*) High solubility of CO_2/O_2 in perfluorinated compounds, (*ii*) High pressure gradient between the blood and liquid membrane of perfluorinated compounds encapsulating O_2 gas[11] helps the diffusion of O_2 through the membrane to the blood.

Studies on interaction of fluoro-carbon compounds with blood *in vivo* as well as *in vitro* showed no significant alterations in the chemical composition of blood except some change in the coagulation factors[5,11]. It has been reported that the presence of inert particulate matter in the blood stream also led to agllutination of platelets[12] and the formation of pulmonary amboli and thrombi. Thus, although it is established that perfluoro-carbon compounds transport O_2/CO_2 *in vivo*, but their presence in considerable amount in blood usually causes unwanted phenomenon and death and thus they do not behave as completely inert. As described earlier, perfluoro-carbon compounds have very low surface tension and hence unusul surface properties. As the surface energy of the liquid perfluoro compounds is very low the dispersed particles having large surface area interact with the substances involved in the the blood coagulation.

Experiments carried out on the cause of death by the use of fluoro-carbons ruled out[13] two possibilities *viz.* (*i*) mechanical blocking by fluoro-carbons. (*ii*) release of histamine and serotonin.

Death due to agglutination of blood platelets was supported by the experiments carried out on frogs. As there are no platelets in frogs they survived after infusion of these artificial substitutes[13]. Death could also occur due to intravascular fibrin formation which is accompanied by a decrease in concentration of platelets. Experimental studies carried out on rabbits by infusion of the perfluoro-carbon compounds gave sufficient deviations in concentration of coagulation factor X, XI and XII from the normal.

Low Vapour pressure. Highly fluorinated compounds usually have low boiling points i.e. very high vapour pressure, which causes a great difficulty in using these compounds as blood substitutes because high volatality of these compounds interferes with the respiration and causes pulmonary gas embolism which can be observed by seeing the expanded lungs[14]. Perfluoro-octane and perfluorobutyl tetrahydrofuran have high vapour pressure, 50 and 51 respectively at body-temperature (37.5° C) and they caused death of warm-blooded animals when administered as intravenous emulsions[14]. This factor eliminated a number of perfluoro-carbon compounds to be used for this purpose. Compounds with lower vapour pressure proved successful for example perfluoro-decalin (V. P. 14 at 37.5° C) and perfluorotributylamine having (V.P. 2.5 at 37.5°C) gave good results. It might be possible that certain perfluoro-compounds having other required properties can be used by reducing their vapour pressure. V. P. of perfluoro-carbon compounds can be decreased by increasing their molecular weight by methods such as halogenation, methylation, etc. For example perfluoro-octane has vapour pressure 50 at normal body temperature while its brominated product perfluoro-octyl bromide has vapour pressure of only 14. Similarly perfluoro-decalin V. P. 14 at 37° C on methylation gives perfluoro-methyl-decalin having vapour pressure of only 5 at 37° C. But it was observed that these substitutions increase their toxicity. For example, perfluoro-octane 3 day LD 50 is about 30 ml/kg, while perfluoro-octyl-bromide has 3 day LD 50 about 150 ml/kg.

Dispersibility to form Emulsion. As discussed earlier, these perfluoro-carbon compounds cannot be used as such to substitute the blood, because of their inability to perform the function of blood plasma[5]. They are used as substitute to erythrocytes. It is necessary to prepare their emulsion before they can be used as artificial blood substitutes. Emulsion[15] consists of two immiscible liquids, one is present in the form of small droplets as internal phase also called as discontinuous phase, into the other, known as continuous or external phase[16]. Perfluoro-carbon compounds are present in the form of small droplets as internal phase. While the other that is external phase is aq. saline solution. In order to get a stable emulsion of these two immiscible liquids some stabilising agent is necessary which is known as emulsifying agent. An emulsifier may be defined as a chemical compound that reduces the interfacial tension between two dissimilar surfaces, thereby allowing a large increase in interfacial area. We can classify emulsions in two types depending on the size of droplets (*i*) microemulsion—particle size more than 1 μm but usually less than 1-2 μm and it is transparent (*ii*) macroemulsion—it is on opaque emulsion having particles of size about 2 μm as in case of natural erythrocytes. Erythrocytes are approximately 8-10 μm in diameter.

Important requirements of an emulsifier to be used for synthetic blood purpose are[17] :

(*a*) It should be non-toxic, that means, it should not be toxic to living system and it must not produce any toxic substance on aging in the body.

(*b*) It must be stable in aqueous solution at *pH* 7.4 and in physiological saline.

(*c*) It must be chemically inert or rather it should not interact with the components of the blood to disturb in its normal functions.

(*d*) It must be stable to O_2/CO_2 and

(*e*) It must be readily excreted from the body.

Egg phospholipids[14], because of their wide acceptance as intravascular lipid emulsifier and their ability to emulsify perfluoro-decalins, have been accepted as good emulsifiers for perfluorinated organo-compounds. Another good emulsifier is Pluronic[18] F 68, because of its ability to reproduce the oncotic pressure similar to that provided by blood proteins. Further the size of droplets in the emulsion also plays an important role. Fine emulsions have the advantage over the coarse, because the half life span of blood level of fine emulsion is far larger than that of coarse emulsion.

Emulsification is usually carried out by sonication. But the formation of emulsion by sonication led to the generation of fluoride ions[13]. This difficulty was overcome by providing CO_2 atmosphere which has a number of advantages[20].

(*a*) It provides an inert atmosphere and therefore diminishes possible oxidation of emulsifier.

(*b*) Being very soluble in the fluorocarbons it facilitates their emulsification perhaps by enhancing cavitation.

(*c*) Most important function of CO_2 is to prevent the generation of fluoride ions. It reduces the generation of fluoride ions by about 1/10 as compared to CO_2 free atmosphere.

Finally an important requirement in using these perfluorinated carbon compounds as artificial erythrocytes is their elimination from the body. It is seen that certain perfluorinated-carbon-compounds remain attached to the liver of the animal for many years, rather for life long. Thus the perfluorinated compounds which remain intact in the tissue cannot be used even as temporary substitutes. Taking this criterion *FC*-47, *FC*-43, PIB, PIID and Freon E series and number of others have been eliminated from further consideration[14]. A very successful perfluorinated carbon compound, perfluorotributyl amine[20] which has all the important requirements and forms a stable emulsion with pluronic-polyol had to be eliminated because of its retention in the body tissue for a very long time, may be even life long. Thus several perfluorinated carbon compounds have to be eliminated because of their lacking in one or other property.

Studies have been carried out on total replacement of natural blood by artificial preparation. A typical composition of the artificial blood currently being used for complete blood replacement studies is as follows :[20]

Fluoro-carbon *FC*-47	12 ml
Pluronic polyol	2.5 g
Hydroxy ethyl starch	3.0 g
Glucose	0.1 g
KCl	32 mg
$MgCl_2$	7 mg
NaH_2PO_4	9.6 mg
$NaCl$	54 mg
$CaCl_2$	18 mg
Na_2CO_3	to *pH* 7.44
H_2O	to 100 ml

Mixture is gassed with 95% O_2 and 5% CO_2 and final *pH* was adjusted to 7.4. Certain antibiotics such as streptomycin, penicillin and phenol red may be added. The oncotic pressure was adjusted by pluronic polyol and hydroxy ethyl starch equals to that of rat serum. Emulsification was done by sonication after providing CO_2 atmosphere, in order to reduce the fluoride ions generation. The only disadvantage of this preparation was the retention of *FC*-47 in the body tissue. It was suggested that certain changes in the above formulation and decrease in the size of perfluoro-carbon-compound may be helpful. In the above sample particles diameter was between 1.0 μm to 1.5 μm. But the retention of its perfluorinated compound was not found to produce any abnormality, however long retention in the tissue may produce certain abnormalities.

Another important compound perfluorodecalin has the advantage over *FC*-47 in that it is eliminated very soon from the tissue but on the other hand it is very difficult to prepare its stable emulsion. Egg phospholipid was used for preparing its emulsion[21]. An emulsion of 25% wt/vol perfluorodecalin, with 4% phospholipid was prepared. It contained average size particles of 1.25 μm containing about 30% of particles larger than 2 μm. It was not found stable at body temperature. If perfluorodecalin can be emulsified it will be the most successful compound of all the known compounds till today.

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