A new formulation for blood substitues

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ABSTRACT

The synthesis of a novel microemulsion system composed of a fluorinated oil C_8F_{17}-CH_2-CH=CH-C_4H_9 with a biocompatible hydrogenated surfactant, Montanox 80, is described.

Investigation of the solubility of oxygen in these microemulsions showed that they absorbed more oxygen than Fluosol-DA, currently used as an oxygen transporter in biological systems. Oxygen absorption was comparable to that of blood.

Light scattering studies showed that these systems are composed of small-sized aggregates which should, in principle, be compatible with blood. The toxicity of the oil C_8F_{17}-CH_2-CH=CH-C_4H_9 was tested after intraperitoneal injection in rats. No toxic effects were observed at doses up to 5 g/kg.

This study opens new perspectives for the development of oxygen-transporting compounds for biomedical applications.

INTRODUCTION

The supply of human blood for transfusion and other medical applications is now faced with new exigencies of biocompatibility and sterility, as well as those inherent in the economics of the collection and storage of samples. Recent concern with AIDS has highlighted the well recognized problem of viral contamination of blood, especially that due to viral hepatitis.

The choice of a synthetic blood substitute depends to a large extent on the particular requirement :

1) conservation of blood volume can be achieved using solutions of plasma expanders

2) preservation of ionic balance, for which solutions of electrolytes are often adequate

3) transport and distribution of oxygen, and elimination of carbon dioxide.

The third function is a more difficult but nonetheless vital requirement, and forms the subject of this report.

Blood is a complex liquid containing a large number of components serving different functions, of which the most important is the dissolution of respiratory gases. Various formulations can be envisaged which satisfy a number of these requirements, although in each case gas exchange must not be impaired. This latter consideration is so important that gas exchange needs to be optimized even at the expense of other capacities which can usually be provided by additional components of the mixture.

Respiratory gases can be dissolved in

1) artifical solutions of hemoglobin

2) solutions of fluorocarbons

Commercially available preparations are in fact based on the latter approach. Perfluorinated derivatives combined with a suitable oncotic agent are currently used as blood substitutes. Emulsions of perfluorinated compounds have now been tested in several hundreds of subjects throughout the world (1-3).

The essential qualities of fluorocarbons (1,4,5) are

high capacity to dissolve gases, especially oxygen and carbon dioxide

low toxicity resulting from the rapid elimination of the unmetabolized substance from the organism.

The capacity to dissolve gases is explained by the lack of structure of perfluorinaged liquids. They appear to contain ca-

vities which can trap oxygen and carbon dioxide. However, perfluorinated compounds cannot be used in the pure state, and they need to be formulated as emulsions. A suspension of fine particles of fluorocarbon can be produced with the aid of a suitable surfactant.

At present, the commercially available systems of this type are based on Fluosol DA (an emulsion of perfluorodecalin and Pluronic F 68 : the latter is a copolymer of ethylene and propylene oxide with a molecular weight of 8,350) (2,3). However, these emulsions lack long term stability, and must be kept refrigerated.

One way round this problem is to use microemulsions. They have the triple advantage of forming spontaneously, remaining stable for months or years, and have a range of particle sizes which are compatible with blood.

However, the formation of microemulsions (like emulsions) comes up against problems due to the structure of the perfluorinated derivatives themselves. A microemulsion contains three essential components (6) : water - surfactant - oil (in this case a perfluorinated compound).

The intrinsic hydrophobicity of perfluorinated derivatives hinders microemulsification, due to the phenomenon of segregation between perhydrogenated and perfluorinated chains (7,8). Attempts to make these two components mutually compatible can be divided into two main strategies, based on

i) structural modification of the perfluorinated derivatives, or
ii) selection of the surfactant

For the completely fluorinated compounds, various structures have been tested, including amines, ethers, cyclanes, olefins, etc... The various studies have shown that the presence of a functional group or heteroatom generally leads to slower elimination from the organism with a corresponding increase in toxicity. This is particularly marked for the amines. The best

derivatives thus appear to be represented by alkanes, cyclanes or olefins (1).

The structure of the surfactant is also crucial for production of suitable microemulsions. Much research is devoted to this aspect, especially on the synthesis of surfactants which are compatible with perfluorinated alkanes and alkenes lacking heteroatoms.

In view of the problems due to segregation between hydrocarbon and perfluorinated chains, research has concentrated on perfluorinated surfactants (5,9).

However, amphiphilic molecules of this type will inevitably present the same disadvantages as the perfluorinated oils. In all cases, they are compounds with a polar head (ionic or not) and a perfluorinated tail. These surfactants are eliminated slowly from the organism, often being degraded to toxic fluoride ions. Much effort is now being put into the development of non-toxic fluorinated surfactants. This approach can be summarized as :

The development of biocompatible fluorinated surfactants to microemulsify perfluorinated oils.

In view of the above mentioned considerations, we felt that this approach was fraught with difficulties, and we opted for an approach which can be summarized as :

The adaptation of the oil to a known biocompatible surfactant. This is effectively the converse of the first approach.

At present, only hydrogenated non-ionic surfactants (amphoteric) have been found to be biocompatible. We thus decided to synthesize and test mixed oils sufficiently fluorinated to dissolve gas and be eliminated rapidly, but sufficiently hydrogenated to enable them to be microemulsified using biocompatible hydrogenated surfactants.

METHODS AND RESULTS

Synthesis of mixed oils

The mixed oils synthesized were of general formula $R_F\text{-}CH_2\text{-}CH{=}CH\text{-}R_H$. A method for synthesizing compounds of this type developed in this laboratory enabled the balance between the fluorinated and hydrogenated parts to be altered at will(10,11) :

$$(C_6H_5)_3P + R_FCH_2CH_2I \longrightarrow (C_6H_5)_3P^+CH_2CH_2R_F,\ I^-$$

$$(C_6H_5)P^+\text{-}C_2H_4,I^- + R_H\text{-}CHO \longrightarrow R_F\text{-}CH_2\text{-}CH{=}CH\text{-}R_H + (C_6H_5)P{=}O$$

($R_F = C_2F_5$, C_4F_9, C_6F_{13}, C_8F_{17}, $C_{10}F_{21}$)

$C_8F_{17}CH_2\text{-}CH{=}CH\text{-}C_4H_9$ produced in 74% yield was tested. This compound was found to be a good gas solvent. Determination of oxygen solubility using the Clark electrode showed that it dissolved 43 ml of oxygen per 100 ml. This is comparable to that of F-decalin (the oil employed in Fluosol DA).

From the various compounds synthesized, the mixed oil

Microemulsification of the mixed oil $C_8F_{17}\text{-}CH_2\text{-}CH{=}CH\text{-}C_4H_9$

Various hydrogenated surfactants were tested in order to produce microemulsions with this oil. We optimized an aqueous system with $C_8F_{17}\text{-}CH_2\text{-}CH{=}CH\text{-}C_4H_9$ as oil, and the biocompatible surfactant, Montanox 80 (see Fig. 1). This surfactant is used in the formulation of vaccines by the Institut Pasteur (Paris, France) (12).

The diagram in Figure 1 shows the microemulsion zone at 37°C (body temperature). The microemulsions are of the oil-in-water type, which are well suited for use as blood substitutes, since they are readily diluted in blood after intravenous admi-

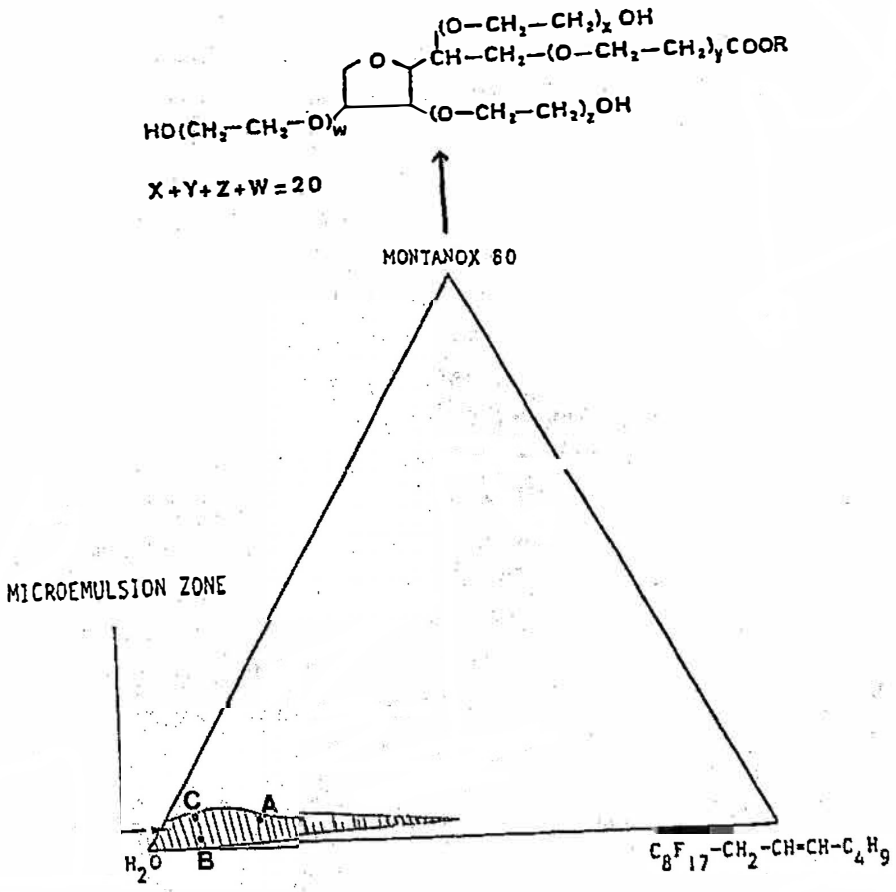

Fig. 1 - Phase diagram of the system
H_2O - Montanox 80 - C_8F_{17}-CH_2-CH=CH-C_4H_9

Properties of the microemulsions

Measurement of viscosity and light scattering at 37°C demonstrated that the microemulsions were of the oil-in-water type, and were composed of small-sized aggregates (mean diam. 50-60 A). Aggregates of this size do not, in principle, favor formation of emboli after injection into the general circulation.

The values of viscosity, and oxygen solubility measured with the Clark electrode are presented in Table I.

DISCUSSION

All the microemulsions tested dissolved more oxygen than FLuosol DA (11 ml O_2/100 ml). Oxygen solubility was comparable to that of blood (20.6 ml O_2/100 ml).

It should be emphasized that the theoretical values of oxygen solubility (given the amount of oil in the microemulsion) are much lower than the measured values. The excess solubility was around 500%. This would tend to indicate that the structure of the microemulsions (existence of micellar cages) increases their capacity to dissolve gas. This phenomenon has been observed, although to a lesser extent (around 200%), for perhydrogenated oils (13). It should also be noted that the presence of a fluorinated oil is required to observe this phenomenon of solubilization. Corresponding micellar solutions (without oil) only took up small amounts of oxygen (around 6 ml/100 ml) similar to that observed for water (3 ml/100 ml).

Preliminary toxicological studies in the rat after intraperitoneal administration have demonstrated the lack of toxicity of these systems (12). Further trials are in progress using intravenous administration.

Table I - Properties of the microemulsion system
$C_{18}F_{17}$-CH_2-CH=CH-C_4H_9 - water - Montanox 80

Microemulsion	Oxygen solubility (ml/100ml)	Viscosity (Cp)	Agregate diam. (A)
A (16% oil)	32	1.0	130
B (9% oil)	21	0.9	87
C (7% oil)	9	1.1	72

Microemulsion a was used for these experiments. It is a good gas solvent, and it could be readily formulated to be compatible with blood by the addition of salts. No hemolysis has been observed in the use of these formulations.

These results demonstrate the feasibility of production of microemulsions using fluorinated oils and hydrogenated, biocompatible surfactants. Such systems could be employed as blood substitutes. An immediate application would be in the formulation of culture media.

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