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# New formulation of blood substitutes: optimization of novel fluorinated microemulsions

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**Summary** — The synthesis of a novel microemulsion system composed of a mixed fluorinated and hydrogenated oil  $C_8F_{17}-CH_2CH=CH-C_4H_9$  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 which is currently used as an oxygen transporter in biomedical applications. Oxygen absorption was similar to that of blood. Light scattering studies showed that the system was composed of small sized aggregates which should in principle be compatible with blood. The toxicity of the microemulsions was tested after intraperitoneal injection in rats, and in mice after intravenous administration. The microemulsions appeared to be well tolerated. These results show promise for the development of oxygen transporting compounds.

**Résumé** — Nouvelle stratégie dans la formulation de substituts du sang: optimisation de nouvelles microémulsions fluorées. Pour la première fois, nous décrivons des systèmes de microémulsions d'une huile mixte fluorée et hydrogénée  $C_8F_{17}CH_2-CH=CH-C_4H_9$  à l'aide d'un tensioactif hydrogéné biocompatible: le Montanox 80. Les études de solubilité de l'oxygène dans ces microémulsions montrent qu'elles absorbent davantage l'oxygène que l'émulsion Fluosol DA, actuellement utilisée dans le domaine biomédical des transporteurs d'oxygène. Cette absorption s'effectue dans des proportions analogues à celles du sang. Du point de vue de la toxicité de ces systèmes, des mesures de la diffusion de la lumière mettent en évidence des agrégats de petite taille compatibles théoriquement avec le système sanguin. La toxicité des microémulsions a été testée en i.p. chez le rat et en i.v. chez la souris. Les microémulsions semblent bien tolérées. Le travail décrit ici constitue donc une ouverture d'une nouvelle voie de recherche dans le domaine des transporteurs d'oxygène.

fluorinated microemulsions / oxygen transporting compounds

## Introduction

All body organs and tissues require a constant supply of oxygen. At present, blood transfusion is the only effective way of restoring oxygen in situations where the supply is seriously impaired, although it is not indicated in all cases. The development of an artificial transporter of respiratory gases would be of great value especially if it was devoid of immunological and infectious risks, chemically stable, and readily utilizable, especially outside a hospital environment.

Fluorocarbons are the best gas solvents known, and they are also chemically and biologically stable. Aqueous emulsions of these compounds have thus been considered as potential blood substitutes for the transport of  $O_2/CO_2$ . Non-transfusional indications include the treatment of cardiac or cerebral ischemia, for cardioplegia,

angioplasty and in the radio- or chemotherapy of cancer where oxygen has been shown to have a synergistic action. Such blood substitutes would also be of value for perfusion of isolated organs and in cell culture, etc [1–7].

A commonly used preparation is represented by Fluosol-DA, an emulsion of F-decaline and Pluronic F68, a copolymer of repeating ethylene propylene oxide units with an average m.w. of 8350. However, it is rather unstable, and must be kept refrigerated [9–11].

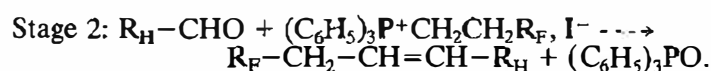
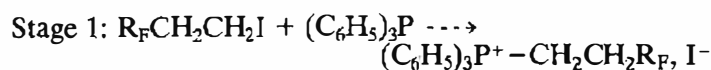
The use of oil in water microemulsions using perfluorinated oils would overcome this obstacle. These microemulsions have the double advantage of forming spontaneously and remaining stable for periods of up to several years [12–14]. However, production of a microemulsion with a perfluorinated oil requires the use of a fluorinated surfactant due to segregation between the fluorinated and hydrogenated chains [15, 16]. Unfortunately some of

these surfactants are eliminated slowly from the organism, and they are degraded to toxic fluoride ions [17].

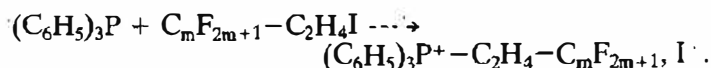
In this study, a different approach was adopted by using hydrogenated surfactants which are known to be biocompatible. The mixed oils must be sufficiently fluorinated to dissolve gases and be eliminated rapidly from the organism, but sufficiently hydrogenated to enable microemulsification with hydrogenated surfactants.

### Synthesis of mixed hydrogenated and fluorinated oils

The mixed oils were of the type  $R_F-CH_2-CH=CH-R_H$ . Using the following synthetic scheme the balance between the fluorinated and hydrogenated parts of the molecule could be altered:



*First stage: synthesis of the fluorinated phosphonium salt*  
This was carried out by reacting triphenylphosphine with the fluoroalkyl iodide at 95°C in the absence of solvent.



Three compounds were synthesized, starting from different fluoroalkyl iodides. The results are summarized in Table I.

**Table I.** Synthesis of fluorinated phosphonium salts  $R_F-CH_2-CH_2P^+(C_6H_5)_3, I^-$ .

Compounds	No.	Yield (%)	MP (°C)
$(C_6H_5)_3P^+-CH_2-CH_2-C_4F_9, I^-$	1	95	104
$(C_6H_5)_3P^+-CH_2-CH_2-C_6F_{13}, I^-$	2	95	170
$(C_6H_5)_3P^+-CH_2-CH_2-C_8F_{17}, I^-$	3	85	180

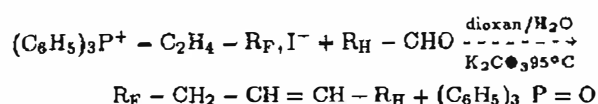
*Second stage: synthesis of the olefin by condensation of the phosphonium salt with an aldehyde*

This was first tried under strongly alkaline conditions, but yields were low:

- n*-butyl lithium / THF / 0°C
- n*-butyl lithium / THF / -40°C
- sodium hydride / THF / 25-60°C
- lithium diisopropylamide / THF / -70°C

Under all these conditions the bases attacked all the protons in the phosphonium salt indiscriminately, since the presence of the fluorinated chain tended to acidify the protons  $\beta$  to the phosphorus atom, and the expected ylide not formed.

In order to get round this problem, the phase transfer method developed by Escoula *et al.* [17] in our laboratory was employed.



Water acts as a catalyst and liberates carbonate ions by solvating  $K^-$ . Under these mild alkaline conditions the yields were considerably increased since  $CO_3^{2-}$  selectively removes the proton  $\alpha$  to the phosphorus atom. There is no degradation of the phosphonium salt.

Four mixed olefins with different  $R_F-R_H$  balance were thus synthesized (*cf.* Table II).

**Table II.** Synthesis of mixed fluorinated and hydrogenated olefins  $R_F-CH_2-CH=CH-R_H$ .

Compounds	No.	Yield (%)
$C_8F_{17}-CH_2-CH=CH-C_4H_9$	4	74
$C_6F_{13}-CH_2-CH=CH-C_4H_9$	5	35
$C_4F_9-CH_2-CH=CH-C_6H_{13}$	6	30
$C_4F_9-CH_2-CH=CH-C_8H_{17}$	7	60

*NB.* These mixed olefins contained 70% of the *Z* isomer and 30% of the *E* isomer (determined by  $^{13}C$  NMR) [17, 18]. The predominance of the *Z* form can be explained by the formation of non-stabilized ylides during the reaction. The isomeric mixture was used for the subsequent stages.

### Selection of the olefin

The solubility of oxygen was used as a criterion for selection of the olefin for the preparation of the microemulsions. Among the various methods for measurement of oxygen solubility (van Slyke, NMR, polarography, Clark electrode [19]), we selected the Clark electrode which had given good results for fluorinated compounds and emulsions in previous studies [20].

In order to quantitate the results, the electrode was calibrated with commercially available fluorinated oils whose oxygen solubilities were known [9]. This also evaluated the reliability of the method. Five perfluorinated or partially fluorinated oils were chosen, and the amounts of oxygen absorbed were measured at 25°C and 37°C (*cf.* Fig. 1). The method was found to be accurate to  $\pm 2$  ml  $O_2/100$  ml (recorder error).

*F*-decalin was selected as a reference with a value of 40 ml  $O_2/100$  ml. The  $\Delta O_2$  values for the other compounds could thus be converted into volumes of oxygen dissolved by the other compounds. The results are shown in Table III.

The results in the table show that the measured values were close to those reported in the literature. The method was considered to be reliable, and the compounds  $C_4F_9-$

CH=CH-C<sub>4</sub>F<sub>9</sub> and F-decalin were chosen as references at 37°C. The absorption of oxygen by compounds 4 and 7 at 37°C are shown in Table IV.

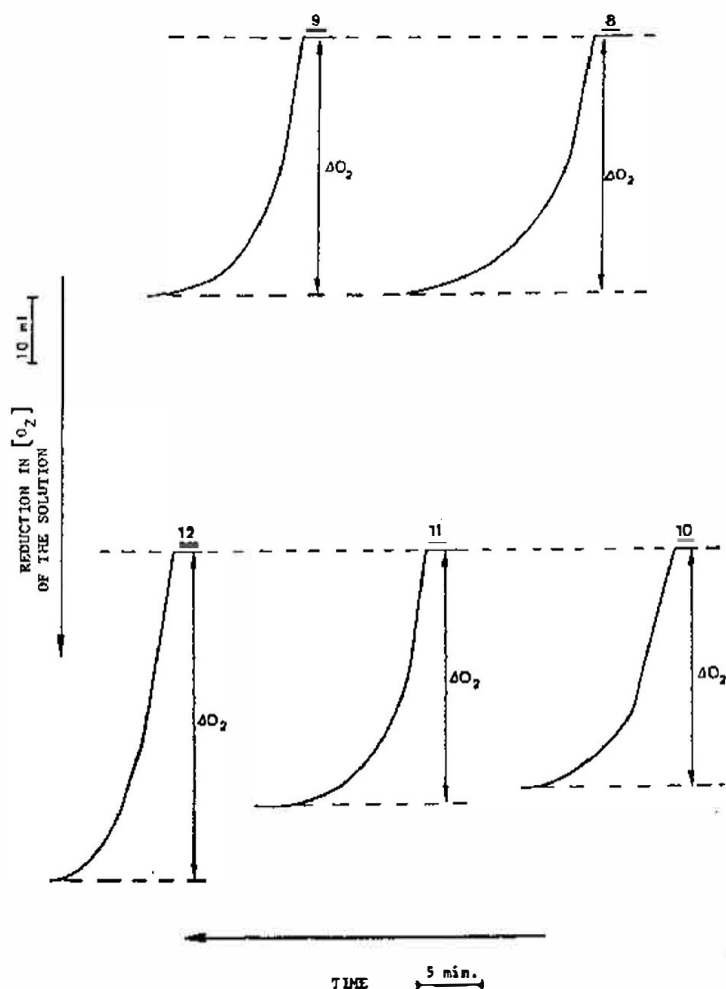


Fig. 1. Absorption of oxygen in 5 commercially available fluorinated oils. Calibration curves.

Table III. Calibration of Clark electrode ( $\pm 2$  ml / 100 ml).

Compounds	No.	T (°C)	Absorption O <sub>2</sub> (5) (ml/100 ml)	Measured O <sub>2</sub> absorption (ml/100 ml)
F-decalin	8	25	40	40
		37	-	43
C <sub>8</sub> F <sub>17</sub> C <sub>2</sub> H <sub>5</sub>	9	25	38	40
C <sub>8</sub> F <sub>17</sub> CH-CH <sub>2</sub>	10	25	38	38
C <sub>6</sub> F <sub>13</sub> CH-CH-C <sub>6</sub> F <sub>13</sub>	11	37	40	40
C <sub>4</sub> F <sub>9</sub> CH-CH-C <sub>4</sub> F <sub>9</sub>	12	37	50	50

Table IV. Absorption of oxygen in mixed oils ( $\pm 2$  ml / 100 ml).

Compound	No.	Absorption (ml/100 ml)
C <sub>8</sub> F <sub>17</sub> -CH <sub>2</sub> -CH=CH-C <sub>4</sub> H <sub>9</sub>	4	43
C <sub>4</sub> F <sub>9</sub> -CH <sub>2</sub> -CH=CH-C <sub>8</sub> H <sub>17</sub>	7	31
C <sub>4</sub> F <sub>9</sub> -CH=CH-C <sub>4</sub> F <sub>9</sub>	12	50
F-decalin	8	43

The results indicate that:

-the mixed oils 4 and 7 are good oxygen solvents: C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub> is particularly interesting, since it has a larger fluorinated part than C<sub>4</sub>F<sub>9</sub>-CH<sub>2</sub>-CH=CH-C<sub>8</sub>H<sub>17</sub>;

-the compounds 4 and 12, although possessing less fluorine than F-decalin dissolve oxygen to as great an extent. In fact, C<sub>4</sub>F<sub>9</sub>-CH=CH-C<sub>4</sub>F<sub>9</sub> dissolved more oxygen than F-decalin.

This shows the importance of steric factors in the solubility of oxygen in the fluorinated compounds. The double bond in the center of the molecule appears to lead to a steric hindrance that favors the dissolution of oxygen. C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub> thus appears to be a viable alternative to F-decalin, the principal component of Fluosol-DA.

Various microemulsions with C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub> were produced, and all experiments were carried out at 37°C in order to approximate physiological conditions.

### Microemulsions with C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub>

#### Use of ethoxy nonylphenols (NPn) as surfactants

These surfactants of general formula C<sub>9</sub>H<sub>19</sub>-C<sub>6</sub>H<sub>4</sub>-(OC<sub>2</sub>H<sub>4</sub>)<sub>n</sub>OH are widely used in the production of microemulsions. In the range of compounds NP2 to NP15 only the NP14 derivative produced a monophasic zone with the mixed oil C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub> (Fig. 2). The

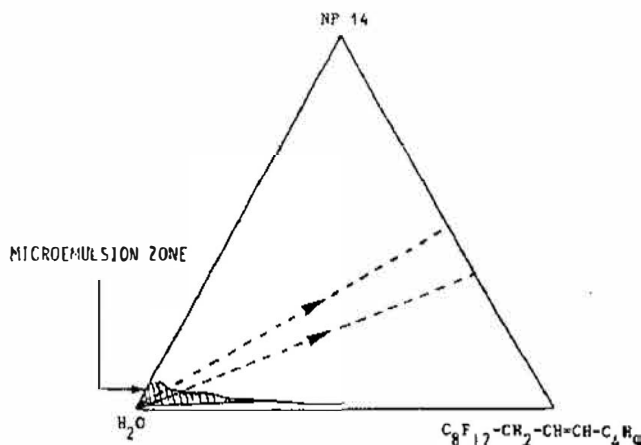


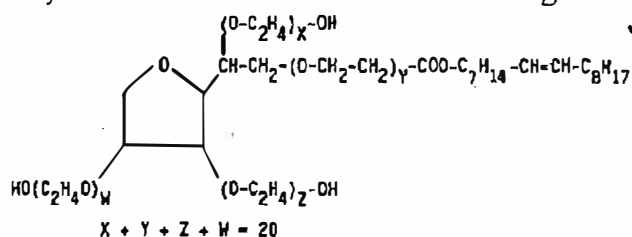
Fig. 2. Pseudoternary phase diagram of the system H<sub>2</sub>O-NP14-C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub> (NP14 = C<sub>9</sub>H<sub>19</sub>-C<sub>6</sub>H<sub>4</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>14</sub>OH).

microemulsion zone was observed in the water-rich region, making it suitable for intravenous administration. However, in view of the potential toxicity of this aromatic non-ionic surfactant (NP14) we investigated non-toxic analogues based on the HLB criteria (hydrophile-lipophile balance).

The HLB value can be determined experimentally or theoretically. The theoretical methods described by Griffin [21] and Davies [22] are commonly employed. Thus the HLB for NP14 is calculated to be  $\approx 14.5$ . Surfactants with an HLB of around 14 were selected.

#### Microemulsification of $C_8F_{17}-CH_2-CH=CH-C_4H_9$ using Montanox 80

Montanox 80 is a non-ionic surfactant manufactured by Seppic with an HLB of 14. It has a very low toxicity (oral  $LD_{50} > 16$  ml/kg) and is used in the formulation of vaccines by the Institut Pasteur. It has the following formula:



Montanox 80 produced a microemulsion zone similar to that obtained with ethoxy nonylphenol (NP14) described above (cf. Fig. 3). The microemulsion zone is found in the water-rich regions up to systems containing equal amounts of oil and water (point B), which would make it suitable for use in physiological conditions.

These properties thus encouraged us to attempt to develop oxygen transporting systems. Structural investigations (viscosity and light scattering) of various microemulsions of the system Montanox 80-water- $C_8F_{17}-CH_2-CH=CH-C_4H_9$  were therefore carried out. These were followed by oxygen absorption and toxicological studies.

#### Structural investigation of the microemulsions $C_8F_{17}-CH_2-CH=CH-C_4H_9$ -water-Montanox 80

Four microemulsions were selected (points B, C, D and E in the phase diagram of Figure 3). Their compositions are shown in Table V.

Table V. Characteristics of microemulsions B, C, D, and E of the system  $H_2O$ -Montanox 80- $C_8F_{17}-CH_2-CH=CH-C_4H_9$ .

No.	Composition of microemulsions (% weight)				Viscosity (cp) $\pm 0.1$ cp	Micelle radius (Å) $\pm 1$ Å	Oxygen absorption (ml/100 ml)	
	Montanox 80	$C_8F_{17}-CH_2-CH=CH-C_4H_9$	$H_2O$				Measured ( $\pm 2$ ml/100 ml)	Calculated
B	2.6	48.7	48.7	70.0	—	23	17.0	
C	5.5	16.6	77.8	1.1	64	32	5.4	
D	7.15	7.15	85.7	1.1	36	9	2.3	
E	1.3	9.1	89.6	0.9	43	21	2.8	

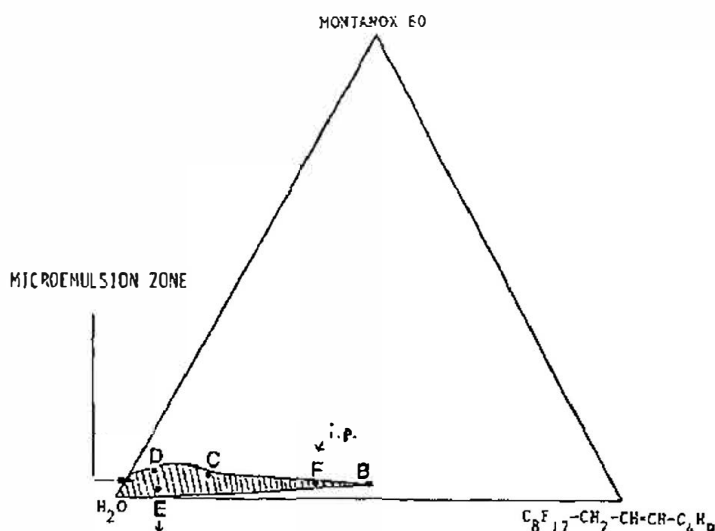


Fig. 3. Pseudoternary phase diagram of the system  $H_2O$ -Montanox 80- $C_8F_{17}-CH_2-CH=CH-C_4H_9$ .

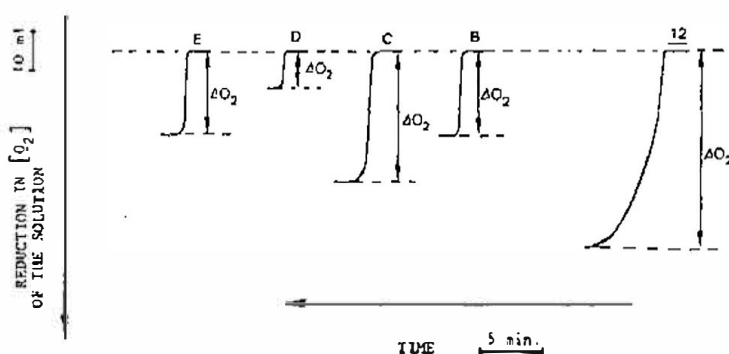


Fig. 4. Curves of absorption of oxygen in microemulsions B, C, D and E (reference  $C_4F_9-CH=CH-C_4F_9$ ).

#### Viscosity

Viscosity was measured in a coaxial cylinder type viscosimeter [23] (cf. Experimental protocols). The results are also listed in Table V.

It can be seen that the microemulsions C, D and E have a viscosity close to that of water (1 cp), a further indication that they are rich in water (O/W type). However, the microemulsion B containing equal amounts of water and oil had a particularly high viscosity (70 cp). It is not a true microemulsion (the viscosity of the oil itself is only 3.15 cp), but it is probably represented by a lamellar structure.

### Quasi-elastic light scattering

The quasi-elastic light scattering of the microemulsions C, D, and E (water as continuous phase) was measured (cf. Experimental protocols). This gives an estimate of the size of the spherical aggregates [24]. The results are shown in Table V.

These physicochemical studies showed that the Montanox 80 - water -  $C_8F_{17}-CH_2-CH=CH-C_4H_9$  microemulsions in the area rich in water are composed of small-sized aggregates of the oil in water type. They are thus likely to be suitable for blood substitutes.

### Solubility of oxygen in the microemulsions $C_8F_{17}-CH_2-CH=CH-C_4H_9$ - water - Montanox 80

Measurement of the absorption of oxygen were carried out at 37°C with  $C_4F_9-CH=CH-C_4F_9$  as reference as for the fluorinated oils. It should be noted that the method measured the solubility of oxygen in the microemulsion after dilution in the sample cell. In some respects this mimics the dilution of the agent in blood after intravenous administration. We also calculated the expected absorption of just the oil fraction of each of the microemulsions. The results are summarized in Figure 4 and Table V. These results showed that apart from microemulsion D, the other microemulsions dissolved oxygen to a greater extent than Fluosol-DA (7.5 ml  $O_2$ /100 ml) and, to a similar extent, to that of blood (20.6 ml  $O_2$ /100 ml).

It should also be noted that the theoretical values of oxygen solubility (taking into account the proportion of oil in the microemulsion) are much less than the measured values in microemulsions C, D and E which have a true micellar structure. The excess solubility was in fact  $\approx 500\%$ . This would indicate that the structure of the microemulsion (presence of micellar cages) increases their capacity to take up oxygen. This phenomenon has been observed, albeit to a lesser extent (excess solubility of around 200%) with perhydrogenated microemulsion [19].

It should be emphasized that the presence of a fluorinated oil is required to observe this phenomenon of solubilization. The corresponding micellar solutions (without oil) only took up low proportions of oxygen ( $\approx 6$  ml/100 ml). Moreover, the solubility of oxygen appeared to depend on the size of the micelles. The larger the micelles, the higher the solubility (cf. Table V).

It would appear that solubilization of gas is favored by the oil in water nature of these microemulsions using Montanox 80. Toxicological studies were thus carried out in the rat after peritoneal injection and in the mouse after intravenous administration.

### Toxicology of the microemulsions $C_8F_{17}-CH_2-CH=CH-C_4H_9$ - water - Montanox 80

#### Toxicity in Wistar rats after intraperitoneal administration

The initial objective was to evaluate the toxicity of the fluorinated oil  $C_8F_{17}-CH_2-CH=CH-C_4H_9$  itself. We thus administered microemulsion F (57% water, 40% oil and 3% Montanox 80).

#### Doses and route of administration

Male and female Wistar rats were treated by intraperitoneal injection. The animals were housed in plastic cages, and had *ad libitum* access to food and water. They were left to habituate for 8 days the experiments. Two 15-day trials separated by an interval of one week were carried out on the same groups of animals. Each group consisted of 5 males and 5 females. The doses used for the 2 trials are summarized in Table VI.

Table VI. Amounts of solute [26] (5 ml/kg) injected i.p.

Trial	Male rats		Female rats	
	Treated	Controls	Treated	Controls
1	0.7 g oil	—	0.7 g oil	—
	0.05 g Mx 80	0.05 g Mx 80	0.05 g Mx 80	0.05 g Mx 80
	1 g water	1.7 g water	1 g water	1.7 g water
2	1.4 g oil	—	1 g oil	—
	0.1 g Mx 80	0.1 g Mx 80	0.07 g Mx 80	0.07 g Mx 80
	2 g water	3.4 g water	1.4 g water	2.4 g water

#### Parameters evaluated

**Weight gain.** The animals were weighed at the same time on each day during the trials. Weight gain was comparable between the 2 groups.

**Autopsy.** At the end of trial 2, the rats were anesthetized with ether, and killed by aortic puncture. Blood was collected on lithium heparinate, and centrifuged at 12 000 rpm for 10 min. The various body organs were examined macroscopically. Administration of these microemulsions by the intraperitoneal route was not found to lead to any macroscopically observable lesions.

**Biochemistry.** Plasma samples were analyzed in the clinical chemistry department at Purpan Hospital (Toulouse). Student's *t*-test was used to compare the results (Table VII).

Statistically significant alterations were observed in both males and females in: albumin, calcium; and in females only in: phosphorus,  $\gamma$ -glutamyl transferase and iron. These alterations were slight, and were observed in some cases as elevations and in others as decreases with respect to control levels. They were assumed to be of no toxicological significance. The fluorinated oil  $C_8F_{17}-CH_2-CH=CH-C_4H_9$  can thus be considered to be well tolerated after intraperitoneal administration.

#### Toxicity in $CDF_1$ mice after intravenous administration

**Doses and route of administration.** In order to prepare a solution suitable for intravenous administration (neutral pH, isotonic and isoionic), salts and glucose were added in the same proportions as those used in Fluosol-DA (Table VIII).

**Table VII.** Biochemical parameters and statistical comparisons: i.p. toxicological studies.

Females:		Na	K	Cl	Alk.*	Alb.	TP**	Urea	Creat.	Ca	P	Alk.ph	Bll	GTP	GOT	Uric	vGT	LDH	Chol.	Trig.	Gluc.	Fe
Cnt	m	142.8	3.66	105	21.4	570	67.4	8.32	40.2	2.6	1.55	205.8	1.4	57.2	71.8	41.4	1.4	398	2.18	0.368	10.26	55.2
	SE	0.45	0.31	1.23	2.0	28.1	2.19	1.59	2.86	0.06	0.25	62.71	0.55	5.68	9.45	19.82	0.55	342	0.36	0.17	0.31	8.87
Exp	m	142.0	5.56	103	22.8	618	72.2	9.50	39.0	2.79	2.03	187.0	1.0	47.2	97.2	48.6	0.2	1004	2.56	0.65	9.56	85.6
	SE	2.0	1.35	1.58	1.64	26.8	5.81	1.49	5.61	0.09	0.34	42.2	0.71	16.5	52.4	39.6	0.45	1118	0.53	0.32	1.60	16.4
	t	0.87	1.45	2.24	1.23	2.76	1.73	1.21	0.43	3.97	2.51	0.53	1.0	1.28	1.07	0.36	3.79	1.16	1.33	1.77	0.87	3.64
	df	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

Males:		Na	K	Cl	Alk.*	Alb.	TP**	Urea	Creat.	Ca	P	Alk.ph	Bll	GTP	GOT	Ur. A	vGT	LDH	Chol.	Trig.	Gluc.	Fe
Ctn	m	142.6	4.36	99.6	25.8	531	64.9	9.54	36.6	2.72	2.36	517.0	1.4	73.4	98.2	47.0	0.0	682	2.02	1.49	9.94	38.4
	SE	1.34	0.64	1.67	0.84	8.22	1.30	0.73	4.88	0.03	1.81	91.8	0.55	6.19	23.8	18.7	0.0	636	0.34	0.86	0.86	8.56
Exp	m	143.0	4.18	100	26.4	510	62.8	8.54	33.2	2.65	2.37	463.0	2.4	73.2	90.4	35.8	0.2	425	1.58	1.51	9.54	46.0
	SE	1.09	0.48	1.41	1.14	18.4	3.27	0.61	7.05	0.04	0.25	59.55	1.52	9.20	20.4	15.6	0.45	553	0.29	0.44	0.81	5.57
	t	0.78	0.51	0.41	0.95	2.33	1.27	2.28	0.89	3.43	0.65	1.11	1.39	0.04	0.57	1.03	1.0	0.63	2.18	0.07	0.76	1.66
	df	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

\*Alk. = alkaline reserve.  
\*\*TP = total protein.

**Table VIII.** Amounts of salts and glucose (same proportions as Fluosol DA) added to the microemulsions (g/100 ml).

NaCl	0.600
KCl	0.034
MgCl <sub>2</sub>	0.020
CaCl <sub>2</sub>	0.028
NaHCO <sub>3</sub>	0.210
Glucose	0.180

Since microemulsion E dissolved as much oxygen as blood (cf. Table V) we prepared an homologous microemulsion E' with the following characteristics:

**Table IX.** Characteristics of microemulsion E'.

Composition (% weight)			Micelle radius Å (± 1 Å)	Measured oxygen absorption ml/100 ml (± 2 ml/100 ml)
Oil*	Mx 80	Sterile water Salts + glucose	43	20
9.1	1.3	89.6		

\*C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub>

It should be noted that the presence of the salts and glucose did not affect the size of the aggregates or the solubility of oxygen in the microemulsion.

Microemulsion E' had a pH of 7. None of the solutions were as hemolytic as the solutions of physiological saline (0.9% NaCl) used as controls. Microemulsion E' was thus injected into female CDF<sub>1</sub> female mice via the tail vein at

various doses (0.1, 0.2, 0.3 ml/10 g body weight). All animals were given *ad libitum* access to food and water, and were weighed regularly.

#### Parameters evaluated

**Body weight.** The mice were weighed daily. Weight gain for treated and controls animals was not significantly different.

**Autopsy.** The mice were killed by i.p. injection of sodium pentobarbital (50 mg/kg) at the following times: 8 days, 15 days, 1 month and 3 months after treatment. No macroscopic lesions were observed in any body organ. More detailed toxicological studies are in progress to investigate elimination of the constituents of this microemulsion.

#### Conclusion

The results show that microemulsions can be produced using a partially fluorinated olefin and a biocompatible hydrogenated surfactant. The oil C<sub>8</sub>F<sub>17</sub>-CH<sub>2</sub>-CH=CH-C<sub>4</sub>H<sub>9</sub> was microemulsified using Montanox 80, a non-ionic, non-toxic surfactant.

Determination of the solubility of oxygen in these microemulsions showed that they absorbed larger amounts of oxygen than Fluosol-DA which is widely used in biomedical applications. Oxygen absorption was in fact comparable to that of blood.

Light scattering studies demonstrated the small size of their constituent aggregates.

The results after intraperitoneal injection in the rat indicated that these microemulsions are well tolerated. For intravenous use, the microemulsions were optimized by addition of salts and glucose. The isotonic preparations of neutral pH also appeared to be well tolerated after intra-



venous administration in mice. These encouraging results are being followed up by further toxicological studies *via* the intravenous route in mice.

## Experimental protocols

The NMR spectra were recorded: for  $^1\text{H}$  at 60 MHz on a Varian T 60, and at 90 MHz on a Bruker WH 90 instrument using TMS as internal references; for  $^{19}\text{F}$  at 90 MHz on a Perkin-Elmer R 32 with  $\text{CF}_3\text{COOH}$  as external reference; for  $^{13}\text{C}$  at 300 MHz on a Bruker AM 300 WB with TMS as internal reference.

The chemical shifts are expressed in ppm with respect to the reference, and the signals were characterized as: s (singlet), d (doublet), t (triplet), and m (multiplet).

The infra-red spectra were recorded on a Perkin-Elmer 683, and the frequencies of absorption are expressed in  $\text{cm}^{-1}$ .

Microanalyses were carried out by the central facilities of the CNRS. The melting points were determined on a Koffler block. Viscosity was measured with a coaxial cylinder type viscosimeter (Contraves Lowshear 2) [23].

Light scattering in the microemulsions was measured using a Malvern-K 7025 128 channel correlator coupled to a helium-neon laser ( $\lambda = 6323 \text{ \AA}$ ) [24].

### Preparation of mixed hydrogenated and fluorinated oils

#### Preparation of phosphonium salts

**General method.** 0.1 mol of the fluoroalkyl iodide and 0.1 mol of triphenylphosphine are heated in the absence of a solvent to 90–100°C under constant stirring; overnight for  $\text{R}_F = \text{C}_4\text{H}_9$ , and all day for  $\text{R}_F = \text{C}_6\text{F}_{13}$  and  $\text{C}_8\text{F}_{17}$ . The reaction is stopped when the mixture solidifies. After cooling the solid residue is washed in toluene followed by anhydrous ether. The white solid is dried under vacuum at 45°C for a whole day.

#### Preparation of $(\text{C}_6\text{H}_5)_3\text{P}^+-\text{C}_2\text{H}_4-\text{C}_4\text{F}_9$ , 1-1

Yield: 95%; MP: 104°C; NMR  $^1\text{H}$   $\text{CD}_3\text{COCD}_3$ : 2.7 (2p, m,  $\text{CH}_2\beta\text{P}$ ); 4–4.5 (2p, m,  $\text{CH}_2\alpha\text{P}$ ); 7.6–8.3 (15p, m,  $(\text{C}_6\text{H}_5)_3$ ). NMR  $^{19}\text{F}$ ,  $\text{CD}_3\text{COCD}_3$ : 5.5 (3f, t,  $\text{CF}_3$ ); 3.8 (2f, m,  $\alpha\text{CH}_2$ ); 48 (2f, m,  $\text{CF}_2\beta\text{CH}_2$ ); 50 (2f, m,  $\text{CF}_2\alpha\text{CF}_3$ ). Found: C = 45.98, H = 3.07, F = 25.19, P = 4.83, I = 18.61.  $\text{C}_{22}\text{H}_{19}\text{F}_9\text{PI}$  requires C = 45.28, H = 2.99, F = 26.89, P = 4.87, I = 19.97%.

#### Preparation of $(\text{C}_6\text{H}_5)_3\text{P}^+-\text{C}_2\text{H}_4-\text{C}_6\text{F}_{13}$ , 1-2

Yield: 94%; MP: 170°C; NMR  $^1\text{H}$   $\text{CD}_3\text{COCD}_3$ : 2.6 (2p, m,  $\text{CH}_2\beta\text{P}$ ); 4.2 (2p, m,  $\text{CH}_2\alpha\text{P}$ ); 7.4–8.4 (15p, m,  $(\text{C}_6\text{H}_5)_3$ ). NMR  $^{19}\text{F}$ ,  $\text{CD}_3\text{COCD}_3$ : 5.8 (3f, t,  $\text{CF}_3$ ); 37.8 (2f, m,  $\alpha\text{CH}_2$ ); 46.5 (2f, m,  $\text{CF}_2\beta\text{CH}_2$ ); 47.4 (2f, m,  $\text{CF}_2\gamma\text{CH}_2$ ); 51 (2f, m,  $\text{CF}_2\alpha\text{CF}_3$ ). Found: C = 42.58, H = 2.63, F = 31.84, P = 3.98, I = 17.57.  $\text{C}_{26}\text{H}_{13}\text{F}_{13}\text{PI}$  requires C = 42.37, H = 2.59, F = 33.55, P = 4.21, I = 17.25%.

#### Preparation of $(\text{C}_6\text{H}_5)_3\text{P}^+-\text{C}_2\text{H}_4-\text{C}_8\text{F}_{17}$ , 1-3

Yield: 85%; MP: 180°C; NMR  $^1\text{H}$   $\text{CD}_3\text{COCD}_3$ : 2.7 (2p, m,  $\text{CH}_2\beta\text{P}$ ); 4.2 (2p, m,  $\text{CH}_2\alpha\text{P}$ ); 7.9–8.5 (15p, m,  $(\text{C}_6\text{H}_5)_3$ ). NMR  $^{19}\text{F}$ ,  $\text{CD}_3\text{COCD}_3$ : 5.3 (3f, t,  $\text{CF}_3$ ); 37.8 (2f, m,  $\alpha\text{CH}_2$ ); 46.9 (2f, m,  $\text{CF}_2\beta$  and  $\gamma\text{CH}_2$ ); 46.1 (6f, m,  $\text{CF}_2\beta$ ,  $\gamma$  and  $\delta\text{CF}_3$ ); 50.5 (2f, m,  $\text{CH}_2\alpha\text{CF}_3$ ). Found: C = 49.78, H = 2.21, F = 37.80, P = 3.82, I = 15.10.  $\text{C}_{28}\text{H}_{19}\text{F}_{17}\text{PI}$  requires C = 40.18, H = 2.27, F = 38.62, P = 4.31, I = 15.18%.

### Preparation of the mixed oils by a Wittig reaction

**General method.**  $2.5 \cdot 10^{-2}$  mol of the fluorinated phosphonium salt,  $2 \cdot 10^{-2}$  mol of aldehyde,  $3 \cdot 10^{-2}$  mol of potassium carbonate,  $1.7 \cdot 10^{-2}$  mol of water or formamide, and 20 ml of anhydrous 1,4-dioxane are placed in a flask. The mixture is maintained at 95°C under constant stirring for 4 h. The reaction mixture is then filtered to remove the  $\text{K}_2\text{CO}_3$ . The solvent is evaporated under vacuum without heating, and the residue is taken up in ether to precipitate most of the triphenylphosphine oxide. This precipitate is filtered, and the ether is evaporated. The residue is taken up in hexane, and extracted 2 to 3 times with water to remove the remaining dioxane. The organic phase is dried over sodium sulfate and the hexane is evaporated. A colorless liquid is obtained after purification by rapid distillation.

#### Preparation of $\text{C}_8\text{F}_{17}-\text{CH}_2-\text{CH}=\text{CH}-\text{C}_4\text{F}_9$ , 4

Yield: 74%; NMR  $^1\text{H}$   $\text{CDCl}_3$ : 0.6–2.2 (9p, m,  $\text{C}_4\text{H}_9$ ); 2.4–3.2 (2p, split t,  $\text{CH}_2\alpha\text{C}_8\text{F}_{17}$ ); 5–6 (2p, m,  $\text{CH}=\text{CH}$ ). NMR  $^{19}\text{F}$ ,  $\text{CDCl}_3$ : 6 (3f, s,  $\text{CF}_3$ ); 39–40 (2f, s,  $\text{CF}_2\alpha\text{CH}_2$ ); 48–50 (10f, m,  $(\text{CF}_2)_5$ ); 53 (2f, s,  $\text{CF}_2\alpha\text{CF}_3$ ). Found: C = 35.67, H = 2.76, F = 63.31,  $\text{C}_{15}\text{H}_{11}\text{F}_{17}$  requires C = 34.88, H = 2.52, F = 62.59%.

#### Preparation of $\text{C}_6\text{F}_{13}-\text{CH}_2-\text{CH}=\text{CH}-\text{C}_4\text{F}_9$ , 5

Yield: 35%; NMR  $^1\text{H}$   $\text{CDCl}_3$ : 0.6–2.2 (9p, m,  $\text{C}_4\text{H}_9$ ); 2.4–3.2 (2p, split t,  $\text{CH}_2\alpha\text{C}_6\text{F}_{13}$ ); 5–6 (2p, m,  $\text{CH}=\text{CH}$ ). NMR  $^{19}\text{F}$ ,  $\text{CDCl}_3$ : 6 (3f, s,  $\text{CF}_3$ ); 39–40 (2f, s,  $\text{CF}_2\alpha\text{CH}_2$ ); 48–50 (6f, m,  $(\text{CF}_2)_3$ ); 53 (2f, s,  $\text{CF}_2\alpha\text{CF}_3$ ). Found: C = 37.24, H = 3.33, F = 58.93.  $\text{C}_{13}\text{H}_{13}\text{F}_{13}$  requires C = 37.50, H = 3.12, F = 59.37%.

#### Preparation of $\text{C}_4\text{F}_9-\text{CH}_2-\text{CH}=\text{CH}-\text{C}_6\text{F}_{13}$ , 6

Yield: 30%; NMR  $^1\text{H}$   $\text{CDCl}_3$ : 0.9–2.2 (13p, m,  $\text{C}_6\text{H}_{13}$ ); 2.4–3.4 (2p, split t,  $\text{CH}_2\alpha\text{CF}_9$ ); 5.2–6.1 (2p, m,  $\text{CH}=\text{CH}$ ). NMR  $^{19}\text{F}$ ,  $\text{CDCl}_3$ : 6 (3f, s,  $\text{CF}_3$ ); 39–40 (2f, s,  $\text{CF}_2\alpha\text{CH}_2$ ); 48–50 (10f, m,  $(\text{CF}_2)_5$ ); 53 (2f, s,  $\text{CF}_2\alpha\text{CF}_3$ ). Found: C = 45.87, H = 5.15, F = 50.25.  $\text{C}_{13}\text{H}_{17}\text{F}_9$  requires C = 45.35, H = 4.94, F = 49.70%.

#### Preparation of $\text{C}_4\text{F}_9-\text{CH}_2-\text{CH}=\text{CH}-\text{C}_8\text{F}_{17}$ , 7

Yield: 60%; NMR  $^1\text{H}$   $\text{CDCl}_3$ : 0.8–2.2 (17p, m,  $\text{C}_8\text{H}_{17}$ ); 2.4–3.2 (2p, split t,  $\text{CH}_2\alpha\text{C}_8\text{F}_{17}$ ); 5.2–6 (2p, m,  $\text{CH}=\text{CH}$ ). NMR  $^{19}\text{F}$ ,  $\text{CDCl}_3$ : 6 (3f, s,  $\text{CF}_3$ ); 39–40 (2f, s,  $\text{CF}_2\alpha\text{CH}_2$ ); 48–50 (10f, m,  $(\text{CF}_2)_5$ ); 53 (2f, s,  $\text{CF}_2\alpha\text{CF}_3$ ). Found: C = 48.50, H = 5.98, F = 45.48.  $\text{C}_{15}\text{H}_{21}\text{F}_9$  requires C = 48.38, H = 4.64, F = 45.96%.

### Determination of microemulsion zones

The microemulsion zones were determined by direct observation after addition of a mixture or a pure constituent. The sample suddenly becomes transparent on formation of a microemulsion. Increasing amounts of water were added to mixtures of surfactant and oil. The experimental points lay within the dotted lines on the phase diagram (*cf.* Fig. 2).

### Clark electrode: measurement principle and procedure

The Clark electrode consists of 2 electrodes: one of silver (anode) and the other of platinum (cathode). The 2 are connected *via* a semi-saturated solution of potassium chloride. A voltage of 0.8 V is applied between the 2 electrodes which produces a depolarization current corresponding to the reduction of oxygen at the cathode. The solution under test is placed in a thermostated holder in contact with the Clark electrode through an oxygen-permeable Teflon membrane.

The rate of diffusion of oxygen across this membrane is relatively slow with respect to the rate of depolarization of the electrode. The depolarization current thus gives a measure of the rate of diffusion of oxygen across the membrane. This rate of diffusion is dependent on the oxygen concentration of the solution, and so the current is proportional to the oxygen concentration.

The thermostated sample holder is filled with water previously saturated with oxygen (1.5 ml). The oxygen concentration is recorded. Then 20  $\mu\text{l}$  of the degassed fluorinated oil or microemulsion is added, and the reduction in the oxygen concentration of the solution is recorded. This gives an estimate of the oxygen consumption of the system.

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