A physicochemical evaluation of perfluorochemicals for oxygen transport applications

Jeffry G. Weers
Alliance Pharmaceutical Corp., 3040 Science Park Road, San Diego, CA 92121 (USA)

(Received June 20, 1992; accepted November 3, 1992)

Abstract

The history of perfluorochemicals as oxygen transport agents and a comparison of potential second generation perfluorochemicals is outlined. 1-Bromoperfluorooctane (perflubron, perfluorooctyl bromide, PFOB), a linear aliphatic perfluorochemical, has unexpected lipophilic properties relative to other perfluorochemicals. The enhanced lipophilicity leads to the unique combination of excellent emulsion stability and an exceptionally short organ retention time. Other medically desirable properties of PFOB include: high purity and definition; excellent gas solubility; superior compatibility with egg yolk phospholipids; the ability to provide contrast; low vapor pressure; low kinematic viscosity; and a positive spreading coefficient on a saline solution.

Introduction

Perfluorochemicals are cyclic or aliphatic hydrocarbon molecules in which all the hydrogen atoms have been replaced with fluorine. Heteroatoms such as nitrogen or oxygen may be included. The extremely strong C–F bond (116 kcal mol^{-1}) makes perfluorochemicals stable both chemically and biologically. They are white solids below their melting points and colorless, odorless liquids, with a specific gravity of *c.* 2, above. Many perfluorochemicals are well suited to medical applications because they have low toxicity and are not metabolized. The perfluorochemicals used in medical applications should not be confused with the chlorofluorocarbons used as aerosol propulsors, foam producers and in refrigeration. The range of possible medical applications of perfluorochemicals is astounding.

Their ability to act as vehicles for respiratory gases (i.e. oxygen and carbon dioxide) is of particular importance. For example, concentrated perfluorochemical emulsions have the capability of providing temporary maintenance of oxygen delivery to tissues during surgical procedures. Perfluorochemical emulsions are also able to deliver oxygen to the heart in such cardiovascular applications as balloon angioplasty, and are being evaluated for cardioplegia and treatment of myocardial infarction. Significant decreases in the rate of tumor growth have been observed in animals when concentrated emulsions are used in conjunction with oxygen and cancer chemo- or radiotherapy. Other potential oxygen therapy applications for perfluorochemical

0022-1139/93/$6.00

emulsions include the treatment of cerebral ischemia, as well as organ and limb preservation. Neat perfluorochemicals have been investigated as a medium for liquid ventilation, which has potential as a unique treatment for adults or neonates who are afflicted with respiratory distress syndrome. The neat perfluorochemical may also provide a medium for delivering drugs into the lung. Perfluorochemicals (in either neat or emulsion forms) are being evaluated for use as contrast agents for nuclear magnetic resonance and ultrasound imaging, and if an X-ray dense atom such as bromine is present in the molecule, the entire range of X-ray diagnostic procedures is potentially available. Perfluorochemicals have also been investigated for use as tamponade agents in the treatment of retinal detachments.

Historical perspective

The road to the development of perfluorochemicals for medical applications began in 1966 when Clark and Gollan performed the classic experiment in which a mouse immersed in neat perfluorochemical liquid (FX-80) continued to live while breathing the liquid [1]. Later that year, Gollan and Clark published a paper describing an experiment in which an isolated rat heart continued to beat when perfused with neat perfluorochemical [2]. These pioneering experiments demonstrated the ability of perfluorochemicals to transport and deliver oxygen for biomedical applications, and also the low toxicity and high biocompatibility of these substances.

In 1967, Sloviter and Kamimoto formulated the first perfluorochemical-in-water emulsion for use as a plasma substitute [3]. Later, in 1973, Geyer succeeded in nearly totally exchanging perfluorochemical emulsion for blood (hematocrit less than 1%) [4]. The severely hemodiluted rats not only survived, but lived their normal lifespan with no apparent negative effects. Unfortunately, all of the perfluorochemicals used up to this time suffered from prolonged liver and spleen retention.

The independent discovery by Naito *et al.* [5] and by Clark *et al.* [6] in 1973 that perfluorodecalin (FDC) is excreted more rapidly from the body than previously studied compounds paved the way for the development of the first commercial perfluorochemical emulsion usable in man, Fluosol®-DA.

First generation emulsions

In 1979, the Green Cross Corp. (Osaka, Japan) began clinical trials with Fluosol-DA. Although a Green Cross subsidiary, Alpha Therapeutic Corp., attempted to obtain Food and Drug Administration (FDA) approval for this emulsion as a red cell substitute in the treatment of anemia, long-term efficacy could not be shown because the brief intravascular persistence and low perfluorochemical content of the emulsion provided only minimal and short-lived effectiveness in patients. In 1990, the FDA approved Fluosol-DA as an oxygen carrier for use during high-risk coronary balloon angioplasty procedures, thereby breaking ground for regulatory approval of future perfluo-

rochemical formulations (the trade-name was shortened to Fluosol® at that time).

Fluosol contains 20% w/v (*c.* 11% v/v) perfluorochemical, of which 14% w/v is FDC and 6% w/v is perfluorotripropylamine (FTPA). FTPA is added to enhance the extremely poor emulsion stability of FDC. Pluronic F-68, an ethylene oxide–propylene oxide block-copolymer, is the primary emulsifier.

The problems associated with this first generation emulsion are numerous and have been described in detail previously [7–9]. First, the physical stability of Fluosol is poor. In spite of the fact that FTPA is added to improve the stability, the stem emulsion must still be shipped and stored in a frozen state. Second, FDC and FTPA suffer from poor chemical purity (98% and 96%, respectively). Third, FTPA has a prolonged retention time in the organs [half-life ($t_{1/2}$) in liver and spleen of 65 d]. Fourth, the product is cumbersome to use due to the thawing procedure and the need to mix it with two annex solutions prior to administration. Fifth, the emulsion cannot be heat-sterilized due to phase separation at sterilization temperatures, a result of the low inherent cloud point of Pluronic F-68. Finally, and most importantly, the emulsion has low oxygen-carrying capacity due to its low perfluorochemical content. This seriously limits its range of medical applications.

Two other first-generation emulsions have also been administered to humans: Emulsion N° II, which is manufactured in China and is similar in composition to Fluosol [10], and Ftorosan (Russia) which contains 15.2% w/v FDC and 7.6% w/v of an emulsion-stabilizing additive, perfluoromethylcyclohexylpiperidine ($t_{1/2} > 60$ d) [11]. The name of the Russian formulation has recently been changed to Perftoran and the emulsion now contains 14% FDC and 6% FMCHP. These emulsions are stabilized by either Pluronic F-68 or the related block-copolymer Proxanol. Both Emulsion N° II and Perftoran have received extensive clinical evaluation in their respective countries of origin. Their limitations are similar to those of Fluosol.

Second generation emulsions

The goal of finding perfluorochemicals for second generation emulsions was outlined in a review article by Riess in 1984 [12]:

"The achievement of stable emulsions that are also rapidly excreted from the organs is the issue that deserves most of our concern in future research... Unfortunately an inverse dependence between these two characteristics appears to exist. Those perfluorochemicals that give stable emulsions, such as perfluorotributylamine and perfluorodihexylether, are retained in the organs for years, whereas those that display the most satisfactory elimination characteristics, such as FDC and *F*-bicyclo(5.3.0)decane, do not give stable enough emulsions. The considerable efforts that have been devoted in the last decade to finding perfluorochemicals that satisfy both requirements simultaneously, have disappointingly led only to the confirmation that this inverse relationship is a general feature."

After the development of Fluosol, the initial search for improved second generation perfluorochemicals focused on heterocyclic molecules. It was hypothesized that the presence of rings would be an aid in decreasing the organ retention time, while the presence of heteroatoms, such as nitrogen, would improved physical stability. It became apparent quickly that neither hypothesis was correct. The organ retention time was found to be more or less proportional to the perfluorochemical molecular weight and emulsion stability was related to the perfluorochemical's water solubility (which is inversely related to the molecular weight) [13].

The most promising heterocyclic perfluorochemical still in development by the Green Cross Corp. is perfluoro-*N*-methyldecahydroisoquinoline (FMIQ). When emulsified with egg yolk phospholipid, FMIQ exhibits improved emulsion stability relative to Fluosol, yet still has an acceptable organ retention time of 11 d [14, 15]. The difficult and costly preparation and purification required for FMIQ has greatly slowed its development.

Perfluoromethyladamantane (FMA), a cyclic perfluorochemical which appeared promising in terms of its emulsion properties and organ retention time, was being developed by the Adamantech Division of Sun Oil [16, 17]. Unfortunately, what is termed FMA is a complex mixture containing many degradation products. This effort has probably been abandoned.

Even though FDC emulsions stabilized by egg yolk phospholipid and/or Pluronic F-68 have poor emulsion stability, FDC continues to be pursued as a perfluorochemical for second generation emulsions. This is because its excellent biocompatibility characteristics and large-scale commercial availability outweigh its poor emulsion stability. Novel methods have been proposed for the stabilization of FDC emulsions. Included among these is the addition of a small amount of a high molecular weight, high boiling point, polycyclic perfluorochemical to reduce Ostwald ripening (the main mechanism of particle coarsening in perfluorochemical emulsions) [18]. The disadvantage of this method is that the minor components typically have unacceptably long organ retention times. The use of triglycerides [19, 20] or fluorinated surfactants [21–23] to improve the cohesiveness between the lipophilic portion of the emulsifier and the dispersed phase has also been reported. Egg yolk phospholipid has replaced Pluronic F-68 as the surfactant of choice due to its improved biocompatibility and use in FDA-approved injectable fat emulsions.

Bis(perfluorobutyl)ethene, F-44E, has also been proposed as a second generation perfluorochemical [24]. Although F-44E has some positive attributes, including excellent purity and definition, good emulsion stability and a short organ retention time, it is not available commercially on a large scale.

The single most important advance in second generation perfluorochemical emulsions came in 1986 with the development by Long *et al.* of concentrated, heat-sterilizable, injectable emulsions with up to 100% w/v perfluorochemical [25]. These emulsions are now being developed by Alliance Pharmaceutical Corp. under the trade name, Oxygent™. The formulations have approximately five times the gas-carrying capacity of Fluosol and, in addition, have far

superior physical stability. They are based on a linear aliphatic perfluorochemical, 1-bromoperfluorooctane (perfluorooctyl bromide, perflubron or PFOB). Initial research focused on the development of neat PFOB as an X-ray imaging agent because the terminal bromine atom makes the perfluorochemical radiopaque [26, 27]. The presence of the bromine atom has also been found to confer enhanced lipophilicity upon the molecule, which in turn leads to the unique combination (for perfluorochemicals) of a short organ retention time and excellent emulsion stability. A detailed discussion of its versatility and advantages versus other perfluorochemicals in both emulsion and neat perfluorochemical applications follows (the physical properties of first and second generation perfluorochemicals are reviewed in Table 1).

Advantages of PFOB in emulsion applications

Perfluorochemical purity and definition

The large doses that may be associated with administration of perfluorochemical emulsions as blood substitutes necessitates high purity and also the identification and validation of each molecular species within the drug substance. For example, in a 70 kg individual, a 3 ml kg^{-1} dose of a 90% w/v perfluorochemical (PFC) emulsion results in 189 g PFC being administered. Thus, impurities in the range of a single per cent result in gram quantities of impurity being injected into the patient. Although it is clear from the argument above that a high level of purity and definition of the perfluorochemicals are prerequisites for their use as injectable oxygen carriers, the components of the first generation product, Fluosol, do not reach the desirable standard of purity.

The poor definition of the first generation perfluorochemicals stems from the synthetic route by which they are made (i.e. by C–H/C–F exchange). In these substitution reactions, the hydrogen atoms are progressively replaced by fluorine atoms. Several methods are used, including electrochemical reaction in anhydrous HF, reaction with heavy metal fluorides (e.g. CoF_3) or controlled reaction with F_2. These reactions are intrinsically nonselective and highly exothermic (the difference in C–H versus C–F bond energies is *c.* 15 kcal mol^{-1}). The large amount of heat generated drives many side-reactions, including fragmentation, isomerization and elimination reactions [28]. The result can be an inextricable mixture of compounds, with an incompletely determined composition. Purification of these mixtures is usually difficult and expensive, and the presence of a small amount of a toxic component in a complex mixture of nontoxic material can be masked, although the toxic effect may appear at a later time [29].

Some of the proposed second generation perfluorochemicals (i.e. FDC, FMA and FMIQ) are synthesized by substitution reactions. FDC can be prepared in reasonably pure fashion via substitution reactions (*c.* 98%) because of its symmetric structure. Although the Green Cross Corp. appears

TABLE 1

Physical properties of first and proposed second generation perfluorochemicals

Perfluorochemical	Mol. wt. (g mol^{-1})	$t_{1/2}$ (d)	$[O_2]$ at 37 °C [ml O_2 (100 ml PFC)$^{-1}$]	$[CO_2]$ at 37 °C [ml CO_2 (100 ml PFC)$^{-1}$]	T_c (K)
F-decalin (FDC)	462	7[a]	43	140	22
F-tripropylamine (FTPA)	521	65	45	165	44
F-methylcyclohexylpiperidine (FMCHP)	595	60	–	–	40
F-methyldecahydroisoquinoline (FMIQ)	495	11	43	–	32
F-methyladamantane (FMA)	474	5	40	–	–
bis(*F*-butyl)ethene (F-44E)	454	7	50	210	25
F-octyl bromide (PFOB)	499	4	53	210	*c.* −20

[a]There is a discrepancy between the 3.4 d organ half-life obtained by Yamanouchi *et al.* [15] for FDC with the more oft-reported 7 d half-life [24, 33, 36, 60]. For purposes of comparison, the 3.4 d value is used in Figs. 1 and 2 alongside the other half-life data obtained by Yamanouchi *et al.* in the same study. PFOB, which was not reported in the Yamanouchi *et al.* study, is scaled on Figs. 1 and 2 to reflect the relative reported difference between it and FDC (i.e. $t_{1/2} = 4/7 \times 3.4$ d = 1.9 d). The reported $t_{1/2}$ value for F-44E is 7 d. This agrees well with the data of Yamanouchi *et al.* Unfortunately, this translates into an anomalous difference in half-life between F-44E and FDC as depicted in Figs. 1 and 2.

to be pursuing development of a 25% w/v FMIQ emulsion for medical applications, the high cost associated with FMIQ purification, and its poor long-term efficacy due to the emulsion's low perfluorochemical content, has probably slowed its development. The development of FMA has probably been abandoned for similar reasons.

It would, therefore, be ideal to find selective, reproducible, synthetic routes which provide highly purified perfluorochemicals of known composition. The best way to accomplish this for noncyclic perfluorochemicals is to start with already fluorinated building blocks (e.g. tetrafluoroethylene) and combine them by telomerization reactions [30]. This procedure has few side-reactions and results in well-defined compounds. In addition, the small amount of impurities are generally other chain length homologues (i.e. in the case of PFOB, the six carbon and 10 carbon homologues) which are easily separated and purified by distillation. Purities are often as high as 99.9%. The limitation with this method lies in the small number of building blocks available as starting materials. PFOB and F-44E appear to be the only second generation perfluorochemicals for medical use which have been synthesized in this manner in significant amounts; however, F-44E is not available commercially.

Biocompatibility/organ retention

Intravenously administered perfluorochemical emulsion droplets are cleared from the blood in large part by the reticuloendothelial system (RES). In this process, RES organ (liver, spleen and bone marrow) macrophages phagocytize the perfluorochemical emulsion particles. Microscopic observations of vacuolated macrophages in the liver and spleen are indicative of the clearance of the emulsion particles by the RES [31]. Extensive uptake of the perfluorochemical emulsion by macrophages results in cellular hypertrophy and enlargement of the liver and spleen. The effects are temporary and reversible, however, and no injury to the macrophages or surrounding cell-types are evident [32]. Further, microscopic examination of the liver, spleen and bone marrow of rats subjected to almost total exchange perfusion were indistinguishable from those of controls when the animals were sacrificed six months later [32, 33].

Once in the RES organs, the perfluorochemical is probably dissolved in lipid carriers in the blood (e.g. chylomicrons or red blood cell membranes) which transfer it to the lung, where it is removed from the body in expired air. The retention time in RES organs is directly related to the molecular weight of the perfluorochemical, with an optimum range between 460 and 550 g mol^{-1} (Fig. 1) [12]. The lower limit is related to the onset of pulmonary emboli, which occur at perfluorochemical vapor pressures greater than 20 Torr. The upper cutoff limits the organ retention half-life to less than 3 weeks, which is an acceptable limit for single-dose drugs in medical applications. There is poor correlation between organ retention time and perfluorochemical structure; thus, the presence of cyclic moieties or heteroatoms has little effect. The notable exception to the linear dependence of half-life versus molecular weight is PFOB, which has an unusually short organ half-

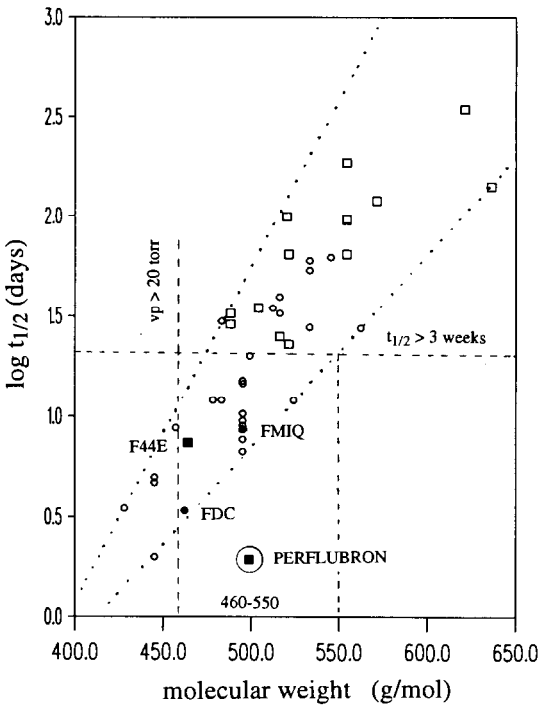

Fig. 1. Plot of the organ retention time versus molecular weight for a number of perfluorochemicals (data from Yamanouchi *et al.* [15]). The squares and circles represent aliphatic and cyclic perfluorochemicals, respectively. The preferred molecular weight range for medical applications is 460–550 g mol^{-1}. Note that PFOB falls outside the projected linear range and has a shorter organ retention time than would be predicted for its molecular weight. The value for the half-life of PFOB for the purposes of the plot was obtained by scaling PFOB relative to FDC where the accepted values are 4 and 7 d, respectively.

life for its molecular weight [12]. It should be noted that the preferred molecular weight range is very narrow. This severely limits the number of possible chemical structures that are appropriate for medical applications, and most of the possible structures have already been tested.

Clark was the first to note a correlation between the perfluorochemical lipophilicity (as measured by the critical solution temperature with hexane) and its RES retention time (Fig. 2) [34]. A number of other researchers have also studied the relationship between organ retention time and perfluorochemical properties [15, 24, 34–36]. Riess *et al.* argue that the measurement of the critical solution temperature offers no advantage over a simple consideration of the molecular weight in determining the suitability of a perfluorochemical for medical application, because plots of molecular weight versus critical solution temperature yield a straight line [24]. However, critical solution temperatures may be directly related to organ retention times due to the mechanism of removal [15, 35, 36].

Yamanouchi *et al.* investigated the relationship between organ retention and a number of thermodynamic properties of the perfluorochemical, including molecular weight, solubility parameter, vapor pressure, critical solution temperature, molar volume, molecular connectivity, van der Waals' volume, van der Waals' area and molar attraction constants [15]. They found the best correlation of retention time to be with a linear combination of critical solution temperature and vapor pressure.

Direct evidence of the importance of perfluorochemical lipophilicity in the removal process comes from the work of Obraztsov *et al.* [36], who propose a two-step removal mechanism. According to their model, the first step is the molecular diffusion of perfluorochemicals through the cytoplasm of the RES cells to the blood stream. This process occurs in a time span of minutes to hours. The second (rate-determining) step involves mass transfer of perfluorochemical from the RES organs to the lungs by lipid carriers, a process which depends critically on PFC lipophilicity. In an elegant experiment, Obraztsov *et al.* found that the organ retention time of perfluorochemicals can be decreased significantly by intravenous injection of a lipid emulsion [36]. The lipid emulsion is, therefore, able to provide a lipid sink in the blood to remove the PFC from the organs and carry it to the lungs.

It is clear from plots of critical solution temperature versus half-life (Fig. 2) that PFOB is unique: of all of the proposed second generation perfluorochemicals is has the lowest organ retention time and the lowest critical solution temperature. The enhanced lipophilicity of PFOB is clearly demonstrated by its moderate solubility in olive oil (Table 2). The lipophilicity observed for PFOB is a result of the substitution of a single bromine atom in the terminal position.

Emulsion stability

The discovery by Long *et al.* that PFOB is excreted rapidly and also produces stable concentrated emulsions opened the door to the formulation of improved second generation emulsions which have overcome the problems associated with their first generation counterparts. The large differences in physical stability between Fluosol and PFOB emulsions are apparent in their emulsion storage requirements: whereas Fluosol must be shipped and stored in a frozen state, PFOB emulsions are stable at room temperature for periods greater than 1 year [33].

Ostwald ripening is the predominant mechanism for particle growth in perfluorochemical emulsions [37–40]. Ostwald ripening results from the isothermal distillation of perfluorochemical molecules from smaller emulsion droplets to larger ones through the continuous phase. Kelvin observed that the solubility of the dispersed phase (i.e. perfluorochemical) increases with an increase in the radius of curvature of emulsion droplets [41]. Thus, large droplets tend to grow at the expense of the small ones.

Lifshits and Slezov have developed a theory to describe the Ostwald ripening process in crystalline dispersions [42]. Their theory was later modified by Higuchi and Misra [43] specifically for emulsions. According to the theory,

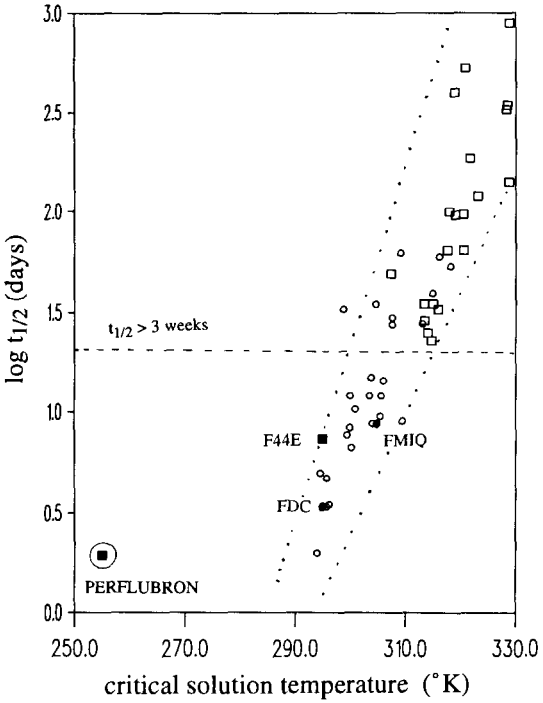

Fig. 2. Plot of the organ retention time versus critical solution temperature for a number of perfluorochemicals (data from Yamanouchi *et al.* [15]). Squares and circles represent aliphatic and cyclic perfluorochemicals, respectively. PFOB exhibits an unusually low critical solution temperature and organ retention time relative to other perfluorochemicals. The value for the half-life of PFOB for the purposes of the plot was obtained by scaling PFOB relative to FDC where the accepted values are 4 and 7 d, respectively.

TABLE 2

Comparison of various lipophilic properties of perfluorochemicals with organ retention times (data from Obraztsov *et al.* [36])

Property	PFOB	FDC	FTPA
$t_{1/2}$ (d)	4	7	65
T_c versus octane (°C)	4	22	43
Solubility in olive oil (mM)	110	26	5.1

the cube of the mean number radius of the particles increases linearly with time at a rate ω given by:

$$\omega = da^3/dt = 8\sigma V_m CD/9RT \quad (1)$$

where a is the mean number radius of the particles, σ is the interfacial tension, V_m the molar volume, and C and D are the concentration and diffusion coefficients of the perfluorochemical in water. The quantity C is

expressed as a volume fraction of solute and is dimensionless; R and T are the molar gas constant and absolute temperature, respectively.

In general, the solubility of perfluorochemicals in water decreases with increases in the molecular volume and/or molecular weight of the perfluorochemical [13, 38]. Hence, emulsion stability increases as follows: FTBA (molecular weight = 671 daltons) > FTPA (molecular weight = 521 daltons) > FDC (molecular weight = 462 daltons). Although their molecular weights are similar, F-44E produces more stable emulsions than FDC, presumably due to the significantly increased molecular volume of F-44E relative to FDC [40]. A comparison of the Ostwald ripening rates of PFOB relative to FDC and F-44E is presented in Fig. 3. The stability can be assessed by the slope of the lines, and represents a growth rate in units of μm^3 $month^{-1}$ (S = growth rate). Emulsion stability increases in the order PFOB > F-44E > FDC.

The molecular diffusion formalism developed by Lifshits does not take into account the role of the emulsifier in the stabilization process, aside from the interfacial tension term. Significant improvements in perfluorochemical emulsion stability have been observed for emulsions stabilized by egg yolk phospholipids versus Pluronic F-68 [40]. Emulsifiers perform two

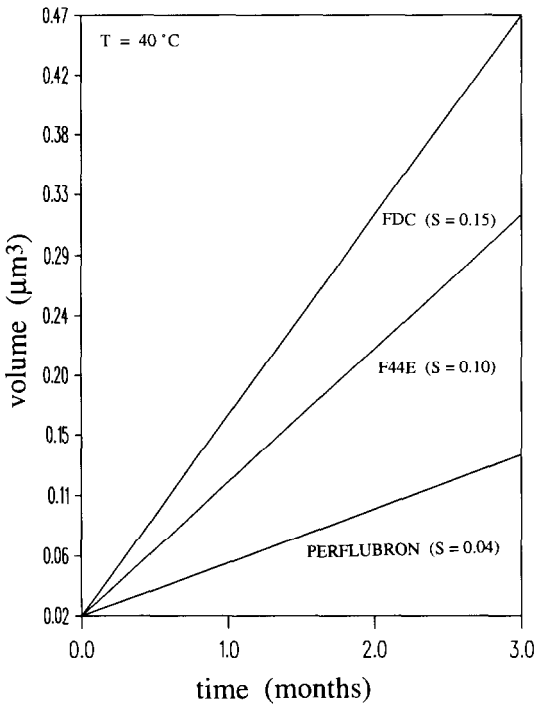

Fig. 3. Plot of growth rates for 90% w/v perfluorochemical emulsions stabilized by egg yolk phospholipids (storage temperature, 40 °C). The growth rates (S parameter) are expressed as the increase in droplet volume per month in accordance with Lifshits–Slezov theory. Emulsion growth rates fall in the order PFOB < F-44E < FDC.

functions in emulsions. First, they lower the interfacial tension at the PFC/water interface, thereby decreasing the work required to form the emulsion; however, decreases in interfacial tension do not necessarily lead to increases in emulsion stability. A stabilizing effect is the second function of the emulsifier. The surfactant must form an interfacial film that is condensed in terms of lateral interactions between the components of the film (i.e. for a perfluorochemical in water emulsion, the hydrophobic groups must have strong lateral interactions). The strength of the interfacial film, and therefore emulsion stability, is related to the film's rheological properties (i.e. to its viscous and elastic character).

The hydrophilic–lipophilic balance (HLB) method provides a correlation between the chemical structure of the surfactant and its emulsifying power [44, 45]. The HLB number of a particular surfactant is related to the balance between the hydrophilic and lipophilic portions of the molecule. In practice, an emulsifying agent, or better a combination of emulsifying agents, is selected whose HLB number is approximately the same as the ingredients to be emulsified. HLB numbers range from 0–40, with the more lipophilic surfactants (e.g. oleic acid, HLB = 1) having low values and the more hydrophilic surfactants (e.g. potassium oleate, HLB = 20) having high values. In general, surfactants with HLB values in the range 4–6 are good emulsifiers for water-in-oil emulsions, while values in the range 8–18 are typical for good oil-in-water emulsifiers. Although the HLB system as developed by Griffin [44] provides a qualitative understanding of emulsion stabilization, the work of Winsor [46], and later Beerbower and Hill [47], helped put the theory on a more quantitative, thermodynamic basis. In a thermodynamic sense, the enhanced stabilization afforded by different surfactants is due to improved 'cohesion' at the perfluorochemical/water interface.

Cohesive energies control the mutual solubility of two liquids. In the case of binary mixtures of perfluorochemical and water, three cohesive energies are involved. The cohesive energy of the water for itself (C_{ww}), of the perfluorochemical for itself (C_{ff}) and of the water for the perfluorochemical (C_{wf}). The quantity C_{ww} contains both a term for the London dispersion energy and additional terms related to its extensive hydrogen-bonding capability, and is equal to *c.* 550 cal cm^{-3}. The quantity C_{ff} contains only dispersion energy contributions and is, therefore, much weaker (c. 20–50 cal cm^{-3}) depending on the perfluorochemical. The quantity C_{wf} also contains only dispersion components and is very small in relation to C_{ww}. Hence, perfluorochemical and water are mutually insoluble.

In the case of an emulsion stabilized by a surface-active agent which contains both hydrophilic (h) and lipophilic (l) components, there are 10 possible cohesive energies as listed in Table 3. The relative magnitudes of these cohesive energies control the phase relationships and miscibility in the three-component perfluorochemical/water/surfactant system. The surfactant, due to its amphiphilic character, tends to locate at the interface between the perfluorochemical and water, with resultant cohesive energies between the surfactant lipophile and perfluorochemical, C_{fl}, and surfactant hydrophile

TABLE 3

Ten possible cohesive energies for surfactant/perfluorochemical/water ternary mixtures

C_{ff}	C_{hw}	C_{ll}	C_{hh}
C_{fh}	C_{ww}	C_{fw}	C_{wl}
	C_{fl}	C_{hl}	

and water, C_{hw}. Winsor states that C_{fl} and C_{hw} are the most important of the 10 cohesive energies [46]. The Winsor quantity R is simply equal to C_{fl}/C_{hw}, and has been found to be qualitatively proportional to the surfactant HLB value. The selection of the appropriate emulsifier molecule is now simplified to finding one in which the solubility parameters of the hydrophilic moiety and the aqueous phase match, as do the parameters of the lipophilic moiety and the oil phase. In the case of the required HLB of the perfluorochemical (HLB_0), only C_{fl} contains contributions from the specific perfluorochemical of interest. Hence, it is logical to assume that HLB_0 may be related to C_{fl}. The C_{fl} term is expected to be especially important in the perfluorochemical emulsions, since it involves interactions between the hydrogenated chains of the surfactant and the perfluorochemical for which the mixing is highly nonideal [48].

Following Beerbower and Hill [47], it is possible to define these cohesive energies (and hence R) in terms of the concept of the solubility parameter as introduced by Hildebrand and Scott [49]. The solubility parameter of a liquid, δ, has been used with great success to describe the thermodynamic properties of dilute solutions. The solubility parameter, or the closely related cohesive energy density, has also been related to other physical properties of nonpolar liquids such as the surface tension, wettability and the required hydrophilic–lipophilic balance [50].

For low molecular weight nonpolar liquids, the solubility parameter is simply the square root of the cohesive energy density, which is related to the heat of vaporization, ΔH_v, and the molar volume, V. For perfluorochemicals, the cohesive energy density, C_{ff} is given by:

$$(C_{ff})^{1/2} = \delta_{ff} = (\Delta H_v/V - RT/V)^{1/2} \quad (2)$$

The quantity C_{ff} is the energy required to separate all the molecules in a cubic centimeter to a distance at which they will no longer interact, and is thus a measure of the cohesive energy. As a general rule, for two phases to be miscible (i.e. cohesive), the two fluids must have comparable values of δ. The magnitude of C_{fl} is, therefore, related to the interaction parameter A_{fl} between the lipophile and perfluorochemical as expressed in eqn. (3):

$$A_{fl} = (\delta_{ff} - \delta_{ll})^2 \quad (3)$$

Thus, as the value of A_{fl} approaches zero, the value of C_{fl} increases.

The value of A_{fl} can be determined experimentally from measurements of the critical solution temperature as outlined below:

$$RT_c = 2A_{fl}[(v_f/v_l)/(v_f^{1/2} + v_l^{1/2})^2] \text{ (Flory–Huggins approximation)} \quad (4)$$

where T_c is the critical solution temperature, and v_f and v_l are the molar volumes of perfluorochemical and lipophile, respectively. It is clear from eqn. (4) that the key to finding a perfluorochemical with both a fast excretion rate and excellent emulsion stabilities lies in increasing the lipophilicity of the perfluorochemical.

The required HLB values (HLB_0) for three different perfluorochemicals are presented in Table 4 along with the HLB values for Pluronic F-68 and egg yolk phospholipid emulsifiers [51–53]. Such HLB_0 values were determined using an HLB test kit (available from ICI America Inc.). The surfactants used were Tween 80, a polysorbate (HLB value 15.0) and Span 80 (sorbitan monooleate) (HLB value, 4.3). Test emulsions were made at various HLB values (obtained using mixtures of Tween and Span) and the physical stability with time was observed. If no differences for a range of HLB values are observed, the total surfactant concentration is decreased until differences become apparent. The emulsions were processed with an Ultraturrax (G-25 head) for 2 min at 8500 rpm. Differences were detected by changes in the amount of free fluorocarbon observed.

The decreased HLB_0 values for PFOB relative to FDC and FTBA again reflects its increased lipophilicity relative to other perfluorochemicals. In theory, the best emulsions are obtained when the values of the emulsifier HLB and perfluorochemical HLB_0 match. In light of this, it is exciting to note the fortuitous match between the HLB_0 value for PFOB and the HLB value of egg yolk phospholipid [52]. It is also clear that Pluronic F-68 is a rather poor emulsifier for perfluorochemicals, and hence it is not expected to be bound extensively to the surface of the emulsion particles. Nevertheless, Pluronic F-68 is capable of stabilizing perfluorochemical emulsions, presumably by acting as a steric stabilizer. Strong stabilization can be achieved when Pluronic F-68 is associated by hydrogen bonds with fluorinated surfactants [54].

In summary, PFOB is capable of forming improved emulsions relative to other perfluorochemicals of similar molecular weight due to its enhanced lipophilicity, which improves the cohesion between its fluorinated groups

TABLE 4

Hydrophilic–lipophilic balance of perfluorochemicals and intravenous surfactants

Perfluorochemical	HLB_0 value	Surfactant	HLB value
PFOB	6.0 [52]	egg yolk phospholipid	6–7[a]
FDC	9.5 [51]	potassium oleate	18.0 [53]
FTBA	10.3 [51]	Pluronic F-68	29.0 [53]

[a]The HLB value of egg yolk phospholipid is estimated to be slightly lower than the value of 8.0 assigned to Clearate-WDF (soya lecithin) [53] due to reduced levels of charged lipids such as phosphatidylethanolamine and phosphatidylinositol.

and the hydrogenated lipophilic groups of the emulsifiers. The enhanced lipophilicity of PFOB is due solely to the presence of the terminal bromine atom. The improved cohesion between EYP and PFOB should also be noted in terms of the excellent match between the HLB value for EYP and the HLB_0 value for PFOB.

One approach to stabilizing perfluorochemical emulsions is to improve the cohesion between the lipophile and the perfluorochemical by fluorinating the amphiphile. As discussed by Riess and Postel, fluorinated surfactants reduce the interfacial tension at the perfluorochemical/water interface thereby decreasing the tendency to phase-separate [55]. Reduced interfacial tensions also decrease the rate of molecular diffusion, as described by eqn. (1). In addition, the introduction of fluorinated amphiphiles might lead to increases in the interfacial film elasticity, an effect which opposes droplet coalescence.

Another approach to improving the cohesion between lipophile and perfluorochemical is to use linear mixed perfluorochemical–hydrocarbon amphiphiles ('dowels') in conjunction with egg yolk phospholipids to 'reinforce' the interface [55–57]. The dowel is oriented with its fluorinated component extending into the perfluorochemical phase and its hydrocarbon component providing better cohesion with the lipid chains. Reductions in interfacial tension and improvements in film elasticity have been observed, which lead to remarkable increases in emulsion stability [33, 55, 58].

Oxygen solubility

Much of the potential medical utility of perfluorochemicals is related to their ability to dissolve large amounts of gases, including oxygen and carbon dioxide. As discussed previously, in order for two fluids to be miscible they must have comparable δ values. Whereas δ values for perfluorochemicals are generally in the range 6–7, hydrocarbons are 7–9 and water is 23.4 hildebrands. Oxygen has a δ value of 5.7 hildebrands and is therefore a good match with perfluorochemicals, resulting in the excellent solubility characteristics observed.

The mechanism of oxygen delivery in perfluorochemical emulsions is fundamentally different from that of hemoglobin (see Fig. 4). For hemoglobin, the sigmoidal shape of the binding curve indicates that the four subunits of hemoglobin bind oxygen in a cooperative manner. In the case of perfluorochemicals, the gas molecules simply occupy cavities within the liquid solvent (i.e. no binding occurs) [59]. The solubility of oxygen in perfluorochemicals obeys Henry's law; the solubility increases linearly with partial pressure or concentration [60]. Oxygen solubility in a perfluorochemical emulsion depends primarily on the total quantity of the perfluorochemical and to a lesser extent on the nature of the perfluorochemical. Gas solubility differences among perfluorochemicals have been noted and these differences may be related to the molecular structure and shape of each perfluorochemical. Of the proposed second generation fluorochemicals, the linear aliphatic perfluorochemicals (i.e. PFOB and F-44E) have the highest solubility for oxygen and carbon dioxide, dissolving *c.* 20–25% more oxygen than their

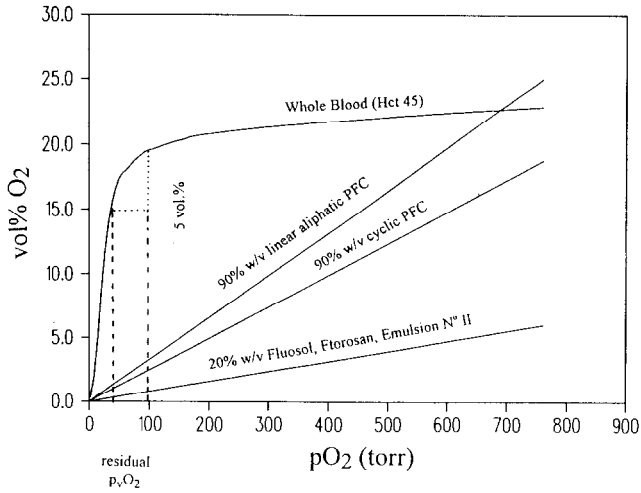

Fig. 4. Plot of the oxygen content (vol.%) versus p_{O_2} (Torr) for whole blood and various perfluorochemical emulsions. Linear aliphatic perfluorochemicals (e.g. PFOB and F-44E) are capable of dissolving *c.* 20–25% more oxygen than their cyclic counterparts (e.g. FDC, FMIQ and FMA).

cyclic counterparts (FDC, FMIQ and FMA) [8, 28]. This can be rationalized in terms of the cavities formed within the perfluorochemicals upon incorporation of solute molecules. Gas molecules occupy large channels in linear aliphatic perfluorochemicals while more closely interlocked channels with a decreased ability to include solute molecules are formed for planar cyclic structures [59].

A comparison of oxygen content of blood and several perfluorochemical emulsions is shown in Fig. 4. At a hematocrit of 45% and a p_{O_2} value of 100 Torr, blood is capable of binding approximately 20 vol.% O_2. The dotted line at 40 Torr represents the mean tissue value of p_{O_2} after oxygen delivery to the tissues. Thus, blood delivers *c.* 5 vol.% O_2 (assuming a normal cardiac output of 5 l min^{-1} in normal physiological conditions). In comparison, first generation perfluorochemical emulsions (based on 20% w/v perfluorochemical) can deliver only *c.* 0.8 vol.% O_2 under similar conditions and require a p_{O_2} value of 633 Torr to deliver 5 vol.% O_2 [61].

New concentrated perfluorochemical emulsions with up to 100% w/v PFOB are currently being developed by Alliance Pharmaceutical Corp. These formulations allow 5 vol.% O_2 to be delivered at p_{O_2} values near those of room air. This improved efficacy afforded by concentrated PFOB emulsions has opened up a new era for the development of perfluorochemical-based oxygen carriers in a number of medical applications.

Although whole blood is capable of binding more oxygen at lower oxygen partial pressures, it is not as effective as perfluorochemical emulsions at unloading the oxygen [61]. For perfluorochemical emulsions, the absence of chemical binding, the smaller size of the particles (hence greater surface

area) and the higher rate of oxygenation and deoxygenation all lead to more efficient oxygen delivery relative to whole blood. In an early clinical study with Fluosol, it was shown that while perfluorochemicals accounted for only 27% of the oxygen flux, they provided more than 50% of the oxygen consumed [62]. Similar ratios have been obtained for concentrated PFOB emulsions [63].

Imaging characteristics

Another advantage of PFOB over other perfluorochemicals (as well as other contrast agents currently on the market) is the unique ability of PFOB to provide contrast for all three major imaging modalities [61, 64]. First, the presence of the heavy terminal bromine atom makes the PFOB molecule radiopaque, thereby facilitating its use as a contrast agent for X-ray and computed tomography (CT). Second, because PFOB contains no hydrogen atoms, it provides a signal void in proton magnetic resonance imaging (MRI). Third, sound waves are reflected efficiently by perfluorochemical droplets due to their compressibility characteristics, making PFOB appropriate for sonography. Linear aliphatic perfluorochemicals such as PFOB, are preferred for ultrasound applications due to their ability to form channels within the liquid, which gives them greater compressibility than cyclic molecules such as FDC, FMIQ or FMA.

The development of concentrated emulsions has improved the efficacy of contrast agents by reducing the inherent volume limitations of low content PFC emulsions. This has enabled the development of agents for potential applications such as blood pool imaging.

Advantages of PFOB in neat PFC applications

Imaging applications

Due to its unique imaging characteristics, neat PFOB is the only known contrast agent in development for both MRI and X-ray studies of the gastrointestinal tract.

Liquid ventilation

The classic liquid ventilation experiments of Clark and Gollan [1] have sparked research in the treatment of respiratory distress syndrome with neat perfluorochemical liquids [65, 66].

Table 5 compares five proposed perfluorochemicals for liquid ventilation and PFOB stands out in these applications as the perfluorochemical of choice [67]. PFOB is preferred over the perfluoroalkyltetrahydrofuran-based compounds (e.g. FC-77, RM-101, FC-75) due to its greater degree of purity and definition and its significantly decreased vapor pressure. The lower vapor pressure of PFOB is important in liquid ventilation for two reasons. First, the vapor pressure of the perfluorochemical must be low enough to eliminate the risk of intravascular gas–vapor emboli in the event of leakage into the

TABLE 5

Comparison of the physical properties of perfluorocarbons for liquid ventilation

Property	Water	FC-77	RM-101	FC-75	FDC	PFOB
boiling point (°C)	100	97	101	102	142	143
density at 25 °C (g cm^{-3})	1.00	1.78	1.77	1.78	1.95	1.92
kinematic viscosity at 25 °C (cS)	1.00	0.80	0.82	0.82	2.44	0.96
vapor pressure at 37 °C (Torr)	47	85	64	63	13	11
surface tension at 25 °C (dyn cm^{-1})	72	15	15	15	19	18
wettability on saline at 25 °C (dyn cm^{-1})	n/a	8.5	6.9	6.9	−1.5	2.7
[O_2] at 37 °C [ml O_2 (100 ml PFC)$^{-1}$]	3	50	52	52	43	53
[CO_2] at 37 °C [ml CO_2 (100 ml PFC)$^{-1}$]	57	198	160	160	140	210

blood. Vapor pressures less than 40 Torr are thought to minimize the potential impact of microbubble embolism with blood substitutes [68], although the lower value of 20 Torr has been recommended [12]. Thus, the vapor pressures of greater than 60 Torr observed for the perfluoroalkyltetrahydrofuran series preclude their use in liquid breathing applications. The second advantage of lower vapor pressure is economic and has to do with the increased cost associated with loss of perfluorochemical during treatment due to vaporization. Since the doses are expected to be high, the cost savings afforded by decreasing the vapor pressure would be significant.

FDC and PFOB are preferable to the other perfluorochemicals with high vapor pressures because they have already been subjected to extensive biocompatibility testing. Direct comparisons between FDC and PFOB for liquid breathing applications reveal the following: first, PFOB can be prepared to a higher degree of purity due to the telomerization synthetic route. Second, the kinematic viscosity of PFOB is significantly less than that of FDC [67]. This is important because airway resistance to liquid flow increases approximately in proportion to the kinematic viscosity, and in order to facilitate PFC flow within the lungs it is imperative that a liquid be chosen with the lowest possible kinematic viscosity. Third, gas solubility in PFOB is significantly greater than in FDC, which is notable for reasons discussed previously [32]. In fact, PFOB is capable of dissolving *c.* 20% more O_2 and 33% more CO_2 than FDC. Effective ventilatory gas exchange depends on both high O_2 and CO_2 gas solubilities. The CO_2 solubilities may, in fact, be more important than O_2 solubilities for liquid ventilation fluids, since CO_2 exchanges must occur across a smaller arterial–alveolar concentration gradient [67]. Fourth, PFOB spreads spontaneously on a air/saline interface (the air/saline interface approximates the aqueous hypophase which may be covering the respiratory membrane in a surfactant-deficient infant), while FDC does not [69]. These spreading properties for PFOB (which are again unmatched for its molecular weight) allow PFOB to form a PFC layer spontaneously over the respiratory membrane [69]. Finally, PFOB offers the additional potential advantage for pulmonary applications due to its additional ability to act as an imaging agent (X-ray) for the lungs [26, 27].

Retinal applications

Low viscosity PFC liquids such as FDC, perfluorotributylamine and perfluorooctane have been investigated for use as intraoperative tools during vitreoretinal surgery for trauma-induced retinal detachment. The high degree of purity, low viscosity and excellent biocompatibility characteristics of neat PFOB also makes it an excellent potential candidate for retinal applications [70].

Conclusions

PFOB is currently the perfluorochemical of choice for the development of many products for medical applications. Its superiority to other perfluorochemicals results from the presence of the bromine atom which gives it both radioopacity and lipophilicity. Due to its lipophilic character, it is the only perfluorochemical with both a short organ retention time and excellent emulsion stability. The formulation of concentrated PFOB emulsions has led to improved oxygen solubility relative to other currently proposed formulations. Other positive attributes of PFOB include superior purity and definition, commercial availability of the raw material, excellent biocompatibility, enhanced compatibility with egg yolk phospholipids, low vapor pressure, low kinematic viscosity, a positive spreading coefficient and imaging capabilities in all three major imaging modalities. These qualities make the bromine-containing perfluorochemical PFOB the ideal candidate for many medical procedures including oxygen transport, diagnostic imaging and liquid ventilation.

Acknowledgments

The author wishes to thank Ms Gwen Rosenberg and Professor Jean Riess for their dedicated proof reading of the manuscript.

References

1 L.C. Clark and F. Gollan, *Science, 152* (1966) 1755.
2 F. Gollan and L.C. Clark, *Physiologist, 9* (1966) 191.
3 H. Sloviter and T. Kamimoto, *Nature (London), 216* (1967) 458.
4 R.P. Geyer, *N. Engl. J. Med.*, (1973) 1077.
5 H. Okamoto, K. Yamanouchi, T. Imagawa, R. Murashima, K. Yokoama, R. Watanabe and R. Naito, *Proc. Intercompany Conf.*, 1973.
6 L.C. Clark, F. Becattini, S. Kaplan, V. Obrock, D. Cohen and C. Becker, *Science, 181* (1973) 680.
7 J.G. Riess, *Curr. Surg., 45* (1988) 365.
8 J.G. Riess, *Proc. Int. Symp. Artif. Blood Substitutes,* Bari, 1987; *Trasfus. Sang., 32* (1987) 316.
9 J.G. Riess, *UNO Ind. Dev. Symp. Rep. US/RAS/85/230/27* (1987).

10 R. Xiong, R.A. Zhung, H.F. Chen, W.Y. Huang, C.P. Luo and W.T. Cao, *Chin. J. Surg., 19* (1981) 213.
11 F.F. Beloyartsev, E.I. Mayevsky and B.I. Isiamov, *Ftorosan; Oxygen Carrying Perfluorochemical Plasma Substitute*, Acad. Sci. USSR. Pushchino, 1983.
12 J.G. Riess, *Artif. Organs, 8* (1984) 44.
13 A.S. Kabalnov, K.N. Makarov and O.V. Shcherbakova, *J. Fluorine Chem., 50* (1990) 271.
14 T. Mitsuno, H. Ohyanagi, K. Yokoyama and T. Suyama, *Biomat. Artif. Cells Artif. Organs, 16* (1988) 365.
15 K. Yamanouchi, M. Tanaka, Y. Tsuda, K. Yokoama, S. Awazu and Y. Kobayashi, *Chem. Pharm. Bull., 33* (1985) 1221.
16 L.C. Clark, E.W. Clark, R.E. Moore, D.G. Kinnett and E.I. Inscho, *Advances in Blood Substitute Research*, Alan Liss Inc., New York, 1983, p. 169.
17 K.C. Lowe, *Adv. Mater., 3* (1991) 87.
18 S.K. Sharma, K.C. Lowe and S.S. Davis, in T.M.S. Chang and R.P. Geyer (eds.), *Blood Substitutes*, Marcel Dekker, New York, 1989, p. 447.
19 F.K. Schweighardt and C.R. Kayhart, *Eur. Pat. Appl. EP282 949* (1988); [*Chem. Abs., 110* (1988) 199 223].
20 R.J. Kaufman, *Eur. Pat. Appl. WO 89/10118* (1989); [*Chem. Abs., 113* (1989) 46 356].
21 C. Santaella, P. Vierling and J.G. Riess, *J. Colloid Interface Sci., 148* (1976) 288.
22 J.G. Riess, C. Santaella and P. Vierling, *Proc. XXIIth Meet. Surfactants*, CED Ed., Barcelona, 1991, p. 157.
23 S.K. Sharma, K.C. Lowe and S.S. Davis, in T.M.S. Chang and R.P. Geyer (eds.), *Blood Substitutes*, Marcel Dekker, New York, 1989, p. 447.
24 J.-J. Grec, J. Riess and B. Devallez, *Nouv. J. Chim., 9* (1985) 637.
25 D.M. Long, D.C. Long, R.F. Mattrey, R.A. Long, A.R. Burgan, W.C. Herrick and D.F. Shellhamer, in T.M.S. Chang and R.P. Geyer (eds.), *Blood Substitutes*, Marcel Dekker, New York, 1989, p. 411.
26 D.M. Long, C.B. Higgins, R.F. Mattrey, R.M. Mitten and F.K. Multer, in R. Banks (ed.), *Preparation, Properties, and Industrial Applications of Organofluorine Compounds*, Ellis Horwood, Chichester, 1982, p. 139.
27 D.M. Long, M. Liu, P.S. Szanto, D.P. Alrenga, M.M. Patel, M.V. Rios and L.M. Nylus, *Radiology, 105* (1972) 323.
28 J.G. Reiss and M. LeBlanc, in K.C. Lowe (ed.), *Blood Substitutes; Preparation, Physiology, and Medical Applications*, Ellis Horwood, Chichester, 1988.
29 R.P. Geyer, in R. Frey, H. Beisbarth and K. Stosseck (eds.), *Oxygen Carrying Blood Substitutes: 5th Int. Symp. Perfluorochemical Blood Substitutes*, Mainz, 1981, W. Zuckschwerdt Verlag, Munich, 1982, p. 19.
30 M. Le Blanc and J. G. Riess, in R. Frey, H. Beisbarth and K. Stosseck (eds.), Oxygen Carrying Blood Substitutes: *5th Int. Symp. Perfluorochemical Blood Substitutes*, Mainz, 1981, W. Zuckschwerdt Verlag, Munich, 1982, p. 43.
31 K.C. Lowe, *Comp. Biochem. Physiol., 87A* (1987) 825.
32 K.C. Lowe, in M. Moenizuki, C.R. Honig, T. Koyama, T.K. Goldstick and D.F. Bruley (eds.), *Oxygen Transport in Tissue*, Plenum, New York, 1988, Vol. X, p. 655.
33 J.G. Riess, *Biomater. Artif. Cells Immob. Biotechnol., 20* (1992) 183.
34 L.C. Clark, *US Pat. 4 289 499* (1981); [*Chem. Abs., 95* (1981) 209 719].
35 R.E. Moore and L.C. Clark, *Int. Anesthesiol. Clin., 23* (1985) 11.
36 V.V. Obraztsov, A.S. Kabalnov and K.N. Makarov, *J. Fluorine Chem., 54* (1991) 376.
37 S.S. Davis, H.P. Round and T.S. Purewal, *J. Colloid Interface Sci., 80* (1981) 508.
38 A.V. Pertsov, A.S. Kabalnov and E.D. Shchukin, *Kolloidn. Zh., 46* (1984) 1172.
39 A.S. Kabalnov, A.V. Pertsov and E.D. Shchukin, *Kolloidn. Zh., 46* (1984) 1108.
40 C. Varescon, C. Arlen, M. Le Blanc and J.G Riess, *J. Chim. Phys., 86* (1989) 2111.
41 W. Thomson (Lord Kelvin), *Philos. Mag., 42* (1871) 448.
42 I.M. Lifshits and V.V. Slezov, *Sov. Phys., JETP, 35* (1959) 331.
43 W.I. Higuchi and J. Misra, *J. Pharm. Sci., 51* (1962) 459.
44 W.C. Griffin, *J. Soc. Cosmet. Chem., 1* (1949) 311.

45 ICI Americas Inc., *The HLB System*, Publ. 0-510M, Wilmington, DE, 1980.
46 P. Winsor, *Solvent Properties of Amphiphilic Systems*, Butterworth, London, 1954.
47 A. Beerbower and M. Hill, *Am. Cosmet. Perfum.*, *87* (1972) 85.
48 R.L. Scott, *J. Phys. Chem.*, *62* (1958) 136.
49 J.H. Hildebrand and R.L. Scott, *The Solubility of Nonelectrolytes*, 3rd Edn., Dover Publ. Inc., New York, 1964.
50 A.F.M. Barton, *Handbook of Solubility Parameters and Other Cohesion Phenomena*, 2nd edn., CRC Press, Boca Raton, FL, 1991.
51 K. Meguro, K. Ogihara, S. Yoshikawa, K. Esumi and M. Ueno, *J. Colloid Interface Sci.*, *90* (1982) 549.
52 J.G. Weers, unpublished results.
53 *McCutcheons Emulsifiers and Detergents*, North America edn., McPublishing, Glen Rock, NJ, 1991.
54 L. Zarif, A. Manfredi, C. Varescon, M. Le Blanc and J.G. Riess, *J. Am. Oil Chem. Soc.*, *10* (1989) 1515.
55 J.G. Riess and M. Postel, *Biomater. Artif. Cells Immob. Biotechnol.*, *20* (1992) 819.
56 M.P. Turberg and J.E. Brady, *J. Am. Chem. Soc.*, *110* (1988) 7797.
57 H. Meinert, R. Fackler, A. Knoblich, J. Mader, P. Reuter and W. Rohlke, *Proc. Int. Symp. Blood Substitutes*, Montreal, 1991, in press.
58 J.G. Riess, L. Sole-Violan and M. Postel, *J. Disp. Sci. Technol.*, *15* (1992) 349.
59 J.J. Delpuech, M.A. Hamza and G. Serratrice, *J. Magn. Reson.*, *36* (1976) 173.
60 J.G. Riess and M. Le Blanc, *Pure Appl. Chem.*, *54* (1982) 2383.
61 J.G. Riess, *Vox Sang.*, *61* (1991) 225.
62 K.K. Tremper, R. Lapin, E. Levine, A. Friedman and W. C. Shoemaker, *Crit. Care Med.*, *8* (1980) 738.
63 N.S. Faithfull, in W. Erdmann and D.F. Bruley (eds.), *Oxygen Transport to Tissue XIV*, Plenum, New York, 1992, p. 441.
64 R.F. Mattrey, *Am. J. Radiol.*, *152* (1989) 247.
65 T.H. Shaffer, *Undersea Biomed, Res.*, *14* (1987) 169.
66 J.S. Greenspan, M.R. Wolfson, S.D. Rubenstein and T.H. Shaffer, *J. Pediatr.*, *117* (1990) 106.
67 K.M. Sekins, presentation at the Ross Conference, December, 1991.
68 L.C. Clark, *Prog. Clin. Biol. Res.*, *19* (1978) 69.
69 J.G. Weers, unpublished results.
70 M. Flores-Aguilar, J.A. Crapotta, D. Munguia, G.D. Bergeron-Lynn, D.A. Long, C.A. Wiley, J.G. Weers and W.R. Freeman, *Retina*, in press.