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A fluorocarbon emulsion with a high solubility for CO_2

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Matthews, W. H., and J. A. Kylstra. A fluorocarbon emulsion with a high solubility for CO_2. 1976. Undersea Biomed. Res. 3(2):113-120.—A stable emulsion can be prepared by subjecting a mixture of 30% (by volume) FC-80 fluorocarbon; a 0.3-M THAM solution titrated to pH 7.4 with HC1; and 0.04 g F68 Pluronic surfactant per milliliter FC-80 to ultrasonic energy. The emulsion has a density of 1.24 g/ml; an absolute viscosity of 2.4 centipoise; and an approximate fluorocarbon-droplet diameter of 3μ. The approximate CO_2 content of the emulsion at partial pressures ranging from 30 to 60 mm Hg is 132 ml (STPD)/liter + (5.5 × P_{CO_2}). The O_2 content in ml (STPD)/liter equals 0.213 × P_{O_2} (mm Hg).

liquid breathing
fluorocarbon emulsions
fluoride ions

Various fluorocarbon liquids have been used in liquid breathing experiments (Clark and Gollan 1966; Saga, Modell, Calderwood, Lucas, Tham, and Swenson 1973). These liquids have, in general, a high solubility for carbon dioxide. Nevertheless, the elimination of carbon dioxide through the lungs of fluorocarbon-breathing mammals is inadequate, mainly as a result of mechanical limitations. For this reason, it has been proposed to increase the CO_2-carrying capacity of a fluorocarbon liquid by adding tris (hydroxymethyl) aminomethane (THAM) to it (Kylstra 1974). When dissolved in water, this substance is capable of binding large amounts of CO_2 (Nahas 1962). Although fluorocarbon liquids do not mix with water, it is possible to make stable emulsions of fluorocarbon-liquid droplets suspended in aqueous solutions with the aid of suitable emulsifiers. Such emulsions have already been employed as organ-perfusion media (Sloviter 1975) and as blood substitutes in animals (Geyer 1975). This paper is a report on the preparation of a stable fluorocarbon in THAM emulsion and several physical and chemical characteristics of such an emulsion.

METHODS AND MATERIALS

Preparation of the emulsion

The emulsion, prepared in batches of 150 ml, consisted of 30% (by volume) FC-80 fluorocarbon (3M Company, St. Paul, MN) dispersed in an isotonic 0.3-molar (M) THAM solution to which Pluronic surfactant F68 (BASF Wyandotte Corporation, Wyandotte, MI) had been added. The 0.3-M THAM solution, made up from Certified Primary Standard

THAM (Fisher Scientific Company, Pittsburg, PA) and distilled water, was adjusted to pH 7.40 with 10-N HC1. The total amount of F68 Pluronic used for a particular batch was first dissolved in 105 ml of a 0.3-M THAM solution. This solution was then poured into a glass beaker containing 45 ml of FC-80 fluorocarbon. The mixture was circulated by a positive-displacement Master-flex tubing pump (Cole Parmer, Chicago, IL) through Tygon tubing from the beaker to a continuous-flow chamber attached to a model W185 sonifier (Branson Sonic Power Company, Danbury, CT) and back to the beaker by gravity (see Fig. 1). The continuous-flow chamber was kept cold during emulsification with circulating water cooled to approximately 5°C. Each batch was emulsified for 1 hr at an energy output of 50-60 watts (W) at the sonifier horn.

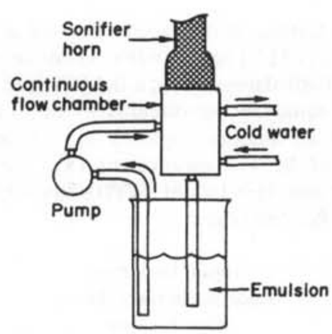

Fig. 1. Arrangement for preparing fluorocarbon emulsions with Branson Model W185 Sonifier.

CO_2 capacity

THAM solutions, in 150 ml batches, were made up in 0.1-M, 0.2-M, and 0.3-M concentrations. Each solution was titrated to pH 7.40 with 10-N HCl, in room air. The pH measurements were made with a #26 radiometer (Radiometer A/S, Copenhagen, Denmark) using a combined pH electrode. During titration, the THAM solution was kept at 37.0 ± 0.1°C in a 200-ml beaker immersed in a waterbath. The temperature of the waterbath was kept constant with a model E52 constant-temperature circulator (Haake Instruments, Inc., Saddle Brook, New Jersey). The pH-calibration buffers were also kept in the same waterbath. The temperature of the THAM solution was measured with a thermometer (Ever Ready Thermometer Company, New York) calibrated against standards certified by the National Bureau of Standards. After titration, the solution was equilibrated in a Farhi tonometer for 2 hr at 37.0 ± 0.1°C with O_2-CO_2 gas mixtures containing 1% to 10% CO_2. Each change to a different gas mixture was followed by a 2-hr equilibration period before samples were removed anaerobically from the tonometer. Five-ml samples of the THAM solution were taken with 10-ml glass syringes and analyzed for pH and P_{CO_2} with a pH/blood-gas analyzer #113 (Instrumentation Laboratory, Inc., Lexington, MA). The waterbath surrounding the two IL electrodes was maintained at 37.0 ± 0.1°C as measured with the same thermometer used to measure the temperature of the THAM solution.

From the pH and P_{CO_2} data, the CO_2 content (C_{CO_2}) in ml (STPD)/liter of the THAM solution at a given P_{CO_2} was computed with the Henderson-Hasselbach equation using a solubility coefficient for CO_2 of 0.7303 ml (STPD)/liter · mm Hg^{-1} at 37°C (Altman and Dittmer 1971). The total CO_2 content of an FC-80 in THAM emulsion was computed by adding the amount of CO_2 dissolved in the FC-80 using a solubility coefficient of 2.105 ml (STPD)/liter · mm Hg^{-1} at 37°C (Sargent and Seffl 1970) and the amount of CO_2 bound by the THAM solution.

Droplet size

The approximate size of the droplets in the emulsion was estimated by forcing it through filters (Millipore Corporation, Bedford, MA) with different pore sizes. The smallest pore size through which an emulsion passed freely and completely was taken to indicate the largest droplet diameter.

Demulsification

Because of the expense of FC-80 fluorocarbon liquid, it may be desirable to recover it in pure form once the emulsion has been used. The emulsion can be demulsified by shaking with ethanol in a separatory funnel. The separated FC-80 is then washed with water to remove any ethanol which may have dissolved in the FC-80. Water is insoluble in FC-80 and the difference in the densities allows separation by gravity.

Fluoride ion concentration

Geyer (1975) and Clark, Wesseler, Kaplan, Miller, Becker, Emory, Stanley, Becattini, and Obrock (1975) have reported that ultrasonically prepared fluorocarbon emulsions contain free fluoride ions, but that the concentration of these ions can be minimized by bubbling CO_2 through the ingredients before and during the process of emulsification. We have repeated these experiments and also tested the effectiveness of other gases in reducing fluoride-ion concentration in ultrasonically prepared fluorocarbon in THAM emulsions.

The fluoride-ion concentration in the aqueous phase of each batch of emulsion was measured with a model 619 Fluoride ion meter (Corning Glass Works, Medfield, MA). The emulsions for this part of the study were prepared using an F68-Pluronic concentration of 0.04 g/ml FC-80. All other parameters were the same as described above. One of six different gases was bubbled through the ingredients contained in a beaker covered with Parafilm for 30 min prior to and during the 1-hr emulsification. Two 50-ml samples of each emulsion were then centrifuged for 1.5 hr at 1870 g in a model HNS centrifuge (International Equipment Co. Needham Heights, MA). We removed 10 ml of the aqueous supernatant from each sample, added it to 10 ml of Corning FAD buffer in separate plastic cups and measured the fluoride-ion concentration. This procedure was repeated twice for each gas. The reported values are the means of four measurements.

RESULTS AND DISCUSSION

Emulsions prepared with a concentration of F68 Pluronic greater than 0.025 g/ml FC-80 were stable at room temperature for at least a week; that is, no phase separation occurred within that period of time. Emulsions prepared with F68 concentrations less than 0.025 g/ml FC-80 were not stable and separated into two distinct phases within several hours after emulsification.

The relationship between the concentration of F68 Pluronic surfactant and the absolute viscosity of 30% FC-80 fluorocarbon in 0.3-M THAM emulsions is shown in Fig. 2 An increase in the F68 concentration causes an increase in the absolute viscosity of the emulsion. There appears to be an abrupt increase in viscosity at an F68 concentration of approximately 0.10 g/ml FC-80. The relationship between droplet size and F68 concentration is shown in Fig. 3. The droplet diameter decreases with increasing F68 concentration up to 0.10 g/ml FC-80. At this concentration, the droplet size tends to stabilize at a diameter of approximately 0.80 μm. These results are in agreement with the general principles of emulsion chemistry (Becher 1965; Schmolka 1970). Increasing the emulsifier concentration causes a decrease in droplet diameter and, therefore, an increase in viscosity, if all other factors (such as the amounts of the continuous and discontinuous phases and the energy output of the emulsification equipment) are kept constant. A further increase in the F68 concentration causes a sudden increase in viscosity (Fig. 2). Since the fluorocarbon-droplet diameter remains the same, the increase in the absolute viscosity at F68 concentrations greater than 0.10 g/ml FC-80 is probably due to an increase in the number of particles by formation of surfactant micelles.

The relationship between P_{CO_2} and pH of the 0.1-M, 0.2-M, and 0.3-M THAM solutions that had been adjusted to pH 7.40 in air is shown in Fig. 4. These data were used to calculate the CO_2 content of the THAM solutions at a given P_{CO_2} using the Henderson-Hasselbach equation. For example, the pH of the adjusted CO_2 free 0.3-M THAM solution (initially

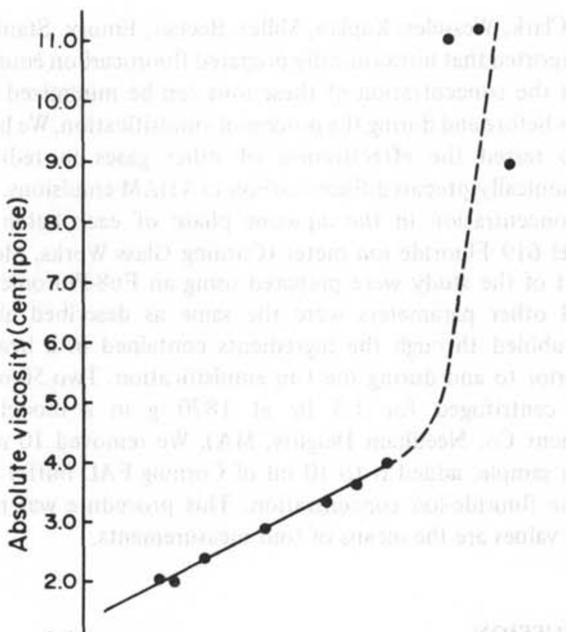

Fig. 2. Relationship between surfactant (F68 Pluronic) concentration and viscosity of 30% (by volume) FC-80 in 0.3-M THAM emulsions at 37°C.

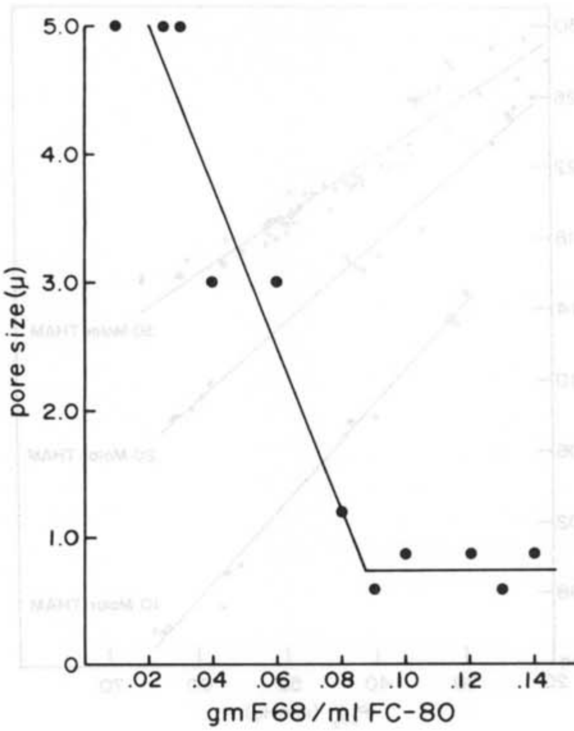

Fig. 3. Relationship between surfactant (F68 Pluronic) concentration and approximate droplet diameter of 30% (by volume) FC-80 in 0.3-M THAM emulsions. Pore size refers to Millipore filter (see text). Regression lines drawn by eye.

7.400) was 7.225 after equilibration with CO_2 at a partial pressure of 40 mm Hg. Substituting the pertinent values in the Henderson-Hasselbach equation yields:

$$7.225 = 6.10 + \log \frac{(\text{total } CO_2) - (0.7303 \text{ ml/liter} \cdot \text{mm Hg}^{-1})(40 \text{ mm Hg})}{(0.7303 \text{ ml/liter} \cdot \text{mm Hg}^{-1})(40 \text{ mm Hg})}$$

Hence C_{CO_2} (total CO_2) = 419 ml (STPD)/liter.

The CO_2 content of 1 liter of a 30% FC-80 fluorocarbon in 0.3-M THAM emulsion at a P_{CO_2} of 40 mm Hg equals the CO_2 physically dissolved in the FC-80 [2.105 ml (STPD)/liter $\cdot$ mm Hg^{-1} × 40 mm Hg × 0.3 liter] plus the CO_2 bound chemically by the 0.3-M THAM solution [419 ml (STPD)/liter × 0.71] which equals 318 ml (STPD) C_{O_2}/liter emulsion.

Similar calculations were made for emulsions composed of 30% FC-80 in 0.1-M, 0.2-M, and 0.3-M THAM solutions which, in equilibrium with room air, had been adjusted to pH 7.40. The results are shown in Fig. 5 and are compared to the CO_2 content of FC-80, air, and 0.9% NaCl. The almost rectilinear relationship between P_{CO_2} and C_{CO_2} over the range of 30-60 mm Hg facilitates estimation of the CO_2 content of a particular emulsion by using the regression equations $C_{CO_2} = (2.8 \times P_{CO_2}) + 133$ ml (STPD)/liter; $(4.0 \times P_{CO_2}) + 132$ ml (STPD)/liter; and $(5.5 \times P_{CO_2}) + 94$ ml (STPD)/liter for 0.1–, 0.2–, and 0.3-M THAM emulsions, respectively.

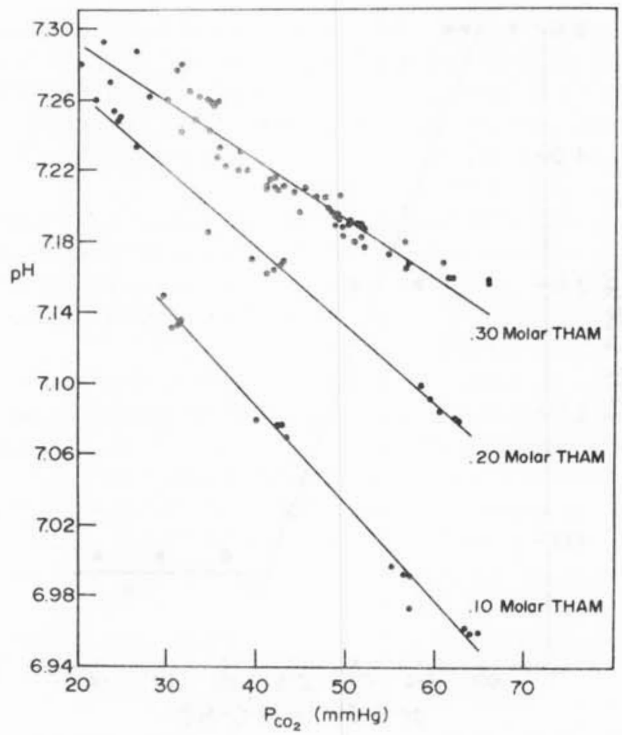

Fig. 4. Relationship between P_{CO_2} and pH of different THAM solutions at 37°C. The pH of the solution equilibrated with room air had been adjusted to 7.40 with 10-N HCl. Regression lines calculated by method of least squares.

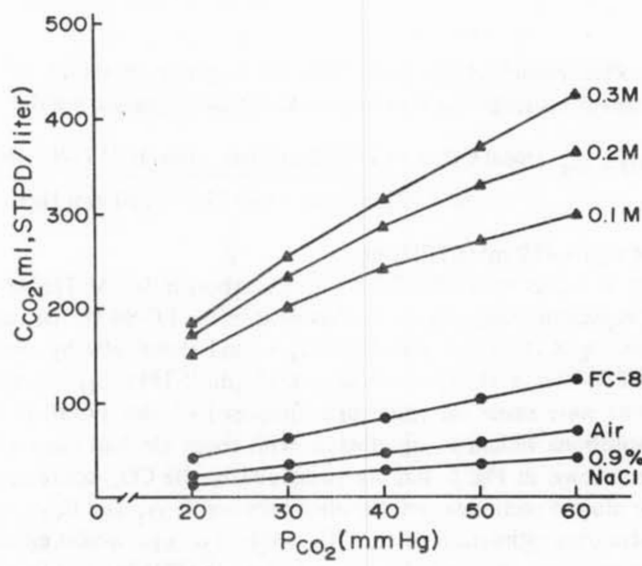

Fig. 5. Relationship between CO_2 content (C_{CO_2}) and partial pressure in 0.9% NaCl, air, FC-80 (○) and emulsions of 30% (by volume) FC-80 in 0.1-, 0.2-, and 0.3-M THAM solutions (Δ) at 37°C (see text).

The oxygen capacity of 1 liter of a 30% FC-80 in 0.3-M THAM emulsion at 37°C is the O_2 dissolved in the FC-80 [0.638 ml (STPD)/liter ··mm $Hg^{-1} \times P_{O_2} \times$ 0.3 liter] (Sargent and Seffl 1970) plus the O_2 dissolved in the THAM solution [0.031 ml (STPD)/liter ··mm $Hg^{-1} \times P_{O_2} \times$ 0.7 liter], or 0.213 ml (STPD)/mm Hg $\times P_{O_2}$. The solubility coefficient of 0.031 ml (STPD)/liter ··mm Hg^{-1} for O_2 in water (Altman and Dittmer 1971) was used to calculate the O_2 dissolved in the THAM solutions.

Schoenfisch and Kylstra (1973) determined the CO_2 content of 0.1-M and 0.3-M THAM solutions (which had been preadjusted to pH 7.40 and then equilibrated with CO_2 by the Van Slyke manometric technique). Their values are somewhat lower than the ones reported here. This is probably due to the fact that acidification of a THAM solution with lactic acid fails to drive off all of the chemically bound CO_2.

The fluoride-ion concentration in the aqueous phase of the ultrasonically prepared FC-80 in THAM emulsions ranged from 1.1 to 15.9 ppm, depending upon the gas dissolved in the ingredients before and during the emulsification process (Fig. 6). The lowest fluoride-ion concentration was measured in the CO_2-equilibrated emulsions. This is in agreement with the results reported by Geyer (1975) and Clark et al. (1975). Geyer (1975) found a concentration of less than 4.0 ppm in 50% (by volume) FC-45 and distilled-water mixtures that had been subjected to ultrasonification. The highest fluoride-ion concentration was found in oxygen-equilibrated emulsions (Fig. 6). Geyer (1975) also reported the highest concentrations in oxygenated ultrasonified FC-45 in distilled-water mixtures. The fluoride-ion concentration measured by us in tap water was, on the average, 1.5 ppm. The concentration of fluoride ions in the distilled water used to prepare the FC-80 in THAM emulsions was 0.5 ppm.

There is an inverse correlation ($r = -0.87$) between the solubility of the gases in water (Radford 1964) and the fluoride-ion concentration in the ultrasonically prepared emulsions. It seems likely that bubble formation could dampen the mechanical impact of the sonifier tip on the fluorocarbon molecules and thus minimize molecular disruption. If this were true, then the more gas molecules there are present in solution, the less likely it is for the carbon-fluorine bond to be broken. The findings reported in this paper are in agreement with such an hypothesis.

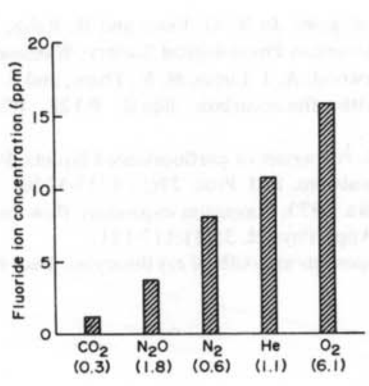

Fig. 6. Mean (n = 4) fluoride-ion concentration (ppm) in aqueous phase of 30% (by volume) FC-80 in 0.3-M THAM emulsions equilibrated with different gases before and during ultrasonic emulsification. Numbers in parentheses are standard deviations. Differences between means for CO_2, N_2O, N_2, and He are significant ($P < .05$).

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Matthews, W. H., and J. A. Kylstra. 1976. Emulsion de fluorocarbone pour la respiration des liquides. Undersea Biomed. Res. 3(2): 113-120.—Il est possible de préparer une émulsion stable en mélangeant le fluorocarbone FC-80 (30% par volume) avec une solution 0,3-m THAM titrée avec de l'acide chlorhydrique à un pH de 7,4 et avec 0,04 g/ml du FC-80 de surfactant Pluronic; et en exposant ce mélange à l'energie ultrasonique. L'émulsion possède une densité de 1,24 g/ml; une viscosité absolue de 2,4 cP; le diamètre approximatif des gouttlettes de fluorocarbone est de 3 mu. Le contenu approximatif en CO_2 de l'émulsion à des pressions partielles entre 30 et 60 mm Hg est de 132 ml (STPD)/litre + (5,5 × P_{CO_2}). Le contenu de O_2 en ml (STPD)/litre est 0,213 × P_{O_2} (mm Hg).

respiration de liquides
émulsions de fluorocarbones
ions de fluorures

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