Emulsions of Perfluorinated Compounds for Use in Quality Control of Blood Gas Analyses

Robert Rej and Joanne Schröder

We prepared emulsions of perfluorinated compounds and examined their utility as liquid matrices for tonometry in quality control of blood pH and gas analyses. Emulsions of perfluorotributylamine and perfluorodecalin, prepared by sonication, consisted largely of particles not exceeding 0.2 μm in diameter. Unlike most aqueous solutions these materials were not easily contaminated by room air and can be reliably used for tonometry liquids. Results for pCO\textsubscript{2} and pO\textsubscript{2} agreed well with calculated values. Average day-to-day coefficients of variation for emulsions of perfluorotributylamine were 2.9% for pH (within the range 7.11–7.60), 4.4% for pCO\textsubscript{2} (2.88–9.17 kPa), and 1.6% for pO\textsubscript{2} (7.76–40.67 kPa).

Additional Keyphrases: tonometry • blood pH • matrix effects • control materials

Interlaboratory and intralaboratory studies have documented the need for effective quality control of blood pH and gas determinations (1–5). Commercially available materials for this purpose include ampules of known gas tensions, prepared with either aqueous or bloodlike matrices (6–9). However, the effectiveness of several of these materials, in particular their ability to simulate authentic patients’ specimens, has been questioned (8–10).

Alternatives to the use of ampouled control materials are liquids that are tonomerized within the laboratory (or in nearby facilities) with gases of known concentration. Both aqueous buffers (11, 12) and whole blood (13, 14) have been suggested as suitable matrices for the preparation of such tonomerized specimens. Aqueous media have the distinct disadvantage of poor oxygen solubility, and the relative ease with which they can be contaminated with atmospheric oxygen is a significant problem (9, 15, 16). Whole blood is an ideal matrix in the sense that it possesses many of the same properties of the patient’s specimen, but also has several shortcomings: poor stability (particularly at increased temperatures), difficulties in reproducibility of pools, poor properties for control of pH, formation of clots, potential biohazard, and formation of bubbles and foam during tonometry. The relative merits and disadvantages of several of these materials have been reviewed (8, 9).

The excellent oxygen solubility of fluorocarbons is well known (17, 18), and recent advances have been made in the preparation of emulsions of perfluorinated compounds for use as in vivo substitutes for blood (19). We have examined the use of these emulsions for quality control of in vitro blood gas analyses, in particular for pO\textsubscript{2} determinations.

Materials and Methods

The following perfluorinated compounds were used in this study. Perfluorodecalin (octadecfluorodecahydroxynaphthalene) was obtained at 95% purity as a mixture of cis- and trans-forms from Aldrich Chemical Co., Milwaukee, WI 53233.

Perfluorotributylamine [(CF\textsubscript{3}CF\textsubscript{2}CF\textsubscript{2}CF\textsubscript{2}N], purity about 95%, was obtained as Fluorinert FC-43 (20) from the 3M Co., St. Paul, MN 55101. Another 3M product, Fluorinert FC-72, was also identified as a perfluorinated compound (b.p. 56 °C). Commercially prepared emulsions of perfluorotributylamine (250 g/L) and perfluorodecalin (175 g/L) were obtained as Fluosol-43 and Fluosol-DA, respectively, from Alpha Therapeutic Corp., Los Angeles, CA 90032. Tris(hydroxymethyl)aminomethane (Tris) was purchased from Sigma Chemical Co., St. Louis, MO 63178. Pluronic F-68 was obtained from BASF-Wyandotte Chemical Corp., Wyandotte, MI 48192. Glycerol (reagent grade) and propylene glycol (USP grade) were from J. T. Baker Chem. Co., Phillipsburg, NJ 08865.

The following commercial blood-gas control materials were also studied: G.A.S. (General Diagnostics, Morris Plains, NJ 07960), Quantra (DADE Div., American Hospital Supply Corp., Miami, FL 33152), ContrIL (Instrumentation Laboratory, Lexington, MA 02173) Qualicheck (Radiometer Copenhagen, London Co., Cleveland, OH 44145), Prime (Diagnostics Division, Fisher Chemical Co., Orangeburg, NY 10962).

Freshly drawn blood was collected in the presence of heparin. Where indicated, blood specimens were frozen at −20 °C or colder.

pH, pO\textsubscript{2}, and pCO\textsubscript{2} were measured with an Instrumentation Laboratory Model 813 blood-gas analyzer. The instrument was modified to allow recording of the analog signal from the O\textsubscript{2}-sensing electrode on a potentiometric recorder. The electrode response was measured in the microsampling mode, to retain the specimen in the sample chamber for the desired length of time. Calibration gases and buffers were obtained from Instrumentation Laboratory. The pH adjustments were verified by using solutions of SRM 186-I-c and 186-II-b phosphate salts, prepared as directed, from the National Bureau of Standards, Washington, DC 20234. All measurements were at 37 ± 0.05 °C, verified with a Model 45CU electronic thermometer and a gallium melting-point cell (Yellow Springs Instruments, Yellow Springs, OH 45387).

To prepare the emulsions, we used a Model 200 Sonifier (Branson Sonic Power Co., Danbury, CT 06810). Particle sizes of the emulsions were determined with the “Nanopar” analyzer and apparatus described by DeBlois et al. (21). For tonometry we used a Dynex gas/liquid equilibration system (Analytical Products, Belmont, CA 94002) at 37 ± 0.3 °C; the temperature of tonometry was verified as described above. Tonometry gases were obtained from Instrumentation Laboratory and Analytical Products.

Means and SD for pH measurements were calculated as
Results

The ability of whole blood and various aqueous materials to retain low oxygen tension when tonometered with O2 at 84 mL/L and presented to the sample chamber of the IL 813 is shown in Figure 1. In contrast to the response of whole blood, where a stable baseline was reached, each buffer solution exhibited a short-lived nadir, followed by increases in the PO2 readings. Minimum values for the aqueous buffers were typically 1 to 2 kPa greater than that calculated and found for whole blood (Figure 1).

Similar studies were performed with commercially available quality-control materials (Figure 2). For each manufacturer’s product we examined the specimen with the lowest oxygen tension. The three aqueous-based materials (G.A.S., Qualicheck, Contr) resemble tonometered aqueous liquids, and the two blood-based products (Prime and Quantra) resemble whole blood (Figures 1 and 2).

Emulsions of perfluorotributylamine and perfluorodecalin were prepared by adding 10 mL of the perfluorinated compound (about 19 g) and 1 g of Pluronic F-68 to 50 mL of Tris buffer (50 mmol/L, pH 7.5). Each mixture was sonicated at room temperature and 70 W (pulsed at a duty cycle of 60% sonication) for 12 min. When these solutions were tonometered, they resembled whole blood in their ability to retain low oxygen tension (Figure 3).

The properties of freshly obtained venous blood, blood previously stored frozen, an emulsion of perfluorodecalin, and a solution of phosphate plus glycerol were examined as described above; O2 electrode response is presented in Figure 4. The response of the perfluorodecalin emulsions most closely resembled that of fresh whole blood. Emulsions of perfluorotributylamine and FC-72 gave results similar to those found with perfluorodecalin.

In addition to a decreased contamination with oxygen at low PO2, emulsions of the perfluoro compounds retained high oxygen tensions longer than did aqueous solutions or frozen blood. Figure 5 presents the O2 electrode responses over time for three materials—Tris buffer, perfluorotributylamine emulsion, and frozen blood—at four concentrations of oxygen. The most stable oxygen measurements were obtained with the emulsion and, at lower oxygen concentrations, whole blood.

We further examined these three materials as routine quality-control materials. Liquids were prepared and tonometered as above, but we used the normal syringe sampling technique and data read-out of the IL 813 blood-gas analyzer. A minimum of seven replicate assays were performed over four days for each material and gas concentration (Table 1). PCO2 and PO2 results agreed well with calculated values, and average day-to-day CVs for emulsions of perfluorotributylamine were 2.9% for pH (within the range 7.11–7.60), 4.4% for PCO2 (2.88–9.17 kPa), and 1.6% for PO2 (7.76–40.67 kPa).

A photomicrograph of a typical emulsion of perfluorotributylamine is shown in Figure 6. The particle size distribution of this emulsion was largely in the 60–200 nm range, as determined with the “Nanopar” apparatus (21).

Discussion

Inadequacies of commercial control materials and of some tonometered aqueous solutions prompted us to examine alternatives for quality control of blood-gas analyses. The excellent solubility of gases in perfluorinated compounds suggested that such compounds may be useful for this purpose. The solubility of oxygen at 101 kPa is 32 mL/L for water, 400 mL/L for erythrocytes, 400 mL/L for perfluorodecalin, and 390 mL/L for perfluorotributylamine (17, 22).

All emulsions of perfluorocompounds tested were superior to aqueous solutions for tonometry in PO2 measurements.
Fig. 5. Oxygen electrode response with time for three liquids tonometered with O₂ at four concentrations.
Tris, 50 mmol/L, pH 7.5; other liquid matrices for tonometry are as described in Fig. 3. Oxygen concentrations (mL/L) were: 1, 450 (balance N₂); 2, 167 (balance N₂); 3, 70 (also contained CO₂, 70 mL/L; balance N₂); 4, 0 (tonometry gas was CO₂, 100 mL/L; balance N₂).

| Table 1. Replicate Measurements of pH, pCO₂, and pO₂ for Three Tonometered Liquids (Mean ± 1 SD) |
|-------------|-----------------|---------------|---------------|-----------------|---------------|---------------|---------------|---------------|
| Gas, mL/L  | pH              | pCO₂, kPa     | pO₂, kPa      |                 |               |               |               |               |
| CO₂        | O₂              | Tris b        | Emulsion c    | Blood d         | Tris b        | Emulsion c    | Blood d         | Calc.          | Tris b        | Emulsion c    | Blood d         | Calc.          |
| 100        | 0               | 7.15          | 7.11          | 7.06            | 8.66          | 9.17          | 9.69          | 9.90          | 4.05          | 1.35           | 0.20           | 0             |
| 70         | 70              | 7.26          | 7.21          | 7.14            | 6.87          | 6.47          | 6.70          | 6.65          | 0.28          | 0.23           | 0.53           | 6.65          |
| 79.5       | 84              | 7.22          | 7.18          | 7.12            | 7.02          | 7.41          | 7.42          | 7.68          | 10.44         | 9.12           | 9.60           | 8.12          |
| 51         | 118             | 7.31          | 7.29          | 7.20            | 4.79          | 4.87          | 4.80          | 4.93          | 13.33         | 12.50          | 13.49          | 11.40         |
| 29.7       | 167             | 7.43          | 7.40          | 7.28            | 2.83          | 2.88          | 2.91          | 2.88          | 16.84         | 16.40          | 17.58          | 16.20         |
| 0          | 450             | 7.59          | 7.60          | 7.55            | 0.93          | 0.59          | 0.37          | 0             | 36.44         | 40.67          | 41.82          | 42.77         |

* Each liquid was tonometered as described with gases at these concentrations. Balance was nitrogen in each case. Tris buffer, 100 mmol/L, pH 7.8. Emulsion of perfluorotributylamine (FC-43) prepared as described in Tris buffer, 100 mmol/L, pH 7.8. Heparinized blood was stored at < -20 °C before use.

(Figures 1, 3–5; Table 1). Aqueous solutions in our hands were best suited for tonometry gas concentrations near that of atmospheric air, resulting in a P₀₂ of about 20 kPa. At low and high oxygen tensions, contamination with room air was rapid and prevented accurate measurement of P₀₂. A recently proposed selected method (23), which suggests the use of gas with an oxygen content of 210 mL/L, is consistent with our experience. Addition of glycerol or propylene glycol had little effect on measured results with aqueous solutions (Figure 1).

The emulsions gave more reproducible P₀₂ values (CV = 1.6%) than whole blood (4.4%). This imprecision with blood was somewhat greater than that reported by Steiner et al. (14). Tonometered Tris buffer solutions, although producing the greatest error from calculated values, exhibited essentially the same precision as the emulsions for P₀₂ and P₀₂.

An emulsion of perfluorotributylamine (FC-43), which combined high precision for all three measurements (pH, P₀₂, P₀₂), and agreement with calculated values, appears to be well suited for use as a tonometry solution in blood-gas quality control.

Similarly prepared emulsions of perfluorotributylamine (FC-43) and perfluorodecalin were judged to be equivalent.
as quality-control materials, but the relatively high cost of perfluorodecalin (~US $1/g) precludes its use as a routine control material. FC-43 is available, at this writing, at about US $50/kg and is economical to use in a daily quality-control program. The similar oxygen solubilities of these two perfluoro compounds support our finding.

The emulsions, once prepared, were reasonably stable and the distribution of particle sizes changed only slightly with time (over six months). There was some settling, but vigorous shaking or 1 min of sonication restored the material to its original particle size and O2 solubility. Although the emulsion consists largely of particles of <0.2 μm in diameter, a significant number of larger particles are also present (Figure 6), without apparently affecting the quality-control properties of the emulsion. The commercially available emulsions of these compounds (Fluosol) were also acceptable control materials; however, Tris base should be added to them so that useful pH values can be obtained after tonometry.

We initially reported this work in 1980 (24); simultaneously, Cormier et al. (25) reported the usefulness of emulsions of the perfluorocompound FC-77. During preparation of this present manuscript, a commercial tonometered material—presumably similar to that reported by Cormier et al. (25)—has appeared. We should also note that Maas et al. (26) as early as 1977 suggested the use of fluorocarbons (although not perfluro- rinated compounds) for use as control materials.

An ideal quality-control material mimics the behavior of the biologic fluid to be routinely examined (27). Although frozen blood satisfies many of these criteria, it is not identical to the freshly obtained specimen, usually having undergone hemolysis and some degradation. Aqueous solutions behave so differently from freshly obtained patients’ specimens, in particular at tensions >5 kPa from atmospheric oxygen, that their use is significantly compromised. There is thus a need for an alternative or supplementary material for blood-gas quality control.

We believe that emulsions of perfluorocarbons meet this need. In particular, perfluorotributylamine is inexpensive, can be easily prepared in large quantities in most laboratories, and can be stored at room temperature. It behaves like fresh blood and possesses no toxic and no significant biologic risk. It is easily tonometered without excessive bubble formation, and results with this compound agree well with calculated values for gas content, with a high degree of reproducibility.

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References