Dual pH Durability Studies of Man-made Vitreous Fiber (MMVF)

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Dissolution of fibers in the deep lung may involve both extracellular and intracellular mechanisms. This process was modeled in vitro for each environment using an experimental flow-through system to characterize both total dissolution and specific chemical changes for three representative MMVF's: a glass wool, a slag wool, and a refractory ceramic fiber (RCF). Synthetic physiological fluids at pH 4 and at pH 7.6 were used to simulate macrophage intraphagolysosomal, and extracellular environments, respectively. Actual commercial fiber, sized to rat-respirable dimension, having an average fiber diameter of 1 μm and an average length between 15 and 25 μm, was used in the experiments. Fiber dissolution was monitored through change in chemistry of the fluid collected after percolation at a constant rate through a thin bed of sample. There are great differences in total fiber dissolution rates for the different fibers. Slag wool and RCF dissolve more rapidly at pH 4 than at pH 7.6, while the reverse is true for glass wool. Dissolution is sometimes accompanied by a noticeable change in fiber morphology or dimension, and sometimes by no change. There is strong dependency on pH, which affects not only total fiber dissolution, but also the leaching of specific chemical components. This effect is different for each type of fiber, indicating that specific fiber chemistry largely controls whether a fiber dissolves or leaches more rapidly under acidic or neutral conditions. Both total dissolution rates and calculated fiber composition changes are valuable guides to interpreting in vivo behavior of man-made vitreous fibers, and demonstrate the usefulness of in vitro acellular experiments in understanding overall fiber persistence. — Environ Health Perspect 102(Suppl 5):61-65 (1994)

Key words: MMVF, fiber durability, dissolution, leaching, extracellular fluids, intramacrophage environment, pH

Introduction

The ability of a fiber to persist within the lung, and to exert its toxic potential, is largely dependent on both its physical and chemical durability (1). Fiber durability may involve processes occurring both within cells (specifically, intraphagolysosomal) and within the extracellular or interstitial fluids; therefore, any in vitro modeling of man-made vitreous fibers (MMVF) dissolution and breakup in the body must take both environments into account. Extracellular conditions in the lung can be represented by simulated physiological saline solutions buffered to a constant pH 7.2 to 7.6 (2). Chemical conditions within macrophage phagolysosomes are less well characterized and more difficult to simulate, but are believed to differ primarily in having lower pH (3,5,6) and in containing hydrolytic enzymes released during phagocytosis (4) that may be capable of specific reactions at the fiber surface.

In vitro acellular systems that employ Gamble's simulant or other model physiological fluids have been developed to pre-

| Table 1. MMVF properties. |
|---------------------------|
|                           |
| **Initial fiber chemistry (wt-%)** | **MMVF11** | **MMVF22** | **RCF-1** |
| SiO₂                     | 63.4       | 38.35      | 47.4      |
| Al₂O₃                    | 3.88       | 10.55      | 48.0      |
| Fe₂O₃                    | 0.25       | 0.30       | 0.97      |
| TiO₂                     | 0.06       | 0.45       | 2.05      |
| Na₂O                     | 15.45      | 0.38       | 0.54      |
| K₂O                      | 1.32       | 0.45       | 0.16      |
| CaO                      | 7.45       | 37.5       | 0.07      |
| MgO                      | 2.92       | 9.9        | 0.08      |
| B₂O₃                     | 4.45       | —          | —         |
| SO₃                      | 0.33       | 1.8        | —         |

**Physical properties**

|                          | **Surface area, m²/g** | **Mean diameter, μm** | **Mean length, m** | **Density, g/cm³** |
|--------------------------|------------------------|-----------------------|-------------------|-------------------|
|                          | 1.5                    | 0.9                   | 19.0              | 2.5               |
|                          | 2.0                    | 1.2                   | 21.4              | 2.5               |
|                          | 2.6                    | 1.1                   | 17.8              | 2.7               |

*Measured using BET methods.*

In both of the above environments. Samples were chosen from three of the constituent groups comprising the MMVF family (9): glass wool, slag wool, and refractory ceramic fiber (RCF). In addition, the samples selected were the same as those used in in vivo fiber durability studies reported previously (10), so that the results from the in vitro and in vivo studies may be compared. Specific objectives of this work were to compare rates for total fiber dissolution as a function of both fiber type.
(composition) and pH: to obtain an indication of the fiber leaching process under both pH conditions through change in specific fiber chemistry with time; and to document morphological changes to the fibers resulting from the dissolution and leaching processes.

Materials and Methods

The fibers chosen for this evaluation were MMVF11 (a glasswool), MMVF22 (a slagwool), and RCF1 (a refractory ceramic fiber), all sized to rat-respirable dimensions (11) with an average fiber diameter of approximately 1 μm and an average length of 15 to 25 μm. Their chemical compositions and physical characteristics are given in Table 1 (see also 10).

Fluid simulants used were modified Gamble’s solutions (6). Sodium azide (0.5 mg/l) was added to both solutions as a biocidal agent. The extracellular fluid simulant was saturated with and kept under constant pressure of 5% CO2/95% N2 to maintain pH 7.6 for the duration of the experiments. For the solution at pH 4, HCl was added in place of sodium bicarbonate and the level of sodium chloride adjusted to achieve the desired pH and maintain the same total cation concentration as that of the solution at pH 7.6.

Experiments were performed in an in vitro flow-through system as described previously (6). In this system, weighed portions of each material are fixed within half-inch spacers between 0.2-μm polycarbonate membrane filters in modified air monitors which serve as the sample chambers. Fluid is pumped at a constant rate through individual polyethylene lines into the sample chambers where it is allowed to react with the fibers and the effluent is collected in individual bottles for each time increment. Aliquots of each solution are then removed for analysis. Nominal conditions used for this study were: 0.5 g fiber at a 10 ml/hr flow rate for 21 days and at a constant temperature of 37°C. Duplicate runs were made for each sample at each pH. Solutions were analyzed by inductively coupled plasma (ICP) (6) to quantify the concentrations of the elements extracted from each fiber sample (in mg/L). The elements measured included both major and minor components of each fiber, as well as phosphorous which may be taken up from the fluid by some types of MMVF (3). To calculate a rate constant for total fiber dissolution, these data were summed and resect in terms of mass loss per unit time \[1-(M/M_0)^{1/2}\], where \(M_0\) is the initial fiber mass, and \(M\) is the calculated mass at a given time during the experiment. Calculated rate constants, \(k\) are given as ng/cm²/hr, mass dissolved per unit surface area per unit time \((t)\), according to the relation

\[ k = 2 \left[1-(M/M_0)^{1/2}\right]/(A_j)(t) \]

where \(A_j\) is the average surface area per unit mass of the fibers comprising the sample. \(k\) may then be readily derived from the slope of a plot of \((1-M/M_0)^{1/2}\) versus time. Details of derivations used for these calculations have been given previously (3,12).

Calculated rate constants may, however, be influenced by diffusion-limiting conditions resulting from the buildup of leached layers or high concentrations of dissolution products at the fiber surface. That may occur if flow rates are insufficient to keep pace with the dissolution process. With more rapidly dissolving fibers, leached layers may develop quite early in the dissolution process. Rates controlled by breakdown of the glass structure are approximated by linear kinetics, while those strongly influenced by diffusion, typically vary with some fractional power of time \((t)\), often near \(t^{1/2}\) (13). To ensure that the rate constants were as close as possible to the true rate of breakdown of the glass in toto and not a lesser rate influenced by diffusion, only data from the initial linear, or approximately linear, portions of the dissolution curves were used in the calculations of \(k\), where flow-independent conditions are believed to exist (14). In some cases, values were obtained over the full 21 days, but for the more soluble fibers, where tendency towards nonlinearity was noted, the data were obtained from a shorter period.

The data were also used to monitor the leaching of specific elements or components of the fiber as a function of time, which made it possible to calculate a final leached fiber composition after the 21-day exposure. Use of solution data as the measure of fiber dissolution is quite sensitive

**Figure 1.** Rate constants \((k)\) for total fiber dissolution at pH 4 and at pH 7.6

**Figure 2.** Relative change in residual fiber composition with time as calculated from solution data: (a) MMVF11, pH 4; (b) MMVF11, pH 7.6; (c) MMVF22, pH 4; (d) MMVF22, pH 7.6; (e) RCF1, pH 4; (f) RCF1, pH 7.6.
Table 2. Average fiber compositions after 21-day exposure to synthetic physiological media (wt.-%, major components only).

| Component | MMVF11, original | MMVF11, ph 4 | MMVF11, ph 7.6 | MMVF22, original | MMVF22, ph 4 | MMVF22, ph 7.6 | RCF1, original | RCF1, ph 4 | RCF1, ph 7.6 |
|-----------|------------------|--------------|----------------|------------------|--------------|----------------|--------------|------------|------------|
| SiO₂      | 63.4             | 66.8         | 65.8           | 38.4             | 58.3         | 44.5           | 47.7         | 47.9       | 47.8       |
| Al₂O₃     | 3.8              | 4.1          | 6.9            | 10.6             | 27.8         | 12.8           | 48.0         | 48.4       | 48.5       |
| Fe₂O₃     | 0.3              | 0.3          | 0.5            | 0.3              | 0.7          | 0.4            | 0.97         | 0.9        | 0.9        |
| Na₂O      | 15.5             | 12.6         | 13.0           | 0.4              | 0.9          | 3.5            | ND           |            |            |
| K₂O       | 1.3              | 1.3          | 1.2            | 0.4              | 0.4          | 0.1            | ND           |            |            |
| CaO       | 7.4              | 7.2          | 8.4            | 13.0             | 8.3          | 29.3           | 7.9          |            |            |
| MgO       | 3.1              | 2.6          | 2.6            | 9.9              | 1.8          | 7.9            |              |            |            |
| B₂O₅      | 4.2              | 4.1          | 2.5            | —                | —            | —              | 2.05         | 2.1        | 2.05       |
| TiO₂      |                  |              |                | 2.8              | 2.6          | 2.6            |              |            |            |
| Total mass lost, % | 2.8 | 40.6 | 61.9 | 16.2 | 1.6 | 0.2 |

ND, not detected. *Glass* components only, corrected for uptake of phosphate.

since many of the elements leached from the fiber are not present in the initial fluid.

Morphological and physical changes to the fibers that accompanied the leaching and dissolution process after 21 days of exposure were studied by scanning electron microscopy (SEM), and included evidence of reduction of diameter or length of fibers, depth of corrosion and leached layer development, surface exfoliation, and formation of surface precipitates.

**Results and Discussion**

Calculated rate constants for total dissolution of each of the three fibers at pH 4 and pH 7.6 are given in Figure 1. For slag wool MMVF22, total dissolution rates were over 30 times greater at pH 4; dissolution of RCF 1 was also faster under more acidic conditions, with a rate constant of 1.4 mg/cm²/hr compared with 0.3 mg/cm²/hr at pH 7.6. For glass wool MMVF11, however, the trend was reversed, with a total dissolution rate of 3.7 mg/cm²/hr at pH 4 and 85.0 mg/cm²/hr at pH 7.6.

In addition to total dissolution, data from these experiments provided information on compositional changes that occurred, so it was possible to construct dissolution profiles indicating the amount of a given element or component dissolved (leached) from a fiber as a function of time. Using these profiles, residual compositions of each fiber were then constructed after each sampling interval and compared with the original composition to give an indication of the congruency of the dissolution process and its variation with time (Figure 2, a–f). This is useful when comparing results from *in vitro* experiments to analyses of MMVF recovered from the lungs of laboratory animals. The final calculated fiber chemistry after the full 21-day exposure is also given for each fiber at each pH condition (Table 2).

Results indicate that, as with total dissolution, compositional changes occurring in a particular fiber vary not only as a function of initial composition, but also with pH of the fluid. MMVF22, and to a lesser extent MMVF11, shows at least two significant changes: a progressive enrichment in both silica and alumina in the residual fiber, and loss by leaching of network-modifying alkali and alkaline earth cations. Leaching of network-modifying cations and concomitant enrichment in alumina, silica, and in some cases iron oxide was also found in fibers recovered from animal lungs from *in vivo* fiber durability studies on various MMVFs (10,15). Although the magnitude of the effect appears greater *in vivo*, the trends *in vitro* appear similar.

Unlike the other fibers, RCF1 shows virtually no change in composition with time under either pH condition. Dissolution appears highly congruent in both cases. Slag wool, in contrast, shows the greatest variation between the two pHs. Leaching of cations and enrichment in alumina occur far more rapidly and extensively in the more acidic environment. The opposite appears true for glass wool MMVF11, although the difference is not as pronounced.

These data do not show how much of each component is leached, only residual concentrations. For a complete characterization, the overall dissolution rates (Figure 1) and total mass losses (Table 2) must be taken into consideration. For example, even though much more alumina is leached from MMVF22 at pH 4 than at pH 7.6, it is not leached at a faster rate than other components, so that the net effect is an enrichment of alumina in the fiber. This may not be reflected by simple examination of dissolution profiles.

The combined effect of component leaching and network dissolution is shown in morphological changes to the fibers after 21-day exposures (Figures 3, 4). For MMVF11, which dissolves more congruently, little difference could be seen between the overall fiber morphologies and lengths before and after 21-day exposure. Even at pH 7.6, despite the loss of 40% of its mass, only the development of a subtle surface texture in the fiber was observed (Figure 3). The primary manifestation of dissolution appears to have been the more complete dissolution of the finest fraction of these fibers and the development of a fine porous structure within the fiber which probably reflects partial hydration and "gelatinization" through countermigration of water (or hydronium ions) into the structure of the glass comprising them.

In MMVF22, more significant morphological changes are observed, at both pH 4 and pH 7.6. At pH 4, extensive corrosion of the slag wool results in the development and eventual exfoliation of relatively thick leached layers from the fiber surface, and some fragmentation of the fibers themselves (Figure 4). Corrosion at pH 7.6 appears less severe (without exfoliation).
tion), but it is still more extensive than is observable in MMVF11 under the same conditions—despite the significant disparity in overall rate constants, which at pH 7.6 is 10 times as great for MMVF11. This finding supports the contention that the resultant fiber morphology and its inherent physical and surface chemical properties are a function, not just of its dissolution behavior, but also of its initial chemistry and the specific leaching reactions that occur with the physiological simulant. Predictably, there was little effect of leaching or overall corrosion for RCF1 or for MMVF11 at pH 4.

Chemical changes (Figure 2, Table 2) do not include changes to the fiber chemistry resulting from incorporation of phosphate or other components from the solution. This was significant, in particular for MMVF22 at pH 7.6, where countermigration of phosphate and probably of sodium occurred. Such effects, however, were not observed in MMVF recovered from in vivo studies (10,15).

These differences in leaching behavior also correlate with pathology results of chronic inhalation studies with these MMVF in rats. They showed that three compositions of RCF (including RCF1), which induce pulmonary fibrosis and significant increases in lung tumors and mesotheliomas (16), whereas MMVF11 and another glasswool of roughly similar composition (MMVF10) produced neither fibrosis nor tumors (17). MMVF22 has produced no fibrosis after 18 months in an ongoing inhalation study.

**Conclusions**

Rate constants for total dissolution vary significantly among different MMVFs, and are a function of both initial fiber composition and pH of the medium. Under conditions simulating an extracellular fluid environment, pH 7.6, total dissolution rates vary in the order glass-wool (MMVF11) > slagwool (MMVF22) > refractory ceramic fiber (RCF1). At pH 4 at similar ionic strength, which simulates the environment encountered by a respired fiber after phagocytosis by macrophages, the order is MMVF22 > MMVF11 > RCF1. Chemical changes to the fiber accompanying the dissolution process include selective leaching of specific components, which also may vary significantly with initial fiber composition and fluid pH. Dissolution may proceed with or without evident morphological changes to the fiber; slag-wool fibers (MMVF22), for example, underwent appreciable changes to their surfaces even with limited dissolution, while glasswool fibers (MMVF11) showed little morphological change even after loss of over one third of their original mass. Exposure to either intracellular or extracellular fluids produces compositional and physical changes to MMVF fibers, which in turn may alter both their physical and surface chemical properties—a consideration in the evaluation of their overall biological reactivity.

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