In Vitro Dynamic Solubility Test: Influence of Various Parameters

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This article discusses the dissolution of mineral fibers in simulated physiological fluids (SPF), and the parameters that affect the solubility measurement in a dynamic test where an SPF runs through a cell containing fibers (Scholze and Conradt test). Solutions simulate either the extracellular fluid (pH 7.6) or the intracellular fluid (pH 4.5). The fibers have various chemical compositions and are either continuously drawn or processed as wool. The fiber solubility is determined by the amount of SiO_2 (and occasionally other ions) released in the solution. Results are stated as percentage of the initial silica content released or as dissolution rate *v* in *nm*/day . The reproducibility of the test is higher with the less soluble fibers (10% solubility), than with highly soluble fibers (20% solubility). The influence of test parameters, including SPF, test duration, and surface area/volume (*SA*/*V*), has been studied. The pH and the inorganic buffer salts have a major influence: industrial glasswool composition is soluble at pH 7.6 but not at pH 4.5. The opposite is true for rock- (basalt) wool composition. For slightly soluble fibers, the dissolution rate *v* remains constant with time, whereas for highly soluble fibers, the dissolution rate decreases rapidly. The dissolution rates believed to occur are *v*1, initial dissolution rate, and *v*2, dissolution rate of the residual fibers. The *SA* of fibers varies with the mass of the fibers tested, or with the fiber diameter at equal mass. Volume, *V*, is the chosen flow rate. An increase in the *SA*/*V* ratio leads to a decrease in the dissolution rate. Test conditions corresponding to high values of *SA*/*V* may lead to saturation of the species released in the solution, slowing the dissolution process. The *in vitro* solubility test described here is used to investigate the dissolution rate of mineral fibers of various chemical compositions. The test parameters must be clearly defined since they have a major effect on the dissolution rate measured. Experimental conditions must be chosen so that the dissolution rate measured *in vitro* is in good agreement with *in vivo* experiments. — Environ Health Perspect 102(Suppl 5):91–96 (1994)

Key words: vitreous fibers, *in vitro* test, solubility, physiological fluid, dissolution rate, glass leaching

Introduction

The *in vivo* solubility of fibers is considered to have a major influence on their health effect. A number of *in vitro* tests have been developed (*1*) to measure the solubility of fibers in simulated physiological fluids (SPF) and correlate it with *in vivo* experiments. The present work describes an *in vitro* dynamic solubility test and the influence of various test parameters on the solubility measurement; the parameters include SPF itself, test duration, and surface area/volume ratio (SA/V).

Experimental Procedure

The test used (Figure 1) is a dynamic test developed by Scholze and Conradt (*2*) in which SPF runs through a cell containing the fibers, at 37° and at a flow rate set with a peristaltic pump. The effluent solution is analyzed systematically for SiO_2 and occasionally for CaO and other ions by inductively coupled plasma.

The test lasts from 3 to 6 weeks, but the effluent solution is analyzed at least weekly. After the test the fibers are checked for weight loss and examined by secondary electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and energy dispersive X-ray (EDX) analysis.

Table 1 presents the two solutions used. One solution, which simulates the extracellular fluid used by Scholze and Conradt (*2*), is buffered at pH 7.6 ± 0.2 with an N_2/CO_2 gas (95/5 volume) constantly bubbled through; the other, simulating the intracellular fluid, the acidity of which may be due to the action of the lysosomes, is at pH 4.5.

The fibers tested were of various chemical compositions and were either continuously drawn (average diameter 10 µm) or processed as wool with different diameters (average diameters 1, 5, and 20 µm).

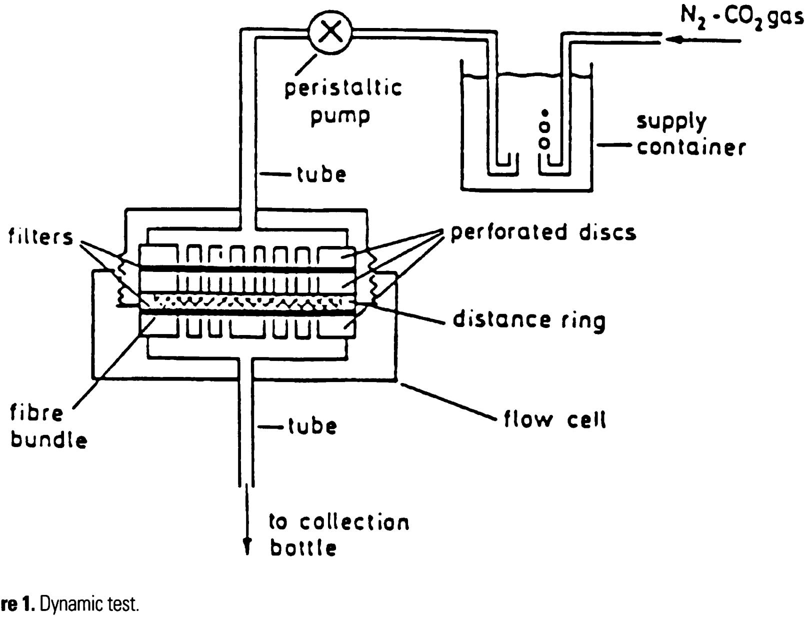

Figure 1. Dynamic test.

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Solubility Measurements: Expression of Results

The amount of silica released in solution, (% SiO_2)sol, is given by the equation

$$(\%\ SiO_2)_{sol} = \frac{[SiO_2] \times V}{m \times (\%\ SiO_2)_{glass}} \quad [1]$$

where $[SiO_2]$ = SiO_2 concentration in the effluent solution (mg/l), V = volume of solution (1), m = mass of fibers tested (mg), (% SiO_2) glass = initial SiO_2 content of fibers (weight percent).

The values of $(\%\ SiO_2)_{sol}$ were determined for each weekly batch of effluent solution and the results for each batch were aggregated.

An analysis of all glass oxides in the effluent solution can be made (except for Na_2O, since Na^+ concentration is too high in the SPF), and the results expressed in the integrated equation

$$\sum_i (\%\text{oxide})_i = \sum_i \frac{[(\text{oxide})_i] \times V}{m \times (\%(\text{oxide})_i)_{glass}} \quad [2]$$

The dissolution rate, v, is assumed to be proportional to the surface area (where $v = -\delta d/2\delta t$) (nm/day and where d = fiber diameter) is for a single fiber:

$$v(J) = \frac{d}{2t}\left(1 - \sqrt{1 + \frac{\Delta J}{J_0}}\right) \quad [3]$$

for fibers with a diameter distribution characterized by d_5, d_{50}, d_{95}

[4]

$$\frac{\Delta J}{J} = 100 \times \left(\begin{array}{l} 1 - \frac{erf * E1}{2} + \exp\frac{\sigma^2}{4}\frac{2vt}{d_{50}} \\ erf * E2 \text{ - } 2\exp\sigma^2\left(\frac{vt}{d_{50}}\right)^2 \\ erf * E3 \end{array} \right)$$

$$where: s = 0.43\ \ln(d_{95}/d_5)$$

$$E1 = \frac{1}{s}\ln\frac{2vt}{d_{50}}, E2 = E1 + s/2, E3 = E1 + s \quad [5]$$

$$erf * x = erfx - 1 = \frac{2}{\sqrt{\Pi}}\int_{-\infty}^{+\infty} \exp(-x2)dx - 1 \quad [6]$$

where, if J = amount of silica present in the fibers at time t,

then

$$\frac{\Delta J}{J_0} = -(\%SiO_2)_{sol} \quad [7]$$

$$v = \frac{d}{2t}\left(1 - \sqrt{1 - (\%SiO_2)_{sol}}\right) \quad [7a]$$

v represents the dissolution rate of the silica network, which is the global dissolution rate of the fiber when one assumes that the dissolution is congruent (i.e., that there is no selective leaching).

or, if J = M (fiber mass remaining at time t), then

$$v = \frac{d}{2t}\left(1 - \sqrt{\frac{M}{M_0}}\right) \quad [8]$$

v is the global dissolution rate of the fiber.

or, if J = amount of all glass components present in the fibers at time t, then

$$v = \frac{d}{2t}\left(1 - \sqrt{1 - (\sum_i \%\text{oxide})_{i_{sol}}}\right) \quad [9]$$

v also represents the global dissolution rate of the fiber.

For some authors (*3*), the dissolution rate constant k is defined by

$$\frac{dM}{dt} = -kA \quad k(ng/cm^2h) \quad [10]$$

where M = fiber mass, A = reaction surface area.

There is a simple relationship between k and v:

$$k = \frac{\rho}{0.24}v \quad (\rho: \textit{fiber density}) \quad [11]$$

Table 1. Composition of the simulated physiological fluids.

Composition, g/l	Extracellular fluid	Acid solution
NaCl	6.415	3.210
$NaHCO_3$	2.703	
NaOH		6.000
Citric Acid		20.800
$CaCl_2$	0.193	0.097
Na_2HPO_4.12 H_2O	0.358	0.179
Na_2SO_4	0.079	0.039
$MgCl_2$.6H_2O	0.212	0.106
Glycine	0.118	0.059
Na_3 citrate.2H_2O	0.153	0.077
Na_3 tartrate.2H_2O	0.180	0.090
Na lactate	0.175	0.085
Na pyruvate	0.172	0.086
Formaldehyde	1 ml/l	1 ml/l

Reproducibility

The reproducibility of the test has been determined for various experimental conditions with fibers continuously drawn or processed as wool and fibers with different chemical compositions. In all cases, the reproducibility depends on the solubility of the fibers. In general, for slightly soluble fibers for which the value of $(\%\ SiO_2)_{sol}$ is <20% after 42 days, the dissolution rate is determined with a confidence interval (at a 95% level) of less than 10%; while for highly soluble fibers, where $(\%\ SiO_2)_{sol}$ is >40% after 42 days, the confidence interval (at a 95% level) is less than 20%.

Influence of the Different Test Parameters

Simulated Physiological Fluid

The influence of pH on the solubility was tested using two solutions, one simulating extracellular fluid with pH 7.6 ± 0.2 and the other, intracellular fluid at pH 4.5. For example, when comparing industrial insulation glasswool (C3) and rock- (basalt)

Table 2. Ponderal composition of the glasses used in this study.

	C3	Basalt	CM38	CM44	MMVF 10	MMVF 11	MMVF 21	MMVF 22
SiO_2	65.00	46.50	62.00	61.30	57.50	63.40	46.20	38.40
SO_3	0.25		0.20	0.04	0.12	0.33	0.23	1.81
Fe_2O_3	0.17	12.70	0.06	0.08	0.07	0.25	7.00	0.30
Al_2O_3	3.40	13.25	0.15	1.00	5.10	3.90	13.00	10.60
CaO	7.00	10.70	16.00	6.70	7.50	7.50	16.90	37.50
MgO	3.00	8.95	3.00	2.70	4.10	2.80	9.30	9.90
Na_2O	15.80	3.05	15.20	15.50	15.00	15.50	2.60	0.40
K_2O	0.70	1.80	0.10	0.70	1.10	1.30	1.30	0.50
B_2O_3	4.50		3.20	11.00	8.8	4.50		
TiO_2	0.20	2.65					3.00	0.50
P_2O_5		0.30		1.00			0.20	
F					0.80			

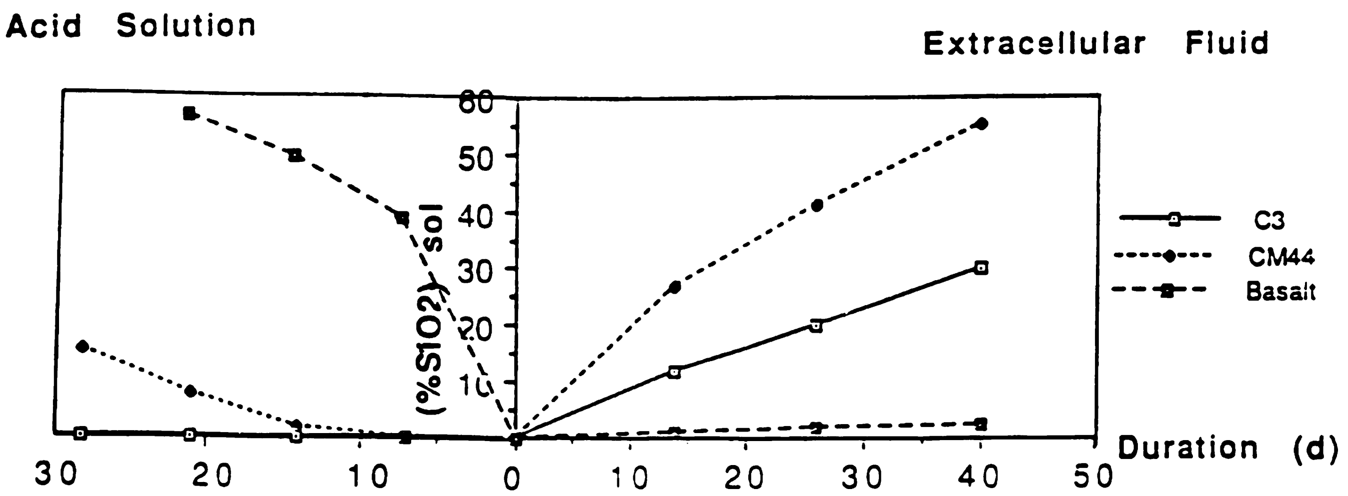

Figure 2. Influence of the SPF on the solubility of C3, CM44, and basalt textile fibers (Flow rate: 40 ml/day).

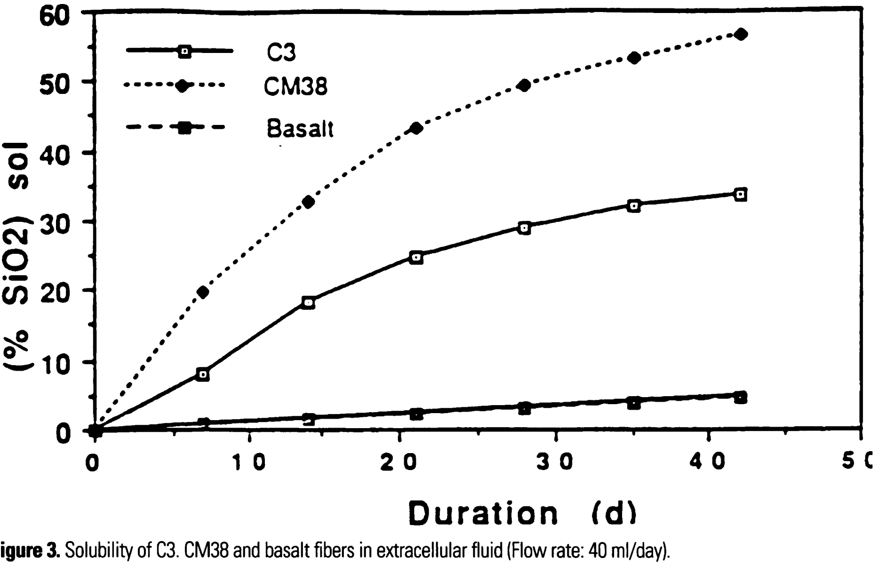

Figure 3. Solubility of C3. CM38 and basalt fibers in extracellular fluid (Flow rate: 40 ml/day).

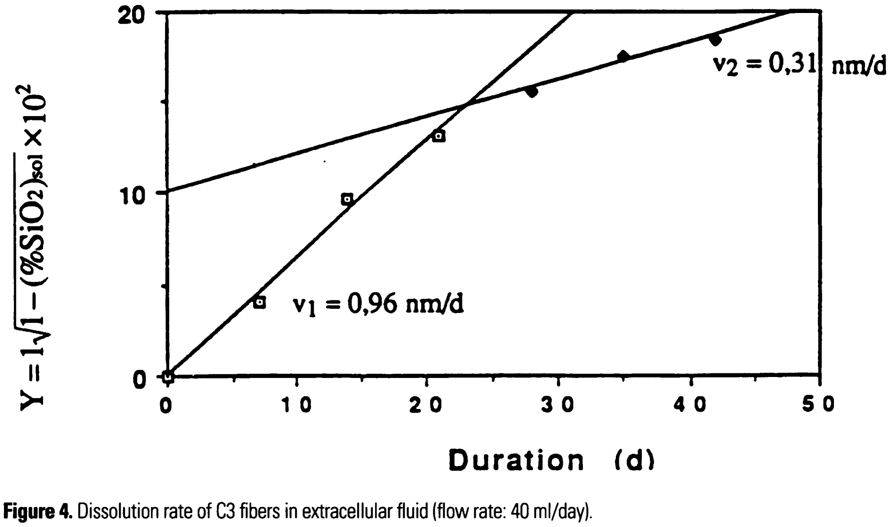

Figure 4. Dissolution rate of C3 fibers in extracellular fluid (flow rate: 40 ml/day).

wool (Table 2), the former is soluble in extracellular fluid but not in intracellular fluid, while for the latter the opposite is observed (Figure 2). The high solubility of basalt in an acidic medium, and particularly in the presence of citrate salts (which probably have a strong chelating effect), has already been observed by Mogensen (*4*). For CM44, a modified glass composition (Table 2), the dissolution rate is higher in both media compared with industrial glass (Figure 2).

Test Duration

If fibers are assumed to have a uniform diameter and to dissolve congruently at a low dissolution rate, i.e., $(\%\ \mathrm{SiO_2})_{sol} \ll 100$), then, from equation [1],

$$v = \frac{d}{4t}(\%\ \mathrm{SiO_2})_{sol}$$

and plotting $(\%\ \mathrm{SiO_2})_{sol}$ against t (test duration) gives a straight line, with

$$\text{slope}\ \frac{4v}{d}.$$

Basalt fibers (obtained by the TOR process) fit this model (Figure 3). The calculated dissolution rate (assuming a uniform diameter equal to d_{50}) is 0.18 nm/day.

For more soluble fibers such as glasswool (C3) or modified glasswool fibers (CM38) (composition given in Table 2), the amount of silica released in the effluent solution, $(\%\ \mathrm{SiO_2})_{sol}$, is not linear with respect to test duration (Figure 3), as would have been expected from equation (*3*), indicating that the dissolution rate is not constant with time,

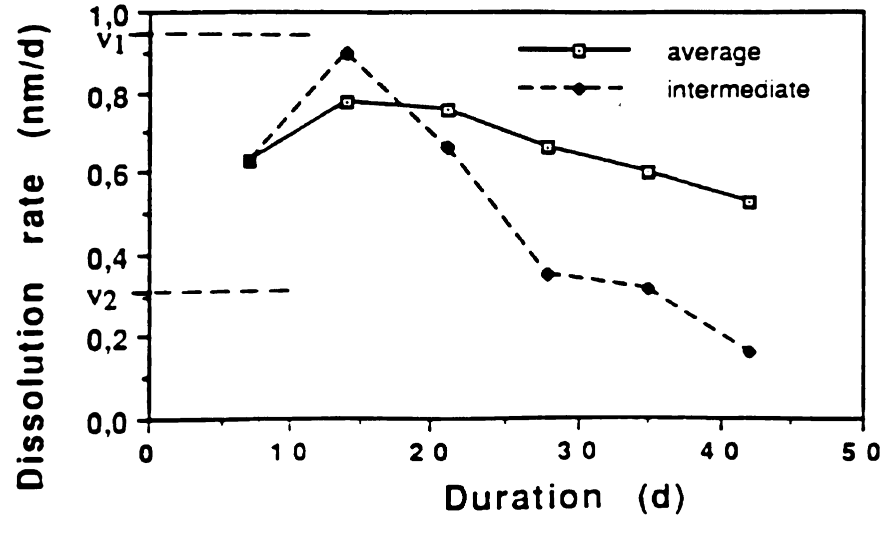

Figure 5. Solubility of C3 fibers in extracellular fluid (Flow rate: 40 ml/day).

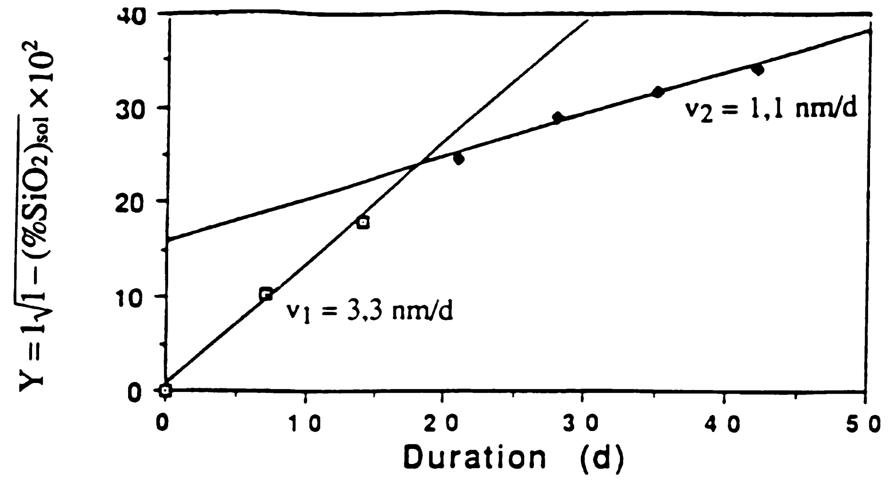

Figure 6. Solubility of CM38 in extracellular fluid (Flow rate: 40 ml/day).

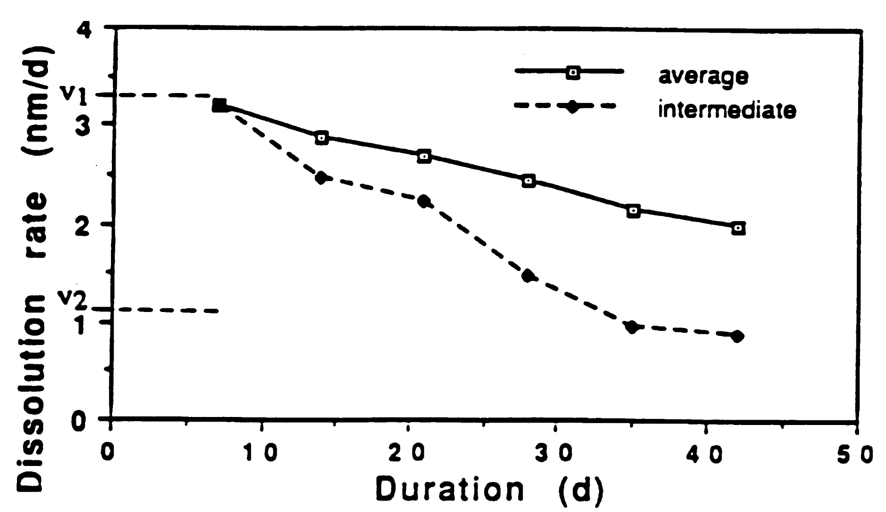

Figure 7. Dissolution rate of CM38 fibers in extracellular fluid (flow rate: 40 ml/day).

i.e., $v = \frac{d}{2}S$ where S = slope.

For glasswool fibers (C3 TOR processed fibers), with a test duration of <25 days, $S_1 = 6.41 \times 10^{-3}$ corresponding to v_1 = 0.96 nm/day. For $t > 25$ days the slope is less (2.07×10^{-3}) corresponding to v_2 = 0.31 nm/day (Figure 4). The SEM and EDX analyses performed on the fibers after the test show a surface layer enriched in Si and Al (*5,6*) and it is the formation of this layer that leads to a decrease in the dissolution rate.

The dissolution rate, obtained through a complete calculation taking into account the real fiber diameter distribution is presented in Figure 5. The continuous line represents an "average" dissolution rate, calculated after a test of n days, and the dotted line represents an "intermediate" dissolution rate, calculated for each time interval. The v_1 and v_2 values obtained from the curve $Y = f(t)$ (Figure 4) are close to the initial and final values of the "intermediate" dissolution rate, differences observed being mainly due to the assumption of a uniform diameter, made for the v_1 and v_2 calculation.

For highly soluble compositions, such as CM38 glass with a low alumina and a high alkali and alkaline-earth content, similar phenomena are observed. Two dissolution rates can also be determined. For t < 20 days, v_1 = 3.3 nm/day, and for > 20 days, v_2 = 1.1 nm/day (Figure 6).

After the test the fibers are covered with a uniform precipitation of calcium phosphate, which has slowed down the dissolution process (*5*).

The evolution of the "average" and "intermediate" dissolution rates are shown in Figure 7.

Surface Area/Volume

The ratio of the surface area developed by the fibers to the volume of the solution (i.e., the flow rate in dynamic experiments) is an important parameter for the determination of the dissolution rate. The kinetic parameter defined by Bauer (*7*) as *SA/V* (expressed in $cm^{-1} \cdot hr$) depends on the flow rate, the mass of fibers and the diameter of the fibers.

Flow Rate. The four man-made vitreous fibers (MMVFs; Table 2) that had been tested *in vivo* at Research and Consulting Company (RCC), were tested at three flow rates (40, 300, 1600 ml/day). For the glasswool and rockwool fibers (MMVF10, MMVF11 and MMVF21), where silica is the major com-

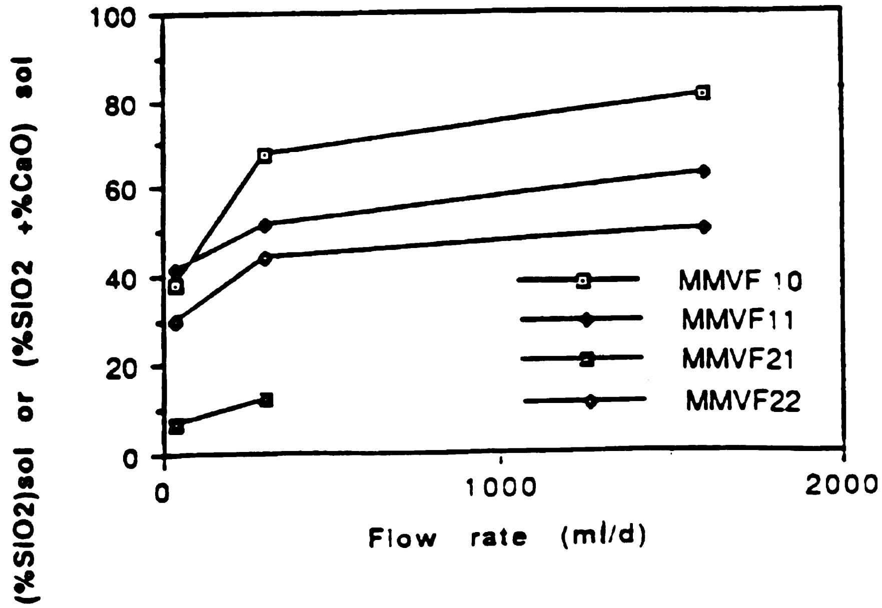

Figure 8. Solubility of MMVF fibers in extracellular fluid at different flow rates. Test duration - 28 days.

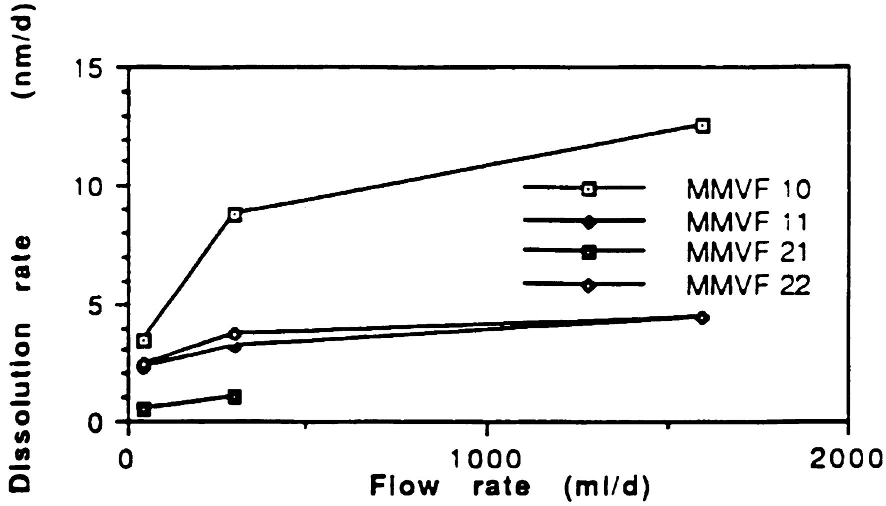

Figure 9. Dissolution of MMVF fibers in extracellular fluid at different flow rates. Test duration, 28 days.

pound or the dissolution is fairly congruent, the silica released in solution, $(\% \mathrm{SiO_2})_{\mathrm{sol}}$ is a good indicator of the global dissolution. In contrast, the dissolution of the slagwool MMVF22, is highly noncongruent with a rapid leaching of calcium. The solubility, therefore, is given by the sum of the silica and calcium released in the solution, $(\% \mathrm{SiO_2} + \% \mathrm{CaO})_{\mathrm{sol}}$.

The $(\% \mathrm{SiO_2})_{\mathrm{sol}}$ or $(\% \mathrm{SiO_2} + \% \mathrm{CaO})_{\mathrm{sol}}$ measured after 28 days increases with the flow rate (Figure 8) indicating that the dissolution is greater at a higher flow rate. The increase is greatest for MMVF10, the most soluble composition. For MMVF21, the silica concentration, at 1600 ml/day , is lower than the detection limit. No value is given at this flow rate.

The SEM and EDX observations made on the fibers after test show corrosion patterns that vary with the flow rate, (due to formation of calcium phosphate at the higher flow rate) (*5*). Dissolution mechanisms also change with change in the flow rate, which may increase leaching or formation of fine deposits.

In general, increasing the flow rate (i.e., by decreasing the value of *SA*/*V*) increases the dissolution rate (Figure 9).

Table 3. Solubility of MMVF11 fibers in extracellular fluid.[a]

Mass of fibers	% SiO_2	v, nm/day
200 mg	41.9	3.1
50 mg	66.3	6.6

[a] Flow rate: 300 ml/day; test duration: 21 days.

Table 4. Solubility of modified slag of different diameters in extracellular fluid.[a]

Fiber	Diameter, mm	% SiO_2	v, nm/day
Code 110	0.78	82.7	5.21
4/5 g	3.66	35	4.88
20 mm	19.7	9.4	9.05

[a] Flow rate: 40 ml/day; test duration: 42 days.

Mass of Fibers. MMVF11 was tested at 300 ml/day with two different fiber masses. The $(\% \mathrm{SiO_2})_{\mathrm{sol}}$ and the dissolution rate were greater with the lower mass of fibers (i.e., with lower *SA*/*V*) (Table 3).

Fiber Diameter. To assess the influence of the fiber diameter on the dissolution rate, fibers with the same chemical composition (a modified slag) were produced by the TOR process with different average fiber diameters, 0.8, 3.7 and 19.7 µm. Dissolution experiments were performed with different masses, thus keeping SA constant.

The $(\% \mathrm{SiO_2})_{\mathrm{sol}}$ after 42 days decreased with increasing fiber diameter (Table 4). The dissolution rate was roughly the same for fibers with an average diameter of 0.8 or 3.7 µm, but was double for fibers with an average diameter of 19.7 µm (Table 4). This difference might be due to actual differences in surface area, resulting from unreliable determination of the mass of fibers used, or to a difference in the surface chemistry or surface structure of the fibers.

Dissolution Rate as a Function of Surface Area and Flow Rate v = f(*SA*/*V*)

Figure 10 shows the evolution of the dissolution rate of MMVF11 under different experimental conditions of flow rate and mass. An increase in *v* was observed with small values of *SA*/*V*, that is, when there are few fibers and high flow rates. This may be due to a rapid transport of the species released in solution. The maximum value of *v* was observed, however, with the smallest mass and an intermediate flow rate, suggesting that a modification of *SA*/*V* through flow rate may not be equiva-

Table 5. k (ng/cm^{2}hr) of MMVF fibers in extracellular fluid.[a]

SA/V, cm^{-1}•hr	MMVF10	MMVF11	MMVF21	MMVF22
16				147*
37	131			
53		46		
71		69		
182			12.1	
198	91			43
284		33		
1370			6.3	
1480	32			25
2130		22		

[a] Duration 28 days, except for *, 8 days.

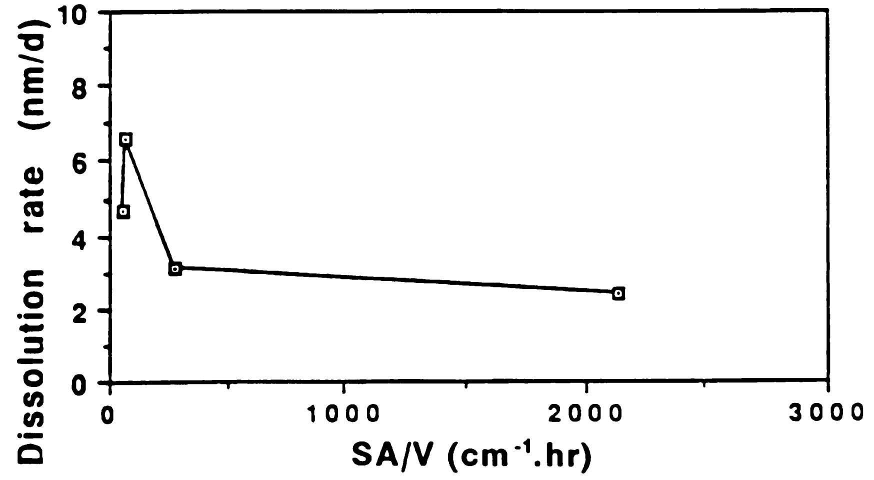

Figure 10. Dissolution rate of MMVF11 fibers in extracellular fluid.

lent to a modification through change of mass.

Table 5 gives the k values, where k = \f(ρ,0.24) v that were obtained for the 4 MMVF fibers under different experimental conditions of flow rate and mass. The k values increase, as SA/V decreases, with the exception of one value for MMVF11, which corresponds to the maximum value of v observed in Figure 10.

Under the given experimental conditions, the rockwool fiber (MMVF21) was the least soluble, and of the glasswool fibers, MMVF10 was more soluble than MMVF11, while the slagwool fiber (MMVF22), due to the rapid leaching of calcium, was as soluble as a glasswool fiber. Since the values of k vary with SA/V, it is clearly important to define the experimental conditions under which solubility measurements were carried out, including the mass of fibers, fiber diameter, and the flow rate.

Conclusion

The experimental solubility test was used to determine the dissolution of fibers of various chemical compositions and to estimate the influence of some of the test parameters on the measured dissolution rate. These included, among others, the pH and the chemical composition of the SPF. As examples, glasswool is soluble at pH 7.6—equivalent to extracellular fluid—but basalt wool is insoluble. In acid solution, the converse is true.

In addition, the dissolution rate depends on the test duration. If this is short, the dissolution rate corresponds to that of the original fiber, whilst with longer test durations, a lower dissolution rate is obtained, corresponding to the dissolution of a modified fiber that has been leached or has surface deposits. The solubility also depends on the SA/V ratio, which may be decreased by increasing the flow rate or by decreasing the mass of the fibers; either will increase the dissolution rate. The choice of experimental conditions will determine the extent to which there is agreement between the dissolution rates measured *in vitro* and *in vivo*.

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