Original Paper

New type of stonewool (HT fibres) with a high dissolution rate at pH = 4.5

Torben Knudsen

Institute of Mineral Industry, The Technical University of Denmark, Lyngby (Denmark)

Marianne Guldberg, Vermund R. Christensen and Søren Lund Jensen

Rockwool International A/S, Hedehusene (Denmark)

A representative, the HT-3 fibre, of a new type of stonewool (HT fibres), characterized by a relatively high content of alumina and a relatively low content of silica, has recently been tested in-vivo by Bellmann et al. to have a low biopersistence after intratracheal instillation in rat lungs. Several advantages of this fibre were found:

- a half-time T_{50} which is 2 respectively 4 times lower than that for the reference fibres glasswool and stonewool (after correcting to 1 µm median diameter),
- a better ultimate clearance after 18 months, resulting in 3 % remaining for the HT-3 fibre compared to 32 % for normal stonewool,
- an effective clearance of fibres with a diameter <0.5 µm. Fibres with a diameter belonging to this class are generally thought to be those with the highest potency for tumour formation.

The HT fibres have a relatively low in-vitro dissolution rate at pH = 7.5, similar to that of normal stonewool fibres, but a high in-vitro dissolution rate at pH = 4.5. The explanation of the favourable in-vivo properties of the HT-3 fibre is based on the recent investigations of the ability of alveolar macrophages to dissolve certain fibres, coupled with the observation of the high dissolution rate of the HT fibres at the pH value of 4.5 to 5 prevailing inside the macrophages. All HT fibres have been shown to have comparable, high dissolution rates at pH = 4.5 to 5.

Neue Steinwolle-HT-Fasern mit hoher Auflösungsgeschwindigkeit bei einem pH-Wert von 4,5

Steinwolle-HT-Fasern sind ein neuer Typus von Steinwollefasern, die sich gegenüber herkömmlichen Fasern durch einen relativ hohen Aluminiumoxid- und einen relativ geringen Siliciumoxidgehalt auszeichnen. Für einen Repräsentanten, die HT3-Faser, wurde kürzlich von Bellmann u.a. in einem In-vivo-Versuch, bei dem Fasern intratracheal in Rattenlungen instilliert wurden, eine niedrige Biopersistenz nachgewiesen. Insbesondere zeichnen sich die HT3-Fasern in diesem Versuchsmodell durch folgende Eigenschaften aus:

- Die Halbwertszeit T_{50} ist zwei- bzw. viermal geringer als für Referenzfasern aus herkömmlicher Glas- bzw. Steinwolle (nach Korrektur der Werte auf einen Standarddurchmesser von 1 µm).
- Die totale Clearance nach 18 Monaten ist mit 3 % in der Rattenlunge verbleibender Fasern besser als für herkömmliche Steinwollefasern, für die nach dieser Zeit 32 % der ursprünglich instillierten Fasern nachgewiesen wurden.
- Die Faser-Clearance ist für Fasern mit einem Durchmesser <0,5 µm besonders effektiv. Für Fasern dieses Durchmessers wird allgemein eine höhere kanzerogene Potenz angenommen.

Die HT-Fasern weisen ähnlich wie herkömmliche Steinwollefasern eine relativ niedrige In-vitro-Auflösungsrate bei einem pH-Wert von 7,5 auf. Sie besitzen jedoch eine hohe In-vitro-Auflösungsrate bei einem pH-Wert von 4,5. Eine mögliche Erklärung für die in-vivo ermittelte hohe Biolöslichkeit ergibt sich aus neueren Untersuchungen zur Rolle der Makrophagen bei der Auflösung bestimmter Fasertypen. Bei dem innerhalb der Makrophagen bestehenden pH-Wert von 4,5 bis 5 weisen die HT-Fasern eine besonders hohe Auflösungsrate auf. Allen Steinwollefasern des neuen Typus HT ist die hohe Auflösungsrate bei einem pH-Wert von 4,5 bis 5 eigen. Sie liegt für alle HT-Fasern etwa in derselben Größenordnung.

1. Introduction

The clearance of man-made vitreous fibres (MMVF) from lungs depends on different mechanisms. Two mechanisms have been recognized to be of importance: the dissolution in the lung fluid and the mechanical macrophage-mediated clearance. But in addition to these, the dissolution by the macrophages of fibres partly or fully engulfed by the macrophages seem to play an im-portant role for fibres, which have a high dissolution rate in the acidic environment (pH = 4.5 to 5 [1]) prevailing inside the macrophages [2 to 4].

The extracellular dissolution in the near-neutral lung fluid (pH = 7.4 to 7.8) has so far been proposed to be the only important clearing mechanism for long fibres [5 and 6]. However, it has been observed [2 to 4 and 7] that also long fibres with a low in-vitro dissolution rate at pH = 7.5 can sometimes have a low biopersistence. This is observed for fibres with a high in-vitro dissolution rate at pH = 4.5 and is suggested to be

Glastech. Ber. Glass Sci. Technol. 69 (1996) No. 10

331

due to the above-mentioned intracellular dissolution. Fibres too long for complete engulfment by the macrophages seem to be broken into shorter pieces by partial engulfment and dissolution.

The half-time T_{50} used in the evaluation of in-vivo biopersistence experiments assumes a first-order kinetics for the clearing of fibres. It is customary in some of these investigations to present time constants for different length fractions. Although an extensive survey of several in-vivo studies [8] indicates that especially the thin fibres with diameters less than 0.5 µm are those with the highest potency for tumour formation, it is not customary to present the time constants belonging to different fibre diameters. In the present paper, T_{50} for fibres <0.5 µm is suggested to be an important parameter.

Since first-order kinetics is seldom exactly observed in in-vivo experiments it may be argued that further time constants such as T_{75} or even T_{90} may be relevant. A short time for clearing 50 % of the fibres seems without significance if a substantial number of fibres are remaining for longer periods of time. In the extensive investigations by Bellmann et al. [4 and 9] using the intratracheal test, a high percentage of fibres remain even after 18 months for most of the fibre types tested. This behaviour violates the assumption of first-order kinetics. The clearance of the stonewool HT-3 fibre, a representative of the new type of stonewool to be described in this paper, remains a first-order kinetics for the entire time, thus, leaving a very low percentage of fibres after 18 months.

The effective clearing observed with the stonewool HT-3 fibre is suggested to be due to the above-mentioned mechanism of dissolution inside the macrophages. The macrophages have been assumed to be responsible mainly for the mechanical clearance of the fibres. Recently Luoto et al. [10 to 13] have demonstrated the dissolving ability of macrophages on fibres. The observation that fibres with a high dissolution rate in acidic environment actually disappear quickly from the lungs suggests that the in-vitro dissolution rate at pH = 4.5 should be regarded as equally important as that at pH = 7.5, when explaining and predicting the in-vivo behaviour of different fibre compositions.

This paper describes measurements of the in-vitro dissolution rates at pH = 4.5 using a flow-through method. The results obtained for the HT-3 fibre are presented along with results for other related fibre compositions, which proved to have comparable dissolution rates at pH = 4.5. All fibres are characterized by having a high content of alumina and a low content of silica compared to normal stonewool fibres.

2. Materials and methods

2.1 Production and characterization of the fibres investigated

The HT fibres were produced either full-scale or at a pilot plant at the Rockwool factories in Denmark. Binder and oil were not added. Fibres from the NAIMA

(North American Insulation Manufacturers Association, Alexandria, VA 22314 (USA)) fibre repository were used as reference samples (MMVF21, 22, 11 and RCF1). The chemical compositions and the dimensions of the fibres are given in table 1.

The fibres which passed a 63 µm sieve were used for the measurements (the reference fibres were not sieved). The chemical composition was determined by means of X-ray fluorescence spectrometry (XRF) using the Philips 1404 equipment (Philips, Eindhoven (The Netherlands)). The length-weighted diameter distribution of the fibres was determined by means of Scanning Electron Microscopy (SEM) using a Leica Instrument S360 (Leica, Cambridge (UK)) and by applying the intercept method (described in [14] as the probabilistic length-weighted method), enabling also a calculation of the specific surface of the fibre sample tested.

2.2 Measurements of rate of dissolution

The measurements and calculations were carried out in principle as described by Guldberg et al. [15]. Weighed amounts of fibres were placed in polycarbonate filter holders (diameter 50 mm) between a 0.8 µm micropore filter on the inlet side (top) and a 0.2 µm micropore filter on the outlet side (bottom). For each experiment two or three filter holders were mounted in parallel. The flow rate of the modified Gamble's liquids (table 2) was kept constant at (105 ± 5) ml/d. The set-up was maintained at 37 °C. The exact amounts of the effluents and their pH values were determined after 1, 4, 11, 18 and 25 d. Aliquots of these collections were analyzed with respect to silicon, aluminium, calcium and magnesium by means of Atomic Absorption Spectrophotometry (AAS), using a Perkin-Elmer AS5000 (Perkin Elmer, Norwalk, CT (USA)). Measurements were carried out at different ratios between the flow rate (F) and the initial surface area of the fibre sample (A), (F/A in µm/s). All fibres were measured at pH = 4.5 and some HT fibres and the reference fibres at pH = 7.5.

The dissolution of the fibres was assumed to be surface reaction-controlled, with a rate constant defined by

$$-\Delta D = 2 v t \tag{1}$$

where t is time, ΔD is the constant decrease of diameter, and v the rate constant.

Since the different components making up the composition of the fibre are not always dissolved at the same rate, one rate constant v_{Si} was calculated for the dissolution of SiO_2 and one rate constant v_{all} as an average for all major oxides. When v_{Si} and v_{all} are very different, the dissolution is incongruent. Although the rate of diffusion of leaching components may play a role in this case, diffusion control was not taken into account in the calculations.

When M_{i0} is the mass of component i in the sample at time zero and ΔM_i the mass dissolved at time t, the following equation has been used for the calculation of v_i:

Table 1. Chemical compositions (in wt%), KI index, and dimensions (in μm) of the investigated fibres

fibre types	SiO_2	Al_2O_3	TiO_2	FeO	CaO	MgO	Na_2O	K_2O	B_2O_3	KI	D_{16}[1]	D_{50}[1]	D_{84}[1]
stonewool HT-3	38.7	31.9	1.7	6.8	15.3	2.0	0.3	0.7		−46	2.2	4.6	7.8
HT-A	39.2	24.1	1.8	3.2	21.5	6.6	1.8	0.8		−18	1.9	3.8	6.2
HT-B	35.4	25.7	1.9	4.8	17.3	10.6	1.0	1.1		−23	1.3	3.0	5.8
HT-C	37.7	24.6	1.7	3.0	24.5	5.5	0.7	0.8		−19	1.7	3.7	6.9
HT-D	37.4	24.2	1.8	4.5	17.1	11.1	1.2	0.8		−19	1.3	3.2	6.8
HT-E	42.1	19.5	1.6	4.9	16.2	11.2	1.2	0.8		−10	1.6	3.7	7.1
HT-F	42.5	19.5	1.5	3.3	24.3	5.5	1.0	0.8		−8	1.7	4.2	6.9
HT-G	42.4	18.2	3.5	5.2	15.9	9.4	1.7	0.7		−9	1.8	4.4	7.5
HT-H	39.4	21.4	2.0	7.4	17.0	10.5	1.5	0.8		−16	1.3	3.3	6.8
HT-I	42.1	21.7	0.7	9.3	17.4	5.1	0.6	1.4		−19	1.7	4.0	7.2
HT-J	33.8	27.5	1.8	3.2	24.9	5.5	0.6	0.8		−24	1.4	3.0	5.4
stonewool, MMVF21	46.2	13.0	3.0	6.3	16.9	9.3	2.6	1.3		3	0.8	1.3	2.1
slagwool, MMVF22[2]	38.4	10.6	0.5	0.3	37.5	9.9	0.4	0.4		27	0.6	1.1	1.8
slagwool, MMVF22[3]	39.8	10.9	0.5	0.4	36.4	9.6	<0.1	0.5		25	0.9	2.6	5.5
glasswool, MMVF11	63.9	3.9	<0.1	0.2	7.5	2.8	15.5	1.3	4.5	23	0.5	1.2	2.2
ceramic fibre, RCF1	47.7	48.0	2.1	1.0	0.1	1.0	0.5	0.2		−94	0.6	1.3	2.5

[1] The characteristic diameter fractions based on a length-weighted fibre diameter distribution. [2] Fine fibre fractions. [3] Coarse fibres.

Table 2. Composition of the modified Gamble's liquid (in mg/l)

pH level of liquid	7.5/4.5
$MgCl_2 \cdot 6H_2O$	212
NaCl	7120
$CaCl_2 \cdot 2H_2O$	29
Na_2SO_4	79
Na_2HPO_4	148
$NaHCO_3$	1950
Na_2-tartrate $\cdot 2H_2O$	180
Na_3-citrate $\cdot 2H_2O$	152
90% lactic acid	156
glycine	118
Na-pyruvate	172
formalin ⎫ addition in ml	1 to 2
HCl 1:1 ⎭	≈3.7 (to pH = 4.5)

$$M_{i0} - \Delta M_i = \frac{\varrho_i L_0 \pi}{4} \int_{2v_i t}^{\infty} (D - 2v_i t)^2 \, dL(D) . \quad (2)$$

Here ϱ_i is the density of component i, L_0 is the accumulated fibre length at time zero and $L(D)$ the length-weighted cumulative diameter distribution function of the original fibre sample.

The right-hand integral is solved by numerical integration, by dividing the cumulative distribution function $L(D)$ into small intervals. v_i is now increased in a stepwise manner until the right-hand side and the left-hand side agree within a predetermined accuracy.

The values thus determined for the periods 1, 4, 11, 18, and 25 d are often seen to vary in a systematic manner. The values found for the period 0 to 25 d (or until 75 % of the fibres were dissolved, when this was reached before the 25 d) have been used in table 3, and a standard deviation was calculated to give an indication of the lack of constancy of the dissolution rates.
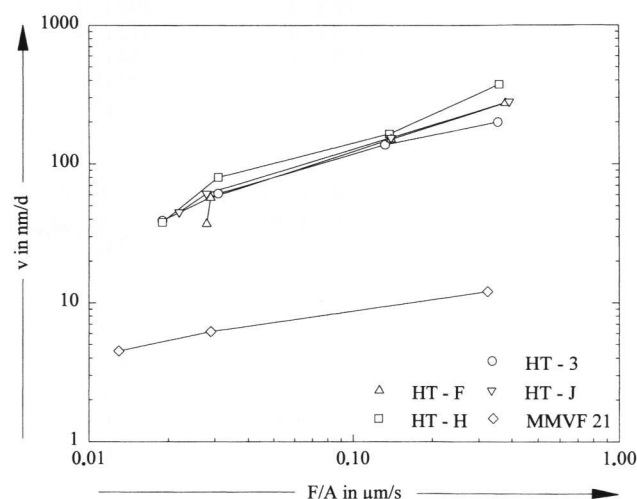

Figure 1. In-vitro dissolution rates at pH = 4.5 as a function of F/A.

At the end of the experiment the remaining fibres were rinsed in de-ionized water, dried and weighed, and the remaining mass compared to the results of the calculation. When the remaining mass of fibres were not too low (>25 %), the difference between the weighed and the calculated amounts never exceeded 10 % relative. The deviation of the results of the different cells involved in each experiment was typically found to be 5 to 15 %.

3. Results

The dissolution rate v at pH = 4.5 found with the flow-through method at different F/A ratios can be seen in figure 1 for the stonewool HT-3 fibre tested in-vivo [2 to 4] and for other HT fibres. For comparison a normal stonewool fibre, MMVF21, has been included. All HT fibres have clearly higher dissolution rates than normal stonewool.

Table 3. Dissolution rates at pH values of 4.5 and 7.5, respectively

fibre type	v_{all} in nm/d	v_{Si} in nm/d	F/A in μm/s	pH values in the effluent[4]	duration of exposition in d[5]
pH value = 4.5					
HT-3	39 (5)	38 (6)	0.019	5.8/5.8	25
HT-3	61 (13)	57 (14)	0.031	5.6/5.3	18
HT-3	137 (22)	134 (22)	0.133	5.3/5.8	11
HT-3	199 (−)	193 (−)	0.357	5.3/5.0	4
HT-H	38 (5)	37 (5)	0.019	6.0/5.8	25
HT-H	80 (20)	77 (21)	0.031	5.8/5.3	25
HT-H	164 (−)	164 (−)	0.138	5.3/5.3	4
HT-H	372 (−)	369 (−)	0.361	5.1/5.0	4
HT-F	37 (6)	34 (7)	0.028	5.3/5.5	25
HT-F	57 (17)	52 (17)	0.029	5.5/5.3	25
HT-F	150 (23)	141 (26)	0.140	5.0/5.0	11
HT-F	273 (−)	268 (−)	0.361	5.0/5.0	4
HT-J	45 (7)	44 (7)	0.022	5.9/5.5	25
HT-J	61 (8)	61 (8)	0.028	5.8/5.3	25
HT-J	154 (−)	164 (−)	0.140	5.5/5.5	4
HT-J	279 (−)	298 (−)	0.381	5.0/5.0	4
HT-A	205 (−)	201 (−)	0.163	5.3/5.3	4
HT-B	186 (−)	193 (−)	0.163	5.5/5.3	4
HT-C	208 (−)	212 (−)	0.170	5.5/5.3	4
HT-D	229 (−)	234 (−)	0.160	5.5/5.3	4
HT-E	130 (14)	129 (13)	0.162	5.3/4.7	11
HT-G	111 (20)	102 (20)	0.162	5.0/4.7	11
HT-I	191 (−)	177 (−)	0.170	5.3/4.7	4
MMVF21	4.5 (0.2)	3.9 (0.6)	0.013	4.7/4.7	25
MMVF21	6.2 (1.0)	4.5 (1.2)	0.029	5.2/4.7	25
MMVF21		12 (2.3)	0.325	4.5/4.7	25
MMVF22[6]	28 (−)	14 (−)	0.014	−	25
MMVF22[6]	43 (−)	20 (7)	0.029	6.6/4.7	11
MMVF22[7]	106 (59)	29 (8)	0.164	5.8/4.7	11
MMVF22[7]	140 (104)	54 (20)	0.455	5.3/5.0	11
MMVF11	0.7 (0.5)	0.5 (0.1)	0.013	4.7/4.7	25
MMVF11	0.9 (0.6)	<0.1	0.029	5.1/4.7	25
RCF1	2.6 (0.3)	2.6 (0.6)	0.035	4.7/4.7	25
pH value = 7.5					
HT-3	3.8 (1.6)	3.1 (1.5)	0.030	7.9/8.0	25
HT-H	3.4 (0.4)	3.5 (0.1)	0.011	8.5/8.5	25
HT-H	4.4 (1.9)	3.9 (1.6)	0.030	7.9/7.9	25
HT-H	6.6 (2.4)	6.6 (1.4)	0.087	8.5/8.5	25
HT-F	2.0 (0.3)	1.8 (0.2)	0.011	8.2/8.3	25
HT-F	2.0 (0.8)	1.3 (0.7)	0.030	7.9/7.9	25
HT-J	4.2 (0.3)	4.1 (0.3)	0.009	7.7/8.1	25
HT-J	8.3 (3.3)	7.4 (2.8)	0.029	7.9/7.9	25
MMVF21	2.1 (−)	1.9 (−)	0.014	7.7/7.7	25
MMVF21	1.6 (0.6)	1.6 (0.6)	0.029	8.0/7.9	25
MMVF21		6.0 (2.5)	0.328	7.7/7.7	25
MMVF11	17 (3)	13 (2)	0.013	8.0/7.9	25
MMVF22[6]	12 (3)	4.8 (0.7)	0.014	−	25
MMVF22[6]	12 (5)	4.9 (2.1)	0.029	8.0/8.0	25

[4] pH value of the effluent at the first day and the last day of the experiment. Inlet pH value = 4.5 and 7.5, respectively.
[5] Duration of experiment: 25 d or until 75 % of the fibres has dissolved, if this happened before 25 d.
[6] Fine fibre fraction.
[7] Coarse fibres.
Explanations: Figures in brackets are the standard deviations between v in the different sampling periods.
MMVF21 = normal stonewool, MMVF22 = American slagwool, MMVF11 = normal glasswool.

In table 3 the values of v for the different HT fibres based on all components and on silicon (network dissolution) are given. For comparison results for normal glass-, stone- and slagwool are included, along with some results at pH = 7.5. The dissolution rate at pH = 4.5 of all HT fibres is higher than that of glasswool and stonewool and on the same level as or higher than that of slagwool, dependent on the F/A ratio.

4. Discussion

4.1 Methods of measurements

Mattson [16] found increasing dissolution rates, v, with increasing flow rate/surface area (F/A) up to a limit, where a rate constant v was obtained. Her measurements concentrated on pH = 7.4 Gamble's liquid. however, the authors of the present paper have made similar observations as to the impact of increasing F/A using liquids with a pH value of 4.5.

The reason for the influence of the F/A ratio on the determined dissolution rate constant v is not well understood. The finding of F/A being a determining parameter for the overall dissolution rate in a flow-through experiment meets with the paradox that this ratio increases significantly during a dissolution experiment and that this increase of F/A is different, depending on the dissolution of the fibre type in question.

At least three physico-chemical effects must be taken into consideration to clarify the effect of F/A:

- when the F/A ratio is very low compared to the dissolution rate of the fibre, a saturation level is maintained during the elapse of the measurements, and the dissolution rate will respond almost proportionally to an increase of F/A;
- even when the F/A ratio has been raised sufficiently to avoid saturation, diffusion through a leached layer or a membrane may control the dissolution rate. A higher F/A value will lead to lower concentrations in the surrounding liquid, and thus, to higher dissolution rates;
- maintaining the intended pH value may present a severe problem at low F/A, as the dissolution of the fibres will cause the pH value to rise. An increased level of pH may lead to higher or lower dissolution rates, depending on the fibre type in question.

The possible role played by diffusion has already been touched upon by Mattson [16] and by Touray [17]. The impact it may have on a mathematical model for dissolution has not yet been evaluated. Work is in progress in the laboratory of Rockwool International to establish a more comprehensive model including the effects of saturation, reaction-controlled dissolution and diffusion-controlled dissolution.

4.2 Effect of chemical composition

In a recent work by Christensen et al. [7] it was indicated that SiO_2 and CaO govern the dissolution rate at pH = = 4.5, the dissolution rate increasing with increasing content of CaO and decreasing content of SiO_2. In the present work a series of flow-through tests were carried out, and the dominant role of SiO_2 was confirmed.

The influence of alumina in the fibre composition is very different at pH values of 7.5 and 4.5. From the work of Mattson et al. [16 and 18] and of Christensen et al. [7] the content of alumina appears to be the single most important element at pH = 7.5: increasing content of alumina significantly decreases the dissolution rate. At pH = 4.5 the content of alumina seems to increase the dissolution rate to the same extent as e.g. the oxides of calcium, magnesium, iron and sodium. This means that the dissolution rate is negatively correlated to the content of silica. The dominating factor for the high dissolution rate at pH = 4.5 of the new type of stonewool is thus believed to be a low silica content. This is further illustrated when looking at the refractory ceramic fibres (tables 1 and 3). These fibres have a low dissolution rate at both pH values, due to a high content of alumina as well as a high content of silica.

The HT fibres have a congruent dissolution at pH = = 4.5, i.e. all elements are dissolved at the same rate. This can be observed from table 3, where the dissolution rate calculated based on all elements and on silicon is the same within the uncertainty of the determination. With slagwool (MMVF22) this is not the case. In this case the basic oxides of calcium and magnesium are dissolving at a much higher rate than SiO_2, leaving behind a silica-enriched leached layer. This selective leaching puts slagwool in a group of its own. The dissolution rate based on the basic oxides of this slagwool is similar to that of the HT fibres, whereas the dissolution rate based on silicon (network dissolution) is lower.

HT fibres and other fibre types were exposed to 70 °C and 100 % relative humidity for 21 d. After this treatment, the HT fibres were practically unaffected. Some fibre types with a high dissolution rate at pH = 7.5, as e.g. the Exp. 3 fibres [4] with a KI index >40 (see below) were clearly corroded after this treatment. The different behaviours at these conditions are related to the dissolution behaviour of the different fibres. When water is present on a fibre surface, the pH value of this layer will rise. Due to the relatively low dissolution rate at neutral or weak alkaline conditions, the HT fibres do not deteriorate rapidly when exposed to humid atmosphere at elevated temperatures.

4.3 In-vitro/in-vivo dissolution

It is recognized that fibres in a lung are exposed to both the environment of the near-neutral lung fluid and to the more acidic environment inside the macrophages [1]. For fibres which easily dissolve in near-neutral liquids, the dissolution in the lung fluid may be a highly contributing factor to the clearing of such fibres. The KI index introduced in the German guideline TRGS 905 [19] is based upon results of intraperitoneal tests (i.p. tests), but is also reflecting the conclusions of an investigation of the in-vitro dissolution rate at pH = 7.4 of different borosilicate glasses [18]. The KI index is defined as the sum of

Torben Knudsen; Marianne Guldberg; Vermund R. Christensen; Søren Lund Jensen:

Table 4. In-vivo results from intratracheal tests [4, 9 and 20]

fibre type	reference	T_{50} WHO fibres[8]	T_{50} (1 μm)[9] WHO fibres[8]	content of WHO fibres[8] (in %) remaining after 18 months	content of thin fibres (in %) after 6 months[10] (<0.5 μm)
M-475	[4 and 20]	183/240	1307/706	10/–	24/–
M-753	[4]	45	214	6[11]	9
M-E	[4]	218	1282	18	33
MMVF11	[4]	199	212	13	17
TL-Wolle	[4]	188	369	14	60
X607	[4]	46	31	6[11]	6
M-Stein	[4]	116	138	5	54
M-Schlacke	[4]	81	105	1	30
RCF1	[4]	343	404	30	92
B-01/09	[4 and 20]	32/39/104	46/62/76	2[11]/–/–	1/–/–
Crocidolite	[4 and 9]	689/1000	3828/7692	70[12]/–	52/–
MMVF21	[9]	326	466	32	41
stonewool HT-3	[9]	110	117	3	2
MMVF31	[9]	254	249	27	48
MMVF30	[9]	357	470	38	69
Exp3	[9]	32	29	2[13]	3[13]

[8] Fibres according to the definition by WHO, i.e. $L \geq 5$ μm, $D < 3$ μm, $L/D \geq 3$.

[9] Corrected from the actual median diameter, D_{50}, of the stock fibres tested to a median diameter of 1 μm by $T_{50}(1 \text{ μm}) = T_{50}/D_{50}$.

[10] Accumulated length of fibres with a diameter less than 0.5 μm after 6 months, in percentage of accumulated lengths of fibres with a diameter less than 0.5 μm at the start of the test.

[11] After 6 months. [12] After 12 months. [13] After 3 months.

the weight% of $CaO+MgO+Na_2O+K_2O+BaO+B_2O_3$ minus twice the weight% of Al_2O_3 with higher KI indices meaning higher dissolution rates at pH = 7.5 according to [18]. According to the recommendations in [19], all fibres with a KI index <30 should be classified as "probably carcinogenic", fibres with a KI index between 30 and 40 should be classified as "possibly carcinogenic", whereas fibres with a KI index >40 should not be classified.

For fibres which easily dissolve in an acidic environment, the dissolution by the macrophages is likely to be important for the clearance. For such fibres the KI index does not even remotely predict the behaviour of the fibre in-vivo.

For instance, the stonewool HT-3 fibre tested in-vivo [2, 3, 4 and 7] has a high dissolution rate at pH = 4.5 and a negative KI index. The in-vivo results are shown in table 4, along with data for other fibres tested [4, 9 and 20]. It appears that T_{50} for the HT-3 fibre is 2 respectively 4 times lower than T_{50} for normal glasswool and stonewool (MMVF11/TL-Wolle, MMVF21), when correcting the T_{50} values to a median diameter of 1 μm. When looking at the fraction of fibres left after 18 months, it is found that there is 3 % left of the HT-3 fibres. This was also found for the fibres with very high dissolution rates at pH = 7.5 (Bayer, Exp3, X607), and for other fibres with a high dissolution rate at pH = 4.5 (M-slag, M-Stein). For all the other fibre types including normal glass- and stonewool 10 to 40 % is left after 18 months.

The macrophages may dissolve either fully engulfed short fibres, or they may dissolve that part of the long fibres, which is engulfed. In [21] the observation is reported that the macrophages, when unable to completely engulf the fibres, attach themselves onto the fibres like "pearls on a string". Also in-vitro cellular tests [11 and 13] have shown that macrophages are capable of engulfing the long fibres locally. The morphological peculiarities with local decrease in fiber diameter observed on SEM photos of the stonewool HT-3 fibres recovered from the rat lung after one year [2 to 4] are taken as a visual indication for a local dissolution of long fibres. Local dissolution is probably an important factor for fragmentation of fibres.

Since the clearing of fibres in the diameter range 0 to 0.5 μm could be of special significance for the tumour potency of fibres [8], details in the development of the fibre diameter distribution with time should be given special attention. In the investigations described in [4 and 9], a clear picture of this is presented in the form of 3D graphs of the amount (length) of fibres in the different diameter classes (up to 2 μm) at intervals 1, 3, 6, 12, and 18 months after instillation. The general picture is that the values of T_{50} for fibres belonging to the 0 to 0.5 μm diameter class are equal to those of other diameter classes. It generally shows no visible shift of the mode (maximum frequency) of the distribution to lower values of diameter. Two extremes may be noted. One is characterized by a significant accumulation of fibres in the diameter interval 0 to 0.5 μm during the first three months, whereafter a gradual decline can be seen. The second is characterized by the opposite trend, a significantly faster clearance of the fibres belonging to the 0 to 0.5 μm diameter interval compared to the clearance of all other fibre diameters. The latter was observed only for the HT-3 fibre. In this case, a concomitant shift of the mode of the distribution to higher values can be observed. It is proposed that this observation is due to a fragmentation of thin HT fibres into particles.

336

Glastech. Ber. Glass Sci. Technol. 69 (1996) No. 10

5. Conclusion

The in-vitro dissolution rates at pH values of 4.5 and 7.5 are both important in evaluating the biopersistence of MMVF. The dissolution rate at pH = 4.5 reflects the dissolution inside the macrophages, whereas the dissolution rate at pH = 7.5 reflects the dissolution in the lung fluid.

The KI index introduced in the proposed German regulation [19] is only indicative of the dissolution rate in the near-neutral lung fluid. When dissolution and breakage caused by the macrophages are the dominant clearance mechanisms, other chemical parameters are governing. Thus, the stonewool HT-3 fibre tested to have a low biopersistence has a negative KI index.

In addition to a low T_{50} value, this HT-3 fibre has further advantages:

- a very low level of remaining fibres (3 %) in the lung is reached after 18 months;
- the diameter distribution is changed during clearing, leaving only few thin fibres. This is considered an advantage, since thin fibres have been shown to be those with the highest potency for tumour formation in animal tests;
- the fibres have been found to have high resistance to humidity at elevated temperatures.

The above-mentioned in-vivo properties are assumed to be due to dissolution by the macrophages (pH = 4.5 to 5). All HT fibres have comparable, high in-vitro dissolution rates at pH = 4.5, much higher than those of normal stone- or glasswool fibres. Therefore, it is expected that all HT fibres have the same in-vivo behaviour as the HT-3 fibre already tested. Preliminary results of ongoing in-vivo investigations of other HT compositions than that of the HT-3 fibre confirm the low biopersistence of the HT type of fibres.

6. References

[1] Oberdörster, G.: Deposition, elimination and effects of fibres in the respiratory tract of humans and animals. VDI-Ber. (1991) no. 853, p. 17−37.

[2] Bellmann, B.; Muhle, H.: Investigation on the durability of the stone wool HT in rat lungs. Report from Fraunhofer-Institut für Toxikologie and Aerosolforschung (Fh-ITA), Hannover, to Rockwool International A/S, Hedehusene (Denmark) 1994.

[3] Bellmann, B.; Muhle, H., Kamstrup, O. et al.: Investigation on the durability of man-made vitreous fibres in rat lungs. Environ. Health Perspect. 102, suppl. 5 (1994) p. 185−189.

[4] Bellmann, B.: Investigation on the durability of man-made vitreous fibres in rat lungs. Final report by Fraunhofer-Institut für Toxikologie and Aerosolforschung (Fh-ITA), Hannover, to Rockwool International A/S, Hedehusene (Denmark) 1994.

[5] Eastes, W.; Hadley, J. G.: Role of fibre dissolution in biological activity in rats. Regul. Toxicol. Pharmacol. 20 (1994) p. S104−S112.

[6] Morgan, A.; Holmes, A.; Davison, W.: Clearance of sized glass fibres from the rat lung and their solubility in vivo. Ann. Occup. Hyg. 25 (1982) no. 3, p. 317−331.

[7] Christensen, V. R.; Lund Jensen, S.; Guldberg, M. et al.: Effect of chemical composition of man-made vitreous fibres on the rate of dissolution in-vitro at different pHs. Environ. Health Perspect. 102, suppl. 5 (1994) p. 83−86.

[8] Rossiter, C. E.: Animal evidence of critical fibre diameter for carcinogenic potential. In: Health risks from exposure to mineral fibres. North York (Canada): Captus Univ. Publ., 1993. p. 334−347.

[9] Bellmann, B.; Muhle, H.: Biobeständigkeit verschiedener Mineralfasertypen in der Rattenlunge nach intratrachealer Applikation. Endbericht für: Bundesanstalt für Arbeitsschutz, Dortmund. 1994.

[10] Luoto, K.; Holopainen, M.; Savolainen, K. et al.: Dissolution of man-made vitreous fibres in rat alveolar macrophage culture and Gamble saline solution. Staub-Reinhalt. Luft 52 (1992) p. 419−423.

[11] Luoto, K.; Holopainen, M.; Savolainen, K.: Scanning electron microscopic study on the changes in the cell surface morphology of rat alveolar macrophages after their exposure to man-made vitreous fibres. Env. Res. 66 (1994) p. 198−207.

[12] Luoto, K.; Holopainen, M.; Karppinen, K. et al.: Dissolution of man-made vitreous fibres in rat alveolar macrophage culture and Gamble's saline solution: Influence of different media and chemical composition of the fibres. Environ. Health Perspect 102, suppl 5 (1994) p. 103−107.

[13] Luoto, K.; Holopainen, M.; Savolainen, K.: Durability of man-made vitreous fibres as assessed by dissolution of silicon, iron and aluminium in rat alveolar macrophages. Ann. Occup. Hyg. 39 (1995) no. 6, p. 855−867.

[14] Koenig, A. R.; Hamilton, R. D.; Laskowski, T. E. et al.: Fibre diameter measurement of bulk man-made vitreous fibre. Anal. Chim. Acta 280 (1993) p. 289−298.

[15] Guldberg, M.; Christensen, V. R.; Krøis, W. et al.: Method for determining in-vitro dissolution rates of man-made vitreous fibres. Glastech. Ber. Glass Sci. Technol. 68 (1995) no. 6, p. 181−187.

[16] Mattson, S.: Glass fibres in simulated lung fluid: Dissolution behaviour and analytical requirements. Ann. Occup. Hyg. 38 (1994) no. 6, p. 857−877.

[17] Touray, J.-C.; Baillif, P.: In vitro assessment of the biopersistence of vitreous fibres: State of the art from the physical-chemical point of view. Environ. Health Perspect. 102, suppl. 5 (1994) p. 25−30.

[18] Potter, R. M.; Mattson, S. M.: Glass fiber dissolution in a physiological saline solution. Glastech. Ber. 64 (1991) no. 1, p. 16−28.

[19] Technische Regeln für Gefahrstoffe: Verzeichnis krebserzeugender, erbgutverändernder oder fortpflanzungsgefährdender Stoffe. Bekanntmachung des BMA nach § 52 Abs. 4 Gefahrstoffverordnung (TRGS 905). BArbBl (1994) no. 6.

[20] Bellmann, B.; Muhle, H.; Pott, F.: Untersuchung zur Beständigkeit chemisch unterschiedlicher Glasfasern in Rattenlungen. Zbl. Hyg. 190 (1990) p. 310−314.

[21] Morimoto, Y.; Yumato, H.; Kido, M. et al.: Effects of inhaled ceramic fibres on macrophage function of rat lungs. Occ. Environ. Med. 51 (1994) p. 62−67.

Addresses of the authors:
T. Knudsen
Institute of Mineral Industry, Building 204
The Technical University of Denmark
DK-2800 Lyngby

M. Guldberg, V. R. Christensen, S. L. Jensen
Rockwool International A/S
Group Environment
Hovedgaden 584, Entrance C
DK-2640 Hedehusene