Discussion Paper

Method for determining in-vitro dissolution rates of man-made vitreous fibres

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A "state-of-the-art" method for determining the in-vitro dissolution rate of man-made vitreous fibres (MMVF) is described. The dissolution rate is determined using an artificial lung fluid, adjusted to pH values of 7.5 or of 4.5, reflecting that the dissolution in-vivo takes place both in the near-neutral lung fluid and in the acidic environment of the macrophages. The method is based on flow-through equipment and prescribes well-defined conditions using a ratio of the flow rate to surface area of 0.03 µm/s. The results obtained with this method correlate to results from in-vivo tests, and thus the method provides a tool for a comparative evaluation of the biodurability of different fibre types. The described method seems suitable to be established as a provisional standard test, until further investigations allow the definition of a final standard test. The empirically derived carcinogenicity index (KI) extrapolates the in-vitro results obtained for some fibre types at the neutral pH value to other very different fibre types. The proposed in-vitro method avoids these uncertain generalizations, as it is based on actually measured dissolution rates at pH values 7.5 and 4.5, respectively.

Verfahren zur Bestimmung der In-vitro-Auflösungsraten von künstlichen Mineralfasern

Es wird eine Methode zur Bestimmung der In-vitro-Auflösungsrate von künstlichen Mineralfasern (KMF) beschrieben, die auf dem gegenwärtigen Stand der Kenntnisse basiert. Die chemische Auflösung von KMF kann in-vivo sowohl in der nahezu neutralen Lungenflüssigkeit als auch im sauren Milieu der Makrophagen erfolgen. Dementsprechend wird die In-vitro-Auflösungsrate in einer künstlichen Lungenflüssigkeit ermittelt, deren pH-Wert an 7.5 oder 4.5 angepaßt ist. Die Messung erfolgt im kontinuierlichen Durchfluß und schreibt ein definiertes Verhältnis zwischen der Durchflußrate der Flüssigkeit und der Probenoberfläche von 0,03 µm/s vor. Die Ergebnisse dieser Methode lassen sich mit denen von In-vivo-Tests korrelieren, und die Methode macht deshalb eine vergleichende Beurteilung der Biobeständigkeit verschiedener Fasertypen möglich. Die beschriebene Methode kann als vorläufiger Standardtest dienen, bis weitere Untersuchungen die Formulierung eines endgültigen Standardtests erlauben. Der empirisch ermittelte Kanzerogenitätsindex KI beruht auf der Extrapolation der Resultate eines relativ einheitlichen Fasertyps, gemessen beim neutralen pH-Wert, auf sehr unterschiedliche Fasertypen. Das vorgeschlagene In-vitro-Verfahren vermeidet dagegen unsichere Verallgemeinerungen, da es auf tatsächlich gemessenen Auflösungsraten sowohl bei pH 7,5 als auch bei pH 4,5 basiert.

1. Introduction

The health effects of inhaled man-made vitreous fibres (MMVF) depend strongly on the biodurability of the fibres, i.e. the biological lifetime within which the fibrous particles may be able to cause diseases. Different physical and chemical removal mechanisms affecting the biodurability are well-known. The mechanisms are in general simultaneously active, and the analysis of the impact of each single mechanism by animal studies is scarcely practicable [1 and 2]. The chemical dissolution seems to be one of the main elimination processes. The chemical dissolution behaviour of inhaled MMVF is believed to define a so-called minimal elimination rate and the interaction by further mechanisms should increase the clearance of fibres. The important role of the chemical elimination on evaluating the potential health impact of different fibre types is reflected by the formulation of the so-called carcinogenicity index (KI) [3], which is based on an investigation of the in-vitro dissolution behaviour of numerous glass fibres [4]. Different methods for studying the in-vitro dissolution rates of MMVF in-vitro have been used recently [4 to 12]. Most of the investigations were conducted at two pH values, because the in-vitro dissolution rates at the pH values 7.5 and 4.5 are thought to reflect the biodurability of fibres in-vivo, i.e. the dissolution in the near-neutral extracellular lung fluid and in the acidic intracellular environment of the macrophages, respectively. The influence of different measuring parameters and the correlation between in-vitro dissolution rates and in-vivo results are being further investigated in more detail in ongoing and planned projects [13 and 14]. The exact formulation of an in-vitro dissolution standard test will depend on the final assessment of the results of those investigations. Since some aspects of the formulation of KI remain unsatisfactory, an alternative or additional method for grading of MMVF should be introduced in the discussion. This paper describes a "state-of-the-art"

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method for the in-vitro investigation of the chemical dissolution behaviour of MMVF, which allows a comparative evaluation of different fibre types. It gives reproducible results, which can be correlated to results from in-vivo tests. The method is to be re-evaluated when the mentioned projects have been finalized.

2. Critical aspects of the carcinogenicity index

In chronic inhalation tests at very high doses, with concentrations of up to 1000 times larger than those found in the workplace, no significant excess of tumours was found for MMVF11 and MMVF21 (standard glass- and stone wool) [15 and 16]. The sensitivity of these tests has been questioned by some authors [17], and the German authorities have therefore introduced guidelines which urge the production of less biopersistent fibres. For that purpose the KI value has been introduced [3]. It results from an empirical comparison between the chemical composition of MMVFs and the carcinogenic potency of those fibres in intraperitoneal (i.p.) tests [18] and is calculated by

$$KI \equiv (c_{Na_2O} + c_{K_2O} + c_{B_2O_3} + c_{CaO} + c_{MgO} + c_{BaO}) - 2 \cdot c_{Al_2O_3}, \quad (1)$$

where $c_{\text{metal oxide}}$ are the oxide contents of the fibre in wt%. KI values of ≥40 lead to the exoneration of the respective fibre. Equation (1) shows a strong similarity to some conclusions of Potter and Mattson [4], reporting measurements of the in-vitro dissolution rate at pH 7.5 of a range of boron oxide-containing soda–lime–silica glasses. The general qualitative conclusion of the KI that a high amount of alkaline oxides and boron oxides decreases the chemical durability as well as a high amount of Al_2O_3 increases the chemical durability of soda–lime–silica glasses in aqueous solutions at a near-neutral pH value, is in agreement with the literature. However, the general quantitative description without defined limits of validity of the durability of amorphous materials in such a simplified manner must lead to faulty results in many cases. Already a few aspects demonstrate the difficulty.

The general qualitative assessment of the influence of components of the specifically investigated glasses in [4] implied that there is a simple quantitative linear correlation between the composition and the dissolution rate. Recently, the authors stated clearly that a relationship was found by a linear fit of the logarithm of the dissolution rate to the weight percent of glass components. This statistical evaluation implies different coefficients of the respective oxide contents, reflecting the different impacts of the components [11]. The values of the coefficients depend significantly on the number of regarded experiments and on the type of vitreous fibres (glass, slag, stone or refractory ceramic fibres (RCF)), constant experimental conditions understood. Thus, it is not possible to apply a statistical evaluation of the behaviour of soda–lime–silica glasses on calcium–aluminosilicate or aluminosilicate glasses.

It is possible to describe some physical properties of glass within well-defined composition limits based on empirical coefficients. An extended review of the different methods is given by Volf [19]. The chemical properties of glass show a multifunctional dependency of the structure (surface structure, phase separation), composition of the glass and that of the attacking agent, and the physical experimental conditions (temperature, attacking time, surface area, etc.) [20 to 22]. Thus, up to now, no general scientific description of the relationship of glass composition to chemical durability is known. The determination of chemical durability as well as of its mathematical model concentrates on the physical conditions, e.g. the temperature or reaction time. All other conditions have to be defined and understood as constant.

3. Proposed in-vitro test

The knowledge of the chemical behaviour of glass against attacking solutions leads to the conclusion that the comparative evaluation of the durability of MMVFs has to be performed by an investigation of each individual fibre type under constant standard experimental conditions. Although a general improvement of all experimental conditions is ongoing and will take more time, the formulation of a standard test should be possible, based on recent experiences. The following proposal has to be re-evaluated when the ongoing and planned investigations are finished.

3.1. Principle of measuring

A fibre sample is subjected to a continuous constant flow of an artificial lung fluid (modified Gamble's solution), adjusted to pH values of 7.4 to 7.6 and 4.5 to 4.7, respectively. The fibre sample is characterized with respect to its chemical composition and specific surface area. The flow rate of the artificial lung fluid per initial surface area (F/A) for the measurements is 0.030 ± 0.005 µm/s. The amounts of silicon and other main glass components dissolved are determined at regular intervals for a period of at least 25 d, or until 75 % of the fibres have dissolved if this is reached before the 25 d. The dissolution rate v (decrease of radius with time, in nm/d) is calculated for the first 25 d, or until 75 % of the whole fibre mass or 95 % of the individual components have dissolved if this is reached before the 25 d.

3.2. Equipment and reagents

An outline of the set-up is shown in figure 1. The cells, the tubes, the storage and the collecting bottles should be stable against the liquid used. The peristaltic pump should be able to deliver a flow constant to ±10 %. The filters in the cell should be micropore filters, 0.4 to 0.8 µm on the inlet side and at least 0.4, preferably

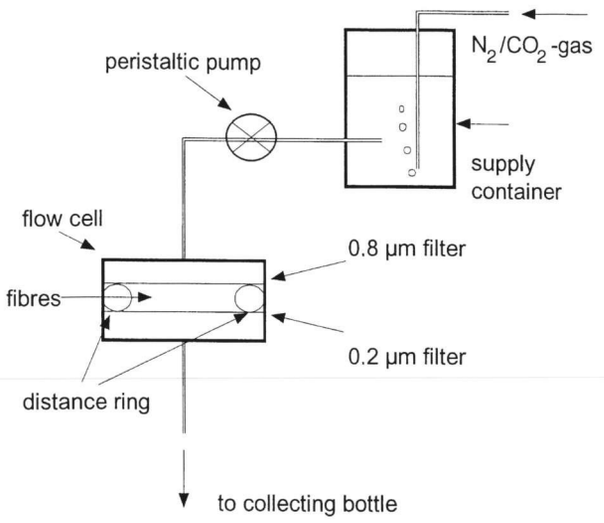

Figure 1. Flow-through test set-up.

Table 1. Composition (in mg/l) of the modified Gamble's liquid (after [9])

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	1 to 2
HCl 1:1 } addition in ml	≈3.7 (to pH 4.5)

0.2 µm, on the outlet side, to prevent particles from leaving the cell. During the test all equipment and containers should be maintained at (37 ± 1) °C. The composition of the liquid is shown in table 1. In principle, the same liquid is used for both pH values. pH 7.4 to 7.6 is maintained by bubbling with 95/5 N_2/CO_2 in the storage container; pH 4.5 to 4.7 is obtained by adjusting with hydrochloric acid (HCl) and omitting any bubbling.

3.3. Characterization of the fibre sample

The fibre sample is prepared from wool samples without oil or binder. Possible contents of oil and binder are removed by low-temperature oxidation (e.g. cold-ashing using ozone). The fibres are sieved and the fraction below 63 µm used for the measurements. Specially prepared fine fibre samples may also be used, if they are produced by the common manufacturing techniques. Fibre samples are stored dry in closed glass flasks. The length-weighted fibre diameter distribution is determined using Optical Microscopy (OM) or preferentially Scanning Electron Microscopy (SEM, necessary for fine fibre samples), either by a measurement of the length and diameter of every fibre counted or by the "intercept" method (= probalistic length-weighted method [23]). At least 400 fibres are measured. This determination allows a calculation of the specific surface of the sample (in m^2/g), using also the density of the fibres. The density is calculated from the chemical composition or determined using the air comparison pycnometer method. The chemical composition of the fibres is determined using X-Ray Fluorescence Spetrometry (XRF) and wet chemical methods (e.g. for boron). Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) or Atomic Absorption Spectrometry (AAS) may also be used.

3.4. Performance of the experiment

The amount of fibres needed to give the required relation F/A is weighed with an accuracy of ±0.2 mg. The amount of fibres is at least 30 mg per cell. The flow rate is normally 100 ml/d approximately, but could be in the range of 50 to 200 ml/d. The exact amount should thus be chosen so that the ratio of the flow rate to the initial surface area is 0.030 ± 0.005 µm/s. The weighed fibres are filled into the cells together with the modified Gamble's liquid and ethanol and are dispersed evenly in the cell, e.g. by means of ultrasonification. Three cells with fibre samples and one blind cell are run for each pH value and flow rate.

The effluent is collected, and the exact amount and its pH value are determined after 1, 4, 11, 18, 25, and 32 d. For very soluble fibres an increased frequency may be adequate. Aliquots of these collections are analyzed with respect to silicon, calcium and other main elements by means of ICP-AES, AAS or graphite furnace-AAS.

The pH values of the liquid used and of the effluent leaving the cells are measured every day. After the experiment the remaining fibres are rinsed in de-ionized water and dried to constant weight. The weight loss is compared to the calculated value. It is useful to inspect the morphology of the corroded fibres in relation to the initial fibres by means of SEM.

3.5. Calculations

The chemical composition of the fibres, the fibre diameter distribution, and the amounts dissolved are used for the calculation of v. The amounts dissolved are the mean values of the three cells at each measuring point, for individual components and as a total.

The dissolution rate, v, is calculated either:

a) from a least-squares best fit to the measured amounts of dissolved material, assuming a dissolution rate constant with time, i.e. proportional to the available surface, or

Table 2. Comparison of different modified Gamble's liquids (in mg/l)

	Christensen et al. [9]	Kanapilly (cited in [8])	Scholze (cited in [8 and 10])	Thélohan/Meringo [10]
pH level of liquid	7.5/4.5	7.6	7.6	4.5
$MgCl_2 \cdot 6H_2O$	212	=	212	106
NaCl	7120	6780	6415	3210
$CaCl_2$	–	–	–	97
$CaCl_2 \cdot 2H_2O$	29	29	255	–
Na_2SO_4	79	–	79	39
Na_2HPO_4	148	–	148	–
$Na_2HPO_4 \cdot 2H_2O$	–	188	–	–
$Na_2HPO_4 \cdot 12H_2O$	–	–	–	179
$NaHCO_3$	1950	2268	2703	–
Na_2-tartrate $\cdot 2H_2O$	180	–	180	90
citric acid	–	–	–	20800
Na_3-citrate $\cdot 2H_2O$	152	59	153	77
90 % lactic acid	156	–	–	–
Na-lactate	–	–	175	85
glycine	118	450	118	59
Na-pyruvate	172	–	172	86
NH_4Cl	–	535	–	–
H_2SO_4	–	49	–	–
NaOH	–	–	–	6000
formalin } addition in ml	1 to 2	–	=	1
HCl 1:1 } addition in ml	≈3.7 (to pH 4.5)	–	–	–

b) successively for each interval of effluent sampling, and using the obtained data for calculation of a mean value for the entire experiment (25 d, or till 75 % of the whole fibre mass or 95 % of the individual components have dissolved if this is reached before the 25 d).

The dissolution rate v is here, as usually done, assumed to be constant with time, corresponding to understanding the dissolution to be reaction-controlled. However, also diffusion and even in some cases saturation of the liquid may influence the dissolution rate measured. For very soluble fibres the dissolution rate v measured at F/A 0.03 µm/s may be too low, as the dissolution is slowed down due to high concentrations of dissolved elements in the liquid. The more complex phenomena of fibre dissolution can only be fully elucidated, when more extensive measurements, e.g. at more F/A values or in well-designed stationary set-ups, are made.

The dissolution rate v is given in nm/d. The values may be converted to the commonly used dissolution rate k in ng/(cm^2 h), using the formula:

$$k = 100\, v\, \varrho/24\,, \qquad (2)$$

where ϱ is the fibre density in g/cm^3. In general, the relation between the values of v and k is accordingly: $k \approx 11\, v$.

The value of v is given as a mean value ± the arithmetical deviation. With reference to the two calculation methods already given, the deviation is determined:

– as the two v values obtained when fitting the curve to the measuring points mostly deviating (+ and –) from the fitted curve,

– as the arithmetic deviation of the periodic dissolution rates (weighted with the length of the period), which are used for the calculation of the mean dissolution rate for the experiment.

To characterize a measurement of the dissolution rate, the ratio F/A used and the duration of the experiment (used for calculation of v) have to be stated.

4. Discussion

4.1. pH value and composition of the liquids

The two pH values, pH 7.4 to 7.6 and pH 4.5 to 4.7, are representing the extracellular and the intracellular environment, respectively [1]. The commonly used compositions are given in [4 to 12]. A schematic overview of some of the different compositions is given in table 2. The composition chosen in this method has a relatively low content of calcium, as laboratory tests indicate that a higher content of calcium according to [5] can initiate precipitation in the system.

The different buffering agents may have a clear impact on the dissolution rate, as they are also acting as complexing agents. For instance, it is known [24] that using high amounts of citrate at lower pH values may increase the dissolution rate significantly.

4.2. Flow rate/surface area

The dissolution rate v has been shown to be increasing with increasing F/A, at least up to a certain level, where

the level of v seems to be almost constant [11]. The ratio F/A of 0.03 µm/s is chosen as a reference point here, as this is the ratio where the dissolution rate of almost all of the normal standard insulation fibres, such as MMVF10, 11, 21, and 22, are at a level where a further increase of F/A does not increase the dissolution rate significantly [25]. Some fibre types may not have reached the level at F/A = 0.03 µm/s, where a further increase of F/A does not increase the dissolution rate [11]. A measurement at 0.03 µm/s would thus for some fibre types underestimate, but never overestimate the value of v.

The dissolution rate, v, is generally assumed to be constant, i.e. proportional to the surface available. That is, a reaction-controlled dissolution kinetic is assumed. However, for some fibre types diffusion through a leached layer may be the controlling mechanism. The clear impact of increasing F/A is probably due to an increasing concentration gradient over the leached layer. In the planned ACEL-project [13] and in the German BMBF/HVBG-project [14], an F/A value of 0.03 µm/s is approximately the mean of those F/A values planned to be investigated. These projects will hopefully contribute to a more detailed understanding of the mechanisms involved in fibre dissolution.

4.3. Calculations

At the beginning of the systematic investigations of the in-vitro dissolution of MMVF, the calculation of the dissolution rate v was based only on silicon, representing the network dissolution. Recently, the dissolution rate v has been based on all major components. For most fibres the two values obtained do not differ much, i.e. the network dissolution will be representative of the overall process. However, some fibre types, with a high degree of leaching of e.g. earth alkali or alkali, may be underestimated with respect to their behaviour, if only the network dissolution is taken into account.

The deviation range given along with the mean value of v is covering partly the experimental uncertainties, partly the variation of v with time, i.e. the deviation from the assumption of v being constant with time. For evaluation of the experimental variation v may be calculated for each individual cell, and the mean and deviation of v may be based on the three individual sets of measurement.

4.4. In-vivo – in-vitro dissolution

Based on already conducted measurements, it has been suggested that the dissolution rate at pH 7.5 correlates with the biopersistence [26]. However, this is not the case for all fibre types, as e.g. described in [27]. The clearance of fibres is influenced by the different environments in the lung. According to Oberdörster [1], both the near-neutral extracellular environment and the acidic environment of the macrophages play a role. Even fibres too long to be fully engulfed by the macrophages will be partly engulfed as described by Morimoto et al [28]. Such long fibres will be partly subjected to the near-neutral environment and partly to the acidic environment. Those fibres with a high dissolution rate at pH 7.5 will be dissolved/weakened on that part of the fibre which is outside the macrophage, whereas fibres with a high dissolution rate at pH 4.5 will be dissolved/weakened on the part which is inside the macrophages. The result of this weakening is in both cases an increased tendency to breakage of the long fibres into shorter fragments, which can then be either mechanically cleared or dissolved by the macrophages.

Bellmann [27] found a low biopersistence for the stonewool HT fibre, i.e. a half-time similar to the glass fibre B-1M (Bayer AG, Leverkusen (Germany)), which was about 75 % thicker and which has the same chemical composition as the glass fibre B-2L (Bayer), with which Pott et al. [29] did not find a tumour indication from the intraperitoneal test (i.p. test) at 10^9 fibres/lung. The reason for the low biopersistence of the stonewool HT fibre is suggested to be its high dissolution rate at pH 4.5 [27].

It has been found [30] that after dosing the number of free macrophages containing fibres is much less in the peritoneal cavity than in the lung. No free fibres were observed in lung lavages, all fibres were within, or partly within, alveolar macrophages. Given that fibres associated with alveolar macrophages may be within the acidic conditions of macrophage phagolysosomes (pH ≈ 4.5), this suggests that there may be significant differences in the environment to which fibres are exposed within the lung compared to that within the peritoneal cavity. The KI values have been related to the results of i.p. tests. For fibres with a high dissolution rate at pH 4.5, the dissolving effect of the macrophages in the lung seems to be a highly contributing factor to the clearance of the fibres.

Table 3 illustrates the relevance of the dissolution rate at both pH values.

4.5. Future regulations

Beside the guideline for classification by the German authorities, the classification of MMVF is also discussed at the European Union level [31], including the possibility of an exoneration clause. An exoneration clause could be based on the in-vitro dissolution rates, either at pH 4.5 or 7.5. With a method established for measuring the in-vitro dissolution rates, also the use of a simplifying index like KI becomes superfluous.

With F/A of 0.03 µm/s and an experiment duration of 25 d the data in table 4 are obtained for standard insulation wools [25]. If new fibre types should be developed fulfilling a minimum dissolution rate (exoneration limit) of 300 ng/(cm² h) ≈ 30 nm/d for either the pH value 4.5 or 7.5, it is estimated based on [27 and 32] that

Table 3. In-vitro dissolution rates and results from intra-tracheal experiments

pH level	v in nm/d		T_{50}[1])	KI[2])
	7.5	4.5		
MMVF21 (normal stonewool)	2 to 4	4 to 8	461	4
MMVF 11/TL (normal glasswool)	11 to 17	0.5 to 1.5	212/369	24/25
HT fibre	3 to 8	35 to 50	117	−48
M-slag/MMVF22	20 to 25	35 to 45	105	28/27
Exp. 3	120 to 140	40 to 60	29	47
crocidolite	0.01 to 0.05	0.01 to 0.05	3828	–

[1]) Fibres whose diameters were corrected to a median diameter of 1 µm [27 and 32].
[2]) According to equation (1).

Table 4. In-vitro dissolution rates for standard insulation wools (F/A = 0.03 µm/s, 25 d)

pH level	v in nm/d		k in ng/(cm² h)	
	7.5	4.5	7.5	4.5
normal stonewool MMVF21	3 (± 1)	6 (± 1.5)	33 (± 11)	65 (± 15)
normal glasswool MMVF11	14 (± 3)	1 (± 0.5)	150 (± 34)	8 (± 4)

Values in brackets stand for the standard deviation.

such fibres would disappear 2 to 3 times faster than the standard glasswool fibres and 3 to 4 times faster than the standard stonewool fibres.

Even lower biopersistence might be obtained, if the dissolution rates were even higher, such as for e.g. the EXP3 fibre [27], where also KI is >40. However, these fibres are not suitable as an insulation material due to, first, a very low melt viscosity, resulting in poor mechanical properties, second, poor fire resistance compared to normal stonewool, and most importantly, third, poor moisture and ageing resistance.

5. Conclusion

A method for measuring the in-vitro dissolution rate of MMVF is described. The method is based on state-of-the-art equipment and procedures and is well-defined in parameters known to influence the results obtained. The method may be used for determination of the in-vitro dissolution rates at pH 7.5 and 4.5, which could serve as parameters for estimating the biodurability of different fibre types.

The method is thought to be applicable for all MMVFs which are based on a silicon network, i.e. including all presently known fibres (glass, stone, slag and amorphous RCF). The method provides a conservative limit value of the dissolution rate, which may underestimate but not overestimate the dissolution behaviour. The proposed limit value is based on really measured data and not on an uncertain statistical extrapolation. For the assessment of a new fibre, experimental results from a relatively inexpensive test method have to be obtained. The described method seems to be suitable to be established as a provisional standard test for fibres, until further investigations allow to define a final standard test.

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