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Correlation between *in vitro* and *in vivo* dissolution behaviour of stonewools by nonlinear modelling techniques

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Abstract

Toxicology studies have indicated that stone wools with different compositions have different levels of biopersistence. It is however not easy to carry out a large number of *in vivo* experiments. It is also known that *in vitro* dissolution rates at two pH values correlate with two dissolution phenomena in lungs. In this work, nonlinear models of *in vitro* dissolution rates at pH 4.5 and at pH 7.4 have been developed for stone wools of widely different compositions from a limited amount of experimental data. These *in vitro* dissolution rates, in combination with some other variables, have then been correlated with *in vivo* retention half times. It is expected that once the models predicting half times are reliable enough, testing on animals could be reduced to a negligible fraction of its amount today.

The dissolution rates of stone wools depend on their composition in a complicated manner. Not only are the effects nonlinear, there are strong cross effects of combinations of variables. Therefore, the conventional linear techniques are not effective. Phenomenological modelling is hardly possible since very little is known about the kinetics of the potential surface reactions taking place at different pH values.

New techniques of nonlinear modelling have made this kind of model development feasible, as it is illustrated in this paper. These techniques have opened up new possibilities in materials science, including fibre technology and ceramics.

Keywords: Al_2O_3; TiO_2; Alkali oxides; Chemical properties; Fibres

1. Introduction

Man-made vitreous fibres, synthetic mineral fibres and synthetic vitreous fibres are generic terms indicating amorphous fibres including glass fibre, mineral wools and ceramic fibre products. Mineral wool includes stone wools, glass wools and slag wools. Stone wools are often the best choice as insulation materials, because of low thermal conductivity and good high temperature resistance. If the composition of stone wools is right,[1] they have very little biopersistence implying a low carcinogenicity and a generally lower pathogenicity. Crystalline silicate fibres are known to be capable of causing pathologies including pulmonary fibrosis, lung cancer, mesothelioma and pleural plaques. Stone wools like MMVF 21 are typically made from basalt or dolomite. Stone wools with lower biopersistence can be produced by modifying the composition in some ways. Alumina content, for example, is known to significantly influence biopersistence. Biopersistence can be reduced by additions or reductions of some other oxides also, and alumina is not the main determining factor in biosolubility.

For about 20 years, a lot of vitreous fibres of various compositions have been tested *in vitro* for their solubility at acidity levels found in the extracellular environment in the lungs (pH 7.4), and in the alveolar macrophages (pH 4.5).[1–6] These are supposed to reflect the biosolubility in the lungs. The Directive 97/69/EC on the Classification, Packaging and Labelling of Dangerous Substances put mineral wools in carcinogenic category 3 but included exoneration criteria based on composition, biopersistence and dimensions. This has further activated the research on biopersistence. A smaller number of *in vivo* tests have also

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been carried out. The *in vivo* tests are expensive, time consuming and involve ethical questions. Epidemiological studies published during the past 15 years provide no evidence of increased risks of lung cancer or of mesothelioma from occupational exposures during manufacture of these materials, and inadequate evidence overall of any cancer risk. Therefore the International Agency for Research on Cancer (IARC) has reevaluated mineral fibres in 2001 and considers mineral wool fibres, glass wool and stone wool not classifiable as to its carcinogenicity to humans (group 3). Luoto et al.[2] also report a method in which macrophages and fibres are placed on a membrane through which culture medium is allowed to flow, and dissolution rates of silica, alumina and iron oxides are measured.

There are however no models which can reliably predict the *in vitro* solubility of the stone wool fibres from the composition of the fibres. There are no models which predict the biopersistence of the fibres from the composition of the fibres, for a good reason. The dissolution rates of stone wools depend on their composition in a complicated manner. The effects are not only not linear, there are strong cross effects of combinations of variables. Conventional linear statistical techniques are not effective at describing these effects, while the new techniques of nonlinear modelling are still not common. These new techniques have made development of such models feasible. An attempt has also been made to correlate these results with the small amount of *in vivo* data. Once we have sufficiently good nonlinear models correlating the biosolubility with composition, or models correlating biosolubility with *in vitro* solubility, it is envisaged that the *in vivo* testing will be redundant to a large extent.

This paper describes the results of modelling of total *in vitro* dissolution rates of fibres of different compositions with moderate to high alumina, followed by a preliminary correlation between *in vitro* dissolution rates and *in vivo* half time for WHO fibres. It is envisaged that the next paper of the authors will deal with separate *in vitro* dissolutions rates for silica and alumina, and a correlation of those dissolution rates with *in vivo* half times.

2. Experimental data

Experiments were carried out to measure the dissolution rates at pH 4.5 and at pH 7.4 of stone wool fibres of a number of different compositions. All *in vitro* measurements at pH 4.5 and pH 7.4 have been performed using the flow-through measurement method. The reliability and comparability of the results have been improved in this study by using measurements from just one laboratory, and using a reference fibre in all series of measurements. The dissolution rate, K_{dis}, for each fibre has been put in relation to the corresponding dissolution rate of the reference fibre. The *in vivo* measurements for measuring fibre clearance from laboratory animals have been performed at the Fraunhofer Institute using the intratracheal installation method.

Data was available for 66 different compositions for dissolution rates at pH 4.5 and for 38 compositions at pH 7.4. Alumina contents varied from low levels to as high as 24%. The maximum content of magnesium oxide was about 20% and the maximum content of iron oxides was over 10%. Sodium and potassium oxide contents were relatively low among most of the fibres in this study. Like any data set from the real world, the distribution of the data is not very good from a modelling point of view. There are internal correlations among input variables, which make empirical modelling more difficult. Sodium oxide and magnesium oxide correlate strongly in the available data. In these situations, it becomes more difficult to distinguish between the effects of the correlated variables. There are lone points, far from where the majority of the data is located, which can either be very valuable in terms of information content, or can dominate the model formation in wrong ways. Fortunately, the harmful effects of internal correlations were minimal for pH 4.5, but were overwhelming for pH 7.4. It was still possible to develop moderately good models by imposing certain restrictions on the partial derivatives or by combining some input variables.

The data was preprocessed and analysed by the NLS Preprocessing Software, which has several facilities, including simpler things like calculating the basis statistics of the data set, filtering observations with missing measurements or variables beyond the range of interest, calculation of correlation matrices, showing the plots of every variable against every other, and more advanced features like clustering, calculating sets of observations with maximum or minimum similarity, and dividing the data into training, test and validation sets with desired forms of imbalance.

3. Nonlinear modelling

Nonlinear modelling has successfully been used for a large number of processes and materials in several sectors of process industries including polymers, plastics processing, ceramics, cement, concrete, pulp, paper and board, power generation, semiconductor processing, water treatment, chemical production, food processing, etc.

Nonlinear modelling can roughly be defined as empirical or semi-empirical modelling which takes at least some nonlinearities into account. Nonlinear modelling can be performed in many ways. The simpler ways include polynomial regression and linear regression with nonlinear terms. One can also use basis functions and splines, and in cases where the form of the nonlinearities is known, nonlinear regression can be used. Artificial neural networks are a set of efficient tools for nonlinear modelling, particularly because of the universal approximation capability[7] of feed-forward neural networks. The user does not need to know the type and severity of nonlinearities while developing the models.

3.1. Why nonlinear modelling?

It is not possible to use physical modelling in many situations. Even if it is possible, physical models tend to compute the output more slowly than empirical or semi-empirical models. Development of physical models is time consuming. Nonlinear modelling tends to be expensive, but physical modelling usually costs even more. Physical models involve assumptions and simplifications. Thus empirical modelling is often a better alternative.

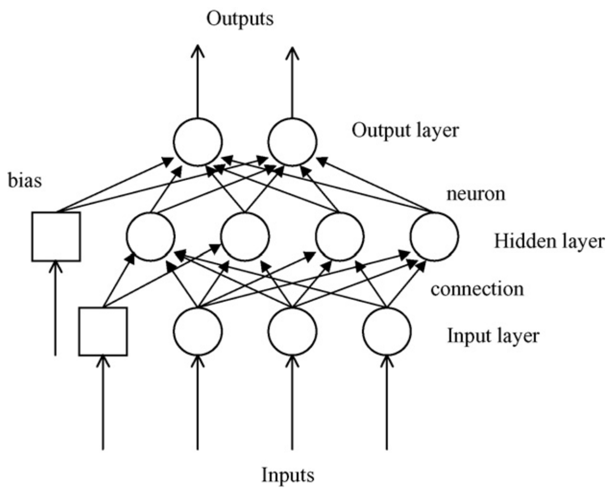

Fig. 1. A typical feed-forward neural network with one hidden layer.

Nothing in nature is absolutely linear. Therefore, you obviously do better by taking nonlinearities into account than by ignoring them. Earlier, the means for nonlinear modelling were modest, and the computational power was limited which prevented significant use of nonlinear modelling. Proponents of linear techniques base their arguments on simplicity of linear models, and on the possibility of using quadratic or other terms in linear models. Nature does not always fit the simplicities we try to force it in. Just as nothing in nature is linear, nothing is quadratic either. Feed-forward neural networks, on the other hand, make no assumptions about the nonlinearities involved, and are capable of approximating all continuous, bounded, first-order differentiable functions.

3.2. Artificial neural networks

Artificial neural networks resemble structurally and to a smaller extent functionally the networks of neurons in biological systems. Like the networks of neurons in the brains, artificial neural networks of some kinds also consist of neurons in layers directionally connected to others in the adjacent layers (see Fig. 1).

There are many different types of neural networks, and some of them have practical uses in process industries.[8,9] Neural networks have been in use in process industries for about 10 years. The multilayer perceptron, a kind of a feed-forward neural network, is the most common one. Most neural network applications in industries[10–14] are based on them.

There are a variety of training methods in use today. Backpropagation used to be the most common training method about 10 years back. Today, most people use good optimisation methods instead, like the Levenberg–Marquardt method.[15,16]

3.3. Applications of nonlinear modelling in materials science

Materials science has been making good progress in the last few decades. New materials with better and better properties are invented all the time. For all materials, a set of some mechanical, chemical, thermal, electrical, optical and other properties are important, which make them useful. Material properties of all kinds depend on the microstructure of the material, the composition and fraction of each phase, the size and shape of the crystals and amorphous regions, the molecular weights of the molecular chains, etc. Material properties can thus empirically be correlated with its microstructure. Empirical or semi-empirical models of this kind are valuable for scientific and academic purposes. Seen in another way, the microstructure is a consequence of the bulk composition of the material, or the mix of raw materials used to produce the material, and the process by which it is produced. Empirical or semi-empirical models of this kind (see Fig. 2) are more practical for industrial applications, since it is normal for industries to measure the composition of the material and material properties as a part of their quality control activities, and process variables are recorded in almost all plants which are not too old. Sometimes, dimension variables like fibre diameter are also taken into account in the input vector.

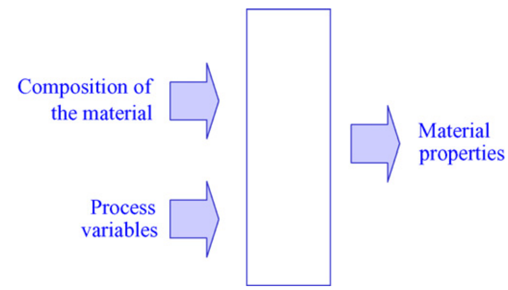

Fig. 2. Material properties depend on composition and process variables.

This approach which was earlier not so feasible with linear techniques is now a very practical approach and has successfully been used for a wide range of materials including metals (alloys), polymers/plastics, pulp/paper/board, concrete, ceramics and even food materials. In some cases, the composition is not an issue (for example, extrusion, where the same composition is used). In some cases, process variables are not an issue (for example, where the process variables are determined entirely by the composition of the material). Sometimes dimension variables, like particle sizes, thicknesses or diameters, are taken into account in addition to composition or process variables.

4. Results

The models developed during this work are plain feed-forward neural networks with a single hidden layer (like the one in Fig. 1) of sigmoidal activation functions. The models have been developed using the NLS 031 nonlinear modelling software, which has several advanced features for monitoring the activity of each neuron, each weight, and for restricting weights. It is possible to follow the partial derivatives of the output variables with respect to any of the input variable, as long as the number of weights is not very large.

Although this work is done from a single data set, the numbers of observations available for pH 4.5 and pH 7.4 are different, and hence separate data subsets were created for model development.

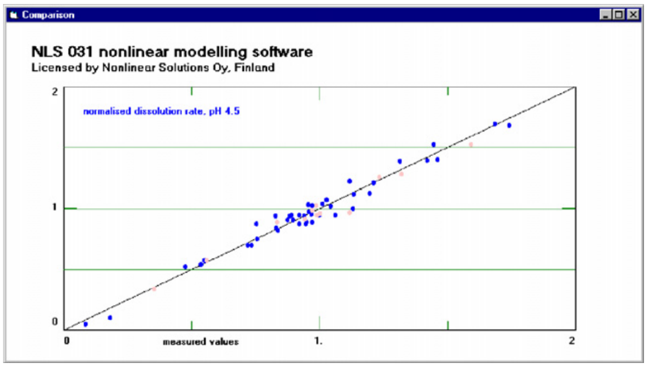

Fig. 3. A comparison of measured values with predictions from the nonlinear model for pH 4.5 (correlation of 96.3%).

This data was divided into training and test sets, but the final models are retuned from the undivided data sets for each pH. No validation set was created. A third data set, with 16 observations, was separated out for the *in vivo* data. This amount is too small to be divided into training and test sets.

4.1. Models for dissolution rates at pH 4.5

The 66 available observations were divided into a training set of 53 observations and a test set of 13 observations. The final models, however, are retuned based on all the 66 observations. A large number of models were attempted with different configurations of feed-forward neural networks, with a single hidden layer, with three different activation functions. One or more of the free parameters or weights of many of those models were restricted. This is particularly necessary because we have a very limited amount of data. Most of the better models showed the same qualitative features, with a high degree of correlation. Fig. 3 shows the comparison of the predicted (on the vertical axis) and measured values for training (in blue) and test (in pink) observations. Fig. 4 shows a histogram of the prediction errors for the training set (in blue) and test set (in pink). The model shows the effects of the different oxide contents as can be expected. Fig. 5 shows the effect of titanium dioxide content on the dissolution rate at pH 4.5; Fig. 6 shows the effect of alumina on dissolution rate at high, medium and low FeO content.

The rms error of this model was 0.05783. The effects of increasing the amounts of most of the oxides on dissolution

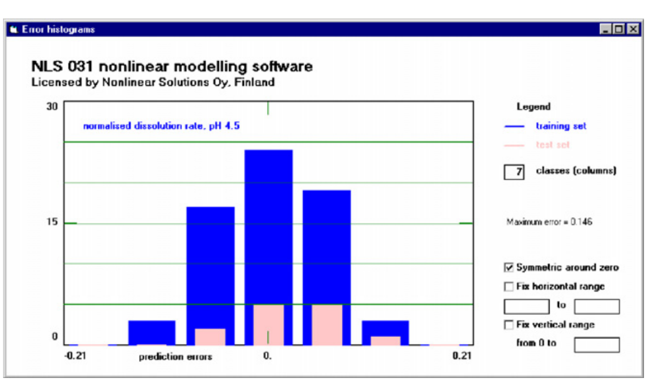

Fig. 4. A histogram of the prediction errors from the nonlinear model.

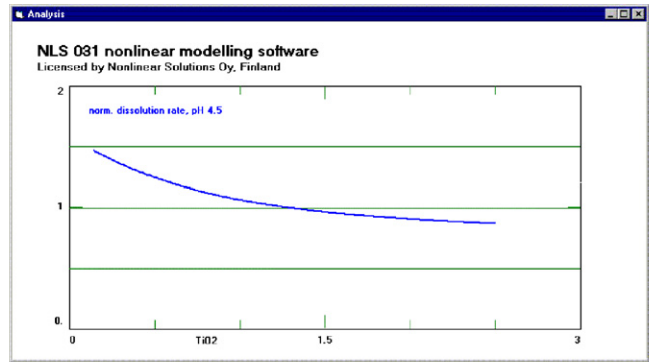

Fig. 5. Effect of titanium dioxide content on the dissolution rate at pH 4.5 according to the nonlinear model.

rates at pH 4.5 were positive. The effects of oxides of silicon and titanium were negative according to the nonlinear model developed during this work.

4.2. Models for dissolution rates at pH 7.4

The number of available observations puts an upper limit on the number of free parameters we can use in the empirical models. With only 38 observations, it is highly desirable, almost essential, to maintain the number of free parameters well below 20. This turns out to be a significant restriction for modelling from this data. More data would improve this situation. However, as the results show, the models derived from this data are still fairly good.

The 38 observations were divided into 31 training observations and 7 test observations. The situation was not very good, and it was not easy to divide the data set in a balanced manner. The division had to be revised a couple of times. The final model is based on all the 38 observations. With more experimental data, this model will be improved.

4.3. Correlating in vivo retention halftime with in vitro dissolution rates

For correlating *in vivo* retention half times with *in vitro* dissolution rates, only 16 observations were available. It is possible

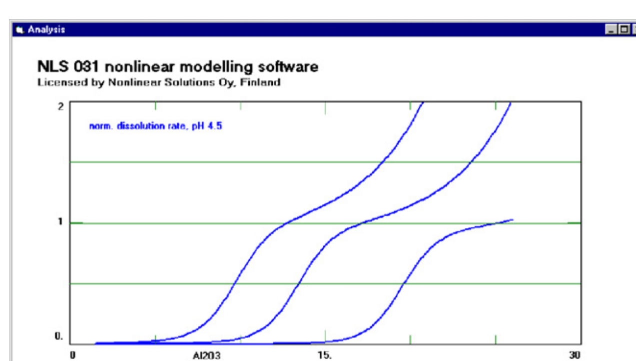

Fig. 6. Effect of alumina on dissolution rate at high, medium and low FeO content.

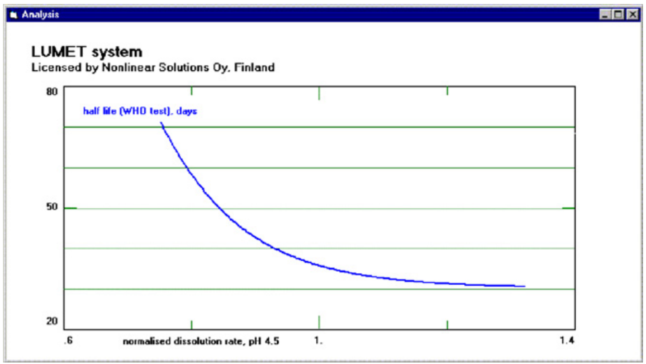

Fig. 7. Retention half time correlates inversely with dissolution rate at pH 4.5.

to see from the data that half times (for WHO fibres as well as for long fibres) are lower when the dissolution rate at pH 4.5 is higher. Such a correlation is not visible for pH 7.4. It was possible to develop preliminary correlations between the half times and the *in vitro* dissolution rates in combination with the mean fibre diameter. It is difficult to see how good these correlations are, since the statistical measures from 16 observations are not likely to be sufficient indications of the quality of the correlations.

The correlation developed during this work was implemented in a *LUMET* system which allows facile use of nonlinear models or correlations by people not familiar with nonlinear modelling. Fig. 7 shows the relation between the dissolution rate at pH 4.5 and the half time in days.

There seems to be a general agreement that the half times of fibres in the lung are dependent on both the dissolution rate at pH 4.5 and 7.4. Fibres of some compositions dissolve better in a neutral environment and some in an acidic environment.

In order to get a better correlation between the *in vitro* dissolution rates and the *in vivo* half times of fibres, further scientific investigations should be carried out or more test data must become available, so that the combined effect of the two dissolution mechanisms can be better combined and correlated with the behaviour in the lung. Another issue which needs to be taken into account is the length of the fibres. The fibres are broken down by phagocytosis, which can be abnormal if the fibres are relatively long. Since in any *in vivo* test, the lengths of fibres will vary, some measure of the fibre length distribution would be a desirable input variable for a more accurate *in vivo* correlation.

5. Conclusions

In this work, nonlinear models predicting dissolution rates in pH 4.5 and pH 7.4, and a correlation between these dissolution rates and biopersistence were developed. The nonlinear model for dissolution rate at pH 4.5 is quite good and shows certain phenomena which are known from theory. Nonlinear modelling, which has successfully been utilised for various materials and processes, was the only feasible approach for this work. The effects of most oxide concentrations were found to be clearly nonlinear, with strong cross-term effects, which makes linear modelling techniques unsuitable. The effects of each oxide on dissolution rates vary depending on the level of other oxides in the fibres. A simple comparison shows that a fibre with 18% alumina and 15% lime has a 30% lower predicted dissolution rate than a fibre with 13% alumina and 25% lime.

The nonlinear model for dissolution rate at pH 7.4 is not as good, primarily because the amount of observations available for model development was too small. The amount of observations for *in vivo* half times is even smaller, and the correlation developed in this work is a preliminary result, which will be refined in further work. With some more data, advanced nonlinear models will permit us to predict *in vivo* half times based entirely on the composition of the fibres.

These models should make it possible to reduce the number of animal experiments by a large fraction. Animal testing will essentially be replaced by *in vitro* experiments. It will be easier to develop new grades of stone wools with lower biopersistence, without substantially increasing the cost of the stone wool, without deteriorating the thermal and mechanical properties, without necessarily increasing the alumina content. The results of this work also demonstrate the strength of the nonlinear modelling approach.

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