DURABILITY OF GLASS AND CERAMIC FIBRES WITHIN THE LUNG

by

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ABSTRACT

The durability in the lung of inorganic fibrous materials, such as asbestos and man-made mineral fibres, appears to be a major determinant of their pathogenic potential. However, studies have been inadequate in explaining differences in the physiological durability of such inorganic fibres.

This study used an iterative approach to determine key factors affecting physiological durability of a soda-lime silicate bulk glass, A-glass, E-glass, Lead-glass, Cemfil and alumino-silicate ceramic fibres. The aims were to develop a) the current theoretical understanding of durability and b) a suitable in-vitro screening test for durability.

Materials were exposed to simulations of the lung environment, which included a) exposure to Gamble's fluid, water, serum and other simulated fluids, b) long-term exposure to the intra-macrophage environment and c) exposure to rat lung.

Durability was characterised by scanning electron microscopy (SEM) in conjunction with energy dispersive X-ray microanalysis (EDXA). The use of secondary ion mass spectrometry (SIMS) was also explored, though further development was required in this area.

Fibre behaviour depended on fibre composition and the nature of the exposure environment. The ceramic was durable in all environments, whilst A-glass, Lead-glass and the soda-lime silicate were prone to nucleophilic attack and leaching. The effects of in-vivo exposure were consistent with the response in-vitro. However, exposure to the intra-macrophage environment in-vitro did not affect fibre durability and this surprising result should be investigated.

Physiological durability was related to the ability of the fibre to resist nucleophilic attack and a hybridization bonding model was examined in order to explain the behaviour of some silicate glasses. It was recommended that models based on the molecular bonding were developed to encompass a wider range of materials.

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ABSTRACT

Occupational exposure and inhalation of asbestos fibre can cause lung disease and although the mechanisms of asbestos pathogenicity remain uncertain, attention has also focussed on the potential effects of other inorganic fibres.

Comparative studies on behaviour of these materials in the lung have strongly implicated the durability and hence lifetime of the fibre to be a major determinant of the pathogenic potential. However, durability studies have generally been inadequate in explaining differences in physiological durability of inorganic fibres and hence provision of theoretical models.

This study used a novel iterative approach to determine key factors affecting physiological durability of a range of glass and ceramic materials. The objective was to develop the theoretical understanding of the durability of inorganic fibrous materials in the lung and appraise in-vitro methods for determination of fibre durability to validate a suitable screening test.

The durability of a range of glass and ceramic materials has been characterised using in-vitro and in-vivo simulations of the human lung environment; novel exposure systems have been developed and durability behaviour has been characterised by application of traditional analytical methods and by development and application of secondary ion mass spectrometry (SIMS) techniques.

Appraisal of in-vitro simulations revealed that fibre behaviour depended upon fibre composition and exposure conditions; durability of the fibres in-vitro was related to model fluid pH. Fibre response in-vivo was rationalised by assuming localised pH variation.

This work supports the use of a range of in-vitro exposure conditions to identify key determinants of fibre durability and to characterise chemical behaviour, and is critical of the use of single in-vitro screening tests which will reflect fibre behaviour under specific conditions.

Resistance of the inorganic fibre network to hydrolytic attack was suggested as a key determinant of durability and a theoretical model was developed to predict this.

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CONTENTS

1. INTRODUCTION

1.1 CONTEXT

It has been established (Selikoff and Hammond [eds] 1979) that inhalation of asbestos fibre may result in various lung diseases, including fibrosis, lung cancer and mesothelioma. Attention has also been focused on the health hazards posed by inhalation of man-made mineral fibres (MMMF) (Kuschner and Wright 1976, Stanton et al 1977). These materials include rock and slag wool and glass and ceramic fibres, many of which were originally introduced as asbestos substitutes (Pye 1979).

Differences in the pathogenic potential of asbestos and MMMF have been identified. For example, it has been established that inhalation of long (> 10 micron), thin (< 1 micron) fibres of asbestos presents a high risk of lung disease (Pott 1978, HMSO Report 1979), whereas, in marked contrast, epidemiological evidence and in-vivo inhalation experiments using rats and hamsters infer a relatively minor risk from glass fibre (Gross 1970 and 1986, WHO/IARC Conference 1986). An understanding of the basis of these differences in pathogenic potential is seen as important in assessing the health hazard posed by new and novel inorganic fibres and providing a better understanding of the hazard posed by the extensive range of the current, commercially available materials.

Known key factors, identified as important with regard to the pathogenic potential of inorganic fibres, are: a) inhaled fibre dose, b) fibre dimensions, c) fibre durability in the lung and d) chemical composition (Leineweber 1979, Spurny 1987).

Whilst a combination of these factors is probably responsible for determining the health hazard associated with exposure to a type of inorganic fibre, durability is of major interest; it determines the residence time of a fibre type in the lung and hence the potential to initiate lung disease. Furthermore, the nature of durability warrants particular investigation as our present understanding of the durability of inorganic fibres in the lung is very limited.

In contrast, the role of fibre dimensions and dose, with regard to the potential health hazard, is well defined. The significance of fibre dimensions is that only thin fibres are capable of penetrating to the alveolar region and if they are greater than approximately 10 microns in length they cannot be readily removed by translocation. The significance of fibre dose is obvious; the larger the dose the greater the potential exposure to long, thin fibres and the greater the potential for initiation of lung disease. The effect of chemical composition is of note, being intrinsically linked to durability (Section 2). The durability of inorganic fibres is likely to be related to

the chemical composition of the fibre. However, the surface chemistry and chemical behaviour may also influence the pathogenic potential directly by interaction with the lung. It may be considered, therefore, that fibre dimensions and dose regulate the number of long, thin fibres that may reach the alveolar regions; fibre durability controls the lifetime of these long, thin fibres in the alveolar regions and the chemical composition may determine the nature of the fibre interaction with the lung tissue.

The significance of fibre durability has been recognised (Leineweber 1979, Morgan 1986) and a number of in-vitro and in-vivo studies have been undertaken (Section 2). These previous studies are critically reviewed (Section 2.2 and 2.3) and durability chemistry is discussed in relation to the behaviour of glass in the lung's environment (Section 2.5). This analysis, a) demonstrates the over-simplification of reported in-vitro studies in relation to the complexity of the alveolar environment and the general failure to develop the theoretical model of glass durability in the lung, b) provides a basis for applying durability chemistry to the characterisation of fibre degradation and c) shows the necessity for further study of the durability question.

1.2 AIMS AND OBJECTIVES

The nature of fibre durability and the effects of chemical composition of a fibre on the lung are not well understood. However, the significance of fibre durability and the associated implications with respect to pathogenic potential have been strongly emphasised (Leineweber 1979, Morgan 1986). In view of this, one of the major aims of this reported programme of work was to identify the key determinants of glass and ceramic fibre durability within the lung and to provide a greater understanding of the potential health hazard associated with inhalation of these materials.

The specific aims and objectives were:

a) development of a theoretical model for the durability of glass and ceramic fibres in the lung, to improve the understanding of the durability behaviour of the current range of glass and ceramics and to enable a theoretical assessment of novel glass and ceramic materials,

b) development and appraisal of an in-vitro screening test for fibre durability, providing a cost effective alternative to in-vivo screening tests,

c) development of analytical techniques for characterisation of the physiological durability of glass and ceramic materials,

d) characterisation of the physiological durability of a novel range of glass and ceramic compositions.

1.3 APPROACH AND METHODOLOGY

The programme of work reported was designed to identify the key chemical, biochemical and physical processes responsible for the degradation of silicate glass and ceramic fibres within the lung. The methodology employed an iterative series of in-vitro experiments where the complexity of the exposures developed in the following ways:

a) bulk to fibrous form of samples.

b) simple to more complex chemical composition of samples.

c) exposure of samples in simple physiological fluids to exposure in the intra-macrophage environment.

The samples, bulk or fibre, were characterised to assess the effect of exposure conditions and the results were compared with models for the degradation of glass. For comparison purposes, fibres were also exposed in-vivo in rats lungs.

This approach required:

a) the study of fibrous materials not previously examined, to the best of our knowledge, in physiological durability experiments (for example, Cemfil fibre).

b) the development of novel exposure systems (for example, providing exposure to the intra-macrophage environment).

c) the development of analytical methods to characterise fibre degradation (for example, secondary ion mass spectrometry [SIMS]).

1.4 RESUME OF CONTENTS

Published in-vitro and in-vivo studies of the durability of glass fibres in the lung are critically reviewed. Results for the exposure of a bulk soda-lime silicate glass and a range of glass and ceramic fibres to simulated lung fluids and for the in-vitro exposures of glass-fibres to macrophages are presented and discussed. Data is also presented for in-vivo exposures of glass fibres to rat lung. The development of analytical procedures is reported and recommendations for the further development of the project are made.

2. CRITICAL REVIEW OF IN-VITRO AND IN-VIVO DURABILITY STUDIES OF MAN-MADE MINERAL FIBRE (MMMF) IN THE LUNG

2.1 HEALTH RELATED ASPECTS OF FIBRE DURABILITY

Epidemiological evidence shows that exposure to asbestos can cause severe lung disease (Selikoff and Hammond [eds] 1979). In-vivo exposures of animals to dusts have substantiated the evidence supporting asbestos fibre toxicity - as have in-vitro studies, aimed at determining the mechanisms of toxicity (Stanton and Wrench 1972, Wright and Kuschner 1977, Brown et al [eds] 1980).

Epidemiological studies and in-vivo animal studies have also been undertaken to identify the risk of lung disease from other inorganic fibrous materials.

Whilst in-vivo studies, using intratracheal instillation or pleural implantation to administer fibre doses, have shown glass fibre to be capable of inducing biological activity in animals' lungs (Wright and Kuschner 1977), epidemiological data for human exposure to glass fibre and other man-made mineral fibre (MMMF), has shown the health risk to be relatively low (Enterline and Marsh 1979, 1980). This was substantiated by inhalation experiments using animals (Gross 1970, 1982, 1986). It is currently thought that this apparent discrepancy is associated with the unnaturally high fibre doses that are introduced by instillation or implantation (which by-passes the filtering action of the upper respiratory tract [Lippman 1980]); this is in contrast to inhalation, where the exposure dose is

likely to be far lower. It is important, therefore, that factors responsible for the differing health risks associated with the various types of inorganic fibrous material are determined, particularly with regard to providing a basis for assessing the health risk posed by the wide range of existing fibrous materials as well as by new materials as they are developed.

The biological response to inhaled fibres is recognised to depend on a number of possible contributory factors. Fibre size, fibre dose and fibre composition are all recognised as important and the durability of fibres within the lungs environment has been recognised as a potentially major determinant of the long-term health risk (Leineweber 1979, Morgan 1986).

"Long, thin" asbestos fibres may be inhaled and retained within the lung for long periods of time, thereby representing a major hazard (HMSO Report 1979). Asbestos fibres of sub-micron diameter and length greater than 10-20 microns have been most strongly implicated and this led the National Institute for Occupational Safety and Health (1977) to recommend working limits for fibrous glass exposure of 3 fibres/ml of air, where the fibre dimensions were: length > 10 microns and diameter < 3.5 microns. Noting that many types of inorganic fibrous material include fibres of similar dimensions (Rood and Streeter 1985, Rood 1988) yet appear to have different pathogenic potentials, many authors have considered this to be

associated with different fibre durabilities within the lung. Consequently, fibre durability may be important in assessing the associated health risk (Leineweber 1979, Wright and Kuschner 1977, Morgan 1986).

The biochemical/chemical factors affecting the degradation of an inorganic fibre within the lung are not well characterised and the need for a better understanding of the processes involved has been identified (Spurny et al 1983).

2.2 IN-VIVO DURABILITY STUDIES OF MMMF

A series of reported in-vivo animal studies has highlighted the differences in corrosion resistance between natural fibres and MMMF. The studies involved administration of fibrous materials to the animal by implantation, instillation or inhalation. After the desired exposure the animals were sacrificed and the fibre recovered and prepared for analysis.

Wright and Kuschner (1977), using intratracheal instillation, examined the effects of varying lengths of asbestos and glass fibre on lung tissue. They demonstrated the fibrogenicity of long, thin glass-fibres as well as the fibrogenicity of equivalent doses of long, thin crocidolite asbestos. It was noted that short fibres of both materials did not cause a significant fibrogenic response and that glass, generally, was far less fibrogenic than asbestos.

Fragmentation of long glass fibres was observed, with small fragments being found in the lymph nodes. This led to the conclusion that the lower pathogenic potential of the glass, compared with asbestos, was a consequence of its lower durability in the lung's environment and thus its shorter lifetime in the alveolar region of the lung. Whether the fragmentation was predominantly the result of chemical or physical processes is unclear.

Intratracheal instillation has been criticised as a method of assessing tissue response in the lung as it by-passes the clearance and filtration mechanisms of the upper respiratory tract, gives a different deposition pattern when compared with inhalation and can result in unusually high, non-physiological doses of test materials (Pritchard et al 1985). However, for the purpose of assessing fibre durability ,as opposed to toxicity, the general conclusions may be considered valid - provided the lung's response to the high instilled dose did not result in an atypical exposure environment for the fibre.

Morgan et al (1982) carried out a well controlled series of experiments and using intratracheal instillation, introduced radio-labelled glass fibres of known dimensions into rats to study clearance and solubility. Intratracheal instillation was purposely used to provide a sufficiently high fibre dose as previous data from inhalation studies indicated the difficulties in administering suitable doses (Morgan et al 1980). It was assumed that neutron-activation labelling of the glass had no effect on solubility.

The samples included 5, 10, 30 and 60 micron lengths x 1.5 micron diameter (0.5 mg dose) and 10 and 60 micron lengths x 3 micron diameter (1.0 mg dose). By digesting the lung and counting the residual fibres it was found that the majority of 10 and 5 micron length fractions were cleared after 12 months but the longer fibres persisted in the

lung. It was concluded that the shorter fibres were engulfed by alveolar macrophages and transported from the alveolar region to the terminal bronchioles from where they were removed on the mucociliary escalator. After 18 months the remaining fibres showed corrosion, with the 30 and 60 x 1.5 micron fibres reduced by over 50% in diameter. These longer fibres, which could only be partially engulfed by the macrophages, were the most heavily corroded and in several cases the longer fibres showed "thinning" at both ends to form a point. Leaching of sodium (Na) from the fibres was also reported.

It was concluded that glass fibre was far less durable in the lung than asbestos and, consequently, did not constitute such a health hazard. It was suggested by Morgan that differences in dissolution rates may be associated with previously reported differences in the pH of the intra- and extra-macrophage environment (Laman et al 1981). However, as solubility may depend on the chemical composition of the material, it was recommended that a study of this should be given high priority.

Bernstein et al (1984), again using intratracheal instillation of neutron-activated fibres, introduced up to 20 mg of 5 micron length x 1.5 micron diameter and 60 micron length x 1.5 micron diameter glass fibre into rats.

The glass fibre was manufactured by Johns Manville Inc. using the described method (Bernstein et al 1980), and was

of composition (mol %): silicon oxide, 63.2; boron oxide, 5.4; aluminium oxide, 5.5; sodium oxide, 14.8; potassium oxide, 1.1; calcium oxide, 6.0 and magnesium oxide, 3.1.

Recovery of the fibre and subsequent examination using the scanning electron microscope (SEM) showed that after 18 months of exposure the most significant reduction in fibre diameter (67.3%) occurred in 60 micron fibres that were partially engulfed by macrophages. Long fibres not associated with macrophages showed only a 26% reduction in diameter over the same time period. Short fibres exposed in saline, short fibres in tissue, and long fibres in saline showed 8.3, 13.0 and 13.2% reductions in diameter respectively. Thinning of the ends of long fibres was not reported.

These results suggest that macrophages can contribute significantly to the dissolution of long, glass fibres. However, it could be inferred that macrophages bind to thinner fibres preferentially, after other factors have resulted in their corrosion. Bernstein suggested that proteolytic enzymes from the macrophage may play a significant role in the corrosion process.

Johnson et al (1982), in an attempt to simulate environmental exposure conditions typical of those experienced by a factory worker, exposed rats to aerosols of microfibre, rockwool and glasswool (10mg/ml), for 5 days per week and 7 hours per day over a 1 year period. The

rats were subsequently sacrificed; the location of the fibre was identified and changes in the chemical composition and physical nature of the recovered fibres were assessed experimentally.

All types of fibre showed surface etching, the extent of which increased with lung residence time. It was noted that rockwool displayed less etching than either glasswool or glass microfibre.

The glass microfibre and rockwool were found mainly within alveolar macrophages and to a lesser extent in interstitial macrophages. They were either resident in phagosomes, free in the cytoplasm, or protruding from the cell.

It was noted that X-ray microanalysis showed that digestion of lung tissue using sodium and potassium hydroxide altered the X-ray peak ratios for constituent elements in the glass fibres, thus making this digestion technique unsuitable. As an alternative, low temperature ashing (LTA) of lung samples was also used; this is a technique known to be suitable for elemental analysis of biological samples (Gleit and Holland, 1962).

Hammad (1982), using inhalation techniques, exposed rats to ceramic fibre and mineral wool. Groups of rats were sacrificed periodically up to 270 days and the lungs ashed by LTA. The fibres recovered were sized and counted.

It was observed that ceramic fibres were cleared at a slower rate than mineral wool and he suggested that this was associated with the greater chemical resistance, and hence physiological durability, of the ceramic fibre. In other words mineral wool was cleared by dissolution and by translocation, whereas ceramic fibre was cleared primarily by translocation.

Morgan et al (1984) examined the dissolution of rockwool following its administration to rats by instillation. It was noted that dissolution tended to develop from the ends, rather than evenly along the fibre length and that the dissolution rate was much less than for glass fibre. This suggested a higher risk from rockwool inhalation.

2.3 IN-VITRO DURABILITY STUDIES OF MMMF

Several workers have examined the in-vitro durability of MMMF. These studies have focused on exposing fibres to water and to simulated interstitial lung fluids, notably Gamble's fluid (Gamble 1951), at body temperature (37°C) and under static or continuous flow conditions. Analytical techniques employed to characterise the effects of exposure have been scanning and transmission electron microscopy (SEM and TEM) in conjunction with energy-dispersive X-ray microanalysis (EDXA) for examination of morphological and chemical changes, and spectroscopic methods to monitor chemical changes in the fluids.

Oberdorster et al (1980) monitored the dissolution rates of trace elements from fibrous materials exposed to Gamble's fluid and citrate solution under continuous flow conditions. Neutron activation analysis was used to quantitatively identify the leached species.
It was observed that neutron activation could significantly increase the leach rate for glass fibre, amosite and chrysotile. For example, in the case of glass fibre an average 26x increase in leach rate was observed for 21 day neutron activation, in comparison with 3 day activation. It was noted that for crocidolite and slagwool, Co, Zn, Si, Mn and Cs were rapidly leached in citrate, but only minimally in Gamble's fluid. The corrosive effect of citrate was attributed to it being a complexing agent and therefore inhibiting the formation of protective, insoluble layers on the fibre surface. Conclusions confirming leaching of Mg in acid-treated chrysotile and by citrate are made. The significance of leaching of species from glass fibres was not emphasised, though possible inaccuracies through the use of the neutron activation technique were stressed.

Klingholz and Steinkopf (1982) examined the physical and chemical changes in slag, glass, rock and basalt wool, after exposure to Gamble's fluid and water. Both static and continuous flow experiments were carried out. Fibres were characterised by SEM and EDXA and dissolved elements characterised by atomic adsorption spectroscopy. It was

generally seen that all fibre types were leached by exposure and clear signs of attack were noted. In the case of the static exposure of fibres to Gamble's fluid, adequate control of pH was not maintained and non-physiological pH values were observed.

Physiological pH was better maintained in continuous flow experiments, where Gamble's fluid was gassed with 5% CO_2. Continuous flow exposure of slag wool showed rapid leaching of calcium and magnesium and the apparent formation of an alkali aluminosilicate "gel" on the surface. It was suggested that the entire fibre eventually converts to this gel, with sodium being adsorbed. Exposure to distilled water gave a similar, though weaker, response - presumably because of the absence of cations in the exposure fluid, which in the case of the Gamble's fluid encouraged the formation of gel layers on the fibre.

In the case of glass fibre, continuous flow exposure to Gamble's fluid resulted initially in leaching of sodium and potassium. Later, some leaching of calcium and SiO_2 occurred, as the alkali metal leaching rate decreased.

Exposure of rock wool to Gamble's fluid under continuous flow produced only weak leaching, though the production of thin oxide gel layers were observed. Exposure to distilled water appeared to have a negligible effect. Basalt wool appeared to show significant loss of SiO_2, and the formation of a thin gel layer was apparent. Pitting of the

fibre could be seen below this gel layer, indicating local corrosion. X-ray microanalysis did not show changes in relative analyte levels, presumably as layers were dissolving as a whole, rather than preferential leaching occurring. Pure water was also seen to dissolve SiO_2.

Leineweber (1982) used the continuous flow method with Gamble's fluid and water to assess the durability of ceramic fibre, mineral fibre and four types of glass fibre (Table 2.1).

He described the durability of the fibrous materials in terms of the dissolution rate constant, calculated from mass loss measurements.

It was observed that all materials were at least partially soluble in both Gamble's fluid and water. The decreasing order of solubility in water was refractory fibre, glass D, glass B, mineral wool, glass C and glass A. In Gamble's fluid the decreasing order of solubility was glass D, refractory fibre, mineral wool, glass C, B and A. SEM analysis indicated that gel layers had formed on many of the fibre samples and that those more readily attacked showed more extensive surface layers. Chemical analysis of glass A exposed to water showed sodium and zinc enriched surface deposits, and exposure to Gamble's fluid produced surface deposits rich in calcium and phosphorus. X-ray diffraction analysis indicated the presence of $Ca_5(PO_4)_3OH$ which is a highly insoluble compound.

Leineweber noted that gel layers were not observed in-vivo. This may have been because of their removal by chelating agents. The effect of non-physiological temperature and the fact that pH in water was not correlated to fibre dissolution makes further comment difficult.

Element	Glass A	Glass B	Glass C	Glass D	Mineral Wool	Refractory Fibre
SiO^2	55.25%	57.6%	52.70%	54.8%	40.3%	53.75%
Al^2O^3	3.93	5.24	5.40	14.7	9.5	45.84
B^2O^3	9.68	8.0	6.00	6.95	-	-
Fe^2O^3	0.10	0.11	0.11	0.37	12.4	0.07
CaO	11.32	7.88	5.49	18.5	25.5	0.10
MgO	0.12	4.32	2.79	2.88	6.3	0.02
SrO	-	-	-	0.40	0.02	-
BaO	-	-	-	-	-	-
ZnO	1.50	-	0.03	-	1.31	-
Na^2O	17.22	14.9	13.81	0.59	1.05	0.29
K^2O	0.73%	1.14	1.10	0.22	0.92	0.05
CuO	-	-	-	-	0.62	-
MnO	-	-	-	-	0.49	-
TiO^2	-	-	-	0.61	0.85	-
F	-	0.97	-	-	-	-

Table 2.1 Glass composition prior to exposure (Leinweber 1982)

Leineweber was unable to satisfactorily relate dissolution to fibre composition, or to explain differences between dissolution rates for the two fluids, though he noted a correlation between alkali oxide content and durability.

Forster (1982) reported a varied series of tests which examined the response of rock, slag, basalt, glass wool and asbestos to a range of fluids (Gamble's, Ringer's, water, potassium hydroxide and sulphuric acid). General reactivity of non-asbestos fibre was noted, as was the formation of gel layers. It was also observed that distilled water was apparently more corrosive towards mineral fibre than Gamble's fluid and that diluted Gamble's fluid was more corrosive than the undiluted solution.

Spurny et al (1983) investigated the chemical changes in exposed asbestos and MMMF, both in-vivo and in-vitro. Chrysotile, crocidolite, glass fibre, rockwool, slagwool and basalt wool were exposed to HCl, H_2SO_4, NaOH, water, and blood serum. Changes in fibres administered to rats and rabbits were also studied. The fibres were categorised by SEM, EDXA, X-ray diffraction (XRD) and mass spectroscopy.

Chrysotile was unstable in acidic solutions and in lung tissue, with magnesium being rapidly leached to leave, in the case of exposure to strong acids, only a silica shell. It was noted that certain chrysotile fibres were preferentially leached. MMMF and especially glass fibre

were unstable in animal tissue and in acids and alkali. MMMF were seen to be depleted of Ca and Zn after acid exposure, and microporous surfaces were observed. A greater degradation of fine, as opposed to coarse, fibre was observed.

Spurny proposed, from his measurements, the following durability ranking for the fibres:
amphiboles > glass fibre > other MMMF > chrysotile

He concluded that further research to relate biological effects to physical and chemical changes in the environment was needed.

Scholze and Conradt (1987) examined the durability of a wide range of siliceous fibres (Table 2.2). Exposures were only carried out using a fluid derived from Gamble's fluid and using a continuous flow rig based on that used by Klingholz and Steinkopf (1982). The exposure rig consisted of a fibre sample (0.2-0.9g) sandwiched between two polycarbonate filters (0.2 micron pore size), through which the exposure fluid was passed. The pH of the exposure fluid was maintained by bubbling with 5% CO_2 before it was passed through the sample cell and the temperature maintained at 37°C using water baths.

The exposure fluid was passed through the cell at a rate of 40 ml/day for up to 120 days. The silica content of the exposure fluid passing through the cell was determined by inductively coupled plasma spectrophotometry and used to

calculate the dissolution rate for the fibres examined. This was expressed in nm/day and used to determine fibre lifetimes.

It was concluded (Table 2.2) that MMMF exhibited relatively poor durability (lifetimes of 0.4-6.5 years), whilst natural asbestos fibres were very resistant to dissolution by the exposure fluids (> 100 years).
There was no attempt to relate these findings to the chemical composition of the fibres concerned.

Table 2.2 Estimated fibre lifetimes in simulated lung fluid

Material	Etch rate (nm per day)	Lifetime (years)
glass wool 475 JM 104	0.9	1.7
glass wool E JM 104	0.21	6.5
glass wool TEL	3.45	0.4
superfine glass	1.4	1.0
diabase wool	1.14	1.2
basalt wool	1.11	1.2
slag wool	0.69	2.0
refractory fiberfrax R	0.27	5.0
fiberfrax H	0.28	4.9
silica	1.10	1.2
chrysotile	0.005	<100
crocidolite	0.011	<100
erionite	0.0002	<100

2.4 CRITICAL APPRAISAL OF REPORTED IN-VIVO AND IN-VITRO STUDIES

With regard to an assessment of the durability of fibrous materials within the lung, the in-vivo and in-vitro studies discussed have been of a pioneering nature.

The in-vivo studies have aimed to determine the response of the lung to various fibrous materials and have identified gross features of their degradation and clearance. The tests have mainly employed rats and have focused on specific types of fibrous material (for example man-made mineral fibres, ceramic and asbestos fibre).

Fibres of known dimensions have been used by certain authors (Morgan et al 1982, Bernstein et al 1982) in an attempt to quantify the fibre degradation. However, in both these cases neutron activation of the fibre was used and it has been suggested (Oberdorster et al 1980) that this technique can greatly influence the chemical behaviour of the fibre. Obviously, any in-vivo experiments should involve the use of suitable controls to determine the nature of processes such as neutron activation on the behaviour of the fibre.

Another criticism relating to the design of certain in-vivo experiments has been the use of chemical digestion methods for recovery of fibre from lung tissue. Bleach, sodium and potassium hydroxide have been used for this purpose (Morgan et al 1982, Johnson et al 1982), however, the effects on the fibre were identified and LTA used instead. Whilst it was

shown that LTA had no significant effect on fibre diameters, it was not made clear whether LTA had any significant effect on analyte levels.

Only limited attempts were made to rationalise in-vivo results in terms of the physical/biochemical/chemical processes underlying fibre degradation and of the detailed physical and chemical structure of the fibrous materials concerned.

The advantage of in-vivo animal studies is that the conditions are probably much closer to those found in human lung, although there are presumably certain physiological differences. The use of fibres of known dimensions has shown the lesser durability of glass fibre relative to asbestos and suggested the role of the alveolar macrophage in degrading glass fibre. The leaching of modifying ions such as Zn and Ca from the glass network has also been demonstrated. The disadvantages of in-vivo studies are long exposure times and the high cost of testing each fibrous material. In addition, these studies demonstrate the overall effect of the lung's environment on the fibrous material and therefore make it difficult to identify degradation processes and to assess the contribution of individual factors. This in turn makes it difficult to relate fibre degradation to fibre composition. To summarise, in-vivo studies have the potential to provide an accurate assessment of the durability of different fibre types, but are time-consuming and expensive and each

different and new fibrous material has to be separately tested.

The in-vitro studies have focused on the exposure of inorganic fibrous materials, of similar types to those examined in-vivo, to conditions intended to simulate the environment of the lung.

A major criticism of these studies is that the lung simulation conditions employed are of such a simplified nature that their validity as an accurate model must be questioned. This is highlighted by the extensive use of Gamble's fluid by many workers (Forster 1982, Leineweber 1982, Klingholz and Steinkopf 1982, Scholze and Conradt 1987). It should be realised that this fluid (an artificial interstitial fluid) only represents one aspect of the potential environment for an inhaled fibre. Therefore, Gamble's fluid exposure should not be used as the definitive screening test of durability unless or until it has been shown that exposure to such fluids in the lung is the key determinant of fibre durability.

Another problem associated with the use of Gamble's fluid as an exposure fluid has been the inconsistency of results from different workers. Whilst it is accepted that in-vitro studies using model fluids and water tend to show the formation of gel layers and leaching of modifying ions from MMMF generally, there are inconsistencies in data interpretation. For example, Klingholz and Steinkopf (1982)

concluded that Gamble's fluid was generally more corrosive than water, whereas Forster (1982) concluded water to be the more corrosive. Leineweber, on the other hand, suggested that corrosion was greater for certain fibre compositions in Gamble's fluid and for certain compositions in distilled water. The more recent work of Scholze and Conradt (1987) ignores the use of exposure fluids other than Gamble's fluid altogether, to produce a "durability ranking" of a range of inorganic fibrous materials during Gamble's fluid exposure. No attempt was made to explain the differences in durability of the test samples or why only Gamble's fluid was used. Furthermore, localised corrosion associated with the intimate packing of the fibres in this system may be possible.

Such inconsistencies demonstrate another failing of in-vitro studies and that is the difficulty in identifying the key factors that control the durability behaviour of inorganic fibrous materials.

It should be noted that one particular factor, pH, has been identified by glass chemists (El-Shamy 1972) as one of the major factors affecting glass fibre durability. In view of this fact in-vitro investigations, and especially those involving Gamble's fluid, require adequate maintenance and monitoring of pH so that corrosion may be related to it. This is particularly important when closed systems are used, as exposure to water may result in a solution pH increase and a consequent increase in fibre corrosion.

Recent work by Conroy et al (1987) has suggested the corrosive properties of Gamble's fluid towards simple soda-lime silicate glass are primarily a feature of the exposure fluid pH and that the higher the pH the greater the dissolution rate.

It is therefore possible that the previously discussed inconsistencies in durability behaviour for exposures of inorganic fibres to Gamble's fluid and water (Forster 1982, Leineweber 1982, Klingholz and Steinkopf 1982, Scholze and Conradt 1987) could be explained by unmonitored pH variations occurring within the exposure test rig.

Both in-vitro and in-vivo studies have demonstrated the chemical reactivity of MMMF in physiological environments. It appears that certain MMMF, such as glass fibre, are far less durable than asbestos fibre and this has been linked to the greater health hazard associated with asbestos inhalation. By both in-vivo and in-vitro methods, the degree of degradation is shown to be sensitive to the type of fibrous material concerned and an approximate ranking of chemical reactivity of the materials is given as:

asbestos < ceramic < other MMMF

It is very important to realise that there will always be exceptions to generalisations and they can not be applied to untested, novel MMMF.

As was the case with in-vivo animal studies, little attempt has been made to rationalise in-vitro findings in terms of degradation processes and the physical and chemical structure of the fibrous materials. Despite these criticisms of previous in-vitro studies, we consider that a carefully designed series of in-vitro experiments offers the potential to identify the key processes and determinants affecting fibre durability.

Unlike previous studies, such a programme of work would be iterative in nature, initially involving exposure of simple soda-lime silicate bulk glass to simple exposure fluids. The work would then develop to include more complex exposure fluids, designed to simulate different individual aspects of the lung's environment and more complex fibre compositions. Data would be interpreted by reference to and development of existing theoretical models of glass durability in aqueous environments and by reference to in-vivo durability studies. This approach would potentially enable,

a) identification of key determinants of fibre durability

b) development of theoretical models of fibre durability

c) development and appraisal of an accurate in-vitro screening test of fibre durability.

In-vivo animal studies can provide an essential control for assessment and confirmation of an in-vitro screening test. Once developed, an in-vitro screening test will potentially provide a quicker, cheaper method of assessing fibre durability than in-vivo methods. Furthermore, the use of

an iterative approach to develop a theoretical model of fibre durability would potentially provide a means of assessing the durability of novel inorganic fibrous materials based on their composition.

2.5 THE CORROSION OF INORGANIC FIBROUS MATERIALS IN THE LUNG ENVIRONMENT

2.5.1 INTRODUCTION

The development of a model describing, and enabling prediction of, the durability of inorganic fibrous materials within the lung requires an understanding of:

a) the physiological conditions in the lung,

b) the structure and composition of the test materials examined,

c) the mechanisms of interaction between the lung environment and the test materials.

This study focuses on the durability of glass and ceramic fibres which have a significant silica content; it aims to identify and characterise the determinant factors of the lung environment and the physical and chemical properties of the fibrous materials which affect fibre durability.

2.5.2 THE NATURE OF THE GLASS AND CERAMIC TEST MATERIALS

The chemical compositions, physical forms and applications of the test samples studied in this programme of work are summarised in Table 3.2.

It is considered important that the durability can be related to the chemical and physical properties of the

fibre. In order to be fully aware of these properties an understanding of the manufacture and processing of the materials studied is required.

The fibres examined were either bulk glasses, "wools" or continuous filaments (Table 3.2). Preparation of the bulk glass is described in detail elsewhere. Processing of this glass consisted of annealing the solidified "melt", then cutting and polishing test samples. Wools are produced by blowing or spinning processes, which gives rise to fibres of a wide range of diameters. The A-glass, lead-glass and alumino-silicate ceramic were all wools. There is little in the actual manufacturing process that would be likely to modify the physiological durability of the wool and bulk glass samples studied, other than surface area effects associated with differences in surface finish.

Continuous filament fibres undergo a very different manufacturing process, potentially more likely to modify the physiological durability.

The E-glass fibre and Cemfil-fibre studied were continuous filaments and consequently the manufacturing process is described in more detail (Proctor 1986).

The raw materials of the glass are melted and fed along refactory canals to a heated, platinum-rhodium, alloy bushing. Hundreds of individual filaments are then drawn simultaneously from tips in the base of the bushing. The molten glass flows through the tips, the rate determined by

the head of molten glass and tip diameter, and the glass beads with fibres attached are collected and wound onto a revolving drum (a "collet"). The diameter of the fibres formed in this way is regulated by the drawing speed. During this process, prior to being wound onto the collet, an aqueous polymeric emulsion called a "size" is applied to the filament to protect it from abrasion. Once wound onto the collet the coated filaments are heated to dry and cure the polymeric emulsion. This process binds the individual filaments together. The application of a polymeric coating is an integral part of the production of continuous filament fibres, having three main functions:

i) prevention of abrasive damage during all stages of production and subsequent processing.

ii) binding of fibres to enable easier processing and handling.

iii) improving the the properties of the final composite, principally by increasing the fibre/matrix bonding and by **improving resistance to degradation in moist conditions.**

These coatings generally have three main components:

i) a film forming polymer for binding and formation of a protective coating, for example, polyvinyl acetate, alkanolamine/epoxy, acrylic and polyester polymers.

ii) a lubricant, often with a cationic head to bind to the anionic glass and an aliphatic tail.

ii) a coupling agent to bind fibre to matrix, generally a complex organosilane with two functional groups ($X_3Si(CH_2)nY$) - one binding to the glass and one to the matrix.

Obviously the application of these polymeric coatings during production of continuous filament fibres could greatly modify the physiological durability of the fibre.

Consequently, particular attention was given to the interpretation of durability data for the continuous-filament E- and Cemfil- fibres.

The use of these coatings, and in particular the coupling agents, may also have important consequences with respect to fibre toxicity if cell/fibre bonding is modified by their presence. It is recommended that the effect of fibre coatings on toxicity should be fully investigated, although it is accepted that the unwillingness of manufacturers to release the exact composition of the coatings may compromise such studies.

2.5.3 THE CORROSIVE ENVIRONMENT OF THE LUNG

Recent in-vitro studies have been limited in their ability to simulate the true exposure conditions of fibres within the respiratory system and, notably, within the alveolar region of the lung.

The physiological environment of the respiratory system is considered here in order to identify aspects of that environment which may influence the durability of inhaled glass and ceramic fibre.

Particulate matter, including fibres, may enter the respiratory system via the nose or mouth. Mucous membranes and nasal hair prevent larger particles entering the lung (Lippman et al 1980). The conducting airways consist of a series of bifurcations, with each larger airway

progressively dividing into two smaller airways. The airways terminate in the alveolar region where gaseous exchange occurs. It is seen that the conducting airways, but not the alveolar regions, are ciliated and contain many mucous producing cells.

The alveolar region is of particular interest as this is where the first signs of particle-induced lung disease occur (Brody et al 1984) and an anatomical knowledge of the alveolar-capillary unit (Simonescu 1980) provides an understanding of the potential environment for inhaled material. Air in the alveolar sac is saturated with water vapour to keep the lung tissue moist and prevent infection. The alveolus itself consists of a thin epithelial and endothelial layer, with various amounts of connective tissue joining the two. The epithelium comprises two major cell types: types I and II. Type I cells are flat and cover approximately 95% of the epithelial surface and are as thin as 0.2 micron in places which assists gaseous exchange; type II cells are thought to be associated with production/ regulation of the surfactant layer, which lines the alveolar sac. The surfactant has a turnover of approximately 14 hours and it is noted that buffering properties have been attributed to this layer. The surfactant layer is very thin (sub-micron) in order to maintain short diffusion pathways, and a major component of its composition is the phospholipid, diapalmitoyl phosphatidyl choline.

The capillary endothelium is of a continuous type, the main feature being the presence of many vesicles, probably for transport across the endothelium. The endothelium is connected to the epithelium of the alveolus by a thin basement membrane. Other features characteristic of the alveolus are regions of connective tissue, particularly at the tip where smooth muscle cells, elastin and collagen help to maintain the open structure of the alveolus.

It has been observed that fibres of less than 5 micron in diameter may penetrate the airways, reaching the alveolar regions. Preferential deposition occurs at bifurcations in the terminal bronchioles (Brody et al 1984). Larger fibres are filtered by the nose or impact on the ciliated mucous membranes higher up the lung (Lippman et al 1980). The ciliated membranes beat in a unidirectional manner carrying inhaled material out of the lung. However, it has recently been suggested (Stahlhofen et al 1987) that there may be dead zones in this muco-ciliary escalator. Presumably, fibres impacting on these dead zones will not be cleared by muco-ciliary action.

long fibres (>10 micron length) appear to present major problems in terms of alveolar clearance. It is thought that fibres smaller than this can be completely engulfed by alveolar macrophages and cleared via migration to the muco-ciliary escalator or the lymphatic system. Actin-containing microfilaments are thought to translocate small fibres to the lymphatic system: to the lumina of

capillaries or to components of alveolar connective tissue. Long, thin fibres, however, are able to penetrate the epithelial lining but are too large for physical removal by macrophages, or effective translocation. It is apparent that these long fibres would have to be fragmented for effective removal, or alternatively, totally dissolved. It has been suggested that partial ingestion of certain MMMF by macrophages can contribute to such processes (Bernstein et al 1982).

It is possible to identify a number of potentially corrosive situations for long, thin fibres resident in the alveolar regions:

(a) Fibre penetrating the alveolar epithelium may be subject to the action of interstitial fluids. Gamble's fluid (Gamble 1951) used extensively in in-vitro durability studies is an artificial interstitial fluid.

(b) Fibre may be exposed to the surfactant layer which is rich in phospholipid and thought also to provide physiological buffering.

(c) Long fibres projecting into air spaces may be subjected to the action of water vapour, though the surfactant layers do have a buffering action.

(d) Chelating agents may be present throughout the alveolar region which may help in the degradation of MMMF. For example, the chelating agent, citrate, has been shown

in-vitro to accelerate corrosion of MMMF (Oberdorster et al 1980). Chelating agents would increase corrosion by binding to positive ions, and thus removing protective layers from the fibre surface. Whether the levels of citrate (an intermediate of Kreb's cycle) or other potential chelating agents are high enough in-vivo to actually affect fibre degradation is unknown.

(e) The intracellular environment of alveolar macrophages has been strongly implicated as corrosive and Bernstein (1982) has suggested that proteolytic enzymes may be responsible for glass fibre degradation.

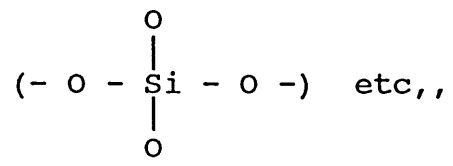
(f) Other factors might include physical damage of the fibres associated with breathing, or the more extreme case of coughing, or with the binding of macrophages and translocation.

2.5.4 THE POTENTIAL INTERACTION BETWEEN INORGANIC FIBROUS MATERIALS AND THE LUNG'S ENVIRONMENT

Of the MMMF, the durability of glass fibre in aqueous solutions is best understood. In particular, the response of alkali silicate glasses to distilled water, and the effect of solution pH, is well characterised (Paul 1982, Hench 1978, Newton 1984). The response of MMMF in water and Gamble's fluid has been characterised (Section 2.3) but the detailed relationship between behaviour and chemical composition has not been successfully pursued.

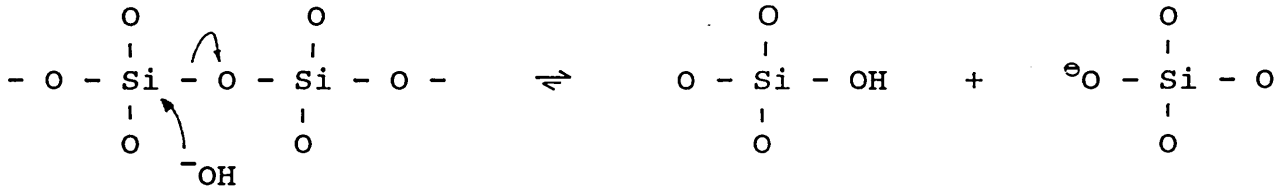
An appropriate approach to the problem of understanding the durability of silicate glass fibres in the lung environment would therefore be to examine the relatively well understood response of simple, bulk glasses to aqueous solutions, and attempt to relate and develop this model to fibrous glasses of more complex compositions, and to more complex exposure environments.

Silicate glass fibres consist of a silicate backbone,



into which may be incorporated other covalently bound network-forming species such as aluminium or boron (Rawson 1967). Many glasses also contain modifying cations such as Na, K, and Ca which are incorporated into the network by ionic or electrostatic bonding. Under certain aqueous conditions these modifying cations may readily partake in

ion-exchange, leading to changes in the glass composition and structure (Conradt et al, 1984). Inhaled fibres may be exposed to the action of water vapour in the airways and the alveolar regions. When alkali-containing glasses, for example A-glass fibre, are exposed to aqueous solutions with pH < 7, conditions are favourable for ion-exchange, and alkali ions may be replaced by H^+ ions, with H_2O simultaneously entering the glass (Conradt et al 1984). When silicate glasses are exposed to high pH (> 9) total dissolution of the silicate network may occur (El-Shamy 1972). All silicate glasses are susceptible to degradation at high pH to some extent because OH^- (aq) acts as a nucleophile, breaking the Si-O bond. For a soda-glass at a pH of 10 the following would tend to occur:



Alkali-silicate glasses with simple composition would be expected to be particularly susceptible to corrosion by aqueous solutions. Ion-exchange processes are likely to leach loosely held cations such as Na and K from the glass and at the same time a localised increase in pH would occur as H^+ ions are removed from the aqueous environment; this does not take into account potential buffering of the

aqueous environment. If the glass was high in alkali content, the ion-exchange processes would leave a weakened silica shell, susceptible to degradation by OH^- (aq).

Alkali glasses of simple compositions are therefore likely to be readily degraded by the action of water vapour in the lung.

In-vitro tests to examine the effect of water on MMMF using a closed system and a relatively high quantity of alkali rich fibre are likely to show a strong chemical reaction as the pH of the environment rises. However, only the simplest of glass fibres are likely to be susceptible to dissolution in water. In the case of more complex glasses (Newton 1984, Paul 1982), which often have a low alkali content, leaching results in a dense silica-rich layer at the surface, which acts as a barrier against further diffusion and the corresponding local pH increase. Often aluminium and boron are incorporated into the network of "durable" glasses to retard attack by aqueous solutions. Species such as aluminium are thought to impart durability by the formation of insoluble aluminosilicate complexes on the fibre surface (Hench 1978, Newton 1984). The most durable types of glass (excluding pure silica) form what are known as type III surfaces (Figure 2.1).

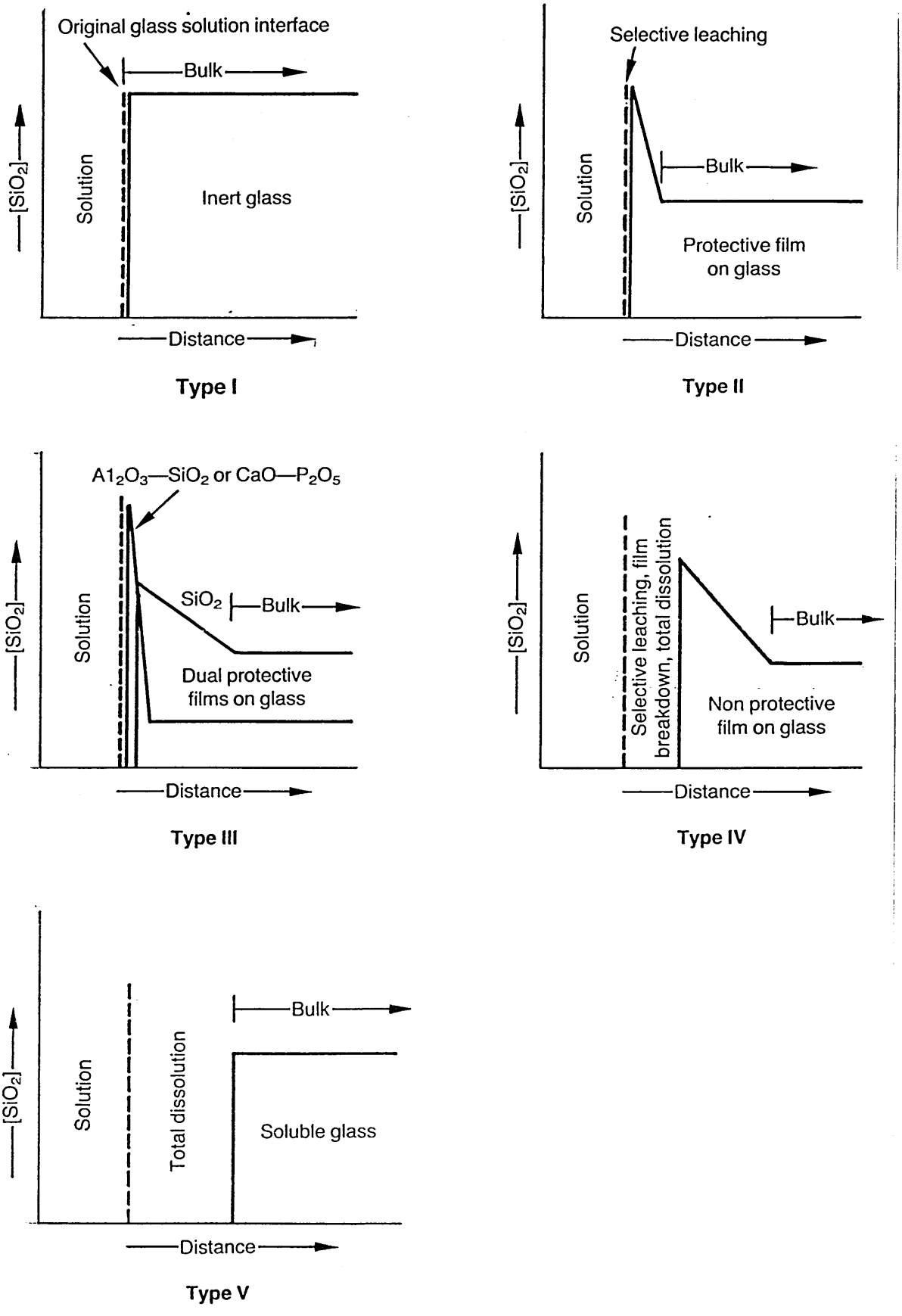


Figure 2.1 Glass surfaces as described by Hench (1978)

These surfaces have been described by Hench (1978 and 1982). Type IIIA surfaces form double protective films, associated with the addition of aluminium oxide, or phosphate to the glass. The effect of water on these glass fibres is to generate a protective alumino-silicate, or calcium phosphate layer, above a second silica-rich protective layer. The Al or P required to form these layers may also be provided by the aqueous environment. The type IIIB surface is slightly more complex, consisting of multiple protective layers of oxides, hydroxides and hydrated silicates. Once more, these layers may be formed by the action of species present in the aqueous environment.

As a further attempt to predict the durability of glass fibres when exposed to aqueous solution in the alveolar regions, multioxide diagrams may be considered (Figure 2.2).

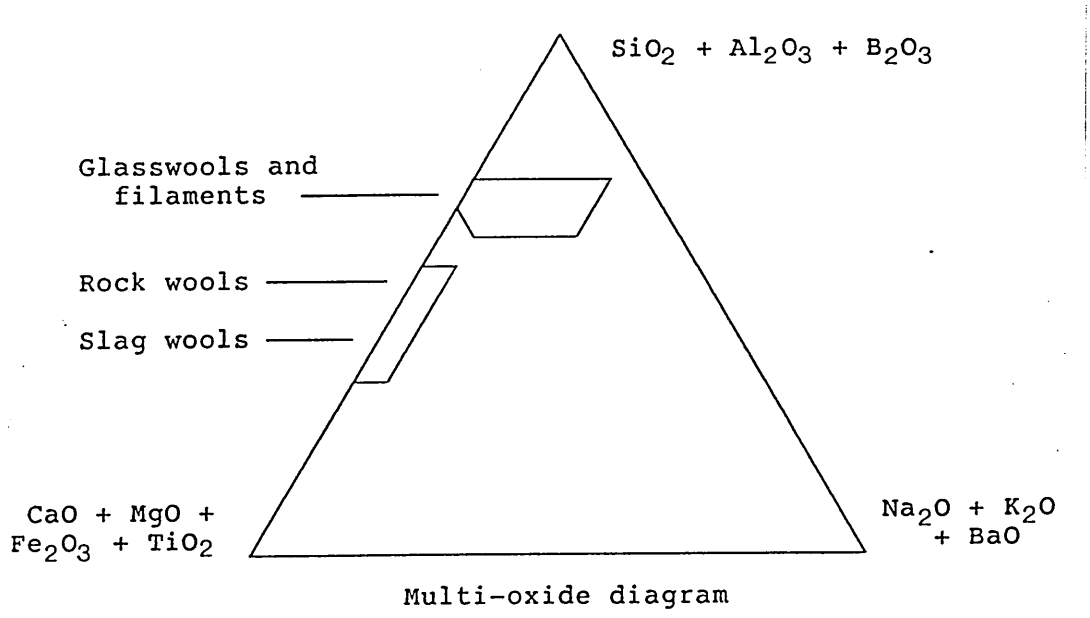


Figure 2.2 A multi-oxide diagram (Klingholz 1977)

Klingholz (1977) has suggested the use of such diagrams to predict biological durability of a given glass fibre. Such diagrams are limited in that they reflect acid resistance, which would only be one aspect of biological exposure. An index of the acid resistance is given by the sum of the mol % of each of the acidic oxides, divided by each of the basic and amphoteric oxides. It is generally seen that as the ratio increases so does the acid resistance. Reference to the multi-oxide diagram (Figure 2.2) shows acid oxides at the top; at the left are oxides which improve resistance and at the right the oxides which increase solubility.

Another somewhat less empirical aid to prediction of the behaviour of glass durability is based on the thermodynamic and kinetic stability of the component oxides (Paul 1982). This approach appears to be useful, and helps to explain how addition of specific species such as Al or Zr oxides increases the durability of the glass; however, it is not clear if this approach can be applied to complex glasses containing a wide range of oxides where the free energy of the glass will not be well defined.

These models are useful when applied to basic or well defined situations, for example resistance of a soda glass to acid or water. However, they are limited in their ability to predict the effects of a complex environment - as would be expected for biological exposure. As the exposure environment increases in complexity from water to model

fluids, the important factors affecting fibre dissolution again become more complex. Attention has been paid to the effects of Gamble's fluid on MMMF. Various types of glass and mineral fibre are reactive in this solution and comparisons have been made with the reactivity in water, although differences in behaviour have not been adequately explained. In a recent study (Conroy et al 1987) it was suggested that for glass fibre of simple composition, the effects of Gamble's fluid were primarily dependent on pH. It has been observed in-vivo that the gel-layers characteristic of in-vitro studies of MMMF durability are not formed, and it has been suggested that, in-vivo, MMMF may be exposed to chelating agents, capable of degrading insoluble protective layers on the fibre (Leineweber 1979). Chelating agents such as citrate, or EDTA, can be quite aggressive towards glass (Newton 1984) as they react with surface species forming soluble complexes, thus re-exposing the glass surface for attack. It is difficult to say whether the levels of citrate, or other potential chelating agents, in the alveolar regions are high enough to significantly assist the degradation of fibre, but such factors have to be considered as a potential cause of fibre degradation.

To date there has been no attempt to evaluate by in-vitro methods the long term effect of the intra-macrophage environment on MMMF. Macrophages have been implicated, in-vivo, as capable of degrading glass fibre (Bernstein et al

1982) and it has been suggested that proteolytic enzymes are involved. If enzymic degradation was occurring, systematic evaluation of enzymic effects would require identification of the enzymes responsible and investigation of the response to specific silicate substrates.

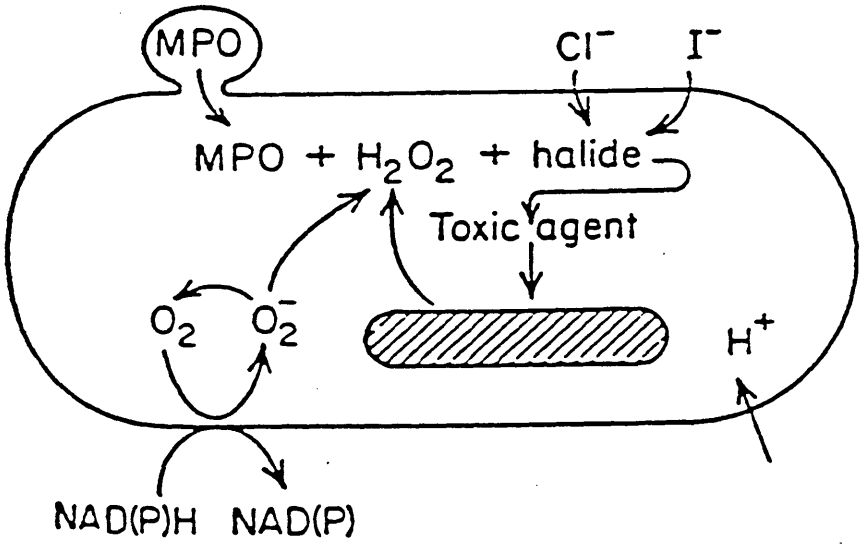
Presumably, the suggested enzyme would have to be capable of degrading the glass lattice, that is, breaking the Si-O bond. This does not seem likely in view of the fact that microbial damage to bulk window glass does not occur and that other siliceous materials, for example asbestos, are durable in the lung. Furthermore, enzymes are usually highly specific with respect to their normal substrates, making it hard to accept that a proteolytic enzyme is also capable of degrading a silicate glass substrate.

Other factors to be considered as potentially relevant during the macrophage/fibre interaction are a) cytoplasmic pH (7.1), b) lysosomal pH (3-6, [Allison 1974]), c) products of the respiratory burst and d) potential chelating agents. Lysosomal pH is fairly acidic because the acid hydrolases and proteases require this optimum pH to degrade proteolytic material. Presumably when fibre is partially engulfed by macrophages, it will be subjected to lysosomal contents and pH. Low pH is known to cause ion exchange or leaching of cations from MMMF and chrysotile. Spurny (1983) showed from <u>in-vitro</u> studies that acid conditions leached Mg from chrysotile and Ca and Zn from the MMMF under study.

It is possible that MMMF containing high levels of alkali species or Zn will be leached under such conditions. This may, in extreme cases, leave a weakened silica shell susceptible to dissolution.

Products of the respiratory burst may also contribute to the dissolution of mineral fibre. When alveolar macrophages engulf foreign material, oxygen is metabolised and eventually reduced to water (Klebanoff 1980). However, partial reduction may occur with the formation of highly reactive intermediates. The enzyme system responsible for this respiratory burst is thought to be a reduced NADPH (nicotinamide adenine dinucleotide, reduced form) or NADH (nicotinamide adenine dicnucleotide phosphate, reduced form) oxidase and is considered to be localised on the outside of the cell's plasma membrane. Phagocytic activity will result in invagination of the cell wall and the formation of a vacuole, with the enzyme forcing in and acting on oxygen in the vacuole. The oxygen consumption is thought to be involved in establishing an anti-microbial defence system. A model for this is shown by Figure 2.3:

Figure 2.3 The respiratory burst



This is thought to occur in the monocytes and neutrophils, but not generally in resident alveolar macrophages. In the case of monocytes and neutrophils, oxygen is reduced via reactive radical intermediates to hydrogen peroxide (H_2O_2) which in the presence of myloperoxidase (MPO) acts on halide ions to produce toxic agents. Strong oxidising agents, for example hypochlorous acid, are produced by this system capable of brominating, chlorinating and iodinating the bacterial wall.

Alveolar macrophages, monocytes and neutrophils all generate highly reactive, oxygen free radicals. The effects of oxygen radicals (O_2^-, HO_2, .OH) on MMMF

structure may warrant examination, particularly as O_2^- has nucleophilic, as well as radical properties (Lee-Ruff 1977). However, a mechanism proposed by Haber and Weiss (1934) concerning the formation of ·OH radicals also involves the generation of OH^- :

$$H_2O_2 + O_2^- \rightarrow O_2 + {}^{\cdot}OH + \underline{OH}^-$$

This suggests that MMMF ingested by alveolar macrophages will be subjected to OH^- and other nucleophiles. These chemical species are known to disrupt Si-O bonds in the glass network.

3. EXPERIMENTAL DETAILS

3.1 EXPERIMENTAL DESIGN

The effects of *in-vitro* and *in-vivo* simulations of the lung environment on the durability of a range of glass fibre types have been reported (Section 2). In these studies, durability has been used as an index of the persistence and hence lifetime of the fibres in the lung, and is associated with the chemical solubility and physical resistance to degradation. The durability of an inorganic fibre within the lung will therefore be determined by its chemical and physical behaviour. However, there is difficulty (i) in relating fibre durability to the chemical and physical conditions of the environmental simulation, (ii) in relating fibre durability to its chemical and physical properties and (iii) in explaining the observed differences between the effects of *in-vitro* and *in-vivo* exposures.

It was considered that these shortcomings were the result of a lack of a sufficiently methodical approach, which could be used to identify the first order determinants of fibre durability in the lung. Consequently, this program of work has been based on an iterative approach that involved increasing exposure sophistication and increasing complexity of the chemical composition and physical form of the test material.

The development of the project may be summarised by reference to Table 3.1, which shows the range of exposure conditions and test materials examined.

Table 3.1 Conditions of exposure

SAMPLE FORM	EXPOSURE	EXPOSURE TIME/MONTH	
Bulk glass	1. water	0.75	
	2. Gamble's fluid	0.75	
	3. pH 10	0.75	
A-glass fibre	1. water	6	12
	2. Gamble's fluid	6	12
E-glass fibre	3. pH 10		12
	4. RPMI 1640	6	12
Cemfil fibre	5. PBS		12
	6. blood serum	6	
	7. BALF	6	
	8. monocytes	6	
	9. rat lung	6	
Lead-glass fibre	1. water		12
	2. Gamble's fluid		12
	3. pH 10		12
	4. RPMI 1640		12
Ceramic fibre	5. PBS		12
	9. rat lung	6	

Key: BALF (broncho-alveolar lavage fluid)

PBS (phosphate-buffered saline)

RPMI 1640 (cell-culture medium)

Much of our understanding of glass durability in aqueous environments has been derived from previous studies involving the use of bulk glasses. It was therefore decided that initial experimental work would focus on a bulk, soda-lime silicate glass (Section 4.1). This formed the first stage of an iterative process, providing a relatively well understood system which could be related to, and used to develop, existing models of bulk glass durability.

Experimental work progressed to the use of fibrous samples, which included A-glass fibre of similar composition to that of the bulk glass. This aspect of the iterative development was designed to highlight the effects of fibre morphology and how, if at all, processing of the bulk material to produce fibres would affect the durability of the material.

Ideally, exposures of sub-micron fibres of the same diameters should be carried out, because of the following:

i) sub-micron diameter fibres demonstrate the greatest pathogenic potentials.

ii) the use of fibres of the same diameter enables the mean diameters of exposed fibres to be compared with the original diameters in order to provide a quantified assessment of the degradation of the fibre.

iii) thin, sub-micron fibres will potentially provide greater sensitivity than thick fibres, with respect to degradation.

iv) sub-micron fibres can be analysed more easily and with greater sensitivity than thicker fibres, using EDXA techniques (Section 3.4.2).

However, a supply of sub-micron sized fibre, covering the compositional range required for an iterative study of this nature, could not be obtained. An attempt to address this issue was made by the preparation of sized fibres, of known diameters, from continuous-filament fibres and where possible from fibre "wools" (Section 3.2).

Iterative development in terms of the chemical composition of test materials involved the use of simple soda-lime silicate bulk and A-glass fibre, through to Cemfil, a zirconia based glass fibre. It was noted that certain fibre types examined had a chemical coating, introduced during manufacture. This was not removed by heat treatment prior to our experiments. The fibres used were of a commercial grade and the bulk glass was specially prepared, based on the composition of window and bottle glasses. The materials were chosen to give a systematic progression in chemical complexity, so that it would be possible to relate differences in durability to compositional differences between the materials.

The iterative development of in-vitro exposure conditions provided progression from water to simulated lung fluids and physiological fluids to cultures of human monocytes. This was designed to determine the effects of key aspects of the lung's chemical and biochemical environment. Fibres were also exposed, in-vivo, in rat lung to provide for critical assessment of in-vitro exposure conditions.

The initial bulk glass experiments were of only a few weeks duration, whereas the latter studies involved more realistic 6 and 12 month exposures.

Due to the long-term nature of much of the experimental work and the associated time restriction on repeating unsuccessful work, planning was required to:
(a) provide accurate sample and exposure fluid preparation,
(b) design realistic exposure conditions,
(c) determine the most suitable methods of analysis.

Exposures were designed to simulate in-vivo exposure conditions; this demanded the accurate control of physiological parameters such as temperature and pH.

As previously defined, fibre durability is an index of the long-term residence time within the lung and may be influenced by the chemical and physical interaction of the fibre with the lung environment. In order to assess the durability of the test materials, specific analytical and statistical methods were therefore adopted and where necessary developed to determine the nature of any chemical and physical changes in the test material.

The chemical interaction of a fibre with the lung environment has a profound effect on the fibre's durability and the types of interaction that may occur have been discussed previously (Section 2.5).

To summarise, glasses generally demonstrate one of two distinct types of chemical reaction in aqueous conditions: i) ion-exchange, or ii) network hydrolysis. The type of behaviour is determined primarily by the pH of the aqueous environment and potentially modified by the chemical composition of both glass and aqueous environment. Note that this modification may often be associated with the formation of surface layers on the glass, of different composition to the bulk of the sample (Hench and Clark 1978).

Therefore, accurate analysis of the type of chemical reaction will potentially enable an accurate determination of: i) the corrosive nature of the exposure environment and ii) the effect of chemical composition in modifying the chemical behaviour of the test sample.

Changes in the chemical composition of samples, potentially caused by leaching and surface layer formation, were monitored by scanning electron microscopy (SEM) in conjunction with energy dispersive X-ray analysis (EDXA) of the sample and where possible by inductively coupled plasma spectrophotometry (ICPS) of the exposure fluids. Secondary ion mass spectrometry (SIMS) was also used in an attempt to determine the chemical behaviour of the surface atom layers of fibrous samples.

Potential signs of network hydrolysis, characterised by physical degradation/dissolution of the samples, were identified by morphological and sizing studies using SEM.

Note that factors leading to network hydrolysis of the samples were of key significance because network hydrolysis can result in complete dissolution of the sample.

The formation of surface layers was determined using EDXA and SEM (chemistry and morphology) and it should be noted that stable surface layers have the potential to modify the durability of a sample.

The physical behaviour of the samples was characterised by morphological studies using SEM in order to determine whether splitting or fragmentation occurred during exposure. Fragmentation would potentially result in the formation of small, easily cleared particles, whereas longitudinal splitting could result in the formation of potentially hazardous sub-micron diameter fibres from larger parent fibres.

3.2 PREPARATION OF BULK AND FIBROUS MATERIALS

The bulk and fibrous materials studied are identified in Table 3.2. With the exception of the bulk glass all the materials were of a commercial grade obtained from the manufacturers.

Table 3.2 Sample composition prior to exposure

Material	Alkali glass	A-glass	E-glass	Cemfil
Form	bulk glass	fibre wool	filament fibre	filament fibre
Coating	none	none	starch	organic
Application	Window /bottle	Battery mat	Yarn	Re-inforced concrete
Diameter/um	-	a range	6	12
Comp/mol %				
SiO_2	74	72.5	54	71
B_2O_3			8	
Al_2O_3		1.5	15	1
Na_2O	16	13.0	1	*
K_2O		0.3		>1
Fe_2O_3		0.1	>1	
CaO	10	9.3	21	*
MgO		3.0	1	
ZrO_2				16
Li_2O				

* total alkali-oxides, 15%.

Table 3.2 continued...

Material	Lead-glass	Kaowool
Form	Fibrous wool	Fibrous wool
Coating	No	No
Application	?	Refractory insulant
Diameter/um	a range	a range
Comp/mol %		
SiO_2	45-55	43-46
B_2O_3		
Al_2O_3		
Na_2O		
K_2O	10-15	
Fe_2O_3		
CaO	0-7	52-56
MgO		
Lead oxide	20-35	

The compositional data was obtained from the manufacturer, except for the Lead-glass data which was determined by EDXA.

The bulk soda-lime silicate glass was prepared (University of Sheffield, Department of Glasses, Ceramics and Polymers) from a melt of its component oxides. The glass was cast in a rectangular mould and air cooled, then cut into thin rectangular sections ($\sim$ 1 x 1 x 0.3 cm^3) using a diamond cutting wheel.. The sections were polished to a 0.5 micron finish on a polishing wheel, washed with distilled water and then annealed by heating to 600°C for 1 hour and gradually cooling to room temperature, to relieve internal stresses.

Fibrous materials were prepared from commercial-grade, continuous filament and wool samples. The E-glass and the zirconia-based Cemfil fibre were continuous filaments and the Lead-glass and Kaowool were "wools". Wools contain a wide range of fibre diameters, whereas the diameters of continuous filament fibres are relatively uniform.

For 12 month in-vitro exposures, small 0.01g test samples were taken direct from the parent sample and placed in plastic tissue-culture flasks prior to sterilisation and subsequent exposure.

For the 6 month in-vitro exposures, sized fibres of known diameter were prepared so that assessment of physical degradation would be possible by monitoring changes in fibre diameter. This was achieved by isolating a 10-20 cm length of fibre from the parent sample and cutting it into short, say 2mm lengths, with the aid of a scalpel and fine

point tweezers. These lengths were transferred, using a hair, to 24-well culture trays prior to sterilisation and exposure.

For in-vivo exposures, continuous filament fibres were cut to 100 (± 20) micron lengths using a hand-held benchtop microtome, whilst wool samples were prepared by grinding with a pestle and mortar.

The bulk and fibre samples were handled using aseptic procedures and sterilised prior to exposure. Gamma irradiation was used to sterilise the fibre samples (courtesy of Swann-Morton, dose: 2.5 megarads) after they had been placed in plastic culture trays and the bulk glass was sterilised with 70% ethanol solution.

3.3 EXPOSURE CONDITIONS

3.3.1 IN-VITRO EXPOSURES TO MODEL FLUIDS

In-vitro studies allowed examination of individual features of the in-vivo environment and a range of model fluids was used to provide in-vitro simulations of various aspects of the alveolar environment of the lung. The composition of these fluids is given in Table 3.3

The exposure fluids were prepared from analytical-grade reagents, except for the RPMI 1640 which was purchased at a 10X concentration. Blood serum was obtained from a human donor and the bronco-alveolar lavage fluid was obtained by irrigating the lungs of a freshly killed rat with distilled water. An incision was made exposing the rat's trachea, and a cut was made across the trachea, but not so deep as to cut completely through. The end of a glass Pasteur pipette was then broken off, so that when the pipette was inserted into the cut and along the trachea towards the lungs an airtight fit was made. Lavage was carried out by pipetting water backwards and forwards into the lung. The lavage fluid was filtered (0.1 micron millipore) to sterilise it and remove macrophages.

Table 3.3 Composition of model fluids

FLUID	CONSTITUENT	CONCENTRATION (g/dm^3)
Gamble's fluid	$MgCl_2.6H_2O$	0.160
	NaCl	6.171
	KCl	0.311
	Na_2HPO_4	0.148
	Na_2SO_4	0.079
	$CaCl_2.2H_2O$	0.060
	$CH_3COONa.3H_2O$	1.065
	$NaHCO_3$	1.950
Phosphate buffer	K_2HPO_4	5.3
	KH_2PO_4	1.183
Phosphate buffered saline		
pH 10 buffer	$NaHCO_3$	
	Na_2CO_3	
Distilled water		
Blood serum		
Lavage fluid		

Table 3.3 continued...

FLUID	CONSTITUENT	CONCENTRATION (g/dm^3)
RPMI 1640	$MgSO_4.7H_2O$	0.400
	NaCl	6.000
	KCl	0.400
	Na_2HPO_4	0.800
	$NaHCO_3$	2.000
	$Ca(NO_3)_2$	0.069
	L-Arginine	0.200
	L-Asparagine	0.057
	L-Aspartic acid	0.020
	L-Cystine	0.059
	L-Glutamic acid	0.020
	L-Glutamine	0.300
	Glutathione	0.001
	Glycine	0.010
	L-Histadine	0.015
	L-Hydroxyproline	0.020
	L-Isoleucine	0.050
	L-Leucine	0.050
	L-Lycine HCL	0.040
	L-Methionine	0.015
	L-Phenylalanine	0.015
	L-Proline	0.020
	L-Serine	0.030
	L-Threonine	0.020
	L-Tryptophan	0.005
	L-Tyrosine	0.025
	L-Valine	0.020
	Biotin	<0.001
	D-Calcium pantothenate	<0.001
	Choline chloride	0.003
	Folic acid	0.001
	I-Inositol	0.035
	Nicotinamide	0.001
	P-Aminobenzoic acid	0.001
	Pyridoxine HCL	0.001
	Riboflavin	<0.001
	Thiamine HCL	0.001
	Vitamin B12	trace
	D-Glucose	2.000
	Phenol red	0.005
	Foetal calf serum	5%
	Glutamine	1%

The in-vitro exposure fluids were designed to represent specific physiological components of the alveolar environment. The physiological significance of the model fluids may be summarised:

(a) **Double-distilled, de-ionised (pure) water:** representative of the water-saturated atmosphere of the alveolar region, and a useful control as the effects of water on glasses are relatively well documented.

(b) **Potassium phosphate buffer (pH 7.4):** representative of the physiological pH of the interstitial regions of the lung.

(c) **Bicarbonate-buffered Gamble's fluid (Gamble 1951):** a simulated interstitial fluid, gassed with 5% CO_2 to achieve physiological buffering.

(d) **Phosphate-buffered Gamble's fluid:** a simulated interstitial fluid, incorporating a phosphate buffer.

(e) **Phosphate-buffered saline:** a physiological buffer (pH 7.4) containing similar levels of sodium chloride to human body fluids,

(f) **RPMI 1640:** a complex fluid used for culturing human cells, providing physiological buffering and being similar in many respects to human plasma. This fluid was also used for culturing monocytes (Section 3.3.2).

(g) **Sodium carbonate/bicarbonate buffer:** providing an alkali pH (pH 10.1) which can potentially result when certain types of glass are exposed to water (Section 2.5) and, therefore, could occur in a localised reaction between a fibre and the water saturated air in the alveolar regions,

(h) **Human blood serum:** to which inorganic fibres embedded in the alveolar regions would potentially be exposed.

(i) **Rat, bronco-alveolar lavage fluid:** which is an aqueous solution of soluble products washed from the lung lining material of the rat.

Aseptic procedure was used throughout to ensure the sterility of the model exposure fluids. Handling of exposure fluids, samples and reaction vessels was undertaken in a laminar-flow safety cabinet and fluids prepared in the lab were autoclaved in Nalgene polypropylene volumetric flasks before use. Drop counts were carried out to check sterility and antibiotics (penicillin and streptomycin) were incorporated into the RPMI 1640.

In order to minimise the risk of microbial contamination during long exposures, closed exposure systems were adopted. Unless otherwise stated, exposures were carried out in sterilised, plastic tissue-culture flasks, or 24-well, multi-well plates. These reaction vessels were maintained at 37°C in a 5% CO_2, 95% air atmosphere (essential for accurate buffering of Gamble's fluid and RPMI 1640) for the specified exposure period and were gently shaken every 3-4 days.

50 ml of the specified model fluid was used for the bulk glass exposures (Section 4.1), 10 ml for the 12 month fibre exposures (Section 4.2) and 2 ml for the 6 month fibre exposures (Section 4.2 and 4.3). Model fluid was introduced to the reaction vessels using sterile pipettes. The use of large solution-volume to sample-mass ratios was to minimise by dilution the effects of any reactive species which were extracted into solution from the glass sample.

3.3.2 IN-VITRO EXPOSURE TO MONOCYTES

Bernstein (1982) and Morgan (1982) attributed corrosion of glass fibre exposed in rat lung to the effect of alveolar macrophages. An in-vitro system was therefore developed to assess the corrosive effects of this aspect of the lung environment.

The ideal system would be an in-vitro method for the exposure of sized, long thin fibres to active human alveolar macrophages over several months. In practice there are difficulties in (a) obtaining and (b) maintaining a phagocytically-active culture over extended periods.

Preliminary studies were aimed at evaluating the potential of cultured P 388 and U 937 cell lines to provide a macrophage-like environment for fibre exposure. Cell lines were found unsuitable in our case because of the poor metabolic and phagocytic activity of the cells examined.

These problems were eventually overcome by using cultured human monocytes obtained from a single donor, which could be constantly replenished (semi-dynamic replenishment). Monocytes and macrophages are described as cells of the mononuclear phagocyte system (Van Furth 1980); the monocyte is the precursor of the alveolar macrophage.

Although there are physiological differences between monocytes and mature macrophages, there are significant similarities in that they contain acidic lysosomes and generate reactive products of the respiratory burst, which

may be important in the degradation of inorganic-fibre for the following reasons:

a) the effects of solution pH on glass corrosion have been studied (El-Shamy 1972, Paul 1982, Newton 1984) and it would be expected that acidic pH would result in leaching of fibre surface layers (Conradt and Scholze 1984) to leave a silicon-rich shell.

b) when mononuclear phagocytes engulf foreign material, oxygen is reduced (Klebanoff 1980). This process, the respiratory burst, involves the generation of highly reactive oxygen radicals (eg : $O_2^{-\cdot}$, $HO^{\cdot}$, $HO_2^{\cdot}$) and inorganic oxidants such as hypochlorite. It is possible that these species could contribute to fibre degradation.

c) in addition, nucleophilic species such as hydroxide have been shown to degrade glasses and can be generated as follows (Haber and Weiss 1934) :

$$O_2^{-\cdot} + H_2O_2 \rightarrow O_2 + HO^{\cdot} + OH^-$$

Nucleophilic degradation, in contrast with the leaching processes generally associated with acidic solutions, disrupts the silicate backbone of the material and leads to total fibre degradation.

The extended exposure of sized fibre to human monocytes was achieved by a process of "semi-dynamic replenishment".

Monocyte culture preparation:

Sized fibre samples were prepared as described (Section 3.2). Controls consisted of fibres exposed to RPMI 1640 and unexposed fibres.

Human blood monocytes were isolated from the same donor throughout. A sample of venous blood (30ml) was heparinised and diluted with 15 ml of phosphate-buffered saline (PBS). This was layered onto Lymphoprep separation media (Nycomed, Oslo, Norway) using a ratio of blood to separation medium of 2:1. The samples were centrifuged at

450 x g for 30 minutes. The milky band containing mononuclear cells (monocytes and lymphocytes) was aspirated with a sterile Pasteur pipette, and the cells were sedimented by centrifugation (650 x g for 5 minutes), washed and suspended in PBS. Cell viability was assessed by trypan blue exclusion and the cells were re-suspended in the culture medium (RPMI 1640, containing 5% heat inactivated foetal calf serum) to give a density of approximately 10^6 monocytes/ml.

The mononuclear cell population isolated by this method contained a high percentage of lymphocytes, which could easily be mistaken for monocytes. Therefore, a preliminary assessment of the numbers of monocytes and lymphocytes was made using a non-specific esterase stain (Hayhoe and Quaglino 1980). This allowed the number of mononuclear cells to be adjusted to give approximately 10^6 monocytes per ml.

Partial purification of the monocytes was achieved using the method of Treeves *et al* (1980). 1 ml of mononuclear cell suspension was pipetted into each well of a multi-well culture tray and incubated for 2 hours at 37°C in a 5% CO_2, 95% air atmosphere. The supernatant from each of the wells was carefully aspirated and the monocytes adhering to the well base were gently washed with PBS and re-suspended in fresh culture medium. In preliminary experiments the number of adherent monocytes was estimated by non-specific esterase staining.

Monocyte cultures were washed weekly with PBS to remove non-adherent cells and re-suspended in culture medium. The decrease in cell population was estimated by daily counting, over at least 10 fields of view, with inverted, phase-contrast microscopy.

After 2 to 3 weeks of culture the monocyte population had been reduced by over 50%. Lost cells were replaced by an equivalent number of freshly isolated monocytes suspended in RPMI 1640 containing 5% foetal calf serum (FCS [hi]).

Superoxide assay:

Generation of O_2^- was monitored in freshly isolated cells by measuring superoxide dismutase-inhibitable reduction of cytochrome C (Babior et al 1973, Rosen and Klebanoff 1976, Johnston et al 1978).

A suspension of approximately 10^6 freshly isolated monocytes was pipetted, in RPMI 1640, into culture wells. After incubation for two hours the cells were thoroughly washed with PBS to remove culture medium. The adherent monocytes were re-suspended in 1ml PBS. 0.3ml of superoxide dismutase (SOD: 300 units activity/ml), an inhibitor of superoxide activity (Lee-Ruff 1984), was added to control wells and 0.3ml of PBS added to the test wells. After incubating for 10 minutes, 0.1ml of cytochrome C (15mg/ml in 30mg/ml glucose solution) was added to all wells and the cells were incubated for a further 45 minutes. 1ml of the supernatant was removed and

centrifuged (5 mins, 450 x g). A sample of the supernatant was pipetted into a micro-cuvette and the absorbance measured against a distilled water blank at a wavelength of 550 nm. Samples awaiting analysis were stored on ice to minimise potential re-oxidation of cytochrome C.

The absorbance difference between test and control assays was determined and superoxide activity calculated from the molar extinction coefficient of cytochrome C (21 x $10^3/M/cm^3$).

Trypan blue exclusion: freshly isolated monocytes were stained with a 0.1% solution of trypan blue in PBS to assess viability. Dead or dying cells stain blue.

3.3.3 IN-VIVO EXPOSURE CONDITIONS

In-vivo exposures were undertaken using intra-tracheal instillation. Each specified fibre sample was suspended in PBS at a concentration of 0.1 mg/ml and a 1ml aliquot was administered to each of three rats for each fibre type. The animals were anaesthetised using ether (analytical grade) before instillation.

3.4 CHARACTERISATION OF EXPOSED MATERIALS

3.4.1 SOLUTION ANALYSIS

Solution analysis was carried out, when possible, to monitor the release of test sample species into the exposure fluid.

Inductively Coupled Plasma Spectroscopy (ICPS) was used for elemental solution analysis as it provided quantitative, multi-element solution analysis; good overall sensitivity and reproducibility and was generally un-affected by matrix interference.

The Jarrel-Ash ICAP 9000 inductively-coupled plasma spectrophotometer was used and, where possible, solution analysis was carried out to determine Si, Ca, and Na concentrations by using a sample flow rate of 2 ml/minute. Multi-element standards were used: Si, 10ppm; Ca, 5ppm; Na, 10ppm (in pure water) and changes in analyte levels were calculated by the difference in concentration before and after exposure.

The detection limits for analysis were (ppm): Si (0.01), Ca (0.02), and Na (0.00005) and it was found that ICPS was of limited use when analyte background levels in exposure fluids were very high.

Solution pH was determined before and after exposure of each sample using the Howe 6031 digital pH meter. pH calibration was carried out against pH 4 and 10 buffer solutions.

3.4.2 ANALYSIS OF BULK AND FIBROUS MATERIALS

Electron optical methods were used for characterisation of the bulk and fibrous samples. These techniques were used to provide elemental analysis and also morphological analysis of the sample.

The methods used for sample preparation were as follows: bulk glass samples, after exposure, were washed in distilled water and dried at 50°C in a warm-air oven. The samples were mounted on aluminium stubs, using conductive carbon cement, and carbon coated (Edwards 306A coating unit) prior to SEM (scanning electron microscopy) and EDXA (energy dispersive X-ray analysis).

Fibrous samples exposed for 12 months were washed thoroughly in double-distilled de-ionised water. Where possible, at least 10 individual fibres were extracted from the sample and mounted, in parallel, on a "flexicarb" support, on aluminium stubs.

The "flexicarb" was mounted on the stub with carbon cement and the fibres were attached at either end to the flexicarb, also with carbon cement (the flexicarb was over 99% carbon and sufficiently thick to prevent interfering X-rays being generated in the stub). The samples were carbon coated prior to EDXA analysis.

The sized fibres exposed for 6 months were recovered from the culture trays using a Pasteur pipette. The fibres, in suspension, were collected by filtration (0.45 micron

millipore filter) and washed thoroughly with double-distilled de-ionised water to remove any soluble species or matter adhering to the fibres. The filter was washed through with water, dried at room temperature and then ashed using a Polaron low-temperature plasma asher to remove organic material (6-12 hours, 100 watts forward power). The fibres were re-suspended in distilled water, ultra-sounded and re-filtered before being mounted on an SEM stub and carbon coated for EDXA analysis.

Fibres exposed in-vivo were also prepared for analysis by low temperature ashing, though longer ashing times were required to remove the organic material. The rats were killed and the lung excised. The lobes were removed and dried at 50°C in a drying oven, then ashed for 48 hours at 150 watts forward power, before being re-suspended in water and filtered. The filters were mounted and carbon coated prior to EDXA analysis.

It was observed, by comparing data for un-ashed controls with ashed samples, that ashing of the samples had no apparently significant effects on the fibre morphology or chemical composition of the materials studied.

Where possible samples were carbon coated simultaneously to ensure a similar thickness of the coating and all samples were gold coated prior to morphological analysis.

Scanning Electron Microscopy (SEM) in conjunction with

Energy Dispersive X-ray Analysis (EDXA) provided a method for elemental and morphological analysis of the sample. Morphological analysis was essential as it allowed a visual method for assessment of sample degradation by monitoring changes in surface morphology and fibre diameter.

In the Scanning Electron Microscope the high energy electrons, incident at the specimen surface, generate secondary electrons which are used to produce an image of the specimen surface and also X-rays, characteristic of the elements present in the specimen, which may be used for elemental identification and quantification.

X-ray analysis was carried out using EDXA; the relative levels of the characteristic X-rays generated were used to determine the relative analyte levels.

SEM and EDXA provide relatively non-destructive, rapid, high-resolution morphological and elemental analysis.

There are certain limitations, particularly with the EDXA technique, which must be considered when interpreting data for fibrous materials:

(a) **Specimen charging:** this is a fundamental problem with insulating samples. To provide a conductive path to earth the surface of the specimen must be coated with a conductive material. Samples were therefore gold coated for morphological studies, which gave good surface resolution, but tended to result in re-adsorption of X-rays characteristic of the lighter elements and a gold X-ray peak in the same region as analyte signals. Consequently, samples were carbon coated for EDXA analysis. This resulted in less X-ray re-adsorption and, furthermore, carbon X-rays are not detected by standard EDXA analysers.
(b) **Elemental detection:** due to their low energy, X-rays generated by elements of atomic mass < 11 cannot penetrate the "window" of the EDXA detector. This can be a problem when examining borosilicate glasses as they contain

significant quantities of boron, which could only be determined if a "windowless" detector were available.

(c) **Elemental sensitivity:** it was observed that signal intensity was dependent on the accelerating voltage of the primary electron beam. The use of a 12kV electron beam was more sensitive for the lighter elements (Na, Mg), whilst a 25kV beam was more sensitive for the heavier elements (K, Ca, Fe) present in the materials studied.

(d) **Surface sensitivity:** 25kV electrons have a greater penetration depth (7-10um for glass and ceramic *) 12kV electrons (2-3 um) and consequently, because of the relative sample excitation volumes involved, 12kV provides greater surface sensitivity. The potential to further increase surface sensitivity by reduction of accelerating voltages was compromised by the associated reduction in X-ray yield. In practice an accelerating voltage of 12kV provides optimum surface sensitivity. It was appreciated that elemental changes occurring in true surface layers would tend to be masked by the characteristic X-rays generated within the total electron penetration volume of the sample.

* penetration depth = $4120/\text{density} \cdot E^{(1.265 - 0.0954 \ln E)}$. E = primary electron energy, MeV, and density is given in g/cm^3 (Link Systems technical data).

(e) **Fibre-geometry:** fibres present analytical difficulties due to their curvature and relatively small size. Most fibres examined were too thick to be electron transparent and hence there was incomplete excitation throughout the fibre diameter. This was significant for two reasons. Firstly, because the fibres were often on the border of being a "thick" or "thin" specimen, they were incompatible with both the ZAF and "Thins" method for quantified X-ray analysis. Thus analysis was carried out in a semi-quantitative manner, showing changes in the relative levels of the analytes. Secondly, preferential re-adsorption of X-rays presented a problem when thick fibres were being examined and was particularly severe in the case of fibres aligned at right angles to the detector. Fibre curvature effectively meant that the fibre had a "dead" side at the edge furthest away from the detector. This is shown in the series of spectra in Figure 3.1. The diagram shows how analysis on the dead side of the fibre resulted in preferential absorption of the lighter element X-rays, producing what appears to be a much stronger Ca peak relative to Si (EDXA: point 3). These problems were satisfactorily overcome by analysing at the apex of the fibre and, where possible, by aligning the fibre point towards the detector (ie) by ensuring that the X-ray path-length was kept constant for different fibres the degree of re-adsorption would also be kept constant.

(f) **Elemental diffusion:** this was a relatively minor problem, accentuated by using higher electron beam

energies. It was observed that readily diffusible elements such as sodium diffuse away from the point of analysis. Ideally a cold stage would be used to reduce elemental diffusion however the problem was overcome by ensuring all analytical variables were kept constant between test and control samples and using fixed integral analysis. Multi-point analysis (3 per fibre) was adopted and it was seen from control sample variance data that this procedure provided a reproducible method for determination of sodium. Clearly the possibility of elemental diffusion must be noted when analysing for light elements such as sodium. The Phillips PSEM 500 was used for SEM and EDXA analysis in conjunction with Link Systems AN 860 X-ray analyser.

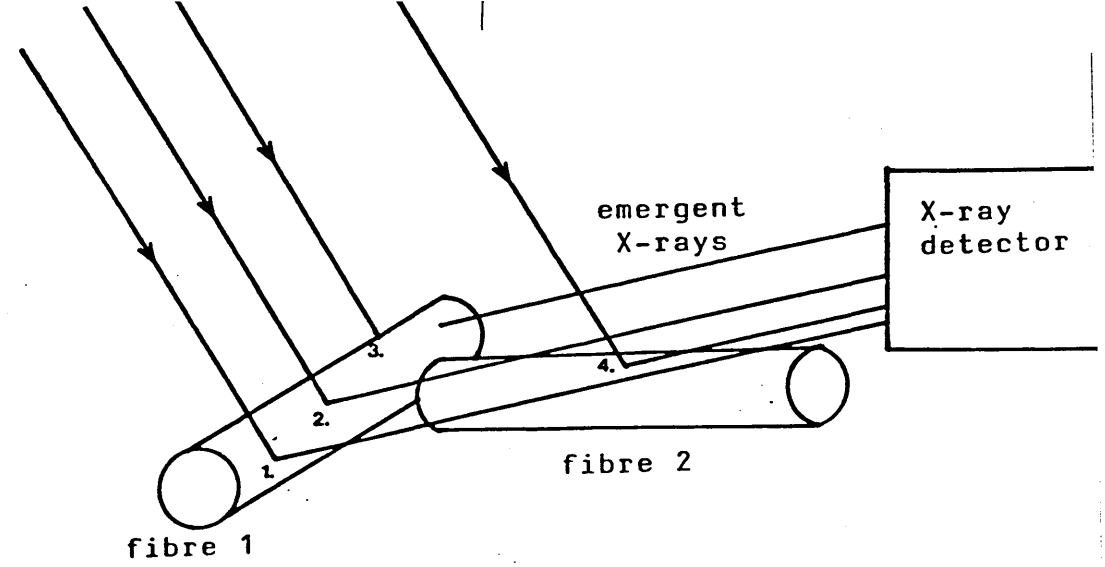
Accurate and reproducible EDXA required that instrumental variables were kept constant. EDXA was carried out at 12 and 25kV on carbon coated samples using a spot size of 0.125 microns. No tilt was applied to the specimen which was analysed pointing towards the X-ray detector with a probe distance of 2 cm. EDXA controls consisted of unexposed fibre samples, carbon coated alongside test samples.

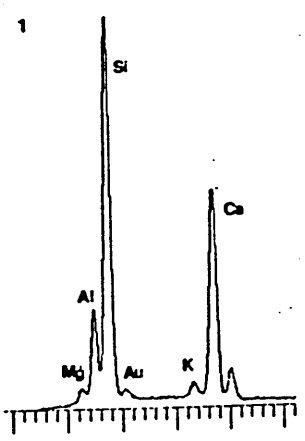
The Link Systems AN860 X-ray analyser programme was used for primary data analysis. Windows were "painted" around characteristic X-ray peaks for the various analytes and the peak area integrated. Typically, window positions were (eV): Na, 920-1140; Mg, 1180-1320; Al, 1400-1560; Si, 1620-1920; Zr, 1980-2300; Pb, 2220-2520; K, 3200-3440; Ca, 3560-3860 and Fe, 6280-6500. A preset count was set for the silicon peak (150000 counts for 25kV analysis and 30000 counts for 12 kV) and used to control the analysis time.

After analysis, peak integrals were obtained for the range of analytes and expressed as a % ratio of the integral value for silicon (analyte peak integral / silicon peak integral x 100%). Background correction was carried out automatically. Analyte peak intensity was expressed relative to silicon peak intensity as silicon is an integral part of the sample lattice and, hence, an appropriate internal standard.

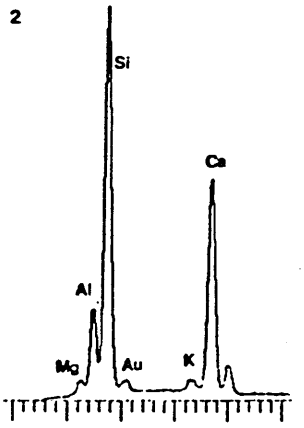
Note that "peak-splitting" software provides a method for resolving severely overlapping peaks and may be necessary for the resolution of some glass spectra.

Fibre sizing and morphological examination was carried out using the Phillips PSEM 500 electron microscope. Size calibration was carried out by reference to suitable calibration standards.

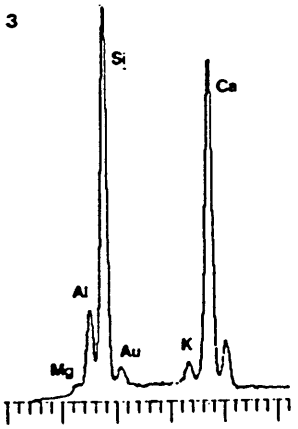




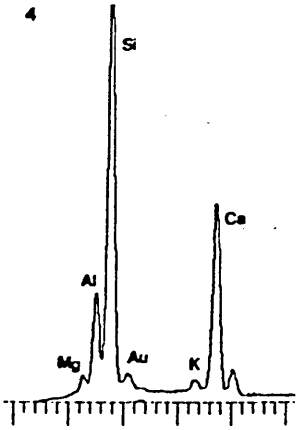
EXDA : point 1



EXDA : point 2



EXDA : point 3



EXDA : point 4

Figure 3.1 The effect of preferential re-absorbtion of characteristic X-rays

3.4.3 STATISTICAL METHODS

Statistical treatment of EDXA data was applied in order to show whether small changes in analyte levels had occurred in the samples during exposure to model fluids.

In the case of the 12 month in-vitro test sample exposures each of 10 test fibres for a given exposure and each of 10 unexposed control fibres was analysed at three separate points along its length. The three point analysis data was then averaged to give a better estimate of the mean analyte ratios relative to silicon for each fibre and the mean analyte ratios for all ten fibres calculated. This procedure was adopted for the six month exposures using "prepared" fibres, but because of the much smaller test and control sample populations only 5 fibres were analysed per sample. A "T" test was applied to test if there was a significant difference between the exposed test sample and unexposed control sample population means, after first checking to determine whether the variance (spread) of the two samples was suitably similar.

F test: to calculate the F statistic,
F = larger variance/smaller variance = larger Std.dev^2/ Smaller Std.dev^2

Null hypothesis (Ho): samples are from normal populations with the same variance (ie) std.dev$_1{}^2$ = std.dev$_2{}^2$
Alternative hypothesis (H_1): samples are from populations with different variances (ie) std.dev$_1{}^2 \neq$ std.dev$_2{}^2$

The calculated F value was tested against $F_{1\%,\ (n1-1),\ (n2-1)}$ from standard F tables (Snedecor 1950). The critical F value was 6.54 for the 12 month exposures, where n = 10, and 23.16 for the 6 month exposures, where n = 5; a calculated F value less than this allows us to accept Ho.

If Ho is statistically valid a "T" test may be carried out to see if the population means of the control data and test data were different. The test was two tailed and the null hypothesis was that the two samples were from the same population, that is, mean 1 = mean 2.

T was calculated from:

$$T = \frac{\text{difference between sample means}}{\text{estimated standard error of difference of means}}$$

$$= \frac{X1 - X2}{\sqrt{\left[\left((n1-1)V1 + (n2-1)V2 \right) / (n1+n2-2) \right] \cdot [1/n1+1/n2]}}$$

The calculated T value was compared with the T table value for $T_{1\%,\ (n1 + n2 -2)}$ (Snedecor 1950).

The critical T value was 2.88 for the 12 month exposures, where the degrees of freedom = 18, and 3.36 for the 6 month exposures where the degrees of freedom = 8; a calculated T value less than this allows us to accept Ho, the means are statistically the same.

If the variances could not be shown to be statistically the same a modified T test was applied where the number of degrees of freedom was determined using the following formula (Davies and Goldsmith eds. 1986):

$$1/dgf = 1/dgf_1 \{(S_1^2/n_1)/(S_1^2/n_1 + S_2^2/n_2)\}^2 + 1/dgf_2 \{(S_2^2/n_2)/(S_1^2/n_1 + S_2^2/n_2)\}^2$$

4. IN-VITRO STUDIES

4.1 THE DURABILITY OF BULK SODA-LIME SILICATE GLASS EXPOSED TO SIMULATED LUNG FLUIDS

4.1.1 METHODOLOGY

Pieces of bulk soda-lime silicate glass were exposed to model fluids designed to simulate the potential chemical environment of the lung. The sections of glass were weighed, sterilised by washing in ethanol solution (70%), allowed to dry and then exposed, one piece of glass per reaction vessel (250 ml Nalgene polypropylene bottles), to 50 ml of each of the exposure fluids.

Time and conditions of exposure are shown as follows:

Table 4.1 Conditions for exposure of glass samples

SAMPLE	EXPOSURE FLUID	EXPOSURE TIME / WEEK
1. 2. 3.	potassium phosphate buffer (pH 7.4)	1 2 3
4. 5. 6.	Gamble's fluid, phosphate-buffered (pH 9.2)	1 2 3
7-9. * 10-12. 13-15.	pure water	1 2 3
16-18. * 19-21. 22-24.	Gamble's fluid, bicarbonate-buffered (pH 7.8)	1 2 3.

* selected exposures were carried out in triplicate to assess reproducibility.

Gamble's fluid exposures required accurate maintenance of physiological buffering. Phosphate buffer (Table 4.1) was incorporated in samples 4-6 to provide a pH of 7.4, whilst samples 16-24 were bubbled with a 5% CO_2:95% O_2 mixture until pH 7.4 was reached, and the reaction vessels were sealed air-tight. Reaction vessels were re-gassed weekly.

During exposure all reaction vessels were maintained in the dark at 37°C, in a shaking water bath. pH and microbial growth (drop count method) were monitored and on completion the glass samples were washed in pure water, re-weighed and stored in a dessicator ready for analysis. Solutions were analysed by ICPS before and after exposure and the glass characterised by SEM and EDXA.

4.1.2 RESULTS

Solution analysis

Microbial growth: bacterial and algal growth were negligible except for samples 22-24 where bacterial growth, but not algal growth, was present. Contamination probably occurred during re-gassing of the reaction vessels, but due to the consistent nature of the analytical data it was assumed that the contamination did not significantly influence the behaviour of the test samples.

Solution pH variation: solution pH was monitored during exposure and is shown:

Table 4.2 Variation in model fluid pH with exposure time

SMPL.	FLUID	EXPOSURE week	pH (MEAN ± HALF RANGE) initial	after exposure
1	potassium	1	7.4	7.5
2	phosphate	2	7.4	7.4
3	buffer (pH 7.4)	3	7.4	7.5
4	phosphate-	1	7.9	9.2
5	buffered	2	7.9	9.1
6	Gamble's fluid	3	7.9	9.2
7-9	pure water	1	5.0	5.2 ± 0.2
10-12	pure water	2	5.0	5.2 ± 0.2
13-15	pure water	3	5.0	5.9 ± 0.2
16-18	bicarb. buffered Gamble's fluid	1	7.4*	7.8 ± 0.1
19-21	bicarb. buffered Gamble's fluid	2	7.4*	7.9 ± 0.05
22-24	bicarb. buffered Gamble's fluid	3	7.4*	7.8 ± 0.0

* The Gamble's fluid was bubbled with 5% carbon dioxide to give a pH of 7.4

Analyte variation: changes in solution analyte levels were monitored, with time, using ICPS and are shown below. Data was corrected for analyte background levels.

Table 4.3 Si, Na and Ca increase in model fluids after 1-3 week exposures

SMPL.	FLUID	EXPOSURE week	ANALYTE (MEAN ± HALF RANGE) [Si]/ppm	[Na]/ppm	[Ca]/ppm
1 2 3	potassium phosphate buffer (pH 7.4)	1 2 3	1.302 1.937 2.886	0.377 0.679 0.885	0.164 0.360 0.530
4 5 6	phosphate buffered Gamble's fluid	1 2 3	8.43 14.85 21.52	- - -	- - -
7-9	pure water	1	*	0.216 ± 0.02	0.110 ± 0.07
10-12	pure water	2	*	0.246 ± 0.04	0.102 ± 0.07
13-15	pure water	3	*	0.270 ± 0.04	0.256 ± 0.28
16-18	bicarbonate-buffered Gamble's fluid	1	0.211 ± 0.13	-	-
19-21	bicarbonate-buffered Gamble's fluid	2	0.816 ± 0.42	-	-
22-24	bicarbonate-buffered Gamble's fluid	3	0.945 ± 0.37	-	-

* below limits of detection

\- background levels of analyte too high for accurate determination

Glass analysis

Glass mass: changes in glass mass during exposure were as follows:

Table 4.4 Variation in glass mass during exposure to model fluids

SAMPLE	FLUID	EXPOSURE week	% MASS CHANGE
4	phosphate	1	- 0.112
5	buffered	2	- 0.205
6	Gamble's fluid	3	- 0.345

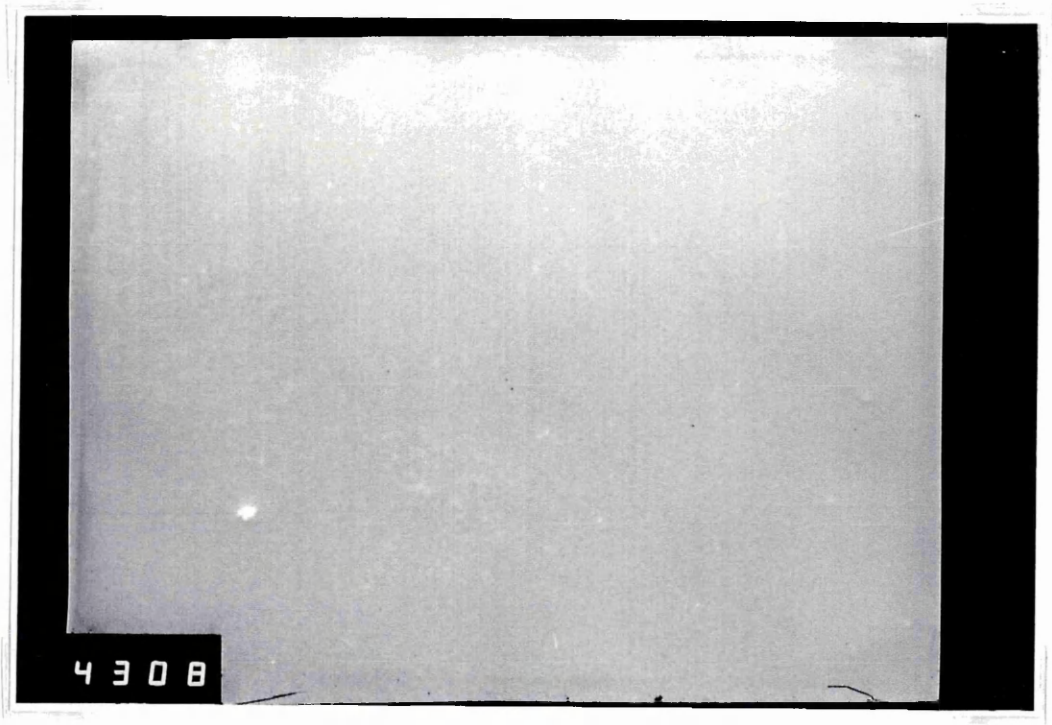
Note that sample mass loss was negligible, or below levels of detection, for other exposure conditions.

SEM and EDXA analysis of glass samples: EDXA data is shown in Table 4.5. Analysis was carried out in triplicate. Micrographs showing the morphology of the exposed glass samples and controls are presented in Figure 4.1-4.

Table 4.5 EDXA analysis of glass samples exposed to model fluids

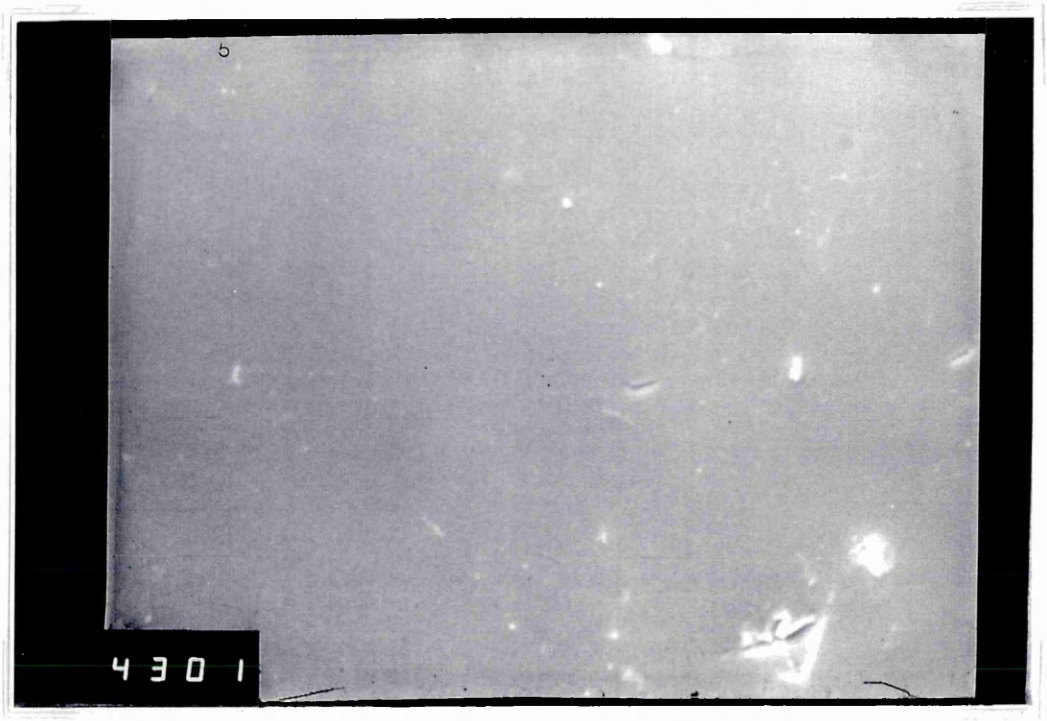
SMPL.	FLUID	EXPOSURE week	% ANALYTE RATIO TO SILICON (MEAN ± HALF-RANGE) [Na]/[Si]	[Ca]/[Si]
1	potassium	1	32.5 ± 0.3	24.9 ± 0.4
2	phosphate	2	32.9 ± 0.5	24.9 ± 0.3
3	buffer (pH 7.4)	3	32.6 ± 0.2	24.7 ± 0.4
4	phosphate	1	33.2 ± 0.4	26.5 ± 0.6
5	buffered	2	33.2 ± 0.3	25.4 ± 0.2
6	Gamble's fluid	3	33.3 ± 0.2	25.0 ± 0.2
7-9	pure water	1	31.8 ± 0.7	24.7 ± 0.5
10-12		2	31.1 ± 0.5	24.5 ± 0.1
13-15		3	29.0 ± 0.7	24.3 ± 0.2
16-18	bicarbonate	1	33.0 ± 0.4	24.7 ± 0.1
19-21	buffered	2	31.9 ± 0.2	23.8 ± 0.5
22-24	Gamble's fluid	3	32.3 ± 0.2	24.8 ± 0.1
cont.	unexposed,	0	33.5 ± 0.5	25.6 ± 0.2
	control		34.6 ± 0.9	26.2 ± 1.5
	samples		35.0 ± 0.4	25.1 ± 0.2

Figure 4.1



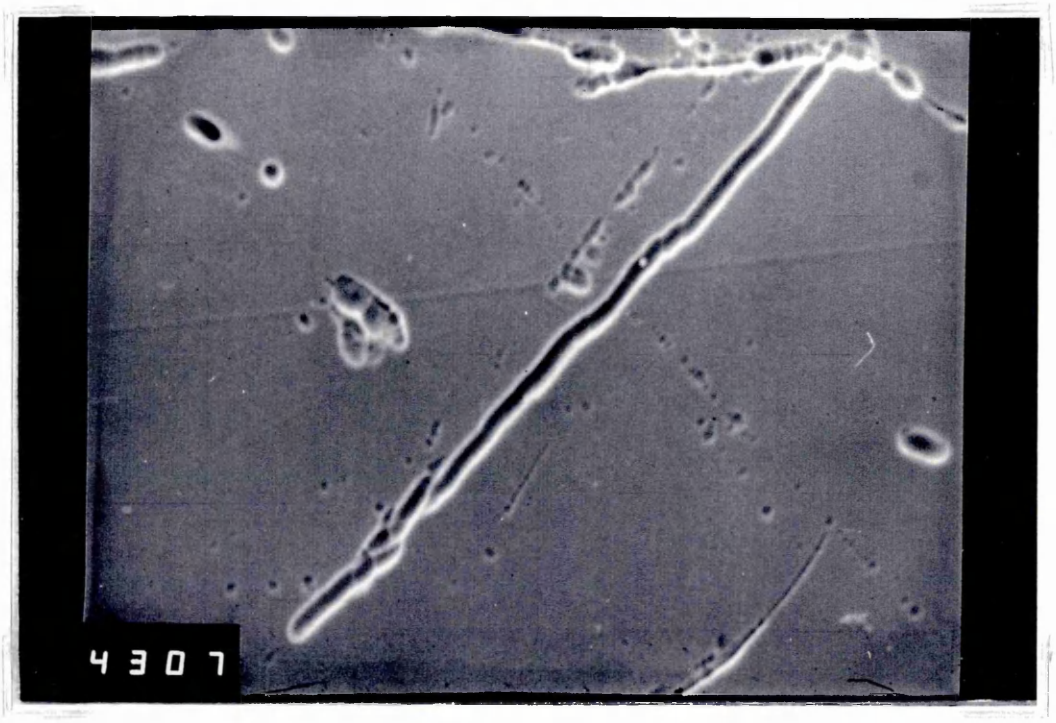
SEM micrograph: unexposed bulk-glass surface (x 1600)

Figure 4.2



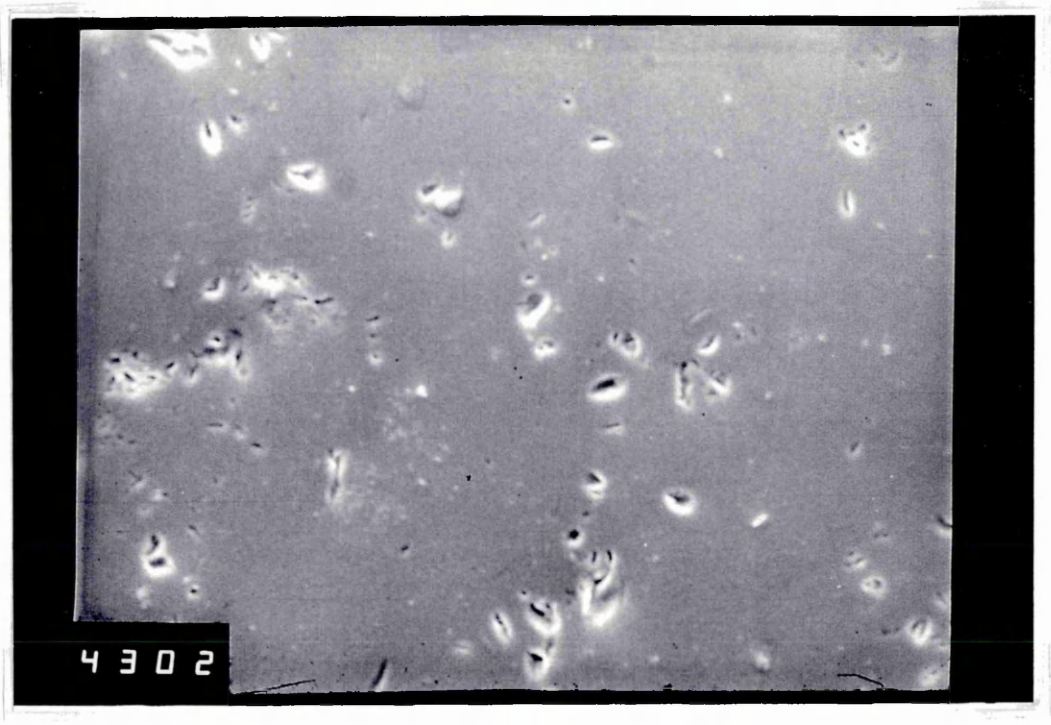
SEM micrograph: bulk-glass exposed to water (x 1600)

Figure 4.3



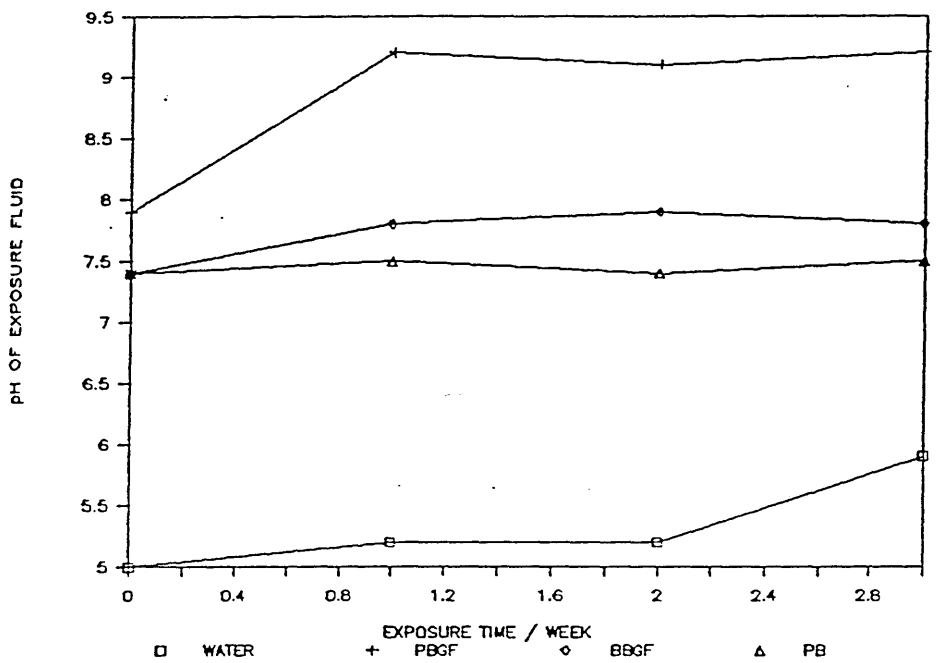
SEM micrograph: bulk-glass exposed to phosphate-buffered Gamble's fluid (x 1600)

Figure 4.4



SEM micrograph: bulk-glass exposed bicarbonate-buffered Gamble's fluid (x 1600)

Figure 4.5



pH variation in exposure fluid

4.1.3 DISCUSSION AND INFERENCES OF RESULTS

Solution analysis:

pH variation (Figure 4.5): the potassium phosphate buffer (PB) presented a stable physiological pH environment for the glass, showing a mean pH variation of 7.45 ± 0.05 over the three week exposure period. In contrast, the Gamble's fluid, incorporating potassium phosphate buffer (PBGF), showed a rapid increase in pH, from 7.9 to 9.2, over the first week of exposure. It was observed that loss of buffering occurred when the solution reached 37 °C and an insoluble, white precipitate formed. Phosphate, Mg, Ca and K ions were identified in the precipitate by spectroscopic methods and it was probable that the majority of the precipitate consisted of magnesium phosphate, which is insoluble in neutral solutions. Loss of physiological buffering would have occurred with precipitation of phosphate. The pure water showed a slight increase in pH (from 5.0 to 5.9) over the three week exposure period, which was probably associated with ion exchange of leachable cations in the glass for hydrogen ions in the water (Hench and Clark 1978, Paul 1982).
The pH stability of the bicarbonate-buffered Gamble's fluid (BBGF) during the three week exposure period (pH 7.4-7.8) demonstrated the success of bubbling the fluid with 5% CO_2 to maintain physiological buffering. This was significant as problems relating to pH maintenance in Gamble's fluid have occurred previously (Klingholz and Steinkopf 1982, Leineweber 1982)

ICPS analysis:

Silicon (Figure 4.6): it was seen (Table 4.3) that the greatest extraction of silicon ions from the glass was during exposure to phosphate-buffered Gamble's fluid. The extraction was directly proportional to exposure time, giving rise to approximately 22 ppm Si in the exposure fluid by the third week. This was over 7 times greater than the levels of silicon extracted by the potassium phosphate buffer - the second most corrosive solution with respect to silicon extraction. Silicon extraction by the bicarbonate-buffered Gamble's fluid was of a similar order to that of the potassium phosphate buffer, both showing an increase in Si extraction with time; no detectable extraction of Si could be observed during exposure to pure water.

This behaviour was consistent with the classical, pH dependent model of glass durability (El-Shamy et al 1972). The high levels of Si extraction, in the case of exposure to phosphate-buffered Gamble's fluid, may be related to the relatively high pH of the fluid (> pH 9). Alkali-silicate glasses are prone to degradation at pH's over 9 - 10, when nucleophilic attack of the Si - O bonds in the glass network occurs:

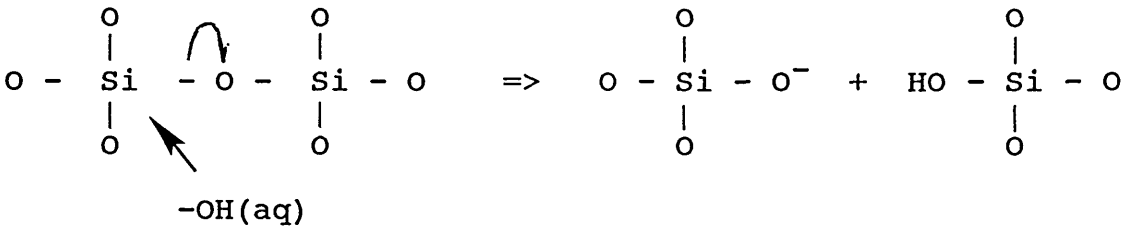
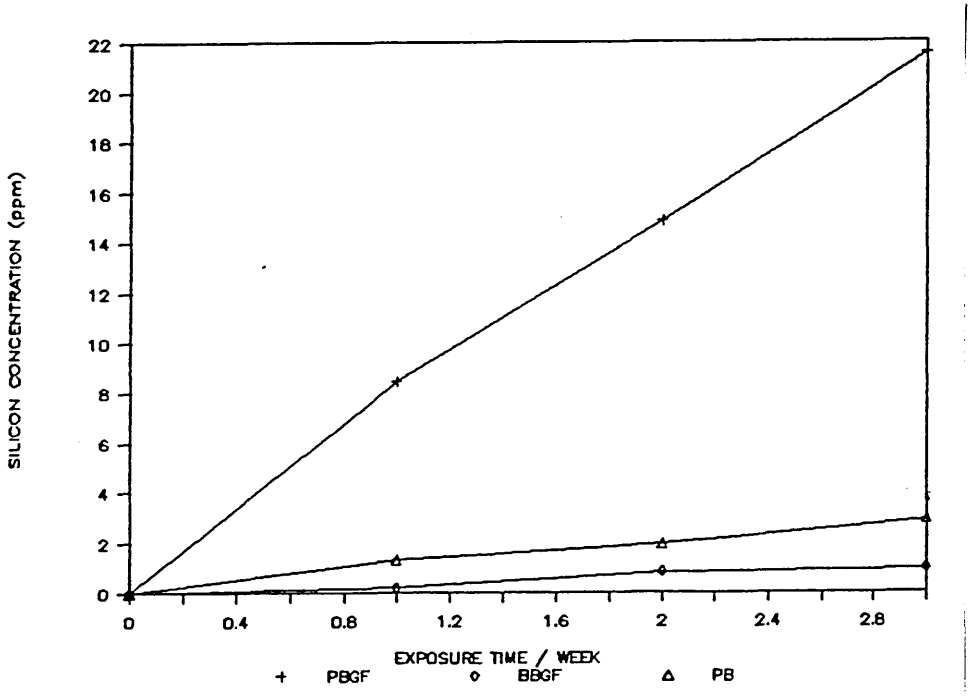
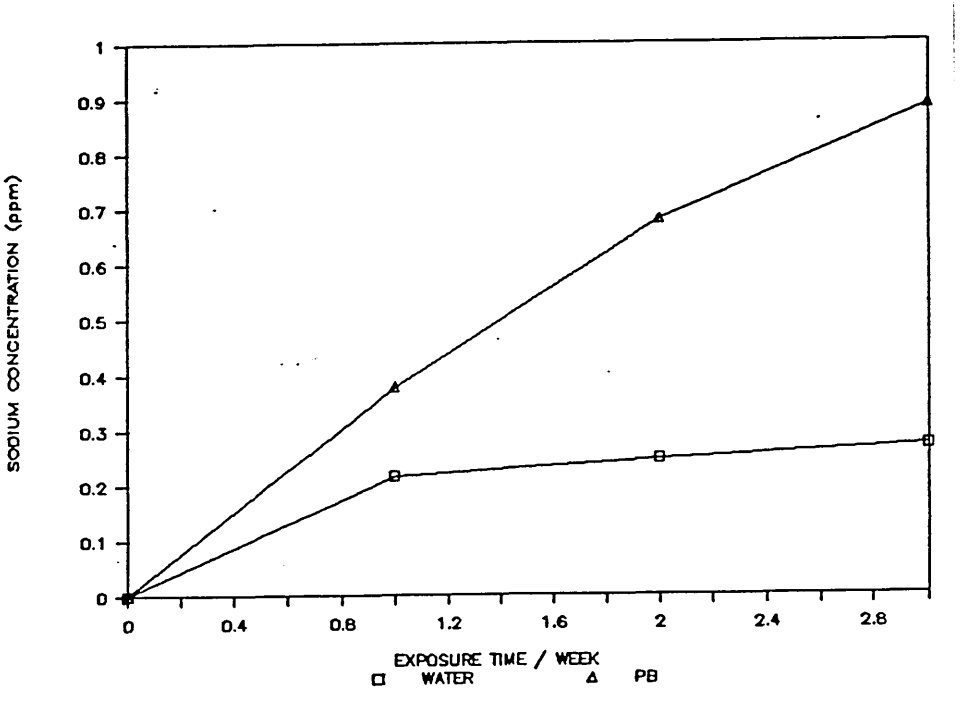


Figure 4.6



Silicon extraction

Figure 4.7



Sodium extraction

The silica network forms the "backbone" of the glass and dissolution results in release of network forming and network modifying species into solution; this process will etch away a non-resistant glass, continually leaving a fresh surface with the same relative concentration of analytes as the unexposed material. Eventually the glass may be totally dissolved by the attacking solution.

Exposure of the glass to pure-water resulted in negligible Si extraction, indicating minimal degradation of the glass lattice. This could be related to the slightly acidic overall pH of the water, under which ion-exchange processes would be predominant and the hydrolytic processes associated with structural corrosion of the glass would be minimal.

It should be noted that the ion-exchange processes involve depletion of cations in the glass in exchange for hydrogen ions in the environment and, therefore, continued exposure may result in the formation of an alkali environment, capable of hydrolysis of the glass network. For this reason care must be taken in evaluating tests, involving static conditions and high glass-surface area to solution volume ratio, where the potential exists for a rapid increase in environmental pH.

Si extraction was minimal during exposure of the glass to the physiologically buffered fluids (phosphate buffer and bicarbonate-buffered Gamble's fluid). This was

consistent with our understanding of simple glass behaviour, from which low levels of hydrolysis of the glass network would be predicted in neutral environments.

Sodium (Na) (Figure 4.7): solution analysis data for Na extraction was of limited value, because Na levels would increase through ion-exchange processes and hydrolysis of the glass network. An increase in the levels of sodium extracted (up to 0.27 ppm.) by pure-water is shown in Table 4.3. This was probably associated with ion-exchange of modifying Na^+ ions for H^+/H_3O^+ ions in the slightly acidic aqueous environment.

Higher levels of Na extraction, up to 0.885 ppm, were observed for exposure to potassium phosphate buffer. This may be associated with the contribution to Na extraction from hydrolysis, indicated by low levels of Si extraction. Note that the hydrolytic processes are relatively weak at neutral pH, but would remain at a constant rate, whilst Na extraction by ion-exchange will tend to slow as ion diffusion distances increase.

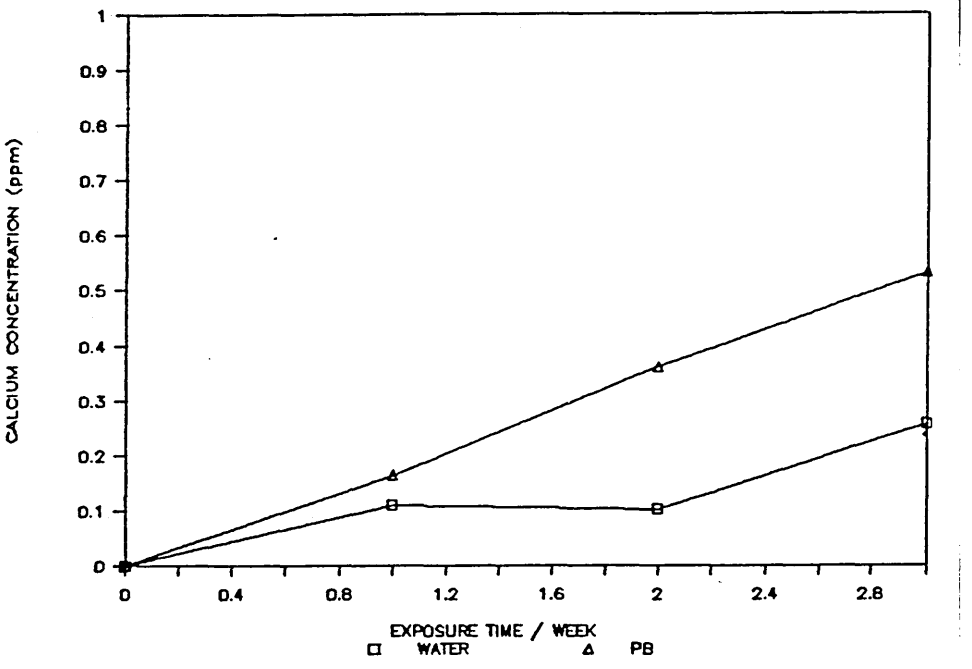
The high Na background in the Gamble's fluid made determination of changes in the analyte concentration impossible in the cases of bicarbonate- and phosphate-buffered Gamble's fluid. It would be predicted that the hydrolytic processes associated with exposure to the phosphate-buffered Gamble's fluid would have resulted in the release of relatively high levels of sodium into solution.

Calcium (Ca) (Figure 4.8): Ca extraction would be expected to follow the same pattern as Na, though in the case of the soda-lime silicate glass studied, the levels of extraction relative to Si would be less. It was found that Ca extraction was greater for exposure to potassium phosphate buffer than for exposure to pure water. As with Na extraction, the contribution from hydrolytic, rather than ion-exchange processes, for the phosphate buffer exposure was probably significant. Ca analysis of the phosphate-buffered and bicarbonate-buffered Gamble's exposure fluids proved impractical due to the relatively high calcium background and also precipitation of Ca, as the carbonate, from solution.

Glass analysis

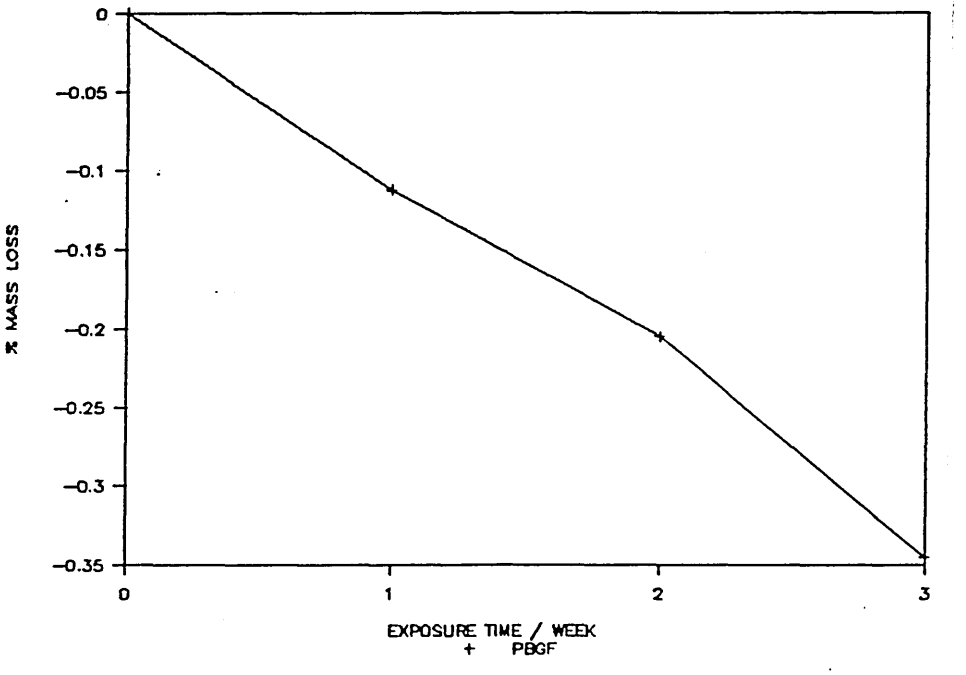
Mass loss (Figure 4.9): exposure of the glass to phosphate-buffered Gamble's fluid produced the only statistically significant mass change in the glass over the three week exposure period. The glass showed a linear mass loss with time and a loss of 0.35% was recorded after 3 weeks of exposure. This mass loss was consistent with expected behaviour at the relatively high pH of the exposure fluid (pH 9), and was associated with hydrolytic breakdown of the glass structure.

Figure 4.8



Calcium extraction

Figure 4.9



Mass loss with time

The mass loss data may be used to predict the lifetime, t, of a theoretical fibre of this composition:

Let the mass removal per unit area per unit time = X kg m^{-2} wk

then volume removal per unit area per unit time = $\frac{X}{p}$ m^3 / m^2 / w

where p = density

and the surface thickness removal per unit area per unit time

$$= \frac{X}{pA} \text{ m / } m^2 \text{ / wk}$$

where A = area.

For a cylindrical fibre:

the area, $A = 2\pi rl$, where r = radius and l = length of fibre.

therefore, $\frac{dr}{dt} = \frac{X.2\pi rl}{p.2\pi rl} = \frac{X}{p}$

and $dt = \frac{p.}{X} dr,$

For the lifetime, t, of a fibre of initial radius, a:-

$$\int_o^t dt = \int_a^o -\frac{p.}{X} dr \quad \text{----->} \quad t = \frac{p.a}{X}$$

and therefore $t = \frac{p.a}{X}$

In this experiment the mass loss of the glass block was 0.35% after 3 weeks of exposure in the phosphate-buffered Gamble's fluid. The original mass was 0.47g and the surface area 3.9×10^{-4} m^2. Hence, mass loss per unit area, $X = 1.41 \times 10^{-3}$ kg m^{-2} wk^{-1}. Assuming the density, p, of the glass was 2351 kgm^{-3} the lifetime, t, of a theoretical, respirable fibre of initial radius, a, of 0.5 micron under these exposure conditions is 0.42 weeks ie. under alkaline conditions a fibre of this material, of 0.5 micron initial diameter, would have a short life time.

EDXA of the glass: to compare the relative levels of the analytes (Si, Ca, Na) in the exposed glass samples the Na and Ca peak intensities were first expressed as a percentage ratio of the Si peak intensities (Table 4.5). A decrease in the value of this ratio reflects a decrease in the concentrations of Na or Ca relative to Si.

Si was used as an internal standard, because it was an integral part of the glass network and therefore not subject to preferential leaching.

Figure 4.10 shows the % decrease of Na in the exposed glass compared to the levels of Na in unexposed control samples. This % decrease was calculated:

$$\% \text{ decrease} = 100\% - \frac{\text{Na/Si exposed sample}}{\text{Na/Si unexposed sample}} \times 100$$

Figure 4.11 shows the control EDXA spectra for the bulk glass.

Pure water resulted in the most significant degree of Na leaching (over 15% decrease after 3 weeks) whilst the least significant degree of leaching (approximately 3%) was for exposure to phosphate-buffered Gamble's fluid.

This behaviour could be rationalised by considering the pH of the exposure fluid. The slightly acidic pH of the pure water would favour the dominance of ion-exchange processes, resulting in preferential extraction of cations such as Na from the glass matrix, whilst the silicate network would be unaffected.

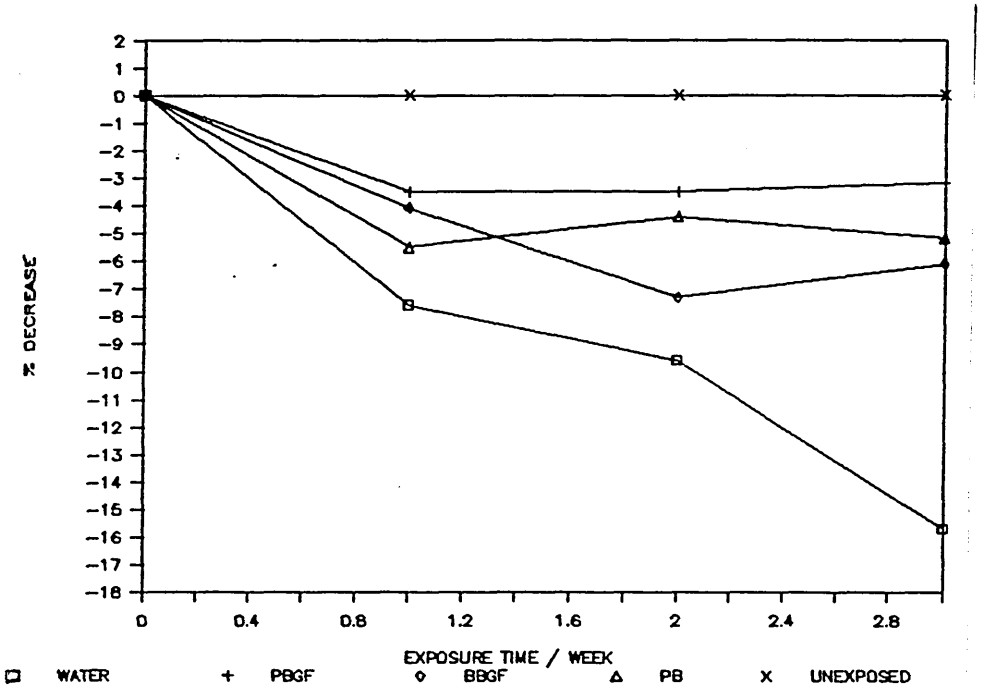
As the solution pH was increased, ion-exchange would be replaced by network hydrolysis eventually causing the release of all species in equal proportions. This explained why the surface compositions of the samples exposed to the relatively high pH of the phosphate-buffered Gamble's fluid were similar to those of the unexposed glass.

Ca leaching was generally less extensive than Na leaching (5.8% ± 0.8 for 3 week exposure to water). This was consistent with established models that state that Na leaching precedes Ca leaching in a soda-lime silicate glass.

SEM analysis of the glass: the electron micrographs (Figures 4.1-4) show extensive physical damage to the glass surfaces exposed to phosphate-buffered Gamble's fluid, whereas the other samples have an appearance more comparable with the unexposed glass samples. Figure 4.4, showing the surface of the bulk glass exposed to bicarbonate-buffered Gamble's fluid did show in one or two areas some evidence of the beginnings of localised corrosion.

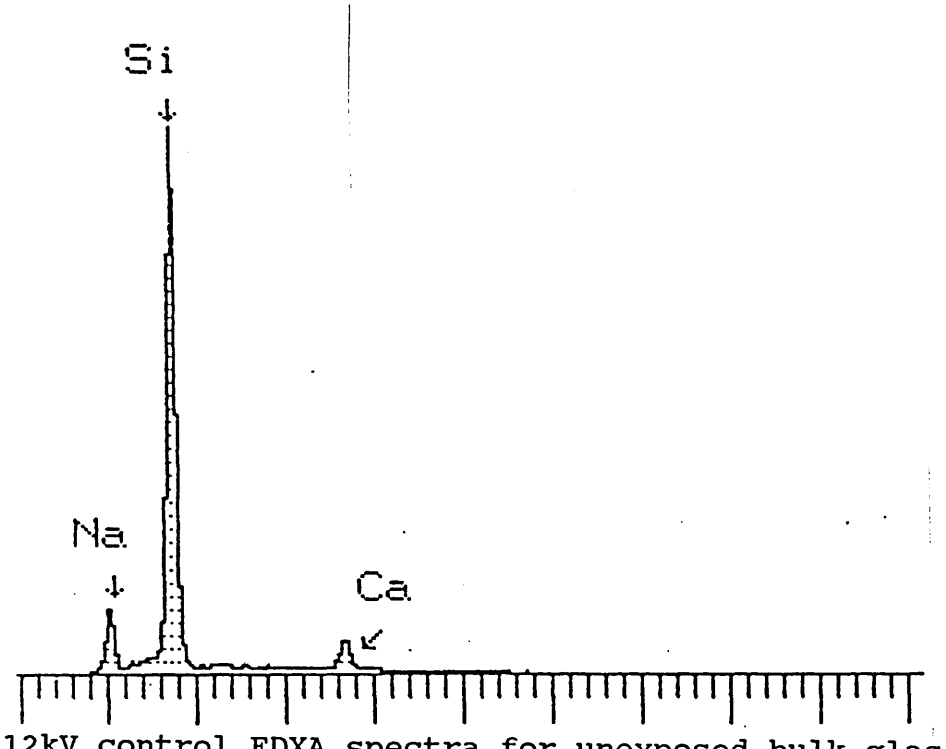
The morphological data provided direct evidence of the corrosive effects of a relatively high pH solution on a simple glass.

Figure 4.10



% sodium decrease relative to silicon

Figure 4.11



12kV control EDXA spectra for unexposed bulk glass

4.2 THE DURABILITY OF GLASS AND CERAMIC FIBRES EXPOSED TO SIMULATED LUNG FLUIDS

4.2.1 METHOD

Fibrous samples (approximately 0.01g in mass) of a range of glass and ceramic fibres were separately exposed to a number of model fluids. Where appropriate, "sized" fibre samples were prepared and also exposed to a range of exposure fluids. Exposure conditions are shown in Table 4.6 and solution pH was measured before and after exposure. SEM and EDXA were carried out on the exposed materials and where possible F and T tests were used to determine the statistical significance of the experimental data. The EDXA data for the 6 month exposures of sized-fibre was compared against the tabular values:

($F_{1\%(4,\ 4)} = 23.16$, $T_{1\%(8)} = \pm 3.36$) for a two-tailed test.

The 12 month data was compared against the tabular values:

($F_{1\%(9,\ 9)} = 6.54$, $T_{1\%(18)} = \pm 2.88$) for a two-tailed test.

EDXA data values given were not absolute concentrations, but analyte signal intensities expressed as a % of the silicon intensity. The data was expressed in this manner to aid determination of the nature of the chemical behaviour of the material.

Morphological studies were also carried out and electron micrographs obtained. Where possible, changes in fibre diameter were monitored, to determine degradation rates of the fibre. For the six-month exposures, where "sized" fibres were prepared, the range and mean of the test-fibre

diameters was compared with the range and mean of the un-exposed controls. For the twelve month exposures only the E- and Cemfil fibre were measured. 50 fibres were measured in each test sample and the mean diameter statistically compared with that of the respective control. The diameter variation for the other fibre types was too great for accurate statistical comparison without a prohibitively large sample population being examined.

Table 4.6 Conditions for exposure of glass samples

SAMPLE	EXPOSURE FLUID	EXPOSURE TIME / MONTH	
		"sized" *	not sized
E glass Cemfil	1. water	6	12
	2. Gamble's fluid	6	12
	3. PBS		12
	4. RPMI 1640	6	12
	5. pH 10 buffer		12
	6. blood serum	6	
	7. BALF	6	
A glass	1. water	6	
	2. Gamble's fluid	6	
	4. RPMI 1640	6	
	6. blood serum	6	
	7. BALF	6	
Lead glass Ceramic	1. water		12
	2. Gamble's fluid		12
	3. PBS		12
	4. RPMI 1640		12
	5. pH 10 buffer		12

* it was only possible to prepare "sized" fibres from the continuous filament or wool samples from which a 5 - 10 cm length of consistent diameter could be extracted.

4.2.2 RESULTS

4.2.2.1 General

CO_2 incubation of the test samples provided physiological buffering of the Gamble's fluid over the exposure period and it was found that solution pH changes for other fluids were minimal after exposure. Microbial growth was generally prevented by the use of aseptic procedure.

4.2.2.2 EDXA and Morphological Analysis

A-glass fibre

This glass is based on a simple soda-lime silicate composition commonly found in window and bottle glasses. Control EDXA spectra (unexposed samples) and electron micrographs are shown in Figure 4.12-15.

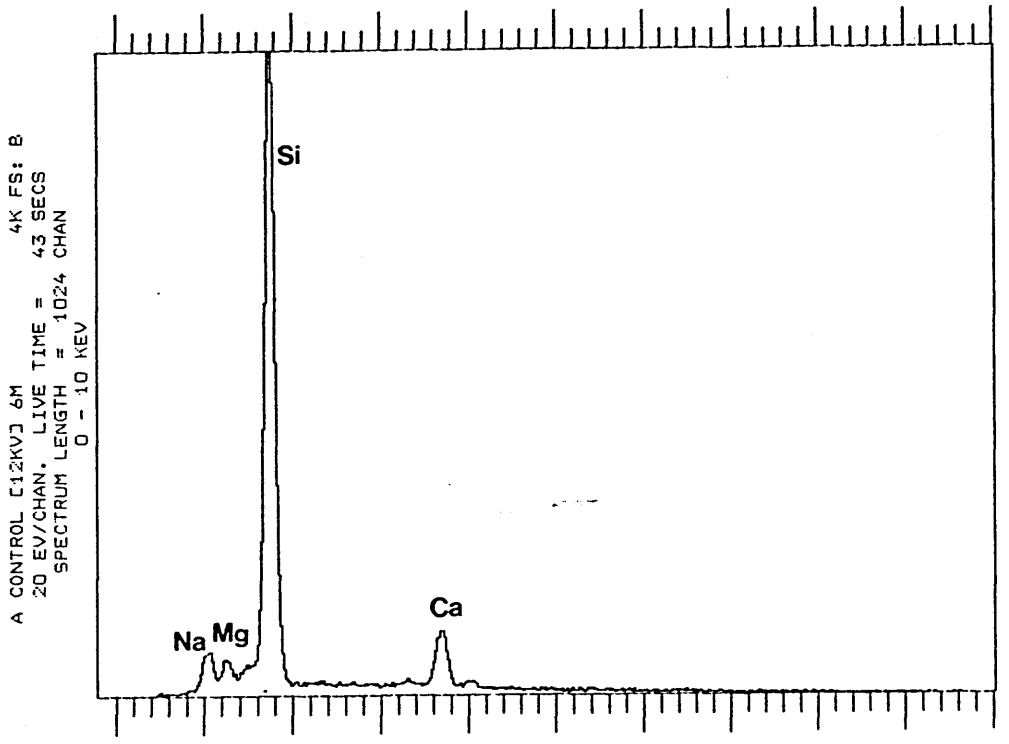
Note that the F and T values were calculated from the absolute values for the sample means and standard deviations (using appropriate computer software). The values given here for mean and standard deviation are rounded to 2 decimal places.

Analysis of A-glass fibre exposed to water

Effect on sodium (Na): after 6 months exposure a large decrease in the relative Na levels was observed. 12kV analysis showed a large decrease of **35.1%**, from **4.47** ± 0.52 (mean ± 2 SD, relative to silicon) to **2.90** ± 0.50 (F=1.05, T=9.65) and 25kV analysis gave a decrease of **36.5%**, from **1.89** ± 0.16 to **1.20** ± 0.40 (F=6.46, T=7.09).

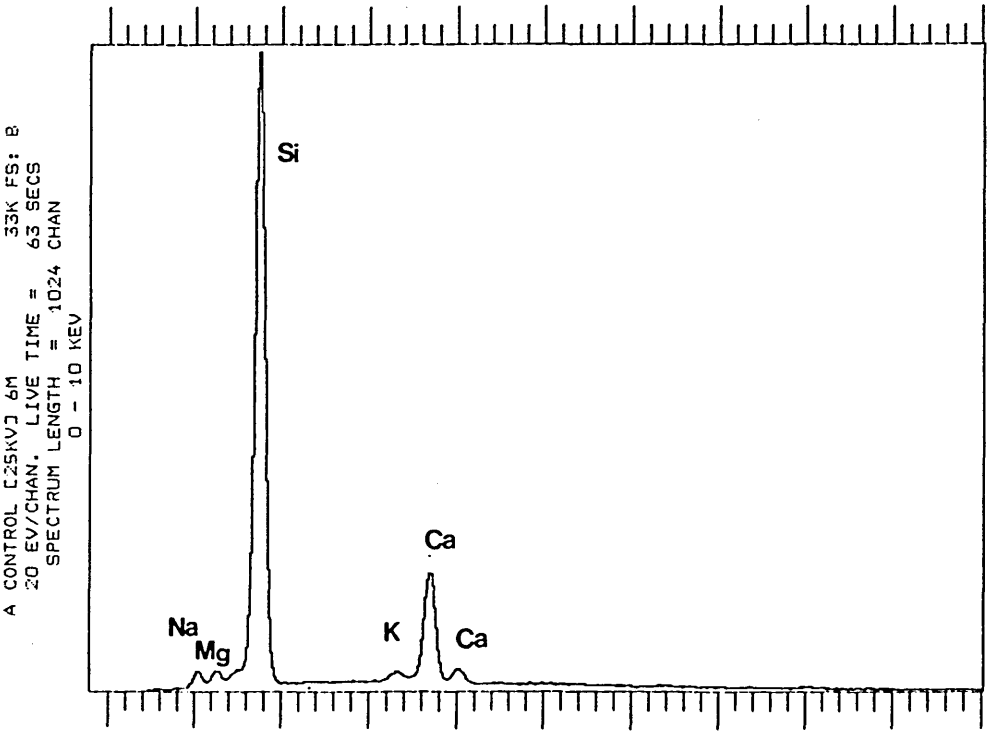
Effect on magnesium, aluminium and potassium (Mg, Al, K): no statistical effect.

Figure 4.12



12kV EDXA spectra of unexposed A-glass fibre

Figure 4.13

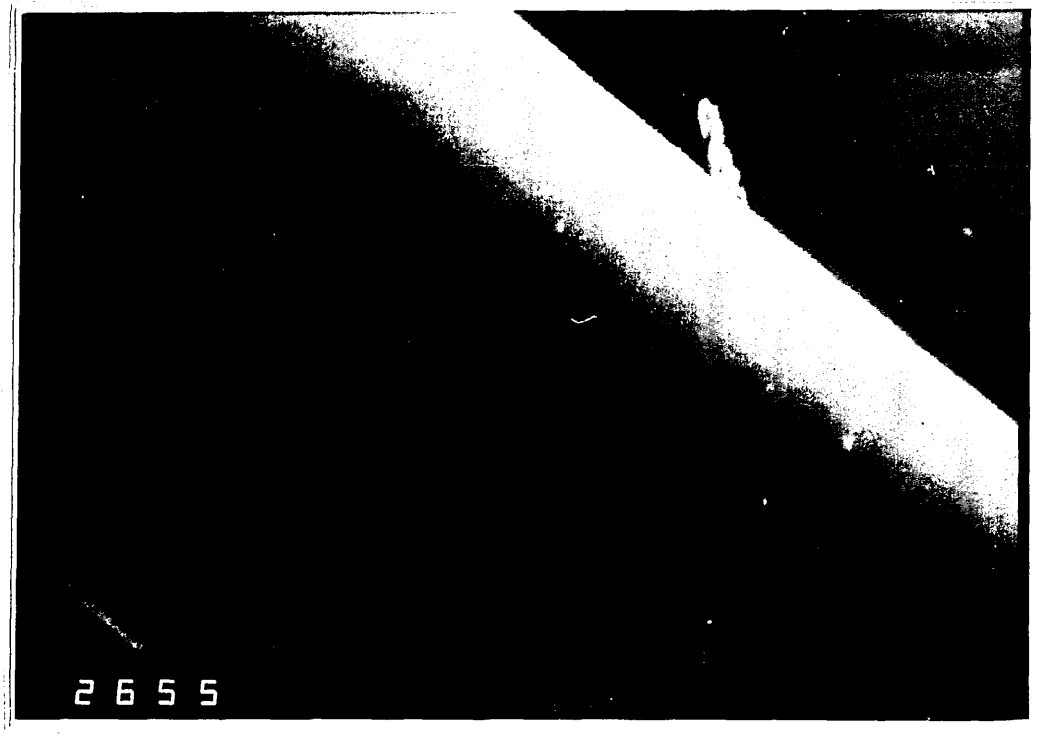


25kV EDXA spectra of unexposed A-glass fibre

Figure 4.14

SEM micrograph: un-exposed A-glass fibre (x 1600)

Figure 4.15



SEM micrograph: un-exposed A-glass fibre (x 12,500)

Effect on calcium (Ca): after 6 months exposure a moderate decrease in Ca levels was observed. 12kV analysis showed a large decrease of **11.1%**, from **9.26** ± 0.28 to **8.23** ± 0.14 (F=4.13, T=14.83) and 25kV analysis gave a decrease of **11.9%**, from **18.49** ± 1.04 to **16.29** ± 1.24 (F=1.43, T=6.05).

Analysis of A-glass fibre exposed to Gamble's fluid

Effect on sodium (Na): after 6 months exposure, 12kV analysis showed no significant change; however, 25kV analysis indicated an increase in Na of **14.8%**, from **1.89** ± 0.16 to **2.17** ± 0.26 (F=2.44, T=-4.22).

Effect on magnesium, aluminium, potassium and calcium: no statistically significant effect.

Analysis of A-glass fibre exposed to RPMI 1640

Insufficient test data was available for an accurate statistical analysis. The qualitative inference from the data obtained was that a moderate increase in Ca levels had occurred.

Analysis of A-glass fibre exposed to serum

Effect on sodium (Na): after 6 months exposure, 12kV analysis showed no significant change; however, 25kV analysis indicated an increase in Na levels of **14.8%**, from **1.89** ± 0.16 to **2.17** ± 0.18 (F=1.31, T=-5.16).

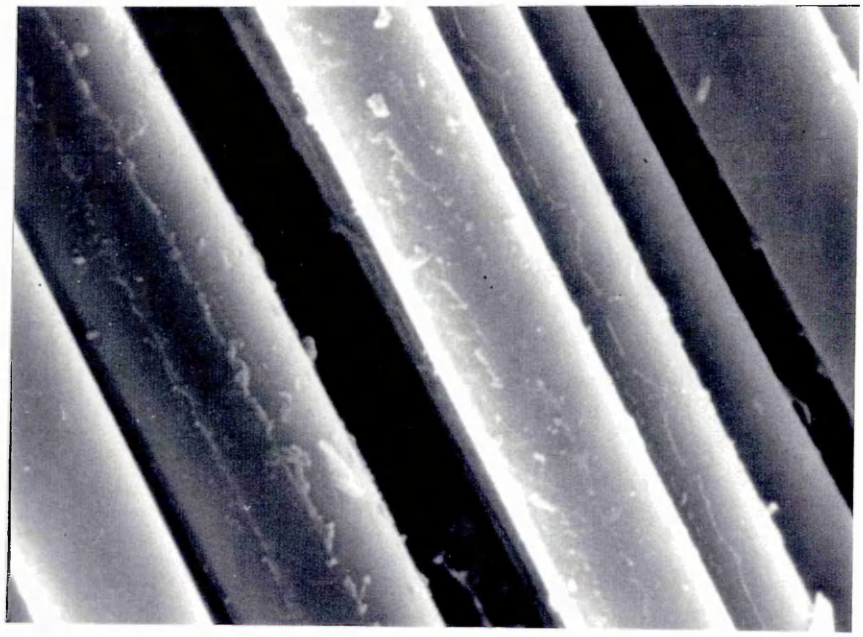
Effect on magnesium, aluminium and potassium (Mg, Al, K): no statistically significant effect.

Effect on calcium (Ca): after 6 months exposure, 12kV analysis indicated a **5.0%** increase in Ca levels, from **9.26** ± 0.28 to **9.72** ± 0.40 (F=2.05, T=-4.26).

Analysis of A-glass fibre exposed to pH10 buffer

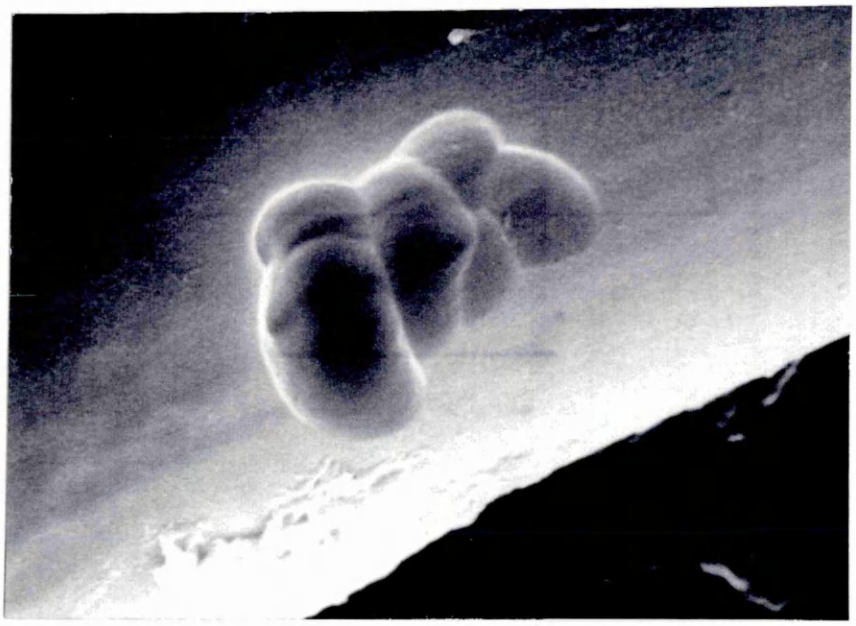
Selective leaching was not observed, however, morphological analysis showed some signs of surface corrosion (Figure 4.16-17).

Figure 4.16



SEM micrograph: hydrolysis of A-glass during exposure to pH 10 buffer (x 1,600)

Figure 4.17



SEM micrograph: hydrolysis of A-glass during exposure to pH 10 buffer (x 12,500)

E-glass fibre

This glass was a calcium boro-silicate and was expected to have good all-round durability because of the lattice stabilising effects of calcium and boron. Furthermore, an organic coating, introduced during manufacture, was present on the fibre surface.

Control EDXA spectra (unexposed samples) and control micrographs are shown in Figure 4.18-21.

Analysis of E-glass fibre exposed to Water

Effect on magnesium, aluminium and potassium (Mg, Al, Ca): no statistically significant effect after 12 months exposure.

Effect on calcium (Ca): after 12 months of exposure, 12kV analysis indicated a small increase in surface Ca levels of **5.1%**, from **30.41** ± 2.04 to **31.96** ± 1.80 (F=1.3, T=-3.61).

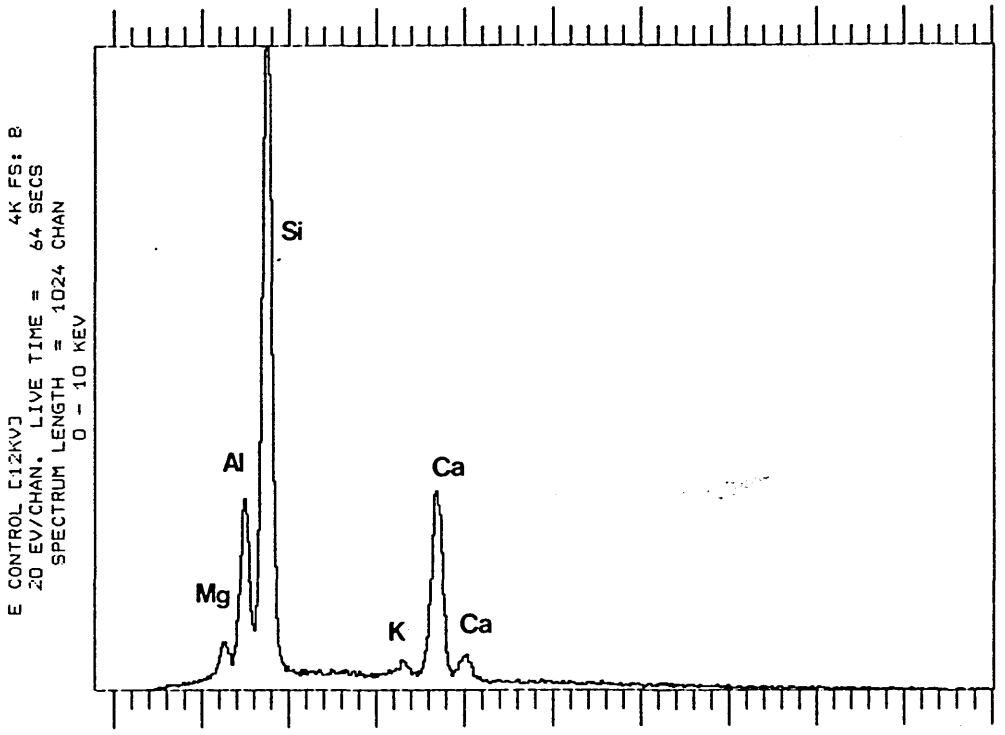
Effect on iron (Fe): no statistically significant effect.

Analysis of E-glass fibre exposed to Gamble's fluid:

Effect on magnesium (Mg): no statistically significant effect.

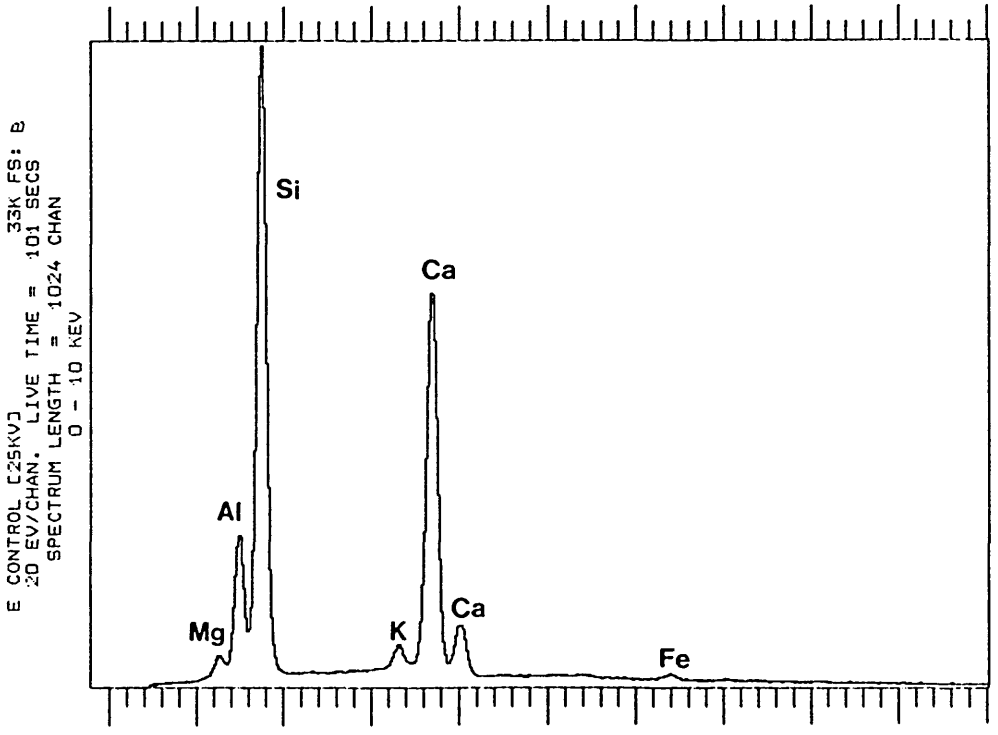
Effect on aluminium (Al): no effect after 6 month exposure, but 12kV analysis after 12 months exposure showed an increase in surface Al levels of **5.7%**, from **13.59** ± 0.86 to **14.37** ± 1.28 (F=2.24, T=-3.22).

Figure 4.18



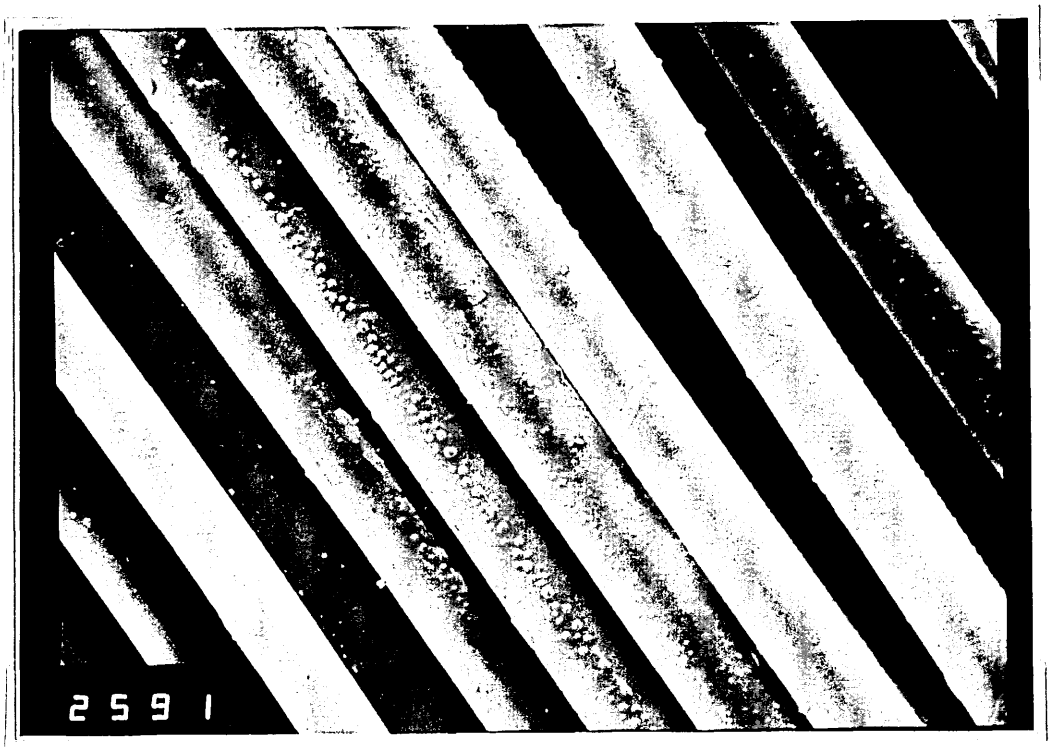
12kV EDXA spectra of unexposed E-glass fibre

Figure 4.19



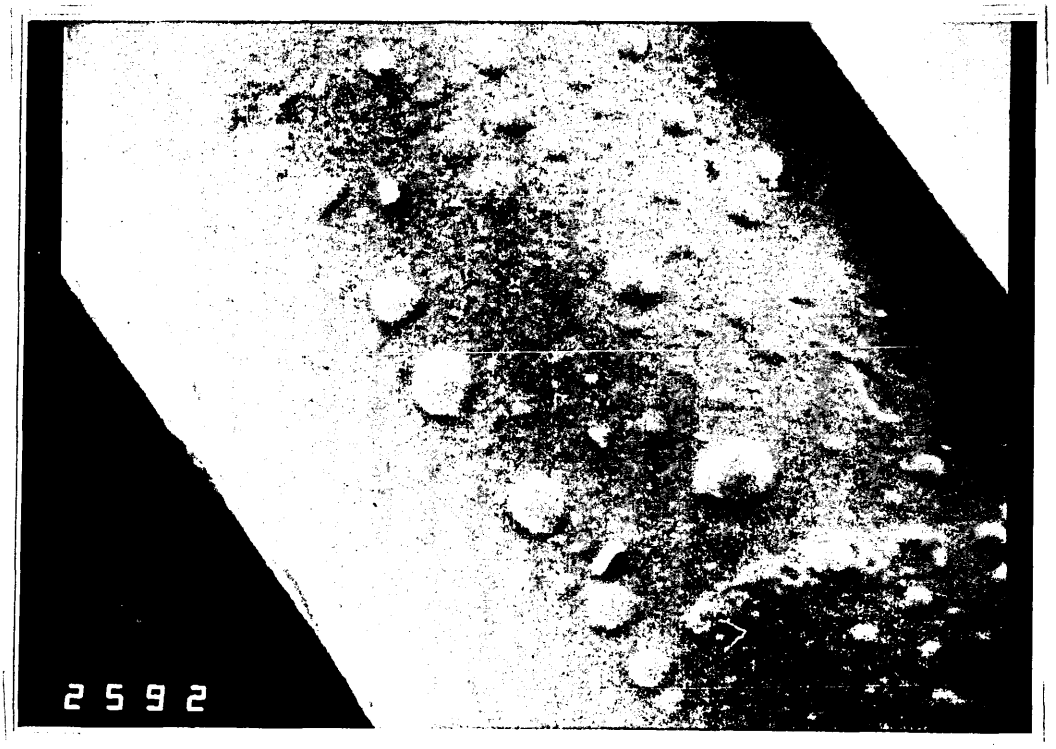
25kV EDXA spectra of unexposed E-glass fibre

Figure 4.20



SEM micrograph: un-exposed E-glass fibre (x 1600)

Figure 4.21



SEM micrograph: un-exposed E-glass fibre (x 12,500)

Effect on potassium (K):

No effect after 6 month exposure. After 12 months, 25kV analysis showed a moderate decrease in K levels of **8.9%**, from **3.05** ± 0.28 to **2.78** ± 0.48 (F=2.84, T=2.99).

Effect on calcium (Ca):

After 12 month exposure, 12kV analysis showed a moderate decrease in surface Ca levels, of **11.3%**, from **30.41** ± 2.04 to **26.96** ± 4.72 (F=5.39, T=4.23). 25kV analysis showed a decrease of **9.1%**, from **65.42** ± 2.22 to **59.45** ± 6.32 (F=8.18, T=5.64), where the calculated degrees of freedom = 11.2 and the critical T value = 3.1.

Effect on iron (Fe): no statistically significant effect.

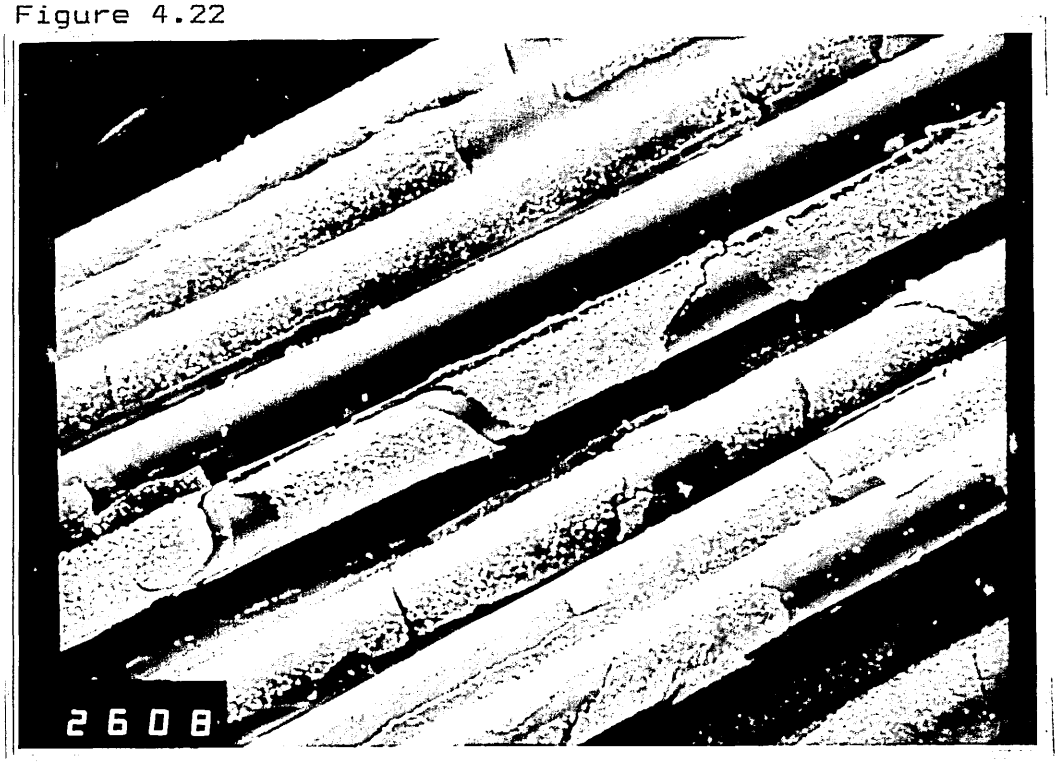
Analysis of E-glass fibre exposed to RPMI 1640

This data was interesting in that very thin, flaky surface layers had formed on the fibres after 12 months of exposure. The layer formation is shown in Figure 4.22 and 4.23

EDXA analysis of "undercoat" areas of the fibre showed no statistically significant change in composition. The composition of pieces of the coating, scraped off the fibre, is shown by the EDXA spectra, Figure 4.24 and 4.25.

The coat was found to be particularly rich in aluminium phosphorus (P) and calcium, with sodium, chlorine (Cl) and potassium also present. Silicon was notably absent from the coating.

Figure 4.22

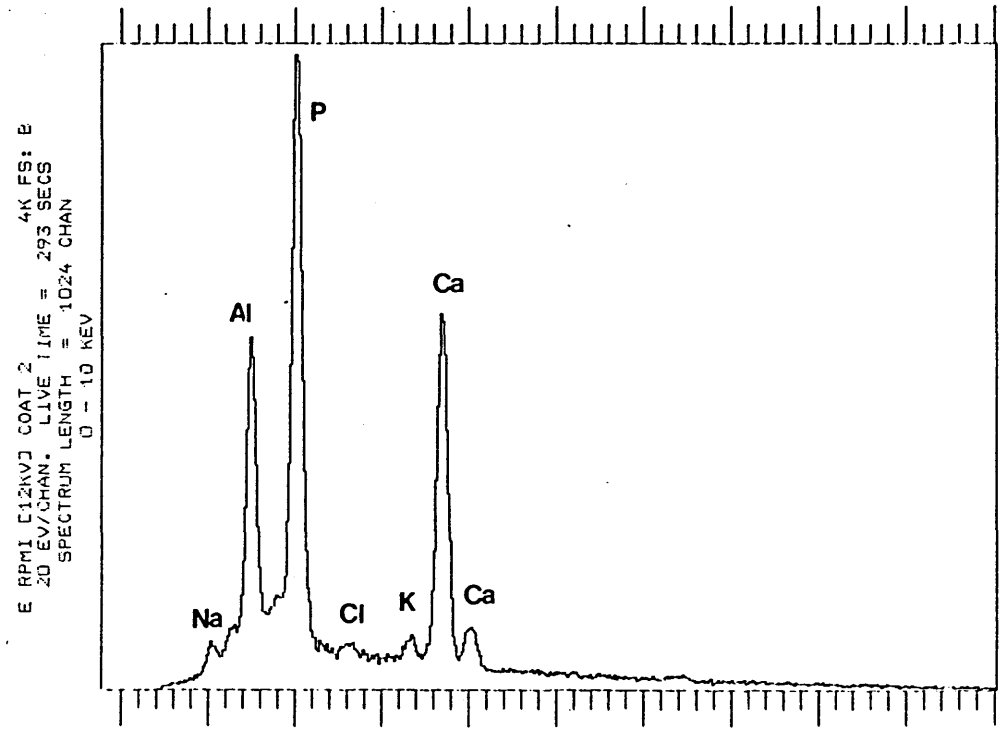


SEM micrograph: coat formation during E-glass exposure to RPMI 1640 (x 1,600)

Figure 4.23

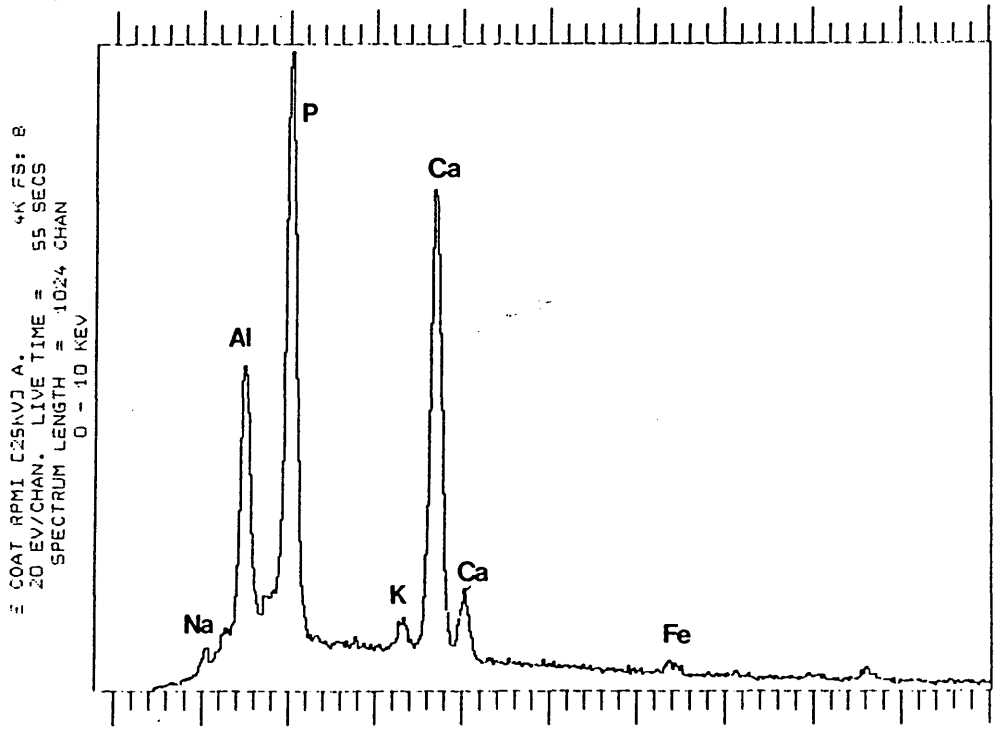
SEM micrograph: coat formation during E-glass exposure to RPMI 1640 (x 12,500)

Figure 4.24



12kV EDXA spectra from coated region of E-glass fibre exposed to RPMI 1640

Figure 4.25



25kV EDXA spectra from coated region of E-glass fibre exposed to RPMI 1640

Analysis of E-glass fibre exposed to PBS

As was the case with 12 month exposures to RPMI 1640, a thin flaky coating had formed on the fibre (Figure 4.26 and 4.27).

12kV analysis of areas of the fibre where coating was absent showed a moderate decrease in Al levels of **8.2%**, from **13.59** ± 0.86 to **12.48** ± 1.04 (F=1.49, T=5.18). 25kV analysis showed a decrease in Al levels of **6.6%**, from **11.79** ± 0.56 to **11.01** ± 0.60 (F=1.18, T=6.08).

Ca levels were apparently increased in the regions where coating was absent, 12kV analysis giving an increase of **12.0%**, from **30.41** ± 2.04 to **34.07** ± 6.54 (F=10.24, T= -3.39) where the calculated degrees of freedom = 10.7 and the critical T value = 3.1. 25kV showed no apparent change in Ca levels.

The composition of the coating is shown by the EDXA spectra, Figure 4.28 and 4.29. The composition of the coating appeared to be similar to the coat formed during RPMI 1640 exposure except that in this case the relative calcium levels were much lower. The major determinable element present were Al, P and Na. Cl, K and Ca were also found.

Analysis of E-glass fibre exposure to pH10 buffer

No change in the analyte levels relative to silicon, after 12 months exposure, except in the case of Ca, where 12kV analysis showed a moderate decrease of **8.2%**, from **30.41** ± 2.04 to **27.93** ± 1.68 (F=1.47, T=5.93).

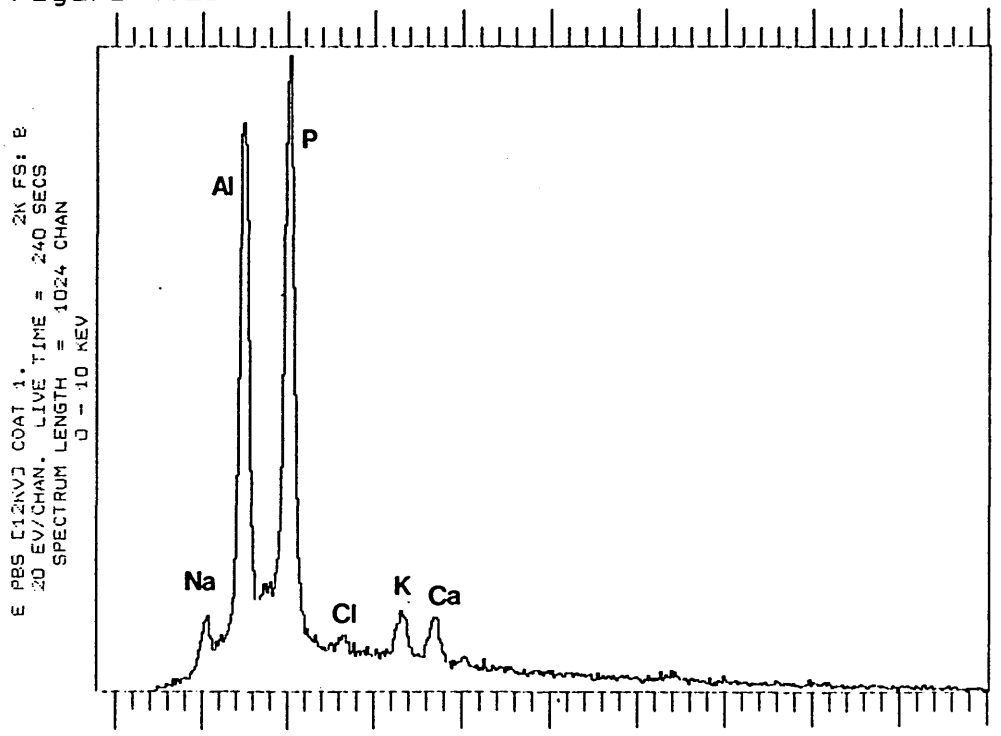
Figure 4.26

SEM micrograph: coat formation during E-glass exposure to PBS (x 1,600)

Figure 4.27

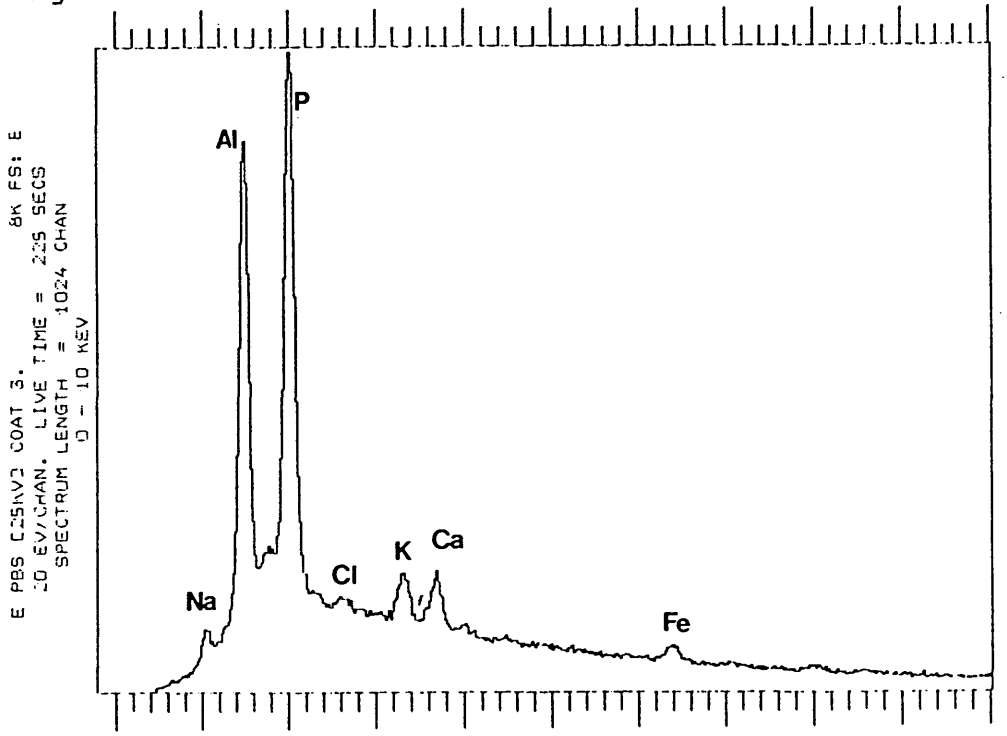
SEM micrograph: coat formation during E-glass exposure to PBS (x 12,500)

Figure 4.28



12kV EDXA spectra from coated region of E-glass fibre exposed to PBS

Figure 4.29



25kV EDXA spectra from coated region of E-glass fibre exposed to PBS

Analysis of E-glass fibre exposure to broncho-alveolar lavage fluid

Effect on magnesium: after 6 month exposure, 12kV analysis showed no statistically significant change in Mg levels relative to silicon. In contrast, 25kV analysis gave an increase of **16.7%**, from **1.14** ± 0.12 to **1.33** ± 0.10 (F=1.24, T=-5.08).

Effect on aluminium (Al): no statistically significant effect.

Effect on potassium (K): after 6 months exposure, 12kV analysis showed no change in potassium levels. 25kV analysis showed an apparently large decrease of **26.6%**, from **3.08** ± 0.24 to **2.26** ± 0.78 (F=10.28, T=4.42).

Effect on calcium: after 6 months exposure, 12kV analysis showed a moderate decrease in calcium levels, of **13.0%**, from **30.79** ± 1.84 to **26.78** ± 3.84 (F=4.32, T=4.21). 25kV analysis also gave a decrease in calcium levels, of **15.5%**, from **65.80** ± 4.18 to **55.62** ± 7.46 (F=3.18, T=5.33).

Effect on iron: no statistically significant effect.

Analysis of E-glass fibre exposed to Serum

No statistically significant effect on the relative levels of any of the analytes of interest.

Cemfil fibre

This glass is used in re-inforced cements and designed to be chemically un-reactive in an alkaline environment. This material also has an organic surface coating introduced during manufacture.

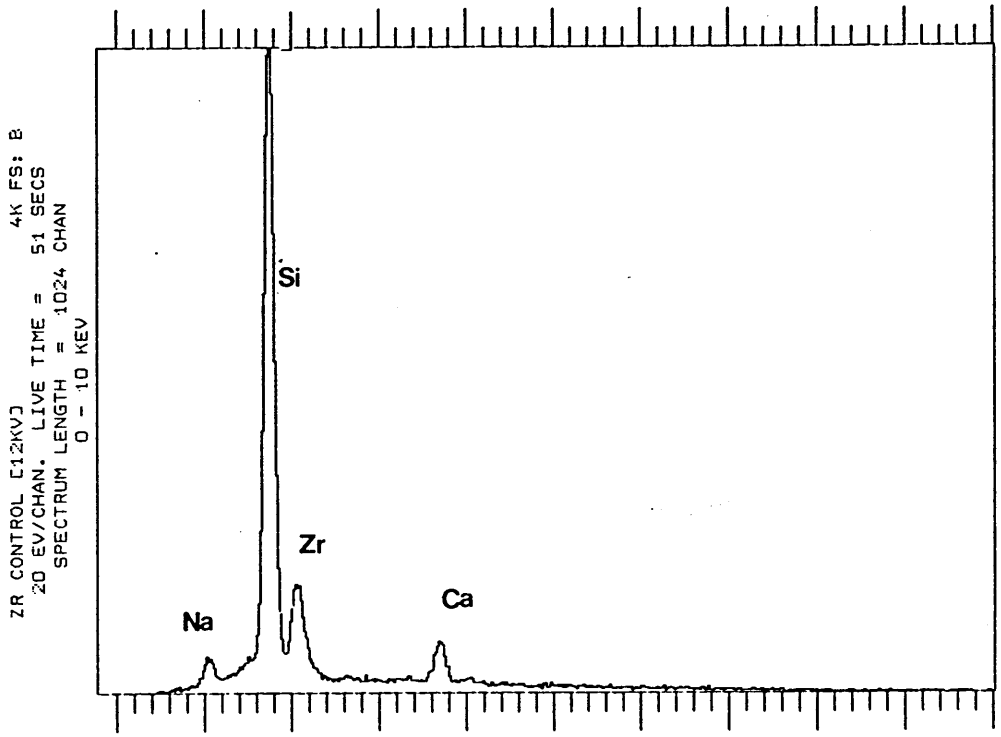
Control EDXA spectra (unexposed samples) and control micrographs are shown in Figure 4.30-33.

Analysis of Cemfil fibre exposed to water

Effect on sodium: it was observed that Cemfil fibre was particularly reactive in double-distilled de-ionised water. 12 kV EDXA of fibres exposed for 6 months to water showed a statistically significant (F=1.84, T=14.42) decrease in Na levels of **54.0%**, from **3.67** ± 0.5 in the controls to **1.69** ± 0.36 in the test samples. 12kV analysis of Cemfil fibres exposed for 12 months to water also showed a large percentage decrease in Na levels of **37.9%**, from **3.80** ± 0.68 in the controls to **2.36** ± 0.60 in the exposed samples (F=1.34, T=10.06). A decrease in Na levels was also observed using 25 kV EDXA of **19.0%**, from **2.05** ± 0.38 to **1.66** ± 0.24 (F=2.43, T=5.59).

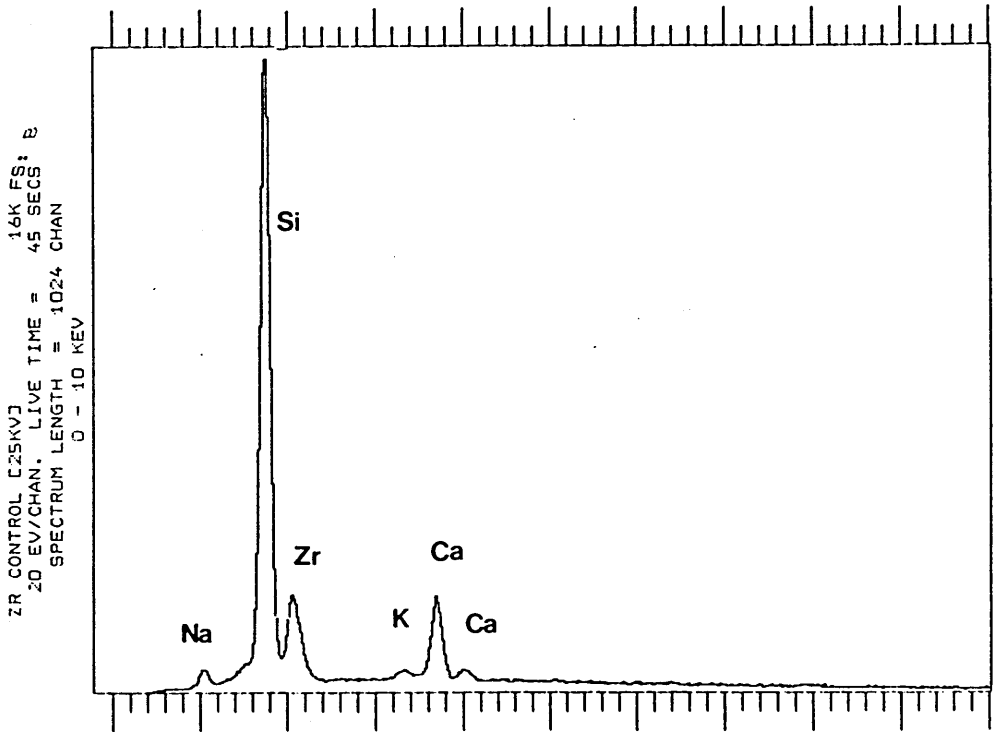
Effect on aluminium: 12 kV EDXA of fibres exposed for 6 months to water showed a statistically significant (F=7.19, T=-3.56) rise in Al levels of **132%** from **0.19** ± 0.10 to **0.44** ± 0.3. The Al increase was not apparent with 25kV EDXA. 12 month exposure showed no statistical change in the aluminium levels.

Figure 4.30



12kV EDXA spectra from unexposed Cemfil glass fibre

Figure 4.31

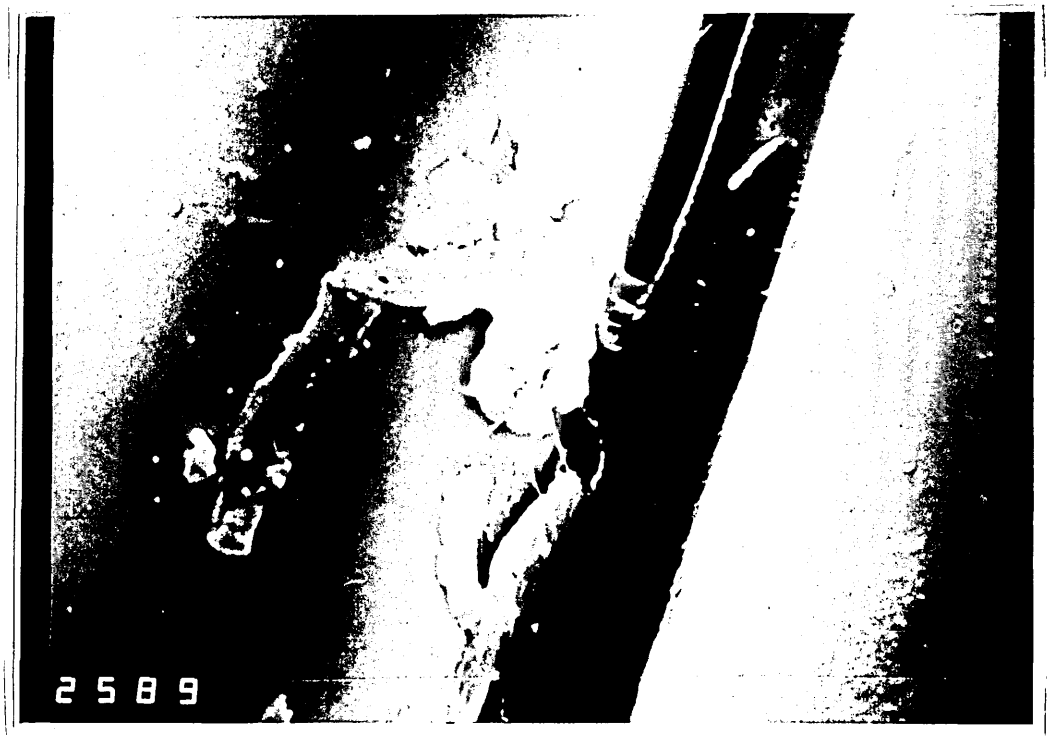


25kV EDXA spectra from unexposed Cemfil glass fibre

Figure 4.32

SEM micrograph: un-exposed Cemfil glass fibre (x 1600)

Figure 4.33



SEM micrograph: un-exposed Cemfil glass fibre (x 12,500)

Effect on zirconium (Zr): 12 and 25kV analyses showed decreased Zr levels in the fibre after six months of exposure. 12kV analysis demonstrated a moderate percentage decrease of **9.8%**, from **8.97** ± 0.36 to **8.09** ± 0.44 (F=1.52, T=6.81). 25kV analysis showed a decrease of **13.1%**, from **8.52** ± 0.26 to **7.4** ± 0.40. In contrast the 12 month data showed an increase in Zr levels for 12kV analysis of **16.4%**, from **7.75** ± 1.36 to **9.02** ± 1.94 (F=2.04, T=-3.38). 25kV data was not statistically different to control data.

Effect on potassium: the K peak intensity was relatively low, making accurate determination difficult, though there were no apparent changes in potassium levels as a result of exposure to water.

Effect on calcium (Ca): the initial effect, after 6 months of exposure was a decrease in Ca. The 12kV data showed a decrease (F=3.7, T=5.56) of **15.6%**, from **6.78** ± 0.40 to **5.72** ± 0.76 and the 25kV data showed a decrease (F=1.16, T=11.49) of **11.2%**, from **13.02** ± **0.38** to **11.56** ± **0.42**. In contrast, after 12 months there was no apparent change in Ca levels.

Analysis of Cemfil fibre exposed to Gamble's fluid

Effect on sodium: after 6 months exposure, there appeared to be an increase in surface Na levels; after 12 months a definite decrease in levels was observed. The 6 month, 12kV data showed an increase of **19.9%**, from **3.67** ± 0.50 to **4.4** ± 0.54 (F=1.17, T=-4.47) whilst 25kV data showed no significant change. In contrast the 12 month, 12kV data showed a **20.3%** decrease (F=1.9, T=4.21) from **3.80** ± 0.68 to **3.03** ± 0.94 and the 25kV data a **17%** decrease from **2.05** ± 0.38 to **1.70** ± 0.28 (F=1.87, T=4.76).

Effect on aluminium: generally no effect, though a large increase observed from 12kV data for the 6 month exposure. The increase was **84.2%** from **0.19** ± 0.10 to **0.35** ± 0.14 (F=1.71, T=-4.05), though the very low signal to noise ratio does mean that this data may be anomalous.

Effect on zirconium: exposure to Gamble's fluid had no statistically significant effect on the Zr levels.

Effect on potassium: no effect after 6 months but a decrease observed after 12 months using 25kV analysis of **26.4%**, from **0.87** ± 0.24 to **0.64** ± 0.26 (F=1.23, T=3.98). 12 month, 12kV data showed no statistically significant change.

Effect on calcium (Ca): as for K. Low levels of Ca leaching was observed after 12 months with 25kV analysis and a **3.4%** decrease occurred from **13.43** ± 0.60 to **12.97** ± 0.34 (F=3.00, T=4.15).

Analysis of Cemfil fibre exposed to RPMI 1640

The 6 month data, which suggested initial increases in the relative levels of Al and Zr, was not used as the amount of Cemfil fibre recovered was insufficient for statistical analysis.

Effect on sodium: 12 month exposures showed a clear decrease in Na levels. 12kV analysis showed a decrease of **27.1%**, from **3.80** ± 0.68 to **2.77** ± 0.90 (F=1.69, T=5.80) and 25kV analysis **33.2%**, from **2.05** ± 0.38 to **1.37** ± 0.42 (F=1.25, T=7.66).

Effect on aluminium, zirconium and potassium: no statistically significant effect was observed.

Effect on calcium: 25kV analysis showed a moderate increase in Ca levels of **5.0%**, from **13.43** ± 0.60 to **14.1** ± 0.90 (F=2.23, T=-3.87).

Analysis of Cemfil fibre exposed to PBS

Effect on sodium: 12 month exposures showed leaching of Na with 12 and 25kV analysis. 12kV analysis indicated a decrease of **32.6%**, from **3.80** ± 0.68 to **2.56** ± 0.62 (F=1.21, T=8.48), and 25kV a decrease of **18.1%**, from **2.05** ± 0.38 to **1.68** ± 0.48 (F=1.58, T=3.85).

Effect on aluminium: no statistically significant effect.

Effect on zirconium: 12 and 25kV data indicated a statistically significant increase in the relative Zr

levels after 12 months of exposure. 12kV analysis gave an increase in Zr levels of **18.1%**, from **7.75** ± 1.36 to **9.15** ± 2.2 (F=2.62, T=-3.41). The 25kV data showed a moderate increase in Zr levels of **9.1%**, from **7.78** ± 0.78 to **8.49** ± 0.56 (F=1.92, T=-4.70).

Effect on potassium and calcium: no statistically significant effect.

Analysis of Cemfil glass fibre exposed to high pH (pH10 bicarbonate buffer)

Effect on sodium: paradoxically, by 12 months of exposure, no change in Na levels was observed using 12kV analysis; this was generally more surface sensitive and also more sensitive to light elements, for example Na, than 25kV analysis. However, 25kV analysis showed an increase in Na levels of **21.0%**, from **2.05** ± 0.38 to **2.48** ± 0.36 (F=1.11, T=-5.28).

Effect on aluminium: no statistically significant effect.

Effect on zirconium: after 12 months of exposure and using 12kV analysis, a statistically significant increase in Zr levels was observed of **12.5%**, from **7.75** ± 1.36 to **8.72** ± 1.20 (F=1.28, T=-3.37).

Effect on potassium and calcium: no statistically significant effect.

Analysis of Cemfil fibre exposed to Broncho-alveolar Lavage Fluid

Effect on sodium: six month exposures, followed by 12kV analysis, showed leaching of Na. A decrease of **57.2%** was observed, from **3.67** ± 0.50 to **1.57** ± 0.56 (F=1.32, T=12.48). No statistically significant change was observed using 25kV analysis.

Effect on aluminium: no statistically significant effect.

Effect on zirconium: six month exposures, followed by 12kV analysis, showed a decrease in the relative levels of Zr. A decrease of **11.4%** was observed, from **8.97** ± 0.36 to **7.95** ± 0.96 (F=6.88, T=4.48). No statistically significant change was observed using 25kV analysis.

Effect on potassium: no statistically significant effect.

Effect on calcium: six month exposures, followed by 12kV analysis, showed a decrease in the relative levels of Ca. A decrease of **8.6%** was observed, from **6.78** ± 0.40 to **6.20** ± 0.60 (F=2.26, T=3.67). No statistically significant change was observed using 25kV analysis.

Analysis of Cemfil fibre exposed to serum

No statistically significant effect.

Lead-glass fibre

This glass was supplied from an experimental batch of fibre used by a commercial company. The fibre varied in composition to such a degree that statistical analysis was unsuitable. However, this glass tended to be very reactive in aqueous solutions so that gross changes in composition could be observed from EDXA spectra. Control EDXA spectra and micrographs are shown in Figure 4.34-37.

Analysis of Lead-glass fibre exposed to Water

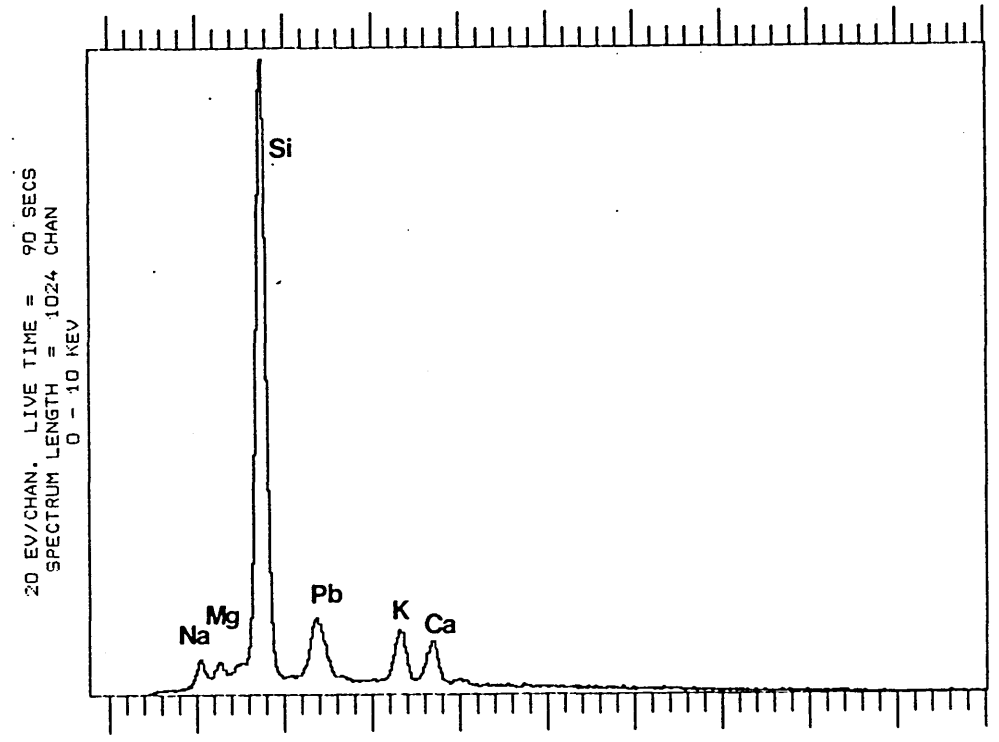
Due to the large variation of analyte levels in the control samples, changes resulting from exposure to water could not be determined.

Analysis of Lead-glass fibre exposed to Gamble's fluid

Test samples were seen from micrographs (Figure 4.38-39) to be heavily coated after 12 months exposure to Gamble's fluid. The 12 and 25kV EDXA spectra of the coat are shown in Figure 4.40 and 4.41, demonstrating the presence of P, Pb, Cl and Ca. The 25kV spectra also shows a Si peak.

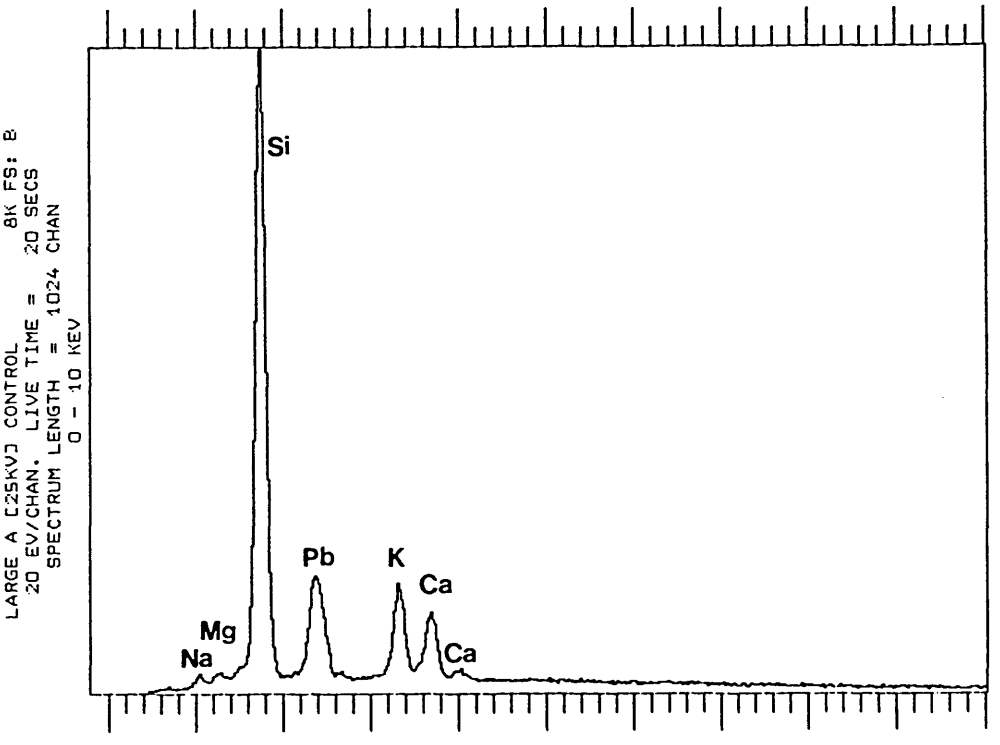
EDXA spectra were also obtained from exposed regions of the fibre surface, where the coating was damaged, or dislodged (Figure 4.42 and 4.43). The 12kV spectra shows that extensive leaching of the fibre surface occurred leaving a silica outer shell.

Figure 4.34



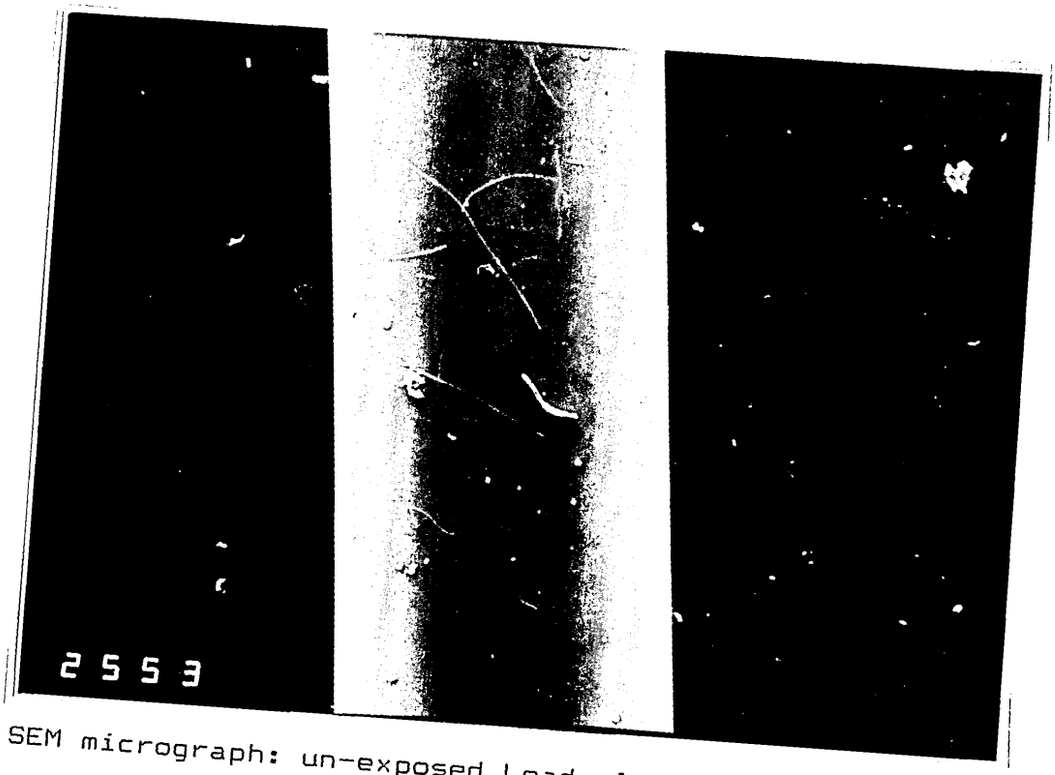
12kV EDXA spectra from unexposed Lead-glass fibre

Figure 4.35



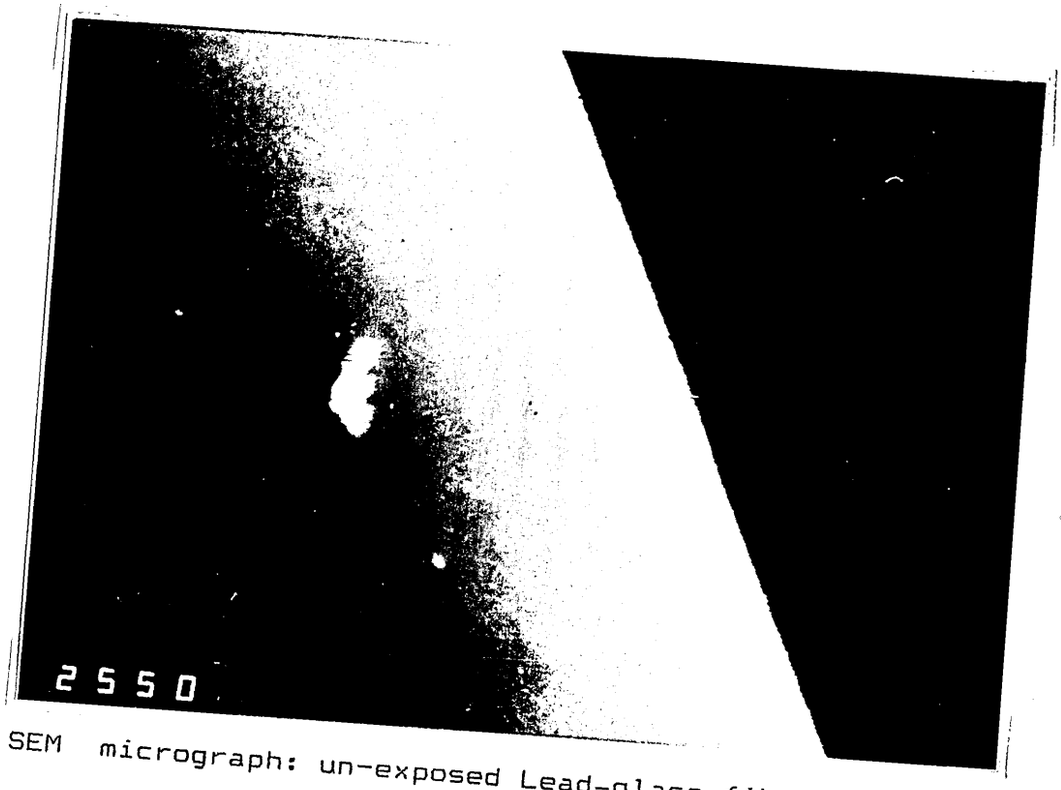
25kV EDXA spectra from unexposed Lead-glass fibre

Figure 4.36



SEM micrograph: un-exposed Lead-glass fibre (x 1,600)

Figure 4.37

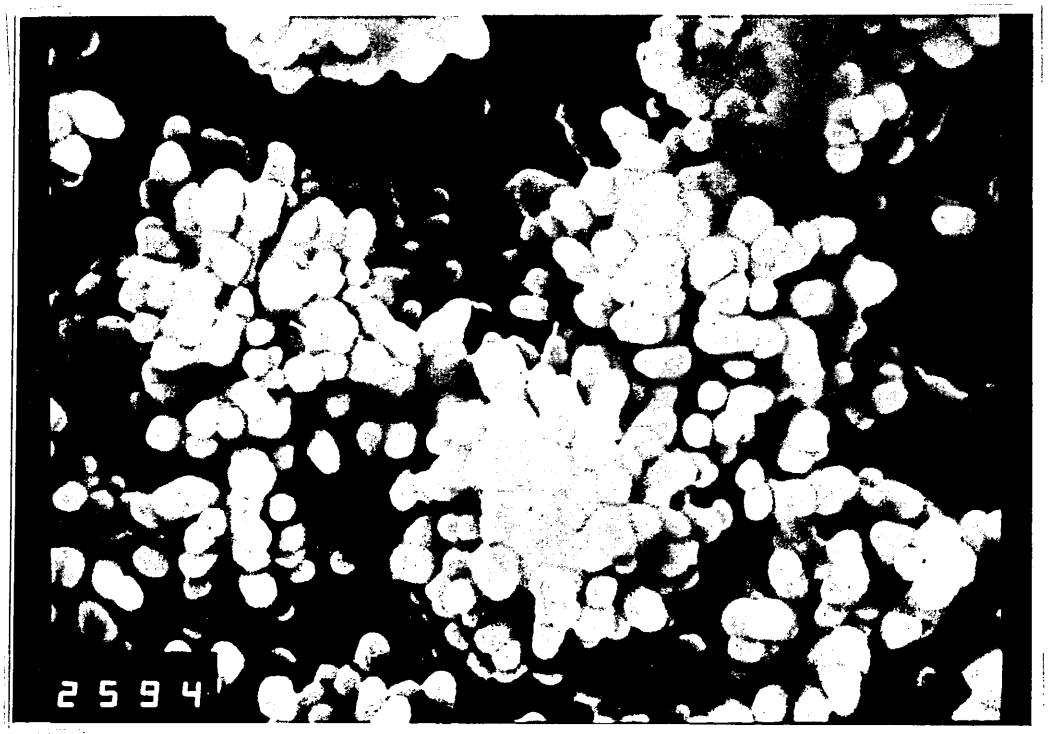


SEM micrograph: un-exposed Lead-glass fibre (x 12,500)

Figure 4.38

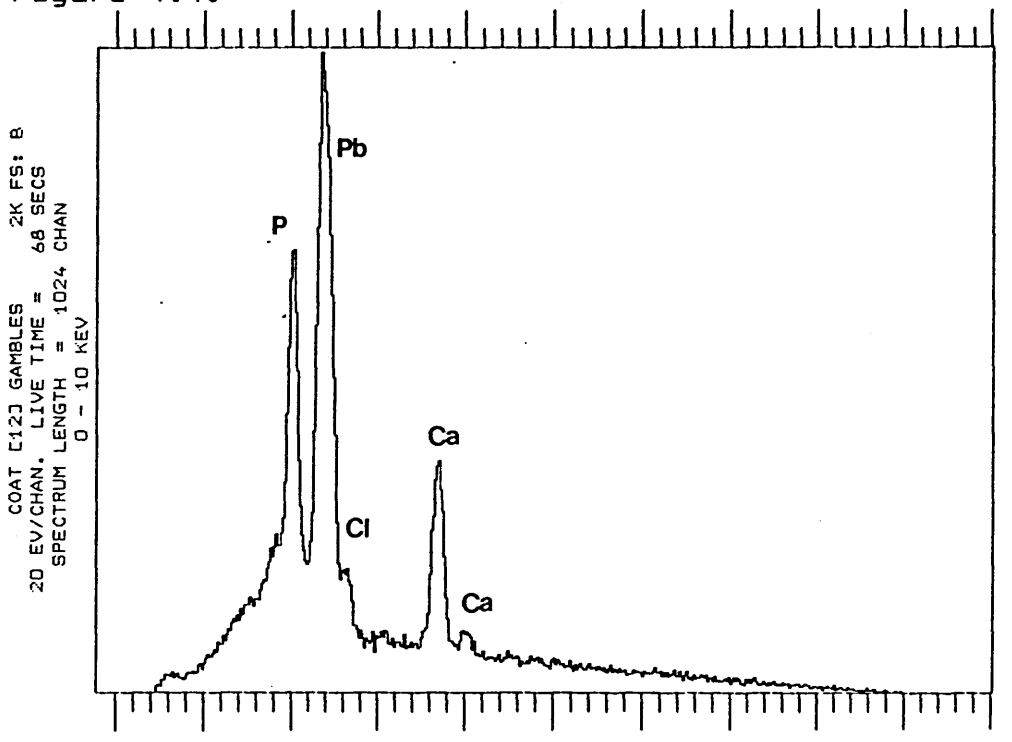
SEM micrograph: coat formation during Lead-glass fibre exposure to Gamble's fluid (x 1,600)

Figure 4.39



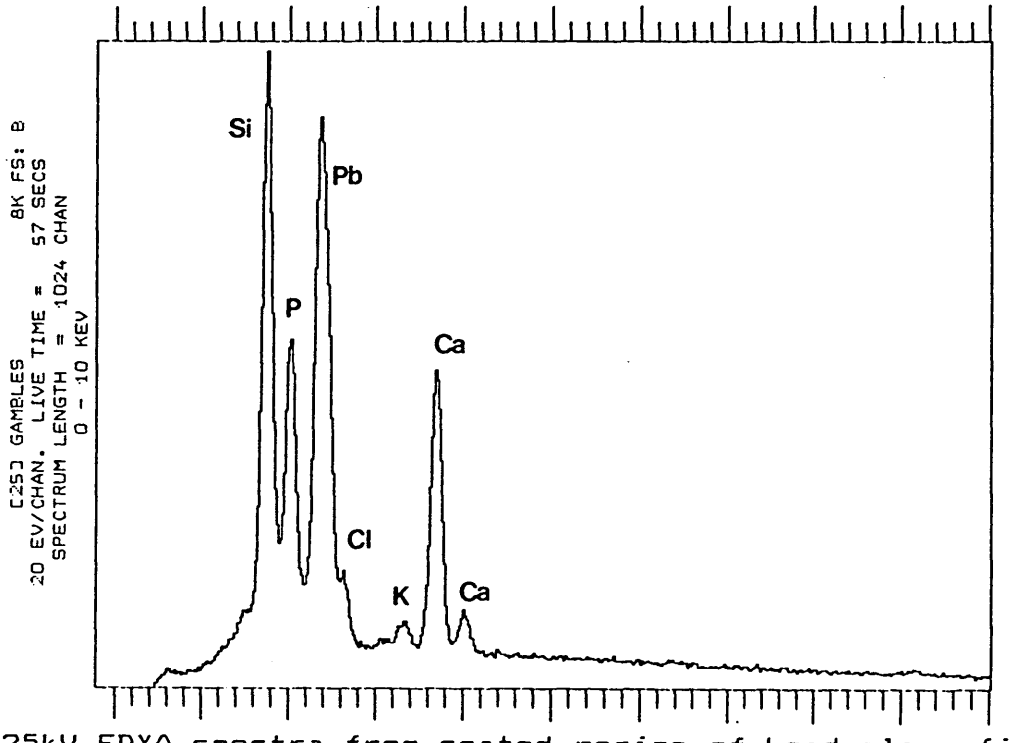
SEM micrograph: coat formation during Lead-glass fibre exposure to Gamble's fluid (x 12,500)

Figure 4.40



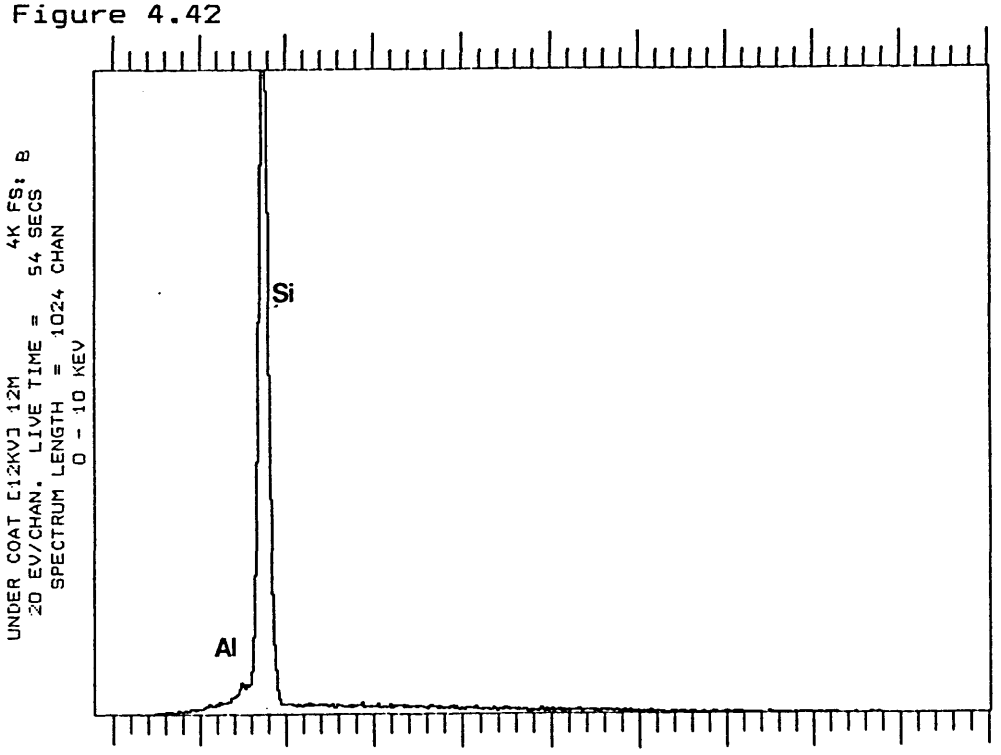
12kV EDXA spectra from coated region of Lead-glass fibre exposed to Gamble's fluid

Figure 4.41



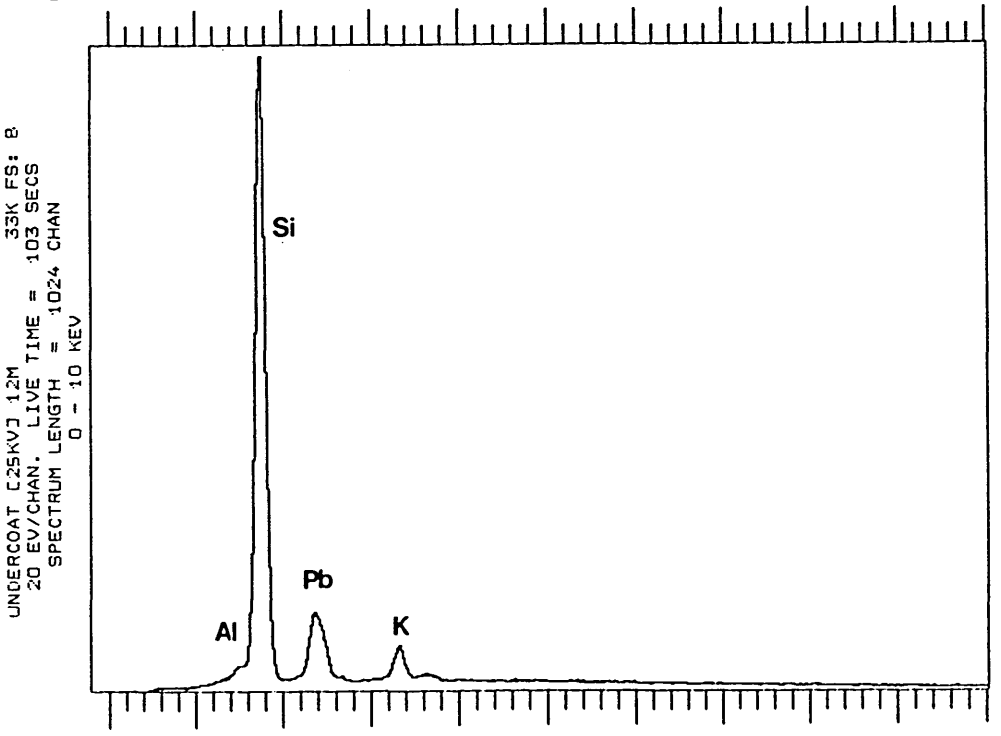
25kV EDXA spectra from coated region of Lead-glass fibre exposed to Gamble's fluid

Figure 4.42



12kV EDXA spectra from uncoated region of Lead-glass fibre exposed to Gamble's fluid

Figure 4.43



25kV EDXA spectra from uncoated region of Lead-glass fibre exposed to Gamble's fluid

Analysis of Lead-glass fibre exposed to RPMI 1640

Exposure to RPMI 1640 also resulted in the formation of a thick coating on the lead-glass fibre. This is shown in the micrographs, Figure 4.44 and 4.45, 12 and 25kV spectra are shown in Figure 4.46 and 4.47. The major determinable elements were P, Pb, Cl and Ca.

12kV EDXA spectra (Figure 4.48-49) of exposed areas of the fibre surface suggested that Na, Mg, Pb and K and Ca levels were reduced relative to silicon.

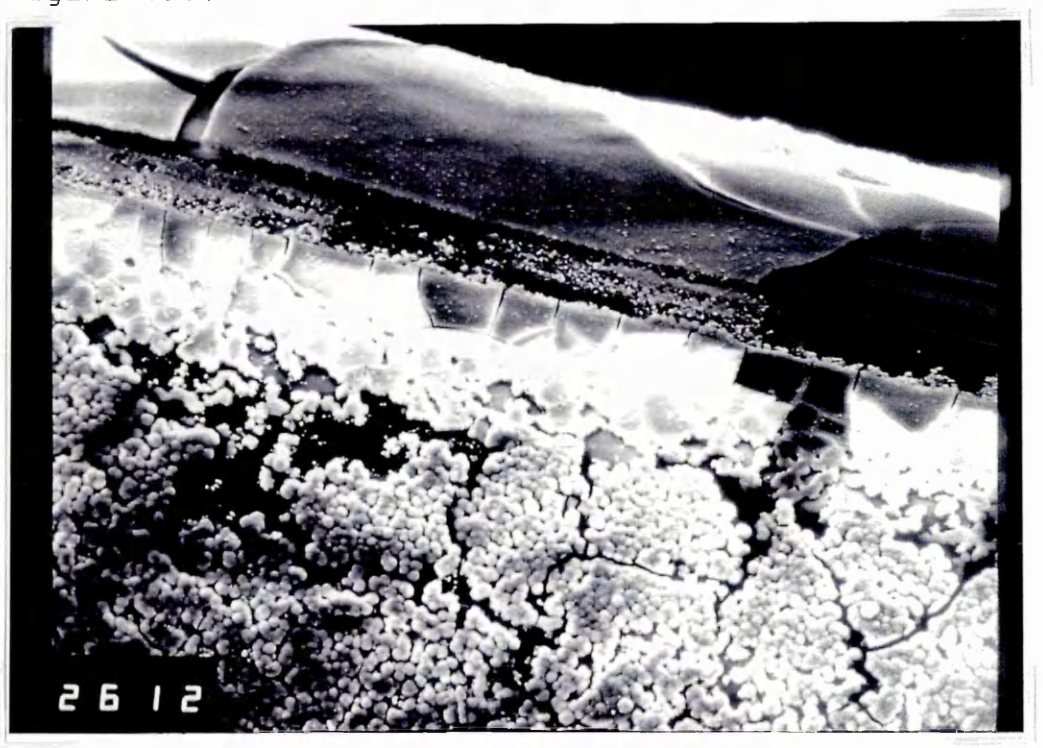
Analysis of Lead-glass fibre exposed to PBS

Extensive coat formation was observed, on the fibre surface (micrographs, Figure 4.50-51). EDXA spectra from coated regions are shown in Figure 4.52 and 4.53. P, Cl and a large Pb peak were present.

Analysis of Lead-glass fibre exposed to high pH (pH 10 Buffer)

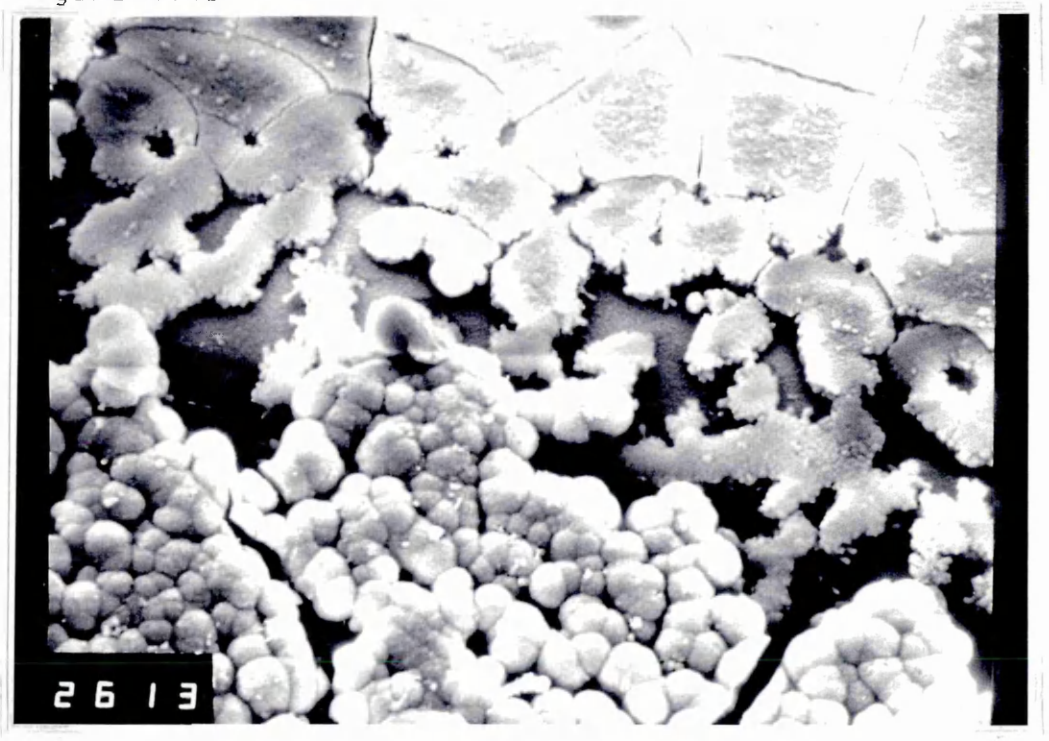
Flaky surface layers were observed on the fibres (Figure 4.54 and 4.55). These appeared to be of the same composition as the underlying fibre and it was thought that hydrolysis of the fibre was resulting in flaking off of surface layers. There also appeared to be regions where localised fibre corrosion was occurring (Figure 4.56-57).

Figure 4.44



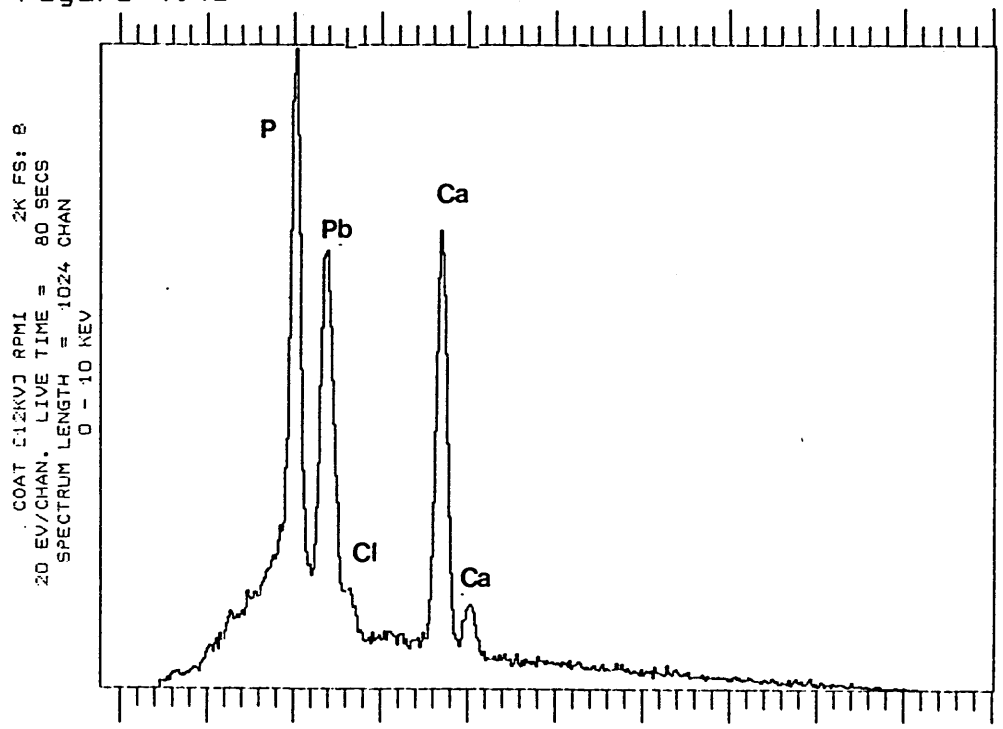
SEM micrograph: coat formation during Lead-glass fibre exposure to RPMI 1640(x 1,600)

Figure 4.45



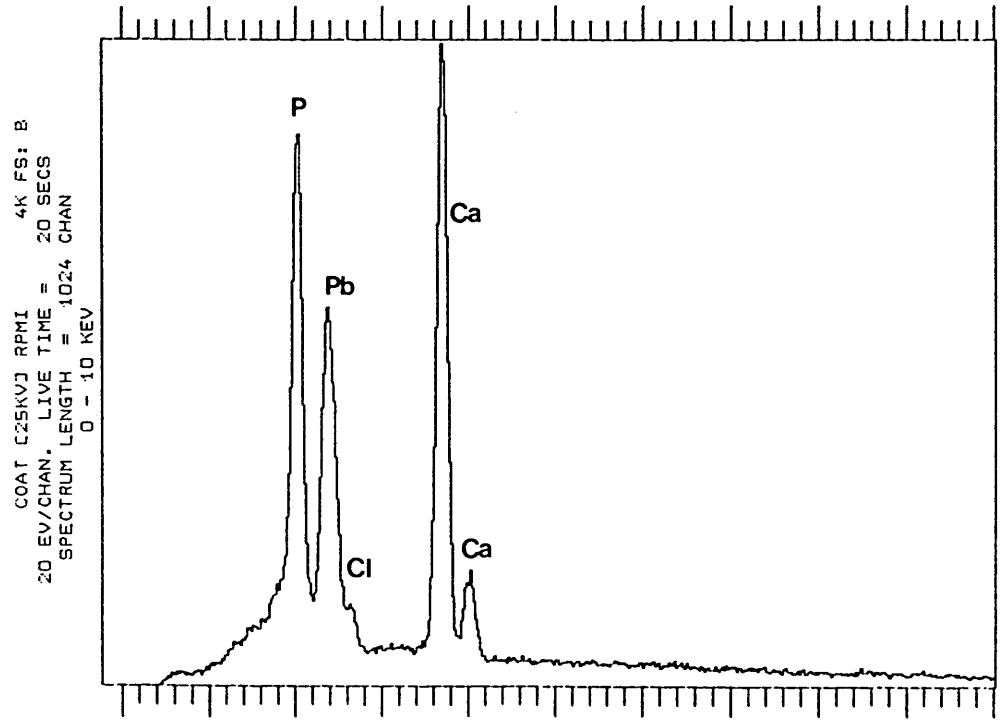
SEM micrograph: coat formation during Lead-glass fibre exposure to RPMI 1640 (x 12,500)

Figure 4.46



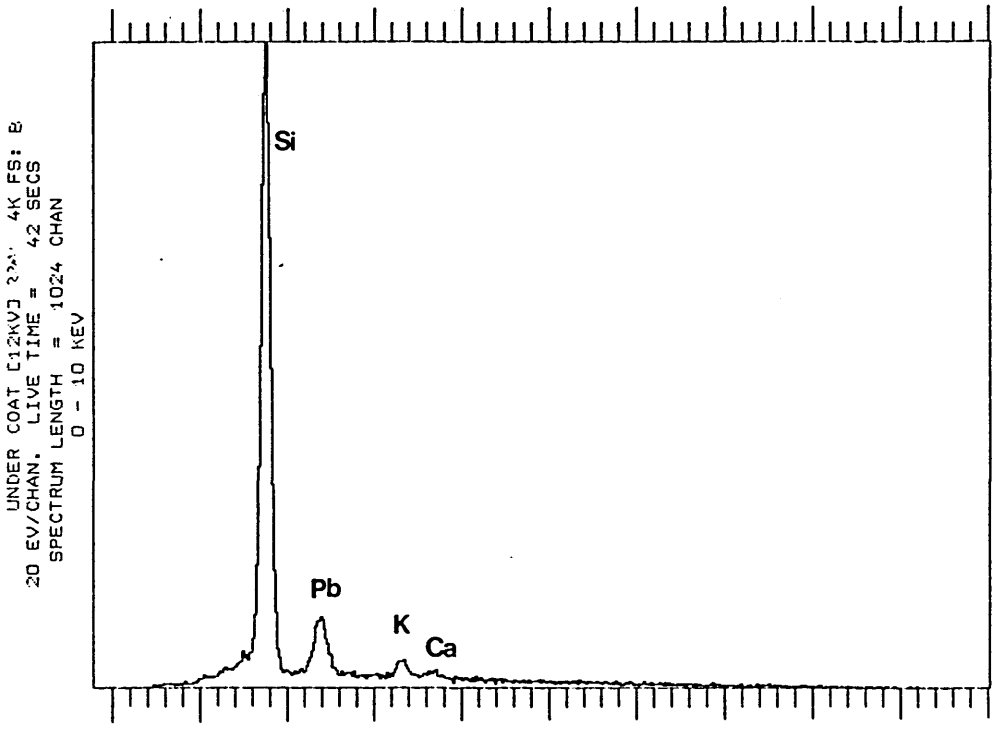
12kV EDXA spectra from coated region of Lead-glass fibre exposed to RPMI 1640

Figure 4.47



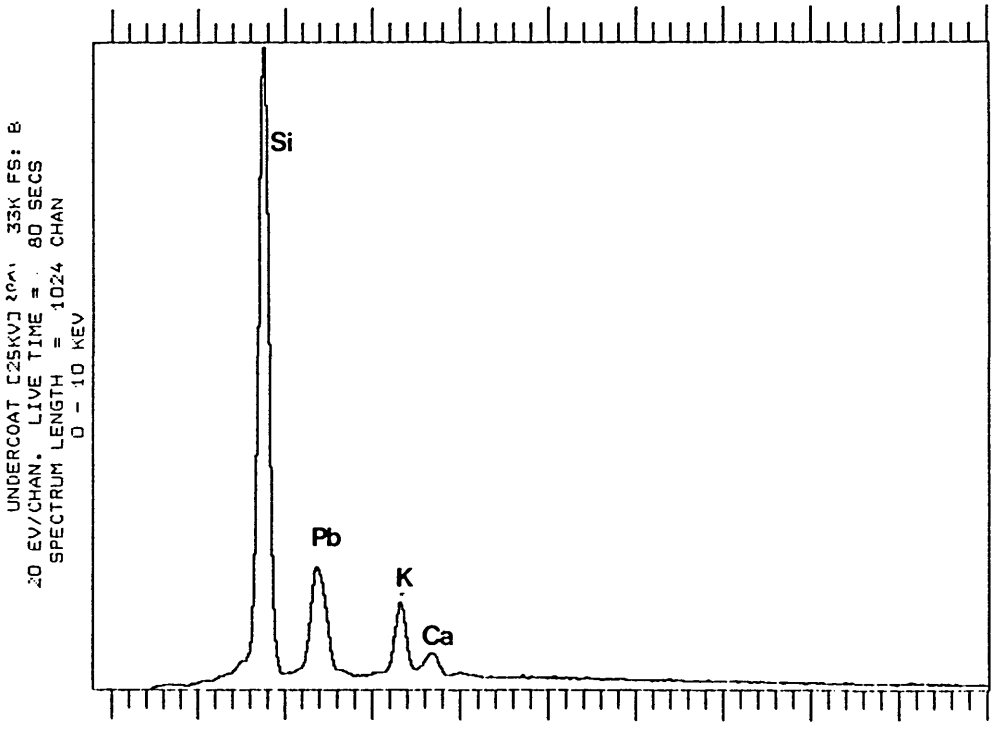
25kV EDXA spectra from coated region of Lead-glass fibre exposed to RPMI 1640

Figure 4.48



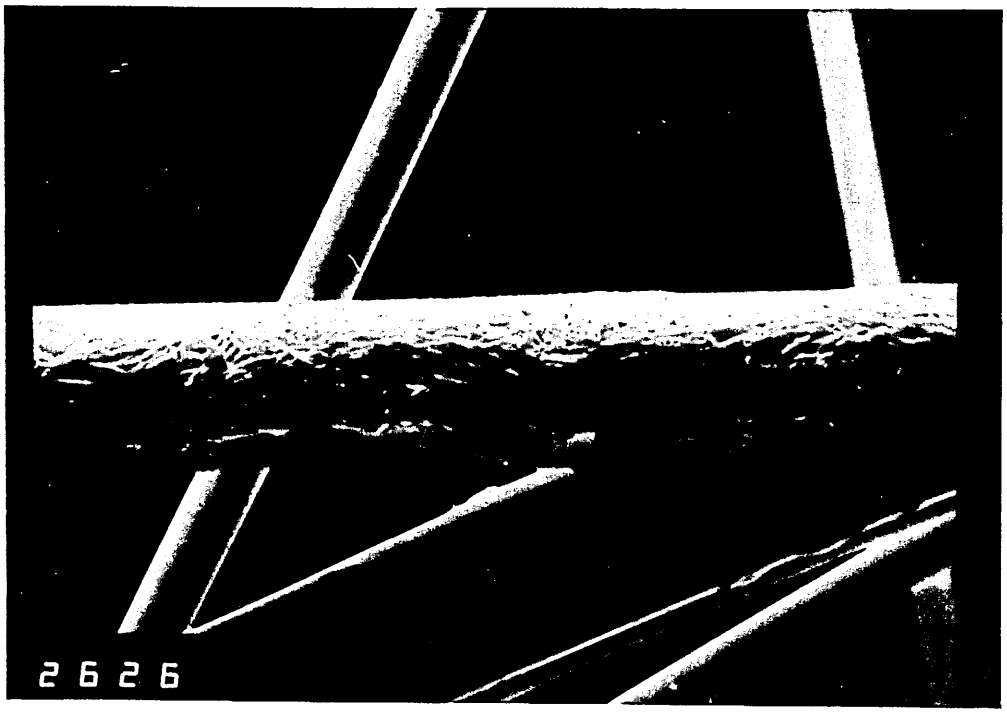
12kV EDXA spectra from uncoated region of Lead-glass fibre exposed to RPMI 1640

Figure 4.49



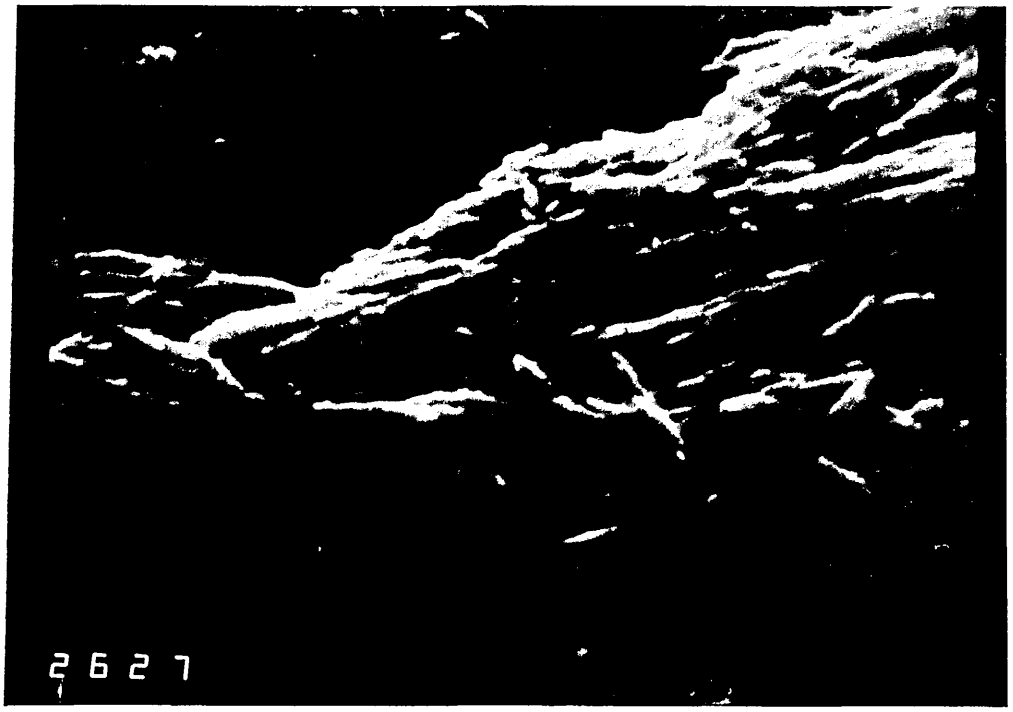
25kV EDXA spectra from uncoated region of Lead-glass fibre exposed to RPMI 1640

Figure 4.50



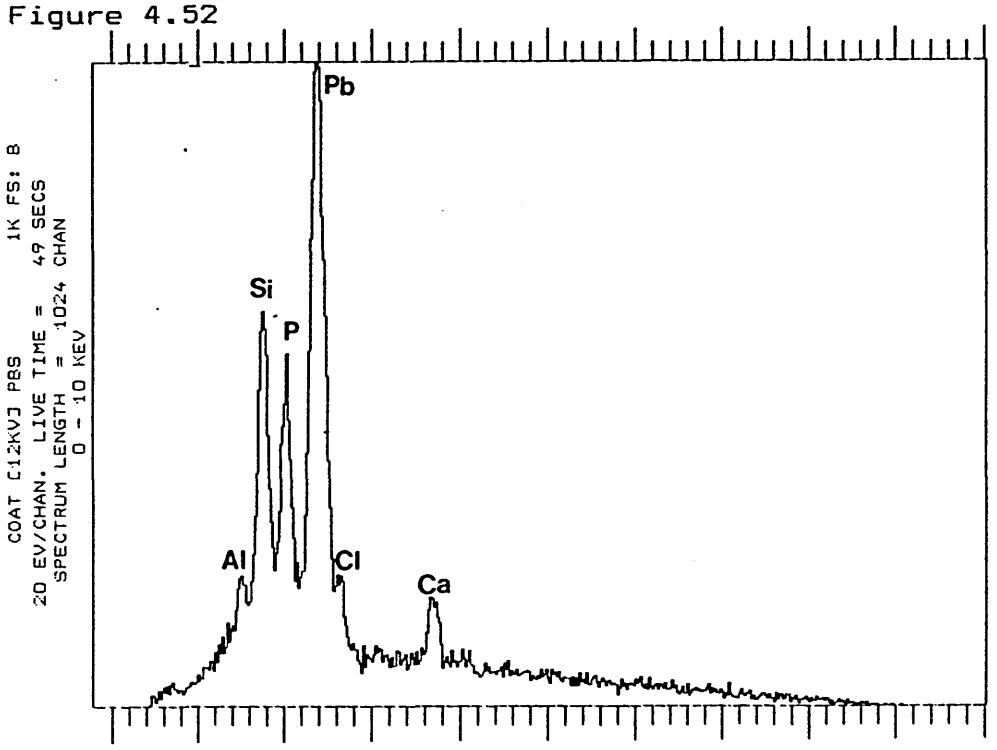
SEM micrograph: coat formation during Lead-glass fibre exposure to PBS (x 1,600)

Figure 4.51



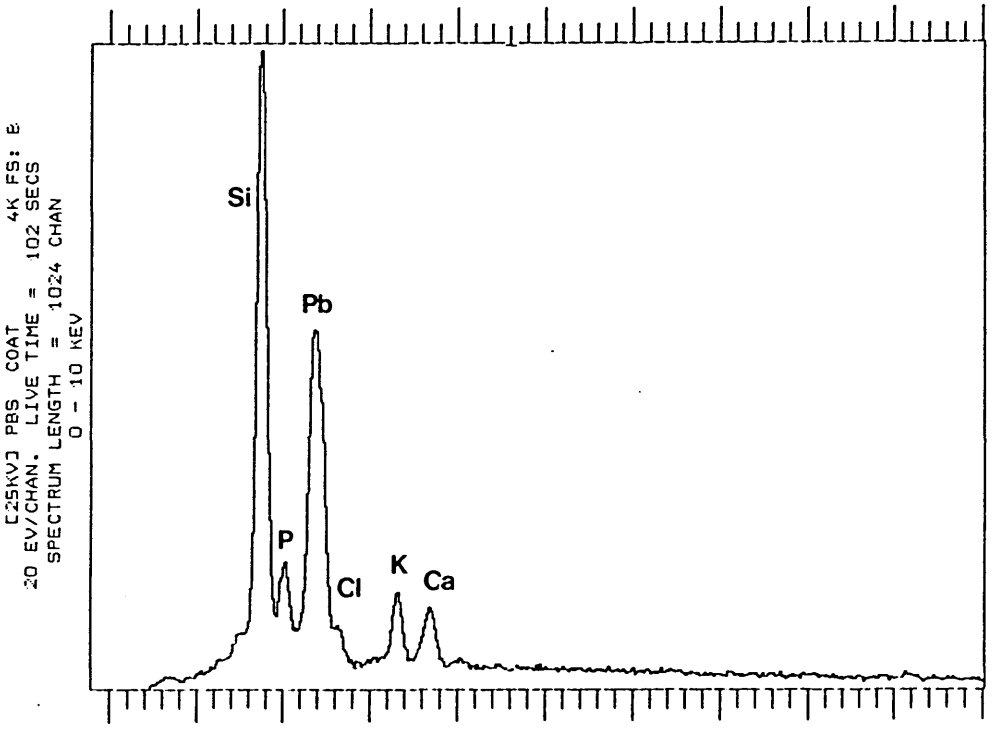
SEM micrograph: coat formation during Lead-glass fibre exposure to PBS (x 12,500)

Figure 4.52



12kV EDXA spectra from coated region of Lead-glass fibre exposed to PBS

Figure 4.53



25kV EDXA spectra from coated region of Lead-glass fibre exposed to PBS

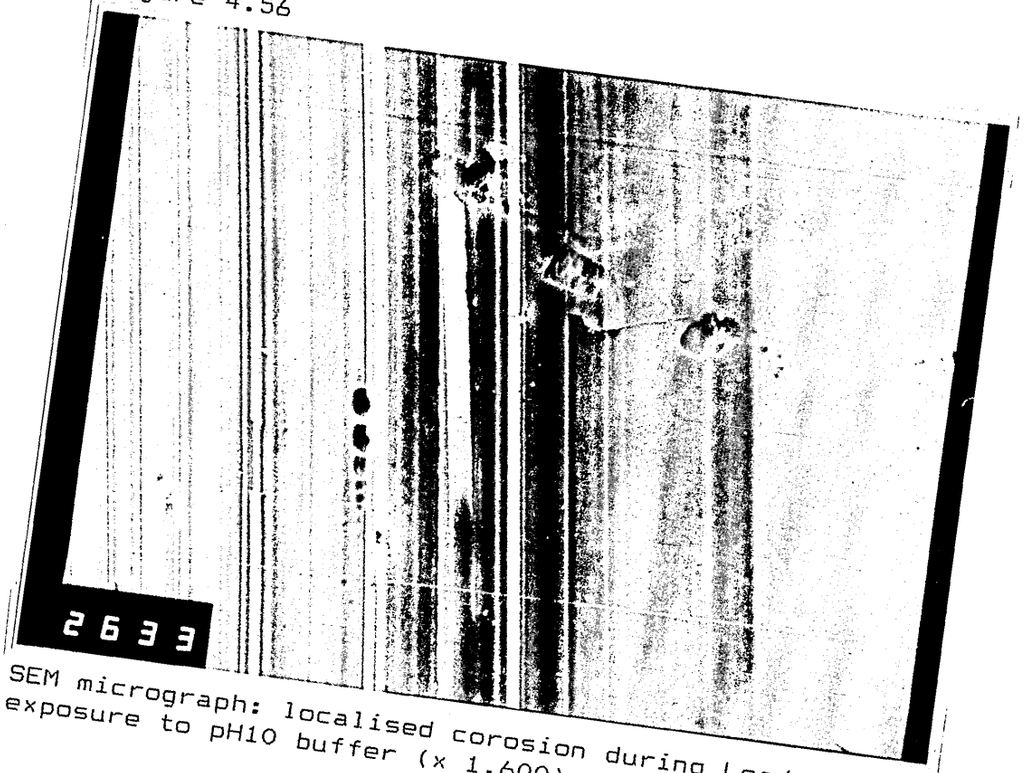
Figure 4.54

SEM micrograph: coat formation during Lead-glass fibre exposure to pH10 buffer (x 1,600)

Figure 4.55

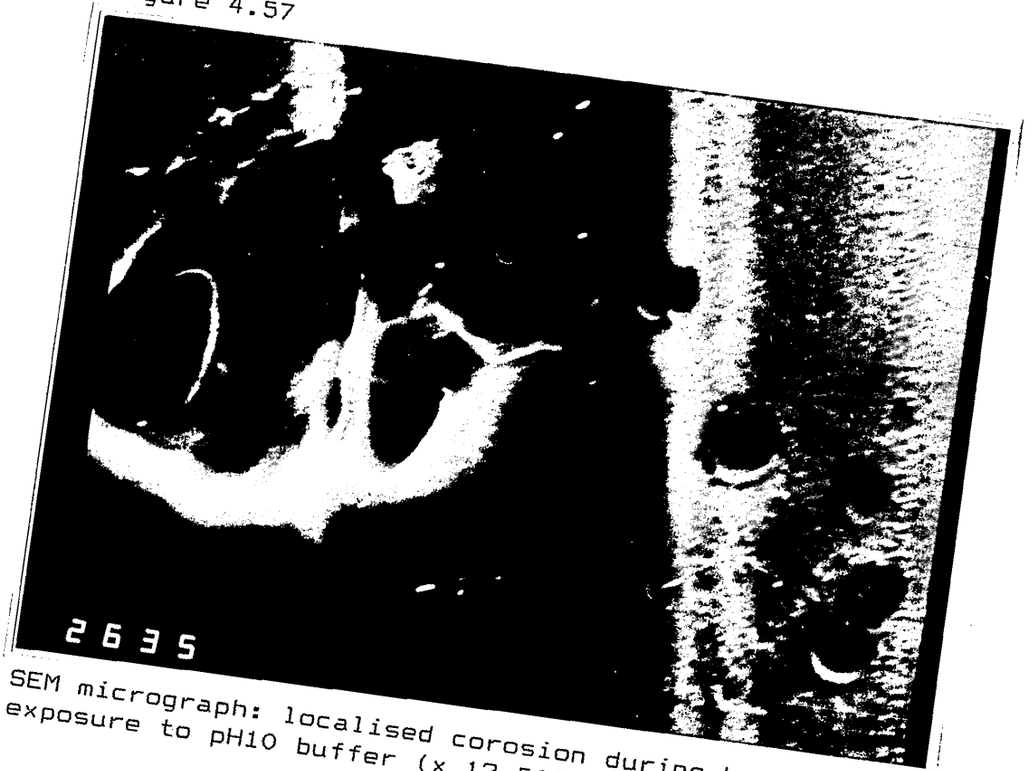
SEM micrograph: coat formation during Lead-glass fibre exposure to pH10 buffer (x 12,500)

Figure 4.56



SEM micrograph: localised corosion during Lead-glass fibre exposure to pH10 buffer (x 1,600)

Figure 4.57



SEM micrograph: localised corosion during Lead-glass fibre exposure to pH10 buffer (x 12,500)

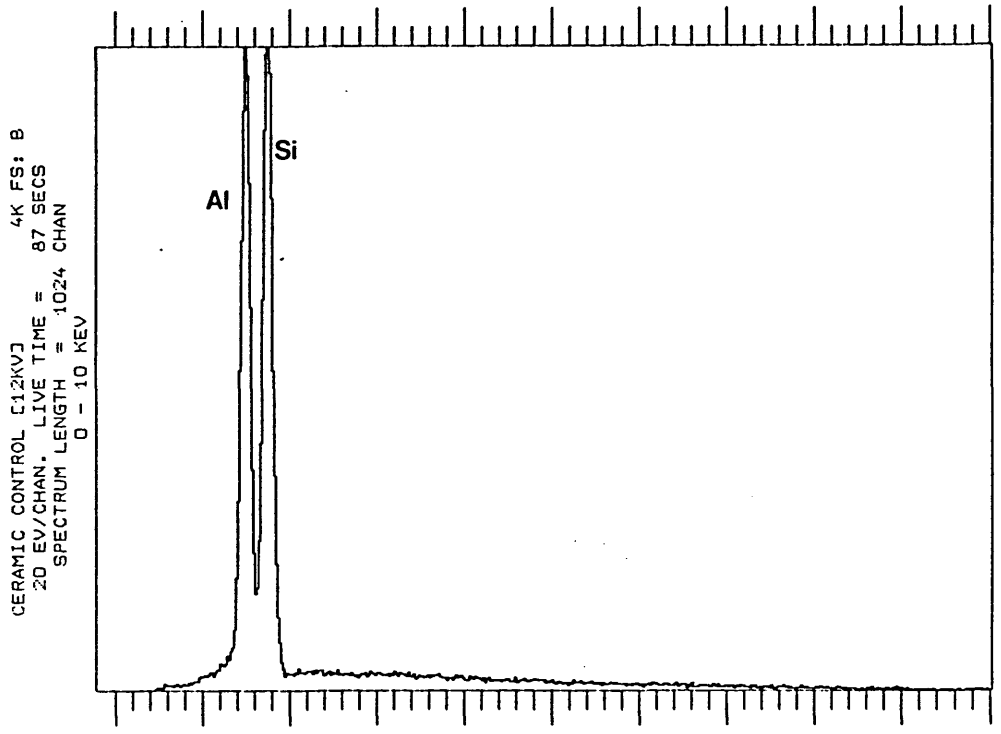
Alumino-silicate ceramic fibre

This fibrous material is commonly used as a refactory insulant and is designed to be stable at high temperatures.

Control spectra and micrographs are shown in Figures 4.58-61.

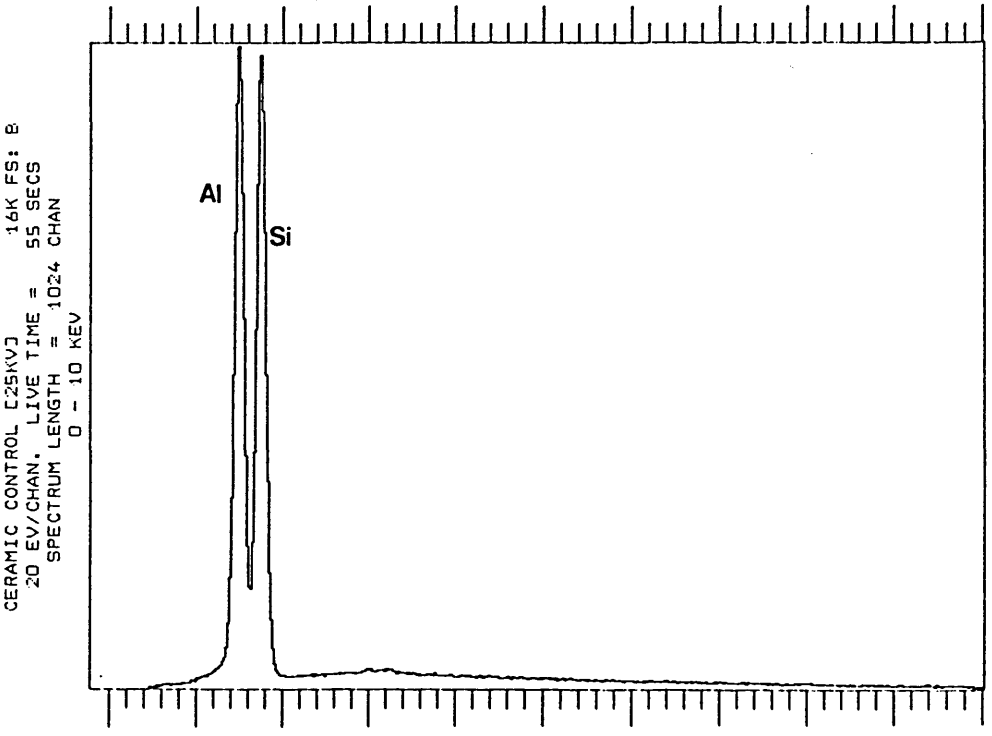
No statistically significant change in analyte levels relative to silicon were seen, after exposure to water, Gamble's fluid, PBS, or pH 10 buffer. In the case of exposure to RPMI 1640 some small, flaky, patches were observed on the fibre surface. These were found to contain P and Ca as shown in EDXA spectra, Figure 4.62 and 4.63.

Figure 4.58



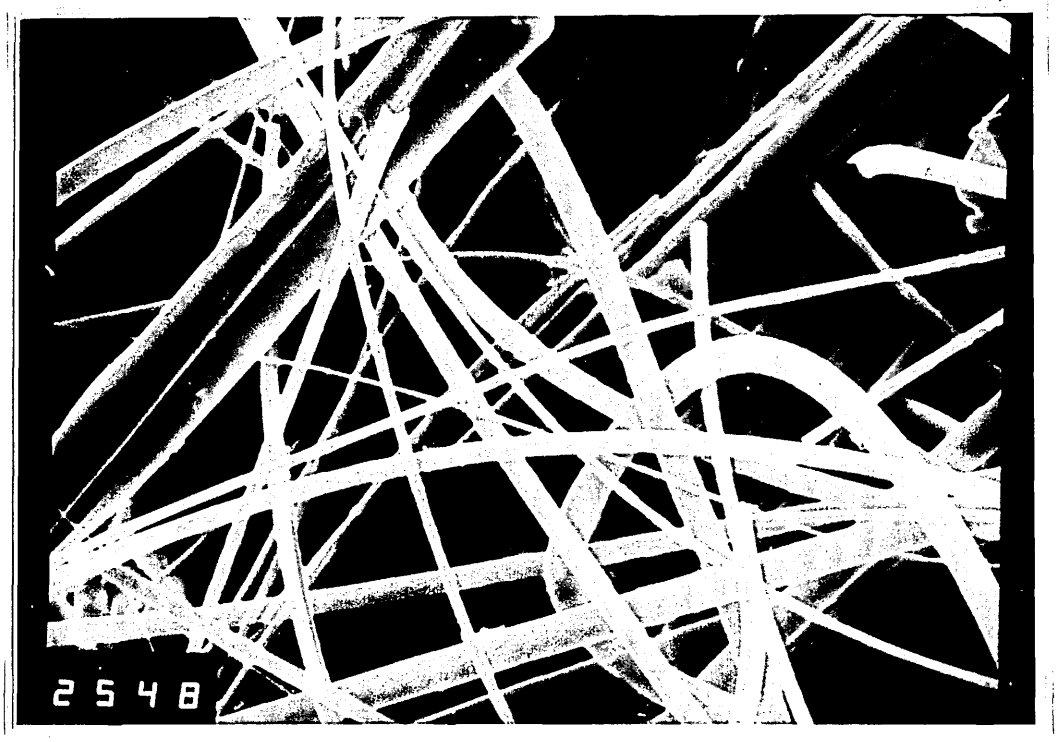
12kV EDXA spectra from unexposed Ceramic fibre

Figure 4.59



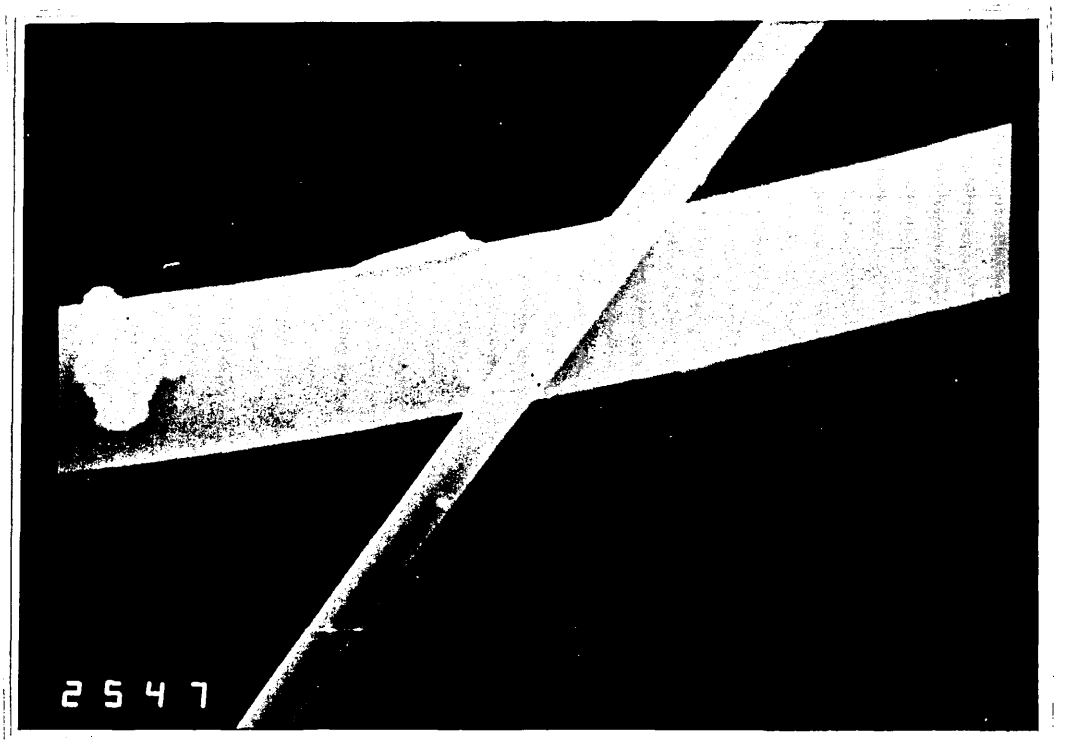
25kV EDXA spectra from unexposed Ceramic fibre

Figure 4.60



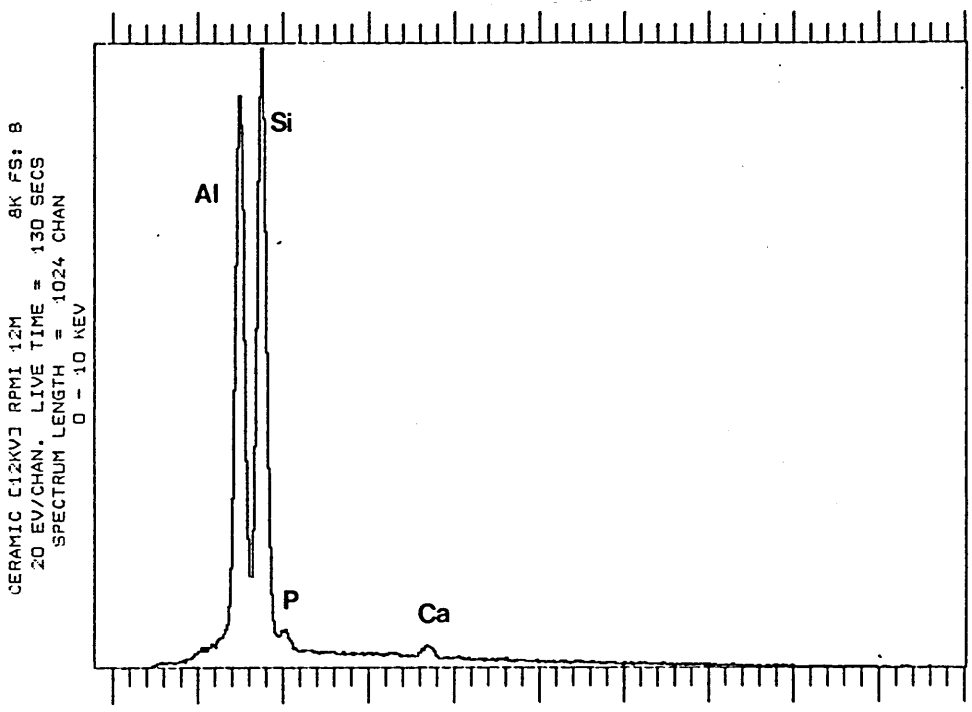
SEM micrograph: un-exposed Ceramic fibre (x 1,600)

Figure 4.61



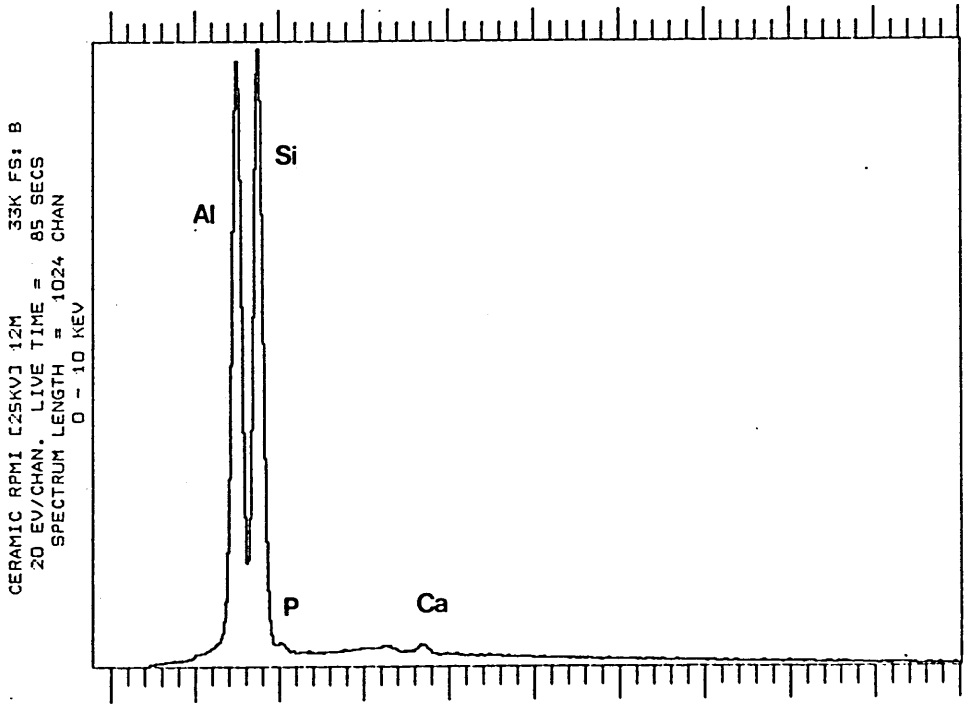
SEM micrograph: un-exposed Ceramic fibre (x 12,500)

Figure 4.62



12kV EDXA spectra from Ceramic fibre exposed to RPMI 1640

Figure 4.63



25kV EDXA spectra from Ceramic fibre exposed to RPMI 1640

4.2.2.3 Fibre Sizing

Changes in fibre diameter were monitored, using the SEM, in order to determine the lifetime of a particular fibre type when exposed to the various exposure conditions. The data for the 6 month and 12 month exposures is summarised in Tables 4.7 and 4.8 respectively.

The fibre diameter data for the six-month exposures of "sized" fibres was inconclusive. In particular, the A-glass data showed that despite using individual fibres the range of diameters within the control population was relatively large (10.00 - 19.75). Even for the continuous filament E- and Cemfil fibre there was a significant range of diameters in the control fibres.

The lead-glass and ceramic fibre were physically unsuitable for this analysis. However, SEM micrographs showed that complete surface layers were being "stripped" from the lead-glass by hydrolytic breakdown during exposure to pH 10 buffer for 12 months (Figure 4.54-4.55).

Fibre diameter data for the six-month exposures of sized fibres

Fibre type	Statistic	Exposure Conditions					
		Control	Water	Gambles' fluid	RPMI	Serum	Lavage fluid
E glass fibre	mean	6.27	-	6.09	5.91	6.13	6.06
	n*	51 (17)	-	27 (9)	24 (8)	36 (12)	9 (3)
	range	5.95 - 6.60	-	5.80 - 6.55	5.8 - 6.05	6.0 - 6.35	6.05 - 6.10
Cemfil fibre	mean	12.61	12.65	12.53	12.49	12.45	12.33
	n	48 (16)	27 (9)	24 (8)	12 (4)	21 (7)	15 (5)
	range	12.1 - 13.0	12.3 - 12.8	12.3 - 12.8	12.25 - 12.75	12.10 - 12.75	12.0 - 12.60
A glass fibre	mean	14.5	13.24	12.14	14.83	11.34	
	n	42 (14)	21 (7)	21 (7)	12 (4)	24 (8)	
	range	10.0 - 19.75	9.55 - 18.8	7.8 - 18.8	10.25 - 17.35	7.80 - 23.25	

* Three measurements of diameter were taken along the length of each fibre. The number of fibres examined is shown in ( ). Fibre diameters are in microns.

Table 4.7

Table 4.8

Fibre diameter data for the twelve-month fibre exposures

Fibre type	Statistic	Exposure Conditions				
		Control	pH10	Gambles	RPMI	PBS
E glass fibre	mean	6.52	6.27	6.49	6.58	6.28
	n	50	50	50	50	50
	std dev	0.46	0.57	0.51	0.59	0.63
	range	5.20 - 7.65	4.55 - 7.80	5.25 - 7.60	5.00 - 8.50	5.20 - 7.95
	F	-	1.51	1.22	1.63	1.87
	T	-	2.37	0.32	-0.58	2.16
Cemfil fibre	mean	12.64	12.02	12.77	12.49	12.72
	n	50	50	50	50	50
	std dev	1.02	0.93	0.99	0.78	0.97
	range	11 - 15.55	10.75 - 15.95	11.05 - 15.00	11.25 - 14.85	11.20 - 15.20
	F	-	1.20	1.08	1.70	1.11
	T	-	3.15	-0.67	0.82	0.40

* Three measurements of diameter were taken along the length of each fibre. Fibre diameters are in microns.

4.2.3 DISCUSSION AND INFERENCES OF RESULTS

Statistical analysis of EDXA data provided a means of confirming the validity of small changes in analyte levels that were not visible from the EDXA spectra. EDXA spectra were particularly useful when relatively major changes in analyte levels had occurred, visual presentation being much clearer than numerical data in these instances.

Morphological analysis and fibre measuring were found to be useful monitors of durability, but only when a significant degree of network hydrolysis had occurred. A qualitative summary of EDXA data and morphological data is presented in Table 4.9. Analyte variation relative to Si is expressed as an increase (+) or decrease (-). The data was summarised from 6 and 12 month exposure and 12 and 25kV EDXA data.

Table 4.9 Qualitative summary of results

The effect of various exposure fluids on a range of bulk glass and fibrous materials:

	BULK	A	E	CEMFIL	LEAD	CERAMIC
WATER	Na --- Ca --	Na --- Ca ---	Ca +	Na --- Zr + Ca -	N/A	NONE
GAMBLE FLUID	Na - Ca -	Na +	Al + K - Ca --	Na -- Ca - K - Al +	COAT P, Pb Cl, Ca	NONE
RPMI 1640	N/A	Ca +	COATED Al,P,Ca Na,Cl,K	Na --- Ca +	COATED P,Ca,Pb Cl	COATED P, Ca
PBS	Na - Ca -	N/A	COATED Al,P,Na Cl,K,Ca	Na --- Zr ++	COATED P, Pb Cl, Ca	NONE
pH 10 BUFFER	Surface attack	Surface attack	Ca -	Na + Zr + Thinning	Surface attack	NONE
SERUM	N/A	Na + Ca +	NONE	NONE	N/A	N/A
BALF	N/A	N/A	Mg + K - Ca --	Na -- Zr -- Ca --	N/A	N/A

Key:

(-), (--), (---): small, medium, large reduction in [analyte]/[Si] ratio.

(+), (++), (+++): small, medium, large increase in [analyte]/[Si] ratio.

N/A: data not available.

If we consider the behaviour of each glass in turn:

A-glass fibre:

Of the fibrous glass samples examined, A-glass fibre had a composition most similar to the soda-lime silicate bulk glass examined in Section 4.1. Also present in the A-glass were low levels of Mg, Al and K (Table 3.2, Figure 4.12-13). Generally, the behaviour was comparable to that of the bulk glass. The most significant degree of selective leaching (ion-exchange) was observed during exposure to pure water and the only noticable signs of hydrolytic attack were seen during exposure to high pH solutions. That is, the pH model of glass behaviour was still relevant, despite the presence in the glass of Mg, Al and K.

12 and 25kV EDXA of A-glass exposures to pure water showed a large decrease in Na levels in the fibre (35.1% and 36.5% respectively). This was significant in that 25kV analysis was more of a bulk analysis technique, with 12kV analysis being more surface sensitive (Section 3.4.3). The agreement between 12kV and 25kV analysis increased the probability that the Na decrease was real and the 25kV data indicated leaching well into the bulk of the fibre. Selective leaching of Ca was also shown by 12kV and 25kV EDXA (11.1% and 11.9% decrease, respectively). The selective leaching of Na and Ca was more extensive than that observed in the bulk glass. However, the exposure time for the bulk glass was only 3 weeks compared with 6 months for the fibrous A-glass. Ca leaching (as was the case with

the bulk glass) was less extensive than Na leaching. It was interesting to note that exposure to the complex physiological fluids, of neutral pH (Gamble's fluid, RPMI 1640, and serum) tended to result in increases of Na and Ca relative to Si. These increases were less definite, being shown only by 12 or 25kV EDXA data, but they may well have been associated with deposition on the fibre surface, or diffusion of Na and Ca from the exposure fluids, with water molecules, into the bulk of the fibres.

Although selective leaching of the A-glass fibre was extensive in pure water and very similar to the behaviour of the bulk glass, the resistance of the A-glass fibre to hydrolytic attack at high pH was apparently much greater. This greater durability may be associated with the smoother surface finish of the fibre as shown in Figure 4.14-15, though it was just as likely that the presence of small quantities of Al in the fibre contributed to the durability at high pH by the formation of protective alumino-silicate surface layers (Hench 1978).

"Gel" layers, or surface coatings, reported in previous in-vitro studies to form on glass surfaces (Klingholtz and Steinkopf 1982, Forster 1982) were notably absent.

Measurement of fibre diameter proved to be an ineffective method of monitoring the A-glass durability, because of the very high statistical spread of fibre diameters in the control samples (Table 4.7). Morphological examination,

however, showed that the A-glass fibres were prone to some surface degradation at high pH (Figure 4.16-17), though it was not possible to quantify this.

E-glass fibre:

This was quite different in composition to the bulk and A-glass fibre, being a calcium boro-silicate. The glass contained only trace levels of Na and was rich in Al, Ca, B (Table 3.2, Figure 4.18-19), all factors which tend to provide resistance to corrosion at high pH. In addition, the organic coating present on these fibres could potentially present a physical barrier to degradation.

The behaviour of the E-glass was distinctly different to that of the soda-lime silicates, showing no selective leaching in pure water and no signs of hydrolytic attack in pH 10 buffer. However, 12 month exposure to the physiologically buffered PBS and RPMI 1640 resulted in the formation of very thin, flaky surface coatings, rich in phosphorus and aluminium. It was noted that 6 month exposure did not show coat formation, indicating that coat formation may have been a relatively slow process. Alternatively, the low-temperature ashing/ultra-sounding/filtering process used to recover the fibre after 6 months, may have dislodged the delicate coat

EDXA analysis of samples of the coating which had become separated from the underlying fibre demonstrated the similarities and differences between the composition of the

coat formed during RPMI 1640 and PBS exposure. Na, Al, P, Cl, K and Ca were present in both cases (Figure 4.24-25, 4.28-29) whilst Si was notably absent. The major detectable difference was the much greater percentage of Ca present in the coat formed through the action of RPMI 1640. It was apparent that Na, Cl and P had deposited from the exposure fluids as they were not present in significant quantities in the E-glass fibre. The much greater percentage of Ca found in the coat formed during exposure to RPMI 1640 was probably due to deposition of Ca from solution. This was supported by the relatively small Ca peak from the coat formed during exposure to PBS, which contained little or no Ca.

The presence of aluminium in the coating was extremely interesting. Al was not present in the exposure fluids, therefore the source of the Al was the glass itself. This was unusual as Al is generally considered to be a "network former", which is a species that is a covalently bound, integral part of the glass network, and thus not considered susceptible to selective extraction. It could be argued that, for example, hydrolytic processes resulted in the release of Al into solution and it was then re-deposited onto the fibre surface, along with other species, to form the coating. However, Si would also be released by such processes and it would be expected that Si would also be present in the coating (possibly in the form of stable alumino-silicate complexes). It was clear from the EDXA

spectra that Si was absent from the coating and thus an explanation was required to explain this phenomenon. Possibly not all of the aluminium was covalently bound in the glass network, existing as the free oxide, or metal, or even as a semi-ionic species associated with non-bridging oxygens. A small amount of Al near the surface of the fibre may then be selectively extracted from the fibre to form a stable surface coating. This possibility was supported by the EDXA data from uncoated regions of the E-glass fibre exposed to PBS, which showed a statistically significant decrease in Al levels of 8.2% and 6.6%, for 12 and 25kV analysis respectively. Analysis of undercoat regions of E-glass fibre exposed to RPMI 1640 did not reflect this decrease in Al levels; however, if only relatively low levels of Al extraction were involved, the EDXA technique may have not been sensitive enough to detect this.

It is important to note that the presence of an organic coat on the E-glass did not prevent the extraction of Al, implying that it is not an integral coating.

The significance of the inorganic coating in terms of the durability behaviour of the glass was uncertain. Possibly this layer provided resistance to aqueous attack, though from its physical appearance it was very frail, often incomplete or cracked and flaking in several areas. It can be surmised that formation of the coat appeared to be associated with exposure to phosphate rich exposure fluids

(P was a major component of the coating) and also, in this case, with the presence of relatively large amounts of Al in the glass. Al may be important for chemical stability of the coating.

SEM examination of exposed "undercoat" regions of the fibres showed no signs of surface corrosion and fibre diameter data (Table 4.6-7) showed no evidence of fibre thinning.

Cemfil fibre:

This glass was similar to the bulk glass and A-glass in that relatively large quantities of Na and Ca were present (Table 3.2, Figure 4.30-31). The Cemfil fibre also contained Zr, which has been found previously to dramatically increase resistance to nucleophilic attack at high pH (Paul 1982). This glass also had an organic surface coating, evidence of which is shown in Figure 4.32-33; these micrographs show that this coating was "patchy" and of poor integrity.

It was interesting to note that in common with the soda-lime silicate glasses, the Cemfil was prone to selective leaching and also that formation of "Gel" layers was absent. The selective leaching confirmed that the organic coating was largely ineffective as a protective layer.

Selective leaching of Na and/or Ca was observed with exposure to water, Gamble's fluid, RPMI 1640, PBS, and BALF. It was also interesting to note that Zr levels

tended to show surface increases, inferring re-deposition at the fibre surface.

Exposure to pure water resulted in Na decreases similar to those found in A-glass fibre. Cemfil showed Na decreases after 12 months with 12 and 25kV EDXA data (38% and 19% respectively) inferring that the presence of Zr in the glass did not significantly reduce selective leaching. Decreases in Ca levels were also inferred, but only by data from 6 month exposures, which indicated 15.6% and 11.2% decreases from 12 and 25kV analysis respectively.

Paradoxically, 12 month exposures to water did not show a Ca decrease, possibly inferring re-deposition of Ca with longer exposure time. The behaviour of Zr was also interesting, showing an initial decrease after 6 months, with 12 and 25kV analysis, of 9.8% and 13.1% respectively, whilst after 12 months the net effect was a surface increase of 16.4% from 12kV data.

As was the case with Ca, this data inferred a "dynamic" situation where the initial effect of exposure was leaching, followed by re-deposition on the surface.

6 month exposure to Gamble's fluid generally had no leaching effect on the Cemfil fibre although 12kV EDXA indicated surface increases in Na (19.9%) and Al (84%). The Al data could not be considered reliable, because the Al peak height was very low and there was a high signal to noise ratio. The phenomenon could also have been

associated with extraction and re-deposition of the analyte. By 12 months of exposure, clear decreases in Na were observed (20.3% and 17% using 12 and 25kV analysis respectively) and 25kV analysis also showed decreases in K and Ca, of 26.4% and 3.4% respectively. It may be expected that 12kV analysis, which was generally more surface sensitive, would also have shown the decreases in K and Ca, however, 25kV analysis was much more sensitive towards these species, which was presumably the most important factor in this case.

12 month exposure to RPMI 1640, as with exposure to water and Gamble's fluid, resulted in selective leaching of Na (27.1% and 33.2%, using 12 and 25kV analysis respectively). 25kV analysis indicated an increase in Ca, of 5%, which may have been associated with deposition from the exposure fluid.

Exposure to PBS resulted, after 12 months, in a large decrease of Na (32.6% and 18.1% using 12 and 25kV analysis respectively), whilst Zr levels showed an increase (18.1% and 9.1% from 12 and 25kV analysis respectively). This implied that whilst selective leaching of Na was occurring, preferential re-deposition of Zr near the fibre surface was taking place. It was surprising that 25kV analysis also showed increases in Zr levels as this was more of a "bulk" analysis method and presumably less sensitive to surface changes than 12kV analysis.

Exposure to BALF was interesting as it was the only exposure fluid that appeared to result in leaching of Na (57%), Ca (9%) and Zr (11%), relative to Si. This appeared to be predominantly a surface effect, as this was only observed using 12kV analysis. It was noted that only 6 month exposure data was available and, as was the case with exposure to water, continued exposure may have resulted in the re-deposition of Zr on the fibre surface.

The effects of pH 10 buffer were also interesting. There were no signs of localised hydrolytic attack, but there were signs of moderate surface increases in Zr after 12 months of exposure (12.5%, 12kV analysis), possibly associated with the formation of zirconia-rich, protective surface layers on the fibre.

The fibre diameter data implied that an average decrease in fibre diameter had occurred during 12 month exposure, of 0.62 microns (Table 4.8). This was unexpected, in view of the fact that Cemfil is designed to be stable at high pH and consequently requires further investigation. It is possible that the fibre only becomes stable at high pH after initial surface dissolution and re-deposition of stable Zr compounds has occurred; this possibility is supported by the increased Zr levels on the surface of the exposed fibre.

It was also noted that the Cemfil fibre surface was very smooth after exposure, showing no evidence

of the organic coating present on the control sample (Figure 4.32-34), or "stripping" of surface layers by hydrolytic breakdown as was the case for the lead-glass at high pH (Figure 4.54-55).

Lead-glass fibre:

This glass contained a relatively large quantity of lead and potassium (Figure 4.34-35). lead glasses are considered to be stable in neutral and acidic solutions (Paul 1982).

In common with E-glass fibre, the lead-glass fibre was associated with the formation of a phosphorus (P) containing surface coat during exposure to phosphate-rich physiological fluids. The lead glass was also susceptible to network hydrolysis at high pH, as were the bulk glass and A-glass fibre.

Coat formation was observed during exposure to Gamble's fluid, RPMI 1640, and PBS (Figure 4.40-41, 4.46-47, 4.52-53). It was noted that all three fluids were buffered at pH 7.4 and contained high levels of phosphate (Table 3.2), a major component of the observed coatings.

Interesting comparisons could be made between the nature of the coatings formed during exposure of E-glass and lead-glass, which inferred that coat formation depends not only upon the nature of the glass but also upon the nature of the exposure fluid.

The presence of phosphate in the exposure fluid appears to be an important determinant in the formation of a stable surface coating and it was observed that in the case of exposure to Gamble's fluid, which contained the least phosphate, coat formation was observed only in the case of lead glass.

The coatings formed during exposure of lead and/or E glass fibre to RPMI 1640 and Gamble's fluid, contained a much greater relative concentration of Ca than the coats formed during PBS exposure. This strongly inferred (because Ca was not present in PBS) that much of the Ca in the coating originated from the exposure fluid.

Silicon was absent from the coating, though Pb was present in relatively large quantities. Obviously Pb was readily extracted by the effects of the exposure fluids to be incorporated into the coating and may (as was suggested in the case of Al extraction during E-glass exposure) be essential for the production of the observed coating.

EDXA analysis of undercoat regions of the fibre showed extensive leaching, leaving, in the case of exposure to PBS, what appeared to be a silica-rich shell (Figure 4.42-43).

Ceramic fibre:

This fibre was very different to the other glass compositions, being a pure alumino-silicate.

This was generally unreactive, showing no significant signs of selective leaching, or of network hydrolysis.

Exposure to RPMI 1640 (Figure 4.62-3) did result in some "patchy" deposits on the fibre surface; these were found to contain P and Ca.

4.3 THE DURABILITY OF GLASS FIBRES EXPOSED TO MONOCYTE CULTURES

4.3.1 METHODOLOGY

Sized samples of A-glass fibre, E-glass fibre, and Cemfil fibre were prepared (Section 3.2) and exposed to monocyte cultures for 6 months. Monocyte cultures were established and maintained by semi-dynamic replenishment, using the method previously described (Section 3.3). Controls consisted of unexposed fibre samples and exposures to RPMI 1640 (the culture medium).

The biochemical activity of the monocytes was assessed by their ability to reduce cytochrome C (Section 3.3.2).

After exposures of up to 6 months the exposed fibres were recovered and prepared using low temperature plasma ashing (LTA) and then analysed using SEM in conjunction with EDXA (Section 3.4.2).

4.3.2 RESULTS FOR MONOCYTE EXPOSURES

4.3.2.1 General

Morphological studies showed that the cultured monocytes were phagocytic towards the glass fibres and this is shown in Figure 7.11. The biochemical activity, measured by the production of oxygen free radicals, was found to be 4.47 ± 1.5 nmols cytotochrome C reduced per 10^6 cells over 45 minutes (Conroy et al 1988).

Data for exposure of Cemfil, E- and A-glass fibre to RPMI 1640 is given in Section 4.2.2.

4.3.2.2 EDXA and Morphological Analysis

A-glass fibre

Effect on sodium: no significant effect using 12kV analysis, but an increase of **8.5%**, from **1.89** ± 0.16 to **2.05** ± 0.04 (F=12.8, T=-4.32), was found using 25kV analysis.

Effect on magnesium, aluminium and potassium: no statistically significant effect.

Effect on calcium: 12kV analysis showed a small increase in Ca levels of **3.9%**, from **9.26** ± 0.28 to **9.62** ± 0.22 (F=1.7, T=-4.61).

Morphological studies showed no signs of network hydrolysis.

E-glass fibre

No statistically significant effect.

Cemfil fibre

No statistically significant effect.

4.3.2.3 Sizing Data

The sizing data is shown in Table 4.10.

Table 4.10 Fibre diameter data for monocyte exposures

Fibre type	Statistic	Exposure Condition Control :	Monocytes
E-glass	mean*	6.27	6.31
	n**	51 (17)	27(9)
	range	5.95 - 6.60	6.15-6.50
A-glass	mean	14.15	12.51
	n	42(14)	30(10)
	range	10.0-19.75	8.8-26.4
Cemfil fibre	mean	12.61	12.62
	n	48(18)	21(7)
	range	12.1-13.0	12.2-12.9

* fibre diameter expressed in microns.
** three analyses per fibre, where the number of fibres is shown ( ).

4.3.3 DISCUSSION AND INFERENCES OF RESULTS

The semi-dynamic replenishment was successful in achieving exposure times of 6 months, and the activity of freshly isolated human monocytes was found to be similar to that of mature macrophages.

However, in contrast to the inferences of Bernstein's <u>in-vivo</u> studies, this data indicated that the macrophage-like environment was not particularly corrosive towards glass fibres during the exposure period and no signs of network attack of the fibres were observed from morphological or sizing studies.

There are a number of possible implications that may be drawn from this:

i) the intra-macrophage environment was not a key determinant of glass fibre durability in the lung,

ii) the 6 month exposure time (in view of the 18 month in-vivo exposure used by Bernstein [1982]) was not sufficient for signs of fibre degradation to be apparent,

iii) human monocytes were not a suitable, representative alternative to mature macrophages,

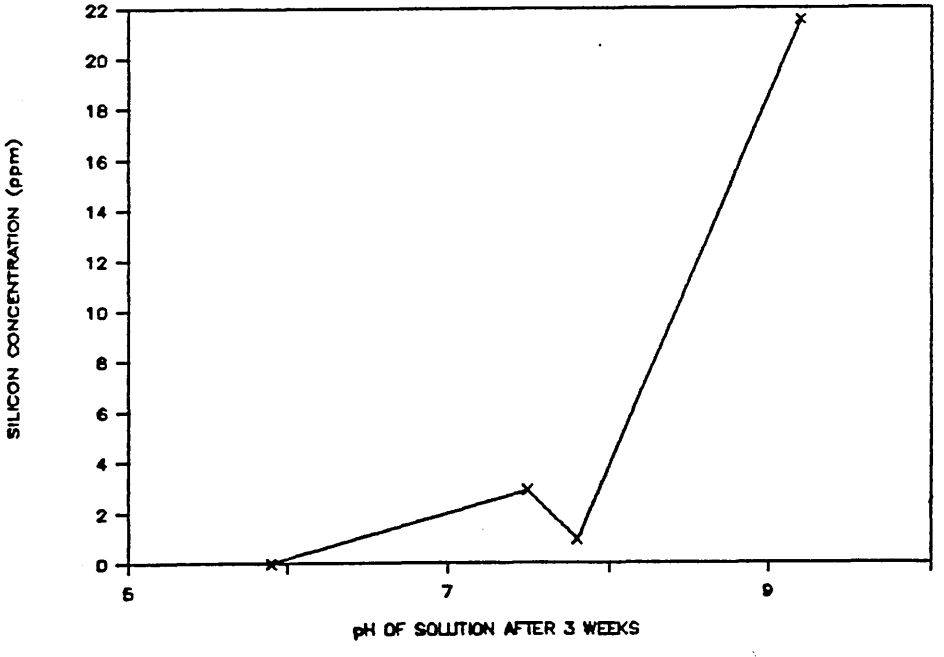
iv) the types of glass fibre studied here, unlike the fibre type studied by Bernstein (1982), were durable.

Recommendations for development of these studies, and a comparison with 6 month in-vivo exposures are made in Section 8.

4.4 CONCLUSIONS FROM IN-VITRO STUDIES

Solution pH appeared to be the major determinant of the behaviour of the bulk soda-lime silicate glass examined. The data was consistent with a classical model for glass behaviour under conditions of different solution pH and is clearly shown in Figure 4.64. This plot, showing the relationship between silicon extraction and solution pH, reflected the increasing degree of network hydrolysis as solution pH was increased - the form of the graph being similar to that reported by El-Shamy (1972). The typical surge in network degradation was seen at pH 8-9.

Figure 4.64



Relationship between Si extraction and exposure fluid pH

The EDXA data for the exposure to phosphate-buffered Gamble's fluid was similar to that of an unexposed material. This highlighted the importance of morphological evidence in demonstrating the damage associated with hydrolytic attack of the glass network.

With respect to the corrosive nature of Gamble's fluid, it was observed that as long as accurate maintenance of physiological pH was achieved the fluid was apparently no more corrosive than other physiological pH buffers towards the soda-lime silicate glass examined.

Consideration of Table 4.9 and the results given in Section 4.3.2 allows a number of general conclusions concerning the behaviour of glass fibres in physiological fluids to be made:

(i) the most un-reactive material in terms of selective leaching and resistance to nucleophilic attack was the ceramic fibre.

(ii) the most corrosive environments, in terms of network attack were those of high pH.

(iii) the most corrosive fluid, in terms of selective leaching, was pure water.

(iv) exposure to the complex, physiological fluids (pH 7.4) (RPMI 1640, PBS, and Gamble's fluid) tended to be associated with the formation of phosphorus rich coatings on the fibres.

(v) sodium rich glasses were prone to selective leaching.

(vi) The soda-lime silicates, whilst prone to both selective leaching and network hydrolysis, were not associated with the formation of surface coatings.

The pH model of glass fibre durability still appeared to be useful. It was seen that network hydrolysis tended to occur at high pH and that pH10 buffer was the most corrosive fluid with respect to network degradation, whilst pure water (the fluid with lowest pH) generally resulted in the most significant degree of leaching. The pH model did not enable prediction of the behaviour of E-glass and lead-glass in the physiological fluids (pH 7.4), where coat formation was observed and selective leaching of Al or Pb occurred. In these cases, particularly for the lead-glass, the driving force for selective leaching could have been the thermo-dynamic stability associated with formation of the coating, rather than any ion-exchange processes associated with the pH of the fluid.

From a health-related view, it could be concluded that the most stable (durable) material over the range of exposure conditions was the ceramic fibre and thus most likely to pose a health hazard, whilst the bulk glass, A-glass and lead-glass compositions (being prone to network degradation) would present less of a health-hazard from the durability point of view. It was also interesting to contemplate the short term toxity of the materials that were prone to selective leaching, and the potential effect of species such as Zr and Pb on the tissues. In order to test the short term toxicity of these materials, a series of toxicity tests were carried out (Section 7).

6 month exposures of A-, E- and Cemfil glass fibre to human monocytes did not appear to cause physical degradation of the glass fibre. This was contrary to Bernstein's suggestion (1982) that exposure of glass fibre to the intra-macrophage environment is a key determinant of fibre durability.

It was intended that the durability of a fibrous material could be accurately related to its composition. An attempt to relate glass stability to the it's constituent species was made (Section 8).

Recommendations for development of in-vitro studies and a comparison with 6 month in-vivo studies are made in Section 8.

5. IN-VIVO EXPOSURES

5.1 THE DURABILITY OF GLASS AND CERAMIC FIBRE EXPOSED TO THE RAT

5.1.1 METHODOLOGY

Fibres were prepared as previously described (Section 3.2) and exposed to rats lungs by intra-tracheal instillation. The fibres used were lead-glass, E-glass, Cemfil and ceramic fibre, and they were exposed for a period of 6 months. Fibres were recovered using low temperature ashing and characterised by SEM and EDXA.

5.1.2 RESULTS

Lead-glass fibre

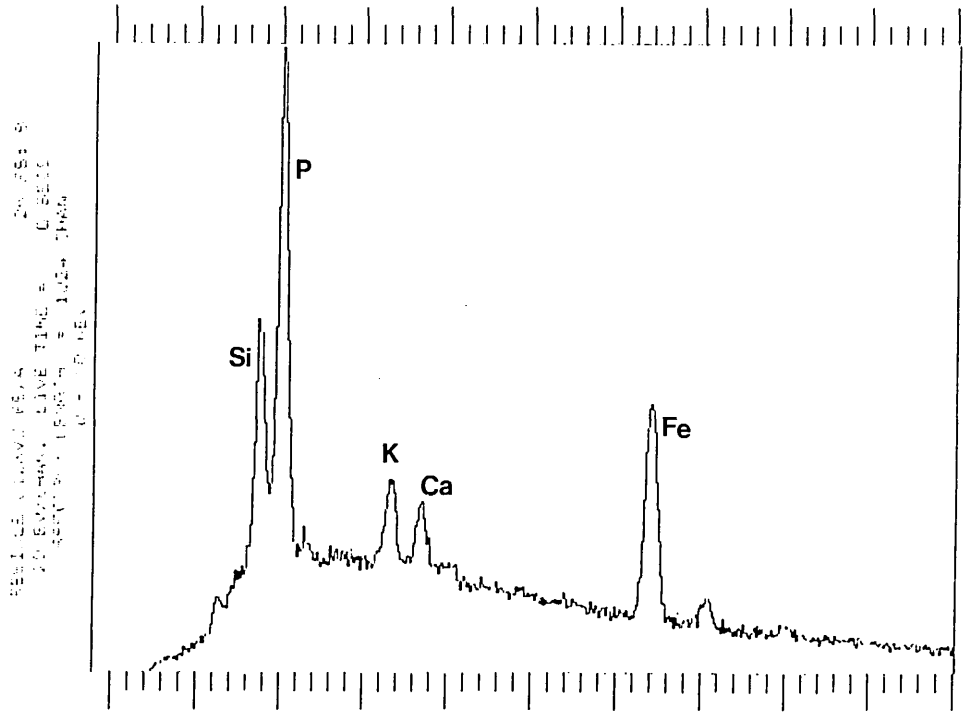
The statistical variation in the levels of the analytes in this glass was very large, preventing accurate analysis. Consequently, it was not possible to determine from EDXA spectra whether selective leaching had occurred. There were no signs of coat formation on the fibre. However, there was some indication of localised corrosion, which is shown in Figure 5.1; this "pitting" corrosion was not apparent on control fibre samples. Furthermore, there was a relatively large amount of residue from the ashing procedure and EDXA of this showed the presence of Si, possibly from the glass (Figure 5.2).

Because of the very wide fibre size distribution in the control sample it was not possible to carry out analysis of changes in the size of the exposed fibre.

Figure 5.1

SEM micrograph showing lead-glass corrosion x 12,500

Figure 5.2



12kV EDXA of ashed lung residue, exposed to lead-glass

E-glass fibre

Effect on magnesium: no statistically significant effect.

Effect on aluminium: 12kV analysis showed a decrease of **8.2%**, from **13.59** ± 0.86 to **12.47** ± 1.42 (F=2.79, T=4.23) and 25kV analysis showed a decrease of **4.6%**, from **11.79** ± 0.56 to **11.25** ± 0.58 (F=1.09, T=4.26).

Effect on calcium: 12kV analysis showed a decrease of **4.4%**, from **30.41** to **29.08** (F=1.05, T=2.88).

Effect on potassium and iron: no effect.

Morphological analysis

Morphological and sizing studies did not reveal corrosion of the E-glass fibre. Sizing of unexposed E-glass control samples gave a sample mean of **6.52** microns and standard deviation of 0.46, where n = 50. The fibres exposed to rats had a sample mean of **6.56** and a standard deviation of 0.47, where n = 50. A statistical comparison of the mean values gave an F value of 1.02 and a T value of 0.43.

Cemfil fibre

Effect on sodium: 12kV analysis showed a large decrease of **26.8%**, from **3.80** ± 0.68 to **2.78** ± 0.72 (F=1.12, T=6.48). No statistically significant effect was observed using 25kV analysis.

Effect on aluminium: no statistically significant effect.

Effect on zirconium: no statistically significant effect from 12kV analysis, however, 25kV analysis showed an increase of **10.2%**, from **7.78** to **8.57** (F=1.7, T=-5.11).

Effect on potassium: both 12kV and 25kV analysis showed statistically significant increases in K levels in the exposed fibre. 12kV analysis suggested a relatively large increase of **193%**, from **0.60** to **1.76** (F=2.92, T=4.88). 25kV analysis suggested an increase of **36.8%**, from **0.87** to **1.19** (F=5.40, T=3.25).

Effect on calcium: no statistically significant effect.

Morphological analysis

Morphological and sizing studies did not reveal corrosion of the Zr glass. Sizing of unexposed Zr glass control samples gave a sample mean of **12.64** microns and standard deviation of 1.02, where n = 50. The fibres exposed to rats had a sample mean of **12.29** and a standard deviation of 0.84, where n = 50. A statistical comparison of the mean values gave an F value of 1.48 and a T value of 1.86.

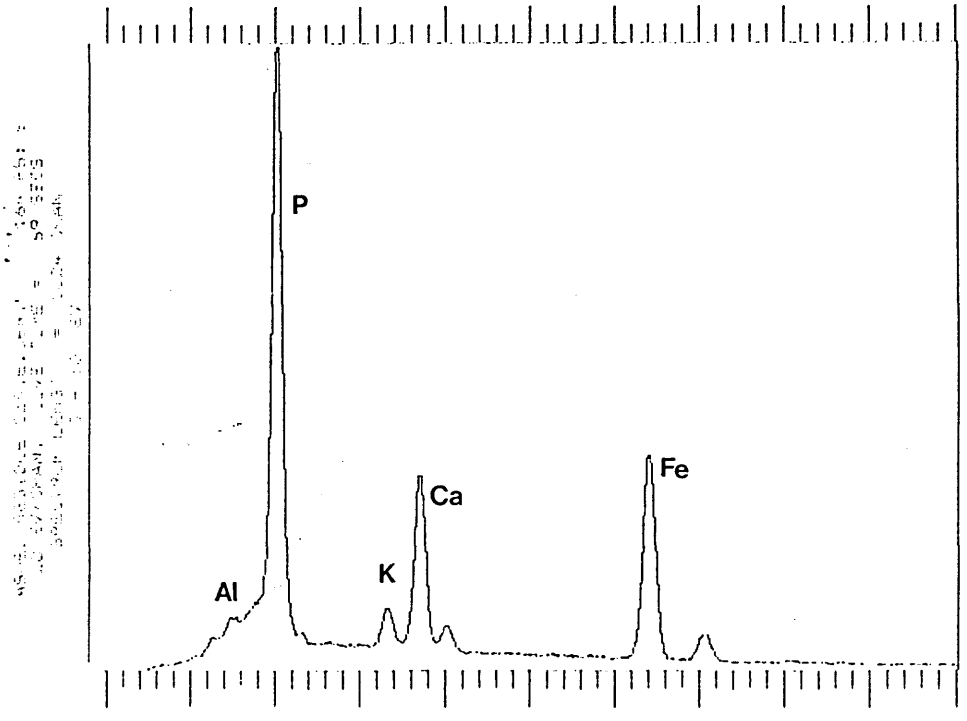
Ceramic fibre

No statistically significant changes apparent from EDXA or morphological analysis. The size distribution of the ceramic fibre control sample was too large for an accurate comparison with the test sample in view of the relatively small amount of fibre recovered after <u>in-vivo</u> exposure.

Low temperature ashing residues

An EDXA spectra of the ash-residue from the lungs of rats exposed to Cemfil, E and ceramic fibre is shown in Figure 5.3. The absence of a Si peak was of interest.

Figure 5.3



12kV EDXA of ashed lung residue, exposed to A, Cemfil and ceramic fibre

5.1.3 DISCUSSION AND INFERENCES OF RESULTS

E-glass, Cemfil and ceramic fibre showed no physical signs of network dissolution. However, E-glass showed a relatively moderate decrease in aluminium levels, apparently extending into the bulk of the fibre, and a small decrease in calcium levels, whilst the Cemfil fibre showed a marked decrease in surface Na (12kV data: 28%) and increases in Zr and K levels.

The lead-glass provided difficulties in analysis. The statistical size distribution and also analyte level variation were prohibitively large for accurate analysis. Furthermore, the degree of residue from the ashing procedure was greater than other samples. However, signs of localised corrosion were present on some of the fibre, indicating network hydrolysis of these samples. This was consistent with our previous studies, which showed that the lead-glass was susceptible to this type of degradation and highlights the poor durability of this material.

Further signs of the dissolution of the lead-glass were provided by EDXA analysis of the ashing residue from lung exposed to this glass, which showed the presence of Si, presumably from the glass network. It was noted that Pb did not appear to be present in this residue although it may have been expected due to hydrolysis of the glass and also if significant lead-rich surface layer formation occurred.

Possibly, any lead in the residue was of too low a concentration for determination by EDXA, alternatively, much of the lead had been selectively leached from the fibres prior to hydrolysis of the silicon rich fibre surface. Particles of silica released by this process could then be trapped in the tissues and retained in the ashing residue. Obviously this is a highly speculative theory requiring further investigation and it is suggested that the use of lead-glass fibre of uniform composition would enable the degree of lead leaching to be quantitatively determined.

The alumino-silicate ceramic appeared to be highly durable, showing no signs of leaching , or network hydrolysis.

Surface layers, characteristic of in-vitro exposures, were not observed, though it was noted that the sample preparation procedure included ultra-sonification to remove ashing-residue from the fibres. This would also remove evidence of fragile surface layers formed on the fibre.

EDXA of the ashing-residue showed clearly the presence of P and Fe, presumably from interstitial fluid and haemoglobin respectively. It was interesting to note that Na was absent from the residue, indicating that highly soluble species were removed during washing.

It should be noted, in relation to the use of LTA, that any traces of organic surface coatings would be removed by this process.

5.1.4 CONCLUSIONS

Generally, these results were consistent with our knowledge of the in-vitro responses of these materials, based on the assumption that localised pH variations in the alveolar region accounts for the behaviour of the materials. It is accepted that this conclusion is based on limited in-vivo data and exposure times of six months. It is, therefore, recommended that longer exposure times and data for a full range of glasses, including soda-lime silicates, should be obtained. The use of fibres of accurately known dimensions would enable improved quantification of such studies.

The high humidity of the alveolar region would account for Na leaching from the Cemfil fibre and the corresponding formation of a potentially protective Zr rich surface; this type of behaviour was observed during in-vitro exposure of Cemfil to water (Table 4.9). In contrast, leaching of Na and Ca from the lead-glass could result in a localised alkaline environment, capable of causing network hydrolysis (indicated in Figure 5.1).

The alumino-silicate ceramic has been shown to be highly stable in-vitro to aqueous environments and not surprisingly showed no signs of corrosion in-vivo.

It is recommended that a more detailed appraisal of the nature of the ashing residue should be carried out, to positively identify the source of the analytes present.

It may also be possible to prepare tissue samples for SEM analysis, avoiding disturbance to the fibre, thus determining whether some types of glass are associated with the formation of surface layers in-vivo.

The exposure time used was 6 months, which would be expected to be sufficient for signs of hydrolytic attack to be determinable by SEM morphological studies (as was the case with the lead-glass), however the use of significantly longer exposure times and fibres of a fixed 1 micron diameter would potentially enable a quantitative assessment of dissolution rates of the more durable glasses.

At the present time, known diameter fibres, of the desired range of compositions are not available and it is suggested that design of a system for the small scale production of such fibres would greatly aid future durability studies.

6. THE USE OF SECONDARY ION MASS SPECTROMETRY FOR THE ANALYSIS OF FIBROUS GLASS SAMPLES

6.1 INTRODUCTION

Several powerful techniques have become available in recent years that provide a means of examining the composition of the outermost atomic layers of a sample surface. Three major techniques are: Auger electron spectroscopy (AES), X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS).

The factor that makes these techniques so powerful is their ability to determine near surface chemistry - a factor which in turn plays a crucial role in the physical and chemical behaviour of the sample.

The opportunity to examine one of these surface techniques, SIMS, was available. SIMS was consequently applied to the analysis of bulk glass and glass-fibre surfaces in an attempt to investigate the relationship between the surface chemistry of the glass and the physiological durability of the glass.

SIMS has already been used successfully for analysis of leached, soda-lime-silicate, bulk-glass samples (Richter et al 1984) and it was hoped that this could provide the basis for development of the technique for the analysis of fibrous samples.

In theory, the SIMS technique is relatively straightforward; in practice, there are many complications which make SIMS a very difficult technique to quantify (Benninghoven et al 1984, Benninghoven et al 1987). SIMS involves the controlled bombardment of the sample with a high energy ion-beam. Bombardment causes the sputtering of secondary ions, characteristic of the sample, from the surface atom layers. Detection by a mass spectrometer enables identification of these species and the ion yield can, in theory, be related to the concentration of the respective analyte.

SIMS offers the potential for extremely sensitive surface characterisation of fibrous glass samples and also a "depth profiling" capability, associated with the continuing sputtering of ions and neutrals from the sample surface by the incident ion beam.

SIMS has been examined for its suitability as a method for the surface analysis of glass fibres and as a method for depth profiling of bulk glasses with the intention of extending this to depth profiling of fibrous samples.

The major advantages of SIMS over the SEM/EDXA techniques, previously described in Section 3. are:

(i) the ability to detect the full range of elemental species, including isotopes, and also molecular species.

(ii) surface sensitivity, allowing analytical resolution of several atom layers, thereby providing potentially important data with respect to surface leaching, and layer formation.

(iii) SIMS analysis, because of its good surface sensitivity, can potentially lead to a reduction in the time required for in-vivo and in-vitro durability studies, currently characterised by less surface-sensitive SEM/EDXA methods.

However, despite the apparently ideal nature of SIMS for surface analysis, glass fibre analysis presented two major problems:

(i) the glass fibres were non-conducting and therefore tended to charge,

(ii) the glass fibre diameter was relatively small in relation to the spatial resolution of the standard ion gun, making it impossible to fully focus the ion beam onto the fibre.

Specimen charging can be overcome in a number of ways. In the study by Richter et al (1984) the approach was to use neutral primary beam SIMS, where neural particles are used instead of ions. This technique was not available, therefore an alternative was adopted and an electron flood-gun was used to saturate the sample surface with electrons.

The resolution problem was approached by using a high-resolution, liquid-metal ion-source. This was used to provide a beam of approximately 5 micron diameter (experimental data) which could be fully focused onto the fibrous samples.

6.2.1 SIMS ANALYSIS OF E-, LEAD-, AND CEMFIL GLASS-FIBRE EXPOSED TO GAMBLE'S FLUID

6.2.2 METHODOLOGY

E-, lead- and Cemfil fibre were exposed to Gambles fluid for six months (Section 4.2). After exposure, test samples were prepared and then analysed using the VG Scientific Microlab 500. Control mass spectra were obtained from unexposed fibre samples.

Samples were prepared by thoroughly washing exposed fibres with pure water. Then individual fibre lengths were isolated using tweezers and mounted so that 1-2mm of the fibre protruded over the edge of a stainless steel specimen stub. Fibres were fixed at two points with carbon paste to hold them securely.

The essential features of the SIMS analysis can be defined as follows:

i) an ultra-high vacuum specimen chamber,

ii) a primary ion-source,

iii) a secondary-ion detector,

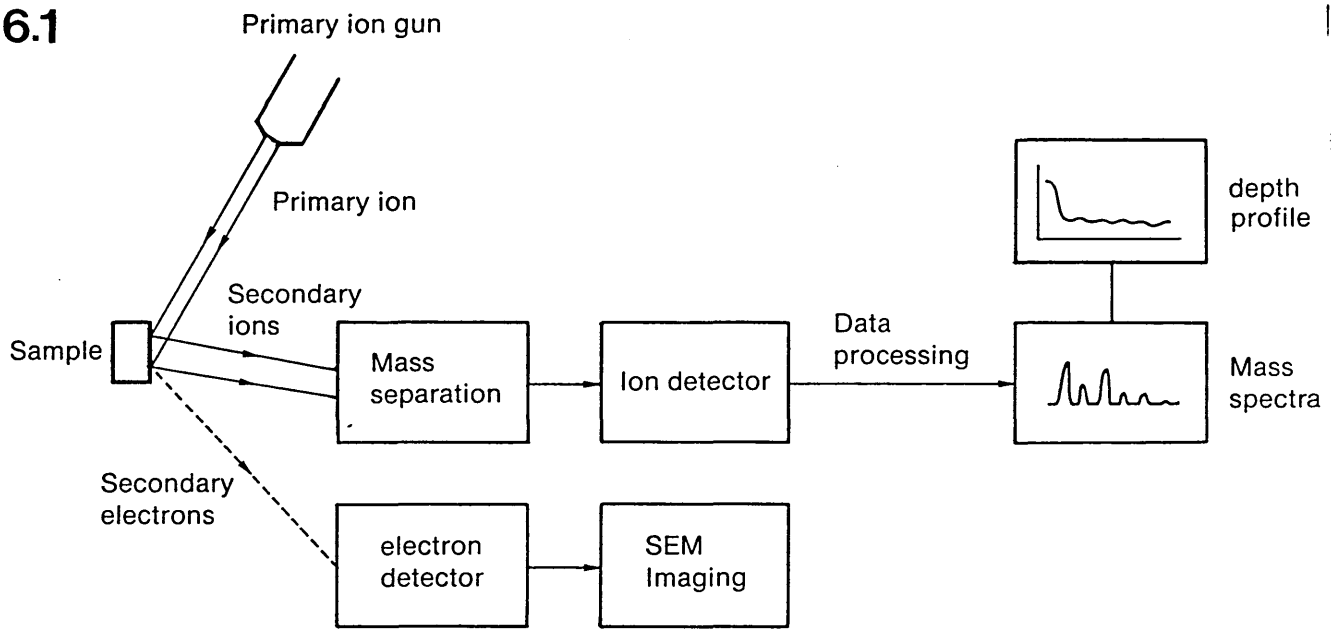
iv) a data processing system.

A schematic diagram showing these features is shown in Figure 6.1.

The ultra-high vacuum conditions ($<10^{-7}$ torr) were essential for the prevention of adsorption of residual gasses onto the sample surfaces (Benninghoven 1987).

The primary ion-source used was a high-resolution liquid-metal ion-gun, utilising positive Gallium ions (Ga+).

6.1



Key Components of SIMS instrument

Using a current density of 10nA and a 10keV accelerating voltage, an ion beam of 5 micron diameter could be achieved (from experimental data). This enabled the ion-beam to be fully focused onto the fibrous samples. A quadrupole mass spectrometer was used for the detection of elemental, isotopic and molecular secondary ions. Mass resolution of 0.1 atomic mass units was possible. Note that positive and negative ions could not be detected simultaneously.

Data processing was carried out using an Apple II micro-computer in conjunction with a VG Scientific software package. The operation of the SIMS instrument was controlled by this software which allowed data presentation in the form of mass spectra or depth profiles, depending on the mode of operation.

The SIMS instrument was operated under the following conditions:

liquid metal ion source: 10kV gallium +ve ion
beam current: 1nA
electron flood: 500eV, 2.5A current, 0.25mA emission

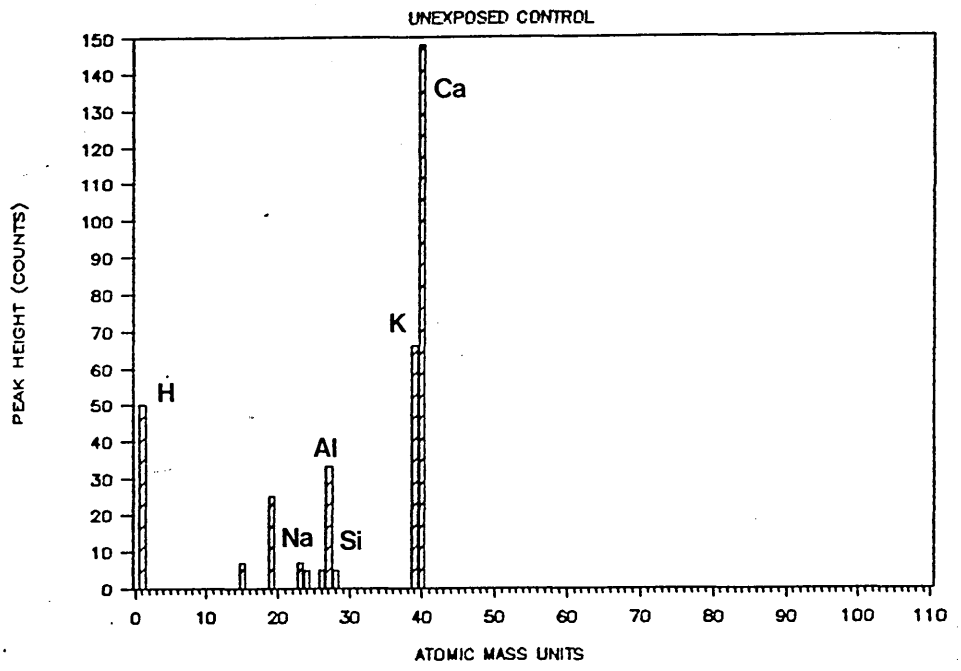
The primary ions were rastered over an area of the sample surface of approximately 25um^2. The quadrupole was set up to scan from 0-10 and 10-110 AMU, after preliminary scans up to 500 AMU had identified that the majority of analytical data was contained in the 0-110 AMU region. Resolution of 0.1 AMU was possible and a data collection time of 0.25 seconds per 0.1 AMU was set. Data was collected in the "static" mode, in the form of mass spectra. Analysis was carried out on the overhanging fibres.

Detection of primary gallium ions on the spectra was found to be a good indication that the sample was being hit by the ion beam. Both positive and negative static spectra from the sample surfaces were obtained and each spectra was obtained from a different region of the same overhanging fibre. Run 1 and run 2 are repeat analysis, also from different regions of the same fibre. Control spectra are from unexposed fibres.

6.2.3 RESULTS

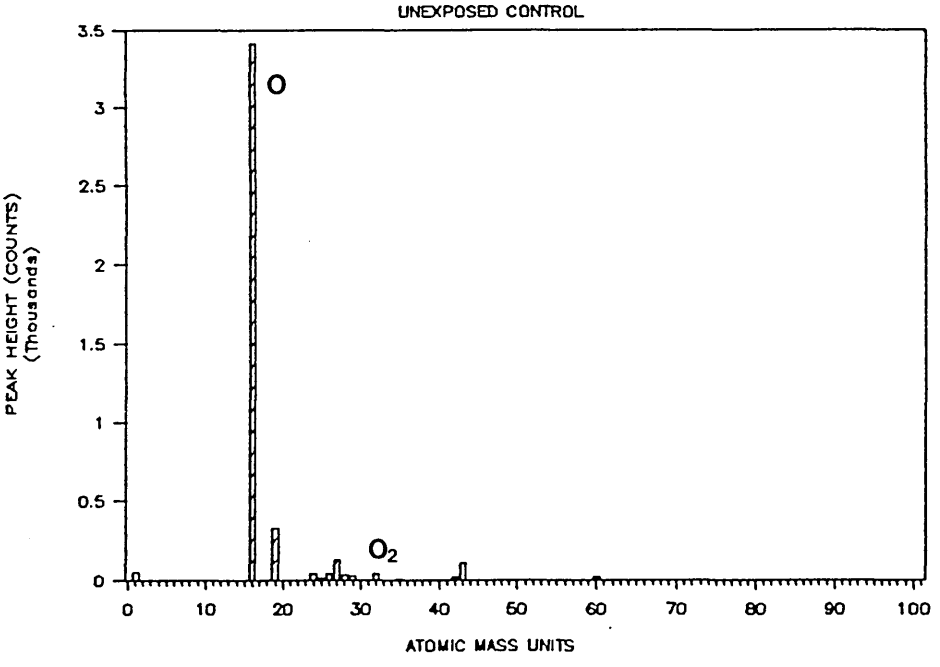
+ve and -ve mass spectra for E, lead and Cemfil glass-fibre exposed to Gambles' fluid and controls are shown by Figures 6.3-24.

Figure 6.3



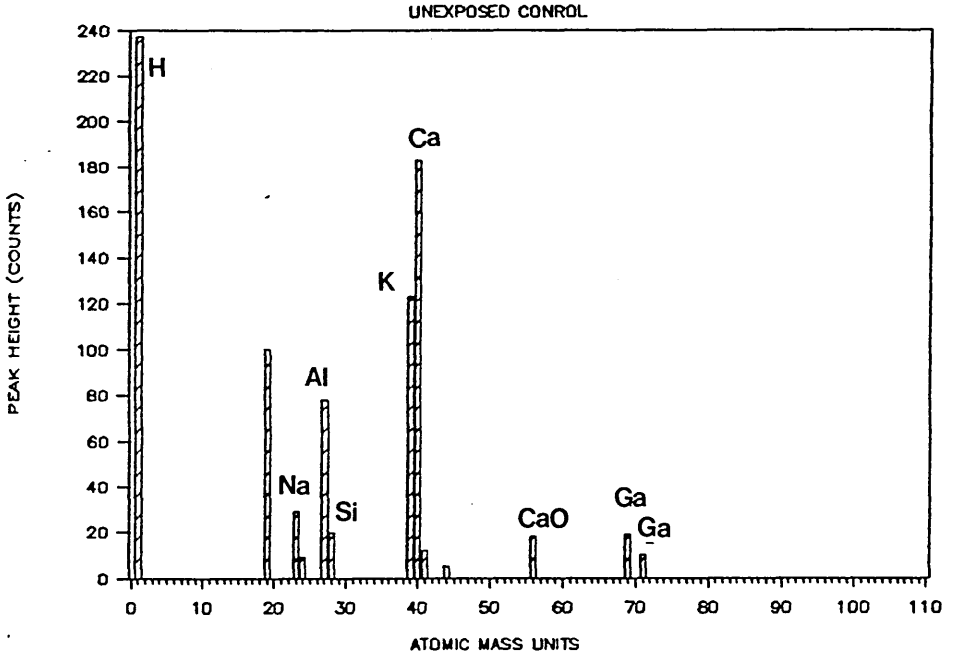
SIMS analysis: unexposed E-glass fibre control, positive mass spectrum (run 1).

Figure 6.4



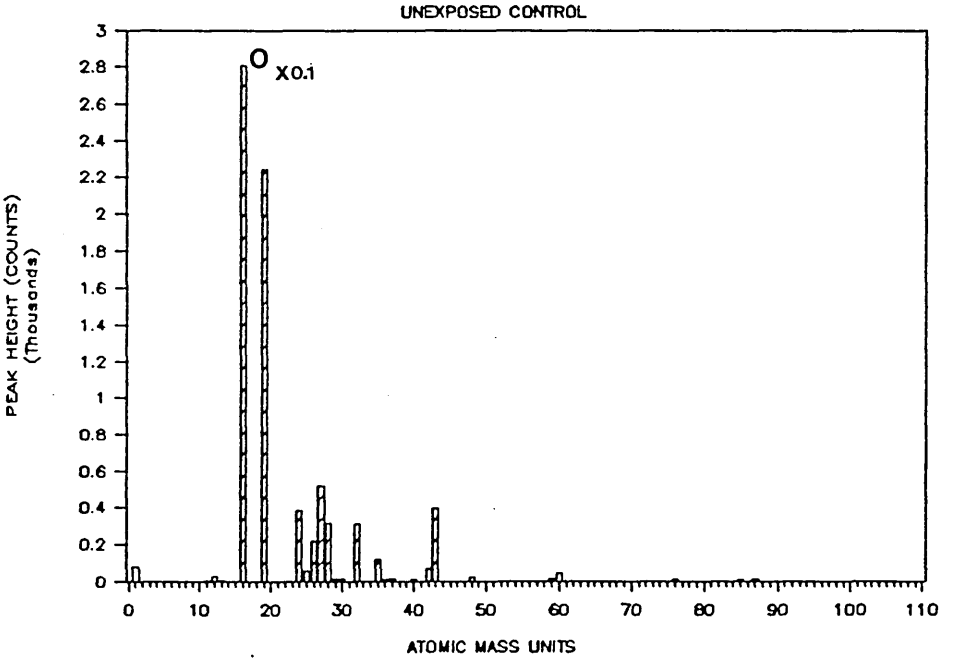
SIMS analysis: unexposed E-glass fibre control, negative mass spectrum (run 1).

Figure 6.5



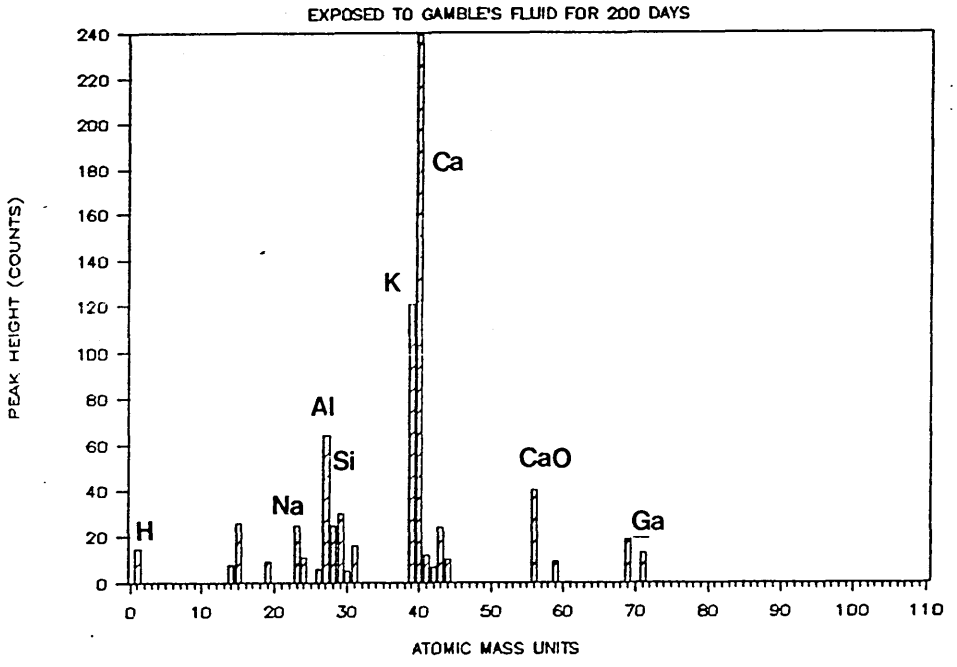
SIMS analysis: unexposed E-glass fibre control, positive mass spectrum (run 2).

Figure 6.6



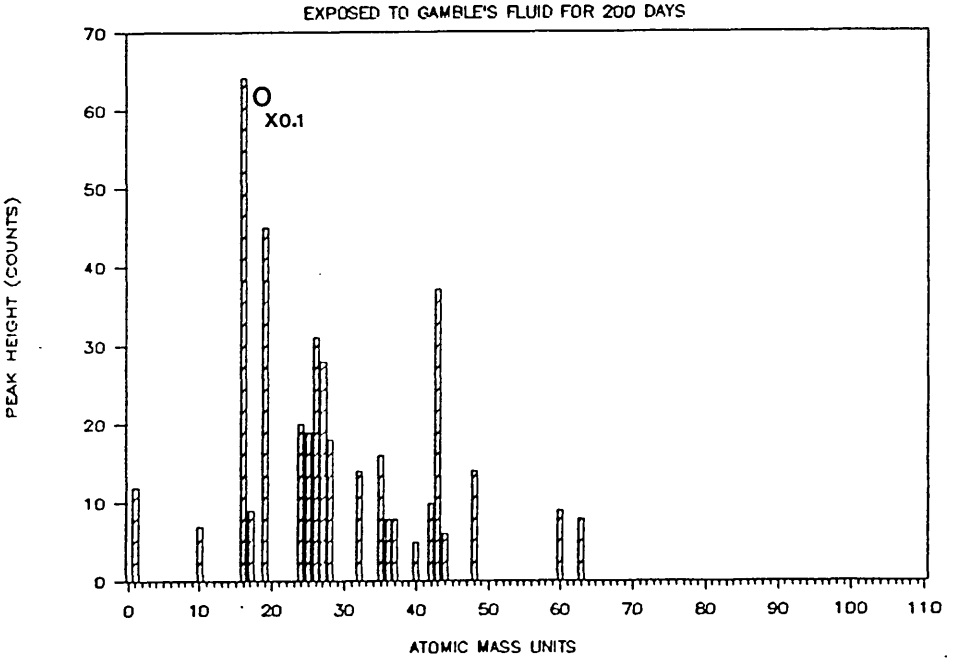
SIMS analysis: unexposed E-glass fibre control, negative mass spectrum (run 2).

Figure 6.7



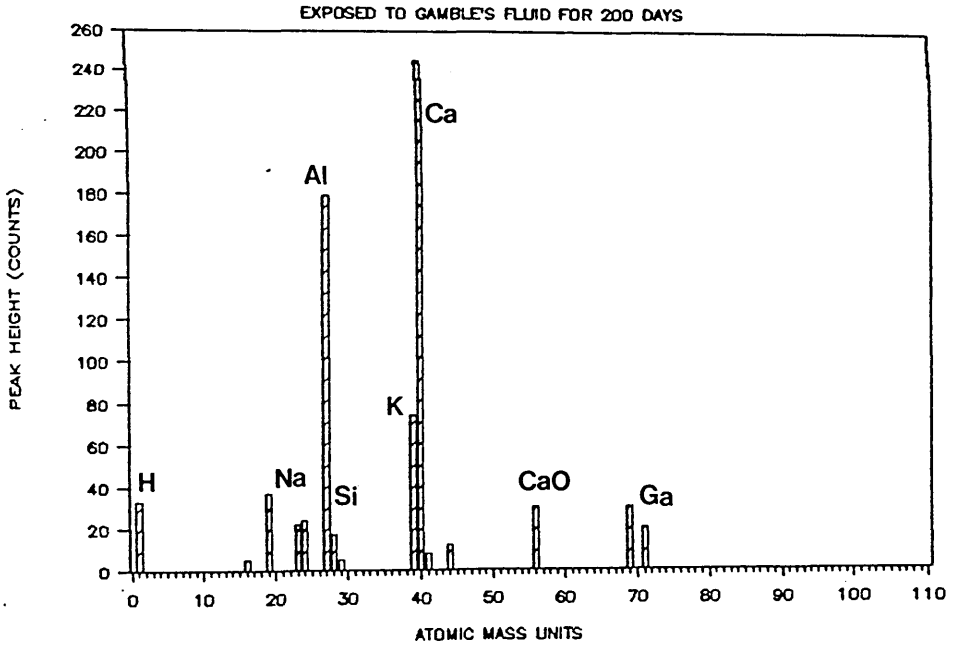
SIMS analysis: E-glass fibre exposed to Gamble's fluid positive mass spectrum (run 1).

Figure 6.8



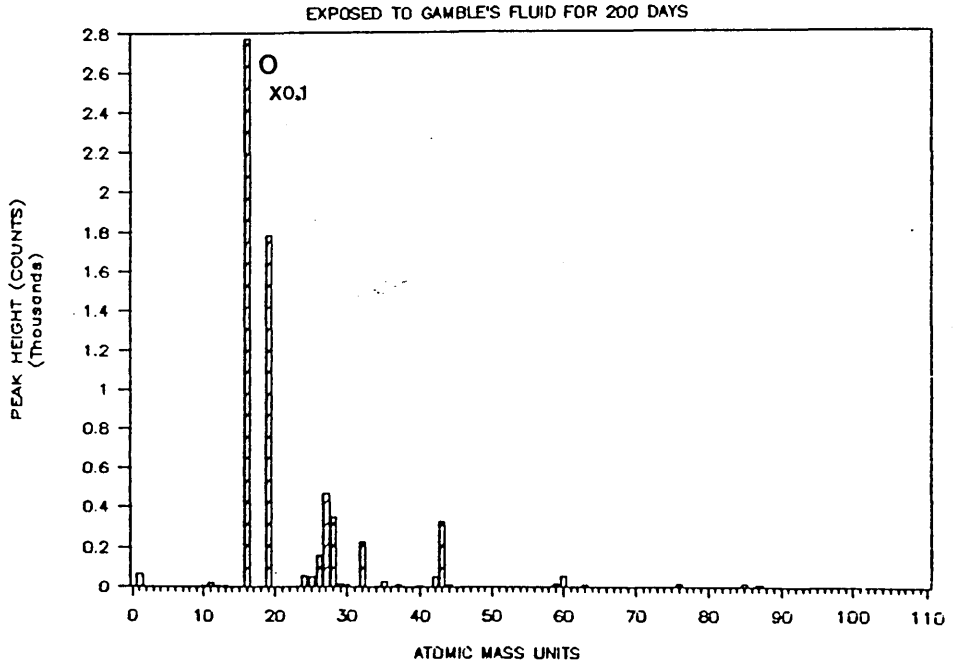
SIMS analysis: E-glass fibre exposed to Gamble's fluid negative mass spectrum (run 1).

Figure 6.9



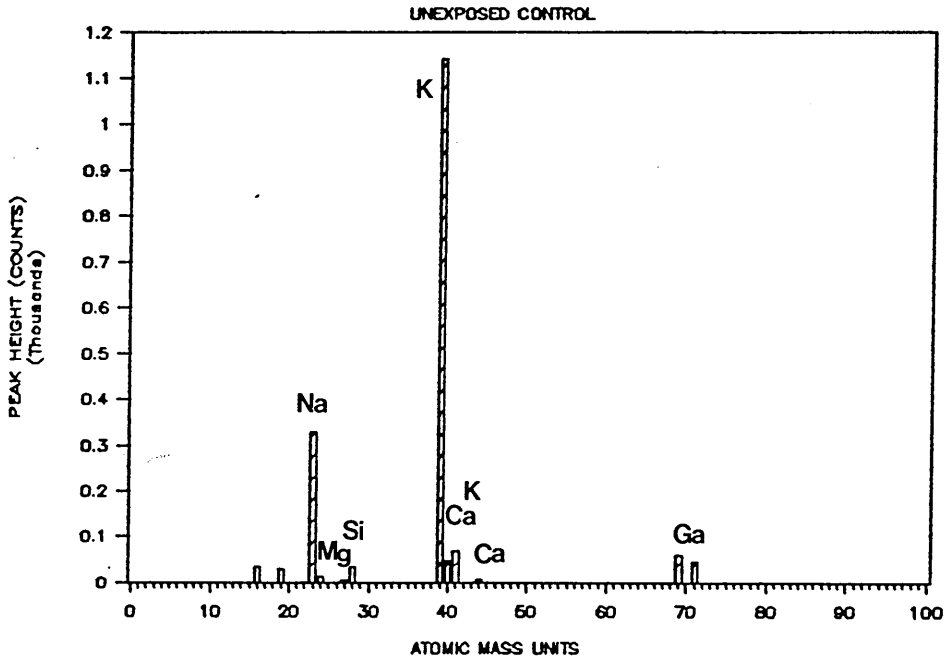
SIMS analysis: E-glass fibre exposed to Gamble's fluid positive mass spectrum (run 2).

Figure 6.10



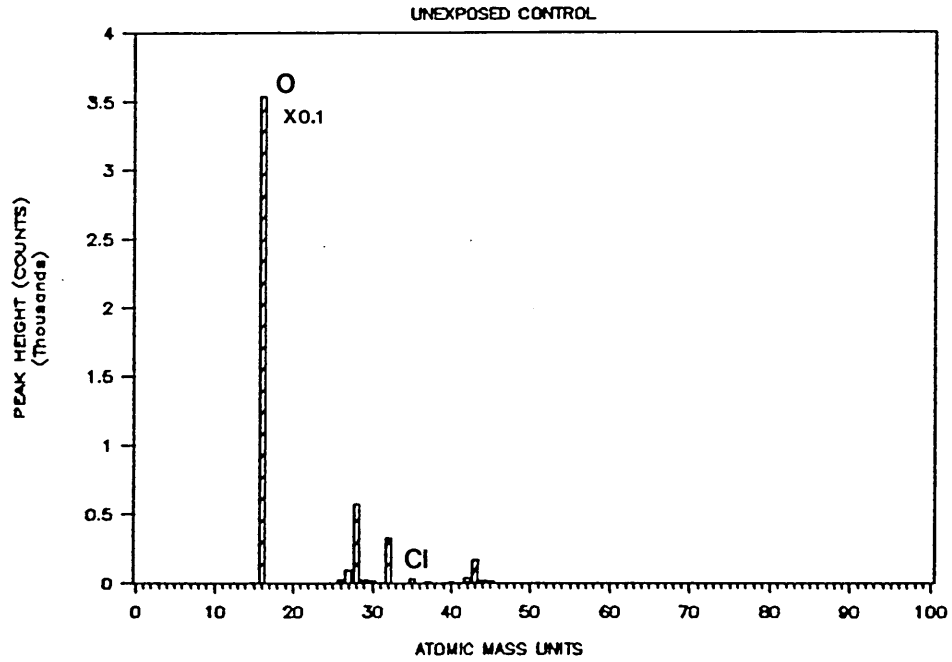
SIMS analysis: E-glass fibre exposed to Gamble's fluid negative mass spectrum (run 2).

Figure 6.11



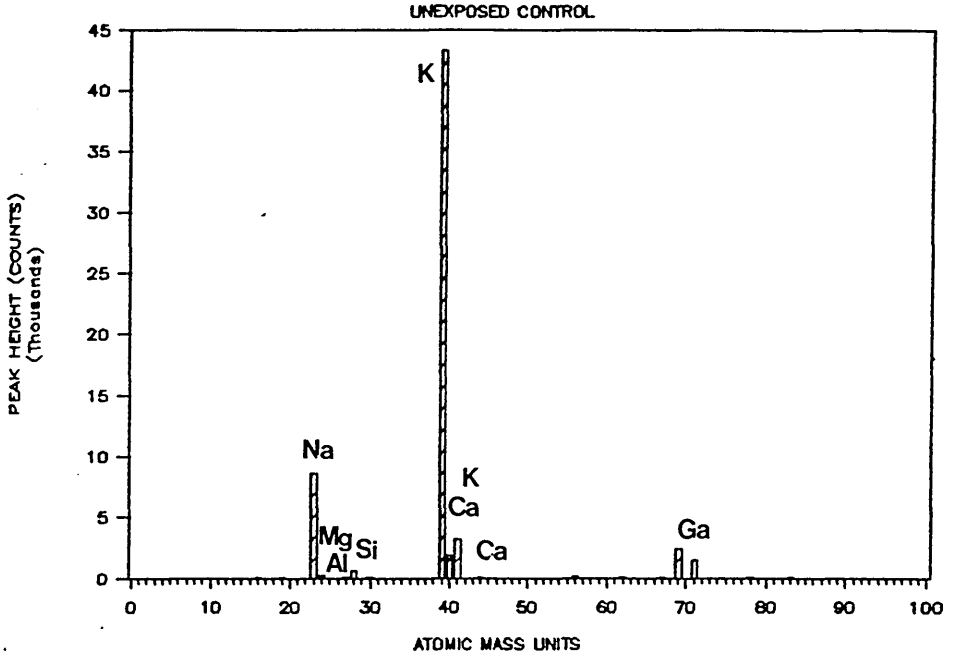
SIMS analysis: unexposed lead-glass fibre control, positive mass spectrum (run 1).

Figure 6.12



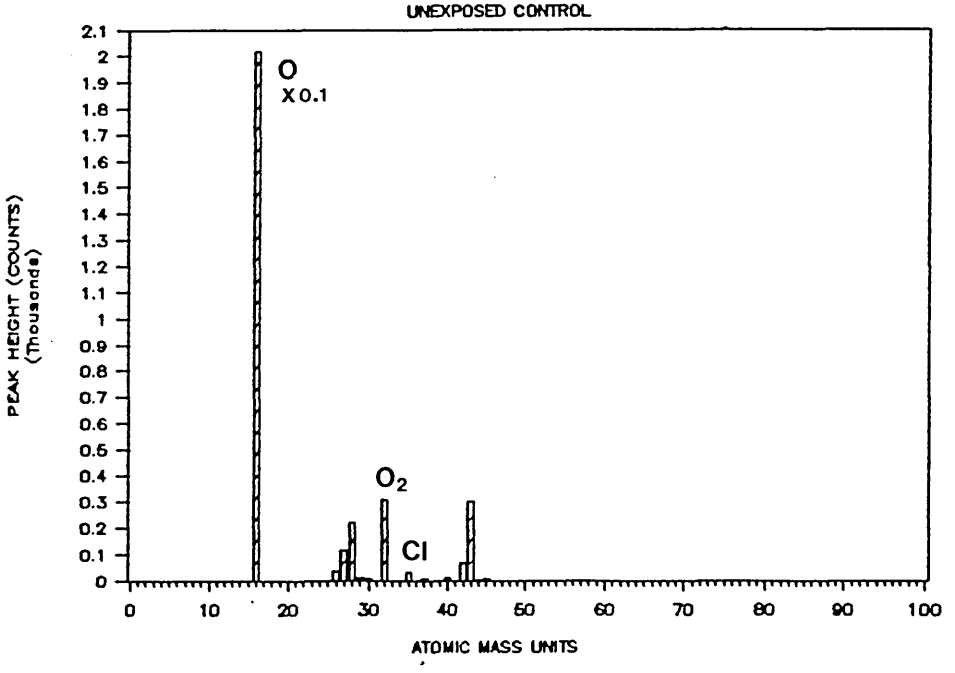
SIMS analysis: unexposed lead-glass fibre control, negative mass spectrum (run 1).

Figure 6.13



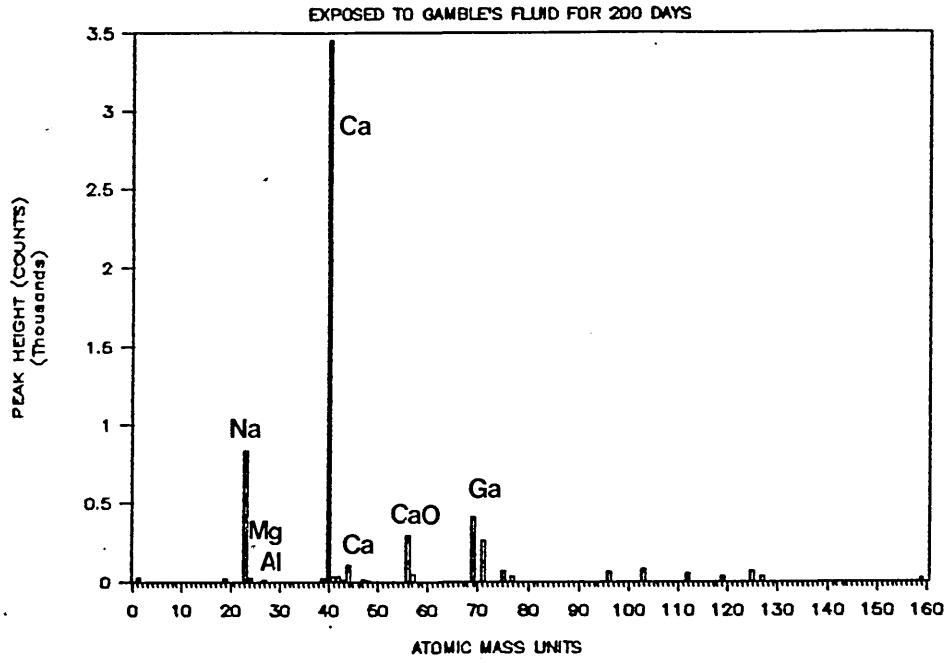
SIMS analysis: unexposed lead-glass fibre control, positive mass spectrum (run 2).

Figure 6.14



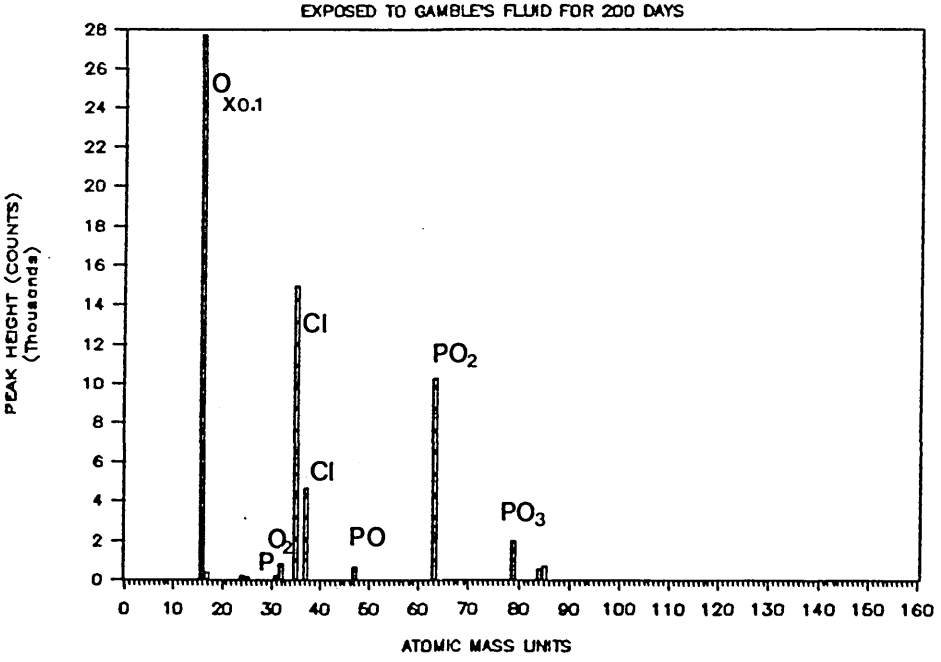
SIMS analysis: unexposed lead-glass fibre control, negative mass spectrum (run 2).

Figure 6.15



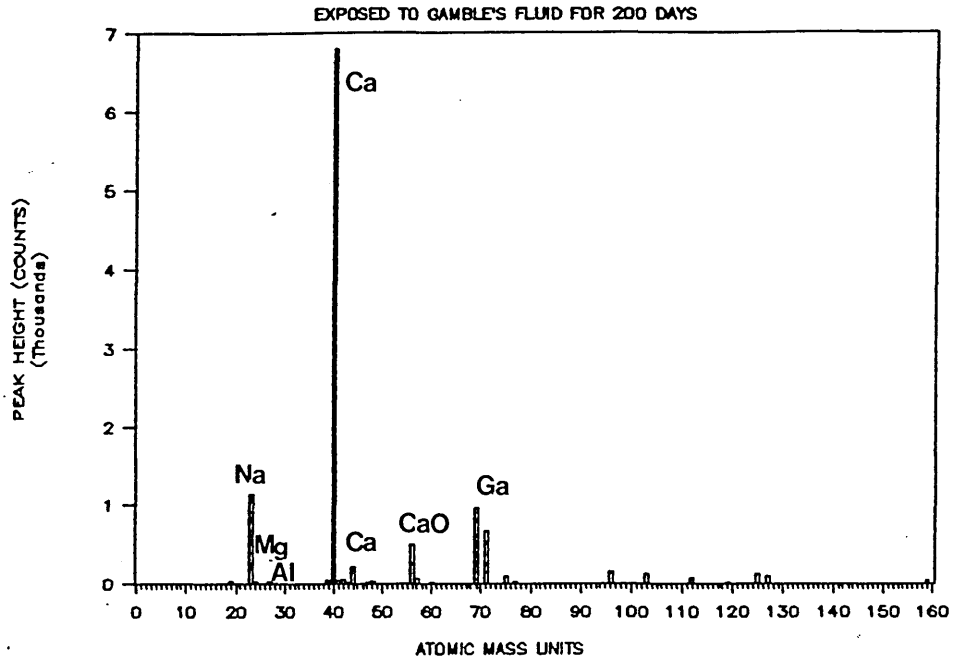
SIMS analysis: lead-glass fibre exposed to Gamble's fluid, positive mass spectrum (run 1).

Figure 6.16



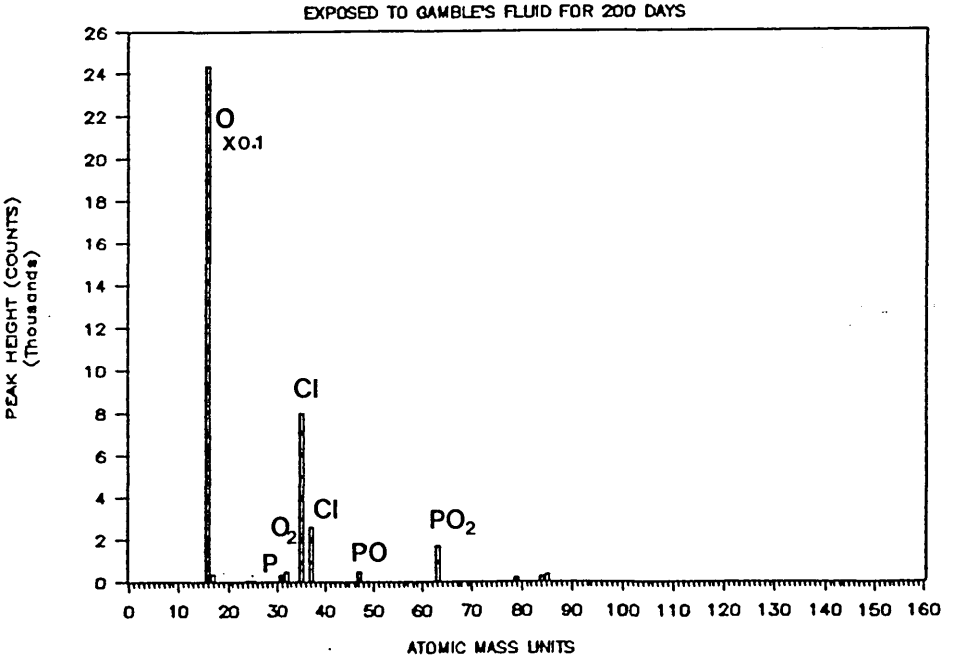
SIMS analysis: lead-glass fibre exposed to Gambles fluid, negative mass spectrum (run 1).

Figure 6.17



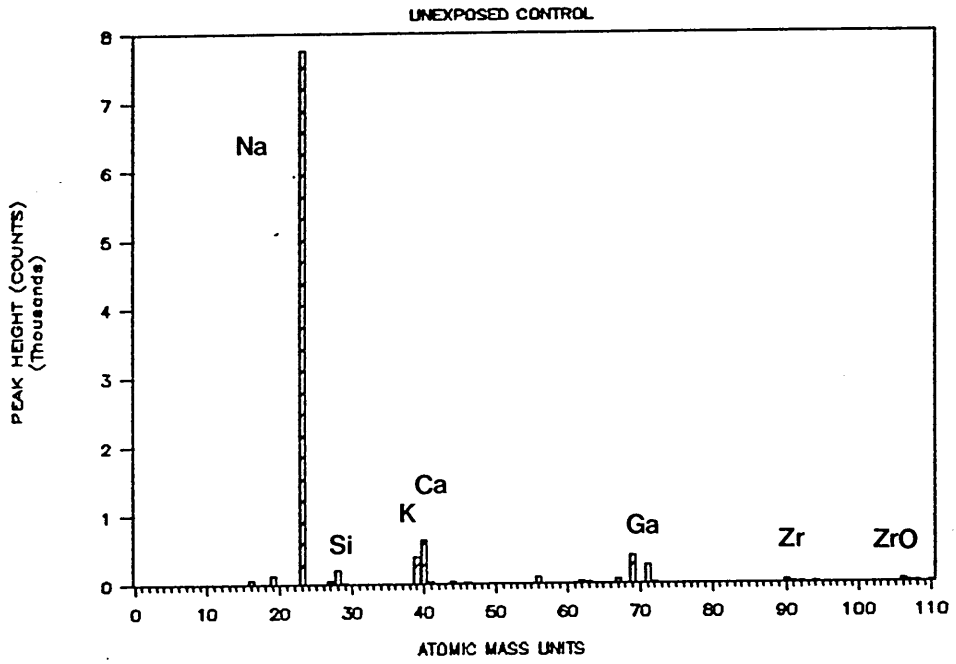
SIMS analysis: lead-glass fibre exposed to Gamble's fluid, positive mass spectrum (run 2).

Figure 6.18



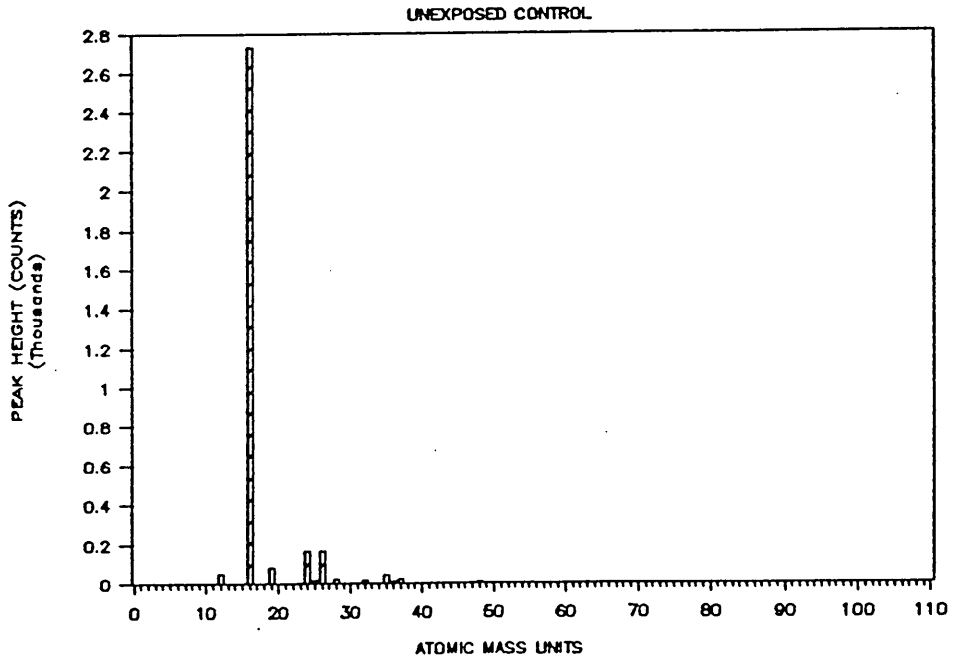
SIMS analysis: lead-glass fibre exposed to Gambles fluid, negative mass spectrum (run 2).

Figure 6.19



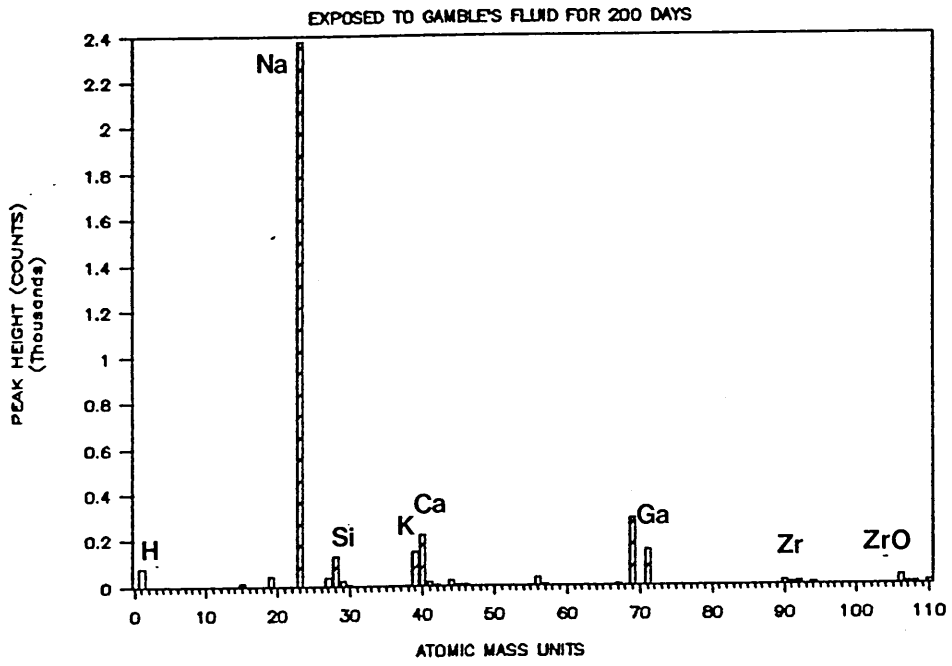
SIMS analysis: unexposed Cemfil glass fibre control, positive mass spectrum (run 1).

Figure 6.20



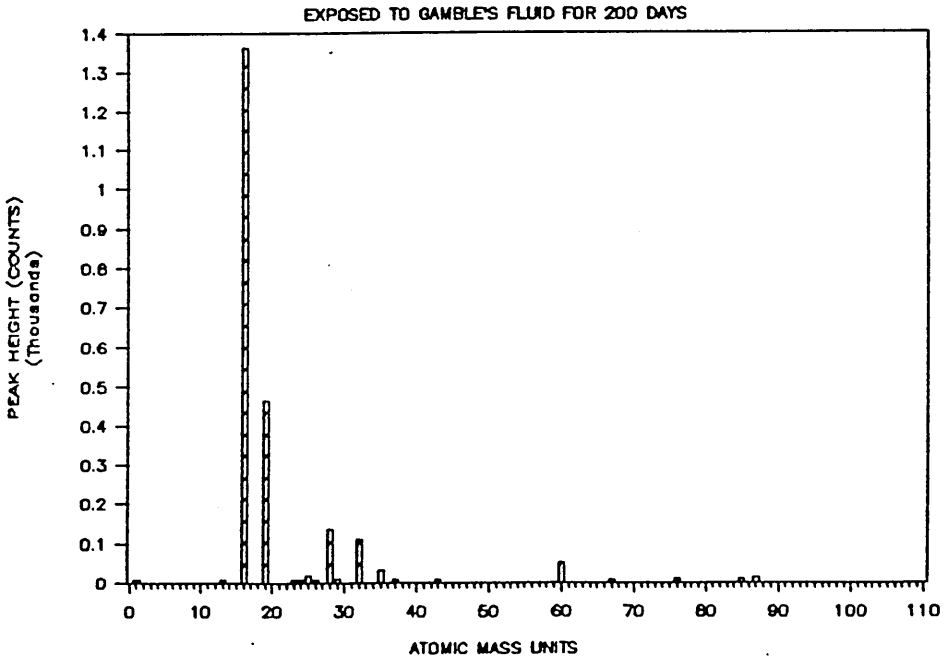
SIMS analysis: unexposed Cemfil glass fibre control, negative mass spectrum (run 1).

Figure 6.21



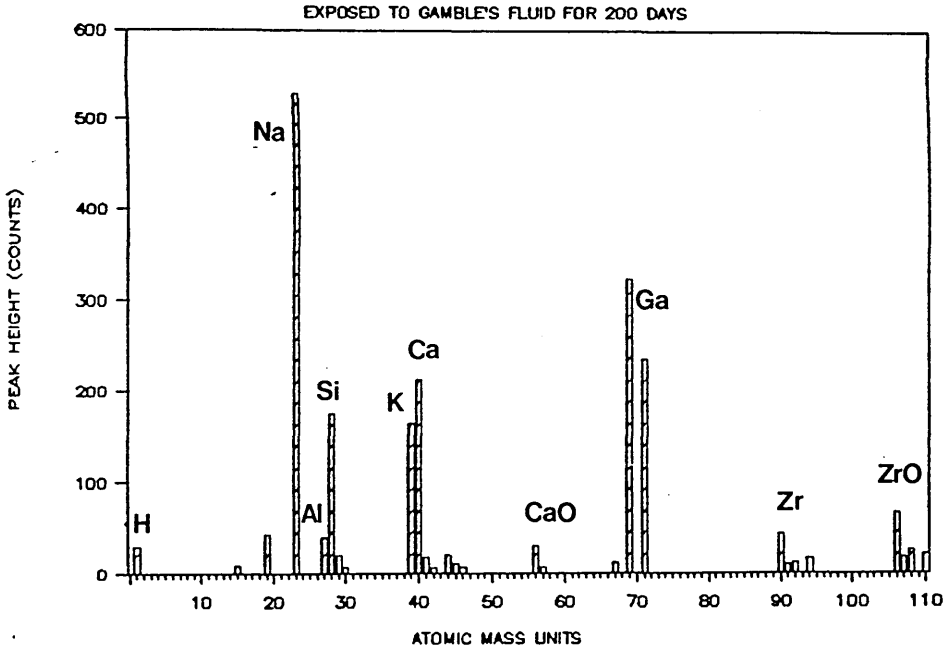
SIMS analysis: Cemfil fibre exposed to Gamble's fluid, positive mass spectrum (run 1).

Figure 6.22



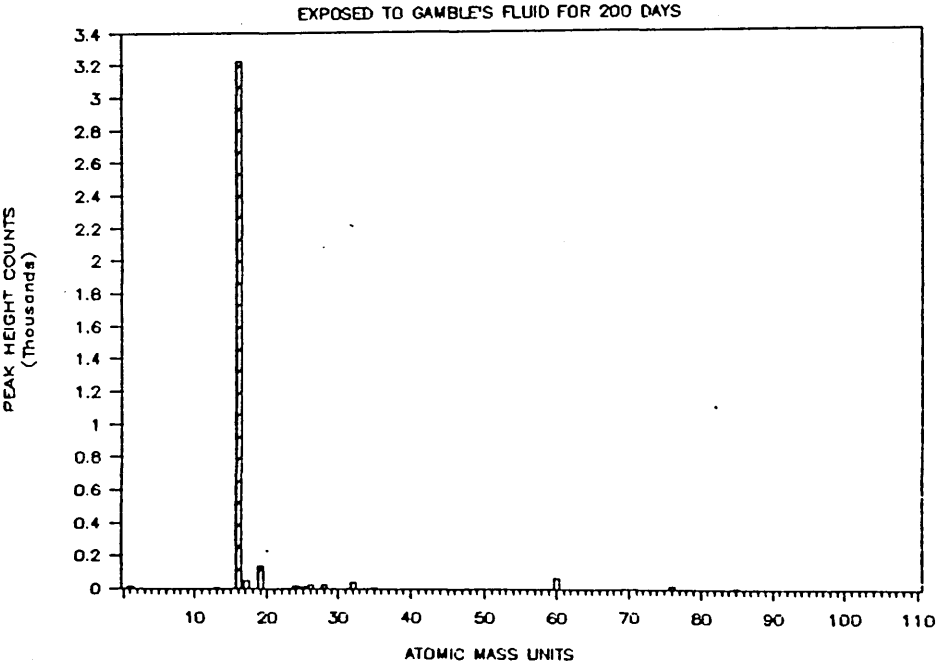
SIMS analysis: Cemfil fibre exposed to Gamble's fluid, negative mass spectrum (run 1).

Figure 6.23



SIMS analysis: Cemfil fibre exposed to Gamble's fluid, positive mass spectrum (run 2).

Figure 6.24



SIMS analysis: Cemfil fibre exposed to Gamble's fluid, negative mass spectrum (run 2).

6.2.4 DISCUSSION AND INFERENCES OF RESULTS

Generally, it was found that the negative SIMS spectra were difficult to interpret and analyte peaks were dwarfed by the large oxygen peak. It was thought that many of the negative-ion peaks were associated with ionisation of residual organic contaminants in the specimen chamber. In the case of the continuous filament E- and Cemfil fibre ionisation of organic coatings introduced during manufacture could also add to the complexity of the negative-ion spectra.

Positive spectra were generally more consistent and the analyte peaks could be related to species known to be present in the control samples.

Total secondary ion count rates were often low, and a range of several hundred (Figure 6.9) to several tens of thousands (Figure 6.13) was encountered for the range of samples. Low total counts can be explained by assuming low etch rates and/or ion yields.

Negative spectra generally had total ion yields an order of magnitude greater than the corresponding positive ion spectra, primarily a result of the relatively large negative oxygen-ion yield.

These observations were probably associated with variable and/or different etch rates and ion yields, associated with i) conditions of analysis and ii) differences in the substrate.

Quantitative SIMS relies on the ability to relate secondary ion yield to the concentration of that species in the substrate. However, it is a well documented fact that etch rates and ion yields are affected by a range of factors, making quantitative SIMS an intrinsically difficult technique to quantify (Benninghoven et al 1987). These factors include i) type of primary ion, energy and angle of incidence ii) composition and structure of the target.

Ion yield and etch rate data is only available for certain well defined systems and liquid metal ion sources have only recently become of interest to researchers. Whilst secondary ion-yield data exists for Cs, In and Sn positive ion-beams, quantitative data is not yet available for the positive ion gallium source used in this study. It is not possible to interpret our data by considering ion yield data for an alternative source because the relative ion yields can be completely different (Benninghoven et al 1987).

Figure 6.3-6.10 are mass spectra from control and exposed E-glass fibre samples. The negative spectra were difficult to interpret, though the oxygen peak (16 AMU) was prominent, being much larger than any of the other negative ion peaks. The other peaks present were possibly associated with negative cluster ions, formed by various combinations of hydrogen (AMU 1) and carbon (AMU 12) (from residual organic contaminants) and oxygen (AMU 16) and other analytes (present in the fibre) or from organic coatings introduced during manufacture. Positive spectra were more consistent and the majority of the peaks could be related to analytes present in the sample and/or expected contaminants.

It was hoped that SIMS would provide a suitable method for the determination of light elements (for which EDXA was unsuitable) and in particular for boron analysis (isotopes of 10 and 11 amu), which was supposedly present in the E-glass (8 mol.%). However, characteristic boron peaks were not present in positive or negative spectra, though negative ion peaks were present at 26, 27, 42 and 43 amu, possibly associated with BO and BO_2 cluster ions. The absence of boron peaks could be associated with low ion yields, though in the absence of reference data it is not possible to comment further.

The positive spectra for both unexposed and exposed E-glass fibres clearly show the presence of the key constituent elements: Al, Si, K and Ca. The presence of Na was also

identified, although this was not detected using EDXA (Section 4.2) which had relatively poor sensitivity towards Na when using the operating conditions defined (Section 3.4.2), as observed experimentally from relevant X-ray spectra (Figure 4.18 and 4.19).

In the absence of SIMS reference data, providing ion-yields for the analytes when determined under the conditions of operation, it was not possible to make a comparison of the relative and absolute sensitivities of the SIMS and EDXA techniques.

It is recommended, in retrospect, that a multi-element calibration standard, containing accurately known concentrations of the components in the test samples, should be prepared and analysed by SIMS and EDXA under the operating conditions defined here and in Section 3.4.2. This would provide basic comparative data for the absolute and relative sensitivity of the two techniques based on ion and X-ray yields.

The following table shows the absolute and relative positive ion yields of H, Na, Al, Si, K and Ca for the unexposed E-glass and E-glass exposed to Gambles' fluid:

Table 6.1 Positive ion data for E-glass fibre.

Exposure condition	Analyte	peak height (counts)	% height rel. Si
unexposed, run 1	H	50	1000
	Na	8	160
	Al	34	680
	Si	5	100
	K	65	1300
	Ca	147	2940
unexposed, run 2	H	238	1190
	Na	33	165
	Al	75	375
	Si	20	100
	K	124	620
	Ca	183	915
Gambles' exposure run 1	H	15	63
	Na	24	100
	Al	63	263
	Si	24	100
	K	120	500
	Ca	240	1000
Gambles' exposure run 2	H	34	227
	Na	23	153
	Al	175	1167
	Si	15	100
	K	72	480
	Ca	245	1633

The data for the unexposed E-glass showed positive ion yields to be low. The ion yields for unexposed E-glass from Run 1 and Run 2 showed poor absolute reproducibility, for example, the Si and Al ion yields were 5 and 34 on Run 1 and 20 and 75 on Run 2 respectively.

Calculation of analyte peak height ratios for the unexposed E-glass fibre showed that the reproducibility of the relative peak intensities was also poor. For example, the Si:Ca peak ratio was 1:29.4 on Run 1 and 1:9.15 on Run 2. This represents a range of ± 53% and a mean of 1:19.3.

The difference between Run 1 and Run 2 was that a different area of the same fibre was being analysed. The differences in relative signal intensities may possibly be explained if:

a) there are regional variations in analyte levels at the fibre surface,

b) if the relative ion yields are affected by regional contaminants on the sample surface.

c) if instrumental faults are occurring.

The data for the E-glass fibre exposed to Gambles' fluid also inferred that the absolute and relative reproducibility of the analysis was poor and that total positive ion yields were low. For example, the Si and Al ion yields were 24 and 63 on Run 1 and 15 and 175 on Run 2 respectively and the Si:Al peak ratio was 1:2.63 on Run 1 and 1:11.67 on Run 2.

The poor absolute and relative reproducibility meant that it was not possible to make a quantitative comparison of the analyte levels in the unexposed and exposed E-glass fibres.

The poor relative reproducibility also meant that a direct comparison of efficiencies of analyte signal emission with EDXA was not possible.

Figures 6.11-18 are mass spectra obtained from exposed and unexposed lead-glass fibre.

Positive-ion mass spectra obtained from the control samples demonstrated the key analytes: Na, Mg, Al, Si, K and Ca. Pb was only just detectable (isotopes at 204, 206, 207, 208 AMU) presumably having a relatively low positive ion-yield when gallium is used as the primary ions source.

This was in contrast with EDXA, which provided good sensitivity with respect to Pb analysis.

The following table shows the absolute and relative positive ion yields of Na, Mg, Al, Si, K and Ca for the unexposed lead-glass and lead-glass exposed to Gambles' fluid:

Table 6.2 Positive ion data for lead-glass fibre.

Exposure condition	Analyte	peak height (counts)	% height rel. Si
unexposed, run 1	Na	320	914
	Mg	15	43
	Al	5	14
	Si	35	100
	K	1150	3286
	Ca	55	157
unexposed, run 2	Na	7000	700
	Mg	500	50
	Al	200	20
	Si	1000	100
	K	43000	4300
	Ca	2500	250

Table 6.2 cont...

Gambles' exposure run 1	Na	800	-
	Mg	50	-
	Al	25	-
	Si	-	-
	K	-	-
	Ca	3450	-
Gambles' exposure run 2	Na	1200	-
	Mg	50	-
	Al	50	-
	Si	-	-
	K	-	-
	Ca	6700	-

The data for the unexposed lead-glass fibre showed the absolute reproducibility to be poor, there being an order of magnitude difference in total ion-yield between the two analyses (Run 1 and Run 2). Generally, however, positive secondary ion-yields were greater than those for the E-glass fibre controls, for example, Si counts of 5 and 20 for E-glass controls, Run 1 and 2, compared with Si counts of 35 and 1000 for the lead-glass controls.

It was thought that poor absolute reproducibility could be associated with fibre geometry, that is, the curvature of the fibre may physically prevent secondary ions reaching the quadrupole, depending on the incidence point of the primary ion beam.

The higher positive ion yields for the lead-glass controls may be associated with:

i) increased sputter rates and ion-yields due to compositional differences in the glass,

ii) the absence of an organic coating on the lead-glass, which could potentially shield the glass.

The relative reproducibility of the positive ion yields for unexposed lead-glass samples was poor, but better than that previously encountered for the E-glass. The largest relative variation in peak height was reflected in a Si:Ca ratio of 1:1.57 on Run 1 and 1:2.5 on Run 2. This represents a range of ± 23% on a mean ratio of 1:2.04.

The positive and negative ion spectra from the exposed samples were considerably different to those of the unexposed samples, indicating qualitative changes in the surface composition during exposure to Gamble's fluid.

Positive ion spectra showed that Si was apparently absent from the surface (consistent with EDXA data, Section 4.2) which appeared to have become greatly enriched with Ca; Na, Mg and Al also appeared to be present on the sample surface. Negative-ion spectra indicated surface enrichment of Cl and P during exposure to Gamble's fluid. These observations were consistent with EDXA data, which confirmed the absence of Si and the enrichment of Ca, P and Cl in the surface layers of the exposed fibres (Figure 4.40-41).

Figures 6.19-24 are spectra from exposed and unexposed Cemfil fibre sample surfaces. The negative-ion spectra were difficult to interpret, however the positive-ion spectra were more consistent, showing the presence of the key analytes: Na, Si, K, Ca and Zr. The spectra showed the characteristic isotopic splitting pattern of Zr and the

molecular species ZrO, also showing isotopic splitting. It was further noted that the ZrO peaks were of greater intensity than the Zr peaks, which indicated the greater ion stability and/or yield of the oxide ion.

The following table shows the absolute and relative positive ion yields of Na, Si, K, Ca and Zr for the unexposed Cemfil fibre and Cemfil fibre exposed to Gambles' fluid:

Table 6.3 Positive ion data for Cemfil fibre.

Exposure condition	Analyte	peak height (counts)	% height rel. Si
unexposed, run 1	Na	7700	2200
	Si	350	100
	K	450	129
	Ca	700	200
	Zr	100	29
Gambles' exposure run 1	Na	2380	1983
	Si	120	100
	K	150	125
	Ca	220	183
	Zr	30	25
Gambles' exposure run 2	Na	535	315
	Si	170	100
	K	150	88
	Ca	210	124
	Zr	35	21

Absolute reproducibility, again, appears to be poor, though it is not possible to comment on relative durability for unexposed Cemfil fibre as only one analysis was carried out (Run 1).

It was interesting to note that the Si:Na peak ratios for Run 1 and 2 of the exposed Cemfil fibre showed a large relative difference (1:19.8 for Run 1 compared with 1:3.15 for Run 2).

6.2.5 CONCLUSIONS

Static SIMS analysis, when carried out using the defined operating conditions, provided a qualitative method for the near surface analysis of glass fibres, clearly showing the different chemical composition of the lead-glass fibre surface after exposure to Gamble's fluid.

The use of a high-resolution gallium ion-gun enabled static SIMS analysis of fibrous samples with diameters as small as 7 microns.

The absolute and relative reproducibility of the technique appeared to be poor and quantitative analysis would not be possible from one or two analysis. Poor relative reproducibility was presumably related to compositional variations at the sample surface.

In certain respects SIMS analysis provided data complementary to EDXA data, with SIMS providing good analytical sensitivity for species such as Na, as well as providing molecular data. Quantitative data is not, however, available to enable a comparison with the EDXA technique.

Whilst SIMS can be accepted as a useful qualitative method for the analysis of glass fibres, a statistical appraisal of reproducibility and reliability was required to determine its usefulness as a quantitative method.

6.3.1 APPRAISAL OF SIMS REPRODUCIBILITY

6.3.2 INTRODUCTION

In view of the initial qualitative success of the SIMS technique used for the analysis of individual fibre samples, an attempt was made to determine the reproducibility of the technique and appraise its potential for use as a semi-quantitative analytical method.

6.3.3 METHODOLOGY

Three point analysis was carried out on each of 5 unexposed Cemfil fibres by the method described previously (Section 6.2.2). This analysis was repeated using Cemfil fibre exposed to water for 12 months.

6.3.4 RESULTS

A summary of a statistical interpretation of the positive-ion data is presented in Table 6.4 and Table 6.5.

"Average" positive-ion spectra are shown in Figure 6.25-26.

Fig 6.25

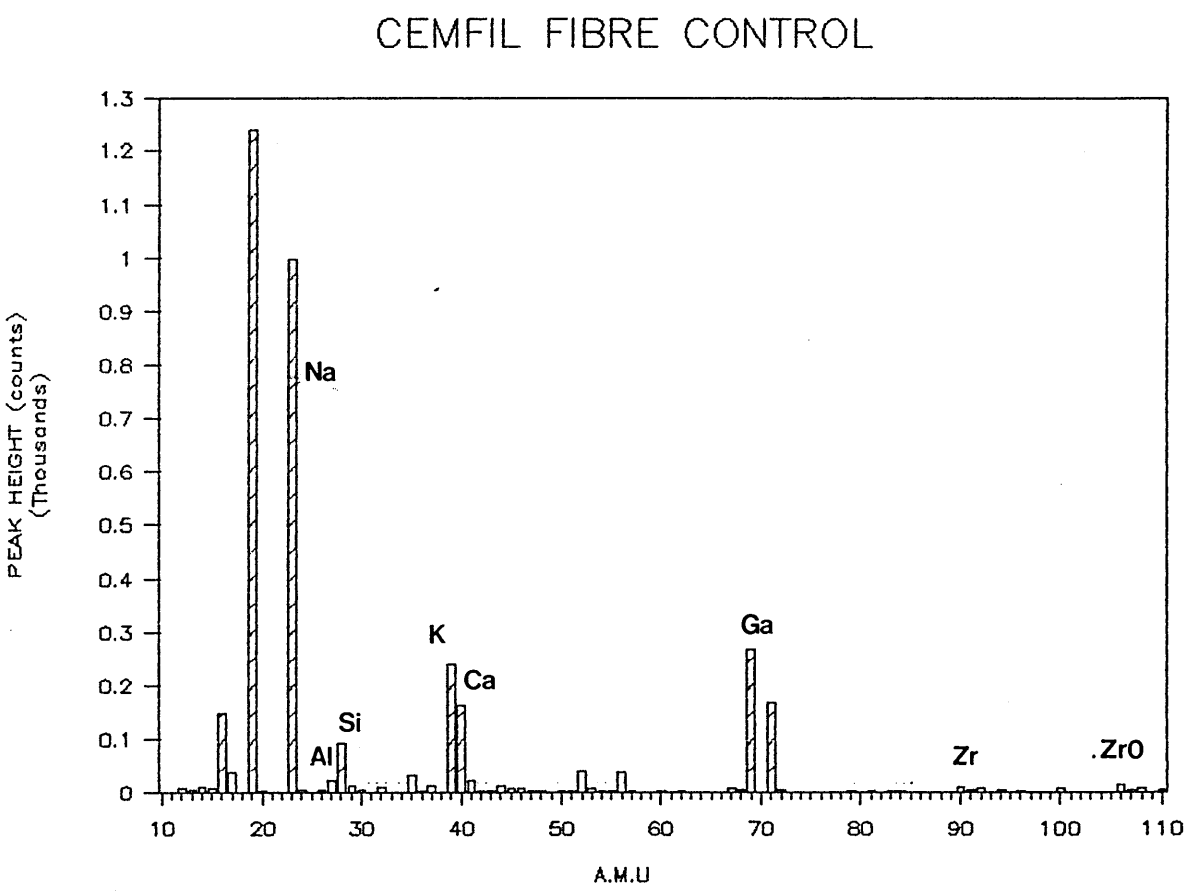


Fig 6.26

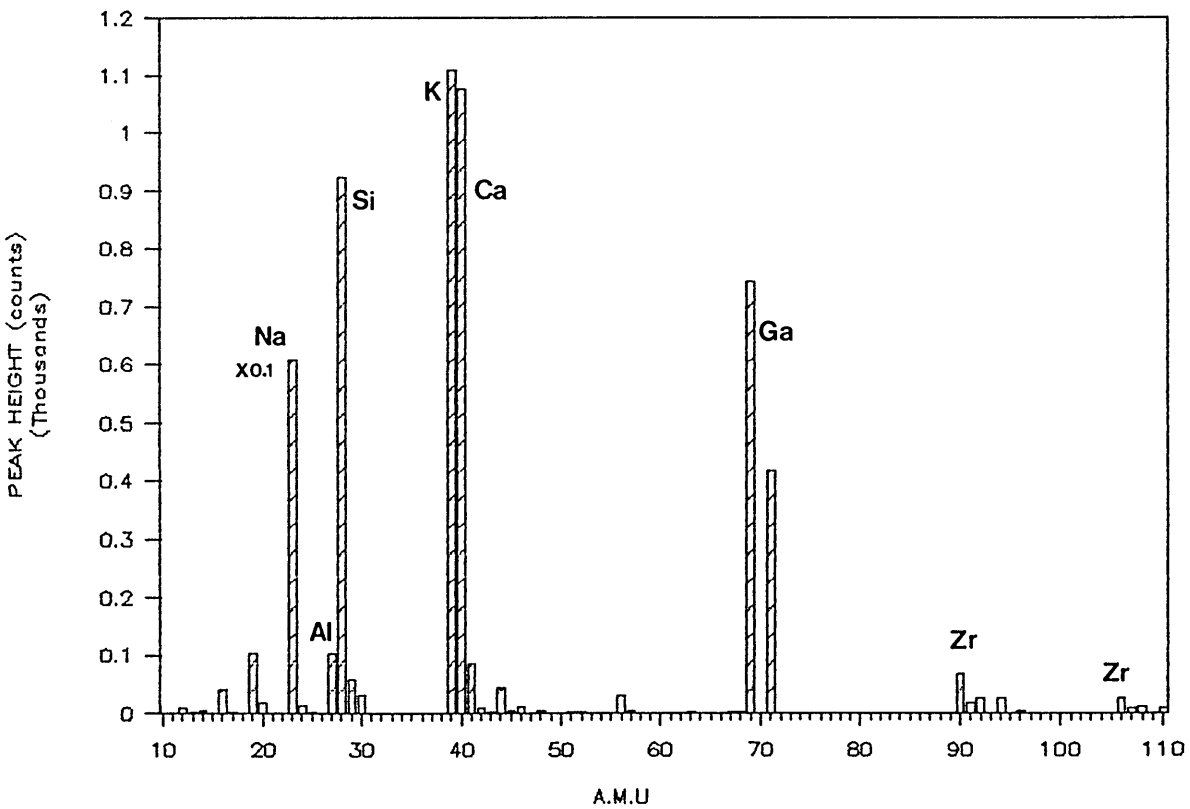


Table 6.4 Summary of +ve ion data for Cemfil controls

A.M.U	Inference	Av.peak ht ± 2.sd	Av.ht rel [Si] % ± 2.sd
19	F ?	1239.93 ± 957.98	1694.55 ± 873.39
23	Na	998.47 ±1046.71	1071.84 ± 607.00
69	Ga	266.40 ± 265.37	346.99 ± 321.05
39	K	238.53 ± 321.26	241.65 ± 187.27
71	Ga	167.80 ± 172.56	219.58 ± 214.51
40	Ca	161.60 ± 196.07	164.36 ± 85.11
16	O, CH_4 ?	147.87 ± 63.63	230.90 ± 266.92
28	Si	91.80 ± 65.04	100.00 ± 0.00
52	Cr ?	39.80 ± 78.50	66.10 ± 168.53
17	OH, NH_3	37.27 ± 23.83	53.66 ± 29.92
56	Fe, CaO	36.53 ± 39.09	47.65 ± 62.61
35	Cl ?	32.47 ± 23.86	45.56 ± 29.59
41	K	22.33 ± 27.14	23.81 ± 13.70
27	Al	22.13 ± 17.78	25.31 ± 6.86
106	ZrO	15.80 ± 9.71	18.58 ± 8.24
37	Cl	12.73 ± 11.50	17.54 ± 15.21
44		12.53 ± 9.40	14.48 ± 2.06
29	Si ?	12.13 ± 7.92	14.64 ± 4.08
32		10.40 ± 4.03	15.35 ± 12.01
90	Zr	10.00 ± 5.09	12.84 ± 7.17
14	CH_2, N ?	9.53 ± 8.09	13.03 ± 7.76
15	CH_3 ?	8.33 ± 9.70	11.33 ± 8.19
12	C	8.13 ± 6.67	10.74 ± 6.97
100		7.60 ± 26.79	11.69 ± 43.47
67		7.47 ± 7.71	8.92 ± 7.99
53		7.13 ± 12.39	10.98 ± 25.94
108	ZrO	6.87 ± 5.11	8.38 ± 7.86
92	Zr	6.87 ± 2.62	9.11 ± 3.75
46		6.73 ± 13.21	6.20 ± 9.22
45		6.67 ± 7.92	8.04 ± 5.59
94	Zr	6.13 ± 3.34	7.78 ± 3.74
110	Zr	5.93 ± 3.61	7.26 ± 6.40
72		5.53 ± 6.23	7.42 ± 10.48
26		5.40 ± 5.44	6.99 ± 3.68
68		5.20 ± 7.84	8.00 ± 17.57
107		4.93 ± 3.85	6.12 ± 5.95
30		4.73 ± 2.84	5.38 ± 2.00
91		4.67 ± 2.39	6.12 ± 3.11
24		4.33 ± 3.70	5.17 ± 5.55
13		4.33 ± 4.77	6.23 ± 3.41
55		3.87 ± 7.79	5.98 ± 13.57
50		3.80 ± 6.55	5.77 ± 14.80
42		3.73 ± 3.30	4.13 ± 1.93
57		3.53 ± 4.25	4.71 ± 6.84
96		3.53 ± 3.81	5.59 ± 9.45
79		3.47 ± 5.28	4.78 ± 6.43
54		3.40 ± 6.04	5.36 ± 12.82
62		3.07 ± 4.70	3.25 ± 4.40

Table 6.5 Summary of +ve ion data for Cemfil fibre exposed to water

A.M.U	Inference	Av.peak ht ± 2.sd	Av.ht rel [Si] % ± 2.sd
23	Na	6090.00 ±180652	2088.61 ±6140.04
39	K	1108.60 ±3394.94	1202.61 ±3941.69
40	Ca	1077.00 ±2431.43	306.88 ± 718.45
28	Si	922.80 ±2049.36	100.00 ± 0.00
69	Ga	743.80 ±1558.29	581.13 ±1936.56
71	Ga	416.00 ± 884.13	326.67 ±1104.24
27	Al	104.80 ± 177.99	32.54 ± 43.54
19	F	103.20 ± 221.27	116.68 ± 280.01
41	K	87.40 ± 229.68	85.87 ± 274.28
90	Zr	69.80 ± 151.19	15.23 ± 25.13
29		59.00 ± 119.23	9.32 ± 6.08
44		44.80 ± 115.64	9.68 ± 16.42
16	O	41.00 ± 57.77	26.96 ± 72.18
30		31.40 ± 66.17	4.55 ± 2.18
56	Fe, CaO	30.40 ± 71.31	7.40 ± 8.55
106	ZrO	26.60 ± 39.61	11.11 ± 20.16
94		26.60 ± 53.78	6.56 ± 12.20
92	ZrO	25.00 ± 38.09	9.49 ± 16.94
20		19.20 ± 36.49	3.65 ± 3.23
91	Zr	19.20 ± 33.79	6.21 ± 9.54
24		13.40 ± 21.53	15.11 ± 46.31
46		11.20 ± 34.90	2.52 ± 5.25
108	ZrO	11.00 ± 16.35	4.49 ± 6.88
110	ZrO	10.40 ± 15.78	4.40 ± 8.79
42		9.60 ± 14.73	4.24 ± 8.95
107	ZrO	9.40 ± 12.87	4.07 ± 6.19
12	C	9.00 ± 16.44	11.71 ± 30.36
96	Zr	4.80 ± 7.31	1.36 ± 2.76
45		4.80 ± 5.99	2.45 ± 6.91
14		4.60 ± 6.14	4.74 ± 11.81
48		4.20 ± 11.76	0.19 ± 0.49
57		4.00 ± 2.19	3.02 ± 5.40

6.3.6 DISCUSSION AND INFERENCES OF RESULTS

The average peak-height data, presented in Table 6.4-5, provided an appraisal of the inter-fibre signal reproducibility with respect to the total ion-count for the respective analytes.

The average peak-height data expressed as a percentage of the Si signal provided an appraisal of relative analyte variation with respect to Si levels.

Considering first the positive-ion data for the Cemfil fibre control: it was observed that the analyte peak-height standard deviations were generally quite high relative to the mean peak-height of the analyte (Si, 91.8 ± 33; K, 238 ± 161 etc..). The signal response showed considerable variation between analyses, despite the fact that analysis conditions were kept essentially constant. This inferred that peak-height could not be directly related, quantitatively, to analyte concentration in the specimen.

The mean analyte peak-height relative to Si also generally showed quite a high degree of variation, indicated by large standard deviations relative to the mean (Si, 100% ± 0; K, 242% ± 94; Na, 1072% ± 304 etc..).

Analyte peaks of particular interest were those that were definitely known, from EDXA, to be present in the sample and it was expected that the relative levels of these analytes would be more constant with respect to each other.

Analytes known to be present in the fibre were: Na, 1072 ± 607 (average % peak height relative to Si ± 2.sd); Al, 25 ± 7; Si, 100 ± 0; Zr, 13 ± 7; K, 242 ± 187; and ZrO, 19 ± 8.

The relative levels of these key analytes show considerable variation, which indicates that semi-quantitative SIMS analysis based on one or two repeat analyses would be very poor.

Similar inferences may be drawn from the data for the exposed fibre samples (Table 6.5), where the data variation was even greater in this case. It was noted that the variation was strongly influenced by the unusually high values obtained for one of the sets of data, emphasising the importance of a full statistical appraisal of SIMS analysis and the necessity to carry out repeat analyses.

The poor reproducibility of the static SIMS technique is likely to be associated with the fact that the method is highly surface sensitive and the first few atom layers represent a "dirty" surface of variable composition. Surface contaminants would lead to unpredictable sputtering effects, as well as compositional variations, resulting in inconsistency of data. One method that could possibly be used to prepare "clean" sample surfaces would be to ion-etch the samples prior to analysis, though such an approach would in itself change the nature of the surface.

Factors such as variation in sample geometry and position must also be considered as a possible influence on the variation of secondary ion signal intensity, though this would primarily influence total ion yield rather than relative ion yield.

6.3.7 CONCLUSIONS

Static SIMS provided a qualitative method for the surface analysis of fibrous materials and was complementary in certain respects to EDXA techniques. In contrast, the technique was unsuitable for semi-quantitative analysis, due to problems with data reproducibility. This was thought to be associated with a) surface contamination and/or b) surface composition variation and/or c) sample position and geometry.

It is suggested that poor reproducibility is an inherent feature of the technique, and SIMS studies should be supported by a relatively reproducible technique such as EDXA. It is accepted that EDXA is more of a bulk technique, though it is probably this feature that makes it more reproducible.

6.4.1 DEPTH PROFILING OF GLASSES USING SIMS

6.4.2 INTRODUCTION

An important inherent feature of SIMS analysis is its depth profiling capability. Depth profiling is possible because of the destructive nature of the analysis, which, at relatively high beam energy and current, results in etching away of the sample surface. Depth profiling would allow characterisation of the variations in chemical composition with depth into the surface. When SIMS is used for depth profiling it is described as "dynamic" SIMS.

Initial experimental work has been carried out to determine the suitability of depth profiling for glass analysis using the VG Scientific Microlab.

6.4.3 METHODOLOGY

The initial work featured the use of bulk glass samples. The basic components of the SIMS instrument were as already described except for the use of an argon ion source to provide the primary ions. An electron flood gun was used to reduce specimen charging and the SIMS instrument was set up to operate in the dynamic mode.

Depth profiling requires determination of the etch rate of the primary beam in order that the corrosion profile can be accurately related to depth. This calibration was achieved by etching a boro-silicate glass coverslip with a primary Ar^+ ion beam for 3139 seconds at 5 kV accelerating voltage and a current density of 100 nA / 0.1 mm^2. The

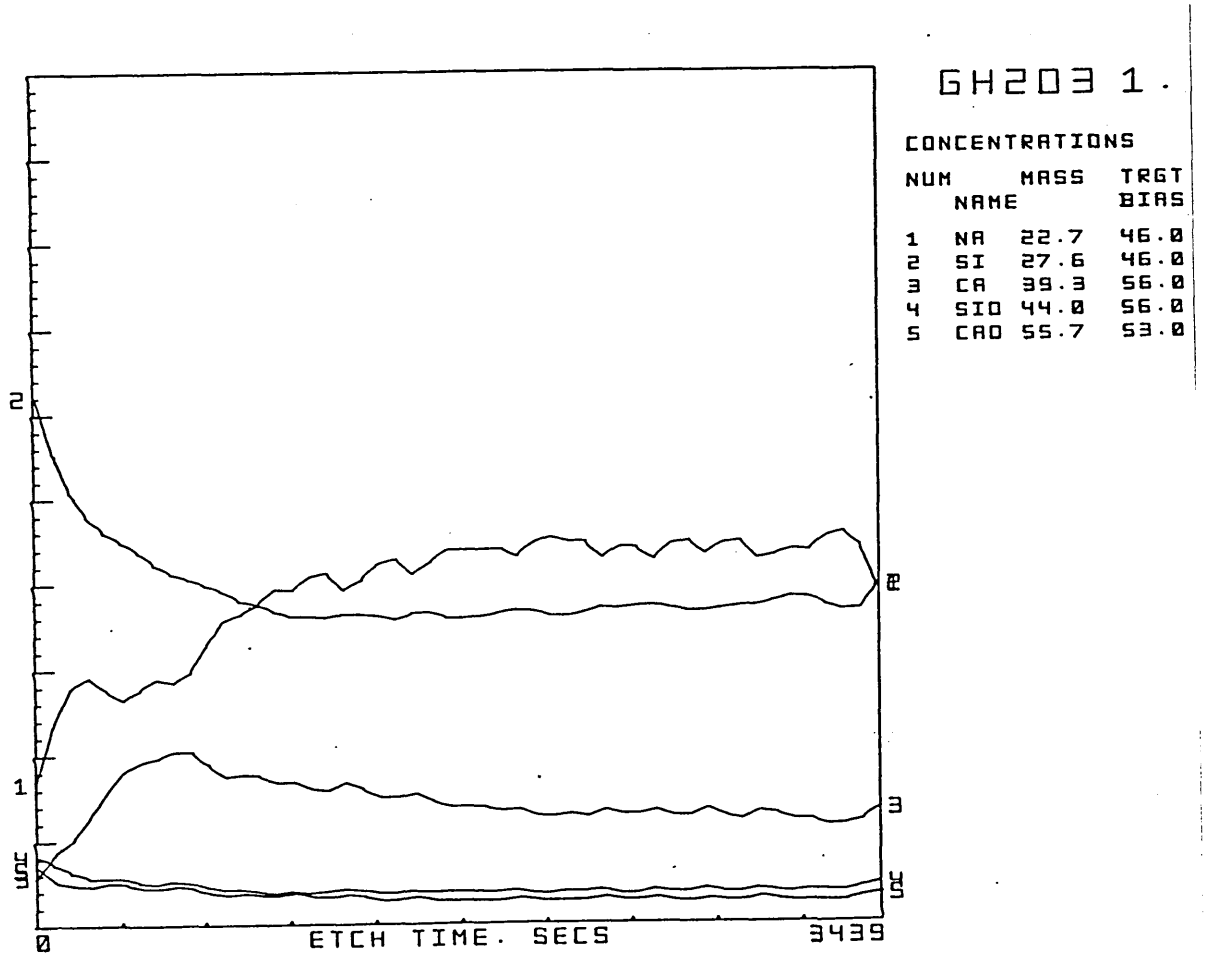
coverslip provided a smooth surface and it was possible to measure the depth the etched crater with a "Talysurf" stylus type instrument. Bulk soda-lime-silicate glass test samples were then profiled using the same operating conditions.

6.4.4 RESULTS

The etch rate was found to be 0.57 microns / hour for the glass samples using the described operating conditions.

Figure 6.27 shows the depth profile for a bulk glass sample exposed to pure water for 3 weeks.

Figure 6.27



6.4.5 DISCUSSION AND INFERENCES OF RESULTS

Whilst a successful calibration of depth was possible, the test sample data was very disappointing. Technical problems arose that led to poor reproducibility of the depth profiles and indecipherable, spiky, analyte profiles were common. To date these problems have not been fully resolved and are thought to be associated, at least partially, with drift in the qradrupole during analysis. The lack of sophistication of the data handling software made compensation for this effect impossible.

Figure 6.27 shows a depth profile for bulk, soda-lime silicate glass exposed to water, which does appear to be consistent with a basic understanding of durability theory. The data appeared to reflect leaching of Na at the surface, though by the time a depth of 0.5 microns into the glass surface was reached, constant levels of the respective analytes was observed.

6.4.6 CONCLUSIONS

Until basic technical problems are addressed, depth profiling using the VG Microlab will not be possible. This was very disappointing particularly in view of the success of other workers who have carried out SIMS depth profiling of glass surfaces (Richter et al 1984).

It should be noted that the successful approach used by Richter et al (1984) involved the use of fast atom bombardment, which prevents charging of glass substrates.

This technique may be inherently more suitable for bulk glass analysis. However, the problem still remains that a technique offering high spatial resolution is required for both static and dynamic analysis of small diameter fibres and this demands a liquid metal ion source.

The suitability of liquid metal sources for depth profiling should be examined, although the previously encountered technical problems will have to be overcome. Improved analytical software and the use of a cooled specimen stage to reduce ion diffusion may provide better reproducibility of the depth profiles.

7. TOXICOLOGICAL EFFECTS OF GLASS AND CERAMIC MATERIALS ON CULTURED MONOCYTES AND V79-4 CELL LINES

7.1 INTRODUCTION

Most of our work has been concerned with characterisation of material durability as an indicator of the pathogenic potential associated with inhalation of the material. Durability studies are aimed at determining the lifetimes of materials in the lung, which are often measured in many years, and hence reflect "long term" pathogenic potentials relating to such long residence times. In contrast, the toxicology studies described reflect the "short term" pathogenic potential of the test material, measured after several days of exposure and associated with chemical composition.

The precise nature of cell/fibre interactions that result in a pathogenic response are not well understood. It may be proposed, as in the case of silica dust, that negatively charged, non-bridging oxygen atoms on the dust surface cause binding and the subsequent disruption of the alveolar-macrophage membrane. Release of cytoplasmic enzymes from the injured/dying macrophage would in turn cause damage to the delicate lung tissue in the alveolar region. A number of studies have shown increased cell membrane permeability and release of factors that could result in tissue damage and disruption of the net balance of the structural proteins (Case et al 1986).

It could also be suggested that release of highly-reactive oxygen radicals and other products of the respiratory burst would result in damage to lung tissue, when foreign particles are engulfed by "patrolling" alveolar macrophages.

It is difficult to accurately determine the causes of dust induced lung disease because of the complexity of the alveolar region and the difficulties in setting up sufficiently accurate in-vitro systems that allow characterisation of the cell/fibre interaction.

Despite these difficulties it can be assumed that the reaction between the alveolar-macrophage and the dust is potentially critical and tissue-culture methods using macrophage/monocyte-like cell lines provide a useful method for examination of cell/fibre interactions.

The following studies examine the survival of the V79-4 cell line and also cultured human monocytes, when exposed to glass and ceramic and the cell affinity of V79-4 cells towards these samples. The V79-4 cell line was derived from hamster lung fibroblasts; fibroblasts are associated with production of collagen, elastin and other components of connective tissue. The V79-4 cell line is of particular interest because previous studies (Chamberlain et al 1979, Brown et al 1980) have provided evidence that V79 cells are only sensitive to mesothelioma causing fibres.

7.2 GLASS AND CERAMIC SAMPLE PREPARATION

In-vivo toxicity studies have shown that fibres of specific dimensions are most strongly associated with initiation of lung disease. A commonly accepted explanation for this is that only fibres below a certain diameter can penetrate the airways and reach the alveolar region and fibres greater than a certain length inhibit removal from this critical region by alveolar macrophages. Previous studies have indicated that respirable fibres of sub-micron diameter and of length greater than 10 microns present the greatest potential-health risk.

Ideally, to eliminate differences in the toxicological effects of fibrous materials which derive from differences in fibre dimensions, fibres of the same size distributions would be used. As sub-micron fibres with the same size distribution are not available in the desired range of compositions, an attempt to eliminate the effects of fibrous morphology was made by preparing particulate samples.

Particulate samples were prepared from fibrous samples of lead-glass, A-glass, Cemfil and ceramic fibre. This was achieved by forming a homogeneous melt of each of the fibrous materials and then using a percussion mortar to prepare a fine powder of each of the samples.

0.5 g of each of the fibrous samples was put into a platinum crucible and melted using a muffle furnace. The

samples were held at approximately 50°C above the initial melting temperature for 2-3 hours, to ensure confluence of the melt, and then air cooled to prevent crystallisation. The melt, when solidified, was powdered in a percussion mortar to provide particulate samples.

SEM micrographs showing the typical range of particle sizes are shown (Figure 7.12-15). Note that some large fragments of glass were present, of up to 150 micron, and also a large number of sub-micron particles. The preparation procedure effectively removed fibrous morphology.

7.3 THE SURVIVAL OF V79-4 CELLS TREATED WITH GLASS AND CERAMIC SAMPLES

The cytotoxicity of the prepared particulate materials was determined from the survival of V79-4 cells when exposed to these materials.

7.3.1 METHODOLOGY

Particulate samples were prepared as previously described. The survival of V79-4 cells was determined (courtesy of MRC Toxicology, Carshalton, Surrey) by the method of Chamberlain and Brown, 1978.

A range of sample concentrations were added to cell suspensions (50 cells/ml) and the resulting mixture was incubated for 6 days at 37 °C in a 5% CO_2 / 95% air atmosphere. After this time cell colonies were fixed (4% HCHO in water), stained with methylene blue (1%) and counted using an automatic colony counter.

7.3.2 RESULTS

V79 survival is shown graphically in Figure 7.1 and by Table 7.1. This shows the % survival of cells incubated with a range of glass compositions at 100, 200, 400 and 800 microgram/ml glass concentrations. The effects of amosite are also shown at 5, 10, 20 and 40 microgram/ml concentrations.

Table 7.1 V79 cell survival after exposure to glass particles

Fibre	% Survival Concentration of fibre (microgram / ml)			
	100	200	400	800
A-glass	92 ± 8.6	79 ± 7.7	56 ± 7.9	25 ± 4.1
A-glass	96 ± 7.3	90 ± 14.6	63 ± 7.8	
Cemfil	92 ± 8.6	87 ± 10.3	78 ± 12.1	38 ± 7.7
Ceramic	108 ± 7.4	106 ± 9.3	99 ± 21	55 ± 4.2
Pb glass	97 ± 9.7	68 ± 9.6	36 ± 9.4	
	5	10	20	40
Amosite	94 ± 13.6	66 ± 9.3	44 ± 11.1	16 ± 9.5

7.3.3 DISCUSSION AND INFERENCES OF RESULTS

Cell survival, after exposure to a potential toxicological agent is a direct method of determining the toxicity of that agent, though this does not tell us the mechanism by which cell death is caused. The method described provided a measure of short term toxicity, relating to a specific type of cell and care should be used in using such data to predict the long term pathogenic effects of respiratory exposure to the dusts described.

It was apparent from the data that amosite fibres were far more toxic towards the V79 cells, than any of the glass samples. The amosite resulted in 84% cell death at a concentration of only 40 microgram/ml compared with 64% cell death during exposure to lead-glass particles at a concentration of 400 ug/ml.

Whilst it was observed that cell survival was dose dependent (with an increase in dose resulting generally in an increase in cell death for the dust concentrations examined) glass doses required to induce a level of cell killing similar to that of the amosite fibre were rather high and would probably never be encountered naturally.

Note that this comparison does not take into account potential differences in toxicity associated with the fibrous morphology of the amosite.

Although there is some question over the validity of comparing fibrous amosite and particulate glasses, direct toxicity comparisons can be made for the different types of glass.

Comparing the % cell survival data at exposure concentrations of 400 ug/ml, it was observed that the lead-glass dust was the most toxic, with only a 36% cell survival rate, whilst the cells exposed to ceramic fibre had a relatively high survival rate of over 95%.

The greater relative toxicity of the lead-glass could be related to the potentially toxic effect of lead being leached from the glass. The A-glass samples and the Cemfil fibre resulted in cell survival of between 55% and 80% at exposure levels of 400 ug/ml.

It may be inferred from the results that exposure to glass particles, albeit at high concentrations, resulted in a pathogenic response in V79 cells and that this response was modified by differences in chemical composition of the glass particles.

It was noted that for a more accurate comparison of the pathogenic potential of the samples in future studies, the particle size distributions should be obtained and a particulate amosite sample should also be prepared.

7.4 THE SURVIVAL OF HUMAN BLOOD-MONOCYTES TREATED WITH GLASS AND CERAMIC SAMPLES

The cytotoxicity of the previously defined materials was determined from the survival of human blood-monocytes when exposed to these materials.

7.4.1 METHODOLOGY

Monocyte cultures (10^6 cells/culture) were established in multi-well trays by a previously described method (Conroy et al, 1988) and after partial purification, were exposed to 0, 100 and 1000 ug/ml concentrations of the glass and ceramic dusts. The cultures were incubated for 48 hours at 37°C in a 5% CO_2 atmosphere, and then cell viability was assessed by the ability to exclude trypan blue.

7.4.2 RESULTS

Cell survival is presented in Table 7.2.

Table 7.2 Monocyte survival after exposure to glass particles

Fibre	% Survival (± 2 sd) Concentration of fibre (microgram / ml)		
	0	100	1000
A-glass	98.8 ± 1.23	96.5 ± 5.40	96.9 ± 2.92
A-glass	98.8 ± 1.23	98.3 ± 2.63	98.4 ± 1.23
Cemfil	98.8 ± 1.23	95.7 ± 9.15	94.1 ± 6.23
Ceramic	98.8 ± 1.23	98.8 ± 1.03	99.5 ± 1.34
Pb glass	98.8 ± 1.23	99 ± 1.25	95.6 ± 3.5

7.4.3 DISCUSSION AND INFERENCES OF RESULTS

Compared with V79 cell survival, the monocytes appeared to have a much better cell survival rate when exposed to equivalent levels of the described glass particles. Because of the high survival rates in all cases it was difficult to comment on the relative toxicity of the glasses. If the 1000 ug/ml data was considered it may be tentatively suggested that the ceramic particles were least toxic, whist the cemfil and to a lesser extent lead-glass were the most toxic.

It was noted that the concentration of particles to cells was much greater in the V79 cell experiments, which may account for the differences in toxicity between the two. Also, the physiological differences between the cell types may have contributed to the observed differences in cell survival.

7.5 THE AFFINITY OF V79-4 CELLS FOR GLASS PARTICLES

The short term affinity of V79-4 cells for a range of glass samples was determined by a density gradient separation method courtesy of MRC Toxicology, Carshalton, Surrey.

7.5.1 METHODOLOGY

Continuous density gradients (specific gravity 1-1.119) were prepared in centrifuge tubes, using ficoll/sodium diazotriate dissolved in phosphate buffered saline.

Lysed cells would not pass through the gradient, whilst cells in complex with the glass or amosite would pass through to the bottom of the gradient during centrifugation. Uncomplexed cells would pass approximately one third of the way through the gradient.

V79 cells were radio-labelled by growing to confluence in a medium containing 1-2 uCi/ml of ^{3}H-Thymidine.

The glass particles and amosite fibres were individually added to cell suspensions (750,000 cells/ml) in silanised flasks to give a dust concentration of 1mg/ml. The mixtures were incubated at 37°C in a shaking water bath and samples withdrawn at 0, 15 and 75 minutes after fibre addition, layered onto density gradients and centrifuged.

The distribution of radio-activity through the density gradients was determined by taking sequential 0.5 ml aliquots, adding scintillant and counting in a liquid scintillation counter.

7.5.2 RESULTS

Counts in each aliquot were expressed as a % of the total and this data was shown graphically in Figures 7.2-7. The "fraction number" was the aliquot number, with fraction 1 being taken from the top of the centrifuge tube.

7.5.3 DISCUSSION AND INFERENCES OF RESULTS

This series of experiments was designed to determine the short term affinity of V79 cells for dust samples. It was clear from the data (Figure 7.7) that amosite fibre was rapidly bound by V79 cells. Figure 7.7 shows that after 15 minutes of contact, over 50% of the total radioactivity was in aliquot 22, meaning that over 50% of the radio-labelled cells had bound to the amosite and had "spun down" the density gradient. It was further observed that by 75 minutes of exposure to amosite, a significant % of radio-activity was present in aliquots 1-4 (7% in aliquot 1), inferring that cell lysis was occurring. This behaviour was consistent with our knowledge that asbestos fibre interactions cause cell membrane disruption.

In contrast, binding of V79 to glass particles was generally not observed, although Figure 7.5 indicated a moderate degree of binding to ceramic particles after 75 minutes (13.5% of the total radio-activity in fraction 22).
It may be inferred from the low short term affinity of V79 for glass that the potential for short term cell/fibre interactions and associated cell membrane disruption would be greatly reduced, that is, it may be expected that the short term toxicity of the glass dusts would be much lower than the amosite, which rapidly binds to the cell membrane.

7.6 THE AFFINITY OF HUMAN BLOOD MONOCYTES FOR GLASS SAMPLES

The affinity of monocytes for glass fibres was determined in a qualitative manner by the use of SEM and phase contrast morphological studies.

7.6.1 METHODOLOGY

Monocyte cultures were established in multi-well culture trays using the method described in Section 3.3.2. Glass fibre was added to the cultures and then incubated at 37°C for 3 weeks in a 5% CO_2 atmosphere.

During incubation, cells were examined using phase contrast microscopy. After 3 weeks samples were also fixed and then gold coated before SEM micrographs were obtained.

7.6.2 RESULTS

The affinity of cultured monocytes for glass fibre is shown in Figures 7.8-11, and Figure 7.11 clearly shows a monocyte attempting to engulf a fibre. It was noted that during incubation the monocytes undergo morphological maturation, becoming macrophage-like. Initially, dense clumps of cells often formed (Figure 7.8) and then tended to disperse. After 1-2 weeks monocytes could be seen attempting to engulf fibres, and it was observed that many of the cells had enlarged from about 10 um to over 20 um in diameter. Often cells were observed to elongate considerably (Figure 7.9) before becoming more round and ruffled in appearance (Figure 7.10).

7.6.3 DISCUSSION AND INFERENCES OF RESULTS

In contrast with V79 cell affinity, the monocytes show a marked phagocytic response to the glass. This may be explained due to V79 data reflecting short term cell attachment occurring over the first 75 minutes of introduction. The monocyte data was longer term and morphological studies did not show a significant phagocytic response towards the glass for at least 1 week.

This difference in response, the relatively rapid binding of amosite to V79 compared with much slower phagocytosis of glass fibre by monocytes, suggests a difference in the mechanisms of the cell/fibre interaction in the two instances. It could, for example, be suggested that the rapid binding to amosite was associated with charge attraction between the fibre surface and charged groups on the cell surface. In contrast, the slower phagocytic response of monocytes to glass resulted from the slow development of chemotactic factors associated perhaps with leaching from the glass, or binding of chemoatractants to the glass.

It was noted that these comparisons were between different cell types, which makes direct comparisons more tentative.

7.7 CONCLUSIONS

This series of toxicity studies highlighted the difficulties in relating different in-vitro studies to each other and also to the in-vivo situation.

The experiments described provided different inferences depending on factors such as time of exposure, cell types, dust type and concentration.

Whilst obvious care is required in interpreting in-vitro toxicity studies and attempts should be made to accurately simulate in-vivo exposure conditions, it was also apparent that a useful insight into cell toxicity was possible from in-vitro studies.

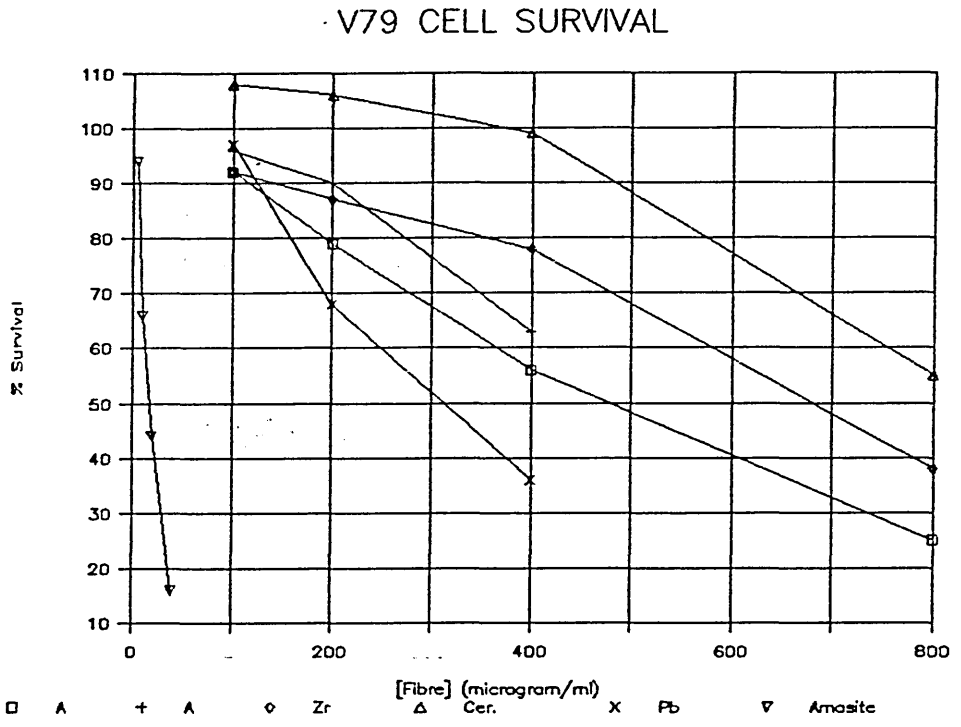
It is suggested that future in-vitro studies should aim firstly at demonstrating their suitability as an alternative to in-vivo studies and then a systematic, comparative study using fibres of accurately known dose, dimensions and composition should be undertaken. It is envisaged that such a study would be facilitated by developing methods for the production of fibres and particles of controlled composition and dimensions.

Differences in glass toxicity, depending on chemical composition, were identified and differences in the mechanism of cell/fibre interactions for amosite and glass were suggested.

It should be further noted that cell survival may not be the best marker of pathogenic potential. A viable, stimulated cell may be more critical and future studies should monitor cell activation and the long term release of potentially pathogenic factors such as products of the respiratory burst.

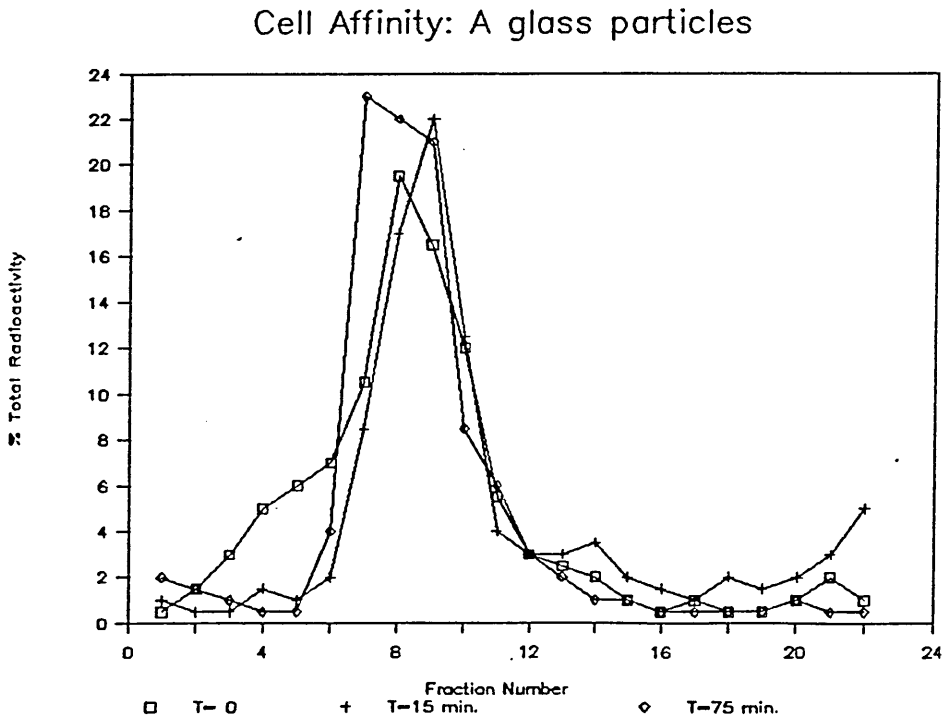
In these studies the organic coating on the Cemfil fibre, introduced during manufacture (Section 2.5.2), will have been removed during preparation of the glass particles. In view of the potential effects of these coatings in modifying the pathogenic potential of the fibre, future work should be directed at the investigation of this factor; fibres should be examined which have not had their organic size coatings removed by heating during preparation and sterilisation.

Figure 7.1



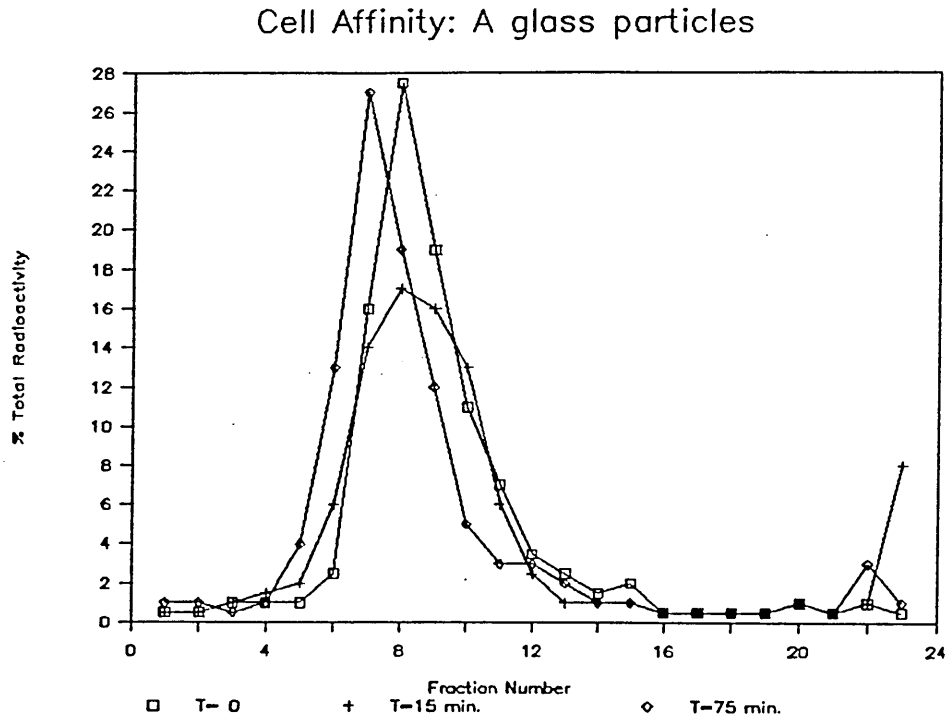
Survival of V79 cells exposed to glass dusts

Figure 7.2



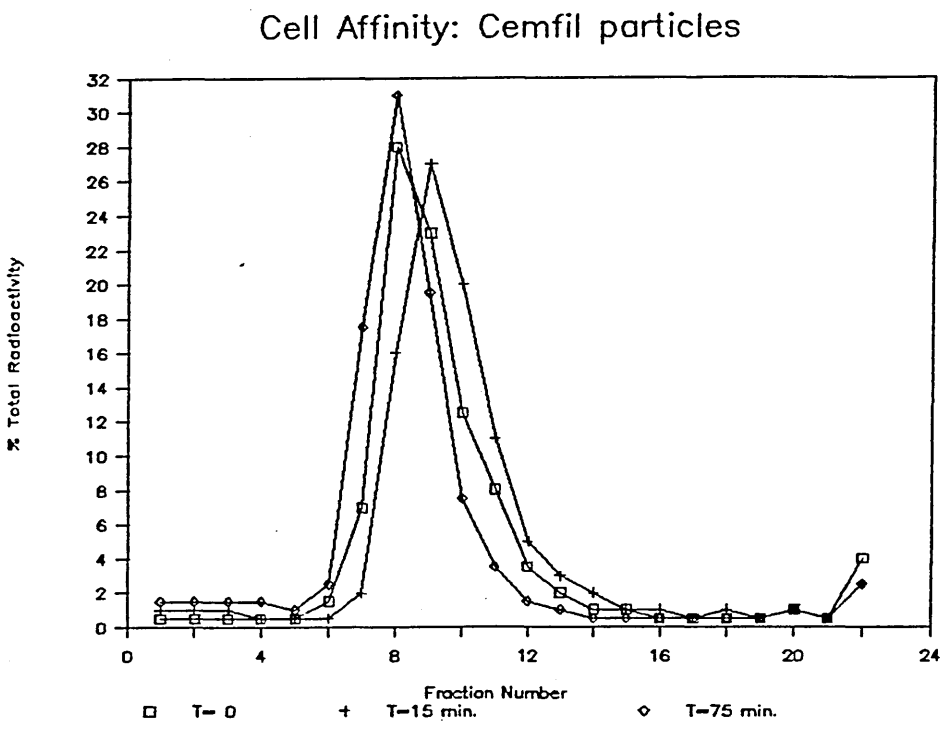
Cell sticking of V79 cells to A-glass

Figure 7.3



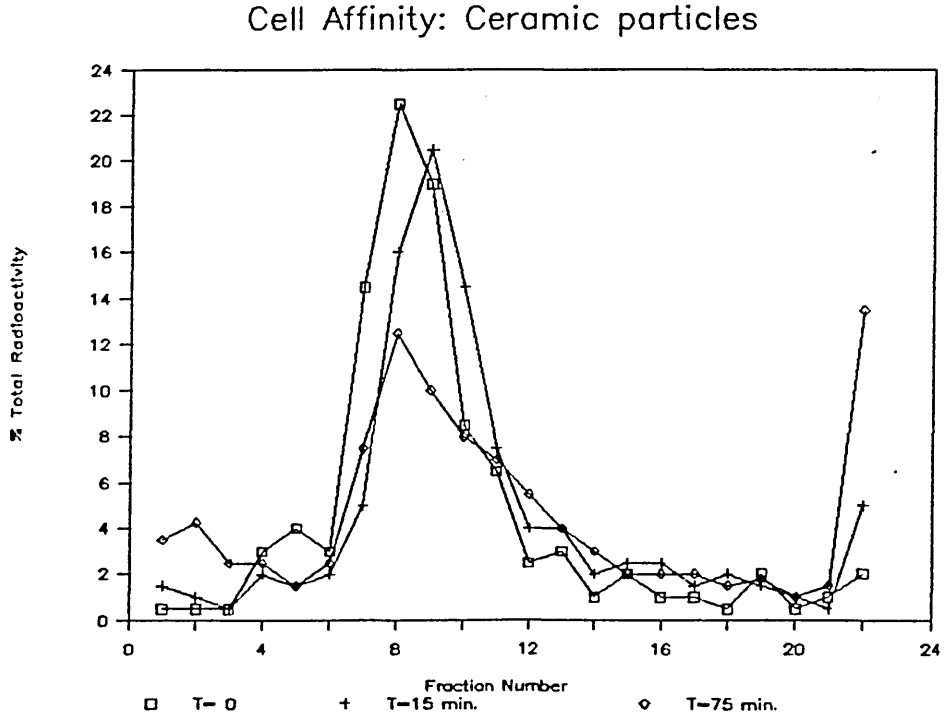
Cell sticking of V79 cells to A-glass

Figure 7.4



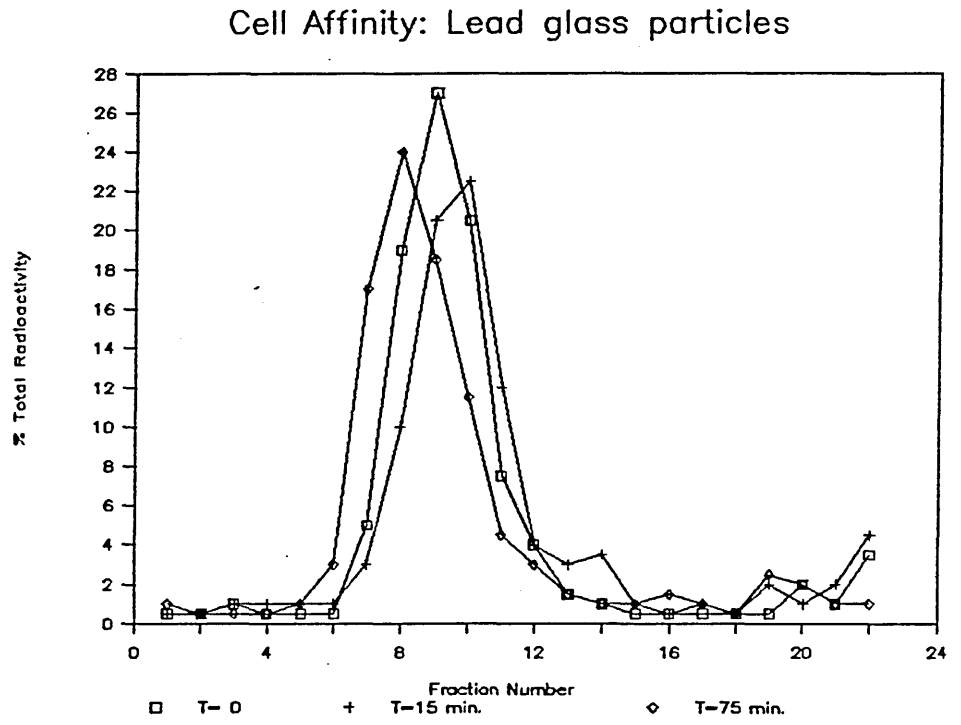
Cell sticking of V79 cells to Cemfil glass

Figure 7.5



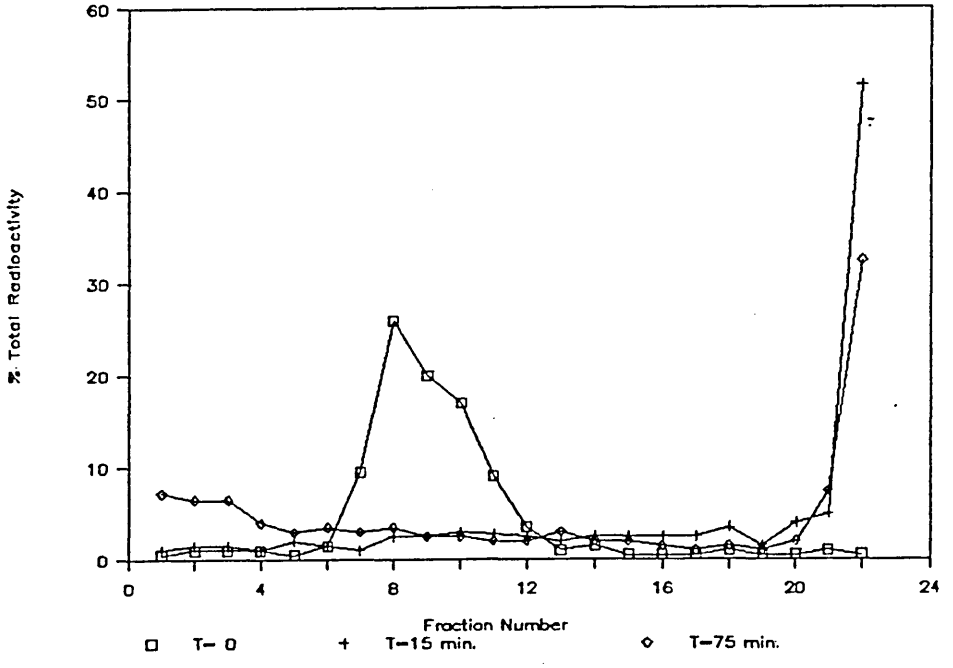
Cell sticking of V79 cells to Ceramic

Figure 7.6



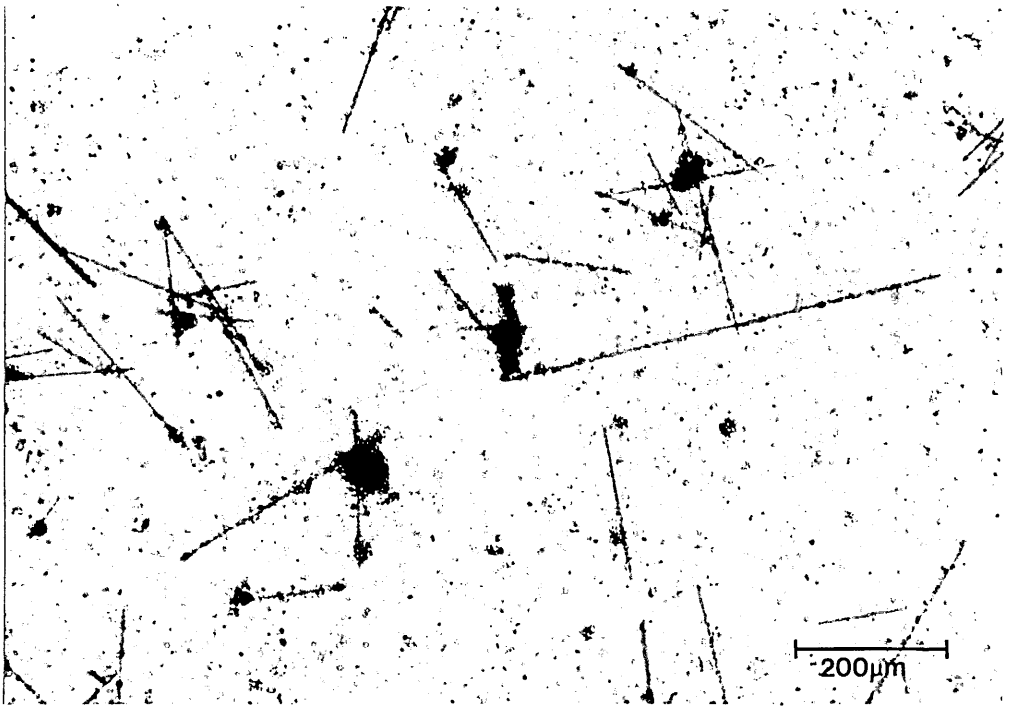
Cell sticking of V79 cells to Lead-glass

Figure 7.7



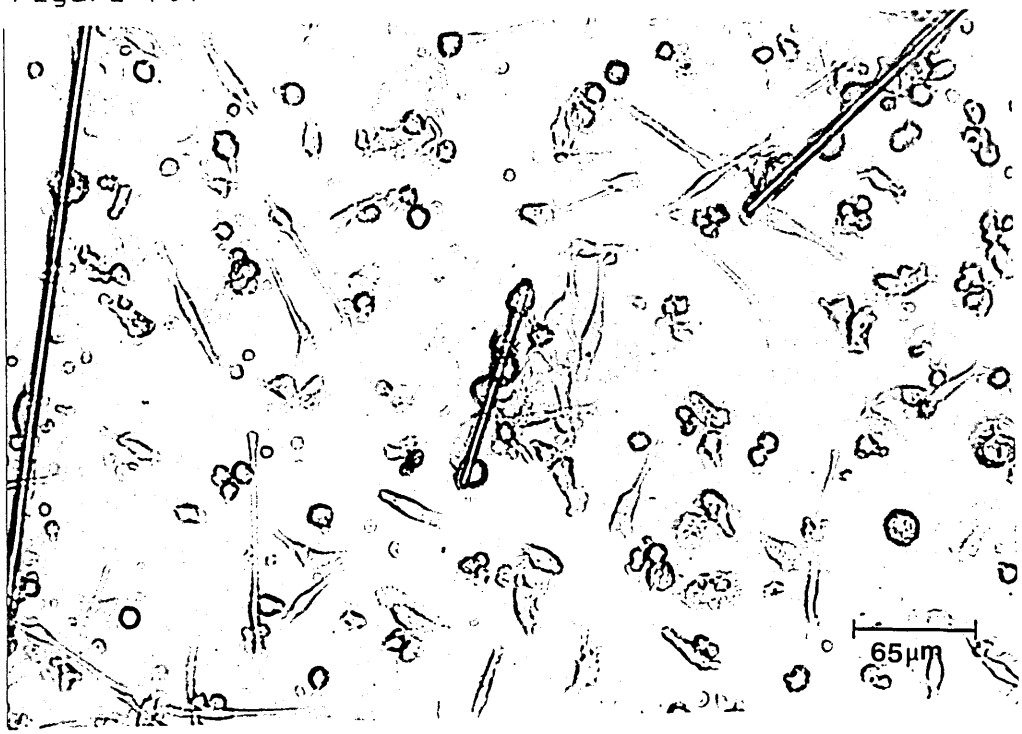
Cell sticking of V79 cells to Amosite fibre

Figure 7.8



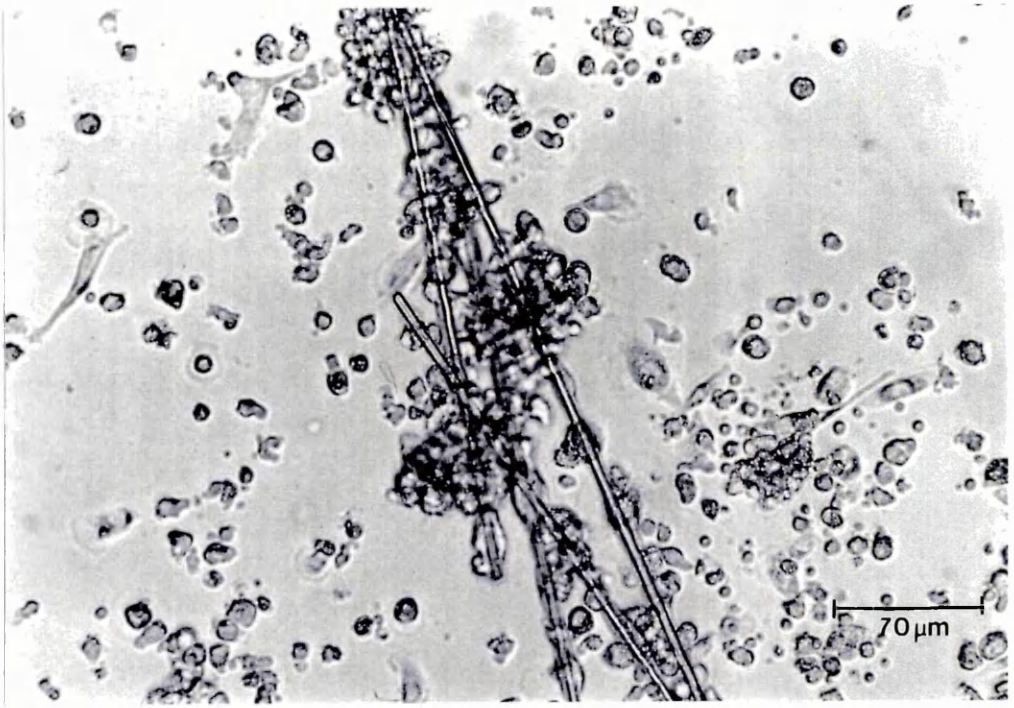
Phase-contrast micrograph showing the initial clumping of cultured monocytes

Figure 7.9



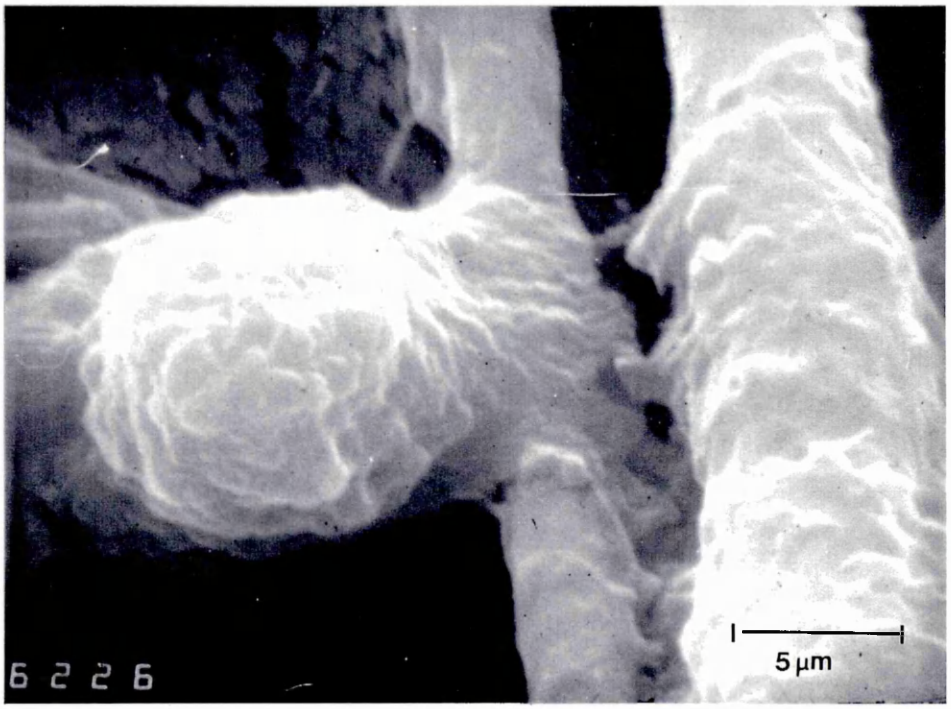
Phase-contrast micrograph showing elongation of cultured monocytes

Figure 7.10



Phase-contrast micrograph showing the affinity of cultured monocytes for glass fibre and their rounded appearance

Figure 7.11

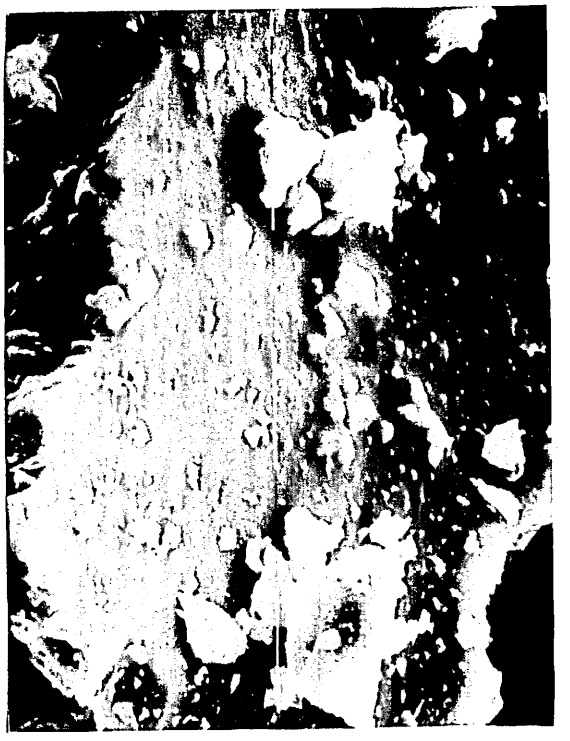


SEM micrograph showing the phagocytic response of cultured monocytes to glass fibres

Figure 7.12

SEM micrograph:
A-glass particles

Figure 7.13

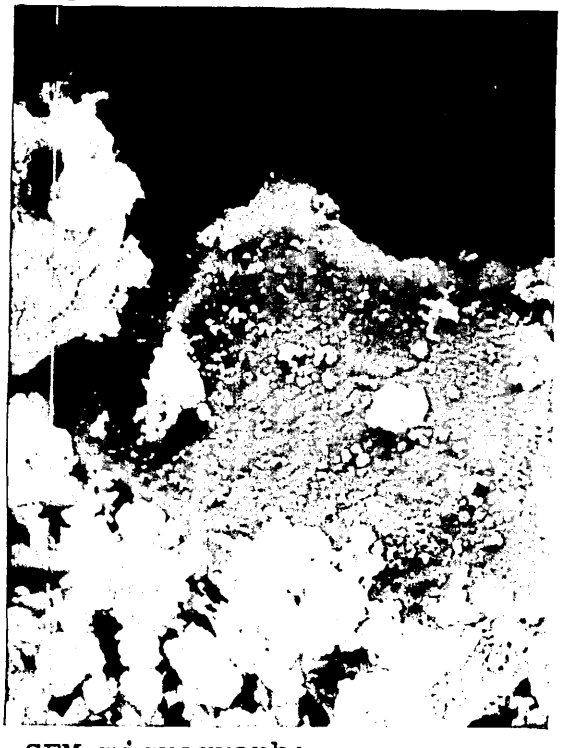


SEM micrograph:
Lead-glass particles

Figure 7.14

SEM micrograph:
Cemfil particles

Figure 7.15



SEM micrograph:
Ceramic particles

8. OVERVIEW: DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

8.1 SUMMARY OF FIBRE RESPONSE

The project was specifically designed to develop i) understanding of the physiological durability of glass and ceramic fibres and ii) a suitable in-vitro test system.

The behaviour of the test materials, in-vitro and in-vivo, is summarised in Table 8.1.

Fibre durability is influenced by the chemical behaviour of the material, and because the test samples demonstrated different types of chemical reactions it was difficult to provide a scale of relative durability. However, as fibre types prone to network degradation can be totally degraded it may be inferred that these materials will have poor durability and consequently a low pathogenic potential.

Certain fibre types were chemically reactive, demonstrating leaching and/or layer formation. This did not necessarily infer poor durability, as such behaviour can result in the formation of protective layers able to prevent network degradation. It is also possible that leachates from chemically reactive fibres are highly toxic and this should be noted when interpreting data for fibres in this category.

It was observed that E-, lead- and Cemfil-fibre were reactive in-vivo and in-vitro, whereas ceramic fibre appeared to be unreactive and durable. The persistence of the ceramic fibre, coupled with the significant proportion of fine

fibres present in alumino-silicate ceramic samples (Rood 1988), inferred a significant long term health risk from inhalation.

The lead rich glass was highly reactive, demonstrating signs of i) leaching ii) surface layer formation and iii) network hydrolysis. The susceptibility to hydrolysis, in-vivo and in-vitro, inferred poor durability, which suggested that persistence in the lung and the associated long term pathogenic potential would be low. However, it should be noted that dissolution and leaching of this fibre would release lead into the tissues and this may itself present a significant hazard.

The behaviour of the E- and Cemfil fibre was particularly interesting. Both were reactive in-vitro and in-vivo, showing signs of surface leaching/layer formation, and the Cemfil fibre was particularly prone to leaching; this was despite the organic "size" coatings introduced during manufacture (Section 2.5.2). The E-glass fibre showed no signs of network hydrolysis from morphological studies or by statistical monitoring of fibre diameters during exposure. It may be inferred from this that the E-glass fibre examined would have a significant persistence time in the lung. The Cemfil fibre, whilst not showing signs of any localised corrosion, appeared from fibre diameter data to be prone to network dissolution when exposed to high pH.

This was an unusual observation in view of the fact that the fibre is designed to be pH stable and consequently this requires further investigation.

In-vitro data for A- and bulk glass highlighted the poor durability and a susceptibility to network hydrolysis as indicated by the surface etching. It would thus be inferred that the lifetime and long term pathogenic potential of these fibres would be small. In contrast to the glass fibres, all of which were susceptible to some kind of chemical reactivity in-vivo or in-vitro, the alumino-silicate ceramic showed very few signs of reactivity, other than traces of formation of phosphorus and calcium rich surface deposits during exposure to RPMI 1640. It can be inferred that the ceramic was very durable and may present a high pathogenic potential because of this.

It was a significant observation that the effects of in-vitro exposures were consistent with the observed effects of in-vivo exposure in rats. In-vivo exposure indicated that the lead-glass was susceptible to network degradation, whereas the ceramic fibre appeared to be chemically inert and durable, consequently having a high pathogenic potential. The Cemfil fibre was found to be prone to leaching of sodium and surface enrichment of zirconium in-vivo, as was the case in-vitro.

The significance of the consistencies between the effects of in-vitro exposure as a whole and in-vivo exposure is that this substantiates the suitability of in-vitro exposures as an alternative to in-vivo exposure. However, it is important to note that a range of different in-vitro exposure conditions is necessary to simulate the complex in-vivo environment and the use of a single in-vitro exposure environment is too over-simplified; for example, Gamble's fluid exposure gave no indication that the bulk-, A- and lead-glass fibres were prone to network hydrolysis.

Exposure to human monocytes, in-vitro, designed to evaluate the effect of an intra-macrophage environment on fibre durability and to potentially provide a single in-vitro durability test, had little apparent effect of the fibres studied. Possibly this was due to insufficient exposure time, or simply that the intra-macrophage environment has little effect on the fibre types studied. More speculatively, the organic coatings on the E- and Cemfil fibre may modify potential interactions between fibre and monocyte.

Table 8.1 Qualitative summary of results

GLASS :	BULK	A	E	CEMFIL	LEAD	CERAMIC
FLUID	summary of effects of exposure on test material					
WATER	Na --- Ca --	Na --- Ca ---	Ca +	Na --- Zr + Ca --	N/A	NONE
GAMBLE FLUID	Na - Ca -	Na +	Al + K - Ca --	Na -- Ca - K - Al +	COATED P, Pb Cl, Ca	NONE
RPMI 1640	N/A	Ca +	COATED Al,P,Ca Na,Cl,K	Na --- Ca +	COATED P,Ca,Pb Cl	COATED P, Ca
PBS	Na - Ca -	N/A	COATED Al,P,Na Cl,K,Ca	Na --- Zr ++	COATED P, Pb Cl, Ca	NONE
pH 10 BUFFER	SURFACE ATTACK	SURFACE ATTACK	Ca -	Na + Zr + THINNING	SURFACE ATTACK	NONE
SERUM	N/A	Na + Ca +	NONE	NONE	N/A	N/A
BALF	N/A	N/A	Mg + K - Ca --	Na -- Zr -- Ca --	N/A	N/A
MONO-CYTES	N/A	Na + Ca +	NONE	NONE	N/A	N/A
IN-VIVO	N/A	N/A	Al -- Ca -	Na --- Zr + K +	SURFACE ATTACK	NONE

Key:

(-), (--), (---): small, medium, large reduction in [analyte]/[Si] ratio.

(+), (++), (+++): small, medium, large increase in [analyte]/[Si] ratio.

N/A: data not available.

8.2 THE ROLE OF pH IN DURABILITY BEHAVIOUR

Fibre durability has been defined as a key determinant of pathogenic potential and the need to relate durability to chemical composition has been identified (Morgan 1982)

Fibre lifetime, as determined by fibre durability, can be greatly influenced by the chemical behaviour of the material. For glasses this chemical behaviour is considered primarily to depend on the pH of the exposure fluid; the role of pH in determining the nature of silicate glass corrosion has long been known by glass chemists (El-Shamy et al 1972). It has been shown that the specific glass and/or exposure fluid composition, can modify the chemical behaviour (Hench and Clark 1978, Clark et al 1979, Paul 1982), whilst temperature and surface area to volume ratio affects the rate of reaction (Clark et al 1979, Etheridge 1979).

In the case of alkali-containing silicate glasses exposed to aqueous environments, the behaviour can be divided into two distinct categories. At neutral and acidic pH, de-alkalisation by leaching is the dominant process (type I), whilst at higher pH (>9) network dissolution by hydrolysis becomes predominant (type II).

The "two process" behaviour may be related to the structure of silicate glasses and the nature of the network forming bonds in the glass. The network of the glass consists of a covalently bound, 3-dimensional, silicate lattice based on

distorted SiO_4 tetrahedra (Zachariasen 1932). This lattice may contain other network forming species such as boron or aluminium. The Si-O bond has a certain degree of ionic character, due to the electron withdrawing effect of oxygen, and is thus susceptible to attack by strong nucleophilic species. It is also noted that the Si-O bond is prone to attack by hydrofluoric acid (HF) and though the exact mechanism is not well understood, it possibly involves a combination of polarisation of the Si-O bond and nucleophilic attack by the fluoride ion. This mechanism has been proposed by Bud (1962), though it is not clear why hydochloric- and hydroiodic acid cannot also degrade the glass lattice (Paul 1982). Possibly, the smaller size, greater charge density and electronegativity of the F^- ion provide greater reactivity, less stearic hindrance and greater nucleophilic character.

Glasses often contain network modifying species, such as Na and Ca, introduced to reduce the melting point of the glass. These species are principally ionic in character and occupy sites in the network lattice, bonded by electrostatic/ionic bonds to non-bridging oxygen. These species have a tendency to be leached from the network (type 1 process) at pH < 7. However, this does not necessarily indicate that the glass network (the silicate backbone) will be attacked and leached glasses can still be durable and have long lifetimes <u>in-vivo</u>.

In contrast to type I processes, glasses prone to network attack (type 2) may be totally degraded, with species present in the glass being released into the attacking solution at the same relative rates. A material prone to this type of chemical reaction is likely to be of poor durability and have a low long-term pathogenic potential.

It was an important observation that much of the behaviour of the glasses examined in this project, in-vitro and in-vivo, could be interpreted by application of the described "pH" model. It is suggested that (i) a measure of the resistance of the fibre network to nucleophilic attack would provide a useful indicator of the physiological durability of the material, and (ii) a suitable in-vitro screening test would at least involve exposure to a pH range.

Assuming that resistance to type II attack (network hydrolysis) is a good indicator of durability, it may be possible to propose models for predicting durability based on factors such as the bond polarity of the glass network, and other factors capable of influencing the susceptibility of the network to nucleophilic attack.

8.3 CLASSIFICATION OF THE GLASS SURFACES FORMED IN-VIVO AND IN-VITRO ACCORDING TO THE DEFINITIONS OF HENCH (1977)

8.3.1 INTRODUCTION

It was observed that the types of glass surface encountered in-vitro and in-vivo may be associated with the categories described by Hench and Clark (1977, 1978).

The five types of glass surface are shown schematically in Figure 8.1. Glass fibres exposed in-vivo and in-vitro may be classified according to these, and an attempt can be made to deduce the durability based on pH models.

8.3.2 SODA-LIME SILICATE GLASS

The bulk glass and A-glass were both based on simple soda-lime silicates. The silica content was in the order of 70%, therefore the network structure would probably consist of continuous, sp^3 hybridised silica tetrahedra and modifying calcium and sodium ions would be ionically bonded to non-bridging oxygens.

Exposed to water, these glasses leach Na and Ca to give a Type II/IV surface. This may be related to the ion-exchange of Na and Ca cations for H_3O+ (aq) ions at the slightly acidic pH of distilled water. These surfaces are characterised by a silica-rich surface film, which may or may not provide protection against further de-alkalisation /dissolution, depending on the alkali concentration; silica concentrations much lower than 50% are associated with a non-continuous network and de-alkalisation can result in fragmentation of the network.

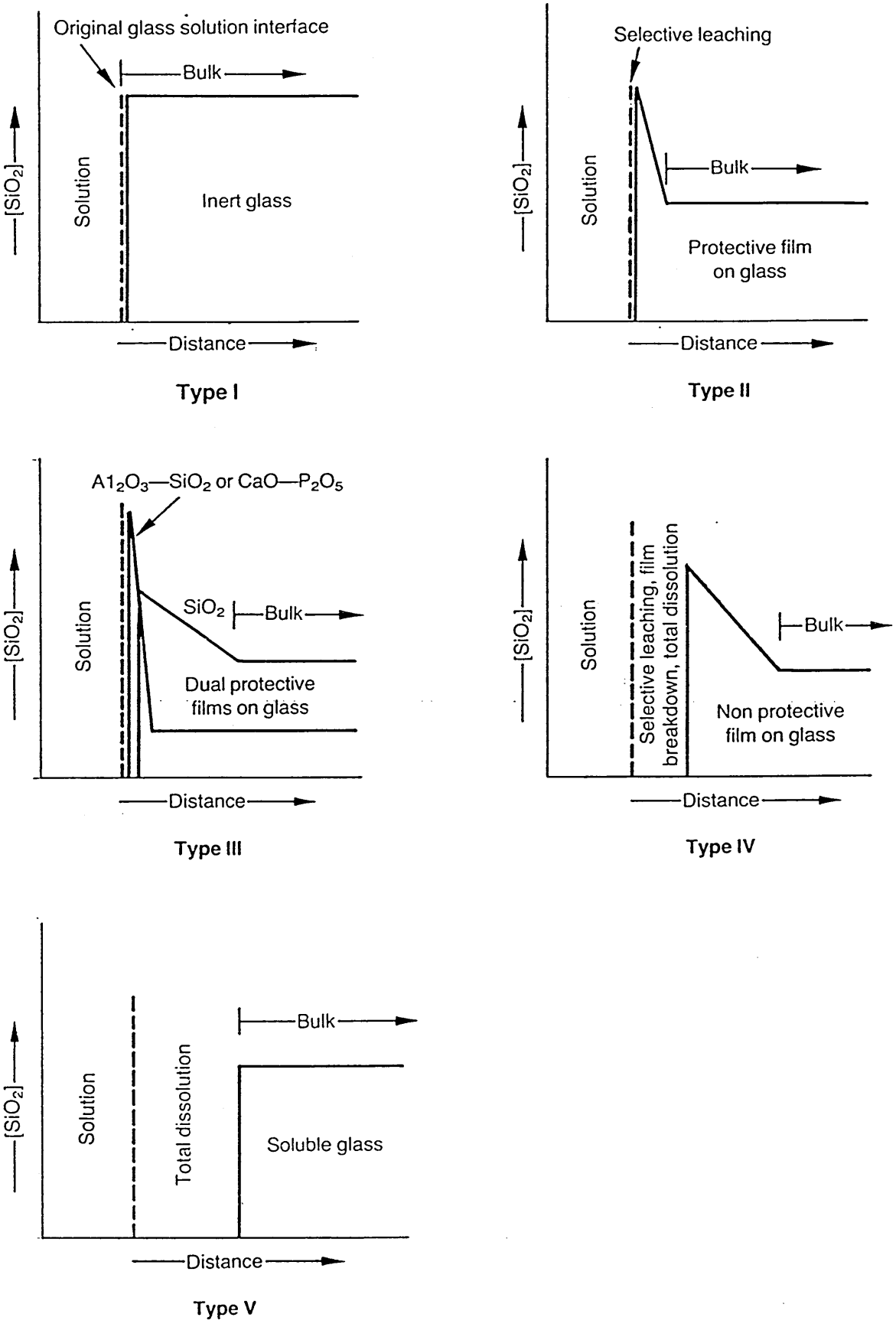


Figure 8.1 Glass surfaces as described by Hench (1978)

Note also that de-alkalisation results in an increase in solution pH and that at pH >9 even alkali-silicate glasses with relatively high silica content may exhibit total dissolution (Type V surface).

When exposed to the physiologically buffered solutions: Gamble's fluid, PBS, serum and RPMI 1640, the glasses were fairly unreactive and tended towards a Type I surface. However, the bulk glass showed some surface leaching, that is, some Type II behaviour, whilst the A-glass demonstrated slight increases in surface Na and Ca, that is, surface layer formation indicative of Type III surface behaviour. The more stable A-glass surfaces were presumably associated with the presence of small quantities of Al and Mg in the fibre, which are species known to improve fibre durability (Paul 1982).

In contrast, exposure to high pH resulted in total dissolution, characterised by a Type V surface and associated with nucleophilic attack of the polar Si-O bonds in the silicate network by OH- (aq).

Data for exposure <u>in-vivo</u> is not available, whilst exposure of A-glass fibre to monocytes resulted in a similar type of surface to that formed during exposure to physiological fluids and showed a weak Type III tendency.

To summarise, as the exposure fluid pH was increased the type of glass surface changed from IV to V. This shows how the type of surface formed is dependent on solution pH, but

note that the addition of certain species to the glass, or exposure fluid can modify the surface behaviour to give Type III surfaces.

8.3.3 LEAD-GLASS

The lead glass presented a much more complicated situation. This material contained Si, Pb, Ca, K, and Na. The Si would act as the main network former, and Pb may also be incorporated into the network (co-ordination: 2) with further Pb acting as a modifier within the large holes in the network (Stanworth 1948). Ca, K and Na will exist as modifiers, bonded ionically to non-bridging oxygen atoms.

Exposure to physiological fluids resulted in extraction of large quantities of lead from the fibres and the formation of very thick (up to micron thickness) surface layers. These layers were not an integral part of the glass fibre and did not appear to offer protection from further leaching. This type of surface was not well defined by the categories of Hench (1978), sharing characteristics of Type III and Type IV surfaces. It could best be described as a modified Type IV surface (IV b) exhibiting a thick non-protective, non-silicate surface coating. The formation of the surface layer <u>in-vitro</u> appeared to be associated with the high levels of phosphate in the exposure fluid, coupled with significant amounts of extractable Pb. The thermodynamic stability of layer formation may in fact have encouraged extraction of Pb, as opposed to extraction purely being a result of passive ion-

exchange processes.

Exposure to pH 10 buffer solution resulted in extensive surface dissolution, characterised by a Type V surface. This highlighted the problems associated with application of general durability models, such as the "thermodynamic stability" model of Paul (1982), to complex glasses. This model bases the durability of glasses on the thermodynamic and kinetic stability of the component oxides. Using this approach the stability diagram for PbO in aqueous solutions at different pH shows that the oxide is stable from neutral pH to pH 14.

Obviously the discrepancy in behaviour reflects the limitations of a model based on the component oxide stability and ignores the fact that the molecular bonding in a multi-component glass network is probably different to that in the component oxide; a thermodynamic model of stability would require the free energy of the glass to be known and hence is only correct for well defined systems, for example, for determining the stability of quartz at various pH values.

Our data provided evidence of Type IV/V surface formation (dissolution) during exposure of the lead-glass <u>in-vivo</u>. This was indicative of hydrolytic breakdown of the glass lattice and can be explained by assuming exposure to the water saturated atmosphere of the alveolar sac.

Long fibres embedded in the lung tissue may be envisaged as protruding into this water saturated atmosphere, and the exchange of cations for H+ ions in the water would possibly result in the generation of a very high localised pH. As determined from exposure of the lead glass to pH 10 buffer, this glass is prone to dissolution at high pH.

An alternative explanation for dissolution of the lead glass involves the release of highly reactive oxygen radicals, such as superoxide which has nucleophilic properties, and possibly the OH- anion, by alveolar macrophages bound to the inhaled fibre (Conroy et al 1988). These are nucleophilic species that may cause degradation of the glass network by disrupting Si-O bonds.

However, the important observation was that a tendency to form Type V surfaces could have been predicted from the susceptibility of the glass to nucleophilic attack in-vitro.

8.3.4 E-GLASS

E-glass fibre contained three network forming species: Si, B and Al, and a significant proportion of Ca which would act as an ionically bonded modifier. Some Al may also act as a modifying species. Si would exist as sp^3 tetrahedra, as would Al; B may exist in sp^3 tetrahedra and sp^2 trigonal planar forms. An organic coating was also present on the surface of these fibres.

It was interesting to note that all of these species are associated with improving the durability of glasses, and this was reflected somewhat in the behaviour of the E-glass. In-vitro exposure tended to result in the formation of Type III surfaces and it was noted that thin and delicate coatings were formed during exposure to RPMI 1640 and PBS. Some extraction of Ca was observed in-vitro indicating some Type II character; this showed that the organic coating was not integral and does not render the glass inert.

Nucleophilic attack was not observed during exposure to pH 10 buffer, or during exposure in-vivo (Type I). This inferred a potentially high health risk from inhalation of long thin fibres of this composition.

It was observed that Al was extracted in-vivo, and also during exposure to RPMI and PBS. The Al was re-deposited in-vitro as a component of delicate surface coatings. This inferred that some Al existed as a glass modifier; extraction was probably not associated with passive ion-exchange processes because extraction in water was not observed. Reaction between Al and species in the environment to form stable complexes at the fibre surface may have encouraged, thermodynamically, the extraction of Al. In-vitro this appeared to require the presence of relatively high phosphate concentrations in the exposure fluid and, in-vivo, Al extraction may have been associated with exposure to phosphate and/or other species in interstitial fluid.

8.3.5 CEMFIL FIBRE

Cemfil glass is basically an alkali-silicate containing a significant quantity of zirconia (16%). The network would consist of continuous sp^3 silica tetrahedra and with Zr acting as a network former and also probably existing in a range of high co-ordination states. Na, Ca and K would be network modifiers, as found in simple alkali-silicate glasses. Cemfil fibre was also coated with an organic "size".

The glass showed no signs of network dissolution in-vivo, which indicated a potential long term health hazard from inhalation of super-fine fibres of this composition. The presence of ZrO_2 is considered to result in the formation of protective Type III surfaces; this was supported by increases in surface Zr levels during exposure to pH 10 buffer and during exposure in-vivo. However, fibre diameter data suggested that the Cemfil fibre was subject to dissolution at high pH, although morphological data showed no evidence of localised corrosion or the stripping of complete surface layers - as was the case with lead-glass fibres. This phenomenon requires further investigation; it is proposed that studies using smaller, say 1 micron, fibres of the same diameter should be carried out and that diameter should be monitored with time in order to determine whether dissolution rates decrease as Zr rich surface layers are formed.

Despite the incorporation of ZrO_2, which is known to

greatly improve glass durability by its being virtually insoluble in acid and alkali (Paul 1982, Cotton and Wilkinson 1980), the Cemfil showed a considerable tendency for the leaching of Na and Ca (Type II surface formation). Leaching of Na was observed in all exposure conditions except for exposure to serum, monocytes and pH 10 buffer. Leaching in-vivo may be explained, therefore, by exposure to water vapour in the alveolar air space, or by exposure to interstitial fluid.

It was apparent, both from these findings and from morphological studies, that the organic coating was not an integral layer and therefore probably of limited effect as a durability enhancer.

8.3.6 CERAMIC FIBRE

The ceramic fibre would consist of a continuous network of sp^3 hybridised tetrahedra. The net negative charge on the AlO_4- tetrahedra would be balanced by modifying Al^{3+}.

It was found that the ceramic was generally very inert, forming a Type I surface except during exposure to RPMI 1640, where surface layer formation occurred (Type III).

8.4 A THEORETICAL BASIS FOR GLASS DURABILITY

Several models have been proposed for prediction of glass durability in aqueous media and these have been reviewed previously (Section 2). These models can be very useful and often provide a useful understanding of glass durability. However, they tend to be rather specific and also empirical in nature, which makes it difficult to apply them to novel situations. Paul (1982) has proposed a more rigorous, theoretical model, based on the thermodynamic stability of the component oxides at different pH values. Paul's model requires an accurate determination of the free energy of the glass and is thus also limited to well defined systems.

It is proposed that a thorough knowledge of the molecular structure and the nature of the chemical bonding could be used in conjunction with empirical models to put glass durability behaviour on a more theoretical basis.

To a certain extent this has already been done for simple alkali-silicate glasses, where the polarity of the Si-O bond makes the Si susceptible to nucleophilic substitution at high pH and this has been used as an explanation of the solubility of these glasses in alkali solutions.

Therefore, it may be possible to predict the durability of glasses based on resistance to nucleophilic attack of the network, which in turn may be related to bond polarity. The use of bond polarity in the network to predict glass behaviour is not new. Stanworth (1948, 1952)

proposed a model which related glass forming ability to the % of ionic character of the network forming bond. This model was not applied to durability behaviour and was criticised (Rawson 1967) as it was only applicable to some oxides and could not explain many other glass forming systems.

However, we are concerned solely with oxide glasses, and the role of bond polarity with respect to glass durability and glass forming ability is somewhat different. Bond polarity is affected by factors such as atom size, polarizability, and electro-negativity. Important criterion that may affect the susceptibility of glasses to nucleophilic attack are summarised in Table 8.2, which shows chemical data for the species present in the glasses examined.

From this table a number of trends can be observed and related to the network forming and network modifying ability of the species. For example, network formers tend to have i) high oxygen bond strengths ii) three or more valence electrons iii) electronegativities of about 2 and iv) similar covalent radii to oxygen.

Table 8.2

Atomic Number	Element	Outer Electronic Configuration	D-29s KJ/mol X-O Bond Strength	Metallic Radii Length pm	Covalent Radii pm	Ionic Radii pm	Electro-Negativity (Pauling Scale)	Status in Glass
8	O	$2s^2$ $2p^4$	498.3		74	2- : 140	3.44	Former
14	Si	$3s^2$ $3p^2$	809.6		118	4+ : 40	1.90	Former
5	B	$2s^2$ 2p	806.3		79	3+ : 23	2.04	Former
13	Al	$3s^2$ 3p	507.3	143		3+ : 53	1.61	Former/Mod
82	Pb	$6s^2$ $6p^2$	377.8	175		2+ : 118 4+ : 78	2.33	Former/Mod
40	Zr	$4d^2$ $5s^2$	759.8	159		4+ : 72	1.33	Former
11	Na	3s	256.6	186	154	1+ : 102	0.93	Modifier
20	Ca	$4s^2$	385	197		2+ : 99	1.00	Modifier
19	K	4s	276	227	196	1+ : 138	0.82	Modifier
12	Mg	$3s^2$	362.3	160		2+ : 72	1.31	Modifier
26	Fe	$3d^2$ $4s^2$	408.8	124		2+ : 78 3+ : 64	1.83	Modifier

Such trends have been noted by a number of authors and used to explain glass forming ability (see Rawson 1967 for reviews of: Smekal's requirement of mixed bonding 1951; Stanworth's elecronegativity criterion 1952; Winter's "p electron" criterion 1955, and Sun's bond strength criterion 1947). Although, these ideas were proposed to explain glass forming ability, they were based on the bonding in the glass which is also likely to be an important factor in durability behaviour.

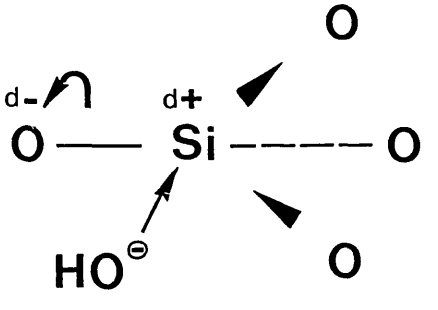
The original models of glass forming have been subject to criticism because of the number of exceptions that exist, which reflects their simplistic nature. However, it was thought that these ideas could be adapted to durability models and, in particular, Stanworth's electronegativity criterion (1952) could be useful for predicting bond polarity of the network former-oxygen bond, and thus related to susceptibility of the network to nucleophilic attack.

It was found that bond polarity (estimated by the difference in electronegativity between oxygen and the network former) was not a good indication of the resistance of the bond to nucleophilic attack. For example, the addition of B and Al to a SiO_2 network improves the resistance to nucleophilic attack, but based on the B-O, Al-O and Si-O bond polarities calculated by the described method, this would not be expected. Obviously factors other than bond polarity affect susceptibility to nucleophilic attack.

It has been observed that simple soda-lime silicate glasses are prone to dissolution at high pH and de-alkalisation at low pH. Empirical data has led to the use of "multi-oxide" diagrams (Klingholtz 1977) and simple rules of thumb that state that an increase in alkali oxide content reduces durability of the glass. Such diagrams are not useful unless basic systems are being examined and do not explain why a loss of durability occurs.

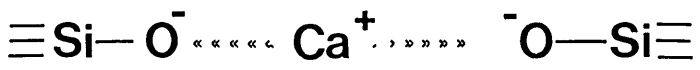
It is suggested that a better understanding of the bonding and the application of a mechanistic approach may improve our theoretical understanding of durability and used in conjunction with empirical models a fairly comprehensive explanation for the behaviour of the glasses studied can be developed.

In alkali-silicate glasses with a [SiO_2] of greater than approximately 70% the network consists of continuous, 3-dimensional tetrahedra. Exposure to acidic solutions de-alkalises the glass by ion-exchange processes, but the continuous network remains unaffected and the glass intact. However, if the solution pH rises significantly through de-alkalisation, or if the glass is exposed to solutions of pH > 9-10, nucleophilic degradation may occur:



The mechanism has been explained in terms of the Si-O bond polarity leaving the Si delta +, that is, electron deficient and therefore susceptible to nucleophilic attack. This process breaks the integrity of the network and the glass dissolves. For silica and simple glasses the dissolution rate at a given pH may be predicted by the use of thermodynamic stability diagrams of the component oxide (Paul 1982).

Bond polarity is not the only criterion for nucleophilic attack, and a fuller explanation for the susceptibility of Si-O to nucleophilic attack can be found from the application of hybridisation bond theory. Using this model it may be proposed that OH- (aq) approaches the electronically neutral sp^3 hybridised silica tetrahedra and then OH- (aq) is able to donate electrons to the delta + Si, via vacant Si 3d electron orbitals, thus forming a "transition state" complex prior to SN2 substitution. In alkali-silicate glasses where SiO_2 is the only network former and there is < 50% SiO_2, then an incomplete network may exist. In such a case de-alkalisation at low pH may result in total dissolution, because some of the structural integrity may be associated with "cross-linking" modifiers, for example:

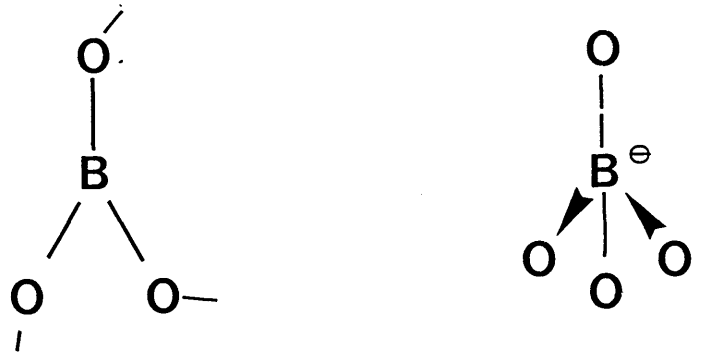


The improvement in alkali-glass durability, by the replacement of mono-valent Na or K for di-valent Ca or Mg cations may therefore be related to the ability of the divalent cation to "cross-link" the discontinuous silica network. Note that Mg has a greater stabilising effect on the glass than Ca (Rawson 1967), and it is suggested that this may be related to the greater Mg^{2+} charge density resulting in stronger MgO ionic bonds within the glass.

It is proposed that the role of alkali, ionic modifiers with respect to durability is greatly reduced when a continuous Si-O network exists, capable of maintaining the integrity of the glass despite de-alkalisation (this behaviour is characterised by Type II surfaces). It is noted that species such as Al that can behave as formers and/or modifiers may be involved in Type III surface formation, thereby establishing potentially protective layers as was observed during exposure of E-glass fibre to RPMI 1640. In contrast, small ionic modifiers such as Na^{+} are highly soluble and mobile and would not be expected to partake in formation of stable Type III surfaces.

Boron and aluminium have both been found, from empirical observation, to greatly improve the alkali resistance of silicate glasses. This has been related to the thermodynamic stability of the oxides and the ability of alumino-silicate glasses to form Type III protective surfaces.

It is suggested that the stability of boro-silicates may be related to the bonding in the network. Boron acts solely as a network former and is incorporated as sp^2 trigonal planar and sp^3 tetrahedral forms in the SiO_2 network, that is:



The B-O bond strength is high (806.3 KJ/mole: CRC Handbook) which helps to explain the thermodynamic stability of the structures, though examination of the polarity of the B-O bond with respect to the Si-O bond, based simply on the electronegativity differences, suggests that the B-O bond is also polar and thus susceptible to nucleophilic attack. However these structures are found to be highly resistant to nucleophilic attack and this may be explained:

(i) unlike silicon, boron is a second row element and thus d electron orbitals are not available for the formation of a transition state during nucleophilic attack.

(ii) in the trigonal planar structure oxygen back donates a pair of electrons to the vacant boron 2p pi orbital, thus stabilising the structure.

(iii) the net negative charge on the B in the sp^3 tetrahedra will repel an attacking nucleophile.

Aluminium is found in two forms in alumino-silicate glasses - as a former and a modifier - and may have a dual role in improving the alkali resistance.

As a network former, Al exists in a negatively charged sp^3 tetrahedra. The net negative charge will discourage attack by OH- (aq), though d orbitals would be available for transition state formation. Note that although Al has the same number of valence electrons as B, a corresponding trigonal planar form of AlO_2 is not encountered, because p pi-p pi back donation is not favourable between the oxygen 2p and aluminium 3p orbitals.

Aluminium also exists as a modifier (Al^{3+}) and can be extracted to form insoluble surface layers on the glass, which may protect against dissolution.

Presumably, in order for Al and B to be effective in stabilising a glass network there must be sufficient present in the glass to be able to form an integral protective layer at the glass surface, and the behaviour of a complex glass containing a wide range of species will depend on the balance between stabilising network formers and de-stabilising modifiers.

From this discussion it can be seen how glass durability may be approached from a theoretical basis, using models for bonding and the associated mechanisms of nucleophilic attack.

Presumably these models can be applied to the cases of Pb and Zr and also novel network forming species in order to help predict the stability of the glass. Difficulties arise in explaining the effect of these species, using a

simple hybridisation bonding approach, because of the availability of bonding orbitals and it becomes extremely difficult to predict the stable structures and determine the nature of the bonding.

Whilst (i) the effects of adding Zr are known empirically to greatly increase the resistance of glasses to acid and alkali attack and (ii) zirconium oxides are extremely stable, it is recommended that an investigation of the bonding could be used to provide a theoretical understanding of the associated increase in durability.

It is suggested that addition of Hafnium (and possibly other transition metals similar to Zr) to glasses would have a similar effect in improving the durability of the network.

8.5 IN-VITRO SURFACE LAYER FORMATION

Our data has shown that inorganic surface layers that are not an integral part of the fibre may be formed in-vitro and that this appears to depend on (i) the composition of the fibre (ii) the composition of the exposure fluid.

This behaviour was only observed for E-glass, ceramic and lead-glass fibre and appeared to be associated with high levels of phosphate in the exposure fluid and with the extraction of either Al (E-glass and ceramic fibre) or Pb (lead-glass fibre) to form an insoluble surface layer. It may be suggested that the presence of an extractable species such as Pb or Al that can act as a "modifier" or "former" and is multi-valent is essential for the formation of this type of surface layer in-vitro.

Previous studies (Morgan 1982, Bernstein 1982) have indicated that surface "gel-layers" are not observed in-vivo. This has been related to the dynamic environment, or the possible presence of chelating agents capable of degrading such layers; however, the formation of "gel-layers" will depend on fibre composition and it should also be noted that these layers are fragile and would not survive recovery procedures involving low temperature ashing and ultra-sonification.

With regard to the dynamic nature of the *in-vivo* environment, some work has been carried out *in-vitro*, which compares the dynamic environment with the static. It would be worthwhile applying continuous-flow to our *in-vitro* systems and some work of this nature has already been carried out (Bugg *et al* 1988). This work examined the response of bulk soda-lime silicate glass and A-glass fibre to water and pH 10 buffer and compared the dynamic response with the static response. The data could be related to the pH of the environment and surface layers were not observed in either case. These trials should be extended to examine the dynamic response of a wider range of materials, with particular reference to factors associated with coat formation.

It should be noted that the design of some of the reported experiments demanded a static environment. In particular, the Gamble's fluid exposures and RPMI 1640 exposures, which had to be incubated in a 5% CO_2 static environment to achieve physiological buffering. The use of a closed static system greatly reduced the chances of microbial contamination, allowing exposures of over a year *in-vitro*.

It is suggested that a dynamic system would probably discourage the formation of the types of layers observed *in-vitro*. It is questionable whether a dynamic *in-vitro* system is a more realistic simulation and it should be noted that the formation of surface layers provides more chemical information.

In effect, the exposure of fibre to monocytes involved a dynamic system. However, it was observed that, if anything, the cultured monocyte exposures had less of an effect than exposure to the culture fluid (RPMI 1640). It was possible that in binding to the fibre the monocytes had protected the fibre surface from the aqueous environment. It was also noted that human serum had little effect on the fibrous materials and this may have been related to protein "sticking" to the fibre and protecting it from the aqueous environment.

8.6 TOXICOLOGY STUDIES

With respect to toxicology studies, it is suggested that in-vitro systems using human monocytes, or cell lines characteristic of the major "target" cells (macrophages and possibly nutrophills, fibroblasts etc..), may provide a suitable alternative to in-vivo studies and it is recommended that further studies should be carried out to assess this.

In particular the effect of phagocytosis of fibres on the respiratory burst of the monocytes requires investigation, because reactive products of the respiratory burst are potentially highly toxic to host tissue. For the same reason an assessment of the effect of glass fibres on the release of digestive enzymes by monocytes is recommended. Furthermore, the use of organic "size" coatings warrants further investigation because such coatings have the potential to modify the cell/fibre interaction as well as increase fibre durability.

As previously discussed (Section 7.7), fibres of the same dimensions and composition would greatly enhance the design of such studies.

8.7 SECONDARY ION MASS SPECTROMETRY

Considerable time was spent developing analytical approaches for characterisation of fibre durability. A statistical approach was applied to EDXA analysis, which allowed confirmation of changes in relative analyte levels of as little as 2-3%. This was particularly useful as such small changes would not be apparent from visual inspection of EDXA spectra.

However, EDXA is primarily a bulk technique providing analytical information from micron-depths into the fibre rather than from just the atomic layers of the surface being analysed. An interest in the near-surface chemistry of the fibres led to investigation of the application of SIMS, a surface sensitive technique potentially capable of determining changes in the surface atom layers of the sample. It was hoped that the technique would also allow depth profiling of the sample to provide better understanding of near-surface behaviour. Furthermore, the potential for improved sensitivity to chemical effects at the surface was seen as a way of possibly reducing fibre exposure times.

The identification of surface species in unexposed and exposed fibres has been achieved, although problems with the reproducibility of analyte signal intensities have made it impossible at this stage to undertake semi-quantitative analysis of the type possible with EDXA. It is recommended that further studies of the application of SIMS to fibre analysis should be carried out, though it is suggested that

features such as a cooled specimen stage and a higher resolution liquid metal ion-gun would be necessary for a significant improvement in the performance of the SIMS instrument used for this investigation. It is also suggested that techniques such as Auger spectroscopy and laser ablation mass spectroscopy may provide the way forward in surface analysis of small fibres.

9. CONCLUSIONS

9.1

An iterative approach has been applied to investigate the durability of a range of glass fibre compositions in-vitro and in-vivo and a "semi-dynamic replenishment" method (Conroy et al 1988) was developed to examine the response of fibres to human monocytes. Maintenance of pH in Gamble's fluid was achieved using CO_2 incubation and sterile conditions were maintained over a one year exposure period by using a closed in-vitro system.

It was observed, generally, that the chemical behaviour of the wide range of test materials, in-vitro and in-vivo, could be related to the pH of the environment, or explained in-vivo by proposing models that may result in generation of high localised pH. This pH effect may be associated with the ability of the glass and ceramic fibre network to resist nucleophilic attack.

9.2

For the range of glass and ceramic fibres studied it was found that their chemical behaviour in aqueous solutions typical of the environment of the lung could be classified under the following categories:

Chemically and physically inert: ceramic fibre

Chemically reactive but not prone to network hydrolysis : E-glass fibre, Cemfil?

Chemically reactive and prone to network hydrolysis : bulk-, A-, lead-glass fibre Cemfil?

It is inferred that materials found *in-vitro* to be chemically and physically inert will be the most durable *in-vivo* and those susceptible to nucleophilic attack (network hydrolysis) will be least durable *in-vivo*.

Note that whilst fibre durability can be used as an index of the long-term pathogenic potential, it does not necessarily reflect the short-term pathogenic potential. For example, if toxic species were released into the lung tissue by a reactive material this may generate a high, short-term pathogenic potential.

9.3

A method was successfully developed which enabled the exposure of fibres to human monocytes, in an attempt to evaluate the effect of the intra-macrophage environment on fibre durability. This *in-vitro* simulation showed that after 6 months of exposure the human monocytes had a negligible effect on the durability of the fibres examined.

This *in-vitro* system requires further investigation to demonstrate that it is characteristic of the *in-vivo* intra-macrophage environment.

The use of human monocytes may also provide a more physiologically accurate alternative to the use of cell-lines for *in-vitro* toxicity studies.

9.4

In-vivo studies using rats produced results consistent with those produced in-vitro. Variations in pH in-vivo were considered to be the major determinant of fibre durability. The in-vivo studies may be improved by the use of longer exposure times and sub-micron fibres of the same dimensions to show more clearly any changes in fibre dimensions.

9.5

A major limitation of commercially available materials, in relation to the requirement of a comparative study of material composition effects on fibre durability, was that a supply of different materials having controlled dimensions and compositions were not available.

Production of fibres of the same sub-micron dimensions and different controlled compositions is seen as essential to improve the comparativity, sensitivity and quantification of both durability and toxicity studies. For example, the preparation of 1 x 20 micron fibres in a range of chemical compositions would enable toxicity studies to be carried out for different fibre types with the elimination of toxicity effects between samples associated with the different size distributions. The sensitivity of durability studies would also be greatly improved because changes in fibre dimension caused by dissolution would be more apparent in a population of fibres with a uniform, relatively small diameter.

It is suggested that design of a system for the small scale production of such fibres would greatly aid future durability studies.

The effect of organic "size" coatings on durability and toxicology requires investigation; this will necessitate an understanding of the formulation of these coatings and release of this information by manufacturers must be encouraged.

9.6

The findings suggest that the durability of glasses _in-vitro_ and _in-vivo_ is primarily related to the ability of the glass network to resist nucleophilic attack. A theoretical model based on molecular bonding has been developed and used to explain the dissolution of the materials examined, and it is suggested (i) that this structure-based model may be developed to appraise the potential durability of other glassy, silicate fibrous materials and (ii) that _in-vitro_ tests exposing fibres to a range of simulated fluids can and should be used to provide a useful first indicator of the durability _in-vivo_.

9.7

Toxicological studies were carried out examining the effects of fibre exposure on human monocytes and V-79 cells. These studies reflected short-term toxicity, associated with the chemical composition of the materials studied and should not be used to measure the long term

pathogenic potential.

In these studies the glass and ceramic fibres were melted and ground into a particulate form. This was intended to eliminate toxicity differences between the samples associated with fibrous morphology.

It was found that the short term toxicity and cell affinity was much greater for fibrous amosite than for particulate glass and ceramic. Of the glass and ceramic samples the lead-glass had the greatest toxicity on average and the ceramic the least. This may be related to the chemical inertness of the ceramic and the toxic effect of lead leaching from the lead-glass. This highlighted the differences between short-term and long-term pathogenic potentials and how durability is not necessarily a good indicator of the short term toxicity of a material. Toxicity studies of this kind are potentially a useful indicator of the short-term toxicity of materials and comparative studies would benefit greatly from the use of materials of the same dimensions.

9.8

Analytical approaches were developed to characterise fibre degradation. These included (i) a statistical treatment of EDXA data which greatly improved the ability to identify significant changes in analyte levels and (ii) the use of SIMS to determine the elements present in near-surface layers of fibres, although further work is required to develop this technique for semi-quantitative analysis.

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APPENDIX 1 STATEMENT OF POSTGRADUATE COURSES OF STUDY AND READING LIST

The following postgraduate courses of study and conferences were attended:

Courses:

i) The Durability of Glass,

ii) The Respiratory System,

iii) Lung Physiology.

These lecture courses were presented by the University of Sheffield (Spring Term 1986).

Conference attendance:

i) 1986 - The Inaugural Conference of the Aerosol Society.

ii) 1987 - Aerosols: Their Generation, Behaviour and Applications; the Aerosol Society first conference, Loughborough University. A paper was presented, Conroy P J *et al*, The durability of inorganic fibrous materials in the lung.

iii) 1987 - Meeting of the British Association for Lung Research, University of Surrey. A paper was presented, Conroy P J *et al*, An *in-vitro* approach to examine the chemical response of mineral fibres to human monocytes.

The following reading list was used:

i) CLARK D E, PANTANO C G AND HENCH L L (1979) Corrosion of Glass. Books for industry, New York, 1979.

ii) DAVIES O L AND GOLDSMITH P L (1986) Statistical Methods in Research and Production. 4th edition, Longman 1986.

iii) MORGAN A AND HOLMES A (1986) Solubility of asbestos and man-made mineral fibres *in-vitro* and *in-vivo*: its significance in lung disease. Environmental Research. 39: 475-484.

iv) PAUL A (1982) Chemistry of Glasses. Chapman and Hall 1982.

v) Proceedings of the WHO/EURO conference of Biological Effects of MMMF. Copenhagen 1982.

vi) Secondary Ion Mass Spectrometry (1984) SIMS IV: proceedings of the 4th international conference, Osaka, Japan. Edited by Benninghoven A, Okano J, Shimuzu R and Werner H W, 1984.