JOURNAL OF Materials CHEMISTRY

Redox state and mobility of iron at the asbestos surface: a voltammetric approach

Laura Prandi,[a] Silvia Bodoardo,[b] Nerino Penazzi[b] and Bice Fubini*[a]

[a]*Dip. di Chimica Inorganica, Chimica Fisica e Chimica dei Materiali, Università di Torino, via Pietro Giuria 9, 10125 Torino, Italy. E-mail: fubini@ch.unito.it*
[b]*Dip. Scienza dei Materiali Ing. Chimica, Politecnico di Torino, c.so Duca degli Abruzzi, 24 I-10141 Torino, Italy*

Received 14th July 2000, Accepted 29th January 2001
First published as an Advance Article on the web 3rd April 2001

The mechanism by which asbestos causes the development of cancer in people exposed to airborne fibres, still unclear at the molecular level, seems to involve iron ions located at particular sites at the fibre surface. In this paper, cyclic voltammetry has been employed to investigate the oxidation state and mobility of iron ions on crocidolite and amosite, the two most common types of amphibole asbestos. Experiments have been carried out at three pH values: 0.5, at which iron is spontaneously released from the solids; neutral (close to the extracellular and cytoplasmatic value); and 4.5, representative of the lysosomial fluid, *i.e.* of the environment to which a phagocytized fibre is exposed. An Fe-exchanged Y zeolite and an Fe-silicalite have been used for comparison as 'model solids'. At neutral pH iron is readily mobilised from FeY upon cycling, in contrast with Fe-silicalite. With both asbestos materials, at neutral pH iron is mobilised during the subsequent cycles and is brought into solution, amosite releasing more iron than crocidolite per unit area under the same conditions. Three couples of redox peaks are seen, centred at *ca.* 0.0, −0.2 and +0.6 V. The first is due to the Fe^{3+}/Fe^{2+} couple in solution, the second is probably related to the Fe^{3+}/Fe^{2+} at the surface of the asbestos particle, and the third is assigned to surface iron in an oxidation state higher than three. At pH 0.5, the couple of peaks due to surface Fe is absent and mobilisation immediately occurs. The release of iron at pH 0.5 from asbestos was also measured photometrically and, in the first two hours, corresponds to that expected for the outermost layer of the minerals. Voltammetric cycling markedly enhances the amount of Fe solubilised. Partial oxidation or reduction of surface iron ions was obtained by keeping the sample at a defined potential before cycling: subsequent voltammograms indicate that the smaller Fe^{3+} cation is the mobilised species.

Introduction

Asbestos encompasses a diverse family of fibrous silicates, which have been largely used in various manufacturing processes in the past because of their exceptional physico-chemical characteristics. Their use is now banned in most Western countries because of the well-established correlation with the development of cancers (pleural mesothelioma and bronchogenic carcinoma) in populations exposed to airborne asbestos fibres.[1] Asbestos forms belong to two main groups: amphiboles (chain silicates) and serpentines (sheet silicates). Their fibrogenic and carcinogenic effects are well established but the molecular mechanisms responsible for these adverse biological responses are not yet fully understood.[2] It is generally accepted that both fibrous habit and chemical composition of the mineral contribute to their toxicity.[3] In particular, great attention has been given in the past few years to the crucial role that iron in the fibre may play in generating ROS (Reactive Oxygen Species) in the proximity of target cells.[2–6] Chrysotile, the main representative of the serpentine group, has the chemical composition $Mg_3Si_2O_5(OH)_4$ and may contain iron in variable amounts up to *ca.* 0.7% by weight only as a substitute for magnesium ions in the octahedral (brucite) sheet formed by edge-sharing $MgO_2(OH)_4$ octahedra. Chrysotile is used in 90% of the applications of asbestos and is less carcinogenic in humans than crocidolite or amosite. Amosite and crocidolite are the best known representatives of the amphibole group. Iron ions are constitutive of these silicates, their chemical formulae being $(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2$ and $Na_2(Mg,Fe^{2+})_3Fe_2^{3+}Si_8O_{22}(OH)_2$, respectively.

The crystal structure of the amphiboles can be described in terms of a basic structural unit formed by a double tetrahedral chain (corner-linked SiO_4 tetrahedra) of composition $(Si_4O_{11})_n^{6n-}$. These silicate double-chains share oxygen atoms with alternate layers of edge-sharing MO_6 octahedra, where M stands for a variety of cations, but mostly Mg^{2+}, Ca^{2+}, Fe^{2+} or Fe^{3+}. The total amount of iron in amosite and crocidolite is in the range 27–29% by weight. Amphiboles exhibit prismatic cleavage: when crushed or milled they fracture along the octahedral layers giving rise to acicular fragments. Individual fibres are usually *ca.* 0.2 μm in diameter and they tend to aggregate into bundles.[1,3]

Many investigations have shown that the biological effects of asbestos were ameliorated in the presence of deferrioxamine, a potent iron chelator, suggesting a crucial role for iron in the overall toxicity.[4–8] Not all iron in the fibre appears to be involved in pathogenicity, but only some few iron ions in specific active sites at the surface, whose coordination and redox state are still to be identified.[2,3,9] Iron may also be mobilised from the fibre by low molecular-weight chelators and reach cellular DNA in this soluble form.[4,10,11] Research in our and other laboratories in the past decade, mostly devoted to the evaluation of the generation of ROS and consequent DNA damage, has evidenced four different iron sites in the amphiboles,[12–16] among which the most reactive ones appear to be those where the iron ion is more isolated,[14] more coordinatively unsaturated[15] and more easily removable from the silicate framework.[4,8,12,13] It has been hypothesised that redox cycling and iron removal and deposition on the fibres might be the mechanisms by which the active iron sites involved

DOI: 10.1039/b005714m

in ROS release may be continuously regenerated *in vivo*.[4,10,11,17–19]

In the present paper we report results obtained with cyclic voltammetry, an electrochemical technique new to the field[20] since only one pioneering paper is available,[21] concerning the redox state and mobility of the iron ions located at the octahedral sites in the silicate framework of amphibole asbestos. We have considered the most common forms, crocidolite and amosite, which are also the richest in iron (30% by weight). Experiments have been carried out at three pH values (0.5, 4.5, 7.0): pH 7 and pH 4.5 are relevant from a biological point of view because during subsequent ingestion/reingestion cycles by alveolar macrophages the fibre is exposed to pH 7.4 (cytoplasm) and to pH 4.5 (phagolysosome) conditions.[2–4,8] At pH 0.5, iron species are released spontaneously (in contrast with neutral pH), so that it is of interest to investigate the effect of pH on the electrochemical behaviour of iron in asbestos.

Because of the complexity of the amphibole structure, where iron is present in two redox states and in different configurations, coupled with the difficulty of making voltammetric measurements on non-conductive solids, we have prepared two 'model solids' which contain iron associated in a simple manner to a silica framework. These were used for comparison (or as a blank) in the experiments with amphiboles. The solids chosen were an Fe-exchanged Y zeolite, successfully employed in our laboratory in previous research to mimic the reactivity of iron in asbestos,[19] and an Fe-silicalite, where iron is a constitutive part of the zeolite framework.[22] Zeolite Y exhibits nearly spherical cavities with a cavity diameter of 10 Å connected with channels of 7.4 Å. The Fe-silicalite considered in this work is a member of the silicalite family, characterised by three-dimensional arrays of TO_4 tetrahedra. T is usually tetravalent silicon (Si^{4+}) which may be partially substituted by tetravalent or trivalent elements such as Al^{3+} or Fe^{3+}. When trivalent substitutes are present (in our case Fe^{3+}), the silicalite skeleton acquires a negative charge. The charge balance is then achieved by counter-cations such Na^+, K^+, H^+, *etc.* The framework-substituted elements are held rigidly in the lattice whereas the charge-compensating cations are mobile and exchangeable.

The two model solids therefore exhibit iron located in two extreme configurations, a mobile position in FeY and strongly held in the framework in Fe-silicalite, and may provide cyclic voltammetric evidence corresponding to these extremes.

Experimental

Materials

Crocidolite and amosite were from UICC (Unione Internazionale Contro il Cancro).[15] Previous outgassing treatments at 400 °C were carried out on the samples placed in quartz cells connected to conventional vacuum lines (residual pressure: 5.0×10^{-5} Torr; 1 Torr = 133.33 Pa) for 2 h in order to eliminate contaminants from the surface.

FeY was prepared by ion exchange by soaking and stirring 1 g of the zeolite NH_4Y in a buffered 0.05 M solution of $FeSO_4$. Particular attention was paid to avoid the spontaneous oxidation of Fe(II) to Fe(III) in air: argon was bubbled through the solution and the pH was kept at 4.5 (acetate buffer). Washed and dried samples were then stored under an inert atmosphere until used. The total amount of exchanged iron, as measured by atomic absorption spectroscopy, was *ca.* 7.1×10^{-4} moles per gram of zeolite.

Fe-silicalite was prepared from an alcoholic solution of $FeCl_3$ and a solution of tetraethyl orthosilicate.[22] The sample was heated in air at 550 °C. Elementary analysis of this material showed that the iron content (as Fe_2O_3) was 0.34 wt% and that the Fe : Si ratio was 366.

The iron chelators 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid (ferrozine) and deferoxamine mesylate (deferoxamine B) were from Aldrich Chemical Co.

Potassium sulfate and sulfuric acid were from SIGMA.

Techniques

Cyclic voltammetry. Zeolites and asbestos are non-conductive powders. In order to carry out electrochemical measurements, a very close contact between the sample and an electronic conductor is required. To prepare the modified electrode, we used the method proposed by El Murr *et al.*[23] with the addition of a preliminary step in which the solid sample is intimately mixed with graphite, in the weight ratio 1 : 4, in an inert solvent (acetone) and sonicated for 15 min (Liarre starsonic 35 80–180 W). The solvent is removed from the paste by heating at 473 K for 2 h and a few drops of 0.2 M K_2SO_4 solution and dodecane are added to the mixture to ensure conductivity.

The working electrode, specifically designed, consisted of a PVC body containing a platinum disk, upon which was placed the asbestos/graphite paste, described above. The electrolytic cell also contained a Pt counter-electrode and a Hg/Hg_2SO_4 reference electrode, termed SSE. All reported potential values are referred to this SSE electrode, the potential of which is 615 mV with respect to the standard hydrogen electrode.

Particular care was also taken to obtain a constant ionic strength in all solutions: pH 7 was obtained by means of a 0.2 M K_2SO_4 solution; pH 4.5 was obtained with a 0.2 M K_2SO_4 solution where the pH was adjusted with dilute sulfuric acid; and pH 0.5 was obtained with a 0.2 M sulfuric acid solution. All electrolytic solutions were prepared using analytical grade reagents and double-distilled water.

Electrochemical measurements were run at ambient temperature by means of an AMEL system 5000 potentiostat. Cyclic voltammetry experiments were performed at a sweep rate of 5 mV s^{-1}, a value which resulted in the best compromise between sufficient signal intensity and peak resolution. The potential was scanned linearly (first in the negative then in the positive direction) between −1000 and +1000 mV/SSE. The redox cycle was repeated several times in order to investigate the shape of the curves and the peak intensity variations during cycling.

Initial oxidation or reduction of surface iron ions was obtained electrochemically by keeping the sample at a defined potential (1 V or –1 V) for 15 min before scanning.

For pre-oxidation, the sample was maintained at +1 V for 15 min before experiment, whereas for pre-reduction the sample was maintained at –1 V for 15 min.

Iron mobilisation measurements. Fibres were incubated at a concentration of 1 mg fibres/1 ml acidic solution (H_2SO_4 0.2 M) for 10 days at 37 °C and were continuously shaken and kept in the dark. The pH was readjusted at regular time intervals throughout the incubation period to prevent alteration of the rates of iron mobilisation. At regular time intervals 1.25 ml samples were taken and centrifuged at 10 000 rpm for 20 min in order to remove the asbestos. The total amount of iron present in the supernatant was determined spectrophotometrically on a Uvikon 930 dual beam spectrophotometer from the absorbance at 428 nm of the complex Fe^{3+}–deferoxamine (ferrioxamine).

Results and discussion

Model solids

The cyclic voltammetric curve obtained for an FeY-modified carbon paste electrode is reported in Fig. 1(a). Only one couple of anodic/cathodic peaks, centred at *ca.* 0.0 V, is present. The redox potential of these peaks suggests that they are due to iron

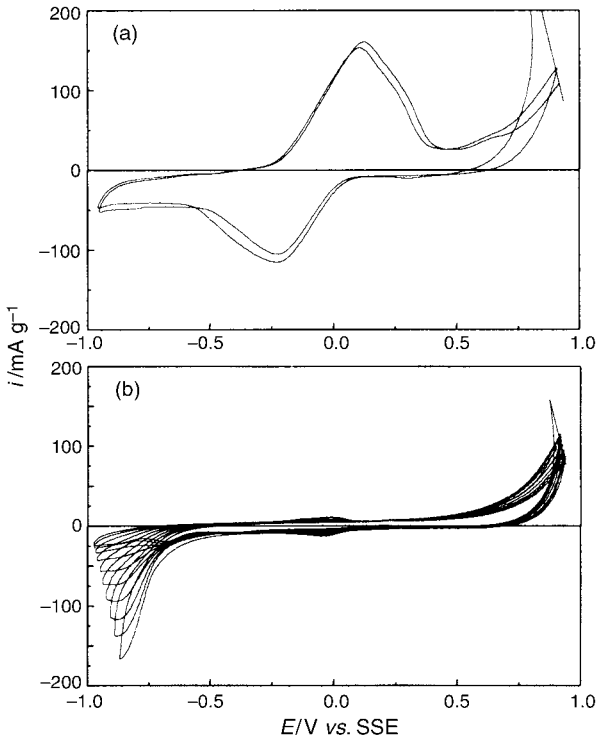

Fig. 1 Cyclic voltammogram of an FeY-modified electrode (a) and of an Fe-silicalite-modified electrode (b) at neutral pH (scan rate = 5 mV s^{-1}).

species in solution, for which $E^{\ominus}$ of the Fe^{3+}/Fe^{2+} couple is 0.013 V/SSE. The high and steady intensity of these peaks in subsequent cycles confirms a full mobility of iron in FeY: the release of iron into the solution is immediate.

The cyclic voltammetric curve obtained for an Fe-silicalite-modified carbon paste electrode is reported in Fig. 1(b). The couple of peaks, typical of iron in solution, is not detectable: only a very weak couple of peaks is present, centred at about −0.2 V. The position and intensity of the peaks is steady in subsequent cycles. As expected, because of the firm position of iron in the Fe-silicalite framework during the electrochemical experiment, iron is not released into the solution. A small fraction of Fe sites in Fe-silicalite undergoes redox cycles: the redox potential of the Fe^{3+}/Fe^{2+} couple on the surface is *ca.* 200 mV more negative than that of the Fe^{3+}/Fe^{2+} couple in aqueous solution.[21]

Asbestos

In order to evidence the effect of pH and to compare the behaviour of the two asbestos forms, the voltammograms obtained at the different pH values with amosite and crocidolite asbestos were obtained and are reported in Fig. 2.

Physiological pH values. Fig. 2(a) shows subsequent cyclic voltammetric curves obtained at neutral pH for an amosite-modified carbon paste electrode. Three couples of anodic and cathodic peaks [indicated in the figure by **(a)**, **(b)** and **(c)**] are seen.

In the initial cycles only the **(a)** couple (centred at *ca.* −0.25 V) is present. As in the preceding case, it can be ascribed to the reduction/oxidation of iron ions in the asbestos structure, progressively released in subsequent cycles. As the number of cycles increases, the current intensity associated with these peaks decreases while a new couple of peaks [couple **(b)**], centred at *ca.* 0.0 V, appears. Both the increase in intensity with the subsequent cycles and the redox potential of these peaks suggest that they could be related, as in the preceding case, to a redox process involving iron ions mobilised into the solution. To confirm this hypothesis further the measurement displayed in Fig. 3 was performed. The experiment on a sample of amosite at pH 7 was run up to a cycle when peak **(b)** attained a high intensity. At the end of the positive scan, the amosite-modified carbon paste electrode was substituted by a graphite-working electrode in the same electrolytic solution. In spite of the absence of asbestos in the electrolytic cell, and hence of iron in the electrode, a voltammetric curve typical of free iron in solution was obtained. Comparing Fig. 3 with Fig. 2(a) a good superposition of the corresponding couple of peaks **(b)** confirms that peak **(b)** in Fig. 2(a) is related to oxidation and reduction of iron ions free in solution. At neutral pH, therefore, iron in subsequent cycles progressively leaves the solid to enter the solution.

As can be seen in Fig. 2(a), the redox peak **(b)** currents increased with the number of cycles as a consequence of a progressive release of iron at each cycle. Only after *ca.* 100 cycles is a steady state obtained (not shown).

Finally, the couple of peaks **(c)** centred at 0.6 mV is present. This result is under investigation at the moment: it is possible that it is due to iron ions stabilised in some way by ligands or a solid state environment. The evident difference of the potential from peaks **(a)** and **(b)** suggests the idea that iron in a higher oxidation state could be involved.[24] The intensity of the redox peak couple **(c)** is associated with the increasing intensity of couple **(b)**, *i.e.* with the presence of iron ions in solution: indeed, it appears after 2–3 cycles.

Results obtained on a crocidolite sample at pH 7 are shown in Fig. 2(b). The cyclic voltammograms obtained on amosite and on crocidolite show an overall similar behaviour, in particular the presence of the three couples of peaks previously described. However, some differences in the intensity of the peaks may be evidenced. Amosite releases iron more quickly than crocidolite: in the case of amosite the intensity of peak **(b)** is higher than that of crocidolite after the same number of cycles. After *ca.* 100 cycles a similar steady state is obtained in both samples.

As expected, both amosite and crocidolite, in which iron is characterised by a similar coordinative position, show an electrochemical behaviour that can be considered as intermediate between that of FeY and Fe-silicalite. This intermediate behaviour is confirmed by the simultaneous presence of the couples of peaks shown by FeY and Fe-silicalite and by the variability of the intensity of these peaks in subsequent cycles. Fig. 2(c) and 2(d) describe cyclic voltammograms concerning amosite and crocidolite at pH 4.5. Changes with respect to pH 7 only consist of a more rapid Fe release in the first few voltammetric cycles; in contrast, remarkable changes are observed when the pH is brought down to 0.5.

Low pH. Fig. 2(e) and 2(f) describe cyclic voltammograms concerning amosite and crocidolite at pH 0.5: in both samples the peak relative to the iron in the solution is greater than that observed in other cases and only the redox couples **(b)** and **(c)** can be detected. This is most probably connected to the fact that the asbestos surface is modified in strongly acidic media.

Indeed, a mere incubation of both crocidolite and amosite in an aqueous solution at pH 0.5 resulted in some iron release. The amount of iron released into the solution at pH 0.5 is reported in Fig. 4 as a function of the time of incubation. For both asbestos types a linear relationship exists between the amount of iron mobilised from asbestos and time in the first three days of incubation: in this range of time the rate of mobilisation can be calculated to be nearly 3 nmol mg h^{-1} for crocidolite and 2 nmol mg h^{-1} for amosite. After this period of time the amount of iron released reaches a plateau (0.18 μmol mg^{-1} for crocidolite and *ca.* 0.08 μmol mg^{-1} for amosite), *i.e.* crocidolite released twice the amount of iron released by amosite in contrast with that found in cyclic voltammetry experiments [Fig. 2(e) and 2(f)].

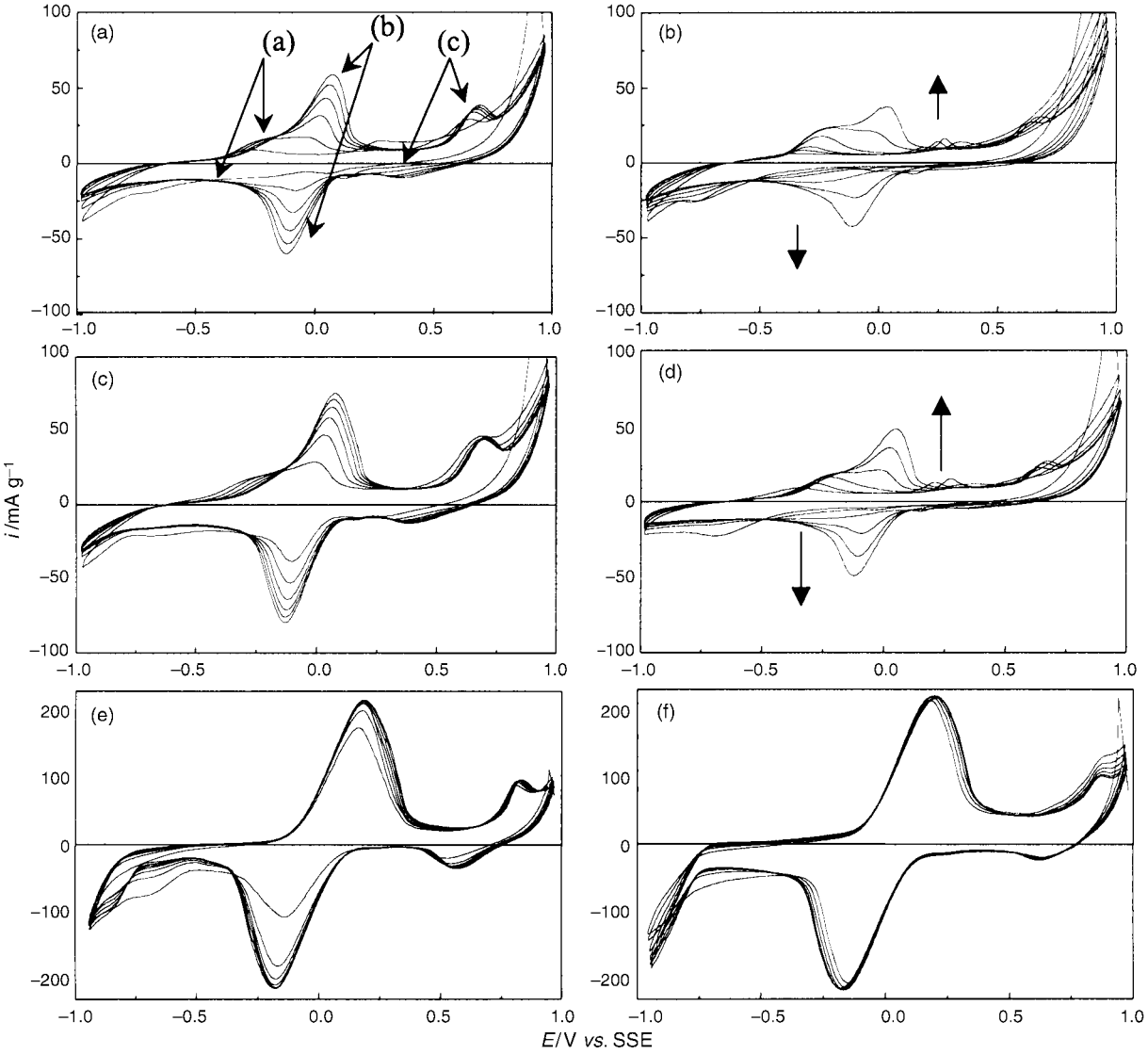

Fig. 2 Cyclic voltammograms of an amosite-modified electrode at pH 7.0 (a), at pH 4.5 (c) and at pH 0.5 (e), and of a crocidolite-modified electrode at pH 7.0 (b), at pH 4.5 (d) and at pH 0.5 (f) (scan rate 5 mV s^{-1}). The arrows indicate the growth direction of the intensity in subsequent cycles.

Iron mobilisation during the voltammetric experiments. The amount of iron released into the solution after six voltammetric cycles (for both samples this is *ca.* 0.6 mM) is twenty-five times greater than that obtained after two hours of incubation (*ca.* 0.024 mM). The mobilisation of iron is strongly favoured by the acidity of the electrolytic solution but the redox cycling is much more effective than mere incubation. Experiments like the one described above were also performed both at pH 4.5 and pH 7. As expected, no iron was detected in the neutral solution and only traces were found at pH 4.5. Therefore, the mobilisation of iron shown in Fig. 2 is related to the redox reactions induced on the asbestos sample. At pH 4.5 both amosite and crocidolite release iron into solution more quickly than at pH 7, and in all cases, including pH 7, amosite seems to release iron into solution more quickly than crocidolite. At very low pH values the difference in the behaviour of the two asbestos varieties is less pronounced than that at neutral pH because in these experimental conditions the pH effect becomes predominant. Nevertheless, the two diagrams [shown in Fig. 2(e) and 2(f)] are not exactly the same. In the case of crocidolite, the peak due to the iron in solution immediately reaches a steady state; with amosite, a steady state is reached only after 2–3 cycles. This difference in the behaviour of the two asbestos types at acidic pH is in agreement with results reported in Fig. 4 for iron mobilisation: at acidic pH amosite releases iron into solution with a rate lower than that of crocidolite.

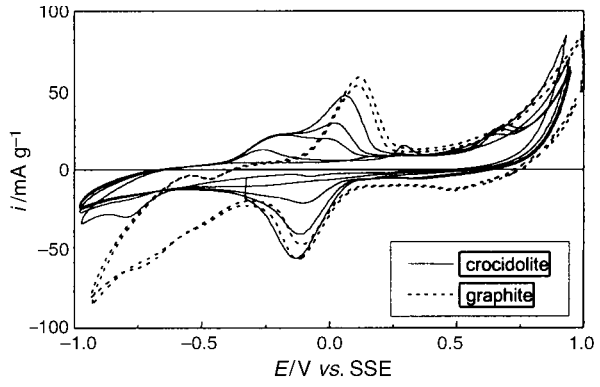

Fig. 3 Cyclic voltammograms of a crocidolite-modified electrode (solid line) and of a graphite-modified electrode (dotted line), substituting for the first one in the same solution, pH 7.0.

The amounts of redox-active iron released into solution at different pH values for crocidolite and amosite were estimated through the total charge passed during the oxidation or reduction of mobilised Fe(II) or Fe(III) from the asbestos fibres. Integration of peaks $\mathbf{b_{ox.}}$ and $\mathbf{b_{red.}}$ gives the total charge resulting from the reactions of oxidation and reduction,

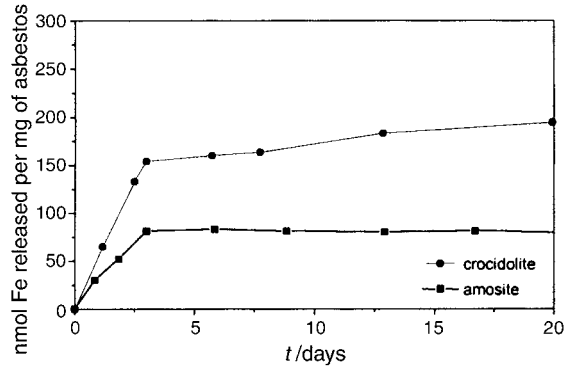

Fig. 4 Release of iron *vs.* time from both crocidolite and amosite suspended in a H_2SO_4 solution at pH 0.5. The amount of iron, determined spectrophotometrically, is expressed in nmoles of iron released per mg of sample.

respectively, of the iron in solution as the potential is scanned. The charges obtained were converted into moles of Fe(II) and Fe(III) (F = Faraday constant):

$$\text{mol Fe(II)} = \text{area } \mathbf{b}_{\text{red.}}/F$$

$$\text{mol Fe(III)} = \text{area } \mathbf{b}_{\text{ox.}}/F$$

Preliminary experiments at different sweep rates provided evidence that the intensity of the current peaks increased along with the sweep rate, with an apparent linear law, which suggested a diffusional behaviour of the voltammetry. Even if peak integration does not give the true amount of mobilised iron, the values measured, though lower than the total amount of iron released, prove clearly that the amount of Fe ions mobilised during the voltammetric measurements is far higher than that released during incubation. As the tests have been carried out under the same conditions, the performance of the two asbestos samples can be confidently compared.

Crocidolite and amosite contain *ca.* 28% iron by weight in the bulk sample. It results that, at pH 0.5, only *ca.* 1.9% of the total iron in the bulk of the sample is solubilized from crocidolite and *ca.* 2.3% for amosite. The percentage of iron solubilized at different pH values for both amosite and crocidolite is shown in Table 1.

The primary elemental constituents detected by XPS at the surface[25] of crocidolite and amosite fibres include O (40–50%), C (12–30%), Si (7–21%) and Fe (4–14%). Despite the different compositions in Fe^{2+} and Fe^{3+} of the two asbestos types, most of the iron at all surfaces was in the Fe^{3+} state because of oxidation in ambient air. There is on average *ca.* 5% more Fe^{2+} on amosite than crocidolite, in accordance with the different Fe^{2+} contents of the two asbestos types. Not all surface iron, however, is oxidised to Fe^{3+} even when samples have been stored in air. One must, however, consider that outgassing treatments at 400 °C were previously carried out on all the samples. There is evidence in the literature that this kind of treatment can be reductive. In order to prove this, both original and outgassed asbestos samples were suspended in 1 mM ferrozine solution. Ferrozine was used for these experiments because of its high affinity for Fe(II) and the high extinction coefficient of the ferrozine–Fe(II) complex (absorbance, 562 nm), which allows detection of iron in the mM range. In the case of the outgassed sample, an immediate colour change to purple can be observed, indicating the formation of the complex Fe(II)–ferrozine. In contrast with non-treated samples, the colour change is detectable only after many hours. This simple result suggests that the amount of Fe(II) is greater at the surface of outgassed asbestos than on non-treated samples.

The data shown in Table 1 may also be related to the surface area of both amosite and crocidolite: at pH 0.5 crocidolite has 2.5 nmoles of iron solubilized per square centimetre, compared with 1.2 nmoles for amosite. If we consider the surface area, the differences in the behaviour of the two asbestos types are more evident.

Amounts of iron released after two hours or ten days of incubation at pH 0.5 for both amosite and crocidolite are also reported in Table 1. The data obtained are reported as a percentage of total iron and are normalised to the surface area of the fibres. The nmoles of iron released at acidic pH per square centimetre from crocidolite are more or less the same as those from amosite both after two hours and ten days. The different behaviour of asbestos after a redox cycling and after a simple incubation in acidic solution suggests that there could be a correlation between the mobility of iron and its redox history: this result could be consistent with a particular solubility of Fe(II) oxidised to Fe(III). Unfortunately, there is no certain means of distinguishing the actual amount of iron of the outermost surface from that located in subsurface layers. To establish approximately whether the iron involved in the redox reaction is only surface iron or not, consider the same data expressed as the number of ions per nm^2 (Table 1). Considering the structure of asbestos, in one nm^2 there could be no more than 4–6 surface iron ions. Comparison of the data in Table 1 with this average value shows that both amosite and crocidolite, after two hours of incubation in acidic solution, release only surface iron into the solution. But a long-term incubation (a plateau is reached after about ten days) gives rise to the mobilisation of iron from one or two subsurface layers (10 ions nm^{-2} for amosite and 12 ions nm^{-2} for crocidolite). Also, the data ascribed to the redox cycling are consistent with a mobilisation of iron from subsurface layers.

A comparative analysis of the voltammetric curves in Fig. 2 indicates that the intensities of the redox peak couples **(c)** and **(b)** are related. The redox couple **(c)** is associated with iron in solution but is seen only in the presence of the solid. This result is currently under investigation.

It could be associated either with a hypothetical further oxidation of Fe^{3+} or with ions which have become isolated as a

Table 1 Iron mobilised in voltammetric cycles and iron released after incubation in acidic solution from both amosite and crocidolite

	Amosite									Crocidolite								
	Mobilisation of iron in voltammetric experiments			Iron released during incubation						Mobilisation of iron in voltammetric experiments			Iron released during incubation					
	6 cycles			2 h			10 d			6 cycles			2 h			10 d		
pH	% iron	nmol cm^{-2}	ions nm^{-2}	% iron	nmol cm^{-2}	ions nm^{-2}	% iron	nmol cm^{-2}	ions nm^{-2}	% iron	nmol cm^{-2}	ions nm^{-2}	% iron	nmol cm^{-2}	ions nm^{-2}	% iron	nmol cm^{-2}	ions nm^{-2}
0.5	2.3	2.5	15	0.09	0.13	0.8	1.5	1.6	10	1.9	1.2	7.5	0.15	0.1	0.6	3.0	2.1	12
4.5	0.61	0.66	4.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.28	0.19	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7.0	0.37	0.4	2.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.22	0.15	0.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = Not detectable.

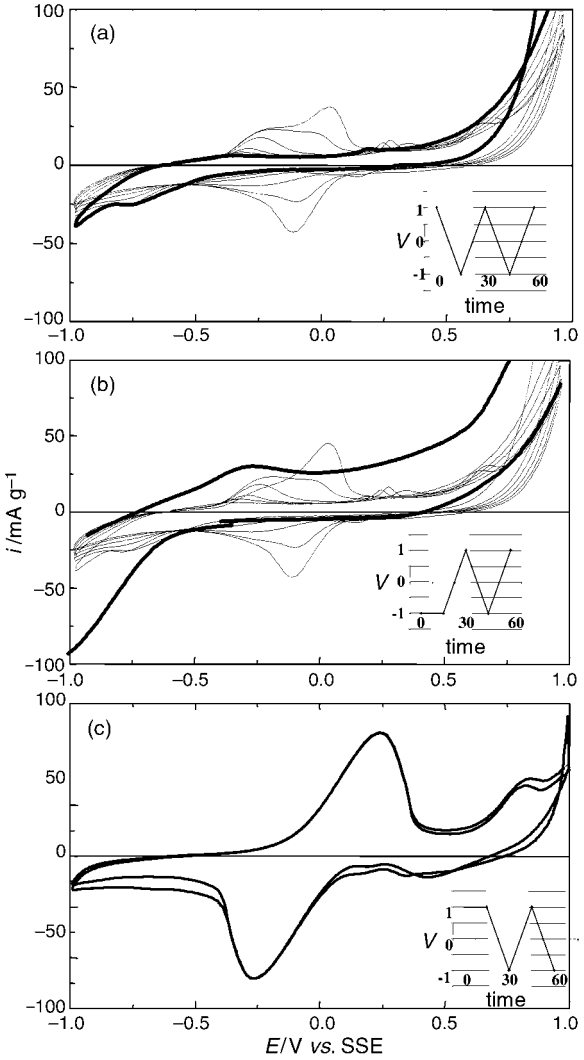

Fig. 5 Cyclic voltammograms of a crocidolite-modified electrode (a) [same curve as reported in Fig. 2(b)], after a pre-reductive treatment (b), and after a pre-oxidative treatment (c), at neutral pH. The first cycle is reported as a bold line.

consequence of release in solution of the neighbouring ones and which have acquired a different redox potential.[14]

To gain a deeper insight into the electrochemical process occurring on the sample, voltammetries preceded by oxidative or reductive pre-treatment were run. Fig. 5 reports the results concerning pre-reduction [Fig. 5(b)] and pre-oxidation [Fig. 5(c)] of crocidolite. Also reported in Fig. 5(a) are the data without any pre-conditioning actually coinciding with the data in Fig. 2(b). In the plots of Fig. 5, the first cycle is evidenced and the variation in the potential is reported in the inset. As in the experiments the reductive peaks of iron fall in the range $+0.5$V to -0.5 V; at -1 V, reduction of all the iron at the surface has probably occurred. After the reductive pre-treatment [Fig. 5(b)], during the first cycle a peak at -0.25 V appears, due to the oxidation of reduced iron, but no traces of iron in the solution are visible before 2–3 cycles. Once the $+1$ V potential has been attained, as in the ordinary experiments [Fig. 5(a)], the peak couple **(b)**, due to iron in solution, appears. After pre-oxidation [Fig. 5(c)], the oxidation of all surface iron probably occurs. At the first potential scan, therefore, only Fe(III) is present at the surface. The couple of peaks **(b)** in this case appears already in the first cycle, *i.e.* iron ions are brought into solution at the first scan, resulting in the behaviour found with FeY [Fig. 1(a)]. The intensity of these peaks, opposite to what happens in all the other cases, does not increase in subsequent cycles, *i.e.* all removable iron is released immediately.

These results indicate a correlation between the redox behaviour of asbestos types (in particular the release of iron) and their redox history. Release of iron into solution, in the absence of chelators, can also be obtained as consequence of a strong oxidative process. In order to estimate the strength of the oxidation process needed for the release of iron, cyclic voltammetry experiments on samples pre-treated at 0.5 V for 15 min have been carried out (data not shown). Scans in the potential range $+0.5$ V to -1 V showed no trace of solubilised iron; therefore, a voltage higher than 0.5 V is required to attain the extent of oxidation whereby iron is solubilised.

Conclusion

Cyclic voltammetry is a useful technique for the study of the state of iron in complex matrices such as asbestos. A comparison of the results obtained with removable iron associated to a zeolite (FeY) and with iron fixed within a silicate framework (Fe-silicalite) with those of the two asbestos types clearly depicts the peculiarities of the iron ions in the amphibole structure. While the exchangeable iron in FeY is easily removed in the first cycle, practically no framework iron is removed from Fe-silicalite, even in subsequent cycles. In the case of asbestos, iron is indeed removed and brought into the solution, but this process (opposite to that of FeY) takes place progressively in subsequent cycles, indicating the possibility of slowly releasing iron over prolonged periods of time.

The complexity of iron sites in asbestos is better revealed at neutral pH where the peak corresponding to removable iron has a different potential value from that already removed into the solution. A peak at a high potential value only appears when a substantial fraction of iron has already been removed, and is related to some processes taking place at the solid surface whose characteristics are still under investigation. Such sites, largely reported for iron complexes in solution,[26] have never been directly evidenced at a solid surface. They have been proposed, however, as possible centres for the peculiar activity of asbestos and other pathogenic solids in the release of ROS even in the absence of OH-generating substances such as hydrogen peroxide.[18,27]

Crocidolite and amosite exhibit similar behaviour when compared with the zeolites; they do show, however, some differences in the mobility of iron ions, which may be explained by the absence or presence of structural Fe(III) in amosite and crocidolite, respectively. In both cases the amount of iron released in subsequent cycles largely exceeds that expected to be at the surface, confirming a substantial mobility of iron through the octahedral parts of the crystal out towards the surface. In this respect, measurements performed after an oxidation or reduction pre-treatment (Fig. 5) reveal that the Fe(III), because of its smaller diameter, may be removed by the crystal framework when oxidised from Fe(II). In contrast, Fe(II) resulting from Fe(III) reduction is not released into solution.

As it concerns the identification of the active sites in asbestos toxicity, we plan to employ the present technique, in association with more conventional ones, to compare the physico-chemical differences among asbestos samples, which have been modified by various ways in order to decrease their pathogenic potential.

Acknowledgements

This work was supported by Regione Piemonte, Italy, in the context of a collective project on Asbestos.

The authors are grateful to Professor P. Spinelli, Dip. Scienza dei Materiali Ing. Chimica, Politecnico di Torino, for enlightening discussions, and to Professor E. Garrone for carefully reading and improving the manuscript.
The Fe-silicalite sample was a kind gift of Enitecnologie through Dr S. Bordiga, Dip. Chimica IFM, Torino.

References

1 B. T. Mossman, J. Bignon, M. Corn, A. Seaton and A. Gee, *Science*, 1990, **247**, 294.
2 A. B. Kane, in *Mechanisms of Fibre Carcinogenesis*, eds. A. B. Kane, P. Boffetta, R. Saracci and J. D. Wilburn, IARC Scientific Publication, Lyon, France, 1996, vol. 140, p. 11.
3 B. Fubini and C. Otero Areàn, *Chem. Soc. Rev.*, 1999, **28**, 373.
4 J. A. Hardy and A. E. Aust, *Chem. Rev.*, 1995, **95**, 97.
5 D. W. Kamp, P. Graceffa, W. A. Prior and S. A. Weitzman, *Free Radical Biol. Med.*, 1992, **12**, 293.
6 B. T. Mossman and J. P. Marsh, *Environ. Health Perspect.*, 1989, **81**, 91.
7 C. C. Chao and A. E. Aust, *Arch. Biochem. Biophys.*, 1994, **308**, 64.
8 B. Fubini, A. E. Aust, R. E. Bolton, P. J. A. Borm, J. Bruch, G. Ciapetti, K. Donaldson, Z. Elias, J. Gold, M. C. Jaurand, A. B. Kane, D. Lison and H. Muhle, *ATLA, Altern. Lab. Anim.*, 1998, **26**, 579.
9 B. Fubini, L. Mollo and E. Giamello, *Free Radical Res.*, 1995, **23**, 593.
10 A. E. Aust and L. G. Lund, *Biological oxidation system*, 1990, **2**, 597.
11 L. G. Lund, M. G. Williams, R. F. Dodson and A. E. Aust, *Occup. Environ. Med.*, 1994, **51**, 200.
12 M. Gulumian, D. J. Bhoolia, P. Theodorou, H. B. Rollin, H. Pollak and J. A. van Wyk, *Suid-Afrikaanse Tydskrif vir Wtenskap*, 1993, **89**, 405.
13 H. Pollak, H. de Waard and M. Gulumian, *Suid-Afrikaanse Tydskrif vir Wtenskap*, 1993, **89**, 401.
14 A. Graham, J. Higinbotham, D. Allan, K. Donaldson and P. H. Beswick, *Occup. Environ. Med.*, 1999, **56**, 606.
15 G. Martra, E. Chiardola, S. Coluccia, L. Marchese, M. Tomatis and B. Fubini, *Langmuir*, 1999, **15**, 5742.
16 A. J. Werner, M. F. Hochella, G. D. Guthrie, J. A. Hardy, A. E. Aust and J. D. Rimstid, *Am. Mineral.*, 1995, **80**, 1093.
17 B. Fubini and L. Mollo, *Toxicol. Lett.*, 1995, **82**, 951.
18 B. Fubini, in *Mechanisms of Fibre Carcinogenesis*, eds. A. B. Kane, P. Boffetta, R. Saracci and J. D. Wilburn, IARC Scientific Publication, Lyon, France, 1996, vol. 140, p. 35.
19 B. Fubini, E. Giamello, L. Mollo, G. Zanetti, S. K. Eborn and A. E. Aust, *Res. Chem. Intermed.*, 1999, **25**, 95.
20 D. R. Rolison, in *Advanced Zeolite Science and Applications*, eds. J. C. Jansen, M. Stocker, H. G. Karge and J. Weitkamp, Elsevier, Amsterdam, 1994, vol. 85, p. 543.
21 Z. Shen, V. D. Parker and A. E. Aust, *Anal.Chem.*, 1995, **67**, 307.
22 S. Bordiga, R. Buzzoni, F. Geobaldo, C. Lamberti, E. Giamello, A. Zecchina, G. Leofanti, G. Petrini, G. Tozzola and G. Vlaic, *J. Catal.*, 1996, **158**, 486.
23 N. El Murr, M. Kekerni, A. Sellami and Y. Ben Taarit, *J. Electroanal. Chem.*, 1988, **246**, 461.
24 B. H. J. Bielski, *Free. Radical Res. Commun.*, 1991, **12–13**, 469.
25 J. Gold, H. Amandusson, A. Krozer, B. Kasemo, T. Ericsson, G. Zanetti and B. Fubini, *Environ. Health Perspect.*, 1997, **105**, 1021.
26 *Encyclopedia of Electrochemistry of the Elements*, ed. A. J. Bard, Marcel Dekker, New York, 1982, vol. 9, p. 229.
27 H. Pezerat, R. Zalma, J. Guignard and M. C. Jaurand, in *Non Occupational Exposure to Mineral Fibers*, eds. J. Bignon, J. Peto and R. Saracci, IARC Scientific Publication, Lyon, France, 1989, vol. 90, p. 57 100.