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Reduction of heavy metals by cytochrome c_3

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Abstract

We report on reduction and precipitation of Se(VI), Pb(II), Cu(II), U(VI), Mo(VI), and Cr(VI) in water by cytochrome c_3 isolated from *Desulfomicrobium baculatum* [strain 9974]. The tetraheme protein cytochrome c_3 was reduced by sodium dithionite. Redox reactions were monitored by UV-visible spectroscopy of cytochrome c_3. Analytical electron microscopy work showed that Se(VI), Pb(II), and Cu(II) were reduced to the metallic state, U(VI) and Mo(VI) to U(IV) and Mo(IV), respectively, and and Cr(VI) probably to Cr(III). U(IV) and Mo(IV) precipitated as oxides and Cr(III) as an amorphous hydroxide. Cytochrome c_3 was used repeatedly in the same solution without loosing its effectiveness. The results suggest usage of cytochrome c_3 to develop innovative and environmentally benign methods to remove heavy metals from waste- and groundwater.

3 Introduction

Biological processes for removing heavy metals from wastewater include biosorption, bioaccumulation and bioreduction (Abdelouas et al. 1998, 1999a; Bustard and Mchale, 1998; Vecchio et al., 1998; Basnakova et al., 1998; Macaskie et al., 1997). However, theses processes can fail to lower the concentrations of metals below regulatory standards due to toxicity to living microorganisms or rapid saturation of sorbing sites of biomass. Using proteins such as cytochrome c_3 eliminate these limitations. Cytochrome c_3 exhibits selective and high-affinity binding sites for electron transfer and catalyze redox reactions in solution.

An innovative way to develop effective and environmentally benign processes for water treatment involves the use of specific enzymes extracted from microorganisms. Lovley et al. (1993) and Lovley and Phillips (1994) showed that cytochrome c_3, purified from *Desulfovibrio vulgaris*, can be used to separate dissolved uranium and chromium species from water by reduction and precipitation of sparingly soluble compounds of these metals. More recently, Lojou et al. (1998) and Lojou and Bianco (1999) showed that several c-type cytochromes including cytochrome c_7 from *Desulfovibrio acetoxidans*, cytochrome c_3 from *D. vulgaris* Hildenborough, *D. desulfuricans* Norway, *D. gigas*, and cytochrome c_{553} from *D. vulgaris* Hildenborough reduce soluble Fe(III), Cr(VI), U(VI) and oxides of Mn(IV), V(V) and Fe(III). The tetraheme protein cytochrome c_3, first identified in sulfate-reducing bacteria, is responsible for the reduction of elemental sulfur to sulfide by these bacteria (Fauque et al., 1979). Cytochrome c_3 was also found in the facultative anaerobic bacterium *Shewanella putrefaciens* (Tsapin et al., 1996). Chapman et al. (1997) described this four heme protein as "the most versatile redox center in biology". Lalonde (1997) described biocatalysts (e.g., enzymes) as the ideal "green" catalyst for redox reactions, producing less waste and consuming less energy. Using cytochrome c_3 rather than living cells of sulfate-reducing bacteria eliminates their intoxication and loss of effectiveness caused by high concentrations of contaminants as described, e.g. by Lovley and Phillips (1992). Also, reduction of metals by cytochrome c_3 was shown to be very fast (Lovley et al., 1993; Lovley and Phillips, 1994). The redox potential of the hemes of cytochrome c_3 can be as low as $E_H \approx -400$ mV (Coutinho and Xavier, 1994), a value low enough to reduce all higher valence states of metals. If not reduced to the metallic state, many metals form insoluble oxides or hydroxides in lower valence states.

The center of each heme molecule in cytochrome c_3 consists of an iron atom. The iron atom changes valence between Fe(II) and Fe(III) during electron transfer. The reaction between the cytochrome c_3 and a given bivalent cation (M^{2+}) can be described by the following reaction (Niki et al., 1977):

$$Fe^{II}\text{cytochrome } c_3 + 2M^{2+} = Fe^{III}\text{cytochrome } c_3 + 2M^0 \qquad (1)$$

assuming the electron of all four hemes is transferred. Cytochrome c_3 can also be reduced electrochemically on platinum, mercury, and carbon electrodes (Niki et al., 1977; Lojou and Bianco, 1999) or chemically, e.g. by sodium dithionite (Favaudon et al., 1978). The catalytic role of cytochrome c_3 can be expressed by reactions (2a-d):

$$2S_2O_4^{2-} + 4H_2O + Fe^{III}\text{cytochrome } c_3 = Fe^{II}\text{cytochrome } c_3 + 4SO_3^{2-} + 8H^+ \qquad (2a)$$

$$SeO_4^{2-} + 8H^+ + 1.5Fe^{II}\text{cytochrome } c_3 = 1.5Fe^{III}\text{cytochrome } c_3 + Se^0 + 4H_2O \qquad (2b)$$

$$3U^{VI}O_2(NO_3)_2 + 1.5Fe^{II}\text{cytochrome } c_3 = 1.5Fe^{III}\text{cytochrome } c_3 + 3U^{IV}O_2 + 6NO_3^- \qquad (2c)$$

$$1.5\ O_2 + 6H^+ + 1.5Fe^{II}\text{cytochrome } c_3 = 1.5Fe^{III}\text{cytochrome } c_3 + 3H_2O \qquad (2d)$$

Reaction (2b) shows reduction to the zero-valence state [Se(VI)→ Se^0]; reaction (2c) reduction to a lower valence state [U(VI) →U(IV)] and formation of sparingly soluble UO_2; reaction (2d) shows reduction of dissolved oxygen. All reactions are mediated by cytochrome c_3. Direct reduction of most metal ions by dithionite is thermodynamically possible but the reactions are too slow to be of practical interest.

The purpose of this work is to study reduction reactions of Se(VI), Pb(II), Cu(II), U(VI), Mo(VI), and Cr(VI). Upon reduction, all of these elements are expected to form sparingly soluble compounds. These reactions may be applied to clean waste- and groundwater.

2. Experimental

Cytochrome c_3 was isolated from *Desulfomicrobium baculatum* [strain 9974] by the procedure of Moura et al. (1988), and the electrochemical potential of each of the four hemes was measured. Relative to the standard hydrogen electrode (SHE) the potentials are E = –70 mV for the heme with the lowest negative potential to E = –350 mV for the one with the highest negative potential and two potentials in between. The reason for selecting this cytochrome c_3 is that it has the highest negative potential compared with those purified from *Desulfovibrio* (*D. gigas*, *D. vulgaris*, and *D. desulfuricans*). Ma et al. (1998) attributed this highly negative potential to several effects, including high ruffling of the heme molecule.

A 0.1 M solution of sodium dithionite ($Na_2S_2O_4$) was prepared in a serum bottle and flushed with argon to remove oxygen. This solution was used to reduce cytochrome c_3. Selenium was used in the form of Na_2SeO_4, lead as $Pb(NO_3)_2$, copper as $CuCl_2 \cdot 2H_2O$, uranium as $UO_2(NO_3)_2 \cdot 10H_2O$, molybdenum as Li_2MoO_4, and chromium as $K_2Cr_2O_7$. Stock solutions of these chemicals were prepared with concentrations between 1 and 18 mM by dissolving high-purity chemicals in a 30 mM sodium bicarbonate buffer solution (pH = 7.3). Only $CuCl_2 \cdot 2H_2O$ was dissolved in pure water to avoid precipitation of carbonates.

Each experiment began with the reduction of cytochrome c_3 and was conducted in a quartz cuvette placed in a Hewlett-Packard 8452 diode array UV-vis spectrometer: 10 μL of 0.1 M sodium dithionite solution were added to 1 mL of 10^{-5} M cytochrome c_3 solution. This yields a molar excess dithionite of at least 100. The spectrum of reduced cytochrome c_3 was recorded. Then an aliquot of a stock solution of one of the metals was added sufficient to oxidize dithionite and cytochrome c_3, and the spectrum of cytochrome c_3 was recorded again. Separate experiments were conducted for each metal. Enough precipitate (reduced metal species) was produced for analysis by transmission electron microscope. Blank experiments were conducted with de-ionized water alone and de-ionized water with nitrate to determine the effect of dissolved oxygen and nitrate on cytochrome c_3 oxidation.

At the end of a reduction/precipitation experiment, the cuvette was removed from the spectrometer and shaken. A few drops of the suspension were deposited onto a carbon-coated grid. Then, the grid was rinsed with de-ionized water to remove soluble salts. The grid was placed into a Jeol JEM-2010 transmission electron microscope (TEM), equipped with an Oxford Link ISIS EDS (energy-dispersive X-ray spectroscopy) system. Precipitates were analyzed for chemical composition, morphology, and crystal structure. Structural information was obtained using selected area electron diffraction (SAED). The microscope was operated at 200 keV.

3. Results and discussion

3.1 Redox reactions with cytochrome c_3 in solution

The results of blank experiments with Fe^{II}cytochrome c_3 in deionized water saturated with oxygen (7 mg/L) and with 10 mM nitrate are shown in Figures 1 and 2. For the experiment with dissolved oxygen, deionized water with 7 mg/L O_2 was added little by little until complete oxidation of dithionite and cytochrome c_3 was achieved (Figure 1). A total volume of 80-100 µL of water was added. The same volume was needed in the presence of nitrate (Figure 2). This shows that cytochrome c_3 does not reduce nitrate.

Figure 3 shows the shift of the cytochrome c_3 spectrum from reduced to oxidized after reduction of selenate (SeO_4^{2-}) and exhaustion of dithionite. SeO_4^{2-} was reduced to Se^0 (equation 2b). In the beginning, Se^0 was in colloidal form and the solution was clear. After a week, the solution became red, and red particles precipitated. This experiment was repeated several times using the same cytochrome c_3 in solution. Whenever dithionite was added cytochrome c_3 was reduced and oxidized instantaneously after a sufficient supply of SeO_4^{2-} was added. The experiments with heavy metals described hereafter have also been repeated several times with cytochrome solution, and the redox shifts of the spectra were always reproducible giving spectra like that shown in Figure 3. These results show that cytochrome c_3 is fairly stable in solution and can be reused.

Results of reduction of Pb(II), Cu(II), U(VI), Mo(VI), and Cr(VI) by cytochrome c_3 gave spectral changes like that shown in Figure 3. Without exception, addition of small quantities of metal (10 µL of stock solution) led to instantaneous oxidation of cytochrome c_3, indicating complete consumption of dithionite and reduction of the respective metal ions.

3.2 Solid reaction products

<u>Selenium</u>: The precipitate resulting from Se(VI) reduction is shown in Figure 4a. The figure shows aggregates of small particles with an average diameter less than 100 nm. Electron diffraction patterns showed the particles are amorphous, and EDS analysis showed the particles consisted of Se with some S and traces of Te as impurities (Figure 4b). The presence of amorphous particles is not surprising because an amorphous modification of red selenium (glassy Se) is known. The observation of nanosize particles will be described in more detail in another publication (Abdelouas et al. 1999b).

Several studies have shown that a variety of microorganisms including sulfate-reducing bacteria can reduce selenate and precipitate Se^0 (Kauffman et al., 1986; Tomei et al., 1995;

Tucker et al., 1998). Our study is the first showing the reduction of selenate by cytochrome c_3 and precipitation of Se^0.

Lead: Figure 5a shows small particles of lead. These particles precipitated instantaneously upon reduction of Pb(II) by cytochrome c_3. Electron diffraction data showed that lead metal had formed (hexagonal structure; *d*-spacing in nm: 0.196, 0.153, 0.14, 0.128 and 0.114). The particle size was less than 40 nm. EDS analysis showed that the metal was pure (Figure 5b). No other solid phases were found.

Precipitation of lead in the presence of *Pseudomonas maltophilia* was reported by Saiz and Barton (1992). The authors showed that a decrease of the Pb^{2+} concentration in solution was accompanied by formation of gray-black Pb-containing colloids, 175 nm in size. The authors did not provide evidence that Pb^0 was present.

Copper: The precipitate from the reduction of Cu(II) is shown in Figure 6a. The figure shows that particles are small (below 50 nm). Electron diffraction data were not conclusive. EDS analysis showed only copper (Figure 6b). The presence of a copper compound, including oxide, hydroxide, and carbonate was excluded. Formation of Cu^0 is suggested.

A review by Lovley (1995) on microbial reduction of metals shows that microbially mediated reduction of copper ions to Cu^0 is possible.

Uranium: Results with U are given in Figure 7. Figure 7a shows the particles precipitated upon reduction of U(VI) by cytochrome c_3. An electron diffraction pattern is also shown (cubic structure of uraninite, UO_2; *d*-spacing in nm: 0.195, 0.127 and 0.107). The presence of pure uraninite was confirmed by EDS measurements (Figure 7b).

Uraninite is the product of reduction of the uranyl (UO_2^{2+}) cation by a variety of microorganisms, including metal- and sulfate-reducing bacteria (Gorby and Lovley, 1992; Abdelouas et al., 1999a). Uraninite is also the product of uranyl reduction in the presence of cytochrome c_3 purified from *Desulfovibrio vulgaris* Hildenborough (Lovley et al., 1993).

Molybdenum: The precipitate from Mo(VI) reduction is a poorly crystallized phase (Figure 8a). The precipitate was brown-back. There may have been colloidal particles as precursors of the precipitate. This was suggested by the brown color of the solution after reduction. The electron diffraction pattern showed that the precipitate was amorphous. EDS analysis showed Mo and O as the only constituents of the precipitate (Figure 8b). The chemical form of Mo is not known exactly but it is most likely MoO_2 or a hydrated form of MoO_2.

Recently, microbial reduction of Mo(VI) to Mo(IV) has been observed under sulfate-reducing conditions (Tucker et al., 1998; Chen and Clayton, 1999). In this case, the reduced molybdenum precipitated as MoS_2.

Chromium: Figure 9 shows the results for chromium. Figure 9a shows that Cr precipitates as aggregates of tiny particles. Electron diffraction patterns indicated that the precipitate was amorphous (Figure 9a). The EDS spectrum (Figure 9b) showed that the particles contained Cr and O. These findings suggest that an amorphous Cr-hydroxides [$Cr(OH)_3$] formed. Amorphous $Cr(OH)_3$ is presumably the product of Cr(VI) reduction by numerous microorganisms under aerobic or anaerobic conditions (Lovley, 1995).

3.3 Potential application of cytochrome c_3

We have shown for the first time that Se, Pb, Cu, and Mo are directly reduced by cytochrome c_3. Enzymatic reduction by sulfate-reducing bacteria, presumably involving cytochrome c_3 has been demonstrated for Se, U, Mo, and Cr (Lovley et al., 1993; Lovley and Phillips, 1994; Tucker et al., 1998). Cu and Pb were shown to be reduced by microorganisms (Saiz and Barton, 1992; Lovley, 1993).

Our results suggest that cytochrome c_3 may be used as an efficient reducing agent in waste- and groundwater remediation. Even metal recovery may be considered. The redox reactions with cytochrome c_3 are very fast. Electrochemical reduction of cytochrome c_3 can avoid addition of chemicals to the system to be treated. Furthermore, we found that after several cycles of reduction-oxidation, cytochrome c_3 conserved its integrity and could be reused for metal reduction. Stability of cytochrome c_3 in aqueous solution, without significant loss of activity, is likely to be enhanced when immobilized (Yildirim et al., 1994; Somers et al., 1997; Lalonde, 1997; Katchalski-Katzir, 1993; Tischer and Wedekind, 1999; Tischer and Kasche, 1999).

Cytochrome c_3 can reduce metals in dissolved (this work, Lojou and Bianco, 1998) or in immobilized form, i.e. fixed on a substrate (Lojou and Bianco, 1998). Besides the couple hydrogenase-H_2 as electron provider for cytochrome c_3 (e.g., sulfate-reducing bacteria), electrons for cytochrome c_3 reduction can be provided chemically by dithionite or electrochemically when immobilized on carbon electrodes (Lojou and Bianco, 1998). The gene for cytochrome c_3 from *D. vulgaris* has been cloned and expressed in the closely related *D. desulfuricans* (Voordouw et al., 1990), as well as the genetically distant *Rhodobacter sphaeroides* (Cannac et al., 1991), suggesting the possibility of engineering an organism to overexpress cytochrome c_3 to enhance metal reduction. More recently, Aubert et al. (1998) showed that the gene encoding cytochrome c_7 from *D. acetoxidans* was expressed in *D. desulfuricans* that produces a relatively large quantity of cytochrome c_7.

No measurements of final concentrations of the elements in solution were conducted. However, the precipitates observed and identified here are all known to be sparingly soluble. It is likely that solution concentrations are controlled by the solubility of the respective compounds after precipitation . Table 1 lists the precipitates. Their solubilities are compared with the United States Environmental Protection Agency's drinking water standards, indicating that the standards would be met in all cases. The stability of colloids (here: particle sizes of less than 100 nm) and their contribution to final concentrations in solution must be further investigated.

References

Abdelouas, A., Lu, Y., Lutze, W. and Nuttall, H. E. (1998) Reduction of U(IV) to U(IV) by indigenous bacteria in contaminated ground water. J. Contam. Hydrol. 35, 217-233.

Abdelouas, A., Lutze, W. and Nuttall, H. E. (1999a) Uranium contamination in the subsurface: characterization and remediation. In: *Uranium: Mineralogy, Geochemistry and the Environment.* Reviews in Mineralogy, 1999b, Vol. 38 (P. C. Burns and R. Finch, eds.), 433-473.

Abdelouas, A., Gong, W. L., Lutze, W., Shelnutt , J. A., Franco, R. and Moura, I. (1999b) Using cytochrome c_3 to make selenium nanowires. Submitted to Chem. Mater.

Aubert, C., Lojou, E., Bianco, P., Rousset, M., Durand, M. C., Bruschi, M. and Dolla, A. (1998) The *Desulfovibrio acetoxidans* triheme cytochrome c_7 produced in *Desulfovibrio desulfuricans* retains its metal reductase activity. Appl. Environ. Microbiol. 64, 1308-1312.

Ball, J. W. and Nordstrom, D. K. (1998) Critical evaluation and selection of standard state thermodynamic properties for chromium metal and its aqueous ions, hydrolysis species, oxides, and hydroxides. J. Chem. Eng. Data 43, 895-918.

Basnakova, G., Spencer, A. J., Palsgard, E., Grime, G. W. and Macaskie, L. E. (1998) Identification of the nickel uranyl phosphate deposits on *Citrobacter* sp. Cells by electron-microscopy with electron-probe X-ray-microanalysis and by protein-induced X-ray-emission analysis. Environ. Sci. Technol. 32, 760-765.

Bustard, M. and Mchale, A. P. (1998) Biosorption of heavy-metals by distillery-derived biomass. Bioprocess. Engineer. 19, 351-353.

Cannac, V., Caffrey, M. S., Voordouw, G. and Cusanovich, M. A. (1991) Expression of the gene encoding cytochrome c_3 from the sulfate-reducing bacterium *Desulfovibrio vulgaris* into the purple photosynthetic bacterium *Rhodobacter sphaeroides*. Arch. Biochem. Biophy. 286, 629-632.

Chapman, S. K., Daff, S. and Munro, A. W. (1997) Heme : the most versatile redox center in biology. Structure and Bonding 88, 39-70.

Chen, G. and Clayton, C. R. (1999) X-ray photoelectron spectroscopy analysis of Mo metal surface exposed to sulfate-reducing bacteria. Surface Interface Anal. 27, 230-235.

Coutinho, I. B. and Xavier, A. V. (1994) Tetraheme cytochromes. Methods Enzymol. 243, 119-140.

Fauque, G., Herve, D. and Le Gall, J. (1979) Structure-function relationship in hemoproteins: the role of cytochrome c_3 in the reduction of colloidal sulfur by sulfate-reducing bacteria. Arch. Microbiol. 121, 261-264.

Favaudon, V., Ferradini, C., Pucheault, J., Gilles, L. and LeGall, J. (1978) Kinetic of reduction of cytochrome c_3 from *Desulfovibrio vulgaris*. Biochem. Biophys. Res. Commun. 84, 435-440.

Federal Register (1995) Environmental Protection Agency (EPA), CFR 40 Part 192, Groundwater Standards for Remedial Actions at Inactive Uranium Processing Sites, Table 1, p. 2866, November, 1995.

Gorby, Y. A. and Lovley, D. R. (1992) Enzymatic uranium precipitation. Environ. Sci. Technol. 26, 205-207.

Katchalski-Katzir, E. (1993) Immobilized enzymes –learning from past successes and failures. Trends Biotechnol.11, 471-478.

Kauffman, J. W., Laughlin ,W. C. and Baldwin, R. A. (1986) Microbiological treatment of uranium mine waters. Environ. Sci. Technol. 20, 243-248.

Lalonde, J. (1997) Enzyme catalysis: cleaner, safer, energy efficient. Chem. Engineer. September 1997, 108-112

Lide, D. R. (1991) *Handbook of Chemistry and Physics*. 71st edition 1990-1991, CR Press, Boston.

Lojou, E. and Bianco, P. (1999) Electrocatalytic reduction of uranium by bacterial cytochromes: biochemical and chemical factors influencing the catalytic process. J. Electroanal. Chem. 471, 96-104.

Lojou, E., Bianco, P. and Bruschi, M. (1998) Kinetic studies of the electron transfer between bacterial c-type cytochromes and metal oxides. J. Electroanal. Chem.452, 167-177.

Lovley, D. R. (1993) Dissimilatory metal reduction. Annu. Rev. Microbiol. 47, 263-290.

Lovley, D. R. (1995) Microbial reduction of iron, manganese, and other metals. In: Donald L, Sparks L (Eds). Advances in Agronomy 54, Academic Press, New York, 175-231.

Lovley, D. R. and Phillips, E. J. P. (1992) Bioremediation of uranium contamination with enzymatic uranium reduction. Environ. Sci. Technol. 26, 2228-2234.

Lovley, D. R. and Phillips, E. J. P. (1994) Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. Appl. Environ. Microbiol. 60, 726-728.

Lovley D. R., Widman, P. K., Woodward, J. C., Phillips, E. J. P (1993) Reduction of uranium by cytochrome c_3 of *Desulfovibrio vulgaris*. Appl. Environ. Microbiol. 59, 3572-3576.

Ma, J. G., Zhang, J., Franco, R., Jia, S. L., Moura, I., Moura, J. J. G., Kroneck, M. H. and Shelnutt, J. A. (1998) The structural origin of nonplanar heme distortions in tetraheme ferricytochromes c_3. Biochem. 37, 12431-12442.

Macaskie, L. E., Yong, P., Doyle, T. C., Roig, M. G., Diaz, M. and Manzano, T. (1997) Bioremediation of uranium-bearing waste-water : Biochemical and chemical factors influencing bioprocess applications. Biotechnol. Bioengineer. 53, 100-109.

Moura, I., Teixeira, M., Huynh, B. H., Legall, J. and Moura, J. J. G. (1988) Assignment of individual heme electron-paramagnetic-res signals of *Desulfovibrio baculatus* (Strain 9974) tetraheme cytochrome-c_3 : a redox equilibria study. Eur. J. Biochem. 176, 365-369.

Niki, K., Yagi, T., Inokuchi, H. and Kimura, K. (1977) Electrode reaction of cytochrome c_3 of Desulfovibrio vulgaris, Miyazaki. J. Electrochem. Soc. 124, 1889-1892.

Saiz, B. L. and Barton, L. L. (1992) Transformation of Pb II to lead colloid by *Moraxella bovis*. Amer. Soc. Micro. Meet. Abst. 347.

Somers, W. A. C., Van hartingsveldt, W., Stigter, E. C. A. and Van der Lugt, J. P. (1997) Electrochemical regeneration of redox enzymes for continuous use in preparative processes. Trends Biotechnol. 15, 495-500.

Tischer, W. and Wedekind, F. (1999) Immobilized enzymes: methods and applications. Topics Current Chem. 200, 95-126.

Tomei, F. A., Barton, L. L., Lemanski, C. L., Zocco, T. G., Fink, N. H. and Sillerud, L. O. (1995) Transformation of selenate and selenite to elemental selenium by *Desulfovibrio desulfuricans*. J. Indus. Microbiol. 14, 329-336.

Tsapin, A. I., Nealson, K. H., Meyers, T., Cusanovich, M. A., Van Beuumen, J., Crosby, L. D., Feinberg, B. A. and Zhang, C. (1996) Purification and properties of a low-redox-potential tetraheme cytochrome c_3 from *Shewanella putrefaciens*. J. Bacteriol. 178, 6386-6388.

Tucker, M. D., Barton, L. L., Thomson, B. M. (1998) Reduction of Cr, Mo, Se and U by *Desulfovibrio desulfuricans* immobilized in polyacrylamide gels. J. Industr. Microbio.l Biotechnol. 20, 13-19.

Vecchio, A., Finoli, C., Disimine, D. and Andreoni, V. (1998) Heavy-metal biosorption by bacterial-cells. Fresenuis J. Anal. Chem. 361, 338-342.

Voordouw, G., Pollock, W. B. R., Bruschi, M., Guerlesquin, F., Rapp-Giles, B. J., Wall, J. D. (1990) Functional expression of *Desulfovibrio vulgaris* Hildenborough cytochrome c_3 in *Desulfovibrio desulfuricans* G200 after conjugational gene transfer from *Escherichia coli*. J.Bacteriol. 172, 6122-6126.

Wagman, D. D., Evans, W. H., Parker, V. B., Schumm, R. H., Halow, I., Bailey, S. M., Churney, K. L. and Buttall, R. L. (1982) The NBS tables of chemical thermodynamic properties. Selected values for inorganic and C1 and C2 organic substances in SI units. J. Phys. Chem. Ref. Data 11, suppl 2, 392.

Wolery, T. J. (1992) *EQ3NR, A computer program for geochemical aqueous speciation-solubility calculations*. Theoretical Manual, User's guide, and Related Documentation, version 7.0. UCRL-MA-110662-PT-IV, Lawrence Livermore National Laboratory, Livermore, CA.

Yildirim, O., Akbulut, U., Arinc, E. and Sungur, S. (1994) Stability and storage-conditions of NADH-cytochrome b_5 reductase cross-linked into gelatin by chromium(III) acetate. Biomaterials 15, 587-592.

Table 1. Phases precipitated by cytochrome c_3, solubility in water at 25°C and EPA drinking water standards.

Solid phase	Solubility (M)	U. S. EPA standard (M)*
Se^0 (red)	10^{-90} (Wagman et al., 1982)	$6.3 \cdot 10^{-7}$
Pb^0	10^{-12} (Ball & Nordstrom, 1998)	$2.4 \cdot 10^{-7}$
Cu^0	10^{-44} (Wolery, 1992)	$2 \cdot 10^{-5}$
UO_2	$1.9 \cdot 10^{-8}$ (Wolery, 1992)	$1.8 \cdot 10^{-7}$
MoO_2	10^{-57}(Lide, 1991)	10^{-6}
$Cr(OH)_3$	10^{-60} (Wolery, 1992)	$9.6 \cdot 10^{-7}$

*(Federal Register, 1995)

Figures captions

Figure 1. Oxidation of cytochrome *c*$_3$ (Cyt c$_3$-reduced) in a sodium dithionite solution after addition of a 30 mM-NaHCO$_3$ buffer solution containing 7 mg/L O$_2$.

Figure 2. Oxidation of cytochrome *c*$_3$ (Cyt c$_3$-reduced) in a sodium dithionite solution after addition of a 30 mM-NaHCO$_3$ buffer solution containing 7 mg/L O$_2$ and 10 mM NO$_3^-$.

Figure 3. Oxidation of cytochrome *c*$_3$ (Cyt c$_3$-reduced) in a sodium dithionite solution after addition of a 30 mM-NaHCO$_3$ buffer solution containing 7 mg/L O$_2$ and 10 mM SeO$_4^{2-}$.

Figure 4. (a) TEM image of Se^0 particles formed by reduction of SeO$_4^{2-}$ by cytochrome *c*$_3$. (b) EDS spectrum of Se^0 particles with S and Te impurities. The Cu lines are from the grid.

Figure 5. (a) TEM image of Pb^0 particles formed by reduction of Pb^{2+} by cytochrome *c*$_3$. (b) EDS spectrum of uraninite particles showing Pb. The Cu lines are from the grid.

Figure 6. (a) TEM image of Cu^0 particles formed by reduction of Cu^{2+} by cytochrome *c*$_3$. (b) EDS spectrum of uraninite particles showing Cu. Some of Cu is attributed to the grid.

Figure 7. (a) TEM image of uraninite particles formed by reduction of UO$_2^{2+}$ by cytochrome *c*$_3$. (b) EDS spectrum of uraninite particles showing U and O. The Cu lines are from the grid.

Figure 8. (a) TEM image of amorphous Mo-rich particles formed by reduction of MoO$_4^{2-}$ by cytochrome *c*$_3$. (b) EDS spectrum of Mo-rich particles showing Mo and O. The Cu lines are from the grid.

Figure 9. (a) TEM image of amorphous Cr-rich particles formed by reduction of CrO$_4^{2-}$ by cytochrome *c*$_3$. (b) EDS spectrum of Cr-rich particles showing Cr and O. The Cu lines are from the grid.

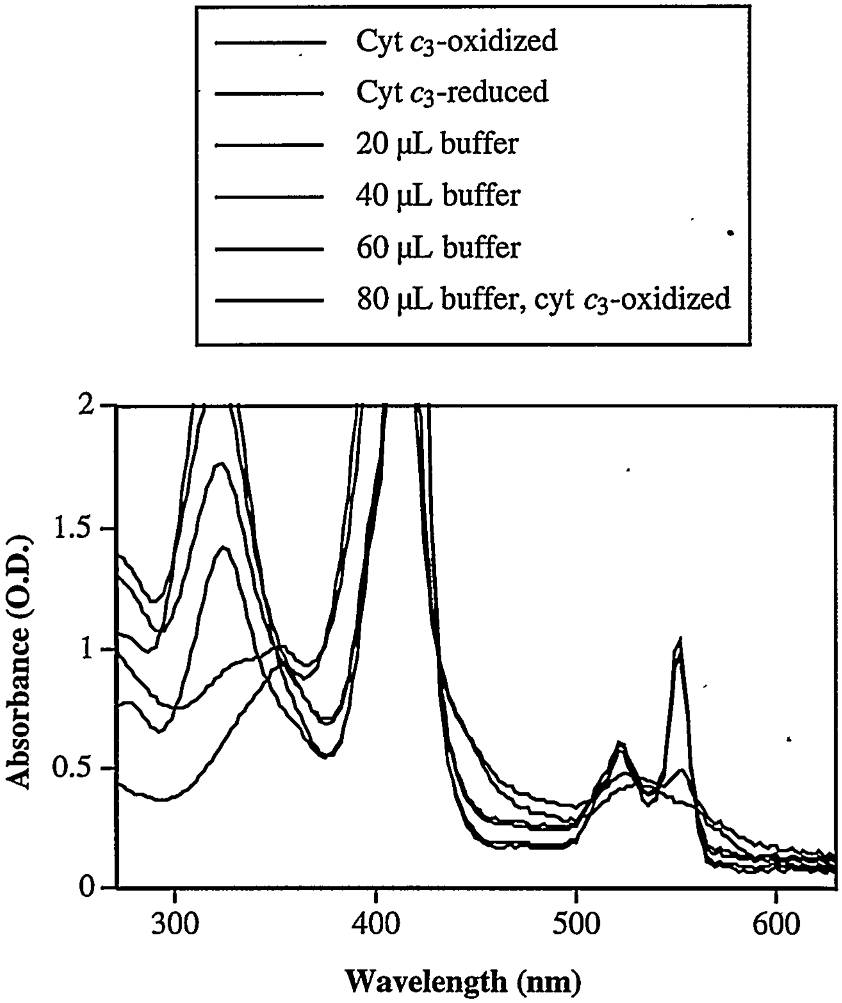

Cyt c_3-oxidized
Cyt c_3-reduced
20 µL buffer
40 µL buffer
60 µL buffer
80 µL buffer, cyt c_3-oxidized
Absorbance (O.D.)
0
0.5
1
1.5
2
300
400
500
600
Wavelength (nm)

Fig. 1

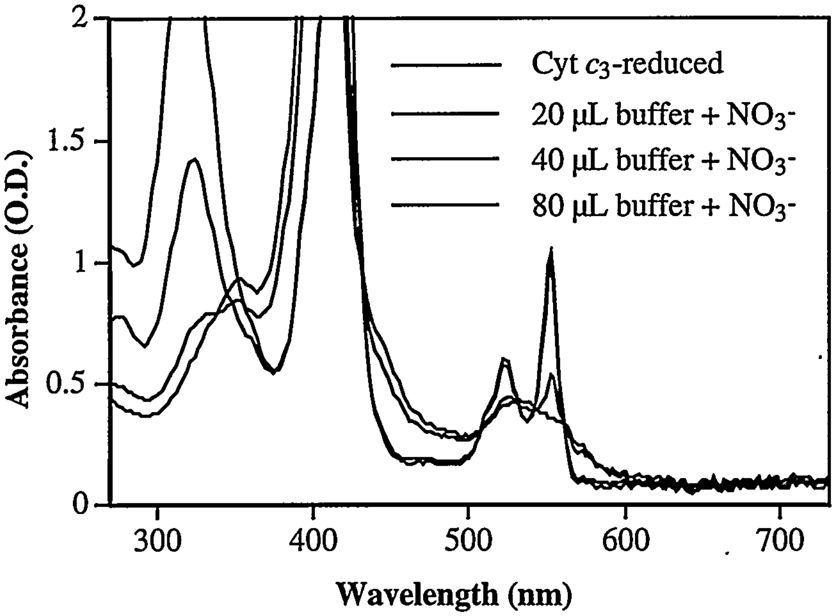

2
1.5
1
0.5
0
Absorbance (O.D.)
300
400
500
600
700
Wavelength (nm)
Cyt c_3-reduced
20 μL buffer + NO_3^-
40 μL buffer + NO_3^-
80 μL buffer + NO_3^-

Fig. 2

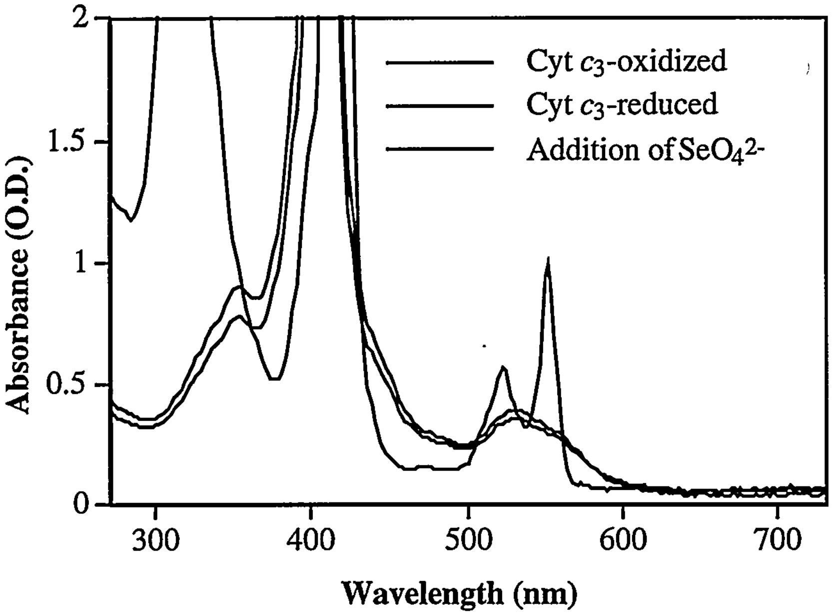

2
1.5
1
0.5
0
Absorbance (O.D.)
300
400
500
600
700
Wavelength (nm)
Cyt c_3-oxidized
Cyt c_3-reduced
Addition of SeO_4^{2-}

Fig. 3

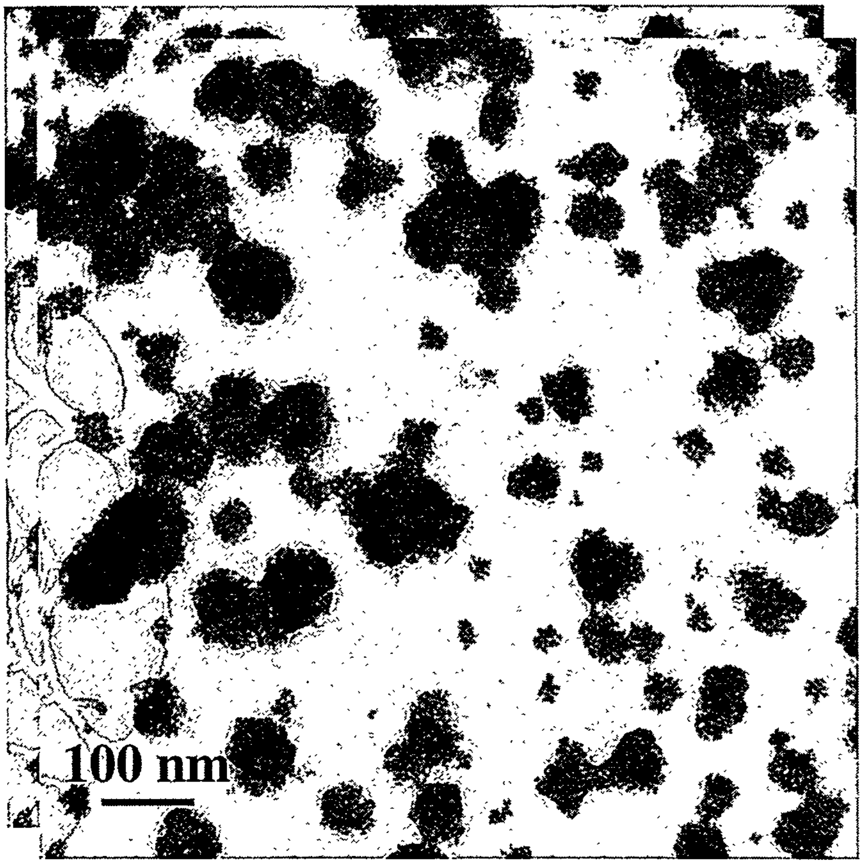
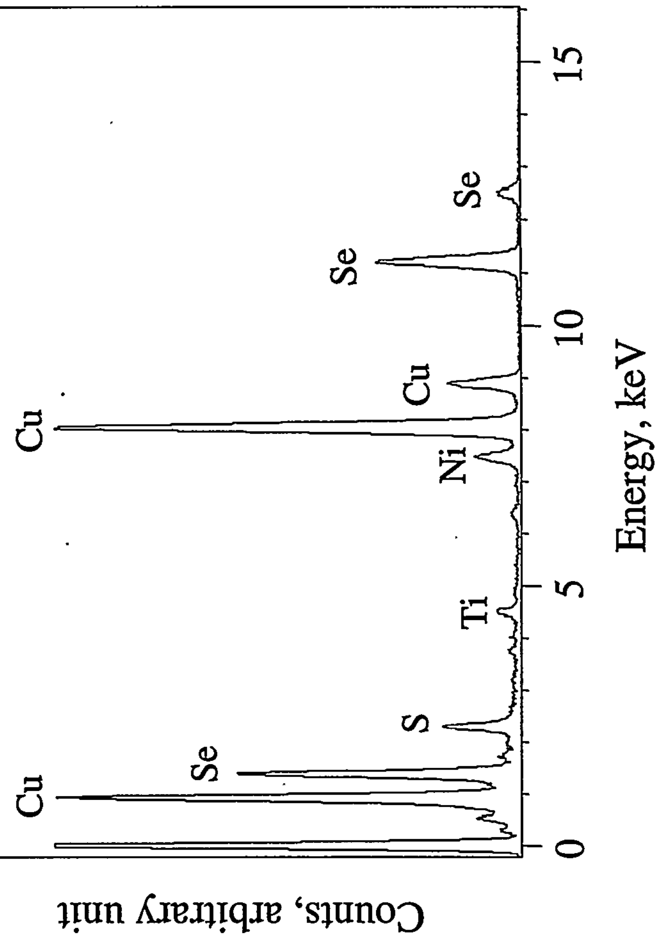
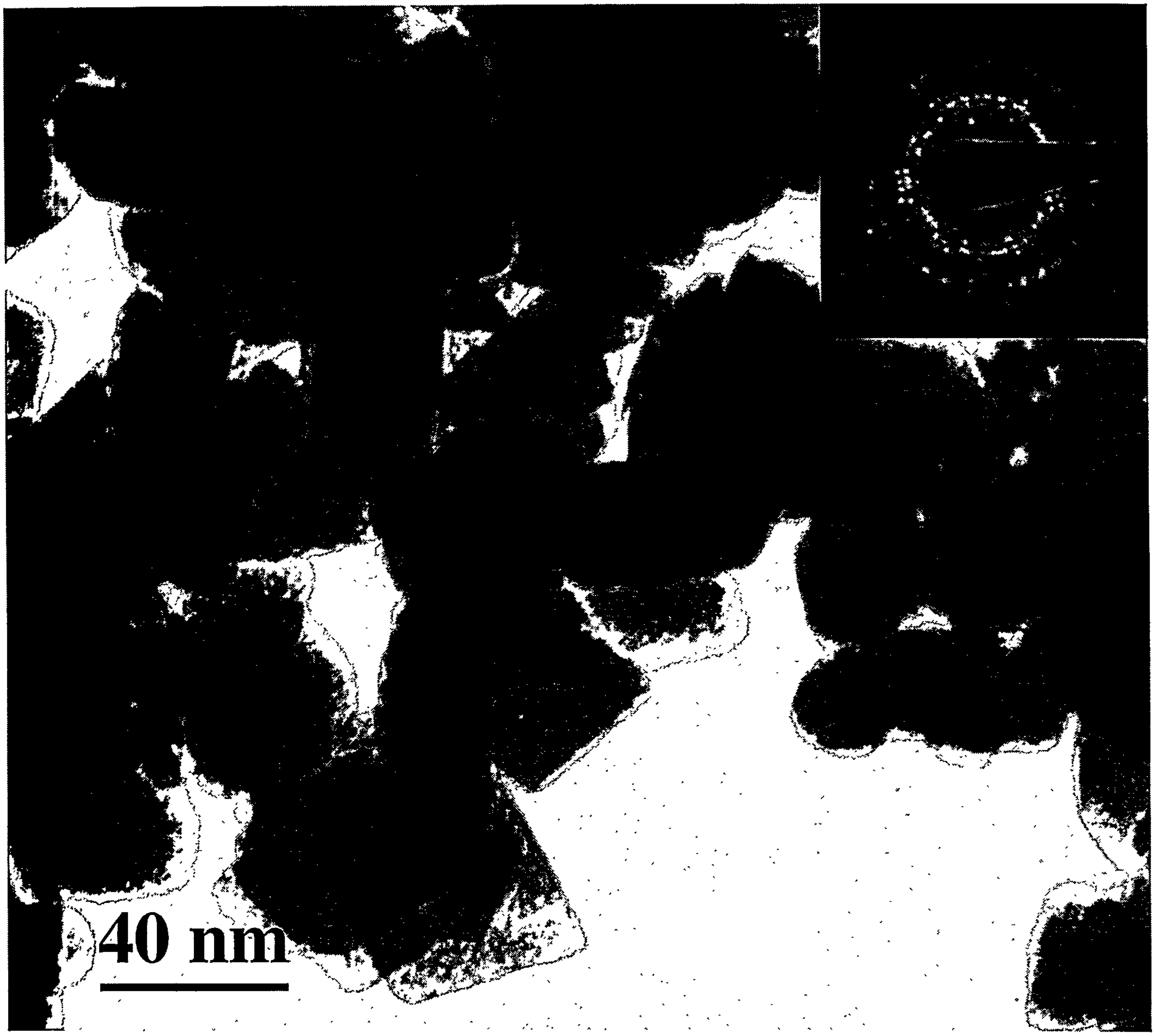


Fig 4(a)

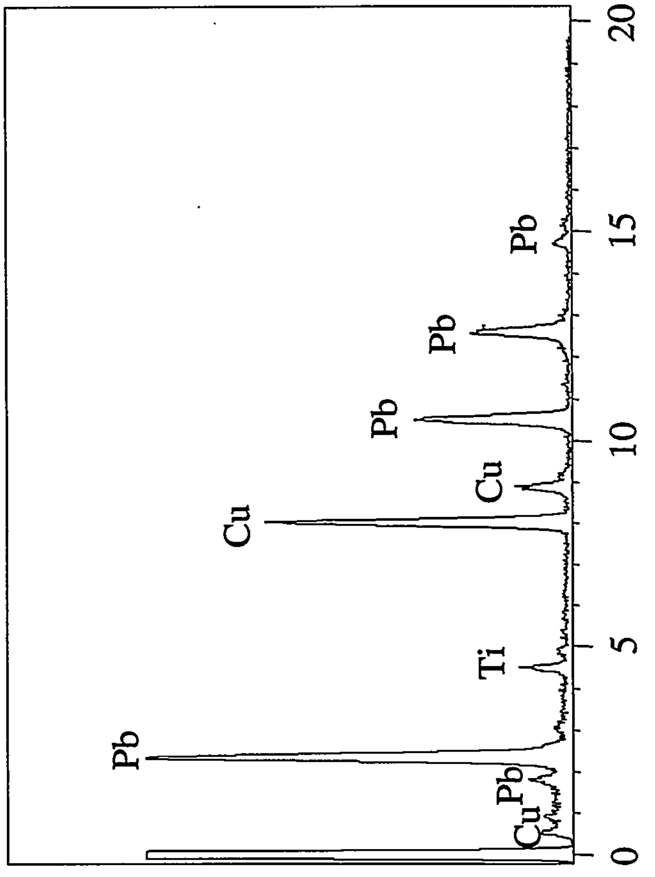

Counts, arbitrary unit
Energy, keV
0
5
10
15
Cu
Se
S
Ti
Ni
Cu
Cu
Se
Se

4(b)



Metallic Lead nanoparticles

Fig 5(a)



5(b)

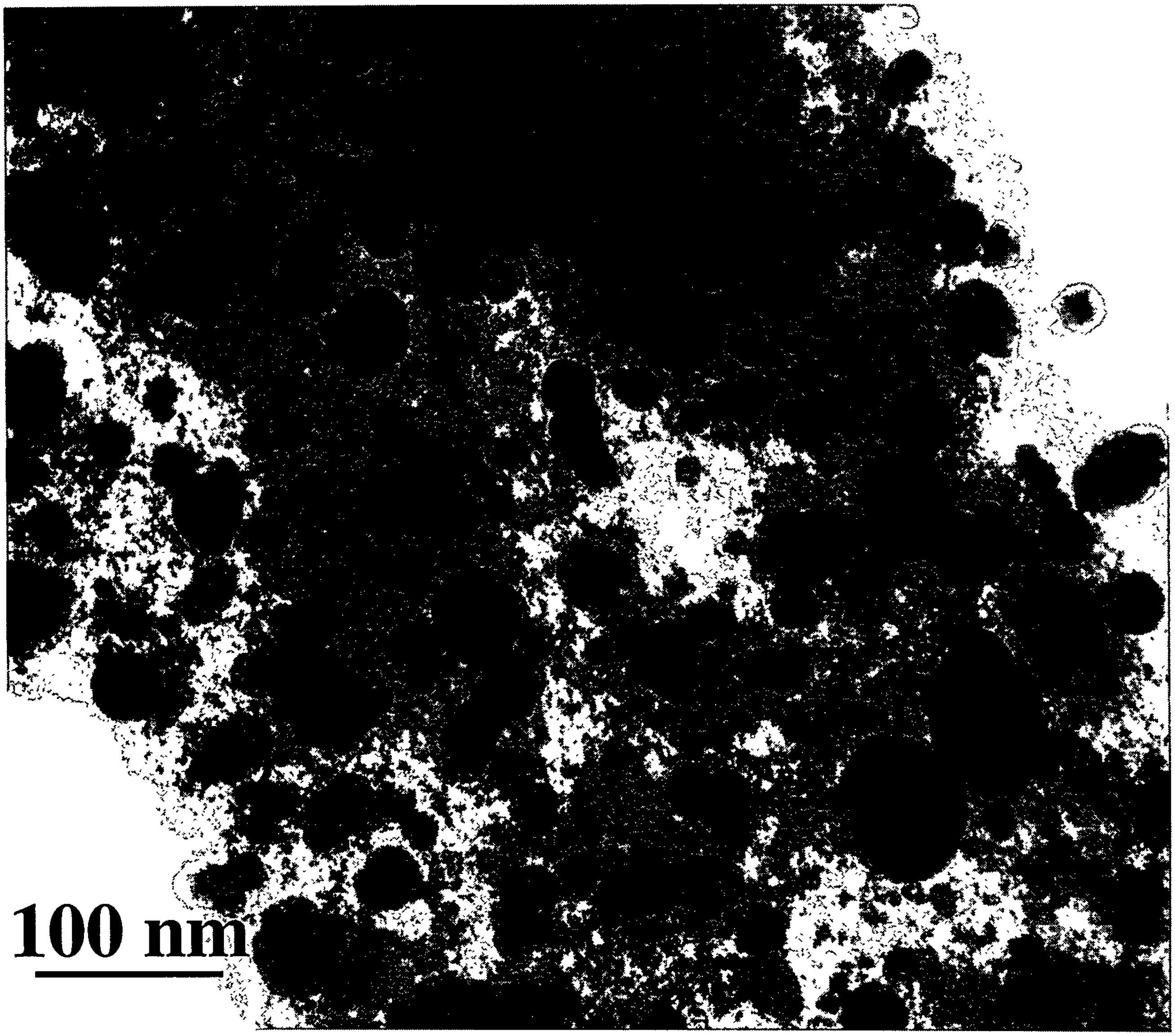
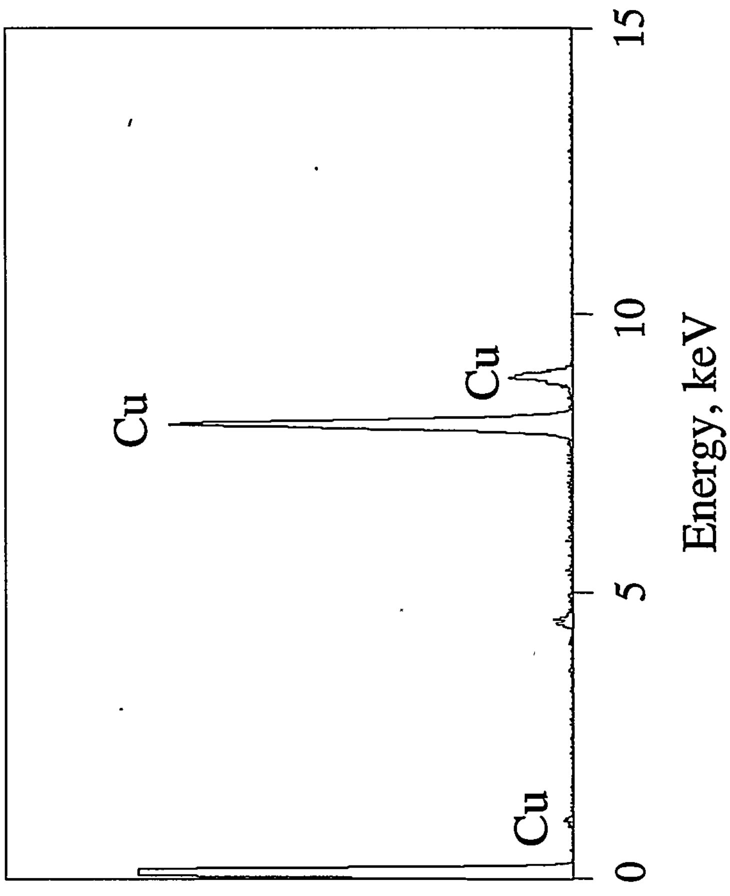


Fig 6(a)



6(b)

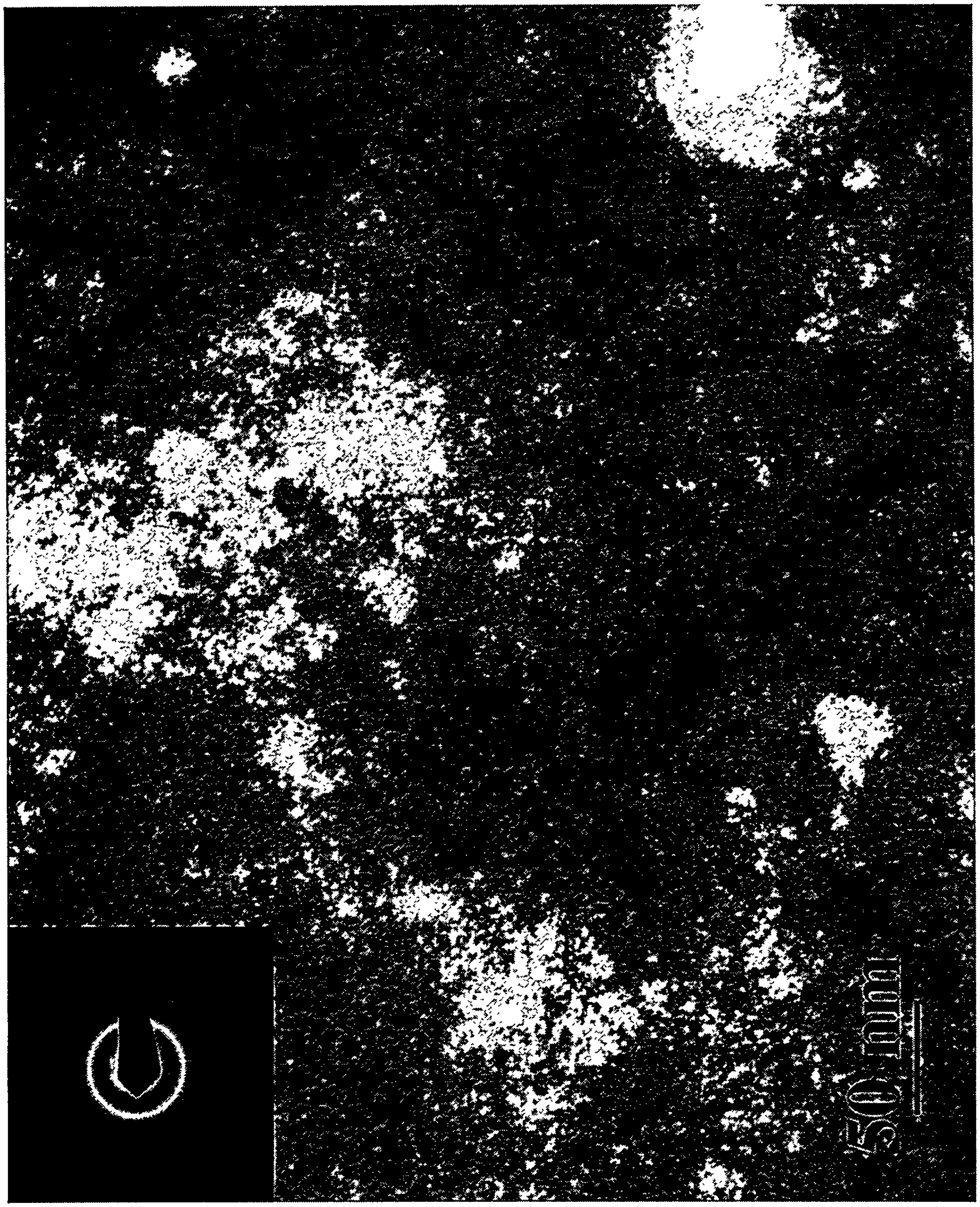
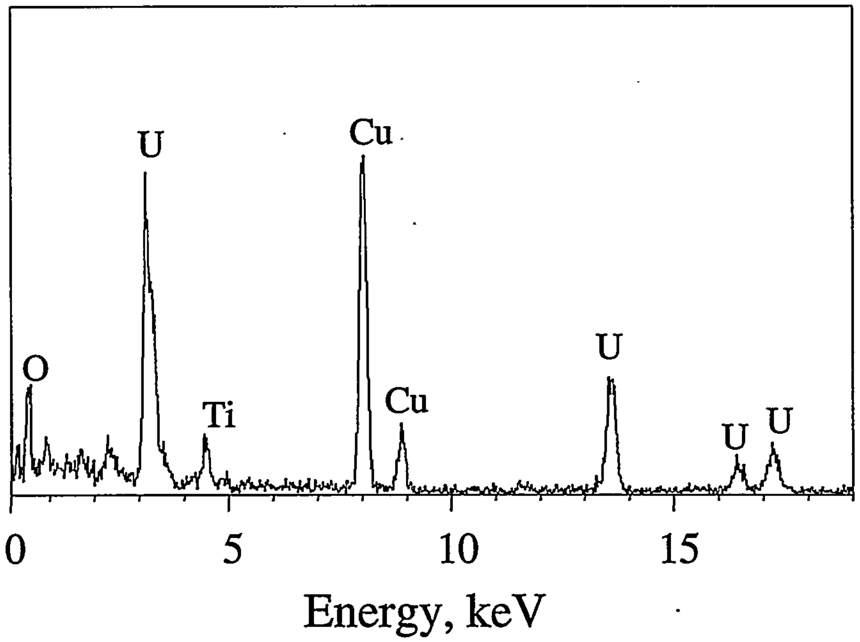


Fig. 7(a)



7(b)

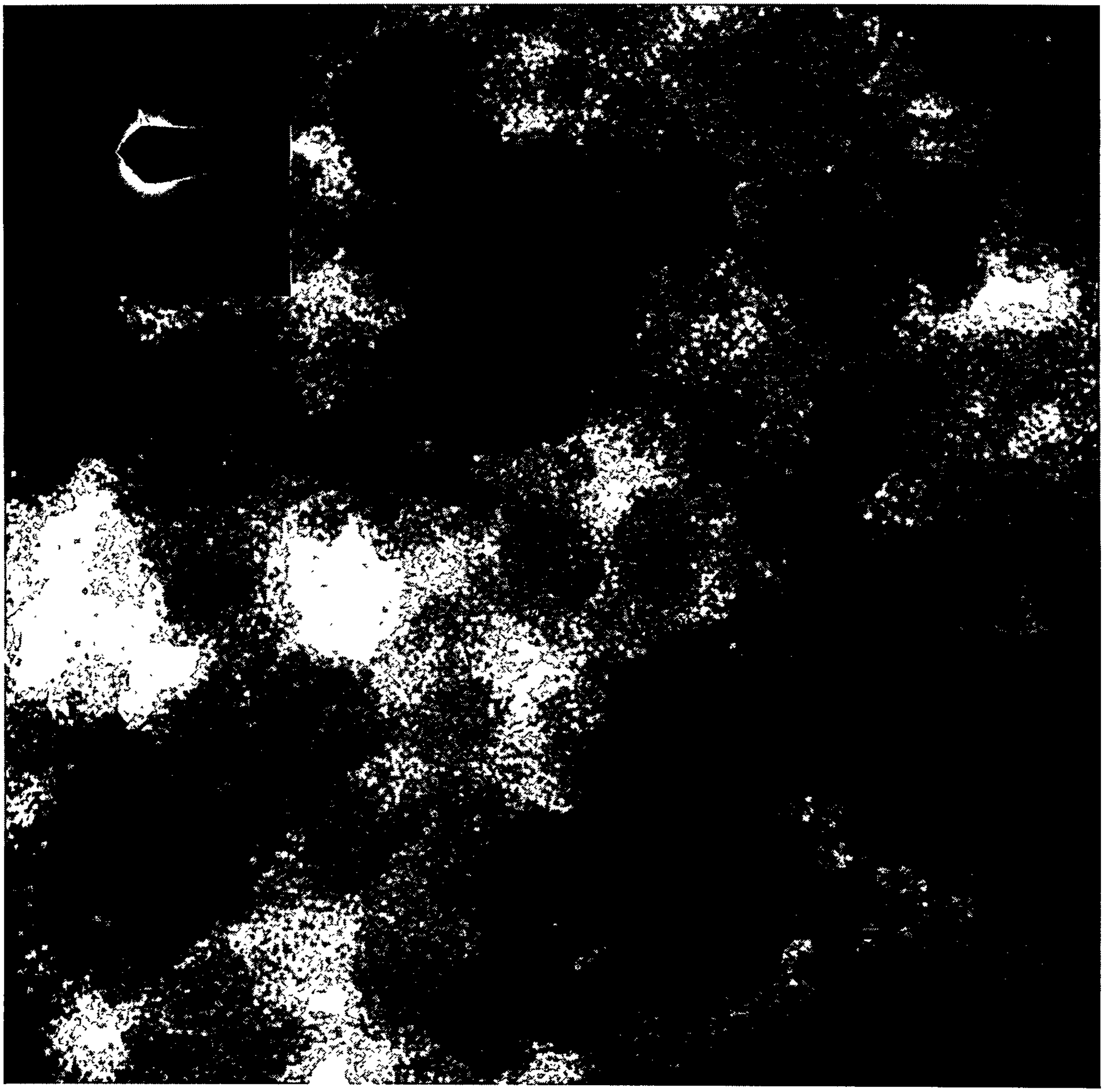
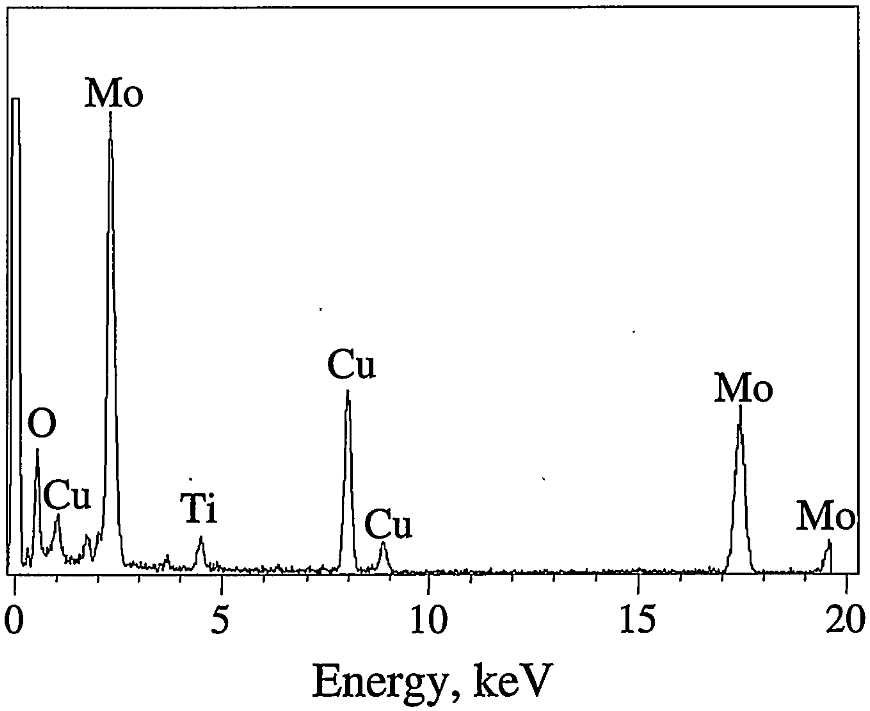


Fig 8(a)



8(b)

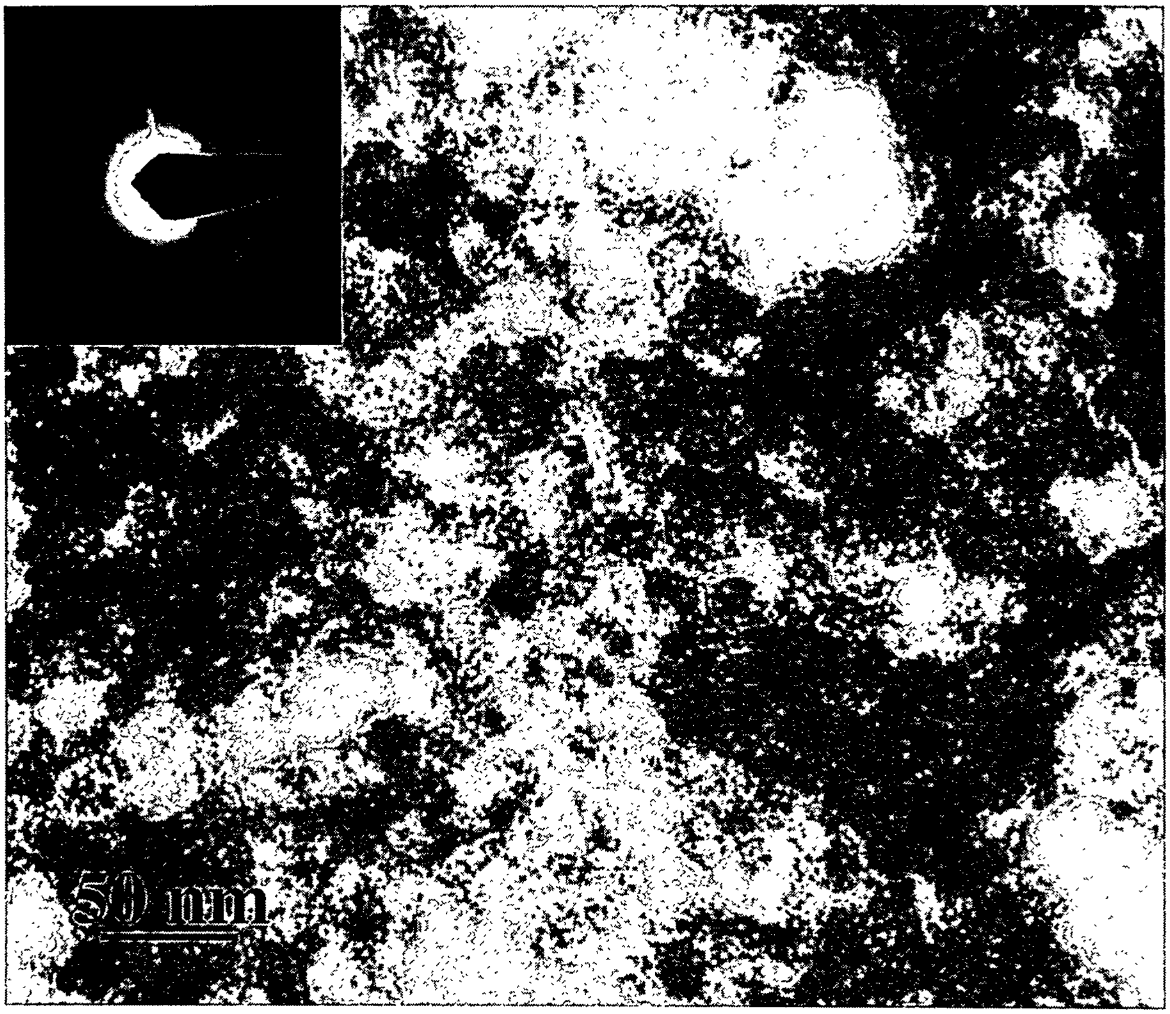
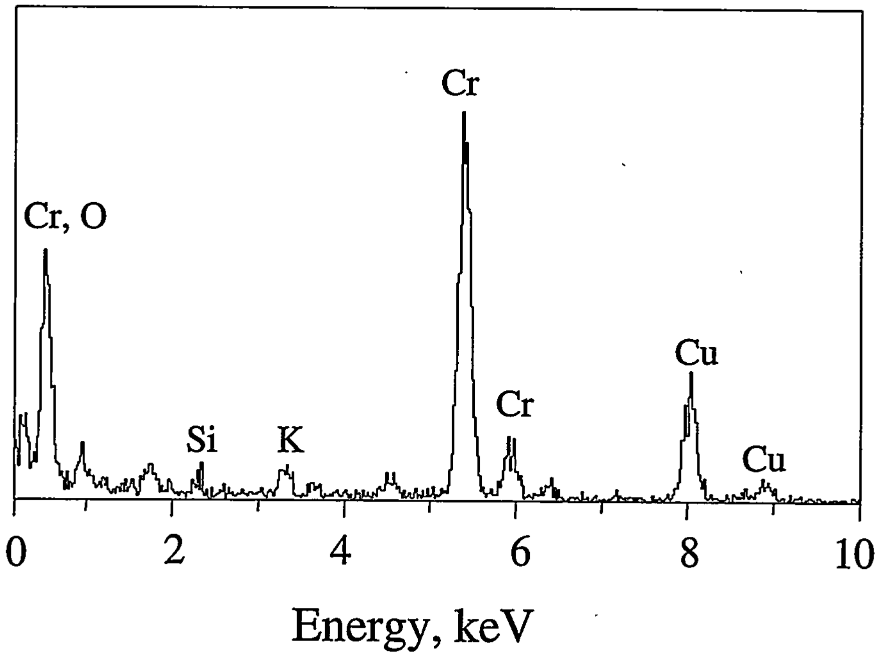


Fig 9 (a)



9(b)