revealed that 90% of the erythrocyte copper is bound in superoxide dismutase. Only 10% of the copper is found in the high molecular fraction following gel filtration of the haemolysate. All other copper proteins migrate in the region near $M_r = 32\,000$.

The yield of both superoxide dismutase and Cu_2–$(haem_b)_2$–protein clearly shows that the copper of the red blood cell is distributed in these proteins. Taking into account minimal losses of intracellular copper in the course of the fractionation steps no copper proteins other than the preceding ones were detectable. It appears that Cu_2Zn_2 superoxide dismutase or cuprein is the major intracellular copper protein.

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S15

Attempts to Detect and Locate Platinum Metals in Plant Tissues by a Variety of Microscopic Techniques

MARGARET E. FARAGO and PATRICK J. PARSONS

Department of Chemistry, Royal Holloway and Bedford Colleges, Egham Hill, Surrey TW20 0EX, U.K.

Root and leaf samples of *Eichhornia crassipes* (water hyacinth) containing the platinum metals were examined by a number of techniques.

(1) *Light microscopy*. This classical technique is of little use, except where the concentrations are unusually high. However, morphological changes caused by toxic elements can be studied. The palisade mesophyll of the leaf of a Pt-treated plant was found to be distorted in contrast to that in control or Rh (non-toxic) treated plants.

(2) *Scanning electron microscopy with energy dispersive X-ray analysis* (SEM + EDXA). Control root and leaf specimens showed high concentration of Ca and smaller amounts of P and S. Roots treated with $[PtCl_6]^{2-}$ were covered in electron dense deposits. EDXA showed Ca (K_α, 3.96 keV) and Pt (M_α, 2.05 keV; L_α, 9.44 keV; L_β, 11.07 keV). X-ray photo-electron spectra of these deposits showed a corrected Pt 4f binding energy of 76 ± 0.5 eV, indicating Pt^{4+} in the deposits.

(3) *Electron microscope analysis* (EMPA). Examination of Pt-treated roots showed clearly that platinum accumulated in the epidermal region, lesser amounts are in the cortex. Ruthenium however, was distributed more evenly.

(4) *Scanning transmission electron microscopy with energy dispersive X-ray analysis* (STEM + EDXA). Problems arise with Pt-treated root materials, which show widespread electron dense deposits. In order to avoid interference with Pt, sections were viewed unstained, hence cytological detail is absent. EDXA confirmed the presence of Pt.

S16

The Uptake and Accumulation of Platinum Metals by the Water Hyacinth (*Eichhornia crassipes*)

MARGARET E. FARAGO and PATRICK J. PARSONS

Department of Chemistry, Royal Holloway and Bedford Colleges, Egham Hill, Surrey TW20 0EX, U.K.

The biological effects of the platinum group metals on plants have been studied by treating plants with chlorocomplexes of each metal at a range of concentrations. The vascular aquatic plant *Eichhornia crassipes* (water hyacinth) was selected for detailed study because of its remarkable ability to assimilate high levels of transition elements from solution. Water hyacinths are capable of recovering platinum group metals even from dilute solution though to varying degrees depending on the metal:

$$Pt^{2+} > Pd^{2+} > Os^{4+} \approx Ru^{3+} > Ir^{3+} \approx Rh^{3+}$$

decreasing % recoverey at the 0.05 ppm level

When this is compared to the relative order of toxicity at the 10 ppm level for each metal, some similarities emerge:

$$Pt^{2+}(5d^8) \approx Pd^{2+}(4d^8) > Os^{4+}(5d^4) \approx Ru^{3+}(4d^5) > > Ir^{3+}(5d^6) > Pt^{4+}(5d^6) \gg Rh^{3+}(4d^6)$$

decreasing order of toxicity at the 10.0 ppm level

The relationship between phytotoxicity and position in the periodic table is tenuous but appears to be linked with the oxidation state and hence electron configuration of the metal ion. The two least toxic ions, Ir^{3+} and Rh^{3+}, have the electron configuration (d^6); it is significant too that $Pt^{4+}(d^6)$ is far less toxic than $Pt^{2+}(d^8)$. A similar relationship has been found for the phytotoxicity of 1st row elements:

$$Cd^{2+}(4d^{10}) \gg Zn^{2+}(3d^{10}) > Ni^{2+}(3d^8) > Co^{2+}(3d^7)$$

decreasing relative toxicity, decreasing softness

Included in the soft acid classification is Pt^{2+} and Pd^{2+} whilst Rh^{3+} and Ir^{3+} are considered borderline between hard and soft, along with Fe^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}. This approach goes some way to explaining

the relative toxicity of the platinum metals. The anomaly is Pt^{4+}, which is classified as soft, but which is relatively non-toxic; however, some softness is lost when Pt^{2+} is oxidized to Pt^{4+}.

The most prominent toxic symptom at low levels was the appearance of reddish-brown streaks in the leaves of *Eichhornia crassipes*. Such phytotoxic symptoms have been observed in beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*) treated with high quantities of zinc. Cd^{2+}, Co^{2+} and Ni^{2+} are reported to cause similar symptoms. In contrast to the toxic effects of Pt^{2+}, Rh^{3+} appears to exhibit a tonic effect. When treated with 10 ppm Rh^{3+} applied as $Na_3[RhCl_6]$, water hyacinth increased its biomass some 6.7% more than control plants, grown under the same conditions. When the South African grass *Setaria verticillata* was treated with 0.5 ppm Pt^{2+} (as $K_2[PtCl_4]$), vascular discolouration was absent and the roots were growth stimulated some 65% more than controls. Thus the phytotoxic symptoms of platinum vary according to which species is treated, though with water hyacinth, some stimulation of vegetative reproduction was apparent with platinum complexes, at low levels.

When applied as the antitumour complex *cis*-$[Pt(NH_3)_2Cl_2]$ at low levels, some 47.9% of the platinum found in the leaves of water hyacinth was associated with α-cellulose and lignin; 16.1% was removed by the proteolytic enzyme pronase and 20.8% found with water soluble pectates. A similar distribution of platinum was found in the floats of water hyacinth. In the roots of treated plants, the values were 35%, 9.5% and 14.2% respectively; in addition to this, a further 23.1% was removed with low molecular weight alcohol soluble materials and 12.0% with polar water soluble materials. Thus in water hyacinth, the cell wall acts as a ion exchange column trapping most of the platinum, though some is found bound to water soluble pectates. Together, this accounts for 49.2% of the platinum found in the roots and this figure rises to 68.7% in the leaves. The platinum released by pronase may represent that which is bound to protein from a number of sources including organelle protein, membrane protein and cell wall glycoprotein.

S17

Energetics of the Adsorption of Cu(II) on Activated Sludge

CORRADO SARZANINI

Istituto di Chimica Fisica, C.so M. d'Azeglio 48, Turin, Italy

and LUIGI STRADELLA

Istituto di Chimica Generale Inorganica, Via P. Giuria 9, Turin, Italy

Dried activated sludge (DS) and thermally modified samples (MDS) [1] were used as adsorbents of Cu(II) from water at zero and 0.1 ionic strength. Three mechanisms are essentially implied at the solid-liquid interface:

(i) ion exchange of free metals and hydroxo-complexes with surface protons,

(ii) adsorption and hydrolysis at the surface,

(iii) hydrolysis followed by an adsorption reaction.

The use of activated sludge as adsorbent is also justified by its bacterial nature [2].

Experimental

Two kinds of adsorbent were prepared: the samples obtained from a pilot plant were thickened and dried at 100 °C in air, powdered and sieved in the 42–200 mesh range (DS), and heated at 110 °C in air for 24 h (MDS). The BET surface evaluation resulted in 2 ± 0.1 m²/g for DS and 0.5 ± 0.1 m²/g for MDS; this may be interpreted assuming that grain agglomeration takes place.

The calorimetric experiments were carried out using a SETARAM rotating C.R.M.T. calorimeter [3], with a cell we constructed because of the impossibility of any handling from outside after introduction of the sample. The method has already been described in detail [4].

Results and Discussion

In Fig. 1 we have reported the enthalpies of displacement, $-H_{12}$ (J/g), as a function of the specific quantities of Cu(II) adsorbed, n_{2s} (mg/g).

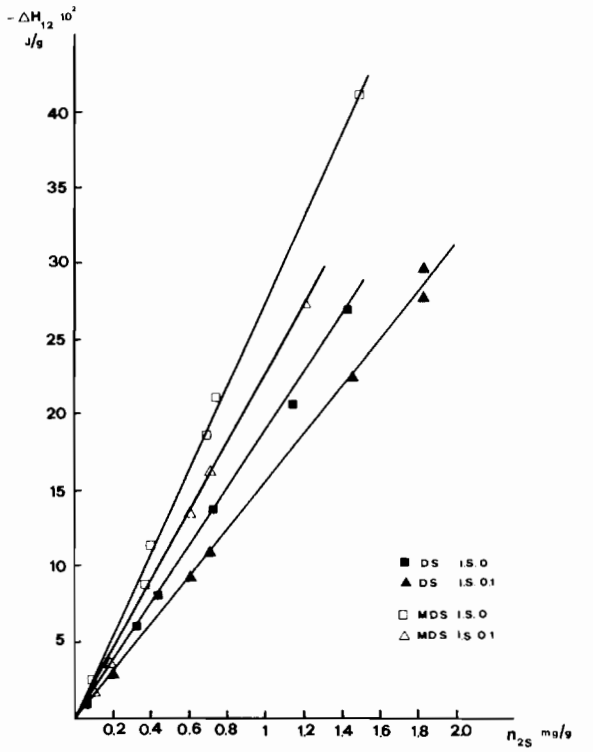

Fig. 1. Enthalpies of displacement *vs.* Cu(II) adsorbed.