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The *In Vitro* Adsorption study of Isoniazide and Pyrazinamide on Indigenous Bentonite

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In the research series on the interactions of some drugs with bentonites, having a special interest for the pharmaceutical science we studied the adsorbing capacity of revived and HCl activated bentonites from Valea Chioarului. The adsorbed drugs were isoniazide and pyrazinamide. The adsorbing capacity was expressed in the amount of drug adsorbed per unit mass of adsorbent.

The practical application of the experimental data was made using the liquid phase adsorption isotherm of Freundlich and of Langmuir.

The characteristic parameters of the bentonite–drug system were obtained processing the experimental data by regression analysis. The adsorption coefficient (a) and the adsorption capacity at the equilibrium concentration equivalent to unity (b) were determined. The spreading of the data show obedience to the Langmuir pattern.

The derivatographic study of the bentonite drug system allowed the calculation of the activation energie (*E*) and of the reaction order (n) characteristic parameters of the dehydration kinetics. For this purpose the differential method of Freeman and Carroll was used.

The electronic spectra of bentonite–drug system in KBr pellets, shows hypsochromic shifts of the 265 nm band characteristic for isoniazide and the 269 nm band characteristic for pyrazinamide.

The IR spectra for bentonite–drug system show significant changes in the 1700–1500 cm^{-1} range characteristic for $\nu_{C=O}$ and δ_{H_2O} modes.

TABLE

System		Freundlich Method	Langmuir Method	n	*E* (kcal/mol)
Bentonite–	a	0.69	0.298		
Isoniazide	b	0.0075	0.0019	0.7	10.67
	s^2	4.84 × 10^{-5}	0.87 × 10^{-5}		
Bentonite–	a	0.13	0.87		
Pyrazinamide	b	2.99	3.01	0.7	6.54
	s^2	0.041	5.39 × 10^{-5}		

The Nature of the Type 2 Copper Binding in Blue Oxidases

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Of the three different forms of copper usually contained in the blue oxidases, the Type 2 Cu is a divalent paramagnetic form characterized by a 'normal' EPR spectrum, similar to that of tetragonal copper complexes, with $A_{\parallel}$ of 150–200 gauss [1]. The Type 2 Cu site is open to solvent access and binds dissolved monovalent anions, such as azide, cyanide and fluoride [1]. These anions have an inhibitory effect on the oxidase activity much stronger than their affinity for the resting enzyme would predict. For tree laccase the affinity of the Type 2 Cu for azide increases on reduction of the Type 3 Cu [2], a likely event during turnover. All oxidases show in the presence of azide an intense absorption band at 400 nm and a shoulder at about 500 nm. The latter is never observed in mononuclear Cu(II)–azide complexes and is assigned to a charge-transfer from the bridging azide to the Cu(II) in the Cu(II)–N_3–Cu(I) group of azide-bound met-hemocyanine [3]. This suggests that a similar type of binding might be present in the oxidases. The EPR spectra of the Type 2 Cu(II)–N_3 and –CN adducts of tree laccase, measured under reducing conditions, are very similar to those of the corresponding complexes of the Cu(II) substituted carbonic anhydrase, where the Cu(II) is believed to be pentacoordinated in a distorted square pyramidal geometry [4]. They are characterized by low $A_{\parallel}$ in the range 106–137 gauss. The metal ion is bound to three hystidyl residues and one water molecule, besides the anion, in the Cu(II) carbonic anhydrase [4], to at least one water [1] and one hystidyl residue [5] in the tree laccase.

Selective removal of the Type 2 Cu abolishes the oxidase activity. In the tree laccase both the rate of reduction and of reoxidation of the reduced enzyme are affected [6]. Removal of the Type 2 Cu has no effect on the spectroscopic properties of the Type 1 Cu, below pH 7.0, but decreases the enzyme absorbance at 330 nm, usually assigned to the Type 3 Cu, and in the 650–800 nm region. The latter decrease amounts to 250–300 M^{-1} cm^{-1}, a rather high value for a 'normal' type of Cu(II) [6]. Similar results were obtained on removal of the Type 2 Cu from ascorbic acid oxidase [7]. The redox potential of the remaining copper ions and the stability of the tree laccase were also affected by removal of the Type 2 Cu.

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Correlation of K-Absorption Edge of Cobalt Derivatives of Some Metalloproteins

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The recent availability of a stable and intense X-ray source using synchroton radiation from high energy electron storage rings has allowed meaningful measurements to be made on dilute systems.

In this report we present measurements, carried out at the ADONE Synchroton (Frascati), of the K-edge absorption spectrum of cobalt derivatives of some metalloproteins.

A K-edge absorption spectrum of a transition metal ion is usually characterized by a first weak absorption assigned to the 1s→3d transition, a second absorption present either as a shoulder or a peak about 9–10 eV higher in energy assigned to the 1s→4s transition, and an intense absorption still higher in energy assigned to the 1s→4p transition.

The K-edge of the cobalt derivative of superoxide dismutase where the cobalt is substituted for the native zinc shows all of these three features. The striking property of this spectrum is the quite intensity of the first peak due to the 1s→3d transition. It has been already shown by Shulman *et al.* [1] that departure from configurations that have inversion symmetry such as octahedral or square planar toward tetrahedral symmetry produces such an effect. Therefore these measurements confirm the previous suggestion obtained by EPR and optical data [2], that the cobalt substituted for the native zinc retains the distorted tetrahedral structure.

A correlation between the K-edge spectrum of this derivative, of that where the cobalt is substituted for the copper in the native superoxide dismutase [3] and of several cobalt derivatives of metalloproteins of known structure such as stellacyanin and carbonic anhydrase will be discussed.

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The Binding of Copper Ions to Copper-Free Bovine Superoxide Dismutase. Applied Aspects

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The catalytic activity, $k = 3 \times 10^9\ M^{-1}\ sec^{-1}$ [1], close to the diffusion limit and the very high relaxivity of $^{19}F^-$ nucleus, $R_F \sim 10^7\ M^{-1}\ sec^{-1}$ [2], due to the copper in the native site of Cu–Zn superoxide dismutase, make possible the measurements of very low concentrations of these enzymes.

In connection with the high sensitivity in the enzyme detection the reconstitution of the native site from Cu^{++} and the copper-free enzyme besides, to offer a good insight into the interaction copper–protein [3–5] presents some interesting applied aspects.