

Synthesis and characterization of zeolite encaged enzyme-mimetic copper histidine complexes

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Histidine was complexed with copper cations and immobilized in zeolite Y by an ion exchange procedure. The influence of the initial copper concentration in the ion exchange solution and the ion exchange time on the structure of the final zeolite encaged copper complexes was studied. Two different copper complexes were found on the zeolite: a mono-histidine complex (**A**) and a bis-histidine complex (**B**). The complex ratio **A/B** decreases with increasing copper loading in the ion exchange solution. The dynamics of the exchange was also studied. The **A/B** ratio does not change during this process. The exchange process itself is very fast, as it takes place within an hour. After that the it is slowed down by orders of magnitude.

1. INTRODUCTION

Histidine, a naturally occurring amino acid, was complexed with copper cations and incorporated inside the supercages of zeolite Y [1,2]. The obtained complexes mimic the active center of natural enzymatic counterparts, such as *galactose oxidase*. The pore geometry of the zeolite induces shape selectivity in reactions and allow for *intra*-particle transport of reactants and products. The zeolite host material also induces additional stability of the incorporated active center, thereby expanding the range(s) of operating temperatures and pressures as well as solvents. The zeolite encaged copper histidine (CuHis) complex has already been shown to exhibit promising catalytic activity for the epoxidation of alkenes with peroxides [3].

ESEEM experiments on the zeolite occluded CuHis systems showed the presence of (at least) two different CuHis complexes (**A** and **B**) on the zeolite [4,5]. Complex **A** exhibits ²⁷Al modulations, which indicates that the Cu(II) coordinates to zeolite framework

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oxygen. Complex **B**, however, shows no ^{27}Al modulation. Complex **A** is proposed to be a mono-histidine complex where both the amino and imino nitrogens of the histidine are coordinated to Cu^{2+} , whereas the other equatorial ligands are provided by a zeolite oxygen and a water molecule. The complex is stabilized by the presence of protons in the zeolite framework. Complex **B** is proposed to be a bis-histidine complex, situated in the center of the supercage. The two histidine molecules coordinate to Cu^{2+} in a square planar geometry: the amino and imino nitrogens of one histidine molecule and the imino nitrogen and carboxylate oxygen of the second histidine molecule.

A number of parameters might affect the A/B ratio. These parameters are:

1. The pH of the exchange solution;
2. The copper concentration in the ion exchange solution;
3. Duration of the ion exchange.

The effect of the first parameter has been discussed in a previous paper [4]. In this paper, we investigate the effect of the other two parameters. Once it is possible to set the A/B complex ratio by tuning the synthesis conditions during the ion exchange procedure, the next step is to relate this ratio to catalytic yield and/or selectivity of the resulting heterogeneous Cu(II)-catalyst.

2. EXPERIMENTAL

2.1. Preparation

Aqueous solutions of CuHis complexes, prepared in bi distilled water with a His:Cu(II) ratio of 5:1 at pH 7.3 were used for ion exchange with NaY (ZEOCAT, Si:Al = 2.71). A series of zeolite samples, differing in their amount of CuHis complexes, were prepared using solutions with different copper concentrations (0.1, 0.25, 0.50, 1.0, 1.5 and 4.5 copper/unit cell (Cu/UC)), while keeping the His:Cu(II) ratio in the solution at 5:1 and the pH at 7.3. The pH was adjusted with 0.1 M NaOH and/or 0.1 M HCl solutions. All samples were stirred for 24 hours at room temperature. The pH of the exchange solution was measured regularly and adjusted if needed. All samples were dried at 333 K after washing and filtration.

The duration of ion exchange affects the copper concentration of the zeolite: longer exchange times lead to higher copper concentration. The exchange dynamics was studied by taking a series of zeolite samples from the ion exchange solution at different points of time.

2.2. Characterization

CW-EPR X-band measurements were performed on a Bruker ESP 300E Spectrometer at a temperature of 120 K. The CuHis complexes are paramagnetic due to the $S=1/2$ spin of the Cu^{2+} ion. Nitrogen physisorption was performed with a Micromeritics ASAP 2400 apparatus. Measurements were done at 77 K. Prior to the measurements the zeolite samples were degassed for 24 hours at 373 K in vacuum. Micropore volumes and pore size distributions were determined with standard BET and BJH theory. Diffuse Reflectance Spectroscopy of the CuHis complex encapsulated zeolite samples were taken on a Varian Cary 5 UV-Vis-NIR spectrophotometer at room temperature. The DRS spectra were recorded against a halon white reflectance standard in

the range 2500-200 nm. Atomic Absorption Spectrometry (AAS) measurements for quantitative analysis of Cu^{2+} in the zeolite samples were performed using an Instrumentation Laboratory Inc. apparatus with a nitrous oxide-acetylene flame. Measurements were done at a wavelength of 324.7 nm using a hollow cathode lamp. The amount of Cu^{2+} was determined after dissolution of known quantities of ion-exchanged zeolite materials in $\text{HF}/\text{H}_2\text{SO}_4$.

3. RESULTS AND DISCUSSION

The X-band CW-EPR spectra, recorded at 120 K, are shown in Fig. 1. The EPR intensity is proportional to the number of copper ions taken up by the zeolite during the ion exchange. Each of the spectra consists of two distinct EPR subspectra, which can be attributed to two different complexes, viz. complex **A** and complex **B**.

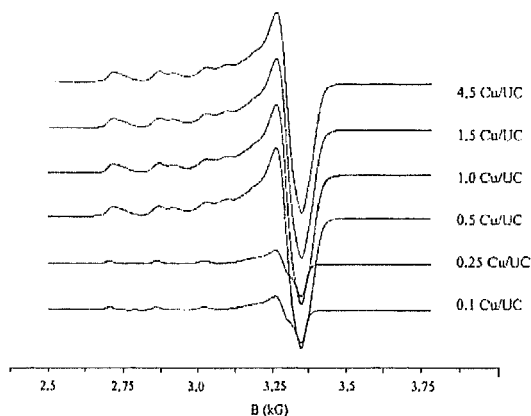


Fig. 1. CW-EPR spectra as a function of the external copper concentration in the ion exchange solution.

A change in the relative amounts of these two subspectra becomes visible upon going to higher copper concentrations in the ion exchange solution. At low copper concentrations only the subspectrum of complex **A** is visible, but at higher copper concentrations also the subspectrum of complex **B** appears. The shape of the spectrum is particularly sensitive to the values of g_{\parallel} and A_{\parallel} . The larger g_{\parallel} and smaller A_{\parallel} of complex **A** indicate lower density of the unpaired electron at the site of the copper nucleus. It suggests that the electronic orbital is affected by fewer nitrogen atoms in the first coordination sphere of the $\text{Cu}(\text{II})$ ion compared to complex **B**.

The EPR spectra can be simulated as a superposition of two different subspectra: **A** respectively **B**. The shape of each sub spectrum is chosen as a molecule with an axial Zeeman interaction plus an axial hyperfine interaction to the $I = 3/2$ copper nucleus.

Table 1.
Calculated EPR parameters of the zeolite-Y encapsulated CuHis complexes.

Complex A	Complex A	Complex B	Complex B
$g_{//}$	$A_{//}$	$g_{//}$	$A_{//}$
2.32	154	2.27	173

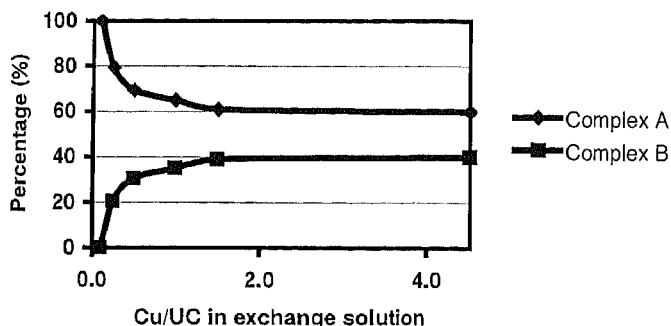


Fig. 2. Relative amounts of complex A and complex B on the zeolite as a function of the copper concentration in the ion exchange solution (estimated from EPR spectra).

Hyperfine couplings to nitrogen are omitted because they are experimentally not resolved. The calculated values for $g_{//}$ and $A_{//}$ for both complexes are presented in Table 1. The experimental accuracy did not require simulation as a mixture of $^{63}\text{Cu}/^{65}\text{Cu}$ isotopes.

The relative amounts of complex A and complex B on the zeolite can be estimated from the EPR spectra in Figure 1. These amounts are depicted in Fig. 2 as a function of the copper concentration in the ion exchange solution. At the lowest copper concentration only complex A is found on the zeolite. With increasing copper concentration in the ion exchange solution an increasing amount of complex B is found, with a maximum of approximately 40 % for the highest copper concentrations.

The amount of copper in the zeolite was measured by quantitative analysis. The results are presented in Fig. 3 as a function of the copper concentration in the ion exchange solution. The EPR intensity also gives an indication of the amount of copper exchanged onto the zeolite. These results are also depicted in Fig. 3. At low copper concentrations (up to 1 Cu/UC) the amount of Cu(II) exchanged onto the zeolite increases linearly with the copper concentration.

In this region, all the copper in the exchange solution is deposited on the zeolite. At higher copper concentrations in the exchange solution (above 1 Cu/UC), almost no extra copper can be deposited on the zeolite. An explanation for the observed behavior might be the congestion of the zeolite crystals by immobilized histidine or CuHis complexes in the outer pore system and supercharges of the zeolite crystals.

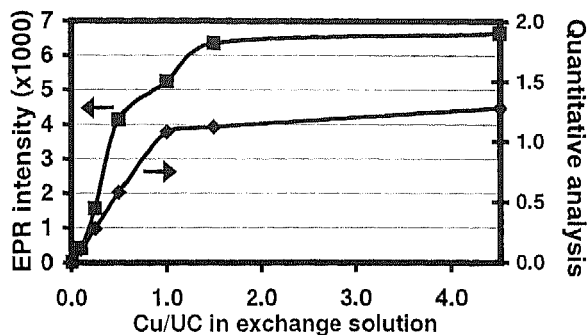


Fig. 3. EPR intensity and quantitative analysis (in Cu/UC) of the zeolite occluded copper complexes as a function of the copper concentration in the exchange solution.

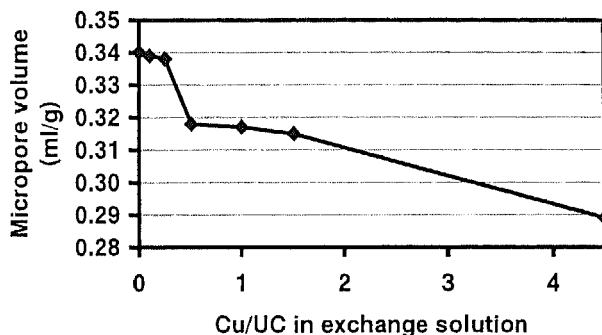


Fig. 4. Micropore volume as a function of the copper concentration in the ion exchange solution.

The process of ion exchange will affect the available pore volume inside the zeolite. The micropore volume has been measured by N_2 physisorption. The N_2 physisorption isotherms of the CuHis loaded zeolite samples are of Langmuir type I. The evolution of the micropore volume as a function of the initial copper concentration in the ion exchange solution is presented in Figure 4. The micropore volume decreases from 0.34 ml/g for a pure Y zeolite to 0.29 ml/g for the highest copper loading. The curve displays a bend in going from an initial copper concentration of 0.25 Cu/UC to an initial copper concentration of 0.50 Cu/UC. This phenomenon may be attributed to an increase in the relative amount of the more bulky complex **B** in the pore system of the zeolite.

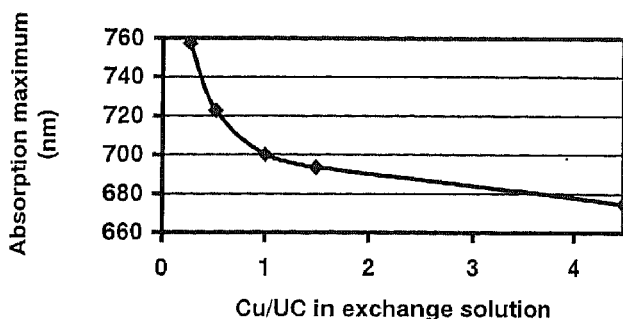


Fig. 5. Maxima of the absorption band in the DRS spectra of the CuHis complexes in zeolite Y as a function of the copper concentration in the ion exchange solution.

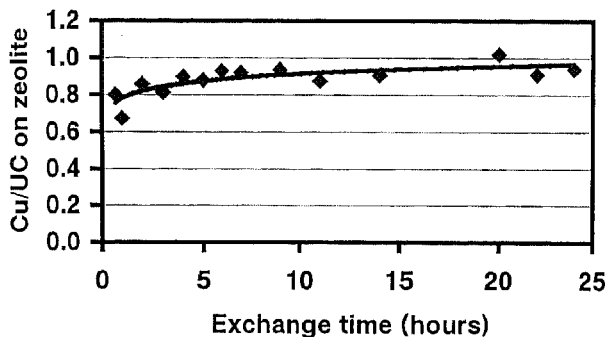


Fig. 6. Copper loading on the zeolite as a function of the duration of exchange reaction. External copper concentration is 1.5 Cu/UC.

At higher copper loadings the micropore volume is decreasing with the higher copper concentrations. This indicates an increasing fill up of the pore system and/or an increasing congestion of the pore system.

Diffuse Reflectance Spectroscopy (DRS) in the UV-Vis-NIR region was used to measure the ligand field strength of the different samples. The maximum of the d-d absorption band shifts from 758 nm to 675 nm with higher copper concentrations in the ion exchange solution (see Figure 5). This shows that the ligand field strength of the Cu(II) in complex B is higher than that in complex A. This is an indication for an increase in the number of nitrogens in the first coordination sphere of the Cu(II) ion and is in agreement with the trend observed in the EPR parameters.

The influence of the ion exchange time on the A/B ratio in the resulting zeolite samples was studied by CW-EPR. It was found that this ratio was independent of the ion exchange time (data not shown). The results of the quantitative analysis of the samples are presented in Figure 6. In this figure the amount of copper exchanged onto the zeolite is represented

as a function of time. The figure shows that the ion exchange is a fast process. Most copper is exchanged onto the zeolite within the first hour of the ion exchange process. The exchange process slows down considerably in the later stages, presumably due to partial congestion in the pore system of the zeolite.

4. CONCLUSIONS

Two different copper complexes were found on the zeolite, a mono histidine complex (**A**) and a bis-histidine complex (**B**). The influence of the initial copper concentration in the ion exchange solution on the **A** to **B** ratio was studied. A high **A/B** ratio was found at low copper concentrations and *vice versa*. For high copper concentrations the ratio leveled off at a value of $A/B = 1.5$. Full exchange was only achieved at low concentrations up to 1.0 Cu/UC. At higher copper concentrations the copper was no longer totally exchanged on the zeolite and the **A/B** ratio became independent of the external copper concentration.

We also studied the dynamics of exchange. The **A/B** ratio does not change during this process. The exchange process itself is very fast, as it takes place within an hour. After that the exchange process is slowed down by orders of magnitude.

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