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Metal complexes immobilised in/on porous matrices – possible enzyme mimics[*]

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Cu(II) histidine or Cu(II)-diethylene-triamino-μ-imidazolato-Zn(II)-*tris*-aminoethylamine triperchlorato complexes were prepared and immobilised in/on porous matrices (montmorillonite and silica gel) *via* (i) adsorption/hydrogen bonding, (ii) electrostatic forces or (iii) grafting with covalent bond. It was found that immobilisation increased catalytic activity and catalyst lifetime in the decomposition reaction of hydrogen peroxide relative to the matrix-free complexes. The immobilised materials were characterised by experimental and computational methods.

Key words: *Cu(histidine) complexes; Na-montmorillonite; silica gel; host-guest substances; FT-IR spectroscopy; immobilisation; anchoring*

1. Introduction

Homogeneous catalysts are generally more selective and occasionally more active than their heterogeneous counterparts. Nevertheless, they also have several disadvantages. Just two of them are their tedious and often unsuccessful separation from the reaction mixture and their shorter lifetime. Both of these unfavorable features may be eliminated if the homogeneous complexes are heterogenized. Complexes immobilised in solid or semi-solid matrices may mimic enzymes [1, 2], which may mean high ac-

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tivity at low temperature and very high selectivity: typical features of environmentally friendly procedures.

If one intends to model enzymes or wants to exploit their high activity and (or) selectivity, a viable way is trying to anchor the prosthetic group(s) found or assumed to be responsible for these parameters onto rigid or semi-rigid supports. When Cu-amino acid or Cu-Zn–N-containing multidentate ligand complexes are immobilised on supports, enzyme mimics may be obtained, which might be efficient and selective catalysts in a large variety of enzyme-catalysed reactions [3]. Immobilisation can be done in several ways, for a detailed description of various possibilities [4]. In this work, we show some feasible methods for the preparation. The synthetic methods and the resulting hybrid materials are described in the present paper. The immobilised complexes were characterised by various experimental and computational methods and some of them were used as catalysts in the decomposition reaction of H_2O_2.

2. Experimental

2.1. Synthesis of the complexes and methods for immobilisation

Materials. For the synthetic work $CuCl_2$ or $Cu(NO)_3$ (products of REANAL), L-histidine (Aldrich), montmorillonite (Bentolit-H, Laporte, ion-exchange capacity: 80 meq/100 g, BET surface area: 90 m^2/g (as-synthesised) or silica gel (Aldrich, TLC high-purity grade, average particle size: 5–25 μm, BET surface area: ~500 m^2/g, Fe^{3+}: 0.001 %, Cl^-: 0.003 %, pH [10% aqueous suspension] ~ 6.8, average pore diameter: 6 nm) were used. For the preparation of the Cu(II)-diethylene-triamino-μ-imidazolato-Zn(II)-trisaminoethylamine triperchlorato complex (denoted as **C** in the present paper) zinc and copper perchlorate (products of REANAL), imidazole, diethylene triamine and tris(2-aminoethyl)amine (products of Aldrich) were applied.

Immobilisation *via* adsorption/hydrogen bonds. During this form of immobilisation a calculated amount of L-histidine was adsorbed in montmorillonite or on silica gel at neutral pH. Then, the metal ions were introduced from solution, and finally, the empty coordination sites of the central ions were filled in by added L-histidine. During and after applying the method, the host material was washed free of excess amino acid. Anchoring the amino acid was by adsorption first, and then by using the electrostatic field of the Cu^{2+} ions.

Immobilisation *via* electrostatic forces. The Cu–L-histidine complex was prepared *ex situ* and then it was introduced into the pore system of montmorillonite. In this procedure the copper salt was dissolved in water, and then the ligand was added under continuous stirring. During the formation of the complex changes in pH were registered. The starting solution was acidic (pH = 3.2 for the copper solution) becoming close to neutral by the end of the reaction. Then, the complex was crystallised by cooling and evaporating the excess of solvent. The preformed complexes were introduced into montmorillonite using isopropanol as a solvent. The procedure applied was ion exchange of the bulky complex ion in the pre-swollen montmorillonite (0.6 mmol complex ion/g montmorillonite).

In the second case, Cu(II) ions were introduced by ion exchange into (onto) montmorillonite or silica gel and they served as starting materials. Ion exchange was performed twice at 343 K for 12 h each time. The concentration of the exchanged ions was the same as when the procedure was performed by the preformed complexes. The complexes were synthesised *in situ* inside the pores by adding ligand molecules, enough for the presumed fourfold coordination. The synthesis was performed using isopropanol solution of the ligands under reflux for 24 hours.

Before immobilisation the Cu(II)-diethylene-triamino-μ-imidazolato-Zn(II)-tris-aminoethylamine triperchlorato complex was prepared *ex situ* following the general recipe published in ref. [5]. In this procedure the zinc or the copper perchlorate was dissolved in water and then the ligands (imidazole and diethylene triamine for the copper salt solution and tris(2-aminoethyl)amine for the zinc salt solution) were added under continuous stirring. For preparing the bridged binuclear complex, the two complex solutions were added in 1:1 molar ratio to a quarter of that amount of free Zn(II) in ethanol under vigorous stirring. The stirring was maintained for 24 h and then, the bridged binuclear complex was allowed to crystallise. The immobilised complex catalyst was prepared using isopropanol. The preformed complex was dissolved in isopropanol and this solution was introduced into montmorillonite suspended in isopropanol. Immobilisation occurred with cation exchange.

Immobilisation using *via* covalent bonding. For covalent grafting 3-chloropropyl-functionalised silica gel (Aldrich) was used. Approximately 8% of the OH groups carried the chloropropyl functionality. To enforce bonding at the N-terminal, L-histidine methylester hydrochloride (Aldrich) was the amino acid derivative. The coupling was achieved under base conditions after reflux for 2 hours. Then, the product was divided into two parts. One part was treated with $Cu(NO_3)_2$ solution and the amino acid ester, resulting in Cu–L-histidine methylester complex covalently anchored to silica. The other part was subject to acid hydrolysis and then to subsequent treatment with $Cu(NO_3)_2$ solution and L-histidine, resulting in covalently anchored Cu–L-histidine complex. Before any further use, the immobilised materials were washed free of excess Cu(II) ions as well as ligands and were air-dried.

2.2. Methods of characterisation

The host-free complexes were examined by derivatography, UV and IR spectroscopies. The host materials and the immobilised materials were characterised by powder X-ray diffraction and IR spectroscopy, thermal analysis and BET measurements. The thermal behaviour of the substances was investigated with the use of a Derivatograph Q instrument. The samples were studied under the following conditions: mass sample 100 mg, heating rate 10 deg/min, temperature range 300–1273 K in air. X-ray diffractograms were registered on a DRON 3 diffractometer. BET measurements were performed in a conventional volumetric adsorption apparatus cooled to the liquid nitrogen temperature (77.4 K). Prior to measurements the host samples were pretreated in vacuum at 573 K for 1 h. Neither BET surface area nor basal distance was significantly altered compared to those of the host material (montmorillonite: 90 m^2/g, 1.46 nm (as synthesized)).

Substances obtained by covalent grafting were studied by FT-IR spectroscopy, either by means of the KBr technique or the self-supported wafer method. The KBr pellets (1.2 mg of the substances in 200 mg KBr) or the self-supported wafers (10 mg/cm^2) were pressed from the materials and these were applied for monitoring changes in the IR spectra of the samples. The FT-IR spectra of the host and guest materials and the host–guest complexes prepared by both the two-step and three-step methods were taken and compared. The 3800–480 cm^{-1} range was investigated. The spectra were recorded by a Mattson Genesis I spectrophotometer with 2 cm^{-1} resolution. For a spectrum 126 scans were collected. Spectra of the KBr pellets were taken at 298 K (every host–guest complex was studied) in air. Self-supported wafers (not all but only few host–guest complexes were investigated due to experimental difficulties encountered when making the wafers) were degassed at 348 K, 373 K, 423 K, 573 K, and 673 K for 1 h at each temperature and the IR spectra were recorded after each pretreatment temperature. The spectra were evaluated by the Win-IR package.

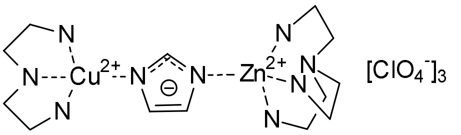
The covalently grafted complexes were modelled by the PM3 semi-empirical quantum chemical code [6] included in the HyperChem package [7].

Many of the immobilised samples as well as the effects of host-free complexes were tested in the decomposition of hydrogen peroxide. The reaction was performed in the liquid phase at 298, 313 or 333 K with the Cu–L-histidine containing and at 333 K with the Cu–**C** containing composite. The transformations were followed by UV-VIS spectroscopy (Perkin Elmer). The well-stirred reaction mixture contained 100 mg of the catalysts and 20 cm^3 of 30% hydrogen peroxide aqueous solution.

3. Results and discussion

3.1. Host-free complexes

UV-VIS spectroscopic results revealed that the copper ions were complexed (absorptions relevant to the free ions disappeared form the spectra of the complexes). The structure of the binuclear complex is shown below [5]:



The thermal behaviour of this complex was relatively simple (Fig. 1a). Up to 473 K water was removed in an endothermic process (probably it is the physisorbed and the crystal water). Between 473 K and 573 K the complex decomposed vigorously. This process was exothermic. Above 573 K the organic ligands were removed gradually and continuously.

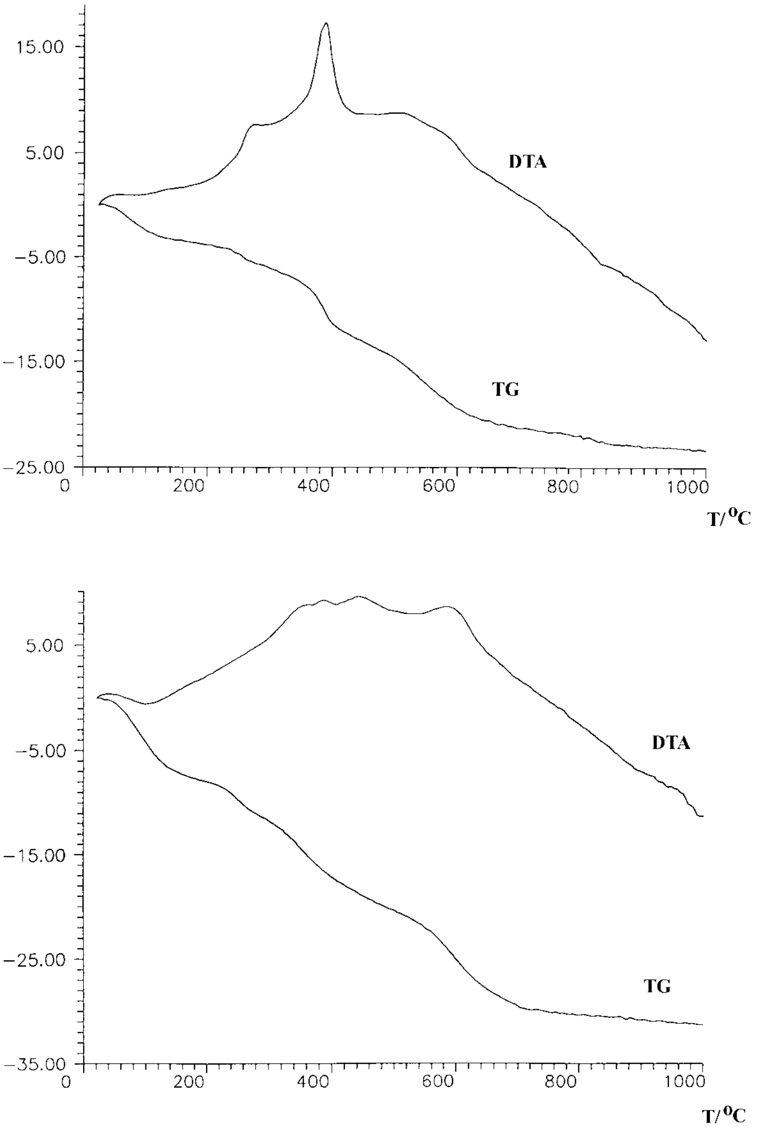


Fig. 1. Thermogravimetric (TG) and differential thermal analytic (DTA) curves of (a) the bare Cu(II)-diethylene-triamino-μ-imidazolato-Zn(II)-*tris*-aminoethylamine triperchlorato complex (**C**) and (b) the **C** complex immobilised in montmorillonite

3.2. Host materials

A great deal of information is available concerning the structural characteristics of the host materials [8]. Let us summarize in a couple sentences the main features relevant to this work.

Montmorillonite is a layered material of the cation exchange ability. It is capable of swelling in a large variety of solvents, such as water, various alcohols, etc. Therefore, it can accommodate the complexes in various positions. If intercalation is too dense, free traffic of the reacting species may be hindered, thus, optimisation of pillaring is of great importance.

Silica gel is a material containing surface OH groups capable of ion exchange. Although it has pore structure, as a first approximation we can consider it as a planar material, at least relative to montmorillonite. The surface OH groups may be functionalised, thus, extended structures may be built if the spacer groups contain reactive groups.

3.3. Intercalated substances

Substances immobilised *via* electrostatic forces. When the complexes investigated were incorporated into any of the porous matrices studied, they became more stable, loosing all ligands at higher temperature (around 1100 K) than the free complexes (around 900 K). Between 373 K and 473 K the dehydration could be observed for the host materials.

As far as the Cu–C complex immobilised in montmorillonite is concerned, ligands are removed in steps (they are combusted since measurements were performed in air). No abrupt changes were observed, indicating that immobilization occurred mainly with ion exchange (Fig. 1b).

In the case of the immobilised Cu–L-histidine complexes, it became clear on the basis of weight losses measured by derivatography that two histidine molecules were bonded to the central ion. Nevertheless, fourfold coordination may be assumed, since histidine generally acts as a bidentate ligand [9, 10]. On heating the composite materials, a multiple weight loss occured, indicating gradual loss of the ligands. By 773 K the immobilised complexes were completely decomposed.

Substances immobilised *via* adsorption/hydrogen bonding. In the Cu–L-histidine-montmorillonite composite (Fig. 2) the loading was low, and the band structure of the host in the low wave number region (1350–400 cm^{-1}) was clearly seen in the spectra of the hybrid materials. Probably due to a low loading, the bands of the carboxylic OH were hardly seen, and the bands of the OH groups in montmorillonite were also not completely used. It seems as if the carboxylic group of histidine, after deprotonation, took part in complex formation and only few Cu^{2+} ions interacted with the OH groups of montmorillonite. Possibly, the complex was immobilised between the layers of montmorillonite by adsorption and (or) hydrogen bonding.

Only a small amount of Cu–L-histidine complex could be anchored to silica gel with this method (Fig. 3).

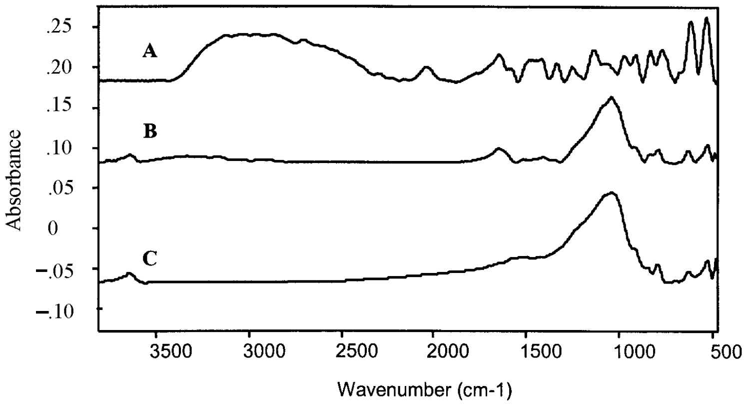


Fig. 2. FT-IR spectra of A) L-histidine, B) the Cu–L-histidine complex immobilised in montmorillonite by the adsorption/hydrogen bonding method, C) the air-dried montmorillonite

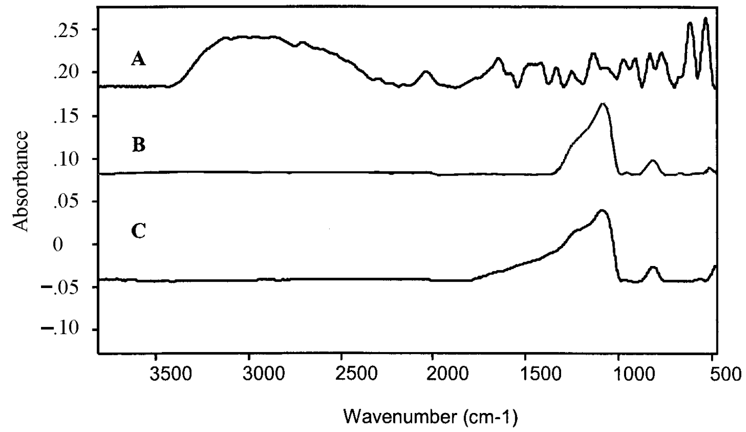


Fig. 3. FT-IR spectra of A) L-histidine, B) the Cu–L-histidine complex immobilised on silica gel by the adsorption/hydrogen bonding method, C) the air-dried silica gel

Substance immobilised *via* covalent bonding. In our view, the most promising anchoring method is covalent grafting. The complex is immobilised indeed, leaching is minimal if occurs at all, and the synthesis method provides some control over the structure of the anchored complex. Moreover, the linker group allows some flexibility for the complex, which is surely advantageous for use as a catalyst. At the beginning of an extended research project silica gel was chosen to be the first host. It contains large amount of OH groups suitable for functionalisation. The chloropropyl-modified version (also available commercially) undergoes easy reaction with either the N-terminal or the C-terminal of an amino acid. The reaction can be controlled by choosing a suitably protected amino acid. We used C-protected L-histidine, thus an amide was synthesized first. Then, the ester group was hydrolysed and the complex was allowed to form by adding Cu(II) ion-containing solution and unprotected L-histidine to the slurry. The obtained materials were studied by FT-IR spectroscopy. The relevant spectra are shown in Fig. 4.

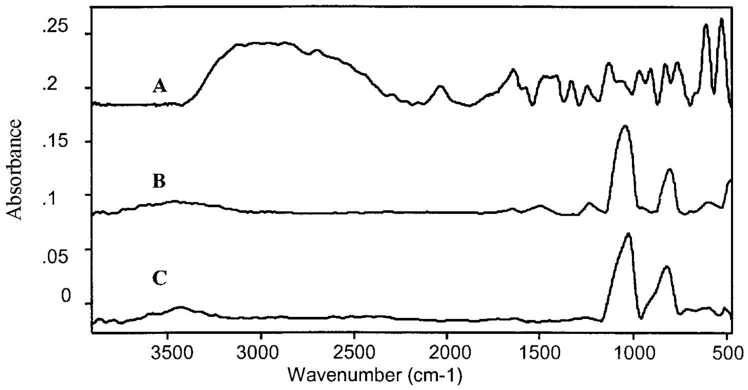


Fig. 4. FT-IR spectra of (A) L-histidine, (B) the Cu–L-histidine complex covalently grafted onto 3-chloropropyl silica gel, (C) the air-dried 3-chloropropyl silica gel

Unfortunately, FT-IR spectra are rarely enough for giving accurate structure for the anchored complex. Some information may be obtained, however. The band in the OH stretching range indicates the presence of uncomplexed OH groups. The large red shift of the C=O band may be taken as the sign of co-ordinative (carbonyl) O–Cu bond. The other two co-ordination sites are probably filled in by the nitrogens of the amino groups, since the relevant bands also moved towards lower wave numbers.

Fig. 5. Molecular model of Cu–L-histidine complex covalently anchored to silica gel *via* the reaction of amino group of one amino acid and t he 3-chloropropyl funcionality of the silica gel

Molecular modelling facilitates visualisation of the possible structure (Fig. 5). In order to obtain a more realistic picture, relatively large silica cluster was chosen. Energetic data may also be obtained by e.g., DFT calculations, however, at present the computational exercise is not tractable by our means.

3.4. Decomposition of hydrogen peroxide

The catalytic activities of complexes (Cu–L-histidine prepared by the in situ as well as the ex situ methods and the Cu–**C** complex) immobilised in montmorillonite were investigated. First, blank reactions were made using the metal salts, the host-free complexes, and the complex-free host and, then, the variously prepared host-guest materials were studied.

While the Cu(II) solution was inactive in the decomposition reaction of hydrogen peroxide ($H_2O_{22}= H_2O + 1/2\ O_2$) at any of the temperatures used, in the presence of the complexes the reactions started, although after long induction periods (20 min for Cu –L-histidine and 30 min for the Cu–**C** complex). The montmorillonite was completely inactive, only the Cu(II)-montmorillonite showed a slight decomposition activity at the highest temperature used (333 K).

Measurements revealed that the immobilised Cu–L-histidine complex was able to decompose the peroxide compounds without induction period and, thus, more rapidly than the aqueous complex could do. The activity of the immobilised complex was found to depend on the method of sample preparation, too (Table 1). The *in situ* prepared immobilised complex was found to be more active than the preformed and then introduced one.

Table 1. Rate of hydrogen peroxide decomposition on Cu(II) complexes immobilized in montmorillonite

T/K	Decomposition rate (molecules/central ion/h)		
	Cu–L-histidine		Cu–**C**
	ex situ	*in situ*	*ex situ*
298	0	0	–
313	0.24	0.42	
333	1.46	2.50	97.6 (71.6[a])

[a] Rate of decomposition in the presence of the host-free complex.

The Cu–**C** complex immobilised in montmorillonite was also found to be active in the decomposition reaction, the induction period, however, was 70 min. Nevertheless, the turnover rate for the immobilised material was higher than that of the host-free complex (Table 1, last column). Moreover, the immobilised substance could be reused at least three times without any appreciable loss of activity, while the bare complex decomposed and lost its activity by the end of the first run.

4. Conclusions

Cu complexes were prepared and immobilised in montmorillonite or on silica gel by various methods using primary bonding (ionic or covalent) or secondary forces (adsorption/hydrogen bonding) for anchoring the complexes through either their ligands or their central ions. The immobilised complexes are promising catalysts, and after some optimisation and some further structural studies they can be used as enzyme mimics and may allow better understanding how certain enzymes work.

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