Mimicking catalase and catecholase enzymes by copper(II)-containing complexes

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Abstract: An imidazolate-bridged copper(II)–zinc(II) complex (Cu(II)-diethylenetriamino-µ-imidazolato–Zn(II)-tris(2-aminoethyl)amine perchlorate (denoted as “Cu,Zn complex”) and a simple copper(II) complex (Cu(II)-tris(2-aminoethyl)amine chloride ( “Cu-tren”) were prepared and immobilised on silica gel (by hydrogen or covalent bonds) and montmorillonite (by ion exchange). The immobilised substances were characterised by FT–IR spectroscopy and their thermal characteristics were also studied. The obtained materials were tested in two probe reactions: catalytic oxidation of 3,5-di-tert-butyl catechol (DTBC) (catecholase activity) and the decomposition of hydrogen peroxide (catalase activity). It was found that the catecholase activity of the Cu,Zn complex increased considerably upon immobilization on silica gel via hydrogen bonds and intercalation by ion exchange among the layers of montmorillonite. The imidazolate-bridged copper(II)–zinc(II) complex and its immobilised versions were inactive in hydrogen peroxide decomposition. The Cu(II)-tris(2-aminoethyl)amine chloride complex displayed good catalase activity; however, immobilisation could not improve it.

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1 Introduction

Immobilisation of the synthetic structural mimics of the active centres of metalloenzymes on various supports is nowadays a frequently used method to obtain artificial enzymes working efficiently [1–5]. Sometimes their activity can reach that of the native enzyme. Working in this field, we recently synthesised and investigated in detail the imidazolate-bridged Cu(II)-diethylenetriamino–μ-imidazolato–Zn(II)-tris(2-aminoethyl)amine perchlorate (“Cu,Zn complex”) and some of its building blocks such as Cu(II)-tris(2-aminoethyl)amine chloride (“Cu-tren”) complex (Fig. 1). They were immobilised on various supports (silica gel or montmorillonite) by different methods. The properties of the non-supported complexes are known.

Catalase enzymes are important antioxidant metalloenzymes that catalyse the decomposition of hydrogen peroxide to water and dioxygen. This type of antioxidant function is essential for all organisms exposed to dioxygen since they readily form hydrogen peroxide both enzymatically, through the action of oxidases, and non–enzymatically, as a side product of respiration or autooxidation of cell components. Two families of catalases are known: (i) one having a heme cofactor and (ii) a structurally distinct family containing nonheme manganese [6]. Recently, a number of manganese containing nonheme catalases were isolated and characterised. Since then, several manganese(II-IV)-containing complexes were investigated as structural models for dimanganese catalase [7–9]. The immobilisation of the native enzyme has been published as well [10]. The catalase mimicking properties of homo– and/or heterobinuclear copper(II) complexes have also been investigated, but reports on this activity of copper(II)-containing systems reported are relatively scarce [11–13]. Catechol oxidases are ubiquitous plant enzymes catalysing the oxidation of a broad range of o-diphenols to o-quinones in the presence of dioxygen. They belong to the family of type-3 copper proteins containing a binuclear copper active site that is EPR silent in the native form, due to a strong antiferromagnetic coupling between the copper atoms [14]. A number of mono- and binuclear copper(II) complexes have been investigated as biomimetic catalysts for catechol oxidation [15–21]. Binuclear copper(II) complexes are generally found to be more reactive than the mononuclear ones. In addition the catecholase activity of montmorillonite-immobilised native tyrosinase enzyme [22] and some silica-supported copper(II) complexes [23] were also investigated.

The imidazolate-bridged Cu,Zn complex and Cu-tren (Fig. 1) were immobilised on silica gel by two methods: via hydrogen bonds or via covalent linkage. The montmorillonite-immobilised version of the Cu,Zn and Cu-tren complexes was also prepared and investigated. The properties of the non-supported Cu,Zn and Cu-tren complexes in aqueous solution have been studied by pH potentiometry, visible and EPR spectroscopy, mass spectrometry, cyclic voltammetry and published in one of our previous works [24]. The superoxide dismutase activities of these unsupported complexes have also been investigated [24]. EPR and FT–IR spectroscopic characterisation and the investigation of thermal behaviour and superoxide dismutase activities of the silica- (immobilisation oc-
curred via hydrogen bonds) and montmorillonite-supported Cu,Zn complex have been published as well [25]. These materials performed rather well as superoxide dismutase mimics. Now, we want to screen the scope of reactions in which they can be used as enzyme-mimicking catalysts. For this reason we tested their catalase and catecholase activities. To screen the scope of reactions in which they can be used as enzyme-mimicking catalysts we tested the catalase and catecholase activities of these unsupported and immobilised Cu,Zn and silica- and montmorillonite-supported Cu-tren complexes. Results of this work are communicated herein.

![Fig. 1 Structure of the investigated complexes: Cu(II)-tris(2-aminoethyl)amine (A) and Cu(II)-diethylenetriamino–μ-imidazolato–Zn(II)-tris(2-aminoethyl)amine (B).](image)

In accordance with those mentioned above, the aim of the present work is to study the catalase and catecholase activity of the unsupported and silica and montmorillonite-immobilised imidazolate-bridged Cu,Zn dimuclear complex and the Cu-tren complex.

2 Experimental part

2.1 Materials

For the preparation of the complexes, Cu(ClO₄)₂·6H₂O (product of Fluka), CuCl₂·2H₂O (Reanal) and Zn(ClO₄)₂·6H₂O (Fluka), imidazole (Aldrich), diethylenetriamine (Fluka), tris(2-aminoethyl)amine (Aldrich) were applied. As hosts, montmorillonite (Bentolite-H, Laporte, ion-exchange capacity: 1.05 mol/g, BET surface area: 90 m²/g), silica gel (for immobilisation via hydrogen bonding, Aldrich, TLC high-purity grade, average particle size: 5-25 μm, BET surface area: ~500 m²/g, Fe³⁺ ≤ 0.001%, Cl⁻ ≤ 0.003%, pH [10 % aqueous suspension]: ~6.8, average pore diameter: 6 nm), 3-chloropropyl functionalised silica gel (for immobilisation of Cu-tren complex via covalent linking, Aldrich, 2.5 % Cl, ~ 8 % functionalised) and 3-(diethylenetriamino)propyl-functionalised silica gel (for the immobilisation of Cu,Zn complex via covalent linkage, Aldrich) were used. For the probe reactions, hydrogen peroxide (Sigma), Na₂HPO₄·2H₂O (Sigma), KH₂PO₄ (Sigma), 3,5-di-tert-butyl catechol (Fluka) were applied. All the chemicals were of reagent grade and were used without further purification.
2.2 Preparation of the host-free complexes

The Cu,Zn complex was prepared on the basis of the general procedure published in ref. [26]. In this procedure the zinc(II) or copper(II) perchlorate was dissolved in ethanol, and then the ligands (imidazole and diethylenetriamine for the copper(II) salt solution and tris(2-aminoethyl)amine for the zinc(II) salt solution) were added under continuous stirring. For preparing the imidazolate-bridged binuclear complex the two complex solutions were added in 1:1 molar ratio under stirring. The obtained blue material was filtered and dried in vacuum.

For the preparation of Cu-tren complex tris(2-aminoethyl)amine was added to copper(II) chloride dissolved in isopropanol. The ratio of the amino compound to copper(II) was 1:1. Colour changes were observed (the green colour changed to dark blue), indicating that chemical reaction occurred. After this procedure the solution was partially evaporated and the solid material precipitated. It was collected, washed with a small amount of isopropanol and dried in vacuum at 80 °C.

2.3 Preparation of the immobilised complexes

Immobilisation on silica gel via hydrogen bonding

The silica-supported (immobilisation occurred through hydrogen bonding) Cu,Zn (denoted as SG–h–Cu,Zn) and Cu-tren (denoted as SG–h–Cu-tren) complexes were prepared essentially the same way. First, calculated amount of the host-free complex was dissolved in 100 mL suspension of isopropanol and silica gel (0.5 g). The mixture was stirred for a day at room temperature. After that, the solid material was filtered and suspended again in 100 mL isopropanol and stirred for a day to remove weakly connected complex molecules. The obtained solid material was filtered and dried in vacuum at 80 °C.

Immobilisation on silica gel via covalent grafting

The covalently linked Cu,Zn complex (denoted as SG–c–Cu,Zn) was prepared according to the following procedure. 1.50 g 3-(diethylenetriamino)propyl-functionalised silica gel (having 1.84 mmol diethylenetriamino group) was suspended in 125 cm³ ethanol containing 0.68 g Cu(ClO₄)₂·6H₂O (1.84 mmol copper(II) ion). The mixture was stirred for one hour, and then 0.13 g imidazole (1.84 mmol) was added and stirred for three hours at room temperature. At the same time 0.69 g Zn(ClO₄)₂·6H₂O (1.84 mmol zinc(II) ion) was dissolved in 30 mL ethanol and for this solution 0.20 mL tris(2-aminoethyl)amine (1.84 mmol) was added and the solution was stirred for one hour at room temperature. After these, the 30 mL solution of zinc(II)-tris(2-aminoethyl)amine complex was added to the suspension detailed above. The mixture was stirred for one hour, and then filtered and the obtained blue solid material was dried in vacuum at 80 °C.

For the preparation of the covalently grafted Cu-tren complex (denoted as SG–c–Cu-tren) first, the tris(2-aminomethyl)amine functionalised silica gel was prepared (Scheme 1). 2.72 mL tris(2-aminoethyl)amine (18.17 mmol) was added to the suspension of pyridine
and 5.00 g 3-chloropropyl functionalised silica gel (3.63 mmol chloropropyl group) and the mixture was boiled and stirred under reflux for one day. After this, the solid material was filtered and washed in water for a week to remove pyridinium chloride evolved. The obtained material was filtered and dried in vacuum at 80 °C.

![Scheme 1 Immobilisation of tris(2-aminoethyl)amine on 3-chloropropyl functionalised silica gel by covalent bonds (SG indicates the silica gel part).](image)

After this procedure 40 mL (0.09222 M) copper(II) chloride in isopropanol was added to the suspension of 60 mL isopropanol and 1.00 g of the obtained tris(2-aminomethyl)amine functionalised silica gel (0.73 mmol amino group from the results of nitrogen determination on the silica gel). The mixture was stirred for one day, then filtered and suspended again in 100 mL isopropanol. After stirring for a day at room temperature, the light blue solid material was filtered and dried in vacuum at 80 °C.

### Immobilisation in montmorillonite

The montmorillonite-immobilised (immobilisation occurred by ion exchange) Cu,Zn (denoted as Mont–i–Cu,Zn) and Cu-tren (denoted as Mont–i–Cu-tren) complexes were prepared essentially the same way. In either case calculated amount of the host-free complex dissolved in isopropanol was added to the suspension of 100 mL isopropanol and 0.50 g montmorillonite. The mixture was stirred for one day at room temperature. After this, the solid material was filtered and suspended in 100 mL isopropanol. After stirring for one day, the light blue solid material was filtered and dried in vacuum at 80 °C.

### 2.4 Characterisation of the catalysts

FT–IR spectra were recorded on a BIO-RAD Digilab Division FTS–65 A/896 spectrophotometer with 2 cm⁻¹ resolution. In FT–IR measurements the KBr/nujol technique was applied in reflectance mode. The spectra of the host and guest materials and the host–guest compounds were taken and compared. The 400–4000 cm⁻¹ range was investigated and the spectra were evaluated by the Win–IR package.

The host-free and the immobilised complexes were also studied by thermal (TG, DTA) methods. The thermal behaviour of the substances was investigated by a Derivatograph Q instrument in air atmosphere (mass of the sample ∼100 mg, heating rate 10 °C/min, temperature range 30 to 1000 °C).

The amounts of zinc(II) and copper(II) on/in the solid hosts were measured by atomic absorption spectrometry (AAS – Perkin Elmer 3110 instrument). Before measurements the solid materials were dissolved in aqua regia.
2.5 Catecholase activity measurements

The reaction of the host–free and the immobilised complexes with 3,5-di-tert-butyl catechol (denoted as DTBC) was monitored as follows. The reactions were studied in a stirred and thermostated cell with 1 cm path length at 25 ± 0.1 °C. In every case, methanol was used as solvent. Under the catalytic oxidation of DTBC to 3,5-di-tert-butyl quinone (DTBQ), the formation of DTBQ was followed by UV–Vis spectrophotometry (by a Hewlett Packard 8452A Diode Array spectrophotometer) through the development of the corresponding band at 400 nm (ε = 1900 M$^{-1}$ cm$^{-1}$). The noise during the measurement was reduced by reading the difference in absorbance between 400 nm and 800 nm (at 800 nm the absorbance variation during the reactions is only due to the noise). In every case, the reaction mixture (3.00 mL) contained 10.0 mM DTBC and 0.1 mM copper(II) ion in methanol. During the catalytic reaction the absorbance was read at 2, 5, 10, 15, 30, 45 and 60 min and the initial rate of the oxidation was obtained from the DTBQ concentration versus time plots. In order to make initial rates and conversions comparable, the reaction mixture always contained the same amount of Cu$^{2+}$.

2.6 Catalase activity measurements

The catalase activities of the host-free and the immobilised complexes were measured on the basis of the conversion of hydrogen peroxide to water and dioxygen. Several methods [8, 27, 28] are known to follow the amount of the hydrogen peroxide in the catalytic reaction. Here, the amount of the hydrogen peroxide was measured by spectrophotometry (Hewlett Packard 8452A Diode Array spectrophotometer) through the disappearance of the corresponding band at 240 nm. The concentration of hydrogen peroxide was calculated from the absorbance using a calibrating curve. All reactions were performed in 30 mL samples in air, the samples were thermostated at 25 ± 0.1 °C and the stirring rate was constant. The reaction mixture contained 20.1 mmol hydrogen peroxide and 0.21 mmol copper(II) ion in aqueous phosphate buffer (Na$_2$HPO$_4$ and KH$_2$PO$_4$, 50.0 mM, pH=6.86). The probe reaction was run up to 120 min and at every 20 min 0.5 ml sample was withdrawn from the reaction mixture. After this, the sample was filled to 10.0 ml with 0.1 M HCl solution in order to stop the catalytic reaction. The absorbance of the diluted sample was measured at 240 nm and 800 nm (thus the noise was reduced) and the amount of the hydrogen peroxide was calculated using the calibration curve. From the experimental data, conversions and the initial rates were calculated. In order to make initial rates and conversions comparable, the reaction mixture always contained the same amount of Cu$^{2+}$. 
3 Results and discussion

3.1 Characterisation of the catalysts

Cu,Zn complex and its immobilised forms

We have published on the equilibrium structure of the Cu,Zn complex and its sub-
species in aqueous solution of various acidities recently [24]. In that paper the properties
of the complex were investigated by pH potentiometric titration (determination of the
complexes formed and their stability constants), UV–Visible and EPR spectroscopies
(characterisation of the structure of the evolved complexes), mass spectrometry (deter-
mination of the molecular weight of the Cu,Zn complex). In addition, its electrochemical
behaviour (by cyclic voltammetry) and superoxide dismutase activity were also studied.
It was found that imidazolate-bridged, heterobinuclear Cu,Zn complex was stable in the
pH range 7-11, although deprotonation of this complex occurred above pH 8. The elec-
trochemical and superoxide dismutase activity results revealed that due to its appropriate
electrochemical properties it displayed good superoxide dismutase activity.

The Cu,Zn complex and its immobilised (SG–h–Cu,Zn and the Mont–i–Cu,Zn) forms
were investigated in the solid state [25]. The structure of these substances were charac-
terised by FT–IR and EPR spectroscopy. Their thermal behaviour (by TG, DTA meth-
ods) and superoxide dismutase activity were also studied. Those results indicated that
immobilisation of the Cu,Zn complex was successful on silica gel (via hydrogen bonding)
and in montmorillonite (through ion exchange). EPR measurements indicated that the
structure of the Cu,Zn complex did not change upon immobilisation in montmorillonite.
However, two types of copper(II)-containing species were found on the silica-immobilised
material: one had similar EPR parameters to the montmorillonite intercalated Cu,Zn
complex; for the other species, stronger ligand field was discovered. Dramatic increase
was observed in the superoxide dismutase activity of the Cu,Zn complex immobilised on
silica gel.

We also have published on the FT–IR spectroscopic characterisation of the Cu,Zn
complex covalently bonded to silica gel (SG–c–Cu,Zn) very recently [29]. After dissolving
the solid substance, a zinc(II) to copper(II) ratio of 1:1 was found in the solid material
by atomic absorption spectroscopy. This result and the FT–IR characterisation clearly
demonstrated that immobilisation of the Cu,Zn complex indeed occurred. The thermal
behaviour of the SG–c–Cu,Zn was also studied by TG (thermogravimetry) and DTA
(differential thermal analysis) methods. The measured thermal decomposition curves are
displayed in Fig. 2. Between 120 °C and 220 °C dehydration could be observed for the
host material. From around 230 °C the organic ligands started to depart from the silica
gel-immobilised sample. This immobilised complex is thermally more stable than the
host-free complex, which fully decomposes by 300 °C.

Cu-tren complex and its immobilised forms

The structure of the host-free Cu-tren complex was studied earlier in solution and
also in the solid state [24, 30–33]. In addition, its superoxide dismutase activity [34] and its ability of hydrolysing phosphodiesters [35] are known. In these works it was confirmed that tris(2-aminoethyl)amine coordinated to the copper(II) ion through four nitrogen atoms under suitable conditions (pH, temperature, etc.). It also displayed good superoxide dismutase and phosphodiester hydrolysing activities.

The amount of copper(II) in the immobilised Cu-tren complexes were determined after dissolution in aqua regia by atomic absorption spectroscopy (1.66 mmol/g for SG–h–Cu-tren, 0.49 mmol/g for SG–c–Cu-tren and 1.05 mmol/g for Mont–i–Cu-tren). The FT–IR spectroscopic characterisation of the silica-immobilised (both via hydrogen bonding [SG–h–Cu-tren] and covalent linkage [SG–c–Cu-tren]) Cu-tren complexes has been published in our previous work [29]. The FT–IR spectra of the host-free complex, the immobilised forms and the host material were measured and compared. The Cu-tren complex immobilised in montmorillonite was characterised by FT–IR spectroscopy as well. In all cases the success of immobilisation was confirmed. Now, the FT–IR spectrum of the montmorillonite-intercalated Cu-tren complex (Mont–i–Cu-tren) was measured and displayed in Fig. 3. Besides the bands of the host material, those of the Cu-tren complex could also be identified in the spectrum of the host-guest material (see e.g. $\nu$ (NH)$_{as}$ and $\nu$ (NH)$_{s}$ in the range 3000-3550 cm$^{-1}$ and $\beta$(NH$_2$)$_{as}$ at $\sim$ 1480 cm$^{-1}$). This is an indication that immobilisation really occurred among the layers of montmorillonite.

The thermal decomposition of the Cu-tren complex and the host–guest materials (SG–h–Cu-tren, SG–c–Cu-tren, Mont–i–Cu-tren) were also studied by thermogravimetry (TG) and differential thermal analysis (DTA). The measured curves are seen in Figures 4-7. The thermal decomposition curves of the supported complexes indicate that immobilisation was successful both on silica gel and in montmorillonite. Namely, dehydration could always be observed between 100 and 220 °C. The thermal decomposition of the organic

Fig. 2 TG and DTA curves of the Cu,Zn complex immobilised on silica gel by covalent bonds (SG–c–Cu,Zn).
ligand occurred between 220 and 420 °C. This process took place in an easily distinguishable step for Cu-tren (Fig. 4), SG–h–Cu-tren (Fig. 5) and SG–c–Cu-tren (Fig. 6). The thermal decomposition of the ligand for the montmorillonite-immobilised Cu-tren complex (Mont–i–Cu-tren, Fig. 7) completed at higher temperature (500 °C) than for the other materials. Finally, the full decomposition of Cu-tren (host-free and immobilised) was over by 800 °C.

### 3.2 Catecholase activity

The catecholase activities of the host–free and immobilised complexes were tested in the catalytic oxidation of 3,5-di-tert-butyl catechol (DTBC) to 3,5-di-tert-butyl quinone.
Fig. 5 Thermal decomposition (TG and DTA) curves of the silica-immobilised \((\text{via hydrogen bonding, } \text{SG–h–Cu–tren})\) Cu-tren complex.

Fig. 6 Thermal decomposition (TG and DTA) curves of the silica-immobilised \((\text{via covalent bonds, } \text{SG–c–Cu–tren})\) Cu-tren complex.

(DTBQ) in the presence of dioxygen (Scheme 2). The amount of the evolved DTBQ was determined by UV-Visible spectrophotometry \((\lambda = 400 \text{ nm}, \epsilon = 1900 \text{ M}^{-1}\text{cm}^{-1}\) in methanol).

The ability of heterogenised systems (SG–h–Cu,Zn, SG–c–Cu,Zn, Mont–i–Cu,Zn, SG–h–Cu-tren, SG–c–Cu-tren, Mont–i–Cu-tren) to catalyse the oxidation of DTBC was evaluated and the results were compared to those found for the Cu,Zn and Cu-tren complexes. The initial rates \(v_o\) and the conversion were calculated from the resulting concentration of DTBQ versus time graph (see Fig. 8 for the Cu,Zn complex and its immobilised
forms). In all cases low conversion values (< 3 %) were observed. The initial rates for the host-free and the immobilised complexes are given in Table 1.

DTBC oxidation measurements reveal that the complexes – bare or immobilised – all show catecholase activity. The calculated initial rate values of the host-free complexes (Cu,Zn and Cu-tren) are fairly similar. The activity of the Cu-tren complex hardly changed upon immobilisation. Some increase was observed with SG–h–Cu-tren and Mont–i–Cu-tren, however, the catecholase activity of SG–c–Cu-tren decreased relative to the bare complex. However, the catecholase activity of the Cu,Zn complex changed significantly upon immobilisation on either silica gel (SG–h–Cu,Zn and SG–c–Cu,Zn) or in montmorillonite (Mont–i–Cu,Zn). The most active material was the SG–h–Cu,Zn having nearly 50-fold higher activity than in the case of the host-free complex.

3.3 Catalase activity

The catalase activities of the host-free and immobilised complexes were tested by meas-
uring the absorption band of hydrogen peroxide at 240 nm with a Cu$^{2+}$:H$_2$O$_2$ ratio of
Material & $v_o \times 10^5$ (mM min$^{-1}$)$^a$ & $v_o/v_{uncat}^b$ \\
Cu,Zn complex & 32.9 & 13.7 \\
SG–h–Cu,Zn & 1506.7 & 627.8 \\
SG–c–Cu,Zn & 234.8 & 97.8 \\
Mont–i–Cu,Zn & 808.6 & 336.9 \\
Cu-tren complex & 36.8 & 15.3 \\
SG–h–Cu-tren & 63.5 & 26.5 \\
SG–c–Cu-tren & 27.2 & 11.3 \\
Mont–i–Cu-tren & 40.6 & 16.9 \\

$^a$ The standard error was within ±1%. 
$^b$ $v_{uncat}$ = uncatalysed oxidation of DTBC under the same conditions.

| Table 1 | Initial rates of DTBC oxidation catalysed by the host–free and immobilised complexes (SG–h–...= immobilisation on silica gel via hydrogen bonding, SG–c–...= immobilisation on silica gel via covalent links, Mont–i–...= immobilisation in montmorillonite by ion exchange; the reaction mixture always contained the same amount of Cu$^{2+}$). |

| Fig. 8 | Catecholase activity of the Cu,Zn complex and its immobilised forms described by the concentration of DTBQ versus time plot ([DTBC]=10 mM, [Cu$^{2+}$]=0.1 mM, T=298 K). |

1:96 and copper(II) concentration of 0.21 mM. The pH value was kept at 6.9 adjusted with the aqueous phosphate buffer. It was found that the Cu,Zn complex and its immobilised forms had no catalase activity under these reaction conditions. Nevertheless, the host-free and the immobilised Cu-tren complexes performed well in hydrogen peroxide decomposition. In these cases, decrease in absorbance at 240 nm and oxygen evolution were observed. The conversion and initial rate values (calculated from the concentration
of $\text{H}_2\text{O}_2$ versus time graph) are given in Table 2 and Fig. 9.

| Material            | Conversion (%) | $v_o$ (mM min$^{-1}$)$^a$ |
|---------------------|----------------|---------------------------|
| Cu-tren             | 48.1           | 7.5                       |
| SG–h–Cu-tren        | 22.0           | 2.1                       |
| SG–c–Cu-tren        | 42.4           | 3.7                       |
| Mont–i–Cu-tren      | 31.9           | 5.1                       |

$^a$ The standard error was within $\pm$1%.

Table 2 Conversion and initial rate values for disproportionation of $\text{H}_2\text{O}_2$ by the host-free and immobilised Cu-tren complexes (the reaction mixture always contained the same amount of Cu$^{2+}$).

Fig. 9 Catalase activity of the host-free and immobilised Cu-tren complexes described by the conversion (calculated for 120 min reaction time) and initial rate values for disproportionation of $\text{H}_2\text{O}_2$.

The host-free Cu-tren complex was the most active material in hydrogen peroxide decomposition. Somewhat higher activity was found than for the copper(II)-containing complexes previously reported [13]. The $\text{H}_2\text{O}_2$ decomposition rates decreased upon immobilisation regardless whether silica gel or montmorillonite was used. Conversion values provide with the following reactivity order:

Cu-tren > SG–c–Cu-tren > Mont–i–Cu-tren > SG–h–Cu-tren

The reactivity sequence differs somewhat when the initial rates are compared:

Cu-tren > Mont–i–Cu-tren > SG–c–Cu-tren > SG–h–Cu-tren

On the basis of the initial rates, Mont–i–Cu-tren is more active than SG–c–Cu-tren, but its conversion value is lower. Perhaps dioxygen leaving was sterically hindered from
montmorillonite, which is a more closed system due to the smaller pore size than silica gel.

4 Conclusions

In the present paper the catecholase and the catalase activities of the host-free and immobilised copper(II)-containing complexes, termed Cu,Zn complex and Cu-tren, were investigated. These complexes were immobilised on silica gel (via hydrogen bonding or covalently) and in montmorillonite (by ion exchange). On the basis of the results of FT–IR spectroscopy, atomic absorption spectroscopy and thermogravimetry, it was found that immobilisation was in all cases successful. Each complex showed catecholase activity; moreover, the activity increased considerably on immobilisation of the Cu,Zn complex on silica gel via hydrogen bonds (SG–h–Cu-tren). The Cu,Zn complex and its immobilised forms were completely inactive in the hydrogen peroxide decomposition (lack of catalase activity), but the host-free and supported Cu-tren complexes were active, although, (omit comma) the activity decreased upon immobilisation.

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