Catalytic Activity of Copper(II) Enzyme-like Catalysts, Prepared by Molecular Imprinting Technique in Oxidation of Phenols

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Summary: Molecularly imprinted polymer copper(II) catalysts were prepared by suspension polymerization of 4-vinylpyridine, trimethylolpropane trimethacrylate (series A) and additional monomer – acrylonitrile (series B) in the presence of Cu(II) ions and template: 4-metoxybenzyl alcohol; two samples were also prepared by surface molecular imprinting technique utilizing W/O emulsion. The catalytic activity was tested in model oxidation reactions of hydroquinone and 2,5-di-*tert*-butylhydroquinone using hydrogen peroxide. The imprinted catalysts were more effective in both reactions than non-imprinted but their activity strongly depended on Cu(II) loading. Surface imprinted samples showed the highest activity (L_H up to 100%).

Keywords: biomimetic; molecular imprinting; metal-polymer complexes; phenols oxidation

Introduction

For many years the enzymes have constituted an inspiration for researching into their synthetic equivalents. Their unique properties (remarkable region- and stereoselectivity, high specificity, great rate acceleration, mild reaction conditions) are ascribed to well-organized three-dimensional structures with both catalytic and binding sites for a target molecule. But at the same time the practical use of enzymes is often limited by their sensitivity and instability against pH, temperature, organic solvents etc. Therefore the synthesis of organic compounds possessing specific recognition centre and mimicking natural biomolecules have been recently of great interest for organic chemistry and pharmacy.

One of the most promising approaches to design biomimetic molecules with artificial binding sides able to specific recognition of target molecules is molecular imprinting technique (MIT).[1–4] This process involves formation of the complex between functional monomer and template molecule and, in the presence of cross-linking agent, the complex polymerization. Removal of the template leads to the remaining of the specific recognition cavities in the polymer matrix, spatially and functionally compatible with the target molecule,. The molecules such as catalytic reaction product or its analogue may be used as the template. However, to obtain the most active catalyst the transition state analogue (TSA) is preferred.[3] Depending on the polymerization method and the template molecule, various driving forces are applied for binding of template to functional monomer: hydrogen bonds,[3,5] covalent bond[3] and metal coordination.[6,7] In recent years molecular imprinting technique has been applied to obtain molecular imprinting polymer (MIP) metal-complex catalysts possessing unique properties in comparison to the simple metal-complex catalyst (Scheme 1). They are attached to solid and rigid material, surrounded by polymer matrix with shape-selective space close to metal ion. The

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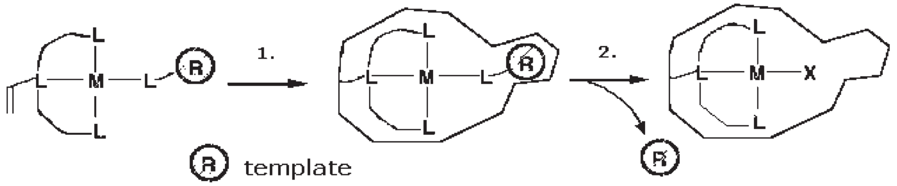


Scheme 1.
Schematic representation of molecular imprinting of metal complex. Template molecule constituted ligand-® coordinated to metal centre: (1) polymerization of metal-template ligand complex; (2) removing of template molecule (X-coordinating group).

nature of metal ion and its interactions with ligands within an active centre determine the activity and selectivity of the designed catalysts.[6] Tada and Iwasawa reviewed the novel metal-complex catalysts prepared by molecular imprinting polymerization.[8]

MIP catalysts, like the enzymes, show high regio- and stereoselectivity. They can utilize the same interactions that are presented in biological systems and accelerate the reaction rate. Additionally, they have more rigid structure and are very tolerant to conditions damaging biopolymers structure.[4] However, there are still some challenging basic problems to be resolved i.e. slow reaction kinetics due to slow inner diffusion of imprint molecules towards the active centres located deeply inside the polymer matrix. To overcome this problem Uezu and coworkers proposed *surface molecular imprinting technique*.[9–13] The imprinted polymer was obtained by polymerization of W/O (water-in-oil) emulsion with host molecule, template and crosslinking monomer. In this method W/O emulsion organic-aqueous interface was utilized as a recognition field for target molecule. Orientation of functional molecule was fixed at oil-water interface. After polymerization, the recognition sites complementary to the template were situated at the inner cavity surface of obtained polymer. Also in recent years, growing interest on polymers obtained using surface active monomers was observed. The presence of surfmer in polymer structure increases emulsion stability during polymerization and on the other hand helps to make imprinting more effective by interactions among various functional groups.[14]

The aim of this work is the synthesis of traditional and surface molecularly imprinted polymer catalysts containing complexed Cu(II) ions and determination of their catalytic activity in model oxidation reactions of hydroquinone or 2,5-di-*tert*-butylhydroquinone using hydrogen peroxide. In our studies we would like to combine promising surface imprinting technique with application of surface active monomer.

Experimental Part

Materials

All used materials were purchased from Sigma-Aldrich. 4-vinylpyridine was purified by distillation prior to use and other chemicals and solvents were of analytical grade. Oxidation substrates: hydroquinone and 2,5-di-*tert*-butylhydroquinone from Aldrich (99% GC) were used.

Preparation of Imprinted Polymers

Two series of molecularly imprinted polymers (MIPs) were prepared by suspension polymerization of 4-vinylpyridine (VP), trimethylolpropane trimethacrylate (TMPMA) (series **A**) and additional monomer – acrylonitrile (AN) (series **B**) in the presence of cyclohexanol (Table 1). Prepolymerization copper(II) complex was prepared by mixing the functional monomer VP with imprinting molecules: 4-metoxybenzyl alcohol (MBA) and Cu(II) ions ($Cu(CH_3COO)_2 \cdot H_2O$) in cyclohexanol. Then the rest of monomers and the initiator: benzoyl peroxide (BPO; series **A**) or 2,2′-azobis-isobutyronitrile (AIBN; series **B**) was added and organic phase was suspended in 1.0 wt.% of polyvinyl alcohol

Table 1.
The composition and characteristics of copolymers.

Sample	Monomers		Cu(II)	Cu(II)/MBA	υ	W	Z_N
	Symbol	vol.%	mmol/g	mmol/mmol	vol/vol	g/g	mmol/g
A-1	VP,	7	0.13	0.45	1.4	2.15	0.39
	TMPMA	93					
A0-3	VP,	10	—	—	0.9	0.70	0.65
A0-4	TMPMA	90	—	—	2	1.76	0.54
A-6			0.05	0.10	0.9	0.16	0.49
B0	VP,	5	—	—	1.8	2.75	8.29
B-7	TMPMA,	40	0.08	0.44	1.8	3.20	7.20
B-8	AN	55	0.03	0.20	1.8	3.15	7.20
B-9[a)]			0.03	0.20	1.8	2.25	5.56
B-10[b)]			0.03	0.20	1.8	2.29	6.18

[a)] 0.2 wt.% VOBS
[b)] 0.5 wt.% VOBS
Cu(II) – amount of copper given to polymerization (per organic phase), υ – diluents to monomers ratio, W – water regain, Z_N – nitrogen content

(PVA) solution containing sodium chloride. After purging the mixture with gaseous nitrogen, polymerization was carried out at temperature ranged from 60 to 95 °C for 10 hours with stirring at 240 rpm. Obtained beads were washed with acetone and 1 M HCl solution repeatedly until colorless solution was seen. The water regain W (g/g) was measured using centrifugation technique (5 min, 3000 rpm). Nitrogen and copper(II) content was checked by elemental analysis (Table 1). The FT-IR spectra were recorded on SPECORD M-80 CARL ZEISS JENA (KBr pellets). For both series non-imprinted blank samples were prepared using the same procedure. Two samples of series **B** surface molecular imprinted polymers were prepared utilizing W/O emulsion. Cu(II) ions solution in acetate buffer (pH 4.7) was added to organic phase composed of VP, TMPMA, AN, MBA, surface active monomer 4-vinyloxybutylstearate (VOBS; 0.3–0.8 wt.%) and cyclohexanol and then rapidly mixed for 20 min to obtain W/O emulsion. After adding AIBN initiator, the mixture was polymerized under a flow of nitrogen using the procedure mentioned above.

Preparation of Catalysts

Sorption of Cu(II) ions was performed using batch method.[15–17] The resins were swollen in distilled water then placed in 20 ml of copper acetate solution (5×10^{-4} and 5×10^{-3} M) in acetate buffer pH 5.0. After 48 h shaking at room temperature and separation of the loaded samples, the Cu(II) concentration in solution was determined by atomic absorption spectrophotometry (AAS) on a AAS-30 Perkin Elmer Aanalyst 100. Sorption of copper S (mmol/g) was calculated from the difference of Cu(II) concentration in solution before and after sorption. The structure of Cu(II) active centre was studied by EPR method at 77 K on swollen samples after filtration and air-drying. The EPR spectra were recorded on a Bruker ESP 300E spectrometer operating at X-band frequency and equipped with the Bruker NMR gaussmetter ER 035M and the Hewlett-Packard microwave frequency counter HP 5350B. The spectra were analyzed by computer simulation using Bruker's WIN-EPR SimFonia software.

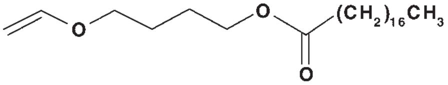
Oxidation Reactions

Catalysts swollen in 20 ml hydrogen peroxide solution (5.4×10^{-2} mol/l in acetate buffer pH 5.0) were placed in polyethylene flasks. Mixture was purged with gaseous nitrogen then 20 ml of 4×10^{-3} mol/l hydroquinone (H_2Q)[15,16] or 1×10^{-3} mol/l 2, 5-di-*tert*-butylhydroquinone ($DTBH_2Q$, series A) was added. The Cu(II) to substrate molar ratio was 1:10. The entire mixture was shaken (350 cycles/min) in water bath at 35 °C (H_2Q) or at room temperature ($DTBH_2Q$) for 80 min. Samples were taken at given time interval and diluted with distilled water or ethanol for H_2Q and $DTBH_2Q$, respectively. The concentrations of unreacted substrates and products: *p*-benzoquinone (Q) and 2,5-di-*tert*-butylbenzoquinone (DTBQ) were determined by UV-Vis spectrophotometry using Jasco 570c spectrophotometer. Characteristic absorption maxima were: $\lambda = 289$ nm for H_2Q, $\lambda = 246$ nm for Q, $\lambda = 292$ nm for $DTBH_2Q$ and $\lambda = 255$ nm for DTBQ. Oxidation degree of substrate (L_S; %) and yield of product (Y_P; %) were defined as a decrease of substrate concentration and an increase of product concentration, respectively, with relation to the initial substrate concentration. Selectivity of product (S_P; %) was defined as a ratio of product concentration to the reacted substrate concentration.

Results and Discussion

Synthesis and Characteristic of Catalysts

Two series of molecularly imprinted polymers with different composition were prepared by suspension polymerization. Series **A** composed of 4-vinylpyridine (VP) and trimethylolpropane trimethacrylate (TMPMA) copolymers made up with Cu(II) ions and 4-metoxybenzyl alcohol (MBA) as a template or without imprint (blank samples A0). Conception of such system modelling was based on interactions between Cu(II) ions, pyridine nitrogen and hydroxyl group of metoxybenzyl alcohol. Additionally, MBA molecules were well solvated by cyclohexanol, thus creating porous structure during the polymerization of VP and TMPMA. The use of TMPMA matrix guaranteed porous structure of support with pores complementary to the template shape and with higher hydrophilicity than in the copolymers with traditionally used DVB as a crosslinker.[18] After washing with hydrochloric acid, weak interactions between VP and Cu(II) were destroyed, leaving imprints free. Adding of acrylonitrile (AN) to monomer mixture (series **B**; samples B-5 to B-8) was aimed to preserve the rigid, porous structure and further planned modification of acrylonitrile to amino groups. The composition and characteristics of obtained supports are presented in Table 1. To synthesize surface imprinted polymer samples (B-9, B-10) W/O emulsion was prepared from VP, AN, TMPMA, MBA, Cu(II) ions and surface active monomer - 4-vinyloxybutylstearate (VOBS; **1**). Addition of higher amounts of VOBS increased stability of W/O emulsion during synthesis and facilitated metal ions removal.



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The structure of supports was confirmed using FT-IR method. In all samples a strong peak at about 1724 cm^{-1} exists as the result of C=O group vibration of TMPMA (Figure 1). After addition of acrylonitrile, a peak at 2242 cm^{-1} attributed to nitrile C=N group is observed (Figure 1, B0). The presence of Cu(II)-VP complex is confirmed by shift the bands characteristic for pyridine, to 1652 cm^{-1} (C=N) and to 1596 cm^{-1} (CH=CH). Additionally, the C=N band reveal significant increase of intensity. In the region of 820–740 cm^{-1} the peaks characteristic for aromatic C–H deformation vibration of pyridine ring appear (Figure 1, arrows).

Sorption of copper(II) ions led to catalysts with Cu(II) loading ranged from 0.02 to 0.39 mmol/g. The Cu(II) ions may be coordinated to pyridine nitrogen donor and/or oxygen donors (from hydroxyl or carboxyl group formed during TMPMA hydrolysis in polymerization process or from water molecule). Sorption of Cu(II) higher than pyridine ligand concentration is connected with physical absorption in the porous structure as well as chemisorption on polar TMPMA ester structure. The EPR spectra are typical of the tetragonal symmetry of the complexes formed between Cu(II) ions and functional groups of supports (Figure 2). An anisotropic

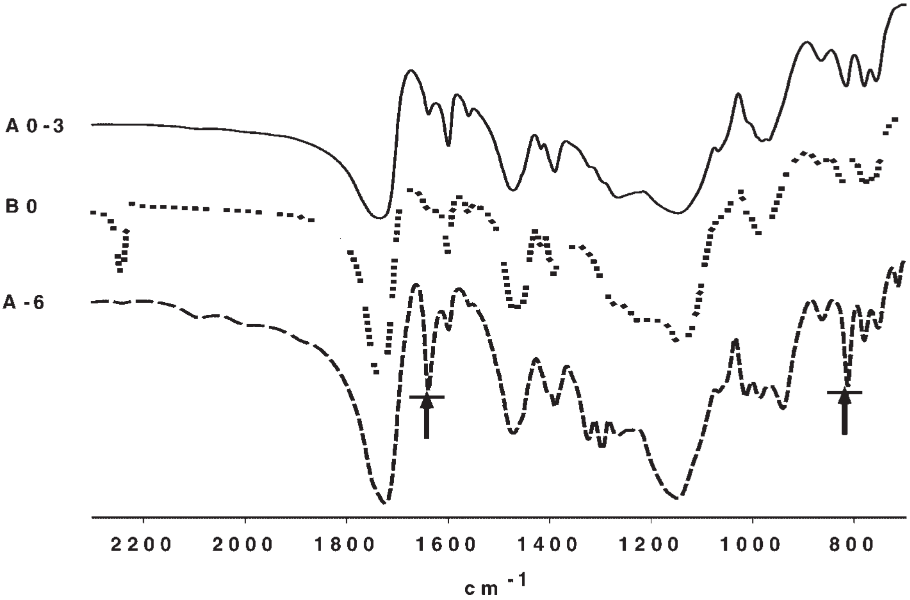

Figure 1.
FT-IR spectra of chosen samples: A0-3, B0, A-6.

character of the spectra at room temperature confirms the presence of Cu(II) complexes immobilized by ligands of the polymer matrix. As an example, at neutral pH the EPR parameters for sample B-7 ($A_{||} = 160 \times 10^{-4}$ cm^{-1}, $g_{||} = 2.300$) and B-9 ($A_{||} = 168 \times 10^{-4}$ cm^{-1}, $g_{||} = 2.313$) are close to those assigned to the N2O2 donor set in Cu(II) plane due to coordination of two monodentate pyridine ligands [19] incorporated into polymer matrix (Figure 3). Similar complex is formed in sample B-10 as indicated by $A_{||} = 167 \times 10^{-4}$ cm^{-1}, $g_{||} = 2.303$ (Figure 7).

Catalytic Activity in Model Reactions

As it results from our previous investigations on enzyme-like catalysts with aminoguanidyl ligands, the catalytic activity depends not only on specificity of metal

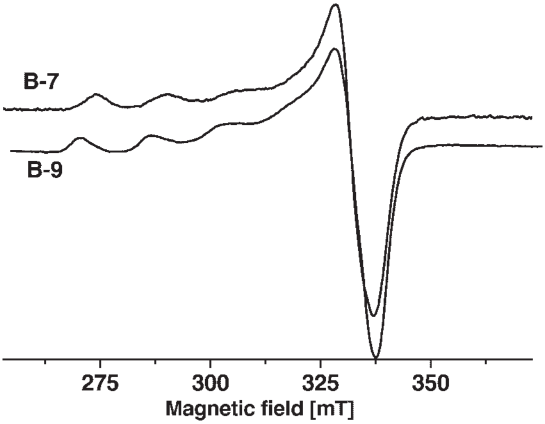

Figure 2.
EPR spectra of the Cu(II) complexes in B-7 and B-9 catalysts at 77 K.

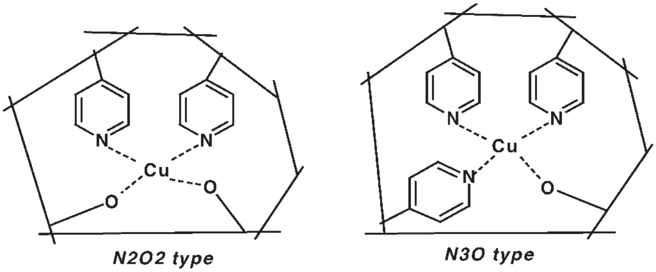

Figure 3.
The structures of Cu(II) complexes (charges are omitted).

complex inside the active centres but also on the complex environment formed by free groups.[16] The catalytic activity of all samples was measured in model oxidation reaction of hydroquinone (H_2Q) to *p*-benzoquinone (Q) using hydrogen peroxide (Figure 4). This reaction is appropriate to characterize the catalytic properties of the tested samples due to mild reaction conditions, short time and simple measurement procedure. Details concerning the UV-Vis spectra parameters and catalytic oxidation were discussed earlier.[15]

The results of hydroquinone oxidation are given in Figure 5 and 6. Oxidation degree after 60 min ranged from 11 (blank sample) to 100% (for surface imprinted sample) depending on catalyst type. Catalysts obtained with MBA as a template were more active in the tested reaction. Molecular imprinting technique led to higher oxidation degree and benzoquinone yield. However, for almost all samples from series **A** an improvement of reaction selectivity was unsatisfactory: $S_Q = 26\%$ for blank sample A0-4, $S_Q = 75\%$ for sample A-1. Catalytic activity observed for the samples with small Cu(II) loading seems to be mainly due to complex formation. But when sorption of copper was high (sample A0-4; 0.39 mmol/g) the catalytic properties ($L_H = 11\%$) may be also caused by uncomplexed Cu(II) ions.[15] Low activity of sample A-6 results probably from small amount of solvents used during polymerization.

Addition of acrylonitrile (series **B**) increased significantly the loss of hydroquinone as well as reaction selectivity (from 43% for B0-5 to 100% for B-10) in comparison to series **A**. It was probably due to increasing porosity and high polarity of CN groups, which strengthened substrate–support interactions. The catalytic activity of all samples strongly depends on Cu(II) loading (Figure 6). The samples with small amounts of well isolated copper active centres (lower loadings) showed the highest activity.

The samples B-9 and B-10 prepared in the presence of surface active monomer VOBS showed higher activity than the

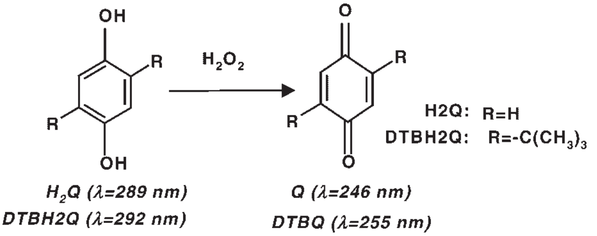

Figure 4.
Scheme of hydroquinone and 2,5-di-*tert*-butylhydroquinone oxidation.

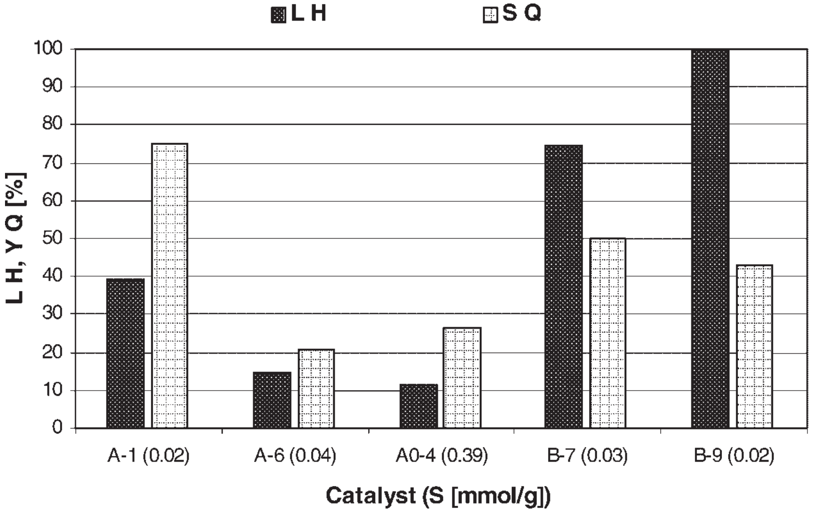

Figure 5.
Influence of catalyst type on loss of hydroquinone (L_H) and selectivity of *p*-benzoquinone (S_Q) (S – Cu(II) loading, blank sample – A0).

remaining samples (Figure 5). An increase of surfmer amount led to more stable W/O emulsion during synthesis and to increasing stability of Cu(II)-ligands complexes at the polymer beads surface causing improved activity of the catalyst. Oxidation degree for sample B-10 (Cu(II) loading 0.02 mmol/g) and selectivity were 85% and 100%, respectively. Unfortunately, in the case of sample B-10 after hydroquinone oxidation, a partial destruction of active centres took place which is seen in the EPR spectra of catalysts after reaction (Figure 7); the spectrum intensity is about four times smaller than that for the

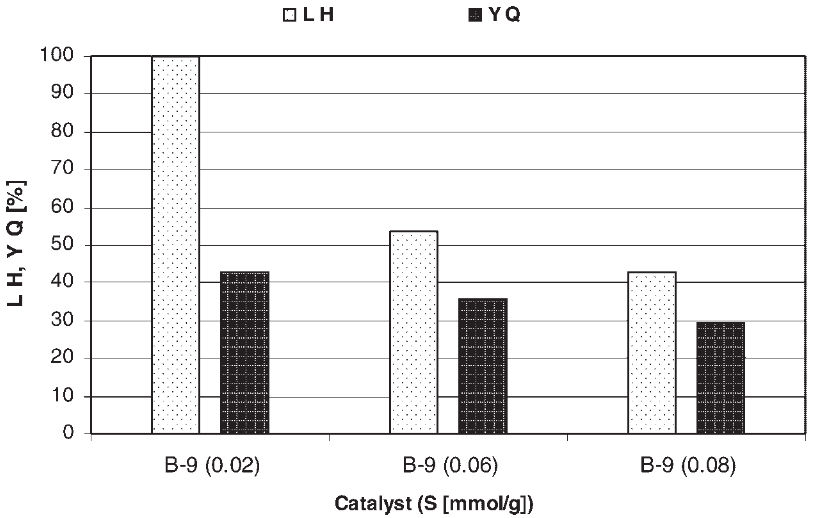

Figure 6.
Influence of Cu(II) loading (S) on loss of hydroquinone (L_H) and yield of *p*-benzoquinone (Y_Q) for catalyst B-9.

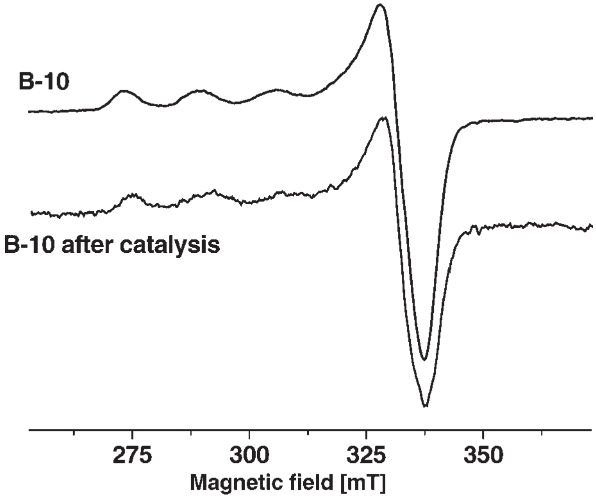

Figure 7.
EPR spectra of B-10 catalyst (S = 0.02 mmol/g) before and after hydroquinone oxidation. The spectrum of the sample after catalysis is about four times amplified as compared to the spectrum of B-10 sample.

initial B-10 sample. It suggests a decrease of Cu(II) complex concentration. Furthermore, the change of EPR parameters for B-10 sample after catalysis is observed. The parameters $A_{||} = 171 \times 10^{-4}$ cm^{-1}, $g_{||} = 2.289$ are suggests N3O donor set of the ligands around Cu(II) plane. These effects may be caused by washing out of Cu(II) ions or reorganization of imprints during catalysis. Further work on this topic should be carried out.

The catalytic activity of catalyst series **A** was also measured in a new model oxidation reaction of 2,5-di-*tert*-butylhy-

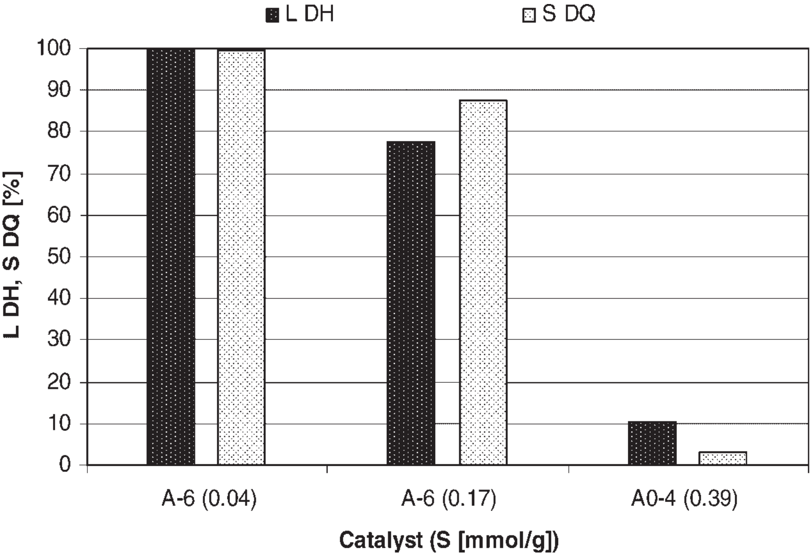

Figure 8.
Influence of catalyst type on loss of 2,5-di-*tert*-butylhydroquinone (L_{DH}) and selectivity of 2,5-di-*tert*-butylbenzoquinone (S_{DQ}) (S – Cu(II) loading, blank sample – A0).

droquinone ($DTBH_2Q$) to 2,5-di-*tert*-butylbenzoquinone (DTBQ) using hydrogen peroxide (Figure 4). The $DTBH_2Q$ was easier oxidized than H_2Q, which made more difficult the selection of an efficient catalyst. Even sample A-6 almost not active in hydroquinone oxidation showed high catalytic activity towards $DTBH_2Q$. The prepared catalysts were more effective in second model reaction: oxidation degree was close to 100% (for sample A-6) with almost 100% selectivity. The presence of imprints with active centres improved significantly catalytic activity of tested samples (Figure 8). One can see a weaker influence of Cu(II) loading on activity of catalysts in $DTBH_2Q$ oxidation.

Conclusion

The catalysts prepared using molecular imprinting technique with Cu(II) ions and 4-metoxybenzyl alcohol exhibited high activity in both model diphenols oxidation reactions. Their activity depended on copolymer composition, its structure and Cu(II) loading. Activity of MIP-samples was improved by addition of other mer – acrylonitrile. Utilizing surface imprinting technique with 4-vinyloxybutylstearate as a polymerizing surfactant is profitable because it stabilizes W/O emulsion during synthesis and gives additional interactions in the polymer matrix. The surface imprinted polymer-catalysts constitute promising target for further investigation due to their efficiency in model reaction. However, an appearance of deformation of imprinted cavities in the most effective samples needs to be examined in details. Very interesting issue for further investigation is also the possibility of surface transformation of CN groups into amino ones under mild conditions without changing porous structure of the polymer.

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[1] K. Mosbach, O. Ramström, *Bio/Technology* **1996**, *14*, 163.
[2] W. B. Mortherwell, M. J. Bingham, Y. Six, *Tetrahedron* **2001**, *57*, 4663.
[3] G. Wulff, *Chem. Rev.* **2002**, *102*, 1.
[4] C. Alexander, L. Davidson, W. Hayes, *Tetrahedron* **2003**, *59*, 2025.
[5] K. Haupt, K. Mosbach, *Chem. Rev.* **2000**, *100*, 2495.
[6] K. Severin, *Curr. Opin. Chem. Biol.* **2000**, *4*, 710.
[7] S. Striegler, *J. Chromatogr. B.* **2004**, *804*, 183.
[8] M. Tada, Y. Iwasawa, *J. Mol. Catal. A: Chem.* **2003**, *199*, 115.
[9] E. Toorisaka, K. Uezu, M. Goto, S. Furusaki, *Biochem. Engeen. J.* **2003**, *14*, 85.
[10] M. Yoshida, Y. Hatate, K. Uezu, M. Goto, S. Furusaki, *Colloid. Surf. A* **2000**, *169*, 259.
[11] K. Tsukagoshi, K. Yu Yu, M. Maeda, M. Takagi, T. Miyajima, *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3095.
[12] M. Yoshida, K. Uezu, M. Goto, S. Furusaki, *J. Appl. Polym. Sci.* **1998**, *73*, 1223.
[13] E. Toorisaka, M. Yoshida, K. Uezu, M. Goto, S. Furusaki, *Chem. Lett.* 1999, 387.
[14] I. Fujiwara, M. Maeda, M. Takagi, *Anal. Sci.* **2000**, *16*, 407.
[15] I. Owsik, B. N. Kolarz, *J. Mol. Catal. A: Chem.* **2002**, *178*, 63.
[16] I. A. Owsik, B. N. Kolarz, *Catal. Today* **2004**, *91–92*, 199.
[17] I. A. Owsik, B. N. Kolarz, D. Jermakowicz-Bartkowiak, J. Jegierska, *Polymer* **2003**, *44*, 5547.
[18] B. N. Kolarz, D. Jermakowicz-Bartkowiak, A. Trochimczuk, *Eur. Polym. J.* **1997**, *34*, 1191.
[19] G. P. Vishnevskaya, L. S. Molochnikov, R. Sh. Safin, "*EPR in ion exchangers*", 'Nauka', Moscov 1992, p. 88.