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Miniemulsion polymers as solid support for transition metal catalysts

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ABSTRACT

A pentadentate salen-type ligand was immobilized in a poly[(styrene)]-*co*-(butyl acrylate)] matrix by miniemulsion polymerization. The obtained polymer beads revealed a particle size of 50 nm in the dry state by transmission electron microscopy. Dynamic light scattering experiments in methanol and water showed a solvent-dependent average particle size with a mean particle diameter of up to 233 nm in methanol. These results provide valuable insights for the optimization of macromolecular oxidation catalysts and their future use as enzyme-like entities in aqueous media. The particle stability was demonstrated over a wide pH range (3–11) by gel permeation chromatography, and initial results for the metal ion binding ability were obtained.

1. Introduction

In the last decade, considerable interest has been given to the immobilization of transition metal complexes combining the advantages of homogeneous catalysts with those of their heterogeneous counterparts [1–5]. Highly cross-linked, but soluble polymers prepared by emulsion or miniemulsion polymerization were recently introduced as solid support for such catalysts [6–14].

Miniemulsion polymers offer several advantages over other commonly used solid supports, such as ease in their preparation [10]; the use of environmentally benign aqueous solution during their synthesis; low viscosity at high molecular weight; robustness due to a highly cross-linked matrix with a large surface area; very low sensitivity to impurities during preparation; and most importantly, uniformity of the particles in shape, size, size distribution, and composition [15,16]. The size of particles prepared by miniemulsion polymerizations is typically between 30 and 500 nm and depends directly on the amount of surfactant [16]. Therefore, transition metal catalysts immobilized on these soluble, submicron particles offers a very promising new strategy in achieving enzyme-like transformations of substrates in green solvents (including water) that were not accessible before [12,13,17–20].

We and others have hypothesized that the macromolecular environment of a catalyst contributes to its overall catalytic performance [7,21]. As a proof of this concept, we prepared a macromolecular catalyst based on a binuclear Cu(II) complex by miniemulsion polymerization. A salen-type pentadentate ligand *N*, *N*′-1,3-bis[(2-hydroxy-4-vinylbenzyloxy)benzylideneamino]propan-2-ol (**1**) was co-polymerized with styrene (**2**) and butyl acrylate (**3**) by miniemulsion polymerization (Fig. 1) [7]. Binding of Cu(II) ions to the immobilized pentadentate ligand **1** activated the dormant catalyst polCu_2**1** thereafter [7].

Excellent catalytic performance of polCu_2**1** was observed for the oxidation of 3,5-di-*tert*.-butyl catechol into 3,5-di-*tert*.-butyl quinone in methanol and compared to the performance of its small molecular weight analog, complex Cu_2**1** [7]. The oxidation was about 460,000-fold accelerated over the background reaction (without catalyst) when using polCu_2**1** as the catalyst, but was only about 60,000-fold accelerated when using the small molecular weight entity Cu_2**1** under identical conditions [7]. Further evaluation of the small molecular weight complex in aqueous solution revealed excellent catalytic performance of the binuclear catalysts with a reaction acceleration of up to 160,000-fold over the background [22]. However, the catalytic oxidation ability of Cu_2**1** is about one order of magnitude higher in a macromolecular environment than in free solution [7], which distinguishes the prepared entities from other work on immobilized transition metal catalysts.

This finding prompted us to conduct a detailed characterization of the polymer matrix prior to any further use of such macromolecular catalysts for reactions performed in methanol, or eventually, in aqueous solution. We therefore give herein a detailed report on the synthesis of the poly[(styrene)]-*co*-(butyl acrylate)–**1**] microgel beads and their control polymers, the characterization of the polymer size distribution in methanol and water, and an account for the investigation of the polymer stability in acidic and alkaline solutions. Furthermore, we evaluated the metal binding properties

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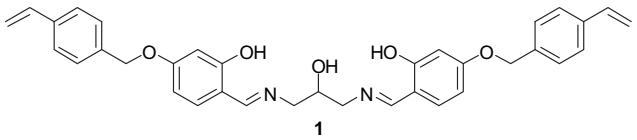

Fig. 1. Structure of the pentadentate salen-type ligand **1**.

in methanol, and while metal binding properties in aqueous solution are still a topic of ongoing investigations, some preliminary data on this matter are also included.

2. Experimental details

2.1. Reagents

All chemicals were reagent grade or better and were used without further purification unless stated otherwise. Styrene (**2**), butyl acrylate (**3**), methyl acrylate (**4**), pyrocatechol and potassium persulfate (KPS) were obtained from Acros Organics. Sodium dodecyl sulfate, dimethyl sulfoxide (DMSO), Tween 20 and decane were obtained from Sigma Aldrich. All monomers were distilled immediately prior to use. The polymerizable ligand *N*, *N*′-1,3-bis[(2-hydroxy-4-vinylbenzyloxy)benzylideneamino]propan-2-ol (**1**), [7] and its water-soluble analog *N*, *N*′-1,3-bis[{2-hydroxy-4-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}benzylideneamino]propan-2-ol, TEGbsdpo, **1a**, [22] were synthesized as described.

Nanopure water was obtained from an EASYpure® II water system from Barnstead (18.2 MΩ/cm). The 200 mesh carbon or formvar coated Cu grids for TEM measurements were obtained from Electron Microscopy Sciences. The dialysis membrane Spectra/Por Biotech (16 × 10 mm; 0.79 ml/cm; volume/length) had a molecular cutoff of 15,000 and was obtained from Spectrumlabs; Micropore ultracentrifugal filters from Amicon with a cutoff of 10,000 were used for ultracentrifugation experiments.

2.2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer (400.2 MHz for ^{1}H and 100.6 MHz for ^{13}C). Chemical shifts (δ) are expressed in ppm. Deuterated acetone was used as solvent, and the chemical shift values are reported relative to the residual signals of this solvent ($\delta = 2.05$ ppm for ^{1}H NMR, and $\delta = 29.9$ ppm for ^{13}C NMR). IR spectra were recorded using a Shimadzu IR Prestige-21 FT-Infrared Spectrometer with IR solution software Vers. 1.10 as thin films in CCl_4 on a KBr disc; ν in cm^{-1}. Atomic absorption spectroscopic (AAS) data were acquired on a Varian AA240 flame atomic absorption spectrometer using the SpectraAA version 5.01 PRO software. Isothermal titration calorimetry (ITC) was performed on a VP isothermal titration calorimeter (Microcal® Inc, Northampton, MA) at 300 K, and the reference cell was loaded with nanopure water. Electrospray Ionization (ESI) Mass spectrometric experiments were performed on a Q-ToF Premier (Waters) on positive ion mode by injecting a sample aliquot directly into the ion source and scanning between 100 and 700 Da. The UV/Vis spectra were recorded on a Cary WinUV, Vers. 3.0, Analysis Suite with Suprasil standard cells (200–2000 nm) of 1 cm thickness and 4.5 ml volume at 30 °C over a range of 200–900 nm. The pH value was measured using a Beckman Φ 250 pH meter equipped with refillable long Futura pH electrode of 0.7 mm thickness. The pH meter was calibrated before each set of readings with standard buffer solutions for pH 4, 7 and 10.

Transmission Electron Microscopy (TEM) imaging was performed in the Advanced Microscopy & Imaging Laboratory at Auburn University on a Zeiss EM 10C 10CR Transmission Electron Microscope operating at 60 kV. Dynamic light scattering (DLS) experiments were performed with a submicron particle size analyzer (Model 380, PSS Santa Barbara, USA) equipped with a He–Ne laser source at 632.8 nm at a fixed scattering angle of 90°. The data were analyzed using the intensity-weighted Nicomp distribution fitting provided by the NICOMP software package version 1.60. Samples for elemental analysis were sent to Atlantic Microlab Inc., Atlanta, GA. Samples for the determination of the molecular weight distribution by gel permeation chromatography (GPC) were sent to the Materials Research Laboratory at the University of California, Santa Barbara, CA or obtained on a Shimadzu SEC system with a Phenogel column 10^3 (300 × 7.8 mm, 5 μm) in THF with a flow rate of 1 mL/min and UV–Vis detection at 254 nm.

2.3. Miniemulsion polymerization, typical procedure

The poly(acrylate–styrene)–**1** beads (pol**1**) were prepared by mixing of 3 g (23.4 mmol) butyl acrylate, 3 g (28.8 mmol) styrene and 500 mg decane with 48 mL water containing 140 mg (0.5 mmol) sodium dodecyl sulfate (SDS). Ligand **1** (140 mg, 0.24 mmol) dissolved in 1 g of DMSO was added and the resulting mixture stirred vigorously for 1 h followed by sonication for 2 min. The polymerization was initiated at 72 °C by addition of 4 mL aqueous potassium peroxysulfate (200 mg, 0.74 mmol) solution and was allowed to proceed for 3 h. The solution of the miniemulsion was separated from a gummy precipitate that formed in the presence of **1** around the magnetic stirrer. The CHN analysis of the precipitate did not reveal any N-content (found C 75.36, H 8.92, N 0.0%). The supernatant was divided into two portions (28 mL each) and purified by ultracentrifugation or dialysis as described below.

For control experiments, poly(acrylate–styrene) beads (pol$_{\mathbf{blank}}$) were prepared by the same procedure in the absence of ligand **1** (but in presence of the DMSO). The formation of a precipitate was not observed during the work-up procedure for the control polymer.

2.4. Polymer formation over time

The solid content during the formation of the miniemulsion polymers pol**1** and pol$_{\mathbf{blank}}$ was determined to follow the polymerization reaction and to establish a reasonable reaction time for all experiments. Towards this end, 2 mL sample aliquots of the polymerization mixtures were taken after 2, 4, 6, 10, 15, 20, 30, 45, 60, 120, and 180 min, treated with 2 mg pyrocatechol in order to terminate the reaction and allowed to dry for 2 weeks at ambient temperature. The remaining solid content was determined gravimetrically and corrected for the amount of pyrocatechol added. The polymer formation is given in weight percent of the solid formed and calculated by using equation (1),

$$P[\%] = \frac{m_{\text{solid}}}{m_{\text{Mo}} + m_{\text{E}}} \cdot \frac{m_{\text{all}}}{m_{\text{aliquot}}} \cdot 100 \qquad (1)$$

where m_{solid} is the mass of the remaining solid corrected for the pyrocatechol added, m_{Mo} corresponds to the mass of all used monomers, m_{E} equals the mass of the emulsifier, m_{all} equals the overall mass of all compounds and m_{aliquot} is the mass of the aliquot taken.

2.5. Miniemulsion polymerization, variation of the surfactant

The miniemulsions obtained after the addition of SDS (280 mg, 1.0 mmol) in two-fold molar amount relative to the original recipe

were not sufficiently stable and separated into two layers after 20 min. Likewise, phase separation was visible for the naked eye after 5 min when the SDS emulsifier was replaced by equimolar amounts (595 mg, 0.5 mmol) of Tween 20. Unstirred miniemulsions prepared with 70 mg (0.25 mmol) or 140 mg (0.5 mmol) of SDS were stable for at least 1 h.

2.6. Polymer purification by ultracentrifugation, typical procedure

The aqueous polymer miniemulsions prepared above were extracted twice with 16 mL of chloroform, and separated from the chloroform layer by centrifugation. The aqueous layer was then diluted with 20 mL of water and centrifuged, which also resulted in separation of a gummy residue at the bottom of the centrifuge tube. Elemental analysis of this second residue did not reveal any presence of the polymerizable N-containing ligand **1** (found: N 0.0%). The polymer emulsion was subsequently purified by 5 ultracentrifugation–dilution circles using micropore filters with a 10,000 MW cutoff and nanopure water. The aqueous polymer stock solution pol**1** had a final volume of 48 mL and a solid content of 18.2 mg/mL which was determined gravimetrically after freeze-drying a sample aliquot. The aqueous polymer stock solution of $\mathbf{pol_{blank}}$ had a final volume of 48 mL and a solid content of 29.7 mg/mL. The polymers purified by ultracentrifugation were used for the analysis of the chemical composition by NMR, IR, and elemental analysis.

2.7. Polymer purification by dialysis

Dialysis membranes with a molecular weight cutoff of 15 kDa and a nominal diameter of 10 mm were soaked in nanopure water for 30 min prior to immediate use and filled with 25 mL sample aliquots of the microgel stock solution. The tubing was subsequently closed and dialyzed against nanopure water three times for 8 h each. A sample aliquot (52 mL) of the purified miniemulsion polymer was diluted with the same volume of nanopure water. The nominal concentration of the ligand in the resulting polymer pol**1** is 2 mM. The control polymer $\mathbf{pol_{blank}}$ was treated identically. The polymers purified by dialysis were used for TEM imaging, GPC analysis, DLS analysis and metal binding studies.

2.8. Sample preparation prior to NMR analysis

A sample aliquot of pol**1** was freeze-dried and dissolved in acetone-d_6 prior for NMR analysis.

2.9. Sample preparation prior to IR analysis

The freeze-dried sample aliquots of pol**1** and $\mathbf{pol_{blank}}$ were dissolved in CCl_4 and measured as thin films on KBr disks.

2.10. Sample preparation prior to UV/Vis analysis

Freeze-dried sample aliquots of pol**1** and $\mathbf{pol_{blank}}$, and a sample aliquot of **1** were dissolved in THF for UV/Vis analysis.

2.11. Sample preparation prior to elemental analysis

Elemental analysis of freeze-dried sample aliquots of the miniemulsion was calculated for pol**1**: C 78.83, H 8.53 and N 0.11%; found: C 77.47, H 8.30 and N 0.29%; and for $\mathbf{pol_{blank}}$: C 77.5 and H 8.68%; found: C 77.34 and H 8.41%.

2.12. Sample preparation prior to GPC analysis

Dialyzed sample aliquots of pol**1** and $\mathbf{pol_{blank}}$ were suspended in aqueous solution and the pH was adjusted to 1, 3, 5, 7, 9, 11 and 13 using 0.1 M $HCl_{(aq)}$ or 0.1–1 M $NH_4OH_{(aq)}$. The solutions were equilibrated for 24 h. Subsequently, the solvent was removed by freeze-drying and the samples were equilibrated in THF for another 24 h, filtered by using 0.2 μm syringe filters and analyzed by gel permeation chromatography (GPC). The number average (M_n), the weight average (M_w), the *z* average (M_z) and the nominal molecular weight (M_p) of the polymer samples were determined in comparison to polystyrene standards. The polydispersity (D) of pol**1** and $\mathbf{pol_{blank}}$ was calculated from the values obtained ($D = M_w/M_n$).

2.13. Sample preparation prior to DLS analysis

A 100 μL sample aliquot of the polymer stock solution purified by dialysis was diluted into 20 ml of nanopure water or methanol immediately prior to analysis. All measurements were performed at ambient temperature.

2.14. Sample preparation prior to TEM imaging

For morphological observations by transmission electron microscopy (TEM), a 1 mL sample aliquot of the polymer stock solution purified by dialysis was diluted to a 100 mL solution with nanopure water. One drop (~5 μl) of the diluted solution was placed on a 200 mesh carbon or formvar coated Cu TEM grid and allowed to dry for 48 h prior to analysis. No additional contrasting was applied. The particle size distribution analysis from the TEM images was obtained by using the *ImageJ* program.

2.15. Sample preparation for metal ion binding

Typically, 10 mL of pol**1** were dialyzed against 100 mL of a 5–10 mM transition metal ion solution at ambient temperature for 12 h (Cu(II) 10.37 mM; Ni(II) and Mn(II) 5.37 mM). The use of 10 mM Ni(II) or Mn(II) solutions led to polymer precipitation. The excess of unbound metal ions in the miniemulsion polymer was then removed by repeated dialysis against 1 L of nanopure water. Sample aliquots (200 μL) of the metal ion loaded polymer polM_2**1** (M = Cu(II), Ni(II) or Mn(II)) were taken after 0, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h and subjected to AAS analysis. The nanopure water for dialysis was replaced in 1 h intervals during the first 8 h of the dialysis and every 2 h thereafter. A sample aliquot of the control polymer $\mathbf{pol_{blank}}$ was treated under identical conditions.

2.16. Sample preparation for isothermal calorimetry titrations (ITC)

(i) *experiments in methanol*: A 0.55 ml aliquot of the purified polymer solutions pol**1** or $\mathbf{pol_{blank}}$ was diluted into a 20-fold larger volume of methanol to a nominal concentration of 0.015 mM. The polymer solution was loaded into the sample cell and titrated subsequently in 5–10 μl aliquots with a 0.3 mM Cu(II) acetate in methanol. Dilution effects were determined by titration of methanol into the polymer solutions, and then subtracted from the initial polymer–metal complex titration curve to obtain the final binding curve. The obtained data were fitted using the subsequential binding site model that is implemented into the data acquiring Origin® software, version 7.0. (ii) *experiments in nanopure water and aqueous buffer*: A 1 ml aliquot of the polymer stock solution was diluted into 10 ml of nanopure water, 50 mM MES buffer solution at pH 7.0 or 50 mM tricine buffer at pH 8.0 yielding a 0.13 mM solution of pol**1** and $\mathbf{pol_{blank}}$, respectively.

2.17. UV/Vis experiments

All experiments were performed in thermostated 4 ml UV/Vis cuvettes in aqueous solution at a constant ionic strength (0.1 M $NaClO_4$) at 30 ± 0.1 °C. Typically, a 2 ml solution containing 1 mM Cu(II) acetate and 0.5 mM TEGbsdpo (ligand **1a**) was titrated with 10 μl aliquots of a 0.005–5 M aqueous NaOH solution. After mixing and equilibration of the solution, UV/Vis spectra were recorded as a function of the pH at a range of 220–800 nm. The spectral data of complex formation were then computed from the titration data by using the Series Model Fit and the global model procedures implemented in the computer program SPECFIT [23].

2.18. Electrospray ionization mass spectrometry

Sample aliquots (1 μl) of a 1 mM aqueous solution of water-soluble ligand **1a** were diluted into 1 ml of 0.1 M aqueous hydrochloric acid at pH 1, concentrated ammonium hydroxide solution at pH 11, and nanopure water at pH 5.6 as the control reaction. After 24 h, 10 μl aliquots of the reaction mixtures were diluted into 100 μl of MeOH and subjected to immediate analysis mass spectrometry.

2.19. Sample preparation prior to AAS analysis

Calibration curves for each metal ion were prepared prior to sample analysis using appropriate standard solutions (Cu(II), Ni(II) and Mn(II) nitrates, each 1000 ppm). Each measurement refers to the average of three instrument readings. The given metal ion amount corresponds to four (4) independent sample analyses for Cu(II), two (2) for Ni(II) and one (1) for Mn(II). The slit was kept constant at 0.5 nm for Cu(II) and 0.2 nm for Ni(II) and Mn(II) ions. The wavelength for the analysis was set to 324.8 nm for Cu(II), 232.0 nm for Ni(II) and 279.5 for Mn(II) ions.

2.20. Sample preparation for degradation studies

Sample aliquots of pol**1** and $pol_{\mathbf{blank}}$ were exposed to equal volumes of 0.01, 0.02, 0.05, 0.1, 0.5, 1 and 6 M aqueous hydrochloric acid and 0.1, 0.5, 1 or 6 M of ammonia solution and shaken for 24 h at ambient temperature. Subsequently, the solutions were neutralized by addition of appropriate amounts of base or acid, respectively and freeze-dried. The solids obtained were subjected to IR analysis.

3. Results and discussion

3.1. Formulation of a stable miniemulsion with styrene and acrylate monomers

The polymerizable ligand **1** contains vinylbenzyl groups that will react during the polymerization as styrene analogues (Fig. 1, see above). The formation of miniemulsion polymers was therefore initially studied using styrene (**2**) in mixtures with butyl acrylate (**3**) and methyl acrylate (**4**) and sodium dodecyl sulfate (SDS) as surfactant. Addition of potassium peroxysulfate at 72 °C initiated the miniemulsion polymerization after sonication and preheating of the compound mixture. To follow the reaction, sample aliquots were taken from the reaction mixture in regular time intervals. The solid content of the miniemulsion polymerization was determined gravimetrically after drying of the sample aliquots at ambient temperature.

The miniemulsion polymerization of methyl acrylate (**4**), butyl acrylate (**3**) and styrene (**2**) in a 11:7:1 (*g*:*g*:*g*) mixture allows the formation of a miniemulsion polymer with a steady conversion of the monomers in more than 95% yield within 3 h. However, exchanging **2** partially against polymerizable ligand **1** failed to yield stable miniemulsions or polymer particles. Instead, unstable miniemulsions were observed with visible phase separation within 3 h or less. Similar results were obtained for attempts to polymerize equimolar mixtures of **1** and **2** with excess of **4**. Attempts to polymerize ligand **1** with **2** in the absence of **3** or **4** lead to incomplete polymer formation (75–80 wt%) and were therefore not further pursued.

3.2. Immobilization of the pentadentate ligand *1*

Subsequent efforts focused on the formation of stable miniemulsions in the presence of ligand **1** and styrene (**2**) and butyl acrylate (**3**). More than 97% conversion of the overall monomer concentration was achieved in less than 160 min reaction time in the absence of methyl acrylate (**4**) using a 1:1 (*g*:*g*) mixture of **2** and **3**. In subsequent polymer formulations, the polymerization reaction was allowed to proceed for 3 h to obtain the highest monomer conversion possible (Fig. 2).

The described monomer mixture 2:3:1 = 3:3:0.144 (*g*:*g*:*g*) forms a stable miniemulsion after addition of SDS (140 mg, 0.5 mmol) and sonication, and does not show phase separation visible for the naked eye when unstirred for about 45 min. By contrast, unstable miniemulsions with apparent phase separation in less than 5–20 min were observed when the amount of surfactant was increased by two-fold or when SDS was replaced with equimolar amounts of Tween 20. Attempts to further optimize the polymerization conditions by varying the surfactant and/or its concentration were therefore not pursued further at this point. The control polymer $pol_{\mathbf{blank}}$ was prepared under identical conditions omitting only ligand **1**, but not the DMSO that was used for dissolving **1** during the preparation of pol**1**.

3.3. Polymer purification

The polymer particles obtained were purified prior to analysis by washing of the aqueous polymerization mixture with chloroform to remove unreacted monomers. The separated aqueous layer was then subjected to repeated ultracentrifugation–dilution circles with water using Micropore® filters with a 10,000 MW cutoff to remove any small molecular weight impurities. The obtained polymer stock solution was used subsequently without further

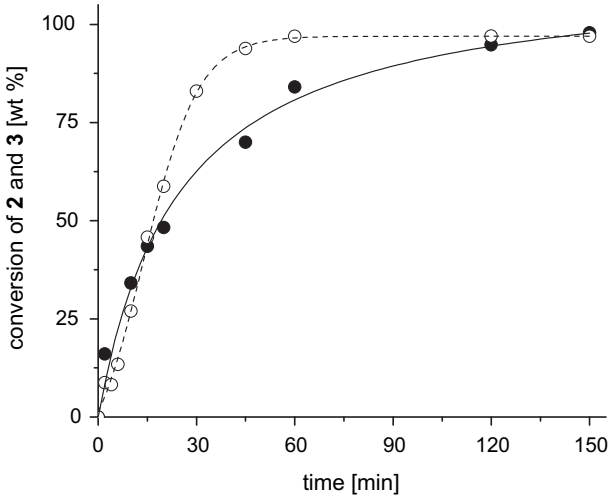

Fig. 2. Co-polymerization of **2** and **3** in (a) the presence **1** (black circle), and (b) the absence of **1** (open circle) over time; the miniemulsion polymer pol**1** was prepared from styrene (**2**):butyl acrylate (**3**):**1** = 3:3:0.144 (*g*:*g*:*g*).

purification. A control polymer was prepared and purified under comparable conditions in the absence of ligand **1**, but in presence of the same amount of DMSO solvent.

3.4. Polymer characterization – chemical composition

The incorporation of salen-type ligand **1** into the polymer matrix was confirmed by elemental analysis of freeze-dried sample aliquots. The analysis of pol**1** revealed an overall presence of 0.29% of nitrogen, which is in an acceptable range of error with comparison to the calculated amount of nitrogen (N: 0.11%). As ligand **1** is the only source for N atoms during the synthesis of pol**1** (Fig. 3), all nitrogen content found by elemental analysis has to be ascribed to **1**. The pentadentate ligand **1** is therefore nearly quantitatively incorporated into the microgel. Preliminary experiments with other monomer mixtures had frequently shown a separation of a yellow ligand **1** containing layer on top of a white appearing miniemulsion solution. During all our efforts to immobilize **1**, precipitation of **1** was not observed. In line with these observations, the gummy precipitates removed during the purification of pol**1** did not contain any measurable nitrogen content.

Attempts to further verify incorporation of ligand **1** into the microgel by using ^{1}H and ^{13}C NMR spectroscopy were not conclusive due to the very small content of **1** (0.6 mol%) with respect to the co-polymerized monomers (99.4 mol%). ^{1}H NMR spectroscopy revealed the composition of the obtained copolymer to be approximately 1:1 for **2** and **3** by comparison of the integrated resonance signals of the phenyl protons in **2** between 6.6 and 7.2 ppm and of the methylene protons of **3** at 3.5 ppm. Unfortunately, the protons of ligand **1** could not be observed using ^{1}H NMR spectroscopy presumably due its low content (0.6 mol%) in the polymer matrix (Fig. 4).

The ^{13}C NMR spectra revealed signals assigned to the carbonyl C atoms of butyl acrylate esters at 175 ppm. However, resonance peaks for the carbonyl C atoms of the monomer **3** or for the imine carbon atoms of **1** are absent (^{13}C NMR data not shown). Indication for the successful incorporation of **1** into the polymer matrix was subsequently obtained by comparison of the UV/Vis spectra of pol**1** and pol$_{\mathbf{blank}}$ (Fig. 5). The shoulder and absorbance at 311 nm shows

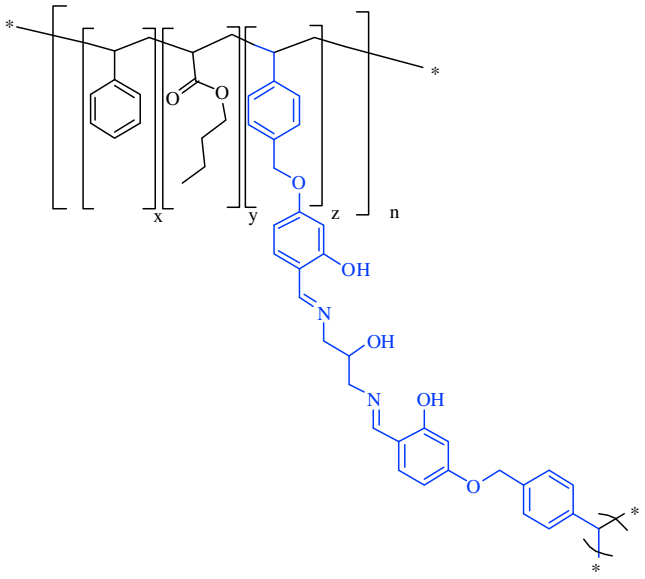

Fig. 3. Composition of the miniemulsion polymer pol**1** prepared from styrene (**2**):butyl acrylate (**3**):**1** = *x*:*y*:*z* = 28.8:23.3:0.3 (mol:mol:mol).

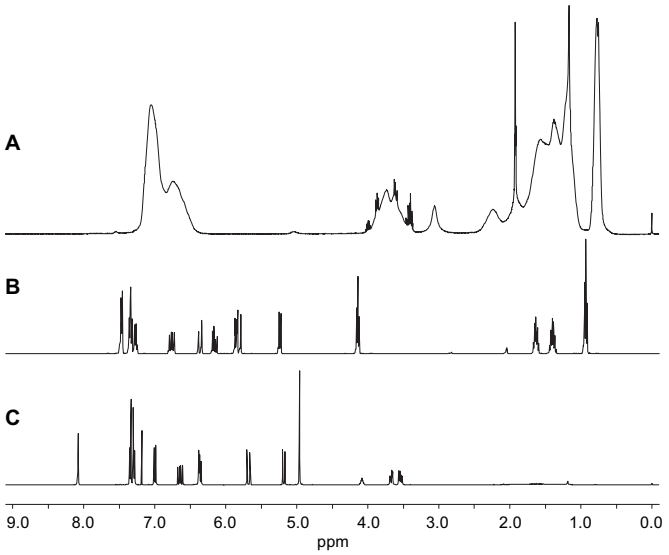

Fig. 4. ^{1}H NMR spectra of (A) freeze-dried miniemulsion polymer in acetone-d_6, (B) a 1:1 (wt/wt) mixture of styrene (**2**) and butyl acrylate (**3**) in acetone-d_6, and (C) ligand **1** in $CDCl_3$.

an $n \rightarrow \pi^*$ transition indicating the C=N bond and the non-bonding electrons at N in **1**, which are present in pol**1**, but absent in pol$_{\mathbf{blank}}$.

While the degree of cross-linking in pol**1** and pol$_{\mathbf{blank}}$ was not quantified, a comparison of the IR spectra of pol**1** to the monomers **2** and **3** revealed a negligible amount of remaining C=C double bonds in the miniemulsion polymer. The bands for the C=C stretch vibration of **2** (1630 cm^{-1}) and **3** (1636 and 1617 cm^{-1}) are not observed for pol**1** (Fig. 6).

3.5. Particle characterization in solution – dynamic light scattering

The volume average particle diameters of pol**1** and pol$_{\mathbf{blank}}$ were determined by dynamic light scattering (DLS) in nanopure water and methanol and the data obtained were analyzed with the intensity-weighted Nicomp model.

The observed mean particle diameter of pol**1** varies significantly dependent on the solvent choice. While the mean particle diameter of pol**1** in water is 75 nm, it increases to 232 nm in methanol

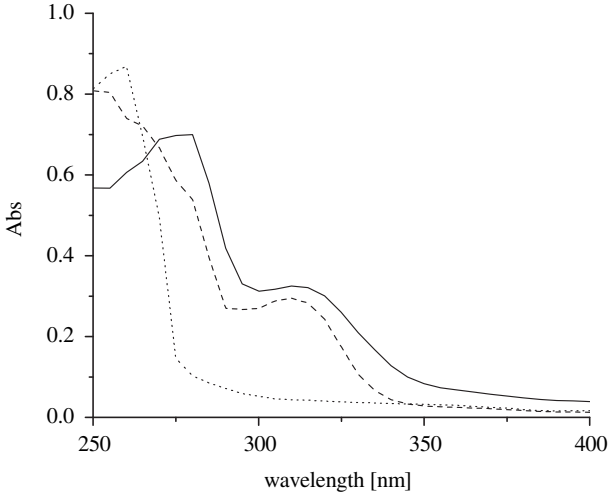

Fig. 5. UV/Vis spectra of pol**1** (solid), ligand **1** (dashed) and pol$_{\mathrm{blank}}$ (dotted) in THF.

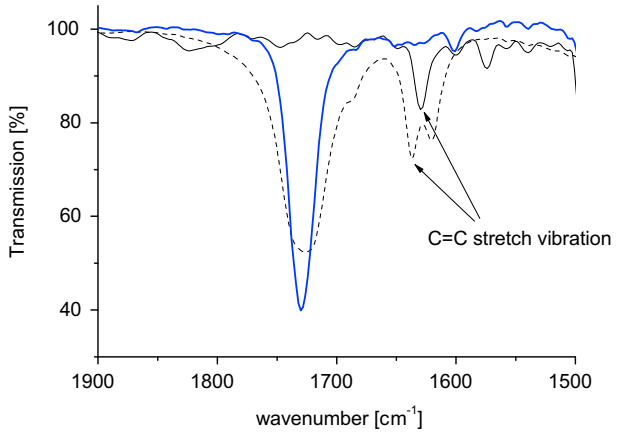

Fig. 6. IR spectra of **2** (solid black), **3** (dashed black) and pol**1** (solid blue) in CCl_4.

indicating possible aggregation of the polymer under these conditions. Interestingly, we observed precipitation of pol**1** when handled in methanol at concentrations above 2 mM [7]; however, polymer precipitation was not observed when handling pol**1** in water under comparable conditions. In contrast, the control polymer $pol_{\mathbf{blank}}$ shows mean particle diameters in water (66 nm) and methanol (72 nm) of the same order of magnitude (Fig. 7). The cause for the swelling of pol**1** remains unclear at this point and requires further investigation. We moreover hypothesize that filling of the void volume in the polymer matrix around the incorporated planar ligand **1** might account for the particle swelling.

3.6. Particle characterization in dry state – TEM imaging

We subsequently obtained transmission electron microscopy (TEM) images of pol**1** and $pol_{\mathbf{blank}}$ and analyzed the mean particle diameter in the dry state (Fig. 8). The average size for the particles in pol**1** is 50 nm and thus smaller than in nanopure water or methanol. In contrast, the average diameter for the control polymer $pol_{\mathbf{blank}}$ revealed a median diameter of 71 nm in the dry state, which is comparable to the results obtained by DLS experiments after equilibrating the polymer in nanopure water or methanol. The swelling of the control polymer is thus independent of the solvent, whereas the diameter and the solvent uptake of pol**1** varies remarkably. The considerably different particle size of pol**1** is ascribed to the presence of ligand **1** and unlikely to be caused by the sample preparation prior to TEM or DLS analysis.

3.7. Polymer stability in acidic and alkaline media

For future use of the microgels as catalysts, evaluation of the overall matrix stability in acidic and alkaline solution is crucial. This will be particularly relevant when pH dependent reactions are catalyzed, when Lewis acidic metal ions are involved in transformations or when the product isolation requires acidic or alkaline work-up conditions. We therefore characterized the chemical stability of pol**1** in acidic and alkaline conditions by degradation studies. Towards this end, sample aliquots of the miniemulsion polymers were exposed to diluted aqueous hydrochloric acid or ammonia solutions at ambient temperature for over 24 h. Subsequently, the mixtures were neutralized by addition of appropriate amounts of base or acid, freeze-dried and analyzed by IR spectroscopy.

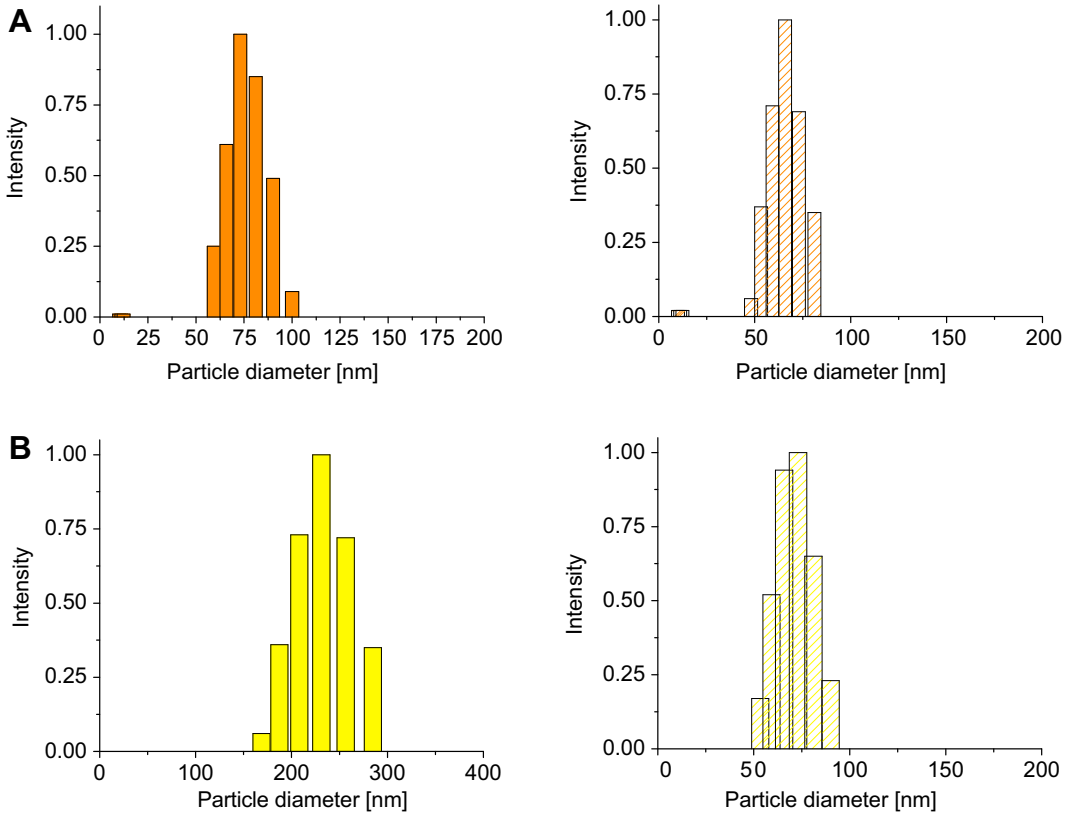

Fig. 7. A. Volume average particle diameter of pol**1** (solid) and pol_{blank} (sparse) in nanopure water; the mean diameter for polymer pol**1** is 75 nm and for pol_{blank} 66 nm. B. Volume average particle diameter of pol**1** (solid) and pol_{blank} (sparse) in methanol; the mean diameter for polymer pol**1** is 232 nm and for pol_{blank} 72 nm.

Table 1
Average molecular weight distributions for pol**1** and pol$_{\mathbf{blank}}$ from pH 1–13.

Entry	pH	Average molecular weight of pol**1** (g/mol)				Average molecular weight of pol$_{\mathbf{blank}}$ (g/mol)			
		M_n	M_w	M_p	D	M_n	M_w	M_p	D
1	–[a]	47,000	62,000	76,000	1.3	50,000	66,000	79,000	1.3
2	1	44,000	57,000	75,000	1.3	54,000	73,000	84,000	1.4
3	3	48,000	62,000	77,000	1.3	57,000	74,000	83,000	1.3
4	5	47,000	60,000	77,000	1.3	54,000	73,000	84,000	1.4
5	7	55,000	68,000	79,000	1.3	56,000	73,000	84,000	1.3
6	9	49,000	61,000	77,000	1.3	58,000	75,000	84,000	1.3
7	11	44,000	55,000	77,000	1.3	54,000	73,000	84,000	1.3
8	13	44,000	56,000	76,000	1.3	53,000	71,000	82,000	1.3

[a] Untreated polymer.

Sample aliquots of the pol**1** stock solution coagulate and precipitate immediately, when exposed to 0.25 M or higher concentrated aqueous hydrochloric acid. A visible precipitate is not formed upon treatment with 50 mM or less concentrated aqueous hydrochloric acid or under alkaline conditions in up to 3 M ammonia solution. IR spectra of freeze-dried sample aliquots of pol**1** and pol$_{\mathbf{blank}}$ do not reveal degradation of the polymers exposed to acidic or alkaline conditions equivalent to a pH range from 1 to 13. The analysis of the average molecular weight distributions by gel permeation chromatography (GPC) showed reasonable matrix stability for both polymers after 24 h equilibration time (Table 1).

Slightly decreasing values of the number average (M_n) and the nominal molecular weight (M_p) are noted for pol**1** after treatment with acidic or alkaline solutions. However, the nominal molecular weight of pol**1** is, within the experimental error, close to 77,000. The control polymer pol$_{\text{blank}}$ shows almost constant molecular weight distributions with a nominal molecular weight of 84,000. The polydispersity ($D = M_w/M_n$) remains constant at 1.3 for both polymers and is comparable to those observed for other miniemulsion polymers [10]. While particle dissolution during the 24 h experiments is not expected for any of the investigated acidic or alkaline conditions, prolonged use of a putative polCu$_2$**1** catalyst in aqueous solutions with a pH below 3 or above 11 might lead to unwanted alterations of the support material, and thus influence the catalytic performance. Further experiments are also needed to clarify, whether or not the polymer matrix is sufficiently stable on the atomic level to ensure an unaltered polymer structure around the metal complex during catalysis, which will be topic of future investigations. The degradation studies disclose nonetheless reasonable matrix stability between pH 3 and 11, implying that the support of a transition metal catalyst in miniemulsion polymers is feasible for enzyme-like reactions without alteration of the surrounding matrix in physiological and moderate alkaline conditions.

Since the salen ligand **1** is not water-soluble, we exposed a water-soluble analog of **1**, *N*, *N*′-1,3-bis[{2-hydroxy-4-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}benzylideneamino]propan-2-ol, TEGbsdpo, **1a** (Fig. 9), [22] to the same acidic and basic conditions as used during the degradation studies of pol**1**. The study provided information on the stability of the imine bond of **1**.

The solutions of **1a** in aqueous hydrochloric acid at pH 1, in nanopure water and in concentrated ammonium hydroxide solution at pH 11 were diluted into methanol and subjected to analysis by electrospray ionization mass spectrometry in the positive ion mode. The mass spectrometric data do not show apparent decomposition of **1a** in neutral (Fig. 10A) or acidic conditions (Fig. 10C), but reveal the formation of **1b** after exposure of **1a** to ammonium hydroxide solution at pH 11 for 24 h (Fig. 10B)

A detailed investigation of the decomposition of **1** in pol**1** or its putative protection by the polymer matrix in alkaline conditions is therefore topic of future investigations. Towards this end, polymers with a higher content of **1** in comparison to the monomers will be prepared to follow the degradation over time by ^{1}H NMR spectroscopy on the polymer itself.

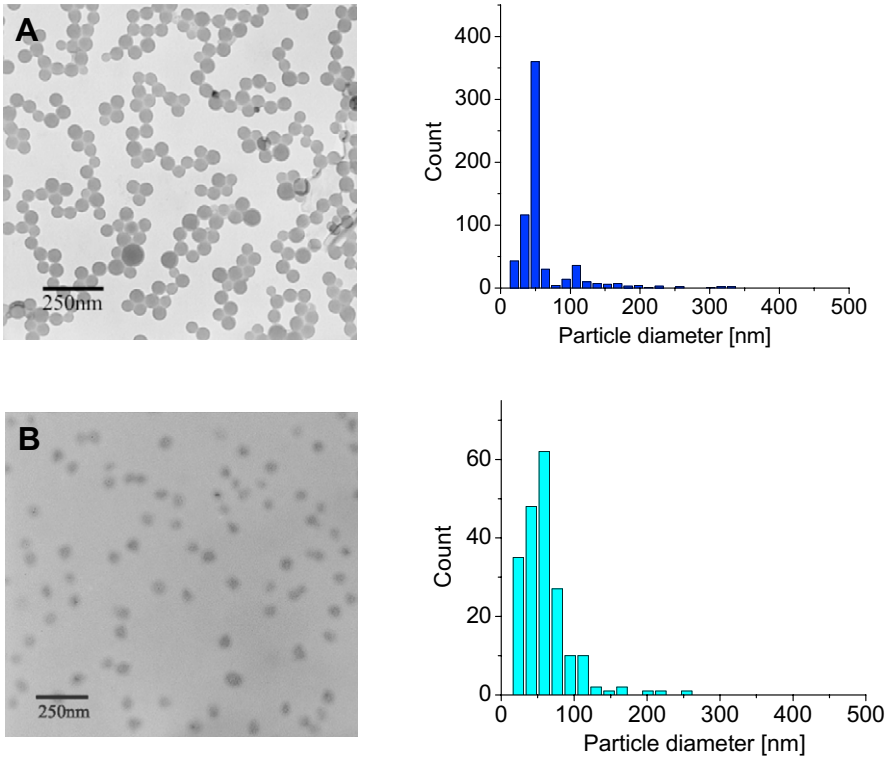

Fig. 8. TEM micrographs (left) and particle size distribution analysis (right) of (A) pol**1** and (B) pol$_{\text{blank}}$.

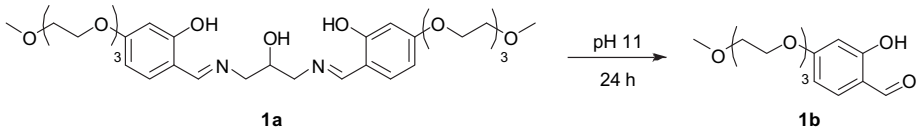

Fig. 9. Cleavage of the imine bond in **1a** forming **1b**.

3.8. Particle characterization – binding of metal ions

The immobilization of the pentadentate ligand **1** in a mini-emulsion polymer following the described recipe is nearly quantitative as concluded from the elemental analysis data for the composition of pol**1** (see above). The nominal concentration of **1** in the stock solution of pol**1** was calculated as 2 mM. pol**1** was subsequently explored for its ability to bind transition metal ions, such as Cu(II), Ni(II), Mn(II), and Fe(III). All initial efforts focused on activating a Cu(II) catalyst in methanol and latter experiments focused on the activation of the catalyst in aqueous solution.

For binding of Cu(II) ions to pol**1** in methanol, we diluted a sample aliquot of the aqueous pol**1** solution into a 20-fold higher volume of methanol. The binding of Cu(II) acetate to pol**1** was then explored by isothermal titration calorimetry. The obtained data were fitted with a two site binding model to reveal a binding constant K_1 of 11500 ± 5300 for the coordination of the first mol of Cu(II) ions to the immobilized **1**. The reaction is exothermic with a binding enthalpy ΔH_1 of −12580 ± 5190 kcal mol^{-1} and a binding entropy ΔS_1 of −23.3 cal mol^{-1} K^{-1}. The association of the second mol of Cu(II) ions is endothermic and about one order of magnitude stronger with a binding constant K_2 = 83900 ± 3200; the corresponding binding enthalpy ΔH_2 is 41040 ± 4960 kcal mol^{-1} and the binding entropy of ΔS_2 is 159 cal mol^{-1} K^{-1}. A comparable titration with the control polymer pol$_{\mathbf{blank}}$ showed only release of heat corresponding to dilution and consequently no Cu(II) ion binding. The study disclosed accessibility of the Cu(II) ions to the ligand core and quantitative complex formation with immobilized **1** in a 2:1 molar ratio (Fig. 11) [7].

Further addition of metal ions after saturation of the metal ion binding sites does not release additional heat indicating no more uptake of Cu(II) ions by the polymer matrix. Thus, the addition of appropriate amounts of Cu(II) acetate to pol**1** converts the polymer quantitatively into polCu_2**1**. The use of this macromolecular catalyst for the oxidation of 3,5-di-*tert*.-butyl catechol into 3,5-di-*tert*.-butyl quinone in methanol has been previously described and contrasted to the small molecular weight analog catalyst Cu_2**1** in detail [7], and will be not discussed herein.

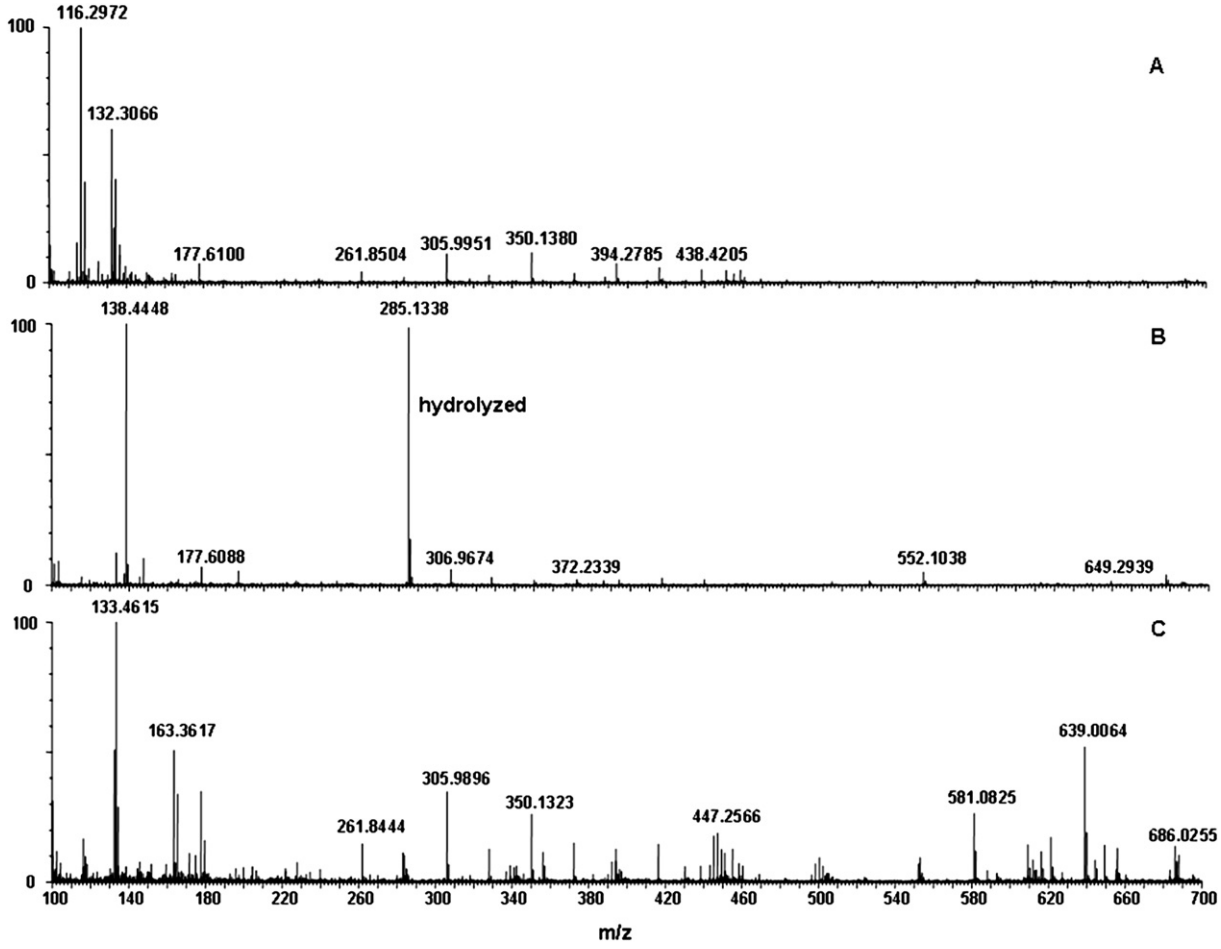

Fig. 10. ESI mass spectra of **1a** after exposure to (A) nanopure water, control, (B) alkaline conditions at pH 11, and (C) acidic conditions at pH 1 for 24 h.

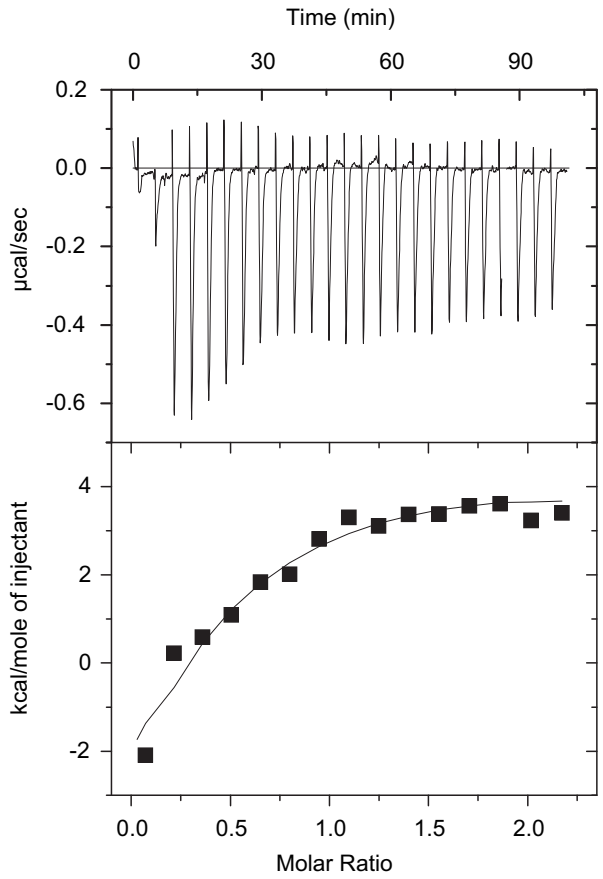

Fig. 11. Isothermal titration of $Cu(OAc)_2$ solution into pol**1** in methanol at 300 K.

Subsequently, we focused on the uptake of Cu(II) ions by pol**1** in aqueous solution. Repeated isothermal titration experiments did not disclose binding of Cu(II) acetate in nanopure water, MES or tricine buffer at pH 7 and pH 8, respectively. Attempts to load Cu(II) acetate at higher pH led to precipitation of Cu(II) hydroxide and was therefore not further pursued.

To achieve saturation of the metal ion binding sites despite this shortcoming, a 10 mL sample aliquot of the 2 mM pol**1** solution was dialyzed for 12 h against a 5-fold higher concentrated aqueous Cu(II) acetate solution. The dialysis was then continued in nanopure water to rinse the excess of unbound Cu(II) ions out of the polymer matrix. The rinsing process was monitored by the determination of the Cu(II) ion content in sample aliquots, which were taken in regular time intervals using atomic absorption spectroscopy (AAS) (Fig. 12).

Substantial amounts of unbound Cu(II) ions are removed from both pol**1** and $pol_{\mathbf{blank}}$ during the first 3 h of the dialysis in water. After 8 h, the Cu(II) ion content determined in pol**1** corresponds to half of the theoretical value expected for the formation of a macromolecular Cu(II) complex $polCu_2$**1**, while the control polymer $pol_{\mathbf{blank}}$ indicates less, but still significant retention of Cu(II) ions under comparable conditions. A substantial amount of the Cu(II) ions in $polCu_2$**1** is thus not coordinated to ligand **1**, but rather randomly retained in the matrix. The dialysis was therefore continued to lower the amount of unspecifically matrix-bound Cu(II) ions. After dialyzing $polCu_2$**1** for 12 h overall, 41% of the theoretical Cu(II) content (2.1 mM) are still retained in the polymer, while the amount of randomly distributed Cu(II) ions in $pol_{\mathbf{blank}}$ is less than 13% (0.5 mM). The ability to bind Cu(II) ions by pol**1** is thus higher than for $pol_{\mathbf{blank}}$ and has to be attributed to coordination of Cu(II) ions to the immobilized pentadentate ligand **1**. The overall metal ion binding ability of pol**1** is, however, poor and insufficient

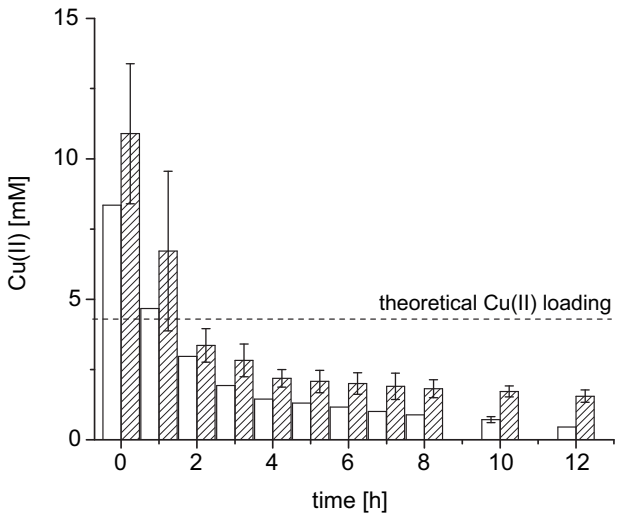

Fig. 12. Plot of the concentrations of Cu(II) ions in pol**1** (shaded) and pol_{blank} (blank) during dialysis of the Cu(II)-loaded microgels against nanopure water; the given values are averages of four independent experiments.

for the intended use as a macromolecular catalyst in aqueous solution. This observation is strongly contrasting previous isothermal titration calorimetry experiments which had confirmed a nearly quantitative formation of $polCu_2$**1** in methanol [7]. The binding of other metal ions by pol**1**, such as Ni(II) or Mn(II), are found to be negligible as well and the binding of Fe(III) ions is hampered by polymer precipitation under the used conditions.

This lack of Cu(II) ion binding to pol**1** in aqueous conditions prompted us to investigate the complexation of the water-soluble derivative of **1a** with Cu(II) acetate in more detail [22]. Toward this end, we used the spectrophotometric method developed by Zuberbühler et al. [23] and measured UV/Vis spectra of a solution containing **1a** and Cu(II) acetate in a molar ratio of 1:2 in dependence of the amount of sodium hydroxide solution added. Computation of the titration data allows the calculation of the speciation curve for the complex formation in dependence of the pH (Fig. 13).

At pH 5, 100% of **1a** is free in solution, while the major species above pH 5.5 is a mononuclear $[CuL]^{2+}$ complex. Coordination of another mol of Cu(II) ions to $[CuL]^{2+}$ leads to a dinuclear species $[Cu_2L_{-2H}]^{2+}$ above pH 6.5 that undergoes further deprotonation above pH 8.5, forming $[Cu_2L_{-3H}]^{+}$ (Fig. 14).

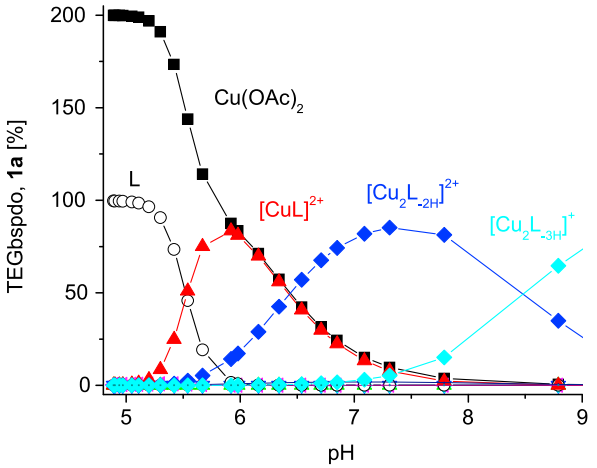

Fig. 13. Distribution of species derived from water-soluble ligand **1a** and Cu(II) acetate in a 1:2 molar ratio from pH 5 till 9.

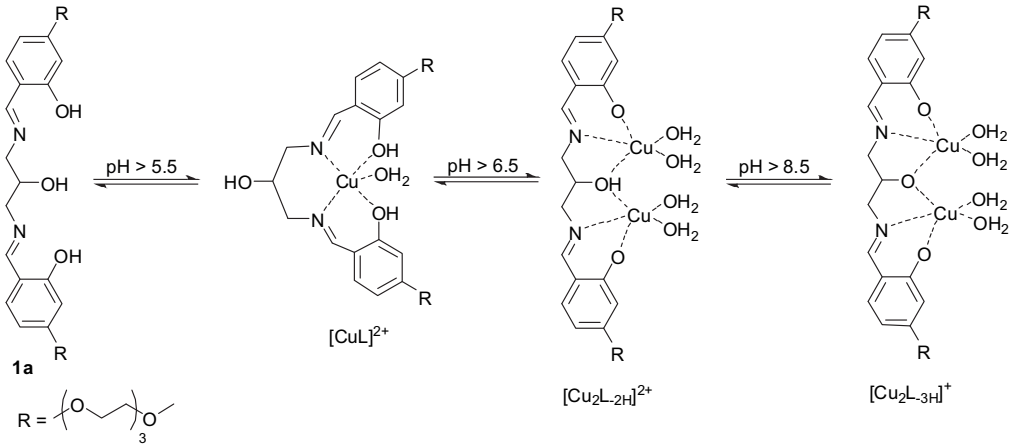

Fig. 14. Putative species formed from ligand **1a** and Cu(II) acetate in a 1:2 molar ratio between pH 5 and 9.

The pH of a 10 mM solution of Cu(II) acetate in nanopure water is 5.85, at which 80% of the ligand formed the mononuclear species $[CuL]^{2+}$, 13% the dinuclear species $[Cu_2L_{-2H}]^{2+}$, and 6% of the ligand remain uncoordinated. Thus, these conditions do not favor the binding of Cu(II) ions to pol**1** and will not activate a dormant polCu_2**1** catalyst. Instead, a mononuclear complex is preferentially formed, which is in very good agreement with the amount of metal ion bound during dialysis.

To achieve Cu ion binding in aqueous solution, current efforts focus on the development of polymerization procedures that allow miniemulsion polymerization in the presence of complexes with paramagnetic metal ions. This task might involve masking the radical character of the paramagnetic metal ions during radical polymerization by coordination of another radical or by a change of the polymerization mechanism that is insensitive to the presence of radicals. Alternatively, we focus on evaluation of other pentadentate Cu(II) complexes ions that favor the copper(II) binding in aqueous solution, particularly at neutral pH. Further results along these lines will be reported in due course.

3.9. Summary of results

A pentadentate salen-type ligand was immobilized in a poly-[(styrene)]-*co*-(butyl acrylate)] matrix by miniemulsion polymerization in aqueous solution. The conversion of the styrene and butyl acrylate monomers was almost quantitative in less than 3 h without phase separation visible to the naked eye. The chemical composition of the polymer was determined by elemental analysis and suggests an almost quantitative incorporation of the salen ligand based on its nitrogen content. Activation of a dormant catalyst for catechol oxidation in methanol by coordination of Cu(II) acetate to the immobilized ligand is stoichiometric indicating the formation of binuclear polCu_2**1**. However, the coordination of the same metal ion salt in aqueous solution corresponds to half of the quantitative amount, suggesting the formation of a mononuclear complex pol**Cu1**. This observation can be rationalized by the speciation data obtained from a water-soluble analog (**1a**) of the salen ligand **1** and Cu(II) acetate. The formation of a binuclear complex is not favored at slightly acidic or neutral pH, which was used for metal ion loading into the polymer in aqueous solution. Higher pH values that would favor formation of a binuclear complex with the free ligand lead, however, to precipitation of Cu hydroxide.

Moreover, the obtained polymer beads have a particle size of 50 nm in the dry state, but show swelling in water to 75 nm and in methanol to 233 nm. The swelling of the control polymer without salen ligand is considerably less, and its mean diameter equals about 70 nm in both solvents. The particles are demonstrated to be stable over a wide pH range (3–11) by gel permeation chromatography. The polymer matrix may therefore protect the immobilized ligand against hydrolysis on the atomic level, while the free ligand is stable under acidic and neutral conditions, but decomposes in alkaline solution. Further studies are needed with polymers at higher ligand loading and will be reported in due course.

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