Emulsion and Miniemulsion Polymers in Catalysis

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Abstract: Microgels obtained by miniemulsion polymerization are highly cross-linked but soluble polymer particles with sizes between 10 and 600 nm. This property renders them useful not only as platforms for molecular recognition and sensing of biomolecules, but also as colloids that participate in catalysis. Recent approaches employing catalytically active templated and non-templated microgels are highlighted.

Keywords: Catalysis, microgels, emulsion polymerization, templated polymers.

1. INTRODUCTION

The development of new and efficient catalysts plays a central role in chemical research [1]. While a wide variety of synthetic catalysts are known to promote common organic reactions, such as C-C bond formations, oxidations, reductions or hydrolyses in organic solvents, progress in the preparation of catalysts that facilitate reactions with sufficient turnover numbers in 'green' solvents remains slow. Moreover, the development of catalysts promoting common reactions regio-, stereo- or enantioselectively in aqueous solution is still in its infancy. This might be in part because enzymes can be engineered by directed evolution to allow their usage in aqueous organic solvents at elevated temperatures or for non-natural substrates [2-4]. However, limitations associated with the use of proteins include, among others, a narrow pH range of operation, a difficult, costly and/or time-consuming preparation, and/or a limited shelf-life. These obstacles might be overcome by designed synthetic entities that exhibit enzyme-like selectivity and high turnover numbers during any possible reaction. Very recent efforts along these lines are summarized below. This review does not include a discussion of the templating of proteins onto the surfaces of emulsion polymers [5, 6].

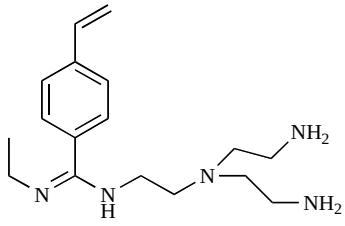
2. TEMPLATED CATALYSTS

The Template Polymerization Principle

A very promising strategy towards the design of synthetic entities with enzyme-like properties is the template polymerization approach. Particularly the design of soluble templated microgels is very appealing and of high current interest. In a general protocol for preparation of catalytically active templated polymers by bulk polymerization, functional monomers are preorganized around a template in solution to form a monomer-template assembly; subsequent cross-linking of the three-dimensional po-lymeric material stabilizes this arrangement. After template removal, the remaining matrix contains immobilized functional monomers and cavities that are complementary to the template. If the template resembles an analogue of the transition state of a reaction of interest, the prepared material can be used to catalyze this reaction involving the targeted substrate [7-10].

Catalysts Prepared by Templated Bulk Polymerization

Efficient templated catalysts for ester or carbonate hydrolysis were prepared by bulk [7-10] or emulsion polymerization [11, 12]. The highest acceleration of a reaction with templated catalysts reported so far has been achieved for carbonate hydrolysis promoted by a Cu(II) complex under <u>*heterogeneous*</u> reaction conditions (k_{cat}/k_{uncat} = 110,000) [7, 13], and is two orders of magnitude higher than that reported for corresponding catalytic antibodies (k_{cat}/k_{uncat} = 810) [7, 13, 14].



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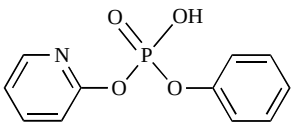

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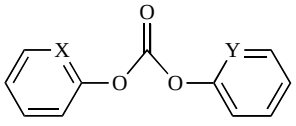

3 X = Y = C
4 X = C, Y = N
5 X = Y = N

Early attempts to prepare functional models of carboxypeptidase A focused on templating a stable transition-state analogue in close proximity to an amidine function and a Zn(II) binding site. Towards this end, the combined use of functional monomer **1** and template **2** was found to provide the most active catalysts [13]. The monomer **1** contains an amidine and a triamine functionality for binding a metal ion. The amidine group is designated to coordinate to the tetrahedral transition state for carbonate hydrolysis similar to the guanidine moiety of Arg 127 in carboxypeptidase A. The enzyme also contains an active Zn(II) ion playing an important role in catalysis [13].

After these very promising initial results, the same authors replaced the Zn(II) ions by Cu(II) ions. With substrates **3**, **4** or **5**, they observed a dramatic increase in the catalytic activity of the obtained imprinted sites (Fig. **1**) [7]. They ascribed this effect to the improved stability of the complexes formed from the triamine moiety of **1** and the stable transition state analogue **2** during the imprinting procedure. Most importantly, the Cu(II) ions generate a more nucleophilic hydroxyl group compared to Zn(II) ions during catalysis. At the same time, the prepared heterogeneous polymers show typical enzyme properties such as selectivity, Michaelis-Menten kinetics and competitive inhibition [7].

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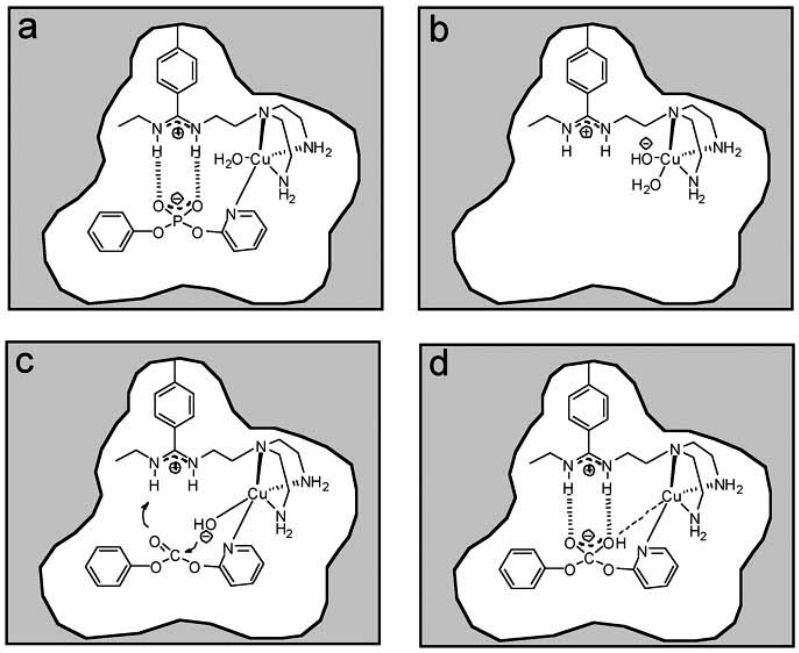


Fig. (1). Preparation and function of an imprinted catalyst prepared by bulk polymerization; (**a**) molecular imprinting with template **2** and monomer **1** in the presence of Cu(II) ions. (**b**) a cavity after removal of the template **2**; (**c**) substrate **4** bound in the cavity of b; (**d**) intermediate in the catalysis; after release of the products (phenol, pyridone and CO_2), structure b is regenerated (adapted from Ref. [7]).

Catalytically active templated polymers based on transition metal complexes are therefore attractive alternatives to catalytic antibodies, and might be particularly interesting in applications involving unnatural reactions that enzymes do not catalyze. Excellent reviews on catalysis with templated polymers prepared by bulk polymerization are available in the literature and will be not further discussed herein [1, 15-29].

The disadvantages of the templated catalysts based on bulk polymerization include their insolubility and the heterogeneity of the active sites [30]. Additionally, a very high degree of cross-linking might hamper substrate or product transport as well as the turnover properties of the material, while decreased cross-linking promotes a certain degree of matrix solubility at the cost of its selectivity [31].

To provide a templated matrix in a homogeneous system, imprinted sites inside dendrimers have been prepared and explored for their use in molecular recognition [32-37]. The method, requires elaborate syntheses of the dendrimer synthons, while the apparent substrate discrimination with such templated dendrimers is rather modest. Other approaches used 'molecular footprinting' or 'surface imprinting' to synthesize surface-imprinted silica or polymer particles [6, 15, 18, 38-40]. Additionally, templated polymer shells based on immobilized chiral templates have been proposed to prepare catalytically active polymers [41]. Detailed discussions of these studies are outside of the focus of this review article; they are discussed in the literature [41, 42].

To overcome the outlined challenges of the use of templated catalysts prepared by bulk polymerization, the preparation of catalytically active templated microgels by emulsion polymerization has been suggested as a promising approach to promote further advances in the field [11, 12, 43-46]. Recent efforts in the preparation and evaluation of catalysts prepared by emulsion polymerization are summarized below.

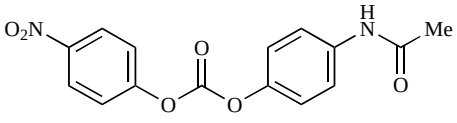
3. MICROGELS AND EMULSION POLYMERIZATION

Microgels are highly cross-linked, soluble polymer particles with sizes between 10 and 600 nm, which render them useful for homogeneous catalysis [11]. One of the main advantages of these macromolecules is that they give rise to colloidal systems when dissolved in the appropriate solvent system, thereby acquiring a fluid and flexible nature [12]. They exhibit additionally a low viscosity and can be conveniently isolated by precipitation, ultracentrifugation, or ultrafiltration.

Catalysts Prepared by Radical Emulsion Polymerization in Homogeneous Solution

In one of the earlier attempts to prepare efficient catalysts by emulsion polymerization, the hydrolysis of *p*-nitrophenyl carbonates **6** was studied in acrylamide microgels templated with phosphate **7** [11, 12]. These microgels used derivatized arginine **8** and tyrosine **9** as functional monomers [11, 12]. Possible interactions between **7** and the side chains of the functional monomers **8** and **9** are indicated in complex **10**. The interaction between the positively charged guadinium group and the phosphate anion was expected to play a significant role during the imprinting process [12].

The radical polymerization to immobilize **10** was initiated with AIBN in the presence of large excess of DMSO as porogenic sol-



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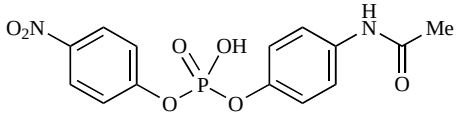

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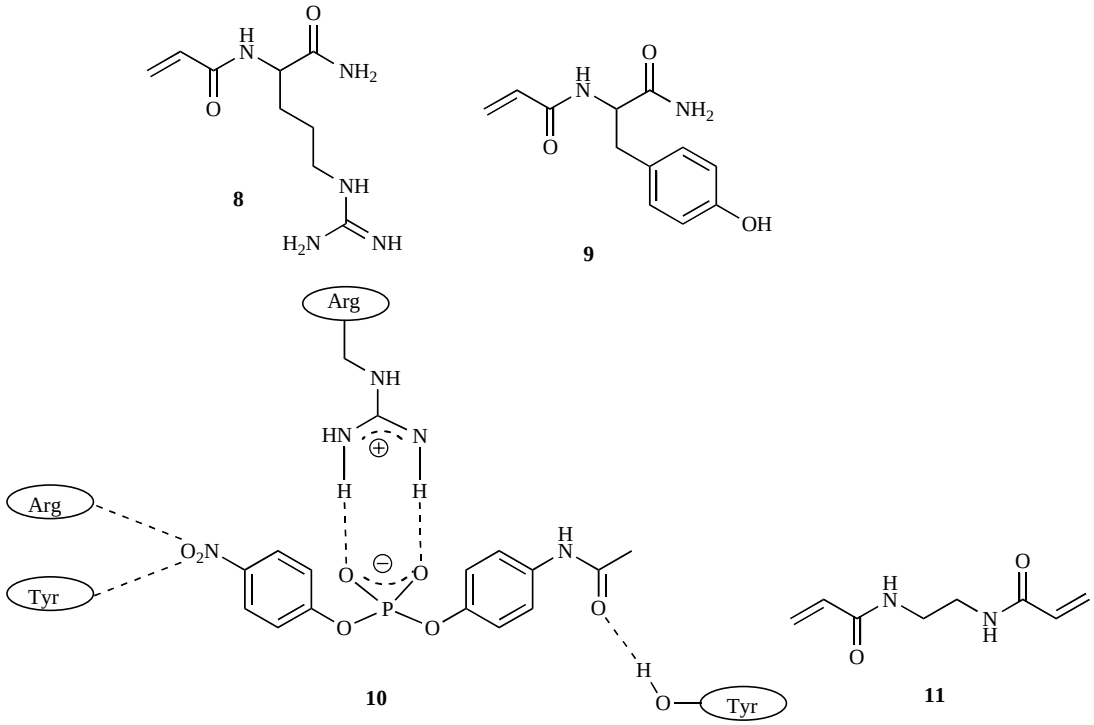


vent and *N*, *N*'-ethylenebis(acrylamide) **11** as cross-linker, and continued at 80 °C for 4 days under a nitrogen atmosphere. The polymer was precipitated by adding diethyl ether to the reaction mixture and filtered off. The resulting microgel was dialyzed against water, freeze-dried, and stored until use.

The relative molecular weight M_R, the coil density and the particle size were determined for microgels synthesized with 70, 80 or 90 % of cross-linker **11** in reference to control polymers prepared in absence of phosphate template **7** [12]. The relative molecular weights M_R of the microgels prepared in this study range between 23,000 and 100,000 g/mol [12]. Similar diversity has been observed in other microgel formations using excess solvent by other researchers [47].

The characterization of the catalytic activity of the microgels by kinetic parameters indicated that the critical monomer concentration (C_M) and the percentage of cross-linker strongly influence the catalytic efficiency [12]. Only microgels that contained 70 % cross-linker followed the Michaelis–Menten saturation model ($V_{max} = 1.34 \times 10^{-6}$ ($\pm$ 1.28×10^{-7}) Ms^{-1}, $K_M = 2.38 \times 10^{-3}$ ($\pm$ 3.1×10^{-4}) M) [12]. The catalytic efficiency (k_{cat}/k_m) was calculated to be 53 assuming that 10 % of the estimated arginine residues included in the polymerization mixture of the control polymer are catalytically active [12]. Thus, the catalytic activity of these microgels prepared in high dilution is rather low; only 1 % of the prepared sites are catalytically active [30]. Microgels prepared by this polymerization protocol were subsequently considered to be inadequate for a successful imprinting process, as the size and the molecular weight of the microgels prepared by the emulsion polymerization approach is hardly controllable. Instead undesired "fractal" structures might result that highly impact the selectivity and the catalytic turnover of the templated material [30].

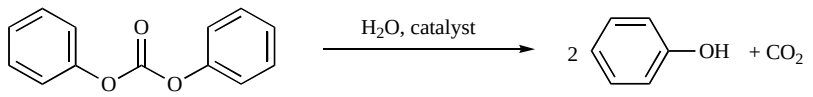
The efforts to develop more efficient microgels subsequently focused on methods to prepare high molecular weight particles with high degrees of cross-linking and low polydispersity, while controlling and avoiding macrogelation. This could be achieved by several methods including (a) increasing the monomer concentration to attain higher catalytic activity while accepting considerably higher polydispersities (M_w/M_n) of the particles; (b) performing polymerization in three stages by increasing the reaction temperature in time intervals, e.g. from 60 to 70 to 80 °C; or (c) by the 'postdilution' method.

In reference to Wulff's previous work, Zhang *et al*. have recently shown that the use of increased monomer concentration during the material synthesis facilitates the hydrolysis of *p*-nitrophenyl acetate in the same way for bulk polymers [48]. The acceleration of the hydrolysis by this imprinted polymer, however, was marginal when compared against the non-imprinted control polymer [48].

Nevertheless, the 'postdilution' method was demonstrated to allow the synthesis of very rigid microgels with better stabilization of the active site by more efficient cross-linking at low polydispersity M_w/M_n [49]. The previously-explored aqueous hydrolysis of diphenylcarbonate **12** was chosen as a model system to investigate the conditions under which highly active microgels could be prepared (Scheme **1**) [49].

Towards this end, the templated catalyst was prepared by immobilization of complex **13**, which consisted of the diphenyl phosphate template **14** as a stable transition-state analogue of the carbonate hydrolysis, and *N*, *N*'-diethyl-4-vinylbenzamidine **15** as functional monomer.

The ratio of the monomers and the content of cross-linker were the same as described for the preparation of insoluble bulk polymers previously [49]. The polymerization mixture consisted of



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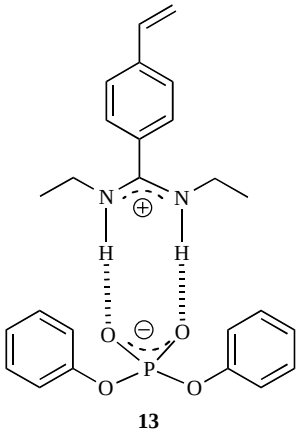
Scheme 1.



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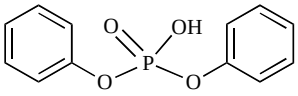

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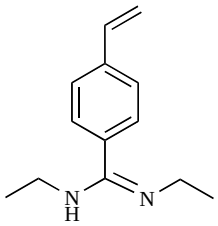

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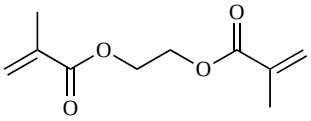

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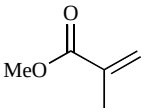

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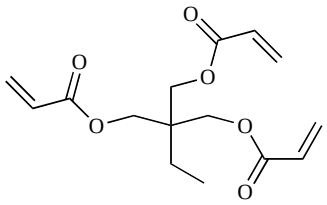

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mers previously [49]. The polymerization mixture consisted of ethyleneglycol dimethacrylate (EGDMA) **16** (80 wt %), methyl methacrylate **17** (11 wt %) and complex **13** (9 wt %). The polymerization of this mixture was initiated after dilution with the same volume of cyclopentanone by addition of AIBN (3 wt % of the monomer mixture) at 60 °C. As the degree of cross-linking of EGDMA reaches its maximum right before macrogelation occurs [50], the polymerization was stopped just prior to this point (after ~ 120 min). The mixture was then diluted with cyclopentanone to keep the concentration of the polymerization solution below the critical monomer concentration C_M (0.1–1.5%). Continued stepwise polymerization at 60 °C (144 h), 70 °C (96 h), and 80 °C (96 h) resulted in the desired increase of the particle density [30]. The obtained microgels were isolated by precipitation with petroleum ether or ultracentrifugation.

The template was subsequently stripped off the matrix by extraction in alkaline aqueous solution. The remaining microgel contained then active sites with a shape complementary to the transition state in close proximity to an amidine group that promotes catalytic turnover. Optimization studies confirmed that the influence of the catalyst solubility on the substrate turnover is important, but microgels with high rigidity and reduced heterogeneity gave superior catalytic turnover rates [30]. This conclusion is furthermore supported by the evaluation and comparison of the three polymers pol**1**, pol**2** and pol**3** that differ in the dilution of the polymer solution in the 'postdilution' polymerization period. While the matrix dilution during the synthesis of pol**1**, pol**2** and pol**3** was altered from 1.0 to 0.5 and 0.1 %, the polydispersity (M_w/M_n) dropped from 6.0 to 3.6 and 1.5; the absolute weight-number average molecular weight $M_{n,abs}$ decreased from 624 to 261 and 44 kDa due to a much lower aggregation probability of the primary particles. The decrease of the polydispersity of the particle is, however, accompanied by a simultaneous drop of the catalytic activity (see below) [30].

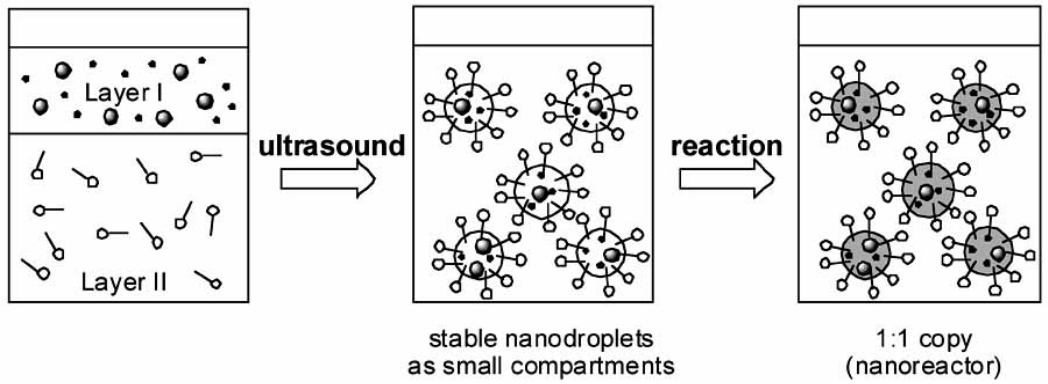
The catalytic activity of the microgels was determined by investigating the rate of hydrolysis of **12** (Scheme **1**) in HEPES buffer (pH 7.3)/ acetonitrile (1/ 1, v/ v) at 10 °C. The rate constant of the carbonate hydrolysis promoted by the polymers pol**1**, pol**2** and pol**3** decreases from 12.8×10^{-7} min^{-1} to about 3.5×10^{-7} min^{-1}. The catalytic efficiency for the catalysis in comparison to the transformation with a non-templated microgel (or the solution catalyst) also decreases from 52.7 (5.5) to 14.3 (2.6). These values are far lower than enzymatic rates for carbonate hydrolyses, which reach a catalytic turnover that is six to eight orders of magnitude higher [51].

While an imprinting effect in the templated catalysts is apparent by comparison of the rate constants (k_{cat}) of the templated catalysts

Table 1. Catalytic Activity of Imprinted Microgels pol1–pol5

Microgel	k_{cat} ($\times 10^{-7}$)[a] [min^{-1}]	k_{cat}/k_{sol}[b]	k_{cat}/k_{contr}[c]	Cavities/Particle[d]
pol **1**	12.8	52.7	5.52	45.7
pol **2**	3.77	15.5	3.2	18.2
pol **3**	3.48	14.3	2.58	1.81
pol **4**	70.9	291.4	18.5	94.5
pol **5**	3.91	16.1	2.43	1.03

[a] Hydrolysis of **12** in a solution of 50 mM HEPES buffer (pH 7.3)/ CH_3CN (1/ 1, v/ v) at 10°C with two equivalents of cavities to one equivalent of substrate; k_{impr} = pseudo-first order rate constant in presence of the imprinted nanogel.
[b] k_{sol}, rate constant in HEPES buffer (pH 7.3)/MeCN (1/ 1, v/v).
[c] k_{contr}, rate constant in presence of the control nanogel.
[d] Available cavities with amidinium groups determined by potentiometric titration in aqueous 0.1N NaCl/ MeCN (1/ 1, v/ v) solution with 0.02 N aqueous HCl.



Scheme 2.

to (a) those in non-imprinted control microgels (k_{contr}), (b) the catalytic activity of complex **12** in solution (k_{sol}), or (c) to the uncatalyzed reaction (k_{uncat}), the effect is most pronounced for pol**4** with a catalytic efficiency of 2990. In contrast to pol**1**–pol**3**, pol**4** was prepared with trimethylolpropane trimethacrylate (TRIM) **18** as crosslinker.

The calculation based on the experimental results shows on average 1.8 active sites per particle for pol**3**. The number of active sites per particle for the polymers with higher molecular weight is higher. Decreasing the amount of template in subsequent efforts allowed the preparation of a microgel pol**5** with a molecular weight of 40 kDa, Michaelis-Menten kinetics during catalytic turnover, a very low polydispersity and on average only one active site per particle. Thus, synthetic entities with properties analogous to natural enzymes, but at much higher stability, were realized for the first time [30]. These enzyme-like polymers did not, however, show significant catalytic activities in aqueous media due to the negative contribution of water molecules during molecular recognition [52].

Taking advantage of the developed solution polymerization method, peroxidase-like imprinted microgels were very recently presented by Chen *et al*. to confirm the above discussed principles [52]. The obtained microgels, had an irregular shape and a relatively large size of about 200 nm. They also displayed apparent aggregation that make any conclusion on equal accessibility of sites or their catalytical activity questionable.

4. MINIEMULSION POLYMERIZATION PROCESS

A continuous system in which small droplets with high stability are created by using high shear is called a 'miniemulsion' [53]. In the first step of the miniemulsion process, small stable droplets of nm size are formed through the shearing of a system containing the dispersed phase, the continuous phase, a surfactant, and an osmotic pressure agent (Scheme **2**) [53]. In a second step, these droplets can undergo a reaction, such as radical polymerization, without changing their identity [53].

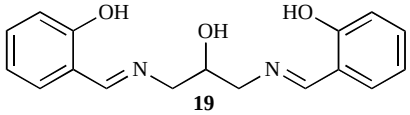
The droplet size for direct (oil-in-water) miniemulsions is determined by the amount of oil and water, the oil density and solubility, and the amount of surfactant used. The droplet size is initially a function of the amount of mechanical agitation. However, the droplets change rapidly in size throughout the sonication process to approach a pseudo steady-state, provided that the required minimum of energy for reaching this state is provided. The polydispersity of the droplets decreases simultaneously due to constant fusion and fission processes. The droplet size remains constant, once a pseudo steady-state is reached. As the droplets' surfaces are incompletely covered with surfactant molecules, micelle formation is not observed in the continuous phase [53].

The efficiency of the molecular imprinting effect for these submicron particles prepared by miniemulsion polymerization was examined using chiral amino acid derivatives. The best enantioselective molecular recognition properties were obtained for poly(MAA-co-EGDMA) nanospheres with a molar ratio of 4 to 1 between ethyleneglycol dimethacrylate (**16**) and methacrylic acid (**17**) [45]. Several investigations furthermore aim at the imprinting of protein surfaces onto miniemulsion polymers, but are out of the focus of this review, and are therefore not further discussed herein [54-57].

Non-Templated Macromolecular Catalysts Employing Miniemulsion Polymerization

As the macromolecular structure of enzymes is known to contribute to their catalytic ability, it was hypothesized that a reagent immobilized in a polymer matrix will support selective interactions between a catalytic site and a substrate due to the rigidity imposed by the polymer backbone [58, 59]. This might be achieved by appropriate building blocks of the macromolecular matrix that enable hydrophobic interactions, π–π stacking and/or hydrogen bonding in addition to the coordinative bonds at a metal complex-containing active site [59].

Along these lines, a low molecular weight and a *non-templated* macromolecular catalyst were very recently prepared and investigated for their ability to oxidize a catechol model substrate under identical conditions [59]. The low molecular weight entity is derived from a pentadentate salen-type ligand **19** that, after addition of copper(II) ions, yields dinuclear copper(II) complex *{N,N'*-1,3-bis[(2-hydroxy-4-vinylbenzyloxy) benzylideneamino]propan-2-ol}ato (μ-acetato) **20**.



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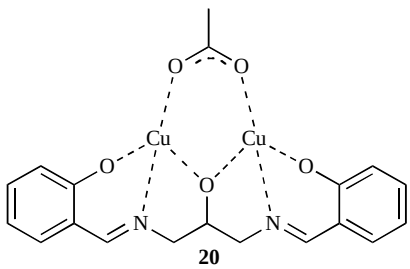

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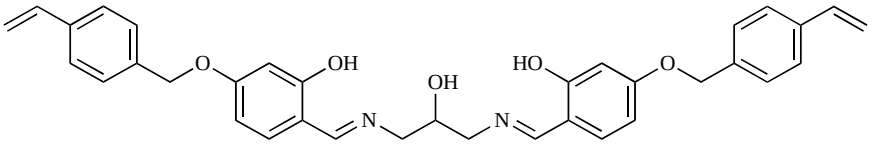

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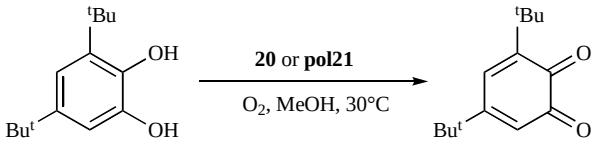
The macromolecular catalyst was derived from a polymerizable salen derivative **21**. As radical polymerizations are inhibited in the presence of paramagentic metal ion radicals, such as Cu(II) ions, ligand **21** was immobilized in a poly(acrylate) matrix by miniemulsion polymerization in the absence of metal ions. Elemental analysis of the dried beads confirmed quantitative incorporation of **21** into the poly(acrylate). TEM imaging revealed formation of spherical particles with a diameter of 50 nm on average. Isothermal titration calorimetry disclosed accessibility of the Cu(II) ions to the ligand core and quantitative complex formation with immobilized **21** in a 2 : 1 molar ratio. The addition of Cu(II) acetate activated the dormant catalyst **pol21** that was subsequently used for the aerobic oxidation of 3,5-di-*tert*-butylcatechol (3,5-DTBC) **22** to 3,5-di-*tert*-butylquinone **23** (3,5-DTBQ) in methanol at 30 °C (Scheme **3**) [59].



Scheme 3.

The initial rates for the formation of **23** promoted by **pol21** were analyzed using the Michaelis-Menten model. The reaction proceeds in the presence of 1.5 μM **pol21** with a rate constant k_{cat} of 3.3 min^{-1} and a Michaelis-Menten constant K_m of 200 μM. The acceleration of the reaction over background is 470,000 in the presence of **pol21**, which was shown to be almost 10-fold faster than for the low molecular weight salen-type catalyst **20**. The macromolecular structure of **pol21** does therefore significantly contribute to its overall catalytic activity [59].

CONCLUSIONS

The design of robust, synthetic entities with enzyme-like selectivity and high turnover numbers for any imaginable reaction is like the 'holy grail' that fascinates and drives many researchers in the field of molecular recognition. The very recent developments in the field discussed here employ miniemulsion polymerization as a new platform, which appears to be a very promising strategy towards this goal. While the turnover numbers in these systems can attain those of catalytic antibodies, high selectivity among substrates is achievable, reactions in water or aqueous organic solutions are within reach, and the employment of known transition metal complex-based catalysts is possible. Recent studies also demonstrate the contribution of the overall macromolecular environment to the catalytic turnover ability of a colloidal catalyst. Combining all these recent achievements in one catalytic system will certainly require a substantial amount of further studies prior to any industrial application, but promises challenges and fun for the near future.

ACKNOWLEDGEMENT

A career award from the National Science Fandation (CHE-0746635) is gratefully acknowledged.

ABBREVIATIONS

AIBN	=	2,2'-azobis(isobutyro)nitrile
Arg	=	arginine
C_M	=	critical monomer concentration
DMSO	=	dimethylsulfoxide
EGDMA	=	ethyleneglycol dimethacrylate
HEPES	=	2-[4-(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid
kDa	=	kilo Dalton
k_{cat}	=	reaction rate constant k of a catalyzed reaction
k_{uncat}	=	reaction rate constant k of an uncatalyzed reaction
k_{sol}	=	reaction rate constant k of a low molecular weight catalyst
K_M	=	Michaelis–Menten constant
M_R	=	relative molecular weight
M_w	=	weight-average molecular weight
M_n	=	number-average molecular weight
$M_{n,abs}$	=	absolute weight number-average molecular weight
M_w/M_n	=	polydispersity
nm	=	nanometer
TRIM	=	trimethylolpropane trimethacrylate
Tyr	=	tyrosine

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Received: April 04, 2008 Revised: November 20, 2008 Accepted: December 03, 2008