

Polymer Catalysts by Molecular Imprinting: A Labile Covalent Bonding Approach

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An imprinting technique with labile covalent interactions has been developed in the design of new polymer catalysts. The template monomer **2** was prepared and copolymerized with DVB or EDMA to provide the polymer with a cavity having the shape of the transition state of the reaction as well as binding sites for the substrate and catalytic functionalities. The rate of hydrolysis of diphenyl carbonate (**1**) in the presence of the imprinted polymer **IP-DVB-THF** was found to be 120 times faster than the background uncatalyzed reaction. A K_m of 32 mM and a k_{cat} of $1.8 \times 10^{-3} \text{ min}^{-1}$ were observed from Michaelis-Menten kinetics with the imprinted polymer **IP-DVB-THF**.

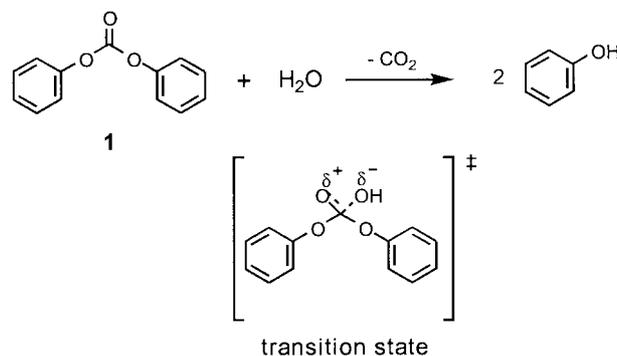
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Introduction

Molecular imprinting has proven to be a very effective method that creates three-dimensional binding sites in polymers.¹⁻⁶ Copolymerization of functional monomers (binding sites) in the presence of a template (or print) molecule and of cross-linkers followed by removal of the template generates specific binding cavities within the polymer matrix complementary to the template molecules. Application of the molecular imprinting technique ranges widely from polymer catalysts to sensor design, artificial receptors/antibodies, and HPLC stationary phases for chiral resolution (see reviews¹⁻⁵).

The development of an efficient artificial enzyme by molecular imprinting has been one of the most challenging subjects in this area and many efforts were directed to achieve this goal.⁷⁻¹² Since the discovery that considerable rate enhancement is observed for a reaction catalyzed by antibodies that were raised against an analog of the transition state of the reaction to be catalyzed,¹³ many research groups have focused on the development of catalytically active imprinted polymers. Thus, various transition state analogs have been prepared and polymerized with functional monomers and cross-linking agents to provide three dimensional binding cavities for the substrate. Recently, a very efficient polymer enzyme mimic has been reported with the rate enhancement of approximately 100 over the background reaction.⁷

Most common approaches in the design of catalytically active imprinted polymers utilize noncovalent interactions such as ionic and/or hydrogen bonding between the template molecule and the functional monomers acting as binding sites and/or as catalytic groups. We now report a polymer catalyst prepared by molecular imprinting using labile covalent bonds between transition state analog and functional



Scheme 1. Hydrolysis of diphenylcarbonate (**1**).

monomers.¹⁴

The main advantage of *covalent imprinting* is that the interaction between the template and functional monomer is much less affected by solvent polarity and temperature during the imprinting process. Furthermore, the functional groups are only situated inside the cavities and not statistically distributed all over the polymer matrix as it is usually the case with noncovalent imprinting in which a large excess of polymerizable binding sites have to be used.^{2,4} We have focused our initial studies on the hydrolysis of diphenyl carbonate (**1**) (Scheme 1).

Experimental Section

Materials. Diphenyl chlorophosphate and *N*-hydroxymaleimide were purchased from Aldrich. AIBN, which was recrystallized from methanol was obtained from Fluka. All other solvents and reagents were laboratory reagents grade or better.

Preparation of template monomer 2. After coupling diphenyl chlorophosphate (2.33 g, 8.67 mmol) with *N*-hydroxymaleimide (0.98 g, 8.67 mmol) in the presence of triethylamine in THF at 0 °C for 30 min, the precipitate was

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removed by filtration. The filtrate was concentrated and the residue was diluted with ethyl acetate. Addition of *n*-hexane to the solution led to precipitation of impurities. The mixture was filtered and the filtrate was concentrated. The above process was repeated until a clean oily residue was obtained (2.40 g). The product was pure enough for polymerization and was characterized by spectroscopic analysis. Attempts to purify the product by column chromatography (silica gel) resulted in decomposition.

^1H NMR (500 MHz) (CDCl_3 , ppm): δ 6.75 (s, 2H), 7.20–7.50 (m, 10H); ^{13}C NMR (CDCl_3 , ppm): δ 120.1, 126.1, 129.9, 132.9, 150.1, 150.2, 163.5; ^{31}P NMR (CDCl_3 , ppm): δ –10.4.

Preparation of imprinted polymers. In a typical experiment, the template monomer **2** (6 wt%) was mixed with styrene (14 wt%) and a large excess (80 wt%) of the cross-linker, 1,4-divinylbenzene (DVB), in THF solvent (1 : 1 weight ratio of monomers to solvent). After degassing the solution with repeated freeze-pump-thaw cycles, a free radical polymerization initiated by AIBN (1 wt% of total monomers) was conducted for 3 days at 60 °C in a fused ampule. A total 10 g of monomers was used for the polymerization. A control polymer without template was also prepared with *N*-hydroxymaleimide as the functional monomer under identical polymerization conditions. The resulting highly cross-linked macroporous polymers were ground and sieved. The particle fraction 45–125 μm in size was collected. After washing with acetone, the polymers were treated with a 1 : 1 mixture of 0.1 N aqueous HCl and THF in a shaker overnight at room temperature. During this process about 70% of diphenyl phosphate was released from the polymer obtained with the template monomer **2**. The polymer was then subjected to more vigorous hydrolysis conditions (24 hr reflux with 1 N aqueous HCl followed by 24 h reflux with 1 N aqueous NaOH). The polymers were thoroughly washed with distilled water until neutral.

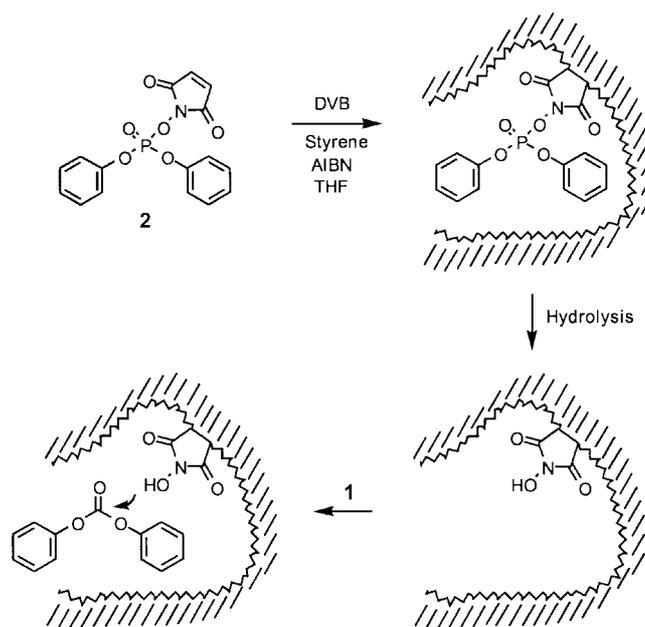
Determination of the specific surface area. Polymer surface areas were determined from multipoint N_2 adsorption isotherms and calculated by using the BET equation. Polymers were degassed *in vacuo* overnight at room temperature before measurement.

Kinetic assay. The hydrolysis of the substrate **1** was carried out in a mixture of 2-[4-(2-hydroxyethyl)-1-piperazino]-ethanesulfonic acid (HEPES) buffer (pH=6.8) and MeCN solvent by monitoring the release of phenol (270 nm) with time by a Waters HPLC system. Propiophenone was used as an internal standard. An example of the experimental procedure is given below: to 89 mg of polymer IP-DVB-THF was added 10 mL of CH_3CN /HEPES (2 : 1, v/v) and the resulting mixture was stirred at 15 °C for 1 h. After equilibration, 100 mL of diphenyl carbonate (100 mM CH_3CN solution) and 100 mL of propiophenone (10 mM CH_3CN solution) were added to the mixture. 100 mL of the reaction mixture was taken at the designated time and filtered to remove the polymer particles and injected to a reverse phase C_{18} HPLC column.

Results and Discussion

In order to generate both substrate binding sites and catalytic functionalities in the polymer matrix, we have designed template monomer **2** (Scheme 2). The diphenyl phosphate moiety of the template monomer should mimic the transition state of the reaction. The maleimide group will be converted to *N*-hydroxysuccinimide moieties under hydrolysis condition and should be able to facilitate the hydrolysis of the substrate by functioning as a nucleophilic catalyst. Template monomer **2** was prepared by coupling commercially available diphenyl chlorophosphate and *N*-hydroxymaleimide.

Template monomer **2** (6 wt%) was copolymerized in the presence of styrene (14 wt%) and a large excess (80 wt%) of the cross-linker, 1,4-divinylbenzene (DVB), in THF (1 : 1 weight ratio of monomers to solvent) by free radical polymerization. A control polymer without template was also prepared with *N*-hydroxymaleimide as the functional monomer under identical polymerization conditions. The resulting highly cross-linked macroporous polymers were ground and sieved. The particle fraction 45–125 μm in size was collected. After washing with THF (nearly no monomers extracted), the polymers were treated with a 1 : 1 mixture of 0.1 N aqueous HCl and THF in a shaker overnight at room temperature. During this process about 70% of diphenyl phosphate was released from the polymer obtained with the template monomer **2**. The polymer was then subjected to more vigorous hydrolysis conditions (24 hr reflux with 1 N aqueous HCl followed by 24 h reflux with 1 N aqueous NaOH). Additional 10% of diphenyl phosphate was released from the polymer in the form of phenol. The polymers were thoroughly washed with distilled water until neutral. The



Scheme 2. Preparation of imprinted polymers with **2** as the template monomer and removal of the template. A possible mechanism for the hydrolysis of diphenylcarbonate is given.

surface area and pore volume values of the imprinted polymer (804 m²/g and 1.29 mL/g) were found to be very close to those of the control polymer (1113 m²/g and 1.27 mL/g) proving that substitution of the functional monomer by hydroxymaleimide in the polymerization mixture of non-imprinted polymeric sample does not influence the polymer morphology. Although IR spectroscopic monitoring of the polymers showed that the *N*-hydroxysuccinimide moieties were found to be stable under the severe hydrolytic conditions, we should not eliminate the possibility of generating undetectable minute amount of completely hydrolyzed dicarboxylate groups in the polymer. In order to investigate the effect of polymer structure to reactivity, polymers were prepared also with methyl methacrylate and with ethylene dimethacrylate (EDMA) as a cross-linker.

Catalytic activity of the polymers was measured in a mixture of 2-[4-(2-hydroxyethyl)-1-piperazino]-ethanesulfonic acid (HEPES) buffer and acetonitrile solvent by monitoring the release of phenol with time by HPLC. Several other buffers, such as phosphate or TRIS, have been employed, but the best results were obtained with HEPES buffer. It is clear from Figure 1 (top) that the rate of hydrolysis of

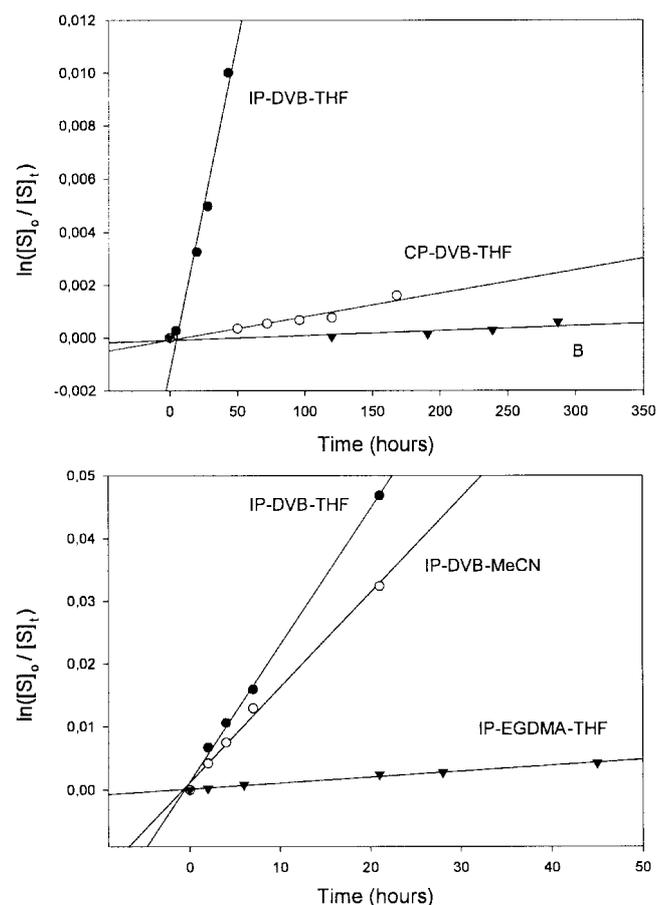


Figure 1. Hydrolysis of diphenyl carbonate in 5% HEPES (20 mM, pH 6.8)-MeCN at 15 °C (polymer: 1 mM available cavities, substrate: 5 mM) [top]. Comparison of catalytic performance among imprinted polymers prepared under different conditions in 66% HEPES (0.1 M, pH 7.3)-MeCN at 20 °C (polymer: 2 mM available cavities, substrate: 2 mM) [bottom].

substrate **1** in the presence of the imprinted polymer prepared with DVB in THF (**IP-DVB-THF**) is definitely larger than that with the control polymer (**CP-DVB-THF**) and that without any polymer in buffer/MeCN (**B**). If the rate of hydrolysis in buffer-MeCN without polymer is defined as 1.0, the rate is enhanced by a factor of 120 with the imprinted polymer. In addition, under these conditions the imprinted polymer catalyzes 28 times faster than the control polymers containing statistically distributed *N*-hydroxysuccinimide residues. Especially this value is quite remarkable since in all other reported cases this value was clearly lower.⁷⁻¹² This shows that the shape of the cavity provides strong catalytic activity.

Comparison of the rate of hydrolysis of the substrate by imprinted polymers prepared under different polymerization conditions is shown in Figure 1 (bottom). The imprinted polymer with DVB as cross-linker, **IP-DVB-THF**, is proved to be a better catalyst than EDMA-based polymer, **IP-EDMA-THF**. In general, it is known that EDMA-based materials are superior to DVB-based polymers in terms of selective binding when imprinted polymers are used as chiral stationary phases. It is to be expected that the rate enhancement in DVB-based catalysts is caused by hydrophobic interactions between the phenyl rings of the substrate and those of the polymer backbone. The solvent used in the polymerization also has an effect on the reactivity. Thus, the imprinted polymer **IP-DVB-THF**, which was obtained with THF as porogen, shows better catalytic performance than the polymer **IP-DVB-MeCN** prepared in the presence of acetonitrile. Acetonitrile as a poorer solvent for the polymer causes another macroporous structure compared to THF as solvent.

The polymer **IP-DVB-THF** imprinted with template monomer **2** exhibits Michaelis-Menten kinetics for hydrolysis of **1** in 66% HEPES-MeCN with a K_m of 32 mM and k_{cat} of $1.81 \times 10^{-3} \text{ min}^{-1}$ (Figure 2). The kinetic parameters for hydrolysis of substrate **1** by **IP-DVB-THF** indicate that the

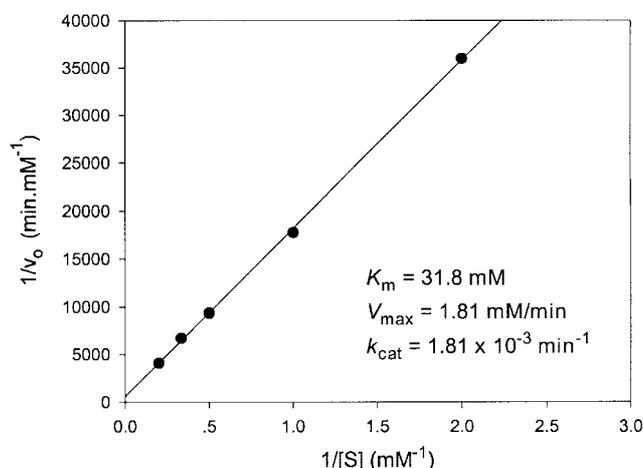


Figure 2. Lineweaver-Burk plot of kinetic data of hydrolysis of substrate **1** (5 mM) with **IP-DVB-THF** (1 mM available cavities in the polymer) in 66% HEPES-MeCN at 15 °C.

polymer is less effective than hydrolytic catalytic antibodies. However, these experiments provide the first example of an imprinted polymer-catalyzed hydrolysis of carbonate by imprinting with labile covalent interactions and demonstrate the feasibility of using this technology to the design of new polymer catalysts. Particularly noteworthy aspects of the results reported above include the following: (1) the design of a template molecule that provides the polymer both with binding sites for the substrate and as well as catalytic functionalities, (2) release of the template molecule from the polymer matrix under relatively mild conditions, leaving the polymer with the designed cavities, and (3) introduction of functional groups which are only located within the cavities.

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