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Biomimic recognition and catalysis by an imprinted catalysts: a rational design of molecular self-assembly toward predetermined high specificity

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This article presents an original work contributing to the rational design of imprinted catalyst by molecular self-assembly toward predetermined high specificity. Assembling with *p*-nitrophenyl phosphate as the transition state analogue (TSA) of *p*-nitrophenyl acetate esterolysis and 1-vinylimidazole as the functional monomer, the imprinted catalyst was prepared. An increase in the amount of assembled monomer results in a higher activity of hydrolysis, which, however, does not lead to an improvement of specificity. The best specificity is shown at the optimal self-assembly (corresponding to a stoichiometric interaction of monomer-TSA). Higher or lower an amount of assembled monomer would lead to a dramatic decrease in this specificity. Related information indicates that these may be a result of increasing specific interaction between the TSA and binding sites, which make the catalyst capable of selectively recognizing the transition state and promoting the conversion from reactant to the transition state.

KEY WORDS: molecular recognition; imprinted catalyst; self-assembly.

1. Introduction

Biomimic recognition and catalysis has been one of hot points in chemical research over the latest years [1–3]. In the wake of a jump from combinatorial method to molecular assembly, the oriented design by crown ethers or cyptands toward molecular recognition is entering into an era of free imprinting [4,5]. Comparable to the recognition of natural biomolecules such as antibody-antigen, receptor-ligand and enzyme-substrate etc, the molecular imprinting recognizes three-dimensionally a specific substrate basing on the same principle. For this reason, molecularly imprinted polymer is also called as 'artificial antibody' [6,7]. To fabricate a shape- and structural-complement between antibody and template (i.e. imprint molecule), this methodology uses usually molecular self-assembly to position the groups of functional monomer around an imprint molecule [8,9]. Subsequently, a photo- or thermal-polymerization in the presence of crosslinker is performed to fix the additive structure. The imprint molecule is then removed from the polymer, leaving behind binding sites complementary to the imprint molecule in terms of the shape and position of functional groups. The recognition of the imprinted cavity constitutes an induced molecular memory, which makes the binding sites capable of specifically recognizing the imprint species. As a rigid crosslinked-polymer, the significant advantages offered by imprinted antibody include physical robustness, high strength, resistance to elevated temperature and pressure, and inertia usually to acid, base, metal ion, organic solvent and so on. Owing to these features, molecularly imprinted antibodies can be used not only partially as the substitute of natural biomolecules but also as the substrate-selective or separation materials under harsh conditions [10–12].

As commonly known, the molecular recognition by imprinted antibody, in essence, is a result of shape- and structural-complement between the arrangement of functional groups (binding sites) and template. Thus, the fabrication of imprinted cavity by molecular self-assembly plays usually a crucial role on predetermining the recognizability of antibody. Unlike as the substrate-selective or separation material, the use of imprinted antibody as a catalyst has to choose the transition state of a specific reaction or a transition state analogue (TSA) as the template (i.e. the imprint species) [13,14]. Reason for this can be related to that activation energy is usually required to overcome the potential barrier between reactant and product (i.e. a conversion from the reactant to the transition state). Since the transition state itself can not be directly used as the imprint species because of its instability, a TSA, usually extremely similar to the transition state is generally required to use. As assembled with monomers, the functional groups of monomers are positioned regularly around the TSA, forming spatially and sterically an order of binding sites. After fixed by polymerization and removed template, the binding sites complementary to the TSA in terms of

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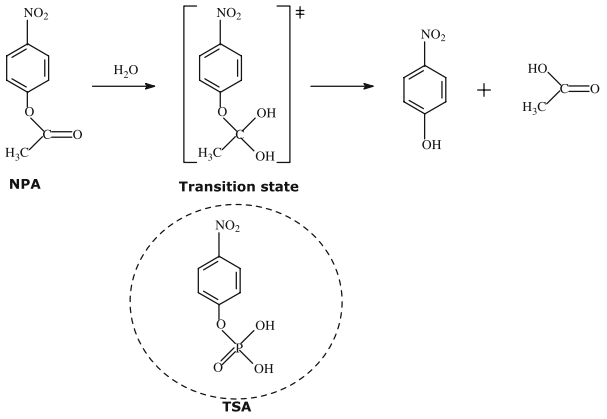
the shape and position of functional groups are left behind. The regular architecture therefore has the ability to recognize the transition state and to promote a conversion from the reactant to the transition state. Since the study of imprinted catalyst is in its infancy, there has not yet been a commonly accepted viewpoint on predetermining the practical function. Based on simple contrasts [15,16], some researchers have concluded that a larger extent of self-assembly can result in a higher specificity. Others have claimed that the shape of imprinted cavity is the main aspect of molecular recognition, and that a change in the shape will result in a lower level of distinguishability [17]. More recently, more and more researches trend to support a modest extent of self-assembly with respect to the best recognizability [18,19]. Clearly, for the rational design of imprinted catalyst toward predetermined high specificity, a specialized work is necessary.

In this article, an original work dedicating to the rational design of molecular self-assembly toward predetermined high specificity was presented. The hydrolysis of *p*-nitrophenyl acetate (NPA) was selected as the test reaction as it has a well-proven TSA (i.e. the *p*-nitrophenyl phosphate; NPP) for the transition state (scheme 1) [20,21]. Assembling with NPP as the template and 1-vinylimidazole as the functional monomer, the imprinted catalyst was prepared and used for substrate-discriminated hydrolysis. For a contrastive presentation, the self-hydrolysis of NPA, along with the hydrolysis of related ester (i.e. *t*-butyl acetate; BA), were also carried out. The aim is to present a complementary study to the general design of imprinted catalysts.

2. Experimental

2.1. Materials

p-Nitrophenyl acetate (NPP) and divinylbenzene (DVB; mainly *p*-isomer) were purchased from Acros (Belgium). *p*-Nitrophenyl phosphate is the product of Biocity (UK). 1-Vinylimidazole and *t*-butyl acetate (BA) were obtained from Aldrich (USA). 2,2'-Azobis (isobutyronitrile) (AIBN) were purchased from Peking Chemical Reagent Plant (China) and other chemicals concerned are commercially available products of reagent grade.



Scheme 1. General process involved by NPA hydrolysis.

2.2. Preparation of imprinted catalysts

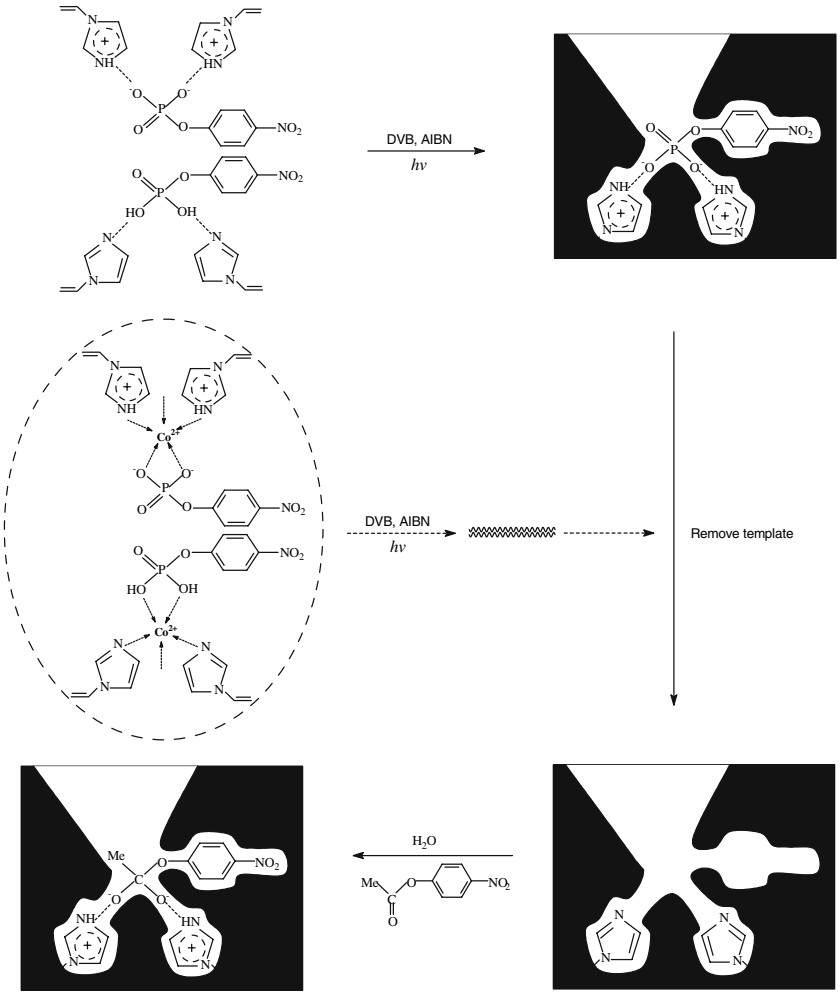
Scheme 2 presents the technical outline for preparing imprinted catalysts [20]. 0.11 g (0.5 mmol) of NPP, 1.4 mL (9.87 mmol) of DVB, 0.6 g (3.65 mmol) of AIBN and a certain amount of 1-vinylimidazole (stated additionally in text) were dissolved in a mixture of acetonitrile (5mL) and dimethyl sulphoxide (5mL). After deoxygenation with sonication and nitrogen, the system was irradiated by ultraviolet light (365 nm) at ice water (0 °C) for 5 days. The resulted polymer (mentioned as catalyst precursor) was crushed roughly and subsequently washed by a mixture of methanol and acetic acid (in a volume ratio of 9:1) with many cycles to remove the template. The final polymer (i.e. imprinted catalyst) was dried in a vacuum vessel (20 °C) and then ground into 40–50 mesh for further study.

2.3. Catalyzed hydrolysis

In a batch format, the catalyzed hydrolysis of NPA was performed in phosphate buffer (pH 7.0) at a 30 °C water bath [18,20]. The initial concentration of NPA is 0.01 μmol/mL (totally 10 mL). The solid content of catalyst was 3.3 mg/mL in each operation. The sample solutions (in identical quadruple) were stirred for a regular time. The released *p*-nitorophenolate was monitored at 400 nm. The hydrolysis amount per gram catalyst was obtained from the average value of quadruple samples. For a contrastive study, the hydrolysis of BA was determined under comparable conditions. To subtract the effect of self-hydrolysis, the hydrolysis of NPA and BA in the absence of catalyst was also carried out.

2.4. Temperature-programmed desorption

Temperature-programmed desorption (TPD) experiments were carried out to evaluate the interaction of imprinted catalyst and related substrates [22]. In an apparatus including gas chromatography, TCD detector and data processing system, 0.2 g of catalyst was placed into an online quartz U-shaped tube (4 mm I.D.). After pre-adsorbed with 10 μL of substrate (0.01 μmol/mL-acetonitrile), the imprinted catalyst was heated in a flow of nitrogen (40 mL/min; 0.24 MPa) from the room temperature to 280 °C with a rate of 10 °C/min. The signal for substrate desorption was simultaneously recorded by the data processing system.



Scheme 2. Schematic presentation of the preparation and recognition of the imprinted catalyst.

3. Results and discussion

3.1. Template-monomer interaction and molecular self-assembly

As already mentioned, the molecular self-assembly between monomer and TSA is a premise for the fabrication of imprinted cavity. Normally, depending on this template-monomer interaction, the imprint is achieved by arranging polymerizable functional monomers around TSA. Figure 1 presents the titration of 1-vinylimidazole to NPP under the monitoring of UV spectrum. With the proportional recipe as the control and initial basis (section 2.2), the monomer 1-vinylimidazole is titrated into the TSA. A titration of 1-vinylimidazole results in a shift of UV absorption-band. The shift is evidenced by an increase in the titration amount. The shift in the absorption band achieves a maximum when the titrated 1-vinylimidazole reaches a critical value (corresponding to 1.53 mol/mol of monomer-TSA ratio). Beyond the critical point, no additional shift in the absorption band is observed except for an increasing absorbency. This indicates that there is an interaction existing between the monomer and the TSA, and that this interaction is subsequently saturated by adding a chemically stoichiometric amount of monomer. As perceived from the general mechanism of imprinted catalyst (scheme 2), too high an amount of assembled monomer will render an imprinted cavity with spatial and steric mismatch to the transition state, due to an over abundance of functional groups that distribute randomly throughout the polymer. Too low an amount of assembled monomer will deliver the polymer with an insufficient quantity of functional groups to achieve a complete self-assembly. Thus, only a stoichiometric amount of assembled monomer could be anticipated to achieve the best catalytic specificity. In the present case, the optimal amount of assembled monomer is shown at 1.53 mol/mol. Thus, with this amount, the prepared catalyst is normally expected to present the best molecular recognition. According to the basic recipe as presented in section 2.2, the expected catalyst can be prepared with the use of 0.765 mmol of 1-vinylimidazole. In this context, the further study is performed and the details are shown below.

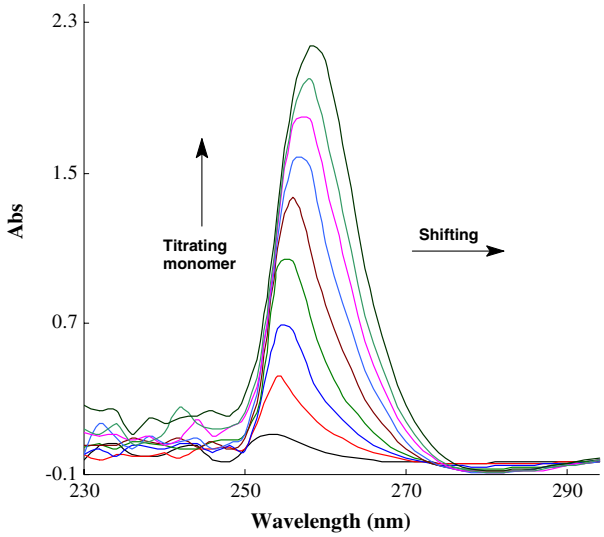


Figure 1. The curve of titrating 1-vinylimidazole to TSA under the monitoring of UV.

3.2. Characterization of imprint

Figure 2 presents the infrared spectrum of catalyst prepared (i.e. Cat). Beyond the fingerprint area, there are two main absorption-bands existing in the spectrum, distributing respectively within the ranges between 2800–3100 and 3200–3600 cm^{-1}. In basic condition, as already displayed [23], the absorption band ranged from 2800 to 3100 cm^{-1} can be related to the stretching of whole imidazole-group. The peek with the range of 3200–3600 cm^{-1} may be responsible for the stretching of C–O (linked to carboxyl group or benzene cycle). For a purpose of clarification, the spectra of catalyst precursor, TSA and the blank polymer as prepared without TSA are also enclosed in figure 2. As observed, relative to the blank polymer, the adsorption band (ranged from 3200 to 3600 cm^{-1}) of the catalyst precursor is obviously stronger. After washing, this adsorption band is dramatically decreased to almost the same level as that of the blank polymer. Figure 3 presents the morphological difference of catalyst and the blank polymer. The blank polymer shows a slippery surface. However, there are obviously some cavities existing within the prepared catalyst. These strongly indicate that an imprint of template is formed within the catalyst. As also noted, after washing, the spectrum of catalyst shows no visible difference from that of blank polymer. This indicates that almost all TSA are removed from the precursor, which thus presents a convenience for subsequent use.

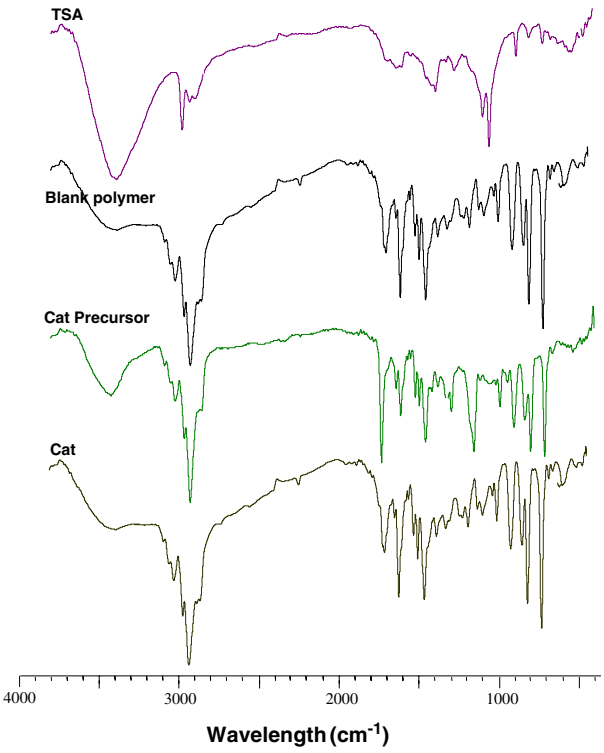


Figure 2. IR spectra of catalysts before and after removing template.

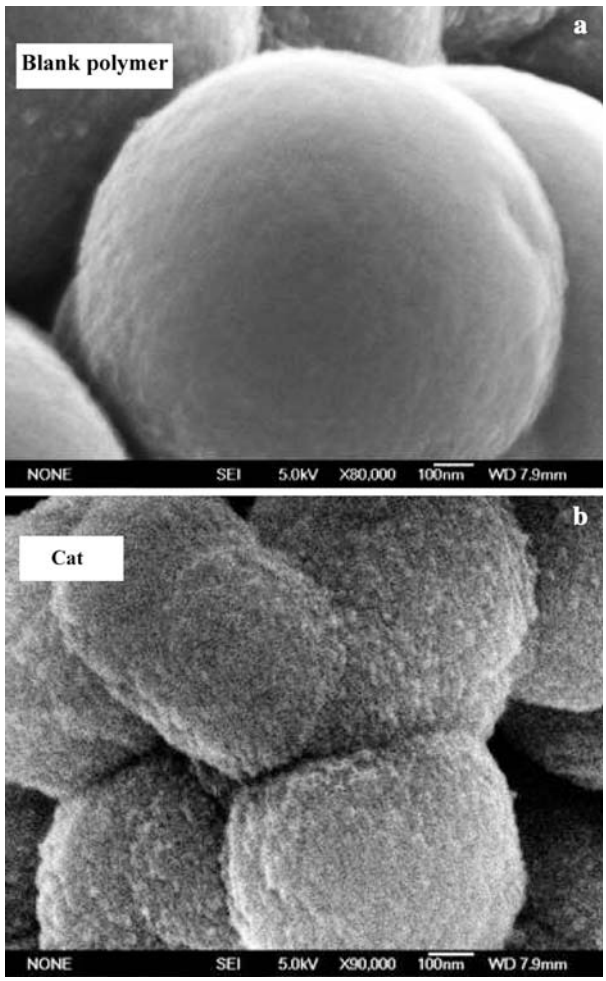


Figure 3. SEM profile of catalyst prepared ((**a**) blank polymer (**b**) Cat).

3.3. Catalyzed hydrolysis and molecular recognition

Figure 4 presents the hydrolysis curves of Cat (in which the line represents NPA and the break one corresponds to BA). An increase of time results in a larger hydrolysis. Relative to the self-hydrolysis, the hydrolyzed NPA and BA in the presence of Cat is higher. The Cat plays obviously a catalyst role. As specially noted, in the self-hydrolysis, the conversion of BA is higher than that of NPA. In the presence of Cat, the order is reversed—the hydrolyzed NPA being higher than the BA. This indicates that the catalyzed hydrolysis by Cat can be a substrate-discriminated process. As subtracted the effect of self-hydrolysis from the hydrolysis curves, figure 5 presents a clear-cut outline for the catalyzed hydrolysis. The Cat shows obviously a preferential promotion for NPA hydrolysis. For an evaluation of catalysis specificity, the hydrolysis curves of two control catalysts as prepared under comparable conditions (except for the amount of assembled monomer) are also enclosed in figure 5. In the preparations of Cat-L and Cat-H, the monomer-TSA ratios used are 0.77 and 3.06, respectively lower and higher than 1.53 of the Cat. In the whole hydrolysis spectrum, an increase in the amount of assembled monomer results in a higher catalysis activity. Like the Cat, both controls show also preferential promotions for NPA hydrolysis. However, the relative preference of both controls is much lower than that of the Cat (figure 6). These strongly indicate that there is a high specificity of interaction existing between the Cat and the NPA, and that the changing monomer-TSA ratio can play an important role on influencing this specificity. As explained previously, the specific catalysis by imprinted catalyst, in essence, is a result of matching arrangement between the transition state and binding sites. As assembled with chemically stoichiometric monomer, all groups of monomer positioned regularly around the TSA- forming three-dimensionally an order arrangement of binding sites. Relatively higher or lower

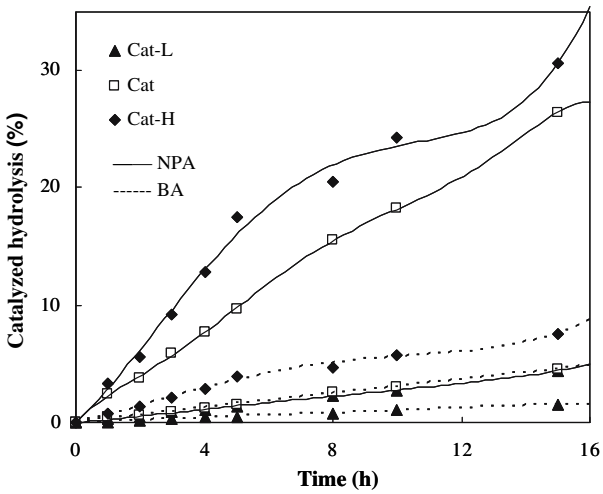


Figure 5. Catalyzed hydrolysis curves.

an amount of assembled monomer would lead to a spatial mismatch of binding sites to the transition state. Thus, only the use of stoichiometric monomer could achieve normally the highest loyalty to the transition state. As a result, the highest specificity of hydrolysis is shown at the Cat.

3.4. Simulation of kinetics and fitting

To simulate hydrolysis of esters, the pseudo-first-order kinetic process is in common use [24,25]. Reason for this can be related to an over abundance of water as solvent and reactant. In generalization, the pseudo-first-order kinetics is also used in the present case:

$$-\frac{dC}{dt} = kC \tag{1}$$

Correlating the relationship in the cases of NPA and BA would present the hydrolytic specificity of catalyst:

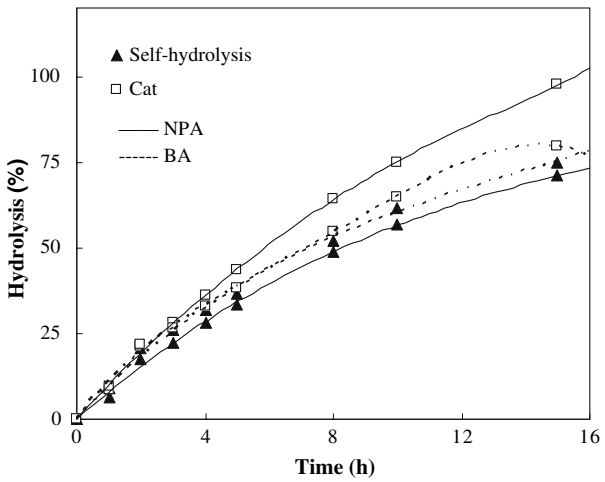


Figure 4. Hydrolysis curves.

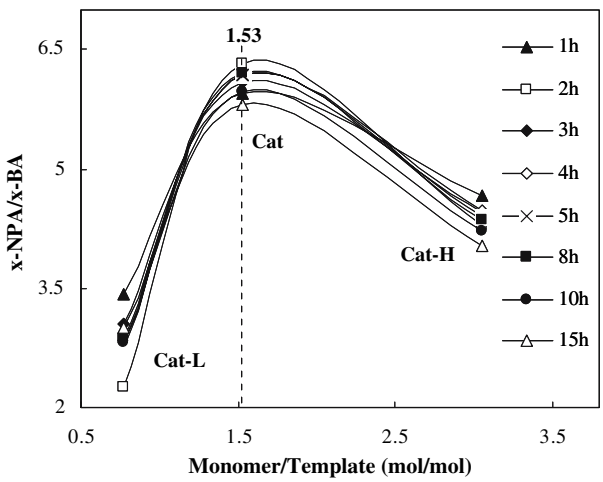


Figure 6. Effect of monomer-template ratio on catalytic specificity.

$$\frac{dC_{NPA}}{dC_{BA}} = \frac{k_{NPA}}{k_{BA}} \cdot \frac{C_{NPA}}{C_{BA}} = \frac{k_{NPA}}{k_{BA}} \cdot \frac{C_0(1-x_{NPA})}{C_0(1-x_{BA})} = \frac{k_{NPA}}{k_{BA}} \cdot \frac{1-x_{NPA}}{1-x_{BA}} = \frac{dx_{NPA}}{dx_{BA}} \quad (2)$$

Here C is the concentration of substrate, k the rate constant of hydrolysis and x is the conversion of substrate. The subscripts 'NPA' and 'BA' are related to NPA and BA. Now, defining a constant A_r, reflecting the relative reactivity of NPA and BA, as follows

$$A_r \equiv \frac{k_{NPA}}{k_{BA}} \quad (3)$$

One can seek equation (2) into such a form:

$$\frac{dx_{NPA}}{1-x_{NPA}} = A_r \cdot \frac{dx_{BA}}{1-x_{BA}} \quad (4)$$

which thus gives

$$\ln(1-x_{NPA}) = A_r \ln(1-x_{BA}) \quad (5)$$

In this equation, plotting ln $(1-x_{NPA})$ versus ln $(1-x_{BA})$ is normally expected to be a straight-line. From the slope, the A_r-value can be obtained. As shown in figure 7, the Cat leads to a largest A_r-value among these mentioned catalysts. This indicates that the hydrolytic specificity of Cat is the best among these catalysts. As already explained, the molecular recognition and specific catalysis by imprinted antibody, in essence, is a result of shape- and structural-complement between the arrangement of binding sites and template. Since the molecular self-assembly with stoichiometric monomer can present relatively a higher loyalty to the transition state, the best specificity for NPA hydrolysis is expectant and learnable.

3.5. Specific interaction and binding framework

To evaluate the interaction of catalyst-substrate, the temperature-programmed desorption (TPD) was carried out (figure 8). At 198 °C, the main desorption of BA is occurred. The main desorption of NPA is, however, at 164 °C. For a contrastive presentation, the TPD profiles of both controls are also enclosed. In the whole spectrum, the increasing amount of assembled monomer results in a larger remaining-temperature. However, the largest discrimination for NPA and BA is shown at the Cat. The difference between NPA and BA on the remaining temperature can be 39 °C. These indicate that there is a high specificity of interaction existing between the Cat and NPA, and that the changing amount of assembled monomer plays an important role on influencing this specificity. As already interpreted, the molecular recognition by imprinted antibody can be due to the matched arrangement between binding sites and template. Since the self-assembly with stoichiometric monomer can present exactly a matched framework to the template, a relative higher cooperative-induction from these binding sites can be achieved. As a result, the largest discrimination for NPA and BA is shown at the Cat. As correlated to the previous study, these reveal that the specific catalysis by imprinted catalyst is an external embodiment of these highly specific interactions, which makes the catalyst capable of specifically promoting the conversion of NPA.

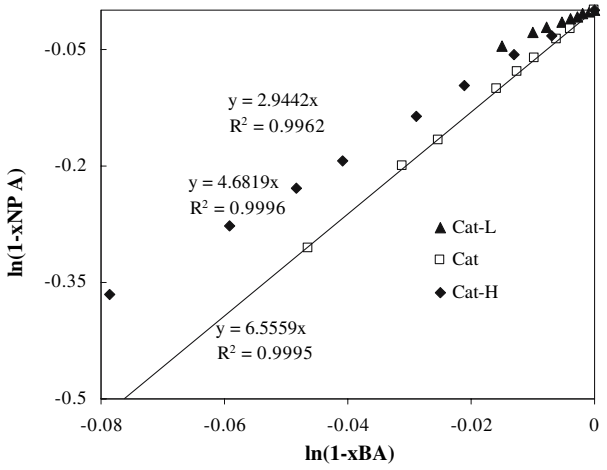


Figure 7. Coupling of first-order kinetics.

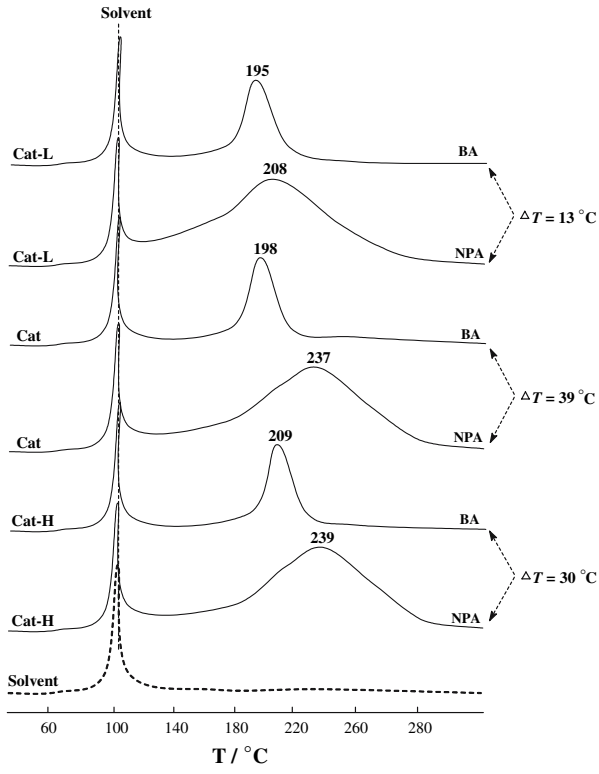


Figure 8. TPD profile for catalysts prepared.

4. Conclusive remarks

This article presents an original work dedicating to the rational design of imprinted catalyst by molecular self-assembly toward predetermined high specificity. Chemically stoichiometerically assembling with *p*-nitrophenyl phosphate as the TSA of *p*-nitrophenyl acetate (NPA) esterolysis, 1-vinylimidazole as the functional monomer, the imprinted catalyst was prepared and used for substrate-discriminated hydrolysis. For a contrastive clarification, two controls were also enclosed in the experimental spectrum. The use of stoichiometric monomer plays a positive role on ameliorating the prepared catalyst, increasing significantly the level of specific hydrolysis. Higher or lower an amount of assembled monomer would result in a dramatic decrease of this specificity. Related information indicates that these may be a result of increasing specific interaction between the TSA and binding sites, which make the catalyst capable of selectively recognizing the transition state and promoting the conversion from reactant to the transition state. In content, this work presents a complementary study to the general design of imprinted catalyst. It is also necessary to point out that these results are preliminary and that further work is necessary regarding a clearer understanding.

Acknowledgments

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