CHEMICAL REVIEWS

Volume 102, Number 1 January 2002

Enzyme-like Catalysis by Molecularly Imprinted Polymers

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Received April 2, 2001

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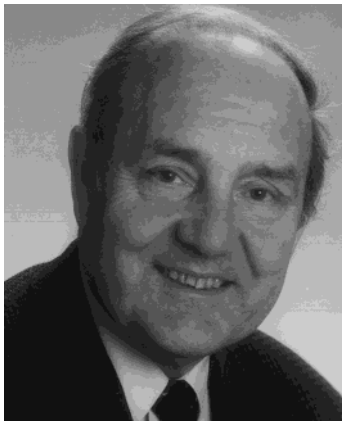
I. Introduction

The development of new and efficient catalysts plays a central role in chemical research. The progress in synthetic work, both scientifically and technically, depends greatly on the quality of the catalysts. In the preparation of such catalysts, a promising approach is to translate the principles of enzyme catalysis for the design of new catalytic materials. Thus, artificial enzyme analogues might be synthesized that possess a high catalytic activity and also show substrate, reaction, and stereoselectivity comparable to enzymes. At the same time, they might be better accessible, more stable, and catalyze a larger variety of reactions. In addition, such enzyme mimics offer the opportunity that the characteristics of enzyme catalysis can be studied in greater detail by systematically varying and simplifying the functional groups in the active site. This can help us to gain a better understanding of the whole process.

Recent years have seen remarkable progress in the design of enzyme mimics based on low molecular weight substances. Cram[1] and Lehn[2] made use of crown ethers or cyptands as the molecular hosts providing cavities for specific binding. Furthermore, it was possible to include catalytically active functional groups within the cavity in the correct vicinity to reacting groups of the bound substrate. Remarkable enhancements in rate and selectivity were observed, though turnover numbers of such reactions were mostly poor. Other hosts have been used in the form of cyclodextrins (for reviews, see refs 3−5), large ring systems (for reviews, see refs 6−8), or in the form of certain concave molecules (for a review, see ref 9) for performing such type of selective chemical operations. General reviews[10,11] are available, and an in-depth discussion of the underlying principles has recently been given by Kirby.[12]

10.1021/cr980039a CCC: $39.75
Published on Web 12/18/2001



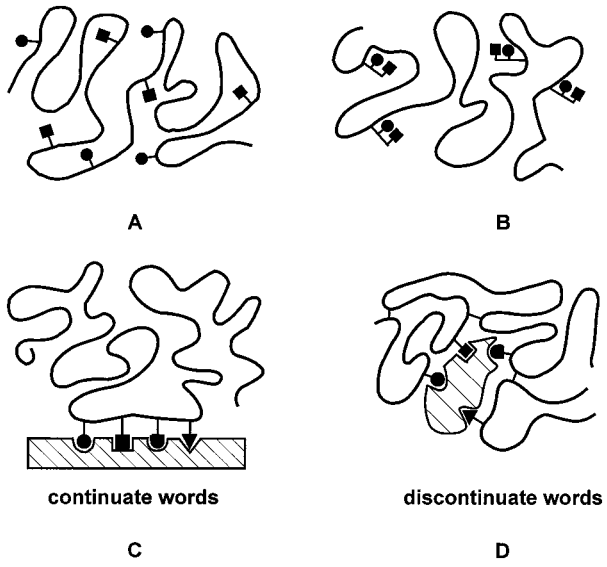
Günter Wulff is professor emeritus for organic and macromolecular chemistry at the Heinrich-Heine-University in Duesseldorf, Germany. He studied chemistry in his native city Hamburg, where he received his diploma. He was awarded his Ph.D. at the University of Bonn in 1963 for a dissertation on the isolation and characterization of glycosidic natural products from plants under the supervision of R. Tschesche. His habilitation, dealing with glycoside synthesis, was completed in 1969 in Bonn. Since 1979 he has been a full professor at the Heinrich-Heine-University. His research interests include the synthesis of polymers with enzyme analogues properties through imprinting with template molecules, the synthesis and investigation of optically active (main-chain chiral) vinyl polymers, and the application of renewable resources (monosaccharides and starch) for the synthesis of specialty polymers.

Of special interest in preparing enzyme mimics would be the use of synthetic polymeric substances, since these compounds are usually very stable against heat, chemicals, and solvents, and they can easily be fabricated in a form suitable for industrial application. Basically, the use of polymers makes the system more complicated compared to its low molecular weight counterparts, since the support needs to be prepared in a defined three-dimensional structure. On the other hand, polymers offer certain advantages if the macromolecular nature of enzymes is taken into consideration. In fact, many of the unique features of enzymes are directly related to their polymeric nature. This is particularly true for the high cooperativity of the functional groups and the dynamic effects such as the induced fit, the allosteric effect, and the steric strain exhibited by enzymes.

There has been a long tradition of using synthetic polymers as the backbone of enzyme models (for reviews, see refs 13−19). Especially when the mechanism of the enzyme catalysis of chymotrypsin in the late 1960s became clear (for reviews, see refs 11, 20, 21), a lot of activity in the polymeric field started.[22,23]

For obtaining polymeric catalysts as enzyme mimics, catalytically active groups were introduced into polymers mostly by copolymerization of the appropriate monomers bearing the desired catalytic functionalities (e.g. imidazole, OH, and COOH). This method provides a polymer with randomly distributed functional groups (Scheme 1A). Another possibility involves the attachment of side chains, containing the desired arrangement of functional groups, onto the parent polymer (Scheme 1B) (see, e.g., ref 24). A third possibility is the polymerization or polycondensation of monomers with the desired linear arrangement of functional groups. In this case, the groups are localized in the main chain one after another, as in certain hormone receptors (Scheme 1C) (see, e.g., ref 25). All

Scheme 1. Possible Arrangements of Functional Groups in Synthetic and Natural Polymers (reprinted with permission from ref 29. Copyright 1973 Elsevier Science)

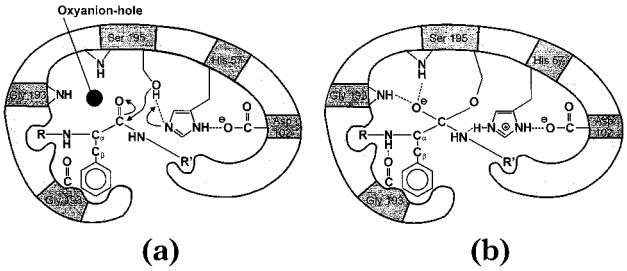


these attempts brought rather limited success, since it was not possible to orient a binding site and certain catalytic functionalities in a defined three-dimensional neighborhood. In some cases, however, polymers even only with statistical cooperativity gave surprisingly high catalytic activities (see, e.g., refs 26 and 27).

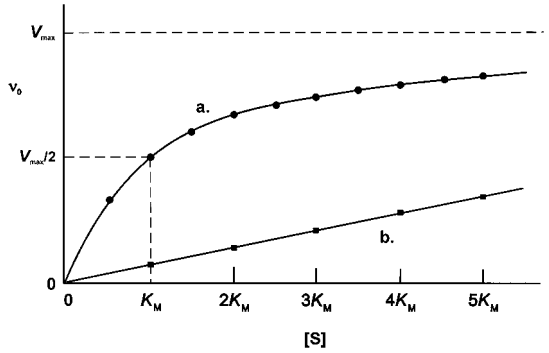
Natural enzymes and antibodies possess quite another arrangement of functional groups responsible for the specificity. These are located at rather distant points from each other along the peptide chain and are brought into spatial relationship as a result of specific folding of the chain. In this case, both the functional group sequence in the chain and the peptide's tertiary structure, i.e., its topochemistry, are decisive (Scheme 1D). This type of arrangement in enzymes has been termed as the "discontinuate word" by Schwyzer.[28] It leads to a complex, three-dimensional, steric arrangement of the functional groups.

Improved polymers as enzyme mimics require the use of a "discontinuate word" arrangement of functional groups, and more of the typical features of the enzyme action have to be verified. Some specific features of enzyme action are therefore briefly discussed (for more details, see, e.g., ref 20). By looking at the well-known serine-protease chymotrypsin, these features will be illustrated. Scheme 2 shows a schematic picture of the active site of chymotrypsin. The shape of this site and the arrangement of suitable binding sites is complementary to the chemical structure of the substrate, and therefore, the substrate, in this case an L-phenylalaninamide, is bound selectively (a). Such binding of the substrate usually produces an appreciable change in the three-dimensional conformation of the peptide chain and of the amino acid residues in the active site. This phenomenon is called "induced fit".[30] The substrate−enzyme binding interactions are rather complex and consist of a combination of electrostatic interactions, hydrogen bonds, hydrophobic interactions, and others.

Scheme 2. Schematic Picture of the Mechanism of Chymotrypsin Action Hydrolyzing an L-Phenylalaninamide (adapted from ref 31)



Scheme 3. Schematic Diagram of Michaelis−Menten Kinetics (a), in Comparison to Simple Catalysis, e.g., by Protons (b)



In a proper orientation with respect to the substrate, a charge relais system of catalytically active groups—a hydroxyl group from serine, an imidazole group of histidine, and a carboxyl group from asparaginic acid—attacks the carbonyl group of the amide. The newly formed tetrahedral transition state is even more firmly bound than the substrate, since the active site can accommodate the tetrahedral transition state more precisely and the oxyanion hole is now occupied (b). The preferred binding of the transition state lowers the activation energy of the reaction and has thus a catalytic effect on the reaction rate. This was already postulated by Pauling[32] and later discussed more in detail by Jencks.[33] The concept was shown to be correct by Lerner and by Schultz, independently, by generating antibodies against a stable transition state analogue of a reaction (for reviews, see refs 34 and 35). These antibodies showed considerable catalytic activity. Thus, antibodies prepared against a phosphonic ester (as a stable transition state analogue for alkaline ester hydrolysis) enhanced the rate of ester hydrolysis by 10^3-10^4-fold.

Enzymes show—unlike most catalysts used in chemical laboratories or in industry—a peculiar kinetic behavior. Scheme 3 shows the typical Michaelis−Menten kinetics.

The substrate is bound to the enzyme in an preequilibrium step. The bound substrate is converted under catalysis of the enzyme to products, which are afterward released. If the reaction is performed with increasing amounts of substrate (see Scheme 3), the rate of the reaction first increases with increasing substrate but then levels off and at higher substrate concentration, when all active sites are occupied, it remains constant, i.e., it is zero-order with respect to substrate concentration (saturation kinetics). In a simple case, the kinetics can be described by the Michaelis−Menten equation (see, e.g., ref 31).

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

$$\frac{d[P]}{dt} = -\frac{d[S]}{dt} = k_{cat}[ES] = \frac{k_{cat}([E] + [ES])}{1 + K_m/[S]}$$

if $[S] \gg ([E] + [ES])$ and $[S] \ll K_m$

$$-\frac{d[S]}{dt} = \frac{k_{cat}}{K_m}[S]([E] + [ES])$$

where E = enzyme, S = substrate, P = product, ES = enzyme−substrate complex, EP = enzyme−product complex, k_{cat} = rate constant of the catalyzed reaction, and K_m = Michaelis constant.

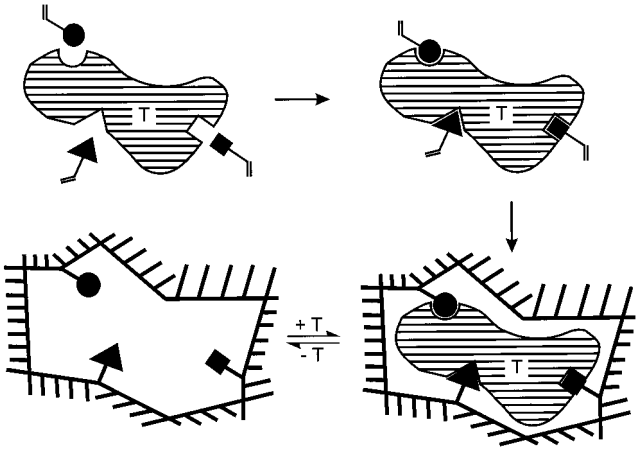
Under standard conditions, the rate of the catalytic reaction is proportional to k_{cat}/K_m. K_m reflects the affinity of the enzyme for its substrate, formally it is derived from a dissociation constant. The lower the value of K_m (usually given in mM) the more tightly the substance is bound. An ordinary noncatalyzed reaction or a chemically catalyzed reaction (e.g., by acid) shows, under the same conditions in nearly all cases, a straight line (see Scheme 3) relative to substrate concentration.

Looking at this enzyme mechanism, a number of prerequisites have to be fulfilled for the preparation of synthetic polymeric materials showing enzyme-like catalytic activity. First, a cavity or a cleft has to be made with a defined shape corresponding to the shape of the substrate or, even better, to the shape of the transition state of the reaction. At the same time, functional groups have to be introduced that act as binding sites, coenzyme analogues, or catalytic sites within the cavity and are in a defined stereochemistry. Since binding and catalysis in enzymes is a rather complex procedure, simplified structures have to be found that can be handled more easily. In this case it is not necessary that types of binding or catalysis are identical to enzymes; only the overall characteristics should be analogous. In the case of coenzymes, closer analogies might be possible.

To fulfill these prerequisites, we have introduced a novel strategy for obtaining "enzyme-analogue built polymers" quite some time ago.[29,36,37] For this, a cross-linked polymer is formed around a molecule that acts as a template. The monomer mixture contains functional monomers that can interact with the template through covalent or noncovalent interaction. After removal of the template, an imprint containing functional groups in a certain orientation remains in the highly cross-linked polymer. The shape of the imprint and the arrangement of the functional groups are complementary to the structure of the template (see Scheme 4).

In this review the present status and the problems in the preparation of enzyme-like catalysts made by imprinting will be discussed. This topic has also been covered to some extent in a number of general review articles on molecular imprinting. The topic was

Scheme 4. Schematic Representation of the Imprinting of Specific Cavities in a Cross-Linked Polymer by a Template (T) with Three Different Binding Groups (adapted from ref 38)



treated in detail by Davis.[39,40] One of his special interests concerned the preparation of catalysts by imprinting in silica and zeolites.

Most papers on catalytically active imprinted polymers appeared during the last 10 years. As there are very few before this period, they too will be included in this discussion.

II. Some Basic Considerations on Molecular Imprinting in Polymeric Materials for the Preparation of Catalysts

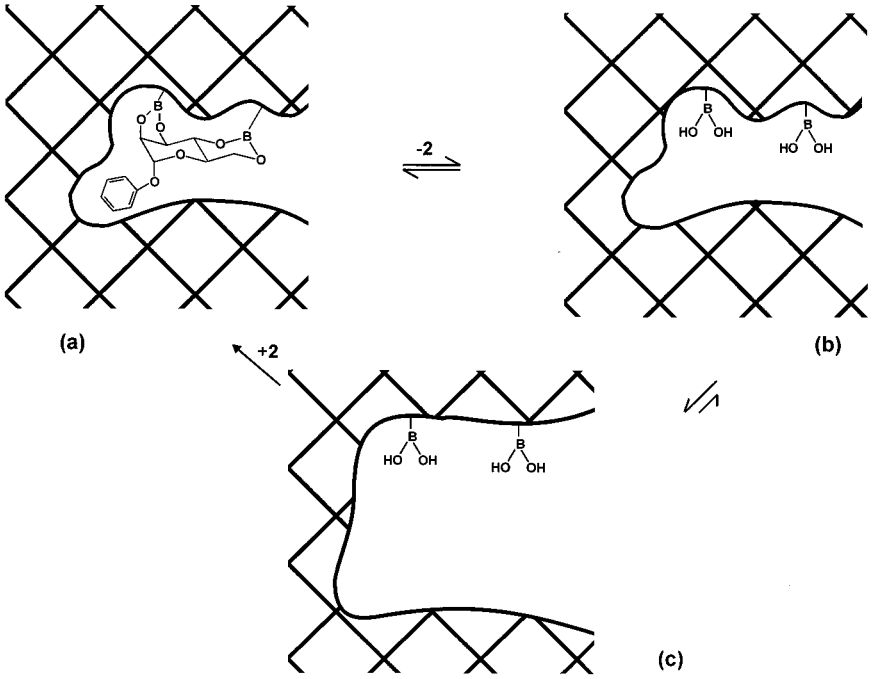
A. An Example of Covalent Imprinting

Numerous reviews on the molecular imprinting procedure have been published during the last years (see, e.g., refs 41−56) as well as two books.[57,58] The reader is referred to these quite detailed descriptions. In this paragraph some details will be discussed that are especially important for the application of imprinted polymers as catalysts.

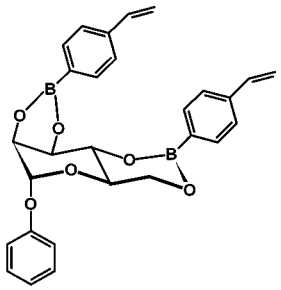
Polymerizable functional groups are usually bound by covalent or noncovalent interaction to a suitable template molecule (see Scheme 4). The template monomer is then copolymerized by radical initiation in the presence of a large amount of cross-linking agent and a certain amount of an inert solvent (acting as a porogen). This procedure furnishes macroporous polymers with a permanent pore structure and a high inner surface area. Under proper conditions the template molecules can be split off in high percentages. Optically active templates have been used in most cases during optimization. In these cases the accuracy of the structure of the imprint (the cavity with binding sites) could be measured by its ability for racemic resolution, which was tested either in a batch procedure or by using the polymeric materials as chromatographic supports.

One of the early examples of imprinting is shown in Scheme 5. In this case, phenyl-α-D-mannopyranoside **2** acts as the template.[38,60] Two molecules of 4-vinylbenzeneboronic acid are bound by covalent diester linkages to this template. Monomer **1** is copolymerized with a large excess of ethylene dimethacrylate as cross-linker. After the template has been split off by the addition of water or methanol to an extent of up to 95%, the polymer is equilibrated in a batch procedure with a solution of the racemate of the template. The enrichment of the antipodes on the polymer and in solution is determined and the separation factor α, i.e., the ratio of the distribution coefficients of D and L compound between polymer and solution, is calculated. After extensive optimiza-

Scheme 5. Schematic Representation of a Cavity Obtained by Polymerization of 1[a] (adapted from refs 41 and 59)



[a] The template **2** can be removed from (a) with water or methanol to give (b). Solvation of the functional groups leads to swelling of the cavity (c). When the template binds again, the original shape is restored (in analogy to an "induced fit").



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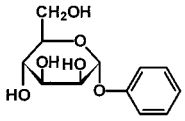


2

tion of the procedure, α-values between 3.5 and 6.0 were obtained. The selectivity in a chromatographic procedure is also high (separation factors up to $\alpha = 4.6$) and, at higher temperature with gradient elution, resolution values of $R_s = 4.2$ with baseline separation have been obtained.[41,61,62]

The optimization of the racemic resolution procedure produced a number of interesting results. Even though they concern primarily the selectivity in binding studies, certain aspects are also important for catalysis. It is now known under which conditions higher or lower selectivity is expected, and important conclusions with respect to the kinetics of the mass transfer can be drawn from the chromatographic studies. Complete separations can be accomplished in a few minutes. This clearly shows that a quick mass transfer between solution and selectively occupied cavities at the polymer is possible. The mass transfer is strongly dependent on the temperature, indicating that for effective catalysis higher temperatures should be used. In chromatography, selectivity at higher temperature is improved, since more cavities with higher selectivity become available. This might be similar in catalysis by imprinted polymers, though, usually selectivity in catalysis is favored at lower temperatures.

B. Optimization of the Polymer Structure

Optimization of the polymer structure was rather complicated. On one hand, the polymers should be rather rigid to preserve the structure of the cavity after splitting off the template. On the other hand, a high flexibility of the polymers should be present to facilitate a fast equilibrium between release and reuptake of the template in the cavity. These two properties are contradictory to each other, and a careful optimization became necessary. Furthermore, good accessibility of as many cavities as possible is required, as well as high thermal and mechanical stability. Since the initial experiments on imprinting, most examples until now are based on macroporous polymers with a high inner surface area (100−600 m^2/g) that show, after optimization, good accessibility as well as good thermal and mechanical stability.

The selectivity is mainly influenced by the kind and amount of cross-linking agent used in the synthesis of the imprinted polymer.[63,64] Figure 1 shows the selectivity dependence for racemic resolution of the racemate of **2** on the structure of polymers of the type described in Scheme 5. Below a certain amount of cross-linking in the polymer (around 10%), no selectivity can be observed, because the cavities are not

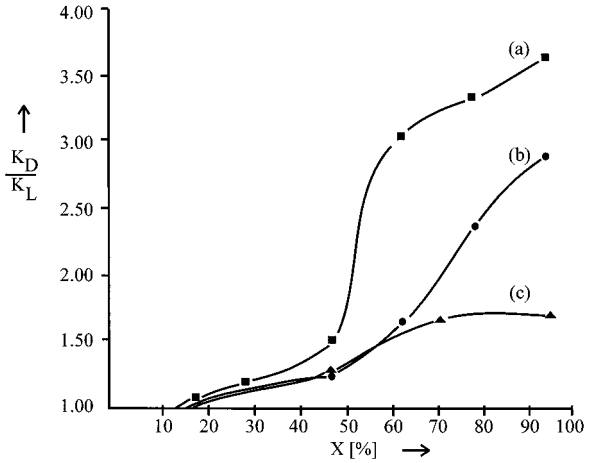


Figure 1. Selectivity of imprinted polymers as a function of the type and amount (X) of the cross-linking agent.[63] Polymers are prepared from **1**. Selectivity for racemic resolution of the racemate of **2** is measured in the batch procedure. Cross-linking agents: (a) ethylene dimethacrylate, (b) tetramethylene dimethacrylate, (c) divinylbenzene. (Adapted from ref 63.)

sufficiently stabilized. Above 10% cross-linking, selectivity increases steadily. Between 50 and 66%, a surprisingly high increase in selectivity takes place, especially in the case of ethylene dimethacrylate as a cross-linker. This cross-linking agent is now preferred by most groups working in the field. Cross-linking with divinylbenzene (either the commercial mixture or pure regioisomers) results in reduced selectivity, yet this cross-linker has the advantage of higher chemical stability (bonds are not hydrolyzable) and less interaction with functional groups.[64] It is a matter of discussion whether a similar trend with respect to the cross-linking degree is expected in catalysis. A range of catalyses with slightly cross-linked, imprinted polymers has been described. This problem will be discussed in more detail in the last section of this review.

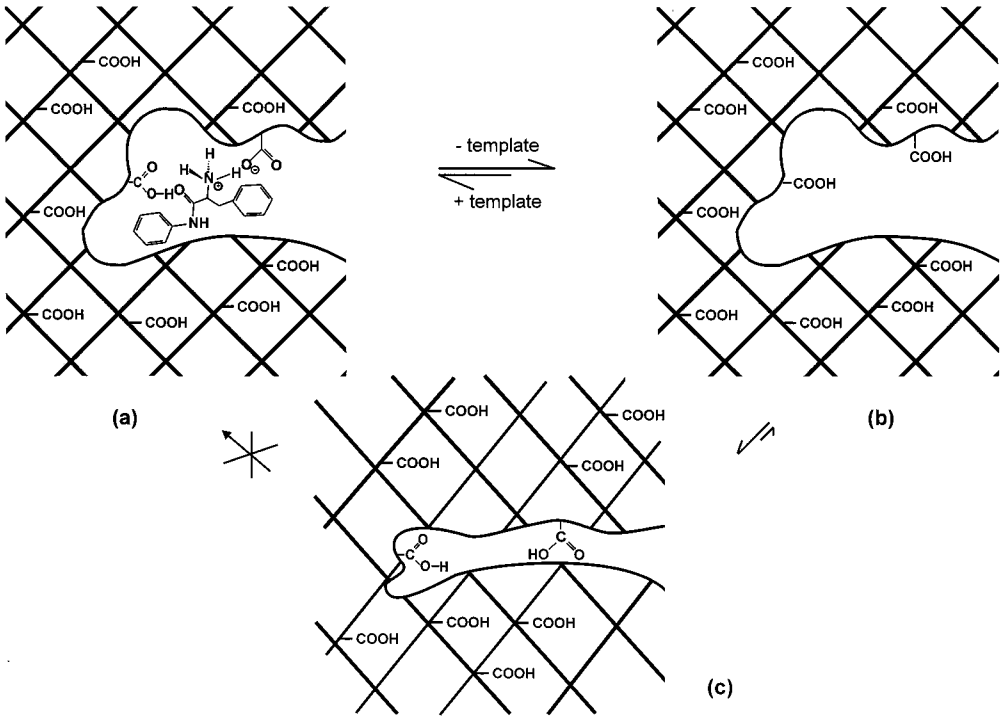
C. Binding Site Interactions

Aside from the cross-linker content, a very critical point in optimizing imprinted polymers for applications in catalysis is the design of the binding site interaction between the template (the imprint molecule) and the functional groups in the formed polymer.

Whereas most of the imprinting was initially performed with covalent binding,[29,36,38,51,56,65−68] in subsequent years the number of groups entering the field greatly enlarged and a considerable number of different binding site interactions have been employed. The group of Mosbach started work especially with noncovalent binding (electrostatic interaction and hydrogen bonding[45−47,69−72]). For example, they used L-phenylalanineanilide as template and acrylic or methacrylic acid as noncovalent binding sites. In Scheme 6a, a schematic picture of such binding is shown.

As a result of the efforts of many research groups, many different types of templates have been used as well as a considerable number of different binding site interactions. A lot of information on the impor-

Scheme 6. Imprinting by Noncovalent Interaction with L-Phenylalanine Anilide as Template and Methacrylic Acid as Binding Site[70] (adapted from refs 59, 70, and 73)[a]



[a] After removal of the template, a considerable portion (around 85%) of the cavities cannot be reoccupied by the template,[73] because they might show shrinking.[59] Owing to a strong excess of carboxylic acid binding sites, most of the carboxyl groups are situated in a statistical manner in the matrix of the polymer.

tance of the role of the binding sites is therefore now available.[59,75−77]

Detailed investigations have shown that the selectivity depends both on the orientation of the functional groups inside the cavities and the shape of the cavities.[78−81] The dominant factor, however, is the orientation of the functional groups inside the cavity.[80] If the template has two binding sites, several single-point bindings can occur, but only one two-point binding.[41] It is the two-point binding that provides high selectivity. Therefore, this portion may be increased by raising the temperature.[82]

Another problem in the binding procedure is the rebinding of template molecules in the cavity. In the case of covalent binding and all other types of stoichiometric binding, after splitting off the original templates, binding sites are only situated inside the cavity. After removal of the template, this leads usually to a swelling of the cavities due to a solvation of the binding sites,[41,83,84] which guarantees a high proportion (90−95%) of reuptake after the first removal. At the same time, it facilitates a quick mass transfer during equilibration of the template with the polymer. On reuptake of the template, the cavity shrinks to its original volume (induced fit) (see Scheme 5).[41]

In the usual noncovalent interaction (electrostatic and hydrogen bonding), association constants are rather low. Therefore, in a 1:1 molar ratio, only a small part of the template is bound (see Table 1 for examples). Only multiple hydrogen bonds show higher association constants. Therefore, noncovalent imprinting, e.g. with acrylic acid, requires at least a 4-fold molar excess of binding sites to completely saturate the functionalities at the template molecule in order to ensure good selectivity. The binding sites are therefore not only distributed inside the cavity but throughout the polymer (see Scheme 6). As was found,[73,75] only 15% of the cavities can reuptake a template under these conditions; the remaining 85% are lost irreversibly for use in separation. This might be due to a shrinking of a majority of the cavities (see Scheme 6c).[41,59,74] Such imprinted polymers are hence less suited for preparative separations or for catalysis.

Catalytically active imprinted polymers require that the functional groups responsible for binding during imprinting and for binding and/or catalysis later on should show favorable properties during different steps of their use:

(1) During polymerization, the interaction between binding site and template needs to be stable. Template and binding site should be present in a stoichiometric ratio. Therefore, stoichiometric binding with stable covalent bonds or with noncovalent interactions and high association constants are desirable. The binding interaction should possess a specific geometric directionality, as present in covalent bonds or hydrogen bonds.

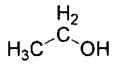
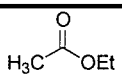
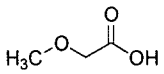
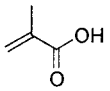
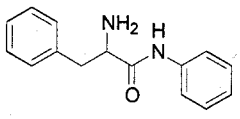
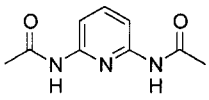
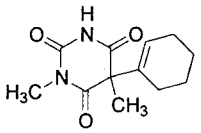
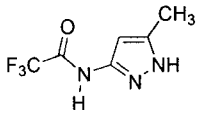
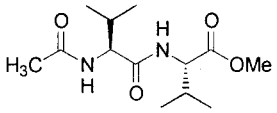
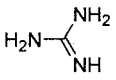
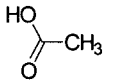
(2) The template should be able to split off under mild conditions and as completely as possible.

(3) Equilibration with substrates and catalysis has to be rapid.

(4) There must be a favorable binding equilibrium.

In catalysis, mostly covalent (a), noncovalent (b), stoichiometric noncovalent (c), and coordinative bonding (d) have been used during the imprinting procedure.

Table 1. Association Constants for Noncovalent Interactions (model systems) That Can Be Used in Molecular Imprinting Preparing Catalysts; Determined by ^{1}H NMR if Not Indicated Otherwise

binding site	template	K_{ass} (M^{-1})	solvent[a)]	T (°C)	complexation (%)[b)]	refs
		1.7 [c)]	CCl_4	25	12.9	85
		3.3 [c)]	ACN	25	20.7	86
		3.0×10	ACN	23	56.6	70
		5.5	ACN	60	28.3	70
		2.0×10^2	$CDCl_3$	22	80.0	87
		8.9×10^2	$CDCl_3$	25	90.0	88, 74
		7.9×10^3 [d)]	DMSO	25	96.5	89

[a] Deuterated solvents have been used for NMR experiments. [b] Calculated percentage of formed complex of template and monomer at concentrations of each 0.1 mol L^{-1}. [c] Determined by IR spectroscopy. [d] Calorimetric determination.

Table 2. Association Constants of Different Acids with Amidine 3 (data from refs 59, 95)

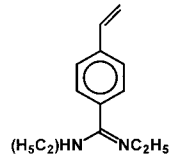
	solvent	K_{ass}[a] (M^{-1})	complexation (%)[b]
carboxylic acid[c]	chloroform	3.4×10^6	99.9
carboxylic acid[c]	acetonitrile	1.2×10^4	97.2
phosphonate[d]	acetonitrile	8.7×10^3 (25 °C)	97.7
		7.6×10^3 (60 °C)	96.4
phosphate[e]	acetonitrile	4.6×10^3	95.4

[a] Association constants determined by ^{1}H NMR spectroscopy at 25 °C. [b] Complexation percentage at equimolar concentrations (0.1 mol L^{-1}) of acid and amidine **3**. [c] 3,5-Dimethylbenzoic acid. [d] 3,5-Dimethylbenzylphosphonic mono(3,5-dimethylphenyl) ester. [e] Bis(3,5-dimethylphenyl) phosphate.

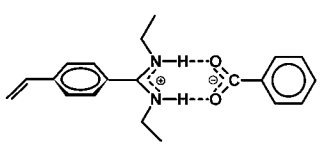
(a) Covalent interaction during imprinting fulfills the first point of the above-mentioned requirements most perfectly. Unfortunately, there is only a limited number of covalent interactions that can easily be split afterward to a high percentage, such as boronic diesters or Schiff bases (see, e.g., ref 59). Boronic acids, diols, as well as aldehydes (and acids after oxidation) and amines can thus be introduced in the cavity for use in binding or catalysis. With boronic acids it was evident from chromatographic experiments that a very quick mass transfer with diols is possible at higher temperature in the presence of certain bases.[59,90]

(b) The use of noncovalent interactions during imprinting is the easiest way to introduce functional groups acting as binding and/or catalytic site into a cavity (for a review, see ref 75). Mostly electrostatic interactions and hydrogen bonding have been employed in this respect. Hydrogen-bonding interactions are particularly important in noncovalent polymer imprinting, due to their specific geometric directionality. Binding sites are distributed all over the polymer and not only inside the cavity. Only if the catalytic activity inside the cavities is much higher than among the statistically distributed ones is a clear effect in catalysis to be expected. These polymers behave as if they are the catalyst and the control polymer (with statistically distributed functional groups) at the same time.

(c) Of special interest are stoichiometric noncovalent interactions.[59,74,91] Multiple hydrogen bonds can have very high binding constants ($K = 10^3-10^7$) (see Tables 1 and 2), so they behave during the imprinting like a covalent bond. We have used, for example, the amidine group to bind carboxyl groups, phosphonic monoesters, and phosphates. Since these complexes tend to be insoluble in the polymerization mixture, a number of derivatives have been prepared.[44,74,92] The *N*,*N*′-diethyl-4-vinylbenzamidine **3** is especially



3



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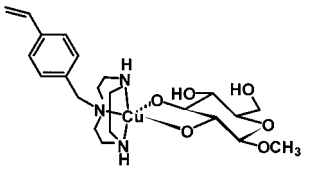
suitable.[93,94] Association constants with carboxylic

acids (see **4**) are typically well above 10^6 L mol^{-1} in $CHCl_3$ (see Table 2).[95] Templates are easily split off from an imprinted polymer, and equilibrations are rapid. Sufficient binding occurs even in aqueous solutions. In contrast to usual noncovalent interactions, a reuptake of nearly 100% is easily possible.[96] A reverse interaction of an amidine-containing drug (pentamidine) and acrylic acid has been reported previously.[97] Guanidinium binding sites have also been used to prepare imprints of phosphates and phosphonates in SiO_2 xerogels.[98]

(d) Coordinative binding is an interesting type of interaction for molecular imprinting (for a review, see ref 76), and closely resembles that in ligand-exchange chromatography (see, e.g., ref 99). The advantage of this kind of bond is that its strength can be controlled by experimental conditions. Definite interactions can occur during polymerization under proper conditions, and an excess of binding groups is unnecessary. This method was first used for imprinting to produce enantioselective polymers by Fujii et al.[100] Especially Arnold et al. have later developed this method by using mostly copper complexes of polymerizable iminodiacetic acid.[50,54,101] With this binding site group, imidazole groups can be bound. Another promising approach uses triazacyclononane−Cu^{2+} chelates for binding.[102] Such a binding site is able to bind, e.g., glucose (**5**) as well as free amino acids.[103]



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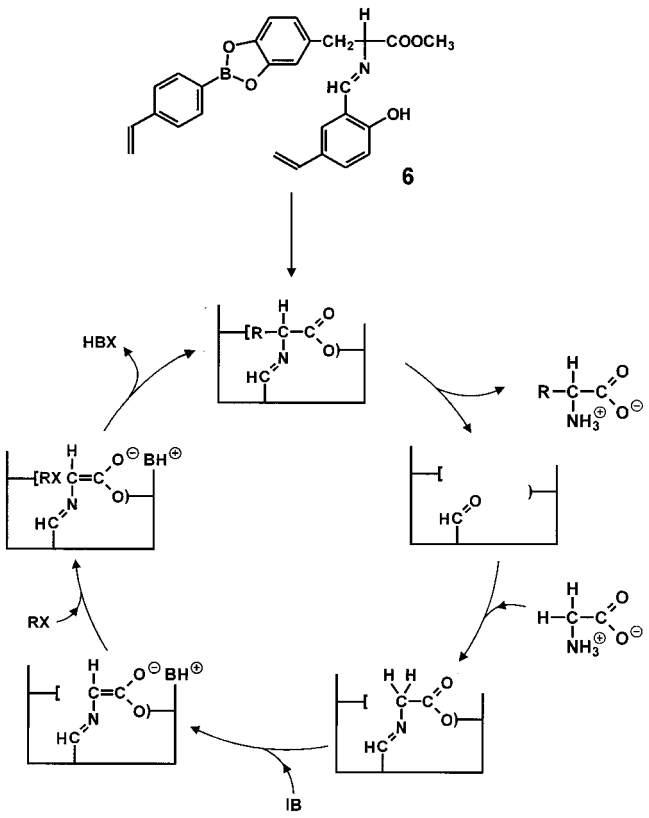
To overcome the problems with noncovalent interactions, a covalent bond is used during the imprinting procedure, whereas, after splitting off the template, the newly generated group remains accessible for noncovalent interaction[29,83] (for reviews, see refs 59 and 77). Another example was reported by Whitcombe et al.,[77,104] who employed carbonate ester for the imprinting step and hydrogen bonding during equilibration. When Schiff bases are used during imprinting, removal of the aldehyde template leaves a polymer-bound amine behind that can interact electrostatically with carboxylic acids.[41,59]

III. Preparation of Catalysts by Imprinting in Synthetic Polymers

A. Imprinting with the Product or an Analogue of the Reaction Product (Microreactors)

In the early years of imprinting, use of imprinted cavities for selective reactions was attempted. In a strict sense, these were not catalysts. The aim is the preparation of microreactors for regio- and stereoselective reactions. To achieve this, the cavity was first imprinted with one possible product of the reaction and a precursor was then embedded into the cavity. The idea was that product should be favored that was used as template if the reaction was run within the imprinted cavity. The first experiments were carried out by the research groups of Shea[66,67] and Neckers,[65] who performed cycloadditions that led to cyclopropanedicarboxylic and cyclobutanedicarboxylic acids, respectively. The latter were obtained with remarkable regio- and diastereoselectivity. Belokon et al.[105] used Schiff base formation for binding in templating with an amino acid. They established that, on removal of a proton from the amino acid template located in the cavity, the intermediate carbanion maintains its original configuration (unlike in solution) and therefore reacts under retention. Sarhan and El-Zahab[106] were able to apply this strategy in performing a stereospecific inversion of the configuration of mandelic acid. Similarly to the work of Belokon,[105] use of pyridoxal−coenzyme analogues for imprinting and catalyzing the deprotonation of bound amino acids was tried.[107]

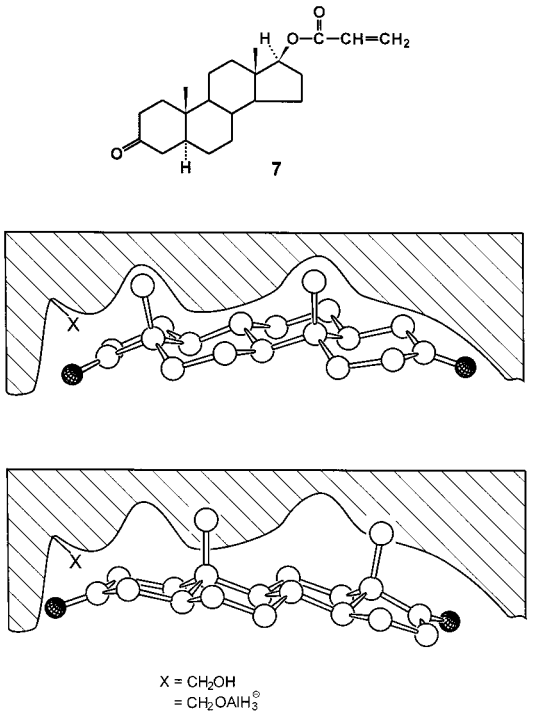
Scheme 7. Schematic Representation of the Synthesis of a Chiral Amino Acid in an Imprinted Polymer Acting as a Microreactor[a] (adapted from ref 110)



[a] In the example given[108,109] the polymer was prepared by imprinting with the L-DOPA template monomer **6**. After removal of the template, glycine was bound, deprotonated, and then alkylated in the chiral cavity to yield an optically active amino acid that can be released from the cavity. The cycle can then be repeated by binding again glycine.

The first asymmetric syntheses in a chiral cavity were achieved in our research group.[108,109] Enantioselective C−C bond formation was attempted inside a chiral cavity with the preparation of optically active amino acids as the objective. The synthetic approach is outlined in Scheme 7. Imprinting was carried out with template monomer **6** with L-DOPA as template

Scheme 8. Another Example for the Use of an Imprinted Polymer as a Microreactor[a] (adapted from ref 111)



[a] The scheme shows a representation of the embedding of androstane-3,17-dione in a cavity produced by imprinting with template monomer **7**, X = COOR, → CH_2OH → $CH_2OAlH_3^-$, after two transformations. The two structures show binding of the 3,17-dione when the 17-keto group (top) or the 3-keto group (bottom) is in close proximity to the X-group. The latter shows a poor fit.

possessing boronic acid and salicylaldehyde binding sites.

After removal of the template, glycine was embedded in the cavity, deprotonated, and alkylated. The obtained enantiomeric excess (36% ee) represented for a long time the highest enantioselectivity reported with imprinted polymeric reagents. This enantiomeric excess is purely a result of the asymmetric induction caused by the chiral cavity.

Very remarkable regio- and stereoselective reactions were performed by Byström et al.[111] They used an ester bond for binding a steroid during imprinting (see monomer **7**). The template was removed by reductive cleavage, thus producing a cavity with a CH_2OH group in a particular position. The hydroxyl group was then converted to an active hydride species with $LiAlH_4$. On reacting androstane-3,17-dione with this polymer, nearly exclusively the carbonyl group in position 17 was reduced to the alcohol, whereas in solution or with a polymer having statistically distributed hydride groups position 3 was reduced exclusively. Scheme 8 shows that the shape of the cavity favors indeed reduction only at position 17. A considerable stereochemical preference (ratio of α- to β-groups formed) was observed as well.

Whitcombe and co-workers[56] used steroids with polymerizable boronophthalid binding sites attached to their 3β- and 12α-OH groups. This diester served as the template monomer for the imprinting in synthetic polymers. Afterward, the steroids can be removed, and other steroids having additional hydroxyl groups aside of 3β- and 12α-hydroxyl groups can be placed in these cavities. The cavity functions as a protecting group only for the 3β- and 12α-OH groups and the other groups can be selectively esterified. High regioselectivity of the esterification reaction is obtained in this way.

B. Imprinting with a Transition State Analogue (TSA) of the Ester Hydrolysis

1. General

In view of the remarkable results obtained with catalytic antibodies generated against a stable analogue of the transition state of a reaction,[34,35] molecular imprinting should be an excellent method to prepare catalytically active imprinted polymers in a similar fashion. Thus, antibodies prepared against a phosphonic ester (as transition state analogue for alkaline ester hydrolysis) enhance the rate of ester hydrolysis by 10^3-10^4-fold. The enhancement is due to the preferred binding of the transition state of the reaction. The antibodies obtained constitute a vast number of different compounds (polyclonality), and only a very tedious screening and isolation procedure provides—if at all—catalytically active antibodies that then have to be produced by standard techniques to provide uniform antibodies (monoclonal antibodies).

The preparation of catalytically active polymers should be much easier, though, up till now, no procedure is available to prepare these in a "monoclonal" state. Instead, a complex mixture of different (polyclonal) catalytic sites is generally obtained (see section VI.D).

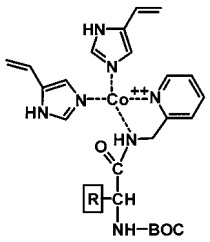
In addition to a preference for binding of the transition state of the reaction, it was shown that special catalytically active functional groups in antibodies can considerably enhance the catalytic reaction. It was therefore of particular interest to prepare active sites with the shape of the transition state and to locate catalytically active groups in the correct position inside the active site to catalyze the reaction. Since most of the experience in antibody chemistry concerned ester hydrolysis, these reactions were first investigated in imprinting, too. The main problem was to find a suitable template array containing both the stable transition state analogue as well as binding sites and catalytically active groups in the desired position. Catalysts were prepared using different types of binding site interactions during the imprinting procedure. This will be discussed in the next paragraphs.

2. Metal-Coordination during the Imprinting Procedure

To orient different functional groups during the imprinting procedure in a definite orientation to each other and the template molecule, metal coordination was used in many cases. In some investigations Co^{2+} was the coordinating metal. This type of coordination

is not easy to control (see ref 112), since Co^{2+} forms mostly either octahedral or tetrahedral complexes, which are in equilibrium with each other. Some bidentate monoanions can also give rise to planar complexes. If different ligands are offered to the metal ion, an equilibrium exists between different coordinated species. If Co^{2+} is reacted with equimolar amounts of four different ligands (A, B, C, D) and if all ligands have the same complexing reactivity, less than 5% of a [(ABCD)Co(II)] complex is expected, assuming only tetracoordination is occurring. More than 95% of the Co(II) complexes would have another composition. If the reactivity of the ligands is different (as it usually is), even considerably less of that complex is expected. The present available analytical methods do not allow one to directly distinguish in solution between the many possible species. A careful choice of two different bidentate ligands and control of the complexing procedure may improve the situation, and reasonable amounts of the desired complex may be obtained. Unfortunately, nearly all papers discussed in this section are short communications, lacking a proper identification of the underlying complexes.

The first attempts to prepare catalytically active imprinted polymers using metal complexes did not use the transition state of the reaction but the desired product as template. Thus, Mosbach and Leonhardt[113] polymerized a complex whose structure was assumed to be mainly **8** and, after removal of the



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amino acid derivative, this polymer exhibited esterolytic activity for the corresponding amino acid *p*-nitrophenyl ester.

This polymer showed a 2−3 times higher catalytic activity than a control polymer prepared without template. Furthermore, substrate selectivity was observed. If different amino acid templates were used for imprinting, the corresponding template *p*-nitrophenyl ester was hydrolyzed easier than by other imprinted polymers.

The first catalyst prepared with a transition state analogue (TSA) template came from the same group.[114] A poly[4(5)-vinylimidazole] was cross-linked with dibromobutane in the presence of $CoCl_2$ and *p*-nitrophenyl methylphosphonate as the transition state analogue. Under these conditions, it was not possible to completely orient the imidazole groups selectively, and only a small enhancement in rate (1.6-fold compared to a control polymer) for the hydrolysis of *p*-nitrophenyl acetate was observed after removal of the template.

The procedures of Mosbach[114] have been followed by Ohkubo.[115−117] He used only polymers with a low degree of cross-linking; some of these were even soluble polymers. No control polymers were tested, and the enhancement of 6−7-fold was observed against the corresponding solution without polymers. Further work has to be performed to prove that really an imprinting effect is responsible. The calculated thermodynamic data[117] could not be verified from the figures given.[39]

Even more complex systems have been reported by Kulkarni et al.[118,119] for the imprinting. Here, four different ligands have been used with Co^{2+} as the coordination center. Since the esterolytic activity of the imprinted polymers obtained seems promising, further investigations on the structure of the template monomer, on the mechanism, on control polymers, and on polymers with different degree of cross-linking are desirable.

3. Noncovalent Interaction during the Imprinting Procedure

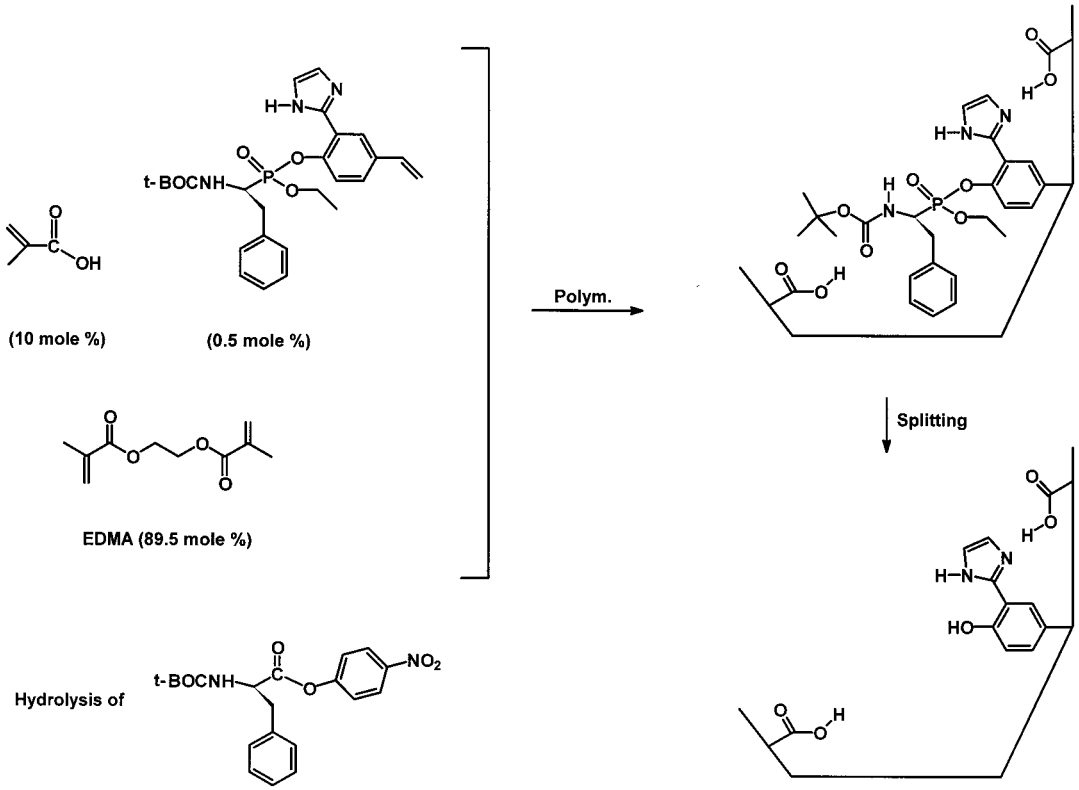
In addition to their work with metal coordination, Ohkubo et al.[120] investigated stable phosphonates as transition state analogues and noncovalent interaction for the imprinting of a polymer. Phenyl-1-benzyloxycarbonylamino-3-methylbutyl phosphonate was used as template. One equivalent of *N*-acryloyl-L-histidine methyl ester was added as a catalytic site. It was assumed by the authors that template and binding site, being present in a 1:1 molar ratio, should give a high yield of hydrogen-bonded complex. A water-soluble polymer was obtained with 8.4% of the cross-linker ethylene bis(acrylamide). Catalytic activity and substrate selectivity for the hydrolysis of amino acid *p*-nitrophenylester is claimed. Unfortunately, no control experiments have been performed and no control polymers prepared. It is therefore uncertain whether a real imprinting effect was achieved. Enantioselectivity can be explained by the L-histidine residues at the polymer. Similar investigations on soluble as well as on insoluble polymers were reported in other papers.[121−124]

In a recent paper[125] the catalytic hydrolysis of 4-nitrophenyl acetate is performed by an imprinted polymer that was prepared from *p*-nitrophenyl phosphate as the template and vinylimidazole as the binding site. In this case, an insoluble salt is formed, which is polymerized in the presence of a high amount of divinylbenzene (∼60%). A control polymer without template, but with vinylimidazole, was also prepared. After removal of the template, the imidazole-containing imprinted polymer showed a 2-fold enhancement in rate compared to the control polymer and an 85-fold enhancement compared to a solution at the same pH.

4. Covalent Interaction during the Imprinting Procedure

The advantage of covalent interaction during the imprinting procedure is the exact control during the introduction of the functional groups in a certain vicinity. It is not necessary to employ an excess of functional monomers, and these groups are therefore only positioned inside the cavity. While the cleavage of the template from the polymer might be problem-

Scheme 9. Schematic Representation of the Preparation of a Catalyst by a Transition State Analogue Using Labile Covalent Binding and Noncovalent Binding (adapted from refs 126, 127)



atic, relatively labile covalent interactions have to be used in this case.

Sellergren and Shea et al.[126,127] prepared highly cross-linked polymer catalysts for the hydrolysis of an amino acid ester (*N*-*tert*-butoxycarbonyl phenylalanine-*p*-nitrophenyl ester). They made use of a stable transition state analogue for ester hydrolysis in the form of a chiral phosphonate analogue of phenylalanine. To this phosphonate were attached a catalytically active phenol and imidazole-containing vinyl monomer through a labile ester linkage. After polymerization in the presence of methacrylic acid and removal of the template, catalytically active sites were obtained that contained an enantioselective binding site, a site complementary to a transition state structure, and a hydroxyl, imidazole, and carboxylic acid group (similar to chymotrypsin) (see Scheme 9). The carboxyl groups were positioned during the imprinting procedure by hydrogen bonding to the basic imidazole and the carbonyl group (see Scheme 9).[127] Carefully designed control polymers were also prepared and investigated. The maximum rate enhancement, when compared with a nonimprinted control polymer, was 2.5 and 10 when compared to a solution containing the phenol imidazole monomer. Enantioselectivity for the hydrolysis of L- and D-phenylalanine derivatives was surprisingly high, with $k_D/k_L = 1.85$. This clearly shows a substrate selectivity that in most other investigations was not present or not investigated.

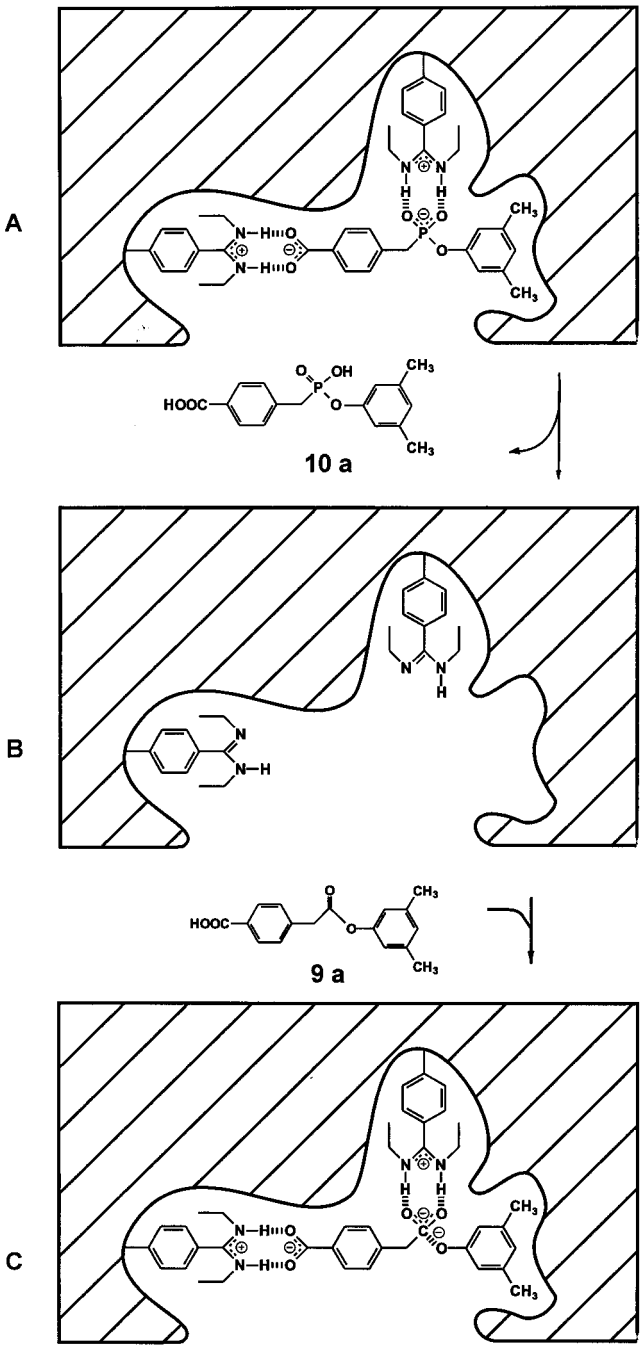
5. Stoichiometric Noncovalent Interaction during the Imprinting Procedure

Noncovalent interaction with high association constants combine the advantages of covalent and noncovalent interaction during the molecular imprinting procedure. As already pointed out earlier, the amidine group binds strongly to carboxylic acid and phosphonic acid groups. Thus, it is possible to fix a transition state analogue template during imprinting and at the same time, position the amidine groups in the correct position for catalysis. In this respect it is interesting to note that the most active species among catalytic antibodies also contain a guanidinium group (of the amino acid L-arginine) that plays an important role catalyzing the basic hydrolysis of esters.[128]

The amidine groups were therefore applied both for binding and catalysis in the investigation of the alkaline hydrolysis of ester **9**.[74,93,94] Phosphonic monoester **10** served as a stable transition state analogue for templating. Addition of 2 equiv of the new binding site monomer **3** furnished a soluble bisamidinium salt. By the usual polymerization using 80% ethylene dimethacrylate as cross-linker, workup, and removal of the template, the resulting active sites contained two amidine groups each. Owing to the stoichiometric interaction of the binding sites, the amidines are only located in the active sites (Scheme 10B). The idea was that one of the two amidine binding sites inside the cavity acts as anchor for the reactant at the carboxylic acid site, while the other brings about the base-catalyzed ester hydrolysis. There is some similarity looking at the mechanism of carboxypeptidase A with two guanidinium groups (in contrast, in our imprinted catalyst, no Zn^{2+} is used).

The imprinted polymer accelerated the rate of hydrolysis of ester **9a** (see eq 1) compared to the

Scheme 10. Schematic Representation of the Polymerization of Template 10a in the Presence of 2 equiv of Binding Site Monomer 3 (see A), Removal of 10a (see B), and Catalysis[a] (adapted from ref 93)



[a] Alkaline hydrolysis of **9a** occurs through a tetrahedral transition state (see C). Actually 10 is not a transition state analogue but the analogue of a reaction intermediate. Due to the fact that the transition state and the intermediate are on a similar energy level they are structurally similar [Hammond Postulate]. Actually in nearly all investigations based on transition state stabilization of ester hydrolysis actually antibodies and imprinted polymers are imprinted with the tetrahedral intermediate analogue.

reaction in buffer of the same pH by more than 100-fold calculated with respect to the formation of acid **11** and 235-fold calculated on the formation of phenol **12a** (see Table 3). This difference shows that some

Table 3. Relative Reaction Rates for the Basic Hydrolysis of the Ester 9a[a] (data from ref 93)

pH value	solution + amidine vs solution	control polymer vs solution	TSA imprinted polymer vs solution
7.0	1.5	10.9	49
7.6	2.4	20.5	102.2 (235.3)[b]
9.0	1.9	4.7	17.6

[a] Kinetics according to eq 1 followed by HPLC determining the diacid **11** released. The ratios refer to the reaction in buffer/acetonitrile at the same pH. This value is defined as 1.0. [b] Relative enhancement on determination of the phenol **12a** released.

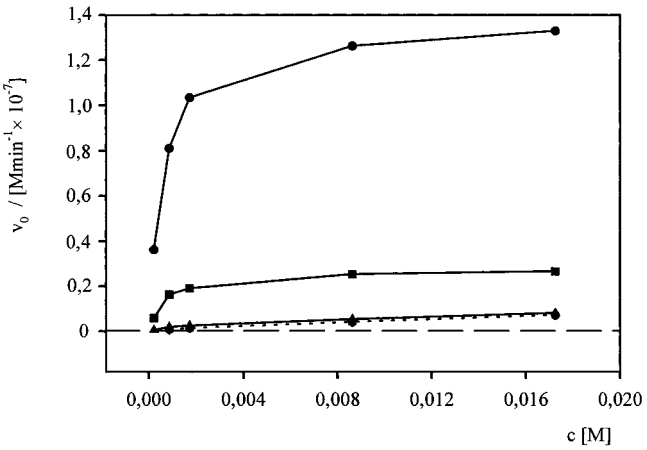
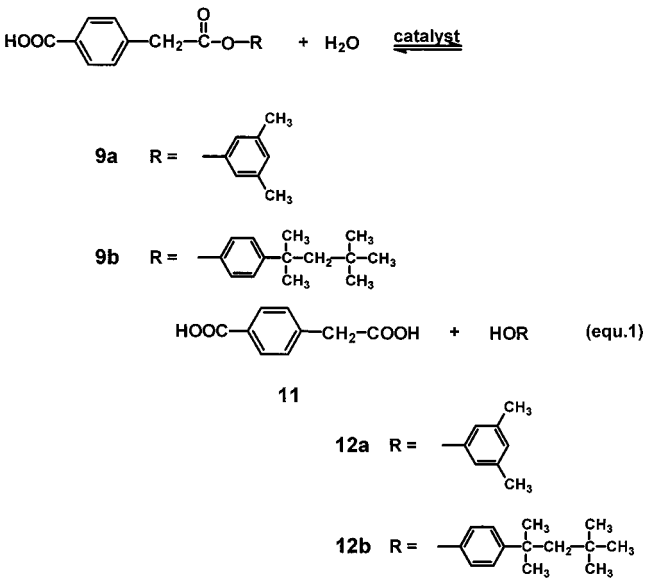


Figure 2. Michaelis−Menten diagram for the hydrolysis of the model ester **9a** in acetonitrile/ pH 7.6 buffer 1:1 in the presence of (●) polymer imprinted with transition state analogue **10a**, (■) polymer imprinted with amidiniumbenzoate, (▲) an equivalent amount of amidine, (- ● -) acetonitrile/ pH 7.6 buffer 1:1 as a control. Hydrolysis according to eq 1. The initial rates versus substrate concentration are plotted. (Adapted from ref 93.)

of the acid is not liberated from the polymer (product inhibition).



Addition of an equivalent amount of monomeric amidine instead of imprinted polymer to the buffer solution increased the rate only slightly (see Figure 2). Polymerization of the amidinium benzoate (pro-

Table 4. Relative Reaction Rates for the Hydrolysis of 9a and 9b Catalyzed by Polymers Imprinted with 10a and 10b (pH 9) (cross-equilibration) (data from ref 93)

substrate	solution	imprinted with **10a**	imprinted with **10b**	factor
9a	1.0	17.6	11.8	1.5
9b	1.0	40.6	52.4	1.3

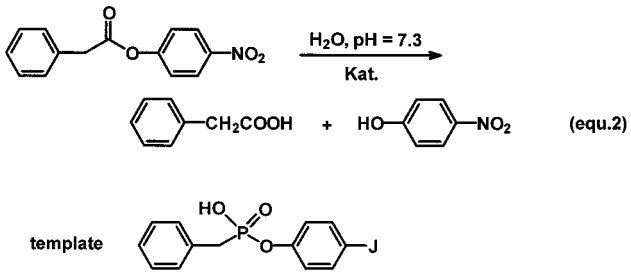
viding a statistical control polymer) gave a somewhat stronger enhancement in rate compared to the solution. In conclusion, these results show a very strong catalytic effect of the imprinted polymers. It is remarkable that these effects occur with nonactivated esters and not, as in nearly all other reported examples, with activated 4-nitrophenyl esters.

To see whether these polymers show typical enzyme analogue properties, we investigated the kinetics of the catalyzed reaction in the presence of varying excesses of substrate with respect to the catalyst. Figure 2 shows typical Michaelis−Menten kinetics as it was observed. Saturation phenomena occur at higher concentrations, demonstrating that all active sites are then occupied and that the reaction becomes independent of substrate concentration. In contrast, a much slower linear relationship is found in solution and in solution with the addition of amidine. The amidinium benzoate shows also some type of Michaelis−Menten kinetics. The benzoate acts therefore as a less effective template.

The Michaelis constant (K_m) was determined to be 0.60 mM. Turnover numbers are relatively low ($k_{cat} = 0.4 \times 10^{-2}$ min^{-1}) but are definitely present. Furthermore, we found that the template molecule itself is a powerful competitive inhibitor, and with $K_i = 0.025$ mM, it is bound more strongly than the substrate by a factor of 20. It is remarkable that such a strong binding of substrate and template occurs in water−acetonitrile 1:1. Binding in aqueous solution by usual electrostatic interaction or hydrogen bonding is much weaker than binding in the active site in this case.

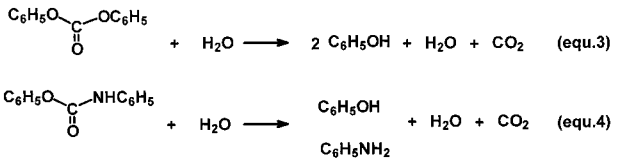
These enzyme models show a remarkable substrate selectivity, as deduced from the cross-selectivity of the polymers imprinted with **10a** and **10b**. The determination of the rates of alkaline hydrolysis of **9a** and **9b** with both polymers proves that each polymer hydrolyzes its "own" substrate significantly better (factor 1.3−1.5) (see Table 4), although both are phenol esters and the cavities have only a slightly different shape caused by the small structural differences of the substituents in the aromatic rings.

The catalytic activity of the imprinted polymers is dependent on many parameters. It is therefore difficult to perform a systematic investigation on the influence of all of them. This is especially true since the preparation of each of these single polymers is rather tedious by preparing these polymers in bulk in ampules, crashing them to small particles, sieving them, removing the template, and measure the kinetics of the reaction in question. Therefore, a combinatorial chemistry approach with automated parallel synthesis of many samples at the same time and the subsequent handling and kinetic investigation would enable a much larger number of samples to be investigated. A first step in this direction was done by Takeuchi et al.[129] They used a synthesis robot for the preparation of the polymers and tested the selectivity for separation by HPLC of each sample. An even simpler method was used recently in our group for the investigation of catalytic activity.[130,131] In this case, the catalysis of the hydrolysis according to eq 2 was investigated. The imprinted polymers



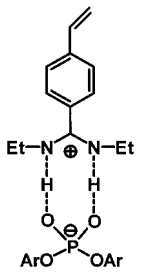
were prepared with the corresponding phosphonic ester as a template, but instead of the nitro group, the isosteric iodo group was used, since the nitro group can act as an inhibitor for the radical polymerization. A commercial synthesis robot (Gilson 222 XL) was equipped with a double-bored needle in order to be able to simultanuously fill small vials capped with septums and flush the vials with inert nitrogen. From more than 20 different stock solutions the vials can be filled with high accuracy. Overall mixtures from 10 to 200 mg per vial were used. Usually 96 polymers are prepared at once. All steps (washing, removal of the template, temperature programs, addition of buffer and substrate) are computer-controlled. After the reaction (eq 2) the solutions are transferred by a multipipet to a rack also containing 96 compartments, and the intensity of the yellow color is measured in a microtiter plate reader within 5 s, so that a high-throughput screening is really possible. With this methodology, a vast number of different polymers and different reaction conditions can be investigated simultaneously. Thus the optimization of the reaction is done and will be further performed.

One drawback in the reactions of eqs 1 and 2 catalyzed by imprinted polymers is the product inhibition similar to that observed for catalytic antibodies.[34] Again, as with catalytic antibodies, less product inhibition is expected for the hydrolysis of carbonates and carbamates (eqs 3 and 4). Imprinted



polymers were prepared from a complex **13** consisting of diphenyl phosphate as the template and *N*,*N*′-diethyl-4-vinylbenzamidine (**3**) as the functional monomer.[91] In this case, in addition to the usual bulk polymerization, polymer beads were also prepared by classical suspension polymerization. This was pos-

sible since the complex **13** between diphenyl phosphate and amidine **3** was not hydrolyzed and the components did not leach into the aqueous phase in the course of polymerization. Removal of the template leads to cavities with one amidine group each.



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The hydrolysis of diphenyl carbonate (see eq 3) and diphenylcarbamate (see eq 4) was investigated in the presence of buffer solution, buffer and imprinted polymer, and buffer and statistical polymer. Substrate hydrolysis was treated, as usual, as a pseudo-first-order reaction, and rate constants k of the initial reaction were determined (similar as in the ester hydrolysis). The ratio of k_{impr}/k_{sol} showed enhancements of 588 in the case of carbonate and 1400−3860 in the case of carbamate. The enhancement with respect to nonimprinted polymers containing statistically distributed amidine groups was clearly lower, being 10-fold for carbonate, 5.8-fold for carbamate bulk polymers, and up to 24-fold for imprinted polymer beads.[91]

These values are among the best published until now for molecularly imprinted catalysts. The activity for carbamate hydrolysis in this example is on the same order of magnitude compared to catalytic antibodies[132] (see also Table 6 in section VI.A.). This is surprising considering the fact that the polymers are insoluble and have a rigid structure and "polyclonal" cavities.

In a similar fashion, imprinted polymer catalysts were prepared for the hydrolysis of cholesterol carbonates.[133] The aim of this investigation was to test the possibility of generating catalytic sites in the polymer with a relatively extended and flat template molecule with a freely rotating alkyl side chain. *O*-Chlolesteryl(3)-*O*-(4-nitrophenyl)phosphate was used as template molecule, which was polymerized with the amidine monomer **3**. The hydrolysis of the corresponding carbonate was enhanced by the catalyst 27-fold over the background buffer uncatalyzed reaction and 2.4-fold compared to the control polymer.

C. Catalysis of Elimination Reactions

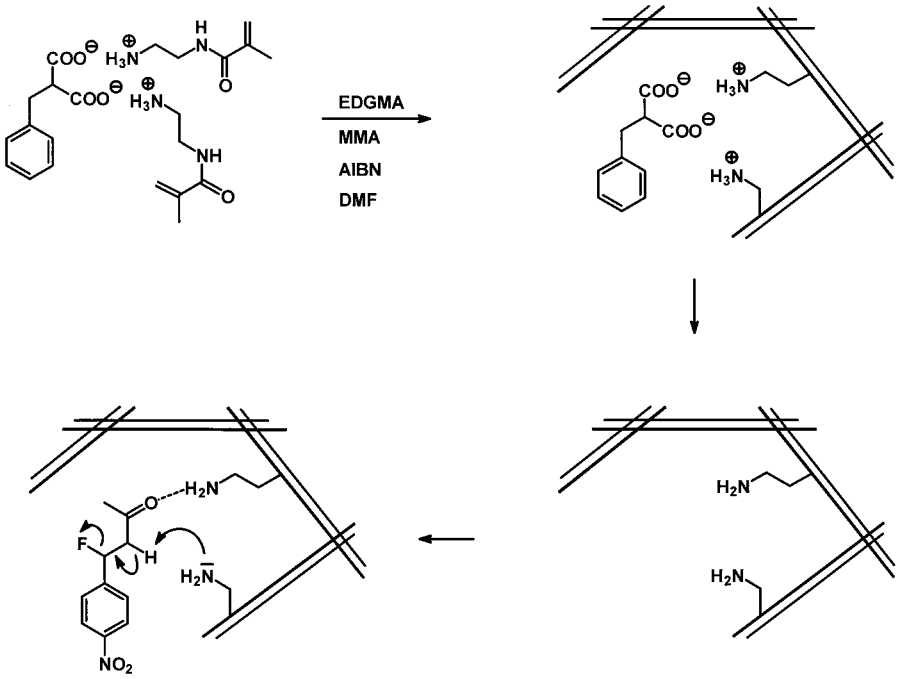
Quite successful were investigations on elimination reactions catalyzed by antibodies generated against stable transition state analogues of the reaction. Since the structure of the substrate (or the transition state) of the reaction is quite different from that of the product, no product inhibition is expected.

Imprinted polymers have been prepared in analogy to investigations on the dehydrofluorination reaction catalyzed by antibodies.[134] Mosbach et al.[135] were the first to use this approach. Their system consisted of a secondary amine as template and carboxylic acids as binding and catalytic sites. An enhancement of the defluorination reaction by a factor of 2.4 was found compared to a control polymer prepared without the template. This system has recently been investigated in chemical reaction engineering processes.[136] A more sophisticated approach was described by Shea et al.[137] With the aid of a dicarboxylic acid acting as the template, two amino groups were introduced in the active site in a certain stereochemical relation to each other. In analogy to site-directed mutagenesis, the functionalities at the catalytic site were manipulated by changing the template dicarboxylic acid. The catalytic activity of the imprinted polymers changes strongly if the carboxylic acid groups were in a different position to each other. The most active imprinted polymers were obtained with benzylmalonic acid as the template (see Scheme 11). The imprinted polymer shows a rate constant (pseudo-first-order) 7.5 times greater than that observed with the control polymer prepared without template. Compared to the homogeneous solution, the rate enhancement of the imprinted polymer is 12.8 times. This imprinted polymer exhibits Michaelis−Menten kinetics in benzene, and the template acts as a competitive inhibitor, though with relatively low activity.

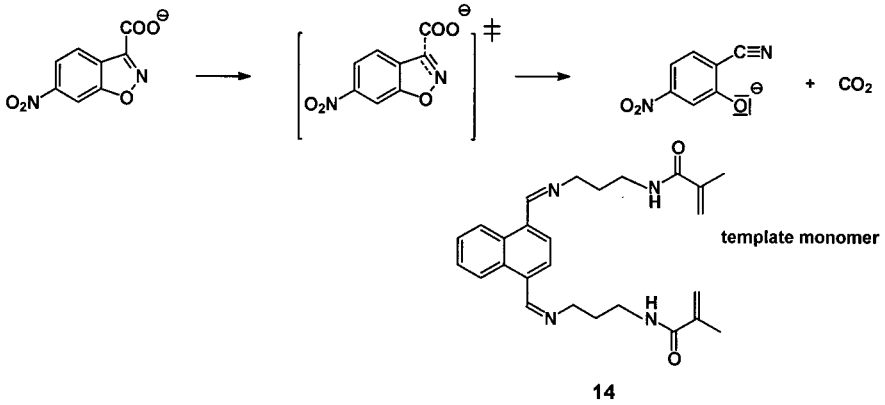
Recently, Shea and Kato[138] investigated covalent interaction during imprinting to position two amino groups in the active site. In this way previous problems with noncovalent interactions were overcome, since excess acid was avoided. To catalyze the decarboxylation of 5-nitro-3-carboxybenzisoxazole (a substrate that has been used before with antibodies[139]), template monomer **14** with two Schiff-base bindings was introduced (see Scheme 12). Enhancements of 10.7 times in the catalytic activity of imprinted polymer were observed in relation to a control polymer. Linear kinetic behavior over 80 turnovers is realized. It resulted in Michaelis−Menten kinetics, as well as a strong competitive inhibition caused by the template molecule.

A similar reaction was investigated by Mosbach's group,[140] who studied the isomerization of benzisoxazole to 2-cyanophenol (see Scheme 13). The substrate analogue indole was used during the imprinting procedure to position a pyridinyl group by a relatively weak hydrogen bonding in the active site. After removal of the template, a considerable enhancement in pseudo-first-order rate constants by the imprinted catalyst was observed, whereas solution and control polymers show nearly the same rate. An enhancement for the imprinted polymer of 7.2-fold was found. The control polymer was made with indene (instead of indole), since it cannot position the pyridinyl groups in the correct position. This appears to be a very appropriate control polymer. Michaelis−Menten kinetics was observed. Interestingly, nearly no competitive inhibition of the template molecule indole occurred. The reason is that the binding of the substrate benzisoxazole is much higher than that of the template indole.

Scheme 11. Catalyst for the Dehydrofluorination Prepared by Imprinting Using Noncovalent Interaction (adapted from ref 137)



Scheme 12. The Decarboxylation of 5-nitro-3-carboxybenzisoxazole[a] (adapted from ref 138)



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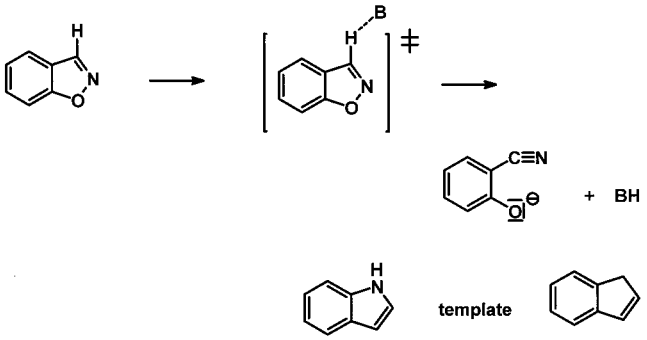
[a] The decarboxylation of 5-nitro-3-carboxybenzisoxazole occurs from the carboxylate anion and is known to be a concerted, intermediateless elimination. The template monomer **14** is used to position two amino groups in the active site of the imprinted catalyst.

Scheme 13. Isomerization of Benzisoxazole Similar to Scheme 12[a] (adapted from ref 140)



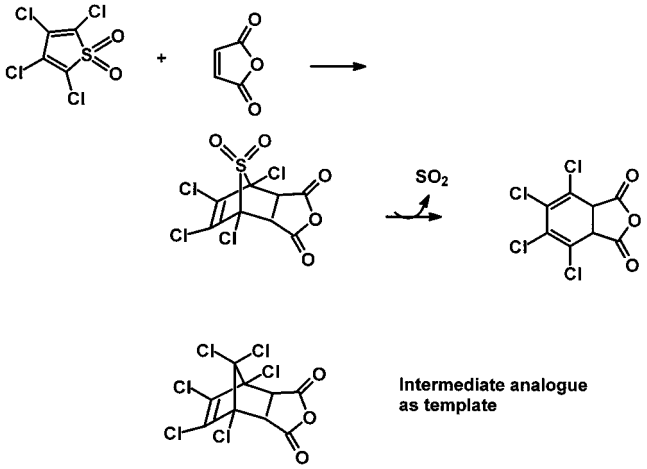
[a] While indole is the template, indene is used for the preparation of the control polymer.

D. Catalysis of Carbon–Carbon Bond Formation

The catalysis of carbon−carbon bond formation is a special challenge in organic synthesis. Unlike hydrolysis and elimination, it proceeds through an unfavorable pathway for entropic reasons. Initial attempts have been tried using imprinted polymers. Mosbach et al.[141,142] tried to mimic class II aldolase-like activity as found in primitive cells, such as yeast and bacteria. A complex between dibenzoylmethane (as an analogue of the reaction intermediate) and 4-vinylpyridine with Co^{2+} was used for imprinting. This polymer showed activity in catalyzing the reaction of acetophenone and benzaldehyde to produce chalcon. The reaction proceeds very slowly, even at 100 °C, and some degradation of the polymer takes place at this temperature over time. Compared to a control polymer, a 2-fold increase in rate and compared to the solution an 8-times enhancement is observed. This catalysis shows turnover.

Another C−C bond formation, a Diels−Alder reaction, has been studied by the same group.[143] The same type of stable intermediate analogue has been used for templating, as previously with antibodies.[144]

Scheme 14. Diels–Alder Reaction of Tetrachlorothiophene Dioxide with Maleic Anhydride[a] (adapted from ref 143)



[a] The intermediate expels sulfur dioxide. Chlorendic acid is used as intermediate analogue template molecule.

The idea is that the reaction forms an intermediate initially that then undergoes a spontaneous rearrangement, leading to formation of stable products by expulsion of sulfur dioxide (see Scheme 14).

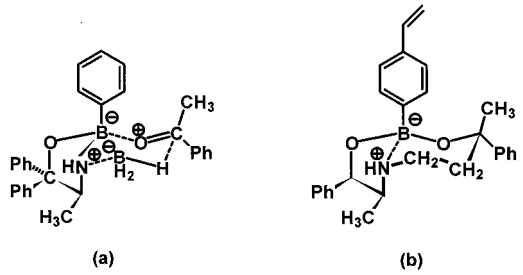
The imprinted polymer shows a significant enhancement in rate compared to solution or control polymer. A comparison of quantitative data is difficult, since the kinetic data show a longer inhibition period (e.g., even after 20 h, the reaction in solution is faster than that catalyzed by the imprinted polymer).

E. Catalysis of Oxidation and Hydrogen Transfer

Applications of molecularly imprinted polymers for the catalysis of oxidation reactions have already been described from 1980 on by Efendiev and Kabanov (for reviews, see refs 145 and 146). This group synthesized first functional polymers, e.g., copolymers of diethyl ester of vinylphosphonic acid and acrylic acid, and then prepared Co(II) complexes under addition of the template (either the substrate, the end product, or an intermediate of the reaction). This prearranged polymer was then cross-linked with *N*,*N*′-methylenebis(acrylamide) to a network. For comparison, a control polymer without template was prepared, too. If the polymer is, for example, imprinted with ethylbenzene (the substrate of the oxidation), the polymer had 2−3 times the catalytic activity for the oxidation of ethylbenzene than a control polymer. The activity and selectivity for the formation of acetophenone was even better when phenylethyl hydroperoxide, the reaction intermediate, was used as template for imprinting.[145,147]

In a similar manner, poly-4-vinylpyridine could be complexed with Ni^{2+} and cross-linked with *N*,*N*′-methylenebis(acrylamide) in the presence of allyl alcohol as template. After removal of the template, the polymer can be used as catalyst for the hydrogenation of allyl alcohol, and it is reasonably more active than the control polymer. The substrate selectivity is, however, quite low.[148]

Scheme 15. Enantioselective Reduction of Prochiral Ketones Using Oxazaborolidines as Catalysts[a] (adapted from refs 59, 150)

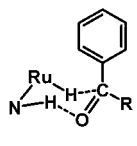


[a] The transition state of the reduction of acetophenone (a) and a stable, polymerizable transition state analogue (b) are represented.

More recently, Lemaire et al.[149] studied the hydride transfer reduction of prochiral ketones using Rh(I)-based catalysts. The asymmetric catalysis was investigated using imprinting with the optically active reaction product. Since the catalyst ligand was also optically active, the results of the reduction of propiophenon are not clear-cut. Nevertheless, the reduction with the imprinted catalyst gives somewhat higher enantiomeric excess than with the nonimprinted one. The reaction rate of the reduction is increased by a factor of 5−10 times with the imprinted catalyst. It would be of special interest to see the enantioselectivity after imprinting with optically active templates but with achiral ligands instead.

We have conducted similar experiments on the borane reduction of ketones (CBS reaction).[59,150] In Scheme 15 the assumed transition state of the reduction of acetophenone is shown (a). A stable transition-state analogue (b) of this reaction was prepared (Scheme 15). After polymerization, the template molecule can be released by hydrolysis, leaving behind polymer-bound boronic acid groups, which can subsequently be coupled with optically active amino alcohols to give polymer-bound oxazaborolidines embedded within chiral cavities complementary to the transition state of the reaction. However, in this case, enantioselectivities in reduction of acetophenon never exceeded the value obtained with the same optically active oxazaborolidine in solution. An achiral oxazaborolidine bound in the chiral cavity as catalyst gave a negligible imprinting effect.[150]

Catalytic reduction of ketones was also studied by Polborn and Severin.[151−153] A similar transition state as in the preceding example with a six membered ring having a chair conformation (**15**) is assumed in



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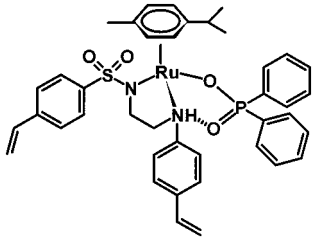
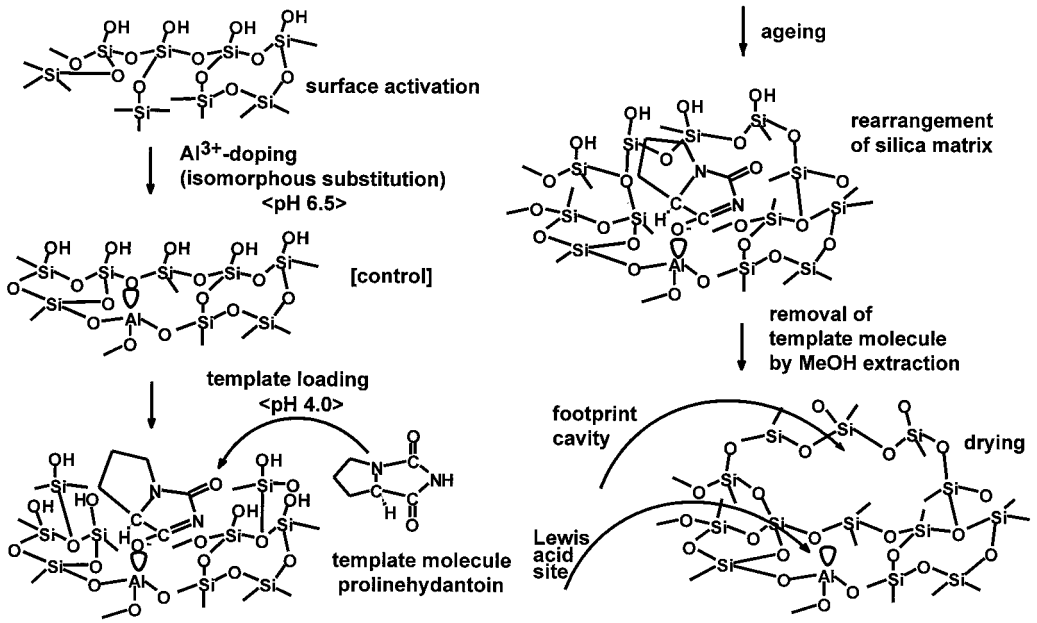


16

the case of the Ru(II)-catalyzed transfer hydrogenation of ketones. In principle, it is the same general

Scheme 16. Schematic Representation of the Preparation of "Footprints" on the Surface of Silica[a] (adapted from ref 159)



[a] After doping with Al^{3+}, proline hydantoin is used as the imprint molecule. For details, see the text and refs 163, 168

reaction investigated by Lemaire,[149] but in this case achiral catalysts and the catalytically more active Ru(II) are used instead of Rh(I). Moreover, the stable transition state analogue (template monomer) is first isolated and fully structurally characterized (i.e., by X-ray single-crystal structure determination). The catalyst shows a 7-fold increased rate in hydrogenation relative to the control polymer. A significant substrate selectivity is observed at the same time. So, with the polymer prepared from a transition state analogue for the reduction of benzophenone (**16**), this ketone is preferably reduced out of a mixture of benzophenone and acetophenone. The opposite selectivity is observed with the control polymer. This type of catalyst can also be used for asymmetric reductions.[154] Both the activity and the selectivity of the imprinted catalysts is further improved if the catalyst is attached to the polymer by a 2-fold connection as in **16**[152] instead of a single connection.[151]

IV. Catalysis with Imprinted Silicas and Zeolites

A. "Footprint" Catalysis

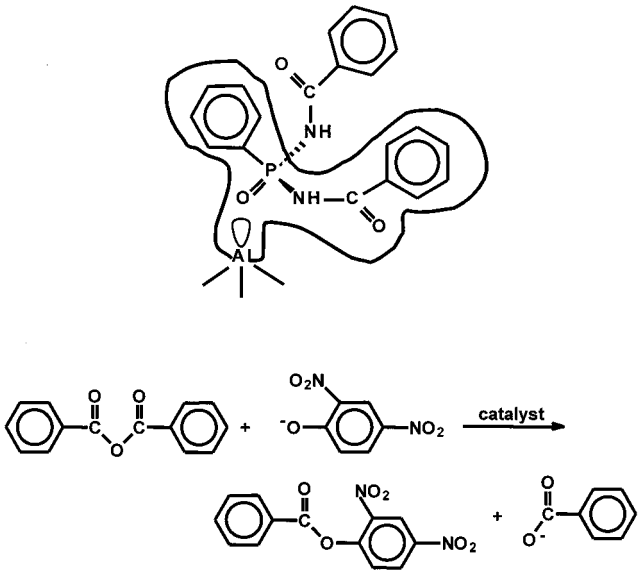
The first attempts to obtain imprinted materials were achieved with silicas by Dickey.[155,156] He precipitated silica gel in the presence of dyes. After drying, the dyes were washed out, and the as-prepared silica showed an increased affinity for the template compared to similar compounds. During this approach, the formation of the rigid silica gel matrix around the template facilitates shape selectivity, and additional silanol groups might be arranged such that interactions with the template can occur. No directed interactions between template and the growing silica chains were tried in these first experiments. One should expect a high stability of these arrangements in silica, but the selectivity disappeared readily.[157] Especially racemic resolutions have been studied for some time with imprinted silicas (see, e.g., reviews in refs 41, 158).

Much later, investigations of imprinted silicas started anew. The first to use this approach for the synthesis of catalytically active silicas was Morihara (for a review, see ref 159). He used a quite unconventional approach to obtain catalytically active silicas by surface imprinting. This procedure was called "footprint catalysis". From 1988 onward many examples were published.[160−172]

In Morihara's procedure (for details, see refs 159, 163, and 168), commercial silica gel is treated at pH 6.5 with Al^{3+} ions. The incorporation of Al^{3+} in the silica matrix by isomorphous substitution of silicate with aluminate causes the formation of surface Lewis acid groups (see Scheme 16). The surface is then loaded at pH 4.0 with template molecules that contain Lewis base groups. The silica is then aged and dried under highly controlled conditions. It is assumed that in this way the silicate matrix rearranges around the acid−base complexes by depolymerization of the silicate matrix and repolymerization under thermodynamic control. The complexes are stabilized by forming a maximum number of interactions between the acid−base complex and the surrounding silicate matrix. Afterward, the template is removed with methanol.

Mostly acyl transfer reactions are investigated, since substrates and transition state analogues in this case are readily available. Scheme 17 depicts the surface imprinting with the transition state analogue phosphonic acid diamide (*N*,*N*′-dibenzoylbenzenephosphone diamide). The substrate was an acid anhydride, and the nucleophile was 2,4-dinitrophenolate, the consumption of which was followed photometrically. In this case (one of the best in a large series), k_{cat} increases by a factor of 10, and K_m is improved by a factor of 3 with regard to nonimprinted materials.[163] A comparison with other imprinting

Scheme 17. Schematic Representation of Imprinting with a Phosphonic Diamide as a Stable Transition State Analogue Template[a] (adapted from ref 163)



[a] The catalysis of the nucleophilic attack of a phenolate at benzoic anhydride is investigated.

methods with regard to k_{cat} and K_m is difficult; since the exact number of active sites is not incorporated in the calculations, second-order rate constants are used for k_{cat} and the reported K_m values are indeed mostly K_{ass} (M^{-1}) values. Therefore Morihara's k_{cat}/K_m values do not refer to the usual Michaelis–Menten kinetics. In comparison with his own control silicas, though, the imprinted silicas show a remarkable catalytic activity.

In a number of examples, substrate specificity is shown. If an optically active template is used as a transition state analogue for imprinting, these catalysts cause enantiomers to react at different rates (kinetic racemic resolution). The substrate enantiomer corresponding to the template enantiomer reacts 2–4 times more rapidly than the other.[164,165,167,170,172]

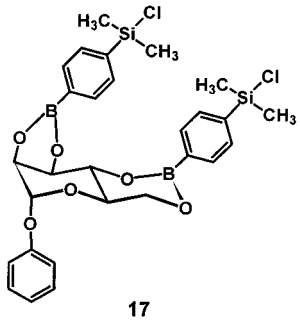
Other reactions, such as crossed aldol condensation, enantioselective racemization, and asymmetric reductions, have also been investigated,[159] though no details are available at present.

The footprint cavity approach has reached quite promising results. At the moment it is not easy to understand the mechanism completely. Apart from the Lewis acid–Lewis base interaction, no defined interactions between silica and template are known, and no investigations in this directions have been made. Only small shape selectivity should be possible in shallow cavities. Unfortunately, no other research groups have used this method until now. In view of the interesting results, more detailed knowledge of the method is desirable.

B. Other Examples for Catalytically Active Imprinted Silicas

Imprinted silicas for catalysis were also prepared by Heilmann and Meier.[173] They used 1-triethoxysiloxy(phenyl)methanephosphonic ethyl hexyl ester as a transition state analogue for a transesterification reaction. After a sol–gel process with this compound and tetraethoxysilane, the template was removed by calcination at 523 K, leaving behind cavities with a shape of the transition state of the transesterification. An acceleration of the transesterification was indeed observed,[173] but more detailed investigations showed that this effect was not due to an imprinting effect but to phosphoric acid left at the polymer and in solution.[174,175]

More similar to the usual imprinting method in polymers, silicas with defined binding sites have been prepared that were introduced by polycondensation of functionalized silanes together with tetraethoxysilanes. In this case the polymerizable double bond has been replaced by functionalized silanes (see, e.g., **17**). Selective recognition was observed.[41,43,68] This



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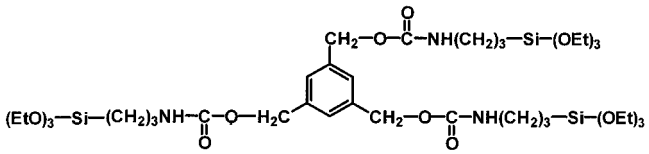
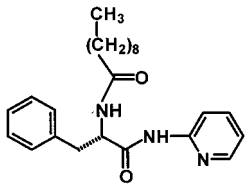


18

method has also been used to prepare catalytically active silicas.[176] Via carbamate moieties to a template molecule, either one, two, or three amino groups (see e.g. **18**) are introduced in cavities during a sol–gel process. Initial results (without experimental details) indicate catalytic activity for a Knoevenagel condensation.

In several recent papers Markowitz et al. reported on a very original molecular surface imprinting method.[177–179] This method involves surface imprinting of silicas during the formation of the particles in a sol–gel process. A surfactant derivative of the imprint molecule is used as the template during the polycondensation of tetraethoxysilane in the presence of added functionalized organosilanes. First, the surfactant imprint molecule, e.g., *N*-decanoyl-L-phenylalanine-*N*-pyridin-2-ylamide (**19**), is incorporated



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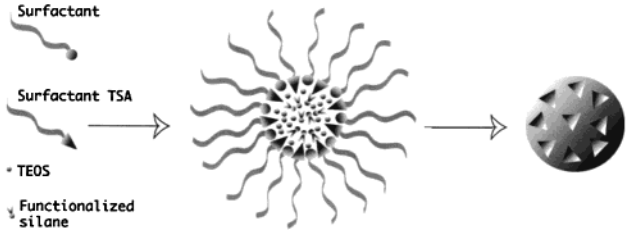
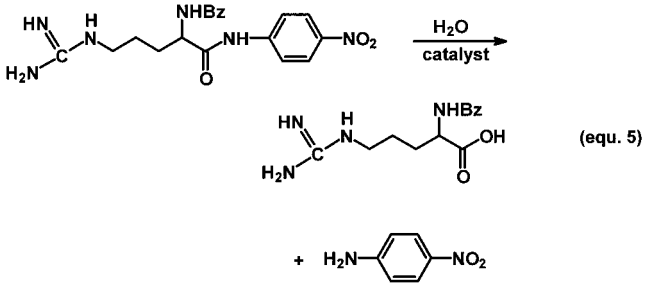
Scheme 18. Template-Directed Molecular Imprinting of Silica Particles (reprinted from ref 177. Copyright 2000 American Chemical Society)



in a water-in-oil microemulsion with added nonionic surfactant. Tetraethoxysilane and a mixture of amine, dihydroimidazole, and carboxylate-terminated organosilanes were added to commence base-catalyzed surface imprinting and particle formation (see Scheme 18). Since the silica particles are formed by a microemulsion process, the imprint molecule, which acts as the headgroup of the surfactant, should be positioned at the surfactant−water interface of the reverse micelles within which the silica particles are formed. As a consequence, catalytic sites should only be formed at the surface and they should all have the same orientation with regard to the surface. l-Phenylalanine-*N*-pyridin-2-ylamide can be regarded as a stable transition state analogue of the α-chymotrypsin-catalyzed amide-fission of peptides.

Although the catalyst was imprinted with a phenylalanine derivative, the imprinted silica catalyzed best the hydrolysis of an arginine derivative (see eq 5). An enhancement in initial rate of 4.8-fold is



(equ. 5)

obsered compared to a nonimprinted control having the same functional groups (see Table 5). The most remarkable result is the enantioselectivity as silica imprinted with a L-phenylalanine derivative catalyzes the hydrolysis of the D-enantiomer of an arginine derivative 34 times faster than the L-enantiomer.[177] This is by far the highest enantioselectivity for imprinted materials published to date. The reversed enantioselectivity (catalyst imprinted with L-enantiomer hydrolyses the D-substrate more actively) might be due to the surfactant moiety in the template. If the D-enantiomer is used as template instead of the L-enantiomer, the opposite enantioselectivity is observed. This result is to be expected, but it rules out possible experimental errors. Further development of this method might substantiate an important new way toward catalytic imprinted silicas and clarify some open problems.

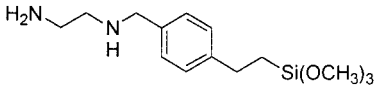
C. Imprinting in Zeolites

Zeolites consist of a crystal lattice having defined pores and cavities throughout. Zeolites with very different cavity diameters are known. A very detailed review article[180] deals with the numerous possibilities for using these compounds in catalysis. Until now, no direct imprinting in zeolites has been published. There are reports on the synthesis of zeolites in the presence of certain organic molecules acting as "templates" in order to control the type of lattice being formed. This is no molecular imprinting in the original sense. Zeolites should be interesting candidates for imprinting inside the holes. This might reduce the polyclonality of the cavities. A problem can be foreseen with regard to the mass transfer inside the zeolites containing additional organic or inorganic polymers inside the pores and holes. Further possibilities of application of zeolites in the imprinting procedure are discussed by Davis et al.[39]

Table 5. Data of Initial Rates for the Hydrolysis According to Eq 5 Catalyzed by Surface-Imprinted Silica Particles (data from ref 177)

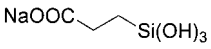
composition of polycondensation mixture[a]		hydrolysis reaction		
amount of template[b] (mol %)	amount of silanes[c] (wt %)	substrate[d]	initial rate (μM/mg/min) $\times 10^5$	rate enhancement compared with control
0	0	D,L	0.47	
0	5	D,L	0.83	control
20	0	D,L	1.37	1.6-fold
20	5	D,L	3.98	4.8-fold
20	5	D	4.80	5.8-fold
20	5	L	0.17	0.2-fold

[a] Preparation under standard condition. [b] Template **19**. [c] Mixture of PEDA

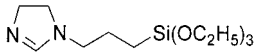


,

CTES



and IPTES



[d] Benzoylarginine-*p*-nitroanilide (either D,L; D; or L).

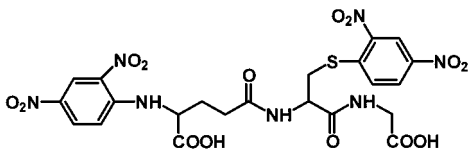
V. Bioimprinting

Biopolymers can be used as the matrix for the imprinting procedure (for a review, see ref 181). This procedure is quite different from those discussed until now. Keyes et al.[182−185] prepared semisynthetic enzyme analogues. Their starting materials are different types of proteins that were imprinted with suitable substances. The resulting compounds can sometimes have similar properties to that of natural enzymes. First, the protein is partially denatured and then brought into contact with a modifying agent (the template). This may, for example, be an inhibitor for the enzyme to be modeled. The protein is subsequently covalently cross-linked with glyoxal or another suitable reagent. Then the template is removed. In this way products with new types of enzymatic activity are obtained, although the starting proteins had either no activity (for example, albumin and

concanavalin A) or a completely different type of activity (ribonuclease, trypsin, glucose oxidase, α-amylase) before being converted to catalysts that mimic glucose isomerase or chymotrypsin.

These semisynthetic enzymes act in aqueous solution. In one example, transformation of a trypsin-active to a chymotrypsin-active protein is described, with indole as inhibitor acting as template and glutaraldehyde as cross-linking agent. The new product showed an increase in chymotrypsin activity of 4-fold and a decrease in trypsin activity of 14%. The partially denatured protein is assumed to bind the template, which leads to the formation of a new active center of a specific shape and functional groups in a proper orientation similar to that of the enzyme to be modeled.

A similar procedure was also used to produce a biocatalyst with glutathione peroxidase activity.[186] A denatured egg albumin was equilibrated with the glutathione derivative **20** to form a new conformation



20

via hydrogen bonds, ion pairing, etc. The new conformation is then fixed by cross-linking with glutaraldehyde. After removal of the template, the serine residues in the binding site are converted into selenocysteines. This imprinted protein shows an 80-fold higher activity compared to a protein treated the same way but without template.

Another type of bioimprinting has been introduced by Klibanow et al.[187] and in a somewhat different manner by Mosbach et al.[188] In recent years, the application of enzymes in organic media has received much attention. In these systems, no or nearly no water is present, and the enzymes are used in an insoluble form. Water-insoluble substrates can thus be transformed, and the enzymes show higher stability. One problem is the lower catalytic activity. By a certain type of bioimprinting, catalytic activity as well as selectivity and stability can be increased.

For this, the enzymes to be modified (proteases, lipases etc.) are lyophilized in the presence of a substrate analogue, e.g., *N*-Ac-L-Phe-NH_2. The template is afterward removed under anhydrous conditions. The modified enzyme possesses under anhydrous conditions 1−2 orders of magnitude higher catalytic activity compared to nonimprinted lyophilized enzymes. Similarly, other structurally quite different compounds, such as sugars or poly(ethylene glycol)s, show the same effect if they are used as templates in the imprinting procedure. This effect does not occur if these substances are just present in the organic solvent during catalysis. It is assumed that the template stabilizes the enzyme against denaturation upon lyophilization,[189] i.e., it acts as a lyoprotectant and thus a special conformation is frozen. If this enzyme comes into contact with water, the imprinting effect disappears. The effect does not seem to be selective, and no data on substrate selectivity are given. It is merely an effect on the assessibility of the active sites. Dordick et al., who lyophilized subtilisin in the presence of different nucleophilic substrates (such as thymidine, ribose, sucrose, deoxyadenosine), investigated the catalytic activity for acylations.[190,191] The acylation of the corresponding template molecules in the presence of the imprinted subtilisin seems to be facilitated,[190−192] but no data for the selectivity in the bioimprinting process are available. Dordick et al.[191] assume that the activation of the enzymes is the result of structural changes in the catalytic triad for the serine protease. Quite a few studies on this effect have been performed by molecular dynamic simulation.

Similar results are obtained on bioimprinting of a chymotrypsin derivative with photoswitchable substituents.[193] The enzyme−substrate complex is obtained after precipitation and drying. This yields a substantially more active biocatalyst in the organic phase.

If lipase is imprinted with (*R*)-1-phenylethanol and coated with a synthetic glycolipid, this product shows after lyophilisation a high activity and a remarkably enhanced enantioselectivity. The glycolipid coating is improving the solubility of the enzyme in organic solvents.[194]

Bioimprinting effects can also be achieved by freeze-drying in the presence of micellar amphiphiles [interfacial activation based on molecular (bio)imprinting (IAMI)].[195,196] In anhydrous media, lipases can be activated for esterification and transesterification by 2 orders of magnitude. It should be taken into account, though, that in all cases of bioimprinting the activity of enzymes is still considerably lower compared to the original enzyme in aqueous solution.

Only Mosbach et al. were able to get a more or less selective conformational imprinting.[188,197] They precipitated chymotrypsin as the enzyme−inhibitor complex (with *N*-acetyl-D-tryptophane) in 1-propanol. This precipitate changed its selectivity after removal of the template. It was then able to synthesize *N*-acetyl-D-tryptophane ethyl ester in cyclohexane, whereas the nonimprinted and the *N*-acetyl-L-tryptophane imprinted enzyme did not give the same enantiomer. This seems to be an attractive example of real imprinting in contrast to the previously mentioned examples. Detailed kinetic studies were not available.

Similar to work with synthetic polymers (see Scheme 11), Ohya et al.[198] have tried to imprint bovine serum albumin by freeze-drying with a transition state analogue of the dehydrofluorination reaction. The imprinted protein shows a 3.3 times stronger catalytic activity compared to the nonimprinted one. Michaelis−Menten kinetics are observed.

Similar results for the catalysis of the dehydrofluorination reaction were obtained by imprinting β-lactoglobulin.[199] In this respect it is interesting to note that even nonimprinted proteins show a remarkably high catalytic activity for the dehydrofluorination reaction.

VI. Concluding Remarks—A Critical Discussion

A. The Problem of Controls in Kinetic Measurements

In this review many examples of catalysts prepared by a molecular imprinting procedure have been referred to. In many cases the catalytic rate enhancement is still moderate, and further attempts are being made to improve the activity and selectivity. The main advantage of the imprinted catalysts is the possibility to obtain a selective catalysis that is quite similar to natural enzyme action. In many papers an enzyme-like Michaelis−Menten kinetics is postulated, whereas in a very few cases substrate selectivity is actually observed. Quite a number of papers postulate molecular imprinting effects, but the benefit provided by molecular imprinting is difficult to assess at the moment. The following critical remarks on imprinted catalysts are made in the hope of providing a more general methodology in this approach.

The catalytic activity usually is measured by determination of the pseudo-first-order rate constant of the reaction in question. In these cases only the initial rates are measured to avoid effects of the back-reaction in an equilibrium. Furthermore, product inhibition, if present, will be less pronounced. It is an important question whether real turnover is present, which can only be measured if conversion is studied for a longer time. Product inhibition can easily be studied if the initial rates are measured in the presence of different amounts of products. It has thus been shown that the product of the hydrolysis of the ester **9** (this is the diacid **11**) shows product inhibition,[93] but HCO_3^-, the hydrolysis product of **14**, does not.[91]

A transition state analogue imprinted polymer should show competitive inhibition by the template, and this is actually observed in many cases. Often this compound shows much better binding compared to the substrate. It is a good indication for a molecular imprinting effect. The competitive inhibition should occur with an imprinted catalyst, whereas it should not occur or occur less strongly with a non-imprinted control polymer.

Whether or not the catalysis can be described by a Michaelis−Menten kinetics is usually evaluated by measuring the initial catalytic reaction with increasing amounts of substrate relative to catalyst, as this leads to a saturation kinetics. The solubility of the substrate has frequently not been good enough to really give the full curve. It is quite dangerous to apply in such a case a calculation program to obtain the kinetic data, since the error might be extremely large. Only if saturation is really observed will a meaningful kinetic analysis be possible. Computer calculations or a graphical analysis provide the following figures: K_m = the Michaelis constant; k_{cat} = catalytic rate constant (also called the "turnover number"); v_{max} = the maximal (saturation) rate of the enzyme; and k_{cat}/K_m as a measure of the catalytic efficiency. If the kinetics are measured in the presence of different amounts of a competitive inhibitor, also K_i (the inhibition constant) can be obtained. These values can only be obtained if the number of active sites at the catalyst is known with some accuracy. It is not sufficient to calculate the number of active sites from the amount of template released from the polymer, since in some cases only a smaller part of the active sites is really accessible, as was discussed section II.C. In Table 6, values for different catalytic systems obtained by molecular imprinting are given. For comparison, also the values for corresponding catalytic antibodies are shown. Natural enzymes show similar K_m values, but k_{cat} values are considerably higher. Thus the enhancement in rate compared to a solution of the same pH can be as high as 10^8-10^{12}-fold.

The catalytic efficiency of enzymes or catalytic antibodies is usually measured in relation to a solution of the same buffer at the same pH value. In the case of imprinted polymers, rate enhancements k_{impr}/k_{sol} of up to 3800 have been observed, which is on a similar order compared to catalytic antibodies but much less when compared to enzymes. If rate enhancements of different catalysts are compared, it is very important to compare the kinetics of reactions of the same reaction order. All rate constants in this paper are calculated as pseudo-first-order rate constants. If for an identical reaction a second-order rate constant is derived, the apparent rate enhancement can be much larger for mathematical reasons.[140] Many papers on enzyme models report k_{cat}/K_m values. These data show the efficiency of the system.

An important question arises as to the reason for the rate enhancement caused by imprinted polymers. In enzyme models there is a good chance that these factors can be investigated simply by changing the catalytic systems. In enzymes and antibodies this is by far more difficult.

The original idea is that the rate enhancement is based only on the imprinting effect. This means it is based on the exact shape of the cavity and on the correct orientation of the functional groups inside the cavity. The catalysis inside the imprinted cavity might be caused by different effects of the imprinting, and some other effects, regardless of the imprinting, might be present as well:

(a) The heterogeneous polymer structure might cause nonselective catalysis by surface effects (adsorption, activation, etc.).

(b) The catalytically active functional groups might cause catalysis regardless of their position inside or outside the cavity.

(c) It is known that functional groups a certain distance to each other can show neighboring group effects in catalysis, which can be rather high.

(d) The polarity of the medium inside a cavity might be very different from that in solution. In such a case, the pH, the concentration of salts, or added organic solvents might be different inside and outside the imprinted cavities. This behavior is similar to that of natural enzymes.

(e) It should be mentioned that the pK_a values of functional groups are different at a polymer (e.g., an ion exchanger) and in solution.[201,202] Therefore, at the same pH, a functional group might be in a different type of dissociation (and catalytic activity) at the polymer or in solution. Amidine groups show an

Table 6. Examples of Michaelis−Menten Kinetics of Imprinted Polymers and Their Biological Counterparts

type of reaction	scheme/ eq[a]	cross-linking[b] (%)	binding type[c]	solvent of catalysis	enhancement of rate k_{obs} vs solution	enhancement of rate k_{obs} vs control plmr	K_m (mM)	$10^3 k_{cat}$ (min^{-1})	K_i[d] (mM)	refs
		synthetic polymers								
ester hydrolysis	S 9	87	A + C	MeCN/buffer 1:1	10	2.5	1.96	0.019	−	126, 127
ester hydrolysis	S 10/eq 1	88	D	MeCN/buffer 1:1	102−235	5.0	0.6	0.08	0.025	93
carbonate hydrolysis	eq 3	80	D	MeCN/buffer 2:1	588	10	5.0	12	0.1	91
carbamate hydrolysis	eq 4	80	D	MeCN/buffer 2:1	1400−3860	5.8	3.3	22	0.3	91
dehydrode fluorination	S 11	∼88	B	benzene	12.8	7.5	27	11	ci	137
decarboxylation	S 12	78	A	diethyl ether	25.4	10.7	0.84	320	ci	138
isomerization	S 13	∼90	C	buffer/EtOH 3:1	−	7.2	0.48	205	−	140
		bioimprinting								
dehydrode fluorination	S 11	albumin freeze-drying with template	B, C	ethyl acetate	−	3.3	189	267	57	198
acetylation of thymine		subtilisin freeze-drying with template	B, C	THF	−	51.8	4.9	1.5×10^6	−	191
		antibodies								
ester hydrolysis		raised against TSA		buffer	960	−	0.0019	1620	0.00016	200
carbamate hydrolysis		raised against TSA		buffer	3000	−	120	0.018	ci	132
dehydrode fluorination		raised against TSA		buffer	8.8×10^4	−	0.182	193	0.0003	35, 134

[a] The reaction is represented in a scheme (S) or an equation (eq) of this paper. [b] The synthetic polymer is prepared with a certain percentage of cross-linking agent. [c] Binding type during the imprinting procedure: A = covalent interaction, B = electrostatic interaction, C = hydrogen bonding, D = stoichiometric noncovalent. [d] K_i = inhibition constant with the template as competitive inhibitor. ci = competitive inhibition observed, no quantitative data.

apparent pK_a in solution of 10.6, whereas the apparent pK_a of polymeric amidines in the same solution range from 9.02 to 8.62. Even the average pK_a value of a carboxyl group inside a specific cavity (apparent pK_a = 8.9) is somewhat different from a group in a statistical position outside the polymer's cavity (apparent pK_a = 9.1).[203]

(f) It is quite clear that even in solution buffers, added salts, different pH, different solvent composition, and different temperature all influence the rate of catalysis.

(g) Measuring the rate of the reaction at different temperatures is usually not a valid way to elucidate for imprinted polymers the activation parameter of the reaction, as is done in solution chemistry. A higher temperature causes swelling of imprinted polymers, and more cavities become available. Thus, the number of active sites is not constant with temperature.

It is desirable to elucidate the different contributions to the overall catalytic effect. A good possibility for this evaluation is the use of proper control systems. Some important possibilities are given below:

(a) In the preceding discussion, the enhancement by catalysis is determined by comparison to a reaction in buffer solution. The same reaction with identical concentrations is performed in the same buffer solution without the imprinted catalyst. The ratio of the pseudo-first-order rate constants is then determined. This comparison is identical with the enhancement that is determined for antibodies and enzymes. Usually this method gives by far the highest values for the enhancement in rate.

(b) To elucidate the influence of the catalytic groups that have been introduced into the imprinted polymer, the buffer solution with addition of the same molar concentration of these groups in soluble form is used as a control.

(c) A control consisting of the same buffer solution containing a nonfunctionalized heterogeneous polymer shows influences of possible surface effects. The nonfunctional polymer might show an enhancement in rate by catalytic effects of the surface by adsorption and enrichment of substrate concentration. It can also cause a slower reaction rate if the reaction is not catalyzed at the polymer, and the concentration in solution is thus diminished. It is important that this polymer should consist of the same polymer matrix and should have the same inner surface area and porosity. In some cases such a control polymer already gives a remarkable enhancement.

(d) A further option for a control uses the buffer solution, the soluble catalytic groups, and the nonfunctionalized reference polymer (see point c).

(e) A very important control that is used in many careful investigations is the buffer solution containing the same amount of heterogeneous polymer but with the functional groups (for catalysis or binding) in statistical distribution. Such a control polymer is

not easy to design. Usually it is prepared from an identical composition compared to the imprinted polymer but where the template molecule is omitted. The problem is that the monomer containing the functional group, yet without the accompanying template, possesses a different polarity and maybe another copolymerization behavior. Furthermore, degradation of the free catalytically active group by radicals of the polymerization mixture (especially with monomers forming very reactive radicals) has been observed (e.g., with amidinium groups). In such a case, it is important to use a much simpler compound instead of the template for interaction with the functional group. However, if for example amidines are used with a benzoic acid, benzoic acid already causes some imprinting effects.[93]

The same microstructure of the control polymer compared to the imprinted polymer has to be assured for such a control polymer. It is also very important that the same amount of catalytically active groups is present in the control and in the imprinted polymer. In many cases it is not sufficient to calculate the amount of functional groups from the composition of the polymerization mixture. A quantitative determination of the functional groups in the final polymer has to be performed. This determination is not easy to perform and needs in many cases a considerable amount of experience (see, e.g., ref 204).

The comparison with a proper control polymer of this type yields valuable data on the influence of the shape, the exact orientation of the functional groups, and the suitability of the model system (e.g., a good transition-state analogue, efficient catalytic groups, a proper imprinting system, etc.). This comparison still contains some weaknesses. Even if the control polymer and the imprinted polymer have the same number of functional groups, the accessibility of the groups in the polymers might be very different. If a bulky template is used for imprinting and no template for the controls, just variations in the accessibility of the groups might be responsible for different catalytic activity.

(f) The imprinting effect can be directly proven by determining substrate selectivity. For this, two imprinted polymers with slightly different templates are prepared. For both polymers, the catalytic activity of the two substrates (corresponding to the templates) are then measured (cross-selectivity). If the corresponding substrates react faster with their "own" polymer, this system shows substrate selectivity and therefore proves an imprinting effect. This cross-selectivity is investigated most elegantly in the case of enantiomers of template and substrate. By measuring enantioselectivity, all other effects of different reactivity, solubility, etc. are omitted, and a real proof for the imprinting effect is provided.

The catalytic effects of imprinted polymers should at least be distinguished in three categories:

(1) "catalytic activity" (ratio of rates of imprinted polymer and solution),

(2) "imprinting selectivity" (ratio of rates of imprinted polymer and control polymer), and

(3) "substrate selectivity" (ratio of rates of different substrates, cross-selectivity).

B. The Influence of the Cross-Linking Degree

As outlined in section II.B, the ability for selective separation by imprinted polymers is highest with highly cross-linked polymers. Polymers with a low degree of cross-linking show poor or no selectivity, e.g., for racemic resolution. The question has now been raised whether in the preparation of catalysts appropriate conditions can be found under which even with low or no cross-linking efficient and selective catalysis is possible.[205,206] It was postulated that it might thus be possible to equilibrate monomers with different functional groups around a template molecule and to polymerize them quickly so that the functional groups during polymerization have not sufficiently moved from their equilibrium position. If the energy of this conformation at this equilibrium is lower than that of all other conformations, it was expected that there might be a thermodynamical trend to renature to this very conformation, even if no cross-linking is present in the polymer. At present no convincing experimental data[207] are available to substantiate this hypothesis, which was also discussed in another context.[18] It should be taken into account that even natural enzymes, if denatured, are not able to spontaneously rebuild the original biologically active conformation.

In fact, some papers appeared in which molecularly imprinted catalysts have been prepared with a rather low degree of cross-linking.[115−122] Polymers with a low degree of cross-linking show a higher flexibility, and thus, the template can be removed more easily. Furthermore, the exchange equilibria and the catalytic action might be higher. No case of an imprinted catalyst with low cross-linking (e.g., below 10%) and selectivity of catalytic action has been reported. With today's knowledge and the usual technique of imprinting, no substrate selectivity (cross-equilibration) is expected and imprinting selectivity is equally doubtful.

C. The Influence of the Type of Dispersion

In most cases catalytically active imprinted polymers have been prepared by bulk polymerization in an ampule, crushing the polymer, and sieving. Thus irregularly broken particles with relatively low yield of the desired particle size are obtained. In the meanwhile, for selective recognition many attempts have been made to obtain imprinted polymers directly as regular beads[208−210] or for sensor preparation as thin membranes.[46] A general problem in the preparation of regular beads by conventional suspension or emulsion polymerization is the fact that usual noncovalent interactions will be broken by the presence of water. For this reason in some cases more or less complicated procedures have been applied. For the synthesis of catalytically active imprinted polymers, only one example is known for the preparation of beads.[91] These beads showed a substantially better "imprinting selectivity". Also a higher catalytic activity in such cases is to be expected due to a quicker mass transfer, and this effect should be stronger the smaller the beads are, as was actually found in a very recent investigation.[211] Aside from beads of diameter 8−375 μm by another technique, also those of 100−

200 nm were obtained that showed a good catalytic activity and especially a high "imprinting selectivity".

Of special interest are soluble, highly cross-linked beads of the same diameter as the enzymes exhibit, i.e., 5−10 nm. These macromolecules can be prepared by strong intramolecular cross-linking without intermolecular cross-linking. In a special procedure this is possible in a surprisingly simple way.[212] Thus, soluble but highly cross-linked imprinted polymers have been obtained (diameter 10−40 nm; $M_w = 1.3 \times 10^5 - 6 \times 10^6$) that show selectivity in molecular recognition. The same type of imprinted polymer is now under investigation for catalysis. With the preparation of these tiny beads, we are approaching more and more enzyme analogy. If the number of imprinted active sites is reduced to one per bead, a high similarity to enzymes is reached. This type of polymer may then be separated by affinity chromatography in order to reduce polyclonality and to obtain enzyme mimics with higher catalytic activity.

D. "Polyclonality" of the Active Sites

The catalytically active, imprinted polymers possess, unlike enzymes and monoclonal antibodies, active sites with differing binding ability, differing selectivity in binding, as well as differing catalytic activity. At the moment, no detailed studies on the variation of these properties and the connection to each other are available. More investigations are known on the heterogeneity of the recognition sites in imprinted polymers.

Apparently, there is a remarkable difference between molecularly imprinted polymers prepared by covalent or stoichiometric noncovalent interaction compared to those with standard noncovalent interaction. In the first case, functional groups are only situated inside the active sites, whereas with noncovalent interaction a high proportion is statistically distributed all over the matrix. In addition, in noncovalent interaction a considerable part (ca. more than 80%) of the active sites after removal of the template are not accessible for reagents, possibly due to a shrinking process.

The distribution of active sites in covalently imprinted polymers has been investigated already quite early.[60] With a multisite model the ratio of the binding constants for D- and L-**2** on a polymer imprinted with **1** were investigated with regard to the heterogeneity of the sites. It was found that few cavities show binding but no selectivity in binding (i.e., α values of 1.0). The majority of the cavities show selectivity and have a distribution of selectivities around an average figure, but the selectivities of a smaller proportion can go up very high.[60]

The binding variability in imprinted polymers with noncovalent interaction has been investigated in batch-rebinding experiments by different models (Langmuir adsorption isotherm, two-site- and three-site Langmuir adsorption isotherm, and Freundlich isotherm), as well as by frontal analysis in chromatography (refs 72, 104, and 213; for review, see ref 75). In this case more than 75% of the binding site groups may show binding and some catalytic activity but no selectivity, since they are not situated inside the cavities. The binding heterogeneity can be investigated by a two-site model in which the larger part represents the background activity. As already mentioned, after removal of the template, only a small part (∼15%) of the cavities can be reoccupied by the substrate. The other part (∼85% of the cavities) is irreversibly lost for binding and catalysis. These inactivated sites, though, do not interfere in the later equilibria. It appears that the remaining cavities possess a relatively high selectivity. But also within these active sites there is a distribution of selectivity. In case of 9-ethyladenine, this distribution seems to be rather small.[213] If a continuous heterogeneous distribution of sites over a range of binding affinities is assumed, the application of a site affinity distribution function (SADF)[214] might be advantageous. First results for imprinted polymers have been obtained.[215]

A full characterization of the activity distributions for catalytic systems might be done in a similar mode as with certain technical catalysts where the Langmuir−Hinshelwood formalism has been applied, e.g., for first-order or pseudo-first-order reactions. If saturation phenomena occur, this leads to very similar equations compared to the Michaelis−Menten equation. Another possibility is the Langmuir−Rideal formalism, in which it is assumed that one component is bound to the catalyst and the other reacts directly from the solution (or the gas phase).[216] If there is a variety of activity, the overall rate is the sum of the rates on the various types of site. Usually the more active sites dominate the characteristic of the kinetics.

Table 7. Comparison of Molecularly Imprinted Catalysts versus Antibodies and Enzymes

a	catalytic efficiency	enzymes ≫ antibodies ≥ imprinted polymers
b	binding site homogeniety	enzymes ≃ antibodies ≫ imprinted polymers
c	moderate cost and ease of preparation	antibodies ≪ enzymes < imprinted polymers
d	handling and stability	antibodies ≤ enzymes ≪ imprinted polymers

E. Future Prospects in Catalysis

What has been reached in the preparation of catalysts by molecular imprinting in polymers or silicas? Over the last 10 years a considerable progress in the preparation of efficient catalysts has been made. A comparison of imprinted catalysts, monoclonal antibodies, and enzymes is shown in Table 7.

Enzymes are in every case several orders of magnitude catalytically more efficient, but in a few cases imprinted polymers have reached the activity of catalytic antibodies, e.g., in the hydrolysis of carbamates.[91] This is surprising, since monoclonal antibodies are compared with "polyclonal imprinted" catalysts, and the imprinted materials are insoluble and rigid, whereas antibodies are soluble and more flexible. The binding site homogeneity in enzymes and monoclonal antibodies is high, whereas imprinted polymers, as discussed before, have a broad distribution of activity and there is no method available at the moment to really reduce this broadness. A real advantage of imprinted catalysts is the ease

of preparation and handling. They can be prepared in large quantities by suspension polymerization, and stable particles of uniform diameter can be easily obtained.

Imprinted polymers can be applied directly in chemical processes. Such catalysts can also be prepared, in addition to beads or broken particles, in other very different forms, such as monoliths, microcapsules, membranes, surfaces. At the same time these materials are rather stable. Whereas enzymes and antibodies degrade under harsh conditions such as high temperature, chemically agressive media, and high and low pH, imprinted polymers show better behavior in most cases. They have both good mechanical and thermal stability. Usually they can be used for a long time in a continuous process, or they can be reused many times. As a result of the insolubility of the materials, they can be easily filtered off after a reaction, or they can be placed in a flow reactor. All this brings a lot of advantages in the use of imprinted polymers or silicas.

Though quite some progress has been made in the preparation of catalysts by molecular imprinting, for large application in industry and for broader application in research, further achievements have to be made. On one hand, the imprinting procedure has to be further improved and new approaches in the preparation of catalysts have to be used. At present, the following problems are in the forefront of investigations to improve the molecular imprinting procedure:

(a) molecular imprinting in microparticles during suspension or emulsion polymerization (see section VI.C);

(b) imprinting procedures in aqueous solutions [see, e.g., ref 54 and section V (bioimprinting)];

(c) imprinting with high molecular weight templates, biopolymers, or even bacteria by surface imprinting (see, e.g., ref 217);

(d) development of new and better binding sites in molecular imprinting (see, e.g., section II.C);

(e) improvement of the mass transfer in imprinted polymers (see, e.g., ref 41);

(f) reduction of the "polyclonality" of cavities (see section VI.D);

(g) increase of available active sites, especially with the usual noncovalent interaction (see, e.g., ref 75);

(h) development of extremely sensitive detection methods for use in chemosensors (see, e.g., ref 46); and finally,

(i) development of further suitable groupings for catalysis.

VII. Acknowledgments

Our group has received financial support over a long period from the Deutsche Forschungsgemeinschaft, whom I thank in particular. Also, support from the Fonds der Chemischen Industrie has helped us considerably. I thank Prof. K. J. Shea, Irvine, and Dr. M. A. Markowitz, Washington D. C., for providing me with unpublished results. Thanks are due to Dr. A. Kraft, Edinbourgh, who assisted me with the linguistics of the manuscript, and K. Knorr, Düsseldorf, in preparing the cover art.

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CR980039A