Towards artificial antibodies prepared by molecular imprinting

Richard J. Ansell, Olof Ramström, and Klaus Mosbach*

The new technique of molecular imprinting has increasingly been adopted by research laboratories worldwide during the last few years. We have studied the use of such imprints against drugs as artificial antibody-binding mimics in competitive radioimmuno-style binding assays. The recognition sites “molded” in the polymers mimic the binding sites of natural antibodies in their interactions with the target antigen. Binding constants are as low as 4.0 nmol/L for a small number of well-defined sites, and cross-reactivities are similar to or better than those observed with biological antibodies. In some cases, the polymers have been used to determine drug concentrations in human serum specimens.

INDEXING TERMS: immunoassays • polymers • antigen–antibody interaction • drug assays • resolving racemic mixtures

Antibodies are routinely utilized as analytical reagents in clinical and research laboratories. Perhaps their most common applications are in sandwich-type or competitive immunoassays[1-3] and immunoaffinity chromatography (both for purification[4] and analysis[5]). Interest is also increasing in their use in immunoarrays, in combination with some form of transducer that detects the binding of antigen and antibody directly[6]. These techniques have in common as a first step the binding of analyte to an antibody that, except in a few immunoassay configurations, is immobilized to some form of solid phase. The binding utilizes the exquisite recognition properties of an antibody for an antigen, such that the antigen fits exactly into the antibody binding site, whereas other, even structurally related, compounds are excluded from the site.

Organic chemists have pursued several approaches mimicking natural biological recognition in synthetic macromolecular structures[7]. The design and synthesis of systems capable of binding biomolecules such as nucleotides and peptides with similar affinity and specificity to natural ligands is regarded as one of the greatest challenges in bioorganic chemistry. One technique increasingly being adopted is molecular imprinting [8-10], in which a print molecule or template is allowed to form reversible covalent or noncovalent interactions with polymerizable monomers, which are then polymerized in the presence of a cross-linking monomer and porogenic solvent (Fig. 1). The result is a hard, macroporous polymer. Breaking the reversible interactions between the polymer and the template leaves behind recognition sites with shape and functional groups complementary to the imprint molecule. Using various different experimental approaches, investigators have shown that such synthetically produced recognition sites are capable of rebinding the imprint species in preference to other closely related structures.

Different monomers capable of different interactions with the imprint molecule, and various configurations, such as bulk polymers, beads, membranes, and polymer films, have been used in molecular imprinting. The best results so far, however, have

<table>
<thead>
<tr>
<th>Glossary</th>
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<tbody>
<tr>
<td>Artificial antibody</td>
<td>Synthetically prepared antibody-like entity.</td>
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<tr>
<td>Chiral mixture</td>
<td>Mixture comprising optically active compounds.</td>
</tr>
<tr>
<td>Enantioselectivity</td>
<td>Property of recognition sites capable of discriminating between enantiomeric species.</td>
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<tr>
<td>Epimeric selectivity</td>
<td>Property of recognition sites capable of selectively distinguishing between carbohydrate epimers.</td>
</tr>
<tr>
<td>Imprint species/antigen</td>
<td>Molecule used to produce recognition sites with the molecular imprinting technique.</td>
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<tr>
<td>Molecular imprinting</td>
<td>Preparation of recognition sites in macromolecular matrices by using an imprint antigen in a casting procedure.</td>
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<td>Molecularly imprinted sorbent assay</td>
<td>Radioisotopic assay using molecularly imprinted polymers as recognition elements.</td>
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<td>Polymer monolith</td>
<td>Massive polymer block.</td>
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<tr>
<td>Porogen</td>
<td>Solvent used to introduce porous structure in synthetic polymers.</td>
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been achieved with bulk methacrylate polymers, containing methacrylic acid, which can form noncovalent hydrogen bond or ionic interactions with the print molecule. Because these interactions are weakened by water, the polymerization is performed in a dry organic solvent. This approach is not suited to providing artificial antibodies towards proteins, given the requirement for nonaqueous conditions and the highly cross-linked nature of the polymer, but polymers have been prepared with impressive selectivities towards small molecules such as drugs (Fig. 2).

Molecularly imprinted polymers (MIPs) cannot compete in their present forms with natural antibodies for use in techniques in which they are used in their soluble form, e.g., in immuno-diffusion, immunoelectrophoresis, immunoblotting, and tissue immunofluorescence. However, for techniques such as immunoassay, immunoaffinity chromatography, and immunosensors, which utilize antibodies bound to a solid support, molecularly imprinted materials appear to offer a potential recognition element alternative to natural antibodies. We have used these artificial antibodies in competitive ligand-binding assays [11] and chromatography [12] and as recognition elements in biomimetic sensors [13]. Here, we describe their use primarily in the competitive ligand-binding application.

**Materials and Methods**

*Preparation of artificial antibodies.* In a typical preparation, the imprint antigen (1 mmol) is initially dissolved in the imprinting porogen (3:2 by vol, solvent:monomers) in a glass reaction vial together with methacrylic acid, in a ratio optimized for the chosen antigen (1–10 mmol). An amount of 50–500 μmol of antigen is required per gram of total monomer content when simple methacrylate monomers are used. Cross-linking monomer, e.g., ethylene glycol dimethacrylate (EDMA) or trimethylol propane trimethacrylate, is added at 70–90% of the total monomer content (molar ratio) to secure the outcome of a highly rigid polymer (i.e., 5–50 mmol if EDMA is used). Subsequently, initiating reagent, e.g., azobis-isobutyronitrile (0.2–2 mmol), is added; the resulting solution is carefully degassed by thorough purging or freeze–thawing with nitrogen; and the mixture is polymerized by thermolytic (45–60 °C) or photolytic (366 nm) initiation. After polymerization for 10–20 h, the resulting polymer monolith is subjected to a work-up protocol. Ordinarily, the polymer is crushed and ground repeatedly, with intermittent sieving with water to a desired particle size. For binding assays a particle size of 5–40 μm is suitable; smaller particles lead to poor particle separation from the assay samples. The particles are washed extensively (>500 mL/g) with a solution of ethanol:water:acetic acid (8:7:5 by vol) containing 1 mol/L ammonium acetate, and then with methanol, which

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1 Nonstandard abbreviations: MIP, molecularly imprinted polymer; EDMA, ethylene glycol dimethacrylate; and MIA, molecularly imprinted sorbent assay.
effectively extracts the imprint antigen from the polymer. The more extensive the washing, the more the best recognition sites become accessible. After final drying by desiccation under reduced pressure, the polymers are ready for analytical use. Given the ease of preparation, the reproducibility of the polymers prepared is fairly good. Any performance deviation these polymers exhibit is more a consequence of the assay conditions used than the actual preparation.

**Saturation analysis.** To estimate the binding capacity of the artificial antibodies in a given assay, we perform saturation studies with the antibodies in the assay solvent. In a typical experiment, 1 μg to 20 mg of polymer is incubated with radiolabeled antigen (the least amount possible for detection) in ordinary polypropylene microcentrifuge tubes and allowed to reach equilibrium in the assay solvent (>6 h). After incubation, the samples are centrifuged, the resulting supernatants are added to scintillation liquid, and the samples' radioactivity is counted to estimate the amount of ligand not bound to the polymer. For further assays, the amount of polymer capable of binding ~50% of radiolabeled antigen is adequate for optimal sensitivity in the protocol.

**Ligand competition analysis.** Competition assays essentially follow the same simple strategy. MIP samples in an amount estimated from the saturation studies are incubated with nonradiolabeled analytes, typically at 1 nmol/L to 1 mmol/L, and radiolabeled antigen. After sufficient incubation (>6 h), the samples are centrifuged, aliquots of the supernatants are mixed with scintillation liquid, and the radioactivity is measured. From the values obtained, the binding strength (K_D), the site population density (B_max), and the cross-reactivities may be estimated.

**Calculations of binding characteristics.** Estimations of the binding performances (i.e., K_D and B_max) and the selectivities (i.e., the ability to distinguish between different ligands) of the artificial antibodies are carried out according to routine immunoassay theory. A mathematical model may be used to calculate the cross-reactivities (relative IC_{50}-values, the concentrations of the individual compounds that displace 50% of the bound radiolabel) of artificial antibodies [14]. The K_D values are easily determined by simple Scatchard-type analysis [15]. The polyclonal nature of the artificial antibodies obtained by the imprinting protocol results in nonlinear Scatchard-plots, but a two- or three-site model is usually adequate for a clear estimation of the binding characteristics of the antibodies.

**Results and Discussion**

It is important to consider the nature of the recognition sites in MIPs and the structures of the actual materials. The polymers we have studied are rigid solids ground into small particles (typically 5–25 μm in diameter). These particles are readily suspended in aqueous or organic media and can be separated from solution by simple centrifugation. Each particle is penetrated by a network of pores, the number and size of which depend on the exact conditions used in the manufacture of the materials [16]. The polymer core is highly cross-linked, allowing very limited flexibility in the polymer structure. However, this also gives rise to the great robustness of the materials and their ability to retain their recognition properties even under harsh conditions. The recognition sites may be at the liquid–solid interface in the pores, in which case the binding of the print molecule will occur rapidly, or may be buried within the solid structure, in which case the print molecule will take much longer to diffuse through the rather inflexible polymeric chains. Importantly, the exact arrangement of the polymer chains and functional groups around each recognition site differs. This means that a range of recognition sites are present, recognizing differing features of the print molecule, such that some are highly selective for the print molecule and some less so—a situation akin to that in a sample of polyclonal antibody. The actual interactions contributing to the selectivity of the recognition sites, mainly hydrogen bonding and ionic interactions, are much stronger in organic solvents than in aqueous media. Hence, the recognition specificities observed are higher when these artificial antibodies are used in nonaqueous media. Much research effort continues to be invested in improving the properties of imprinted polymers. These efforts are directed particularly at increasing the number of highly selective recognition sites while reducing the number of less-specific sites, and at improving the recognition properties of the polymers when used in aqueous media. Table 1 summarizes the physical characteristics of the polymer particles.

The wide variety of techniques developed for determination of analytes by immunoassay includes various configurations of RIA [2] and ELISA [3]. Historically, the most decisive step in the development of these techniques was the immobilization of antibody to a solid phase, which simplified enormously the separation of bound antigen–antibody complexes. The first reported solid-phase immunoassay, developed to detect human growth hormone, was based on the competition of unlabeled analyte and radiolabeled antigen for binding to polyclonal antibody immobilized on a polymer [17]; after an incubation period, the polymer was removed, and the radioactivity remaining in the supernatant was recorded. The radioactivity bound to the polymer was inversely correlated to the amount of unlabeled analyte present.

The molecularly imprinted sorbent assay (MIA) is precisely analogous to this early RIA, except that the polymer-bound

<table>
<thead>
<tr>
<th>Table 1. Characteristics of molecularly Imprinted polymers.</th>
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<tr>
<td><strong>Feature</strong></td>
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<tr>
<td>Physical stability</td>
</tr>
<tr>
<td>Chemical stability</td>
</tr>
<tr>
<td>Storage endurance</td>
</tr>
<tr>
<td>Imprint memory</td>
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<tr>
<td>Recovery yield</td>
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* Recovery of imprint species after polymerization by washing.
Table 2. Binding characteristics for artificial antibodies (data fitted to two- or three-site models depending on the degree of "polyclonality" of the binding sites).

<table>
<thead>
<tr>
<th>Print molecule</th>
<th>Imprinting porogen</th>
<th>Assay solvent</th>
<th>High-affinity sites</th>
<th>Low-affinity sites</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline</td>
<td>CHCl₃</td>
<td>MeCN/HOAc</td>
<td>Kᵦ, µmol/L 350</td>
<td>Bₘₐₓ, µmol/g 0.016</td>
<td>65</td>
</tr>
<tr>
<td>Diazepam*</td>
<td>CHCl₃</td>
<td>Toluene/heptane</td>
<td>18</td>
<td>0.006</td>
<td>60</td>
</tr>
<tr>
<td>Morphine</td>
<td>MeCN</td>
<td>Toluene/HOAc</td>
<td>92</td>
<td>1.2</td>
<td>8.9</td>
</tr>
<tr>
<td>Leu-enkephalin anilideᵇ</td>
<td>MeCN</td>
<td>MeCN/HOAc</td>
<td>130</td>
<td>0.017</td>
<td>43</td>
</tr>
<tr>
<td>Cortisol</td>
<td>THF</td>
<td>THF/heptane/HOAc</td>
<td>570</td>
<td>0.21</td>
<td>1590</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>THF</td>
<td>THF/heptane/HOAc</td>
<td>1230</td>
<td>0.37</td>
<td>840</td>
</tr>
<tr>
<td>S-Propranolol</td>
<td>Toluene</td>
<td>Toluene/HOAc</td>
<td>40</td>
<td>2.0</td>
<td>23</td>
</tr>
<tr>
<td>Atrazine</td>
<td>CH₃Cl₂</td>
<td>Toluene/MeCN</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Methyl-α-D-glucoside</td>
<td>CHCl₃</td>
<td>MeCN/HOAc</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Yohimbineᵈ</td>
<td>CHCl₃</td>
<td>MeCN/HOAc</td>
<td>60</td>
<td>0.12</td>
<td>4.8</td>
</tr>
</tbody>
</table>

|                  |                    | Aqueous buffer           | 120                 | 0.06               | 62   |      |

MeCN, acetonitrile; HOAc, acetic acid; THF, tetrahydrofuran; Kᵦ, apparent dissociation constant; Bₘₐₓ, site population density; n.d., not determined.

* Three-site model used with experimental data: Kᵦ = 2.3 µmol/L was estimated for medium-affinity sites corresponding to Bₘₐₓ = 0.17 µmol/g.

ᵇ Leu-enkephalin (underivatized) was used as analyte.

ᶜ Three-site model: Kᵦ = 12 µmol/L and Bₘₐₓ = 0.72 µmol/g for medium-affinity sites.


The antibody is replaced by a MIP. The physical nature of the polymer particles makes them better for use in competitive than noncompetitive assays. The latter are often more sensitive when used with natural antibodies [18] but have the disadvantage of requiring two separate epitopes or recognition sites on the antigen and so are less suitable for small analytes. Thus, in the determination of small molecules such as drugs, which can be readily imprinted but are not suited to noncompetitive immunoassay, MIA may present a useful alternative.

MIA may be used for three different purposes, depending on which species are allowed to compete with the radiolabeled print molecule or antigen for binding to the polymer. First, using known concentrations of different nonradioabeled analytes (usually analogs of the print molecule), we can determine the IC₅₀ values. Being related to the cross-reactivities of the different compounds, these values tell us about the specificities of the polymer recognition sites. Second, using known concentrations of nonradioabeled print molecule, followed by Scatchard analysis, we can obtain information about the binding constants for the recognition sites in the polymer and about the number of recognition sites available. Finally, we can obtain results for unknown solutions by comparison with a calibration curve obtained with known concentrations of the print molecule and get a measure of the concentration of the print molecule present.

Table 2 lists the compounds that have so far been molecularly imprinted and analyzed by MIA. The first examples were the bronchodilator theophylline and tranquilizer diazepam. These drugs were imprinted and the polymers used for measurement of their concentrations in blood samples by radioligand-binding assays [11]. The drugs were first extracted into organic solvents (dichloromethane:isopropanol, 4:1 by vol) and then allowed to compete with radioactivity labeled drug for the recognition sites in the polymer. Calibration curves were prepared by measuring samples to which known drug concentrations had been added, and these samples were measured by using these curves. Detection limits of the assays (defined as the concentration of ligand required to displace 10% of the bound radiolig) were 3.5 µmol/L for theophylline and 0.2 µmol/L for diazepam. Moreover, the results showed excellent correlation with those obtained by Emit® [19] (Fig. 3).

We also tested various related compounds in the MIA and determined their IC₅₀ values. The cross-reactivities were com-

![Fig. 3. Comparison of MIA and Emit in competitive binding assays for determining serum concentration of theophylline in patients' samples (n = 32).](image-url)
comparable with the cross-reactivities of these compounds with natural antibodies (Table 3).

On the basis of this early work, two criticisms might be made of MIAs as a potential tool for actual clinical analyses. First, the requirement that the binding competition take place in nonaqueous media necessitates an extraction step. However, this is not a great problem because some antibody immunoassay techniques already routinely require a clean-up step such as solvent extraction. Second, because the assays require a large amount of polymer (12.5 mg per assay for theophylline), a lot of print molecule (3.2 μmol) is used in the polymer preparation, whereas only a small amount of antigen is present in each assay (up to 9.0 nmol). Hence, the competition involves only a small fraction of the available recognition sites.

Subsequently, both of these issues were addressed in work with polymers imprinted with morphine and the endogenous neuropeptide [Leu²]enkephalin [20]. These compounds are both active at opioid receptors, and antibodies to morphine and [Leu²]enkephalin have been used in studies of ligand–receptor interactions [21, 22]. Radioligand-binding assays were developed in aqueous media and used smaller quantities of imprinted polymer (1 mg for the morphine MIP, 5 mg for [Leu²]enkephalin). Importantly, the cross-reactivities of other alkaloids binding to the anti-morphine polymer, as determined by IC₅₀ values, were similar in aqueous media and in organic solvent and were also similar to those for binding to natural antibodies. For the anti-[Leu²]enkephalin polymer, the cross-reactivities of other enkephalin derivatives in organic solvent were very low; however, they increased in aqueous media.

Recently, MIPs have been prepared with cortisol and corticosterone as target molecules [23]. The corticosteroids are of clinical interest for (e.g.) assessing the functional status of the adrenal cortex and are routinely analyzed by methods such as RIA and ELISA [24]. In addition, these compounds are interesting targets for molecular imprinting because the rigid structure of the fused ring-system means that the number of conformations the molecules may adopt in their interactions with recognition matrices is small, resulting in a low loss of entropy on binding and hence a higher binding strength [25]. However, noncovalent molecular imprinting is highly dependent on polar interactions, and the number of points capable of such interactions in steroids is small. We estimated the binding characteristics for a range of structurally related ligands by MIA analyses in organic solvents. The prepared antibody mimics were highly selective for the ligands that had been used in their preparation, and the cross-reactivities with related structures resembled those obtained in studies with natural antibodies.

Scatchard analyses to estimate the strength of binding of the imprint molecule clearly showed (Fig. 4) that the result of the MIA is a nonlinear Scatchard plot. This result is typical of MIA using imprinted polymers and reflects the heterogeneity of the recognition sites present. To estimate the binding strengths and site populations a two-site model is commonly adopted. For cortisol binding to the anti-cortisol polymer (Fig. 4), the model gives K_D values of 5.7 × 10⁻⁷ mol/L and 1.6 × 10⁻³ mol/L associated with B_max of 0.21 and 280 μmol/g, respectively. As shown in Table 3, as MIAs have been developed for theophylline, diazepam, morphine, enkephalin, cortisol, and corticosterone, the numbers of binding sites involved in the binding of the radiolabeled analyte have increased. This demonstrates an improvement in the recognition properties of the polymers and is also important for practical applications in that less polymer is needed per assay. To further increase the sensitivity of MIA analyses and decrease the quantities of polymer used, it is desirable to increase further the proportion of strong binding sites in the polymer.
Andersson reported a thorough study of the preparation of (S)-propranolol-imprinted polymers and their use in radioligand-binding assays [26]. This enantiomeric β-blocker was marketed originally as a racemate, but the in vitro antagonist action of the (S)-isomer is ∼100-fold more potent than that of the (R)-isomer [27]. Molecular imprinting is particularly effective for resolving chiral mixtures [28]. Such separations also provide the best evidence for the integrity of the imprinting process, because any enantiomer-resolving properties cannot be related to the physical characteristics of the polymers but can result only from the imprinting process. A comparison was made of different cross-linker systems and methods of polymerization initiation. The most promising polymer, prepared with methacrylic acid as the functional monomer and EDMA as the cross-linker in toluene, was used to optimize the composition of the aqueous phase for recognition. MIA with radiolabeled (S)-propranolol resulted in a detection limit in organic or aqueous systems of ∼6 nmol/L. Comparison of IC50 values showed that (R)-propranolol cross-reacted by only 1.5% in organic solvent and 17% in aqueous medium.

Besides drugs, other compounds have been imprinted and the resulting artificial antibodies used in MIAs. A prime example is the pesticide atrazine, which has been studied by several research groups [e.g., 29-31]. For the polymers prepared in our laboratory, comparison of IC50 values showed extremely impressive selectivity. For instance, propazine, which differs from atrazine only in possessing an extra methyl group, cross-reacts with atrazine by only 18%, whereas its cross-reactivity with anti-atrazine monoclonal and polyclonal antibodies is >100%. This apparent difference is probably a consequence of the fact that biological antibodies have to be raised against derivatives conjugated to carrier proteins (usually, a caproic acid linkage when immunizing a host against atrazine). If this linkage is situated at the point where atrazine differs from propazine, biological antibodies may exhibit poor selectivity between the compounds.

Sugar derivatives have also been studied. Sugars have previously been studied extensively with covalently imprinted polymers, the polymers having been used successfully in chromatographic separations. The first report of the imprinting of sugar derivatives included a MIA analysis of the recognition properties of the resulting polymers [32]. A polymer imprinted with octyl-α-D-glucoside bound methyl-α-D-glucoside with impressive epimeric selectivity, the IC50 for methyl-α-D-glucoside being two orders of magnitude lower than that for methyl-α-D-galactoside (which differs only by the orientation of one hydroxyl group). Analyses in this case were performed in organic solvent because of the poor solubility of the analytes in aqueous media. The use of a template for the imprinting procedure that differs from the antigen used in the analysis reflects the better solubility of the octyl-analog. Use of an organic-soluble template may provide a general strategy for the preparation of artificial antibodies to compounds that are soluble only in water.

Recently, the Rawolfia alkaloids yohimbine and corynanthine (α2-adrenoceptor antagonists) have been imprinted, and the resulting adrenoceptor mimics have been analyzed by MIA (Berglund J, Nicholls IA, Lindbladh C, Mosbach K; unpublished results). The two structures differ only by the configuration at the carboxyl-bearing stereogenic center, yet the polymers developed exhibit impressive selectivity. The binding of yohimbine to anti-yohimbine polymer in aqueous phase gave KD values of 120 nmol/L and 62 µmol/L associated with Bmax = 60 nmol/g and 11 µmol/g, respectively. The IC50 for yohimbine was three orders of magnitude lower than that for corynanthine in organic phase, the difference being reduced to two orders of magnitude in aqueous phase.

Conclusions

Detection and analysis of pharmaceuticals provides one of the most interesting areas in which applications of molecularly imprinted materials can be foreseen. All examples of the imprinting of drugs reported to date have involved noncovalently imprinted bulk polymers. The first was the imprinting of the β-adrenergic blocker (S)-(−)-timolol, and the polymer was used for chromatographic separation of the print molecule from its enantiomer and other closely related structures [33]. MIP particles are well suited to packing into chromatography columns for use in HPLC separations. More recently, (S)-naproxen (a nonsteroidal anti-inflammatory drug) has been imprinted and the polymer used in HPLC to separate the print molecule from its enantiomer and from the related compounds ibuprofen and ketoprofen [34].

The results summarized here demonstrate that the interactions of drugs and small compounds with these artificial antibodies are similar to their interactions with biological receptors and antibodies. Recognition sites with dissociation constants in the nanomolar range can be prepared. Closely related compounds exhibit various degrees of cross-reactivity with the imprinted antigen, but these cross-reactivities are usually of similar extent to those exhibited by the natural receptors or antibodies. For theophylline and diazepam, the concentrations of the drugs in real biological samples may be determined by MIA with MIPs by comparison with calibration curves. MIPs specific for other compounds have been studied with the particular aim of improving the recognition and extending the use of MIA to aqueous conditions.

The advantages of MIPs over biological antibodies include their enormously impressive stability. The polymers can be stored at room temperature for years, or treated with acidic or alkaline solutions without any decrease in their recognition properties. Compared with the preparation of an antibody sample, the manufacture of a polymer imprinted with a small molecule such as a drug is very simple and time-efficient. Raising natural antibodies against these compounds sometimes is not easy and may require chemical coupling to a carrier protein for the antibody to be immunogenic. Molecular imprinting is also useful for creating artificial antibodies to immunosuppressant compounds (Senholdt M, Andersson LI; unpublished results) and should be compatible with toxins. In addition, molecular imprinting avoids the use of animals. In these respects MIA should provide a useful complement to traditional immunoassay techniques involving biological antibodies.

The recognition sites in MIPs are, by the nature of their preparation, heterogeneous, with only the most specific and
strongest binding sites being utilized in MIAs. Current research is
directed at reducing the "polyclonality" of the binding sites so
that a higher proportion of the sites may be used. Efforts are also
being made to make the preparation of artificial antibodies less
material- and time-consuming, e.g., by preparing polymer par-
ticles by suspension polymerization—a technique with the
added advantage of yielding more-uniform particles (Mayes AG,
Mosbach K; unpublished results). The major limitation of the
technology used in the examples shown here is that the im-
printed polymers are always formed in organic solvents. For this
and other technical reasons, the preparation of artificial anti-
bodies specific for proteins is more difficult. A major focus of
our current work is on the extension of these techniques to
aqueous media and macromolecules.

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