Review

Advances in the manufacture of MIP nanoparticles

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Molecularly imprinted polymers (MIPs) are prepared by creating a three-dimensional polymeric matrix around a template molecule. After the matrix is removed, complementary cavities with respect to shape and functional groups remain. MIPs have been produced for applications in *in vitro* diagnostics, therapeutics and separations. However, this promising technology still lacks widespread application because of issues related to large-scale production and optimization of the synthesis. Recent developments in the area of MIP nanoparticles might offer solutions to several problems associated with performance and application. This review discusses various approaches used in the preparation of MIP nanoparticles, focusing in particular on the issues associated with large-scale manufacture and implications for the performance of synthesized nanomaterials.

Introduction

The *in vitro* diagnostics market is large ($36 billion USD) and is expanding rapidly, with annual growth of approximately 9% [1,2]. It relies in significant part on the use of antibodies, which are highly specific for various chemical and biological moieties and can be produced on a large scale [3]. Unfortunately, they suffer from relatively poor stability, short shelf-life, high costs and problems with immobilization [2,4–6]. Furthermore, their production against small molecules requires chemical coupling to haptens [3]. Finally, it is difficult to generate antibodies against molecules such as immunosuppressants or toxins because of their adverse effects on the immune response [7]. There is potential to replace natural antibodies with synthetic analogues, and molecular imprinted polymers (MIPs) have shown considerable promise among these (Figure 1) [8,9].

In contrast to biomolecules, MIPs are stable at low and high pH, pressure and temperature (<180 °C). They are less expensive than antibodies, are easier to obtain and can be synthesized for a wide range of substances [10–14]. In addition, they can be used in both organic and aqueous solvents, although imprinting in aqueous solutions is not straightforward. However, they still suffer from the lack of a standard manufacturing procedure [15]. MIPs are usually prepared as bulk monoliths, and are then ground and sieved to obtain irregularly shaped particles of adequate size (typically 5–50 μm) for various applications. Unfortunately, this procedure is time-consuming and causes loss of material. Imprinted sites are heterogeneous and conspicuous amounts of template are required to create high-affinity interactions. Furthermore, this method is unsuitable for industrial application because of the poor heat dispersal. Polymerization reactions are exothermic; thus, for large volumes an increase in temperature can cause solvents to boil and start explosions owing to the high pressure [8,16,17]. The attention of researchers has thus shifted to obtaining regularly shaped imprinted polymers, especially on the nanoscale [18], because of their better properties.

MIP nanoparticles: a revolutionary format

In contrast to bulk monoliths, MIP nanoparticles have higher surface area-to-volume ratios; thus, imprinted cavities are more easily accessible by the templates and the binding kinetics are improved [19,20]. This format fits better with surface imprinting strategies [21], facilitating the design of *in vitro* assays with enzyme-conjugated probes, which are usually too bulky to fit into recognition cavities. In addition, because MIP nanoparticles easily remain in solution, it is simpler to dose them precisely for use in assays [22,23]. MIP nanoparticles have already been used as enzyme substitutes [24,25], drug delivery systems [13,26] and antibody substitutes [2,3,22,27], as well as in capillary electrophoresis [28–31] and in sensors [32–34].

Unfortunately, fabrication of MIP nanoparticles is not easy. Manufacturing aspects such as the degree of cross-linking and requirements for strong template–monomer interactions can narrow the choice of protocols suitable for MIP nanoparticle production [35]. The most popular synthetic strategies include precipitation polymerization, mini- and micro-emulsion polymerization, core–shell approaches (with subsequent grafting) and living radical polymerization processes, such as atom transfer radical polymerization (ATRP) and reversible addition–fragmentation chain transfer polymerization (RAFT). Each of these procedures has its own set of pros and cons, which are discussed in this review and highlighted in Table 1 for MIP nanoparticles and Table 2 for MIP micro- and nanogels. Finally, we consider MIP micro- and nanogels as a new and advantageous technology for creating artificial antibodies and enzyme mimics.

Precipitation polymerization

The precipitation polymerization approach for obtaining MIP nanoparticles was first described in 1999 [36], whereby monodisperse particles were imprinted for 17β-estradiol and theophylline (Figure 2). Precipitation polymerization involves the formation of imprinted nanoparticles in an excess of solvent (monomer concentration 2% v/v). Growing polymer chains do not coagulate, but continue to capture

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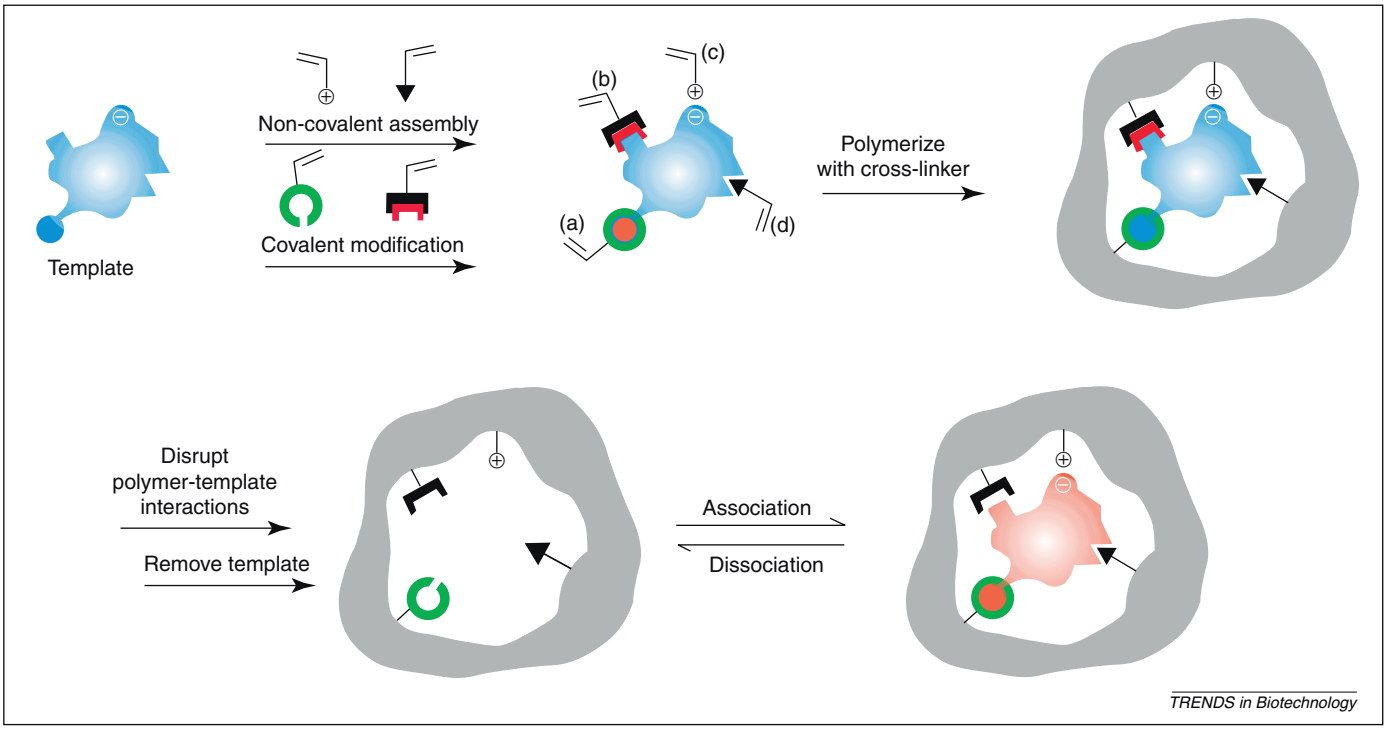

Figure 1. The molecular imprinting process. Reversible interactions between the template (blue) and the polymerizable functional monomer can involve one or more of the following mechanisms: (**a**) reversible covalent bonds; (**b**) covalently attached polymerizable binding groups that are activated for non-covalent interaction by template cleavage; (**c**) electrostatic interactions; and (**d**) hydrophobic or van der Waals interactions. Each of these is established with complementary functional groups or structural elements of the template. Subsequent polymerization in the presence of a cross-linker results in the formation of a porous matrix in which the template sites are located. The template is then removed through disruption of interactions a–d that exist between the template and the polymer, and is subsequently extracted from the matrix. The target analyte (blue) or its analogue (red) can selectively rebind to the polymer into the sites formed by the template, or the imprints. Adapted with permission from [9].

oligomers and monomers from solution, precipitating only when their size makes them insoluble in the reaction medium. Furthermore, the technique is easy, less time-consuming than other procedures, and provides good yields (Table 1).

Throughout the last decade, precipitation polymerization parameters have been investigated for the production of MIP nanoparticles. Optimizing the level of crosslinking and using reduced template concentrations have improved the polymer binding characteristics and reduced the level of non-specific interactions [37]. Increasing either the monomer concentration or the reaction temperature has resulted in larger nanoparticles, but has also interfered with particle size uniformity [38]. An increase in the amount of initiator similarly affected monodispersity [38].

The shape of the reactor also influences the size of MIP nanoparticles, probably because it affects parameters such as the radial diffusion or local concentration of reactants [39]. To accelerate nanoparticle manufacture, a modified protocol for precipitation polymerization that involves a distillation step was applied [40]. However, the high temperatures involved in the process resulted in reductions in the binding affinity and selectivity of the synthesized materials.

The type of crosslinker strongly affects both the final size and yield of MIP nanoparticles [27]. According to this study, when divinylbenzene was used as the crosslinker, polydisperse MIP particles were obtained in low yield. Conversely, trimethylolpropane trimethacrylate (TRIM) led to uniform nanoparticles in high yield (90%). Furthermore, the presence of template (*R*,*S*)-propranolol had a strong influence on the size and uniformity of TRIM nanoparticles, most likely because of the interactions established with the functional monomer, methacrylic acid. However, all the MIP particles obtained showed high template rebinding and low cross-reactivity (<5%), 6–7-fold lower than that obtained for irregular bulk MIP particles.

Applications of MIP nanoparticles

MIP nanoparticles synthesized by precipitation polymerization have been used in several different applications. For sensing purposes, MIP nanoparticles were integrated with a UV fluorescent scintillation monomer [41]. The binding signal was generated by proximity energy transfer arising from the specific binding of ^{3}H-(*S*)-propranolol.

Recently, MIP nanoparticles imprinted with the peptide melittin were prepared using a small combinatorial library of different functional monomers [42]. Only nanoparticles that contained suitable amounts of *N-tert*-butylacrylamide and acrylic acid exhibited high affinity for the peptide; the K_d was 25 pM, comparable to that of natural antibodies for melittin (17 pM). Nanoparticles were obtained in good yield (80–90%) and with uniform diameter. The authors then tested the ability of the particles to bind melittin *in vivo* [43]. In mice treated with the toxin, MIPs reduced mortality by approximately 50%, as well as common melittin toxic effects (e.g. peritoneal phlogosis and weight loss). The next step would be to test anti-melittin antibodies

Table 1. Polymerization approaches used for the preparation of MIP nanoparticles.

Production process	Templates	Comments	Refs.
Precipitation polymerization	17β-Estradiol, theophylline, caffeine	First example of precipitation polymerization applied to MIP nanoparticles.	[36,37]
	(*S*)-Propranolol	Use of a UV fluorescent scintillation monomer for sensing purposes.	[41]
	L-2-Chloromandelic acid	Temperature, initiator amount and polymerization mixture concentration strongly influence the nanoparticle size.	[38]
	Di(2-ethylhexyl)phthalate	The shape of the reactor influences the size of nanoparticles.	[39]
	(*S*)-Propranolol	Addition of a distillation step to the protocol and reduction of the reaction time. High temperatures disrupted the imprinting effect.	[40]
	(*S*)-Propranolol	Template amount and type and crosslinker amount strongly influence the size of nanoparticles.	[27]
	Melittin	Use of a combinatorial library synthetic approach and *in vivo* application of the MIP nanoparticles.	[42,43]
	Human rhinovirus 14	Application of MIP nanoparticles as stencils for sensing layers in chemosensors.	[34]
	5-Fluorouracil	Production of nanosized drug delivery systems with sustained release of 5-FU.	[13]
Mini-emulsion polymerization	L,D-Boc-phenylalanine anilid	First example of mini-emulsion polymerization applied to MIP nanoparticles.	[44]
	(*S*)-Propranolol	Use of a surfactant monomer to achieve surface imprinting; faster rebinding kinetics, which is useful in capillary electrochromatography.	[31]
	Glucopyranoside	Use of a surfactant monomer to achieve surface imprinting in a semi-covalent approach; issues with template removal.	[46]
Micro-emulsion polymerization	GFP-9	Use of a surfactant template for surface imprinting; influence of the carbon chain length on the imprinting effect.	[12]
Core–shell emulsion polymerization	Cholesterol	First example of core–shell emulsion polymerization applied to MIP nanoparticles using a sacrificial spacer; use of magnetic cores.	[48]
	Cholesterol	Use of a polymerizable surfactant and a polymerizable template to achieve surface imprinting.	[49]
	Propranolol	Non-covalent imprinting; porogen influence on the capacity of MIP nanoparticles; fluorescent properties.	[52]
Core–shell grafting approach	Propranolol, naproxen, morphine	MIP grafting using an immobilized iniferter; production of multilayer MIP nanoparticles.	[53]
	2,4,6-Trinitrotoluene	MIP grafting on silica cores; high density of imprinted sites (strong template–core interaction).	[20]
	Bovine haemoglobin	APBA layer for protein imprinting on magnetic cores; fast rebinding kinetics and rapid recovery.	[57]
	Human haemoglobin	Self-polymerization of dopamine in alkaline medium (PDA layer) for protein imprinting on magnetic cores.	[58]
	Estrone	Semi-covalent imprinting of a silica layer on magnetic cores; long production process.	[60]
	2,4-Dichlorophenoxyacetic acid	Surface grafting of MIP layer on magnetic cores via ATRP polymerization.	[61]
	Bisphenol A	Surface grafting of MIP layer on magnetic cores via RAFT polymerization.	[62]

Table 2. Polymerization approaches used for preparation of MIP micro- and nanogels.

Production process	Templates	Comments	Refs.
Extensive grinding and ultrafiltration of monolith	Thylakoid membrane D1 protein	First example of water-soluble MIP nanoparticles with biological activity; low yields.	[68]
High-dilution polymerization	Trypsin	Use of an anchoring monomer to enhance the imprinting effect.	[69]
	TSA of the cross-aldol reaction between 4-nitrobenzaldehyde and acetone	First example of MIP nanogels able to catalyze C–C bond formation.	[25]
Post-dilution method	Diphenyl phosphate TSA	Thermal polymerization produces high-affinity product with an average of one active site per particle; high yields.	[24]
Early termination of iniferter-mediated polymerization	Acetoguanamine	MIP nanogels with high affinity; low yields.	[70]

in vivo under the same conditions to compare the efficacy of these MIP nanoparticles with respect to their natural counterparts.

MIP nanoparticles for human rhinovirus immunoglobulins were recently prepared [34]. These were deposited on microscope slides and used as a template to imprint a polymeric layer on a quartz crystal microbalance. The resulting chemosensor was sixfold more sensitive than the corresponding sensor coated with natural antibodies. This represents a good example of using MIP nanoparticles not directly as sensing elements, but instead as stencils to imprint another polymeric structure.

Precipitation polymerization has also been employed to prepare MIP nanoparticles suitable for drug delivery

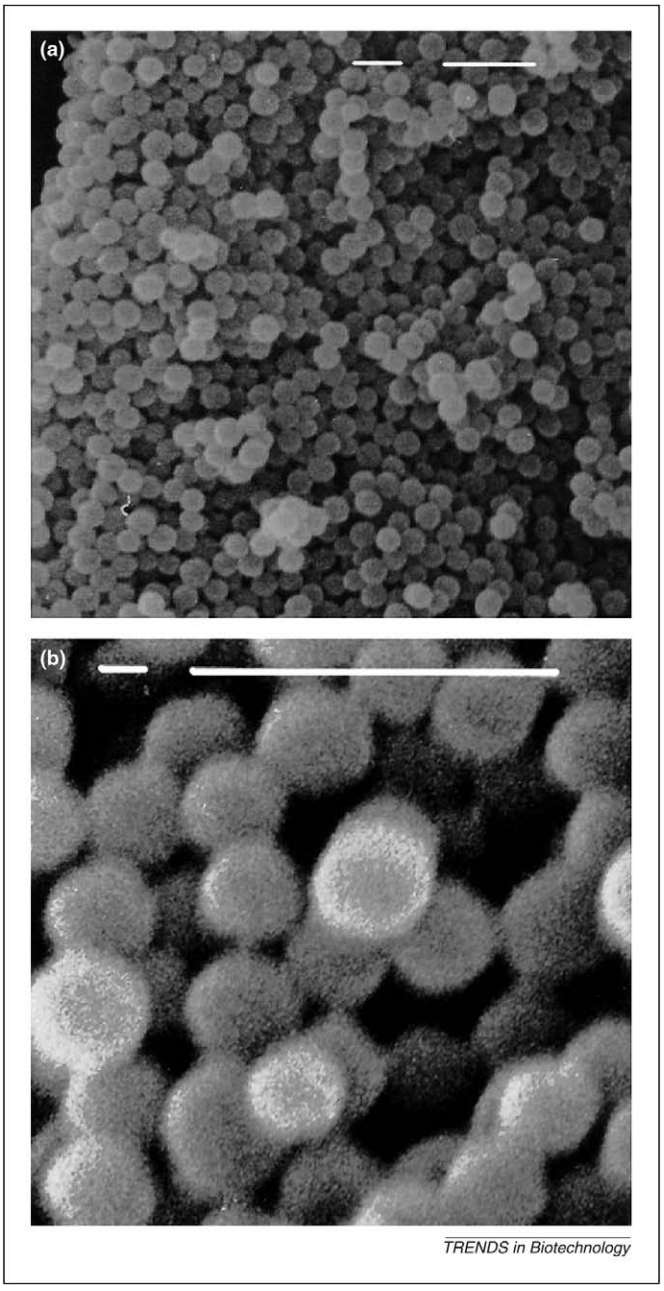

Figure 2. SEM images of MIP nanoparticles prepared by precipitation polymerization. The particles were imprinted for 17β-estradiol for use in radioligand binding assays. (**a**) 7500× magnification. (**b**) 30 000× magnification. Scale bar = 1 μm. Reproduced with permission from Ref. [36].

of 5-fluorouracil (5-FU) [13]. The nanoparticles obtained were 274 nm in diameter, with low polydispersity. They exhibited good affinity properties in both acetonitrile and water, as well as low cross-reactivity for a template analogue, uracil. Finally, they showed a sustained release of 5-FU over 50 h *in vitro*, while non-imprinted polymers completed the release after only 5 h under the same conditions.

Precipitation polymerization is a straightforward approach for obtaining MIP nanoparticles (Table 1) and is suitable for imprinting different types of substances, including peptides, because it does not use surfactants. However, the requirement for high dilutions has a negative impact on the strength of template–monomer interactions. Moreover, in this technique the composition of the imprinting mixture must be matched to the operating conditions of the system (e.g. type of initiation, temperature, reactor shape) to better control the size, shape and imprinting properties of the nanoparticles. Combinatorial libraries or even computational techniques might be helpful in this respect. However, owing to the large impact of various physical and chemical parameters, precipitation polymerization would be difficult to automate and apply as a generic approach for the synthesis of MIP nanoparticles.

Mini- and micro-emulsion polymerization

A method used to obtain MIP nanoparticles in high yield is mini-emulsion polymerization (Table 1) [44], which involves a high-shear homogenization step and use of a co-surfactant to obtain particles of 50–500 nm [45]. A polymeric surfactant monomer can be used to confine imprinting to the surface of synthesized MIP nanoparticles, although this might produce material with inferior affinity compared with microparticles prepared by precipitation polymerization [31]. Nevertheless, synthesized polydisperse particles efficiently resolved a racemic mixture of propranolol.

Recently, the same approach was adopted to synthesize semi-covalent MIP nanoparticles imprinted with glucopyranoside [46]. In semi-covalent imprinting the template is covalently attached to the monomer during the polymerization process, whereas the rebinding step depends only on non-covalent interactions. The synthesized nanoparticles showed good rebinding capacity compared to non-imprinted ones, as well as good selectivity for glucopyranoside versus galactopyranoside (separation factor (α)=6.5). However, complete extraction of the template from the nanospheres was not possible.

Inverse micro-emulsion polymerization was recently used to obtaining 28-nm spherical MIP nanoparticles imprinted with a small hydrophilic peptide, GFP-9, coupled to fatty acid chains of different length (C_5, C_{13} and C_{15}) [12]. Only nanoparticles imprinted with the peptides coupled to C_{13} and C_{15} exhibited specificity and affinity properties. It is likely that the C_5 chain was too short and too hydrophilic to correctly act as a surfactant template, and therefore did not confine the template to the surface of the particles.

Even if mini-emulsion polymerization can produce very small (30–220 nm) spherical nanoparticles, the presence of several chemicals (e.g. surfactants and co-stabilizers) might interfere with the imprinting process, thus broadening the distribution of affinity sites. Semi-covalent imprinting approaches might be helpful, but this also depends on the chemical nature of the template. Moreover, the purification steps required to remove all these chemicals can be long and tedious.

Core–shell approaches

Core–shell approaches – namely, core–shell emulsion polymerization and grafting – have been used to synthesize MIP nanoparticles with complex architectures and controlled sizes specific for target molecules of all sizes (Table 1). These approaches involve deposition of an MIP layer on preformed support nanospheres composed of various materials, such as silica, polymers and magnetite (Fe_3O_4). In this way it is also possible to use cores with specific

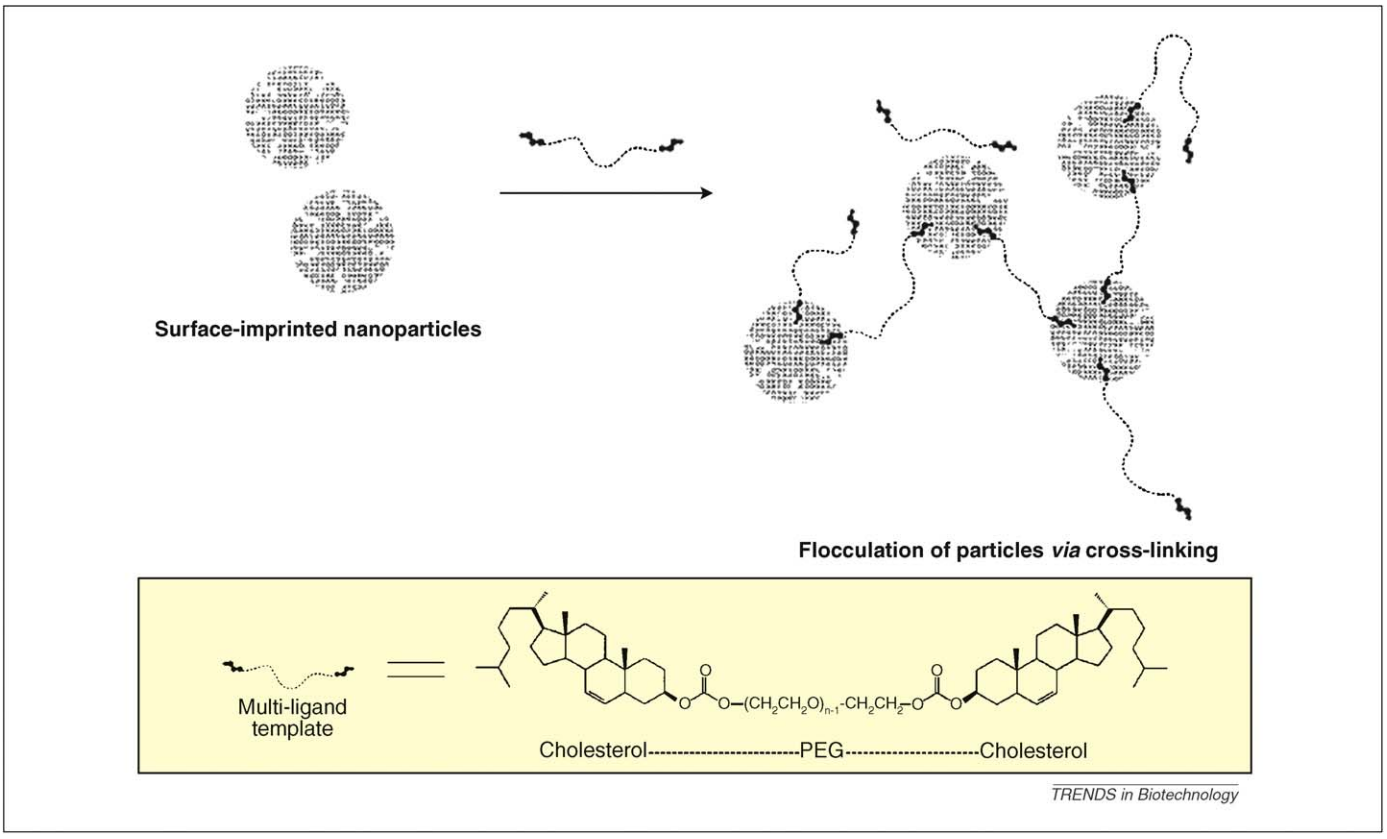

Figure 3. Immunoprecipitation-like separation of surface-imprinted particles in the presence of PEG-bis-cholesterol. Addition of the multi-ligand template resulted in flocculation of MIP particles. Adapted with permission from [49].

properties, which might improve the whole performance of the imprinted nanosystem [21,47].

Emulsion polymerization

The most direct technique for producing core–shell MIP nanoparticles is core–shell emulsion polymerization [48], which is a two-stage process: production of a monodisperse seed latex (0.03–1 μm) and creation of an imprinted shell using emulsion polymerization. Core–shell particles were imprinted for cholesterol using sacrificial spacers and various monomers and crosslinkers [48]. All of the imprinted nanoparticles were small (50–100 nm) and had a high surface area (80–120 m^2/g). Magnetic cores were also investigated, leading to superparamagnetic core–shell MIP nanoparticles (74 nm) that were rapidly recoverable and able to efficiently rebind the analyte. To create binding sites preferentially on the MIP nanoparticle surface [49], the same authors slightly modified this approach, using a polymerizable surfactant, pyridinium 12-(cholesteryloxycarbonyloxy)dodecanesulfate (PyS), and a template surfactant, pyridinium 12-(cholesteryloxycarbonyloxy)dodecanesulfate (TyS). Size uniformity and cholesterol rebinding were influenced by the amount of TyS used, and the presence of surfactants strongly affected the rebinding properties. Nevertheless, the nanoparticles were successfully used in an immunoprecipitation-like reaction in which addition of a multi-ligand template, poly(ethylene glycol) (PEG)-*bis*-cholesterol, resulted in flocculation of MIP particles (Figure 3) [49]. These results are encouraging for the application of MIP nanoparticles in immunoassays.

Non-covalent imprinting approaches have also been exploited in core–shell polymerization [47,50,51]; for example, core–shell nanoparticles were imprinted with propranolol [52]. Yields were very high (98–100%) for all the polymerization mixtures tested. Porogen (toluene) strongly affected shell porosity, leading to high surface area and high rebinding capacity. However, synthetic conditions had to be optimized to avoid secondary nucleation phenomena. Moreover, the presence of an aqueous phase reduced the imprinting effect. The authors also prepared a fluorescent core, thus demonstrating that this method is viable for producing nanoparticles for facile imaging.

Core–shell emulsion polymerization is a good method to obtain surface-imprinted nanoparticles with high yield and improved rebinding capacity and kinetics. It is also more suitable for large-scale applications in industry owing to the efficient heat dispersion. However, the presence of surfactants and an aqueous phase represent serious drawbacks for standardizing the already complex procedure, both in terms of particle dimensions and imprinting effects.

Grafting approaches

Another method for preparing MIP core–shell nanoparticles is grafting of a thin MIP layer onto the surface of prefabricated nanoparticles. This can be achieved in a grafting-from approach, which uses an initiator, such as *N,N′*-diethyldithiocarbamate trihydrate, immobilized on the nanoparticle surface, and usually results in layers characterized by a higher degree of MIP grafting [53]. With this type of initiator (iniferter), one of the radicals arising

from the decomposition step is not capable of initiating polymerization, but is able to terminate the growing polymer chains in solution [54,55], thus providing better control over the polymerization process. The grafted MIP layers were successfully imprinted with different templates. A very thin MIP layer could be deposited onto the particle surface, so three polymer layers with different properties were also constructed, which only slightly reduced the specific binding [53].

Core–shell nanoparticles have also been prepared using silica cores [20]. To increase the density of imprinted sites in the MIP shell, the authors exploited a strong charge-transfer interaction between the nitroaromatic ring of the template and surface amino groups of the core particles. Nanoparticles exhibited very good selectivity and rebinding kinetics (4.5-fold faster than conventional microparticles). However, such a thin imprinted shell (25 nm) might not be suitable for imprinting of bulkier templates, such as proteins.

Protein imprinting can be difficult for several reasons, not least their size and intrinsically poor stability under imprinting conditions. For protein imprinting, the monomer 3-aminophenylboronic acid (APBA) has been particularly useful as a monomer because of its water solubility and suitability for interaction with amino acids [56]. Magnetic polystyrene core–shell nanoparticles were recently imprinted for bovine haemoglobin using APBA [57]. The nanoparticles synthesized exhibited fast rebinding kinetics (30–120 min), as well as good specificity and selectivity. Moreover, magnetic properties ensured rapid product recovery, which is particularly suitable for large-scale production and applications (e.g. large-scale separation of proteins).

An original imprinting approach that involves dopamine self-polymerization at slightly basic pH values was recently used to imprint human haemoglobin on a polydopamine (PDA) layer synthesized onto magnetic Fe_3O_4 nanoparticles [58]. The MIP nanoparticles exhibited good recognition affinity for haemoglobin (K_d=18.13 μg/mL) and good selectivity. It seems that the use of PDA is particularly suitable for protein imprinting because it is hydrophilic, biocompatible, and can easily interact with the macromolecular template owing to its amino and catechol groups. Moreover, the thickness of the PDA layer can be tuned by changing the polymerization time [59].

Core–shell MIP magnetic nanoparticles have also been prepared using a semi-covalent imprinting approach on a silica shell, followed by thermal cleavage of the template (estrone) [60]. This ensures better imprinting properties, together with rapid template removal and product recovery. The authors obtained relatively monodisperse, 150-nm superparamagnetic nanoparticles. Scatchard analysis indicated the presence of homogeneous binding sites (R^2=0.998), with high selectivity. However, the number of synthetic steps required makes this approach unsuitable for large-scale processes, despite the rapid magnetic recovery.

Controlled living radical polymerization methods, such as RAFT [61] and ATRP [62], have also been used to prepare core–shell magnetic MIP nanoparticles. Both methods ensure good control over the thickness of the MIP layer, thus avoiding secondary polymerization phenomena [63,64]. Nevertheless, the ATRP protocol relies on catalytic complexes formed by copper ion and acidic or basic ligands, which might be disrupted by the interaction with the template molecules. Moreover, the metallic catalyst has to be removed from the final product [65]. RAFT seems to give better products than ATRP, even if for both methods the polymerisation conditions should be carefully evaluated. Because their application in molecular imprinting is relatively new, these techniques warrant more studies for full utilization in MIP synthesis.

MIP micro- and nanogels

The term nanogel refers to unimolecular crosslinked polymer particles of a size comparable to the statistical dimensions of a natural enzyme or antibody (~100 nm) that can exist as stable solutions in appropriate solvents. MIP micro- and nanogels (Table 2) overcome mass transport issues typically associated with insoluble bulk materials. Standard techniques available for soluble macromolecules can be used for their separation and characterization [66,67]. MIP nanogels could represent a viable alternative to the biological molecules used in sensors, separation and catalysis, or could be used *in vivo* for drug delivery and diagnostics. Furthermore, it might be possible to combine nanogels with other approaches, such as grafting, to create high-performance MIPs for real-life applications.

The first water-soluble MIP nanoparticles were produced through extensive grinding and ultrafiltration of an MIP monolith [68]. Imprinted nanoparticles were able of enhancing the activity of chloroplasts. However, the yield was very low and the process was long and inefficient for large-scale production purposes. This approach has not been further developed. By contrast, water-soluble MIP nanoparticles capable of inhibiting trypsin enzyme activity were synthesized via polymerization from a very dilute solution [69]. Because benzamidine is a well-known inhibitor of trypsin, a polymerizable derivative was used to complex the template with high affinity and to localize MIP nanogel synthesis on the surface of the enzyme. The K_i value calculated for the imprinted nanoparticles was 79 nM, which is much lower than the value for free benzamidine (18.9 μM). Moreover, the nanoparticles exhibited strong selectivity.

Covalent imprinting techniques have also been applied to the preparation of MIP nanogels under highly dilute conditions. Nanogels capable of catalyzing a cross-aldol reaction, such as that catalyzed by natural aldolase type I enzymes, have been prepared [25]. Imprinted nanogels exhibited 20-fold more catalytic activity than non-imprinted gels. Furthermore, the data revealed a homogeneous affinity distribution for the catalytic sites and good enantioselectivity. Their catalytic activity (k_{cat}=0.25×10^{-2} min^{-1}) was lower than that of natural aldolase; nevertheless, these MIP nanoparticles could be used to complement enzymes under conditions in which they are unstable, such as in organic media or at extreme pH values or temperatures.

Compared with dilute synthesis conditions, a post-dilution method was used to prepare MIP nanogels. This method involves polymerization at high monomer concen-

trations in a suitable solvent, with early termination by diluting the solution. This yielded MIP nanogels that could catalyze carbonate hydrolysis [24]. To imprint the active sites, a diphenyl phosphate template was used as a transition-state analogue (TSA) for the carbonate hydrolysis reaction. After removal, the template left behind binding sites with shape and functional groups suitable for catalyzing the hydrolysis reaction. The MIP nanogels produced were of lower polydispersity (M_w/M_n=1.54) and smaller size (10–20 nm) than those produced under high-dilution conditions. Moreover, they had, on average, one active site per particle.

Although the post-dilution method provides high yields, it relies on thermal polymerization, which might interfere with the imprinting process. In response, soluble MIP nanoparticles imprinted with acetoguanamine have been prepared using early termination of an iniferter-initiated polymerization [70]. As in the post-dilution method, the polymerization was performed at high monomer concentration, but instead of thermal initiation the authors used an iniferter and UV irradiation was applied for only 2.5 min (Figure 4). Iniferter use facilitated adjustment of the reaction kinetics, introducing the possibility to re-initiate the polymerization later. After fractionation through gel permeation chromatography and affinity chromatography, nanoparticles of 90–100 kDa were produced with high affinity and selectivity for acetoguanamine. The K_d value of 6.6×10^{-8} M was comparable to that of mono-

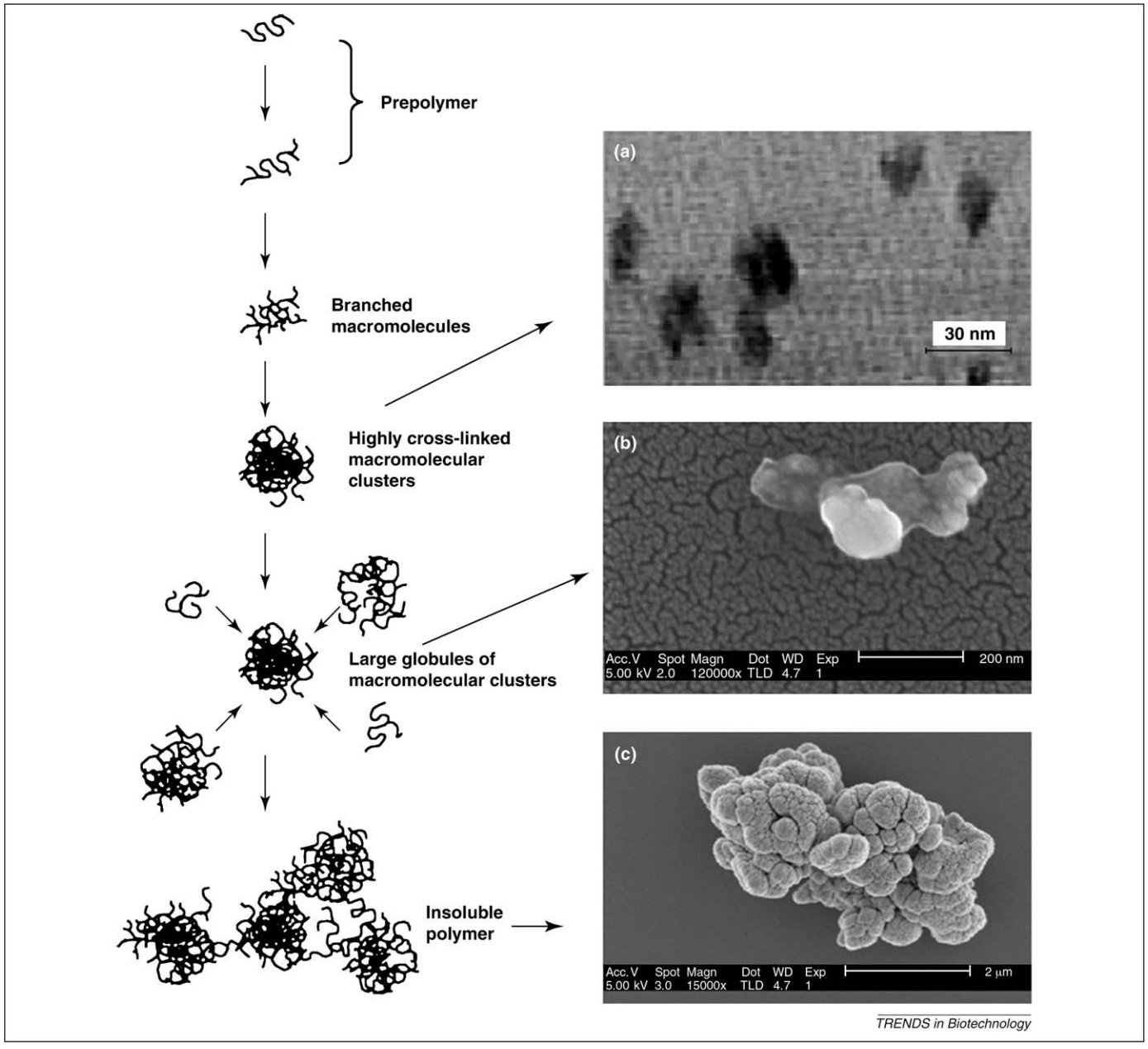

Figure 4. The polymer chain-growth process. Monomers begin to add from solution to form small polymer chains that are poorly branched. The degree of branching then increases owing to intramolecular reactions and the continued capture of monomers and oligomers from solution. Highly crosslinked macromolecular clusters with stable network structures are then formed owing to reactive groups within the molecule that strongly favor intramolecular crosslinking reactions. On further reaction, these macromolecular clusters bind to each other, giving rise to globules and eventually to the insoluble polymer. (**a**) TEM image of nanoparticles formed by 170 s of UV irradiation (magnification 340 000×). (**b**,**c**) SEM images of polymers formed by aggregation of molecular clusters achieved during 180 and 250 s of irradiation, respectively. Adapted with permission from Ref. [70].

clonal antibodies reported for atrazine (3.87×10^{-7} M [71] and 9.20×10^{-9} M [72]). However, the main drawback of this method is the low yield (3% w/w).

Conclusions and outlook

The feasibility of developing MIP nanoparticles has been discussed using examples from the most recent literature, noting methodological advantages and limitations, as well as compatibility with imprinting procedures and requirements for large-scale manufacture. The choice of polymerization approach depends on the characteristics required for the final material, together with the type of template to be imprinted. Despite the quality work discussed in this review, two limitations should be highlighted. First, there is no evidence in the literature of an automatic method for synthesis of MIP nanoparticles. Given the emerging importance of MIP nanoparticles as antibody and enzyme substitutes, automation of their manufacture needs to be investigated. Ideally, similar to peptide and DNA synthesizers, an automated system should guarantee fabrication and purification of high-affinity MIP nanoparticles of a uniform size distribution. Moreover, it should be suitable for imprinting of several template types. To the best of our knowledge, only one reactor for MIP synthesis has been developed to date, related to the fabrication of MIP microparticles [73].

Second, commercial exploitation of molecular imprinting is still in its infancy. MIPs cannot yet provide a total replacement for biological molecules in terms of capacity, selectivity and homogeneity of binding affinity. However, their potential for use in separation and sensing applications is clear, considering their low cost and robustness. Advances in MIP chemistry can be expected in the near future and should facilitate the direct production of MIPs in the form of nanoparticles on a continuous basis. This will then provide the missing impetus for investment in MIPs, leading to a new generation of superior, commercially available affinity materials.

References

1 RNCOS (2010) *Global* In Vitro *Diagnostic Market Analysis*
2 Ansell, R.J. (2004) Molecularly imprinted polymers in pseudoimmunoassay. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 804, 151–165
3 Lavignac, N. *et al.* (2004) Current status of molecularly imprinted polymers as alternatives to antibodies in sorbent assays. *Anal. Chim. Acta* 510, 139–145
4 Hock, B. *et al.* (1999) Stabilisation of immunoassays and receptor assays. *J. Mol. Catal. B Enzym.* 7, 115–124
5 Butler, J.E. (2000) Solid supports in enzyme-linked immunosorbent assay and other solid-phase immunoassays. *Methods* 22, 4–23
6 Omersel, J. *et al.* (2010) Alteration of antibody specificity during isolation and storage. *J. Immunoassay Immunochem.* 31, 45–59
7 Urraca, J.L. *et al.* (2007) Molecularly imprinted polymers as antibody mimics in automated on-line fluorescent competitive assays. *Anal. Chem.* 79, 4915–4923
8 Mayes, A.G. and Whitcombe, M.J. (2005) Synthetic strategies for the generation of molecularly imprinted organic polymers. *Adv. Drug Deliv. Rev.* 57, 1742–1778
9 Alexander, C. *et al.* (2006) Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003. *J. Mol. Recogn.* 19, 106–180
10 Esen, C. *et al.* (2009) Highly selective ion-imprinted particles for solid-phase extraction of Pb^{2+} ions. *Mater. Sci. Eng. C* 29, 2464–2470
11 Ogiso, M. *et al.* (2007) DNA detection system using molecularly imprinted polymer as the gel matrix in electrophoresis. *Biosens. Bioelectron.* 22, 1974–1981
12 Zeng, Z. *et al.* (2010) Synthetic polymer nanoparticles with antibody-like affinity for a hydrophilic peptide. *ACS Nano* 4, 199–204
13 Cirillo, G. *et al.* (2009) Imprinted hydrophilic nanospheres as drug delivery systems for 5-fluorouracil sustained release. *J. Drug Target.* 17, 72–77
14 Jenik, M. *et al.* (2009) Sensors for bioanalytes by imprinting-polymers mimicking both biological receptors and the corresponding bioparticles. *Biosens. Bioelectron.* 25, 9–14
15 Piletsky, S.A. and Turner, A. (2006) A new generation of chemical sensors based on MIPs. In *Molecular Imprinting of Polymers* (Piletsky, S.A. and Turner, A., eds), pp. 64–79, Eurekah.com/Landes Bioscience
16 Piletsky, S.A. *et al.* (2002) Polymer cookery: influence of polymerization conditions on the performance of molecularly imprinted polymers. *Macromolecules* 35, 7499–7504
17 Piletska, E.V. *et al.* (2009) Influence of the polymerization conditions on the performance of molecularly imprinted polymers. *Macromolecules* 42, 4921–4928
18 Ye, L. and Mosbach, K. (2002) Molecularly imprinted materials: towards the next generation. *Mater. Res. Soc. Symp. Proc.* 723, 51–59
19 Tokonami, S. *et al.* (2009) Review: micro- and nanosized molecularly imprinted polymers for high-throughput analytical applications. *Anal. Chim. Acta* 641, 7–13
20 Gao, D. *et al.* (2007) A surface functional monomer-directing strategy for highly dense imprinting of TNT at surface of silica nanoparticles. *J. Am. Chem. Soc.* 129, 7859–7866
21 Tan, C.J. and Tong, Y.W. (2007) Molecularly imprinted beads by surface imprinting. *Anal. Bioanal. Chem.* 389, 369–376
22 Ge, Y. and Turner, A.P.F. (2009) Molecularly imprinted sorbent assays: recent developments and applications. *Chem. Eur. J.* 15, 8100–8107
23 Haupt, K. *et al.* (1998) Assay system for the herbicide 2,4-dichlorophenoxyacetic acid using a molecularly imprinted polymer as an artificial recognition element. *Anal. Chem.* 70, 628–631
24 Wulff, G. *et al.* (2006) Soluble single-molecule nanogels of controlled structure as a matrix for efficient artificial enzymes. *Angew. Chem. Int. Ed.* 45, 2955–2958
25 Carboni, D. *et al.* (2008) The first example of molecularly imprinted nanogels with aldolase type I activity. *Chem. Eur. J.* 14, 7059–7065
26 Ciardelli, G. *et al.* (2004) Acrylic polymeric nanospheres for the release and recognition of molecules of clinical interest. *Biosens. Bioelectron.* 20, 1083–1090
27 Yoshimatsu, K. *et al.* (2007) Uniform molecularly imprinted microspheres and nanoparticles prepared by precipitation polymerization: the control of particle size suitable for different analytical applications. *Anal. Chim. Acta* 584, 112–121
28 Schweitz, L. *et al.* (2000) Molecularly imprinted microparticles for capillary electrochromatographic enantiomer separation of propranolol. *Analyst* 125, 1899–1901
29 Spégel, P. *et al.* (2003) Selectivity toward multiple predetermined targets in nanoparticle capillary electrochromatography. *Anal. Chem.* 75, 6608–6613
30 Nilsson, C. *et al.* (2007) Use of nanoparticles in capillary and microchip electrochromatography. *J. Chromatogr. A* 1168, 212–224
31 Priego-Capote, F. *et al.* (2008) Monoclonal behavior of molecularly imprinted polymer nanoparticles in capillary electrochromatography. *Anal. Chem.* 80, 2881–2887
32 Piletsky, S.A. and Turner, A.P.F. (2008) Imprinted polymers and their application in optical sensors. In *Optical Biosensors: Today and Tomorrow* (Ligler, F.S. and Rowe Taitt, C.A., eds), pp. 543–581, Elsevier Science
33 Reimhult, K. *et al.* (2008) Characterization of QCM sensor surfaces coated with molecularly imprinted nanoparticles. *Biosens. Bioelectron.* 23, 1908–1914
34 Schirhagl, R. *et al.* (2010) Chemosensors for viruses based on artificial immunoglobulin copies. *Adv. Mater.* 22, 2078–2081
35 Pérez-Moral, N. and Mayes, A.G. (2006) MIP formats for analytical applications. In *Molecular Imprinting of Polymers* (Piletsky, S.A. and Turner, A., eds), pp. 1–11, Eurekah.com/Landes Bioscience
36 Ye, L. *et al.* (1999) Molecularly imprinted monodisperse microspheres for competitive radioassay. *Anal. Commun.* 36, 35–38

37 Ye, L. *et al.* (2000) Synthesis and characterization of molecularly imprinted microspheres. *Macromolecules* 33, 8239–8245
38 Li, P. *et al.* (2003) Morphologies and binding characteristics of molecularly imprinted polymers prepared by precipitation polymerization. *Polym. Int.* 52, 1799–1806
39 Lai, J-P. *et al.* (2007) Molecularly imprinted microspheres and nanospheres for di(2-ethylhexyl) phthalate prepared by precipitation polymerization. *Anal. Bioanal. Chem.* 389, 405–412
40 Yang, K. *et al.* (2009) One-pot synthesis of hydrophilic molecularly imprinted nanoparticles. *Macromolecules* 42, 8739–8746
41 Ye, L. and Mosbach, K. (2001) Polymers recognizing biomolecules based on a combination of molecular imprinting and proximity scintillation: a new sensor concept. *J. Am. Chem. Soc.* 123, 2901–2902
42 Hoshino, Y. *et al.* (2008) Peptide imprinted polymer nanoparticles: a plastic antibody. *J. Am. Chem. Soc.* 130, 15242–15243
43 Hoshino, Y. *et al.* (2010) Recognition, neutralization, and clearance of target peptides in the bloodstream of living mice by molecularly imprinted polymer nanoparticles: a plastic antibody. *J. Am. Chem. Soc.* 132, 6644–6645
44 Vaihinger, D. *et al.* (2002) Molecularly imprinted polymer nanospheres as synthetic affinity receptors obtained by miniemulsion polymerisation. *Macromol. Chem. Phys.* 203, 1965–1973
45 Van Herk, A.M. and Monteiro, M. (2003) Heterogeneous systems. In *Handbook of Radical Polymerization* (Matyjaszewski, K. and Davis, T.P., eds), pp. 301–332, John Wiley & Sons
46 Curcio, P. *et al.* (2009) Semi-covalent surface molecular imprinting of polymers by one-stage mini-emulsion polymerization: glucopyranoside as a model analyte. *Macromol. Biosci.* 9, 596–604
47 Pérez-Moral, N. and Mayes, A.G. (2002) Molecular imprinting of polymeric core-shell nanoparticles. *Mater. Res. Soc. Symp. Proc.* 723, 61–66
48 Pérez, N. *et al.* (2000) Molecularly imprinted nanoparticles prepared by core-shell emulsion polymerization. *J. Appl. Polym. Sci.* 77, 1851–1859
49 Pérez, N. *et al.* (2001) Surface imprinting of cholesterol on submicrometer core–shell emulsion particles. *Macromolecules* 34, 830–836
50 Carter, S.R. and Rimmer, S. (2002) Molecular recognition of caffeine by shell molecular imprinted core–shell polymer particles in aqueous media. *Adv. Mater.* 14, 667–670
51 Carter, S.R. and Rimmer, S. (2004) Surface molecularly imprinted polymer core–shell particles. *Adv. Funct. Mater.* 14, 553–561
52 Pérez-Moral, N. and Mayes, A.G. (2004) Noncovalent imprinting in the shell of core–shell nanoparticles. *Langmuir* 20, 3775–3779
53 Pérez-Moral, N. and Mayes, A.G. (2007) Molecularly imprinted multilayer core–shell nanoparticles – a surface grafting approach. *Macromol. Rapid Commun.* 28, 2170–2175
54 Otsu, T. *et al.* (1989) Living radical polymerization through the use of iniferters: controlled synthesis of polymers. *Eur. Polym. J.* 25, 643–650
55 Rückert, B. *et al.* (2002) Molecularly imprinted composite materials via iniferter-modified supports. *J. Mater. Chem.* 12, 2275–2280
56 Bossi, A. *et al.* (2001) Surface-grafted molecularly imprinted polymers for protein recognition. *Anal. Chem.* 73, 5281–5286
57 Li, L. *et al.* (2009) Preparation of novel bovine hemoglobin surface-imprinted polystyrene nanoparticles with magnetic susceptibility. *Sci. China Ser. B Chem.* 52, 1402–1411
58 Zhou, W-H. *et al.* (2010) Mussel-inspired molecularly imprinted polymer coating superparamagnetic nanoparticles for protein recognition. *J. Mater. Chem.* 20, 880–883
59 Lee, H. *et al.* (2007) Mussel-inspired surface chemistry for multifunctional coatings. *Science* 318, 426–430
60 Wang, X. *et al.* (2009) A molecularly imprinted polymer-coated nanocomposite of magnetic nanoparticles for estrone recognition. *Talanta* 78, 327–332
61 Lu, C-H. *et al.* (2007) Surface-imprinted core–shell nanoparticles for sorbent assays. *Anal. Chem.* 79, 5457–5461
62 Lu, C-H. *et al.* (2009) Bifunctional superparamagnetic surface molecularly imprinted polymer core–shell nanoparticles. *J. Mater. Chem.* 19, 1077–1079
63 Wei, X. *et al.* (2005) Surface molecular imprinting by atom transfer radical polymerization. *Biomacromolecules* 6, 1113–1121
64 Titirici, M-M. and Sellergren, B. (2006) Thin molecularly imprinted polymer films via reversible addition–fragmentation chain transfer polymerization. *Chem. Mater.* 18, 1773–1779
65 Ye, L. and Mosbach, K. (2008) Molecular imprinting: synthetic materials as substitutes for biological antibodies and receptors. *Chem. Mater.* 20, 859–868
66 Biffis, A. *et al.* (2001) The synthesis, characterization and molecular recognition properties of imprinted microgels. *Macromol. Chem. Phys.* 202, 163–171
67 Flavin, K. and Resmini, M. (2009) Imprinted nanomaterials: a new class of synthetic receptors. *Anal. Bioanal. Chem.* 393, 437–444
68 Piletsky, S.A. *et al.* (2006) Synthesis of biologically active molecules by imprinting polymerisation. *Biopolym. Cell* 22, 63–67
69 Cutivet, A. *et al.* (2009) Molecularly imprinted microgels as enzyme inhibitors. *J. Am. Chem. Soc.* 131, 14699–14702
70 Guerreiro, A.R. *et al.* (2009) Selection of imprinted nanoparticles by affinity chromatography. *Biosens. Bioelectron.* 24, 2740–2743
71 Grant, S.D. *et al.* (1999) Comparative sensitivity of immunoassays for haptens using monomeric and dimeric antibody fragments. *J. Agric. Food Chem.* 47, 340–345
72 Kramer, K. (2002) Evolutionary affinity and selectivity optimization of a pesticide-selective antibody utilizing a hapten-selective immunoglobulin repertoire. *Environ. Sci. Technol.* 36, 4892–4898
73 Zourob, M. *et al.* (2006) A micro-reactor for preparing uniform molecularly imprinted polymer beads. *Lab Chip* 6, 296–301