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'Molecular Imprinting' as Multidisciplinary Material Science: Today and Tomorrow

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Dedicated to Prof. Peter A. Lieberzeit, (Univesity of Vienna, Austria) on the occasion of his 42nd birthday.

Abstract

The review article focuses 'Molecular Imprinting' a multidisciplinary material view from the window of past trends to present's and future's road map. This is a first critical 'review of reviews' that covers the field from laboratory research to advanced material science in industrial applications. The article gives insight into ideas in the perspectives of step by step past trends, practical discussions and tips, its significance, applications, challenges, the ways to cope the challenges and a possible future or direction of the technique. By using selected case studies, it provides a comprehensive overview and a complete picture of the entire field. It illustrates the key soul of imprinting technology, via screening through pros and cons perspectives. The number of publications and applications of molecular imprinting is increasing exponentially and a common audience feels madness due to vastness in the today's research climate of multidisciplinary nature. In which direction we are moving? It can be hard for a busy scientist to keep pace with innovations or cherry pick useful advice from the mass of literature. In these perspectives, this review is equally suitable for all audiences from beginners to experts of today and tomorrow. The article could provide a launch pad to the future of imprinting technology for mining big information in all domains, its applications and far beyond.

Keywords

Molecular Imprinting, Review of Reviews, Advanced Material, Multi-discipline, Applications, Challenges, Present, Future

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1. Introduction

The 'Molecular Imprinting' is now worth billions of U.S. dollars, the topic attracts the attention across the world and the plethora of technique is condensed into a concise account of the key achievements to date. Although the base of technique goes back to the beginning of 1930s, yet it had an exponential growth only 40–50 years later by Wulff and especially by Mosbach. The technique yields three-dimensional cavities with tailored recognition properties and it has obtained a strong position in materials science. It has expended from sensors to biosensors, electronics to bioelectronics and actuators, from chemical science to technology and from materials to advanced materials and beyond.

In February 1993, Klaus Mosbach group published milestone research in Nature on molecularly imprinted polymers (MIPs) as *'antibody mimics'* using non-covalent binding for the first time [1]. They demonstrated that these synthetic antibodies can be *'a useful and general alternative to antibodies'* in future. Efforts of first 20 years in the perspectives of *'MIPs alternative to antibodies'* has been demonstrated by Bowen et al [2].

Literature survey by Kirsch et al on the MIP technology has been published as first part. This part covers 1450 references

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on the development of MIPs science from the serendipitous discovery of Polyakov in 1931 to 2006 [3]. Second part by Whitcombe et al covers 3779 references from 2004 to 2011. Both parts are substantial contributions to the literature review of the technique [4].

In the early age of MIPs, surface imprinting of solid materials and monolayers got great interest at research levels [5], while stationary phases for chromatography and chiral chromatography were developed applications [6]. MIPs applications to the *'world of real samples'* remained challenging in industrial applications [7]. An overview on number of publications on MIP technology till 2015, according to Scopus, can be seen in Figure 1.

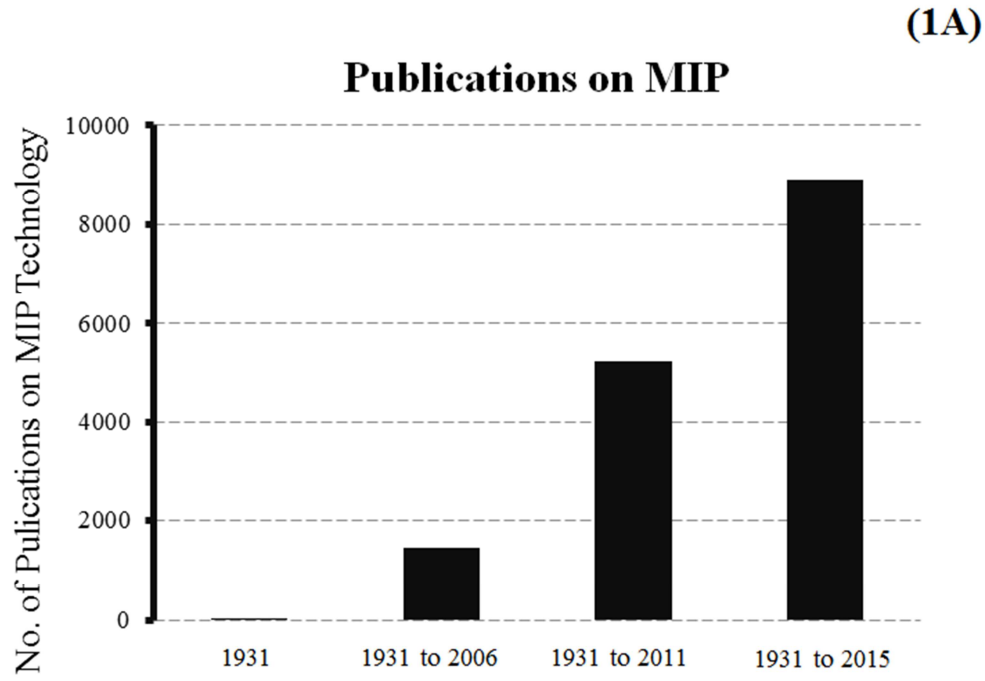

Figure 1A. An overview on number of publications on MIP technology.

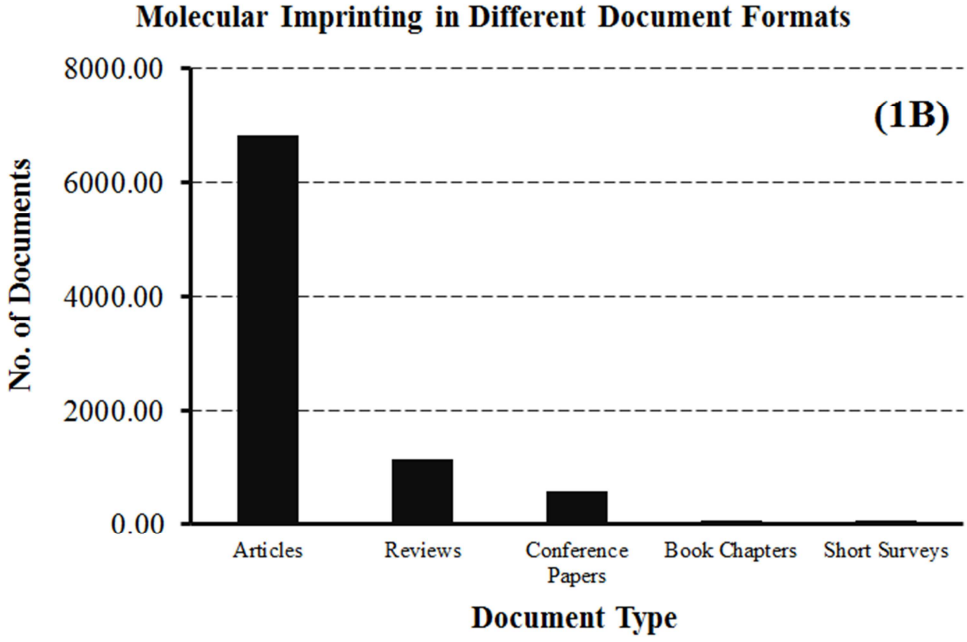

Figure 1B. An overview on number of publications on MIP technology on the basis of document formats.

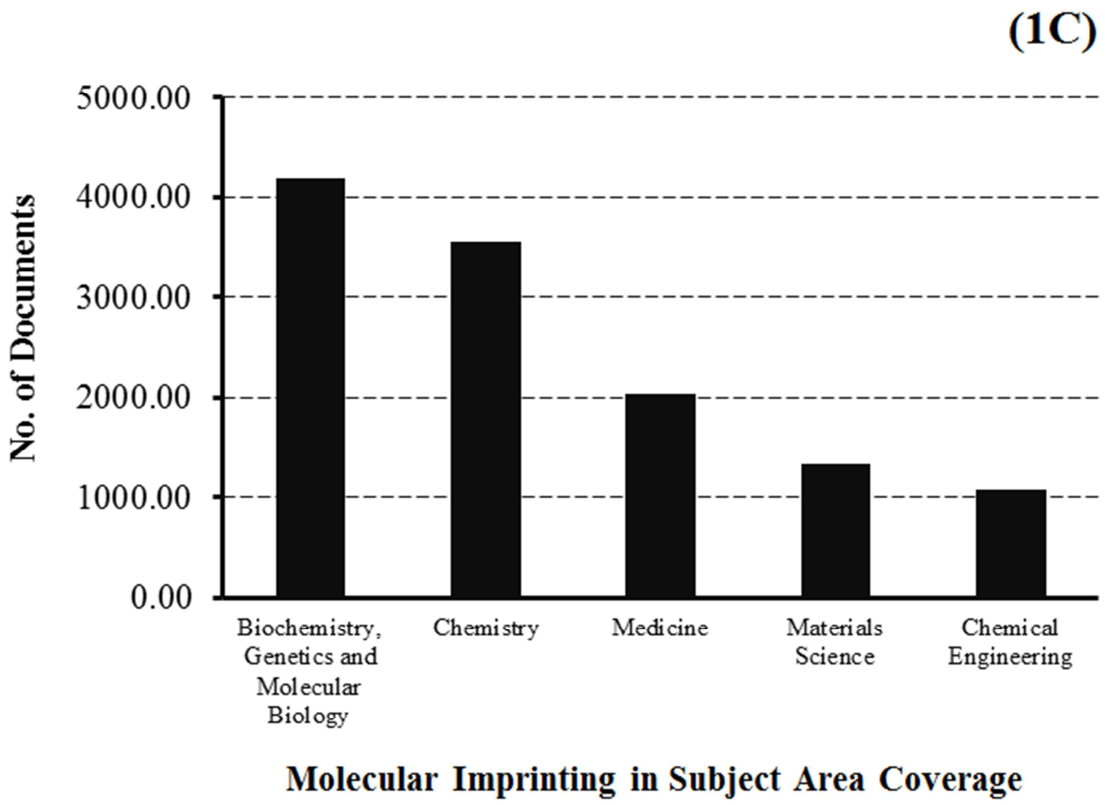

Figure 1C. An overview on number of publications on MIP technology on the basis of subject area coverage.

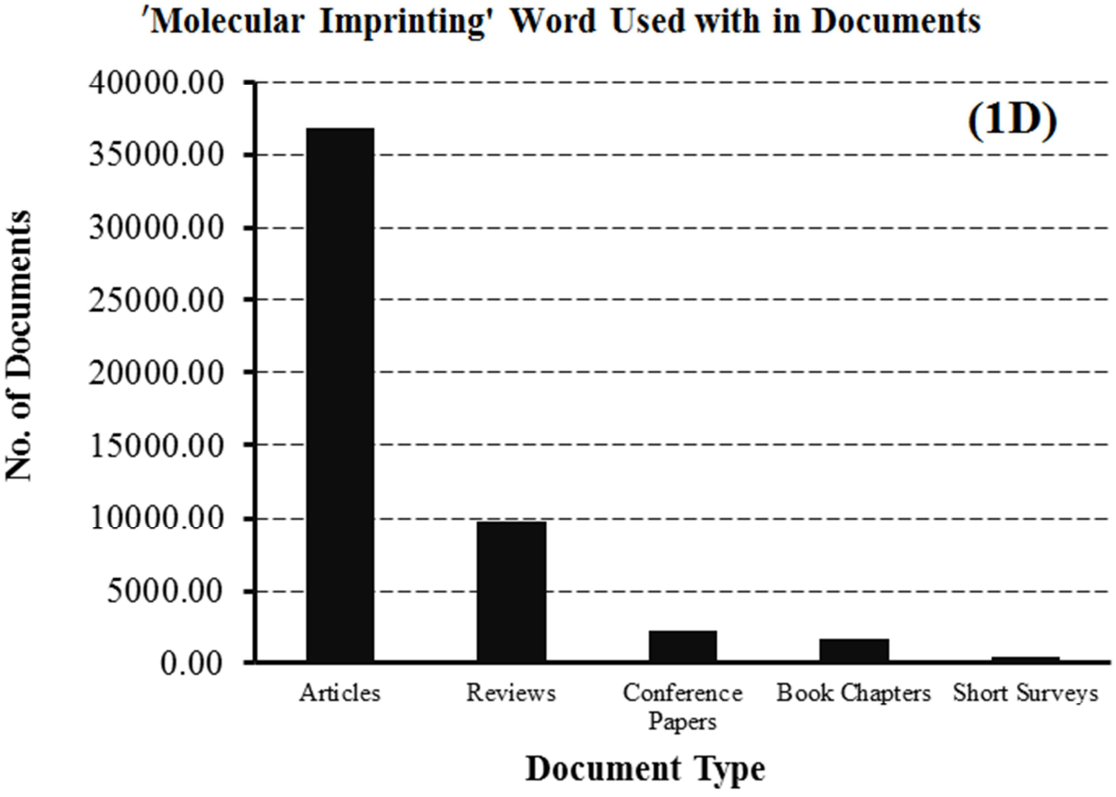

Figure 1D. 'Molecular Imprinting' word usage with in documents, an overview on number of publications on the basis of document types.

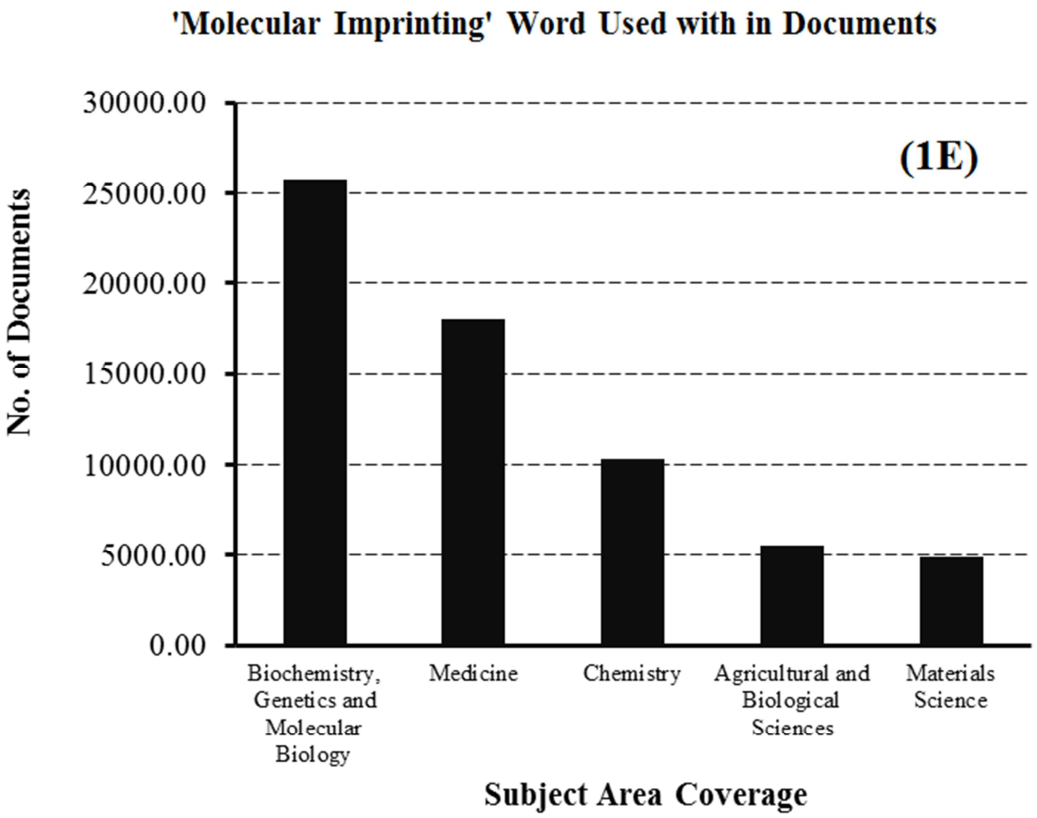

Figure 1E. 'Molecular Imprinting' word usage with in documents, an overview on number of publications on the basis of subject area coverage.

This article is first ideological and critical 'review of reviews' (till December 2014) on MIP technology.

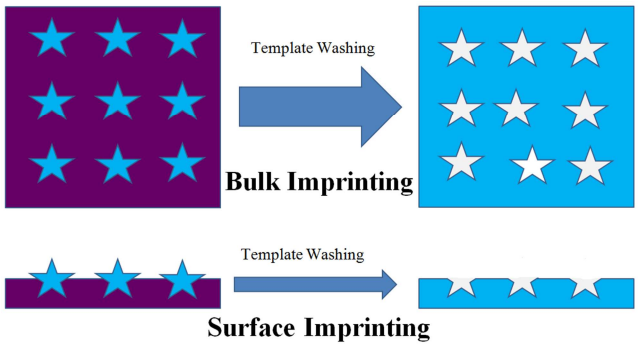

Figure 2. Traditional bulk and surface imprinting.

Figure 2 demonstrates the traditional bulk and surface molecular imprinting approaches. In bulk imprinting, template is mixed into the pre-polymerization mixture containing monomer, crosslinking monomer, solvent and polymerizaiton initiator. After the polymerization and template washing step, the imprints of template molecules homogenously spread in bulk polymer. In traditional surface imprinting approach, template (usually large species such as cells, virus or bacteria) yields imprints on the surface of polymer.

2. General, Current Status, Challenges and Future

Improved MIP catalytic performance in the perspectives of catalyzed reactions and turnover rate would be interesting to mimic the induced fit phenomenon often encountered in enzymatic systems [8]. Merging of molecular imprinting with membrane science is helpful in membrane separation via enhancing the mobility of the target molecule in the membrane [9]. For biosensors, lack of suitable interface between MIP and transducer is barrier in achieving effectiveness of MIPs [10]. MIPs' binding heterogeneity, slow mass transfer, low binding affinity, lack of read out for complexation and trapped template slowly leaching out are challenges. Attempts for a universal approach to MIP can contribute to fundamentals and applied discoveries [11]. MIP can be effectively controlled by using ring-opening metathesis polymerization (ROMP) for homogeneous binding distribution. First MIP arrays as chemical sensors for different MIPs simultaneously were reported by the Shimizu [12, 13], Dickert [14] and Takeuchi [15] groups. This approach could solve the problem of the weak recognition provided by single imprinted polymer replacing with using an array of different MIPs simultaneously. MIP arrays with appropriate chemometrics could ease the analysis of

complicated samples. However, this area still needed more research in developing successful integrated sensors depending on pattern recognition. Integrated sensors based on pattern recognition needed much attention [16]. Merging conductive polymers with MIP is helpful for the generation of semiconducting technology for electronic devices. Surface imprinted polymers (SIP) microrods fabrication for the generation of nanosized MIPs can assist biomedicine and biotechnology [17]. Nanomaterials such as dendrimers, microgels and nanogels, nanofibres, nanowires and nanotubes have successfully used in conjunction with molecular imprinting. These materials are feasible with surface imprinting allowing easy access of target molecules to recognition sites [18].

Selective MIPs for proteins, DNA, viruses and bacteria are challenging because pH and ionic strength complicate the unspecific effects. Non-charged hydrophilic matrices effects on imprinting are still unclear [19]. In nature, high affinity between antibody and antigen or ligand and receptor respectively is due to electrostaticity and hydrophobicity in addition to hydrogen bonding. Recently for peptide (melittin) imprinting in aqueous media, molecularly imprinted nano-particles (MIP-NPs) have been synthesized from N-isopropylacrylamide, N,N'-methylene-bisacrylamide and supplemented with monomers for interesting electrostatic and hydrophobic interactions e.g. acrylic acid and N-t-butylacrylamide etc. MIPs and NIPs should be processed with the same protocol for removal of remainders of detergents e.g. acetic acid and sodium dodecylsulfate in template washing step. Template removal and rebinding should be quantified by validated methods and proper controls via reproducibility, deviations and different batches testing [20]. Novel alternatives of bulk polymerization (such as MIP-NPs) with improved surface morphologies, are substantial for future of different fields especially for catalysis and drug delivery applications [21].

In protein imprinting via surface and epitope-mediated techniques, homogeneous binding sites can be achieved by a semi-covalent and nonhydrogen interaction imprinting approach [22]. Some recent fundamental exploration initiatives are: a) use of advanced equipment for imprinting and recognition mechanism at the molecular level, b) aqueous media polymerization for achieving the level of natural molecular recognition, c) synthesis of new monomers for challenging templates and d) new polymerization approaches for higher imprinting efficiency and binding capacity [23]. This is the stage of proof-of-principle for molecularly imprinted radio, fluoro, enzyme-linked chemiluminescent immunoassay and biomimetic immunosensor because significant issues are unresolved [24]. Discrimination abilities in MIMs are affected by different parameters in polymerization [25].

3. Analytical Chemistry

3.1. Ion-Imprinted Polymers (IIPs)

For analytical perspectives, user friendly instruments/techniques with proper interfacing are prerequisite for MIP based highly sensitive chemo-sensors [26]. Ion-imprinted polymers (IIPs) contributed excellently to the sample preparation part of analytical chemistry. IIPs play substantial role in quantitative analysis of low concentrations of toxicants such as arsenic, selenium, copper, nickel, cobalt, aluminium and complexes of these elements and beyond. Astonishing selectivities pave path for industrial scale clean-up purposes such as in waste water treatment, drinking water treatment and drug delivery [27].

3.2. Small Organic Molecules

The MIP-applications of small organic molecules such as pharmaceuticals, pesticides, amino acids, steroids and sugars are routine for separation and purification of complex samples such as environmental and biological samples via MISPE or MIP-HPLC[28].

3.3. Molecularly Imprinted Sorbent Assays (MIAs)

The sensitivity and selectivity of the micro to nanoscale MIP beads employed in MIAs are comparable to those of conventional MIP-NPs. Selective, simple and direct MIAs in aqueous media are complementary. Challenges of probe size and accessibility in fluorescence-labelled and enzyme-linked MIAs still have not been resolved [29]. Commercial kits of affordable and sophisticated MIAs based assays are expected in future [30].

3.4. Anion Recognition

Positive charges in MIP cavities act as driving force in anion recognition to avoid interference of water molecules. Non-covalent interactions e.g. hydrogen bonding, π-π stacking, hydrophobic interactions and metal-coordination contribute to the recognition and selectivity. Weak interactions between the template and functional monomers require excess of functional monomers for non-covalent MIPs and result in non-specificity and heterogeneity. Stoichiometry can improve the recognition of MIPs by using custom-designed functional monomers in the imprinting process. This generates strong interaction in template monomer complex. MIP and other methodology (e.g. indium-doped tin oxide (ITO), quartz crystal microbalance (QCM), solid phase extraction (SPE) etc.) combinations keep potential for anions sensing [31].

3.5. Chiral Recognition

Liquid chromatographic studies using MIPs as chiral stationary phases for enantiomer separations are attractive due to their friendly applications for aqueous and organic solvent based samples [32].

Enantioselective MIPs are extensively used as efficient scavengers for refinement of chiral intermediates from industrial production streams. These MIPs keep potential in high-through put analysis such as in support of combinatorial discovery of asymmetric (bio)catalysts [33].

Nanostructure fabrications have generated useful new concepts such as the atomic switch, probe-fabrication of molecular arrays and integrated circuit technology. New nanostructures such as nanotubes especially carbon nanotubes, NPs, nanorods, nanosheets, nanowires, nanowhiskers, mesoporous silica, mesoporoous carbon and other mesoporous materials, organic-inorganic nanohybrids and bio-related nanohybrids are attaining popularity. Although these have surprising structural morphologies, orderings and orientations and enhanced surface areas with high functions such as electronic, photonic, magnetic and catalytic properties, yet these are not marketed as chiral sensors [34].

3.6. Environmental and Water Treatment Applications

MIPs are selective materials and promising for long term use. Technically important problem is sensor packaging to avoid biofouling especially sensing in an aquatic environment over an extended period [35].

Carbon-MIP nanocomposites can enhance the adsorption capacity as compared to carbon-MIP because carbon substrates have a larger specific surface area and stability without swelling. Suppressing of non-selective hydrophobic or ionic interactions is an effective approach to achieve high sensitive MIPs for aqueous media. Analogue or fragment templates are short cut to imprint challenging target pollutants. MIP-NPs embedded on MIP are effective for the removal of Highly Toxic Organic Pollutants (HTOPs). This strategy has been extensively used as physical adsorbents or as the molecular recognition elements in composite for enhancing the direct photolysis and biodegradation of HTOPs in waste-water. MIPs coupled with advanced oxidation processes (AOPs) have been successfully employed in preferential photo-catalytic degradation of contaminants. On site or in situ MIPs regeneration can be achieved by removal and destruction of the contaminants via adsorption with MIPs followed by simultaneous extraction and chemical treatment. Water-compatible MIPs development is a critical factor in the newly reported biodegradation of trace target pollutants.

Stability and durability of MIPs in highly reactive environments can be improved via AOP-MIP composites e.g. MIPs-TiO_2 or Fe^{2+}/Fe^{3+} oxidation based composite. Photocatalysts can selectively remove low-level HTOPs in the presence of high level less toxic pollutants. On the other hand, AOPs are reactive due to the strongly oxidizing radical species such as hydroxyl radicals [36].

The capacity of some precious metals such as Ag, Au, and Pt is not large, while MIPs use is focused on selectivity rather than adsorption capacity. MIPs priority should be given to the selectivity and rebinding ability [37].

MIPs having high selectivity and strong affinity for target chemicals could be employed for industrial waste water treatment for removing high concentrations of specific compounds used in manufacturing. NIPs have potential for general water and waste water treatment for removal of various chemical contaminants simultaneously. Toxicity and health side effects of MIPs and NIPs could be drawbacks for drinking water treatment [38]. Pickering emulsion, encapsulation, or immobilization of MIP particles via improved precipitation polymerization on supporting substrates by using electrospinning and click chemistry could yield water-compatible MIPs. NMR, dynamic light scattering and synchronous fluorescence spectra can investigate into the mechanism of molecular imprinting. New approaches, mechanistic studies, synthesis of water-compatible MIPs towards more targets and enhancement of the binding capacity of MIPs by photo-catalytic and biological degradation technologies are required for water treatment [39].

3.7. Characterization

Improved performance of polymers in aqueous media and quantitative characterization for large biomolecules imprinted material are key requirements of good laboratory practice [40].

Principal analytical considerations for good practice for characterization of MIPs and trends in the increasingly rapid for characterization are increasing recent years. UV/VIS spectrophotometry, FT-IR, NMR, thermal methods, chromatography (LC-MS) and allied instruments for morphological analysis have been extensively employed [41].

3.8. Template Removal

Several parameters affect the yield of template recovery such as the amount and MIP-NPs size, solvent and its volume and the operation time. Extraction via ultrasounds, microwaves, or heating under pressure working in less solvents and time

increase the template removal. Drastic conditions lead to distortion in the MIP cavities and affect in rebinding and selectivity. Extraction with subcritical water or supercritical CO_2 is environmental-friendly, but operational costs and instability are disadvantages of this technique. Physically-assisted and subcritical or supercritical fluid extraction with careful controlling can avoid the template removal. This is the limiting step in the MIP synthesis in the perspectives of time spent and of the working of the imprinted cavities. Operation in continuous mode, automation of the template removal process and on-line instrumental integration for real-time monitoring of the template removal can be cost-effective and efficient [42].

4. Macromolecules, Surface Imprinting, Bio-Applications and Food Analysis

4.1. Macromolecules Imprinting

MIP networks have potential for enhanced loading, sustained release and enantioselective release of therapeutic agents (e.g. drugs, amino acids, steroids, nucleotide bases, carbohydrates etc) [43]. Among general approaches for macromolecular imprinting, epitope imprinting is superior because of increased specific recognition and higher affinity [44, 45]. Highly selective and non-conventional sorbents such as affinity sorbents and restricted access materials in SPE, are attracting for urine pre-treatment [46].

Soft contact lenses can improve the bioavailability and can prolong the residence time of drugs. These are astonishing drug carriers for ophthalmic drug delivery [47].

Stability and strength of the monomer-template complex prior to polymerization are substantial for MIP performance. For protein MIPs; hydrogen bonding, electrostaticity and hydrophobicity play important role, while the relative importance of each of these interactions is unclear. These efforts are proof of principles for various templates; thus have main flaw and lack of success to date [48].

4.2. Biological Macro-Templates (Proteins)

MIP is effective for species with molecular weight <1500 and is challenging for larger molecules such as proteins [49]. MIP has to bind to a large flat surface and recognition is based on unique distribution of amino acid residues having various charges, sizes and shapes [50]. Hydrogen bonding, hydrophobic and electrostatic forces are the prominent mechanisms for a template-receptor complexation in aqueous media [51]. MIPs for proteins are substantial for applications in medicine, diagnostics, proteomics, environmental analysis, sensors and drug delivery [52]. MIP thin films for proteins are superior as compared with bulk monoliths [53]. Molecularly imprinted membranes (MIMs) mainly improve mass transfer of templates into and out of the MIP cavities. This is helpful for large templates to avoid encapsulation in three-dimensional networks and for yielding rapid mass transfer of analytes in the order of minutes. MIMs are attractive for sensor applications such as surface analysis methods of surface plasman resonance (SPR) and acoustic wave. Based on nature of the pre-polymer complex, three approaches have been demonstrated for MIMs. The common approach is the mixing of the template and monolayers together in the pre-organized monolayer assembly and subsequently immobilization to the transducer. In second approach, an untemplated monolayer is generated first and followed by the template addition that organizes the monolayer components. The last approach makes use of a linear thin film with interactive groups within the polymer chain which are then template organized into the precursor MIM format. This approach did not require a flat molecule; ultimately it is feasible with a large variety of templates for imprinting. The selectivity in MIMs is based on cavities size and very less on specific interactions with the template [54].

Magnetic NPs, silica NPs, nanowires, quantum dots (QDs) and carbon nanotubes are promising emerging materials for protein and bio-macromolecules imprinting. Merging of new technologies are interesting too but reduction in non-selective binding and improved biocompatibility are still challenges. Computational simulation may assist designing and screening of functional monomers. New hydrophilic and biocompatible monomers in imprinting could help the dispersion of MIP conjugates in aqueous media for proteins studies.

Investigation into protein imprinting mechanism and recognition at the molecular level via computer modeling, docking and advanced experimental methods could be helpful. These advances could help clinical diagnostics, therapeutic monitoring, treatment of diseases, control of bioreactors and detection of microorganisms and toxins [55].

4.3. Surface Imprinting

Surface imprinting has reached a certain degree of maturity in terms of understanding of the imprinting mechanism and various MIP preparations at laboratory levels. Future research will focus developing imprinting systems suitable for large-scale production of MIPs. MIPs approaches with controllable and homogeneous morphologies could replace the conventional imprinting methodology for industrial applications. Surface-MIP spheres of regular, tunable sizes and having fast rebinding kinetics for macromolecules could be appropriate for industrial applications [56].

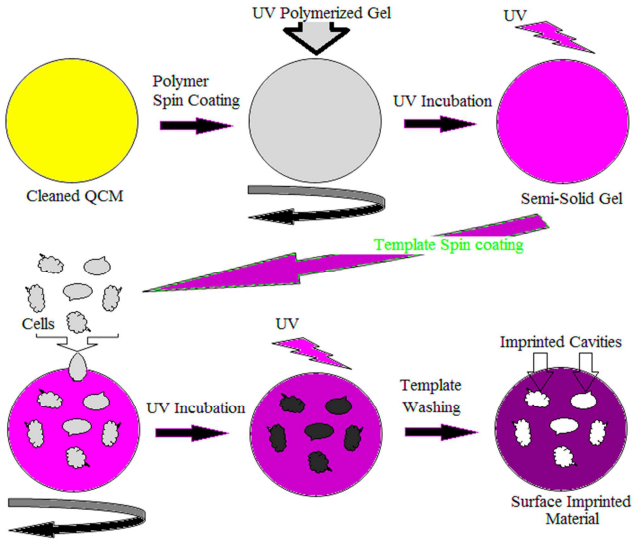

Figure 3. A straightforward approach for cells (platelet) imprinting for heparin induced thrombocytopenia (HIT) platelet aggregation sensing in real patients [57]

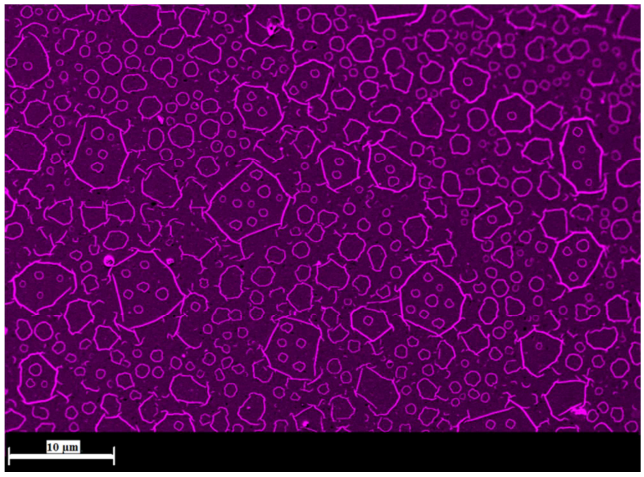

Figure 4. SEM image of activated platelet surface imprinted thin film [57].

4.4. Biomedical, Clinical, Drugs and Pharmaceutical Applications

MIP synthesis is successful for low-molecular weight compounds namely haptens but challenging for macrobiomolecules. Natural body preparation needs conjugation of the hapten to a carrier protein before injection into the animal [58].

Acrylic-based hydrogels are suitable for mucoadhesion due to their nonabrasive and flexibility which reduce damage-causing attrition to the tissues in contact [59].

MIPs are promising for pharmaceutical applications such as separation, extraction, detection, screening, delivery and targeting of drugs or their metabolites. Although similar or sometimes even higher selectivities have been reported on comparing with those of natural antibodies, yet selectivity is a limiting factor [60-63].

The hydrogels based clinical intelligent systems still rely on additional efforts. For example a) synthesis of new biocompatible materials b) controlled synthetic procedures for homogeneity, reproducibility and end quality of the new polymeric materials c) proper reversible systems d) *in vivo* evaluation of new delivery systems [64].

Fast development in MIP technology and modern needs of the drug delivery can yield sophisticated imprinted drug delivery devices for personalized or individual treatment. These devices could yield improved delivery profiles, prolonged releasing times and extended residency of the drugs. Additionally, MIPs could release the drugs in the regulated way for requirements in modern drug delivery systems. This could be interesting application especially for appropriate enantiomeric form of the drugs [65].

Molecular imprints can be helpful to target for delivering given drug to cancer cells and to increase their nuclear and cancer killing abilities. This could be achieved *via* localization of a synthesized MIP on the surface of the target. MIP-NPs and imprinted nanocarriers having complexity and efficacy could yield interesting success. Limited knowledge of the molecular principles and mechanisms for molecular recognition is a main barrier [66].

4.5. Bio-Applications

Nanoparticles (NPs) are attractive due to their contribution to enhancements in sensitivity and selectivity [67, 68]. Natural antibodies opt "induced fit" and optimize analyte-receptor interactions naturally; thus are hard to beat. The established instrumental separation techniques (e.g. modern mass spectrometry offer flexibility and detection limits), are hard to compete [69]. Diagnostics, healthcare or security topics (e.g. illegal drugs sensors) require disposables, while MIPs focus on ruggedness and reusability. Polymerase chain reactions (PCR) are attractive due to better LoDs and amplification properties for microorganisms. The diagnostic tools market is conservative to replace well-established techniques by novel ones because of lack of experience. For large template species, MIPs studies on batch-to-batch reproducibility and upscaling to (pilot) plant levels have not yet established at industrial scales. MIPs can be interesting candidates for applications that require long-term stability e.g. process control or monitoring of air/water quality over extended periods of time and diagnostics area [70]. Large biospecies templates such as viruses with a repetitive surface structure or cells with a relatively rigid cell wall proved successful for imprinting. Flexible surface structures on cells with "fluid" cell membranes are poor templates for imprinting [71].

4.6. Biomolecules Hydrogels

Initial studies on this area focused on the possibility of

hydrogels for MIP strategies, challenges in creating effectiveness of MIPs [72], development of biomaterials (e.g. glucose-sensitive hydrogels), drug delivery systems, enzymatically degradable hydrogels and antigen sensitive hydrogels [73].

The influence of imprinting on the transport of template is extremely complex and got little attention for the gel porosity/tortuosity in the control of template transport and structural analysis. MIPs have led to breakthroughs in controlled and modulated drug delivery, diagnostic sensors and separation. Challenges include gels-characterization, direct template diffusional phenomena within imprinted gels, relation of the complexation contribution of multiple polymer chains and template to thermodynamic theories of polymer network dynamics, controlling and engineering of the network structure, understanding of the template-polymerization reaction and diverse functional monomer incorporation [74].

4.7. Food Analysis

MIPs are attractive for agrofood industry especially in the perspectives of analysis or extraction of components [75]. Challenging detection, clean-up and pre-concentration of natural toxins in complex samples can be achieved by using MIPs. However, still several natural toxins of relevant practical interests show a lack of hypothetical mimicking templates [76]. Some MIP-based SPE cartridges are commercially launched by different companies such as ELIPSA (Germany) and MIP Technologies (Sweden) for sensing of clenbuterol, triazine and chloramphenicol in the food [77].

Mycotoxins such as patulin and moniliformin are challenging for MIPs but interesting for their applications [78]. MIPs have potential in well-developed analytical techniques such as LC for the selective extraction of target analytes from complex matrices of diverse foods [79, 80].

5. Catalysis, Metal Oxide Matrices and Sol-Gel Matrix

5.1. Catalysis

The transition metal catalysts in combination with MIPs significantly yield improved substrates and enantio-selectivity with inherent properties of simplicity and flexibility [81, 82].

Problems and challenges in catalysis-MIP technology are: 1) microparticles formation during suspension or emulsion polymerization 2) suitable groupings for catalysis 3) extremely high sensitivity required in chemo-sensors 4) selectivity with unstable templates 5) mass transfer improvements in MIPs 6) reduction of the "polyclonality" of cavities 7) improvements in availability of active sites in MIPs having non-covalent interactions [83].

5.2. Amorphous Metal Oxide Films

Coordination chemistry and active site engineering in synthetic MIPs share synergy. The structure and reactivity such as stereochemistry of the imbedded metal complexes and catalysts are prerequisite for proper characterization [84]. Metal oxide nanostructures e.g TiO_2-gel or silicate thin films have been employed for three dimensional imprinting of organic templates [85].

Sensitivity can be improved by using electrochemical and electronic detection, while selectivity can be improved via rigidity of polymer system. Metal oxide films as imprinting matrixes compared with cross-linked polymers are advantageous: a) thermal stability due to metal oxide networks b) multifunctional sites from simple and single-component metal alkoxide precursors c) simple imprinting processing by chiral self-dimerization and d) surface functionality with achiral reagents [86, 87].

By using special MIP approaches, soluble, single molecule catalytic NPs with proper size and one functional site per NP could be synthesized. In contrast to catalytic antibodies, MIPs having proper functional groups in a predetermined orientation into a cavity of defined shape could yield effectiveness in the terms of sensitivity and selectivity [88].

MIPs supported metal complexes could be interesting for numerous catalytic reactions such as pharmaceuticals and functional molecules synthesis. The integration of different functional units for a selective catalysis is a substantial issue. For example, imprinting of a transition state structure of a reaction and several molecular binding units in a reaction MIP cavity for highly sensitive and selective molecular recognition[89].

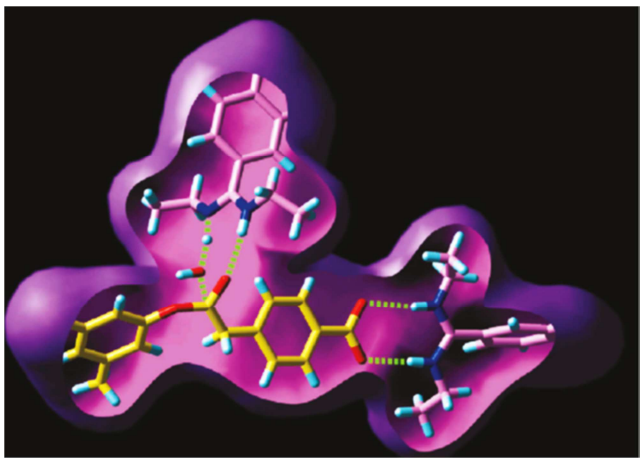

Figure 5. Computer graphic in the Molcad mode of the function of the amidines during the hydrolysis of an ester. One amidinium group binds the additional carboxyl group of the ester and the other group stabilizes the tetrahedral transition state of the ester hydrolysis. (Reprinted with permission from [88] © 2011 American Chemical Society)

5.3. Sol-Gel Matrix

Imprinted sol gel matrix could be superior for selective applications, for example, its use in cigarette filters was able to adsorb ca 11% more nicotine than non-imprinted silica [90]. Organically modified silane precursors (ORMOSILS) for the synthesis of hybrid-MIPs (HMIPs) and their application to surface sol-gel process for imprinting are promising formats [91]. Sol-gel matrix has potential due to mild reaction conditions, processing flexibility, large selection of monomers and cross-linkers and its high surface area [92]. By modifying the precursors, stable and rigid matrix for sensors can be crafted especially for high temperature working environments [93].

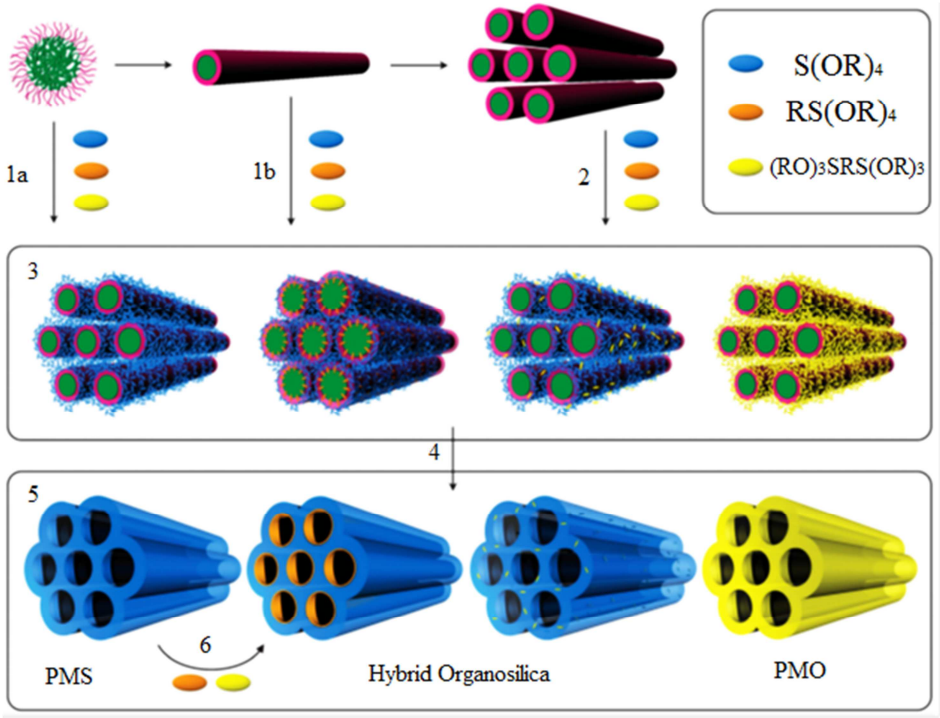

Figure 6. Schematic diagram of the synthetic approach to surfactant-templated mesoporous materials using self-assembly between a template (a typical triblock copolymer) and molecular precursors (blue: tetraalkoxysilane, orange: alkyltrialkoxysilane, yellow: silsesquioxane precursor). (Reprinted with permission from [94] ©The Royal Society of Chemistry 2014).

Shifting from bulk monoliths to NPs to thin films to templated mesoporous materials is advantageous. Diffusion length for template binding governs kinetic binding behaviour and it can be improved by controlling porosity in the form of uniform channels. Commercially available molecular sol-gel precursors are helpful for careful tuning of the imprinting method. Wide range of processing conditions including non-aqueous methods for sol-gel chemistry could yield any imprinting system such as non-covalent, semi-covalent, ionic and coordination imprinting. Covalent imprinting has been ignored due to the energetic cost of binding a target relative to the semi-covalent approach. Morphology of sol-gel materials can be easily controlled regarding diffusion distances down to the nanometer scale as compared to several organic polymer systems employed for molecular imprinting. Orientation of hierarchical imprinting or templating approaches in highly porous molecularly imprinted mesoporous organosilica (MIMO) materials has excellent potential as green chemical system by employing water and alcohol instead of toxic organic solvents. This area could merge successes of eight decades of molecular imprinting and four decades of sol-gel technology to continue the imprinting in organic and inorganic polymers in parallel. Imprinted silica and imprinted mesoporous organosilica have potential towards delicate selectivity and tremendous versatility. For a specialized review on this area refer to citation [94].

To the figure 6, the surfactant forms micelles in solution and its shape (spherical or rod-like) depends on concentration. At low template concentrations, cooperative self-assembly (1a, 1b) between micelles and sol-gel precursors yields the liquid crystalline phase. At high template concentrations, a liquid crystalline micelle phase is formed independently and sol-gel precursors assemble around the micelles on addition to solution (3) Sol-gel via catalyzed hydrolysis and polycondensation followed by template removal (4) produces ordered mesoporous materials of different chemical structure (5) Periodic mesoporous silica (PMS) is pure sol-gel silica. Hybrid organosilica synthesized from co-condensation of a mixtures of precursors (blue + yellow or orange, or yellow+

different yellow or orange (not shown) will have the R' group localized on pore surfaces if it is terminal (orange precursor) or distributed throughout the pore walls if it is bridging between two Si atoms (yellow precursor). It can also be synthesised by grafting an organosilica precursor onto the pore surface of PMS (6) A terminal R' group will dangle into the pore, while a bridging R' group will exist on the surface or dangle into the pore, depending on the grafting method employed. Periodic mesoporous organosilica (PMO) is pureorganosilica in which every silicon atom is bound to a bridging R' group.

6. Sensors, Microfluidic Devices and Relevant Applications

MIMs are not fully established yet and are usually synthesised by UV, thermal or phase inversion techniques. MIMs-based sensors offer high selectivity and stability on comparing to those of biomolecules. Current MIMs-applications focus sensing of water contaminants, drugs, food additives and some molecules in biological fluids. Large biological species or molecules such as viruses are still challenging, these require materials with more flexibility and a higher degree of accessibility [95].

6.1. Biosensors

MIP based array technology can help in commercializing biosensors with a variety of bio-recognition elements for general diagnostic and detection purposes [96]. Novel nanostructure based MIPs, conducting polymers and switchable systems have potential for hybrid devices for new therapeutic, antimicrobial and drug release perspectives [97].

6.2. Piezo-Electric Sensors

This technology is at early stage of development because majority of applications involve the use of buffered and pure solutions rather than real clinical or environmental samples. In piezoelectric sensors, achieving high selectivity is challenging for MIPs for analytes in complex fluids especially in real samples. Overall MIPs' performances and selectivities are poor and donot follow general synthesis-procedure. Optimization of several parameters separately is prerequisite for each template for yielding reproducible and sensor performance [98]. Classical NIPs are attractive for clinical applications of piezo-electric sensors due to their robustness and non-specificity towards real samples [99-104].

Figure 7. A piezoelectric (i.e. QCM with dissipation (QCM-D)) instrument with QCM transducer for laboratory research, world's most robust QCM-D having astonishing fastest stability of baseline and marvelous features, (www.3t-analytik.de). [105, 106].

6.3. Optical Sensors

Novel MIPs via using various optical transduction schemes such as luminescence with time discrimination etc could be interesting for ligand-binding assays of wide range applications [107].

6.4. Electrochemical Sensors

For electrochemical sensors, MIP are attractive due to their stability, compatibility with micro-fabrication and cost-effectiveness. Challenges include general protocol for MIP manufacturing, effectiveness in aqueous media, selectivity and effective immobilization on transducer [108].

Conducting and non-conducting polymers are mainly synthesised by electro-polymerization of electroactive functional monomers such as pristine, derivatized pyrrole, thiophene, aminophenylboronic acid, porphyrin, aniline, phenol, phenylenediamine and thiophenol. MIP immobilization on a transducer surface is still challenging for acrylic- or vinylic-based systems. This can be overcome by deposition of MIP thin films directly on transducers by using electroactive functional monomers effectively. MIPs having electroactive functional monomers are superior over free-radical polymerizated MIPs which are immobilized via drop coating or spin-coating. Proper film layer height can be achieved during electro-polymerization by electrochemical parameters and deposition. Proper solvents and supporting electrolytes tune the viscoelasticity and porosity of the MIPs. They facilitate the charge transport between the electrode substrate and the analyte from MIP cavities. Conductivity of MIP layers can be improved by coupling them with conducting NPs such as Au-NPs or carbon nanotubes. Derivatized electroactive functional monomers containing additional functional groups form stable complexes and π-delocalized with templates during prepolymerization, yield selective ECP-MIPs. ECP-MIPs mainly applied for amino acids, pesticides, drugs and proteins. Non-conducting MIPs are mainly employed for the fabrication of piezo-electric

micro-gravimetry (PM) and capacity chemosensors by employing 1,2-Phenylenediamine (PD) and Phenol (Ph) functional monomers. Nonconducting MIP insulation can be achieved by using self-assembled monolayer (SAMs). SAMs yield improved selectivity and LoD even in the gas phase, which is not possible with normal MIPs [109]. ECP-MIP hybrid approaches are novel for developing highly sophisticated and sensitive devices. Computational or combinatorial tools provide synthetic routes to ECP-MIPs in the perspectives of the conspicuous theoretical and applied knowledge [110]. Graphene (Gr) (an extraordinary carbonaceous material) has potential for the fabrication of electrochemical sensor comprising nanosystems due to its outstanding electrical, physical and thermal properties. Their MIP composites are highly selective for real sample applications [111].

6.5. Microfluidic Devices

MIPs with microfluidic devices have yielded improved capturing efficiency and response times via reducing diffusion. These devices have simple microfluidic architectures via employing surface imprinting approach and require lower sample volumes and easy sample handling [112].

6.6. Thermistor

Thermal biosensors and micro-biosensors in flow injection analysis are mainly based on enzyme thermistor operating with an enzyme column. Other miniaturised devices such as thermal lab-on-chip and MIP based devices for both affinity and catalytic reactions are interesting too [113].

7. Separation (TLC, HPLC, Capillary, Affinity Capillary Electrophoresis) Applications and Chromatographic Characterization

Cheong et al [114] have contributed a specialized review of reviews on MIP applications in separation science. In separation science, MIPs have been established into different formats such as irregularly ground particles, regular spherical particles, NPs, monoliths in a stainless steel or capillary column, open tubular films in capillaries, surface attached thin films, membranes, and composites etc [115-123]. The reviews of last 10 years have been categorized into subgroups and each group demonstrates brief summaries, comments and different scopes with future of prospects.

7.1. General

Successful area of general applications is MIP-chiral stationary phases (MIP-CSPs), which is applied in most chiral analysis techniques for drugs. For affinity separations especially in HPLC and SPE, MIPs are interesting alternative to traditional stationary phase media. MIPs of structurally related analogues can be used for ultra-trace analysis of the targets.

7.2. MIP Characterization, Evaluation and Optimization

Characterization of MIP materials is done by Brunauer-Emmett-Teller (BET) (surface area), Barrett-Joyner-Halenda (BJH) (pore size distribution), FT-IR, solid-state NMR (functional group incorporation, DP) and volumetric methods (MIP swelling). Most applied template to functional monomer ratio is 1:4, functional monomer employed is methacrylic acid (MAA) or 4-vinylpyridine and cross-linker being ethylene glycol dimethacrylate (EGDMA). Initiators have been used at low concentrations e.g. 1 wt% or 1 mol% and 60°C temperature for thermal polymerization. The peak properties of a MIP column such as tailing, retention and broadening can be explained by nonlinear chromatographic-theory.

7.3. Chromatography

For analytical purposes, the monolithic MIP capillary columns are superior to the conventional MIP columns such as ground or sieved bulk MIPs-based. Mono-dispersed MIP spheres and composite MIP beads have proven better media for large-scale separations. Furthermore they have potential for further modifications and improvements for efficient analytical separation. For example, for isolation of aflatoxins can be achieved via novel design and improved separation efficiency, which is not possible by using conventional MIP columns [124].

7.4. CEC Applications

CEC is essentially a hybrid separation technique that combines partition based selectivity of LC and high separation efficiency of CE. The literature mainly focus the applications of MIPs in different formats including packed particles [125, 126], in situ monoliths [127-129], coatings [130], and NPs as pseudo-stationary phases. CEC enhances column efficiency on comparing to that of HPLC. This is due to electrically driven flow with a uniform flow velocity profile across the column diameter, for example in the cases of monolithic MIP-type porous layer open tubular columns. The partial filling CEC technique using MIP-NPs is promising for screening applications. Various approaches for preparation of MIP-CEC capillaries as well as their

applications have been developed and applied. More research is needed in MIP-CEC, especially, on development of sorbents having superior selectivity and strong electro-osmotic flow, quantitative tools for estimating MIP recognition processes, and methods for generation of less multi-clonal binding sites [131].

7.5. SPE and Micro-Extraction-Sample Preparation

SPE-MIPs are mostly employed in on-line or off-line procedure in formats of mini-columns, knotted reactors, membranes, renewable beads, disks, or cartridges based on the analytical goal and flow manifold. Sample preparation is the backbone of the analytical process. Some molecule-imprinted solid-phase extraction (MISPE) cartridges are commercially available in the market for extraction purpose. MAA is mostly employed monomer in bulk polymerization, which limits MISPE application to analytes having interaction by hydrogen bonding with MAA [132]. Proper template structure is prerequisite for MISPE methods for the simultaneous extraction of structurally relevant analytes. For sample screening for whole analyte classes in the cases of extraction of food contaminants, analytes from clinical, environmental and pharmaceutical matrices [133, 134]. The "dummy" imprinting can reduce the bleeding of analyte on compromising with the selectivity. Use of stable-isotope-labelled compounds as templates could eliminate the leakage of template. Furthermore, heating the MIPs and then eluting them with a strongly polar solvent is helpful [135]. Fundamental understanding of the intermolecular interactions among the molecular building blocks during imprinting is required for rational designing of next-generation MIP technology [136]. Success of MIP technology is connected with the introduction of miniaturized analytical systems to utilize smallest sample volumes into laboratory practice with modification of the MIP formats [137, 138].

MIP-NPs and monolith are attractive for sample preparation. Bulk polymerization yields irregular-shape NPs after grinding and sieving of MIP monoliths, but still it is first priority for preparation of MIPs for sample preparation. Modes of MISPE are off line mode, online mode, on-column extraction, SPME and MEPS. Offline MISPE is employed for sample preparation because of no restriction for the selection of washing and eluting solvents, while concentrated or reconstituted elutes are employed for the separation and detection of an analyte in GC, LC or CE [139]. MIPs for wider range of mycotoxins have low selectivity in the aqueous media on compared to natural antibodies. Mycotoxins are costly for the large-scale synthesis of MIPs [140]. Direct coupling of MIP columns in-line with detectors could simplify the routine laboratory processes [141-144].

Stir-bar sorptive extraction (SBSE) is the most widely used technique for the extraction and pre-concentration of pollutants in sorbent micro-extraction, while hollow fiber-protected micro-extraction (HFME) is mostly used in liquid phase micro-extraction (LPME) [145]. Future work on MISPME will target on MIPs for selectivity and sensitivity for a wide range of analytes and performance of established MISPME techniques for implementation of MISPME in analytical laboratories [146]. MISPE is more efficient for extraction and cleanup because of better selectivity on comparing with classical SPE. MIP-based SPE columns have advantages of re-usability without any deterioration, lower organic solvent consumption and cost-effectiveness. MISPEs are mostly used in offline mode for applications in herbicides, fungicides and pesticides. In online mode, purification, pre-concentration and separation are done in only one MIP column and it provides higher accuracy and easy automation. Online MISPE coupled GC or HPLC limits its application in the case of multiresidue analysis of agrochemicals. Multi-template MIPs could be a solution for analysis of agrochemicals [147].

7.6. Specific MIP-Formats

Monolithic MIP stationary phases are simple and cost-effective as compared to other MIP formats. Precipitation or suspension or emulsion polymerized MIPs coupled with surface imprinted MIPs, are attractive for industrial applications. Monoclonal binding with enhanced recognition-selectivity for core-cross-linked NPs can be generated via mini-emulsion polymerization. MIP-NPs format is suitable with surface imprinting for in-vitro assays with bulk enzyme-conjugated probes.

7.7. Future Perspectives

MIPs for analytical separations are producing useful commercial products especially in SPE. In the perspectives of literature, HPLC and SPE applications are mainly focused followed by capillary electro-chromatography (CEC), then open tubular capillary electro-chromatography (OT-CEC) most recently. In performance perspectives, MIP-OT-CEC is the most promising because of cost-effectiveness and simple synthesis procedure. Capillary columns' superior performance is due to enhanced mass transfer kinetics and reduced heterogeneity of binding cavities. The MIP-OT-CEC has not received attention in literature. MIP selectivity is the most effective in the solvent that has been used in MIP synthesis. First choice of porogen is ACN in the most cases because separation sciences are relying on ACN-based mixed solvents. Improved separation efficiency could be achieved by improving molecular recognition, reducing site heterogeneity, better access to the site and enhancing mass

transfer kinetics to eliminate band broadening. A less rigorously prepared MIP can be applied for SPE. MIP-HPLC application for analytical purposes is losing its repute because the separation performance is inferior to that of CEC, while HPLC is superior in preparative scale separation. In HPLC, the monolithic columns have proven for better separation performance than the packed columns. Monolithic columns suffer serious disadvantage of difficult and time-consuming template removal procedure for their industrial application. Large-scale separation with MIP has potential market such as chiral separations in pharmaceutical industry. MIP-HPLC at industrial application is promising in the perspectives of utilizing well-prepared MIP-NPs for packing. Preparative HPLC is possible with mono-dispersed MIP-NPs production via suspension or emulsion polymerization and surface imprinted MIP beads. This area suffers a problem of poor separation efficiency because of agitating action during polymerization and end result of loose or heterogeneous (multi-clonal) cavities. Future progress depends on a deep understanding of MIP-related phenomena, novel functional and cross-linking monomers, algorithms in efficient selection of MIP components and reaction conditions, and advances of relevant techniques such as nanotechnology, microfabrication technology, support materials for surface-imprinted beads, membranes and sensing transducer elements [148].

8. Miscellaneous Advanced MIP Formats

8.1. Stoichiometric Non-Covalent Interaction

Stoichiometric non-covalent interaction demonstrates a useful method for template binding during the molecular imprinting. This method could introduce binding site functional groups inside the imprinted cavities. 98% template re-uptake to the MIP is possible in contrast to non-stoichiometric non-covalent interaction. Stoichiometric non-covalent interaction has advantage of covalent and non-covalent interaction without corresponding drawbacks such as catalysis and separations with higher loading. By using low-molecular weight model substances, the thermodynamics of the association can be investigated via NMR spectroscopy [149].

8.2. Living Radical Polymerization

Only few reports cover designing mineral matrices with imprints. The approach of the surface functionalization by multipoint fixation of complex molecules is confined to some specific cases. Sorbents synthesised via the modification of areas left unoccupied after the template adsorption have limited applications, because the imprints are complementary to the templates on the basis of their geometry. The materials synthesized by polycondensation of siloxanes or polymerisation of organic monomers in the support pores in the presence of template molecules are promising [150].

The use of living radical polymerization (LRP) for MIP synthesis leads to improved binding and transport properties due to improved network architecture, morphology and decreased dead regions in crosslinked polymer networks by controlling polymer chain growth. Minimizing heterogeneity of MIPs with finely controlled architecture is prerequisite for industrial applications [151].

8.3. Deep Desulfurization of Fuel Oils

Recently, inorganics such as silica gel, TiO_2, K_2 Ti_4 O_9 and carbon microspheres have been employed as supports to prepare surface MIPs (SMIPs) for adsorbing dibenzothiophene and benzothiophene. This new approach creates specific recognition sites on the MIP-surface for increasing the adsorption efficiency, removal and rebinding of sulfur bearing molecules. Raw materials or the influence of poisonous remainders of MIPs on the environment is unresolved issue for green chemistry. For example, MAA, ABIN and chloroform etc., are poisonous or hazardous to life. The types and proportion of reactants should be optimized for cost-effectiveness, efficiency and safe synthesis of SMIPs. Characterization of binding sites between SMIPs and template is substantial to cope these challenges [152].

8.4. Signalling MIPs

Signalling functions to MIPs enable them to respond according to specific binding events. Cinchonidine-imprinting by using 2-(trifluoromethyl) acrylic acid (TFMAA) can shift the fluorescent spectra of cinchonidine on being bounded by the polymers. Such MIPs are interesting in simplified analytical applications for avoiding bound-free separation steps by direct monitoring of the fluorescence spectra shift of the template binding. Novel competitive binding assays for various targets could be established via using different fluorescent or fluorescence-labelled molecules. The signalling MIPs prepared from fluorescent monomers are capable of the formation of multiple hydrogen bonding e.g. 2,6-bis(acrylamido)pyridine and 2-acrylamidoquinoline yield enhanced fluorescence intensity by the specific binding of a template. Fluorescence monomers having low background and enhanced emission wavelength shifts due to the binding events, are prerequisite for this area. Many templates possessing coordination to metalloporphyrins yield no characteristic of absorbance or fluorescence. Present metalloporphyrin-based readout systems perform specific detection for several templates

which have no significant spectroscopic properties. Signalling MIPs could be interesting for new types of stable, selective and sensitive sensors for biomolecules [153].

8.5. Photo-Polymerization

Photo-induced polymerization is comparatively more appropriate for non-covalent imprinting of synthetic polymers because of suitability at low temperature and imprinting efficiency. MIP polymerization via optical methods has potential and is comparatively new as compared to counterpart strategies such as soft lithography or mechanical micro-spotting.

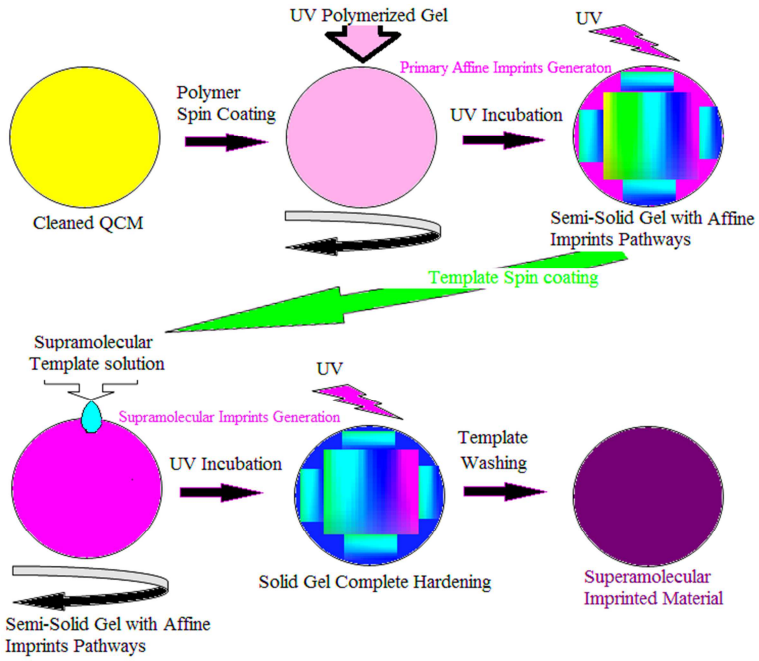

Figure 8. Supramolecular (e.g. heparin) imprinting using photo-polymerization [154]

Contact and proximity printing, micro-stereo-lithography, projection photo-lithography and near-field assisted optical lithography could be easily merged with MIPs. This could yield innovative high-resolution approaches with improved specificity and selectivity [155].

8.6. Tailor-Made Receptors

Different categories of cyclodextrins (a-, b-, and c-CyDs) having different cavity sizes could be merged with other host molecules (calixarene, cyclophane and crown ether) for better recognition. Hydrogen-bonding sites incorporation to the polymeric receptors could yield more precised recognition. Hydrogen bonds could be formed in aqueous media via regulation of chemical environments. Molecular conformations in the host-guest adducts could be exactly immobilized in the polymeric receptors via photo-polymerization at low temperature [156].

8.7. MIP-Nanomaterials

MIP nanomaterials such as NPs, core-shell/hollow NPs, nanowires, nanotubes and nanofilms can be generated via controlling nanotechnologies and surface chemistry. This approach leads to improved removal of templates, the binding capacities, kinetics of molecular recognition, higher physical/chemical stability and a better engineering possibility. The challenges are: a) improvement in selectivity by the design of MIP nanostructure, b) protocols for MIP nanomaterials having uniform shape and size, c) MIP nanoarrays engineering with transducer d) multisensors manufacturing with multiplexing capabilities and integration through the use of nanofabrication [157].

The MIP-NPs selectivity in electrochemical and optical sensors can be improved by improving their binding to the transducer and developing a straightforward understanding of the primary signal transduction mechanism [158]. Enzyme, DNA, RNA, catalytic antibody, aptamer, or labelled biomolecule could be merged in nanostructured films for assembling different properties in the same device such as optical, electrical or electrochemical [159]. Recent efforts are to impart MIP-NPs especially silica NPs, magnetic NPs, Au NPs, and QDs with additional capabilities by introducing NPs size-control, stimuli-responsiveness, biocompatibility and optoelectronic properties [160].

8.8. Rational, Computational, Theoretical and Coarse-Grained

Rational studies on imprinting started with the birth of MIP technology in different domains [161, 162] and are still

increasing dramatically with the development of computer hardware and software technologies [163]. Parameters of MIP synthesis such as the affinity on functional and crosslinking concentration, buffer nature, pH, ionic strength and temperature need more studies [164].

The current applications of computational and theoretical techniques for describing, predicting and analysing MIP systems are increasing in therapeutics or medical devices [165]. The computational models in the MIPs' design can be categorized into two approaches namely screening for possible functional monomers for a template and macromolecular models for the structural characterization of the MIPs. The computational models are based on molecular mechanics calculations of low energy pre-polymerization complexation between the template and functional monomers for screening the most suitable monomers for a specific template. The computational models are helpful but lack important aspects of the MIPs such as functional site heterogeneity, template aggregation and restructuring of the imprinted pore due to the limitation of computational power. Coarse-grained models give substantial feedback such as the intricate balance between stoichiometry, equilibrium versus kinetics and MIP performance for improvement of its design especially for imprinting of macromolecules (e.g. proteins). The role of molecular shape on MIP selectivity is ignored area in both experiments and theory [166].

For challenging proteins templates, the successful approach employs small to medium peptides as the template, called as an epitope imprinting. These selected epitopes are linear peptides and similar in sequence to the terminal peptide chains of the target protein. This is a narrow definition of epitope in the perspective of the regions of proteins recognised by antibodies. In the approach for designing epitope templates, the recognition properties for surface regions or loops of the target can be interesting but it is unexplored. The entrapment and covalent immobilisation of bulk imprinting of proteins demonstrate that only surface imprinting approaches, at interfaces or by stamping or printing approaches, can beat those based on epitope imprinting. For polymer systems, soft hydrogels are unsuitable for rigid systems especially in sensor applications. Nano-structured MIP materials can be called as direct replacements for natural antibodies, these have been demonstrated to act as such in vivo. For epitope imprinting selection of monomer can be handled with computational and combinatorial. In major cases a small amount of ionisable or ionised monomer enhances selectivity and specificity, while higher amounts lead to detrimental effect. The majority of functional monomers employed are neutral such as acrylamide could impart recognition properties for a wide range of peptide and protein templates. For the sensing, a reagentless sensor platform is preferable for proteins and it involves least form of liquid handling. Transducers such as SPR and QCM-based sensors and their allied techniques are promising, while sensitive and compact electrochemical detectors are attractive for electroactive proteins but very few proteins are likely to be redox active for such detectors. Other electrical properties, such as capacitance or impedance, can be employed for the sensitivity purpose. For a specialized review on this area refer to citation [167].

Protein imprinting for rational studies still faces challenges such as obtaining the template, polymerization and rebinding in aqueous media, heterogeneous binding sites and compatibility. This area needs 1) development of mechanisms of epitope recognition 2) design and synthesis of working compatible MIPs in aqueous media; and 3) new MIP methods and formats to reduce non-selective binding [168].

For new generation of columns such as monolithic or stationary phase columns' performance can be enhanced by optimization of the polymerization and chromatographic conditions from theoretical calculations. The challenge is to prepare monolithic MIPs with a homogeneous format of binding sites similar to monoclonal antibodies. In addition to hydrogen bonding more interactions such as hydrophobic and ionic contributions between templates and functional monomers should be included in the synthesized systems [169].

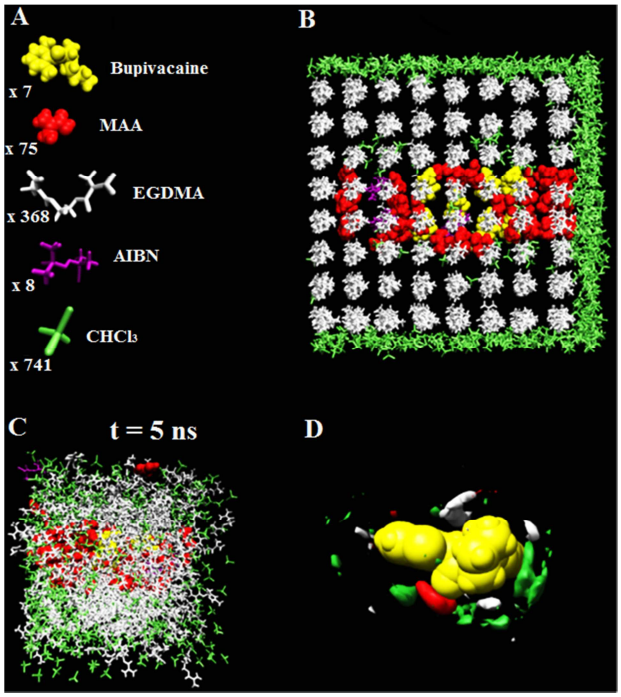

Figure 9. Schematic representation of the steps involved in a typical molecular dynamics simulation of a pre-polymerisation mixture. MIP system having bupivacaine, methacrylic acid (MAA) and ethylene dimethacrylate (EGDMA) in chloroform. (A) Components in the model (B) Model setup (C) Pre-polymerization mixture after equilibration and a 5 ns production phase (D) Statistical analysis of the different pre-polymerization complexes in the reaction. (Reprinted with permission from [163] ©2009 Elsevier).

9. Outlook and Remarks

MIP technology is attractive due to its potential for versatility, sensitivity, reusability, easy production, robustness, cost-effectiveness, high capacity, long life, thermal stability, resistance to harsh environment, chemical inertness and long shelf life at room temperature and humid conditions. MIPs applications for small-molecular-weight analytes species with molecular weight <1500 are successful areas, while their extension to large species regime suffers infancy and challenging for industrial applications. Magnetic NPs, silica NPs, nanowires, quantum dots (QDs) and carbon nanotubes are promising emerging materials for protein and bio-macromolecules imprinting. Rational, computational, theoretical and coarse-grained are helpful for challenging large species e.g. proteins via epitope imprinting. Surface imprinting approaches, at interfaces or by stamping or printing approaches, can beat those based on epitope imprinting.

MIPs for separation (TLC, HPLC, capillary and affinity capillary electrophoresis) applications and chromatographic characterization are the most successful and developed applications. This area has entered into *'the real world'* of industrial applications due to different formats such as irregularly ground particles, regular spherical particles, NPs, monoliths in a stainless steel or capillary column, open tubular films in capillaries, surface attached thin films, membranes and composites etc. In this regard, successful area of general applications is 'MIP-chiral stationary phases' (MIP-CSPs), which is applied in most chiral analysis techniques for drugs. For affinity separations, especially in HPLC and SPE, MIPs are interesting alternative to traditional stationary phase media. MIPs for analytical separations are producing useful commercial products especially in SPE. MIP-based SPE columns have advantages of re-usability without any deterioration, lower organic solvent consumption and cost-effectiveness. Molecule-imprinted solid-phase extractions (MISPEs) are mostly used in offline mode for applications in herbicides, fungicides and pesticides. Stir-bar sorptive extraction (SBSE) is the most widely used technique for the extraction and preconcentration of pollutants in sorbent micro-extraction, while hollow fiber-protected micro-extraction (HFME) is mostly used in liquid phase micro-extraction (LPME). HPLC and SPE applications are mainly focused in literature, followed by capillary electro-chromatography (CEC), then open tubular capillary electro-chromatography (OT-CEC) most recently. In performance perspectives, MIP-OT-CEC is the most promising because of cost-effectiveness and simple synthesis procedure. MIP selectivity is the most effective in the solvent that has been used in MIP synthesis. First choice of porogen is 'acetonitrile' (ACN) in the most cases because separation sciences are relying on ACN-based mixed solvents. Capillary columns' superior performance is due to enhanced mass transfer kinetics and reduced heterogeneity of binding cavities.

Further extensions of MIP-nanostructures to bio-applications, biomedical, clinical, drugs and pharmaceutical applications such as drug delivery systems, are paving path to industrial applications. Soft contact lenses can improve the bioavailability and can prolong the residence time of drugs. These are astonishing drug carriers for ophthalmic drug delivery. Molecular imprints can be helpful to target for delivering given drug to cancer cells and to increase their nuclear and cancer killing abilities. This could be achieved *via* localization of a synthesized MIP on the immune system.

MIPs for environmental applications, waste water treatment and food analysis have entered for industrial scale applications, such as MIP-based SPE cartridges are commercially available now. Ion-imprinted polymers (IIPs) contributed excellently to the sample preparation part of analytical chemistry. IIPs play substantial role in quantitative analysis of low concentrations of toxicants such as arsenic, selenium, copper, nickel, cobalt, aluminium and complexes of these elements and beyond. Enantioselective MIPs are extensively used as efficient scavengers for refinement of chiral intermediates from industrial production streams.

MIPs for sensor applications such as SPR and piezoelectric sensors (e.g. QCM-based sensors) and their allied techniques are promising. QCM-D is attaining popularity due to its robustness, fast stability of baseline and uniqueness of information. Sensitive and compact electrochemical detectors are attractive for electroactive proteins, but very few proteins are likely to be redox active for such detectors. MIPs having electroactive functional monomers are superior over free-radical polymerizated MIPs which are immobilized via drop coating or spin-coating. Molecularly imprinted sorbent assays (MIAs) kits and microfluidic devices are emerging relevant applications.

Merging MIPs with inorganics such as graphene (an extraordinary carbonaceous material), have potential for the fabrication of electrochemical sensor comprising nanosystems due to their outstanding electrical, physical and thermal properties. Similarly, MIP-Silane composites (sol-gel matrixes) are highly selective for real sample applications. Sol-gel matrix has potential due to its mild reaction conditions, processing flexibility, large selection of monomers and cross-linkers and its high surface area. Wide range of processing conditions including non-aqueous methods for sol-gel chemistry could yield any imprinting

system, such as non-covalent, semi-covalent, ionic and coordination imprinting. Orientation of hierarchical imprinting or templating approaches in highly porous molecularly imprinted mesoporous organosilica (MIMO) materials has excellent potential as green chemical system by employing water and alcohol instead of toxic organic solvents. Recently, inorganics such as silica gel, TiO_2, K_2 Ti_4 O_9 and carbon microspheres have been employed as supports to prepare surface MIPs (SMIPs) for adsorbing dibenzothiophene and benzothiophene. This new approach creates specific recognition sites on the MIP-surface for increasing the adsorption efficiency, removal and rebinding of sulfur bearing molecules.

Coordination chemistry and active site engineering in synthetic MIPs share synergy. MIPs supported metal complexes could be interesting for numerous catalytic reactions such as pharmaceuticals and functional molecules synthesis.

Photo-induced polymerization is comparatively more appropriate for non-covalent imprinting of synthetic polymers because of suitability at low temperature and imprinting efficiency. Contact and proximity printing, microstereo-lithography, projection photo-lithography and near-field assisted optical lithography could be easily merged with MIPs. This could yield innovative high-resolution approaches with improved specificity and selectivity.

The use of living radical polymerization (LRP) for MIP synthesis leads to improved binding and transport properties due to improved network architecture, morphology and decreased dead regions in crosslinked polymer networks by controlling polymer chain growth.

Signaling MIPs could be interesting for new types of stable, selective and sensitive sensors for biomolecules

GLP and GMP for robust, straightforward, reproducible, practicable, sensitive and highly selective approaches are prerequisite to cross barrier of the *'world of real samples'* and industrial applications. UV/VIS spectrophotometry, FT-IR, NMR, thermal methods, chromatography (LC-MS) and instruments for morphological analysis have recently got attention for GLP and GMP. Shorter analysis times and cheaper instrumentation in the resulting method should be priorities on industrial scale applications.

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