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Molecular imprinting in sol–gel matrix

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ABSTRACT

Molecular imprinting is a newly developed metho[illegible]ogy wh[illegible]rovides mo[illegible]ular assemblies of desired structures and properties and is being increasingly[illegible]ed for several[illegible]plication[illegible]uch as in separation processes, microreactors, immunoassays and antibody mi[illegible]talysis, artificial[illegible]s, biosensor recognition elements and bio- and chemo-sensors. The ambient pr[illegible]ssing c[illegible]ditions and versa[illegible]ity of the sol–gel process makes sol–gel glassy matrix suitable for molecular imprinting. The p[illegible]ress of sol–gel based molecular imprinted polymers (MIPs) for various applications can be se[illegible]m the growing [illegible]ber of publications. The main focus of the review is molecular imprinting in sol–gel [illegible]trix and applications of m[illegible]ecular imprinted sol–gel derived materials for the development of sensors. Com[illegible]ing sol–gel process with molecular imprinting enables to procure the sensors with greater sensitivity and selec[illegible]ity necessary fo[illegible]ensing applications. The merits, problems, challenges and factors affecting molecular impri[illegible]g in sol–gel m[illegible]ix have been discussed. Considerable attention has been drawn on recent developments like[illegible]e of organically modified silane precursors (ORMOSILS) for the synthesis of hybrid molecular im[illegible]ted polymers ([illegible]IPs) a[illegible]pplying surface sol–gel process for molecular imprinting. The development of mo[illegible]printed sol–gel nanotubes for biochemical separation and bio-imprinting is a new advancement and is u[illegible]er prog[illegible]templated xerogels and molecularly imprinted sol–gel films provide a good platform for various se[illegible]or ar[illegible]cation[illegible]

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1. Molecular imprinting

The concept of molecular imprinting based on molecular interaction is very old but their applications in various fields are emerging recently particularly for sensor applications. The initial examples of molecular imprinting by use of synthetic organic polymers were independently reported by Kiefer et al. (1972) and Wulff and Sarhan (1972) and have since then found applications in separation processes (chromatography, capillary electrophoresis, solid phase extraction (SPE), membrane separations), microreactors, immunoassays and antibody mimics, catalysis and artificial enzymes, biosensor recognition elements and bio- and chemo-sensors (Takeuchi and Haginaka, 1999; Piletsky et al., 2001; Sellergren, 2001). The field of molecularly imprinted polymers (MIPs) will evolve further to include new applications such as recognition elements in intelligent drug delivery devices, in targeted drug delivery applications and in microfluidics devices with applications as analyte sensing micro-valves and microactuators (Mitchell, 2001). The molecular imprinting technique can be applied to different kinds of target molecules, ranging from small organic molecules (eg. pharmaceuticals, pesticides, aminoacids and peptides, nucleotide bases, steroids and sugars) to polypeptides (Andersson et al., 1995; Rachkov and Minoura, 2001), high molecular proteins (Kempe et al., 1995; Shi et al., 1999) and even whole cells (Alexander et al., 2003). The principle underlying molecular imprinting is the assembly of a cross-linked polymer matrix around a template; when the template is removed, recognition sites are created which should be complementary to the template. The principle of molecular imprinting is shown in Fig. 1. Generally, the fabrication of MIPs consists of three main steps (i) pre-arrangement of the monomers around the target molecule, (ii) polymerization in the presence of cross-linker and (iii) removal of the target molecul extraction process. These MIPs can be stable in various cri chemical and physical conditions for a long time and reused witho any alteration to the memory of the template (Kriz and Mosbach 1995; Ciardelli et al., 2006). Usually MIPs have been p in the form of a macroporous monolith, then ground d sieve to the required particle dimensions. Recent improvem ts in the rphology of MIP particles have been achieved usi a ecipitat polymerization procedure that allows to o in micro nanospheres with regular size and shape and partic y able to reb effectively template molecule due to the hig sur /volume rat (Ye and Mosbach, 2001). The use of imprinted films in sor development has attracted a lot of interest rec y because of fa r rate of diffusion through surface and resul into high sensitivit imprinted films also possess high phys and ch ical stability with respect to immobilized biomateri s th ecognition part. Bulk imprinted polymers have been emplo sensors a orous materials. But this design suffered g diff n tim of the target molecule into the polymer n recen years, s groups have dealt with the applicatio imprint films as recognition layers applied on various transducti yste for e , piezoelectric, amperometric, surface plasmon r ance (SPR), fluorimetric and field effect transistors (FET) (Marx and on, 2001). These imprinted films can be *in situ* synthesized at an electrode surface by electropolymerization (Malitesta et al., 1999) or at a non-conducting surface by chemical grafting (Piletsky et al., 2000) or in sandwich configuration (Haupt et al., 1999).

1.1. Noncovalent and covalent imprinting

The molecular imprinting technology is a valid alternative to molecular recognition system present in biological systems, such as those activated by antibodies (Wulff, 1995). MIPs are quite different from antibodies due to their large size, rigidity and insolubility as compared to small size, flexibility and solubility of antibodies except the one common property of ability to selectively bind a target molecule that is why MIPs can be emplo a in unoassay-type binding assays in place of antibodies (upt, 2003). is was first demonstrated by Mosbach's group w a MIP-based ay for the bronchodilator theophylline and th tran illiser diazep n (Vlatakis et al., 1993). The association tween th mprint olecule and monomers is based on two t es of interaction non-covalent and covalent interactions. Th on-coval interacti s occur between specific functional gr ups th polymeri able monomers in a specific spatial ori tation p to poly rization and may be hydrogen bonds ic bonds, hy phob interactions and van der waals forces e Th non-covalent oach has been pioneered by Mosbach (1994). After lymerization and removal of the template, the functional groups of polymeric matrix can then rebind the targe via the same non-cov nt interactions. The limitation of this tec ique is that the template and target must form a sufficient nu ber of non-c alent intermolecular interactions to generate bin g pockets d ring polymerization. Thus, the non-covalent appr h cannot b used for template molecules that lack functional groups r e for strong noncovalent interactions. This limitation be overcome by covalent approach, which utilizes reversible c a bonding between a polymerizable monomer and a template olecule. After polymerization, these bonds are cleaved to liberate the template, but they are subsequently reformed in order to selectively bond the target. Specificity is achieved by the exact location of the reactive groups on the polymer initially positioned by the template. This covalent imprinting technique leads to strong interactions as a result of reformation of the covalent bond between the matrix and the target, but limited by the relatively small number of useful reversible covalent bonding reactions that can be utilized (Wulff, 1995; Shea, 1994). Graham et al. (2002) examined both non-covalent and covalent approach for molecular imprinting of dichloro-diphenyl-trichloroethane (DDT) in sol–gel material and found non-covalent approach unsuccessful because of the lack of strong intermolecular interactions between the DDT and the sol–gel precursors. In 1995, Whitecombe has proposed a new approach that combined the advantages of both covalent and noncovalent imprinting called hybrid molecular imprinting strategy (Whitecombe et al., 1995). In this hybrid strategy, target molecule is imprinted as a stable complex with the functional monomers formed via covalent interactions, whereas upon later use of the MIP, only non-covalent interactions come into play. Following this approach, Klein et al. (1999) have reported the imprinting of a

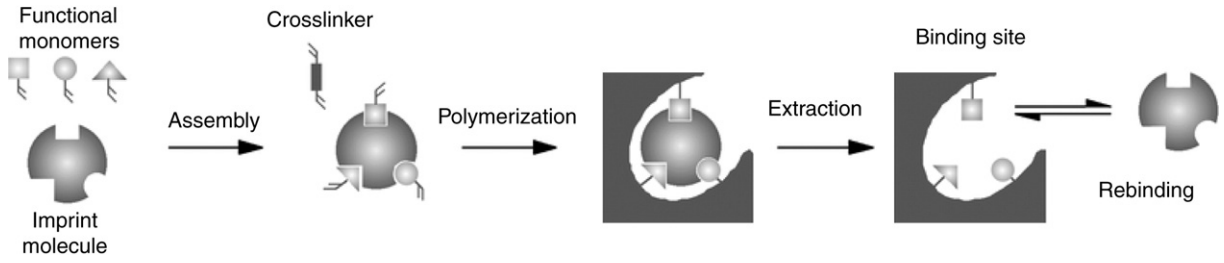


Fig. 1. Schematic representation of molecular imprinting.

tripeptide (Lys-Trp-Asp) using a sacrificial spacer (o-hydroxybenzamide) between imprint molecule and monomer. After polymerization, the covalent bonds between the imprint molecule and the monomers are hydrolyzed leaving precisely positioned carboxy groups. During rebinding, the peptide interacts with the polymer only via non-covalent interactions (Fig. 2).

1.2. Problems and challenges in molecular imprinting

Compared with antibodies, enzymes or biologicals receptors, molecularly imprinted materials possess inherent advantages viz. robustness, low cost and potential utility in cases where no recognizing biomolecule is available. Besides many advantages, molecular imprinting technology still needs to overcome few limitations such as template leakage, poor accessibility of the binding sites, low binding capacity and non-specific binding (Ansell et al., 1996). Since biological recognition mainly occurs in aqueous systems, it is quite important to make MIPs capable of recognition in water in order to mimic biomolecules. Generally MIPs prepared by polymerizing in relatively non-polar organic solvent, exhibit better recognition properties than those prepared using a polar organic solvent. The development of a method for making efficient MIPs in polar organic solvents is of general interest (Yu and Mosbach, 1997). The applications of imprinted polymers are also restricted to the use of organic solvents for the dissolution of organic polymers viz. acrylate or acrylic type polymers which are most commonly used for the preparation of MIPs (Haupt, 2003). Template molecules that are only soluble in aqueous phases are generally not amenable to imprinting in such a manner. Imprinting of larger molecules like polypeptides and proteins is still a challenge requires well-defined protocols. The hydrophobic nature and highly cross-linked structure of the polymer limit access of the imprinting binding sites by the large proteins. The molecular imprinting of enzymes is also less practical, because of requirement of aqueous buffer for the biological activity of the enzymes (Haupt, 2003). The preparation of MIPs in aqueous system very challenging task because of the interference of water molecules. Non-specific interactions between the template and functional monomers or functional oligomers or polymers may also become much weaker or even disappear in aqueous system. Further, many biomolecules are often insoluble or lose activities in organic solvents (Ramström and Ansell, 1998). Thus it is of great importance to develop imprinted materials that can be used in aqueous environment.

Research is going on to overcome these limitations and thrust is to find out new polymeric matrix for molecular imprinting that can be used for environmental and biological applications. Sol–gel imprinting is also one of new emerging field in area of molecular imprinting due to straightforward synthesis of sol–gel materials and particularly useful for the various applications. Further, the availability of a range of pure functional monomers and organically modified silane precursors (ORMOSILS) makes their use in molecular imprinting more attractive.

2. Molecular imprinting in sol–gel matrix

Molecular recognition is the basic concept in nature. Pauling (1940) was the first person to discuss the lock and key mechanism to explain molecular recognition. This conception has been translated into MIPs. Polymeric materials with specific sites are producible through the use of various templates (Wulff, 1995; Mosbach and Ramström, 1996). In literature, mostly MIPs were derived from organic polymers synthesized from vinyl or acrylic monomers by radical polymerization and using non-covalent interaction. These monomers can be basic e.g., vinylpyridine or acidic e.g. methacrylic acid, permanently charged e.g. 3-acrylamidopropyltrimethylammonium chloride, hydrogen bonded e.g. acrylamide, hydrophobic like styrene or metal coordinating etc. (Haupt, 2003). There are very limited reports where inorganic molecularly imprinted polymers (IMIPs) are being used. In 1949, Dickey observed that adsorption of template methyl orange was more on imprinted silica gel as compared to non-imprinted gel. This publication was appeared as the first documented demonstration of molecular imprinting in sol–gel matrix (Dickey, 1949). Pinel et al. (1997) used sol–gel chemistry to generate imprinted gel with (−) menthol as template. In contrast to Dickey's observation, there was no difference between imprinted and non-imprinted gel for the adsorption of template molecule. Hunnius et al. (1999) used sol–gel process to prepare new catalyst materials. Interestingly, they observed that the amorphous microporous oxide remembered the kinetic diameter of imprinted molecule (alcohol). His investigation provided another door for molecular imprinting to enter into inorganic matrices particularly sol–gel materials.

2.1. Sol–gel process

The sol–gel process is a convenient and versatile method of preparing transparent optical materials at ambient processing conditions and also enables entrapment of numerous organic, organometallic and biological molecules (proteins and enzymes) within microporous network of sol–gel derived matrix. The dopant entrapped inside the sol–gel matrix can interact with other molecules or surrounding physical environment. These interactions are easily monitored by appropriate optical methods (absorption and fluorescence). In general, the sol–gel process involves the transition of a system from a liquid 'sol' (mostly colloidal) into a solid 'gel' phase. The schematic of sol–gel process and various products and sol–gel reactions are shown in Figs. 3 and 4, respectively. The starting materials used in the preparation of the sol are usually inorganic metal salts or metal organic compounds such as metal alkoxides [M(OR)n]

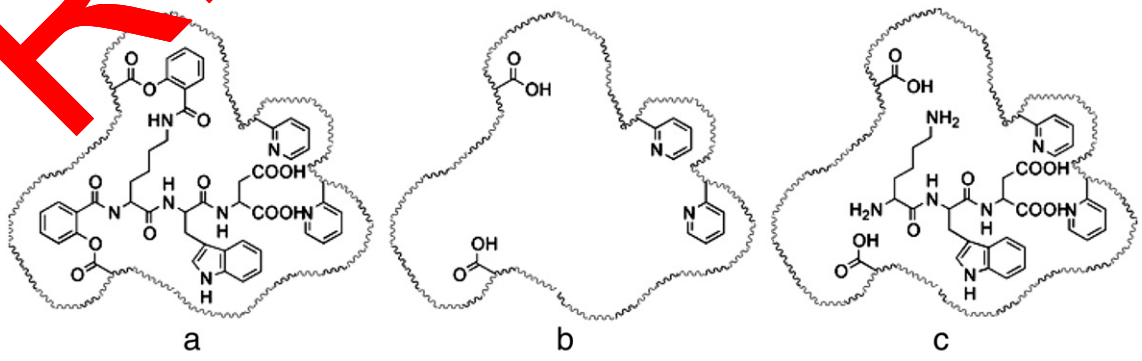


Fig. 2. Molecular imprinting of the tripeptide Lys-Trp-Asp using both covalent and non-covalent interactions. (a) Binding site with covalently bound imprint molecule; (b) binding site after chemical cleavage and extraction of the imprint molecule; (c) rebinding of the imprint molecule *via* only non-covalent interactions. (Reproduced with permission from Klein et al., 1999).

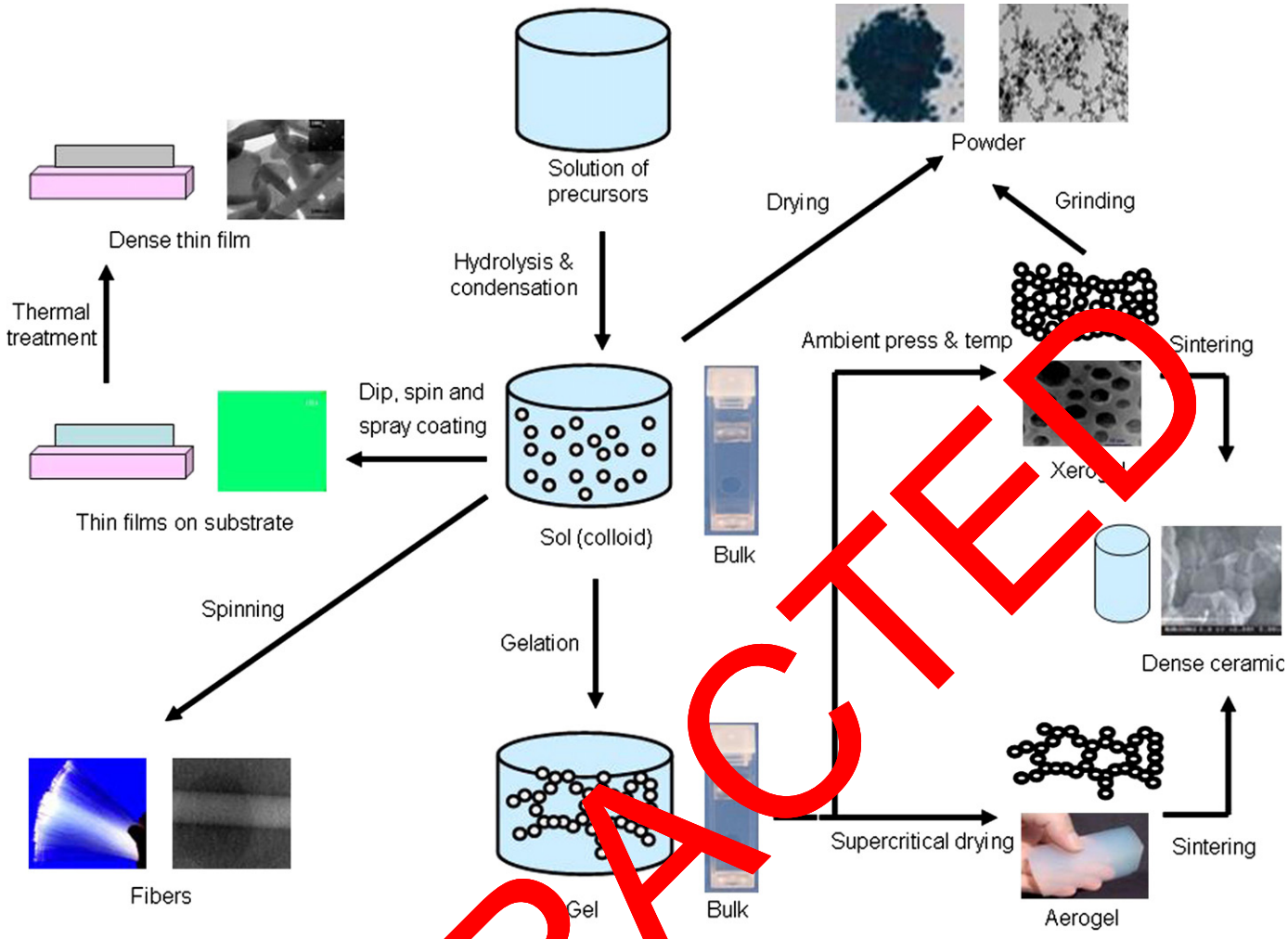


Fig. 3. Schematic diagram showing sol-gel process and various products (Reproduced with permission from Gupta and Kumar, 2008b).

where M represents a network-forming element such as Si, Ti, Zr, Al, B, etc., and R is typically an alkyl group. The most commonly used precursors are tetramethyl-orthosilicate (TMOS) and tetraethyl-orthosilicate (TEOS) in sol–gel process. The basic sol–gel reaction begins when metal alkoxide is mixed with water and a mutual solvent (mostly alcohol) in presence of acid or base catalyst. Generally, both

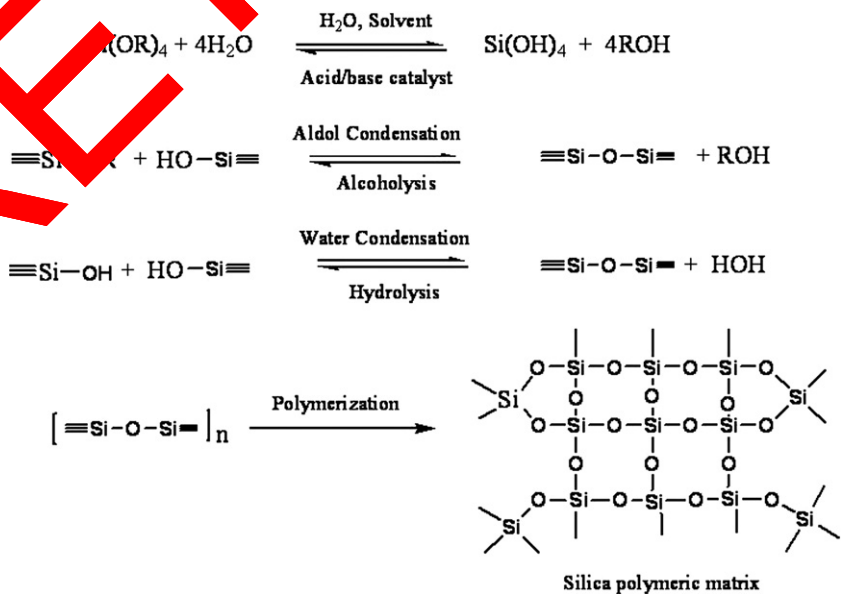


Fig. 4. Chemical reactions in sol–gel synthesis (Reproduced with permission from Gupta and Kumar, 2008b).

the hydrolysis and condensation reactions occur simultaneously once the hydrolysis reaction has been initiated. Hydrolysis leads to the formation of silanol groups (≡Si–OH) and condensation reactions produce siloxane bonds (≡Si–O–Si≡) and resulting into production of alcohol and water as by-products. The chemical reactions occurring during sol–gel process strongly influence the properties and composition of the final material. Further processing of the sol enables one to make sol–gel materials in different configurations. Thin films can be produced on a piece of substrate by various coating techniques like dip, spin and spray coating. During the sol–gel transformation, the viscosity of the solution gradually increases as the sol becomes interconnected to form a rigid, porous network of gel. With further drying and heat-treatment, the gel can be converted into crushed fine powder after grinding. During the drying process (at ambient pressure), the solvent liquid is removed and substantial shrinkage occurs. The resulting material is known as a xerogel. When solvent removal occurs under hypercritical (supercritical) conditions, the network does not shrink and a highly porous, low-density material known as an aerogel is produced. Heat treatment of a xerogel at elevated temperature produces viscous sintering (shrinkage of the xerogel due to a small amount of viscous flow) and effectively transforms the porous gel into a dense glass. As the viscosity of a sol is adjusted into a proper viscosity range, ceramic fibers can be drawn from the sol. Ultra-fine and uniform ceramic powders are formed by precipitation, spray pyrolysis, or emulsion techniques. Sol–gel materials have several advantages for example (a) compatible with many organic and inorganic reagents, (b) chemically, photochemically and thermally stable as compared to organic polymer, (c) optically transparent and thus suitable for various spectroscopic based analytical measurements and (d) can be cast as monolith, coated as thin films on glass slides and fiber and can be ground into p[illegible]. They have controllable surface area and average pore size, c[illegible] be miniaturized to micron or nano size and can also allow contr[illegible] of conductivity through the choice of the metal or metal alkox[illegible] (Brinker and Scherer, 1990; Wen and Wilkes, 199[illegible]; Díaz-García a[illegible] Badía Laíno, 2005; Gupta et al., 2005a,b; Chaudh[illegible]y et al., 2007).

2.2. Merits of using sol–gel polymeric matrix in m[illegible]cul[illegible] imprinting

Silica based materials are extrem[illegible]igid due to th[illegible]igh degree of cross-linking found in the (SiO_2) net[illegible]rk. This pro[illegible]y is very important in the design and synthesis of [illegible]printed materials since both the size and shape of th[illegible]avities created [illegible]he template must be retained after the remov[illegible]of the template. High [illegible]hermal stability of sol–gel derived mate[illegible] provide[illegible]an easy way to remove imprint molecule using high t[illegible]perat[illegible]e such as in calcinations method. In addition, sol–gel glasses a[illegible]ucturally [illegible]ous and can be engineered to have extre[illegible]y [illegible]h surf[illegible] area (~[illegible]0–2000 m^2/g). These properties make [illegible]ca sol–[illegible] matrix [illegible]mprinting host (Dai et al., 1997). The sel[illegible]tion and [illegible]ype of precursor plays an important role in achieving [illegible]electi[illegible]y in t[illegible]ulting sol–gel MIPs (Marx and Liron, 2001). Sol–[illegible]ethod also provides an efficient way for preparing hybrid matrix[illegible] incorporating organic components into inorganic polymers under [illegible]d thermal conditions and controlling the thickness, porosity and surface area as well as selectivity and sensitivity in case of thin films. The ease of preparation of sol–gels coupled with their compatibility with polar environments is advantageous when using lisinopril as a template species (Olwill et al., 2004). From a chemical and material standpoint, sol–gel derived materials have a combination of properties which can hardly be achieved by other materials.

2.3. Factors affecting molecular imprinting in sol–gel matrix

In general, the recognition ability of MIPs is largely governed by factors such as polymerization conditions, nature of cross-linking agents and degree of cross-linking. In addition to these factors, monomers and nature of the print molecule could also influence the ability of the MIPs to recognize the print molecules. The physico-chemical properties of sol–gel derived materials mainly depend on the composition of sol and processing conditions. These include water to precursor ratio, nature of precursor (hydrophilic/hydrophobic), type of catalyst (acidic/basic), pH, nature of solvent and ratio of solvent to precursor, temperature, humidity and aging (storage) conditions. No doubt these factors will play a significant role during imprinting of molecules in sol–gel derived polymeric matrix.

2.3.1. Effect of pH

Hydrolysis is most rapid and complete[illegible]hen ca[illegible]sts (acid/base) are used in sol–gel processing. Although m[illegible]eral acids or a[illegible]monia are most commonly used in sol–gel processing[illegible]ther known cat[illegible]ysts are acetic acids, potassium hydroxide, a[illegible]ines, [illegible]assium fluo[illegible]e, hydrogen fluoride, titanium alkoxide, va[illegible]dium alko[illegible]e and oth[illegible] metal oxides. Acid-catalyzed hydrolysis [illegible]d linear or rand[illegible]ly b[illegible]nched polymeric sol–gel network where[illegible]base–cata[illegible]ed hydro[illegible]s yield more highly branched polymeric [illegible]etwo[illegible] Th[illegible]ondensatio[illegible] rate depends on the pH of the sol and is h[illegible]est at in[illegible]ediate pH[illegible]elation is the point when the condensin[illegible]twork has su[illegible]ient [illegible]ness yet filled with solvent. From this p[illegible]nt, f[illegible]her evaporatio[illegible]ay collapse the film or generate porosity within the f[illegible] Refractive index and pore volume are also very sensitive to pH. Gelati[illegible] time is decreased by factors that increase co[illegible]ensation rate. Increase[illegible] the ratio of water to alkoxide, tempera-t[illegible]e, concentration of alkoxide and decrease in the size of the alkoxy [illegible]oup reduces th[illegible] gelation time. At intermediate pH, gelation time is v[illegible]y low whereas[illegible] low pH, gelation time is very long. Thus, pH plays a ve[illegible] important [illegible]le in determining the structure of sol–gel-derived polyn[illegible]x and also gelation behavior. The consequences of these changes affect the imprinting process as well as original structure of pH [illegible] template molecules and rebinding of template after extraction. Both pH of the analyte solution and pH during synthesis of the sol–gel matrix affect the rebinding of template molecule (Hench and West, 1990; Brinker and Scherer, 1990). Zhang et al. (2005) investigated the effect of solution pH over the range 4.5–9.2 on the frequency response of the L-histidine imprinted sol–gel piezoelectric sensor. The isoelectric point of L-histidine is 7.59. A maximum response was observed at about pH 7.4. At pH lower than 7.59, the protonation of L-histidine occurs and it becomes positively charged, whereas when the solution pH is higher than 7.59, L-histidine dissociates and becomes negatively charged. Both of these two conditions affect the interaction between the L-histidine and the sol–gel film. Li et al. (2008) prepared surface molecular protein imprinted hybrid sol–gel matrix at different pH viz. pH 2, pH 4, pH 7 and pH 9 using 3-aminopropyltrimethoxysilane (APTMS) in combination with TEOS, methyltrimethoxysilane (MTMOS) and glycidoxypropyltrimethoxysilane (GPTMS) and evaluated the influences of pH on resulting characteristics of template removal and re-adsorption. At pH 2, material possessed low bovine serum albumin (BSA) re-adsorption capacities. At pH 7, removal of BSA was highest, whereas re-adsorptions were lowest. In general, materials imprinted

Table 1
Preparation conditions and characteristics of IMIPs prepared with varied amount of lactic acid as pore-forming agent (Reproduced with permission from Wei et al., 2006)

Run No.	Caffeine (g)	Lactic acid (g)	Appearance	Caffeine (μmol/g)	Theophylline (μmol/g)	Selectivity (α)
P-11	0.34	0.00	Black	–	–	–
P-12		0.30	Yellow	–	–	–
P-13		2.58	White	8.1	3.6	2.25
P-14		5.42		13.6	5.6	2.43
P-8		8.32		17.9	8.1	2.21
P-15		16.6		20.6	10.1	2.04
P-16		25.0		15.5	6.4	2.42

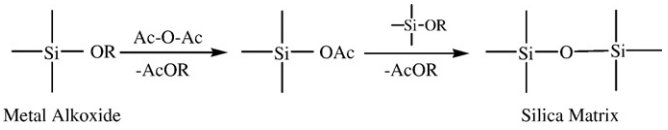


Fig. 5. Novel synthesis of sol–gel silica in acetic anhydride instead of water.

at pH 4 and pH 9 demonstrated easy template removal and considerable BSA re-adsorption. As well known, pH affects gelation kinetics of sol–gel process and three dimensional structure proteins and hence consequently affects template removal and rebinding at different pH.

2.3.2. Effect of temperature

The temperature affects the gelation kinetics of the sol–gel process that influences the physico-chemical properties such as porosity and thickness of sol–gel derived materials (monoliths/films) as well as removal of template molecule when using calcination as an extraction method (Vorotilov et al., 1994; Hegde and Rao, 2006). Zhang et al. (2006) investigated the effect of temperature on frequency response of the imprinted sol–gel sensor over the temperature range 10–50 °C in air/buffer solution (PBS) or with human serum albumin (HSA). No change in response frequency of imprinted sol–gel electrode was observed in air with increment of temperature whereas this increased when immersed in PBS. Increased temperature caused the swelling of film due to penetration of water into the imprinted film. Frequency shift of imprinted sol–gel electrode binding with HSA decreased when the temperature was over 35 °C or lower than 20 °C. The optimum binding temperature for the association of sol–gel sensor with HSA was observed in the range of 20–35 °C. At low temperature, the t structure of the highly shrunken gel was not suitable for bindin HSA; whereas at high temperature, swollen gel and too-loo structure was unfavorable for the binding them resulting in decreas of the frequency shift in both cases.

2.3.3. Effect of pore-forming agent and catalyst

The porosity of the sol–gel derived matrix plays an important role in determining both the sensitivity and response time of sensors and affected by various factors like water to precursor ratio, type of precursor, sol pH and aging time (Brinker and Scherer, 1990; McDonagh et al., 2002). Wei et al. (2006) synthesized TEOS-derived IMIPs for the template molecules caffeine (CAF) and theophylline (TH) by adding pore-forming agent (lactic acid) in base catalyzed (ammonia solution) sol–gel process. By using lactic acid, improvement in porosity of IMIPs as well as the adsorption of CAF (or TH) in IMIPs was observed. Zheng et al. (2000) has also reported such type of investigation. Table 1 showed the characteristics of IMIPs prepared with varied amount of lactic acid. The appearance of IMIPs change from B to yellow with small addition of lactic acid. Further it c nged from y ow to white with increased addition of lactic acid. C or TH) adsorpti into IMIPs linearly increased with increasing amou of lactic aci As further more lactic acid was added int e system s shown T e 1, sudden decrease of CAF adsorption s observed. It w at buted to loose silica network due to ad on of la e quantiti of lactic acid. In contrast, the higher s lecti (r of CAF b und to TH bound) of IMIPs and lower CA sorption s observe base catalyzed sol–gel process than addi pore-forming ent m hod. Lactic acid increased the porosity t re ted into high sorption of CAF whereas by adding base catalyst, t lation time decreased and the shape of CAF captured in the gel, result into better selectivity (Wei et al., 2006).

2.3 Effect of solvent and template

enerally in s gel process, solvents are added in order to fac ate mixing of organic precursors with water and to produce a mor mogeneo sol. Besides homogenization, solvent influences the rat lysis and condensation by increasing or decreasing lytic activity due to hydrogen bonding with solvent molecules or h diluting the sol as well as reducing the concentration of precursor (Brinker and Scherer, 1990; McDonagh et al., 2002). Fujiwara et al. (2004) reported a novel synthesis of sol–gel silica in

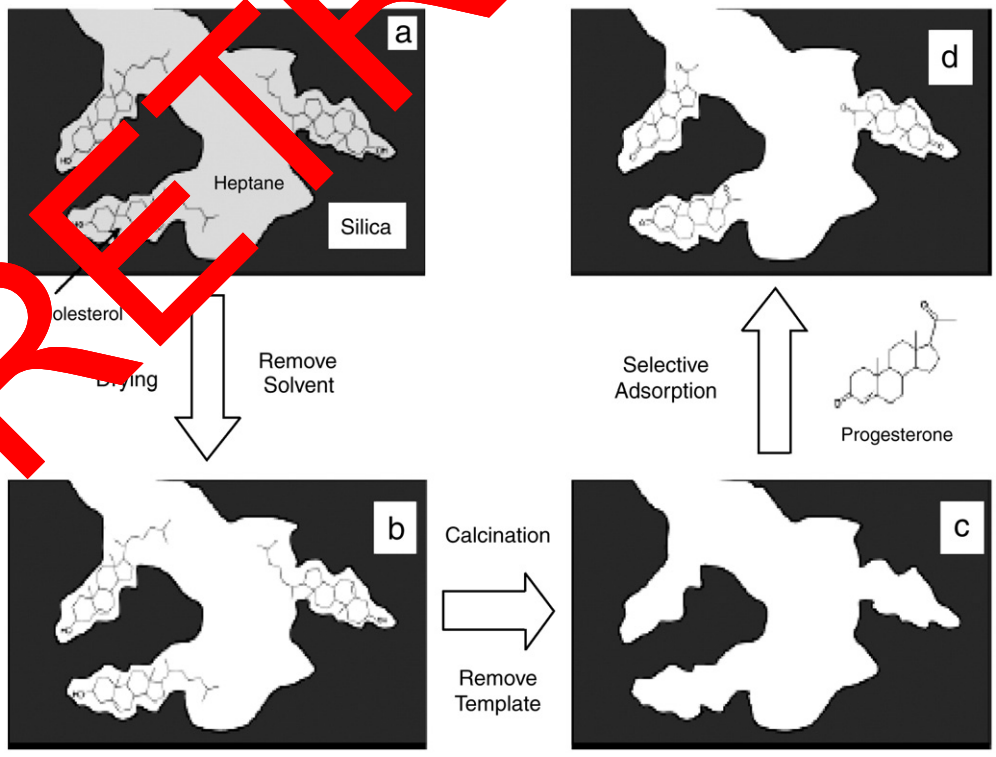


Fig. 6. A plausible mechanism of the formation of pore void suitable for the selective adsorption of steroid skeletons (Reproduced with permission from Fujiwara et al., 2004).

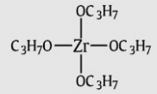
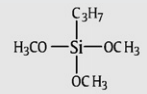
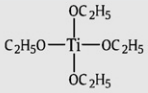
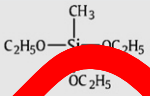
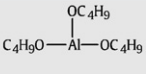
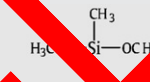
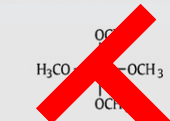
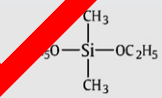
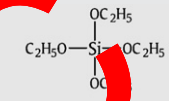
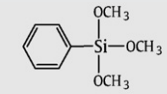
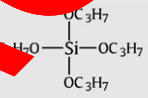
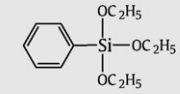
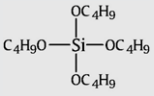
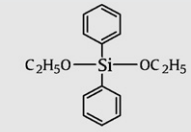
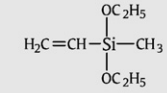
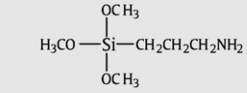
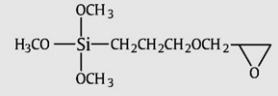
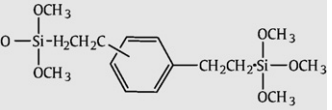
acetic anhydride instead of water as shown in Fig. 5 with the intention to dissolve various organic or hydrophobic molecules in sol solution. Further, the mixing of hydrophobic molecules was enhanced by the utilization of an additional solvent. The imprinting of various template molecules viz. cholesterol, cholesteryl chloride, progesterone and pregnenolone on adsorption ability of modified sol–gel method for steroid hormones was compared and revealed the suitability of cholesterol as template molecule than other analogues. The use of acetic anhydride as condensing agent and addition of *n*-heptane as an additional solvent facilitate the fixing of cholesterol in silica matrix as isolated one, not as an aggregated one. This was due to interaction of its hydroxyl group and long alkyl chain with silica surface and *n*-heptane, respectively. The shape of the imprinted steroid skeleton of cholesterol was retained in pore voids of the silica matrix after the removal of cholesterol by calcination. A plausible mechanism of the formation of pore void suitable for the selective adsorption of steroid skeleton is shown in Fig. 6. Thus, the structure of template molecule and solvent plays an important role for imprinting molecules and effect rebinding.

3. Recent developments in sol–gel molecular imprinting

3.1. Organic–inorganic hybrid MIPs

One of the major advances in sol–gel processing is the possibility of synthesizing hybrid inorganic–organic materials. They combine the properties of inorganic and organic compounds in one material, called as organically modified silanes (ORMOSILS). Thus, there is a wide range of possibilities to vary the properties of the materials by varying the chemical composition of the precursors and the ratio of the inorganic to organic components (Tripathi et al., 2006). Table 2 lists some inorganic precursors and most commonly used ORMOSILS monomers. Chemical incompatibility such as requirement of aqueous sol–gel processing and use of hydrophobic solvent in organic MIPs is the significant obstacle in preparing organic–inorganic hybrid MIPs resulting in phase separation. This would make it difficult to form a true hybrid material. Gill (2001) reported two approaches to overcome the issue of chemical incompatibility: (1) a combination of water-soluble organic polymer with inorganic precursors, followed by polymerization reaction and cross linking or cryo-phase separation or (2) the use a preformed network polymer with in-diffused and subsequently polymerized precursors of the second polymer. Sassi et al. (2002) reported the copolymerization of hydrolyzed and partially condensed TMOS/3-(trimethoxysilyl)propyl methacrylate with methacrylate to yield a 'leather-like' transparent gel. A stepwise or free-radical copolymerization of organic monomers with coupling agents before, during or after sol–gel co-condensation of alkoxysilanes in bulk organic (usually alcoholic) solvent has been reported by Jang and Ha (2002) for preparing transparent monolithic nonporous glasses. In this case, macro-phase separation was prevented by the use of coupling agents, which control the size of the SiO_2 particles that are formed. Recently, much attention has been given to non-hydrolytic route for the formation of ORMOSILS, as these routes have the potential to avoid the use of solvents and to eliminate residual silanol groups in the final product (Arkhireeva et al., 2005). Nonhydrolytic sol–gel synthesis of silica has been reported by Bourget et al. (1998). Generally this procedure involves either the reaction of a silicon halide with an oxygen donor, such as an alkoxide, alcohol, ether, etc. under non-aqueous conditions or the reaction of silicon halides with alkoxysilanes or dialkyl ethers in the presence of a Lewis acid catalyst (Hay and Raval, 2001). To produce a homogeneous hybrid material, it is necessary that the alkoxyoligosilanes/silicates formed in the first polymerization must remain soluble during the subsequent polymerization of the organic components. Due to the poor solvating properties of typical sol–gel formulations, the formation of homogeneous polymer solutions is limited to only a few organic polymers,

Table 2
List of inorganic precursors and commonly used ORMOSILS monomers

S. No.	Inorganic precursors	ORMOSILS
1.	 Tertrapropyl-orthozirconate (TPOZ)	 Propytrimethoxysilane (PTMS)
2.	 Tetraethyl-orthotitanate(TEOT)	 Me triethoxysilane(M
3.	 Aluminium *tert*-butoxide	 ethyldimethoxysilane(DMDMS)
4.	 Tetramethyl-orthosilicate (TMOS)	 Dimethyldiethoxysilane(DMDES)
5.	 traethyl-orthosilicate (TEOS)	 Phenyltrimethoxy silane (PhTMOS)
6.	 Tetra-*n*-propoxysilane	 Phenyltriethoxy silane (PhTEOS)
7.	 Tetrabutoxysilane	 Diphenyldiethoxysilane (DPDES)
8.		 Methylvinyldiethoxysilane(MVDES)
9.		 3-aminopropyltrimethoxysilane(APTMS)
10.		 Glycidoxypropyltrimethoxysilane(GPTMS)
11.		 Bis-(trimethoxysilylethyl)benzene (BTEB)

such as polymethylmethacrylate, which do not precipitate or phase separate once gelation of the sol begins (Wojcik and Klein, 1994). Skrdla et al. (2006) proposed a potential new route for the synthesis of hybrid MIPs through spontaneous hydrolysis and condensation reactions of TMOS in chloroform/methacrylic acid solution without the addition of water (Fig. 7). Formation of the shorter chain alkoxyoligosilanes was monitored by gas chromatography-mass spectroscopy (GC-MS) and possible mechanisms for their formation was proposed. In this study, the important finding was that TMOS undergo sol–gel chemistry in hydrophobic medium, a novel synthetic strategy for the preparation of hybrid MIPs. Silva and Augusto (2006) prepared an organically modified molecularly imprinted silicas (MIS) using APTMS as functional monomer and TEOS as reticulating agent, selective for methylxanthines. A quantitative method for methylxanthines in water, using SPE cartridges packed with the MIS coupled with HPLC-UV was developed with an imprinting factor of (20.5 ± 1.9).

3.2. Surface molecular imprinting technique in combination with sol–gel

In molecular imprinting, imprinted materials exhibit high affinity and selectivity but poor site accessibility to the target molecules because the template and functionality are totally embedded in the polymer matrices. Therefore, the kinetics of the sorption/desorption process is unfavorable and the mass transfer becomes slow. This problem can be overcome by using surface molecular imprinting in which the imprinted materials with binding sites situated at the surface, show many advantages including high selectivity, more accessible sites, fast mass transfer and binding kinetics (Dai et al., 1999; Yang et al., 2005; Fang et al., 2005). The sol–gel technique offers a wide range of processing approaches that can produce three-dimensional matrices in different configurations (bulk structures, thin films and powder) for use as imprinted matrix for sensing applications. Traditionally, surface sol–gel process is used to prepare ultrathin metal oxide films (Ichinose et al., 1996, 1997). Since the sol–gel reactions proceeds only on the surface, process is called surface sol–gel process. In this process, a solid substrate with hydroxyl groups on its surface is allowed to react with metal alkoxides in solution to form covalently-bound surface monolayers of the metal alkoxide. The excessively adsorbed (physisorbed) alkoxide is removed by rinsing. The chemisorbed alkoxide monolayer is then hydrolyzed to give a new hydroxylated surface. The schematic of surface sol–gel process is shown in Fig. 8. A very thin layer (~1 nm) of metal oxide layer can be deposited by using surface sol–gel process and can be repeated many times to give desired multilayers on the surface of support. Various guest molecules like organic, polymeric, biological and metallic materials are readily adsorbable or imprinted using functionalized groups on metal oxide layer and removed by solvent washing (Kunitake and Lee, 2004). Thus, surface sol–gel process combined with molecular imprinting technique provides a new platform for various sensing applications.

Lee et al. (199[illegible] demonstrated that the surface sol–gel process is superior as a means of molecular imprinting to the past imprinting techniques because in conventional sol–gel processes, time consuming procedures of gel formation, pulverization and extraction process create problem in creating imprinted cavities. Han et al. (2005) prepared a new molecularly imprinted amino-functionalized silica gel sorbent with binding sites situated at the surface for the template pentachlorophenol (PCP), by a surface imprinting technique in

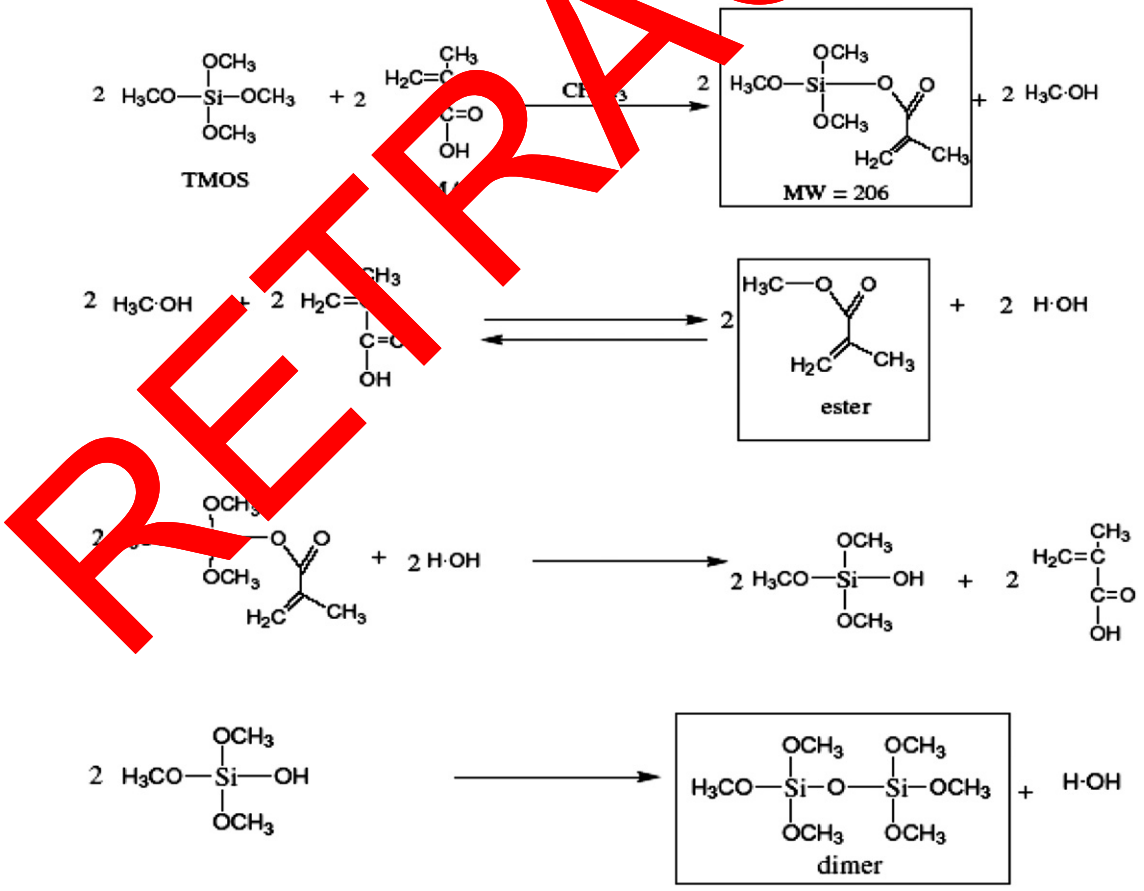


Fig. 7. Proposed secondary mechanism for the formation of silicate polymers in a (non-aqueous) solution containing TMOS, MA and chloroform. Note that the net reaction reduces to that of general sol–gel reaction upon the addition of a single reagent water molecule to the precursor. The highlighted (boxed) intermediates observed experimentally by GC-MS (Reproduced with permission from Skrdla et al., 2006).

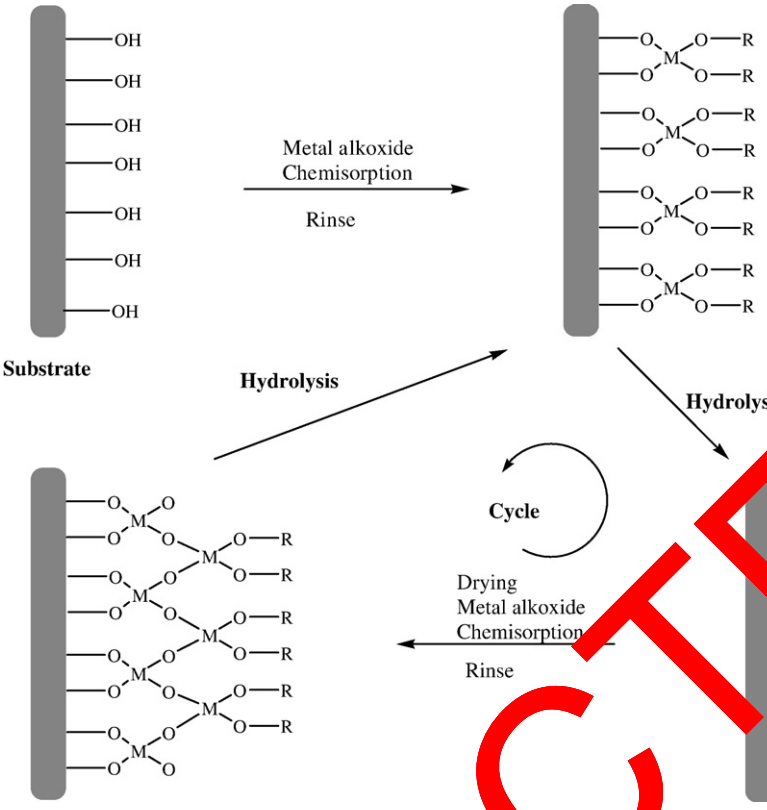


Fig. 8. General scheme of surface sol–gel process (Reproduced with permission from Kunitake and Lee, 2004).

combination with a sol–gel process, and applied it to on-line selective SPE coupled with HPLC for the determination of trace PCP in water samples. PCP is used as a general herbicide in agriculture and as an insecticide for termite control in the preservation of wood. Li et al. (2008) reported surface molecular imprinting in combination with sol–gel for protein recognition. The functional copolymer chitosan (CS) as microsphere were chosen as polysaccharide core for surface imprinting of BSA via covalent linkage. These microsphere were surrounded by APTMS and TEOS derived hybrid sol–gel polymeric matrix in aqueous solution at room temperature (Fig. 9). After template removal, the protein-imprinted sol–gel surface exhibited a preference for the template protein in adsorption experiments, as compared with four contrastive proteins. The complementation in hydrophilicity/hydrophobicity was a major factor affecting the imprinting formation and template recognition. The grafting of imprinted layer through interfacial organic–inorganic hybridization improved stability and reproducibility properties of the final material.

3.3. Molecularly imprinted sol–gel nanotube membrane

Presently, techniques used to prepare molecularly imprinted materials most often result in materials exhibiting high affinity and

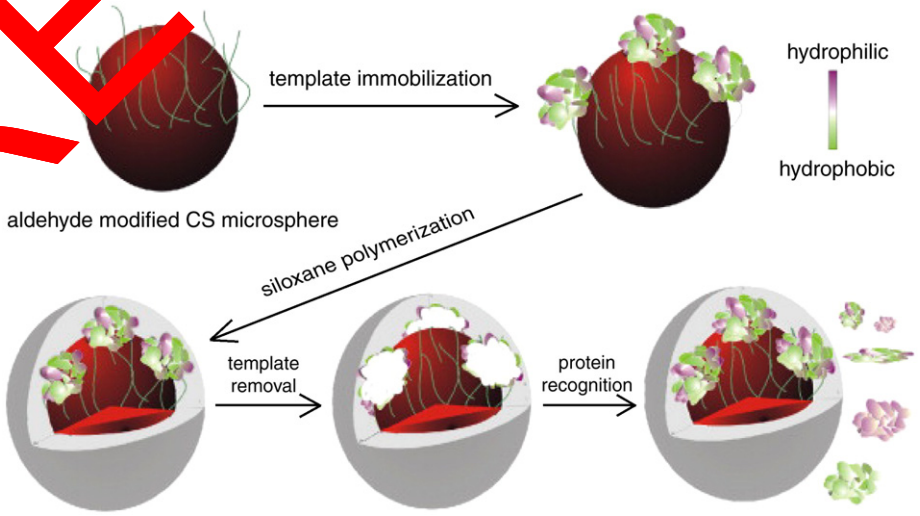


Fig. 9. Schematic representation for synthesis of the protein-imprinted polymer on chitosan microsphere using immobilized protein template (Reproduced with permission from Li et al., 2008).

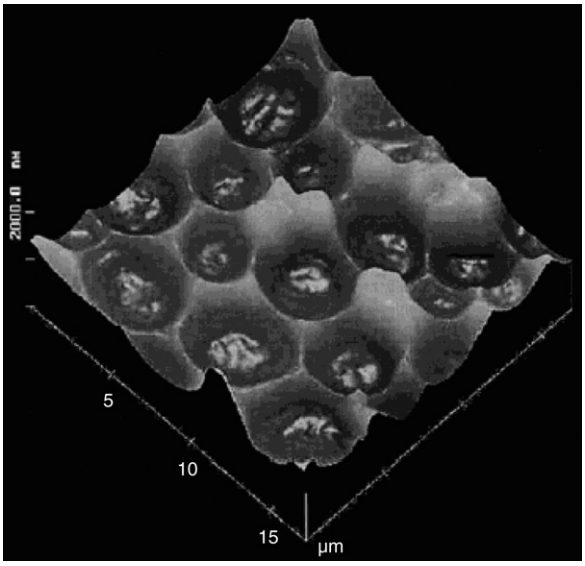


Fig. 10. AFM image showing sol–gel layer from titananium IV ethylate imprinted with *S. cerevisiae*. The coatings are extremely robust and scratch resistant (Reproduced with permission from Dickert and Hayden, 2002).

selectivity but low capacity and poor site accessibility to the target molecules. To advance this technique to the application with preparative-scale or high-efficiency separations, new morphologies and manufacturing techniques need to be developed (Yang et al., 2004). Recently, Martin's group have pioneered a technology, called template synthesis, for preparing monodisperse nanotubes of ne any size and composed of nearly any material (Martin, 1994; Jir et al., 1997; Miller et al., 2001; Martin and Kohli, 2003). The nanotubes offer some interesting advantages for biochemical applica tion. Yang et al. (2004) reported a simple procedur a lying a molecular imprinting technique to imprint functio groups to the walls of the template-synthesized sol–gel nanc es for bi emical separation of estrone. Silica nanotubes are e y t ake ave cro linked structure, and are highly suitable fo e forma of a delicate recognition site.

3.4. Bio-imprinting in sol–gel

Detecting biomolecules roteins, cells and m oorganisms) in different matrices is be ming an i easingly important task in a variety of fields including iopro s control, food technology, health care and environmental a s (Dicker et al., 2003). Although molecular impri chniq is wid developed for imprinting organic mol ules, b this ap n has not been adequately investigat for bio terial appl cations. Very few reports are available o io- i ol–gel. Dickert and Hayden (2002) presented con ation of mass-sensitive transducer with a surface-imprinting techn , as an ideal in situ analytical system for studying the interactions of b olymers or even whole cells with surfaces. The regular patterns of molded polymer and sol–gel surfaces with yeast cells (*Saccharomyces cerevisiae*) as template were prepared that showed honeycomb-like structures as shown in Fig. 10 and monitored cell concentration in the range from 10^4 to 10^9 cells/ml in flowing conditions of 10 ml/min. No unspecific adhesion of microorganisms was detected on non-imprinted polymers under flowing conditions. Lee et al. (2007) imprinted proteins (lysozyme or RNase A) in macroporous polysiloxane (silica) scaffolds using sol–gel processing. The quantity of surface-accessible protein, related to the number of potential binding sites was varied by changing the amount of protein loaded into the sol. Up to 62% of loaded protein was accessible. The imprinted scaffolds exhibited up to three times preference for binding their template molecules, even in the presence of a similar-sized competitor. The scaffolds were highly textured and included micro-, meso- and macropores. Fig. 11a shows higher magnification image of an imprinted scaffold, with noticeable protein embedded on the surface and Fig. 11b shows that submicron-sized pores were revealed on the surface after the scaffold was exposed to protease solution to digest the surface-accessible protein.

4. Applications of molecular imprinted sol–gel materials towards the development of sensors

Research in the field of biosensor development is fast becoming one of the most exciting areas. This has been as ed t a large number of possible applications of these importa evices in a iety of fields such as clinical diagnostics, defence, e ronmental cont , drug and agricultural industries. Sol–gel g ssy i rix has bee used as a potential host matrix in seve sensing lication and in the development of a number c hemical sensor and iosensors as a result of their ability to ly entra arious rec nition elements, such as pH indicators, prote , e mes and ntibodies (Chaudhury et al., 2007; Gupta a Chaudh 2007; G a and Kumar, 2008a,b). The imprinting various mole les i sol–gel matrix has been attempted in nu r of studies. et al. (1997) described the application of imprintin echniques to the synthesis of inorganic silica sorbents which exhibit hi affinity and selectivity for the binding of uran ons. Collinson (1999) viewed the advantages of templating usi sol–gel matrix rather than acrylic systems. Burleigh et al. (2001) sy hesized orga c–inorganic hybrid polymers using 1,2-bis (tri oxysilyl) eth e (BTSE) monomer copolymerized with metal ion mplexes o N-[3-(trimethoxysilyl)propyl]ethylenediamine [(TMS)e presence of supramolecular assemblies of cetyltri- thylammoniumchloride (CTAC), via room temperature hydrolytic s g cedures with ionic recognition characteristics for exploring

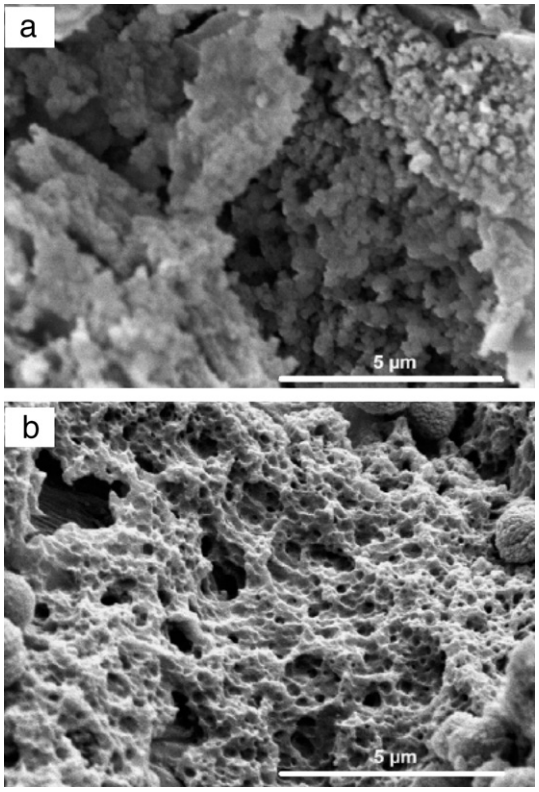
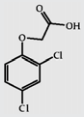
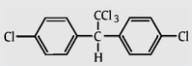
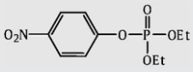
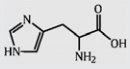
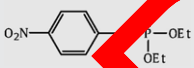
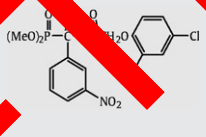
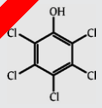
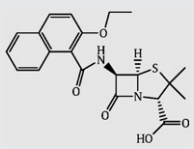


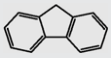
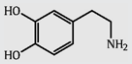
Fig. 11. Higher magnification SEM images of protein (lysozyme)-imprinted scaffolds (a) before and (b) after exposure to protease to digest surface-accessible protein (Reproduced with permission from Lee et al., 2007).

Table 3
Selected examples of sol–gel molecular imprinted sensors

S. No.	Objective	Template molecule and their structure	Sol–gel precursors and configuration	Extraction method and detection mode	Significant results	Ref.
1.	Sol–gel molecular imprinting of 2,4-dichlorophenoxyacetic acid (2,4-D)	2,4-D-(herbicides) 	3-[*N*,*N*-bis(9-anthrylmethyl) amino]propyl-triethoxysilane derived organosilane matrix	Soxhlet extraction/ luminescent photoinduced electron transfer (PET)	a. The MI sol-gel derived luminescent material was found to selectively respond to 2,4-D in aqueous media at pH 7. b. Demonstrated the feasibility of using a PET mechanism as a means of signal transduction for MIP based luminescent sensing of non-fluorescent analytes.	Leung et al. (2001)
2.	Development of chemical sensor for 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT)	DDT (Insecticide) 	Bridged polysilsesquioxane, bis-(trimethoxysilylethyl)benzene (BTEB)-derived sol–gel monolith and films on glass microscopic slides along with sacrificial spacer	Repeated washing with solvents / Fluorescence	a. 7-nitrobenz-2 diazole, (NBD) was us as fluores probe. b. Sol–gel lms were foun quantita y detect DDT in wa to a limit-o tection of 50 ppt h a sponse tim <60 s and re ble.	Graham et al. (2002)
3.	Detection of paraoxon in water using ormosils derived MIP	Paraoxon (toxic metabolite of parathion pesticide) 	TMOS+PTMOS+TMOS-CTAC [3-(trimethoxysilyl (propy] octadecyl dimethylammonium chloride) and TEOS +PTMOS+ APTES derived spin coated films	Soxhlet extraction/ colorimetric assa	Demonstrated ry sens method f the dete mole amou bound para into the thi in relatively short assay using Ellm olorimetric based bu chol esterase (BuCh nhibition.	Marx and Zaltsman (2003)
4.	Development of sol–gel molecular imprinted piezoelectric sensor for L-histidine	L-Histidine (amino acid) 	TEOS+PTMOS+MTMOS-derived imprinted sol–gel film on the surface of a quartz cry electrode	Repeated action in double-di d water for 24 h Piezoelectric sensing	a. A linear range from 5×10^{-8} to 1×10^{-4} M for a detection of L-histidine was observed with a detection limit of 2.5×10^{-8} M and 23.7 μmol/g maximum binding site (Qmax). c. Sol–gel imprinted QCM sensor showed maximum frequency response at pH near to the isoelectric point of L-histidine and good stability, high specificity and excellent stereoselectivity.	Zhang et al. (2005)
5.	Development of electrochemical sensor for the detection of parathion	Parathion (organophosphate pesticides) 	*p-tert*-b dcal 6]-1,4-crown-4 derived s el thin films	Repeated extraction with solvent / Electrochemical sensing	a. A linear response over parathion concentration in the range of 5.0×10^{-9} to 1.0×10^{-4} M was exhibited with a detection limit of 1.0×10^{-9} M with S/N ratio=3. b. Applicable for the determination of parathion in real samples.	Chunya et al. (2005a)
6.	Development of voltammetric sensor for *O*-dimethyl-(2,4-dichlorophenoxyacetoxyl) (3-nitrophenyl) methinephosphonate (Phi-NO_2)	Phi O_2 (organopho ate des) 	TEOS+*p-tert*butylcalix[6]arene derived inorganic–organic hybrid film	Repeated extraction with solvent / Electrochemical sensing	a. The sensor responded linearly to Phi-NO_2 over the concentration range of 2.0×10^{-5} to 1.0×10^{-8} mol L^{-1} with detection of limit of 1.0×10^{-9} mol L^{-1} (S/N=3) and stability for 30 days. b. The sensor was found to be highly sensitive, selective and stable and applicable for a simple and rapid determination of Phi-NO_2 in cabbage samples.	Chunya et al. (2005b)
7.	Determinatio tachlor nol (PCP) in w samp using su imprint technique combinati with gel proces	PC rbicides) 	PCP-imprinted amino-functionalized (APTEOS) silica sorbent along with TEOS-derived sol–gel coating	On-line solid-phase extraction-HPLC determination	a. With sample loading flow rate of 5 ml min^{-1} for 2 min, an enhancement factor of 670 and a detection limit of 6 ng l^{-1} were achieved at a sample throughput of five samples h^{-1} with S/N ratio= 3. b. The MI sorbent was found to be promising for on-line solid-phase extraction coupled with HPLC determination of trace levels of PCP in environmental samples.	Han et al. (2005)
8.	Optosensing of nafcillin using molecular imprinted ormosils	Nafcillin (antibiotic) 	TMOS+MTMOS+PhTMOS+ APTEOS derived imprinted sol–gel particles	Soxhlet extraction and calcinations and Optosensing using room temperature phosphorescence (RTP)	a. The imprinted sol–gel particles showed a linear range up to 6×10^{-5} M nafcillin with detection limit of 5.8×10^{-6} M (*S*/*N*= 3) and response time of 20 s. b. An average decrease of the RTP signal of ≤10% was observed after more than 30 successive nafcillin injections (4×10^{-5} M). Sample throughput was 22 h^{-1}.	Guardia et al. (2006)

(continued on next page)

Table 3 (*continued*)

S. No.	Objective	Template molecule and their structure	Sol–gel precursors and configuration	Extraction method and detection mode	Significant results	Ref.
9.	Development of sensor for fluorene	Fluorene (Polycyclic aromatic hydrocarbon used to make dyes, plastics and pesticides) 	Bis(trimethoxysilylethyl) benzene (BTEB) derived dip coated sol–gel film on microscopic coverslips	Washing with solvents/ Fluorescence	a. Fluorene binding was detected by a change in NBD fluorescence intensity. c. Sensing films showed response times of <60 s and detection limits below 10 parts-per-trillion. e. The sensing design was limited by a lack of reversibility following fluorene binding.	Carlson et al. (2006)
10.	Development of a highly sensitive biomimetic sensor for dopamine (DA)	DA 	TMOS+MTMOS+PTMOS derived dopamine imprinted sol, spin coated on APTES derived (self-assembled) ITO electrode	Electrocycling and Electrochemical sensing	a. Detection limit of electrochemical sensor was found in micromolar range with the linear range from 2.0 mM to 0.8 mM of DA. b. Potential scanning in electrocycling was found a very effective approach for DA extraction. c. Surface modification of the electrochemical transducer with functional monomers was found to be responsible for the development of MIP-based highly sensitive biomimetic sensor.	Gao et al. (2007)

mesoporous materials as hosts for molecular imprinting. Marx and Liron (2001) imprinted and resolved enantiomerically propranolol in thin films of organic–inorganic hybrid sol–gel and acrylic polymers. The sol–gel matrix has been shown to be superior as compared to acrylic matrix in terms of preparation process, faster diffusion times, reduced non-specific binding and sensor applications. Olwill et al. (2004) illustrated the potential of 3-monomer sol–gel system utilizing SPE to imprint the active pharmaceutical compound (lisinopril dihydrate) inspite of its limited solubility in non-polar and most polar porogens. Park et al. (2004) investigated whether the shrinking in HCl solution, after swelling in NaOH solution, is effective in imprinting phenylalanine (Phe) molecules on the copolymer matrix prepared by the wet-phase inversion and sol–gel transition method by measuring the uptake capacity and adsorption selectivity of the beads. The adsorption selectivity of the copolymer was maintained after 11 batches of adsorption/desorption in 1 g Phe solution composed of 5% D-phe and 95% L-phe. They concluded that swelling and shrinking process is one of the most effective methods in improving the adsorption selectivity of MIP polymer. Denkbaş and Katz (2005) synthesized novel imprinted materials via bulk imprinting of silica that have successfully translated proline-based homogeneous catalysts to the synthesis of confined catalytic materials containing isolated sites within hydrophobic microporous and mesoporous environments. Cummins et al. (2005) achieved the successful molecular imprinting of 2-aminopyridine via bulk polymerizations of acrylic and sol–gel based polymers. Both polymeric systems revealed varying degrees of affinity in rebinding the original template as well as a number of structural analogues. Rebinding was conducted in chloroform, acetonitrile and methanol in order to assess the role of hydrogen bonding in imprinting. The acrylic imprinted polymer retained approximately 50% of the template in rebinding studies in chloroform compared to 100% for the sol–gel materials. However, this higher affinity for the sol–gel material was accompanied by a higher degree of non-specific binding. While the acrylic polymer performed poorly in acetonitrile, the sol–gel materials maintained a high degree of discrimination. Jiang et al. (2006) described the preparation of a new enantioselective chitosan (CS)/γ-GPTMS hybrid MI membrane (HMIMs) by sol–gel process in aqueous system using CS as bulk polymer, L-phenylalanine (L-Phe) as imprinting molecules and GPTMS as crosslinking agent. SEM-EDX characterization revealed the dense structure of the membrane and homogeneous distribution of silica in chitosan matrix. The chiral separation ability of CS/GPTMS HMIMs towards the underivatized D,L-Phe aqueous mixture was evaluated by permeation and binding experiments with significantly improved selectivity with a separation factor $\alpha_{D/L}$ up to 1.5.

Molecular imprinting is an exciting and promising technique that is being increasingly used for developing sensors recently. Molecularly imprinted materials appear to offer an inexpensive, robust, and reusable alternative to expensive and labile biorecognition elements. Basically, a sensor or biosensor consists of recognition element/bioselective material/transducer. A chemical or physical signal is generated on the binding of the analyte to the recognition element, which is then translated into a quantifiable output signal by transducer. In molecular imprinted sensors, MIP is used as the recognition element instead of a biomolecule. Certain general and specific properties of the analyte (such as its IR spectrum/fluorescence/electrochemical activity) or changes in one or more physico-chemical parameters of the system (such as mass accumulation or adsorption) upon analyte binding are used for the detection. Reporter groups can be incorporated into the MIP to generate or enhance the sensor response (Haupt, 2001). The sol–gel imprinted polymers for various sensing applications have been investigated in several studies. A brief summary of observations from selected reports is shown in Table 3. Literature indicated that the use of mass-sensitive acoustic transducers such as the surface-acoustic wave (SAW) oscillator and the quartz crystal microbalance (QCM) provide easy approach for the designing sol–gel based molecular imprinted sensors. QCM has been particularly popular probably because of its comparatively low price, robustness, ease of use and direct measurement of adsorbed species. Even though sol–gel based MIPs have found several analytical applications but their commercialization needs more research to be done to make them suitable for real samples in field applications and as a real alternative to biomolecules. The successful imprinting has been seen mostly in monoliths and crushed powder. Recently, films are increasingly used for imprinting and sensing applications. The main factors that are important in development of thin films are the uniformity and thickness of film, its adhesion to the substrate and resistance to cracking, designing of stable internal environment and minimizing the potential of leaching of entrapped species. The thickness of the sol–gel derived films is highly dependent on the gelation behavior of the sol, which depends upon the viscosity of the casting solution. Generally, thin films require high levels of the entrapped species for sufficient signal to be generated. This is one of the main causes of failure of films for commercialization in field applications. Further in subsections, molecular imprinting in xerogels and sol–gel films were discussed as a good platform for various sensing applications.

4.1. Templated xerogels as a good platform for sensor applications

Sol–gel-derived xerogels are attractive for bioengineering and sensor applications because of possibility of tailoring a xerogel's physicochemical properties by altering sol–gel processing conditions (Sanchez and Ribot, 1994; Jin and Brennan, 2002) and exhibiting remarkable stability over time (Bukowski et al., 2005). Molecularly imprint xerogels can also exploit to design luminescence-based chemical sensors for the detection of small molecules (Leung et al., 2001; Graham et al., 2002). Chambers group has prepared molecularly imprinted xerogels for the recognition of protein Ricin and investigated the protein–xerogel interactions using intrinsic fluorescence from the tryptophan residues within Ricin (Lulka et al., 2000). Despite the obvious attraction of luminescence-based detection, xerogels and molecular imprinting, researchers have not developed a protein detection strategy that exploits the power of luminescence, the tunability of xerogels, and molecular imprinting. Tao et al. (2006) reported a new strategy for fabricating protein-responsive chemical sensor elements based on sol–gel derived molecularly imprinted xerogels termed as "protein imprinted xerogels with integrated emission sites (PIXIES)" and described a methodology for rapidly producing and screening a wide variety of sol–gel derived xerogel based formulations and compared the analytical figures of merit for PIXIES to standard antibody-based assays (ELISA). For human interleukin-1, the PIXIES-based sensor elements exhibited the following analytical figures of merit: (i) ~2 pg/ml detection limit, (ii) <2 min response times, (iii) >85 selectivity, (iv) <6% R.S.D. long term drift over 16 weeks of ambient storage, (v) >95% reversibility after more than 25 cycles and (vi) >85% recoveries on spiked samples. The PIXIES platform is completely self-contained, and it achieves analyte recognition without a biorecognition element (e.g., antibody). The PIXIES relies upon sol–gel derived xerogels, molecular imprinting, and the se[illegible] installation of a luminescent reporter molecule directly within the molecularly imprint site. In operation, the templated xerogel selectively recognizes the target analyte, the analyte binds to the template site, and binding causes a change in the physicochemical properties within the template site that are sensed and reported by the luminescent probe molecule.

4.2. Molecularly imprinted sol–gel films as sensor

Molecular imprinting of sol–gel thin film is advantageous over organic-polymer, not only because of its facile preparation, but also due to better kinetics, low non-specific adsorption and high association constants. Adding to it the large library of existing trialkoxy-silane derivatives makes the sol–gel chiral imprinting alternative for thin films as an attractive approach. Fireman-Shoresh et al. (2003) showed that the sol–gel matrix is an excellent substance for tailoring specific film-adsorption and separation characteristics via molecular imprinting. They demonstrated the chiral recognizability of spin coated submicron composite sol–gel films (~700 nm) and their enantioselectivity under steady-state conditions for three enantiomer pairs viz. (*R*)- and (*S*)-propranolol, [illegible] and (*S*)-2,2,2-trifluoro-1-(9-anthryl) ethanol, and D- and L-3,4-dihydroxyphenylalanine (D- and L-dopa). The combination of imprinted sol–gel thin films as the sensing element and electrodes as the transduction element has been investigated by several groups (Gutierrez-Fernandez et al., 2001; Shustak et al., 2003. Marx et al., 2004) and found many applications in different area such as in electrocatalysis, enzymatic catalysis, sensors and biosensors, electrochemiluminescence, batteries, fuel cells and electroactive films. Marx et al. (2004) developed thin films of a molecularly imprinted sol–gel polymer on glass substrates and on glassy carbon electrodes with specific binding sites for parathion in both liquid and gas phase. Gas-phase binding measurements were performed on coated quartz crystal microbalance resonators. The binding of parathion to the imprinted films in the liquid phase was investigated by steady-state experiments with analysis by gas chromatography-flame photometric detection (GC-FPD) and cyclic voltammetry. The difference between molecular recognition in the gas- and liquid-phase imprinted polymer was discussed. Fireman-Shoresh et al. (2005a) fabricated enantioselective surfactant-templated thin films derived from PTMOS, TMOS and propanol. The chiral cationic surfactant (−)-*N*-dodecyl-*N*-methylephedrinium bromide was imprinted in thin films and after its extraction chiral domains were created. Selective adsorption was measured by fluorescence analysis using (*R*)- and (*S*)-propranolol, (*R*)-**2** and (*S*)-**2**, respectively, and (*R*)- and (*S*)-2,2,2-trifluoro-1-(9-anthryl) ethanol, (*R*)-**3** and (*S*)-**3**, respectively, as the chiral probes. In 2005, same group has demonstrated a new application of spin coated thin films (~70 nm) of a molecularly imprinted sol–gel matrix derived from TMOS, phenyl trimethoxysilane (PTMOS), carboxyethylsilanetriol (CTES) sodium salt on ITO electrodes as a highly sensitive and enantiospecific electrochemical sensor for the recognition of chiral molecules. The advantages of the very thin films (70 nm) as compared with the films of 700 nm thickness (Fireman-Shoresh et al., 2003) reflected through larger electrochemical signals, faster removal of the template by solvent extraction and the superior sensitivity of the imprinted films. Two chiral electroactive probes viz. D- and L-3,4-dihydroxyphenylalanine (D- and L-dopa) and (*R*)- and (*S*)-*N*,*N*-dimethylferrocenylethylamine [(*R*)-Fc and (*S*)-Fc] were used as template molecules for the molecular imprinting process. Dopa imprinted films revealed both high sensitivity, by the detection of nM (~2 ppb) concentration and excellent selectivity, when challenged with a set of catechol derivatives. Fc-imprinted films were able to detect ca. 2 ppm of the target molecule, with very good enantioselectivity and low non-specific adsorption. This was the first report of successful molecular imprinting of an organometallic complex like ferrocene derivative (Fireman-Shoresh et al., 2005b).

In conclusion, the molecularly imprinted materials prepared via sol–gel process have great potential for various sensing applications. The interfacing area of sol–gel combined with molecular imprinting provides a new route for improving the properties of conventional [illegible] and in identifying new applications of imprinted polymers. Ongoing research should be directed at enhancing selectivity of sol–gel polymeric matrix for successful imprinting of proteins and even cells that contain more homogeneous binding sites, have a higher affinity for the target analyte and can be routinely prepared and used in aqueous systems. New applications are also emerging, particularly in the field of biosensor for environmental monitoring.

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