Macro, Micro and Nano Imprinted Gels

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Abstract— Molecular imprinting (MI) is a process in which imprinted gels prepared by cross linking of functional monmers with template. Cavities are left in porous material which rebind to other template molecule in specific location and not to different molecule on account of the matching location of the functional group. MIGs are commonly prepared by two methods. One is cross linkers in the presence of template molecule and second by freeradical copolymerization of ligand. MI process consists of three basic steps. (1) Analyte selection as a template. (2) In a polymer network incorporation of the template. (3) Removal of the specific cavities where rebinding of the other template occur. Template, monomers, cross-linkers and solution play important part in synthesis of MIGs. Macroimprinting is more sensitive towards temperature and many environmental factors. Microgels synthesis is more efficient than nanoimprinted gels because it is low yield process. Nanoimprinting could give better yield by postdilution method. Efficiency of MIGs can be measured by swelling ratios. MIGs show deviation in their behavior due to aqueous solvent. MIGs are responsive to pH and temperature and their catalytic activity is efficient at optimal condition but not at extreme condition.

Index Terms— Molecular imprinting, Template, Crosslinkers, Solvent, Rebinding, Synthesis, Chitosan.

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1   INTRUCTION

IN a period of rapid growth of material science and chemistry Klaus and Mosbach studied the combination of the two emerging sciences. Mosbach started his work in Lund University on secondary metabolism. Mosbach and his companions began investigating enzymes entrapment and immobilization in 1970. During their investigation they found that the gels could give the same effect of enzymes. After this Theorised and Mosbach monitered that the gels could be use to create substrate-selective template, due to formation of dense network. Nearly at the same time Wulff presented some enzymes models [1]. These models gave the information about artificially achieving the specific and selective recognition seen in the field of biology and gave the idea of molecular imprinting of gels in MI process. In 1930 Polyakove introduce the imprinting technique to imprint the silica (Si) matrices with benzene. He observed that the natural enzymes were selective and specific in nature but not generally applicable as imprinted gels. Molecular imprinted gels (MIGs) due to their high selectivity, low cost and highly specific behavior are used widely in biological field and material sciences. His discovery gave the key role to the scientist to work in the MI field.

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Molecular imprinting (MI) is a process in which imprinted gels prepared by cross linking of functional group with the templates. Cavities are left in porous material which rebind to the other template molecule in specific location and not to similar molecule on account of the matching location of the functional groups. Molecular imprinted gels (MIGs) are commonly prepared by two methods. One of which is cross-linkers in the presence of template molecule while the second by freeradical copolymerisation of ligand [2]. For the low production cost and easy preparation of imprinted gels, they combine chemical and thermal stability. Macro, micro and nanogels are different on the basis of their particle sizes. The gels of the particle size greater than 0.1µm are called macroimprinted gels. If the particle size of the gel is in range of 0.1-1µm, then gel system is called microimprinted gels and if the particle size of the gel is in range of 0.1-100 nm then gel system is called nanoimprinting gels. Imprinting of macro molecules was problematic for scientist because macro molecules are usually large and soft [3]. Macro molecular imprinting established a strategy for preparing enzyme mimics. In many catalytic reactions natural enzymes suffer from low recovery and poor stability.

To overcome this draw back MI of macromolecule have a great pressure in selection of template. MI have been widely used as catalyst in different chemical reactions [4]. Microimprinted gels and nanoimprinted gels are prepared easily as compared to macroimprinted gels just because of their particle size. Micro and nano sized particles are efficiently fast in the equilibrium with the analyte. The fast equilibrium of these gels enhances the catalytic activity of

the surface area for binding. Smaller sizes of the gel particle have a great efficiency in accessible binding cavities. MIGs are affected by the temperature, pH and bonding between the cross-linker and template. Many artificial enzymes show different pH then natural pH as hemin shows pH value about 11. While for natural horseradish peroxide (HRP) shows the optimum pH value 8.5 in 0.1M Tris-HCl buffer. To find the catalytic activity of enzymes poly-(N-isopropylacrylamide) with its copolymers are used. By changing in temperature, catalytic activity can be modulated [5]. This temperature modulation can provide the binding sites to catalytic activity [6]. Many other factors except temperature and pH also affect the efficiency of the MI process such as concentration of the solution and bonding between the template and functional monomer in the solution. Bonding can cause major effect on imprinting. Cross linkers and monomers attached by covalent and non-covalent bonding. After this the removal of template in the complex matrix is of a great interest. In this report the effect of bonding of analyte with functional monomers would analyzed and also it would be seen how efficiently the separation of analyte occurs. The both effects could be seen by mathematical design. The both effects could be seen by mathematical design. The information about participants (Template, functional monomers and cross-linker) of imprinted process and their importance in the MI of gels would be covered in this report [7].

2 BASIC PRINCIPLES

MIGs can be synthesized by using the strategy of lock-and-key metaphor that the only particular target will bind efficiently to the matching molecular imprint. The hypothesis behind the MI theory is that when an auxiliary molecule is added into a mixture of prior monomers. Then the polymerization, natural diffusion, Brownian motion and a self-assembling reaction cause the polymeric normal structure to form around the added material, adjoining it, and capture it for the network formation. By covalent or by non-covalent forces the functionalities of the ligands or the guest molecules interact with one another. Self assembling based on these binding forces and specific arrangements [8]. From the casting material the host molecule removed to rebind it with the other target molecule which is same in size but different in spatial arrangements. Only the specific molecules are used for imprinting the binding cavities of the imprint matrix will be effective target.

3 BASIC STEPS INVOLVED IN SYNTHESIS OF MOLECULAR IMPRINTED GELS

Three important steps are involved in this process

(a) Analyte selection as a template
(b) In a polymer net work incorporation of the template
(c) Removal of the specific cavities where the rebinding of the other template occur [9].

In the polymerization process experimental variables such as cross-linkers, initiator, solvents, the method of initiator, nature of the template, functional monomers and duration of polymers can be challenge for synthesis and designing MIGs. These variable changings can be discouraging projection to the inexperienced practitioner.
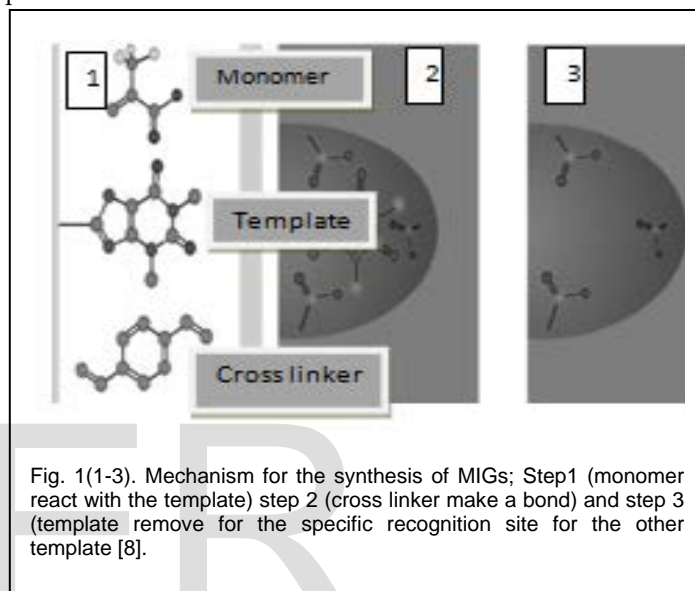

Fig. 1(1-3). Mechanism for the synthesis of MIGs; Step1 (monomer react with the template) step 2 (cross linker make a bond) and step 3 (template remove for the specific recognition site for the other template [8].

The good ''rule of thumb'' have a great come out in literature that is very helpful in this regard. For the efficient polymerization, the basics of free radical polymerization process should be known. Selection of the suitable template and monomers attached to the template. The complete information of the properties of the cross-linkers and the binding templates which bind in the cavities are necessary for the efficient imprinting process.

3.1 Template

Template has great importance in imprinting process. It is the central part of the MI process gives the surface area to the monomers where the monomers attached. Not all the templates use directly in the MI process. The template should ideally be chemically inert for the compatibility under the free radical polymerization. If the template could take part in free radical polymerization or for some reasons unstable under the polymerization then alternative imprinting may have to be used. For the selection of the template for imprinting we should know how much temperature template can bear, which temperature range is suitable for example at around 60 °C, if the Azobis-

dimethyl valeronitrile (AIBN) is being used as the chemical initiators, what is the effect upon exposure to UV irradiation, the capacity to restrain a free radical polymerization (as thiol group and hydroquinone moiety) and ability to bear any polymerisable group.

3.2 Functional Monomers

Functional monomers attached with a template by covalent or non-covalent bond. They are used in excess than the number of templates. They are responsible for the binding sites in MI process. Usually in non-covalent bonding the ratio of the template and monomers is 4:1. For the efficient imprinting and enhance the effect of imprinting functional monomers and templates should have the matching functionalities in a complimentary manner [10]. Matching functionalities of the imprinting help in maximize the more complex formation. We should know the reactivity of the monomers in a very good manner if two or more than two monomers used in the imprinting process as used ''cocktail'' [4] and the reactivity is feasible for co-polymerisation. Template can influence the reactivity of the functional monomers to some degree of perturbations to the electronics or the monomers sterics. Monomers with their structural formula are given in figure 2.
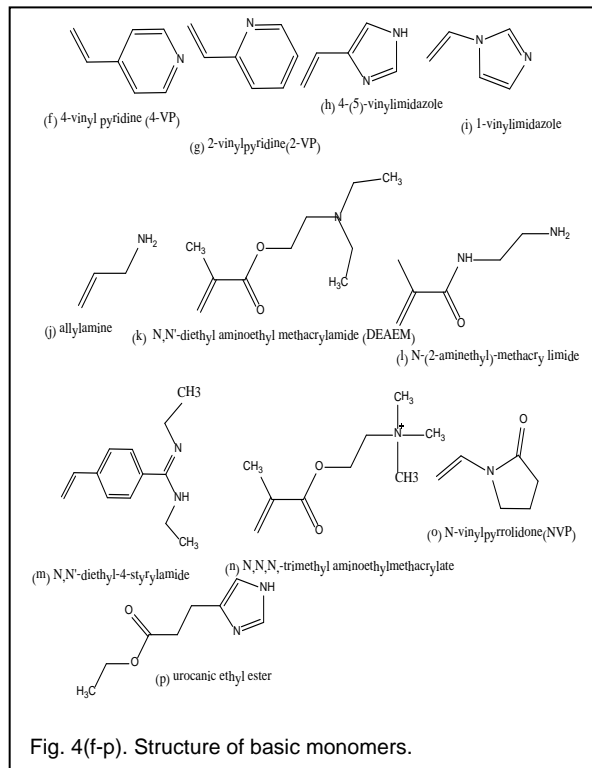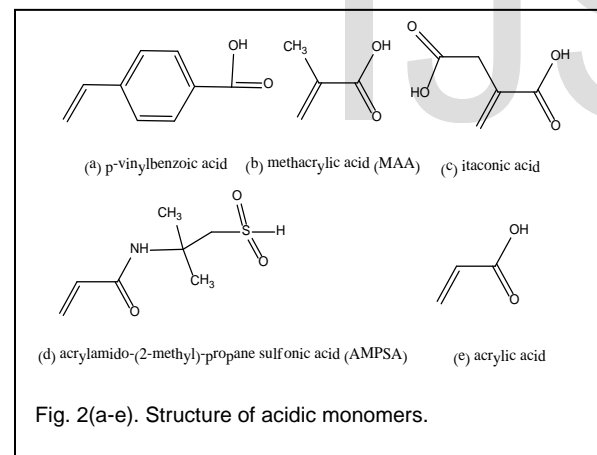

Fig. 2(a-e). Structure of acidic monomers.

Fig. 3(q-x). Structure of neutral functional monomers.
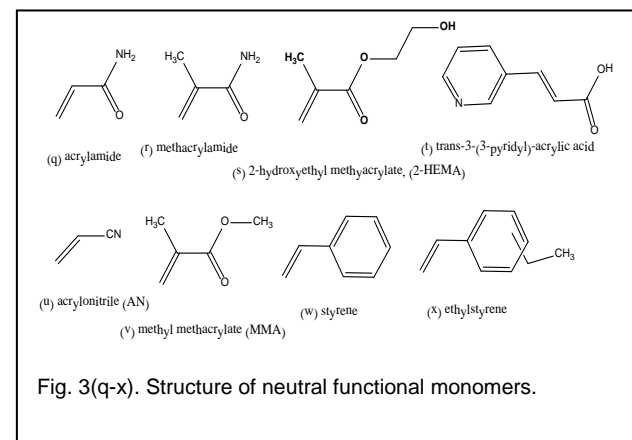

Fig. 4(f-p). Structure of basic monomers.

3.3 Cross-linkers

Molecular imprinting process depends on cross linkers due to their three important functioning in this process. It controls the morphological aspect of the polymer matrix (whether the polymer macro porous, micro porous or gel type), give stability to the imprinted binding site and give the mechanical strength to the polymer matrix.

Cross-linkers make a bond with the functional monomers more closely and some time it reacts with the template and gives the property of functional monomers [10]. Cross-linkers must be ideally reactive and its functionality with the functional monomers should be matched. Sometimes exact reactivity relative amount of the cross-linkers not be well identified and in such cases we assume the reactivity value of the cross-linkers through the structural analogues. Cross linkers like the ratio of templates are more than the number of templates added in the imprinting process to make a bond with the monomers and to give a structural aspect to the imprinted gel. In the cocktail polymerization where more than one type of monomers are used, more than one type of the cross-linker with a known functionality and a known reactivity can be used as well [11].

In case of polymerisation cross-linkers are used with high ratio to provide great stability and porosity to the

matrix. Some well known cross linkers with their chemical formulas are listed in figure (5) and (6).


Fig. 5(a-f). Chemical structure of cross linkers.

Fig. 6(j-q). Chemical structure of cross linkers..
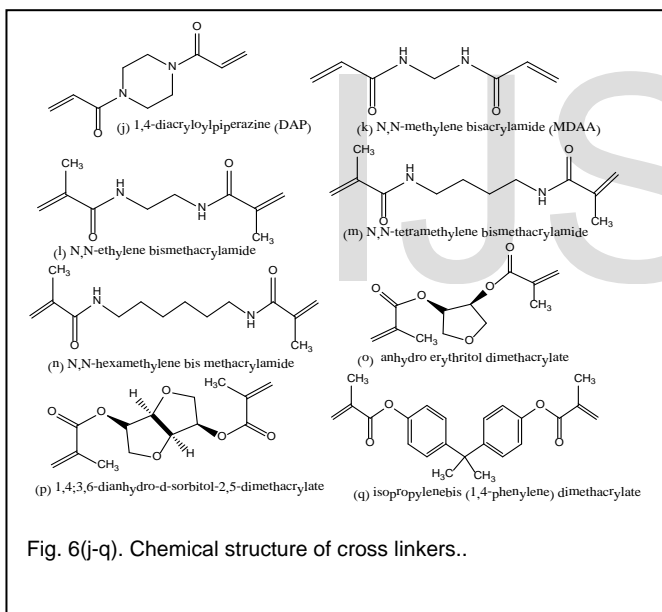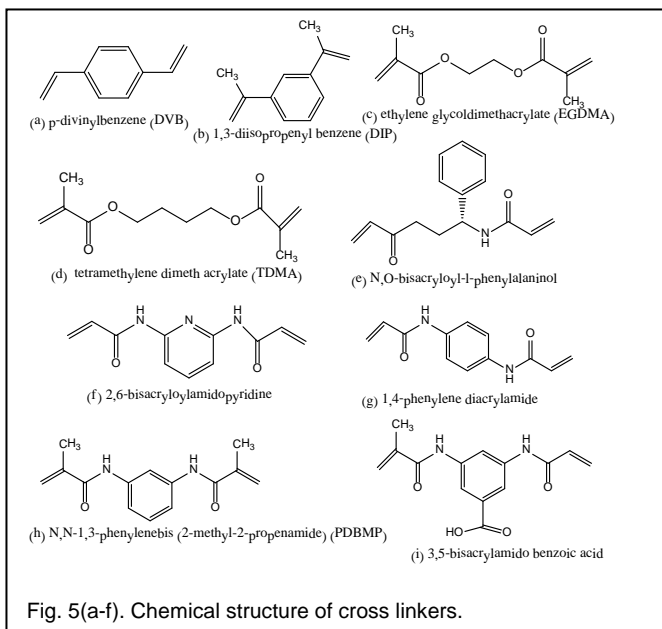
3.4 Solvents

In MI process solvent plays more efficient role by two ways. Firstly solvent combine all templates, cross-linkers, initiators and functional monomers in the imprinting process. Secondly solvent is responsible for the pores in the gel. Solvents called porogens due to their pores formation property. More the solvent gave additional pores in the gel to imprinting. To get efficient imprinting more specific and thermodynamically good solvent used in imprinting. By the choice of a thermodynamically good solvent we get the more specific and well developed pore structure. Solvent

shows dual character as pore maker and as a solvent. It maximizes the likelihood to complex formation of functional monomers and the template. Water can be used as a solvent in imprinting if hydrophobic forces are being used to complexation reactions. Generally polar solvents give raise the recognition in organic or bio-macromolecules [12]. If the non-covalent bond formed in the aqueous solvent then imprinting is difficult to maintain [13].

3.5 Initiators

In any reaction where the reactant do not possess sufficient amount of energy to undergo a chemical reaction, some foreign substance can be used which have ability to energize the reacting molecule to start the chemical reaction at its initial stage is called initiator. In molecular imprinting process some time initiators require to start imprinting. Initiators used in imprinting according to their template nature. If the template is photochemically unstable in nature then photochemical initiator used and if the initiator is thermally unstable then thermal initiator triggered, in order to achieve effective imprinting this is not attractive technique. If the cross-linker make a complex by hydrogen bonding than the preferred temperature is lower and low temperature is favorable in the photochemically active initiator. Some initiators with their chemical formulas are given in figure.

Fig. 7(a-f). Chemical structure of selected chemical initiators [2].
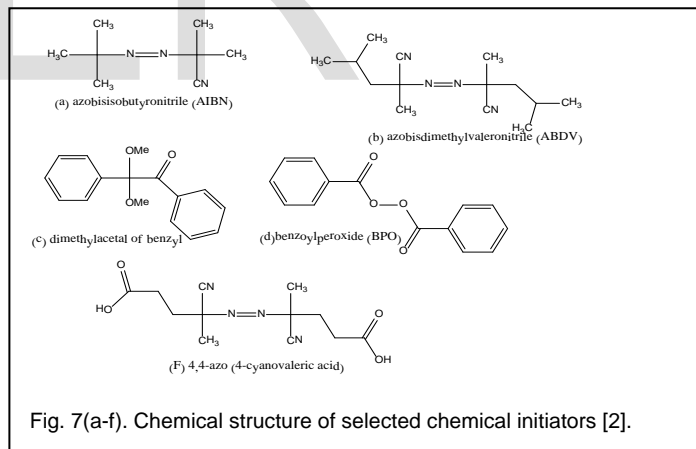
4   SYNTHESIS OF MOLECULAR IMPRINTED GELS

Synthesis of molecular macro imprinted gels is difficult because of the large size of biomolecules. MI of macrogels has a great intrest in the biotechnology and medical field. Template making is problematic in molecular macro imprinted gel, to overcome this issue scientist are working on a large scale. Many natural compounds are used in this process such as protein (amino acids) and chitosen.

In molecular macroimprinting process very high efficiency of cross linkers require because biomolecules are larger in size and show very sensitive behavior to the pH, temperature and other environmental factors. Some selective cross-linkers and functional monomers play an important role in imprinting of macro gels (*N,N'-*methylenebisacrylamide and ethylene glycol dimethacrylate) and (Methacylic acid and vinylpyridiene) are respectively. MIGs are used as antigens to produce artificial antibodies [19]. Some structural formulas of imprinted antigens are given in figure:
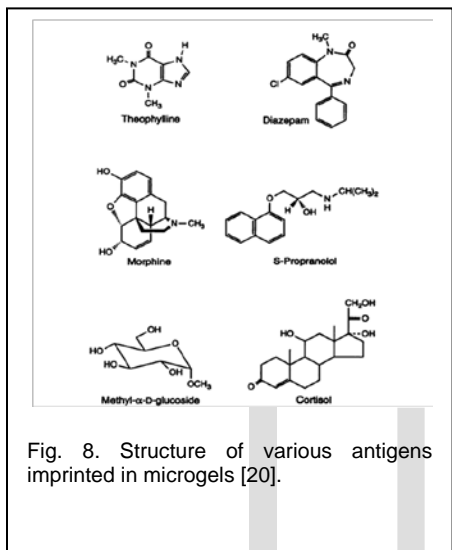

Fig. 8. Structure of various antigens imprinted in microgels [20].

4.1 Protein Imprinting

Proteins have very complex structure. It can be denatured in its 3D arrangements, as a result of changing in temperature, pH changes and some other environmental changing take place. Protein with the large molecular structure is difficult in template recognition sites due to the steric hindrance and when trying to remove the template from the complex. Furthermore complete removal of template is also difficult [21].

The polymerisation methods for MIDs can be used by immoderation of heat or UV light to initiate the reaction and can be determined how to protein response to the environmental effects. If the 3D arrangements of the protein molecular structure occur then imprinting process is difficult to sustain. Some scientist have also used circulardichroism studies to know how commonly functional monomers and cross-linkers used in imprinting process to reveal the better stability and sensitivity [22]. However if the concern protein overcome these problems than this imprinting could be better used and provide the better recognition along with the large molecules. If the large number of sites present in a metrix then separation of

very specific enantiomers and amino acid sequence from one another [23].

4.2 Use of Chitosan in Molecular Imprinting of Macrogels

Chitosan is the most abundant bipolymer in nature and is a linear polyschcride. It is a good biomaterial due to its positive biointeraction and its physical and chemical properties. It can be form sponges, porous scaffolds and gels [24].
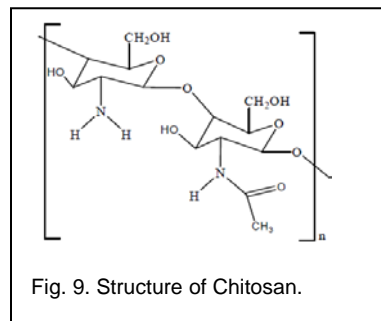

Fig. 9. Structure of Chitosan.

Chitosan is biodegradable and through the enzymatic cleavage chitosan can be broken into glucosamine units. Its biodegradation is quite slow but it is useful for the long time biomaterial for example in drug delivery [25]. Its rate of degradation can be controlled by molecular degree of acylation. Chitosan in molecular imprinting works for recognition matrix as a structural supporter [26]. It can cause hindrance in the imprinting structure that's why it is not widely used in every application [20].

4.3 Functional Monomers

Functional monomers are obligatory for the binding sites in imprinting. In molecular imprinting of macro gels functional monomers should have same stability and specificity to the template. Usually methacrylic acid and vinylpyridien are used in molecular imprinted macrogels preparation because of their rapid kinetics [27].

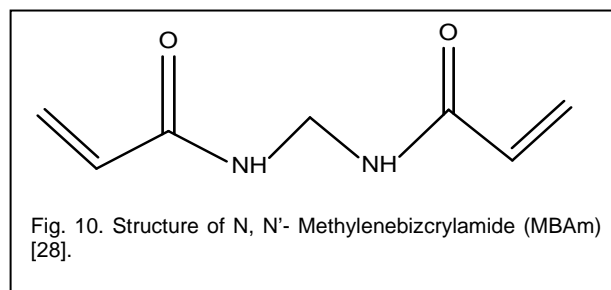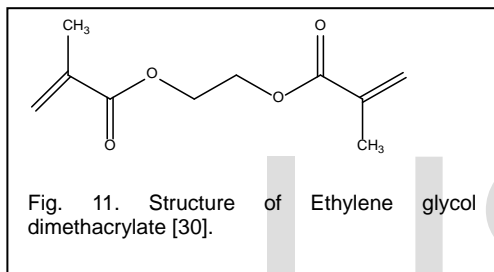## 4.4 Cross-linkers
4.4.1 *N,N'*-Methylenebiscrylamide (MBAm)

Fig. 10. Structure of N, N'- Methylenebizcrylamide (MBAm) [28].

Its structure formula is $C_7H_{10}O_2N_2$. It is chiefly used in the separation of proteins and nucleic acid in molecular biology. MBAm is widely used to prepare polyacrylamide. In the process of formation of polymers bisacrylamide is obtained, further polymerization of bisacrylamide with acrylamide are capable of creating cross-links between the chains of polyacrylamide [28]. Its percentage of cross-linking activity is very high as compared to the other cross linkers in macro gels synthesis. MBAm gives the efficient electrophoretic separation effects and its functional groups have great ability to provide a strong hydrogen bonding, even in polar solvent [29]. Due to its controllable properties polyacrylamide it is widely used as a cross-linker in many applications of the molecular macroimprinted gels [30].

4.4.2 Ethylene Glycol Dimethacrylate (EGDMA)

Fig. 11. Structure of Ethylene glycol dimethacrylate [30].

Its chemical formula is $C_{10}H_{14}O_4$. It is the second most common cross-linker used in molecular imprinting of macrogels. Ethylene glycol diacrylate (EGDA) is the simplest form of it which is also used in MIGs synthesis. Its extensive knowledge is very wide due to di- and tri-esters. It can be formed even very long chain network depends upon the need of cross-linkers in MIGs. Due to the long chain and presence of vinyl group its solubility is very low in water. Acrylate gives the fast kinetics and effective cross-linking activity.

5 SYNTHESIS OF MOLECULAR IMPRINTED MICROGELS

Molecular imprinted microgels are easier to prepare than macro imprinted gels. A reverse emulsion method can be used to prepare this gel [31].

After the modification of this method, this can be used to prepare MIGs. In the preparation of molecular micro imprinted gel template *p*-nitro phenyl phosphate (NPP), dialdehyde dextran-histidien conjugate (PAD-His) as a functional monomers and N-N methylenebisacrylamide (MBA) or N-Isopropylacrylamide (NIPA) as cross-linkers are used in synthesis. The schema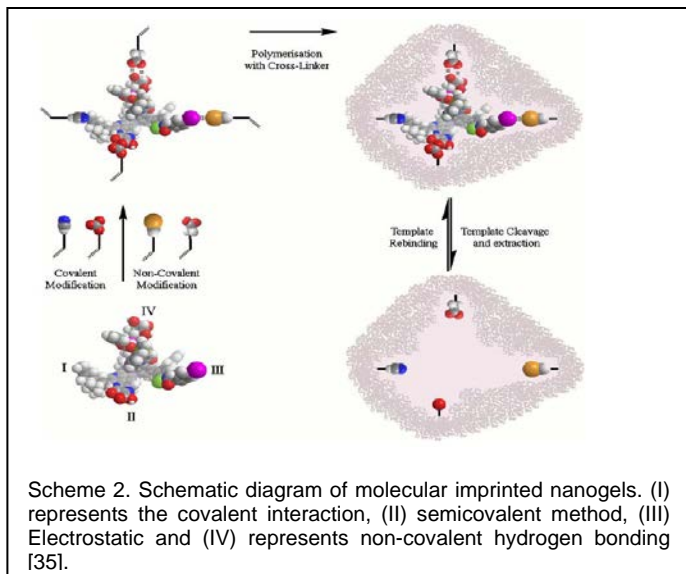tic illustration for the preparation of molecular micro imprinted gels given below. In this synthesis process *p*-nitrophenylacetate used to re bind the cavity. This is also three step mechanism in first step template (NPP) attach with the functional monomers (PAD-His) after this cross linkers (MBA) and (NIPA) added in the solution. Template removes when cross-linker make a bond with the functional monomers and a specific cavity formed in the matrix. *p*-Nitriphenylacetate is used to rebind the cavity. Because it is similar to the (NPP) just has difference in functional group.

Scheme 1. Schematic diagram of synthesis of molecular micro imprinted gels
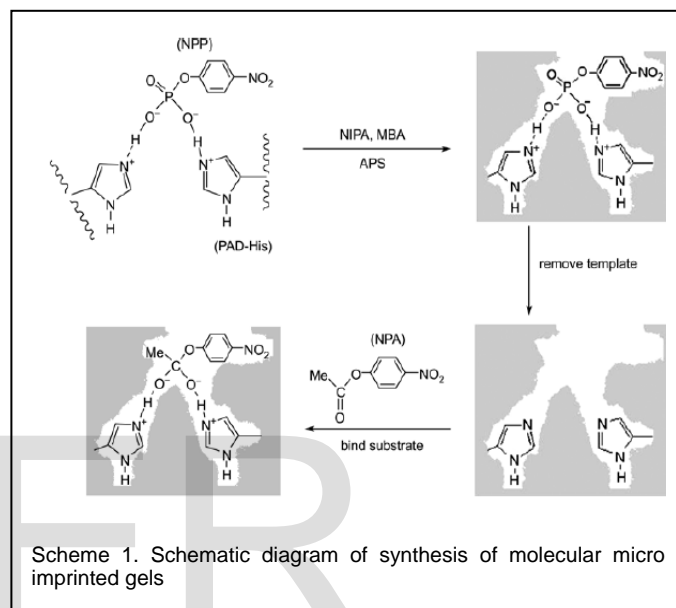
6 SYNTHESIS OF MOLECULAR NANOIMPRINTED GELS

Molecular imprinted nanogels refered to as a unimolecular crosslinked gels having particles of size compareable to the statistical dimensions of a natural enzymes (-100nm) [33]. For the separation of MIGs different methods of separation can be used [34]. In the size range of (10-300 nm) nanogels can be synthesised by polymerization method. Nano gels are easy to synthesize due to small porous size. But on a large production scale polymerization method was inefficient to synthesize. This process gave very low yield and the process was very long. Water soluble MIGs can be prepared by polymerization from a very dilute solution and these gels capable of hindering trypsin enzymes activity. Molecular nanoimprinted gels gave more (20-folds) catalytic efficiency than the non imprinted nanogels. Catalytic activity of molecular imprinted nanogels is less than natural particles. MIGs could be used to balance the enzymeunder the unstable conditions, like in organic media or extreme temperature and pressure. MIGs can be prepared by Post-dilution method (polymerization at high

monomer concentration in a suitable solvent). MIGs produced by this process could be catalysed by carbonate hydrolysis [35]. Post dilution method provides high yield it depends on thermal polymerization, which might hinder with the imprinting process. UV radiations used in post-dilution method as an initiator.

Scheme 2. Schematic diagram of molecular imprinted nanogels. (I) represents the covalent interaction, (II) semicovalent method, (III) Electrostatic and (IV) represents non-covalent hydrogen bonding [35].

This imprinting process usually used theophylline as a template which has many hydrogen bonding sites. Acrylic group as a functional monomer which could form a stable complex. Divinylbenzene used as a cross-linker. For post-dilution method two different thermal initiators were used 2,2′-azobis 2,4-dimethyl-4-methoxyvaleronitrile (ADMV) and azobisisobutyronitrile (AIBN). ADMV is known as a good initiator at low temperatures [36].

7   DETERMINATION OF THE EQUILIBRIUM SWELLING RATIO

Molecular imprinted gels placed in the solution of phosphate buffer to maintain its pH and compare the swelling ratio of the imprinted gel to non-imprinted gel.

$$SR = (M_{MIGs}–M_{NIG}) / (M_{NIG})$$

Where M_{MIGs} and M_{NIG} denote the weight of the imprinted gels and non imprinted gels, respectively. This equation gives the SR difference of the imprinted gels.

Fig. 12. SEM image of molecular imprinted gel of 300nm, microgel spheres under 15,000×magnifcation [32].
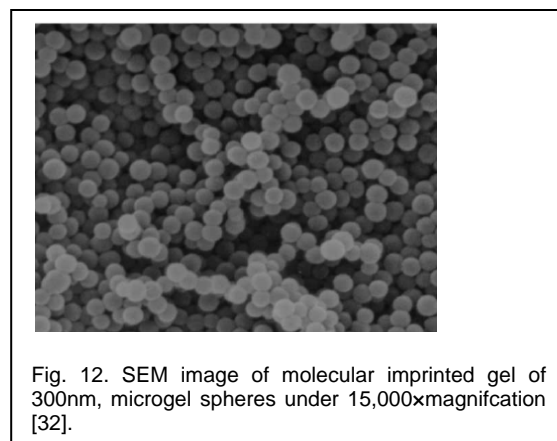
8   EFFECT OF TEMPERATURE ON MOLECULAR IMPRINTED GELS

Molecular imprinted gels show responsive behavior to temperature. MIGs have the temperature range (25-40 °C). Equilibrium swelling ratio (SR) is the function of temperature. Swelling of the gel increases at room temperature (25 °C), As the temperature increases from room temperature the swelling of the imprinted geld decrease gradually (25-30 °C). After 30 °C the swelling ratio of the gel decreases rapidly. As the temperature increases hydrogen bonding between the MIGs and water weighed down by hydrophobic interaction between other hydrophobic groups, obtaining in volume phase transition [37]. Novel hydrophobically modified temperature-sensitive microgels with tunable volume phase transition observed by [38].

9   EFFECT OF pH ON MIGs

Molecular imprinted gels affected by the pH change. By the change of pH catalytic efficiency of imprinted gels should be changed. Different effects of the pH on molecular imprinted gels are shown in figure 13.

Fig. 13. Comparison of pH sensitive ESME images with different pH [31].
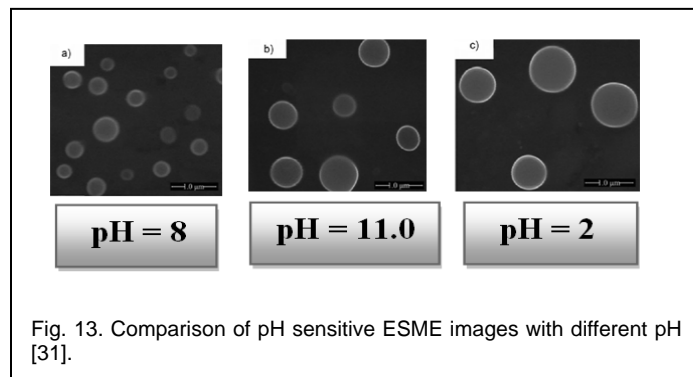
Many artificial imprinted gels show the different behavior to their natural counterparts. For example in the 0.1 M solution of Tris-HCl Natural HPR gives pH=8 and

when the Hemin used as a catalyst it gives pH=11. Catalytic activity of the MIGs increases at pH (8-9) but to the more alkaline side and acidic side catalytic activity of MIGs decreases

      MIGs with the methacrylic acid cross-linkers and with the homovinylic acids gave a brilliant response to pH. The MIGs which shows temperature sensitive behavior also shows good response to pH. Under the optimal condition MIGs accomplish to a brilliant microenvironment within the imprinted nanostructure [39].

10 MOLECULAR IMPRINTING IN AQUOUS SOLVENTS

The great challenge currently occupying much of the contemporary body of molecular imprinting research is realizing the specific recognition of MIGs within an aqueous system [40]. As mentioned, the solvent plays a major role in the prepolymerisation matrix of imprinted gels. Polar solvents like water will naturally disturb the hydrogen bonds customarily exploited in the creation of most non-covalent MIGs are responsible for maintaining the interaction between the monomers and template in polymerisation, as well as the target molecule in remaining binding sides and therefore this can severely disturb the imprint selectivity and specificity [41]. Thus it is more difficult to maintain the imprinting in the non-polar solvent. However, a great majority of chemical and biomedical applications involve molecular targets are water soluble, like amino acids and therapeutics.

12 CONCLUSION

Synthesis of molecular imprinted gels have very wide range field. Micro and nano imprinted gels can be prepared easily but molecular macro imprinted gels is quite problematic to synthesize due to large size and responsive behavior towards environment. Nano imprinted gels give low yield which can overcome by post-dilution method. Proteins (amino acids) with a very long change fluctuate at very small change in pH and temperature. Efficiency of MIGs can be measured by rational designs. By this design, how efficiently separation of the template and how many functional monomers involve in bonding with template can be seen. Swelling ratio of the imprinted gels is a factor of temperature. As the temperature arises from (30 °C) the swelling ratio of imprinted gels decreases because the catalytic activity in the MIGs decreases rapidly. Macromolecular microgels show fluctuation at a very less temperature change and their catalytic activity disturb more by the change of temperature. MIGs are responsive to pH value. MIGs at room temperature and pH value (8-9) give the efficient catalytic activity but at the extreme

parameters MIGs show disturbed catalytic activity. MIGs were difficult to maintain imprinting and extracting of template in aqueous solvents.

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