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# Analytical Strategies for Characterization of Molecular Imprinted Polymers: A current Review

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**Abstract:** The purpose of this short review is to present, in clear English, a summary of the principal analytical considerations pertaining to good practice for characterization of molecular imprinted polymers (MIPs). This review summarizes the previous and current literature regarding the analytical tools employed for characterization of synthesized MIPs. It is our expectation that this will facilitate researchers to plan their own sophisticated analytical pathway for characterization of MIPs in a more logical and structured fashion, and to begin to appreciate the limitations of the present approaches in this molecularly complex area.
**Keywords:** Molecular imprinted polymer; characterization; analytical.

## 1. Introduction

Molecularly imprinted polymers (MIPs) are crosslinked polymeric materials that possess huge binding capacity and selectivity against a target molecule (template) present during the synthesis process [1]. MIPs are highly cross-linked polymeric porous material with specific recognition sites in terms of shape, size and functional groups to the target molecule (template) and capable of mimicking r antibodies and receptors[2].

In past few years, molecular imprinting technology has been used in many fields of chemistry, biochemistry, biotechnology and pharmaceuticals. Given the versatility, high recognition and specificity that can be achieved, the future of MIPs would seem bright. MIPs have been applied in the synthesis of receptors for many analytes like herbicides, drugs, proteins and toxins, etc., and as adsorbents in chromatographic and electrophoretic separation techniques [3].

Some generic analytical techniques, i.e. radioimmunoassay [4], chemiluminescence [5], gas chromatography (GC) [6], high-performance liquid chromatography (HPLC), LC-MS**,** combinations of GC–MS, or HPLC–MS, and morphological techniques like Scanning electron microscope (SEM) have been developed for drug determination. UV–visible spectroscopic analysis, FTIR and 1H NMR study are commonly used to characterize the nature of binding interactions and the extent of complex formation between functional monomers and template molecule in solution[7]**.** Meanwhile, Brunauer–Emmett–Teller (BET) and Scanning electron microscope (SEM) analyses are employed to elucidate the morphological characteristics. These may provide

valuable information for the synthesis and application of the MIPs However, these techniques usually demands laborious procedures involving isolation, clean up,preconcentration and solid phase or liquid-liquid extraction, to concentrate and purify drugs before determination, and reproducibility of the results is seriously affected by sample [8].

## 2. Characterization of Polymers

Crosslinked and macroscopic chain polymers are practically difficult to characterize largely on account of their intractable, insoluble nature. Imprinted polymers are no exception. A degree of characterization is possible, however, we can distinguish between three levels of characterization: (I) chemical characterization, (II) morphological characterization, and (III) characterization of the molecular recognition properties. The molecular recognition aspect has been dealt with numerous times elsewhere, hence we will restrict ourselves to a short consideration of the chemical and morphological characterization aspects, listing a few of the sophisticated techniques available at the disposal of the analyst and the information that can be extracted [9].

### 2.1 Chemical characterization

#### 2.1.1Fourier-transform infra-red spectroscopy (FTIR)

The FTIR spectra of imprinted polymers can be easily acquired e.g. as a KBr disc to extract quantitative information on the composition of the polymer. The method is of particular importance when the different chemical fluctuations in the sample (e.g. arising by the functional monomer and crosslinker in an imprinted polymer) give rise to well resolved, diagnostic signals. It is also possible to use FTIR to probe non-covalent interactions, e.g. hydrogen bonds.

FT-IR spectra gives the fundamental analytical base for rationalizing the mechanisms of recognition during the imprinting process which governing interactions for selective binding spot formation at the molecular level**.** The interaction between the monomer and template molecule during pre-polymerization complex formation and the template incorporation into the imprinted polymer during rebinding can be achieved by the characteristic FT-IR absorption analysis [10].

Xuemin Zhou and coworker [11] reported a FTIR characterization of synthesized MIPs **of** sildenafil and vardenafil in herbal dietary supplements. FT-IR spectra were recorded in the range of 4000–400cm−1

Shunli Ji et al [12] published the characterization of amino glycosides antibiotics in honey by FT -IR spectroscopy. The strong bands appeared at 3441.76 cm−1 corresponding to the OH stretching vibration indicating the removal of template molecules. Bands at 3441.76 cm−1, 1732.5 cm−1 and 1259.65 cm−1 resulted from the carboxyl groups. The absorbance at 1638.63 cm−1 was assigned to stretching vibration of C – C bond. These data indicated slight difference of NIP and MIP with or without removal of template molecule.

Mohd Marsin Sanagi and coworker [13] has reported a method of FTIR characterization of organophosphorus pesticides in fruit samples. FTIR characterization has done to determine the functional groups in MIP before and after the washing stage and also in NIP by using the KBr pellet procedure.

Maryam Shekarchi et al reported [14] FT-IR spectra of grounded polymer of lamivudine extracted from human serum were recorded using KBr pellets in the range of 400–4000 cm−1.

#### 2.1.2 U.V Spectroscopy

UV spectroscopy studies have been performed for spectroscopically measure the saturation of template molecules with functional monomer building blocks by recording changes in absorbance spectra or differential absorption. However recently, IR spectroscopy on pre-polymerization products has provided additional complementary information to UV/Vis and NMR studies by probing the vibrational signatures of the involved molecules and complexes [15]. UV measurement mainly provides information about the binding capacity of functional monomer and the template molecule. As per literature reviewed, several studied have been done for determination of binding capacity of MIPs by UV method. Few are listed below.

Huai You Wang and coworker [16] studied the Binding capacities of MIPs for benzotriazole were determined spectrophotometrically at 252 nm.

Ram B. Gupta and coworker [17] have reported a binding experiment for tetracycline.

The polymer particles (15mg) were mixed with 5 ml tetracycline solution in a 10 ml conical centrifugation tube and sealed. The tubes were oscillated by a wrist action shaker in a water bath for at least 20 h. Then the mixture was centrifuged for 10 min and the tetracycline concentration in the liquid phase was measured by a UV-Vis spectrophotometer. The amount of tetracycline bound to the polymer was calculated by subtracting the concentration of free tetracycline from the initial tetracycline loading. Partition coefficient K=CP/Cs was used to characterize the binding extent, where CP is the concentration of tetracycline inside the polymer, and CS is the concentration of tetracycline in the solution

Ai-jun Tong et al [18] spectrophotometric binding analysis for five steroidal drugs. Fluorescence and UV-VIS absorption properties of the five steroid compounds in different organic solvents were reported. Only the estradiol derivatives, b-estradiol, ethynyl estradiol and estradiol benzoate showed high fluorescence emission in methanol or ethanol solution, testosterone and methyl testosterone do not fluoresce. Adsorption percentages for the first three steroids were thus calculated from the corresponding fluorescence intensity, while the following two compounds were determined by UV-VIS absorption spectra. The excitation and emission wavelengths were chosen as 281 and 307 nm,

**2.1.3 NMR**

NMR techniques fulfill the need to work in solution and therefore enable the NMR spectra of insoluble species to be acquired. As far as MIP work is concerned, solid-state NMR has been relatively under-exploited to date, as has, for that matter, suspended state NMR. Proton NMR titration experiments facilitate observation of hydrogen bond formation between bases and carboxylic acid through hydrogen bonding. These techniques has been introduced in molecular imprinting for investigating the extent of complex formation in prepolymerisation solutions and as a means of identifying the specific sites in interacting structures that engage in complexation. Thus evaluating the shift of a proton signal due to participation in hydrogen bond was used as the selection criterion for complex formation, M/T ratio and interacting forces [19].

Lachlan J. Schwarz and coworker [20] applied NMR technique for the selective recognition of the bioactive polyphone, (E)-resveratrol MIPs. This is a representative of the general approach that has been deployed for these 1H NMR spectroscopy titration experiments. (E)-Resveratrol (23 mg, 0.1 mmol) dissolved in trideuteroacetonitrile was titrated with increasing molar equivalents of 4-vinylpyridine. The 1H NMR spectrum was observed after each addition and the change in aromatic –OH shifts followed until the presence of H bonding interactions was evidenced by the consistent downfield shift of this aromatic–OH signal with increased additions. This process was continued until the aromatic –OH signal was no longer detectable due to peak broadening.

Jianhua Xiong et al [21] described NMR characterization of five sulphonylurea herbicides. A series of solutions was prepared with a fixed concentration of pyrazosulphuron ethyl as template (20 mmol per L) and three different concentrations of MAA (0, 60 and 120 mmol per L) in dichloromethane-d .The 1H NMR spectra were recorded using tetramethylsilane (TMS) as the internal standard.

In this study, the intermolecular interactions between PS and MAA during the pre-polymerization stage were evaluated by 1H NMR analysis, which was performed with various molar ratios of MAA and PS in CDCl3. The two different amino protons present in the template PS molecule were numbered as NH1 andNH2. The chemical shifts of the triazine ring NH1 and the sulphoamino group NH2 were 7.30 and 12.96 ppm, respectively, in the absence of MAA in CDCl3. After the addition of 120 mmol per liter of MAA, and NH1 was chemically shifted from 7.30 to 7.89 ppm; however, the chemical shift of the NH2 in PS was essentially unchanged from 12.96 to 12.98 ppm. These observations suggested that the NH1 was involved in the formation of hydrogen bonds, and the magnitude of the change in chemical shift was related to the concentration of MAA. As the concentration of MAA increased, the hydrogen bond interactions were strengthened. The formation of hydrogen bonds led to shielding effects that increased the chemical shifts of NH1. The NH2 proton was not involved in the formation of intermolecular hydrogen bonds; therefore, the influence of MAA on the chemical shift of NH2 was not observed.

### 3.1 Morphological characterization

The analyte binding capacity, binding specificity and chemical and thermal capacities of these MIPs were found to depend directly on the characteristics of their surface morphology. Several approached have been found to be suitable for the determination of surface morphology of MIPs.

#### 3.1.1 Mercury intrusion porosimetry

Mercury intrusion porosimetry involves mercury being forced, under pressure, into a fixed mass of dry polymer. The information that can be gained from such experiments is similar to that which can be obtained from Nitrogen Sorption Porosimetry, although it is generally more sensitive at probing larger (macro-) pores.

#### 3.1.2 Nitrogen sorption porosimetry

Nitrogen sorption porosimetry deals a fixed mass of dry polymer being exposed to a gas (usually nitrogen) at a series of fixed pressures. By calculating the amount of gas sorbed as a function of pressure, sorption isotherms can be constructed from which, following application of theory (BET) and mathematical models, information on the specific surface area (m2/g), specific pore volume (ml/g), average pore diameter and pore size distribution can be extracted. The method is particularly full of advantage for analyzing in detail medium-sized (meso-) and small (micro-) pores. (The IUPAC definitions of size as employed to pores are as follows: micropores <2 nm; 2 nm < mesopores <50 nm; macropores >50 nm).

#### 3.1.3 Solvent uptake experiments

Macroporous polymers are permanently porous even in the dry state and solvent can be employed to access the pore network. After measuring the amount of solvent up taken by a polymer an estimate can be made of the specific pore volume (ml/g).

#### 3.1.4 Inverse size exclusion chromatography (ISEC)

ISEC enables the porous structure of polymers to be probed in the wet-mode. This is perhaps significant as far as imprinted polymers are concerned because imprinted polymers find applications, more often than not, in the wet state. In ISEC experiment, the porous solid is the stationary phase and the time taken for a series of linear soluble polymer standards of known molar mass to elute through the column is measured at fixed flow-rate. Upon applying suitable mathematical models information on the pore structure of the polymer stationary phase can be extracted. In many aspects ISEC can be viewed as being a complementary tool to nitrogen sorption porosimetry and mercury intrusion porosimetry, with the advantage being that it can operate in the wet-mode.

#### 3.1.5 Microscopy, e.g. Scanning electron microscope (SEM)

Microscopy can be applied in a variety of distinct ways to probe imprinted polymers on a variety of length scales. For instance, light microscopy can be used to verify the structural integrity of polymer beads whereas scanning electron microscopy (SEM) can be used to image macrospores.

### 4.1 Characterization of the molecular recognition properties.

#### 4.1.1 Liquid chromatography–mass spectrometry (LC-MS)

Jun HaginakA et al [22] proposed determination of non-steroidal anti-inflammatory drugs in river water samples by liquid chromatography– mass spectrometry (LC-MS) using molecularly imprinted polymers as a pretreatment column.

Ya-lei Zhang etal [23] reported LC-MS method for diclofenac MIPs derived from water samples The LC–MS/MS analyses of DFC were performed on High Performance Liquid Chromatography coupled with Mass Spectrometry. The separation was conducted on an Agilent Eclipse XDB C18 reversed phase column, with the flow rate of 0.30 mL/min. Methanol (mobile phase A) and water with 0.1% (v/v) acetic acid (mobile phase B) were applied for separation. The injection volume was 10 per L, and the column temperature was 35 °C. The gradient was held at 70% A for 5 min, and increased to 85% A within 5 min and held for 5 min, and then reset

to initial conditions of 70% A in 5 min and held for 5 min. The mass spectrometer detection was carried out in electrospray ionization negative ion mode with selected reaction monitoring (SRM).

## 3. Thermal study

M.V. Gonzalez-Rodriguez and coworkers [24] first proposed thermal method for MIPs**.**The DSC traces and the TGA were obtained to survey the glass transition temperature and thermal stability of the polymers. Dynamic experiments were conducted under Argon atmosphere. The heating rate was 10°C min−1. The temperature range of the experiments was from room temperature to 600°C. The Thermogram as measured in the onset point of the loss mass curve. The DSC measures were carried out using 5–10 mg of the samples under an atmosphere of nitrogen gas. The samples were first heated to 100 °C and held at that temperature for 5 min to remove the thermal history. Then the samples were cooled to −50 °C at the rate of 20 °C min−1, held for 5 min, and again heated from −50 to 250 °C at 20 °C min−1 (second scan). Glass transition values (Tg) were taken as the midpoint of transition in the second scan of DSC thermograms.

## 4. Supercritical fluid technology.

Hun-Soo Byun and coworker [25] Synthesize and characterized a high selective molecularly imprinted polymers for bisphenol and 2, 4-dichlorophenoxyacetic acid by using supercritical fluid technology. The MIPs nanoparticles obtained in this study have binding properties comparable to the general MIPs synthesized with/without conventional organic solvents. The supercritical polymerization process may be applied as a new sophisticated tool of preparing highly functional polymers.

### 5. Swelling studies

The capacity of a functional polymer is governed by the accessibility of the reactive functional groups, which in turn depends on the extent of swelling and solvation. The rate of diffusion of a reagent into the polymer complex mainly depends on the extent of swelling. Hence swelling is an important parameter, which controls the extent of rebinding. The most effective solvent can carry out the percentage rebinding reaction very effectively. The efficacy of swelling can be determined in terms of change in weight. Alternatively, by packing a definite weight of the polymer in a capillary tube and measuring the volume before and after incubation in the solvent, the swelling ratio can be determined in terms of change in volume [26].

## 6. Conclusion

In this paper, recent analytical methods applied for characterization of molecular imprinted polymers (MIPs) were reviewed. Several tools like UV/VIS spectrophotometry, FT-IR, NMR, thermal methods, chromatographic methods ( LC-MS) and morphological analysis tools are the main techniques that have been used, of which it is found a tradition to utilize faster techniques with cost savings and reduction in solvent consumption. From this work, it was observed a trend in the application of techniques increasingly rapid for characterization of MIPs. It is our expectation that this will facilitate researchers to plan their own sophisticated analytical pathway for characterization of MIPs in a more logical and structured manner, and to begin to appreciate the limitations of the present approaches in this molecularly complex area.

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