# *Insight the Rational Design of Molecularly Imprinted Polymer for the Development of Biomimetic Receptors*

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## ABSTRACT

Innovation on clinical devices is essential to enhance the accuracy and efficiency on patients' diagnosis, and how their clinical treatments are monitored. Molecular imprinting technique has gained attention to generate novel biosensor and clinical diagnostic devices with high sensitivity and specificity which demonstrated affinities compared to their natural counterparts. In order to rationally design a hydrogel-based imprinted gel, a method to evaluate complex formation, imprinting synthesis and MIP-ligand rebinding was completed. The extent of the association between hydrocortisone and the functional monomer, methacrylic acid (MAA) was investigated by Nuclear Magnetic Resonance (NMR) spectroscopy. Dissociation constants for the complex formation between hydrocortisone and a functional monomer analogue, acetic acid, as a function of solvent nature were estimated by NMR titration. The stoichiometry of the complex formation between hydrocortisone and an acetic acid on each solvent was evaluated continuous variation method. Dimethyl sulfoxide and ethanol were selected as porogens to assess solvent effect. The dissociation constants obtain for ethanol-$d_6$ reflects a greater proximity of interaction between solution adducts compared to dimethyl sulfoxide-$d_6$. It is consequently associated by their dielectric constant of the solvents 2:1 for dymethyl sulfoxide-$d_6$. The collective analysis of NMR titration and Job plot method indicated that the extent of shifts displacement was proportional to their proximity to the interaction site which is not apparently associated with its stoichiometric capabilities of complex formation. To evaluate synthesis condition, *in situ* free radical copolymerization was monitored by ATR-FTIR spectroscopy with methacrylic acid (MAA) as the functional monomer and tetra(ethylene glycol) dimethacrylate (EGDMA) as the crosslinking agent at different solvents. The synthesis was performed in the presence and absence of the template molecule. The combined set of analysis allowed a better understanding of the recognition events giving rise to the imprinting effect during MIP synthesis and to ligand-MIP binding events. In situ polymerization results demonstrated a delay the onset autoaccelaration during imprinting process. In essence, the propagation kinetics was reduced by the decrease of monomer mobility, which suggested the functional monomer-template complexation, already confirm by the NMR spectroscopic studies. Consequently, this information was applied to the design of thin films MIP. The collapse-swelling transition could be programmed to promote binding capabilities and enhance template diffusion. To this aims, hydrocortisone imprinted polymers were synthesized in aqueous medium. To determine the suitability of hydrogel-based MIPs; swelling behavior, structural parameters (e.g. mesh size, $\xi$) and

template permeation was investigated as function of pH and copolymer composition. The MIP characterization results demonstrated an increase on template permeation influenced by mesh size at pH equal to 5.5 at 37°C and ionic strength of 0.1M. A different behavior was shown for the characterization at pH equal to 6.0 at 37°C and ionic strength of 0.1M. A reduction on the permeability coefficient was observed for MIP with a MAA/EGDMA ratio of 17:1. It suggested the influence of MIP-ligand binding on hydrocortisone transport through the polymeric network synthesized by molecular imprinting technique. Further biding capacity studies will allow the estimation of the association constants of the polymeric receptors. Overall, this work reported the evaluation of the principal factors affecting the stabilization of functional monomer-template complex before and during imprinting process which provides essential information for the rational design of molecularly imprinted polymers.

**KEYWORDS**
Molecular imprinting; hydrogels; NMR titration; in situ polymerization; biosensors; rational design.

## INTRODUCTION

Biosensors have experienced great attention within the field of drug detection, clinical diagnostics, environmental analysis, food analysis, and production monitoring(1). Their convenience for self-testing, small size, fast response, sensitivity and specificity demonstrated the advantageous of developing biosensors (2). Among the different class of biosensors (e.g. enzyme and receptor-based sensor, antibody based sensor), the biomimetic sensors develop by molecularly imprinted polymers (MIPs) have shown great stability and robustness, facilitating their application under extreme pressure, temperature, and in the presence of acid or in organic solvents (3).

The molecular imprinting technique has been an essential tool for the development of highly affinity materials employed as recognition elements of biosensor devices. The technique involves the formation of template-functional monomer complex followed by its stabilization due to the presence of crosslinking agent. The subsequential removal of the template molecule will produce a material with specific recognition sites. MIP sensors have been develop for pesticides, sugars, nucleic acid, amino acid derivatives, drugs, toxins, steroids, proteins, and cells (4).

The forefront of imprinted systems is the development of hydrogel-based MIP which modulates its binding ability (5). Several challenges have to be overcome on the formation of imprinting cavities within hydrogels. It is critical to perform validation protocols for MIP design that will identify the optimal morphology to enhance MIP-ligand binding. It is also desired to enhance MIP performance on aqueous medium; and increase the ratio between specific and non-specific binding sites to increase MIP affinity (6).

Tanaka and collaborators (7) reports imprinted gels with adsorption sites that could be destroyed and reformed upon the swelling behavior of the gel. Wulff et al. (8) attempts to develop highly crosslinked microgels and demonstrated their selective and recognition capacity. Similar reports demonstrated the need of develop strategies to obtain imprinted material which balance the flexibility and rigidity for MIP system to

facilitate the mass transfer through the MIP pores without loosing the imprinting memory. Consequently, great efforts have to be taken to optimize the gel morphology in order to advance at *in vivo* measurements on biological systems. To this end, different approaches have been taken to rational design imprinted polymer. Previous reports have showed optimization strategies which concentrate on the assessment of the pre-polymerization stage or the post-polymerization stage (9). Pre-polymerization stage assessment are focused to understand the stabilization of functional monomer-template complex or to develop a method capable for the simultaneous characterization of several significant factors on MIP synthesis. The extensive used of spectroscopic studies of the self assembly, polymerization and MIP-ligand binding have been critical in order to fully understand the mechanism of imprinting process. Workers in the field have yet to carefully examine MIP-ligand binding by FTIR and NMR spectroscopy. The group of Sellenger has pioneered the self assembly evaluation by NMR studies (10). Shea et al. demonstrated an effective method for quantitative analysis of interaction sites at the MIP (11). Related efforts directed to the identification of MIP-ligand binding capacity have recently been undertaken by other authors (12-13). Several reports have made spectroscopic assessment to analyze the functional monomer-template self association by NMR and UV spectroscopy. Direct evidences have been presented to identify the complex motif and to estimate the association constant (14-16). Nicholls et al proposed a model for the molecular basis for ligand recognition of MIP by employing the NMR method (17). The group of Peppas and collaborators have investigated the effect of template molecule on the conversion and polymerization rates of MIP (18). Results suggest the segmental mobility was reduced due to interaction within the template molecule and the functional monomer.

The post-polymerization approach aims on the modification of binding sites distribution by either chemical or physical means. A good example is the evaluation of the influence of mobile phase composition investigated by Lu et al. (19). Results demonstrated the effect of solvent nature on the interaction between the imprinted material and template molecule which influence the selectivity and sensitivity of the system. In another example, the potential of chemometrics for the proposal of a mechanism controlling MIP rebinding was consider by Nicholls and collaborators (20). Furthermore, they provide a basis for the prediction of MIP-ligand biding.

This study investigates the stabilization of the functional monomer-template complex on a hydrogel system by estimating the dissociation constant at the pre-polymeric mixture. In addition, it will monitor the polymerization rates of the imprinting synthesis. The permeability and swelling capacity of imprinted polymers were estimated to evaluate the MIP-ligand recognition process. The implications of these finding will provide the information require to rational design imprinted materials by a systematically methodological approach such as combinatorial chemistry.

## MATERIALS AND METHODS

The real time polymerization kinetic was measured using ATR-FTIR spectroscopy (IR350 Nicolet Instrument, Madison, WI). *In situ* free radical polymerization was monitored on ZnSe 45° crystal in inert ambient purged with nitrogen for 10 minutes. The reaction was initiated ultraviolet source at room temperature.

Prepolymeric mixtures were prepared with a variation of a monomer/crosslinker molar ratio of 1:1 and 4:1. A solvent dilution of 50% w/w was employed with a mixture of ethanol and water; and dimethyl sulfoxide to evaluate the effect of solvent nature. The prepolymeric mixture was previously purged before stating the experiment. The ATR-FTIR spectrums were recorded at intervals in the spectral range of 4000-600cm$^{-1}$ at a spectral resolution of 4 cm$^{-1}$. Conversion was calculated by measuring the peak height as a function of time associated with =CH2 vibration at 1322 cm-1 and 1318 cm-1 for the mixture of ethanol and water, and for dimthylsulfoxide, respectively.

For the pre-polymeric evaluation, NMR spectrums were recorded with a Bruker 500 spectrometer (Germany) from the mixtures prepared in dymethyl-sulfoxide-$d_6$, and ethyl alcohol-$d_6$. The molar fraction of hydrocortisone and functional monomer was varied from 0 to 1.0 with a constant total concentration of 16 M.

Imprinted and non-imprinted polymers were synthesized by free radical solution polymerization. The monomer/crosslinker ratio was varied from 17:1 to 39:1. A mixture of 50%w/w of ethanol and DI water were employed as solvent. The solvent was added with a 50%w/w dilution. The equilibrium volume swelling was determined by buoyancy studies. The weight of the polymer network was determined: (i) after crosslinking but before swelling, (ii) after complete drying; and (iii) after equilibrium was attained in the swelling medium. Imprinted and non imprinted polymers were also characterized on a side by side diffusion cell at 37°C and pH conditions of 5.5 and 6.0. The permeability coefficient of hydrocortisone was estimated by applying Fick's Law.

## RESULTS AND DISCUSSION

The molecularly imprinted synthesis was monitored by ATR-FTIR spectroscopy. The main objective was to evaluate the molecularly imprinted synthesis. The C=C stretching vibration peak at 1640cm-1 confirm how the polymerization proceed (not shown). Concurrently, the chemical shift of carboxylic acid vibration at 1710 cm-1 was observed. These results will suggest the formation of interaction sites within carboxylic acid functional group. Subsequently, the apparent conversion was estimated by measuring the peak area under =CH2 rocking vibration at 1320 cm-1. The changes on peak area under =CH2 are directly related to the conversion of double bonds presence in the system. The solvent employed was dimethyl sulfoxide (see figure 1) and a mixture of 1:1 mass ratio of ethanol and water. The investigation of molecular imprinting polymerization demonstrated a lower synthesis conversion due the presence of the template molecule. It is suggest a delay on the auto-acceleration of the polymerization cause by the reduction of the functional monomer mobility which is forming a complex with the template molecule. The formation of complex adducts between hydrocortisone and methacrylic acid was confirmed by NMR titration and continuous variation studies (not shown).

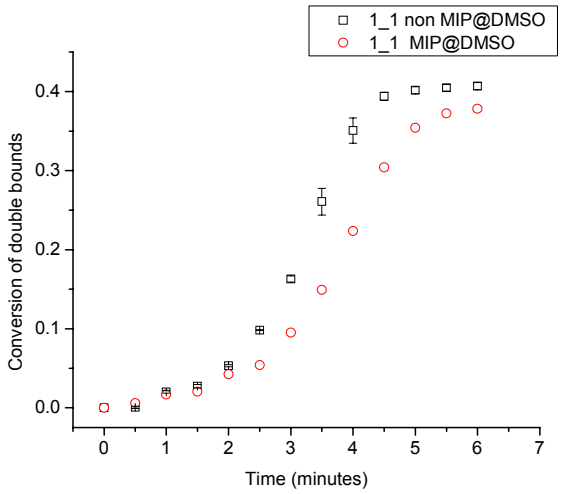


**Figure 1.** Conversion of double bound during in situ polymerization as a function of time for P(MAA-co-EGDMA) at dimethyl sulfoxide

Hydrogels were synthesized in the presence of the template molecule, hydrocortisone, by free radical polymerization at room temperature. Control polymers were also prepared in the absence of the template molecule. The swelling capabilities were studied at different pH values to evaluate the flexibility of the network. The figure 2.a showed the equilibrium volume swelling of imprinted and non-imprinted polymers. Not significant differences on swelling capacities were observed. Even though, the correlation length or mesh sizes were estimated as previously reported (21). As shown on figure 2.a, imprinted polymers have similar swelling behavior compare to non-imprinted polymer. Even though, the cavity formation could be confirmed by the mesh size results (see figure 2.b). The results demonstrated an increment on mesh size was produced by employing by imprinting synthesis.

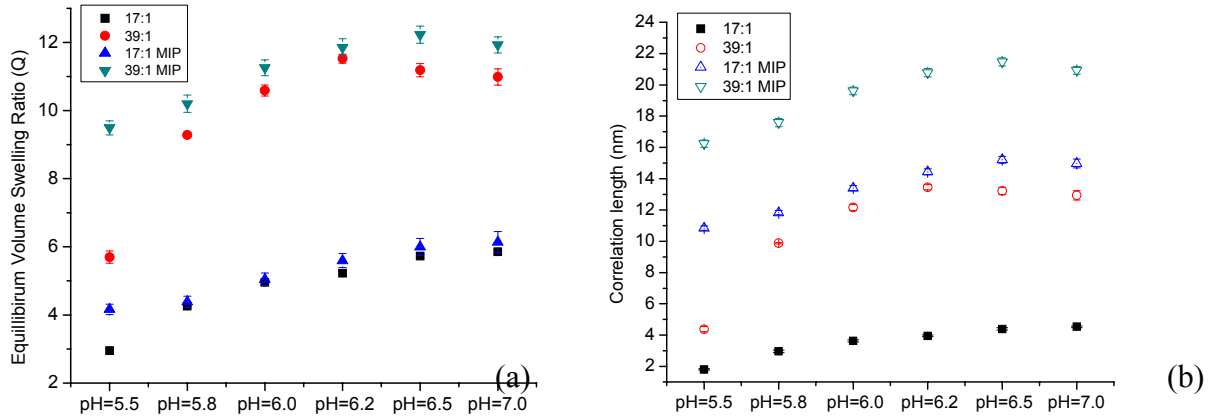


**Figure 2.** (a) Equilibrium volume swelling ratio as a function of pH for P(MAA-co-EGDMA) hydrogels of composition 17:1, and 39:1. (b) Effect on the correlation length (ξ) as a function of copolymers composition and pH of composition 17:1, and 39:1.

Within the purpose to investigate the ability of template molecule to diffuse through imprinted polymers, the solute permeability coefficients were determined from permeation studies using the following equation:

$$\ln\left(\frac{2c_t}{c_o}-1\right)=\frac{2A}{V}Pt \qquad (1)$$

Where $c_t$ is the solute concentration in the receptor cell at time t, $c_0$ is the initial concentration in the donor cell, V is the volume of each of the half cells (7 mL), A is the effective area for permeation (1.803 $cm^2$), and P is the permeability coefficient. The results reveal a reduction on the permeability coefficient at pH equal to 6.0 on the imprinted hydrogel with a morphology using 17:1, MAA/EGDMA ratio (see figure 3). It is suggest the retention of the template molecule on the imprinted polymer due to the MIP-ligand rebinding. It is understood the integrity of the imprinting cavity is maintain for this morphology. Further binding and selectivity studies will confirm the magnitude of interaction and the imprinting specificity.

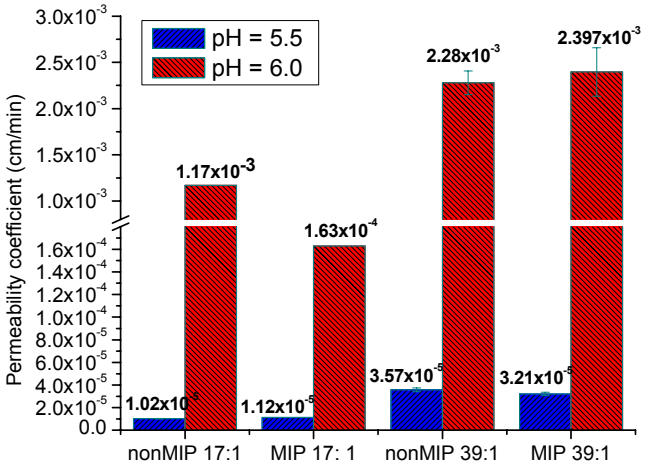


**Figure 3.** Hydrocortisone permeability coefficient through P(MAA-co-EGDMA) hydrogels of composition 17:1, and 39:1

These findings provide an important contribution by describing the nature of recognition in low-crosslinked system. It also demonstrate that evaluating the principal factor affecting the stabilization of functional monomer-template complex will provide essential information for the rational design hydrogel-based MIP. The implication of these findings will be discussed.

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