Changes in the Porosity and Permeability of a Molecularly Imprinted Membrane Induced by the Adsorption of a Trace Quantity of Template

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Abstract: It is known that the diffusive permeability of solutes within a thin layer of molecularly imprinted polymer (MIP) may be affected by specific binding of the MIP with its template molecule. This phenomenon, termed the gate effect, shows promise for the development of novel biomimetic sensors. However, the mechanism underlying this effect is still unclear; although the relationship between the specific adsorption of a template and the corresponding porosity and permeability of the polymeric film or membrane is very important, this association has not yet been examined in detail. We therefore studied this relationship using a molecularly imprinted self-supporting membrane (MISSM) possessing chiral specificity, specially developed as a tool for investigating the gate effect. Both the diffusive permeability and volume porosity of the MISSM were sensitive to the presence of the template compounds (D and L-phenylalanine) at concentrations as low as 5 μM, while, at the same time, insensitive to the enantiomer of the template. The relationship between the amount of adsorbed template and the equilibrium template concentration followed the expected Langmuir isotherm pattern, which indicates the thermodynamic homogeneity of binding sites in the MISSM. This study also demonstrated that the relative concentration of the adsorbed template in the membrane was only 3 ppm and relative site occupation was only 1% following exposure to a 5 μM concentration of the template. These results show that the gate effect may be advantageously exploited during application of MIPs in amplifiers or sensors offering high sensitivity.

Keywords: Molecularly imprinted polymer, membrane, chirality, gate effect, permeability, porosity, adsorption.

1. INTRODUCTION

Molecularly imprinted polymers (MIPs) are synthetic polymers that contain specific binding sites formed by imprinting of a target molecule (or template) during the polymerization process. An MIP layer can be prepared by a simple and economical procedure [1-3]. Before such MIPs may be employed as molecular recognition elements in chemical-sensing devices, however, it is necessary to develop a means of translating specific binding events at the MIP into an electric signal. The so-called gate effect, which refers to changes in the diffusive permeability of solutes within the MIP layer resulting from specific binding at the template, can be used as a mechanism for signal transference based on conductometry, utilizing changes in the ionic permeability of the MIP membrane associated with a specific interaction with the template [4-6]. In this gate analogy, the template corresponds to the key while the MIP site which allows specific rebinding with the template corresponds to the keyhole. An amperometric method is also applicable, using a thin MIP layer grafted onto an electrode, in which the template can be detected by following changes in the faradic current resulting from the change in permeability of a redox marker across the MIP layer [7-11]. The gate effect is a selective process capable of discriminating between reactions of the template even with enantiomers having identical chemical and physical properties [9]. Unfortunately, the mechanism underlying the gate effect is not yet fully understood, partly because the relationship between site-specific adsorption and the associated change in porosity has not been sufficiently elucidated.

Recently, we developed a molecularly imprinted self-supporting membrane (MISSM) composed of poly (methacrylic acid (MAA)-*co*-2-vinylpyridine (VP) -*co*-triethyleneglycol dimethacrylate (TEDMA)) [12, 13]. This membrane possesses sufficient thickness and mechanical robustness to allow measurement of its solution uptake, solute permeability and template adsorption. The permeability and solution uptake of the membrane are both modified by the presence of the template, but insensitive to the template's enantiomer [12], since the template is adsorbed by the membrane, but not the enantiomer. This membrane is therefore an ideal model for basic study of the gate effect.

In this work, we obtained the adsorption isotherms of both the template and its enantiomer on different MISSMs. The effects of template concentration on solution uptake and on the diffusive permeability of the MISSM were also observed and the mechanism underlying the gate effect is discussed based on a comparison of the results.

2. MATERIALS AND METHODOLOGY

Self-supporting membranes imprinted with either D or L-phenylalanine (Phe) were prepared according to a procedure

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described previously in the literature [12]. The functional monomers MAA and VP were copolymerized along with the cross-linking monomer TEDMA, employing polyurethane diacrylate as a plasticizer and 2,2-azobisisobutyronitrile (AIBN) as the initiator. The composition of the prepolymer solution used in membrane preparation is provided in Table **1**.

Table 1. Composition of the Monomer Solution Used for MISSM Preparation [12]

Component	Weight [mg]	Moles [mmol]	Molar Ratio [%]
Template[1]			
L- or D- Phe	20	0.12	0.4
Functional Monomer			
MAA[2]	84	0.98	3.3
2-VP[2]	102	0.97	3.2
Crosslinker			
TEDMA[3]	7847	27.41	91.4
Plasticizer			
PUA	950	0.38	1.3
Initiator			
AIBN	20	0.12	0.4

Porogen: 10 mL acetonitrile:distilled water:acetic acid = 8:1:1 (by volume).
[1]The template was omitted during the preparation of the NIM.
[2]Distilled under reduced pressure before use.
[3]Washed with a 0.1 M aqueous solution of sodium carbonate and then with distilled water and dried over anhydrous sodium sulfate before use.

A mixture of acetonitrile, water and acetic acid was used both as the solvent for dissolving the monomers and the template and as the pore-forming agent (porogen) [12]. Self-supporting membranes were obtained by performing copolymerization under ultraviolet irradiation between two quartz plates separated by a polytetrafluoroethylene spacer. One plate was treated with dichlorodimethylsilane while the second was untreated. Each membrane was subsequently soaked in a large volume of a 50-wt% aqueous methanol solution for more than 24 h in order to extract residual Phe and unreacted monomer, and then air-dried at room temperature. Membranes formed in the presence of L-Phe or D-Phe are referred to as the L-enantiomer-imprinted membrane (LIM) and D-enantiomer-imprinted membrane (DIM), respectively, while membranes prepared without Phe are referred to as non-imprinted membranes (NIMs). The complete removal of the template from the LIM and DIM samples was confirmed by colorimetric analysis using a ninhydrin indicator [13]. The volumetric uptake by each membrane of 0 to 50 mM solutions of Phe in a mixed solvent of methanol and water (1:1 by wt) was subsequently measured at room temperature using a pycnometer according to a procedure described previously [12]. In this manner, the influence of the Phe concentration on the extent of membrane solution uptake was evaluated.

The permeability of the membranes was analyzed by a batch-wise dialysis apparatus composed of two identical chambers, using creatinine as a marker at 37°C, based on a process described in a prior publication [12]. The dialysis test solutions in both chambers were identical and consisted of 0 to 2 mM L- or D-Phe as the guest species, dissolved in a mixed solvent of 1:1 (by wt) methanol and water. Each membrane was soaked in the test solution for 48 h prior to dialysis. Creatinine (2.65 mM) was added to the solution in one of the chambers at the start of each trial as a permeability marker, and the overall mass transfer coefficient of creatinine across the membrane was calculated from the changes over time in the creatinine concentrations in each of the two chambers. The resulting data allowed us to evaluate the dependency of the mass transfer coefficient of creatinine on the concentration of L- or D-Phe.

The amount of adsorption of L-Phe and D-Phe by the membrane material was also estimated using a method outlined in our prior publication [12]. The dried membrane was first pulverized and then suspended in a 1.0 mM racemic solution of Phe in a 1:1 (by weight) mixture of methanol and water, and the Phe concentration in solution was allowed to reach equilibrium by stirring over a 48-h incubation period at 37°C. After equilibration, the L-Phe and D-Phe concentrations in the solution were determined by chiral-discriminative liquid chromatography. The resulting data allowed the calculation of the quantity of each enantiomer which had been adsorbed by the pulverized membrane material. Using the results acquired from these trials, the adsorption isotherm of each isomer on the membrane materials was obtained.

3. RESULTS

3.1. Characterization of the MISSM

The obtained MISSMs were characterized by Fourier transform infrared (FT-IR) spectroscopy using an FTIR-8400S instrument (Shimadzu Co., Ltd., Kyoto). Fig. (**1**) presents the FT-IR spectra of both NIM and LIM in KBr tablets, and it is evident that there are no significant differences between the two spectra. The peaks in these spectra are identified as shown in Table **2**. The results show that all monomers participated in the copolymerization.

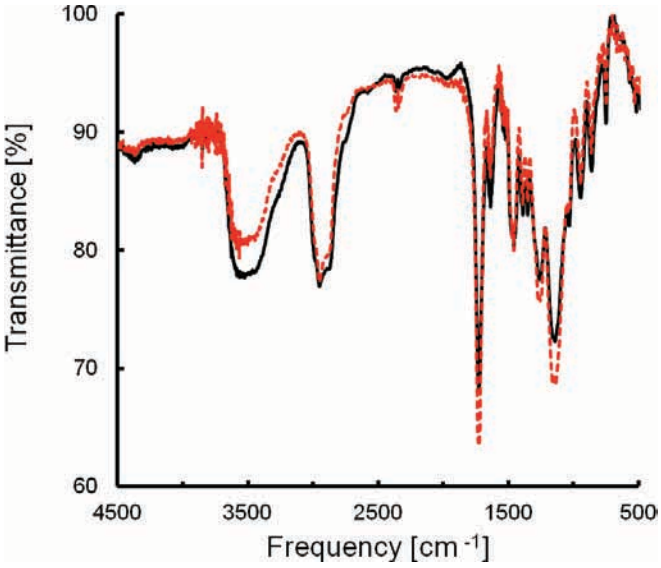

Fig. (1). FT-IR spectrum of NIM (broken line) and LIM (solid line) in KBr tablets.

Table 2. Identification of the Primary Functional Groups Evident in the FT-IR Spectra

Functional Group	Monomer	Peak
Carboxyl	MAA	3600-3420 cm^{-1} (free -OH stretch) 1724 cm^{-1} (associated C-O stretch)
Pyridyl	2-VP	3020 cm^{-1} (CH stretch) overlapped with alkyl group 1635 cm^{-1} (C=C stretch and C=N stretch) 1247 cm^{-1} (ring vibration and C-H scissor) 856 cm^{-1} (ring vibration and C-H scissor) 748 cm^{-1} (ring vibration and C-H scissor)
Triethyleneglycol	TEDMA	1134 cm^{-1} (alkyl ether stretch)

Surfaces and cross sections of the LIM and NIM samples were observed by scanning electron microscopy (VE-9800, Keyence Co., Ltd., Osaka). The corresponding micrographs shown in Fig. (**2**) indicate that both membranes have a fine homogeneous structure free from macropores.

3.2. Template Adsorption Isotherms

We confirmed that the adsorption had reached equilibrium following 24 h of incubation for all adsorbate concentrations tested. The adsorption isotherms for the two Phe isomers onto the pulverized MISSMs (both LIM and DIM) are presented in Fig. (**3**). No adsorption of either of the Phe isomers onto NIM was observed [12].

It is evident from these isotherms that each MISSM sample adsorbed its own template molecule while exhibiting nil or very minimal adsorption of the opposing enantiomer. The adsorption isotherms of the two templates are both well described by the Langmuir isotherm equation given below as Eq. 1:

$$n = \frac{n_{sat} C}{K + C} \quad (1)$$

where n is the molar quantity adsorbed, n_{sat} is the molar quantity adsorbed at saturation, C is the equilibrium concentration of the free template and K is the Langmuir equilibrium constant. The results of regression analyses of the data obtained from template adsorption onto the MISSM according to the Langmuir isotherm equation are summarized in Table **3**, which shows that there were no significant differences between the regression parameters for the two membranes.

The relationship between the amount of template adsorbed on the MIP and the equilibrium concentration of the template can be well fit by the Langmuir isotherm equation, with correlation factors above 0.99. These results are an indication that the imprinted sites on the membranes are thermodynamically homogeneous [14].

3.3. Solution Uptake and Permeability

Solution uptake is the ratio of the volume of solution absorbed by the membrane to the total volume of the wet membrane [12, 13], and this parameter can be considered as a measure of the total volumetric porosity of the wet membrane [15].

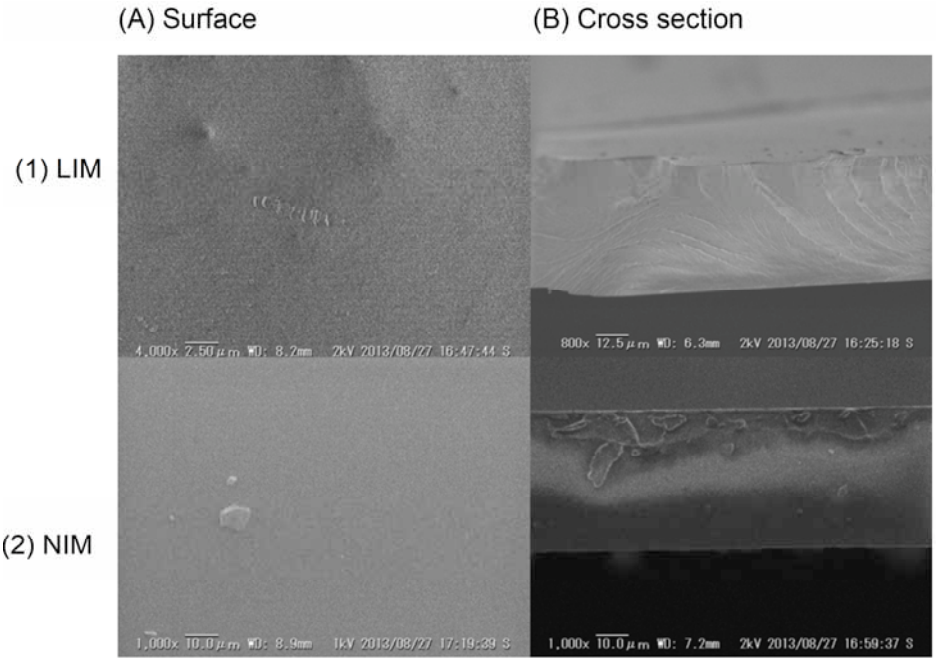

Fig. (2). Scanning electron micrographs of self-supporting membranes.

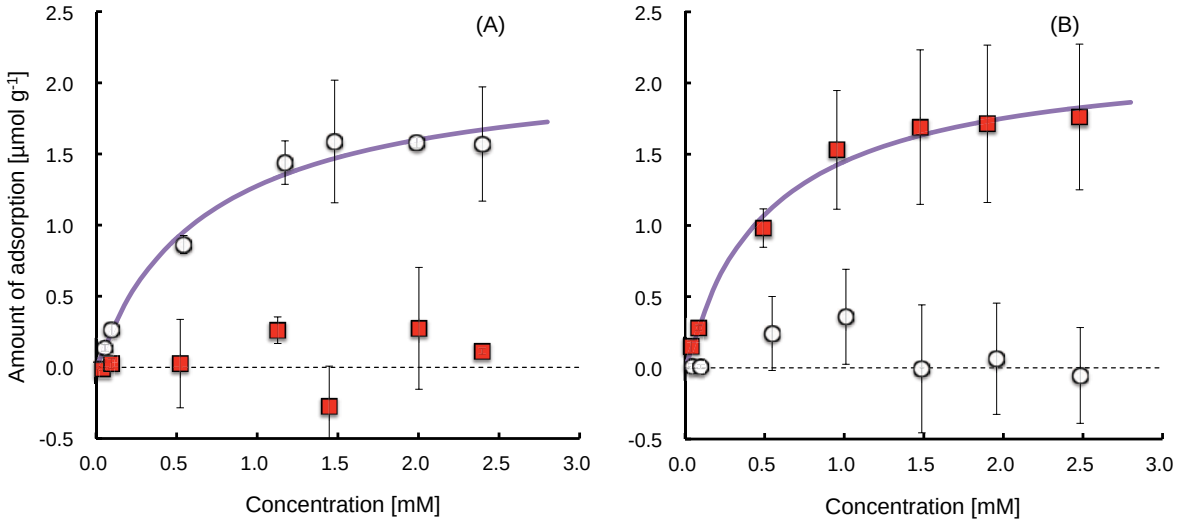

Fig. (3). Adsorption isotherms for L-Phe (circles) and D-Phe (squares) onto LIM (**A**) and DIM (**B**). Solid lines indicate regression fits to the Langmuir adsorption isotherm equation. (Conditions: pulverized membranes in 1:1 (w/w) water/methanol at 37°C).

Table 3. Langmuir Adsorption Isotherm Parameters for Template Adsorption onto MISSM

Membrane	n_{sat} [µmol g^{-1}]	*K* [mM]	Correlation Factor
LIM	2.1	0.68	0.992
DIM	2.2	0.53	0.995

Fig. (**4**) shows the relationship between the solution uptake and concentration of Phe in the solution. Plot (A) of this figure demonstrates that the extent of solution uptake by the NIM was not affected by the concentration of Phe in the solution. The uptakes for the two imprinted membranes, however, increased with increasing template concentration, although both membranes were essentially insensitive to the concentration of the opposite enantiomer. The level of solution uptake appears to level off at a template concentration of 1.5 mM, which is approximately the same concentration at which template adsorption was seen to plateau in Fig. (**3**). These results suggest that the extent of solution uptake for each MISSM is controlled by the specific interaction between the template and the imprinted site. Based on the data in Fig. (**4**), the change in the solution uptake for the LIM or DIM due to the template at concentrations above 5 µM exceeded dramatically the change seen in the NIM due to Phe for all concentrations tested. The changes in the solution uptakes for the imprinted membranes produced by the respective templates were higher than the changes produced by the enantiomer analogues at the same concentrations. The minimum Phe concentration that results in site-specific changes in the solution uptake is 5 µM.

The relationship between the overall mass transfer coefficient of creatinine across the self-supporting membrane during dialysis and the Phe concentration is presented in Fig. (**5**). The transfer coefficient across the two MISSMs increased with increasing template concentration, plateauing at a concentration of 1.5 mM and, once again, each MISSM is shown to be unaffected by the presence of the opposite

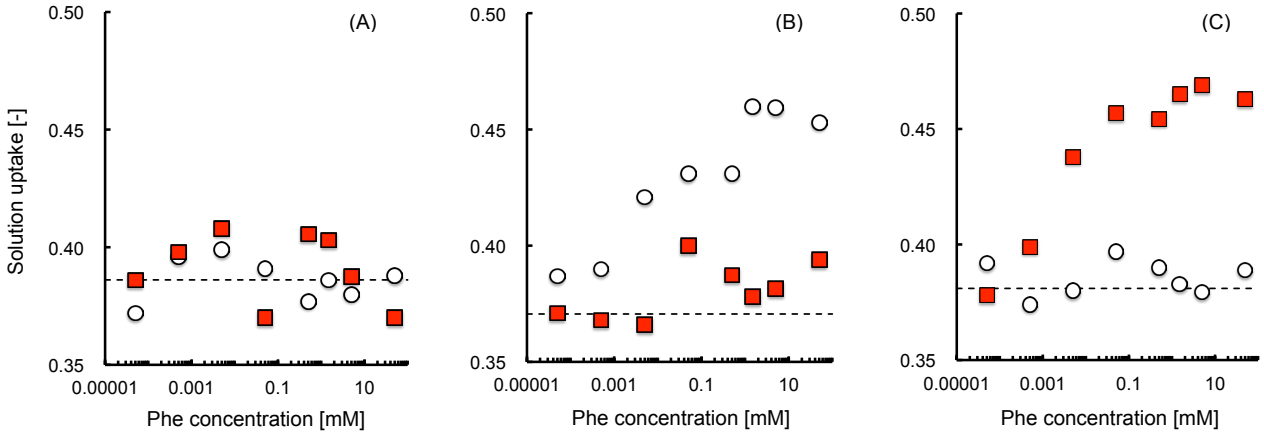

Fig. (4). Relationships between solution uptake for MISSM [(**A**) NIM, (**B**) LIM and (**C**) DIM] and the concentration of L-Phe (circles) and D-Phe (squares) in the solution. (Dashed lines indicate solution uptake in the absence of Phe.) (Conditions: as-formed membranes in 1:1 (w/w) water/methanol at 20°C).

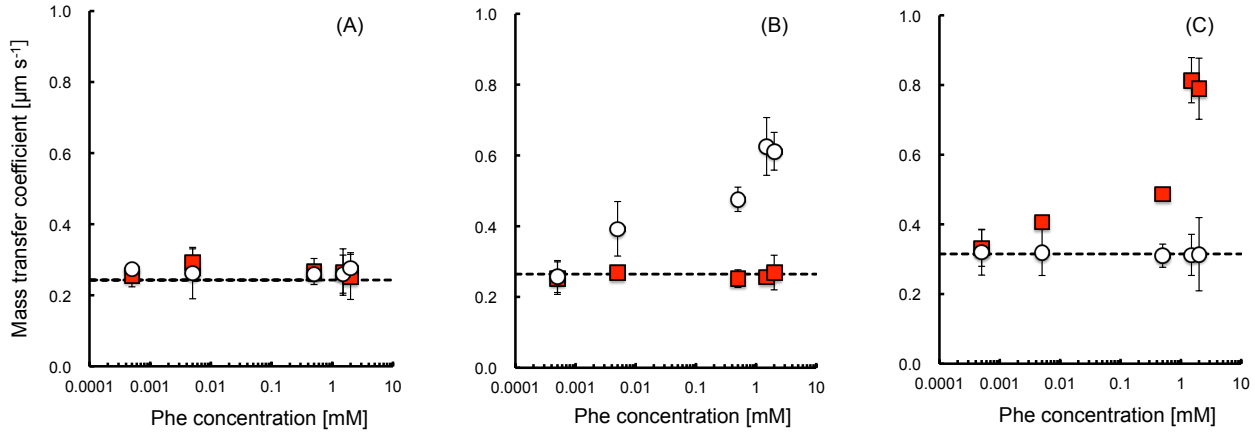

Fig. (5). Relationships between the overall mass transfer coefficient of creatinine across the MISSM [(**A**) NIM, (**B**) LIM and (**C**) DIM] and the concentrations of L-Phe (circles) and D-Phe (squares) in the solution. (Dashed lines indicate solution uptake in the absence of Phe.) (Conditions: as-formed membranes in 1:1 (w/w) water/methanol at 37°C).

enantiomer, since the coefficient across each MISSM was insensitive to the concentration of the opposite template enantiomer. The transfer across the NIM does not demonstrate any dependence on Phe concentration. These results show that the diffusive permeability of each MISSM is also controlled by the specific interaction between the template and the imprinted site. The change in the transfer coefficient for the imprinted membrane exceeded the standard deviation of the data at a concentration of 5 μM. In other words, statistically significant levels of chiral discrimination were observed based on changes in the mass transfer coefficients at Phe concentrations of 5 μM and above.

4. DISCUSSION

Several researchers have suggested that some morphological change occurs in molecularly imprinted polymers during molecular recognition of the template. Wullf *et al.*, for example, reported that the volume of sugar-imprinted poly(ethyleneglycol dimethacrylate-*co*-vinylphenyl boronic acid) [16, 17] is sensitive to the presence of the template. In other work, the volume of a non-covalently imprinted hydrogel was found to be affected by the presence of the template [18, 19]. The occurrence of a morphological change has also been discussed by one of the authors (S.P.) and coworkers, who reported that the conductivity of a molecularly imprinted membrane immersed in an electrolyte solution exhibits variations associated with the presence of the template [4-6]. Another author (Y.Y.) and coworkers found that the faradic current of an MIP-grafted electrode was affected by the template, because the accessibility of the redox marker through the MIP layer was sensitive to the presence of the template [7-11]. In the systems described above, MIPs worked not only as recognition elements, but also as transducer components, suggesting the possibility of creating an integrated sensor which exploits the gate effect in order to allow detection of the template. In spite of this opportunity, applications of the gate effect in sensing devices have not been actively pursued to date, due to our current limited understanding of the mechanisms underlying the effect, which is crucial to the design of highly sensitive and selective sensors. Comprehensive studies of the gate effect which would provide this understanding have not yet been performed.

We have previously developed an MISSM composed of poly (methacrylic acid-*co*- vinylpyridine-*co*-triethyleneglycol dimethacrylate) as a model for studying the gate effect [13, 14], and this membrane exhibited sufficient thickness to allow the measurement of the extent of template adsorption and solution uptake. The membrane also possesses sufficient mechanical robustness to allow its application in dialysis studies during permeability evaluations. (We do not, however, believe that the MISSM itself is applicable to the fabrication of sensing devices, since this material requires more than 24 h to reach equilibrium levels of adsorption due to its thickness of 50 μm. The MISSM is used in this work solely as a means of modeling and investigation of the gate effect.) The results of model experiments have demonstrated that the MISSM readily adsorbs its specific template molecule, but does not adsorb the enantiomer of the template. In addition, both the porosity and permeability of the membrane are sensitive to the concentration of the template in solution, but insensitive to the enantiomer of the template. As a result, this MISSM demonstrates potential for use as a model to elucidate the mechanisms underlying the gate effect.

Taking advantage of these properties of the MISSM [12, 13], the effects of the template concentration on the extent of adsorption, solution uptake and permeability were analyzed in this work. The analysis produced the following results:

1. The relationship between the quantity of template adsorbed and the template concentration in solution followed Langmuir's adsorption isotherm, indicating the homogeneity of the imprinted sites.

2. Substantial changes in both the solution uptake (or volumetric porosity [15]) and mass transfer coefficient (or diffusive permeability) of the MISSMs are induced by the template, beginning at a concentration of 5 μM.

The amount of template adsorption, the fractional occupation of the template sites and the weight occupation of the template in the membrane are given in Table **4**, all at a

template concentration of 5 μM. The adsorbed quantities presented here were calculated from the Langmuir isotherm equation and these quantities were divided by the saturation quantity in order to obtain the relative site occupation values. The relative weight occupation values were calculated by dividing the mass of adsorbed template by the mass of the membrane.

Table 4. Calculated Adsorption, Site Occupation and Weight Occupation at the Minimum Template Concentration (5 μM) Required to Induce the Gate Effect

Membrane	Adsorption [nmol g^{-1}]	Relative Site Occupation	Relative Weight Occupation
LIM	16	0.73%	2.6 ppm
DIM	20	0.92%	3.4 ppm

These results show that the gate effect is induced by the adsorption of very small quantities of template, at site occupation values below 1% and at the equivalent of 3 ppm in terms of weight occupation. It is somewhat surprising that the adsorption of such small amounts of template induces an increase in the solution uptake of approximately 30%, in addition to significantly changing the membrane's diffusive permeability.

From these data, we can readily see the advantages of using the gate effect in MIPs to bring about signal generation and transduction in sensing devices. The quantity of adsorbed template in the imprinted membrane that is sufficient to initiate the gate effect is no more than 3 ppm, which would be difficult to detect directly by other means, such as by measurements of mass increase using a quartz crystal microbalance or by changes in refractive index using a surface plasmon resonance detector. As such, it is evident that highly sensitive recognition is possible through exploiting the gate effect associated with a thin layer of MIPs (several nanometers in thickness for rapid response) immobilized on electrodes with associated amperometric detection of a redox marker. In recent years, the challenge of monitoring very low concentrations of analytes within small spaces, such as in micro total analytical system (μTAS), has arisen. In such cases, a sensor using the gate effect would be highly advantageous since the amount of the template required to trigger the effect is quite small. The high sensitivity of the gate effect also allows the use of very thin MIP layers of only few nanometers, which in turn leads to accelerated kinetics generating the sensor response. As an example, an electrode grafted with a heparin-imprinted polymer layer with a thickness of less than 10 nm was capable of detecting heparin with a response time as short as 15 s [11]. The model developed herein might be useful when studying changes in the conformational and transport properties of MIPs produced by template binding events. A better understanding of these phenomena could lead to improved performance of the sensors as well as the appearance of new devices in which sensing is combined with drug release. Many researchers have been trying to develop MIPs which release their templates as therapeutic |drugs [20, 21] or herbicides [22]. Thus the gate effect could be applicable to the release of drugs in response to a template such as a tumor marker [19].

There are two possible hypotheses for the gate effect phenomenon observed in this work. One is related to changes in the surface and transmembrane potentials induced by the adsorption of the charged template on the surface of the polymer. This possibility has been demonstrated and discussed in a prior publication [23]. The second explanation instead considers conformational changes in the polymer structure, in particular variations in the diameters of nano- and mesopores, associated with adsorption of the template.

Since the absolute mass of Phe adsorbed by the membrane was much less than the quantity of carboxyl and pyridyl groups in the material, it is unlikely that surface adsorption of the template, which does not bear excessive charge, dramatically affects the overall charge of the membrane. In addition, since creatinine is a nonionic solute, its permeability through the membrane is not expected to be affected significantly by variations in the transmembrane potential. The observed changes in membrane permeability therefore more likely result from conformational changes, as indicated by the changes in solution uptake which correspond to variations in the porosity of the membrane. The permeability of creatinine across the MISSM measured during this study is lower than that across a low-flux type hemodialysis membrane (Cuporophane®) [24] with a pore diameter of 2-3 nm [25]. Since no macropores were observed in the MISSM during scanning electron microscopy (as shown in Fig. **2**), the MISSM materials applied in our research must also have a nanoporous structure. The permeability of these membranes will be determined by both pore size and volumetric porosity [15].

We have previously reported that our MISSM has a heterogeneous structure consisting of both sparse and dense domains [13]. The adsorption isotherm data shown in Fig. (**1**), however, fit Langmuir's model rather than Freundlich's (in which the amount of adsorption increases with the adsorbate concentration without saturation), indicating that the imprinted sites in the MISSM which function at template concentrations of 0.05-2.5 mM are thermodynamically homogenous [14]. This suggests that the binding sites are effectively uniform and located only in certain regions of the membrane. If the imprinted sites were situated only in the dense domain, we would expect the activity of the sites to depend on their depth, and thus they would not be homogeneous. Chiral specificity, however, is not possible in the sparse domain, and so the active imprinted sites are thought to exist primarily at the surface of the dense domain. Fig. (**6**) presents diagrams of our proposed membrane structure, based on assembled particles. This structure is a modification of a model developed previously by one of the authors (S.P.) and coworkers [26]. The volumetric porosity, pore size and permeability of a membrane with this structure will all increase as the imprinted sites are occupied by template molecules. This mechanism which we propose to explain the observed increases in porosity and pore size with induced fit agrees both with the results of our own work and with the data presented in the other publications cited herein. Further analysis of this MISSM with different templates

molecules, monomers and solvents would help to clarify the mechanism by which it functions and would also assist in the development of sensors based on the gate effect which exhibit high sensitivity, high selectivity and rapid response. An analysis of the influence of environmental factors (such as pH, ionic strength, temperature and solvent) on the template adsorption, the solution and the permeability of MISSMs will be the focus of further investigations on the gate effect aimed at realizing practical applications, such as in diagnostics.

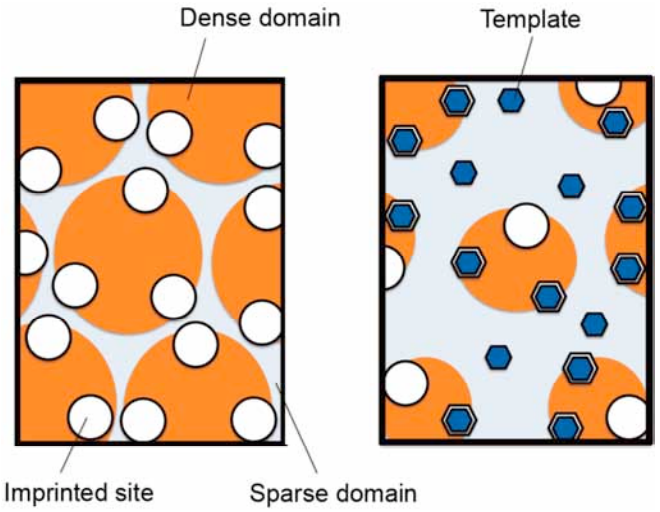

Fig. (6). Proposed model for the gate effect in which the MISSIM is considered as an assembly of dense particles surrounded by sparse domains.

CONCLUSION

A phenylalanine-imprinted self-supporting membrane consisting of poly (triethyleneglycol dimethacrylate-*co*-methacrylic acid-*co*-vinyl pyridine) has been shown to possess homogenous imprinted sites. The gate effect in this membrane is triggered by extremely low concentrations of adsorbed template, on the order of 3 ppm. This membrane represents an ideal model for the study of the gate effect mechanism in molecularly imprinted polymers. The high sensitivity demonstrated by this material suggests that, in future, there will be many opportunities to utilize the gate effect in sensors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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