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A sol–gel derived molecular imprinted luminescent PET sensing material for 2,4-dichlorophenoxyacetic acid

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A novel sol–gel derived molecular imprinted luminescent sensing material has been fabricated by a conventional sol–gel process. 2,4-Dichlorophenoxyacetic acid (2,4-D) was used as template for the imprinting process and a tailor-made organosilane, 3-[*N*,*N*-bis(9-anthrylmethyl)amino]propyltriethoxysilane (**1**), was used as the functional monomer. Luminescent properties of **1** were perturbed by acid–base ion-pair formation with 2,4-D leading to suppression of photoinduced electron transfer (PET) quenching of its anthryl fluorophores. Such PET fluorescent response was preserved after the preorganized monomer–template aggregates were cross-linked by a sol–gel process. The resultant sol–gel derived luminescent material was found to selectively respond to 2,4-D in aqueous media at pH 7. Average binding affinity of recognition sites in the material for 2,4-D was estimated to be $(2.23 \pm 0.5) \times 10^6$ M^{-1}. Selectivity of the MIP was also evaluated. The present study demonstrated the feasibility of using a PET mechanism as a means of signal transduction for molecular imprinted polymer (MIP) based luminescent sensing of non-fluorescent analytes.

Introduction

Induction of highly specific binding sites in synthetic polymers by template-directed cross-linking of functional monomers has aroused considerable research attentions in analytical chemistry and materials science in recent years.[1] The resultant molecular imprinted polymers (MIP) possess outstanding substrate recognition ability as well as good chemical and mechanical stability and are considered prospective materials for applications such as nanostructured catalysts,[2] affinity chromatography and capillary electrophoresis,[3] solid phase extractions[4] and chemical sensing.[5] Among this range of applications, development of MIP based sensors for biomolecules, pharmaceuticals and environmentally important pollutants is perhaps the most challenging.[6] In a way, the use of molecularly imprinted "receptors" for the binding and sensing of target analytes resembles molecular recognition processes brought about by analyte-specific antibodies and/or enzymes in biosensors.[7] Potential advantages offered by MIP based biomimetic sensors include: (a) specificity comparable to biosensors, (b) good robustness and stability even at extreme physical/chemical conditions which are intolerable to biosensors, and (c) the ability to produce sensors for those compounds by which antibodies are difficult to raise. One of the major issues in the development of MIP based biomimetic sensors is signal transduction. Numerous signal transduction approaches, such as voltammetric/amperometric detection,[5c,5i] conductometric detection,[5e] radioisotope labeled binding assay,[5d] colorimetric/fluorometric detection[5f–5h,5j,5k,8] and biomimic bulk acoustic wave detection,[5k] have been explored to convert MIP receptor site–analyte interactions into physically measurable signals. Among these techniques, fluorometric detection is particularly attractive as luminescent signals are very convenient to measure with high sensitivity. However, despite its desirable features, there are only few literature reports on MIP based luminescent sensing and the majority of them involve naturally fluorescing or fluoro-tagged analytes. Successful MIP based luminescent sensing systems capable of direct detection of non-fluorescent analytes are scarce.[5g,5h,9] In order to achieve such a goal, a template-induced luminescent enhancement/quenching mechanism has to be incorporated into the molecularly imprinted receptor sites. In this work, we report a sol–gel derived MIP based luminescent sensing material that makes use of a photoinduced electron transfer (PET) mechanism for the sensing of a non-fluorescent herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). A new organosilane, 3-[*N*,*N*-bis(9-anthrylmethyl)amino]propyltrieth-oxysilane (**1**) (Fig. 1), is synthesized and used as the PET sensor monomer. The sensing MIP material is fabricated by a conventional sol–gel process.

Photoinduced electron transfer (PET) has been a very popular mode of sensing in fluorescent molecular recognition in recent years.[10] PET chemical sensing involves suppression of an intramolecular non-radiative decay pathway for the fluorophore in the sensor molecule, leading to enhancement of luminescence, by binding to a target analyte (Scheme 1). In the present study, the PET sensor monomer (**1**) uses a tertiary amine as the "PET sensor-switch" to control the luminescence of two 9-anthrylmethyl fluorophores. In the absence of analyte, the lone pair of electrons on the tertiary amine is expected to quench the fluorescence of the anthryl moieties *via* photoinduced electron transfer.[11] Upon interacting with a suitable analyte, which is capable of forming hydrogen bonding or an ion-pair with the tertiary amine, the lone pair of electrons on the amine will be tied down, causing a suppression of the PET fluorescent quenching. The result is the enhancement in fluorescence of the anthryl fluorophores. Such a "switch-on"

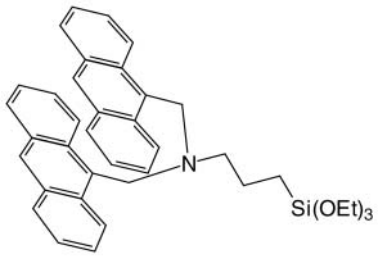

Fig. 1 3-[*N*,*N*-Bis(9-anthrylmethyl)amino]propyltriethoxysilane (**1**).

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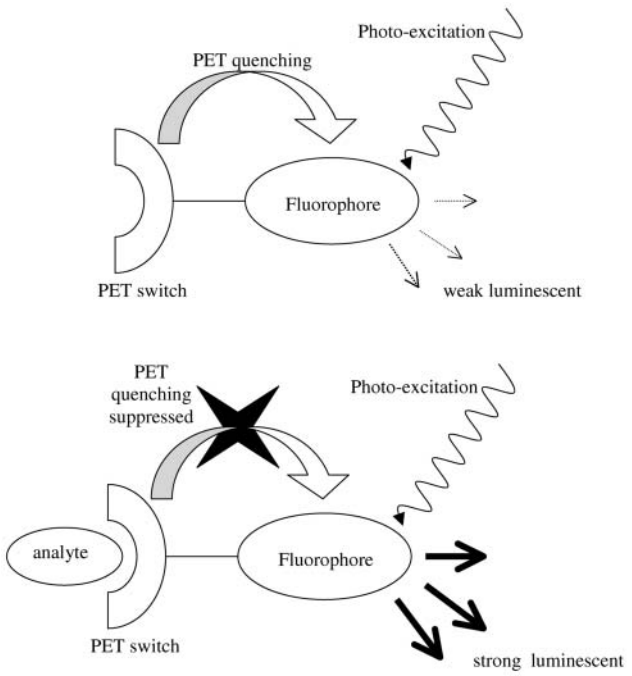


Scheme 1 Principle of photoinduced electron transfer (PET) in chemical sensing.

feature of the PET sensor is advantageous to MIP based luminescent sensing as it facilitates more specific signal transduction and reduces interference from the sample matrix.

In this work, a conventional sol–gel process is adopted to cross-link the pre-organized PET sensor monomer–template aggregates to produce the three dimensional shape selective recognition sites in a bulk organic–inorganic hybrid silica matrix for analyte binding. Although silane-based molecular imprinting has been considered useful in the construction of detection arrays,[12] there have only been a few attempts at using bulk silica as the polymer matrix for molecular imprinting applications.[1c–1d,13]

Experimental

General

Tetraethyoxysilane (TEOS), 3-aminopropyltriethoxysilane, phenyltrimethoxysilane (TMPS) and 9-(chloromethyl)anthracene, 2,4-dichlorophenoxyacetic acid (2,4-D) were obtained from Aldrich. All solvents used were of analytical reagent grade (Riedel-de Haën and BDH). Organic solvents were dried properly before use. Luminescent measurements were undertaken with a Fluoromax-3 spectrofluorometer with built-in magnetic stirrer. ^{1}H NMR spectra were collected using a Varian YH300 300 MHz NMR spectrometer.

Preparation of 3-[*N*,*N*-bis(9-anthrylmethyl)amino]propyltriethoxysilane (1)

A mixture of 3-aminopropyltriethoxysilane (2.10 g, 9.5 mmol), 9-(chloromethyl)anthracene (2.15 g, 9.5 mmol), K_2CO_3 (6.6 g, 0.048 mol) and KI (0.48 g, 2.9 mmol) in 40 ml of acetonitrile was refluxed for 4 h. The resultant mixture was hot filtered and allowed to cool to room temperature. A bright yellow crystalline solid was formed and collected by filtration, washed with small portions of cold ethanol and diethyl ether and dried *in vacuo*. Yield: 3.1 g (54% based on 3-aminopropyltriethoxysilane). ^{1}H NMR [$CDCl_3$, 300 MHz]: δ 8.39 (s, 2H, anthryl-H), 8.35 (d, 4H, $J=9$ Hz, anthryl-H), 7.97 (d, 4H, $J=9$ Hz, anthryl-H), 7.44–7.28 (m, 8H, anthryl-H), 4.60 (s, 4H, anthrylmethyl-CH_2), 3.61 (q, 6H, $J=6$ Hz, ethoxy-CH_2), 2.68 (t, 2H, $J=6.6$ Hz, propyl-CH_2), 1.80 (t, 2H, $J=6.6$ Hz, propyl-CH_2), 1.12 (t, 9H, $J=6$ Hz, ethoxy-CH_3), 0.23 (t, 2H, $J=6.6$ Hz, propyl-CH_2)

Preparation of the sol–gel derived molecular imprinted material

3-[*N*,*N*-Bis(9-anthrylmethyl)amino]propyltriethoxysilane (**1**) (19 mg, 0.032 mmol), TEOS (1.66 g, 8.0 mmol) and TMPS (1.59 g, 8.0 mmol) were dissolved in a solvent mixture containing 5.2 ml of THF, 1.8 ml of ethanol and 1.2 ml of H_2O. The mixture was refluxed for 1 h, cooled to room temperature and 2,4-D (0.074 g, 0.33 mmol) was added. The resultant sol was stirred at room temperature for 15 min to ensure complete dissolution of 2,4-D, transferred into a clean test-tube and incubated at 60 °C for 3 days, then at 100 °C in vacuum for 24 h. The yellow sol–gel material was crushed, grounded into powder, suspended in water and sieved through a 38 μm stainless steel sieve. The resultant fine sol–gel powder was collected by filtration, air dried and soxhlet extracted by a 100 ml acetic acid–methanol (1 : 9) mixture for at least 24 h followed by 100 ml of methanol for another 24 h. The resultant sol–gel derived MIP material was dried *in vacuo*.

Control material was prepared in the same way as described above with the exception that the 2,4-D template was replaced by a similar amount of HCl in the sol.

Spectrofluorometric measurement

Binding of analytes by the sol–gel derived MIP and control materials was studied spectrofluorometrically with 370 nm excitation. Unless stated otherwise, all analyte rebinding assays were performed using 10.0 mg of sol–gel derived MIP or control material suspended in 2.0 ml of aqueous phosphate buffer at pH 7.0 (with the addition of 40 μg of Triton X-114). For rebinding assays in aqueous media, no pH change was observed upon addition of analytes during all binding experiments. Because of limited solubility of the organic acid analytes in aqueous media, analytes were first dissolved in a small amount of methanol before spiking into the aqueous phosphate buffer. In any case, the amount of methanol in the aqueous analyte solutions was less than 0.4% (by volume) and was shown to have no effect on the luminescent responses of the MIP/control materials. In a typical rebinding experiment, increasing amount of an analyte solution was spiked into the MIP/control suspension under vigorous stirring. After each introduction, the suspension was stoppered and allowed to stir for 30 min before spectrofluometric measurement. Stirring was maintained during measurements. Effect of dilution by the analyte solution on the luminescent response of the MIP/control suspension was evaluated separately. The parameter I/I_o, where I_o and I were the fluorescent intensity of the MIP/control suspension at 416 nm before and after addition of analyte solution (with correction for dilution effect), was adopted as the sensing response of the MIP/control materials.

Results and discussion

Synthesis of the PET sensor monomer, 3-[*N*,*N*-bis(9-anthrylmethyl)amino]propyltriethoxysilane (**1**), was straightforward. Fig. 2 shows the emission spectrum of **1** in chloroform. Luminescent properties of **1** corresponded well with the fluorescence of an anthryl moiety.[14] Fig. 3 shows the emission spectra of **1** in aqueous media of various pH. The pH dependence of the luminescent intensity of **1** was in accordance with the PET quenching of the anthryl fluorescence by the lone pair of electrons on the tertiary amine moiety. Upon protonation at low pH, this lone pair of electrons was tied down and no longer able to exert their PET effect. The result was an enhancement of fluorescence of the anthryl fluorophores. The luminescence of **1** at high pH as well as in

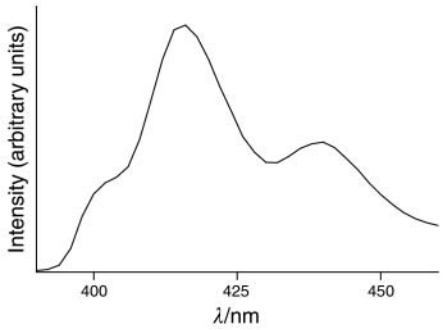


Fig. 2 Emission spectrum of **1** in chloroform (excitation at 370 nm).

chloroform was attributable to the insufficient PET quenching of the two anthryl fluorophores by a single tertiary amine. From the spectrofluorometric titration shown in Fig. 3, the pK_a value of **1** was estimated to be 8.4. Fig. 4 shows the emission spectra of **1** in chloroform at increasing concentration of 2,4-D. Enhancement of luminescence of **1** by 2,4-D in chloroform was ascribable to the acid–base ion-pair formation reaction between 2,4-D and **1** leading to the suppression of PET quenching of the anthryl fluorophores.

The PET sensor monomer–2,4-D aggregates in an ethanol–water mixture were cross-linked by TEOS and TMPS (in 1 : 1 ratio) *via* a sol–gel process. It has been reported that phenyltrialkoxysilane was able to improve optical properties and mechanical stability of sol–gel composite and facilitate homogeneous distribution of hydrophobic components within the composite.[15] The PET sensor monomer to TEOS/TMPS

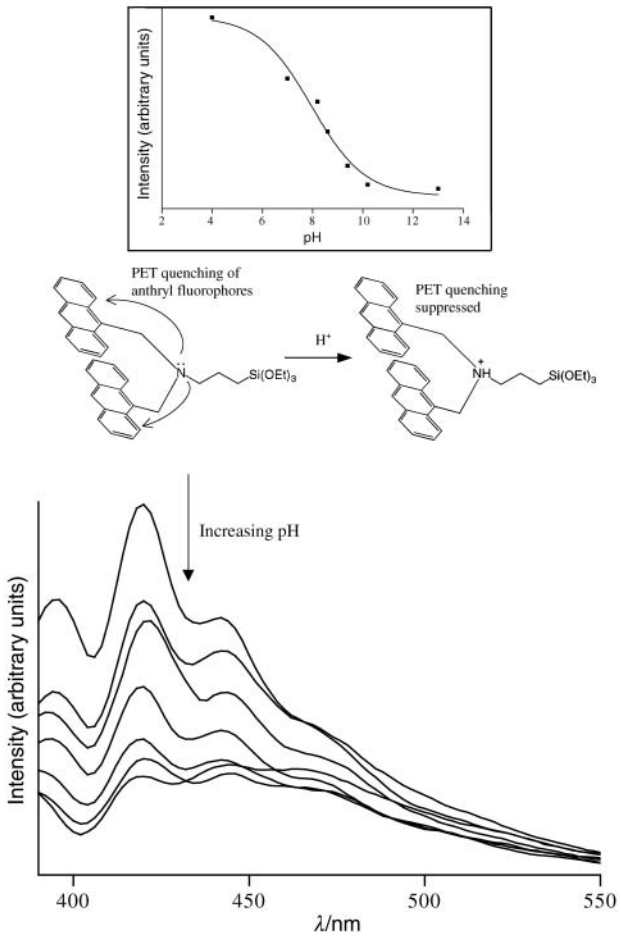


Fig. 3 Emission properties of **1** in aqueous media at various pH (with 370 nm excitation) (from top to bottom: pH 4.0, 7.0, 8.2, 8.6, 9.4, 10.2 and 13.0). The variation of emission intensity at 416 nm with respect to pH is shown in the inset.

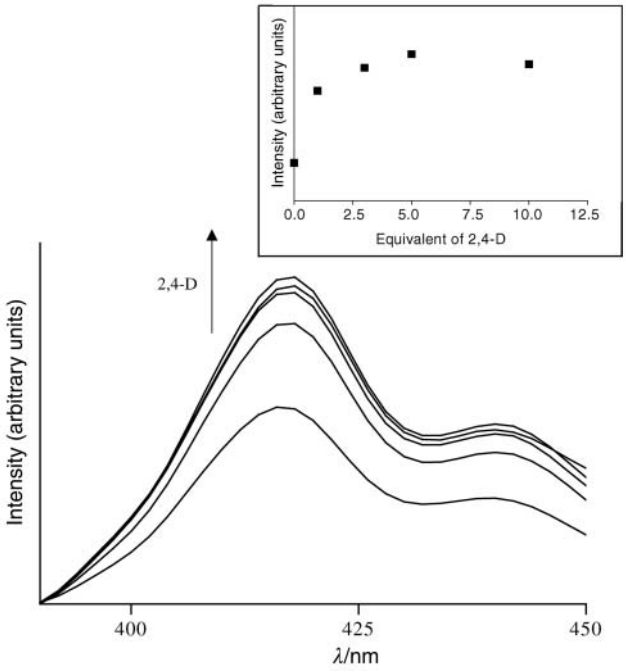


Fig. 4 Emission spectra of **1** (0.4 mM in chloroform) (with 370 nm excitation) with the addition of 0, 1, 3, 5, and 10 equivalents of 2,4-D. Variation of emission intensity at 416 nm with respect to addition of 2,4-D is shown in the inset.

ratio was 1 : 250. Such a ratio was not particularly low compared to other fluorescent MIP based sensors.[5g,5h] Nevertheless, further increases in the amount of fluorescent PET sensor in the MIP may not produce a proportional increase in the sensing response (sensitivity) as self-absorption of emission by ground state anthryl moieties may become significant. A 10-fold excess of 2,4-D with respect to the PET sensor monomer was used to ensure that a substantial proportion of the sensor monomers was engaged in interaction with the template during cross-linking, especially in the polar porogens (ethanol–water) used for the sol–gel process. Nevertheless, judging from the pK_a values of the PET sensor monomer and 2,4-D (*ca.* 2.8),[16] the major monomer–template interaction during the sol–gel process is expected to be ion-pairing between the protonated ammonium cations of **1** and the deprotonated carboxylate anions of 2,4-D. Hydrophobic interaction between the templates and the anthryl moieties of the monomers may also play a part. The importance of ionic and hydrophobic interactions between functional MIP monomers and templates in molecular imprinting in protic porogens has been demonstrated by Haupt *et al.*[17] In their preparation of a noncovalent MIP for 2,4-D using unbuffered aqueous methanol as porogen, 4-vinylpyridine was chosen as the functional monomer in order to allow for ionic interaction with the carboxylate group of the 2,4-D template as well as for hydrophobic interaction with its aromatic ring.

The resultant sol–gel derived MIP material was obtained in the form of a monolithic transparent sol–gel composite. Even spreading of the PET sensor over the material was demonstrated by the homogeneous pale yellow colour of the sensor throughout the material. 2,4-D templates were removed from the MIP material *via* soxhlet extraction with acetic acid–methanol followed by methanol. A negligible amount of the PET sensor monomer was leached from the MIP material during extraction. As our main objective is to develop MIP based biomimetic sensors that can function in neutral aqueous media such as natural waters and biological fluids, aqueous buffer was used in most of the rebinding assays. The emission spectrum of the sol–gel derived MIP material suspended in aqueous media (pH 7) as well as the variation of its luminescent intensity with respect to pH of the media is shown in Fig. 5.

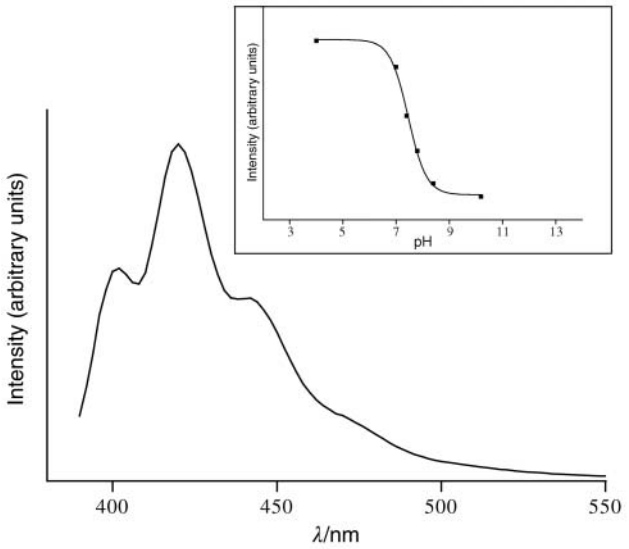


Fig. 5 Emission spectrum of the sol–gel derived MIP material suspended in an aqueous phosphate buffer at pH 7 (370 nm excitation). The variation of the emission intensity at 416 nm with respect to pH is shown in the inset.

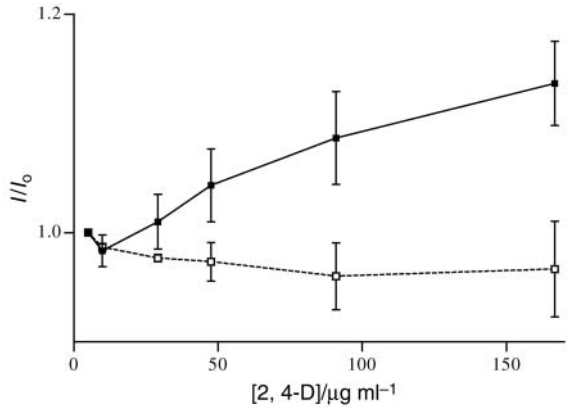
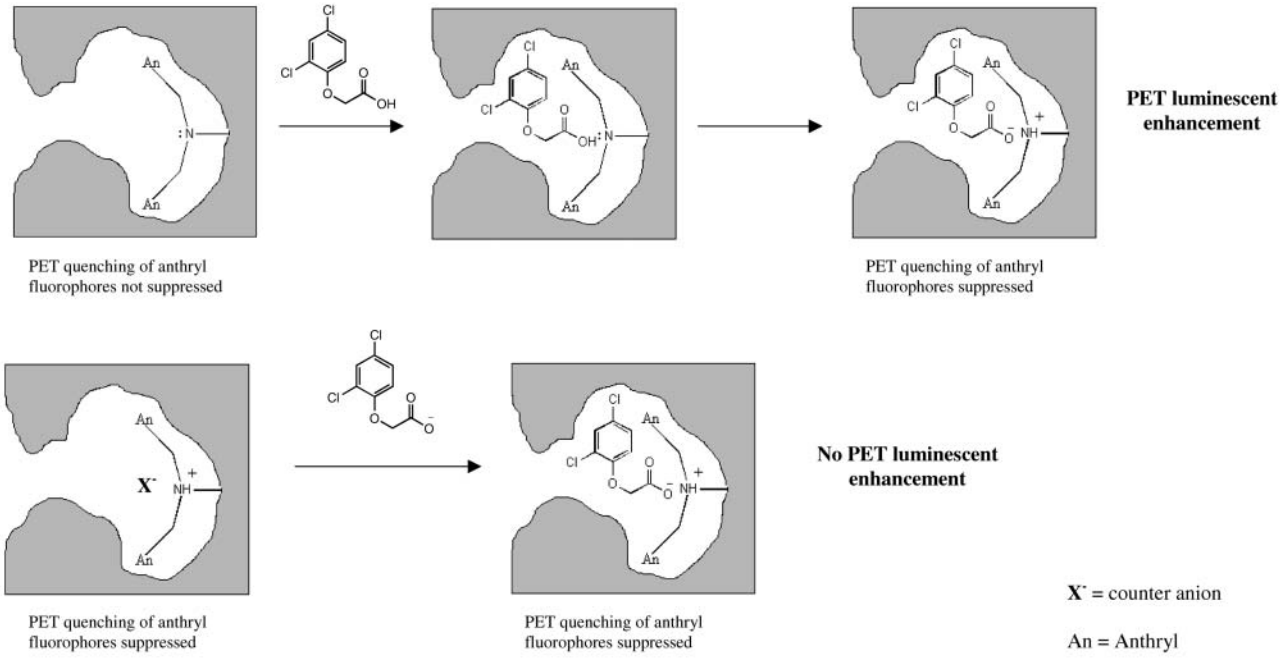


Fig. 6 Fluorescent responses of the sol–gel derived MIP and control materials in the presence of various concentrations of 2,4-D in aqueous phosphate buffer at pH 7. Each data point represents mean ± standard error of the fluorescent response (I/I_o) ($n=3$).

Structured fluorescent peaks of the anthryl groups of the PET sensor monomer **1** were retained with peaks slightly broadened. From the pH dependence of the luminescence properties of the sol–gel material, the average pK_a of the binding sites in the sol–gel material was found to be 7.7, which was considerably lower than that of the PET sensor monomer. Such a decrease in pK_a of protonated functionality upon sol–gel formation could be caused by the much more hydrophobic microenvironment within each binding site, due to the presence of two aromatic anthryl moieties, which did not favour the protonation of the tertiary amine into a cationic ammonium ion. From the pK_a value of the sol–gel material, it is estimated that 20% of the binding sites within the sol–gel matrix remain uncharged, at neutral pH, and were available for the induction of PET responses upon binding with 2,4-D (Scheme 2).

The 2,4-D rebinding and PET fluorescent enhancement properties of the sol–gel derived MIP material were demonstrated by spectrofluorometric titration of suspensions of the MIP, and control materials with sequential addition of 2,4-D standard solution. This method was found to be more convenient, over other batch-type approaches, for rebinding assay especially when measurement of the luminescent response of the MIP material itself was needed. A non-ionic surfactant (Triton X-114) was added to the aqueous media to increase wettability of the MIP/control materials and prevent adsorption of the analyte on the wall of the cuvette.[17] After addition of each portion of analyte into the suspensions, a 30 min equilibration time was allowed before spectrofluorometric measurement was taken. The parameter I/I_o, where I_o and I were fluorescent intensity at 416 nm in the absence and presence of analyte respectively, was adopted as the sensing response of the MIP/control materials. An I/I_o equal to 1.0 indicates no change in fluorescence intensity of the MIP/control materials while $I/I_o > 1$ indicates enhancement of fluorescence. Fig. 6 shows the effect of 2,4-D on the fluorescent response of the sol–gel derived MIP and control materials in aqueous phosphate buffer at pH 7. A general rising trend in luminescent intensity of the MIP material was observed with increasing 2,4-D concentration from 10 to 166.6 μg ml^{-1} (44.8 to 754.0 μM), while that of the control material showed negligible responses. These fluorescent responses were consistent with the



Scheme 2 Interaction of 2,4-D with binding sites in the sol–gel derived MIP material leading to PET fluorescent responses.

suppression of PET quenching of the anthryl fluorophores in the uncharged recognition sites of the MIP material by binding with 2,4-D. The nature of the binding site–2,4-D interaction was expected to be acid–base ion-pairing. Upon diffusion into the uncharged binding site, close proximity of the acidic 2,4-D and the basic tertiary amine functionality facilitated ion-pair formation and subsequently suppression of PET effect of the tertiary amine (Scheme 2). On the other hand, electrostatic interaction between dissociated 2,4-D anions and protonated binding sites should not lead to any PET luminescent enhancement in the MIP material. The lack of responses of the control material demonstrated this point as such non-specific type interaction was also expected to operate in the control material. Nevertheless, this does not imply that such ionic interaction did not exist in the sensor material. In fact, Haupt *et al.*[17] have shown that the nature of binding of the 2,4-D template by MIP derived from a 4-vinylpyridine functional monomer in acidic pH was predominately electrostatic. In our present case, as a large fraction of binding sites in the sol–gel derived MIP material were expected to be protonated at the buffered pH, electrostatic interaction of the dissociated 2,4-D and charged binding sites may also be significant. It was just the case that such ionic interaction did not give rise to PET fluorescent responses. In fact, the I/I_o values smaller than 1.0 observed during the course of titration with control material, as well as at the initial phase of the titration with MIP material where amount of 2,4-D added was small, indicates a small quenching effect of the 2,4-D on the anthryl fluorescence. This may be caused by π–π interaction between the aromatic ring of the 2,4-D and the anthryl moieties of the sensor functionality. From the above consideration of the binding mechanism in the MIP sensing material, it is clear that the relatively low sensitivity of the MIP material towards 2,4-D at neutral pH is attributable to the inevitable protonation of binding sites within the material as well as the dissociation of the acidic analyte, which practically limits the amount of uncharged recognition sites and undissociated analyte to produce a luminescent signal. Higher 2,4-D concentrations were not attempted because of solubility limitation and our intention to maintain the concentration of the organic acid analyte to well below the buffering capacity of the phosphate buffer.

Acid–base ion-pairing of 2,4-D with uncharged recognition sites in the sol–gel derived MIP material that produces the PET fluorescent responses can be modelled by equilibrium processes shown in eqn. (1) and (2):

$$[\text{2,4-D}] \overset{K_a}{\rightleftarrows} [\text{H}^+] + [\text{2,4-D}_{-\text{H}}]^- \quad (1)$$

$$[\text{2,4-D}] + [\text{B}] \overset{K_B}{\rightleftarrows} [\text{B-2,4-D}] \quad (2)$$

where [2,4-D] and $[\text{2,4-D}_{\text{-H}}]^-$ are the concentration of the undissociated and dissociated form of 2,4-D in aqueous buffer, respectively; [B] and [B-2,4-D] are the amount of vacant and bound recognition sites in the MIP material respectively, K_a is the acid dissociation constant of 2,4-D (1.39×10^{-3} M, assuming $pK_a = 2.8$)[16] and K_B is the binding constant. The above model assumes that only the undissociated form of 2,4-D binds to recognition sites and produces fluorescent responses. This assumption is considered reasonable, as only undissociated 2,4-D is capable of inducing ion-pair formation with the PET tertiary amine functionality of the recognition site. As both [B] and [B-2,4-D] contributes to the change in fluorescence of the MIP material during the binding process, the relation between the fluorescent response of the material and concentration of 2,4-D spiked into the media becomes:[19]

$$\frac{I}{I_O} = \frac{K_a + [\text{H}^+](1 + (\frac{k_{\text{B-2,4D}}}{k_B})K_B[\text{2,4-D}]_O)}{K_a + [\text{H}^+](1 + K_B[\text{2,4-D}]_O)} \quad (3)$$

where $[\text{2,4-D}]_o$ is the concentration of 2,4-D spiked into the aqueous buffer solution and $k_{\text{B-2,4D}}/k_B$ is the ratio of quantum efficiency of 2,4-D bound site to vacant site. Normally, this quantity can be determined at high analyte concentration where I/I_o starts to level off. However, as we were not able to observe the leveling off of the I/I_o response within the practical [2,4-D] concentration range, we obtained the $k_{\text{B-2,4D}}/k_B$ ratio from the sensor monomer–2,4-D binding data. From Fig. 4, it was apparent that I/I_o reached a maximum value of 1.59 in homogeneous conditions where all sensor functionalities were engaged in association with 2,4-D. Although quantum efficiency of photophysical processes in a 3-D rigid sol–gel matrix differs greatly from those in homogeneous solution, the ratio $k_{\text{B-2,4D}}/k_B$ should be more or less constant as both vacant and bound sites should be affected to the similar extent. Fig. 7 shows the non-linear regression fitting of the fluorescent responses, I/I_o, of the MIP material to $[\text{2,4-D}]_o$ assuming a $k_{\text{B-2,4D}}/k_B$ ratio of 1.59. Eqn. (3) fitted the experimental data reasonably well except at low 2,4-D concentrations where I/I_o were close to or below 1.0. Such a dip in I/I_o response may be the result of site heterogeneity within the MIP material where a quenching effect caused by the interaction between dissociated 2,4-D and protonated binding sites masked the PET enhancement effect. The best-fitted K_B value was found to be $(2.23\pm0.5)\times10^6\ \text{M}^{-1}$. In spite of the relatively poor fitting at low 2,4-D concentration, this K_B value serves to provide an estimation of the average affinity of uncharged recognition sites in the MIP material for undissociated 2,4-D. Such binding affinity is comparable to other reported MIPs, fabricated from conventional synthetic polymer routines, for various different analytes[2a,5g,20] and even to some of the antibody–antigen interactions.[2a]

Selectivity of the MIP sensing material was evaluated by comparing the luminescent responses induced by 2,4-D to those induced by two other analogous organic acids: benzoic acid and acetic acid. Both analogs contain dissociable carboxylic functionality and comparable molecular size with 2,4-D. Benzoic acid also possesses an aromatic ring similar to 2,4-D. Fig. 8 shows the luminescent responses of the MIP and control materials towards these analytes. The present 2,4-D templated sol–gel derived MIP material clearly shows selectivity towards 2,4-D in neutral aqueous media. One point that is worth noting is that as both organic acid analogs were weaker acids than 2,4-D, under the same total acid concentration, the amount of undissociated acetic acid and benzoic acid in the media was expected to be much greater than that of 2,4-D at neutral pH. Thus, acetic acid and benzoic acid should have induced much stronger PET fluorescent responses, if

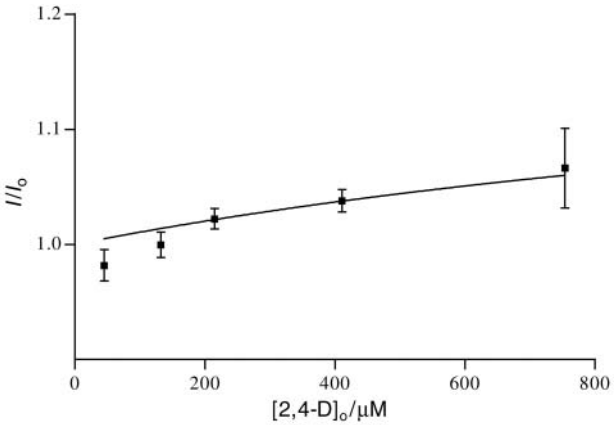


Fig. 7 Non-linear regression fitting of fluorescent responses, I/I_o, of the sol–gel derived MIP material to $[\text{2,4-D}]_o$ using eqn. (3). Each data point represents mean ± standard error of the fluorescent response ($n=3$). The solid line represents the best-fitted curve. The best-fitted K_B value was $(2.23\pm0.5)\times10^6\ \text{M}^{-1}$.

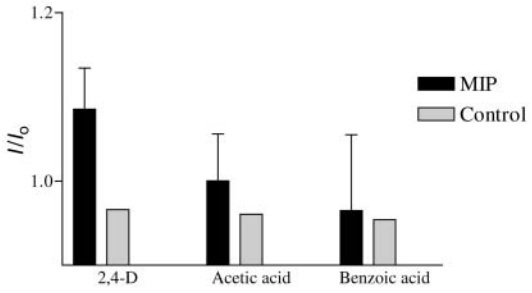


Fig. 8 Fluorescent responses of the sol–gel derived MIP material towards acetic acid, benzoic acid and 2,4-D (all in 0.75 mM) in aqueous phosphate buffer (with addition of 40 μg Triton X-114). 10 mg of MIP material was used.

analyte binding in the recognition sites were solely due to one-point acid–base ion pairing interaction. The selectivity displayed by the MIP material, on the other hand, indicates that ion pairing may not be the only interaction between 2,4-D and its recognition sites in the MIP. Hydrophobic interaction and shape selectivity may be significant as well. Numerous studies have also suggested that shape-selectivity contributes significantly to template binding in MIP, especially in cases of aromatic templates.[1e,5g,21]

Conclusion

The present study demonstrated the feasibility of using photoinduced electron transfer (PET) mechanism as a means of signal transduction for MIP based luminescent sensing of non-fluorescent analytes. Incorporation of organosilane PET sensor monomers into organic–inorganic hybrid MIP material is very convenient and the resultant MIP material is shown to possess significant affinity and selectivity for its target analyte in aqueous media. Sensitivity of the sensing material in neutral aqueous medium, on the other hand, was not particularly high compared to other MIP sensing systems.[5c,5d,5i] This is probably due to the inevitable introduction of binding site heterogeneity by significant protonation of binding sites within the MIP material as well as the corresponding dissociation of the acidic analyte, at neutral pH. This increases the level of background emission and, at the same time, reduces the number of available binding sites capable of giving PET fluorescent responses. Both effects are detrimental to the sensitivity of the sensing material. Works are in progress to explore other sensor monomers and PET mechanisms that would lead to lower background emission and better sensitivity for analyte detection.

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