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Assessment of molecularly imprinted sol–gel materials for selective room temperature phosphorescence recognition of nafcillin

A. Fernández-González [a], R. Badía Laíño [a], M.E. Diaz-García [a,*], L. Guardia [b], A. Viale [b]

[a] *Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, C/ Julian Claveria 8, Oviedo 33006, Spain*
[b] *Department of Chemistry, University of Buenos Aires, Buenos Aires, Argentina*

Abstract

Sol–gel imprinted materials were prepared against nafcillin, a semisynthetic β-lactamic antibiotic employed in the treatment of serious infections caused by penicillinase-producing staphylococci. Two approaches were addressed for preparation of the imprinted materials and the controls: as conventional monoliths, which were ground and sieved to a desired particle size for rebinding analysis, and as films on supporting glass slides. The specific binding sites that are created during the imprinting process are analyzed via selective room temperature phosphorescence (RTP) (sol–gel films) measurements as well as via competitive room temperature phosphorescence ligand assay. Results demonstrated the importance of the physical configuration of the imprinted material for minimizing non-specific binding. The close similarities between the structures of different β-lactamic antibiotics made it possible to interpret the roles of the template structure on specific molecular recognition. In this article, we introduce the use of room temperature phosphorescence as selective transduction method for the template. The imprinted sol–gel films displayed enhanced specific binding characteristics respect to the monolithic sol–gel and can be envisaged for the use as recognition matrices for the screening and rapid selection of antibiotics from a combinatorial library or for the rapid control of nafcillin in biological samples (e.g. milk, serum, urine).

Keywords: Molecularly imprinted sol–gel materials; Phosphorescence recognition; Nafcillin

1. Introduction

Molecular recognition is associated with a close complementarity between the shape of the receptor and the substrate molecule. Taking biological systems (e.g. enzyme-substrate, antibody-antigen, hormone-receptor) as models, molecular recognition elements can be synthesized using molecular imprinting techniques. Molecular imprinting [1–4] (molecularly imprinted polymers, MIP) is now a well established technique for the development of polymers having a high affinity for a target molecule. In a general procedure for creating these materials functional monomers and the template molecule are allowed to self assembly in a medium that maximizes the interactions between the two species. After polymerization of the mixture with an adequate cross-linking agent, the template is removed from the polymer, thus leaving an imprint with a selective "memory" to recognize the original substrate (due to the optimal configuration of the functional groups inside the microcavities). There are two general methods of regulating the interactions between the guest template and the functional monomers, namely: non-covalent and covalent molecular imprinting. Many successful non-covalent imprinted systems used in optical sensing schemes are based on acrylic monomers crosslinked with ethylene glycol dimethacrylate [5,6]. Thus, they have been developed for the determination of drugs [7], herbicides [8] and other biological and environmentally important molecules. Potential advantages offered by MIPs for biomimetic optical sensing include: (i) MIPs are relatively simple and rapid to prepare in large scale, (ii) good mechanical, thermal and chemical stability due to their highly crosslinked nature, (iii) possibility of reuse several times without loss of molecular recognition capability, (iv) the ability to produce recognition materials for those compounds for which antibodies are difficult to raise and (v) possibility to exploit different optical transduction approaches to convert the molecular recognition event into a physically measurable signal.

Based on a similar principle to the early work of Dickey to create nanostructured solids for molecular recognition by

* Corresponding author. Tel.: +34-985-103471; fax: +34-985-103125.
E-mail address: medg@sauron.quimica.uniovi.es (M.E. Diaz-García).

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molecularly imprinting silica gels [9,10], recently, sol–gel derived molecular imprinted materials have been developed for selective optical detection of ricine (potent toxin from castor beans) [11], propranolol [12], DDT [13] and 2,4-dichlorophenylacetic acid [14]. The potential advantages of using the sol–gel technology for developing molecularly imprinted sol–gels (MISG) for optical sensing applications may be straightforward to envision. For example, sol–gel materials are optically transparent and can be prepared as bulk solids, fibers or silica films, organically modified alkoxysilanes can be used to control or modify the matrix structural properties, hydrolysis and condensation take place at mild temperature conditions, the imprint molecules retain much of their physical and chemical properties after the polymerization and both, covalent and non-covalent imprinting are possible.

Among the transduction approaches explored to develop optical MIP sensors, luminescence detection is particularly attractive due to the inherent sensitivity of the corresponding techniques. So, some successful MIP based luminescent sensing systems have been described for both fluorescent and non-fluorescent analytes [15,16]. In our knowledge, no MIP based room temperature phosphorescence (RTP) sensing systems have been reported to date, in spite of the desirable features for transduction that room temperature phosphorescence on solid supports offers [17,18].

Herein, we describe the construction sol–gel molecular imprints against nafcillin, a semisynthetic β-lactamic antibiotic employed in the treatment of serious infections caused by penicillinase-producing staphylococci (e.g. septicaemia, osteomyelitis, pneumonia and endocarditis). Nafcillin has a reasonable phosphorescence quantum yield at room temperature as demonstrated by the possibility to determine it by RTP in a protective medium, such as surfactant micelles [19] or even in fluid solution in absence of protective medium [20]. Because room temperature phosphorimetry affords much greater selectivity than other luminescence techniques, a set of nafcillin imprinted sol–gel materials were examined for their ability to generate specific nafcillin room temperature phosphorescence response in model solutions. The sol–gel materials were further explored in an attempt to elucidate the recognition mechanism. Batch equilibration studies as well as competitive flow-through experiments were performed to evaluate non-specific binding and the importance of the imprinted sol–gel configuration on the recognition event. The purpose of this work is to demonstrate for the first time the possibility of combining the advantageous properties of imprinted materials with the selectivity of room temperature phosphorescence transduction with the aim of eventually exploiting such systems in the construction of nafcillin sensors. Although the results are somewhat preliminary in nature, the indications are that imprinted sol–gel films have potential to extract nafcillin directly from real samples (e.g. milk, serum, urine) with specificity due to the molecular imprinting process. Given that many real samples are of aqueous nature, it is encouraging that imprinted materials show specificity in aqueous-based systems.

2. Experimental

2.1. Reagents and instruments

Nafcillin, amoxicillin and ampicillin were purchased from Sigma and (+)-6-aminopenicillanic acid (6-APA) was from Fluka. 1-Decalone, tetramethoxysilane (TMOS), phenyltrimethoxysilane (PTMOS), methyltrimethoxysilane (MTMOS), tetrabutylammonium iodide (TBAI), tetrabutylammonium fluoride (TBAF), potassium iodide and potassium fluoride were obtained from Aldrich. Hydrochloric acid was obtained from Merck. All solvents were HPLC grade and chemicals were used as received.

Fluorescence and phosphorescence were measured using a Perkin-Elmer LS-50B luminescence spectrophotometer, which has a xenon discharge excitation source (pulse width at peak half-height $<10\,\mu s$). Instrumental parameters and processing data are controlled by the fluorescence Data Manager software.

2.2. Preparation of imprinted sol–gel films

The preparation of the sol–gel polymerization solution was carried out by mixing adequate volumes of TMOS, PTMOS, MTMOS, EtOH, 10^{-3} M nafcillin, additive solution and finally, 0.1 M HCl according to Table 1. The mixture was stirred for 10 min in an ice bath, after which an aliquot of 50 μl was spread over standard microscope glass slides. Each slide was covered with a clean glass slide which was then slithered about on it. This technique, adopted in order to control film thickness during casting, is rapid, convenient and low-cost coating (two coated slides are thus obtained). The glass plates were allowed to polymerize, dry and curing at room temperature for at least 12 days. After the polymerization, the coated glass plates were soaked in methanol ($2 \times 10\,ml \times 30\,min$) and then dried in an air stream (5 min). Control materials were prepared in the same way as described above with the exception that nafcillin was replaced by a similar amount of water in the sol.

2.3. Preparation of imprinted sol–gel monoliths

Sol–gel materials imprinted with nafcillin were prepared according to a standard method using a mixture of TMOS (1.23 ml), PTMOS (0.150 ml) and MTMOS (0.120 ml) as functional precursors. 1.14 ml 0.001 M nafcillin, 1.55 ml ethanol, 0.76 ml TBAF and 0.05 ml HCl 0.1 M were mixed in the test tube. The mixture was allowed to gel and dry for 72 h under ambient conditions and then at 45 °C to constant weight for approximately 2 weeks. The resulting sol–gels were mechanically crushed, sieved in fragmented particles of diameters ranging between 0.16 and 0.08 mm

Table 1
Composition of the polymers

Polymer	MTMOS (μl)	TMOS (μl)	PTMOS (μl)	Ethanol (μl)	Nafcillin, 10^{-3} M (imprinted) water (control) (μl)	Additive 0.025 M (μl)	HCl (0.1 M) (μl)
TM1	375	1230	–	1550	1140	760 (TBAF)	50
TM2	802	802	–	1550	1140	760 (TBAF)	50
TM3	1230	375	–	1550	1140	760 (TBAF)	50
TPM-1	100	1000	123	1000	333	333 (KI)	333
TPM-2	100	1000	123	1000	333	333 (TBAI)	333

and Soxhlet rinsed using a 80:20 (v/v) methanol–acetic acid (80 cycles, 12 min each) to remove the template.

2.4. Flow-through setup for competitive assays

The manifold used in this study is shown in Fig. 1. A Hellma Model 176.52 flow through cell (25 μl) was placed in the sample compartment of the spectrofluorimeter. The MISG was packed into the light path of a flow cell. The flow stream was generated by a Minipulse 2 four-channel peristaltic pump (Gilson, Worthington, OH). The used carrier was a solution of sodium sulphite 0.01 M (oxygen scavenger used to promote RTP), KI 1 M (iodide used as intersystem crossing heavy atom) and NaCl 1 M at a flow rate of 0.5 ml min^{-1}. The samples were introduced by a type 50 PTFE six-way rotary valve (Omnifit, Cambridge, UK) provided with a 125 μl loop. PTFE tubing (0.8 mm i.d.) and fittings were used for connecting the flow-through cell, the valve and the carrier reservoir.

2.5. Direct room temperature phosphorescence binding assay

Coated glass plates were immersed in 20 ml of aqueous 2.4×10^{-5} M nafcillin solution. After 30 s the plates were taken out of the solution, rinsed with distilled water (1×20 ml) and dried at 30 °C in an oven for 30 min. The plates were placed in the sample holder in a right angle, surface detection configuration and the fluorescence ($\lambda_{exc} =$ 256 nm, $\lambda_{em} = 360$ nm) and room temperature phosphorescence ($\lambda_{exc} = 256$ nm, $\lambda_{em} = 470$ nm, $t_d = 0.04$ ms, $t_g =$ 3.00 ms) signals were measured. This configuration allowed reduction of inner filter error for optically dense samples. Control polymers underwent the same procedure. Batches of three different plates were measured for each data point.

3. Results and discussion

3.1. Molecular imprinted sol–gel sensing films

We chose the non-covalent imprinting method, pioneered and extensively developed by Mosbach and Ramström [21], for the bulk of this work. The non-covalent interactions are easily reversed, usually by a wash in aqueous solution of an acid, a base or methanol, thus allowing the removal of the template molecule from the network after polymerization.

The first parameter we investigated was that of the composition of the sol–gel material in order to find the optimum recognition conditions for nafcillin. Thus, we synthesized sol–gels TM1, TM2 and TM3 to investigate the influence of TMOS and MTMOS on the nafcillin recognition. The overall luminescence of the films, given by the performance indices Q_f and Q_p were used to evaluate nafcillin retention/recognition:

$$Q_f = \frac{I^f_{imp} - I^f_n}{I^f_n} \times 100, \qquad Q_p = \frac{I^p_{mp} - I^p_n}{I^p_n} \times 100$$

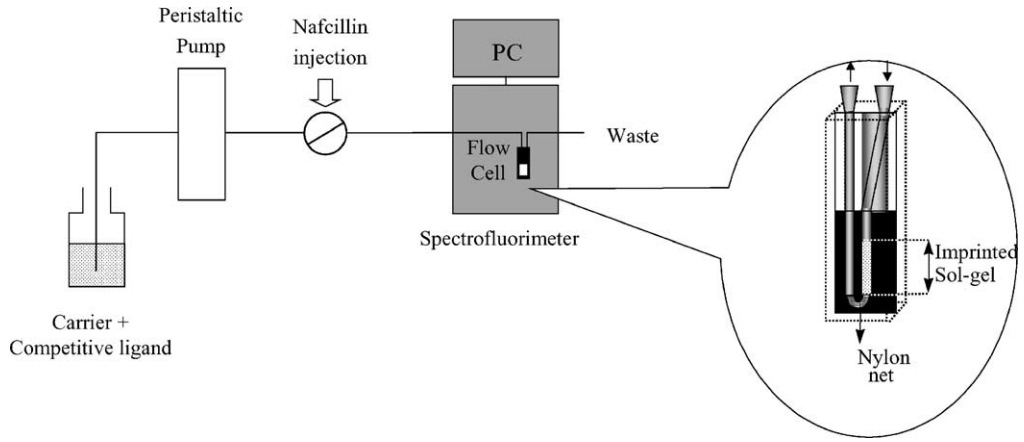

Fig. 1. Flow injection system for competitive binding experiments.

Table 2
Performance indices for sol–gel imprinted films before and after nafcillin removal

	Q_f			Q_p		
	TM1	TM2	TM3	TM1	TM2	TM3
$\lambda_{ex}/\lambda_{em}$ (nm)	256/371	256/363	256/368	256/474	256/470	256/467
Crude films	65.2	300.8	40.9	80.9	62.7	−2.8
Rinsing 1	46.2	159.8	35.6	28.0	69.7	−6.8
Rinsing 2	33.8	136.3	25.4	30.4	79.1	−2.7

Every value was made in triplicate. The R.S.D. between replicates was ≤8%.

In these expressions, I^i_{imp} and I^i_n denote fluorescence or room temperature phosphorescence of the imprinted sol–gel and the non-imprinted one, respectively. In Table 2 the values of Q_f and Q_p for TM1, TM2 and TM3 sol–gel films with varying TMOS/MTMOS weight ratios are shown. These data correspond to the MISGs before use in binding experiments, so reflecting the interaction between the template and the precursors during the sol–gel preparation.

Q_f and Q_p values are consistent with a permanent retention of nafcillin in the imprinted sol–gel, as after exhaustive cleaning [(10 ml MeOH × 30 min) × 2] some nafcillin remains irreversibly bound to the imprinted material. Subsequent rinsings with methanol indicated no further nafcillin extraction (relative signal changes of the imprinted films were less than 2%). The Q_f and Q_p values also reveal the influence of the hydrophilic/hydrophobic balance during the imprinting process. For example, the increase in Q_f and Q_p when moving from TM1 to TM2 can be related with the enhanced hydrophobicity due to the increasing number of methyl groups in the sol–gel backbone, indicating that along with hydrophilic interactions (through Si–OH groups), hydrophobic interactions play a contribution in the interaction with the template. Whilst most of the hydrophobic interactions are usually satisfied by the crosslinking monomer in acrylic-based imprinted polymers, in sol–gel-based materials hydrophobicity can be introduced through the use of organically modified alcoxysilanes (ormosils). A further increase in hydrophobicity (sol–gel TM3) results in a decrease of Q_f and Q_p, which indicates that hydrophilic interactions are also important during the formation of well-organized recognition sites around the template. This is in connection with the fact that three-dimensional gel networks are more difficult to obtain from solutions of organically substituted alkoxysilanes [22].

It was interesting to observe that room temperature phosphorescence (RTP) of nafcillin was not quenched by oxygen when bound to the imprinted materials. In all the described experiences, measurements were taken without oxygen purging and in absence of a heavy atom promoter.

Q_f and Q_p values for TM1, TM2 and TM3 imprinted sol–gels after binding experiments using a 2.4×10^{-5} M nafcillin solution are summarized in Table 3. As can be seen, Q_f data reveal that the TM2 imprinted sol–gel exhibits a high affinity for nafcillin while the binding affinity is low for the more hydrophilic imprinted sol–gel TM1. Q_p data also indicate the high affinity of TM2 for nafcillin, a similar binding affinity of TM1 but a very low binding affinity for the more hydrophobic imprinted sol–gel TM3. A possible explanation for this could be the selectivity of the RTP measurements: while fluorescence allows detection of all bound nafcillin molecules, phosphorescence is more selective for those in a more rigid environment (non-radiational triplet state pathways are minimized). We are currently in the process of evaluate RTP as a tool to discriminate specific and non-specific binding in molecularly imprinted materials.

At this point we investigated the effect of alkyl substituents during the formation of the rigid sol–gel network around the template by developing an imprinted sol–gel (TPM1) and the corresponding control, using a mixture of TMOS and two organically substituted trialkoxysilanes, MTMOS and PTMOS. A TMOS:MTMOS:PTMOS volume ratio of 82:10:8 was used. On the other hand, in order to enhance the RTP emission of nafcillin, a heavy atom promoter (iodide) was used during the imprinted sol–gel preparation. Because iodide does not react with nafcillin, it was directly to the mixture of alkoxysilane functional monomers, prior to acid-catalyzed hydrolysis and condensation. Two different iodide salts were evaluated: potassium

Table 3
Performance indices for sol–gel imprinted films during binding assays

	Q_f			Q_p		
	TM1	TM2	TM3	TM1	TM2	TM3
$\lambda_{ex}/\lambda_{em}$ (nm)	256/371	256/363	256/368	256/474	256/470	256/467
Clean films	33.8	136.3	25.4	30.4	79.1	−3.4
Binding assay	27.5	297.3	80.3	38.1	107.1	3.9
After rinsing	33.8	177.3	25.9	27.8	77.7	−1.0

Every value was made in triplicate. The R.S.D. in the experiments was below 10%.

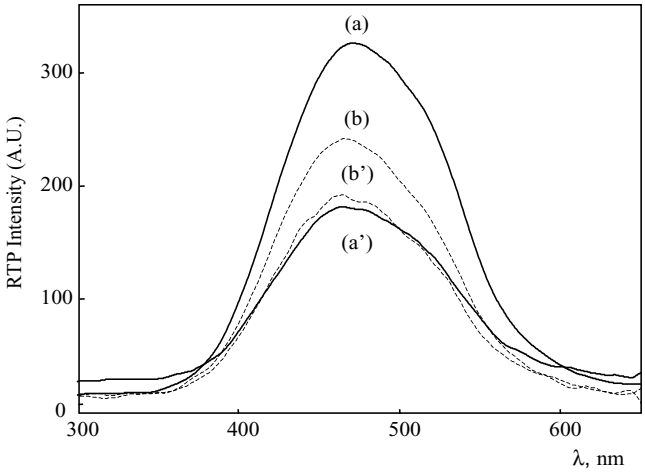

Fig. 2. Room temperature phosphorescence emission spectrum of the TPM1 imprinted sol–gel (—) before (a) and after (a′) removing the nafcillin template. Control sol–gel (- - -) before (b) and after (b′) rinsing. $\lambda_{exc} = 283$ nm, $t_d = 0.04$ ms, $t_g = 3.00$ ms.

iodide and tetrabutylammonium iodide. Fig. 2 shows the room temperature phosphorescence emission spectrum of the TPM1 imprinted sol–gel, before and after removing the nafcillin template. The RTP spectrum of nafcillin bound to the sol–gel shows a maximum emission at 470 nm, while nafcillin in solution shows a maximum at 475 nm and a secondary peak at 505 nm. It is known that virtually all the materials used as support of a luminescent molecule may act as a passive anchor while in others it has an active role. In this particular case, the imprinted sol–gel has an active recognition-interaction role and the spectral changes observed may be related to the different microenvironment experienced by nafcillin in the binding sites compared to that in fluid solution, where every molecule experiences the same average environment.

Data, expressed as Q_f and Q_p values, for two different MISGs prepared are presented in Table 4. As above, these data reflect the nafcillin interaction with precursors during the sol–gel preparation. Although the only difference between the materials is the iodide salt used during their synthesis, the TPM1 seems to have a higher affinity for nafcillin than TPM2. This fact could be ascribed to a loss of hydrogen bonding between the Si–OH groups of the precursors

Table 4
Performance indices for iodide doped organically modified sol–gel imprinted films

	Q_f		Q_p	
	TPM-1	TPM-2	TPM-1[a]	TPM-2
$\lambda_{ex}/\lambda_{em}$ (nm)	256/363	256/352	256/476	256/468
Crude films	1871.5	158.0	148.6	35.1
Rinsing 1	686.2	160.7	34.6	25.6
Rinsing 2	1023.5	179.2	52.6	29.2

[a] Excitation and emission slits were set at 5 nm. Every value was made in triplicate and demonstrated R.S.D. ≤ 8%.

Table 5
Performance indices for iodide doped organically modified sol–gel imprinted films during binding experiments

	Q_f		Q_p	
	TPM-1	TPM-2	TPM-1[a]	TPM-2
$\lambda_{ex}/\lambda_{em}$ (nm)	256/363	256/352	256/476	256/468
Clean films	935.2	179.2	52.6	29.2
Binding assay	1325.1	202.8	77.0	104.3
Rinsed film after binding assay	933.9	146.7	52.1	21.7

[a] Excitation and emission slits were set at 5 nm. All assays are the average of at least three runs, being the R.S.D. ≤ 10%.

and the carbonyl and amine groups of nafcillin in presence of the bulky tetrabutyl ammonium counterion (TBA^+) by steric constraints. The presence of the TBA^+ may lead to changes in the antibiotic structure that cannot be accommodated in the TPM2 imprinted sol–gel.

Binding studies with TPM1 and TPM2 films were carried out according to the recommended procedure at a fixed concentration of nafcillin (2.4×10^{-5} M). Data, expressed as normalized Q_f and Q_p values, are presented in Table 5. Results indicate that the TPM1 sol–gel was successfully imprinted and that non-specific interactions were minor compared to interactions due to specific molecular imprinting. The Q_f and Q_p values obtained before nafcillin binding and after washing it out (2×10 ml methanol $\times$ 1 min) also demonstrate that both, TPM1 and TPM2, imprinted sol–gels function reversibly. The stability of the imprinted sol–gels films has been investigated by repeating measurements of the nafcillin binding by the imprinted sol–gel TPM1 during a month. The imprinted sol–gel films prepared as mentioned above could be stored under room temperature during this time without showing any loss of its binding ability. The response time of the assay was in the order of 32 min (time of incubation, drying and measurement).

3.2. Competitive flow-through assays

In Fig. 3 we can observe the response profile of the MISGRs and the corresponding control to nafcillin in absence of competitive ligands. As can be seen, both the imprinted sol–gel and the control seemed to retain the nafcillin in a permanent manner, probably due to non-specific binding. Even, a slight lower binding of nafcillin was observed for the MISG than for its control. These results contrast with those obtained using the imprinted sol–gel films, where the non-specific binding was not so apparent. A possible explanation for this fact could be the previous history of the recognition material: the bulk sol–gel was ground, sieved and Soxhlet cleaned before use. These processes, involving pressure and temperature changes (e.g. during grinding), may modify the surface of the material, thus creating new non-specific binding points and probably affecting the pore size of both the control and the imprinted sol–gel. All these changes in the material contribute to overshadow the

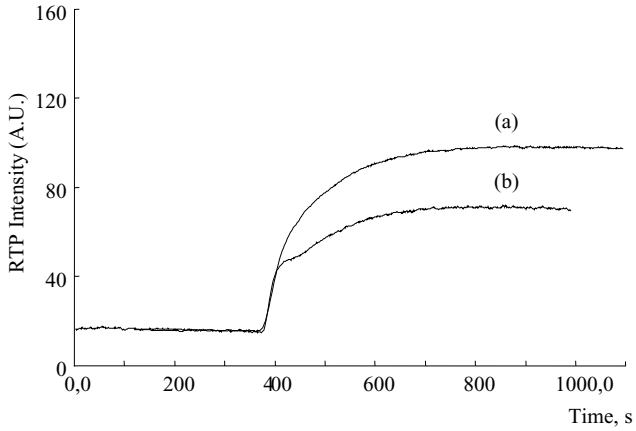

Fig. 3. Response profile of (a) TPM1 imprinted sol–gel and (b) the corresponding control.

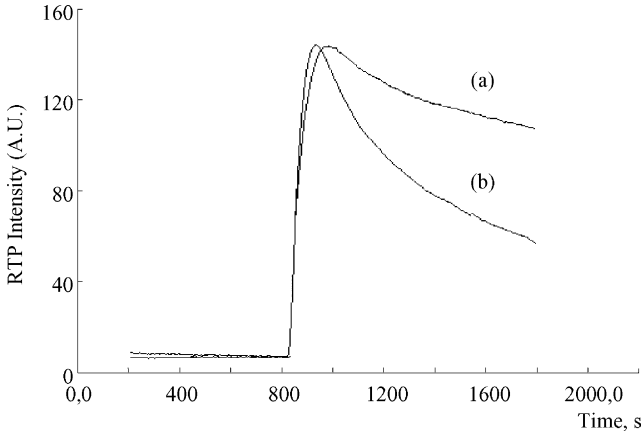

Fig. 5. Response profile of (a) TPM1 and (b) the corresponding control in presence of ampicillin (5×10^{-5} M) as competing ligand for nafcillin (1×10^{-4} M).

imprinting recognition process. On the contrary, the mild conditions used for conditioning the sol–gel films minimizes undesirable creation of non-specific binding points.

In order to evaluate removal of nafcillin from the MISG and the corresponding control, competitive assays with different ligands were carried out. RTP competitive assays were designed in which different non-luminescent ligands (Fig. 4) competed with nafcillin for a limited number of recognition sites in the MISG material. The competing ligand was kept at a constant concentration in the mobile phase (see composition in Section 2). This allowed the recognition sites of the MISG to be permanently saturated by the competing ligand. Elution curves provide an easy means to view the extraction performance of the MISG material, the contribution of both specific and non-specific interactions as well as the nafcillin moiety that is recognized by the imprinted solid. This is illustrated in Fig. 5 for a MISG (TPM1) and a control polymer in presence of ampicillin as competing ligand for nafcillin. As observed, there is an initial similar increase in the RTP signal for the imprinted and the control sol–gels, corresponding to the amount of nafcillin injected. Next, the ampicillin molecules, present in the mobile phase, displaced nafcillin from the imprinted and the control sol–gel at different rate. Finally, a conditioning solution made of sodium sulphite 0.01 M, KI 1 M, NaCl 1 M and MeOH 25% (v/v), is injected to completely remove nafcillin. The signal reached the base line and the system was ready for the next assay.

The different rate of nafcillin elution from the imprinted and the control sol–gel is a measure of the specific imprint-based interactions in the imprinted material. In order to evaluate these interactions we examined the performance of the different ligands as measured by the slope of the competitive elution curves. From the maximum

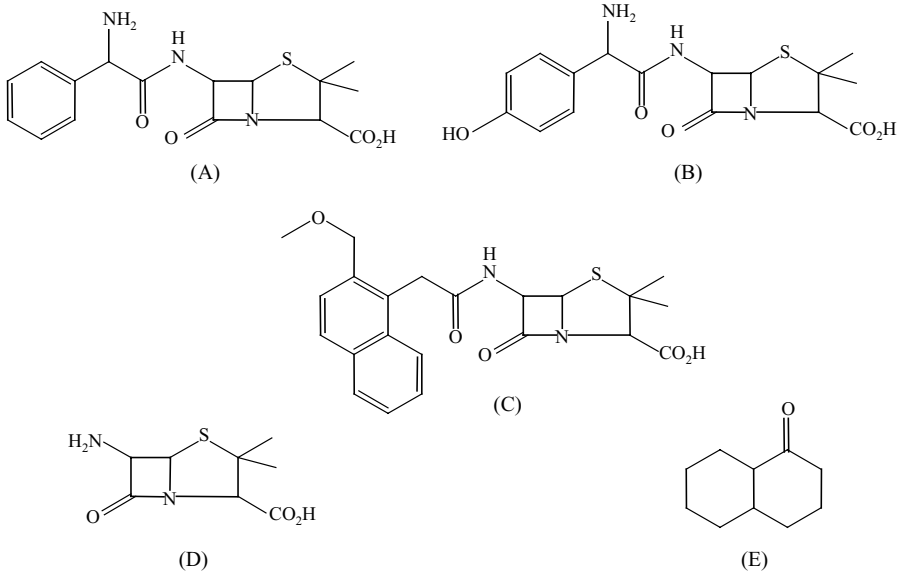

Fig. 4. Structure of the different competitive compounds under study: (A) ampicillin, (B) amoxicillin, (C) nafcillin, (D) 6-APA and (E) 1-decalone.

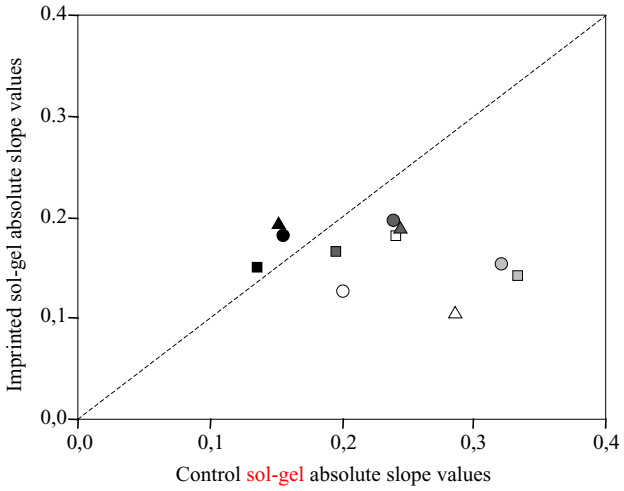

Fig. 6. Absolute slope values of elution curves for each competitive ligand: ampicillin (□, ○, △), amoxicillin (◍, ▣, ◬), 6-APA (●, ■, ▲) and decalone (■, ●, ▲). Ligand/nafcillin molar ratio is indicated by shape: (△) 0.5:1, (○) 1:1, (□) 2:1.

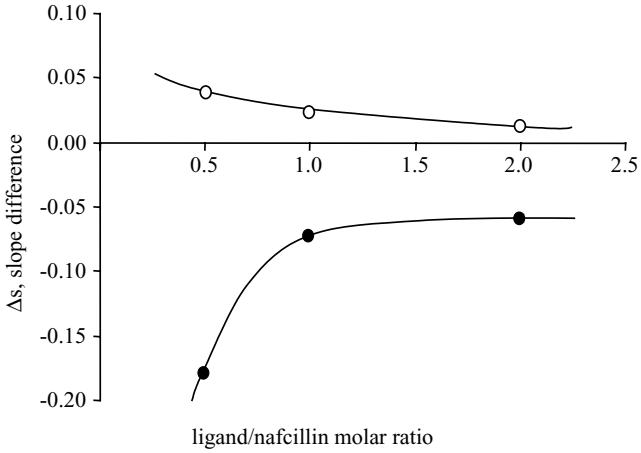

Fig. 7. Ligand concentration influence on the elution curve slope difference: (●) ampicillin and (○) decalone: $\Delta s = S_{imp} - S_c$.

signal obtained, a time interval of 100 s was used for calculations. Thus, a single analytical figure can be used to compare the imprinted material and the control one. The slope values obtained for each competitive ligand with the imprinted sol–gel were plotted against those obtained for the control polymer (Fig. 6), thus giving a measure of the specific retention. An important feature to note from these results is that among the ligands, amoxicillin seemed to compete efficiently for the non-specific points in the materials (the elution slope is the highest for the control and low for the imprinted). On the other hand, decalone seemed to compete efficiently for the specific recognition sites (the lowest slope for the control sol–gel while keeping a high value for the imprinted one). Comparing this behavior to the structure of the ligands it seems that non-specific binding is mainly due to the amino-lactamic moiety of the nafcillin molecule. Amoxycillin, which seems to be a better competitive ligand than ampicillin differs from it in that amoxycillin possesses a hydroxy group on the benzyl group, which may contribute to non-specifically bind to the sol–gels. On the other hand, decalone having two condensed aliphatic rings, seemed to have a higher affinity for specific binding sites in the imprinted polymer and consequently, the slopes of the elution curves for the control and the imprinted sol–gel are substantially close.

Displacement of nafcillin is dependent on the foreign ligand concentration as can be seen in Fig. 7 for two representative ligands, ampicilline and decalone, where the ligand/nafcillin molar ratio was plotted against the difference between the slopes of the elution curves of the imprinted and control sol–gels (Δs). Although the magnitude of these differences are not overly impressive, they are indicative of the binding nature. As the ampicilline concentration increased respect to that of nafcillin, the slopes of the elution curves increased for the imprinted and the control sol–gel. Again, the absolute values of the slopes are higher for the non-imprinted sol–gel than for the imprinted one ($\Delta s < 0$), corroborating the non-specific nature of the ligand binding. The decalone behavior is just the opposite. Strong competition for specific binding sites resulted in a smaller difference between the slopes of the imprinted and the control sol–gel. This difference tend to zero as the concentration of decalone increased which is a direct reflection of how much decalone resemble in terms of shape and size the nafcillin naphtalenic group that is recognized by the imprinted sol–gel (and the responsible for the antibiotic luminescent properties).

4. Conclusions

Analysis of the binding properties reveals that in this case, the naphtalenic group is dominant in determining the affinity of the imprinted sol–gel (receptor) for the substrate, while the β-lactamic moiety is that for non-specific bonding. While sol–gel films bound nafcillin more than 100 times more efficiently than a non-imprinted sol–gel, irregular particles of monolithic imprinted sol–gels are subject to strong non-specific bonding. The ability to prepare receptors (molecularly imprinted materials) with a predetemined selectivity has great implications not only in the construction of biomimetic optical sensors but also in separation methods (chromatography, solid-phase extraction). Current work is directed toward constructing a sensor system using the sol–gel film technology.

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