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Molecular imprinted ormosils for nafcillin recognition by room temperature phosphorescence optosensing

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Abstract

Nafcillin imprinted sol–gels were used as specific binding materials for the room temperature phosphorescence (RTP) recognition of nafcillin using a flow injection system. Selective and reversible binding of the template to imprinted sol–gels could be observed by tailoring the hydrophobic/hydrophilic balance of the materials. Also, the possibility of minimizing non-specific interactions was evaluated by end-capping with trimethyl silyl groups. Control experiments were performed with non imprinted reference sol–gels. The recognition mechanism and the analytical potential of these materials for developing stable, selective and sensitive approaches for nafcillin recognition in real samples are outlined.

Keywords: Molecular imprinted sol–gel materials; Ormosils; Room-temperature phosphorescence recognition; Nafcillin

1. Introduction

During the last 10 years or so, considerable interest has been focused on the design, synthesis and development of specialized materials and systems with improved molecular specificity (Lulka et al., 2000; Marx and Liron, 2001; Graham et al., 2002; Leung et al., 2001; Lin et al., 2003). Among them, materials with tailor-made cavity shapes and sizes are of special interest in analytical applications where molecular or ionic recognition are needed, such as chemical sensing, specific binding assays, chiral separations and solid-phase extraction. Usually, the synthetic schemes to prepare these materials are inspired by biological structures and their functions, and are focused to emulate or duplicate biosystems. Dickey (1949) inspired by the theory of Mudd and Pauling about human immune response (Mudd, 1932), first documented that silica gel synthesized in the presence of a specific dye molecule (methyl orange) had an enhanced affinity for that particular dye over its homologues (ethyl, *n*-propyl and *n*-butyl-orange) once it had been removed from the silica host with methanol. The conceptual process by which silica was provided with specific recognition cavities against methyl orange, is now established and is used in several research groups: molecular imprinting by the sol–gel process.

The imprinting process consists of three steps: (a) selection of the target analyte as template, (b) incorporation of the template into a polymer network, and (c) removal of the template leaving stable, selective cavities that recognize the target analyte. In a non-covalent approach (Díaz-García and Badía Laíño, 2005), the template may be directly added to a sol–gel solution prior to acid-catalysed hydrolysis and condensation. By using a non-polar sol–gel functional precursor and a fairly polar solvent, such as ethanol, imprinted sites are generated by van der Waals, π-stacking, electrostatic, etc. interactions between the template and the sol–gel network (Marx and Liron, 2001). As the solvent evaporates to yield a solid porous material, the imprinted sites are formed by template's affinity for the sol–gel matrix. Following a drying step, the gels are extracted with an adequate solvent to remove the template, thus leaving specific receptor sites capable of rebinding the template molecules.

The application of imprinted sol–gel materials to the production of analytical sensing devices is in its infancy. Although these materials hold considerable promise for the construction of selective chemical sensors, only few studies have dealt with the application of imprinted sol–gels as recognition materials applied on various transduction systems, for example, piezoelectric, fluorescence, voltammetry, etc. (Zhang et al., 2005;

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Pogorelova et al., 2004; Fang et al., 2005; Yang et al., 2005; Cummins et al., 2005; Li et al., 2005).

In a previous paper (Fernández-González et al., 2004) we have reported the production of nafcillin (β-lactamic antibiotic) room temperature phosphorescence-responsive films using the molecular imprinting sol–gel approach. We noticed that control of both sol–gel composition and the material final configuration may provide important insights into the selective recognition process. Our experiments revealed that recognition of nafcillin took place through the naphtalenic moiety of the antibiotic. Preliminary results from these studies indicated that a weak point of imprinted sol–gel materials may be non-specific binding, thus limiting their practical utility. In this paper we focus on the effects of various synthetic conditions (composition, end-capping) and final treatment on the recognition performance of nafcillin imprinted sol–gel particles. The physico-chemical characterization of the imprinted materials was carried out by both dynamic and batch studies and a possible recognition mechanism has been proposed. To our knowledge, this is the first report on the use of imprinted ormosils (organically modified silanes) materials as recognition materials for nafcillin determination in real samples using a flow injection system with room-temperature phosphorescence transduction.

2. Experimental

2.1. Reagents

Nafcillin [6-(2-ethoxy-1-naphthamido) penicillin], chlorotrimethylsilane (99%), 1,1,1,3,3,3-hexamethyldisilazane (99%), ampicillin and amoxicillin were purchased from Sigma. Tetramethyl orthosilicate (98%, TMOS), methyltrimethyl orthosilicate (98%, MTMOS), phenyltrimethyl orthosilicate (97%, PhTMOS), aminopropyltriethyl orthosilicate (96%, APTEOS), (+)-6-aminopenicillanic acid (6-APA), tetrabutyl-ammonium fluoride (97%, TBAF) and potassium iodide were obtained from Fluka. Sodium sulphite and hydrochloric acid were obtained from Merck. All syntheses were carried out using distilled-deionised water (18.0 M mho; Millipore system). All solvents were of analytical-reagent grade and were used without further purification unless stated otherwise. The standard antibiotic solution (1×10^{-3} M) was prepared in water every 3 weeks and was kept under 4 °C to avoid degradation.

2.2. Instrumentation

A Perkin-Elmer LS-50B luminescence spectrophotometer which has a xenon discharge excitation source (pulse width at peak half-height <10 μs) was used for phosphorescence measurement. Instrumental parameters and processing data were controlled by the Fluorescence Data Manager software. The excitation and emission wavelengths were set at 283 and 505 nm, respectively. Excitation and emission slits were set at 15 and 20 nm, respectively. A gate time of 3 ms and a delay time of 0.04 ms were used throughout. Imprinted sol–gels were observed by transmission electron microscopy with a JOEL 2000 ExII-HR microscope operating at acceleration voltage 160 kV and magnification values up to 5000×.

Table 1
Composition of sol–gel materials

Sol–gel	MTMOS (μl)	TMOS (μl)	PhTMOS (μl)	APTEOS (μl)
TPM (a)	120	1230	150	–
TPM (b)	675	675	150	–
TPM (c)	1230	120	150	–
TAM (a)[a]	120	1230	–	150
TAM (b)	675	675	–	150
TAM (c)	1230	120	–	150

[a] TAM (a) could not be prepared as its gelation process started before addition of all reactants.

2.3. Synthesis of molecularly imprinted sol–gel particles

Sols were made by mixing, in that order, 1140 μl of 1×10^{-3} M nafcillin, 1550 μl of ethanol, adequate volumes of TMOS, MTMOS, PhTMOS, APTEOS (according to data in Table 1), 760 μl of 0.025 M TBAF and 50 μl of 0.1 M HCl. All solutions were gentle mixed for 2 min to ensure homogenous mixing. The sol was allowed to gel and dry for 72 h under ambient conditions and then at 45 °C to reach the constant weight for approximately 2 weeks. The resulting sol–gels were mechanically crushed and sieved in fragmented fine particles (0.16–0.08 mesh).

2.4. Conditioning procedures for imprinted sol–gels

2.4.1. End capping treatment

The end capping treatment was performed following the procedure described elsewhere (Lin et al., 2003). Before removing the template molecule, imprinted sol–gel particles (0.1 g) were mixed with 0.55 ml of an equimolar mixture of chlorotrimethylsilane and 1,1,1,3,3,3-hexamethyldisilazane, at room temperature and continuous stirring during 24 h after which the sol–gel particles were washed with THF (5 ml), acetonitrile (5 ml) and methanol (5 ml).

2.4.2. Template extraction

In order to remove the template molecules two approaches were addressed. Sol–gel particles were Soxhlet rinsed using a 80:20 (v/v) methanol–acetic acid (24 h, 120 cycles) and methanol (8 h, 40 cycles). Control sol–gels were prepared in the same way in absence of nafcillin. Removal of the template molecule by calcination was performed in air, using a muffle furnace with a heating rate of 5 °C/min until a maximum temperature of 300 °C was reached. The imprinted sol–gel particles (0.1 g) were left at that temperature for 2 h, after which they were withdrawn, cooled at room temperature and stored before use.

2.5. Measurements systems

2.5.1. Batch analysis

Cleaned imprinted sol–gel (0.01 g) was added into 2 ml of a nafcillin solution (nafcillin contents in the 5×10^{-5} to