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Naproxen-imprinted xerogels in the micro- and nanospherical forms by emulsion technique

Mariana Ornelas, Manuel Azenha*, Carlos Pereira, A. Fernando Silva

Centro de Investigação em Química – CIQUP, Faculty of Science, University of Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal

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ABSTRACT

Naproxen-imprinted xerogels in the microspherical and nanospherical forms were prepared by W/O emulsion and microemulsion, respectively. The work evolved from a sol–gel mixture previously reported for bulk synthesis. It was relatively simple to convert the original sol–gel mixture to one amenable to emulsion technique. The microspheres thus produced presented mean diameter of 3.7 μm, surface area ranging 220–340 m^2/g, selectivity factor 4.3 (against ibuprofen) and imprinting factor 61. A superior capacity (9.4 μmol/g) was found, when comparing with imprints obtained from similar pre-gelification mixtures. However, slow mass transfer kinetics was deduced from column efficiency results. Concerning the nanospherical format, which constituted the first example of the production of molecularly imprinted xerogels in that format by microemulsion technique, adapting the sol–gel mixture was troublesome. In the end, nanoparticles with diameter in the order of 10 nm were finally obtained, exhibiting good indications of an efficient molecular imprinting process. Future refinements are necessary to solve serious aggregation issues, before moving to more accurate characterization of the binding characteristics or to real applications of the nanospheres.

1. Introduction

Molecular imprinting technology allows the preparation of tailor-made binding sites obtained by the co-polymerization or co-condensation of functional precursors and cross-linkers in the presence of a template molecule [1]. The removal of the template leaves a vacant recognition site, with the ability to recognize the shape, the size and the functionality of the template molecule. When both the functional precursors and crosslinkers consist of condensable alkoxides of the general type $M(OR)_{4-n}R'_n$ (M = Si or Ti) molecularly imprinted xerogels (MIXs) may be obtained by a sol–gel process [2] that offers attractive advantages for the preparation of these materials, such as thermal/chemical stability and availability of a large variety of functional trialkoxy-silanes ($Si(OR)_3R'$). Sol–gel technology allows also the easy preparation of different configurations [3] (*e.g.* films, monoliths and spheres).

Typically, MIXs are synthesized in bulk. A monolith is formed and consequently crushed, grounded and sieved to obtain irregularly shaped particles in the desired size range. Obvious limitations of this method are the unpredictable shape of the particles, the intensive time and labour involved and the low yields of material obtained. The resulting irregular particles pack poorly in columns and exhibit lower efficiency and high back pressures when used in chromatography. Additionally, deformation of the binding sites during the crushing process may occur, resulting in loss of selectivity. The direct production of the particles in a spherical format improves the yield of the process, as well as the column packing efficiency. Spherical particles also exhibit better flow properties and are ideal for flow-through applications such as chromatography or solid-phase extraction [4,5]. Different strategies can be found in the literature to prepare MIXs in the spherical format. Grafting of the imprinted sol–gel on the surface of silica microparticles is a typical method to prepare microspherical surface-imprinted silica [*e.g.* 6,7]. The combination of water-in-oil emulsions with the process of molecular imprinting in sol–gel matrix represents another simple way to obtain spherical molecularly imprinted silica. The sol–gel mixture is fractionated in small droplets that offer a unique environment for the sol–gel reaction: they act not only as microreactors but also as steric barriers for inhibiting the condensation of reacting species among the different droplets during the reaction period [8]. Additionally, varying the parameters of the emulsion system and the characteristics of the sol–gel solution it is possible to control the kinetics of particle formation and growth, and consequently their size [9]. Until now, only a few examples of silica imprinted materials prepared through

* Corresponding author.
E-mail address: mazenha@fc.up.pt (M. Azenha).

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emulsion methods were reported. Two of the reports [10,11] describe procedures to prepare surface imprinted catalytic microparticles. They involved the synthesis of a template-derived surfactant, in which the imprint molecule served as the surfactant headgroup. Mixing it together with a non-ionic commercial surfactant (polyoxyethylene (5) nonylphenylether, NP-5) in cyclohexane, a W/O emulsion was formed. The surfactant molecules located in the water-in-oil interface directed the hydrophilic group to the surface of the silica particle being formed and the imprinting occurred only in its surface. The obtained silica particles with a diameter ranging between 400 nm and 600 nm were capable to discriminate D- and L-enantiomers. However, this method can only be applied to molecules that have the capability of being modified by the introduction of an aliphatic chain. We have recently been able to extend this strategy to the preparation of glycylglycine molecularly imprinted silica microspheres [12]. We also came up with a variation of the grafting strategy for the imprinting of naproxen (NAP). The technique consisted of filling up the pores of spherical, mesoporous silica particles with a pregelification mixture by applying pressure [13]. Upon gelification and drying, a layer of MIX was deposited on the mesopores. The present work deals also with the development of NAP imprints, in this case in the microspherical and nanospherical forms, by W/O emulsion and microemulsion, respectively.

Nanomaterials are now among the most researched alternatives to overcome the drawbacks associated with conventional MIX formats. The advantages associated with the nano-size range include [14]: (i) the high surface-to-volume ratio of these materials, facilitating the removal of the template molecules; (ii) the binding sites being located near/closer to the surface, what improves their accessibility as well as the affinity and sensitivity of the materials to the target molecules; (iii) the diffusional length of the target analytes inside the nanoparticles is smaller and a fast equilibration is expected, improving the binding kinetics. *In vivo* applications reachable only in the nanosized domain due to the excellent dispersion and suspension in solution, such as drug delivery or imaging [15,16] can be sought. However, if one searches for papers on imprinted sol–gel nanospheres, their number is reduced (below 40) and the majority of them is related to different core (gold [17] or Fe_3O_4 [18], for example) nanoparticles serving as supports for grafting. Moreover, to the best of our knowledge the preparation of imprinted spherical xerogels through emulsion technique combined with sol–gel method has only been reported for sizes above 300 nm, a diameter that is still within the micro-range. Consequently, the research on this matter requires a serious attention, since the possibilities of combining the advantages of imprinted sol–gel with the simplicity of the W/O emulsion technique can open countless possibilities in the synthesis of new materials with a wide range of applications. For this reason, we approach here, for the first time, concomitantly with the development of imprinted microspheres, the possibility of producing molecularly imprinted xerogels in the nanospherical format by microemulsion techniques.

2. Experimental

2.1. Materials

Naproxen (NAP), ibuprofen (IBU), formic acid and tetramethoxysilane (TMOS), polyoxyethylene (5) nonylphenylether (NP-5), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (Deisenhofen, Germany). Acetonitrile, acetone and methanol (MeOH) of HPLC grade were obtained from VWR International (Carnaxide, Portugal). Ammonia (NH_3, 25–29%) was purchased from Pronalab (Lisbon, Portugal). Absolute ethanol (EtOH) was purchased from Aga (Prior Velho, Portugal) and sorbitane monooleate (Span 80) was obtained from Sigma–Aldrich (Steinheim, Germany). Ultra-pure water of Milli-Q quality (Millipore, Italy) was used. All other reagents used were of analytical grade.

Sodium salt of naproxen (Na-NAP) was prepared by adding an equimolar amount of NaOH (aq.) to naproxen previously dissolved in MeOH, and the reaction mixture was stirred for 2 h at room temperature. The solvent was then evaporated to dryness using a rotary evaporator.

The functional monomer (1-(triethoxysilylpropyl),3-(trimethoxysilylpropyl)-4,5-dihydroimidazolium iodide; DHI^+ iodide) was synthesized according to a procedure described elsewhere [19]. ^1H NMR, ^{13}C NMR and LC–MS characterization of the product was performed, confirming that the synthesis of the functional monomer was successful. Spectral data can be found as Support Information (Figs. S1–S3).

2.2. Synthesis of naproxen templated silica microspheres

The molar ratios of the sol–gel components for the preparation of MIXs used in the study were: 20 (MeOH):1 (TMOS):0.075 (Na-NAP):0.15 (DHI^+ iodide):3 (H_2O). The sol–gel mixture (pH 9) was left to react during 6 h before being emulsified. For the preparation of control microparticles (NIX) the same mixture conditions applied, save for the addition of the template molecule and the use of a NaOH (aq.) solution to adjust the pH. The surfactant solution (0.1 g/mL) was prepared by adding Span 80 to cyclohexane and homogenizing at 2200 rpm for 1 min. Then, the sol–gel mixture was added to the surfactant solution and vigorously stirred to prepare the W/O emulsion using an oil-to-water volume ratio ($V_{o/w}$) of 20. The emulsion was stirred at 2200 rpm for 5 min and the obtained particles were filtered and washed three times with 20 mL of cyclohexane, followed by three times with 10 mL of EtOH, and three times with 10 mL of H_2O. The synthesis yields were found to be above 75% (percentage of the theoretical yield considering complete condensation of the precursors) in all the cases. The microparticles were subsequently washed to remove the template, by soxhlet extraction using MeOH containing 10% of formic acid. The washing proceeded until no NAP was detected by HPLC analysis.

2.3. Synthesis of molecularly imprinted silica nanoparticles

To a 150 mL centrifuge tube, 4.2 g of NP-5 were added to 90 mL of cyclohexane and this mixture was kept under ultrasonication for 40 min. In a 22 mL glass vial, the sol–gel mixture was prepared adding, in this order, 90 mg of DHI^+ iodide, 900 μL of MeOH, 18 mg of Na-NAP previously dissolved in 420 μL of NH_3 catalyst (5.5×10^{-5} mol/L), and 168 μL of TMOS, followed by magnetic stirring at 500 rpm for 1 h. Control nanoparticles were prepared through the same procedure, save for the addition of the template. In this case, the NH_3 solution had a concentration of 9.9×10^{-5} mol/L to assure the medium pH was the same as in the MIX preparations (pH 9). The sol–gel mixture was left to react for 60 min before emulsification. The microemulsion was formed by mixing the surfactant and the sol–gel solutions, and stirring at 10,000 rpm for 30 min. After stirring, 160 mL of acetone were added and the system was stirred at 10,000 rpm for 10 additional minutes. Then, the particles were washed 3 times with 20 mL of acetone, followed by washing with 10% formic acid in MeOH until no naproxen was detected in the supernatant (by HPLC analysis). After the template was removed, the washing procedure continued with redispersing the particles three times in 20 mL of absolute EtOH. For the last 20 mL of EtOH, the dispersion was kept in ultra-sounds for

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