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Molecularly imprinted polymer strategies for removal of a genotoxic impurity, 4-dimethylaminopyridine, from an active pharmaceutical ingredient post-reaction stream

Teresa Esteves [a], Raquel Viveiros [b,c], João Bandarra [c], William Heggie [c], Teresa Casimiro [b], Frederico Castelo Ferreira [a,*]

[a] iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais, 1049-001 Lisbon, Portugal
[b] LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, FCT, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
[c] Hovione FarmaCiencia SA, R&D, Sete Casas, 2674-506 Loures, Portugal

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ABSTRACT

Molecularly imprinted polymers (MIPs) are prepared and evaluated for the effective removal of the genotoxic impurity (GTI), 4-dimethylaminopiridine (DMAP), from a Mometasone furoate (Meta) solution, used as a case study relevant for the pharmaceutical industry. The MIPs formation by bulk polymerization is assessed considering different temperature regimes as well as stoichiometry of template, functional monomer, cross-linker, respectively DMAP, methacrylic acid and ethylene glycol dimethacrylate. A design of experiment (DoE) is performed to establish conditions for a maximum GTI specific binding percentage, validated experimentally at a value of 98% for 5.03 mgGTI/gMIP, for a 256 ppm GTI solution. The MIP robustness and recyclability are successfully evaluated over 6 cycles. Multistep approaches, using MIP alone or in combination with organic solvent nanofiltration (OSN), are discussed aiming to minimize API losses with removal of GTI to reach the threshold of toxicological concern (TTC) for two case studies.

1. Introduction

In recent years, pharmaceutical regulatory authorities have shown increased concerns about impurities – especially genotoxic impurities (GTI) – in active pharmaceutical ingredients (API) due to their adverse effects on human health [1,2]. Sources for organic impurities in the APIs include unreacted starting materials and reagents, intermediates, catalysts, by-products, reagents and degradation products, as extensively reviewed elsewhere [3]. When the formation of GTIs in APIs production cannot be prevented, purification of the APIs must be performed until the GTI is removed to satisfying levels. Conventional separation techniques used in API purification include crystallization, filtration, distillation, the use of adsorbents, resins and column chromatography [2,3]. Recently, the use of organic solvent nanofiltration (OSN) has also been suggested to address this challenge [4,1]. Another possibility involves the use of molecularly imprinted polymers (MIPs), which explores the formation of selective binding sites in polymers to target molecules by using a molecular template [5,6]. MIPs are reported to be robust, insoluble in most media, obtained by easy synthetic methods and have good reusability [7]. These imprinted polymers have been developed as selective materials for several applications on sensors [8], drug delivery systems [9], solid phase extraction [10], chromatography [11,6] and more recently for the removal of potential GTIs from APIs [12,13]. MIPs also have been suggested to be used in an API polish step after removal of 1,3-diisopropylurea by OSN permeation, to remove residual amounts of this urea from the retentate solution [14]. The current study addresses an industrial challenge in the synthesis of Mometasone furoate (Meta), which is the removal of a genotoxic reactant, 4-dimethylaminopyridine (DMAP), used in one of the final steps of this API production. The current study is focused on the removal of DMAP and the research approach taken consists on the use of a synthetic solution prepared by dissolving Meta and DMAP at known concentrations. However, this approach does not consider additional challenges when translated to an industrial setting due to the potential presence on solution of other species, potentially carried out from previous stages, by-products formed or traces of unreacted reagents, which can affect the specificity of the GTI removal.

Meta is a glucocorticoid steroid used topically to reduce inflammation on skin (eczema, psoriasis) or airways (allergic rhinitis, some asthma patients) pathologies [15,1]. The preparation of a

* Corresponding author.
E-mail address: frederico.ferreira@ist.utl.pt (F.C. Ferreira).

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MIP for specific removal of DMAP from a Meta solution in DCM was yet to be developed and inhere is identified the conditions that maximize selectivity of an methacrylic acid based MIP not only in identification of polymerization conditions used in its synthesis, but also in the operations conditions to carry out to remove DMAP. Therefore, a systematic study was followed to select the best conditions to develop a MIP for specific binding of DMAP, using this GTI as the template in the imprinting cross-linking polymer process (Fig. 1); a DoE was applied for the selection of the best key variables for the GTI removal, and; a multi-step approach using either MIPs alone or in combination with OSN was quantitatively discussed for a rational process design aiming to minimize API losses, while reaching the threshold of toxicological concern (TTC).

2. Materials and methods

2.1. Materials

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) and 4-dimethylaminopiridine (DMAP) were purchased from Acros (Belgium). Dichloromethane (DCM), methanol (MeOH), and hydrochloric acid (HCl) were purchased from Fisher Chemicals (USA). 2,2′-azobis(2-methylpropionitrile) (AIBN) was purchased from Fluka (Switzerland). Acetonitrile (ACN) and formic acid solution was purchased from Aldrich (Switzerland). Mometasone furoate (Meta) was kindly provided by Hovione FarmaCiencia SA (Portugal). All chemicals were of reagent grade or higher and were used as received.

2.2. Apparatus and analysis

Specific surface area and pore diameter of the polymeric particles were determined by nitrogen adsorption according to the BET method. An accelerated surface area and porosimetry system (ASAP 2010 Micromeritics) was used under nitrogen flow. FTIR spectra were recorded as KBr pellets on a Nicolet 6700 spectrometer in the 400–4000 cm^{-1} range using 2 cm^{-1} resolution. Visualization of the morphology of the polymeric particles was performed using scanning electron microscopy (SEM) on a FEG-SEM (Field Emission Gun Scanning Electron Microscope) from JEOL, model JSM-7001F, with an accelerating voltage set to 15 kV. Samples were mounted on aluminum stubs using carbon tape and were gold/palladium coated on a Southbay Technologies, model Polaron E-5100. HPLC measurements were performed on a Merck Hitachi pump coupled to a L-2400 tunable UV detector using an analytic Macherey-Nagel C18 reversed-phase column Nucleosil 100–10, 250 × 4.6 mm, with a flow rate of 1 mL min^{-1} and UV detection at 280 nm; eluents, A: aqueous 0.1% formic acid solution, B: ACN 0.1% formic acid solution; method: 0–3 min, 60–20% A; 3–4 min, 20% A; 4–8 min, 20–60% A; 8–15 min 60% A.

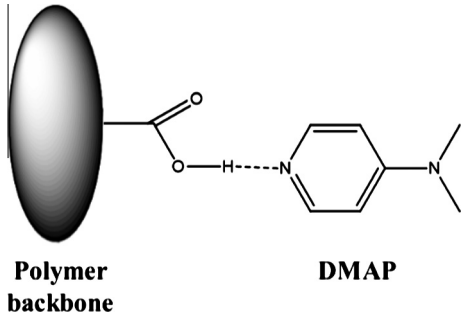

Fig. 1. Predicted hydrogen bond interaction between the functional monomer methacrylic acid (MAA) and the template 4-dimethylaminopyridine (DMAP) in the polymer backbone structure.

2.3. Preparation of polymers

Polymer compositions can be found in Table 1. The model GTI (DMAP) was used as template in the preparation of MIPs, but absent in the preparation of the control polymer labeled as non-imprinted polymer (NIP). The synthesis of MIPs is described below, with quantities exemplified for **MIP2** preparation. MAA (2.323 mmol, 197 μL) was dissolved in DCM (4.35 mL), which works as the porogen. For MIP preparation, the DMAP template (0.581 mmol, 0.071 g) was added to the MAA solution and left stirring for 5 min. EGDMA cross-linker (11.620 mmol, 2.20 mL) and the initiator AIBN (1% wt of total monomers) were added to the polymerization solution, which was purged with a flow of nitrogen for 5 min. The glass tubes were closed and the polymerization was initiated by placing the tubes at 40 °C for 12 h, the temperature was then increased, in increments of 5 °C/30 min, until 65 °C, temperature at which the tubes were left for additional 4 h (method 1). Alternatively, the polymerization was performed isothermally at 65 °C for 16 h (method 2). After polymerization, a white solid was obtained, the tubes were opened and the polymers gently crushed in a mortar. The polymers obtained were washed in a Soxhlet-apparatus with a solution of 0.1 M HCl in MeOH for 48 h for extraction of the template followed by washing with MeOH for 24 h, then the polymers were dried in an oven overnight at 40 °C. The polymers were then grounded in a mechanical mortar and sieved (Resh stainless steel sieves), and the fraction 38–63 μm was used for polymers binding performance evaluation, as well as chemical and morphological characterization. Quantitative analysis of the washing and extraction steps indicated virtually complete template removal. Conditions used are resumed in Table 1.

2.4. Batch binding experiments

Batch binding experiments were evaluated placing 25 mg/mL and 50 mg/mL of each polymer in a 1.5 mL of a 100 and 1000 ppm DMAP solution in DCM in independent vials, which were sealed and stirred for 24 h. The same procedure was performed for a DCM solution of 10 g/L of Meta spiked with 100 ppm of DMAP, as representative solute concentrations and solvent of a post-reaction solution. Binding isotherms and kinetic experiments were performed similarly with a load of 50 mg/mL of **MIP2** stirred in 1.5 mL of DMAP solutions in DCM. Solutions of DMAP with different initial concentrations (5–1000 ppm) were used for 24 h equilibrium experiments to estimate the isotherm. Initial solution of a fixed 100 ppm DMAP concentration was used for kinetic experiments, supernatant samples were taken at 5 min, 15 min, 30 min and at 1, 2, 4, 6, 8, 24 and 33 h and analyzed by HPLC. Experiments to set-up and validate DoE were performed similarly for 24 h using **MIP2** quantity and DCM solutions with DMAP concentrations and volume as reported in the next section. All these sets of experiments were carried out at 60 rpm and 25 °C.

Table 1
Polymer compositions: T – template, MAA – functional monomer, and EGDMA – cross-linker.

	Stoichiometry (mmol)			
	MAA	EGDMA	T	Method
MIP1	0.4	1	0.1	1
MIP2	0.4	2	0.1	1
MIP3	0.4	4	0.4	1
MIP4	0.4	4	0.4	2