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Development of a molecularly imprinted polymer for a pharmaceutical impurity in supercritical CO_2: Rational design using computational approach

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ABSTRACT

Supercritical fluid technology is a green and promising alternative for the development of molecularly imprinted polymers. These affinity polymers are obtained ready-to-use, without organic solvent residues, and with controlled properties which could prompt their use in several areas. In this work, a molecularly imprinted polymer (MIP) with affinity for a model pharmaceutical impurity, acetamide, was pre-designed using a user-friendly computational approach in order to optimize MIP synthesis in supercritical CO_2. Molecular Modelling was performed using SYBYL™ software, introducing for the first time CO_2 as solvent in the rational design of MIPs. A virtual library of functional monomers was created and screened against acetamide. The monomers giving the highest binding energy were selected and used in a simulated annealing (molecular dynamics) process to investigate their interaction with the template acetamide in the presence of CO_2 as the porogen. Itaconic acid and 2-hydroxyethyl methacrylate were selected as the best monomers to interact with acetamide and the molar ratios generated were used in the MIP synthesis in supercritical carbon dioxide. Binding and selectivity experiments were performed to evaluate the affinity performance of the polymers. The experimental results indicate that itaconic acid-MIP, as predicted by SYBYL™, has higher affinity and selectivity to acetamide, highlighting the value of this computational tool in MIP optimization using supercritical fluid technology.

1. Introduction

Acetamide is a pharmaceutical impurity typically present in the last stages of API manufacturing. It has genotoxic potential since it has the ability to interact with DNA (Dow et al., 2013). Increasingly strict regulations have led the Pharmaceutical Industry to attempt different conventional strategies to efficiently remove impurities from APIs, including recrystallization (Stieger and Liebenberg, 2012), chromatography (Maddula et al., 2009), nanofiltration (Székely et al., 2011), resin treatment (Lee et al., 2010), distillation, extraction, activated carbon powder treatments (Székely et al., 2013) and affinity polymers (Székely et al., 2012a, 2012b).

Molecular imprinting technology has been considered a cheap way to produce synthetic affinity structures, especially due to the simplicity and cost-effectiveness of the preparation process. This powerful technique has been used to prepare molecularly imprinted polymers (MIPs) in both organic (Mazzotta et al., 2016; Yang et al., 2016) and alternative solvents such as $scCO_2$ (Soares da Silva et al., 2012a) for application in different fields. This methodology is able to imprint a template within a crosslinked matrix creating empty specific binding sites with unique interaction properties in terms of shape and functionality, which is complementary to the target molecule. The basic concept behind this methodology entails the selection of the functional monomer according to the target template, solvent, crosslinker, and initiator. The interaction complexes formed at the beginning of the polymerization are immobilized by crosslinking. At the end of the polymerization the template molecules are desorbed from the matrices making affinity sites, which are complementary in functionality, size and conformation with the template, available. The key to the success of the imprinting process is the ability of the template to form a strong monomer-template complex which is

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strongly dependent on the nature of monomer and template. In the past, when the library of potential monomers was small, identification of the best monomer candidates for the imprinting process could be done by thermodynamic calculations and combinatorial screening approaches (Chen et al., 2001). Lately, the size of the library has increased substantially with thousands of polymerizable compounds being commercially available, hence new approaches to identify the best candidates in an efficiently and timely manner is required.

Choosing the best functional monomer and crosslinker for each template and the ratios to be used can involve a huge amount of laboratory work. The nature of the solvent employed has to be taken into consideration since the solvent interacts with both the monomer and template. The best solvent should not disrupt the interaction of monomer and template since this is crucial in defining the specificity of the imprinted sites. Molecular modelling (MM) software packages and searching algorithms traditionally used for drug design are a potential solution to the problem (Böhm, 1992). Recently available computational methods have limitations when modulating the complex processes entailed in the preparation of MIPs, particularly the polymerization process. MM software programs such as AMBER, HyperChem 501, GROMACS, Gaussian (Subrahmanyam et al., 2013), MOE, RasMol, QMol, Raster 3D or AGM Build base their computational design on thermodynamic calculations (Piletsky et al., 2001) for multicomponent systems. However, molecular modelling of complex systems including interactions of polymers with template, solvent, and other molecules is difficult due of the high requirements of computational workstations. Moreover, some of these programs cannot generate clear results, thus there is the need of comparing results obtained from different programs, which is very time consuming.

A more straightforward approach involves molecular modelling and focuses on the selection of monomers, which can establish strong complexes with the template (Subrahmanyam et al., 2001; Chianella et al., 2002). Piletsky et al. have pioneered the modelling of monomer mixtures and the interactions between monomers, crosslinkers, template, and solvent using SYBYL™, a user-friendly program typically used in drug discovery, thereby significantly reducing the computational load (Piletsky et al., 2001). The SYBYL program package, which includes the LEAPFROG™ algorithm, is able to address problems related with rational MIP design, such as the selection of the best template-monomer complex and molar ratios to be used. Suitable monomers are chosen from a previously created library containing polymerizable monomers. The template is run against each monomer from the library, and a binding energy for the template-monomer interaction is scored, the highest binding score corresponding to the best template-monomer combination (Subrahmanyam et al., 2013).

This work presents, for the first time, the use of SYBYL™ in a computational approach for the synthesis of a molecularly imprinted polymer (MIP) using $scCO_2$ as solvent. This computational approach is a straightforward way to optimize the construction of these affinity polymers in $scCO_2$.

2. Materials and methods

2.1. Materials

Acetamide (ACET, 99% purity) as template, benzamide (BENZ, 99% purity) and trimethylacetamide (pivalamide, PIV, 98% purity) as analogue molecules, Itaconic acid (ITA, 99% purity) and 2-hydroxyethy methacrylate (HEMA, 99% purity), as functional monomers, ethylene glycol dimethacrylate (EGDMA, 98% purity) as crosslinker and Trifluoroacetic acid (TFA, 99% purity) were purchased from Sigma-Aldrich. Azobis(isobutyronitrile) (AIBN, 98% purity) from Fluka was used as initiator. HPLC grade acetonitrile (ACN) from Carlo Erba was used. Carbon dioxide was obtained from Air Liquide with purity better than 99.998%. All chemicals were used without further purification.

2.2. Computer simulation

The monomer-template interactions were simulated using a Silicon Graphics Octane workstation running IRIX 6.6 operating system, as described elsewhere (Chianella et al., 2002), which was used to execute the software SYBYL 7.6 (Certara Inc.). The computational design was performed in three stages. At stage one: i) ACET was downloaded from the Protein Data Bank (PDB, file: lfjm.pdb); ii) a virtual library of 25 commonly used monomers was designed (Fig. 1); iii) all the monomer structures were inputted using the Gasteiger-Huckel computational method and refined using the molecular mechanics method by applying an energy minimization with the MAXIMIN2 command (Labanowski et al., 1986). In the second stage, each single functional monomer of the library was screened against the template using the LEAPFROG™ algorithm. This algorithm allows the evaluation of binding scores of ligand structures on the basis of their energy (SYBYL, Tripos Associates, Inc., St. Louis). The program was activated for 60,000 iterations sufficient to probe all interactions of the monomers with the template, each one corresponding to a binding score which were then ranked. The monomers giving the highest binding score (Table 1) and capable of forming the strongest complexes with the template were selected as candidates for the preparation of the corresponding polymer. In the third phase, ACET and the monomers were assembled in a pre-computed virtual solvent box, the box saturated with CO_2 as solvent and energy minimized simulations performed. A simulated annealing process was then applied to the box to optimize the arrangement of functional monomers around the template in the presence of CO_2. Annealing conditions were fixed at 300 K with 100,000 iterations. At the end of the program, the number and the position of functional monomers were examined. The number of monomer molecules participating in the complex determines the ratio of template to monomers to be used in the synthesis of the polymer.

2.3. Preparation of ACET-molecularly imprinted polymers in $scCO_2$

Polymerization reactions were carried out using supercritical carbon dioxide technology as reported previously (Casimiro et al., 2005). The MIPs and NIPs were prepared using the ratios, generated from computational simulation, as given in Table 1, along with 1 wt % of the radical initiator AIBN. The reactions were performed in a 33 mL stainless steel high-pressure reactor.

The cell is equipped with two aligned sapphire windows and a Teflon coated magnetic stirring bar. It is immersed in a thermostatic water bath at 65 °C, which is the optimal AIBN initiation temperature. Temperature control was achieved through an open bath circulator Julabo Ed with stability ±0.1 °C. Carbon dioxide was added up to 21 MPa and polymerization proceeded for 24 h under stirring. At these initial temperature and pressure conditions, a single homogeneous phase is assured, with all reactants completely dissolved in the supercritical phase. Non-covalent molecular imprinting occurs since no covalent bonding between template and monomers are formed. At the end of the reaction, the polymer is slowly washed with fresh high-pressure CO_2 for 1 h in order to remove the template molecule and wash any unreacted residues. Non-imprinted polymers (NIPs) were synthesized following the same procedure but no template was added.