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Computational design and synthesis of molecularly imprinted polymers with high binding capacity for pharmaceutical applications-model case: Adsorbent for abacavir

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Abstract

This paper reports the development of selective polymers with high binding capacity suitable for large scale solid-phase extraction (SPE), e.g. for industrial applications. The technology of molecular imprinting was employed in the synthesis of selective molecularly imprinted polymers (MIPs). Abacavir, which is a HIV-1 reverse transcriptase inhibitor, was chosen as the target analyte. An already established computational protocol, developed in our group, was employed to select the best monomers leading to polymers with high binding capacity for the target compound. Three different monomer compositions were chosen for the synthesis. The synthesised materials were then tested for the rebinding of abacavir in solid-phase extraction using several different conditions (buffered/non-buffered solutions and in the presence/absence of organic solvents). The best MIP showed a surprisingly high binding capacity, up to 157 mg of drug/g of adsorbent. The high binding capacity could make this polymer suitable for industrial applications to purify and/or concentrate the drug during its production.

Keywords: Molecularly imprinted polymers; Solid-phase extraction; Abacavir; Binding capacity

1. Introduction

Solid-phase extraction is an analytical tool which is continuously growing in importance as a robust analytical method for sample preparation. SPE in various formats is currently a routine technique employed in numerous pharmaceutical, environmental and bioanalytical applications [1]. Separation for most of the current SPE materials is based on physiochemical retention on the functionalised surface. However typical SPE adsorbents lack selectivity and this constitutes a problem when a selective extraction from a complex matrix has to be performed. Therefore, considerable effort is often expended in the search for more selective SPE adsorbents. An enhancement of the molecular selectivity of SPE can be achieved using molecularly imprinted polymers (MIPs). MIPs are a class of smart materials with a pre-determined selectivity. This is achieved by the copolymerisation of a pre-organised complex, formed between functional monomers and the target molecule (the template), with an excess of cross-linker. The polymer selectivity, specificity and affinity are directly related to the strength of this complex. In this study our aim was the development of a polymer for SPE, which would possess high affinity and high binding capacity for the target analyte. A material with these properties would allow the development of a simple and straightforward SPE method, which could be easily scaled-up for industrial applications.

In order to obtain material with very high affinity, functional monomers able to give very strong complexes with the target analyte need to be chosen. In our group we have developed a computational design method, which enable us to select from a virtual library those monomers interacting strongly with the target analyte [2–5]. In this work abacavir was used as a model target analyte. Abacavir (1592U89) is a drug utilised in the treatment of the infection caused by the human immunodeficiency virus HIV-1, the causative agent of the acquired immunodefi-

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ciency syndrome (AIDS). This drug is a novel nucleoside reverse transcriptase inhibitor with antiretroviral activity against HIV-1 [6]. A few groups [7–10] have already reported the development of analytical methods to measure the concentration of abacavir in complex matrices such as urine and blood samples, using high performance liquid chromatography (HPLC) with UV detection. However all the reported methods based on chromatography techniques are expensive, time consuming and unsuitable for industrial applications. By contrast, the availability of MIPs, which could be used in the isolation, purification and pre-concentration of abacavir by SPE would be very appealing and ideally suited to industrial applications. Many reports of the successful application of MIP based SPE for the extraction and pre-concentration of drugs have already appeared in the literature. Most recently MIPs have been used for the extraction of local anaesthetics [1], naproxen [11], atropine [12], metformin [13] and pyrazinamide [14]. In most of these cases the MIP showed specific binding of the target analytes at very low concentrations even at the ppb level [15]. However, to the best of our knowledge, no report has yet demonstrated the development of MIPs which possessed sufficiently high binding capacity for industrial applications, e.g. for the extraction and purification of drugs from concentrated solutions. Whereas it is relatively easy to develop MIPs with good selectivity, the production of materials with very high binding capacity is not so straightforward. One of the pre-requisites to achieve this is by the rational selection of functional monomers strongly interacting with the target analyte. In this paper our computational method was used to develop MIPs with high affinity, which could lead to polymers with high binding capacity for abacavir. The MIPs were then synthesised and characterised in SPE experiments. As expected some of the polymers tested showed very high binding capacity for the target analyte. The best MIP developed here possessed the requirements necessary for industrial applications aimed at the extraction and purification of abacavir.

2. Material and methods

2.1. Material

Abacavir hemi-sulphate was kindly provided by Glaxo-SmithKline (Manufacturing Operation, Cork, Ireland). The monomers itaconic acid (IA), acrylamide and *N*,*N*-methylene-bisacrylamide (bisacrylamide), the cross-linker ethylene glycol dimethacrylate (EGDMA), the initiator 1,1′-azobis(cyclohexane-carbonitrile), the porogen *N*,*N*-dimethylformamide (DMF) and isopropanol (IPA) were purchased from Aldrich (Poole, Dorset, U.K.). The empty cartridges, filters and vacuum unit used for SPE experiments were from Supelco (Poole, Dorset, U.K.). All solvents were of analytical or HPLC grade and were used as received.

2.2. Computational design of high affinity polymers for abacavir

The computational design performed to optimise the composition of MIPs has been already extensively described elsewhere [2–5]. The workstation used to simulate monomer–template interactions was a Silicon Graphics Octane running IRIX 6.5 operating system. The workstation was configured with two 195 MHz reduced instruction set processors, 1GB memory and a 20GB fixed drive. The system was used to execute the software packages SYBYL 6.9™ (Tripos Inc., 1699 South Hanley Road, St. Louis, Missouri 63144, USA). Regarding the procedure, briefly, the structure of abacavir was drawn and its energy minimised both in vacuum (dielectric constant = 1) and in water (dielectric constant = 80) to get stable conformations in such extremely different environments. A virtual library containing 21 of the most commonly used monomers in molecular imprinting was also constructed and minimised. The LEAPFROG™ algorithm was used to screen the library of functional monomers for their possible interactions with the template. The monomers giving the strongest interactions with the template, minimised both in water and in vacuum, were identified and used for the synthesis of polymers using the general template–functional monomer molar ratio of 1:4. This ratio is a rather commonly used in non-covalent molecular imprinting [16].

2.3. Polymer synthesis

Due to solubility problems in most organic solvents the abacavir hemi-sulphate was converted into the free base by chloroform extraction of an aqueous solution after addition of NaOH. The resulting abacavir free base was then used as a template for the synthesis of MIPs. Three MIPs were synthesised in DMF. Acrylamide and itaconic acid, which were identified as the two best monomers, were used to synthesise two polymers (MIP2 and MIP3, respectively) with template–acrylamide and template–itaconic acid ratios of 1:4. The third MIP (MIP1), containing both acrylamide and bisacrylamide, was synthesised using a molar ratio of template–acrylamide–bisacrylamide of 1:2:2. The composition of the polymerisation mixtures of these three MIPs is reported in Table 1. A corresponding blank polymer, BP3 was also prepared from the same composition using the procedure employed for the MIP3, but in absence of template. For the synthesis of the polymers the oxygen was removed by purging the polymerisation mixtures with nitrogen and the polymerisation carried out by photochemical irradiation for

Table 1
Polymerisation mixtures of synthesised MIPs using abacavir free base as the template

Reagents	Quantity (mg)		
	MIP1	MIP2	MIP3
Abacavir free base	250	250	250
Bisacrylamide	135	–	–
Acrylamide	124	248	–
Itaconic acid	–	–	454
Cross-linker[a]	4490	4500	4300
Initiator[b]	50	50	50
DMF	5000	5000	5000

[a] Ethylene glycol dimethacrylate.
[b] 1,1′-azobis(cyclohexane-carbonitrile).