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Multi-objective optimization and design of experiments as tools to tailor molecularly imprinted polymers specific for glucuronic acid

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ABSTRACT

We present a multi-objective optimization of the binding properties of a molecularly imprinted polymer (MIP) which specifically binds glucuronic acid (GA). A design of experiments approach is used to improve four different parameters that describe the binding properties of the polymer. Eleven different methacrylamide-*co*-ethyleneglycol dimethacrylate polymers imprinted with GA were synthesized according to a full factorial experimental design plan with 3 influencing factors (degree of cross-linking, molar equivalent of monomer to template and initiator concentration). These polymers were characterized by adsorption of the radiolabeled target analyte in methanol:water 9:1. The binding parameters were computed to optimize the polymer composition, taking into account four objective variables: the maximum binding capacity at high (B_{max}) and low (B_2) analyte concentrations, the equilibrium constant K_{50}, and the imprinting factor (IF, binding to MIP/binding to NIP). With the multi-objective optimization method based on a desirability approach the composition of a twelfth "ideal" polymer could be predicted. This predicted polymer with highest "desirability" was synthesized with a composition of 0.65 mol% of initiator and a 1:4:20 ratio of template:functional monomers:cross-linker (T:M:X) (80% of cross-linking), and found to be the overall best MIP. Improvements over the original starting polymer were a 6 times lower K_{50}, which corresponds to higher affinity, 20% higher capacity at low analyte concentration (B_2), 40% higher capacity (B_{max}) and 1.3 times increased imprinting factor (IF). Binding assays were also performed in aqueous solvents. Good binding properties were obtained in pure water with an imprinting factor of 3.2. Thus, this polymer is potentially applicable to biological samples like urine where glucuronides occur.

1. Introduction

In the field of bio- and chemosensing for food, environmental and biomedical analysis there is a continuous need for appropriate recognition elements that specifically detect a target of interest. The requirements for these sensors are dependent on the application, e.g. thermal and chemical stability, reusability, rapidness, high sensitivity, selectivity and specificity [1,2]. In medical diagnostics important targets are sugar derivatives, such as glycosylation sites on cell surfaces like cancer cells [3] or on infectious bacteria [4], as well as glycoconjugate drug metabolites [5].

There is still a lack of suitable biological recognition elements for the sensitive and selective detection of these molecules in technological applications. However, molecularly imprinted polymers (MIPs) are promising synthetic receptor materials in this context. Molecular imprinting of polymers relies on the presence of a molecular template during polymerization that directs the self-assembly of monomers carrying suitable functional groups. In an excess of cross-linking monomers, the functional monomer copolymerize forming a cast-like shell around the template, and subsequent removal of the latter liberates three-dimensional binding sites in the material that are complementary to the template in size, shape and position of the functional groups. Molecularly imprinted polymers are suitable as sensor materials since they are robust, reusable, and often exhibit excellent binding properties, with affinities and selectivities in some cases comparable to antibodies [6]. Therefore, they are sometimes called 'antibody mimics' [7].

The molecular imprinting of glycoconjugates of biomacromolecules, in particular of proteins, is a challenge since their conformation is very sensitive to solvent, temperature, pH and

Abbreviations: AAB, N-acrylamido-benzamidine; AIBN, azobisisobutyronitrile; EDMA, ethylenedimethacrylate; GA, Glucuronic acid; MAM, methacrylamide

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ionic strength [8]. Researchers have therefore suggested to use the so-called 'epitope approach', that is, to imprint a structural epitope of the target molecule of interest, rather than the whole molecule, in order to obtain a synthetic receptor able to recognize both the low molecular weight template and larger molecules that possess the template as terminal part [9]. In the present work, we have chosen to create molecular imprints of a small monosaccharide unit, glucuronic acid, as an epitope of a number of glycoconjugates. Glucuronic acid is part of the glycocalix or intercellular matrix where it mainly can be found as a component of hyaluronan. Recently the group of Sellergren has reported molecularly imprinted polymers tailored with urea functionality against lipophilic derivatives (1,2,3,4-tetra-O-acetyl and 1-O-dodecyl) of glucuronic acid. Glucuronated metabolites could be recognized by the resulting material in a solid-phase extraction (SPE) process in a water containing solvent [10,11].

In our study the template glucuronic acid was used without any derivatization for molecular imprinting. Imprinting underivatized glucuronic acid is a challenge, since both the self-assembly of the template–monomer complex and the recognition of the target by the MIP will have to take place in a polar environment, which is not favorable for the establishment of non-covalent interactions. In addition, we wanted to perform the binding experiments at equilibrium, where the affinity of the sites is even more important compared to chromatographic analysis where multiple association–dissociation phenomena can take place [12].

The development and optimization of a MIP for a particular target can be rather complex as it depends on a large number of variable factors (compositional and operational influencing factors) which are to some extent even dependent on each other. Especially sugar imprinting is not straightforward because of the strong polarity of these molecules and the tendency of their hydroxyl groups to interact with water, if present in the medium, which can interfere with the imprinting process and during recognition of the target by the MIP. Therefore, it is an advantage to perform this optimization with a chemometrics approach like design of experiments (DoE) [13,14], computational methods that model chemical interactions between the target molecule and functional monomers by computer simulations [15] or combinatorial methods for high-throughput synthesis of chemical libraries which can also be coupled with DoE [16,17]. Overviews about these polymer optimization methods are given in [18–20]. DoE is a systematic optimization method for the identification of significant factors that have influence on the process in question, and the modeling of the response with a minimal number of experiments for a determined statistical certainty.

The optimization of molecularly imprinted polymers with experimental design, the approach that we have chosen for the present work, was done for the first time in 2003 by Davies et al. and Navarro-Villoslada et al. [21,22]. To date, only about one dozen publications illustrate the power of DoE for MIP optimization. Different factors and their combinations influencing the analytical properties of the polymers were considered like: the amount of template/monomer(s) [16,22–24], the type of cross-linking monomer [22,25], the influence of the initiator [16,23] and different porogenic solvents [22] as well as the polymerization initiation method (thermal/UV) [16,22]. The types of experimental designs used vary depending on the application: full factorial design [21], central composite design [24,26], fractional factorial design [16] or Doehlert's second order uniform shell design [25]. Since usually many influencing factors are under investigation, a multivariate data analysis is often performed to find cause-effect correlations that are then used to find the optimum polymer composition. To our knowledge, more than one objective parameter was never considered in order to find that composition. This is surprising because to describe the binding properties of a MIP, multiple parameters like affinity, capacity, specificity and selectivity are important.

Here, a multi-objective optimization of the binding properties of a molecularly imprinted polymer on the example of a MIP specific for glucuronic acid is presented for the first time. DoE is used to create the experimental table that corresponds to different MIP compositions representing the design space and to optimize the design variables. The different polymers were characterized by their binding of the radiolabeled target analyte in a methanol:water 9:1 solution in order to compute the objective as well as the response surface functions. The aim of this study, apart from obtaining a MIP for glucuronic acid, is to show that the multi-objective analysis coupled with DoE is a highly promising tool for the optimization of imprinted polymers. The proposed strategy relies on the improvement of 4 dependant variables describing the polymer binding properties: the maximum binding capacity at high (B_{max}) and low (B_2) analyte concentrations, the equilibrium constant K_{50}, and the imprinting factor (IF, binding to MIP/binding to NIP) by varying 3 influencing factors (cross-linking degree, molar equivalents of monomer to template and initiator concentrations). Based on the results obtained for target binding in a methanol:water 9:1 environment, the specific binding of glucuronic acid by the MIP in a purely aqueous environment, which is more relevant with respect to biological samples, was also evaluated.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased either from Sigma-Aldrich (St-Quentin Fallavier, France) or VWR International (Fontenay-sous-Bois, France), unless otherwise stated. Deionised water was obtained from Milli-Q Gradient (Millipore, France). Methanol (AnalaRNormapur) and acetic acid (GPR Rectapur) were obtained from VWR, hydrochloric acid (Analytical grade, Fisher Scientific, Illkirch, France). Ethylenedimethacrylate (EDMA), methacrylamide (MAM) were obtained from Aldrich, D-glucuronic acid (GA) was from Sigma. Azobisisobutyronitrile (AIBN, or VAZO 64) was from DuPont Chemicals (Wilmington, USA). D-[6-^{14}C]glucuronic acid (specific activity: 50–60 mCi/mmol, activity: 0.1 mCi/mL) was from Biotrend Chemikalien GmbH (Germany).

The synthesis of (N-acrylamido)-benzamidine (AAB) was adapted from [27]. AAB was prepared from acryloyl chloride and p-aminobenzamidine. The reaction was conducted in an ice bath by adding 4 ml acryloyl chloride dropwise to 200 mL aqueous solution of sodium acetate (34 g) containing 2 g of p-aminobenzamidine. The addition of acryloyl chloride was completed within 10 min. Afterwards, the reaction was allowed to continue for 1 h. The final product was then precipitated by adjusting the pH to 4 with concentrated nitric acid. After dissolution in hot water, the product was recrystallized by adding dilute nitric acid and then washed with cold water. The compound was dried at 50 °C and the yield was 88.4%.

2.2. Polymerization procedure

MAM-*co*-EDMA-polymers imprinted with GA in the presence of AAB were prepared by polymerization in anhydrous DMSO under dilute conditions. The amounts of precursors were determined by the DoE (Tables 1 and 2). Initially, AAB was dissolved in DMSO in a 4 mL glass vial with an airtight septum under sonication (Elmasonic, Germany) for 30 min, followed by the addition of GA. The solution was left to equilibrate on a rotator (Labinco BV, the Netherlands) for 30 min. Then, the other components (MAM, EDMA and AIBN) were added, the final volume adjusted with anhydrous DMSO, and the mixture was purged