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Selective removal of ATP degradation products from food matrices I: Design and characterization of a dummy molecularly imprinted specific sorbent for hypoxanthine

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ABSTRACT

Specific molecularly imprinted polymers (MIPs) for hypoxanthine (HYP) recognition in aqueous organic media have been developed based upon UV, FTIR and ^{1}H NMR prepolymerization studies in conjunction with batch rebinding UPLC analyzes. The MIPs, which used the template mimics caffeine (CAF) and theophylline (TPH), are prepared in $CHCl_3$ by one step precipitation polymerization from acrylamide (AM), 2-hydroxyethyl-methacrylate (HEMA) and methacrylic acid (MAA) as functional monomers, whereas ethylene glycol dimethacrylate (EGDMA), divinylbenzene (DVB) and trimethylolpropane triacrylate (TMPTA) as cross-linkers. The magnitude of the pre-polymerization binding constants between TPH and AM, MAA and HEMA is consistent with the complex stoichiometry (1:2 and 1:1) and number of interaction points (3-, 2-, 1-hydrogen bonded motif). The strong (1:2) complex between TPH and AM ($K_{11} = 3.36 \times 10^4\ M^{-1}$ and $K_{12} = 1.33 \times 10^2\ M^{-1}$) makes the corresponding MIP the most suitable for HYP recognition. The best performance of the TPH:AM:EGDMA (1:4:20) MIP is reflected in the high IF and high weighted average affinity based on the Freundlich isotherm. Further polymer characterization by ATR–FTIR, elemental analysis, surface area analysis (BET), swelling and SEM yield vital information regarding the degree of polymerization, real monomer:crosslinker ratio, morphology, pore size distribution plus conformational changes on exposure to different solvents.

1. Introduction

Hypoxanthine (HYP) is an essential metabolite to degrade adenine nucleotide, which is an indicator for the quality control of meat or fish products in food industries. Therefore, it is significant to develop a quick and effective analytical method for the determination of HYP. Various methods have been proposed for the evaluation of HYP concentration, such as chromatography, capillary electrophoresis and electrochemistry [1,2]. Owing to the complexity of sample matrices and low levels of the analyte, sample pretreatment and enrichment process become the crucial steps in these analytical procedures. So far, the most widely used sample pre-treatment methods are liquid–liquid extraction, solid-phase extraction (SPE), liquid-phase microextraction, cloud point extraction, ionic liquids extraction and stir bars microextraction, but most of these procedures suffer from several disadvantages such as large amounts of organic solvent, tedious procedure or low enrichment factor [3].

In order to solve these drawbacks molecularly imprinted polymers (MIPs) have been successfully applied as selective phases in solid phase extraction of analytes present in low concentrations or in complex matrices and have lead to enrichments and clean-up of the analytes to levels not achievable with alternative methods. Accordingly, molecularly imprinted solid phase extraction (MISPE) has been widely used in bioanalysis, food, pharmaceutical and environmental analysis in recent years [4–8], MIPs are synthetic polymeric materials with specific and selective recognition sites complementary in shape, size, and functional groups to the template molecule (T), involving an interaction mechanism based on molecular recognition. MIPs can be synthesized either by covalent or by non-covalent procedures. The latter are based on the formation of relatively weak non-covalent interactions between the T and functional monomers (M) before polymerization.

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Normally, the target molecules are used as templates to synthesize MIPs, but when the original T is very expensive or otherwise difficult-to-achieve or purified, involves safety consideration in the manipulation, or when polymerization conditions (thermal or UV irradiation) could result in unwanted compound degradation, a structural analog or dummy template (DT) can be employed for the synthesis. The dummy approach is also selected to avoid the risk of residual T leaking from the polymer and causing erroneous results, particularly in MISPE applied to trace determination of compounds. At last, the strategy of DT can also be an option when the too low solubility of the target analyte does not allow its use for the synthesis of the MIP. Owing to cross-selectivity, the so-called "dummy molecularly imprinted polymer" (DMIP) should give rise to imprints that have the ability to bind the target analyte [5,9,10]. However, DMIPs are often inferior in terms of selectivity for the target analyte, losing recognition and extraction capacities for the compound from the matrices [11,12]. Hence, to achieve both proper affinity and sufficient recovery, the selection of the DT is of great significance. As a pre-condition, the dummy molecule must resemble the target analyte in terms of shape, size and functionalities [5] and should not interfere with its analytical determination.

From another point of view, most studies concern synthesis and new applications of MIPs with less emphasis on understanding the mechanisms and interactions occurring between T and M. Nonetheless, a thorough comprehension of the recognition mechanisms and physical parameters of corresponding polymers are very important to improve extraction. Accordingly, prepolymerization studies on self-assembling systems can be useful for the selection of suitable M and solvents for specific T molecules [13]. UV–Vis, FTIR and ^{1}H NMR spectroscopies are frequently applied to characterize the nature of prepolymerization interactions and the extent of complex formation between M and T in solution [7,14–20]. On the other hand, the porous structure of imprinted materials can be intricate and affects their performance. Brunauer–Emmett–Teller analysis (BET) and scanning electron microscope (SEM) are used to elucidate the morphological characteristics which may provide valuable information for the synthesis and application of the MIPs [13,21,22].

The ultimate goal of our research work is to achieve a MIP for solid extraction and preconcentration of HYP from meat and fish samples which can be used as a simple and rapid method to evaluate freshness. The study has been divided in two articles: part I, the current paper, involving MIP design and characterization and part II describing the application [23]. So far, to the best of our knowledge, there is only one work dealing with HYP based MIP in which the target analyte itself is used as T to prepare a MIP membrane to be applied as electrochemical sensor characterized by its detection speediness [24]. Notwithstanding, the MIP sensor does not tackle the "bleeding" issue which causes serious interferences when applied to quantitative analysis in real samples nor has it been tested in fish samples and its performance in biological matrices is unknown. Besides, our method, which is particularly remarkable for the instrumental simplicity and low cost, allows the selective extraction and purification of inosine in addition to HYP, both ATP derivatives present in fish samples [23].

As a preliminary step, the current study deals with the synthesis and characterization of several non-covalent MIPs, prepared by the precipitation polymerization technique and capable of recognising HYP in aqueous-organic solutions. Caffeine (CAF) and theophylline (TPH) have been selected as DT in order to avoid inherent bleeding and because of solubility problems of closely related compounds such as xanthine or uric acid in commonly used organic solvents. Nonetheless, the two analyte mimics share with HYP a highly delocalized fused ring system containing both a pyrimidine ring and an imidazole ring. The three M, methacrylic acid (MAA), 2-hydroxyethyl-methacrylate (HEMA) and acrylamide (AM) have been chosen because of their ability to form H-bonds and owing to their compatibility with aqueous systems, whereas three C of different functionalities, flexibilities and polarities have been probed namely ethylene glycol dimethacrylate (EGDMA), divinylbenzene (DVB) and trimethylolpropane triacrylate (TMPTA).

The first part of this paper describes the combination of UV, FTIR and ^{1}H NMR spectroscopies to analyze the DT:M interactions in organic solvents. Based on this strategy the porogen, $CHCl_3$, is selected. To further explain the molecular recognition behavior, the synthesized MIPs are thoroughly characterized by ATR-FTIR spectroscopy, elemental analysis, Brunauer–Teller method (BET), swelling experiments and scanning electron microscope (SEM). Prior to rebinding analysis and in order to accurately design batch rebinding experiments, a kinetic assay has been conducted. Finally, the specific binding capability of the MIPs for HYP is evaluated by batch rebinding assays in ACN:water using UPLC in order to determine imprinting factors and adsorption isotherms.

2. Experimental

2.1. Reagents

Theophylline (TPH), trifluoroacetic acid (TFA), 2-hydroxyethyl-methacrylate (HEMA), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), trimethylolpropane triacrylate (TMPTA) and d_1-chloroform (99.96%) ($CDCl_3$) have been purchased from Sigma Aldrich (Steinheim, Germany). Also from Sigma Aldrich, the divinylbenzene (DVB, technical grade 80%) has a monomer content of 80 wt% DVB and 20 wt% ethylvinylbenzene (EVB). The meta- to para- isomer ratio for all monomers is 70 to 30.

Acrylamide (AM), Caffeine (CAF) and 2,2-azobis (2-methylpropionitrile) (AIBN) have been obtained from Fluka (Buchs, Switzerland). Methanol (MeOH), acetonitrile (ACN), chloroform (CH_3Cl), dichloromethane (CH_2Cl_2) and d_4-acetic acid (99.5%) (AA) are from Merck (Darmstadt, Germany). Acetic acid (HAc) has been supplied from Scharlab (Barcelona, Spain) and hypoxanthine (HYP) was obtained from Acros Organics (Geel, Belgium). Water used in the experiments has been purified using a Milli Q Ultrapure water-purification system (Millipore, Bedfore, MA, USA).

2.2. Instruments

2.2.1. UV–Vis spectroscopy

UV–Vis absorption spectra have been recorded on a Cary 100 Conc double-beam UV–Vis spectrophotometer (Varian, USA) at 0.2 cm^{-1} resolution between 200 and 400 nm.

2.2.2. FTIR spectroscopy

The IR data have been recorded on a Bruker Vector 22 spectrometer. The transmission/absorption measurements of the prepolymerization mixtures have been performed in a thin-film liquid cell at room temperature with a 1 mm Teflon spacer and CaF_2 windows (Spepac) in the spectral range of 4000–900 cm^{-1}. Each spectrum is obtained through the averaging of 64 repetitive scans at a resolution of 4 cm^{-1}.

The FTIR analysis of the pre-polymerization mixture in porogen and solid samples have been performed in the attenuated reflection mode (ATR) by using the already described spectrometer equipped with a thermostated MK II Golden GateTM Diamond 45° ATR accessory. The non-polymerized sample solution, the analyte and monomers solved in porogen are flushed into the stainless steel micro reaction flow cell anvil (10,568) clamped on the ATR crystal. For the polymerized samples, the disks are compressed onto the ATR crystal with the Sapphire Anvil (10,531). Owing to