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Synthesis and application of a molecularly imprinted polymer for the voltammetric determination of famciclovir

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ABSTRACT

A molecularly imprinted polymer (MIP) was synthesized and applied as additive within a carbon paste electrode for the cyclic voltammetric determination of famciclovir (FCV). Complementary computational studies were performed to study the intermolecular interactions in the pre-polymerization mixture. Derived from the computational studies, four MIP ratios were synthesized and their performance was evaluated using equilibrium rebinding assays. The MIP with the highest binding capacity was selected. A linear response was obtained in the range of 2.5×10^{-6}–1.0×10^{-3} M with a limit of detection at 7.5×10^{-7} M. Finally, the developed MIP–voltammetry system was successfully applied for the determination of FCV in pure solutions and pharmaceutical preparations.

1. Introduction

Famciclovir (FCV) is a guanine analog antiviral prodrug that is converted by 1st pass metabolism into the active drug penciclovir. Chemically, FCV constitutes 2-[(acetyloxy) methyl]-4-(2-amino-9H-purin-9-yl) butyl acetate. It is successfully used for the treatment of herpes simplex virus (HSV-1), (HSV-2) and varicella zoster virus. More discussion regarding the mechanism of action of FCV has been given in the Supplementary information.

Several methods have been reported for the determination of FCV, such as reversed-phase liquid chromatography (RP-LC) (Mondal and Neeraja, 2013), LC–MS (Brock et al., 2012), liquid chromatography with mass spectrometric detection (LC–MS/MS) (Basu et al., 2011), capillary electrophoresis with electro-chemiluminescence (CE-ELC) (Ye and Chen, 2010), spectrophotometric determination (Reddy and Srikar, 2009), as well as potentiometric (Rezk and El Nashar, 2013) and voltammetric analysis (Chunzhe et al., 2006; Rajeev and Sanjay, 2012). To the best of our knowledge, there is no report published combining electrochemical studies of FCV with molecular imprinted polymers (MIP) as a selective modifier of carbon paste electrodes.

MIPs are artificial receptors providing highly specific complementary binding sites for a certain template in a cross-linked polymer network. Modern molecular imprinting technology was introduced by Wulff and Sarhan (1972) and the team of Mosbach (Arshady and Mosbach, 1981). The synthesis strategy involves the co-polymerization of a monomer and a cross-linker in the presence of a template molecule. After polymerization the template is extracted from the polymer matrix, thus ideally leaving selective recognition sites (i.e., 'imprints') for the template behind (Wang et al., 2014). Compared to natural bioreceptors MIPs offer a number of advantages, in particularly long-term storage stability, potential re-usability, resistance to microbial damage, facile integration into transducers (Karimian et al., 2014), low cost, ease of preparation, and finally, comparable affinity and recognition ability for the target substrate (Wang et al., 2014; Xie et al., 2009). MIPs have a wide range of applications including drug delivery systems (Ruela et al., 2014), stationary phases in HPLC analysis (T. Chen et al., 2014; J. Chen et al., 2014), solid phase extraction (Shaikh et al., 2014), capillary electrochromatography (CEC) (Liu et al., 2013) and sensors (Rizk et al., 2014).

Complementarily, electrochemical sensors offer small dimensions, low cost transducers, low detection limits and easy automation. Electrochemical sensors and biosensors for agricultural,

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environmental, food and pharmaceutical analyses have been increasing rapidly due to electrochemical behavior of drugs and biomolecules and partly due to advances in electrochemical measuring systems (Tajik et al., 2013, 2014).

The combination of MIPs with electrochemical analysis schemes is rather recent, and was predominantly used to combine the intrinsic properties of MIPs with selected electrochemical reactions, in order to improve the response of the electrode (Arvand and Fallahi, 2013; Gholivand and Torkashvand, 2011).

In this study, MIPs were synthesized for FCV with the aid of computer-based studies to optimize the synthesis route using methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linker and methylene chloride (DCM) as porogen. Batch adsorption experiments were performed to evaluate the MIP properties. Thereafter, the MIP with the highest binding capacity was incorporated as a modifier into carbon paste electrodes for enhancing the voltammetric determination of FCV. The use of carbon paste combined with MIPs offered several advantages such as ease of preparation, lower limits of detection, a wide usable concentration range and high selectivity and stability.

2. Materials and methods

2.1. Reagents and materials

All chemicals were of analytical grade and used without further purification, FCV reference standard was purchased from H and Y international group (China). The pharmaceutical preparations Famvir® (250 mg/tablet) (Novartis pharma) and Propencivir® (125 mg/tablet) (Bioriginal international group pharma) were purchased from the local market. MAA, EGDMA, 4-Vinyl pyridine (4-VP), 2,2′-azobisiso-butyronitrile (AIBN), graphite powder ($<20\ \mu m$) and paraffin oil were purchased from Sigma-Aldrich, Germany. The porogen DCM was of HPLC grade and purchased from Sigma, ultra pure water purified in purelab UHQ (ELGA) was used throughout this work. 0.04 M Britton–Robinson (BR) buffer, 0.04 M acetate buffer were used. 0.01 M FCV stock solutions were prepared in buffer solutions or ultrapure water and lower concentrations were prepared by appropriate dilutions.

2.2. Computational optimization and energy calculations

The Gaussian03 software package was used to study the binding interaction between the functional monomers (MAA/4-VP) and template molecules. The structures of monomer, template and template–monomer complexes were established using the gaussview software. All structures were optimized using Hartree–Fock theory with 6–31G(d) basis set.

The binding energy of template–monomer complexes, ΔE, were calculated via $\Delta E = E(\text{template–monomer complex}) - E(\text{template}) - nE(\text{monomer})$ following Roy et al. (2014). Consequently, an increasing value of the interaction energy between two molecular moieties indicates and increased stability of the complex formed between these moieties.

The polarizable continuum model (PCM) was applied to calculate the energy of complex, where the effect of solvent should be considered during energy calculations as it leads to changes in stability and energy of the template–monomer complexes in solvent phase compared to gaseous phase (Roy et al., 2014). In this model, the solvent is modeled as a polarizable continuum instead of individual molecules. The results obtained from computational study of template–monomer interaction provided the basis for selecting appropriate monomers and the choice of monomer–template ratio for synthesis of the MIPs.

Table 1
The chemical composition of the prepared MIPs.

	Template (mmol)	Functional monomer[a] (mmol)	Cross linker (mmol)	Porogen (ml)	Initiator (mmol)
MIP1	1	4	20	4	0.3
MIP2	1	4	40	4	0.3
MIP3	1	8	40	4	0.3
MIP4	1	4	40	4	0.3

[a] MAA was used as a functional monomer except in MIP4 where 4-VP was used.

2.3. Apparatus

Voltammetric measurements were performed in a three electrode cell using a CHI 802 B electrochemical analyzer (CH instruments, Inc., USA). CHI 150 saturated calomel electrode was used as a reference electrode, platinum wire 1.0 mm diameter was used as a counter electrode and a modified carbon paste was used as working electrode. UV-spectroscopic measurements were performed using Jasco V-530 UV/vis spectrophotometer (Japan). Incubation of MIPs with FCV solutions was done in an Eppendorf Thermomixer® (Germany).

2.4. Preparation of the molecularly imprinted polymer

The MIPs were prepared by bulk polymerization using the self-assembly approach (Arshady and Mosbach, 1981). The chemical composition of the synthesized MIPs is summarized in Table 1. More details regarding the synthesis of the polymers has been given in the Supplementary information.

2.5. Equilibrium binding assays

Equilibrium rebinding experiments were carried out for the MIPs and their corresponding NIPs by adding 2 mL of 1.0×10^{-4} M solution of FCV prepared in pure H_2O over 20 mg of MIP or NIP in a 2 mL Eppendorf tube. The tubes were located in an Eppendorf thermomixer and left to shake for 24 h at room temperature followed by centrifugation at 14,000 rpm for 20 min. The resulting supernatant was filtered using a 0.22 μm Whatman syringe filter and the amount of FCV in the clear supernatant was determined by UV/vis. spectrophotometry at 304 nm. The same experiment was conducted using 1.0×10^{-4} M FCV prepared in 0.04 M acetate buffer, pH 5. To determine the binding isotherms and to perform Scatchard analysis for MIP1, 20 mg of MIP1 and NIP1 were incubated with 2 mL solutions of FCV prepared in pure H_2O with concentrations ranging from 1.0×10^{-5} to 1.0×10^{-2} M at the same conditions as stated before; all experiments were done in duplicates.

The amount of FCV bound to the polymers was determined by $Q=[(C_i-C_f)\times V_s\times 1000]/M$, where Q is the binding capacity of the polymer in (μM/g), C_i is the initial FCV concentration in (μM/mL), C_f is the final free FCV concentration at equilibrium in (μM/mL), V_s is the volume of sample solution tested (mL) and M is the mass of polymer used in (mg) (Moreira et al., 2011); the imprinting factor (IF) was calculated as Q_{MIP}/Q_{NIP}.

The Scatchard plot was constructed following $Q/[F] = -(Q/K_d) + (Q_{max}/K_d)$, where Q is the binding capacity of the polymer in (μM/g), $[F]$ is the final free FCV concentration at equilibrium in (mM), K_d is the dissociation constant and Q_{max} is the maximum apparent binding amount, the values of K_d and Q_{max} can be calculated from the slope and intercept of the linear line plotted in $Q/[F]$ vs. Q (Wang et al., 2011).