

Electrosynthesis and characterisation of poly(folic acid) films



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ABSTRACT

Electropolymerisation of folic acid (FA) was performed in different media in order to optimise the best conditions for the formation of a stable poly(folic acid) (PFA) film. Different substrates were also used for PFA deposition. The electrochemical stability of the PFA film was insufficient; therefore, graphene-chitosan (G-Chit) composite was deposited on the top of PFA film in order to prevent it from washing out of the electrode surface. A possible electropolymerisation mechanism was proposed.

The obtained films were characterised microscopically and electrochemically applying atomic force microscopy (AFM) and electrochemical methods such as cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) in different media. The best film was obtained when PFA was electrosynthesised from 0.1 mol L⁻¹ KCl/HCl solution, pH 2.0, covered with G-Chit composite film. The best electrochemical behaviour of such electrode was in the same buffer solution. This electrode will be further applied to (bio)sensor construction.

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1. Introduction

Folic acid (FA) or vitamin B9, an IUPAC name–(2S)-2-[[4-[(2-amino-4-oxo-1H-pteridin-6-yl)methylamino]benzoyl]amino]pentanedioic acid, is a B group vitamin essential for DNA synthesis and the metabolism of homocysteine. FA deficiency is associated with anaemia, unfavourable pregnancy outcomes, cardiovascular disease, and possibly neoplasms of the colon [1]. For these reasons, FA is intensively determined in food [2–4], biofluids [1,5], etc. Moreover, conjugated foliates are used as the targeting drug delivery carriers and disease therapies [6–9] as well as for the diagnosis of some diseases [1,10,11].

Fig. 1 shows the chemical structure of folic acid. As seen, this compound is electroactive as most of the B group vitamins; therefore, this peculiarity allows employing it in electrochemistry for different purposes as well as detecting it electrochemically. Electropolymerisation of folic acid was performed in order to create a sensitive and a selective dopamine sensor [12]. However, this work paid little attention to the electrochemical behaviour of poly(folic acid) (PFA) itself as well as the polymerisation mechanism was discussed briefly.

In order to attract FA to the electrode surface, a polymer or another compound is often deposited on the surface. Chitosan (Chit) is one of such polymers which attracts folic acid due to an interaction of amino groups from chitosan and carboxy-groups from FA; stabilise it and fixes to the surface [13]. Chitosan or β -(1-4)-linked D-glucosamine is a biologically friendly polymer obtained from chitin and it is applied in many fields due to its properties allowing fabricate membranes, thin films, three-dimensional structures, graphene and carbon nanotube immobilisation [14–17]. In some cases, FA by itself can be that particular compound attracting other compounds, for example dopamine [12].

Electrodes are also often modified with materials enhancing an electrochemical signal, for example, electrode for FA determination was modified with ordered mesoporous carbon [18] carbon nanotubes [19–21], metal nanoparticles [20], DNA [22], polypyrrole [23] and imprinted polymers [24,25]. Lately, graphene was also used for such purposes because it showed electrocatalytic activity towards various compounds [26] especially in electrochemical biosensing [27–31].

In this work the electropolymerisation of folic acid is presented. FA was polymerised from different media on glassy carbon electrodes and on HOPG for morphology studies. The electrochemical behaviour of PFA was studied electrochemically employing voltammetric techniques and electrochemical impedance spectroscopy. Finally, the mechanism of PFA formation was proposed. The polymerisation conditions were optimised according to the stability of the PFA film.

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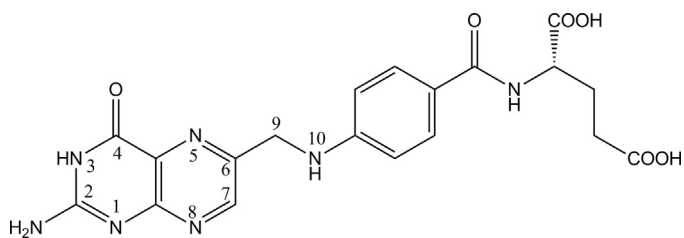


Fig. 1. Chemical structure of folic acid.

2. Experimental

2.1. Chemicals

Folic acid, KCl, chitosan from shrimp shells, H_2SO_4 , HNO_3 , and CH_3COOH were obtained from Sigma Aldrich (Germany). $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, Na_2HPO_4 , NaOH and HCl were from ROTH GmbH (Germany). Graphene flakes with the thickness of 8 nm were purchased from Graphene Supermarket (USA). All reagents used were of analytical grade and used as received, except graphene, which was chemically functionalised as described below. All solutions were prepared with nanopure MilliQ-water (resistivity of 18.2 M Ω cm) directly taken from Synergy 185 unit equipped with a UV lamp (Millipore, USA).

Aqueous chitosan solution of 1% was prepared as described elsewhere [32]. Chitosan was dissolved in aqueous 1% CH_3COOH solution with magnetic stirring for approximately 1 h, pH was adjusted to pH 5.0 with 20% NaOH solution. The solution was filtered using glass fibre filter (70 g m $^{-2}$) (ROTH GmbH, Germany).

Supporting electrolytes used for electrochemical experiments were 0.1 mol L $^{-1}$ KCl/HCl, pH from 1.0 to 3.0, and 0.1 mol L $^{-1}$ sodium phosphate buffer saline (PBS) with 0.15 mol L $^{-1}$ NaCl solutions, pH from 4.0 to 8.0.

2.2. Apparatus

Atomic force microscopy (AFM) images were recorded with the atomic force microscope (CP-II, Veeco Instruments Inc., USA). The imaging of the surface was performed in the contact mode in air under normal laboratory conditions (20 °C, relative humidity 30–60%) using Bruker MLCT probes. The highly oriented pyrolytic graphite (HOPG) 10 × 10 mm (NT-MTD, Ireland) surface was renewed by cleaving the surface with the adhesive Scotch tape prior to AFM investigation.

All electrochemical measurements were carried out with the potentiostat/galvanostat CompactStat (Ivium Technologies, The Netherlands) assembled with the three-electrode system. The working electrode was either bare or modified glassy carbon electrode (diameter of 3.0 mm) or HOPG, a Pt wire served as the counter electrode and an Ag/AgCl (sat. KCl) electrode was as reference. All potentials in this paper are given versus this reference. Electrochemical characterisation was carried out using cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS). The SWV parameters were as follows: the start potential -1.0 V, the end potential 1.0 V, the potential step 5 mV, the frequency 10 Hz and the amplitude 10 mV. EIS investigation was performed at a constant applied potential, chosen at the peak position (see Results and Discussions Section), in a frequency range from 100 kHz to 0.1 Hz, with the potential perturbation of 10 mV.

2.3. Electrosynthesis of poly(folic acid)

Prior to modification, the surface of the glassy carbon electrode was carefully polished to a mirror-like plane with 1.0, 0.3 and 0.05

micron aluminium oxide slurry successively and rinsed with the MiliQ water followed by the sonication in water and ethanol for 1 min after each polishing step. After the mechanical polishing, the electrode was polished electrochemically in 0.1 mol L $^{-1}$ KCl by scanning the applied potential repeatedly between -1.0 V and +1.0 V vs. Ag/AgCl at a scan rate of 100 mV s $^{-1}$ for at least 10 cycles until constant voltammograms were obtained. Then the electrode was successively rinsed with the MiliQ water and transferred to the electropolymerisation solution containing buffer solution, either 0.1 mol L $^{-1}$ PBS, pH 5.0, or 0.1 mol L $^{-1}$ KCl/HCl, pH 2.0, or 1.0 mol L $^{-1}$ HCl, with 0.1 mmol L $^{-1}$ folic acid. PFA was obtained by the repeated scanning of the applied potential between -1.0 and +1.5 V at the potential scan rate of 50 mV s $^{-1}$ for at least 10 cycles. The scan number was optimised according to film stability during the electrochemical characterisation.

When PFA was electrosynthesised on HOPG, the electrode surface was cleaved with the adhesive tape and immediately a droplet of 150 μL of the same solutions containing FA was dropped on the surface. Pt-wire counter electrode and micro Ag/AgCl reference electrode were inserted into the droplet and PFA was synthesised applying the same procedure as in the case of GCE.

PFA was also covered with graphene-chitosan (G-Chit) composite film. Graphene was cast on the electrode surface as described elsewhere [32]. Chemically functionalised graphene was obtained as indicated previously [32]. 50 mg of graphene was sonicated for 20 h in a mixture of 5 mol L $^{-1}$ H_2SO_4 and 5 mol L $^{-1}$ HNO_3 , volume ratio 3:1, at +40 °C; then the mixture was filtrated and washed with the MiliQ water until pH of the washing water was neutral. After that, such functionalised graphene was dried in an oven at 80 °C for 24 h. Finally, this freshly functionalised graphene was added to the 1% acetate solution (pH 5) and sonicated for 2 h, until a homogeneous dispersion was reached. Next, chitosan solution was added in order to get 1 $\mu\text{g mL}^{-1}$ graphene dispersion in aqueous 0.5% chitosan solution. The dispersion was shaken with the Vortex-Genie® 2 (Germany) and then sonicated for 5 min to reach the final homogeneous dispersion. In order to prepare the G-Chit/PFA/GCE, the aliquot of 3 μL graphene dispersion in aqueous 0.5% chitosan solution was dropped onto PFA/GCE surface immediately after sonication and left to dry in air.

PFA was also synthesised on the graphene-chitosan (G-Chit) modified GCE. Graphene was cast on the electrode surface as described above, the only difference—the suspension was dropped onto the pre-treated GCE surface immediately after sonication and left to dry in air. PFA was synthesised in the same way as indicated above.

3. Results and discussion

3.1. Electrosynthesis of poly(folic acid)

First PFA was synthesised by the electropolymerisation of 0.1 mmol L $^{-1}$ folic acid from 0.1 mol L $^{-1}$ PBS solution, pH 5.0, or 0.1 mol L $^{-1}$ KCl/HCl, pH 2.0, either on the GCE or on the HOPG surface by the repeated potential cycling. HOPG was introduced because it was more suitable for microscopic investigations. These solutions were chosen due to the fact that polymerisation of similar heterocyclic compounds such as phenazines usually is catalysed and polymers are stabilised by small anions such as Cl^- , NO_3^- , SO_4^{2-} , etc. [33,34]. The pH values were chosen according to dissociation constants pK' for N(1) and N(10), which were 2.35 and 0.20, respectively at the ionic strength of 1.0 mol L $^{-1}$ [35]. However, at the ionic strength of 0.3 mol L $^{-1}$, $\text{pK}_{\text{a}1}$ (carboxylic group) was 3.23; $\text{pK}_{\text{a}2}$ (N5) was 5.13, and $\text{pK}_{\text{a}3}$ (N3) was 8.39 [36]. The best counterion for folic acid was found to be Cl^- ion. The potential window and the scan rate were optimised for each deposition solution. Since the most stable

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