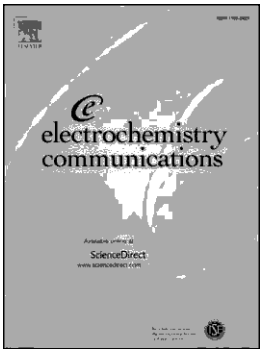

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On/off-switchable electrochemical folic acid sensor based on molecularly imprinted polymer electrode

Najmeh Karimian[a,b], Mohammad Hossein Arbab Zavar[b], Mahmoud Chamsaz[b], Anthony P.F. Turner[a] and Ashutosh Tiwari[a*]

[a]*Biosensors and Bioelectronics Centre, Department of Physics, Chemistry and Biology (IFM), Linköping University, S-58183 Linköping, Sweden*

[b]*Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran*

[*]Corresponding author.
E-mail: ashutosh.tiwari@liu.se; Tel: (+46) 1328 2395; Fax: (+46) 1313 7568

Abstract

The combination of smart polymers with molecular imprinting offers a powerful tool to design more effective sensors and medical devices. In this study, a temperature sensitive amine-terminated poly(*N*-isopropylacrylamide) block with (*N*,*N'*-methylenebisacrylamide) cross-linker along with *o*-phenylenediamine was electropolymerised on a gold electrode in the presence of folic acid (FA) as template to produce an on/off-switchable molecularly imprinted polymer (MIP) affinity sensor for folic acid. Differential pulse voltammetry and cyclic voltammetry were used to characterise the FA-imprinted layer. Incubation of the MIP-modified electrode with FA resulted in a suppression of the ferro/ferricyanide redox process. The highest sensitivity of this temperature gated on/off-switchable folic acid sensor was achieved at 22 ºC. Such switchable affinity materials offer considerable potential for the design of highly selective and controllable biosensors and immunoassays.

Keywords: Molecularly imprinted polymer, Smart polymers, Temperature switchable electrode, Folic acid electrochemical sensor.

1. Introduction

Over the past decade, much attention has been focused on the development of controlled switchable surfaces, also known as "smart surfaces", which switch their physicochemical properties in response to external stimuli [1, 2]. Switching of a surface based on temperature can be realised using thermo-sensitive polymers, which undergo a phase transition at the lower critical solution temperature (LCST), where their behavior switches between hydrophobic and hydrophilic [3]. LCST modulation can be achieved by copolymerisation with other monomers in order to produce a LCST close to physiological temperature. Thus, it could be useful in controllable, temperature-responsive bio-switches for biomedical and biotechnology applications [4-6].

Furthermore, the field of molecularly imprinted polymers (MIPs) has grown exponentially, with numerous papers describing their use as biomimetic receptors in sensors and other devices [7-9]. Combining the properties of a thermo-sensitive polymer with molecular imprinting techniques furnishes a promising strategy for ensuring the system responds more rapidly to an external temperature change. Folic acid (FA) is a well-known biomarker for cancers [3]. It specifically binds with a folate receptor that is significantly overexpressed on the surface of human cancer cells. Due to this region, detection of FA chosen as a model for this study. MIP technology offers one alternative [10,11], but since FA is sensitive to temperature, UV radiation, and other extreme conditions [12], imprinting of FA using bulk polymerisation is problematic and electropolymerisation is more promising. To the best of our knowledge, no study has been published so far reporting the

fabrication FA-imprinted by electropolymerisation using of thermo-sensitive materials. Thus, here we report the preparation of temperature switchable plastic folic acid sensor using co-polymerisation of poly(*N*-isopropylacrylamide) (PNIPAAm) with a cross-linker (*N*,*N'*-methylenebisacrylamide) (MBA) and additional monomer (*o*-phenylenediamine (*o*-PD)), in the presence of folic acid as template (Fig. 1). The analytical performance of the sensor was evaluated by electrochemical methods.

2. Experimental

2.1. Materials and apparatus

o-Phenylenediamine (*o*-PD, ≥98%), poly(*N*-isopropylacrylamide), amine terminated (PNIPAAm, average M_n = 2500), folic acid (≥97%) , *N*,*N'*-Methylenebisacrylamide (MBA ≥98%) and potassium chloride were purchased from Sigma-Aldrich and used as received. Potassium ferrocyanide and potassium ferricyanide were obtained from Merck. All other reagents were of analytical grade and solutions were prepared using Milli-Q water (18.2 MΩ/cm^2). Electrochemical measurements were performed using an Iviumstat potentiostat (Ivium, The Netherlands) controlled by software supplied by the manufacturer. A standard three-electrode configuration was used. A gold disk (2.0 mm diameter), a platinum wire and an Ag|AgCl|KCl (3 M) electrode were used as working electrodes, counter and reference electrodes respectively.

2.2. Preparation of imprinted electrode

In this study, five molecularly imprinted polymer and corresponding non-imprinted polymer were prepared by electrosynthesis as shown in the Table 1. Prior to electropolymerisation, the surface of the gold electrode was polished with 1.0 and 0.05 μm wet alumina slurry followed by 1 minute cleaning in distilled water. Then the electrode was subjected to cyclic potential sweeps between 0.2 and 1.5 V in 0.5 M H_2SO_4 until a stable cyclic voltammogram was obtained. For the preparation of the polymers, the components listed in Table 1 were dissolved in acetate buffer (0.5 M, pH 5.8). After deoxygenating the reaction solution by bubbling nitrogen gas for about 15 min, the electropolymerisation was performed on the surface of the gold electrode, by cyclic voltammetry (20 cycles) in the potential range of 0–1.1 V (versus Ag/AgCl) with a scan rate of 50 mV/s. Then, the polymer film was rinsed in methanol-acetic acid (9:1, v/v) solution for 20 min at 50°C, followed by subsequent washing with methanol to remove the template entrapped in the polymeric matrix. A control electrode (non-imprinted polymer electrode, NIP electrode) was prepared in every case following the same procedure, but in the absence of template molecule.

2.4. FA sensing and temperature switching study

The interaction between FA and MIP film was evaluated by incubating the FA-MIP electrode in the solution containing appropriate concentrations of FA (phosphate buffer at pH 7.4), for 10 min with stirring. Electrochemical measurements to characterise the MIP film were carried out in the presence of 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution

containing 0.1 M KCl. Cyclic voltammograms (CVs) of the imprinted membranes were recorded in the potential range 0.0 to 0.6 V vs. Ag/AgCl, with a scan rate of 50 mV/s. Differential pulse voltammetry (DPV) runs for each concentration of test analyte were quantified over a potential range of 0.0 to 0.4 V at a scan rate of 50 mV/s and pulse amplitude of 25 mV. In order to verify the ability of the MIP to recognise the template molecule, the binding process was examined at different temperatures of 22 and 34 °C.

3. Results and discussion

3.1. Synthesis and characterisation of the MIP

Putative FA-imprinted polymers, consisting of different functional monomers; *o*-PD, PNIPAAm and also MBA cross-linker in the presence of folic acid as template molecule using composition listed in Table 1 were electrosynthesised. As *o*-phenylenediamine and PNIPAAm consist of $–NH_2$ groups which can be generated radical ions. The terminal double bonds of MBA might be cross linked to respected amine terminals during imprinting. Fig. 2a shows a typical cyclic voltammogram recorded during the electropolymerisation of *o*-PD in the presence of PNIPAAm, MBA and folic acid (MIP4) on a gold electrode in acetate buffer (pH 5.8) at room temperature (22°C). A significant decrease in the anodic peak corresponded to irreversible monomer oxidation on the electrode surface during continuous cycling [13]. This illustrates the formation of non-conductive film on electrode surface, which suppresses the anodic oxidation of monomer and is confirmed by the disappearance of the redox peaks of ferro/ferricyanide probe (Fig. 2b). For characterisation of the

polymeric films before and after template removal, differential pulse voltammetry (DPV) and cyclic voltammetry (CV) with using 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1 w/w) solution containing 0.1 M KCl were performed.

3.2. Binding study

To adequately rebind FA, DPV were recorded to monitor the ferro/ferricyanide probe response as affected with and without FA binding on the MIP-modified electrodes (Fig. 2c). After template removal and background response measurements, MIP-modified electrodes were dipped into solution containing of FA in different concentrations for 10 min. Fig. 2d shows the decrease of peak currents and increase of peak-to-peak separation of the ferro/ferricyanide couple for an increase of FA concentration in binding solution. Due to the increasing number of binding sites in the film occupied by FA molecules, the peak current decreased with the increase in FA concentration. Fig. 3a illustrates the different binding with MIP5 compared to the other MIPs (MIP2, MIP3 and MIP4).

The binding isotherm of the MIP-modified electrode was fitted using a model for two types of simultaneous binding (Fig. 3b): on the sites of specific recognition inside polymer film and on the surface of electrode due to non-specific adsorption:

$$\frac{i - i_0}{i_0} = \frac{B_{MAX}\, c}{K_D + c} + N_S c$$

where c is bulk concentration of the target, B_{MAX} – maximum number of binding sites in the MIP, K_D – equilibrium dissociation constant and N_S – binding constant for nonspecific adsorption. The value K_D obtained with fitting is 7.02×10^{-7} M (R^2 0.992). In comparison to

the results obtained when raising the temperature above the LCST (MIP5), the binding capacity (B_{MAX}) of recognition sites was decreased and an increasing in value of non-specific adsorption (N_S) was observed. This behavior could be explained due to a greater dominance of hydrophilic forces within a more hydrated polymer matrix when it had expanded dimensions at below the transition temperature. This process can be applied in a pulsatile manner making the polymer behave as an on-off system when the stimulus is applied or removed.

3.3. On/off- switching

In order to see how temperature changes can affect the binding of folic acid to the different MIP-modified electrodes, the process of polymerisation and binding was performed under different condition. Initially, the influence of high temperature on the structure and also the recognition properties of MIP1 was studied. For this purpose, after template removal it was raised to a high temperature (60 °C) and then the binding experiment was performed. In each step, DPV and CV results were recorded to monitor the ferro/ferricyanide probe response. The results showed, high temperature had no effect on the extent of folic acid uptake by MIP1-modified electrode. Subsequently, the experiment was performed in the presence of different monomers with and without cross-linker under different conditions (MIP2, MIP3, MIP4 and MIP5), The folic acid exhibited considerable binding affinity at 22°C to the MIP- modified electrode produced using the mixture of functional monomers (*o*-PD and PNIPAAm) together with cross-linker (MBA) compared to the other MIPs. *o*-PD and PNIPAAm, employed as functional monomers for imprinting in

this study, contain both amide and amine functional groups, which can interact with the amine and carboxylic acid groups of folic acid via non-covalent and hydrogen binding. In order to verify the effect of temperature on the binding characteristics, MIP5 was synthesised. The concentration dependence of the anodic peak current decrease is shown in Fig. 3c. According to the calibration plot, the MIP–modified electrode (MIP4) displayed a linear response over the concentration range 1.0 to 200 µM, with a detection limit of 0.9 µM. The switching between the two different temperatures is shown in the Fig. 3d. The reason for this could be temperature changes across the LCST of PNIPAAm ranging from 22 to 34°C. Below the LCST the hydrogel is swollen, hydrated and hydrophilic, whereas above the LCST, the hydrogel shrinks and forms a collapsed, dehydrated and hydrophobic state due to the breakdown of the sensitive hydrophilic/hydrophobic balance in the network structure [14, 15]. Hence there may be lower interaction between the print polymer and the template at 34°C.

4. Conclusions

The design and synthesis of smart polymers, their temperature behavior, LCST and also their application was evaluated in this study. The electrosynthesis of molecularly imprinted polymers using thermo-sensitive material such as PNIPAAm and MBA together with *o*-PD in the presence of folic acid as a template was achieved. We demonstrated a novel combination of thermo-responsive polymer with molecular imprinting to generate a molecular recognition material, which could be modulated using an external temperature

change. Such switchable surface performance are suitable candidates for a wide range of applications such as smart sensors, controllable drug release and thermally responsive filters.

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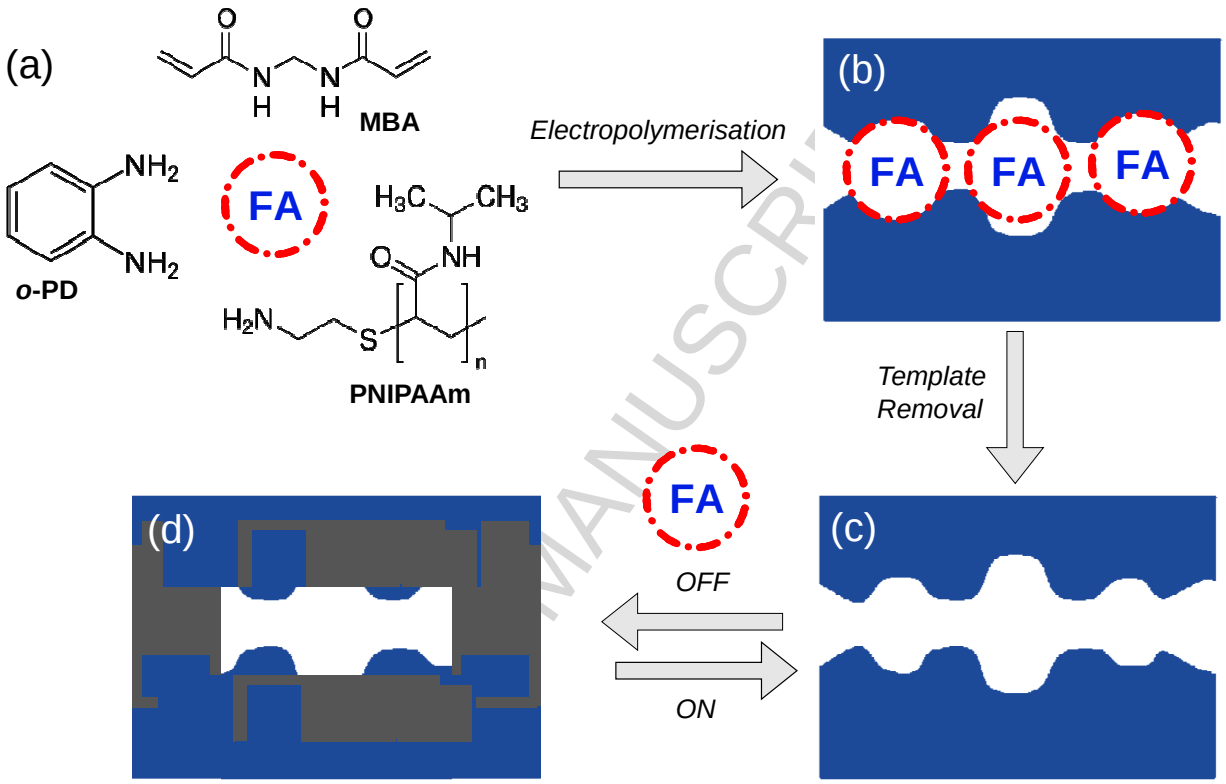

Figure 1. Schematic representation of molecular imprinting and the on/off- recognition of folic acid (a) The folic acid (FA, template), amine terminated poly(*N*-isopropylacrylamide) (PNIPAAm), *o*-phenylenediamine (*o*-PD) and (*N*,*N'*-methylenebisacrylamide) (MBA, cross-linker) interact to form a complex during electropolymerisation, (b) removal of the FA template leaves rebinding cavities, (c) template rebinding, (d) On/Off-switchable behavior of MIP electrode.

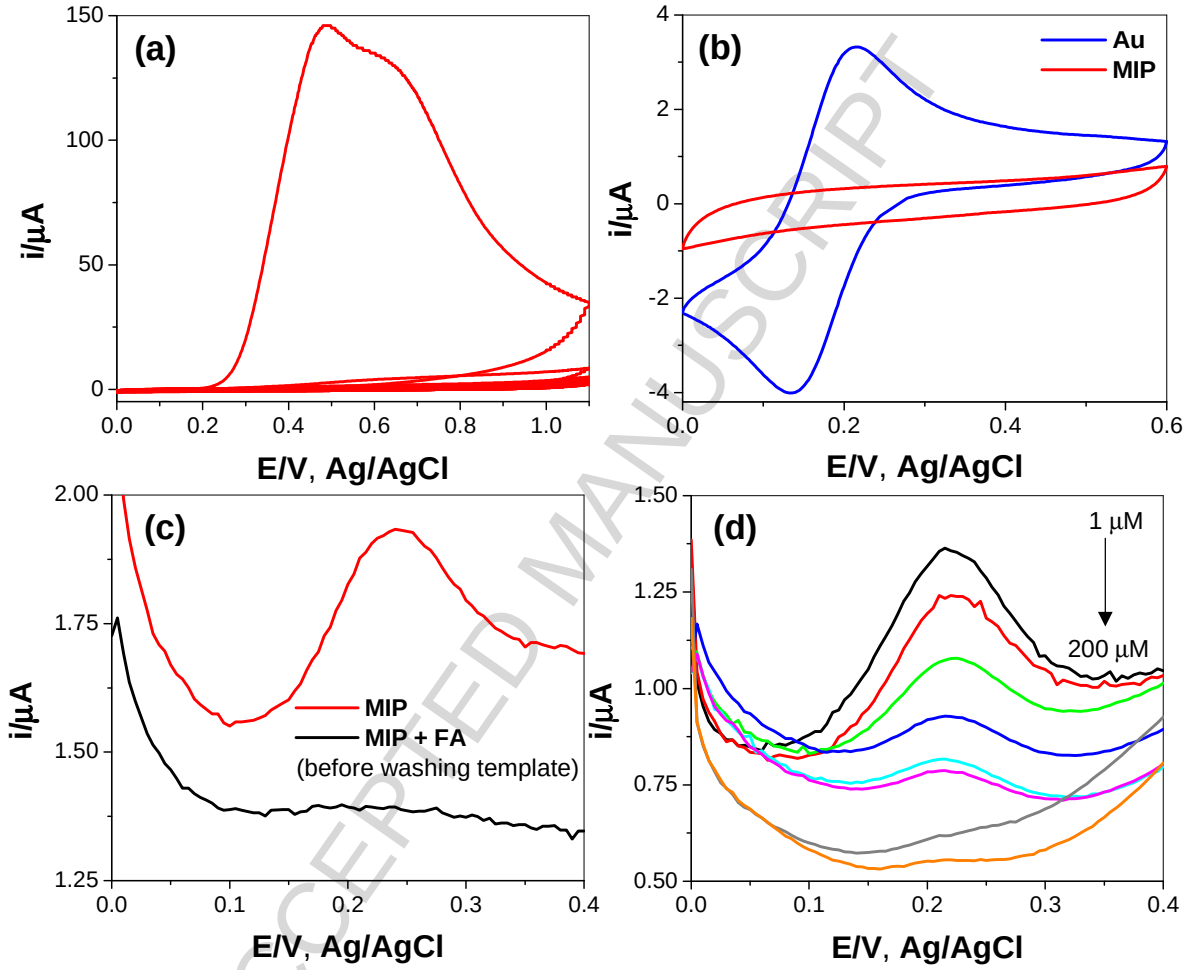

Figure 2. Cyclic voltammogram for (a) *o*-PD electropolymerisation in the presence PNIPAAm, MBA and folic acid at gold electrode (10 mM *o*-PD, 2.5 mM PNIPAAm, 2.5 mM MBA, 0.2 mM folic acid in a deaerated acetate buffer (pH 5.8), 50 mV/s, 20 cycles). (b) cyclic voltammograms were recorded with bare and MIP-modified gold electrode (0.1 M KCl, 0.5 mM $K_3Fe(CN)_6$ and 0.5 mM $K_4Fe(CN)_6$); Differential pulse voltammograms obtained with (c) MIP -modified electrodes before and after template removing and (d) MIP-modified electrode (MIP4) after 10 minutes of incubation in different FA concentrations ranging from 1 to 200 μM containing 0.1 M KCl, 0.5 mM $K_3Fe(CN)_6$ and 0.5 mM $K_4Fe(CN)_6$ solution at scan rate 50 mV/s.

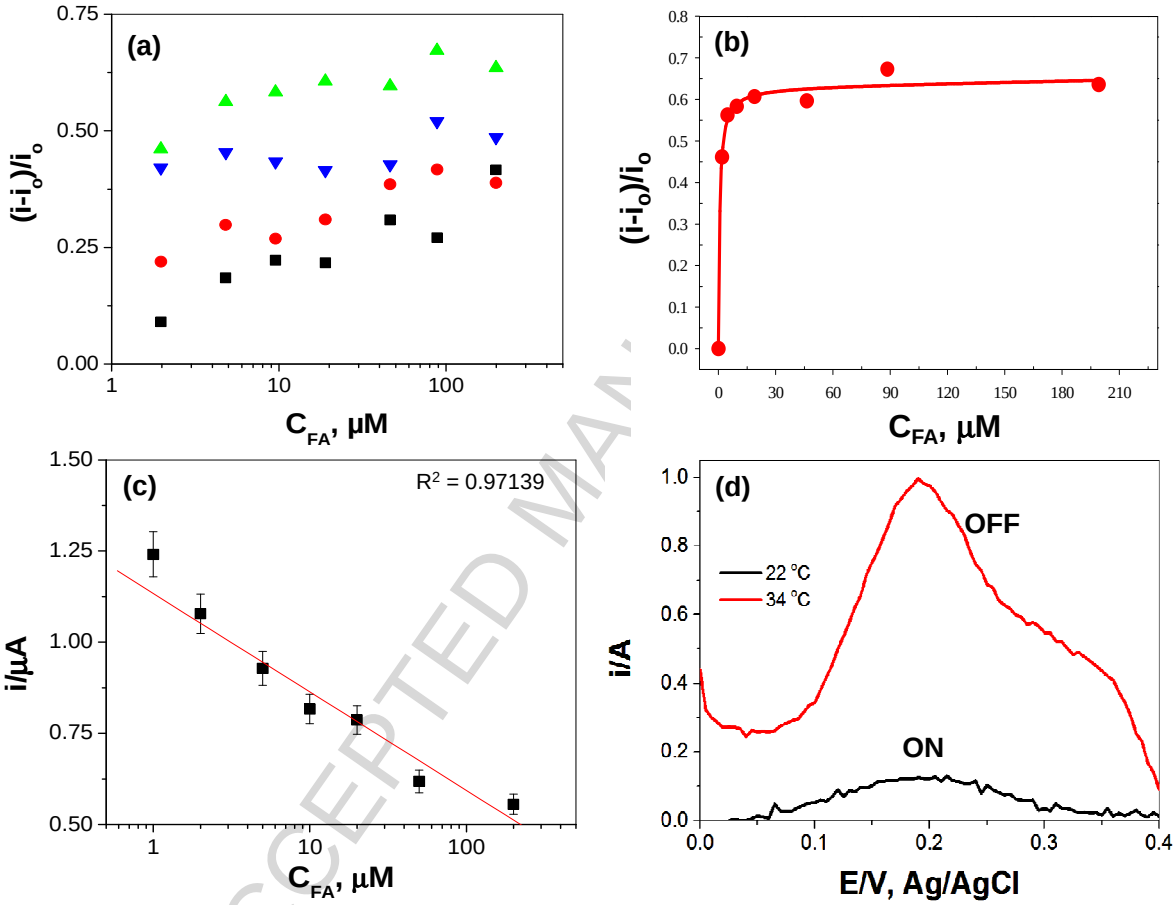

Figure 3. (a) Concentration dependencies of different MIP electrodes response towards FA binding, (■) MIP 2, (●) MIP 3, (▲) MIP 4, (▼)MIP 5. (b) Binding isotherm of MIP-modified electrode at different concentration of folic acid. Solid line – fitting curve for specific binding accompanied with non-specific adsorption. Folic acid binding with MIP (c) the linear relationship between peak currents and the concentration of FA ranging from 1 to 200 µM using differential pulse voltammograms of MIP-modified electrode (MIP4) after 10 minutes of incubation. (d) Folic acid (150 µM) binding with MIP at 22 and 34 ^{o}C. Differential pulse voltammograms have been recorded in 0.1 M KCl, 0.5 mM $K_3Fe(CN)_6$ and 0.5 mM $K_4Fe(CN)_6$ at 50 mV/s scan rate.

Table 1. Polymer composition for preparation of different MIP electrodes.

Composition (mM)	**MIP1**	**NIP1**	**MIP2**	**NIP2**	**MIP3**	**NIP3**	**MIP4**	**NIP4**	**MIP5**	**NIP5**
Folic acid	0.2	-	0.2	-	0.2	-	0.2	-	0.2	-
o-PD	10	10	10	10	10	10	10	10	10	10
PNIPAAm	-	-	2.5	2.5	-	-	2.5	2.5	2.5	2.5
MBA	-	-	-	-	2.5	2.5	2.5	2.5	2.5	2.5
Polymerization Temperature (°C)	21	21	21	21	21	21	21	21	34	34

Highlights

1. Folic acid sensor based on temperature sensitive polymer is reported.
2. The folic acid-imprinted electrode was obtained by electropolymerisation of functional monomers.
3. The analytical performance of the sensor was evaluated by electrochemical methods.
4. A highly specific electrochemical molecular recognition was established on electrode with switching ability.