Evaluation of Kinetic Parameters of Electro Oxidation of Thymine on a Poly(l-Glutamic Acid) modified Glassy Carbon Electrode

Jesny S.[a,b] and K. Girish Kumar [b*]
[a] Department of Applied Chemistry, Cochin University of Science and Technology, Kochi-22, Kerala, India.
*giri@cusat.ac.in
[b] Department of Chemistry, Sree Narayana College, Cherthala, Kerala, India.
jesnysmail@gmail.com

Abstract

The oxidation of the pyrimidine base, thymine was studied on a glassy carbon electrode (GCE) modified with an electropolymerised layer of l-glutamic acid (GA) on the surface. An irreversible oxidation peak was observed for 10 µM thymine at 740 mV with peak current of 5.9 µA in 0.1 MNaOH with square wave voltammetry (SWV).Chronoamperometry (CA) was used to determine the diffusion coefficient of thymine. The charge transfer coefficient, αas well as the heterogeneous rate constant,$k_s$of this electrochemical oxidation was determined making use of linear sweep voltammetry (LSV). The plausible mechanism of electro-oxidation process on the poly (l-glutamic acid) (*p*GA)modified electrode is proposed based on the calculated values.

Keywords: charge transfer coefficient, diffusion coefficient, electro-oxidation,heterogeneous rate constant, thymine.

1. Introduction

The pyrimidine base thymine or 5-methyl uracil is a major monomer unit of DNA together with adenine, guanine and cytosine. Any uncharacteristic change inthe concentration of any of these nucleotides is an indication of mutation in the DNA sequence or disease or disruption in the immune system. Hence the individual determination of these nucleotides is of significance [1].Electrochemical methods on account of their rapid response andcomparatively simple and reasonably priced instrumentation have become popular for the determination of a large variety of target molecules. In addition, the study of kinetics and mechanisms of reactions taking place at the electrode – solution interface is important to understand the controllable changes in the overpotential, i.e.,the energy of activation [2]. In this context the kinetics of the electro-oxidation of thymine on a (*p*GA) modified GCE is studied in alkaline medium.

EXPERIMENTAL

All reagents were of analytical grade and the solutions prepared in Millipore water. Electrochemical measurements were performed on an electrochemical analyser (CH instruments, USA) with a conventional three electrode system. A GCE was used as the working electrode, silver/ silver chloride as the reference electrode and a platinum wire as the auxiliary electrode. Before the experiment, the GCE was mechanically polished with 0.05micron alumina slurry, rinsed ultrasonically with methanol, 50 % nitric Acid and water successively to remove the adsorbed impurities.

GA was polymerized on to the surface of a cleaned GCE using a procedure reported by Yu and Chen [3]. The thickness of the *p*GA layer on the GCE surface was optimized by studying the response towards oxidation of 100 µM thymine. The *p*GA layer formed from 2 mMGA in PBS pH 7 by cycling the potential between -800 mV to 2000 mV for 20 cycles was found to give the best response (lowest oxidation potential) in 0.1N NaOH for the electro-oxidation of 100 µM thymine.

RESULTS AND DISCUSSION

Electrochemical response

The oxidation of 100 µM Thymine on the *p*GA modified GCE(*p*GA/GCE)took place at 740mV with peak current 22.2 µA in 0.1M NaOH in square wave mode.The variation of anodic peak current for the electro-oxidation with concentration of thymine was studied using Square wave voltammetry and was seen that the relationship between current and concentration was linear in the range 30 µM to 1000 µM (Figure 1) with a detection limit of 9.2 µM.

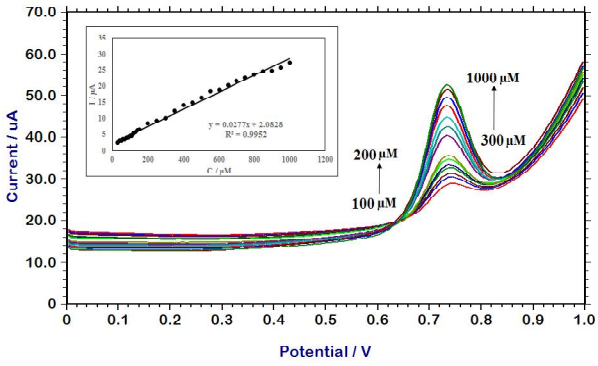


Figure 1. Overlay of SWV of oxidation of thymine. Inset shows the calibration graph

Chronoamperometric study

Double step CA was used to determine the diffusion coefficient of thymine. According to the Cottrell equation [6],

$$I = nFAD^{1/2}C\pi^{-1/2}t^{-1/2} \qquad 5$$

chronoamperograms were recorded for thymine concentrations varying from 12 mM to 24 mM with 1 mM increments. The overlay of the chronoamperograms obtained for 12 mM, 16mM, 20 mM and 24 mM thymine is shown in Figure3.

The plot of Ip vs $t^{-1/2}$was recorded at different concentrations of thymine ranging from 12 mM to 24 mM and the slope of these curves (figure 3 inset a) were plotted against the corresponding concentrations. The slope of this curve (figure 3 inset b) was used in the Cottrell equation and the Diffusion coefficient of thymine on the *p*GA/ GCE in 0.1M NaOH was calculated to be 1.18×10^{-5} cms^{-1}.

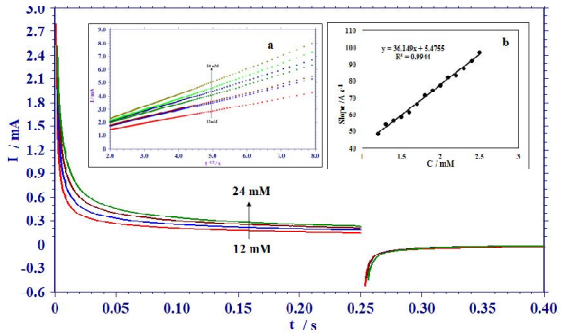


Figure 3. Overlay of Chronoamperograms obtained for the oxidation of 12 mM-24 mM Thymine. Inset a) plot of I vs $t^{-1/2}$ b) plot of slope of I vs $t^{-1/2}$ against corresponding concentration of thymine.

Determination of charge transfer coefficient (α) and number of electrons (n)

For a totally irreversible electrode process, the relation between peak potential $E_p$and half peak potential $Ep_{/2}$[4] is given by,

$$E_p - Ep_{/2} = \frac{47.7\,(mV)}{\alpha n_\alpha} \qquad 1$$

where α is the charge transfer coefficient and n_α is the number of electrons involved in the electrode reaction. For the electro-oxidation of thymine by LSV the E_p and $E_{p/2}$ was obtained as 874 mV and 793 mV respectively so that ¡ was obtained as 0.588.

For a totally irreversible reaction [5],

$$E_p = \frac{b}{2} lnv + constant \qquad 2$$

where

$$b = \frac{RT}{(1-\alpha)n_\alpha F} \qquad 3$$

For the irreversible oxidation of 50µM thymine on the *p*GA/GCE, on varying the scan rate, the peak potential shifted (Figure 3) according to the equation,

($R^2 = 0.9878$) 4

Accordingly, using equations 1, 2 and 3, the value of n was calculated to be 0.9677 ~ 1 and α = 0.6375.

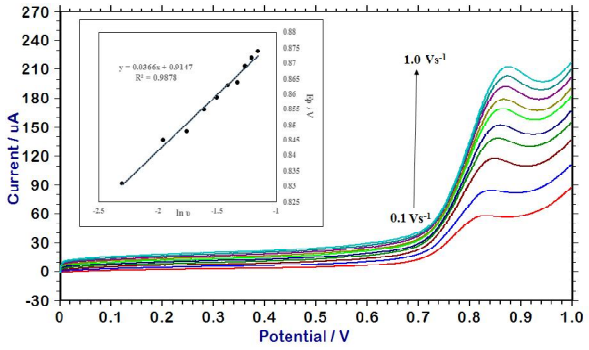


Figure 2. Overlay of LSV of 50 µM thymine at scan rates from 0.1 Vs^{-1} to 1 Vs^{-1}. Inset shows the plot of Ep vs ln υ

Heterogeneous rate constant

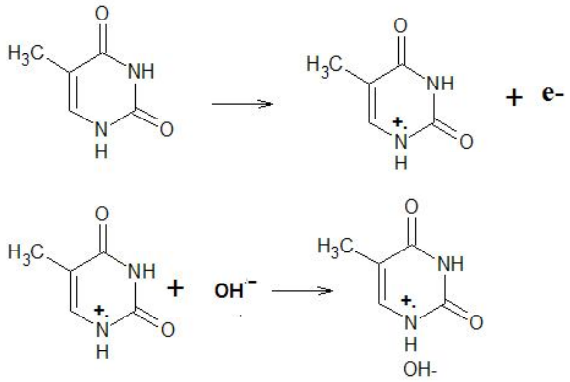
The standard heterogeneous rate constant of an irreversible reaction [7] is given by

$$E_p = E^0 - \frac{RT}{\alpha nF}\left[0.78 - \ln\frac{k_s}{D^{1/2}} + \ln\left[\frac{\alpha n_\alpha F\upsilon}{RT}\right]^{1/2}\right] \quad 6$$

The standard potential E^0 was obtained by extrapolating the plot of E_p vs υ to υ = 0. From the values obtained from equation 4, k_s was calculated to be 3.57×10^{-4} cms^{-1}.

Mechanism of electro-oxidation

In accordance with the scan rate studies, the possible mechanism of the one -electron electro-oxidation can be depicted as shown in the scheme below.



Scheme 1. Mechanism of electro-oxidation of thymine

CONCLUSIONS

The kinetics of oxidation of thymine was studied in 0.1 M NaOH on a *p*GA/GCE. In square wave mode the electro-oxidation response was linear in the concentration range from30 µM to 1000 µM with a detection limit of 9.2 µM. Using chronoamperometric measurements the diffusion coefficient of thymine in 0.1 M NaOH was determined to be 1.18×10^{-5} cms^{-1}. The charge transfer coefficient for the irreversible one electron oxidation of thymine was obtained as 0.6375. The standard heterogeneous rate constant for this irreversible reaction was determined to be $3.57\times10^{-4}cms^{-1}$.

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