Electrochemical behavior of an anticancer drug 5-fluorouracil at methylene blue modified carbon paste electrode

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ABSTRACT

A novel sensor for the determination of 5-fluorouracil was constructed by electrochemical deposition of methylene blue on surface of carbon paste electrode. The electrode surface morphology was studied using Atomic force microscopy and XRD. The electrochemical activity of modified electrode was characterized using cyclic voltammetry and differential pulse method. The developed sensor shows impressive enlargement in sensitivity of 5-fluorouracil determination. The peak currents obtained from differential pulse voltammetry was linear with concentration of 5-fluorouracil in the range $4 \times 10^{-5} – 1 \times 10^{-7}$ M and detection limit and quantification limit were calculated to be 2.04 nM and 6.18 nM respectively. Further, the sensor was successfully applied in pharmaceutical and biological fluid sample analysis.

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1. Introduction

Over the recent past years, electrochemical techniques have found a dynamic position in the fields of medicinal, ecological, industrial, and agricultural analysis [1–6]. The dynamic position obtained by these methods is due to the fact of high efficiency, exceptional selectivity, low cost and low maintenance with time saving process [7,8]. Magnification of the above properties can be achieved by application of chemically modified electrode (CMEs) [9–11]. Electrode modification in analytical chemistry has always been an interesting field. Modifiers effectively transport electrons between the electrodes to analyte. It works by minimizing the over-potential required for the electrode reactions and additionally increasing the sensitivity and selectivity of the electrode [12–14]. After the first usage of carbon paste as the electrode material by Adams, carbon paste electrodes (CPE) have attracted highly the analyst due to its advantage such as steady response, easy renewability, and low ohmic resistance [15,16]. In addition to these beneficial properties, the most beloved property of carbon paste electrode is the practicability of inclusion of different substance during paste preparation, which allows the formation of electrode with preferred composition and pre-determined characters [17]. Such modification of electrode surface can be easily carried out using organic dyes, which display an excellent mediating ability. These included the use of methylene blue [18], methylene green [19], Prussian blue [20], phenazines [21], and thionine [22].

Redox dyes capable of undergoing electro-polymerization are acknowledged one of the excellent electron donors or acceptors. It has been reported that, because of oxidation, MTB forms a polymer semi-conductor films at the electrode surface [23]. Hence, water-soluble cationic dye, methylene blue is used as an excellent electron mediator. With its redox potential very close too many organic drugs, chemical modification of the electrode can enhance the electrochemical behavior of the analyte. Further, early reports have explained the formation of conductive multilayer structures on platinum [24]. The polymer grows through a linkage in one of the carbon atoms of the aromatic system of the monomer. The most probable being one of the ortho carbon atoms with respect to the tertiary amino group. Further, the positive effect of methylene blue has been explained by different methods like, spectro-electrochemistry after adsorption on a platinum electrode at open circuit potential during 15 min [25], methylene blue mixed with Nafion solution and coated on the micro-cylinder electrode by dip-coating method. This led to good results for the determination of haemoglobin in clinical blood samples. The Nafion film served to retain the methylene blue at the electrode surface [26]. It was demonstrated that, as happens with a number of redox dyes, polymerization of methylene blue occurs if a sufficiently positive potential is applied [27]. The formation of poly (methylene blue) by potential cycling was studied using the electrochemical quartz crystal microbalance, as its redox behavior as a function of pH and solution composition [28].

Ribonucleic acid (RNA) consists of a pyrimidine base, uracil that forms base pair with adenine. Nucleic acid biosynthesis in tumors is preferentially carried by using uracil [29]. Halogens when substituted at fifth position of uracil, drastic variations in their biological properties were observed [30]. A large number of uracil derivatives have been reported as anti-cancer agents, amongst which 5-Fluorouracil (5-FU) is of more importance (Scheme 1). 5-FU is effectively used in treatment of solid tumors of breast and rectum [31]. Deoxyribonucleic acid (DNA)

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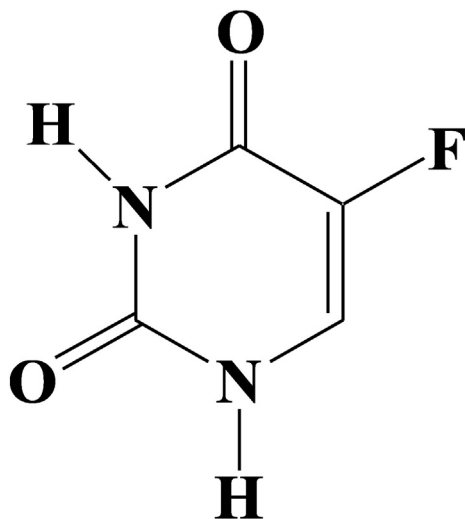
Scheme 1. Structure of 5-Fluorouracil.

and ribonucleic acid (RNA) can be integrated easily by 5-FU leading to cytotoxicity and cell death [32,33]. Since the mechanism of action is not localized to the cancer cells, it also affects all the rapid dividing cells [34]. Some of the side effects of 5-FU include bone marrow depression, gastrointestinal tract reaction, along with leucopenia and thrombocytopenia [35]. In addition to this 5-FU exhibit, varied unpleasant conditions like cardiotoxicity, nephrotoxicity and hepatotoxicity and marked organ toxicity coupled with increased oxidative stress and apoptosis [36].

There are various technique reported untimely for determination of 5-FU. Nevertheless, these techniques fall in the rear when compared for their availability in all analytical laboratories, cost, time consumption, simplicity of reagents, and their selectivity and sensitivity. Considering the adverse affect of 5-FU, there is an eagerness to develop a novel, convenient and a direct method for determination of 5-FU. Literature study reveled that there is a gap in literature for 5-FU determination based on catalytic activity of methylene blue modified electrode. Hence, these works were undertaken.

2. Experimental

2.1. Chemicals and reagents

Stock solution (1 mM) of 5-FU (Sigma-Aldrich, Pune, India) was prepared in double distilled water. Methylene blue (Alpha Chemika, Mumbai, India) Oxalic Acid, Citric Acid, Lactose, Sucrose, Dextrose, Glucose, Gum Acacia, uric acid, xanthine, caffeine, and Starch (SDFCL, Mumbai India). The electrochemical measurements were carried in phosphate buffer solution (PBS) pH 3.0–11.0 prepared according to literature [37]. Utilization of analytical grade solution and double distilled water throughout the experiment was employed.

2.2. Instrumentation

Electrochemical experiments were carried with three electrode system, Ag/AgCl (reference electrode), platinum wire (counter electrode) and methylene blue modified carbon paste electrode (working electrode) connected to CHI Company, USA (Model D630) electrochemical analyzer. The pH of solution was measured with an Elico pH meter (Elico Ltd., India). Contact mode AFM (Model: Nano Surf AG — easy scan; Make: Nano Surf, Switzerland) was used in order to characterize the surface morphology of MTB modified electrode. The measurements were performed, displayed, and evaluated using the SPM Control Software version 3. X-ray diffraction measurements (XRD) for CPE and CPE modified with MTB have been performed using advanced

diffractometer [PANalytical, XPERT- PRO] equipped with Cu kα radiation source. The diffraction data were collected in the 2θ range of 5–90 using a fixed time mode with a step interval of 0.02° and scanning speed of 2°/min.

2.3. Preparation of electrode

By homogenizing 1.0 g of graphite powder with 0.5 ml of paraffin oil in an agate mortar (made of granite) carbon paste electrode was prepared. Cavity of polytetrafluoro ethylene tube (PTFE) was used for the packing of paste prepared. After smoothening, the surface against weighing paper the electrode was transferred to phosphate buffer of pH 7 and activated by cyclic voltammetric sweeps between 0.4 and 1.4 V with a scan rate of 50 mV s^{-1} [38]. Further, the resulting electrode was substituted for modification. Methylene blue solution of 1 mM concentration was prepared by dissolving appropriate amount in 0.05 M H$_2$SO$_4$. The activated CPE was dipped in the solution and cyclic voltammetric sweeps were recorded between −0.8 to 1.4 V at a scan rate of 100 mV s^{-1} for 20 cycles. After this, the same CPE was enforced under sweeping from −0.8 to 1.4 V at a scan rate of 100 mV s^{-1} for 20 cycles in a solution containing 1 mM MTB with 0.01 M NaOH. NaOH was chosen as an activation agent as it is very effective in generating high surface areas at lower activation temperature [39,40].

2.4. Area of electrode

Randles -Sevcik formula was used to calculate the electro-active area of the electrode using 1.0 mM K$_3$Fe (CN)$_6$ as a probe at different scan rates in 0.1 M KCl as supporting electrolyte. At $T = 298$ K and for a reversible process the equation is as follows [41,42]:

$$Ip = \left(2.69 \times 10^5\right) n^{3/2} A_0 D_R^{1/2} \upsilon^{1/2} C_0. \tag{1}$$

In Eq. (1) for 1.0 mM K$_3$Fe (CN)$_6$ and 0.1 M KCl as supporting electrolyte, Ip: anodic peak current, n: number of electron transferred during the electrode reaction $= 1$, A$_0$: surface area of the electrode, D$_R$: diffusion coefficient $= 7.6 \times 10^{-6}$ cm s^{-1}, υ: scan rate and C$_0$: concentration of K$_3$Fe (CN)$_6$. Hence, A$_0$ was 0.155 cm^2, calculated using slope of the plot, Ip vs. $\upsilon^{1/2}$.

3. Results and discussion

3.1. Characterization of electrode

The surface morphology of the electrodes influenced the electrochemical properties of electrode. Hence, surface morphology of unmodified and MTB modified electrode was investigated by AFM in air (Fig. 1). The AFM topographic images provided information on the roughness of the electrode surface. The surface morphology image of unmodified electrode with scan size of 2.03 μm × 2.03 μm indicates the surface with the roughness average value (Sa) = 12.361 nm; mean value (Sm) = −4.339 fm; root mean value (Sq) = 22.292 nm; valley depth (Sv) = −129.81 nm; peak height (Sp) = 157.37 nm; peak value height (Sy) = 287.18 nm. Further, the modification of the electrode investigations with scan size 2.0 μm × 2.0 μm showed the roughness average value (Sa) = 17.614 nm; mean value (Sm) = −4.402 fm; root mean value (Sq) = 21.86 nm; valley depth (Sv) = −100.6 nm; peak height (Sp) = 113.36 nm; peak valley height (Sy) = 214.02. From the reflection of AFM images the roughness average value increased, which may be due to formation of a polymer semiconductor films at the electrode surface. With delta z value for unmodified and modified electrode of 9.081 nm and 44.12 nm respectively, (Delta Z is the difference in the sample height between the start and the endpoint). XRD is a powerful and effective method for the investigation of the interlayer changes and crystalline properties of as-synthesized carbon material. The XRD