Electrochemical characterization of luminol and its determination in real samples

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Electrochemical behavior of luminol as an important molecule in forensic science was studied in Britton-Robinson buffers (pH 2 - pH 13) at a glassy carbon electrode by using cyclic voltammetry and differential pulse voltammetry techniques. Luminol showed a well-defined and irreversible oxidation peak at 0.33 V (vs. Ag/AgCl) in 0.05 mol L^{-1} Britton-Robinson buffer solution at pH 12. The electro-oxidation process of luminol was adsorption-controlled and irreversible over the pH range studied (2.0 – 13). The differential pulse voltammetric determination of luminol was carried out. Under optimal conditions, glassy carbon electrode displayed a linear response over the range from 1.8 $x10^{-8}$ to 4.5 $x10^{-4}$ mol L^{-1} luminol concentration and statistical data were evaluated. A commercially available real sample that contained luminol was analyzed according to the method. The result as luminol percentage of the sample was obtained as 21.2 (± 0.4) %.

Introduction

Luminol ($C_8H_7N_3O_2$, 5-amino-2, 3-dihydro-1, 4-phthalazinedione) is traditionally used by forensic investigators to detect trace amounts of blood left at crime scenes. Luminol is used by crime scene investigators to locate traces of bloodstains which are hidden from naked eye and, for this reason, has been considered important and well-known assays in the field of forensic sciences. When the crime scene investigators spray a solution of luminol and the oxidant, the iron present in blood catalyzes the luminescence to give blue glow lasting for about 30 seconds that allows the detection of even trace amounts of blood [1].

In the previous work, operational use of the luminol reaction, its interferences, and the effect of the luminol reaction on DNA testing were subjected to a lot of studies [2]. Strong oxidants, certain transition metal ions, peroxide effects in the luminol chemiluminescence studies were examined in order to evaluate possible test for blood [3-5]. The studies of electrochemiluminesans (ECL) of luminol on various electrodes have been reported. The catalytic performance of gold nanoparticle modified electrodes on luminol electro chemiluminescence has been investigated by cyclic voltammetry [6]. The ECL features of luminol on platinum or graphite anodes was investigated under potentiostatic and potentiodynamic conditions in order to find out how it varies in time and with the experimental conditions [7]. An ECL sensor for luminol was constructed on the base of a C-doped titanium oxide amorphous semiconductor electrode [8]. The behavior of luminol ECL at a polycrystalline gold electrode was studied by cyclic voltammetry [9].

Luminol has been used to determine some metals in samples. Luminol can be coordinated some metals by the way of its functional groups so that it provides to determine them in samples. Determination of cadmium and copper giving rise to the formation of the complexes with luminol in real samples was carried out by using hanging mercury drop electrode [10]. In an another study it was shown that Mn(III)-containing microperoxidase 8 (Mn(III)-MP8) was an effective catalyst for the cheminluminescent reaction involving the oxidation of luminol with hydrogen peroxide at high pH [11]. The properties of the NADH/NAD+ redox system on hybrid films composed of electro polymerized luminol–flavin adenine dinucleotide (FAD) adsorbed film modified electrodes have been investigated in aqueous acidic solutions [12]. The enhancement mechanism of the Au nanoclusters on luminol CL was studied and a sensitive detection tool was carried out for for the determination of hydrogen peroxide and glucose [13]. The polyluminol modified electrode was prepared by the electro polymerization of luminol monomers in dilute sulfuric acid solution for obtaining a modified electrode to simultaneous determination of ascorbic acid, dopamine, and uric acid [14].

Voltammetric methods are sensitive, selective, and easy to apply and also have the advantage of low cost and rapid results. They require less expensive instrumentation and test materials than the chromatographic and spectroscopic methods [15]. Luminol molecule has a great importance in the area of forensic science. Luminol is usually detected by CL or ECL methods. An ECL determination was carried out by using immobilizing hematin, a catalyst for luminol, to obtain a modified carbon paste electrode for luminol determination from reagent solution containing hydrogen peroxide. Concentration of luminol was found to be linear over the range of from 10^{-8} to 10^{-3} mol L^{-1} [16].

However, to the best of our knowledge, there are no reports in the literature describing electrochemical behavior and voltammetric determination of it. The present work is aimed to investigate electrochemical behavior of luminol by voltammetric methods and propose a simple and rapid determination of it in real samples. For this purpose, in the present study, voltammetric behaviors of luminol were investigated on a glassy carbon electrode (GCE) in aqueous solution and a sensitive voltammetric method was evaluated for determination of luminol in real commercial samples.

Experimental

Apparatus

Voltammetric measurements were carried out with an Autolab PGSTAT 30 (Eco Chemie) potentiostat/galvanostat by using a conventional three-electrode system at the room temperatures. All voltammetric experiments were conducted in a general-purpose cell containing 10 mL buffer solution, a platinum wire as a counter electrode (BAS MW 1032) and an Ag/AgCl/ (3 M KCl) as a reference electrode (BAS MF 2052) were used. The working electrode was a GCE with 3 mm diameter (BAS MF-2012). All solutions were purged for 5 min with pure nitrogen before the experiments to obtain an inert atmosphere. The pH values of the solutions were adjusted using a pH-meter (WTW Inolab pH 720). An ultrasonic bath was used for cleaning glass vessels and preparing the solutions (Bandelin Sonorex RK100 H).

Reagents and solutions

All chemicals used in these experiments were of the highest purity (used without further purification). Luminol (ABCR GmbH & Co. KG, Germany), boric acid, phosphoric acid, acetic acid, hydrochloric acid, and sodium hydroxide (Fluka, Switzerland) were of analytical grade. All solutions were prepared using ultrapure water from a Millipore Milli-Q system (Bedford, USA). Luminol is insoluble in water, but is quite soluble in base solutions. Luminol solutions are very sensitive to light and the presence of metal cations. That is why the stock solution of luminol 1×10^{-2} mol L^{-1} was prepared with 0.1 mol L^{-1} NaOH solution in a dark bottle and stored in a refrigerator at 4°C. The stock solutions were diluted to desired concentration by the Britton-Robinson (BR) buffer solution. BR buffers (pH=2–13) were prepared from boric acid, phosphoric acid and acetic acid and adjusted to the desired pH values with sodium hydroxide.

Pretreatment of the working electrode

The GCE was polished with 0.5 micrometer alumina powder on a polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed and then sonicated in the ultrasound bath and gently dried with a tissue paper.

Electrochemical measurements

Electrochemical behavior of luminol was investigated by cyclic voltammetry (CV) at the potential range between −0.1 and +1.0 V versus Ag/AgCl. 10 mL volume of 0.05 mol L^{-1} BR buffer was transferred into the electrochemical cell and deoxygenated for 5 min with high grade nitrogen to obtain an inert atmosphere in the electrochemical cell. During the CV measurements, first a steady state CV was obtained in blank solution, and then accurate additions of luminol from stock solution were added into the electrochemical cell to obtain luminol voltammogram. DPV performed by scanning the potential in the range from 0.0 to 0.8 V, the peak currents produced as a result of electro-oxidation of luminol were used to obtain luminol quantities. DPV pulses were selected with pulse amplitude of 50 mV, pulse width of 50 ms, potential step of 4 mV, and pulse period of 0.2 s.

Results and discussion

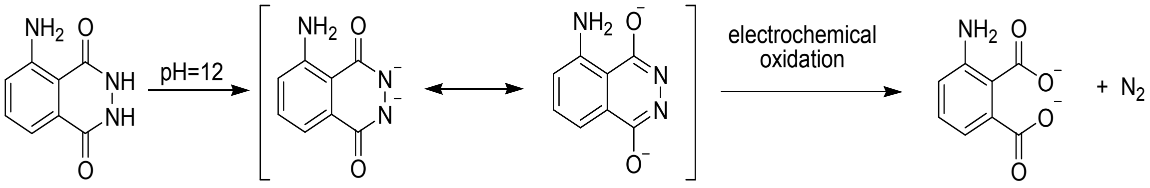
Electrochemical characterization of luminol on GCE

In order to luminol is slightly soluble in neutral and acidic solutions, the preliminary work was carried out in BR buffer at pH 9, at potential range from – 0.4 to + 1.0 V. Luminol exhibited a good electrochemical response, and the voltammograms showed a pair of anodic peaks with a potential value of 0.54 V and 0.90 V. The first oxidation peak at 0.54 V was more prominent compared with the second oxidation peak at 0.90 V. The oxidation peak at 0.54 V at pH 9 has shifted to more negative potentials with the increasing pH. As seen in Fig. 1, it took place at 0.33 V at pH 12. These peaks may be attributed to the dissociation of two hydrogens on the two acyl-hydrazides group of the molecule. The oxidation process of luminol has not been accompanied by a cathodic wave, which indicates that the electrode reaction is irreversible. It was seen that luminol oxidation at pH 12 was very high according to that of pH 9. Fig. 2 displays the CVs in the range of -0.1 V to 1.0 V for 10^{-4} mol L^{-1} luminol at different pHs in 0.05 mol L^{-1} BR buffer. As seen from the voltammograms, luminol oxidation occurs at pH 12 with a high peak current at 0.33 V. DPVs which were obtained at pH 12 have also exhibited well-defined peaks in 0.05 mol L^{-1} BR buffer solution for the different concentration of luminol (Fig 3).

Effect of pH

The effect of the pH of the reagent solution on the oxidation peak current was studied. The effect of pH on the CV response of 10^{-4} mol L^{-1} luminol has been examined in 0.05 mol L^{-1} BR buffer with different pHs. The oxidation of luminol at GCE has been found to be pH-dependent. The oxidation peak current slightly increased with the increasing pH value of the solution until it reached pH 6. However, the oxidation peak current decreased at pH values higher than 6, and then it increased sharply at pH 12 and then began to decrease again (Fig. 4). DPVs which obtained at different pHs in the presence of 10^{-4} mol L^{-1} luminol are seen in Fig.4 as inset. Small peak currents were obtained at acidic pHs. Besides, as can be seen in Fig. 4 inset figure, peak potential is shifted to more negative potentials with increasing the pH value of the solution, indicating that protons take part in the electrode process. When we draw a graph the oxidation peak potential of luminol varies linearly with pH with the equation Ep (V) = - 0.058 pH + 0.924. The obtained slope value of 58 mV per pH is very close to the expected theoretical value of 59 mV per pH indicates that the number of protons and electrons involved in the oxidation of luminol is equal involving two protons and two electrons in electrochemical process.

Previously it was shown that luminol protonation in acidic, neutral, and alkaline solution as LH_2, LH^-, and L_2^- represent the diprotic, monoanionic, and dianionic forms of luminol, respectively [2]. Although some inconsistencies appeared in the literature because of several pKa values are reported for luminol (such as three pka values, discrepancies on the pka values, and lack of agreement is present in the acidity order of luminol's protons) it is common remark that luminol is a diprotic acid (denoted as LH_2) with pKa's of 6 and ~ 13. It is also a general idea that the protons from the amine moiety are relatively stable and are the less acidic according to the acyl-hydrazide protons on the molecule [17]. In our study, the effect of pH on luminol electro-oxidation was obtained as agreeable with previous works so that the maximum peak current obtained pH 6 and 12 can be associated with pka values of luminol corresponding to loss of the two acyl-hydrazide protons, at pKa_1 and pKa_2. It is well known that luminol will have dianionic form in alkaline medium so that can be chemically oxidized easily in the presence of catalyst at pka_2 value to give 3-aminophthalate [2, 18]. In our case accordingly a plausible electro-oxidation mechanism for luminol at pH 12 that a large peak current was obtained can be suggested (Scheme 1).



Scheme 1. Possible electro-oxidation reaction for luminol at pH 12

Effect of scan rate

The effect of potential scan rate (v) on the oxidation peak potential (Ep) and peak current (ip) of luminol were evaluated in Fig. 5. The oxidation peak potential shifted slightly in a positive direction with an increase in the scan rate, and the oxidation peak current (ip) was proportional to the scan rate at lower scan rates 10–250 mV/s. On the other hand, as scan rate increased from 10 to 250 mV/s, the peak potential shifted toward more positive potential as expected for an irreversible oxidation process [19].

A straight line with the slope of 1.0 was observed when ip was plotted against v within the range 10 – 250 mV/s for 10^{-4} mol L^{-1} luminol concentration. Corresponding equation is: Ip (mA) = $1.012\text{x}10^{-8}$ V (mV) + $9.759\text{x}10^{-7}$ (R^2=0.997, Fig. 5 inset). This indicates that the electrode process was an adsorption-controlled process at the GCE surface [20].

Application of voltammetric methods to luminol determination

Based on the voltammetric behavior of luminol, a quantitative method was developed. Differential pulse voltammetric response was investigated to obtain a much more sensitive peak current (Fig. 3). The optimum instrumental conditions like pulse amplitude, pulse width, and scan rate were determined by using 10^{-6} mol L^{-1} luminol solution. DPV pulses were selected with pulse amplitude of 50 mV, pulse width of 50 ms, potential step of 4 mV, and pulse period of 0.2 s. Analytical reproducibility of the method was evaluated from seven different measurements by using 10^{-6} mol L^{-1} luminol solutions. Precision of the developed method in terms of the relative standard deviation (RSD %) were 3.1% that can be considered good reproducibility.

Calibration curve, LOD and LOQ

A calibration graph was obtained under the suitable chemical and instrumental conditions for the voltammetric determination of luminol. The calibration graph was constituted for 0.05 mol L^{-1} BR buffer of pH 12 and was found to be linear in the range from 1.8 $\text{x}10^{-8}$ to $4.5\text{x}10^{-4}$ mol L^{-1} with the equation y = 0.0041 C + $1\text{x}10^{-7}$ (r=0.998, n=6) where y is the current in ampere (A) and C is the concentration of luminol in molar (mol L^{-1}).

The limit of detection (LOD) and quantification (LOQ) were calculated using the equations LOD = 3xSD/m and LOQ = 10xSD/m, where SD is the standard deviation of the intercept, and m is the slope of the calibration curve, (n=5) [21]. The LOD and LOQ for luminol were calculated as $1.1\mathrm{x}10^{-10}$ and 3.3×10^{-9} mol L^{-1} luminol respectively.

Recovery studies

To determine the accuracy and reproducibility of the method and to check the possible interference from the excipients used in the commercial sample, the standard addition method was applied. For this purpose, a known different amount of pure luminol solution was added to a known amount of sample solutions. The mixtures were analyzed as in pure luminol. The percent recovery of the added standard to the assay samples was calculated. The determination of luminol in spiked samples was carried out for three levels of concentration: $5\mathrm{x}10^{-7}$, $1\mathrm{x}10^{-6}$, and $1\mathrm{x}10^{-5}$ mol L^{-1}. The mean recoveries of luminol based on three replicate measurements were found to be 97.6, 98.2, and 99.1 respectively which can be attributed as good recoveries.

Sample analysis

Luminol materials that used for crime scene investigation is sold on the market as to be ready for use. It also contains sodium carbonate, sodium perborate tetrahydrate and the other excipients. The obtained light during the reaction of luminol with blood is proportional to the amount of luminol. Thus, the analysis of luminol content of the commercially sold materials may be important for controlling of the quality of the sample. The luminol sample that was provided from the Security Chief Office of Istanbul accurately weighed and transferred into the flask and solved by using BR buffer solution (pH 12). Then, aliquots of that solution were introduced into the electrolytic cell impending totally 10 mL of BR buffer (pH 12) and sample solution. Differential pulse voltammogram was recorded under the defined optimum conditions to quantify the unknown amount of luminol in the solution. And then luminol content of the sample was calculated. Three replicates were carried out through the developed procedure. The result as luminol percentage of the sample was obtained as 21.2 (± 0.4) %.

Conclusions

The electrochemical behavior of luminol was studied by voltammetric methods at a bare GCE in BR buffer solutions. Luminol oxidation was observed very well at pH 12. A well-defined irreversible peak was obtained for oxidation of luminol that an adsorption-controlled process involving 2 protons and 2 electrons related to the two acyl-hydrazides functional groups of luminol at the pHs lower than pH 12. The currents belonging to the oxidation peak of luminol in different pH values of BR buffers showed max at pH 6 and 12 which can be attributed to the values of luminol pka1 and pka2. A sensitive and reliable differential pulse voltammetric method for the direct determination of luminol was also developed. The peak currents increased linearly with increasing luminol concentration in the range from $1.8\text{x}10^{-8}$ to $4.5\text{x}10^{-4}$ mol L^{-1}. The results obtained by using the voltammetric method are satisfactory for the determination of luminol with a wide linear range, and good reproducibility. The proposed method is very suitable for determination of luminol for quality control process in different commercial forensic science material that contain luminol. Moreover, it has clear advantages when we compare with the reported ECL and spectroscopic methods in point of low cost, simplicity, sensitivity, and short analysis time.

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