DETERMINATION OF LYSINE IN FUNCTIONAL FOOD SAMPLES BY DIFFERENTIAL PULSE VOLTAMMETRY

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TÓM TẮT

XÁC ĐỊNH LYSINE TRONG THỰC PHẨM CHỨC NĂNG BẰNG PHƯƠNG PHÁP VON-AMPE XUNG VI PHÂN

Lysine là một axit amin thiết yếu, đóng vai trò quan trọng trong việc duy trì nâng cao hệ miễn dịch của con người. Lysine được cung cấp cho cơ thể con người qua thức ăn hoặc bổ sung thêm qua thực phẩm chức năng nên việc kiểm tra, kiểm soát các sản phẩm là hết sức quan trọng. Trong nghiên cứu này, lysine được xác định bằng phương pháp von ampe xung vi phân sau khi dẫn xuất hóa bằng formandehit ở pH = 8. Sản phẩm sau khi dẫn xuất hóa có đặc tính khử điện hóa trên điện cực giọt thủy ngân và píc quá trình khử tỉ lệ tuyến tính với nồng độ lysine trong khoảng từ 5.10^{-6} đến 5.10^{-5} mol L^{-1}. Phương pháp có độ nhạy và độ chính xác cao với giới hạn phát hiện của thiết bị (IDL) là $1,3.10^{-6}$ mol.L^{-1}, giới hạn định lượng (IQL) là $4.5x10^{-6}$ mol.L^{-1}. Độ thu hồi khi phân tích lặp lại ở mức nồng độ lysine $5,0x10^{-6}$ mol.L^{-1} là 95,5%, độ lệch chuẩn 3,0% (n=5). Phương pháp nghiên cứu đã được áp dụng để xác định hàm lượng lysine trong một số mẫu thuốc bổ cho kết quả phù hợp với phương pháp sắc kí lỏng hiệu năng cao (HPLC).

Keywords: *Lysine, differential pulse voltammetry, hanging mercury drop electrode.*

1. INTRODUCTION

Lysine, an essential amino acid is used in the biosynthesis of proteins [1, 2]. It contains an α-amino group, an α-carboxylic acid group, and a side chain lysyl (Fig.1). However, human bodies cannot synthesize lysine and need to be supplied from food, drugs, and functional foods. Therefore, determination of lysine in biological and pharmaceutical samples plays an important role in life science.

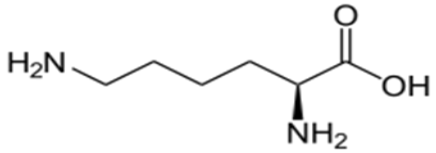


Figure 1. Chemical structure of lysine

Normally, lysine in food has been determined by precolumn derivatization RP-HPLC. However, HPLC is an expensive and requires several time-consuming steps prior to the drug assay compared to the electro- analytical ones. Spectrophotometric method based on the formation of colored compounds has been a poor selectivity and sensitivity [3,4]. The electroanalytical methods are considered as highly selective and sensitive, simple, easy to use, and low cost. In this technique, the hanging mercury drop electrode (HMDE) shows wide range of electroactive, good reproducible, smooth, renewable surface [5,6,7].

In this study, differential pulse voltammetry had been applied to determine lysine followed by its derivatives with formaldehyde in functional food samples.

2. MATERIALS AND METHODS

2.1. Reagents and instrumentation

All reagents were of analytical grade. Stock solution (4×10-3 mol/ L) of lysine was prepared by directly dissolving the required weight of lysine in water and stored in a refrigerator at 0-4^{0}C.

Britton-Robinson (BR) buffers of pH 2 ÷ 11 were prepared from mixtures of 0.04 mol L^{-1} acetic, orthophosphoric, and boric acids and adjusted to the required pH with 0.2 mol L^{-1} sodium hydroxide solution[7]. 0.5 mol/ L of HCHO solution were prepared from the HCHO 37%.

All measurements were performed with an µAutolab type III (Netherlands), interfaced to the electrode assembly 663 -VA Metrohm, Switzerland), and controlled by software 757 VA Computrace. The three-electrode system consists of a (HMDE) as a working electrode, an Ag/AgCl/KCl reference electrode, and a glassy carbon rod auxiliary electrode. A stirring rod provided convective transport during the preconcentration step. All measurements were thoroughly deuterated with high-purity nitrogen for at least 5 min.

2.2. Sample preparation

Solid samples: A portion of the sample containing lysine equivalent to 4×10^{-3} M was accurately weighed, transferred into a 100 mL volumetric flask, and dissolved and reached to the mark with double distilled water. The mixture solution was filtered through 0.45 µm with a Millipore filter (Gelman, Germany).

Liquid drug samples: A portion of liquid sample containing lysine equivalent to 4×10^{-3} M was accurately taken drug and moved into a 50 mL volumetric flask and then diluted to a volume 50.00 mL with distilled water.

2.3. Analytical procedure

The appropriate analytical solutions (50,00 mL) were transferred to the electrical cell, followed by adding 20 mL Britton-Robinson buffer solution and 3.7 mL HCHO 37 %. The solution was deaerated for about 5 min with nitrogen gas. Blank sample was prepared as the sample preparation without lysine. The voltammogram was recorded by differential pulse technique in the range -0.8 V to -1.3 V. The derivative peak current was measured at a potential of - 0.98 V. The lysine concentration in functional food was determined by the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Electrochemical reduction

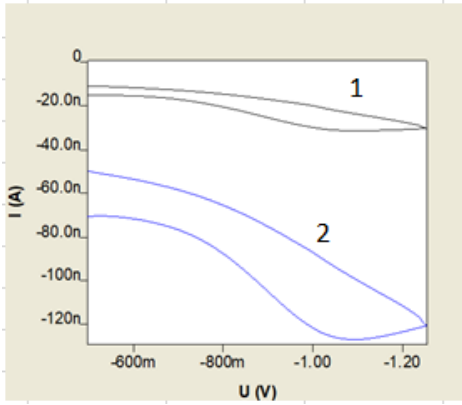


Figure 2. Cyclic voltammograms in BR buffer (pH = 8.0) and scan rate 20 mV s^{-1}, 1) blank; 2) 3×10^{-5} mol L^{-1} lysine

Figure 2 indicated that lysine has not directly electrochemical reaction on the HMDE, whereas the derivative of lysine has an electrochemical reaction [9]. The voltammograms of lysine derivatives were shown in figure 3. Cyclic voltammetry conducted on 3×10^{-5} M solution of lysine showed only a single irreversible reduction peak (Epc = − 1.1 V) in Britton-Robinson buffer (pH = 8.0) solution containing 0.5 M formaldehyde.

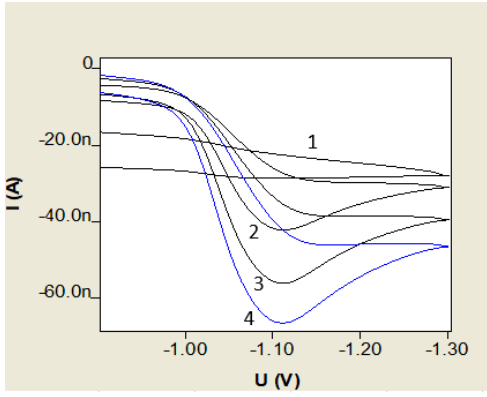
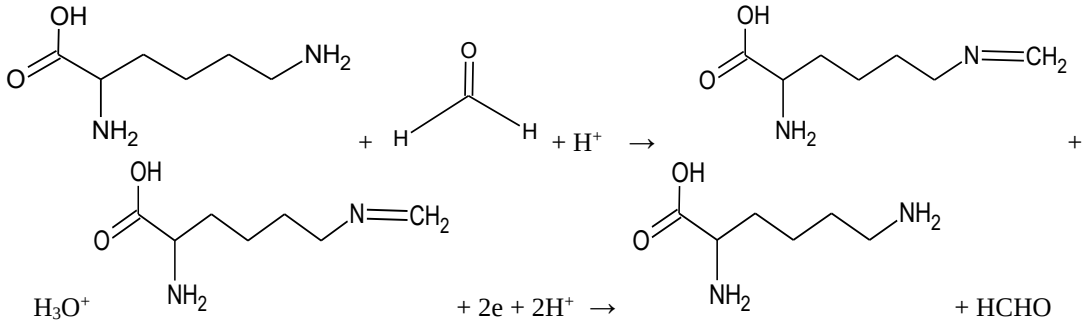


Figure 3. Cyclic voltammograms in BR buffer (pH = 8.0), 0.5 mol L^{-1} HCHO, scan rate 20 mV s^{-1}, 1) blank, 2) 3×10^{-5} mol L^{-1} lysine; 3) 4.7×10^{-5} mol L^{-1} lysine; 4) 6.4×10^{-5} mol L^{-1} lysine



The effect of scan rate on the reduction peak potential (E_{pc}) and peak current (I_{pc}) of lysine at HMDE was examined by cyclic voltammetry as the sweep rate ranged from 10 to 40 mV.s^{-1}.

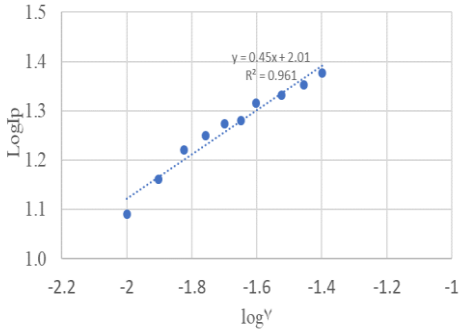


Figure 4. Cyclic voltammetry current (log I_p) vs. logν plot for 3x10^{-5} mg L^{-1} lysine, BR buffer (pH = 8.0), 0.5 mol L^{-1} HCHO, scan rate from 10 mV s^{-1} to 40 mV s^{-1}

Figure 4 confirmed the cyclic voltammograms of 3.10^{-5} mol. L^{-1} lysine in Britton Robinson buffer solution pH = 8.0 depending on the scan rate ranging between 10 and 40 mV.s^{-1}. The electrode reaction was irreversible as shown by the lack of an oxidation peak in the cyclic voltammogram.

The peak current (I_p) increased linearly with scan rates of 10 - 40 mV.s^{-1} according to the equation logI_p = 0.45 logν - 2.01; R^2 = 0.96 (figure 4). For such a relation, the slope values of 1.0 and 0.5 were expected for an ideal surface and solution reaction species [10]. Accordingly, the slope value (0.45) of the relationship (log I_p vs. logν plot) indicated that lysine was not adsorbed on the surface of HMDE. Therefore, the concentration of lysine should be determined by differential pulse voltammetry.

3.2. Optimization of conditions for determination of lysine

3.2.1. Effect of pH on the peak current

pH is a very important factor due on it's effect to the stability of the analyte peak potential peak current. The results showed that pH had affected both the peak current and potential. The largest peak current was obtained in pH ranging from 7.5 to 8.0 (Fig. 5). In this experiment, pH 8 was selected as the best pH value.

As pH increased, the reduction peak potential of lysine shifted to negative values and showed the linear relationship (Fig. 6). The regression equation was E_{pa}= 0.052 pH+0.06 (R^2 = 0.981). The slope value of dEpa/dpH was closed to 0.0592 V pH^{-1} corresponding to the one H^+ needed for one electron transferred.

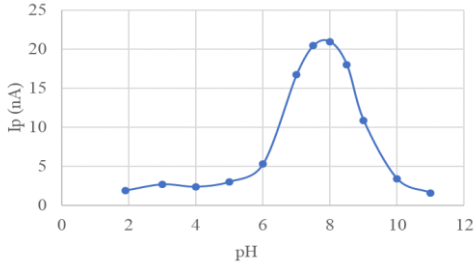


Figure 5. Effect of pH on the Ip of 3x10-5 mg L-1 lysine solution, 0.5 mol L-1 HCHO, scan rate 20 mV s-1, pH from 2.0 to 11.0

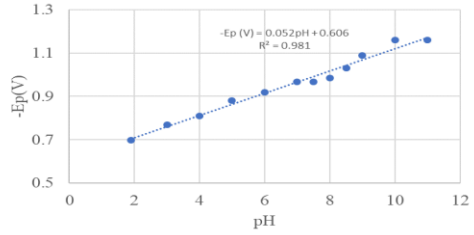


Figure 6. Effect of pH on the peak potential (E_p) of 3x10^{-5} mg L^{-1} lysine solution containing BR buffer and 0.5 mol L^{-1} HCHO; scan rate 20 mV s^{-1}, pH ranged from 2.0 to 11.0

3.2.2. Effect of formaldehyde concentration

Formaldehyde was added to the measurement solutions to form imine from lysine. The

experimental results in figure 7 showed that the peak current of lysine depended on the concentration of formaldehyde. However, as the HCHO concentration was greater than 0.5 mol. L^{-1}, peak current of lysine was insignificant effect. The optimum HCHO concentration was chosen at 1.0 mol L^{-1}.

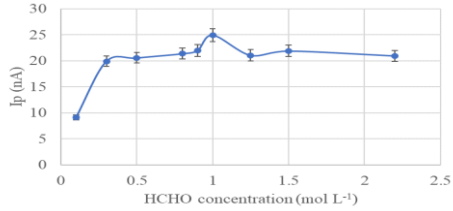


Figure 7. Effect of formaldehyde concentration on the peak potential (E_p) of $3x10^{-5}$ mg L^{-1} lysine solution, BR buffer pH = 8.0, scan rate 20 mV s^{-1}, HCHO concentration ranged from 0.1 to 2.2 mol L^{-1}

3.2.3. Effect of other parameters

To achieve good results such as high sensitivity and selectivity, several instrumental parameters such as scan rate, pulse amplitude were investigated at 1.7×10^{-5} mol L^{-1} of lysine solution (pH = 8.0). As the scan rate and pulse amplitude increased, the peak current increased but the peak width increased. Therefore, the scan rate of 20.0 mV s^{-1} and pulse amplitude of 50 mV should be used to obtain the suitable sensitivity and selectivity of the method.

3.3. Validation of the method

3.3.1. Linearity and IDL, IQL

Figure 8 indicated that the reduction peak currents increased linearly with the increasing amounts of lysine from 5.0×10^{-6} mol L^{-1} to 50.0×10^{-6} mol L^{-1}. The regression equation I_p (nA)=0.83 $C_x\times10^{-6}$ (mol L^{-1}) + 1.87, R^2=0,999 (n=7) showed the good linearity of the method.

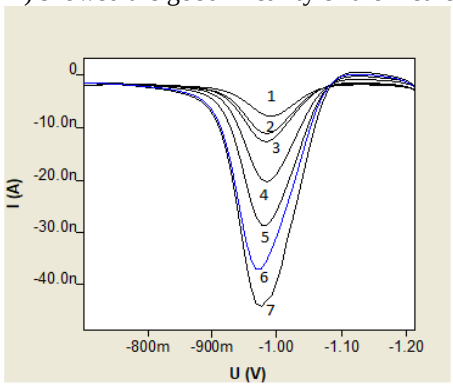


Figure 8. The voltammograms of lysine from 5.0×10^{-6} mol L^{-1} to 50.0×10^{-6} mol L^{-1}, pH = 8, scan rate 20 mv.s^{-1}, pulse amplitude of 50 mV, 1) 5.0×10^{-6}; 2) 8.0×10^{-6}, 3) 10.0×10^{-6}; 4) 20.0×10^{-6}, 5) 30.0×10^{-6}, 6) 40.0×10^{-6}, 7) 50.0×10^{-6} mol L^{-1}

The instrumental of detection (IDL) calculated based on 3×SD of the calibration was 1.3×10^{-6} mol L^{-1}. The IQL was to be found at 3.3×IOD was 4.5×10^{-6} mol L^{-1}.

3.3.2. Accuracy of the analytical method

The repeatability of the voltammetric instrument was assessed through 10 consecutive measurements with the same standard solution 3.0×10^{-5} mol L^{-1} of lysine under the optimum conditions on the same day. The average peak current was 25.91 nA with RSD of 5.3 %. These results confirmed that the proposed method had high repeatability and precision, which could be applied to determine lysine in drug samples.

To determine recovery of the developed method, the spiked sample which was prepared from B vitamin and lysine was added at two levels of concentrations 1.10^{-5} mol L^{-1} and 2.0 mol L^{-1}. The mean recovery of the developed method ranged from 92.5 % to 100.7 %.

4.1. Application of real sample analysis

The developed differential pulse voltammetry method was applied to the direct determination of lysine content in functional food samples by a standard addition method. The analytical results were compared with the HPLC [7] and presented in Table 1.

Table 1. The content of lysine in pharmaceutical formulations (n=3)

Commercial Pharmaceutical Formulations	Proposed voltammetric method (mg)	HPLC method (mg)
Baby green(3000 mg Lysine)	3100± 5	3064 ± 4
IQ Sirup (7500 mg Lysine)	7400 ± 10	7463 ± 7
Bioacimin gold (40 mg Lysine)	39 ± 1	40.3 ± 2.3
Lacto enzyme (500 mg Lysine)	511± 3	493.2 ± 6.8
Diaprid Tablets (4 mg Lysine)	4.0 ± 0.1	4.0 ± 0.4

The contents of lysine in the pharmaceutical formulation determined by the proposed voltammetric method were in good agreement

with standard HPLC method and the labeled values of the products. The calculated values are shown to be in a suitable range for analytical purposes, indicating that the proposed procedure is appropriate for lysine quantification in pharmaceutical formulations and functional food samples.

4. CONCLUSION

A fast, simple, and low cost differential pulse voltammetric method for the determination of lysine in pharmaceutical formulation functional food samples was developed. The method has been successfully applied to the determination of lysine in pharmaceutical formulation samples and functional foods without pretreatment and extraction. The results were in good agreement with the HPLC method and the labeled values. The method could be used in quality control analysis, clinical laboratories, and pharmacokinetic studies.

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