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Determination of Insulin Using a Pretreated Pencil Graphite Electrode

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Abstract: In this paper, pretreated pencil graphite electrode (Pre-PGE) was used for the determination of insulin by anodic stripping differential pulse voltammetry (DPASV). The influences of the pretreatment procedures, pH values, accumulation potential and time on the signal enhancement of insulin were optimized, and a novel electrochemical method for the determination of insulin was described. The currents obtained from DPASV measurements at optimum conditions (phosphate buffer solution pH 7.0), accumulation time (60 seconds), applied potential 400 mV *vs.* Ag/AgCl/3.0 mol L^{-1} KCl) were linearly correlated with the concentration of insulin. Calibration curve was obtained for insulin concentrations in the range of 5.0-200.0×10^{-9} mol L^{-1} .The limit of detection was found to be 2.0×10^{-9} mol L^{-1}.

Keywords: Pencil graphite electrode; Insulin; Anodic stripping differential pulse voltammetry.

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Introduction

Insulin is an important polypeptide hormone which controls the level of glucose in blood [1]. It is considered as a major clinic drug for treatment of diabetes mellitus [2]. Besides, insulin can serve as a predictor of diabetes of insulinoma and trauma [3]. For these reasons, the

determination of insulin has been of a significant interest for analytical and bioanalytical chemists. Recently, various methods and techniques, including eletrophoresis [4], liquid chromatography [5-7], high performance liquid chromatography [8,9] as well as electrochemical techniques [10-20] have been employed for this purpose.

When compared with other carbon based electrodes, PGEs have some advantages such as high electrochemical reactivity, commercial availability, good mechanical rigidity, disposability, renewability, low costs, low technology, and easy of modification [21-23]. Additionally, it was reported that PGEs offer a renewable surface, which is simpler and faster than polishing procedures, common with solid electrodes, and results in good reproducibility for the individual surfaces [24]. A remarkable number of studies on the usage of PGEs in electroanalytical applications have been reported [25-34].

In this paper, we described a simple and rapid method for the determination of insulin using anodic stripping differential pulse voltammetric technique at a pretreated pencil graphite electrode.

Experimental

Chemicals and Reagents

All the reagents including insulin were purchased in Sigma-Aldrich (Czech Republic). Deionized water was used in this study ($G \leq 0.055$ μS). Dissolved oxygen was removed from all the solutions by purging with argon for 15 min (purity 99.99 %, Linde Technoplyn, Prague, Czech Republic).

A 10^{-4} M solution of human insulin (recombinant, expressed in yeast) was prepared freshly with 0.1 M HCl and was kept in a dark bottom during the experiments. Phosphate buffer solution (PBS) was used as supporting electrolyte.

A three electrode system consisting of PGE (working), Ag/AgCl/3.0 M KCl (reference) and platinum wire (counter electrode) connected to PalmSens (Ivium Technologies, Netherland) was used for electrochemical measurement. Pencil leads with a diameter of 0.5 mm and a total length of 60 mm (Faber Castell, Vietnam) and a mechanical pencil Model T 0.5 (Rotring, Germany), which was used as the holder for the pencil lead, were purchased from a local bookstore in Canakkale, Turkey. Electrical contact to the lead

was obtained by wrapping a metallic wire to the metallic part of the holder. For each measurement, a total of 10 mm of lead was immersed into the solutions.

Preparation of the Pre-PGE

The Pre-PGEs were prepared by two different pretreatment methods using chronoamperometric and cyclic voltammetric techniques. In the first technique, the cyclic voltammograms were taken as the procedure reported in [22]. In the second way, the surface of PGE was pre-treated by applying a potential of +1.45 V for 60 s in the supporting electrolyte (0.1 M phosphate buffer solution containing 0.1 M KCl, pH 7.0). It is known that the pre-treatment at the positive potentials can increase the hydrophilic properties of the electrode surface which provides a good adsorption property to PGE, and hence, will increase the effectiveness of the pre-concentration step in the stripping techniques [32].

The results showed insignificant difference between the two techniques. In regarding the ease of the procedure, chronoamperometric method is simpler and quicker than cyclic voltammetric technique. Therefore, it was used for the electrochemical treatment of PG electrode in the subsequent experiments. The effect of pH on the electrochemical treatment of PG electrode was also investigated and pH 7.0 gave the best effect for the electrochemical pretreatment. In all cases, the Pre-PGE electrodes were immediately used after their pretreatment.

Results and Discussion

Enhancement Effect of Pre-PGE on the Oxidation of Insulin

The responses of insulin oxidation on Pre-PGE and PGE without pre-concentration step were compared using differential pulse voltammetry (DPV) in a BPS pH 7.0. As can be seen in Fig. 1, the oxidation peak was obtained from DPV at the potential of 0.63 V for both Pre-PGE (0.58 μA) and PGE (0.12 μA) in the presence of insulin. Besides, the DPV curve on PGE was very low or featureless in the absence of insulin, indicating that the peak at 0.63 V was mostly caused by the insulin oxidation. When the stripping step was applied, the oxidation current of insulin was also observed at the same potential (0.63 V) for both Pre-PGE (1.20 μA) and bare PGE (0.37 μA).

It is evident that the pre-concentration step significantly enhanced the oxidation of insulin since the current obtained from DPASV technique was about 2 times higher than that with only DPV.

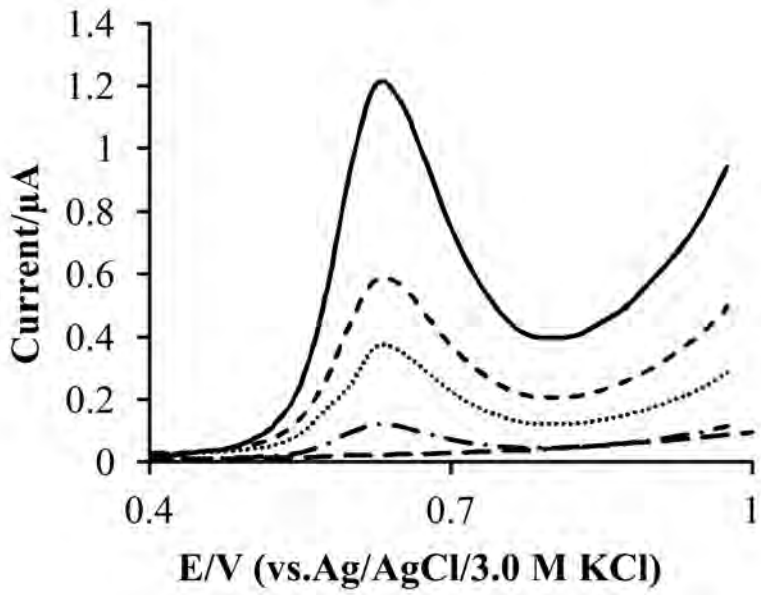

Fig. 1: *Differential pulse voltametry curves of* 0.15×10^{-6} *mol* L^{-1} *insulin (pH 7.0).* Bare PGE (-·-), bare PGE using stripping step (····), pre-treated PGE (----) and pre-treated PGE using stripping step (—) at accumulation potential 0.4 V and time 60 s. Bare PGE response in phosphate buffer solution (pH 7.0) served as control (— —).

The electrochemical behavior of Pre-PGE was investigated by recording cyclic voltammograms in a PBS (pH 7.0) at various scan rates in the presence of 1 μM insulin. As indicated by Fig. 2, the oxidation peak potential gradually shifted towards more positive direction with the increase of the scan rate. The oxidation currents increased linearly with the scan rate (Fig. 2; inlet) (I_a = 0.0056v + 0.0359; R^2 = 0.998) indicating that the electrochemical process of insulin at Pre-PGE was surface controlled.

The Determination of Insulin by DPASV

The variation of peak current with pH within the range of 6.0-8.0 is shown in Fig. 3A. It was found that the oxidation peak currents of insulin on Pre-PGE were insignificant different at pH 7.0 and the lower value. At pH values higher than 7.0, the oxidation current changed

slightly. In regarding to the pH value in physiological plasma, PBS pH 7.0 was therefore chosen as supporting electrolyte for the DPASV of insulin.

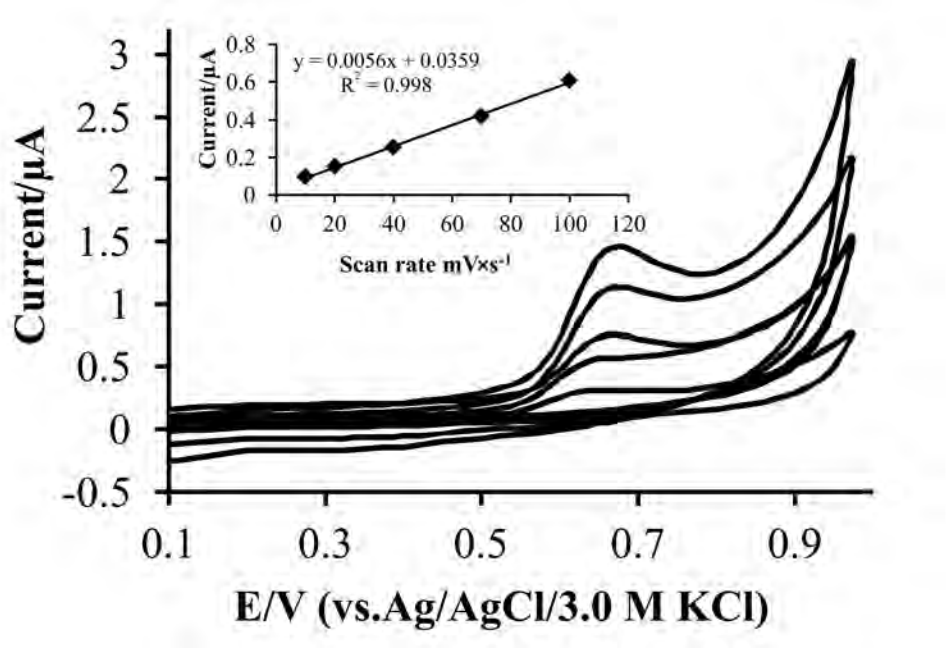

Fig. 2: *Cyclic voltammograms of Pre-PGE at various scan rates (from 10 to 100 mV* s^{-1}*) in the presence of* 2×10^{-5} *mol* L^{-1} *of insulin*. Inlet: the oxidation current of insulin (2×10^{-5} mol L^{-1}) at various scan rates. Experimental condition: Phosphate buffer solution pH 7.0; potential range from 0 to 1000 mV; potential step 25 mV.

The variation of anodic peak current with potential is shown in Fig. 3B. The current gradually increased with the increase of potential and reached the highest value at the potential 0.4 V. After that, the current decreased with the increase of potential. In the literature, it was mentioned that the deposition potential for anodic stripping techniques should be about 0.3 to 0.5 V more negative than the formal potential (E^{0f}) in the case of mercury electrode [35]. In our study, the deposition potential was 0.225 V more negative than E^{0f}.

The influence of accumulation time in the pre-concentration step is shown in the Fig. 3C. The peak current increased rapidly with the increase of accumulation time and reached the highest value after around 60 s. After that, the oxidation current decreased slightly with the increase of accumulation time which can be explained by the decrease of the conductivity of Pre-PGE at long accumulation time.

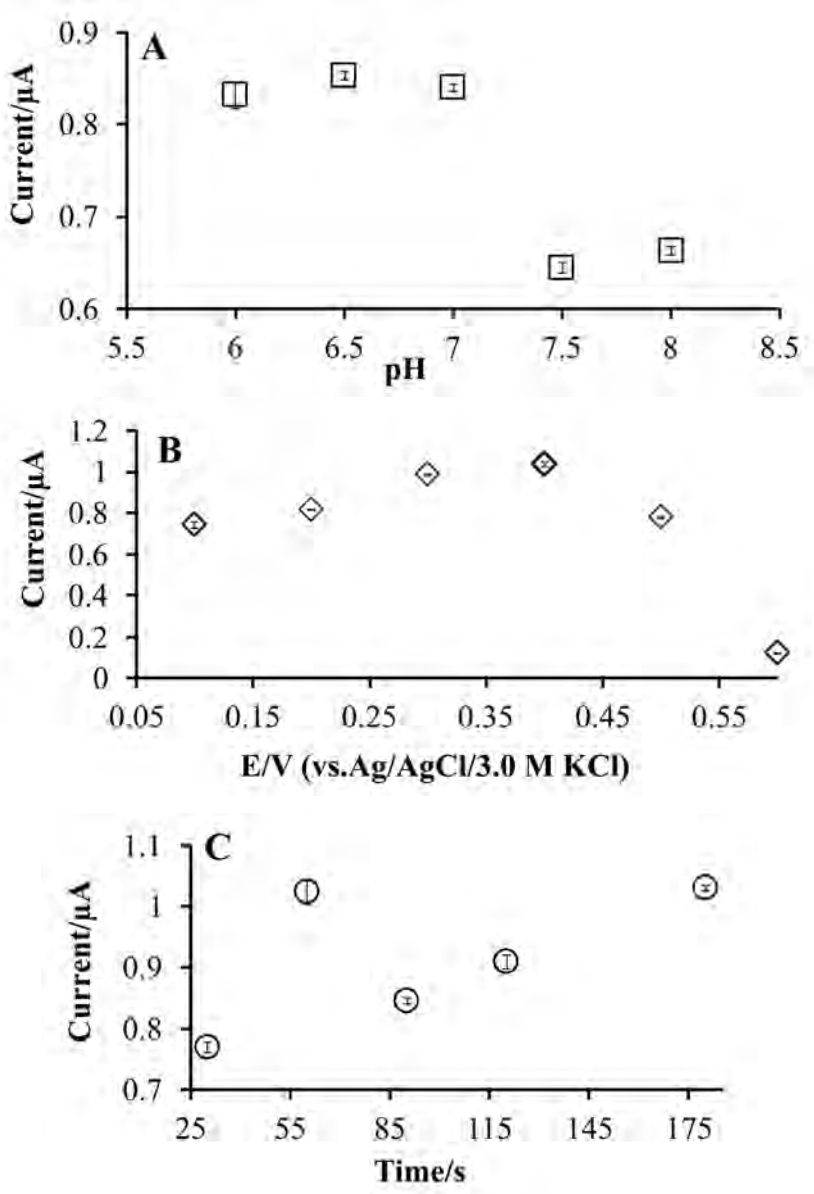

Fig. 3. *The oxidation peak currents of 0.15×10^{-6} mol L^{-1} insulin as a function of A) pH; B) constant potential and C) accumulation time.* Error bar represents the standard deviation of five measurements.

As a result, the optimum conditions for DPASV of insulin were chosen as following: supporting electrolyte (PBS pH 7.0), accumulation potential (0.4 V) and accumulation time (60 s). From these conditions, calibration curve was obtained over the concentration range of insulin: 5 - 200×10^{-9} mol L^{-1} (Fig. 4). The LOD using the equation LOD = 3s_b/m, where s_b is the standard deviation of the blank response and m is the slope of the calibration plot, was found to be 2.0×10^{-9} mol L^{-1}. This Pre-PGE electrochemical sensor is proven to be cheap, extremely sensitive and easy in preparation.

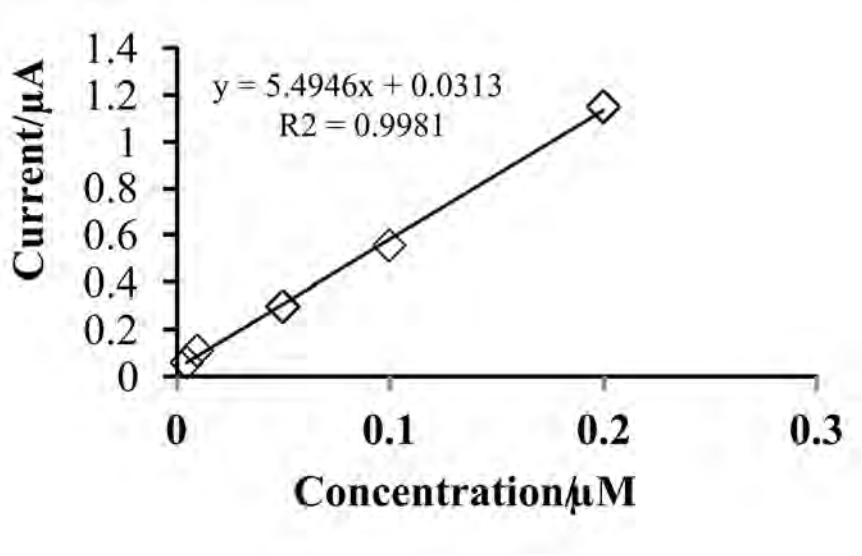

Fig. 4: *The plot of oxidation current vs. concentration of insulin using pre-treated PGE.* Phosphate buffer solution pH 7.0; accumulation time, 60 s; constant potential, 0.4 V.

The repeatability of the method was investigated by the repeated measurements of 50 nM insulin and 100 nM insulin (n = 20) and the relative standard deviation (RSD) was found to be 4.3 % and 5.1 %, respectively.

The linearity ranges and calculated LOD obtained from the Pre-PGE were compared with other modified electrodes. As can be seen from Table 1, the LOD of proposed method was lower than the compared modified electrodes. It could be concluded that the Pre-PGE had a significant effect on the DPASV for the determination of insulin. In addition, the preparation of the proposed method is simpler and cheaper than those compared.

Interferences Studies

The influence of some possible interference in the physiological plasma to the oxidation of insulin was studied. Pre-PGE showed a remarkable selectivity towards insulin oxidation by DPASV. The tolerable concentration ratios, with respect to 0.1 µM insulin were 80-fold for albumin, Ca^{2+}, and Mg^{2+} and 2000-fold for glucose. The determination gives an error less than ± 5.0 %.

Conclusion

In this study the electrochemical determination of insulin on the surface of Pre-PGE by DPASV was firstly investigated. This new kind of electrochemical sensor is proved to be of high sensitivity, excellent selectivity and reproducibility. It was shown that the pretreatment procedure remarkably enhanced the oxidation peak current of insulin comparing with the normal PGE. The detection limit of insulin was estimated to be 2.0×10^{-9} mol L^{-1} in this work.

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