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Research Article

Design of an Insulin Tracer Protein-Based Biosensor for Insulin Determination

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Abstract

Aim: The regulation of blood glucose levels is controlled by insulin, which is produced by the pancreatic beta system. Inadequate synthesis of beta insulin, results in elevated glucose levels, a condition known as diabetes, which can lead to various chronic health issues. In recent times, the diagnosis of diabetes, particularly type 1, has shifted towards the direct measurement of insulin levels. To facilitate this, an immunosensor was created to enable rapid and sensitive examination of insulin levels, with the goal of improving the quality for life for diabetic patients.
Material and Method: For this purpose, an insulin tracer protein based biosensor was designed for the determination of insulin at all solutions. For determination of insulin, electrobiochemical analyses were performed. Optimisation and characterisation studies were performed using differential pulse voltammetry. The performance of bioelectrochemical system was analysed by Receiver Operating Characteristic method.
Results: The insulin biosensor cyclic woltammogram was obtained between -0,1 and 0,6 V potantial. At 0,45 V was found as the anodic peak side for determination the insulin. Optimisation and characterisation studies performed at 0,45 V with differential pulse voltammetry.
Conclusion: The study successfully identified stable and easy-to-use insulin concentrations, indicating the potential of the newly developed immunosensor for applications in clinical biochemistry laboratories.

Keywords: Insulin tracer protein, biosensor, insulin

INTRODUCTION

Insulin is secreted by the beta system of the pancreas to take part in blood glucose level regulation when the plasma glucose level increases (1). An increase in blood glucose levels due to the inability to synthesize sufficient beta insulin is known as diabetes and is seen a cause of chronic kidney disease, cardiological disease and blindness in humans (2). The clinical diagnosis of diabetes is type 1 and type 2, mainly due to transmission (3). While the hyperglycaemic picture seen due to insulin not being synthesised as a result of beta damage marks type 1, decreased insulin secretion or tissue resistance to insulin causes permanent type 2 (4,5). In the early stages of type 2, insulin synthesis increases to regulate the plasma glucose level, but insulin resistance is prevented. The flow of time in the beta system cannot be maintained and hyperglycemia develops. 90% of diabetes cases worldwide are type 2 (3). Those with suspected diabetes or insulin irregularity may face hyperglycemia and related diabetes complications for a long time before diagnosis. Diabetes and the amount it causes can be kept under control by regularly monitoring the amount of insulin (2). For years, they have been trying to protect themselves from the consequences of diabetes by monitoring blood sugar levels using glucose biosensors. However, insulin is an alternative method for controlling blood glucose levels in patients with diabetes, and insulin obtained as a result of incorrectly evaluated glucose levels can cause serious hypoglycemic or hyperglycemic conditions. This will provide a healthier approach for devices that directly monitor diabetes by measuring insulin rather than glucose (6). Biosensor devices have recently taken their place in new studies for use in laboratory examinations due to their high sensitivity, short-time results and low cost advantages. Various studies have attempted to develop insulin detection devices, but insufficient

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sensitivity, difficulty in detecting in the correct range, and stability problems have caused problems in measurement techniques. Laboratory tests used for insulin detection are time-consuming and require expensive equipment and experienced personnel (6).

Glucose monitoring plays a crucial role in the initial diagnosis and ongoing management of diabetes mellitus. However, alongside monitoring glucose levels, keeping track of insulin levels is equally essential as it offers valuable insights into the progression and control of the condition. Therefore, the monitoring of insulin levels holds significant medical significance in the management of diabetes (2,6).

The electrobiochemical method for insulin determination presents novel perspectives for laboratory diagnosis in contrast to conventional approaches. While traditional methods rely on specialized and costly medical equipment, bioelectrochemistry provides the advantage of affordable and portable devices (3). The insulin biosensor investigated in our study holds promise as a model for the development of user-friendly and convenient portable/wearable medical devices.

Therefore, in our study, an immunosensor was developed to perform rapid and sensitive examination of insulin levels to ensure regular insulin monitoring in order to maintain the quality of life of diabetic patients. With the developed immunosensor, shelf-stable and easy-to-use insulin concentrations can detected.

MATERIAL AND METHOD

Chemicals

All biochemical solutions were freshly prepared before the experiment. The source of all the chemicals acquired was SPI bio Inc, a company situated in France. The experiment involved the utilisation of various solution concentrations to identify the most favourable working conditions and to select the optimal potential differences for further investigation. For determining insulin, insulin tracer proteinwas used. Stock insulin ($5x10^{-3}$ M) was diluted 100-fold.

No biological samples from humans or animals were used in the studies. All solutions used are commercially available products. For this reason, ethics committee approval is not required in this study.

Apparatus

In order to conduct the electrobiochemical measurements, the experimental setup involved the utilisation of a gold working electrode, which was paired with the Ag/AgCl reference electrode and the Au/Pd auxiliary electrode. The electrochemical potential was measured using an Ivium potentiostat (Netherlands).

Statistical Analysis

Statistical analysis performed by SPSS 20 program.

Biosensor System Preparation

Before the formation of the bioactive layer, the gold working electrode surface underwent polishing using alumina slurries on microfiber to achieve a pristine surface. The pristine gold working electrode was then washed twice with distilled water to eliminate any remnants of the alumina slurries. Subsequently, the electrode was sonicated in pure ethanol to eliminate any undispersed absorbable particles and was washed twice with distilled water (7). Moving on to the next stage, for the immobilisation of the insulin tracer protein, the gold electrode was initially coated with BSA and gelatine through a self-assembled monolayer (SAM) (8). The Au electrode was then paired with the Ag/AgCl as reference electrode. During the electrode preparation for insulin determination, insulin tracer protein was immobilised on a pre-made Au electrode. To achieve this, 250µl of insulin tracer protein was deposited in an Eppendorf tube.

RESULTS

Immobilisation

The research conducted demonstrated that through the utilisation of SAM in the immobilisation process, a durable bioactive film was established on the substrate, effectively inhibiting the detachment of bioreceptors from the surface. Consequently, this approach led to enhanced stability during prolonged utilisation of the insulin tracer protein.

Electrochemical Characterisation of the Insulin Biosensor

Insulin biosensors were employed to analyse the electrochemical properties of biosensors through the use of cyclic voltammetry. Cyclic voltammograms were employed to identify the potential point of 0.45 V in the study. The biosensor working range cycle woltammogram shown as Figure 1.

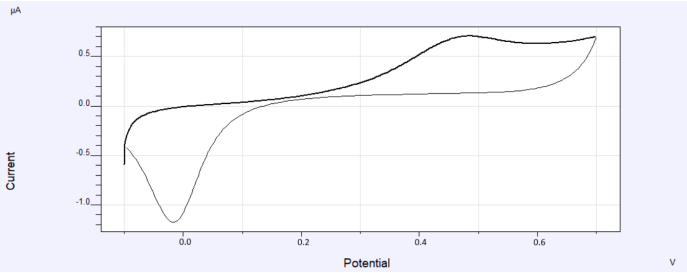

Figure 1. Biosensors cyclic voltammogram (phosphate buffer solution at a 0.3 Vs−1 scan rate)

Optimisation of the Bioactive Layer of the Biosensor

Biologically active films play a crucial role in the operation of biosensors by serving as a medium for detecting and quantifying analytes (9). These layers facilitate the identification and specificity of particular analytes. To enhance the performance of the bioactive layer in biosensors, the impact of insulin tracer protein concentration and polymerisation time on the electrochemical measurement was examined (10). The differential pulse voltammetry curve of the optimised biosensor is shown in Figure 2.

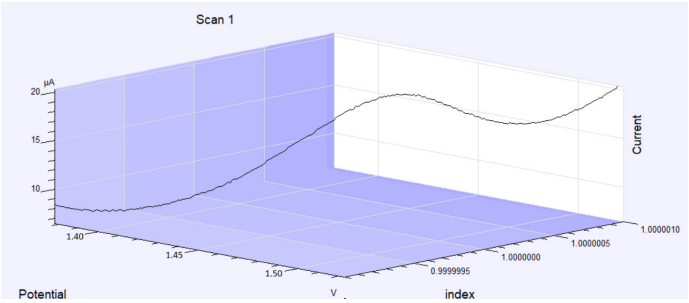

Figure 2. Differential pulse voltammetry of insuline biosensor

Effect of Insulin Tracer Protein Concentration

The concentration of the insulin tracer protein plays a crucial role in determining the sensitivity of the biosensor, as the potentiometric responses of the biosensor rely on the insulin tracer protein's activity (11,12).

In order to investigate the insulin tracer protein's impact on the biosensor's response's, various concentrations of the insulin tracer protein (0.05, 0.1, 0.2, 0.4, and 0.6 mg/mL) were utilised.

The experimental findings revealed that a well-defined calibration curve was achieved when the bioactive layer of the biosensor contained 0.2 mg/mL of insulin tracer protein shown as Figure 3.

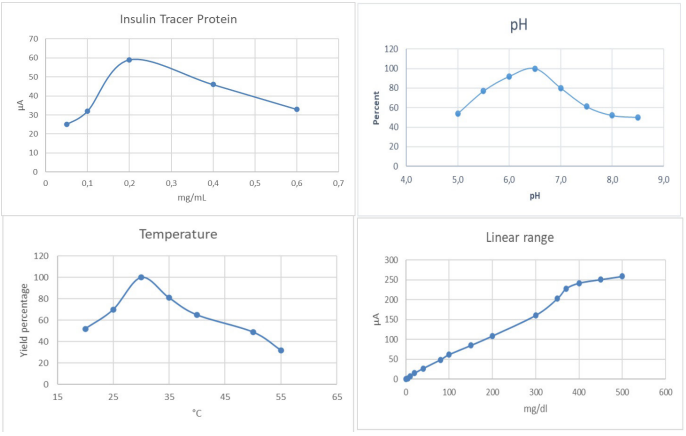

Figure 3. Optimisation and characterisation of insulin biosensor

Biosensor Working Condition Optimisation

pH effect on the biosensor response

The achievement of optimal responses from insulin tracer proteins depends on the presence of a suitable pH medium (13). In order to assess the impact of pH on the biosensor response, various buffers were employed at different pH levels. These buffers consisted of acetate (pH 5.0–5.5) at a concentration of 50 mM, phosphate (pH 6.0–6.5–7.0–7.5), and Tris-HCl (pH 8.0–8.5). The most favorable pH value, determined by a 100% activity rate, was 6.5. Deviations from this optimal pH value, either above or below, resulted in a decline in the biosensor response, as depicted in Figure 3.

According to existing literature, the appropriate pH range for insulin tracer protein is between 6.5 and 7.5. Figure 4 demonstrates that an increase in pH beyond 6.5 reduces in signal intensity due to a loss of insulin tracer protein activity. The structure of is characterized by the presence of three disulphide bridges, which play a crucial role in maintaining the insulin tracer protein's stability and activity. pH values exceeding 6.5 can disrupt the protein structure, causing alterations in the protein structure and subsequent activity loss. Under acidic conditions, the protonation of amino acids may result in a positive charge, thereby influencing the protein's activity through modifications in the electrostatic interactions among charged residues within the insulin tracer protein's active site. Furthermore, a pH under 6.5 inhibits the function.

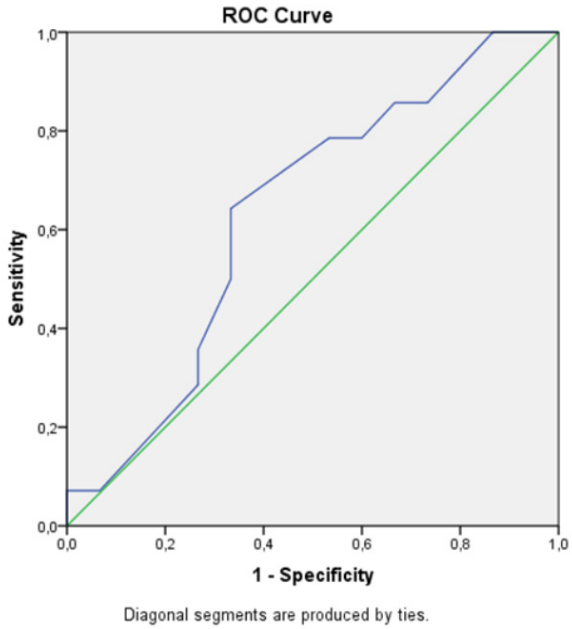

Figure 4. Insulin biosensors ROC curve

Temperature effect

The investigation aimed to assess the impact of temperature on the response of the biosensor (14), thus, a range of temperatures (20-55°C) was analysed. The optimal working temperature for the biosensors was 30°C. Notably, the biosensor response exhibited a direct correlation with temperature up to 30°C, after which any subsequent rise in temperature resulted in a decline in the biosensor response (Figure 3).

Electrobiochemical Analyses Analytical Characteristics

Range of linear detection

The term "linear detection range" pertains to the range of values within which a biosensor is capable of measuring (15). In this study, a linear calibration curve was established by measuring concentrations of insulin ranging from 10 to 370 mg/dL under the most favourable experimental conditions. The quantification and detection limits were determined on the basis of well-defined calibration curves for an insulin concentration of 50 mg/dl, with a sample size of 10 (Figure 3).

Repeatability and Reproducibility

Repeatability refers to the degree of consistency in the detection outcomes of a particular technic within a short

time frame (16). In the case of the biosensor repeatability experiments, the concentration of insulin used was maintained at 50 mg/dl. After conducting 15 consecutive measurements, the standard deviation of the insulin sample with a concentration of 50 mg/dl was 2.9%, where the coefficient of variation was determined to be 11%. On the other hand, reproducibility pertains to the level of agreement in the measurement results of a method across different days. Based on the conducted experiments, it was observed that when the working electrode is stored at a temperature of +4°C, it can be effectively utilized for a duration of 43 days. After 43 days shelf life duration insulin biosensor yield obtained under the 80%.

Receiver Operating Characteristic (ROC) Analyses

The ROC curve serves multiple purposes, including assessing the discriminatory ability of a test, establishing the optimal positivity threshold, monitoring the accuracy of laboratory findings, and comparing the diagnostic efficacy of different tests (17,18). In our analysis, we found that the area under the ROC curve amounted to 63.3% (Figure 4). It is worth noting that if the area surpasses 50% in the ROC curve analysis, it indicates a successful test outcome.

DISCUSSION

Immobilization Efficiency and Stability

The effectiveness and robustness of immobilization techniques play a vital role in enhancing the stability of bioactive films on substrates (19). Utilizing SAM has been shown to be particularly effective in preventing the detachment of bioreceptors, thereby improving the overall stability of biosensors for prolonged durations. This attribute is essential for ensuring the reliability and longevity of biosensors in real-world applications where long-term sensor stability is imperative (20).

Electrochemical Behavior and Optimization

The precise localization of a particular potential site and the enhancement of the bioactive film are crucial stages in attaining a responsive biosensor that is both sensitive and specific (21). The ideal concentration of 0.2 mg/mL for the insulin tracer protein was determined to be optimal, highlighting the significance of adjusting biosensor elements meticulously to improve overall functionality.

Impact of Physicochemical Conditions

The findings regarding the pH and temperature effects are in line with the established understanding that biosensor responses are highly sensitive to environmental conditions (22,23). The optimal conditions identified for pH and temperature are crucial for maintaining the functional activity and structural integrity of the insulin tracer protein, thereby ensuring accurate biosensor readings.

Analytical Performance

The biosensor's analytical reliability is confirmed through the linear detection range, repeatability, reproducibility, and ROC analysis. The biosensor's potential for consistent and reliable measurements is highlighted by the defined linear range, high level of repeatability, and reproducibility. Additionally, the ROC analysis serves to validate the biosensor's diagnostic performance, establishing it as a valuable tool in the analytical field (24,25).

CONCLUSION

When compared to existing methods with our electrobiochemical method for insulin determination, our method offers new insights for laboratory diagnosis. Existing traditional methods need some spesific and expensive medical devices however bioelectrochemistry offers portable low costs devices. The insulin biosensor we were studied on has the potential to be a reference for new simple-to-use portable/wearable devices for medical use.

Biosensors for insulin offer cost-effective, time-efficient, sensitive, and practical solutions. These studies have demonstrated that biosensors are highly sensitive, specific, straightforward, and valuable techniques, typically utilizing immobilised insulin tracer protein forms. The advancement of biosensor technology has significantly enhanced the efficiency of obtaining economical, practical, specific, and sensitive insulin detection results. The newly developed insulin biosensor shows great potential for utilisation in clinical biochemistry laboratories.

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Conflict of interest: *The authors have no conflicts of interest to declare.*

Ethical approval: *No biological samples from humans or animals were used in the studies. All solutions used are commercially available products. For this reason, ethics committee approval is not required in this study.*

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