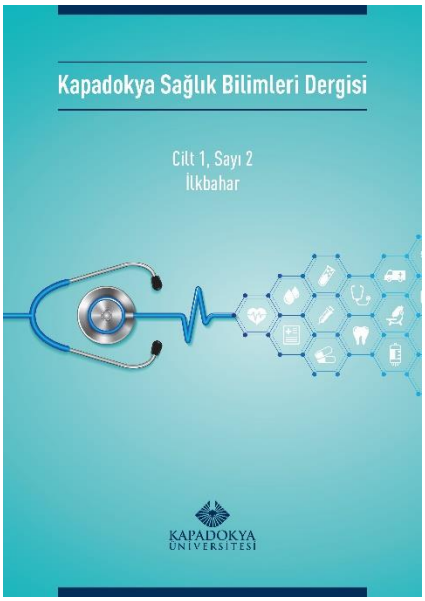

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Research Article

A Preliminary Study of A Lactic Acid Biosensor for the Early Detection of Dental Caries

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A Preliminary Study of A Lactic Acid Biosensor for the Early Detection of Dental Caries

Abstract

Statement of Problem: Many mechanisms in the formation of dental caries, but the common point of these mechanisms is the proliferation of microorganisms in the mouth and mostly on the tooth surface.
Objective: Although there are approximately 200 to 300 different microorganisms in human dental plaque, Neisseria spp. and S. mutans. The common point of these microorganisms is that they produce lactic acid. In this study, it is aimed to use a biosensor, which is a bioelectrochemical measurement system that combines the measurement capability of physical analysis systems and the specificity of biochemical systems.
Material-Method: In this study, lactate oxidase enzyme was immobilised on the gold working electrode surface by means of bovine serum albumin and gelatine. The enzyme was arrested on the electrode surface with the aid of the cross-linking agent glutaraldehyde. Cyclic voltammetry current scanning was performed in lactic acid biosensor preliminary studies. Optimisation studies were conducted in the current range obtained from the peak in the cyclic voltammogram. Within the scope of the optimisation studies, optimum operating temperature and optimum operating pH of the biosensor were tested.
Results: An anodic peak at +0.2 V potential was obtained in the cyclic voltammogram drawn according to the reaction on the working electrode surface. In the optimisation studies conducted, it was found that the optimum operating conditions of the biosensor were at 35 °C and pH 6.5.
Conclusion: According to the data obtained from the pre-study of the bioelectrochemical method, the concentration of lactic acid in the solution can be determined. With the completion of further studies, a portable lactic acid biosensor can be made. As a result, as a result of the preliminary study, the lactic acid biosensors designed to determine in vitro lactic acid for the early diagnosis of dental caries in the future is promising.

Keywords: Biosensor, Dental Caries, Lactic Acid, Lactate Oxidase.

Diş Çürüğünün Erken Tespitine Yönelik Laktik Asit Biyosensörü Ön Çalışması

Öz

Problem: Diş çürüğünün oluşumunda birçok mekanizma vardır ancak bu mekanizmaların ortak noktası mikroorganizmaların ağızda ve çoğunlukla diş yüzeyinde çoğalmasıdır.
Amaç: İnsan diş plağında yaklaşık 200 ila 300 farklı mikroorganizma bulunmasına rağmen, en fazla Neisseria spp. ve S. Mutans bakterileri diş çürüğünden sorumludur. Bu mikroorganizmaların ortak noktası laktik asit üretmeleridir. Bu çalışmada, fiziksel analiz sistemlerinin ölçüm kabiliyeti ile biyokimyasal sistemlerin özgüllüğünü birleştiren bir biyoelektrokimyasal ölçüm sistemi olan biyosensör kullanılması amaçlanmaktadır.
Gereç-Yöntem: Bu çalışmada laktat oksidaz enzimi, altın çalışma elektrot yüzeyine sığır serum albümini ve jelatin yardımı ile immobilize edildi. Enzim, çapraz bağlayıcı ajan glutaraldehit yardımıyla elektrot yüzeyinde tutuklandı. Laktik asit biyosensörü ön çalışmalarında döngüsel voltametri akım taraması yapılmıştır. Döngüsel voltamogram grafiğinden elde edilen akım aralığında optimizasyon çalışmaları yapılmıştır. Optimizasyon çalışmaları kapsamında biyosensörün optimum çalışma sıcaklığı ve optimum çalışma pH'ı test edilmiştir.
Bulgular: Çalışma elektrodu yüzeyindeki reaksiyona göre oluşan döngüsel voltamogramda +0,2 V potansiyelde anodik pik elde edildi. Bu değerde yapılan optimizasyon çalışmalarında biyosensörün optimum çalışma koşullarının 35 °C ve pH 6.5 olduğu tespit edilmiştir.
Sonuç: Biyoelektrokimyasal yöntemin ön çalışmasından elde edilen verilere göre, çözeltideki laktik asit konsantrasyonu belirlenebilir. İleri çalışmaların tamamlanması ile taşınabilir bir laktik asit biyosensörü yapılabilir. Sonuç olarak, yapılan ön çalışma sonucunda gelecekte diş çürüğünün erken teşhisi için in-vitro laktik asit belirlemek üzere tasarlanan laktik asit biyosensörlerinin umut verici olduğu düşünülmektedir.

Anahtar Kelimeler: Biyosensör, Diş Çürüğü, Laktik Asit, Laktat Oksidaz.

INTRODUCTION

Dental caries is the irreversible loss of material caused by organic acids produced in bacterial plaques. Although they do not cause life-threatening pain, pulpitis pain is significant and disturbing to the patient (Borg-Bartolo et al., 2022; Okolo et al., 2022).

The problems caused by dental caries constitute a significant economic burden in many underdeveloped or developing countries. Therefore, early diagnosis and treatment of caries is extremely important (Benn et al., 2022; Matthews, 2022).

It is necessary not to evaluate tooth decay as a simple mineral loss (Okolo et al., 2022; Zhang et al., 2022). It is quite a complex event. In order to explain this complex phenomenon, it is necessary to examine the chemical structures that play a role in dental caries (Acid formation - Reaction environment - Tooth structure) (Folayan et al., 2022; Kimmie-Dhansay and Bhayat, 2022).

In a chemical reaction, the reactant (acid), the reaction medium and conditions (plaque liquid-tooth) and the reaction product are in question. If there is reversal in a chemical reaction, the reaction is reversible, and if there is no reversal, it is irreversible (Benn et al., 2022; Korona-Glowniak et al., 2022; Molina et al., 2022).

In dental caries, acid in the oral environment and dental hard tissues react for a certain period of time, and as a result, caries occur as an initially reversible reaction and later as an irreversible reaction. In enamel and dentine, the inorganic structure is destroyed by acids, whereas the organic structure is destroyed by ferments (Benn et al., 2022; Moriyama et al., 2022; Zhang et al., 2022).

Acids are formed in the mouth for the following reasons:

1) As a result of the activity of oral bacteria, with the breakdown of carbohydrates, (Zhang et al., 2022).

2) People who are in constant contact with acids due to their profession (battery factory workers, laboratory workers, chemists, etc.), breathing acidic air due to air pollution, (Borg-Bartolo et al., 2022).

3) Some acidic beverages (Cola, some fruit juices, some herbal teas, etc.) (Fernandez-Bonet et al., 2022).

4) Frequent vomiting events in pregnancy and chronic alcoholism, gastric juice coming into the mouth in reflux disease, some acidic drugs taken by chewing (Aspirin, some effervescent vitamin tablets, etc.) (Nizami et al., 2022).

Ferments are:

1) They are partly formed by oral bacteria, (Benn et al., 2022)

2) They arise partly from the hard tissues of the teeth, partly from the gingiva and saliva (Benn et al., 2022; Zhang et al., 2022).

The Role of Microorganisms In The Formation of Caries

They are one of the 3 main factors (carbohydrate, microorganism, dental tissue itself) examined in the formation of caries. Several microorganisms are involved in the formation of caries. Approximately 200–300 species of microorganisms in human dental plaque (Benn et al., 2022; Zhang et al., 2022).

The main groups of caries-causing microorganisms are Streptococci, Lactobacillus, Actinomyces.

The first settlers of the tooth surface were mostly Neisseria spp. and Streptococci, including S. mutans. The increase in the number of these precursor species and their metabolism change the conditions of the environment (Ex: pH, coagulation, presence of substrate, etc.) in a way that causes more effective organisms that will form dental plaque to settle here after them. S. mutans, with S. sobrinus, plays a major role in dental caries by converting sucrose to lactic acid with the enzyme Glucansucrase. Determining the intraoral concentration of lactic acid, which is given to the environment by the microorganisms that cause dental caries, will provide information about the formation of dental caries (Assiri et al., 2022; Moriyama et al., 2022; Wei et al., 2022; Zhang et al., 2022).

A system that can detect the presence of dental caries and prevent it with early diagnosis is possible through biosensors. Biosensors are measurement systems in which biochemical mechanisms are combined with physical sensors. Biosensors basically consist of two parts; the first of these is the biochemical sensor and the other is the physicochemical converter (Tsvik et al., 2022). The biochemical sensing moiety can be a biocatalyst (enzyme, microorganism, tissue materials) or bioligand (antibody, nucleic acid, etc.) that interact specifically with the target analyte. The transducer transforms the physical response resulting from the interaction between the target analyte and the biological sensor into a measurable signal (Goode et al., 2015; Kaur et al., 2015). Biosensors, which provide the opportunity to monitor and understand biological and synthetic processes, are one of the most preferred research areas in medical measurement and analysis today (Goode et al., 2015).

In this study, a biosensor was designed to determine lactic acid. The data obtained from this biosensor preliminary study will be used to measure the amount of intraoral lactic acid without intervention in further studies.

MATERIALS AND METHODS

All solutions prepared for use in biosensor preliminary studies were freshly prepared. Bovine serum albumin, gelatine, lactate oxidase enzyme and glutar aldehyde were used in the bioactive layer covering the electrode surface and where the reaction took place. Different concentrations of lactic acid solutions were used as the substrate. All the chemicals were purchased from Merck.

Bovine serum albumin and gelatin, which are used as bioactive layer components to bind the lactate oxidase enzyme to the gold working electrode surface, were applied and bonded to the electrode surface, respectively, to cover the electrode surface. Then, the lactate oxidase enzyme was immobilised to the surface to form a self-forming monolayer. The bioactive layer was attached to the electrode surface by the crosslinking agent glutaraldehyde. In order to remove unbound molecules from the environment, the electrode was washed 3 times with working buffer solution.

When lactic acid reacts with the enzyme lactate oxidase, pyruvate is formed and hydrogen peroxide is formed. Working principle of biosensor; It is based on the principle that the potential change that occurs as a result of the reaction is determined by the biosensor. The enzymatic reaction is given in figure 1.

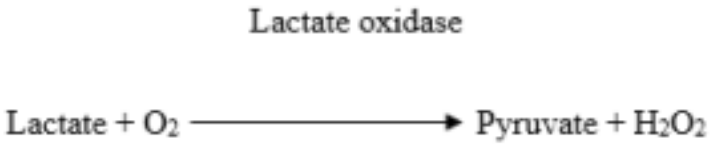

Figure 1. Lactate oxidase enzyme reaction

The prepared working electrode was immersed in the working cell together with the counter platinum electrode and reference Ag/AgCl electrode. In order to find the potential change in response to the constant current, a current scan was performed between -1.0 V and +1.0 V and optimisation studies were carried out at the point where the potential change was observed. In the optimisation studies, the optimum working temperature and optimum working pH of the biosensor were found. In the optimisation studies, the values with the highest potential change were determined as the optimum values.

RESULTS

Determining the Working Range

Cyclic voltammogram scans were performed in the -1.0 to +1.0 V current ranges. According to the results obtained from the cyclic voltammogram, the operating range was found to be between -0.5 and 0.5 V constant current. As a result of these studies, an anodic peak at + +0.2 V potential was obtained. The cyclic voltammogram obtained in the studies is given in figure 2.

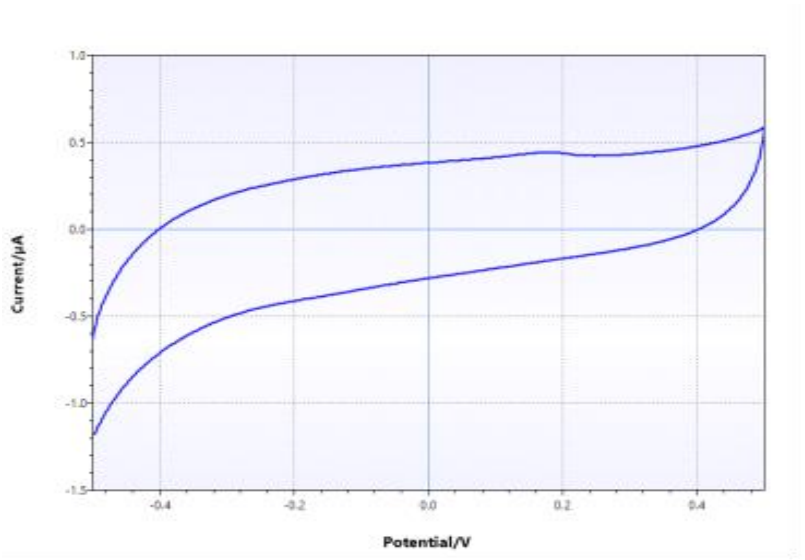

Figure 2. Cyclic voltamogram

Within the scope of optimisation studies, it was aimed to determine the optimum working conditions of the biosensor. Buffers with different pH values were prepared. The optimum working pH was observed as pH 6.5. (Figure 3).

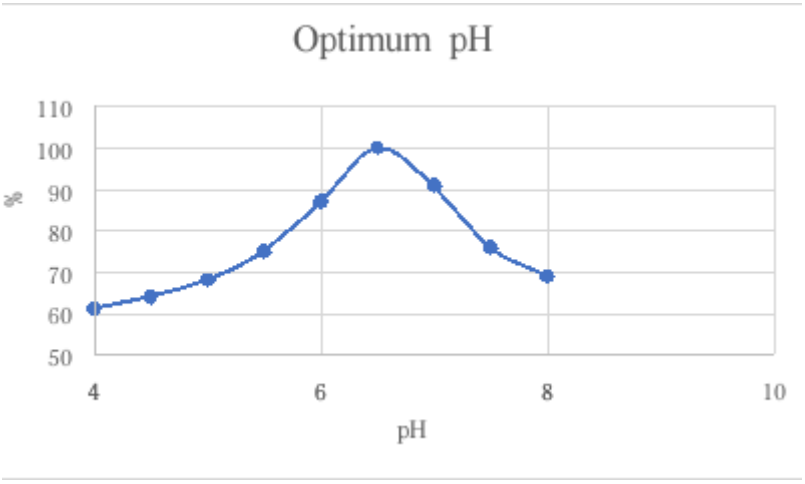

Figure 3. Optimum pH graph.

In the ambient temperature studies carried out to determine the operating temperature, which is another optimisation parameter, a work cell with a water jacket was preferred. The optimum operating temperature was determined by adjusting the degree of water passing through the water jacket. According to the optimum operating temperature studies, the temperature of 35 °C was determined as the optimum operating temperature of the biosensor (Figure 4).

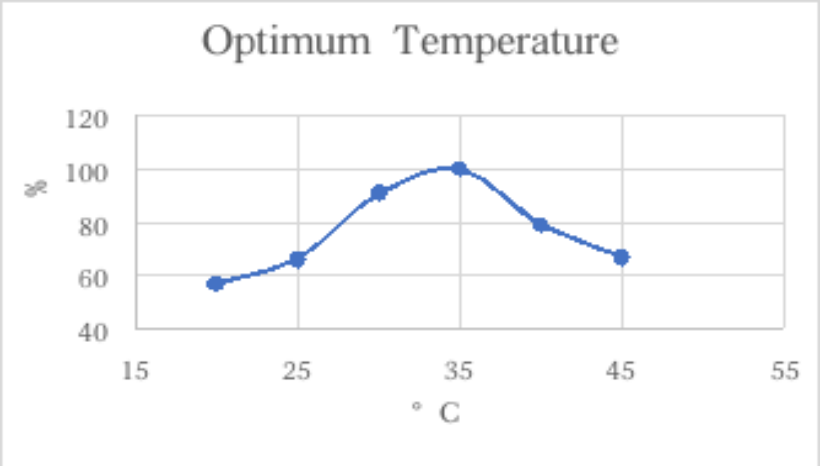

Figure 4. Optimum temperature graph

DISCUSSION

In this study, a pre-study of a biosensor was carried out, which enables in vitro measurement of lactic acid concentration in a shorter time and at a lower cost. The working conditions and optimisation studies of this prepared biosensor were carried out. After the scanning speed and working range were determined, optimum pH and temperature concentrations were found.

In the study, measurements were made between 20 and 45 °C temperature values to determine the optimum temperature. The optimum temperature for maximum activity was found to be 35 °C. Higher temperatures were not increased as there would be enzyme denaturation at higher temperatures. When the studies on the subject in the literature were examined, it was seen that the working temperature of the lactate oxidase enzyme was in the range of 30–40°C (Hamilton et al., 2022; Zhang et al., 2022).

Working environmental conditions are important for the activities of enzymes and the most important factor affecting this activity is the pH of the environment. Determining the pH of the environment after the immobilisation is important in terms of determining the working conditions of the biosensor. Determining the optimum pH value with which the enzymes we use on the surface of the bioactive layer can work together is one of the key factors that increase the sensitivity of the biosensor.

In order to determine the biosensors working pH, measurements were taken at different values between pH 4 and 8 and the optimum working pH was found to be 6.5. In the literature review, it was seen that the pH range of the lactate oxidase enzyme was in parallel with our study (Chaudhry et al., 2022; Leonard et al., 2022).

Order To prevent the decrease in enzyme activity in the prepared biosensor bioactive layer, the number of bonds made with bovine serum albumin and gelatine enzyme was reduced. Enzyme arrest was made with glutaraldehyde, which is used as a crosslinker, to prevent the enzyme with reduced bond number from leaving the bioactive layer.

Nowadays, different spectrophotometric, colorimetric and chromatographic methods are used to determine lactic acid concentrations. Biosensors have more important advantages than other methods. The advantages are practical and economical (Garcia-Rey et al., 2021; Olaetxea et al., 2019; Zhou et al., 2022). Considering the advantages of the lactic acid sensor prepared in this study, it is thought that it can be routinely preferred for in vitro lactic acid determination in serum, urine, saliva and other biological fluids in hospitals and private laboratories. (Hamilton et al., 2022; Mirzaei et al., 2019)

CONCLUSION

In this study, a biosensor was prepared to be used in an in vitro assay for the development of a biosensor for the determination of lactic acid amount. This prepared electrode was immobilised by cross-linking the lactate oxidase enzyme glutaraldehyde on the graphite electrode using BSA/gelatin for specific binding of uric acid. The optimum conditions of the prepared enzyme electrode were determined. The performance of the biosensor in standard solution samples was evaluated and lactic acid quantification was performed.

Selective enzymes were used to be successful in further studies. The as - prepared sensor had high selectivity for lactic acid and is also reproducible.

Biosensor studies attract great importance because of their advantages compared to other methods (Kaur et al., 2015). By using the method we developed in our study, a portable and non-invasive lactic acid determination device can be produced with further studies.

As a result; biosensors, which are fast, portable, economical devices that do not require trained personnel; Today, they are necessary devices to be used in both industry and academic studies in terms of working with too many samples (Goode et al., 2015).

In addition, as a result of the increasing interest in biosensors around the world, this industry is developing rapidly, creating an innovative field academically, from on-site diagnostics to wearable sensors, and adding value to the market shares of countries economically. However, unfortunately, there are no domestically produced commercial biosensors in our country, apart from a few laboratory-scale studies. For this reason, it is important to focus on the production of biosensors that will be marketed as domestic production in the near future. The findings obtained as a result of the study lead to the conclusion that it will contribute to commercial studies.

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