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PREPARATION OF ENZYME BASED STRIPS THROUGH IMMOBILIZATION FOR CALORIMETRIC DETERMINATION OF URIC ACID

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Abstract:

The research was carried out for the development of enzyme based uric acid strips for improved diagnosis of diseases.

***Materials and methods**: In this study, the standard curve for uric acid and hydrogen peroxide by direct and indirect method were developed. The enzyme was immobilized on nitrocellulose membrane and filter paper. Enzyme immobilization was done by chemical method, bovine serum albumen (BSA) and gluteraldehyde (cross linker) and bovine serum albumen (BSA) and gluteraldehyde in combination. For optimization of pH 20mM potassium phosphate buffer of different pH (6, 6.5, 7, 8, 9, 10) were prepared. To determine the effect of temperature on strips storage, immobilized strips were stored at 4ºC and at room temperature and used for analysis after every 24 hours.*

***Results**: In Dip method, enzymes immobilized nitro cellulose membrane was dipped in 30µl of 18mM uric acid. A clear, visible, bright pink color was developed after 30 minutes in strips. Same results were obtained by pouring the sample on the prepared strips of nitro cellulose membrane and filter paper as obtained by dipping the prepared strips in sample.*

***Conclusion**: From this study, it was concluded that more reliable, self-developed and easy to use uric acid test strips were successfully made.*

Keywords: *Uric Acid; Enzyme Based Strips, Biomarker; optimization; diseases*

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INTRODUCTION:

Uric acid is waste produced during the breakdown of purines in human beings. Uric acid is normally carried in the blood, passes through kidneys and eliminated in urine. A high uric acid level may appear prior to the development of high blood pressure, heart disease or chronic kidney disease. A lot of diseases and pathological disorders are related to the variations of uric acid concentration in body fluids (e.g. serum and urine), such as gout, arthritis [1], kidney diseases [2], cardiovascular diseases [3], and neurological diseases (Moallem, Taningo, Jiang, Hirschhorn, & Fikrig, 2002). On the other hand, as a substitute antioxidant, it could be involved in many pathological changes and may play a protection role [4].

The quantitative analysis of serum uric acid is very important in the diagnosis and medical management of various diseases [5]. Since uric acid is found in human serum in relatively low concentrations (0.21 to 0.42 mmol/L in men and 0.16 to 0.36 mmol/L in women), it is necessary to use specific and sensitive methods for its determination [6]. Different methods of uric acid analysis are available such as chemical [7], colorimetric [8], mass fragmentography and Radiochemical–HPLC method [9] and enzyme electrode[10].

The existing methods could be conveniently divided into two groups: reductive and enzymatic. The reductive methods are non-specific and involve the oxidation of uric acid with phosphotungstate reagent to allantoin with resultant blue coloring of tungstate solution. The enzymatic methods are specific and eliminate the interferences intrinsic to chemical oxidation. They involve the catalytic oxidation of uric acid with the enzyme uricase to allantoin with the formation of hydrogen peroxide [11]. The enzymatic colorimetric method employing uricase and peroxidase is more suitable for routine, as it is simple, sensitive and specific and doesn't require expensive apparatus. The method is so popular that its commercial kit is available (Sigma Technical Bulletin. 1997: Kit No. 686-10, St. Louis, MO, USA).

There are two types of the uricase methods, i.e., the direct uricase method that quantifies uric acid itself by the absorbance at 293 nm and the indirect uricase method that quantifies the uricase product hydrogen peroxide by diverse methods. The concentration of H_2O_2 is directly proportional to the concentration of uric acid, which could be determined by a number of methods [12,13] .

$$\text{Uric acid} + 2H_2O + O \xrightarrow{\text{Urease}} \text{Allantoin} + CO_2 + H_2O_2$$

In general, the amount of H_2O_2 produced was spectrophotometrically determined by measuring an amount of dye produced through the following reaction catalyzed by peroxidase. Horseradish peroxidase (HRP) is a heme-protein with the heme group in a ferric state. This protein reacts with H_2O_2 through a complex mechanism in which different reaction intermediates showing different heme oxidation states are involved [14]. The concentration of dye produced as end product is directly proportional to the concentration of uric acid[16].

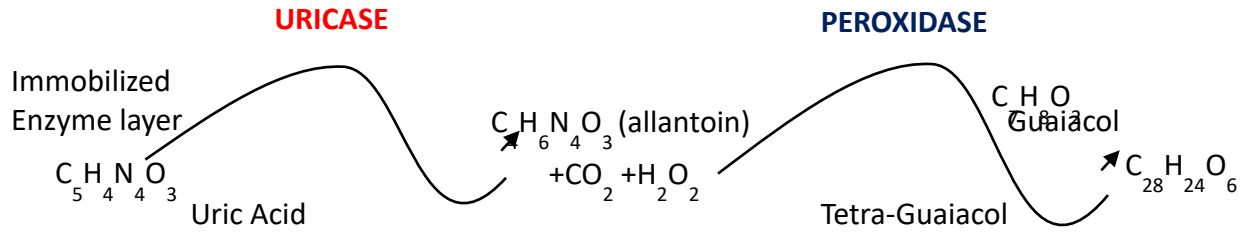
$$\text{Chromogen} + 2H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Dye} + 4H_2O$$

However, the enzymatic colorimetric method becomes expensive when used for a large number of samples that limits there use routine purpose [15].

The use of biosensors with immobilized enzymes has been extensively investigated for hydrogen peroxide analysis based on spectrophotometry[17], fluorometry [18], chemiluminescence (Spohn et al., 1995) and electrochemical techniques (Li et al., 1996). The biosensor has many advantagessuch as simple measurement procedure, short response time, sensitivity and selectivitydue to which they can be used for the analysis of many metabolites (Akyilmaz, Sezgintürk, & Dinçkaya, 2003).Many uric acid biosensors have been made by immobilization of enzyme uricase on egg shell membrane (Wang et al., 2006), alkylamide glass beads immobilized on poly alanine-polypyrole film (Dungchai, Chailapakul, & Henry, 2010). Dispersion of the enzyme molecules by immobilization provides better accessibility and extra stability to the enzymes towards denaturation by the organic medium (Sheldon, 2007).

Point-of-care testing (POCT) has become relatively common place in developed nations as a way to augment traditional medicine and increase patient compliance (Price, 2003). POCT is also needed in developing nations because it can reduce the number of clinical visits, decrease costs to the patient and healthcare system, increase patient satisfaction, improve clinical outcomes, and provide clinical services for people in low resource settings (Myers & Lee, 2008). Paper strip tests are currently used in these scenarios that are commercially available for pregnancy (One Step HCG Urine Pregnancy Test Strip, AI DE Diagnostica Co. Ltd., Shandong, China, 2009), diabetes (Kristensen, Monsen, Skeie, & Sandberg, 2008), drugs of abuse (One Step Drugs of Abuse Test, Core Technology Co., Ltd., Beijing, China, 2009.) and biomarkers of pathogens test

(Mosley & Sharp, 2005) (Zhao et al., 2009).The present study aims to develop the test strips with immobilized uricase and peroxidase for the colorimetric determination of uric acid on the bases of the principle shown in the equation:



Methodology:

Multiple steps that were carried out for strips preparation are as follow (Figure 1).

Uric acid standard curve preparation by direct method

The reaction was set by adding 3.0 ml of 20mM potassium phosphate buffer (pH 9) in control. In test cuvette 2.9 ml of 20mM potassium phosphate buffer (pH 9) and 100µl of 25mM uric acid. To prepare the standard curve of uric acid concentrations of (18mM, 16mM, 14mM, 12mM, 10mM, 8mM, 6mM, 4mM, 2mM) and (0.9mM, 0.8mM, 0.7mM, 0.6mM, 0.4mM, 0.5mM, 0.3mM, 0.2mM, 0.1mM) were used to record the absorbance at 293nm.

Preparation of standard curve of hydrogen peroxide (H_2O_2)

The reaction was set by taking 2.8ml of 20mM potassium phosphate buffer (pH 9), 100µl of peroxidase solution (0.0002U\µl), 50µl of 0.018M Guaiacol in cuvette and then the reaction was started by adding 50µl of 1µM H_2O_2. In control reaction 50µl of 20mM potassium phosphate buffer (pH 9) was added instead of H_2O_2 solution. The absorbancewas recordedat 436 nm. To prepare the standard curve of H_2O_2 concentrations (35µM, 30µM, 25µM, 20µM, 15µM, 10µM, 5µM, 1µM) were used to record the absorbance at 436 nm.

Uric acid standard curve preparation by indirect method

The reaction was set by taking 2.8 ml of 20mM potassium phosphate buffer (pH 9), 20µl of uricase solution (0.001U/µl), 100µl of horseradish peroxidase solution (0.0002U/µl), 50µl of 0.018M Guaiacol, then the reaction was started by adding 50µl of uric acid in sample cuvette. In control reaction 50µl of 20mM potassium phosphate buffer (pH 9) was added instead of uric acid.The absorbance was recorded at 436 nm.To prepare the standard curve, different concentrations of uric acid (0.9mM, 0.8mM, 0.7mM, 0.6mM, 0.4mM, 0.5mM, 0.3mM, 0.2mM, 0.1mM) were used to record the absorbance at 436nm.

Strips preparation

To prepare the test strips for uric acid the two parameters were analyzed: i) Selection of solid support: Nitrocellulose membrane and filter paper were selected and cut in size of 1cm×0.8cm to perform different experiments. ii) Selection of coloring agents: At start the three different coloring agents were analyzed as chromogen for the development of color during reaction that are potassium iodide, o-tolidine and guaiacol. Different experiments were performed with different concentrations but satisfactory results were obtained with guaiacol so it was used for further experiment.

Methods of enzyme immobilization

Different methods of enzyme immobilization were used to immobilize enzymes on nitrocellulose membrane and filter paper.The method used for enzyme immobilization on nitrocellulose membrane is physical adsorption method and for filter paper enzyme immobilization was done by chemical method, bovine serum albumen (BSA) and gluteraldehyde (cross linker) and bovine serum albumen (BSA) and gluteraldehyde in combination.

Enzyme immobilized on nitrocellulose membrane strips

The enzymes were immobilized on nitrocellulose membrane by physical adsorption through i)Dip method: In this method nitrocellulose membrane of size 1cm×0.8cm was dipped in enzyme mixture containing4µl of 0.04U/µl of uricase, 20µl of 0.004U/µl of horseradish peroxidase and 10µl of 0.018M guaiacolfor 30 minutes at room temperature. The strip was removed and air dried. Then 30µl of uric acid of 18mM was applied and the color change was observed on the strip and ii) Pouring method: In

this methodnitrocellulose membrane of size 1cm×0.8cm was taken. The enzyme mixture containing 4µl of 0.04U/µl of uricase, 20µl of 0.004U/µl of horseradish peroxidase and 10µl of 0.018M guaiacol was prepared and 20µl of this mixture was poured on the membrane and air dried. Then the ten pieces of nitrocellulose membrane with the immobilized enzyme were fixed at the one side of the photographic film of size 5cm×0.8cm. The 30µl of 18mM uric acid sample was applied on the enzyme immobilized membrane and the color change was observed.

Enzyme immobilization on filter paper strips
Chemical method
The filter paper of size 1cm×0.8cm was dipped into 1 ml chilled 2 N HCl in a test tube and solid $NaNO_2$ was added to tube and the occasional shaking was done for 30 minutes with on shaker. The strip was removed and washed thoroughly in 20mM potassium phosphate buffer and air dried. The enzyme mixture containing 4µl of uricase (0.04U/µl), 20µl of horseradish peroxidase (0.004U/µl) and 10µl of guaiacol (0.018M). From this mixture 20µl was poured on the membrane. It was kept for 48 hrs at 4ºC with occasional shaking. Then 30µl of 18mM uric acid was poured on the strip and the color change was observed.

Bovine serum albumen (BSA)
The filter paper of size 1cm×0.8cm was dipped in 5% bovine serum albumen (BSA) for 20 minutes. Then removed it from the BSA and raised with 20mM potassium phosphate buffer. The filter paper was air dried. The enzyme mixture containing 4µl of 0.04U/µl of uricase, 20µl of 0.004U/µl of horseradish peroxidase and 10µl of 0.018M guaiacol. From this mixture 20µl was poured on the filter paper and was allowed to dry. Then 30µl of 18mM uric acid was applied and the color change was observed on the strip.

Cross-linking with gluteraldehyde
The filter paper of size 1cm×0.8cm was dipped in 2.5% gluteraldehyde again for 20 minutes. Then removed it and washed with 20mM potassium phosphate buffer. The filter paper was allowed to dry. The enzyme mixture containing4µl of 0.04U/µl of uricase, 20µl of 0.004U/µl of horseradish peroxidase and 10µl of 0.018M guaiacol. From this mixture 20µl was poured on the filter paper and was allowed to dry. Then 30µl of 18mM uric acid was applied and the color change was observed on the strip.

Bovine serum albumen (BSA) and gluteraldehyde in combination
The filter paper of size 1cm×0.8cm was dipped in 5% bovine serum albumen (BSA) for 20 minutes. Then removed it from the BSA and rised with 20mM potassium phosphate buffer. Then the filter paper was dipped in 2.5% gluteraldehyde again for 20 minutes. Then removed it and washed with buffer. The filter paper was allowed to dry. The enzyme mixture containing 4µl of 0.04U/µl of uricase, 20µl of 0.004U/µl of horseradish peroxidase and 10µl of 0.018M guaiacol.From this mixture 20µl was poured on the filter paper and was allowed to dry. Then 30µl of 18mM uric acid was added and the color change was observed.

Methods of sample application
Two different methods were used for sample application that are i) Dip method in which enzyme immobilized nitrocellulose membrane of size 1cm×0.8cm was taken. Then dipped in 30µl of uric acid of 18mM and the color change was observed on the strip and ii) Pouring method in which Enzyme immobilized nitrocellulose membrane and filter paper of size 1cm×0.8cm was taken. Then the nitrocellulose membrane and filter paper with the immobilized enzyme were fixed at the one side of the photographic film of size 5cm×0.8cm. The 30µl of 18mM uric acid sample was applied on the enzyme immobilized membrane and filter paper and the color change was observed.

Optimization of experimental parameters
Different experimental parameters can affect the activity of immobilized enzymes and strips so different experiment were performed for there optimization. For optimization of pH 20mM potassium phosphate buffer of different pH (6, 6.5, 7, 8, 9, 10) were prepared and used in the different experiments. To determine the effect of temperature on strips storage, immobilized strips were stored at 4ºC and at room temperature and used for analysis after every 24 hours. For the optimization of the reagent volume which can carried out successfully on solid support a range of reagent volumes (10µl, 15µl, 20µl, 25µl, 30µl, 35µl) was applied to the solid support.The minimum reagent volume necessary to wet entire detection zone was determined to develop the color on strip.

Different ranges of samples volume (20µl, 30µl, 40µl and 50µl) were used to determine the minimum sample volume requires to develop color on strip. By applying different volumes of samples lower detection limit, optimum response time of strip andthe stability of coloron the strip was also optimized. At the end, Storage stability experiments were made for storage of 15 days period to detect

decreases in the strip response and measurements were carried out periodically after every 2 days. During this period enzymes immobilized on the strip lost their activities as related to time. The prepared strips used for this purpose and were stored at 4ºC.

Results:

Uric acid standard curve preparation by direct method

By using the standard curve of uric acid, the concentration of uric acid in unknown sample can be determined. For the preparation of standard curve different concentration of uric acid were used and absorbance at 293 nm was recorded (Table 1) and used to prepare the standard curve of uric acid (Figure 2a).

Standard curve of hydrogen peroxide (H_2O_2)

For the preparation of standard curve of hydrogen peroxide (H_2O_2) different concentration of hydrogen peroxide (H_2O_2) were used and absorbance at 436 nm was recorded (Table 2) that was used to prepare standard curve (Figure 2b). This standard curve can be used to determine the amount of H_2O_2produced by the action of uricase on uric acid.

Uric acid standard curve preparation by indirect method:

The indirect method was also used for quantifying the uric acid by determining the amount of hydrogen peroxide formed by the action of uricase on uric acid, by taking absorbance at 436nm. After the completion of reaction, a colored product was produced (Figure 3b). Absorption values at 436nm was recorded with different concentration of uric acid (Table 3) and were used to prepare standard curve (Figure 3a). The color development with different concentrations of uric acid with soluble form of enzymes is shown in Figure 3c.

Strip preparation

Enzyme immobilization on filter paper

Firstly, chemical method was used for immobilization. There was no color development on strip by applying 30µl of 18mM uric acid even after 2hours. The enzymes may lose their activity during chemical immobilization so this method was not selected for further study. In Bovine Serum Albumen (BSA), commercially available uricase and horseradish peroxidase were immobilized on filter paper along with BSA. The color did not develop on strip by applying 30µl of 18mM uric acid even after one hour. So, this method was rejected.

Cross linking method using gluteraldehyde was also used.Very light color was developed after 60 minutes by applying 30µl of 18mM uric acid. The developed color was not stable and vanished after short period of time. To increase the efficacy of color development uricase and horseradish peroxidase were immobilized on filter paper BSA- gluteraldehyde in combination. The enzyme mixture was poured on filter paper. Then the 30µl sample was applied to monitor the color development. Bright color was developed on strip after 30 minutes by applying 30µl of 18mM uric acid. The color was stable for 7-8 hours on strip.

Enzyme immobilization on nitrocellulose membrane by physicaladsorption

Enzymes were immobilized on nitrocellulose membrane by dipping the strip in enzyme mixture. When 30µl of 18mM uric acid was applied in strip clear, visible, bright pink color was developed after 30 minutes. Same results were obtained when strips were prepared by pouring the reaction mixture on nitro cellulose membrane as obtained when strips were prepared by dipping in reaction mixture so any method can be used to prepare strips.In the present study, strip preparation by dip/pour method was selected for further experiments.

Color development on strips

In Dip method, enzymes immobilized nitro cellulose membrane was dipped in 30µl of 18mM uric acid. A clear, visible, bright pink color was developed after 30 minutes in strips (Figure 4a). Same results were obtained by pouring the sample on the prepared strips of nitro cellulose membrane (Figure 4b) and filter paper (Figure 4c) as obtained by dipping the prepared strips in sample so any method can be used to prepare strips. Sample application by dip/pour method was selected for further experiments.

Optimization of UA strips

Optimization of enzyme concentrations

In order to optimize the enzyme concentration for strips different concentrations of uricase and peroxidase were used ranging from 0.0001U/µl to 0.04U/µl for uricase and 0.0002U/µl to 0.004U/µl for horseredish peroxidase. It was observed from the results that in case of immobilized enzymes in high concentration 0.04U/µl of uricase and 0.004U/µl of horseredish peroxidase were required for color development. While in case of soluble enzyme color was developed at low enzyme concentration 0.0001U/µl of uricase and 0.0002U/µl of horseredish peroxidase.

Optimization of guaiacol concentration for strips

In order to optimize the guaiacol concentration for strips different concentrations of guaiacol were used

ranging from 5µl of 0.018M guaiacol to 10µl of 0.018M guaiacol. It was observed from the result that in case of immobilized enzymes in high concentration of 10µl of 0.018M guaiacol was required for color development. While in case of soluble enzyme color was developed at low guaiacol concentration 5µl of 0.018M guaiacol.

Optimization of reagent volume

The minimum volume of reagent was determined and toreduced reagents consumption while still making it large enough to wet the entire detection zone and sufficient for analysis. Therefore, 20µl from the 34µl of the reagent solution was used for the further experiments. The result of experiments showed that 20µl of reagent (mixture of enzymes and colored reagents) was required to determine from (low 0.1mM to high 18mM) uric acid concentrations.

Optimization of pH

The 20mM phosphate buffer of various pH (6, 6.5, 7, 8, 9) was used which was used in strip preparation for the preparation of enzyme mixture (Supplementary Table 1). It was found that the response increased when the buffer concentration increased but no further increase when the pH was above 9.0. The optimum pH of reaction was 9.0. Therefore, 20mM potassium phosphate buffer (pH 9) was chosen for further experiments. Color development at different pH is shown in Supplementary Figure 1.

Optimization of temperature

The optimum temperature of UA strips storage was also determined. For this purpose, prepared strips were stored and best results were obtained when strips were stored 4ºC after preparation (Supplementary Figure 2).

Response time:

The enzyme immobilized strips stored at 4ºC were used to optimize the response time (Supplementary Figure 3). The best results were obtained after 30 minutes by applying the different concentrations of uric acid (Supplementary Table 2).

Optimization of sample volume

The minimum sample volume that can spread through the entire was also studied. It was found that 30µl of sample was required to wet all detection zones and strip development of sharp color.

Detection limit

The detection limit of uric acid strips was also investigated. It was observed the experiments that minimum concentration of uric acid which can be determined by these strips was 0.1mM and upper limit of detection was 14mM (Supplementary Figure 4).

Storage stability

Storage stability experiments were made for a long storage period to detect decreases in the strip response. In order to determine the storage stability of the strip at 4ºC, measurements were carried out periodically every 24 hours (Supplementary Table 3). After the storage period, (120 hours) observed that the activity of the strip was not more than 25% of its initial activity.

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Color stability

The color stable on the strip for one day after development.

DISCUSSION:

A method for analysis of uric acid has been developed by using uricase and horseradish peroxidase immobilized nitrocellulose membrane and filter paper. The method is not only economical for routine purpose but also suitable for the clinical laboratories of the developing countries.

In the present study for the preparation of standard curve of uric acid different concentration (from 0.1 mM to 18 mM) of uric acid was used and absorbance at 293 nm was recorded (from 0.21 nm to 2.00 nm) by direct and indirect method. These absorbance values were used to prepare the standard curve of uric acid. A straight line standard curve of uric acid was obtained. For the preparation of standard curve of hydrogen peroxide (H_2O_2) different concentration of hydrogen peroxide (H_2O_2) (from 1µM to 35µM) were used and absorbance at 436 nm was recorded (from 0.05nm to 0.10nm). These absorbance values were used to prepare the standard curve of hydrogen peroxide (H_2O_2). A straight line standard curve of hydrogen peroxide (H_2O_2) was obtained.

For the preparation of standard curve of uric acid different concentrations of uric acid were used and absorbance at 520 nm was recorded by indirect method. These absorbance values were used to prepare the standard curve of uric acid. A straight line standard curves of uric acid was obtained.The content of H_2O_2 generated in the reaction was determined from the standard curve of uric acid (Bhargava et al., 1999).

An entirely new matrix for immobilizing glucose oxidase was reported. The matrix cotton cheese cloth was found to be very useful as highly reproducible enzyme membranes could be prepared. The biosensor

used in the present work consists of GOD immobilized cheese-cloth membrane, which is mounted on a platinum disc inner sensing element. The other advantages of the cheese cloth as a support for the GOD enzyme over other membranes are its easy availability, high porosity, mechanical stability and flexibility, simplicity in the method of immobilization, its ability to incorporate high amounts of enzyme per unit area, besides being highly economical (J.-C. Chen et al., 2005).

A new amperometric biosensor was reported based on the coupling two enzymes, which are urate oxidase and peroxidase for high sensitive, not time consuming and specific measurement of uric acid. Measurements were carried out by standard curves which were obtained by the determination of consumed oxygen level, related to uric acid concentration in the enzymatic reactions. To construct the biosensor, DO probe was covered with high-sensitive teflon membrane by using an O-ring. The biosensor was cross linked with 2.5% glutaraldehyde and a bioactive layer was formed on the DO probe so a new biosensor based on urate oxidase-peroxidase coupled enzyme system which was sensitive and specific for uric acid determination was developed (Akyilmaz et al., 2003).

Uricase was initially covalently immobilized on a fresh eggshell membrane using BSA and gluteraldehyde as cross-linking reagents, and was subsequently mounted onto the surface of an oxygen electrode. The oxygen electrode was then placed in contact with a sample solution for the determination of uric acid. The detection scheme was based on the decrease of dissolved oxygen level in the sample solution. Our proposed method has been successfully applied to determine the uric acid level in serum and urine samples (Zhang et al., 2007).It was found that the sensitivity of the biosensor without BSA was three times less sensitive than that of a biosensor with BSA. The speed of the enzymatic reaction depends greatly on the enzymatic activity. The amount of immobilized enzyme on the solid support would also affect the sensitivity of the biosensor.

The buffer solutions for preparing uric acid standards were 200mM monosodium di-hydrogen phosphate-disodium hydrogen phosphate solutions at pH 8.0 for the Immobilization of uricase on eggshell membrane (Zhang et al., 2007). The Phosphate buffer (pH 6.8) was used as the supporting electrolyte for whole blood glucose determination using glucose oxidase immobilized on cotton cheese cloth (J.-C. Chen et al., 2005).In the study of multiple colorimetric indicators for paper-based microfluidic devices all standard and enzyme solutions were prepared in 0.1M potassium phosphate buffer (pH 6) (Dungchai et al., 2010).The buffers for the biosensor measurements and for storage of the sensor was prepared from potassium phosphate (pH 6.5) for uricase immobilization in poly-N-methylpyrrole (Santha et al., 2002).

To construct the biosensor, DO probe was covered with high-sensitive teflon membrane by using an O-ring 50 mM phosphate buffer (pH 7.5) was used. After this step, urate oxidase (110 U), peroxidase (600 U) and gelatin (20 mg) were mixed in 300ml of 50mM phosphate buffer (Akyilmaz et al., 2003).The optimum pH of reaction was 9.0. Therefore, 20mM potassium phosphate buffer (pH 9) was chosen for further experiments. In the present study the detection limit of uric acid strips was also investigated. The detection limit of uricase immobilized egg shell membrane-based biosensor was estimated 2.0 µM (Zhang et al., 2007). The lower detection limit of the method in which serum glucose determined by using co-immobilized glucose oxidase and peroxidase onto arylamine glass beads affixed on a plastic strip was obtained 5mg/dl and upper detection limit was 100mg/dl. The enzyme immobilized strips were placed at 4ºC and at room temperature from 10 minutes to 60 minutes and then remove after every 10 minutes and 30µl of 18mM uric acid was applied to optimize the response time. The response time of uricase immobilized egg shell membrane-based biosensor was estimated more than 100 seconds (Zhang et al., 2007).

In the present study, storage stability experiments were made for a long storage period to detect decreases in the strip response. In order to determine the storage stability of the strip at 4ºC, measurements were carried out periodically every 24 hours. After the 120 hours observed that the activity of the strip was not more than 25% of its initial activity. The alkylamine bound uricase and arylamine bound peroxidase were stored at 4ºC in the reaction buffers, until use (Bhargava et al., 1999).The biosensor prepared based on urate oxidase -/peroxidase coupled enzyme system for uric acid determination in urine was used for only this purpose and during the period it was stored at 4ºC. After the storage period, it was determined that the remain activity of the biosensor was more than 83% of its initial activity. Storage stability experiments were made for a long storage period to detect decreases in the biosensor response. During this period enzymes used in the strips construction lost their activities as related to time (Akyilmaz et al., 2003). From this study it was concluded that more reliable, self-developed and easy to used uric acid test strips were successfully made.

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