Microcantilever based on flavoenzyme monoamine oxidase (MAO) as a bioprobe

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ABSTRACT

Microcantilevers (MCLs) are cost-effective and highly sensitive devices for biodetection. Adsorption of specific analytes on the microcantilever surface causes the bending of MCL through changing of the surface characteristics. These new bioprobes designed in a way that one side of the microcantilever surface is coated with a selective receptor that absorbs particular molecules. After surface absorption of target, the microcantilever deflects under nano – Newton forces and results in microcantilever bending. In the following work, we have proposed a modifed microcantilever through immobilization of monoamine oxidase (MAO) as a Flavin–Adenosine-Dinucleotide (FAD)-containing enzyme. This enzyme catalyzes the oxidative deamination of amine groups, so interaction between compounds with amine functional group and enzyme is based on biodetection with monoamine oxidase modified microcantilever. In the present study, MAO was immobilized on the microcantilever surface through a cross linker to a monolayer on the gold surface. Following, the Kynuramine solution was used as substrate. The comparative results showed that the enzyme is activated in immobilized state in order to oxidize amine groups and is inhibited in the presence of Methamphethamine as an enzyme inhibitor. Since all processes are performing at room temperature, therefore the design of bioprobe based on modified microcantilever would be highly significant for biodetection.

Keywords: Monoamine oxidase; Microcantilever; Immobilization; Biodetection; Methamphethamine.

INTRODUCTION

Biosensors are powerful devices to monitor molecular interactions between a molecule and an immobilized bio-receptor on a solid surface [1]. Parallel to the development of Micro-Electro-Mechanical-System (MEMS), there was always interest to design low cost analytical methods [2]. Among them, the micro-cantilevers are the simplest MEMS with extensive application in bio-detection [3]. Micromechanical cantilever (MC) based sensors have been investigated to detect chemical and biological species [4,5]. An MC intended for chemical or biological sensing is normally modified by coating one of the cantilever sides with a responsive phase that exhibits high affinity to the targeted ligand. The surface stress changes induced due to the binding of ligand on the sensitized surface is resolved for detection. Potential uses of cantilever transducers in biosensors, biomicroelectromechanical systems (Bio-MEMS), proteomics, and genomics are intriguing trends in advanced biomedical analyse [6-8]. In 1994, it was realized that micro-cantilevers could be constructed extremely sensitive to chemical and physical changes [9]. Therefore, scientists recently were trying to build highly sensitive chemical and biological microcantilevers including DNA [7], alcohol [10], mercury [11,12], antigen [13], organophosphates [14,15], mercaptans [6], etc. These new biosensors designed in a way that one side of the microcantilever surface is coated with a selective receptor such as enzymes, antibodies, DNA fragments, etc that absorbs particular molecules. After surface absorption of target, the microcantilever hanging off the edge of a support piece deflects readily under nano–Newton forces and causes microcantilever bending. The bending of the cantilever also can be sensitively detected by using a number of techniques, such as optical reflection from microcantilever beams [15,16]. Monoamine oxidases (MAOs) are integral

flavoproteins which existed in the outer mitochondrial membrane. These enzymes are responsible for catalyzing the oxidative deamination of a wide variety of xenobiotic and endobiotic primary, secondary and tertiary amines. The general reaction of amine oxidation via monoamine oxidase is as follows:

$$RCH_2NH_2 + O_2 + H_2O \xrightarrow{MAO} RCHO + H_2O_2 + NH_3$$

MAOs are existed in two different isoforms, MAO-A and MAO-B. The sequence alignment of these two types MAOs show 70% sequence identity. The two forms display overlapping distribution in various tissues, but differ in substrate specificity and inhibitor sensitivity. The primary endogenous involves inactivation of monoamine neurotransmitters, such as serotonin and dopamine with MAO; for example, Serotonin and norepinephrine are preferentially catalyzed by MAO-A, so MAO-A inhibitors are used in the treatment of mental disorders such as depression while MAO-B catalysis inactivation of dopamine. MAO-B inhibitors are used in the treatment of neurological disorders such as Parkinson's and Alzheimer's [17,18]. Methamphetamine also known as metamfetamine, meth, glass, tik, N-methylamphetamine, methylamphetamine, and desoxyephedrine is a popular and highly addictive drug of abuse that has raised concerns [19]. These substances are known as MAO inhibitors that are easy to produce at relatively low cost and in large quantities, so design of bioprobe based on modified microcantilever would be highly significant for biodetection of these addictive drugs. In this study, in order to modify microcantilever, MOAs were immobilized on one side of cantilever. The interaction of MAOs with amines and MAO inhibitors cause bending of microcantilever. In the appropriate systems, the immobilized enzymes keep their activity and sensitivity to the inhibitors [20]. Hence, the MOAs were immobilized through glutaraldehyde cross-linker to a self-assembled monolayer of 2-aminoethanethiol on the microcantilever gold surface. We also described enzymatic activities for immobilized and non-immobilized MAO as well as influence of Methamphethamine as enzyme inhibitor addictive drug.

MATERIALS AND METHODS

Reagents and apparatus

One gold side microcantilever was used in this experiment. A human recombinant solution of monoamine oxidase (>50 $UImg^{-1}$), 25% glutaraldehyde, 2-aminoethanethiol, Kynuraminedihydrobromide and Methamphetamine hydrochloride were purchased from Sigma-Aldrich company. Sodium phosphate salts to prepare phosphate buffer and sulfuric acid 98% were purchased from Merck chemical company. All of the reagents were used without further purification. UV absorption of enzymatic reactions was recorded using a Unicum UV-300 spectrophotometer.

Immobilization of monoamine oxidase on the microcantilever

0.01 M sodium phosphate buffer solution at pH 7.2 was prepared with different ratio of 0.01 M of NaH_2PO_3 and Na_2HPO_3 stock solution. The modified microcantilever was prepared in three steps according to known surface conjugation chemistry as follow: (A) A monolayer of 2-aminoethanethiol on the gold surface of a microcantilever was self-assembled by immersing it into a 5×10^{-3} M solution in a 0.01M phosphate buffer for 12 h, and then rinsing with deionized water. (B) The cross-linker, glutaraldehyde, was linked to the amino groups of 2-aminoethanethiol SAM by immersing the microcantilever into a glutaraldehyde solution (2.5% wt) for 12 h. (C) The enzyme monolayer was formed by incubating the microcantilever in an monoamine oxidase solution in 6°C at pH 7.2 for 24 h.

Enzyme activity studies on immobilized and non-immobilized MAO

The 0.5 M sodium phosphate buffer solution was prepared at pH 7.4. The sample silica cuvette were filled by 0.03 mg of Kynuramine, 0.03 ml of monoamine oxidase solution 0.1 ml of 0.5 M phosphate buffer at pH 7.4 and deionized water to a total volume 1 ml. A blank cuvette was prepared in which the Kynuramine was replaced with deionized water. The UV absorption at 290-470 nm was used to monitor Kynuramine as substrate at 358 to 360 nm with concurrent appearance of

new peaks at 315-329 nm for product of amine oxidation. For determination of enzymatic activity on monoamine oxidase modified microcantilever, mixture of 0.003-0.03 mg of Kynuramine, 0.1 ml of 0.5 M phosphate buffer at pH 7.4 and water to a total volume of 1 ml was prepared and contacted with modified microcantilever for appropriate time. The–UV light absorption of Kynuramine solution was recorded at 290-470 nm.

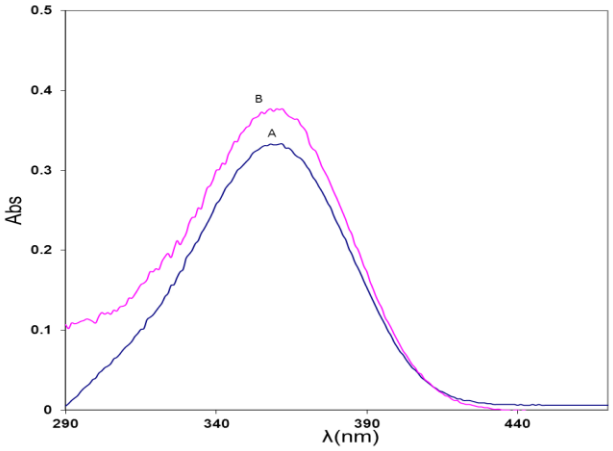

Figure 1. Spectrophotometric spectra of Kynuramine solution (0.03 mg/ml) at 290-470 nm, (A) before incubation with non-immobilized MAO and (B) after 180 min incubation with non-immobilized MAO.

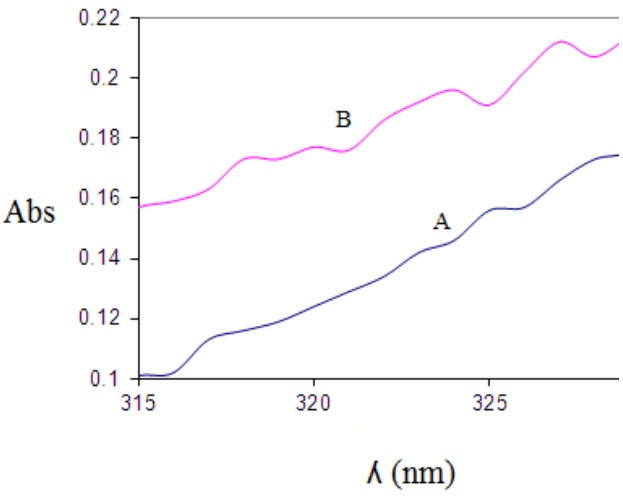

Figure 2. New peaks due to the product of amine oxidation at 315-329 nm, (A) before incubation of Kynuramine solution (0.03 mg/ml) and (B) after 180 min incubation with non-immobilized MAO.

RESULTS AND DISCUSSION

Enzymatic activity on non-immobilized MAO

To investigate MAO-A and MAO-B activities, the Kynuramine solution (0.03 mg/ml) was prepared as substrate for monoamine oxidases. To measure the activity, UV absorption of Kynuramine solution was recorded in the presence of non-immobilized MAO at room temperature for 180 min.

Figure 3. Microcantilever was stabilized in a plastic micro tube and after immobilization of enzyme, subsequent tests were done in contact with immobilized MAO on the microcantilever.

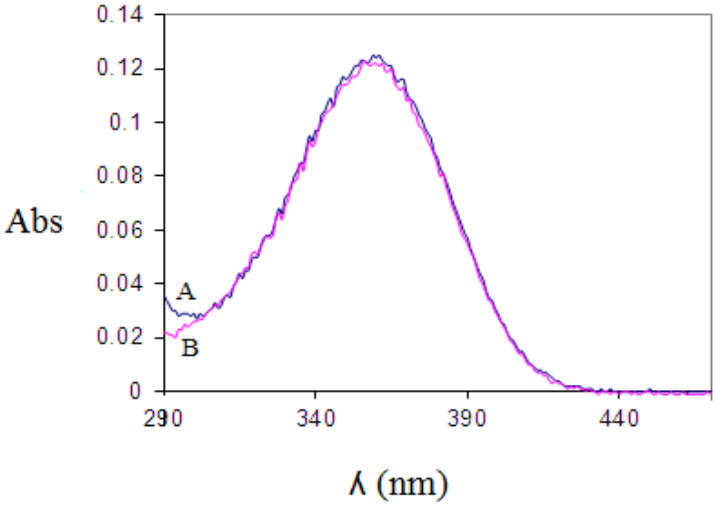

Figure 4. Spectrophotometric spectra of Kynuramine solution (0.006 mg/ml) at 290-470 nm, (A) before incubation and (B) after 30 min incubation with non-modified microcantilever.

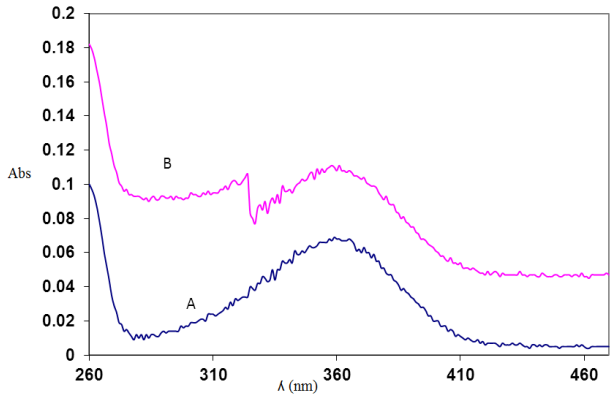

Figure 5. Spectrophotometric spectra of Kynuramine solution (0.006 mg/ml) at 260-470 nm, (A) before incubation and (B) after 30 min incubation with MAO modified microcantilever.

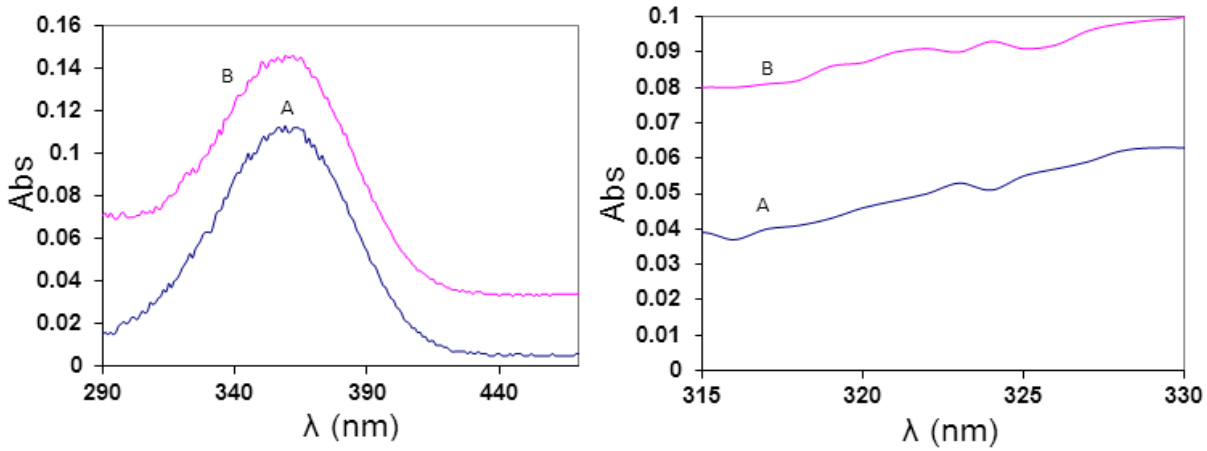

Figure 6. Spectrophotometric spectra of Kynuramine solution (0.012 mg/ml) at 290-470 nm (left Fig) and new peaks due to the product of amine oxidation at 315-329 nm (Right Fig), (A) before incubation and (B) after 30 min incubation with MAO modified microcantilever.

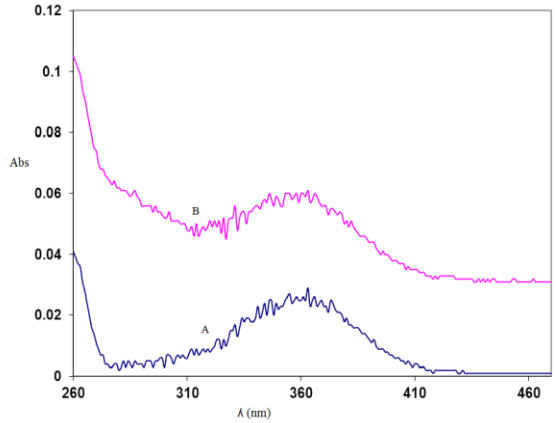

Figure 7. Spectrophotometric spectra of Kynuramine solution (0.003mg/ml) at 260-470 nm, (A) before incubation and (B) after 30 min incubation with MAO modified micro cantilever after 1 week.

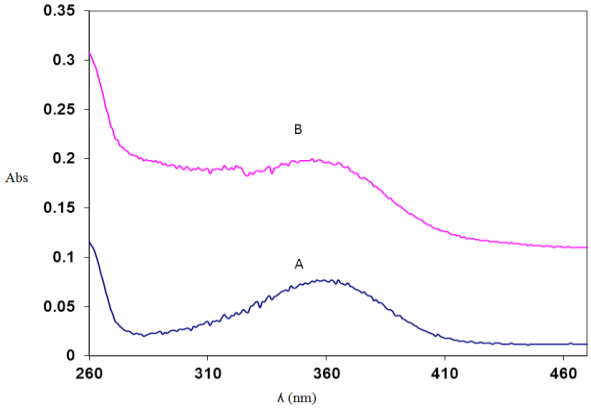

Figure 8. Spectrophotometric spectra of Kynuramine solution (0.006mg/ml) at 260-470 nm, (A) before incubation and (B) after 30 min incubation with MAO modified microcantilever after 1 week.

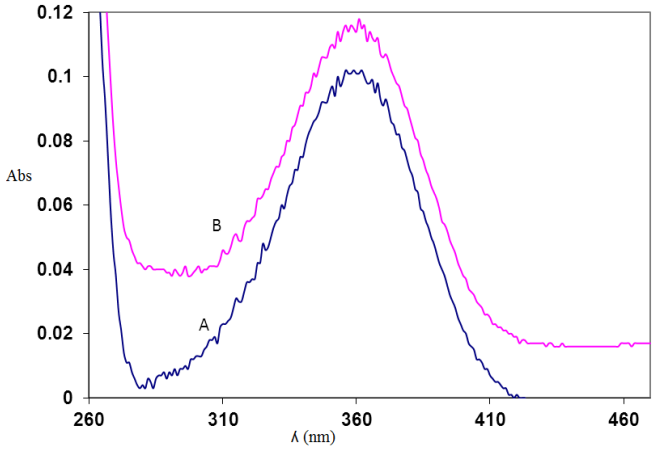

Figure 9. Spectrophotometric spectra of Kynuramine solution (0.012 mg/ml) at 260-470 nm, (A) before incubation and (B) after 30 min incubation with MAO modified microcantilever after 1 week.

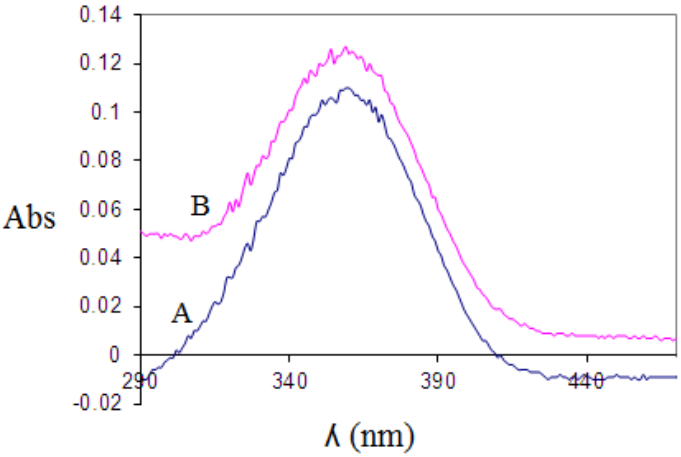

Figure 11. Spectrophotometric spectra of Kynuramine solution (0.006mg/ml) at 290-470 nm, (A) before incubation and (B) after 30 min incubation with MAO modified microcantilever after 5 months.

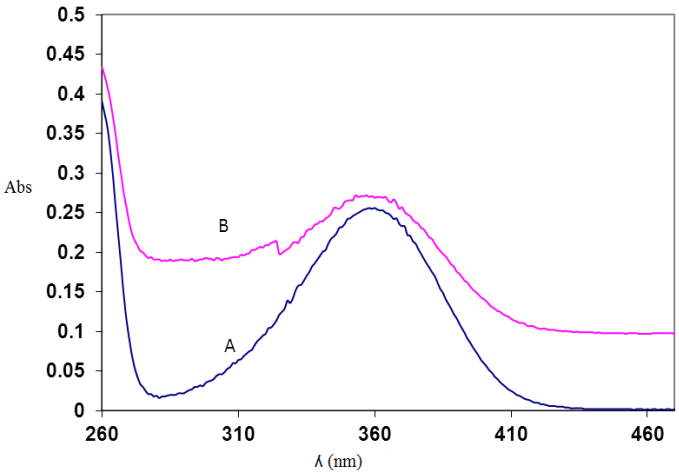

Figure 10. Spectrophotometric spectra of Kynuramine solution (0.03mg/ml) at 260-470 nm, (A) before incubation and (B) after 240 min incubation with MAO modified microcantilever after 1 week.

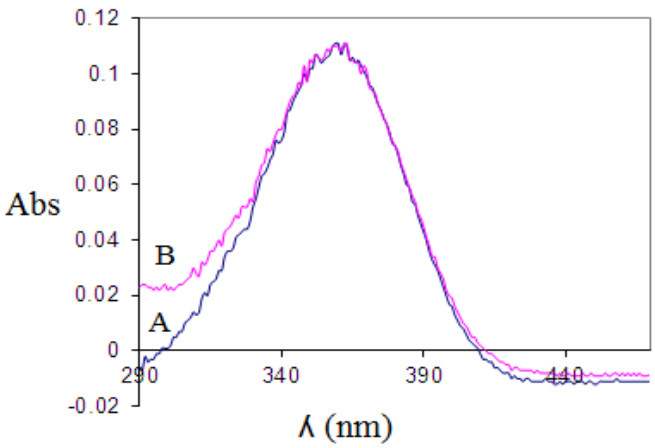

Figure 12. Spectrophotometric spectra of Kynuramine solution (0.006mg/ml) with Methamphethamine solution (0.6 mg/ml) at 290-470 nm, (A) before incubation and (B) after 30min incubation with MAO modified microcantilever after 5 months.

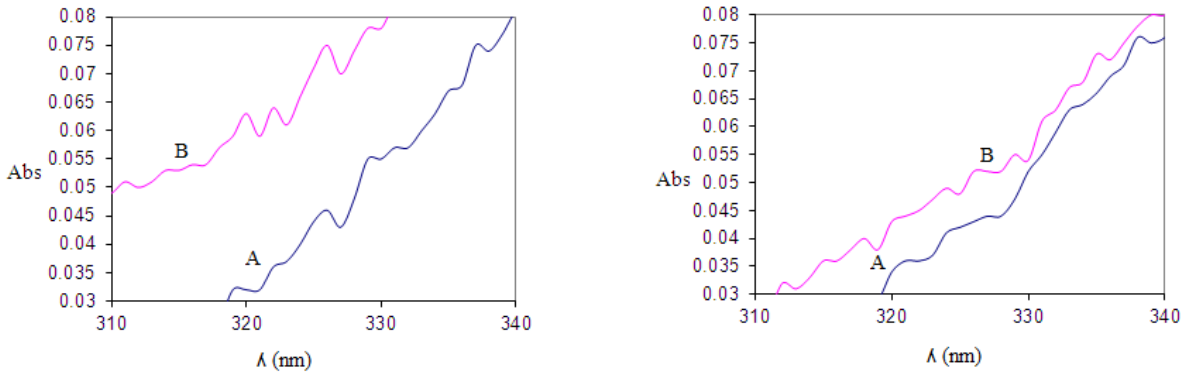

Figure 13. Spectrophotometric spectra of Kynuramine solution (0.006mg/ml) at 310-340 nm, (A) before incubation and (B) after 30min incubation with MAO modified microcantilever after 5 months. (Right: in presence of Methamphethamine solution (0.6 mg/ml) and Left: without Methamphethamine solution).

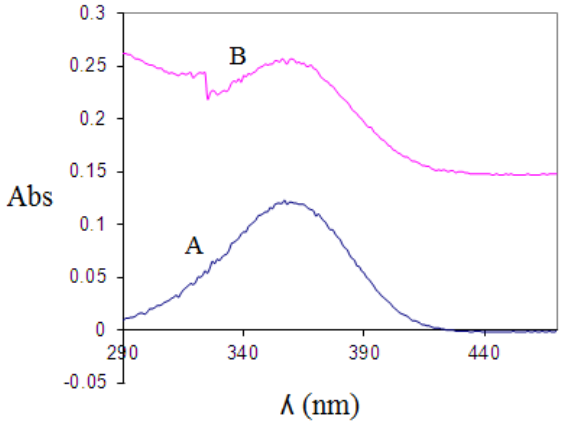

Figure 14. Spectrophotometric spectra of Kynuramine solution (0.006mg/ml) at 290-470 nm, (A) before incubation and (B) after 150 min incubation with MAO recovered microcantilever.

Figure 1 and Figure 2 are showing Kynuramine solution UV spectra at 290-470 nm. The peak at 315-329 nm indicates the amine oxidation of Kynuramine as a substrate by MAO-A. The initial peak that was observed at 358 to 360 nm is due to the substrate, kynuramine.

Enzymatic activity of MAO modified microcantilever

Microcantilever was stabilized in a plastic micro tube (Figure 3) and monoamine oxidase was immobilized on the microcantilever. Kynuramine solution (0.003-0.03 mg/ml) was prepared as substrate for monoamine oxidase enzyme and UV absorption of Kynuramine solution in contact with immobilized MAO for different times was recorded. Comparative results showed that the enzyme is active in immobilized state and is inhibited in the presence of Methamphethamine as an enzyme inhibitor addictive drug. So the bioprobe was design based on MAO modified microcantilever to detect of these addictive drugs. As a blank test, incubation of Kynuramine solution (0.006 mg/ml) with non-modified microcantilever was done for 30 min at room temperature and it's UV spectrum was shown in Figure 4. Incubation of 0.006 mg/ml Kynuramine solution with modified microcantilever at room temperature was done for 30 min and the related UV spectrum shown in Figure 5. Furthermore incubation of 0.012 mg/ml concentration of Kynuramine solution with modified microcantilever was done for 30 min at room temperature and the UV spectra were

recorded. The spectra and new peaks at 315-329 are shown in Figure 6(A) and Figure 6(B). The modified microcantilever was kept under 6-7^{o}C into buffer solution at pH 7.4 for one week, then Kynuramine solution (0.003, 0.006, 0.012 and 0.03 mg/ml) was incubated with it at room temperature. The spectra of Kynuramine solution (0.003, 0.006, 0.012 mg/ml) are shown after incubation for 30 min in Figure 7-9 respectively. The spectrum of Kynuramine solution (0.03 mg/ml) after 240 min incubation is shown in Figure 10. As it shown, the new peaks were observed at 315-329 nm due to the products of kynuramine oxidation.

Enzyme activity study on MAO modified microcantilever after 5 months

The MAO modified microcantilever was kept at the same conditions for 5 months. Then Kynuramine solution (0.006 mg/ml) was incubated with it at room temperature. The UV spectrum is shown in Figure 11. As it shown, the modified microcantilever was remained active after 5 months.

The influence of Methamphethamine as an enzyme inhibitor addictive drug

Since the probe was active after 5 months, we decided to investigate the influence of Methamphethamine as an enzyme inhibitor addictive drug. Thus the mixture of 0.006 mg/ml concentration of Kynuramine and Methamphethamine solutions was incubated with modified microcantilever at room temperature. As it shown in Figure 12 and Figure 13, there is no peak at 315-329 nm that is related to amine oxidation of kynuramine substrate. It means that immobilized MAO was inhibited with Methamphetamine.

Recovery of immobilized enzyme after inhibition

Methamphethamine reversibly inhibited the activity of immobilized MAO, so the enzyme was simply recovered by washing of microcantilever with deionized water and keeping it into the buffer solution at pH 7.4 for a short time. The 0.006 mg/ml concentration of Kynuramine solution was incubated with recovered microcantilever for 150 min and the spectrum shown in Figure 14.

CONCLUSION

In the present study, MAO was immobilized on the micro-cantilever surface through a cross linker to a monolayer on the gold surface. The Kynuramine solution was used as substrate and the comparative results showed that the enzyme is active in immobilized and non-immobilized state for amine oxidation and is inhibited in the presence of Methamphethamine as an addictive drug. These experiments were conformable at room temperature; therefore the design of biosensor based on modified microcantilever would be highly significant for biodetection.

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