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POTENTIAL USES OF MICROBIAL BIOSENSOR FOR DETECTION OF POLYPHENOLIC COMPOUNDS

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Abstract– Microbial principle-based biosensor containing immobilized enzyme tyrosinase has been used for detection of polyphenols in tea solutions. The immobilized tyrosinase based biosensor could detect polyphenols in the concentration range 10-80 mmol L^{-1}. Immobilization of the enzyme by the cross-linking method gave good stable response to polyphenols. The biosensor was tested in the polyphenols compounds determination in vegetables extract and the results were compared with the Folin–Ciocalteau traditional method and the biosensor showed suitability to the quantification of the total polyphenol in the tested samples. In order to enhance the sensitivity and stability of the measurements, the electrodes modified with designable molecules have been used in electrochemical determination of polyphenolic compounds. Although the majority of modified electrodes successfully determined the concentrations of polyphenolic compounds, the fabrication of the electrochemical sensor is still one of the challenging tasks for the researchers.

INTRODUCTION

Polyphenolic compunds increases calcium excretion in the urine and increase the risk of osteoporosis. Biosensor adoption is increasing every year and the number of biosensor applications is continuously growing. In addition reproducibility of the biosensor is very good and the biosensor can be used as an alternative method for routine analysis of polyphenolic compunds. The interference effects of the other compounds were negligible. Developed biosensor gold screen printed electrode (GSPE) was also used in real sample analysis. This study showed minimum response detection ranges with lowest detection limit (LOD) than previous literatures for the same purpose of study and response time was very short just only 40 s by using of isolated screen printed gold electrode for the development of amperometric biosensor against caffeine over concentration range. Caffeine in the soft drinks can be determined sensitively using the biosensor. In this study, it was found that present amperometric biosensor having 95-100% recovery which was average result by comparing with another methods such as Voltammetry, Flow injection, SFC-FTIR, MIP–PMAA/PVC sensor & UV-VIS (AOAC 12.028) (Meena *et al.,* 2018).

Traditional methods for the immobilization of microorganisms include adsorption, encapsulation, entrapment, covalent binding, and cross-linking. Besides these methods, many novel immobilization strategies have been explored in recent years in order to improve the analytical performance and storage stability of the microbial biosensor (Lowe *et al.,* 1984 & North *et al.,* 1985).

Application of microbial biosensor for polyphenolic detection

Biosensors can essentially serve as low-cost and highly efficient devices for this purpose in addition to being used in other day-to- day applications. A "specific biological element" recognizes a specific analyte and the changes in the biomolecule are usually converted into an electrical signal by a

transducer. Biosensors are an important alternative in the food industry to ensure the quality and safety of products and process controls with effective, fast and economical methods. Nowadays, a vast majority of the glucose meters are based on electrochemical biosensor technology. The use of enzymatic biosensor technology in food processing, quality control and on-line processes is promising compared to conventional analytical techniques, as it offers great advantages due to size, cost, specificity, fast response, precision and sensitivity (Meena *et al.*, 2017).

Analytical methods involved in the detection of polyphenolic compounds

The principle of the measurement was based on the determination of the differentiation of biosensor responses in the enzymatic reaction catalyzed by ALP in the absence and the presence of caffeine. Differences between the biosensor responses were related to caffeine concentration which was added in to the reaction medium. Caffeine concentration can be determined accurately between 0.2 and 10 µM using the biosensor. Detection limit (LOD) of the biosensor is 0.1 µM. In the optimization studies of the biosensor, glycine buffer (pH 10.5; 50 mM) and 30 ºC were obtained as the optimum working conditions. The optimum pH value was obtained as 10.5 by using of glycine buffer more comparable with another buffer systems such as Tris/NaOH and borate etc. Below and above this pH value decreases in the biosensor response were observed (Meena *et al.*, 2018).

Situ glucose monitoring by an enzyme sensor with a Clark electrode principle, the linear range of analysis was extended by varying the buffer flow in the dialyser unit. Due to the high specificity of the Clark electrode combined with a lower response time and suitability for detection of a wider range of target analyte, it was chosen for constructing the enzyme electrode. The unstable baseline usually associated with Clark electrode was compensated by having differential measurements (Cleland *et al.*, 1984).

A novel electrochemical sensor for the determination of caffeic acid based on the cobalt oxide microballs modified screen printed electrode. The proposed sensor exhibited superior electrocatalytic activity towards caffeic acid determination in terms of decent sensitivity, a broad dynamic range with a lower limit of detection (LOD) 48 nM. In addition, the proposed sensor offers good selectivity, good reproducibility, and decent stability. Moreover, the real sample analysis were carried out in wine samples and obtained an acceptable recovery rate (Ramki *et al.*, 2018).

Purification and characterization of polyphenolic compounds

Purification and characterization of a novel caffeine oxidase from *Alcaligenes* species. The purified caffeine oxidase with a half-life of 20 min at 50 °C had maximal activity at pH 7.5 and 35 °C. The purified caffeine oxidase had strict substrate specificity towards caffeine (K_m 8.94 ìM and V_{max}47.62 U mg protein^{-1}) and was not able to oxidize xanthine and hypoxanthine. The enzyme activity was not inhibited by para-chloromercuribenzoic acid, iodoacetamide, *n*-methylmaleimide, salicylic acid and sodium arsenite indicating the enzyme did not belong to xanthine oxidase family. The enzyme was not affected by Ca^{+2}, Mg^{+2} and Na^{+}, but was completely inhibited by Co^{+2}, $Cu^{+2}$and Mn^{+2} at 1 mM level (Mohapatra *et al.*, 2006).

A new type of molecular imprinted polymer (MIP) electrode developed for the amperometric detection of dopamine (DA). The MIP electrode was prepared using a screen-printed gold substrate. Thioglycolic acid (TGA) and allyl mercaptan (AM) were immobilized on the gold electrode through covalent bonds, intending to control the TGA density. The mediator, quercetin (Q), was also covalently attached to the carboxyl group of TGA by using the carboxyl activation agent, 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC). Polymerization of molecularly imprinted poly (methyl methacrylate) (PMMA) was accomplished by UV curing. It was observed that different surface distribution densities of TGA have strong influence on the recognition abilities of MIP electrodes. The best selective MIP electrode was made by AM/TGA at the molar ratio of 8 and its selectivities of MDA and ISO were 3.7 and 147.4. The sensitivity and the limit of detection were calculated to be 697.1 mA cm^{-2} M^{-1} and 4.3 ìM, respectively (Chen *et al.*, 2009).

A fluorescent biosensor developed for the determination of xanthine in tea and coffee via enzymatically generated uric acid. The use of rodamine B capped-thioglycolic acid functionalized gold nanoparticles (RB-capped TGA/GNPs) as a novel fluorescence probe for the sensitive determination of xanthine (XA) has been demonstrated. The sensing mechanism was based on the enzymatically generated uric acid (UA)

induced degradation of gold nanoparticles which resulted in the quenching of its fluorescence. By virtue of the specific response, the assay was allowed for the selective determination of XA in the range of 93 nmol/L-0.84 µmol/L with a detection limit of 10.1 nmol/L and that of UA in the range 9.90 nmol/L-65.4 nmol/L with the detection limit being 9.71 nmol/L. In addition, the application of the present approach in real samples like tea and coffee and also in synthetic blood serum were demonstrated (Menon *et al.*, 2017).

Novel phenols biosensor based on polyaniline–polyacrylonitrile composite matrix developed for inhibitive detection of benzoic acid. The electrochemical biosensor functioning was based on the inhibition effect of benzoic acid on the biocatalytic activity of the polyphenol oxidase (PPO) to its substrate (catechol) in 0.1 M phosphate buffer solution (pH 6.5). A potential value of -50 mV versus SCE, and a constant catechol concentration of 20 ìM were selective to carry out the amperometric inhibition measurement. The inhibiting action of benzoic acid on the polyphenol oxidase electrode was reversible and of the typical competitive type, with an apparent inhibition constant of 38 ìM. Inhibition studies revealed that the proposed electrochemical biosensor was applicable for monitoring benzoic acid in real sample such as milk, yoghurt, sprite and cola (Shan *et al.*, 2007).

Piezoelectric quartz sensor for caffeine based on molecularly imprinted polymethacrylic acid. The feasibility of a piezoelectric quartz caffeine sensor based on a molecularly imprinted poly methacrylic acid is described. The SEM micrographs, AFM image and XPS spectra provided evidences for the imprinting process and the recognition of caffeine by the MIP during the sensing process (Ebarvia *et al.*, 2005).

Multi enzymatic biosensor developed for Rapid sucrose monitoring in green coffee samples. Amperometric biosensor utilizing FAD-dependent glucose dehydrogenase (FAD-GDH) for a specific sucrose monitoring in green coffee was described. FAD-GDH was co-immobilized with invertase and mutarotase on a thin-layer gold planar electrode using chitosan. The biosensor showed a wide linearity (from 10 to 1200/ µM), low detection limit (8.4/ µM), fast response time (50/µm), and appeared to be O_2 independent. The good correlation among results of real samples, satisfactory analytical performance and use of the presented biosensor make it suitable for application in coffee industry (Stredansky *et al.*, 2018).

Other conventional study for analysis of polyphenolic compounds

Monitoring of phenolic compounds in the food industry and for environmental and medical applications has become more relevant in recent years. Conventional methods for detection and quantification of these compounds, such as spectrophotometry and chromatography, are time consuming and expensive. Laccase biosensors represent a fast method for on-line and *in situ* monitoring. Laccase production is dominated by a few fungus genera: *Trametes*, *Aspergillus*, and *Ganoderma* (Delgado *et al.*, 2015).

Microbial production of lactic acid is gaining much interest in recent years because of the need for optically pure lactic acid for certain applications like production of poly (lactic acid) (Datta *et al.*, 1995). A process based on immobilized lactic acid bacteria developed in a recycle batch reactor. The immobilized cells are used repeatedly for sugar conversion to lactate. In order to facilitate the progress of fermentation and to enable repeated fermentations without unnecessary time lag, on-line monitoring of the process was desirable (Senthuran *et al.*, 1997).

Protein-based stabilizing agents (PBSA) help to enhance the operational stability of immobilized enzyme-based biosensors. Using this approach, the tyrosinase enzyme (100 IU) was immobilized with different PBSAs such as lysozyme, BSA, and gelatin. Each PBSA (2 mg) was cross-linked with tyrosinase using 5% glutaraldehyde. The biosensor response was measured for repeated analyses using catechin (50 mmol L^{-1}) as substrate over several days with several analyses (Abhijith *et al.*, 2007).

An amperometric principle-based biosensor containing immobilized enzyme tyrosinase has been used for the detection of polyphenols in tea. This biosensor could detect tea polyphenols in the concentration range 10–80 mmol L^{-1}. The tyrosinase-based biosensor gave maximum response to tea polyphenols at 30 °C. The optimum pH was 7.0. This biosensor system can be applied for analysis of tea polyphenols (Abhijith *et al.*, 2007).

CONCLUSION

The main advantages of these devices are their specificity, sensitivity and ease of sample preparation, and the fact that no other reagents

besides a buffer and a standard are usually required. Investigations have been carried out in this work to develop a microbial biosensor for the estimation of polyphenolic compounds in food and beverage samples. The potential uses of biosensors in agriculture and food transformation are numerous and each application has its own requirements in terms of the concentration of analyte to be measured, required output precision, the necessary volume of the sample, time required to prepare the biosensor or to reuse it and cleanliness requirements of the system.

Conflict of Interests

The authors declare that they have no conflict of interests.

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