Sains Malaysiana 51(9)(2022): 2913-2924
http://doi.org/10.17576/jsm-2022-5109-14

A Simple Potentiometric Biosensor Based on Carboxylesterase for the Analysis of Aspartame

(Biosensor Potensiometrik Mudah Berasaskan Karboksilesterase untuk Analisis Aspartam)

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Received: 1 December 2021/Accepted: 13 March 2022

ABSTRACT

A potentiometric aspartame biosensor was fabricated by simply depositing the carboxylesterase (CES)-bonded poly(*n*-butyl acrylate-*n*-acryloxysuccimide) [CES-poly(*n*BA-NAS)] microspheres on a Ag/AgCl screen-printed pH selective electrode. The pH transducer was made from non-plasticized polyacrylate membrane containing a hydrogen ionophore and lipophilic salt. The immobilized CES enzyme catalyzed the enzymatic hydrolysis of aspartame to L-aspartic acid (L-Asp), L-phenylalanine and methanol. Potentiometric determination of aspartame concentration was performed by quantifying the hydrogen ion concentration produced from L-Asp. The potentiometric determination of aspartame exhibited good selectivity with near Nernstian response. The sensitivity of the biosensor was closed to the Nernstian value, i.e., 50-52 mV decade^{-1} with a dynamic linear response range from 10^{-5} to 10^{-2} M and detection limit approaching 10^{-6} M. The aspartame biosensor demonstrated good repeatability and reproducibility with relative standard deviation (RSD) of 1.9% and 1.6%, respectively (n=3). The potentiometric aspartame biosensor was demonstrated to be reliable for determining aspartame content in sweetener samples and was comparable to the conventional high-performance liquid chromatography (HPLC) method for aspartame analysis.

Keywords: Acrylic microspheres; apartamepotentiometric; Nernst; screen-printed electrode

ABSTRAK

Biosensor aspartam potensiometri telah dibangunkan dengan hanya meletakkan mikrosfera poli(*n*-butil akrilat-*n*-akriloksisuksimida) terikat karboksilesterase (CES) [CES-poli(*n*BA-NAS)] pada elektrod terpilih pH cetakan skrin Ag/AgCl. Transduser pH disediakan daripada membran poliakrilat bukan plastik yang mengandungi ionofor hidrogen dan garam lipofilik. Enzim CES terpegun memangkinkan hidrolisis enzimatik aspartam kepada asid L-aspartik (L-Asp), L-phenylalanine dan metanol. Penentuan potensiometrik kepekatan aspartam dilakukan dengan mengukur kepekatan ion hidrogen yang dihasilkan daripada L-Asp. Penentuan potensiometrik aspartam menunjukkan kepilihan yang baik dengan rangsangan *Nernstian* yang hampir. Kepekaan biosensor adalah berhampiran dengan nilai *Nernstian*, iaitu 50-52 mV dekad^{-1} dengan julat tindak balas linear dinamik dari 10^{-5} hingga 10^{-2} M dan had pengesanan menghampiri 10^{-6} M. Biosensor aspartam menunjukkan kebolehulangan dan kebolehasilan yang baik dengan sisihan piawai relatif (RSD) masing-masingnnya sebanyak 1.9% dan 1.6% (n=3). Biosensor aspartam potensiometrik telah ditentusahkan dalam penentukan kandungan aspartam dalam sampel pemanis dan setanding dengan kaedah kovensional kromatografi cecair berprestasi tinggi (HPLC) untuk analisis aspartam.

Kata kunci: Aspartam; elektrod bercetak skrin; mikrosfera akrilik; Nernst; potensiometrik

INTRODUCTION

Sweetness has always been one of the most pleasing tastes to most people. Nowadays, many types of sugar can be produced by the food industry that humans can consume. However, due to the increased health concerns among consumers, they demanded low-calorie sugar. To fulfil consumers' needs, sweeteners with no or fewer calories and do not promote obesity have been extensively

and massively synthesized (Czarnecka et al. 2021). N-L-α-aspartyl-L-phenylalanine methyl ester, also known as aspartame, is one of the most common artificial sweeteners in use today. Aspartame is an artificial sweetener that is low in calories and composed of three chemicals, i.e., L-aspartic acid (L-Asp), L-phenylalanine (L-Phe) (both of which are amino acids) and methanol (Odaci et al. 2004; Pang et al. 2021). Because aspartame is 160-200 times sweeter than sugar, it is widely used as a sugar substitute in many food processing applications (Stegink & Filer 1984), such as cereal, chewing gum, soft drinks, breath mints, yoghurt products and candies.

Furthermore, demand for non-caloric foods or beverages, especially for diabetic and dietetic reasons, and the manufacture of 'tooth-friendly' food and pharmaceutical products have further increased the use of aspartame (Zhou et al. 2018). Even though aspartame is legally approved for consumption in everyday life since 1980, a couple of research showed that aspartame could cause headaches, seizures, mood changes and allergic-type reactions. They are claimed to increase the risk of cancer and harm our blood sugar and gut health. There are also evidences on phenylalanine and methanol contents in the aspartame could considerably mediate neurologic effects (Choudhary & Pretorius 2017).

Various methods can be applied for the analysis of aspartame. Some of which are conventional methods such as high-performance liquid chromatography (HPLC) (Kim et al. 2020; Zhou et al. 2018), capillary electrophoresis (van Vliet et al. 2020), ion chromatography integrated with amperometric detection (Qu et al. 1999), flow-injection spectrophotometric (Fatibello-Filho et al. 1999) and ion chromatography (Chen & Wang 2001; Zhu et al. 2005). However, most of these methods are time-consuming, require expensive equipment, complicated procedure, and pretreatment of samples is always unavoidable.

Biosensors provided more straightforward methods for aspartame analysis. Nevertheless, very few of such biosensors have been developed for aspartame determinations in the past. The majority of these were bienzymes-based and employed amperometric measurements. For instance, a screen-printed amperometric biosensor for aspartame was developed using bienzyme alcohol oxidase and carboxyl esterase (CES) with cobalt-phthalocyanine. The biosensor was incorporated into flow injection analysis (FIA) (Radulescu et al. 2014). In another study, CES and alcohol oxidase enzymes were used in a gelatin membrane on a graphite-epoxy composite electrode where aspartame was quantified via changes of oxygen (Kirgoz et al. 2006; Odaci et al. 2004). Other previously reported amperometric aspartame biosensors involved the bienzyme system containing alcohol oxidase and α-chymotrypsin for aspartame determination via measuring oxygen or hydrogen peroxide (Chou 1996). The bienzymes such as pronase and L-amino acid oxidase had been exploited in amperometric aspartame electrodes in a flow injection analysis system (Male et al. 1993). Apart from the oxygen-based electrode, other gas electrodes such as carbon dioxide- and ammonia-sensitive gas diffusion electrodes were used in aspartame biosensor constructions (Male et al. 1991; Nikolelis & Krull 1990).

The amperometric way of aspartame analysis necessitates the use of a bienzyme system in biosensor construction. Thus, the process of fabricating the biosensor for aspartame is somewhat complicated because of the use of multiple enzymes in a detection system. There is a need to develop a simpler system employing a single enzyme for aspartame biosensor. In this study, a potentiometric aspartame biosensor based on immobilization of an CES enzyme was investigated. The enzyme was immobilized onto poly(*n*-butyl acrylate-*n*-acryloxysuccimide) [poly(nBA-NAS)] microspheres that were coated on top of an Ag/AgCl screen-printed electrode modified with a layer of pH-sensitive ion-selective membrane. The direct detection of the changes of pH due to the reaction of the aspartame with the enzyme enables a much simpler electrode design than amperometric electrode using gas diffusion detection as had been used frequently for aspartame amperometric biosensors.

Materials and Methods

Reagents and instrumentations

A carboxylesterase (EC 3.1.1.1) from rabbit liver, 2-hydroxylethyl methacrylate (HEMA, 98%), Asp-Phe methyl ester (aspartame, ≥ 98%), DL-phenylalanine (98%), L-cysteine (≥ 97%) and L-leusine (≥ 97%) were purchased from Sigma. Sodium hydroxide (NaOH, 99%), hydrochloride acid (HCl, 37%), *N*,*N*-dimethylformamide (DMF, 99.8%) and L(+)-ascorbic acid (99%) were obtained from Systerm. 2,2-dimethoxy-2-phenylacetophenone (DMPP, 98%), potassium tetrakis (4-fluorophenyl)borate dehydrate (KTFPB, 98%), D(+) glucose monohydrate (≥ 99.5%) and hydrogen ionophore I were supplied by from Fluka. In

addition, N-acryloxysuccinimide (NAS, 99%) and L(+)-glutamic acid monosodium salt monohydrate (≥ 99.5%) were procured from Agros. 1,6-hexanedioldiacrylate (HDDA, 80%) and D-sorbitol (99%) were produced by Aldrich. N-butyl acrylic (*n*BA, 99%), sodium dodecyl sulphate (SDS, 95%) and Tris-hydrocloric (Tris-HCl, ≥ 99.8%) were procured from Merck, BDH and Duchefa Biochemie, respectively. All of these chemicals were of analytical grade and used without further purification. UV exposure unit (RS Ltd.) and sonicator (Elmasonic) were utilized to synthesize and photopolymerize acrylic microspheres. Ion meter (Orion) was used for all potentiometric measurements of analyte samples, and pH meter (Metrohm) was used to prepare standard buffer solutions.

SYNTHESIS OF NAS-MODIFIED ACRYLIC MICROSPHERES

NAS-modified acrylic spheres were prepared by mixing appropriate amounts of nBA monomer, DMPP photoinitiator, SDS emulsion agent, HDDA crosslinker and NAS in the deionized water. The colourless solution was then sonicated for 10 min until a milky white suspension was formed. Then, the suspension was exposed to UV light for 500 s to initiate the photopolymerization process. Nitrogen gas was introduced to the suspension during the photocuring process to eliminate oxygen from the polymerization reaction. The size and morphology of the acrylic microspheres were then determined by using scanning electron microscopy (SEM).

IMMOBILIZATION OF CARBOXYLESTERASE ENZYME

Carboxylesterase (CES) enzyme solution was prepared in 0.1 M phosphate buffer at pH 7.0. Some 30 mg of poly(nBA-NAS) micropheres were added to the enzyme solution followed by an adequate amount of DMF. The resulting solution was stored at 4 °C for 24 h to allow succinimide functional groups of acrylic microspheres to react with amine group of enzymes to form amide covalent bond. The mixture was then centrifuged at 4000 rpm for 5 min and washed several times with 0.1 M phosphate buffer (pH 7.0) to eliminate the unbound enzymes and kept at 4 °C when not in use. Bradford reagent was used to determine the percentage of enzyme attached to the NAS-modified acrylic spheres.

FABRICATION OF POTENTIOMETRIC ASPARTAME BIOSENSOR

H^+ ion transducer was developed by depositing a mixture of HEMA monomers and 1.6 wt.% of DMPP photoinitiator on the Ag/AgCl electrode (2 mm diameter, Warner). The mixture was then photocured under UV radiation in a UV exposure unit with a constant nitrogen gas purging for 180 s. The resulting polyHEMA film was hydrated with 0.1 mM Tris-HCl solution for 15 min. A mixture comprised of 100 µL nBA monomers, 1.0 mg DMPP, 0.95 µL HDDA, 7.5 mg hydrogen ionophore I and 1.8 mg KTFPB was then drop-coated on the hydrated poly(HEMA) film and photocured again for another 180 s under nitrogen gas flow. The procedure to prepare a pH sensor using hydrogen ionophore containing self-plasticized poly(nBA) film coated on an inner hydrated poly(HEMA) layer by photocuring technique has also been reported by Nurlely et al. (2021). Finally, the CES-poly(nBA-NAS) microspheres was deposited on top of the H^+ ion sensor.

OPTIMIZATION OF ASPARTAME BIOSENSOR RESPONSE

Orion ion meter was used to measure the potential difference signal (electromotive force, emf) between the H^+ ion transducer and the homemade Ag/AgCl reference electrode. The reference electrode used was a double junction Ag/AgCl gel bridge electrolyte filled with 0.1 M Tris-HCl (pH 7.0) and saturated with AgCl and 0.1 M lithium acetate. The connection of the working electrode and double junction Ag/AgCl reference electrode to the ion meter was similar to those reported earlier (Lee et al. 2022). All the measurements were carried out at ambient temperature (25 °C). 0.01 M aspartame standard stock solution was prepared in 10 mM Tris-HCl at pH 7, and dilution was done using the same buffer solution to the desired concentration. Characterization of the biosensor potentiometric response, including sensitivity, linear response range, detection limit, acrylic microspheres loading effect, pH effect, buffer effect and biosensor shelf life, were studied. The biosensor response in mV unit was plotted against the logarithm concentration of aspartame based on the Nernst equation.

INTERFERENCE STUDY OF ASPARTAME BIOSENSOR

Three types of potential interferents towards aspartame biosensor response chosen for the interference study were sugar, amino acids and common food and drink additives. For examples, sorbitol, glucose, DL-phenylalanine, L-cysteine, L-leucine, L(+)-glutamic acid and L(+)-ascorbic acid. These solutions were prepared at different concentrations ranging from 10^{-5} to 10^{-2} M in 10 mM Tris-HCl (pH 7.0).

MEASUREMENT OF ASPARTAME CONTENT IN COMMERCIAL BEVERAGE AND SWEETENER SAMPLES

Two aspartame-containing food samples were chosen for potentiometric aspartame biosensor validation study, namely low-calorie artificial sweeteners and commercial soft drinks. The sweetener samples were dissolved in 10 mM of Tris-HCl at pH 7.0 before the aspartame analysis by using potentiometric enzyme biosensor. For validation study with a HPLC reference method (Demiralay et al. 2006; Zhou et al. 2018), soft drink samples were used. The pH of the samples was adjusted to pH 7.0 before further measurements with aspartame biosensor and HPLC were carried out. The results obtained from the biosensor tests were then compared with HPLC.

RESULTS AND DISCUSSION

SYNTHESIS OF ACRYLIC MICROSPHERES AND CES ENZYME IMMOBILIZATION

Recently, micro/nanotechnology has been intensively applied in the biosensor field (Xue et al. 2020). The use of microspheres as immobilization phase for biorecognition and chemical reagent attachment offers many advantages. Some examples are considerable increments in assay sensitivity, widening the dynamic range, and lowering the limit of detection (Raja Jamaludin et al. 2018) without adding an electron mediator in the electrochemical detection medium (Aldewachi et al. 2017). The poly(nBA-NAS) microspheres were synthesized with a simple photolithography method and have been successfully used in immobilization of enzymes for optical and amperometric biosensors but has not been used for potentiometric enzyme biosensor (Nik Mansor et al. 2018; Zuki et al. 2018). CES enzymes were attached covalently to the poly(nBA-NAS) microspheres via amide bond, formed between the succinimide functional group of NAS and the amine group of CES enzyme.

The morphology and size of the acrylic microspheres were determined by using scanning electron microscopy (SEM). Based on the SEM image as shown in Figure 1, the size distribution of the as-prepared polyacrylate spheres was observed to be in the range of 300-1000 nm. The use of long alkyl chain sulphate surfactant (SDS) as an emulsion agent has facilitated the formation of the spherical shape of the hydrophobic nBA polymer, which was associated with the thermodynamics interaction between water and nBA monomer (Omari et al. 2021). The small acrylic spheres were also perceived to remain stable in the emulsion phase. Based on the Bradford protein assay, the amount of CES enzymes immobilized on the acrylic microspheres surface was determined to be around 46.5%.

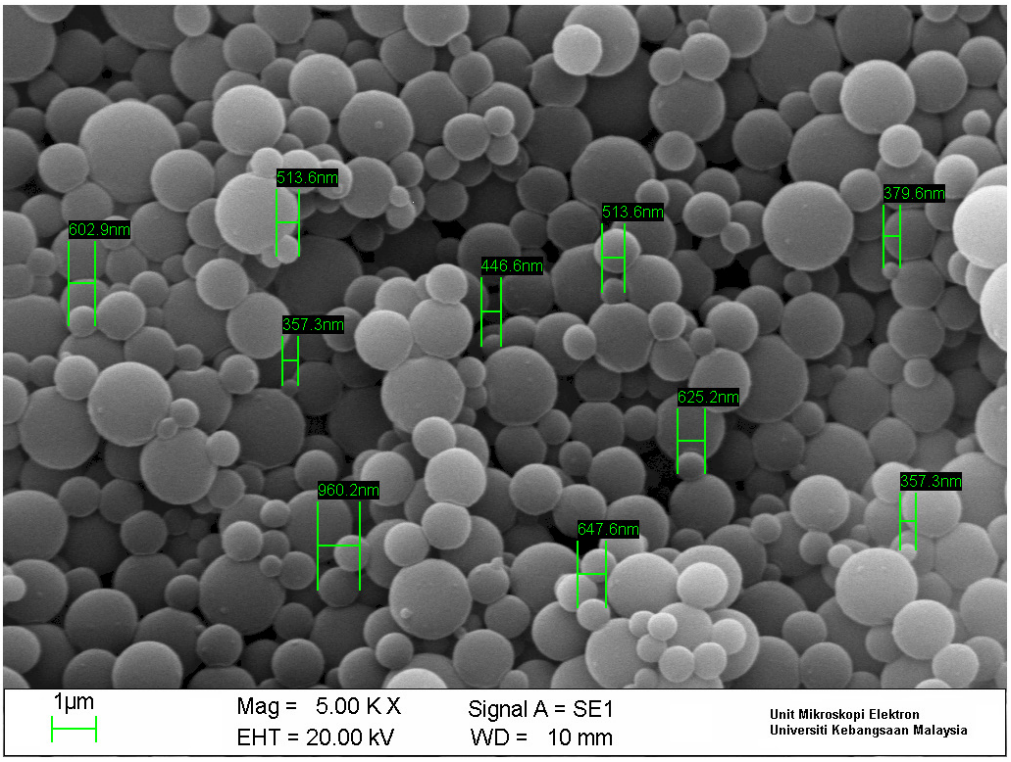

FIGURE 1. SEM image of the microspheres of acrylic polymer in the absence of immobilized CES enzyme (1 µm scale)

The acrylic microspheres are made up of repeating *n*-butyl acrylate monomer ($C_7H_{12}O_2$). The FTIR spectrum in Figure 2 shows the C=O absorption peak at 1734.02 cm^{-1} whilst the stretching band of C-O is at 1164.06 cm^{-1}. The stretching vibration of alkene (C=C) is observed at the absorption band of 1639.06 cm^{-1}, and C-H out of plane bending vibration is between the wavenumber of 1000-650 cm^{-1}. The CH_2 bending peak is at 1457.78 cm^{-1} and the CH_3 bending peak is at 1384.05 cm^{-1}. The absorption peaks at 2960.66 and 3446.50 cm^{-1} are correspond to the OH group and sp^3 CH stretching band, respectively.

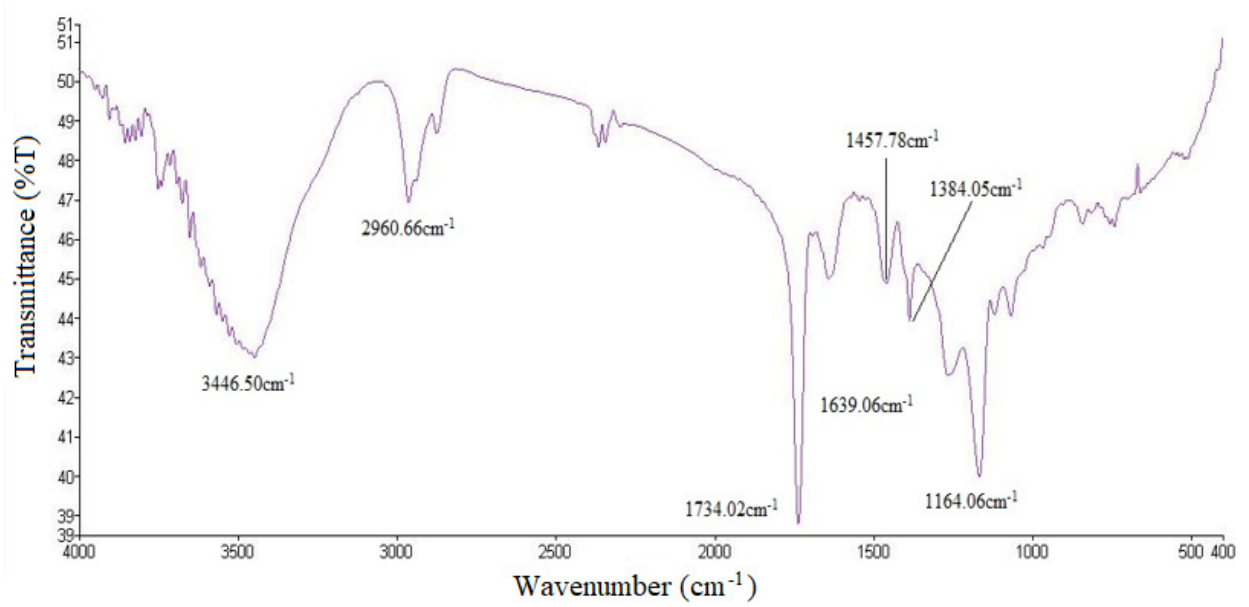

FIGURE 2. The FTIR spectrum of the photopolymerized acrylic microspheres

The use of acrylic micropolymer as an enzyme immobilization matrix for potentiometric biosensing of aspartame can promote the diffusion of reactant and product molecules due to large reaction surface area of the microspheres. Covalent binding of CES enzyme molecules onto acrylic microspheres is proposed in order to prevent the loss of enzyme molecules from the supporting matrix. As such, the analytical performance of the aspartame enzymatic biosensor has enhanced in terms of sensitivity and linear response range.

ANALYTICAL PERFORMANCE OF THE ASPARTAME BIOSENSOR

The determination of aspartame using potentiometric biosensor is performed indirectly via the H^+ ion transducer laid underneath the CES-poly(nBA-NAS) microspheres. The immobilized CES catalyzed the enzymatic hydrolysis of aspartame to L-Asp, L-Phe, and methanol. The dissociated H^+ ion from L-Asp was then diffused into the H^+ ion-selective layer to form a proton-ionophore complex and gave an electromotive force (emf) response to the potentiometer proportional to the concentration of aspartame. The sensing mechanism of the enzymatic biosensor based on CES enzyme and acrylic microspheres pH transducer for potentiometric determination of aspartame is shown in Figure 3.

The acrylic microspheres-based enzymatic biosensor detected proton produced from the dissociation of L-Asp acid for indirect potentiometric determination of aspartame and gave a sensitivity of 51.77±0.81 mV $decade^{-1}$, which is near to the Nernstian value at 59 mV $decade^{-1}$ and a linear potentiometric response range from 10^{-5} M to 10^{-2} M with a limit of detection (LOD) estimated at 4.67×10^{-6} M (n=3) (Figure 4). This linear response range is useful for aspartame analysis in soft drinks as most contain aspartame from 10^{-4}-10^{-3} M (Patz 2015). At low aspartame concentration i.e., from 10^{-10} to 10^{-6} M, the immobilized CES catalyzed the enzymatic conversion of small amount of aspartame into the corresponding L-Asp, L-Phe, and methanol at low levels,

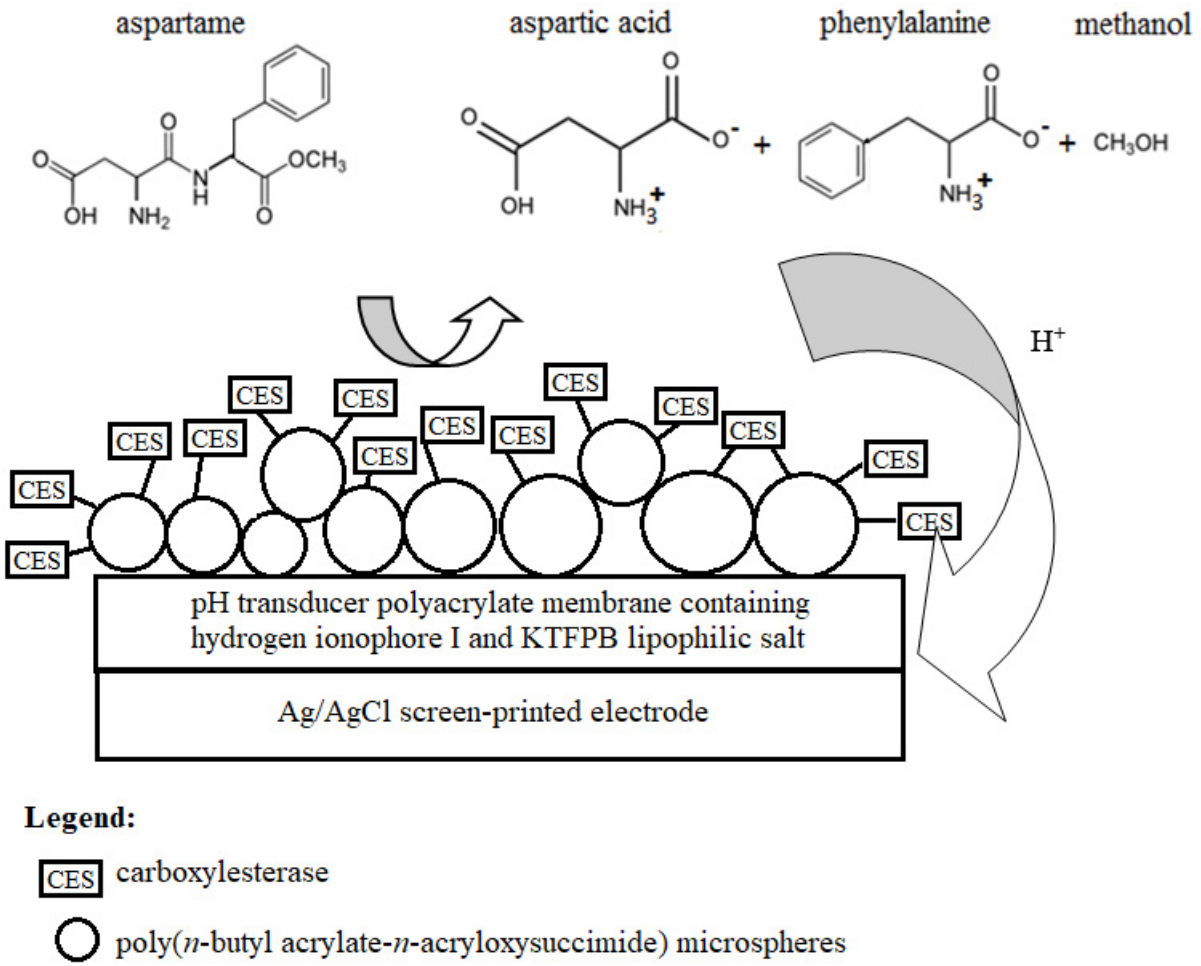

FIGURE 3. The sensing mechanism of the enzymatic biosensor based on CES enzyme and acrylic microspheres pH transducer for potentiometric determination of aspartame

thereby resulted in the production of low concentration of proton and that low potentiometric response was afforded. High aspartame ceoncentration, above 10^{-2} M did not generate a linear emf response as the enzymatic reaction has reached a saturation state in the presence of high substrate concetration.

The amount of NAS-modified acrylic microspheres deposited on the poly(nBA) membrane surface towards potentiometric biosensor response was also investigated as the matrix loading may potentially influence the biosensing performance (Nurlely et al. 2021). The acrylic microspheres loading effect on the potentiometric aspartame biosensor is illustrated in Figure 5. The biosensor sensitivity was observed to decline when the weight of poly(nBA-NAS) microspheres deposited on the pH transducer increased by more than 1 mg. This was most probably attributed to the overloading of the acrylic microspheres and created a barrier restricting the diffusion of aspartic acid into the H^+ ion-selective membrane underneath the polyacrylate microspheres membrane.

The effect of pH on the aspartame biosensor response was studied from pH 5.0 to pH 9.0 at buffer capacities of 100, 10 and 1 mM. The biosensor sensitivity response trend in Figure 6 shows that the aspartame enzymatic biosensor was optimum at pH 7.0 and buffer capacity of 10 mM. The biosensor sensitivity response deteriorated in both alkaline and acidic media because the acids and bases disrupted the salt bridges held together by ionic charges in the protein structure of

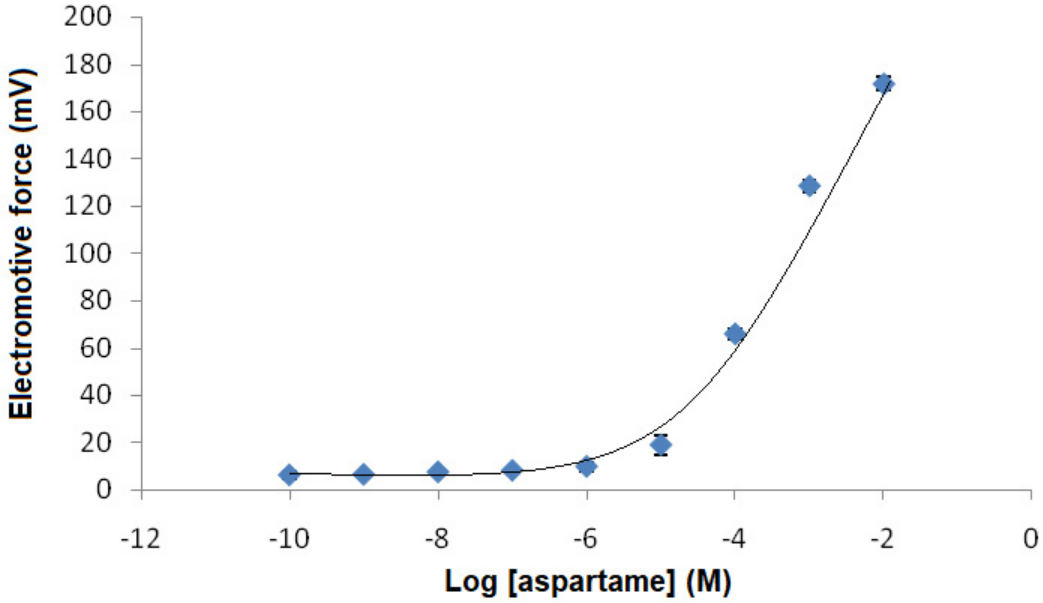

FIGURE 4. The potentiometric response of the aspartame biosensor towards changes of aspartame concentration from 10^{-5} M to 10^{-2} M at pH 7.0

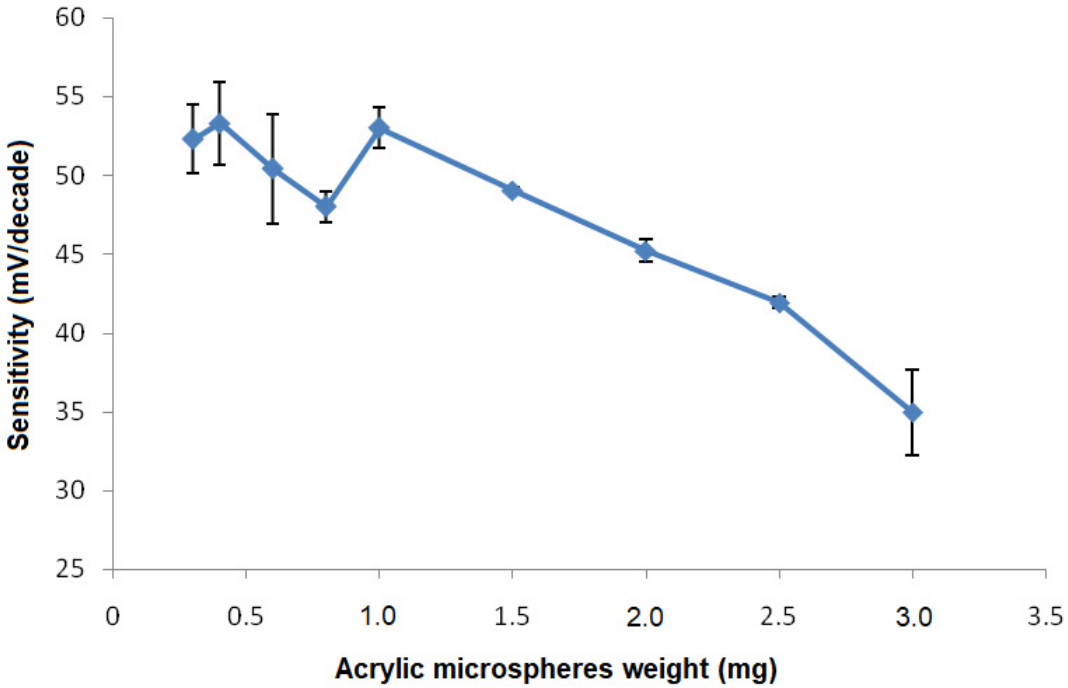

FIGURE 5. The effect of poly(nBA-NAS) microspheres loading between 0.2 mg and 3.0 mg on the potentiometric aspartame biosensor response

the enzyme, and the enzyme underwent denaturation, which reduced the enzyme activity (Nurlely et al. 2021). However, the enzyme sensitivity was better in alkaline conditions than in an acidic medium. This is similar to the previous research findings, where CES enzyme activity reported to be optimum in a more alkaline pH range (Campanella et al. 1995; Odaci et al. 2004; Radulescu et al. 2014). The catalytic activity and stability of the CES enzyme have also been reported to be optimum at pH between pH 7.0 and pH 10.5 (Tomić et al. 1995).

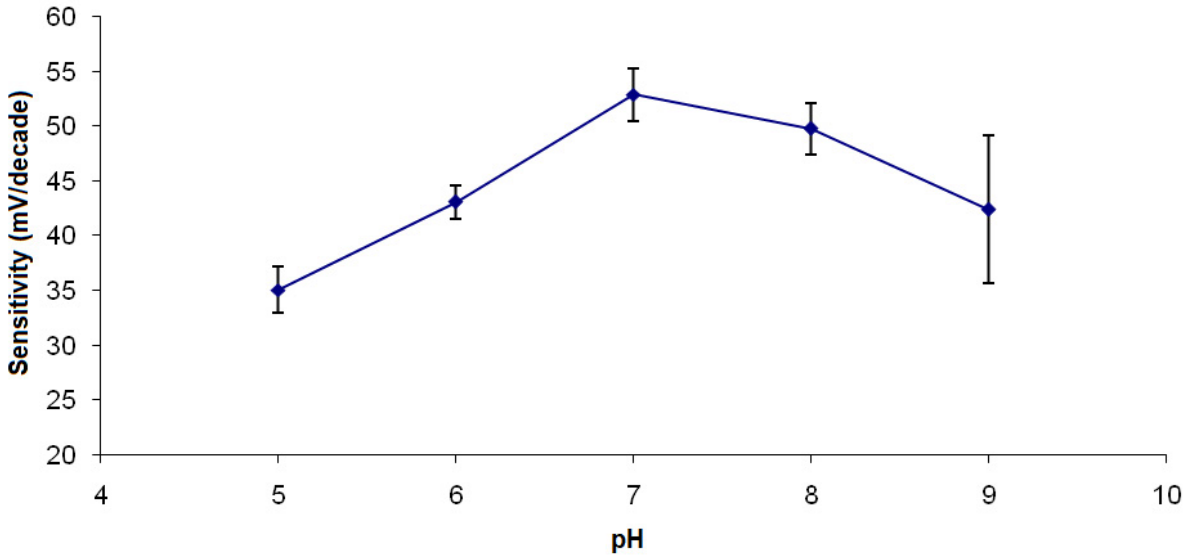

FIGURE 6. The potentiometric aspartame biosensor response trend as a function of pH at a buffer capacity of 10 mM Tris-HCl buffer

The effect of buffer capacity on the aspartame biosensor performance about linear detection range, limit of detection and sensitivity is tabulated in Table 1. The immobilized CES enzyme showed the highest catalytic activity in 10 mM Tris-HCl buffer. The biosensor sensitivity performance declined at 100 mM Tris-HCl buffer due to the high ionic strength of the reaction medium has prevented the reversible binding of H^+ ion to the proton sensitive membrane (Fazial et al. 2018). In addition, interference from H^+ ion was also possible due to protonation reaction at the immobilized CES enzymes' active sites (Hassan et al. 2019; Nurlely et al. 2022). The dynamic linear concentration range of the acrylic microspheres-based potentiometric biosensor was obtained between 10^{-5} M and 10^{-2} M aspartame, which is better when compared to the gelatin membrane-based amperometric aspartame biosensor by using graphite-epoxy composite electrode (10^{-6}-10^{-4} M, 3.9% RSD) (Raja Jamaludin et al. 2018).

TABLE 1. The effect of buffer concentration on the potentiometric biosensor performance in determining series of aspartame concentrations from 10^{-5} - 10^{-2} M

Buffer concentration (mM)	Linear range (M)	Limit of detection (µM)	Sensitivity (mv decade^{-1})
100	10^{-4} - 1^{0}-2	28.5	43.9
10	10^{-5} - 1^{0}-2	4.67	52.75

The shelf life of the biosensor was studied by measuring the sensitivity of the enzymatic electrode on an intermittent basis for 38 days. The fabricated CES-poly(nBA-NAS) microspheres-modified Ag/AgCl

electrode was kept in the refrigerator at 4 °C. The biosensor sensitivity response was stable on the first week with a reproducibility RSD of less than 5%. About 80% of its initial response was still achievable even after a period of 20 days. At 30 days, the response slowly reduced to about 70% of the original response, which could be attributed to the denaturation of the enzyme and resulted in the loss of activity (Fazial & Tan 2021).

INTERFERENCE STUDY OF POTENTIOMETRIC ASPARTAME BIOSENSOR

Three types of interfering agents have been selected for interference study of potentiometric aspartame biosensor, such as sugar, amino acids and other chemical additives commonly used in foods and beverages. Both sugar (sorbitol and glucose) and amino acid (DL-phenylalanine, L-cysteine and L-leucine) showed no interference in the potentiometric biosensor response. This finding is consistent with the previously reported study by Campanella et al. (1995), demonstrating that amino acid provides no relative activity towards the biosensor performance. However, the potentiometric aspartame biosensor responded slightly to ascorbic acid and glutamic acid. However, the selectivity of the biosensor towards aspartame is still good, especially at low concentrations of the interferent, such as ascorbic and glutamic acids, e.g., below 10^{-2} M, the interference is estimated to be <10% (Figure 7). The ascorbic acid content normally available in many commercial soft drinks is less than 10^{-3} M. Thus, the interference of ascorbic acid towards the biosensor is not expected to analyze aspartame in this type of sample.

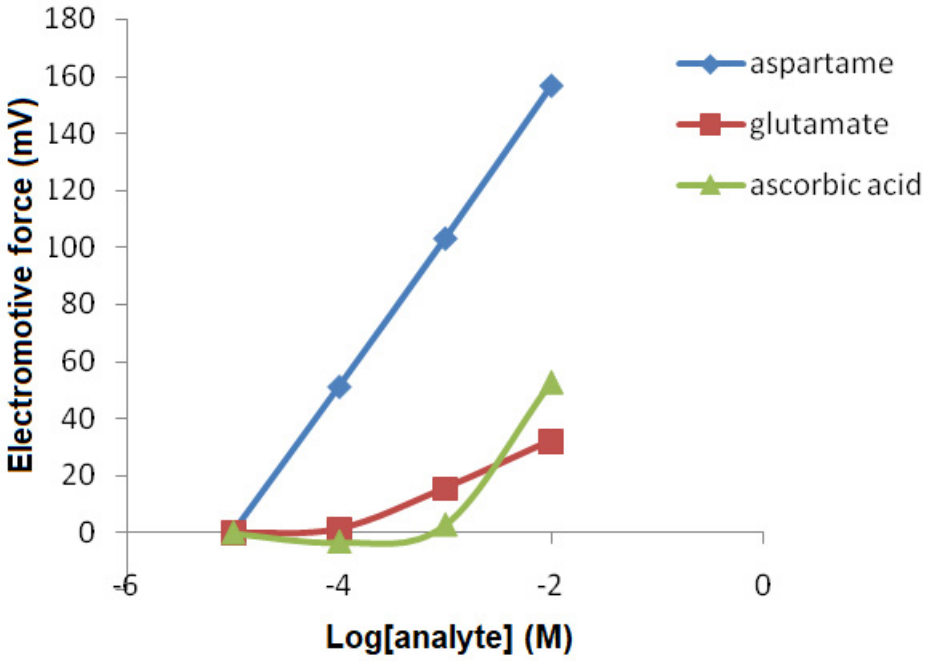

FIGURE 7. Potentiometric response of CES-poly(nBA-NAS) electrode towards determination of aspartame, glutamate and ascorbic acid from 10^{-5} to 10^{-2} M in 10 mM Tris-HCl (pH 7)

REAL SAMPLE STUDY BY USING POTENTIOMETRIC ASPARTAME BIOSENSOR

A calibration curve was constructed before the detection of aspartame in commercial sweetener samples. Based on the result tabulated in Table 2, it is noticed that the aspartame level determined by using the acrylic microspheres-based potentiometric biosensor in low-calorie sweetener samples demonstrated results well agreed with the nutrition facts stated on the samples' packages. This suggests the potentiometric aspartame biosensor based on CES-poly(nBA-NAS) microspheres modified Ag/AgCl electrode can be used as a reliable method for aspartame determination in foods and beverages.

TABLE 2. Comparison between detected aspartame and aspartame content on the commercial sweetener samples (working buffer: 10 mM Tris-HCl, pH 7)

Sample	Label value (mM)	Detectable aspartame (mM)
1	2.45	2.38
2	1.23	1.27

To confirm if the analytical results obtained by the proposed enzymatic biosensor method give reliable results when used for the quantitative determination of aspartame, a comparative study between biosensor and established HPLC method has been conducted. Figure 8 represents the correlation results acquired by using the CES-poly(nBA-NAS) electrode and the HPLC standard method for aspartame concentration analysis. The R^2 value of close to 1 indicates that the results obtained by using the enzymatic electrode are corresponding perfectly to the HPLC method in the quantitative determination of aspartame concentration. This suggests both methods have no statistically significant difference in aspartame determination and that the results generated from both methods are comparable.

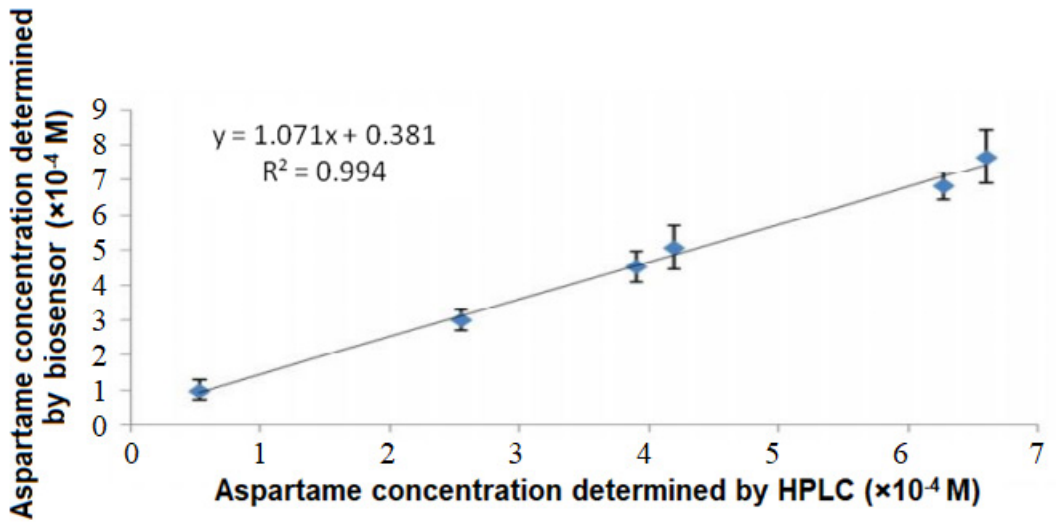

FIGURE 8. Correlation between HPLC and biosensor methods for the determination of aspartame concentration

CONCLUSIONS

A potentiometric biosensor for aspartame has been successfully developed by using a single enzyme. The electrode design that employed a multi-layer solid-state screen-printed and pH-selective electrode is much simpler than many amperometric biosensors for aspartame reported so far. The preparation of CES-poly(nBA-NAS) microspheres electrode involved simple photolithography and drop-coating techniques for electrode fabrication. The aspartame biosensor yielded a linear response range and detection limit useful for directly analyzing aspartame in artificial sweetener and soft drink products. The good agreement between potentiometric biosensor and HPLC standard methods for aspartame analysis indicated that this biosensor could be used for aspartame determination in commercial beverages and sweeteners.

ACKNOWLEDGEMENTS

We acknowledged the research funding from National Biotechnology Directorate, MOSTI Malaysia via research grant UKM-MGI-NBD0021-2007.

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