



Development of Gum-Arabic-based polyurethane membrane-modified electrodes as voltammetric sensor for the detection of phenylalanine

Funda Alışık¹ · Muammer Burç¹ · Serap Titretir Duran¹ · Öznur Güngör¹ · Mahmut Alper Cengiz¹ · Süleyman Köytepe¹

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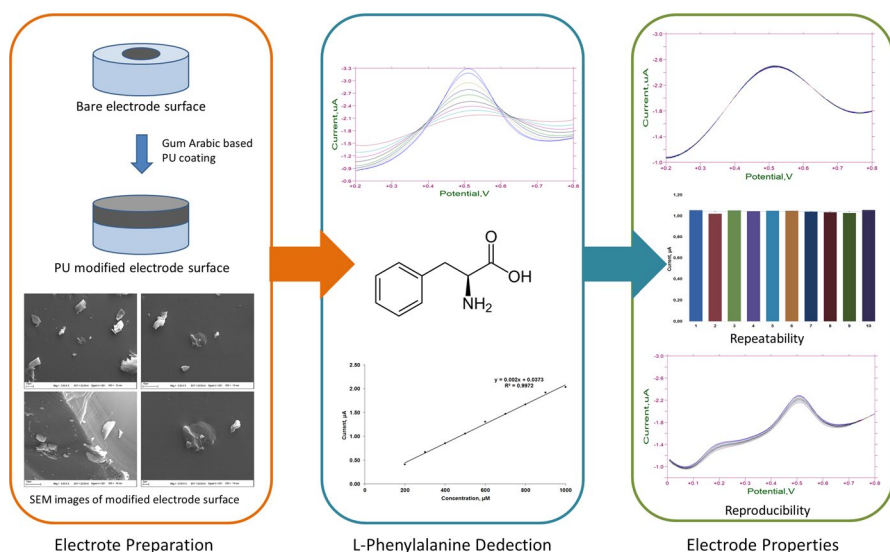
Abstract

In this study, L-phenylalanine (L-Phe) sensors were prepared from Gum-Arabic-based polyurethanes, by the modification of Pt electrode. Firstly, Gum-Arabic-based polyurethanes were synthesized by solution polymerization technique and characterized with Fourier transform infrared spectroscopy, thermogravimetric analysis, differential thermal analysis, scanning electron microscopy and atomic force microscopy techniques. Then, L-Phe selective electrodes were prepared by coating the platinum electrode with different thickness Gum-Arabic-based polyurethane. L-Phe responses of the modified electrodes were investigated by differential pulse voltammetry technique. Sensor parameters such as scanning speed, film thickness, electrolyte type and pH were examined in detailed. Prepared electrodes were showed $R^2=0.9972$ for the concentration range of 200–1000 μM L-Phe. The limit of detection and the limit of quantification of the prepared L-Phe selective modified electrode were calculated as 48.01 μM and as 144.02 μM , respectively. After ten consecutive measurements with the prepared L-Phe selective electrode, 98.8% stable result was obtained between the first and tenth reading and the RSD% value was found to be 1.21% for ten replicates. Consequently, prepared Gum-Arabic-modified sensors can be used successfully for the detection of the L-Phe with large concentration areas, high sensitivity and repeatability.

✉ Serap Titretir Duran
serap.titretir@inonu.edu.tr

¹ Department of Chemistry, Faculty of Arts and Sciences, İnönü University, 44280 Malatya, Turkey

Graphic abstract



Keywords L-phenylalanine sensor · Modified electrode · Polyurethane · Voltammetry

Introduction

Phenylalanine (Phe) is found in the structure of all proteins necessary for the body and is an essential amino acid [1]. Phe is the metabolic precursor of the non-essential tyrosine amino acid and its derivatives [2]. It is used in the production of thyroid hormones, proteins production and neurotransmitters (dopamine, nehrine and norepinephrine). Phe has a hydrophobic property due to the aromatic group in its molecular structure and is in the category of apolar amino acids [3, 4]. In addition, Phe is used as an additive in the pharmaceutical and food industry [2–5]. The phenylalanine hydroxylase enzyme (PAH) synthesized in the liver turns phenylalanine into tyrosine. Phenylketonuria (PKU) occurs with PAH deficiency [6]. PKU is a disease caused by amino acid metabolism disorder. Tyrosine synthesis from Phe cannot occur in PKU disease due to the lack of PAH enzyme activity in the liver. In this situation, Phe and its metabolites accumulate in the blood [6–8]. If the disease is not diagnosed and treated early, phenylketonuria leads to neurological dysfunction, autism, eczema and severe behavior disorder. Early detection can prevent many effects of the disease [7, 8].

Phe is of great importance as an imaging agent during the diagnosis and treatment of PKU, an important genetic disease. Especially in newborns, the early detection of this disease plays a critical role in initiation of treatment [6]. However, the fact that the amount of Phe in the blood is very low and the test sample

is very small requires a procedure such as taking blood from a newborn baby, which significantly reduces the accuracy and sensitivity of this measurement [9–11]. For this measurement in newborns, heel blood is taken and absorbed on a paper and this sample is sent to the big laboratories for Phe determination. This process can lead to experts and some confusion in determination. It also requires a highly equipped laboratory. In particular, Tandem mass spectroscopy is used for Phe analysis [12]. Near infrared (NIR) spectroscopy, UV-visible spectroscopy, gas chromatography, chemiluminescence detection, amperometry, voltammetry, high performance liquid chromatography techniques and biosensors are also used for L-Phe determination [13–19]. The associated analysis requires expensive equipment as well as a multi-step sample preparation procedure and experienced personnel [20, 21]. Electrochemical sensors are a very important alternative to make this analysis more sensitive, rapid and reproducible at lower costs [22–24]. Especially with specially modified electrodes, very low concentrations and high repeatability results can be obtained in very short periods of time.

In this study, phenylalanine sensors have been developed for the rapid, sensitive, accurate and selective determination of phenylketonuria, which is an important disease. Voltammetric measurements are used as the basic measurement principle in the developed sensors. Gum-Arabic-based polyurethane structures, a natural polysaccharide, were used and modified platinum electrodes were prepared using these prepared polyurethane structures. These polyurethane films have good adhesion, coating properties, selective permeability and negative charge surface groups. These negative surface groups facilitate the cumulation of the phenylalanine molecule at the surface and eliminate the interference molecules away from the electrode surface. FTIR, DTA and TGA analysis techniques were used to determine the chemical and thermal properties of the synthesized polyurethane structures before determining the sensor properties of modified electrodes. In addition, SEM method was used to determine the surface properties and morphology of polymer structures. To determine the stability, L-Phe selectivity and sensitivity of modified electrodes, parameters such as scanning speed, type of electrolyte, pH effect and thickness of the polyurethane film layer were analyzed using differential pulse voltammetry (DPV). In conclusion, this study aims to provide a simple, selective, inexpensive high-sensitivity determination of L-phenylalanine.

Materials and methods

Materials

L-Phenylalanine, sodium citrate, citric acid, acetic acid, sodium acetate, boric acid, potassium chloride, sodium hydroxide, sodium chloride, sodium hydrogen phosphate, potassium dihydrogen phosphate, glucose (GL), sucrose (SC), lactose (LC), maltose (ML), uric acid (UA), ascorbic acid (AA) and monomers in synthesis of polyurethanes were purchased from Sigma-Aldrich Chemicals Company.

All chemicals were supplied in analytical purity, and no pre-purification was carried out before the experiments.

Equipment

Fourier transform infrared spectroscopy (FTIR) was used for the structural characterization of the Gum-Arabic-based polyurethanes. PerkinElmer Spectrum Two FTIR device was used for FTIR analysis. FTIR analyses were performed in ATR measurement mode in the wavenumber range of 400–4000 cm^{-1} . SEM analysis was used to determine the surface morphologies of the modified electrodes prepared for L-Phe determination. SEM analyses were performed using the LEO-EVO SEM device, and the samples were analyzed by coating with Au/Pd using a sputter. In addition, TGA, DTG and DTA analysis techniques were used to determine the thermal properties of prepared polyurethane structures used for electrodes prepared by chemical modification. Shimadzu TGA-50 and Shimadzu DTA-50 devices were used in thermal analysis. TGA and DTA analyses were carried out between 25 and 700 $^{\circ}\text{C}$ and were performed in a static air atmosphere at 10 $^{\circ}\text{C}/\text{min}$ heating rate. 10 mg sample and 10 mg alumina standard were used in these analyses. All DTA and TGA analyses were carried out using a platinum sample cuvette.

Voltammetric experiments were performed using a BAS 100B (Bioanalytical Systems, Inc.) electrochemical analyzer. Control of the device was carried out with BAS 100B software running under Windows. Electrochemical measurements were carried out in approximately 10–20 mL Pyrex glass cells.

Electrochemical cells are placed in the C2 (cell stand) cage of BAS company. Thanks to this cell stand, which is used like a Faraday cage, the experimental setup is protected from electrical and magnetic effects in the external environment.

Branson 3510 model ultrasonic bath was used in cleaning the electrodes and making the solutions homogeneous. The pH adjustments of the solutions were made with Orion 4 Star brand pH meter. Kern brand ABJ 220-4NM model scale was used for weighing the chemicals. All glass materials used were dried in a Nuve brand EN400 model oven. 15.8 μS ultrapure water treated with Milli Pore Milli-Q Water Purification System was used for the preparation of solutions and cleaning of the electrodes, and 18.2 μS of deionized pure water was used for cleaning glassware.

In the experiments, platinum wire electrode (BAS brand MW-1032) as auxiliary electrode, Ag/AgCl (BAS brand RE-5B) electrode in 3 M KCl as reference electrode and 1.6 mm diameter platinum (Pt) electrode (CHI102) as working electrode were used.

After the reference electrode was used, it was washed with ultrapure water and stored in 3 M KCl solution. Platinum wire electrode was heated at certain intervals on a naked fire in order to remove possible organic impurities and washed with ultrapure water. The working electrode, on the other hand, was cleaned by dropping a few drops of alumina solution in the size of 0.05–0.1 μM

on a velvet disk, scratching the electrode "8" and then washing it with ultrapure water. In order to completely clean the alumina residues, it was cleaned by waiting in an ultrasonic bath for 1 min in a 1:1 ethyl alcohol: water solution.

Preparation of L-phenylalanine sensors by chemical modification

Gum-Arabic-based polyurethane structures used in chemical modifications were synthesized in DMF/THF mixture using solution polymerization technique. Gum-Arabic, ethylene glycol and hexamethylene diisocyanate as monomer were used in the synthesis of Gum-Arabic-based polyurethanes to be used in the modification of the electrodes. In the synthesis of the pure polyurethane structure (PU), ethylene glycol and hexamethylene diisocyanate as monomer were used. Gum-Arabic-based polyurethane structures were synthesized with Gum-Arabic: ethylene glycol ratios, 1:99 (Gum-PU-1%), 5:95 (Gum-PU-5%) and 10:90 (Gum-PU-10%) mixtures as polyol. During the synthesis, first 0.01 molar polyol mixtures in 10 mL DMF/THF (3/7) were dispersed at room temperature in argon atmosphere. After obtaining a homogeneous solution, 0.01 molar hexamethylenediisocyanate (HMDI) was added as monomer under dynamic argon flow. The monomer mixture was stirred for one hour at 30 °C. Then, the temperature was raised to 90 °C and refluxed for 5 h. The polymeric structure obtained was precipitated by the addition of water. The termination stage of the reaction was determined using FTIR spectrum. The reaction was continued until no free isocyanate peak was seen at 2260 cm^{-1} in the relevant spectrum [24]. This peak is due to free isocyanate groups, and disappearance of the peak indicates that the reaction is terminated. The obtained polyurethane structures have been characterized by FTIR, SEM and thermal analysis techniques. The structural diversity in the polyurethane structure is provided by changing the Gum-Arabic ratio between the polyol groups. Three different Gum-Arabic ratios were used as 1%, 5% and 10%. After the electrode modifications performed, it was determined that the electrode containing Gum-PU-10% structure was ideal and the electrode prepared at this rate was used for advanced electrochemical sensor experiments.

Preparation of phenylalanine sensor with Gum-Arabic-based polyurethane modification

The polymer (Gum-Arabic-based polyurethanes) solutions were prepared by dissolving 0.1 g of Gum-Arabic-based polymer in 1 ml of THF and 0.1 ml of NMP. 4 μL of these polymer solutions was dropped on the platinum electrode surface and dried at room temperature for one day.

Results and discussion

Characterization of Gum-Arabic-based pu structures

For the detection of phenylketonuria disease, Gum-Arabic-based polyurethane-modified L-Phe electrodes were prepared. In order to prepare these electrodes, Gum-Arabic-based polyurethane structures were synthesized and characterized. Then, the synthesized polyurethane structures were coated on the bare Pt electrode surface in different film thicknesses and phenylalanine permeable polymeric film-coated electrodes were obtained.

Structural analyses of Gum-Arabic-based polyurethanes used in chemical modifications of electrodes were made with FTIR spectra. In the FTIR analysis performed, peaks belonging to basic polyurethane groups were determined and the peak arising from the isocyanate monomeric structure was not observed at 2260 cm^{-1} [25]. This result proves that polymerization is complete and no free monomer remains.

In Fig. 1, FTIR spectra of the Gum-PU-1%, Gum-PU-5%, Gum-PU-10% and pure PU structures were showed. In the FTIR spectrum of the pure PU, N–H hydrogen bond peak at about $3400\text{--}3100\text{ cm}^{-1}$, aliphatic C–H stretching vibration at $2850\text{--}2950\text{ cm}^{-1}$, C=O stretching vibration at around 1689 cm^{-1} . C–C stretching vibration at 1540 cm^{-1} and C–N stretching peak at 1450 cm^{-1} were seen. In addition, in FTIR spectra of the Gum-based polyurethanes, peaks were observed stem from both the Gum-Arabic structure and the diisocyanate structure. Carbonyl stretching vibrations in polyurethane structure are clearly seen, especially at 1650 cm^{-1} and 1691 cm^{-1} [26, 27]. In addition, hydrogen bond stress vibrations of free OH and NH groups in the range of $3000\text{--}3500\text{ cm}^{-1}$ are clearly seen. C–N tensile vibrations are seen at 1450 cm^{-1} [26–28]. At 1047 cm^{-1} , C–O–C etheric stretching vibrations

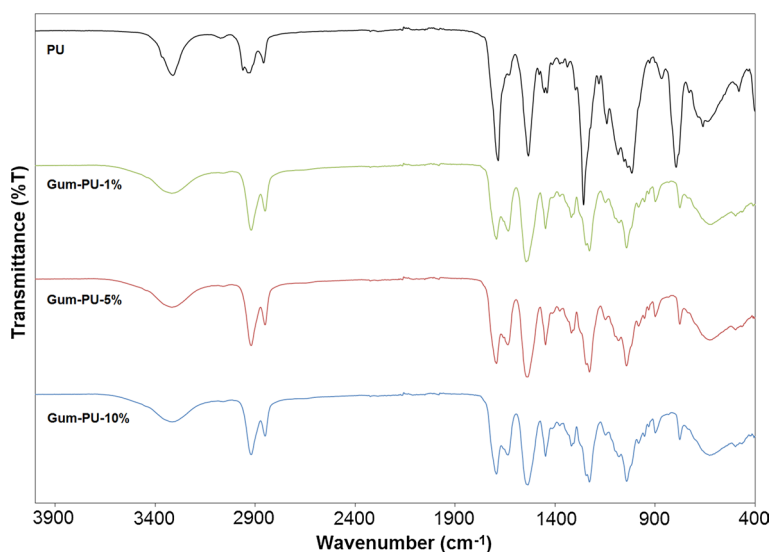


Fig. 1 FTIR spectra of pure PU and Gum-Arabic-based polyurethane structures

are observed. Aliphatic C–H stretching vibrations originating from the Gum structure were observed in the range of $2798\text{--}2975\text{ cm}^{-1}$. The CH_2 rocking vibration was determined at approximately 700 cm^{-1} [29]. All these findings prove that the desired polyurethane structure has been obtained [25–28].

Important properties in polymeric structures to be used in electrode modification are surface morphology and surface porosity. Stable and homogeneous surface structure is generally aimed in sensor studies. SEM images of the polyurethane structures synthesized were taken at $2500\times$, $5000\times$ and $10,000\times$ magnifications. In Fig. 2, comparative SEM images of PU structures containing 1%, 5% and 10% Gum-Arabic are shown. In SEM images at different magnifications, a uniform, homogeneous and additive-free morphology was observed. Synthesized polymer structures have uniform distribution and homogeneous adhesion to the surface. These polymers also exhibit classical film morphology when coated electrode surface. No cracks, foreign molecules and phase are seen on the surface morphology. When the SEM images of the synthesized polyurethane films were interpreted together, it was seen that the SEM image of the Gum-PU-10% structure was stable and homogeneous. Therefore, it has been concluded that this structure is more suitable for use in electrode preparation.

The thermal properties of the synthesized polyurethanes were measured with TGA, DTA and DTG thermograms, and these thermograms are given in Figs. 3 and 4. When the TGA thermogram in Fig. 3 is examined, a weight loss of approximately

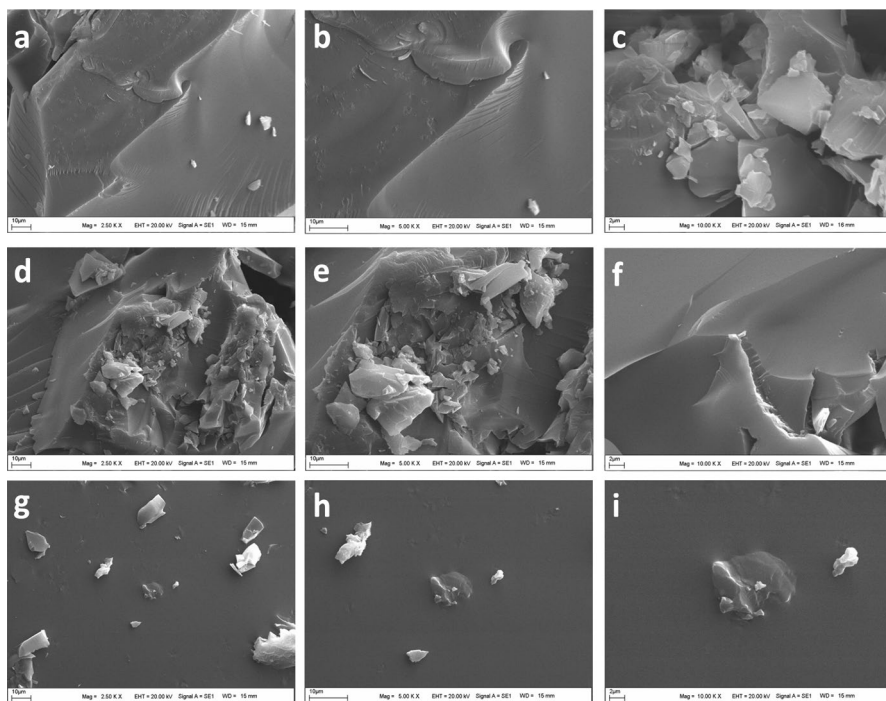


Fig. 2 SEM images of the Gum-PU-1% (a–c), Gum-PU-5% (d–f) and Gum-PU-10% (g–i) structure

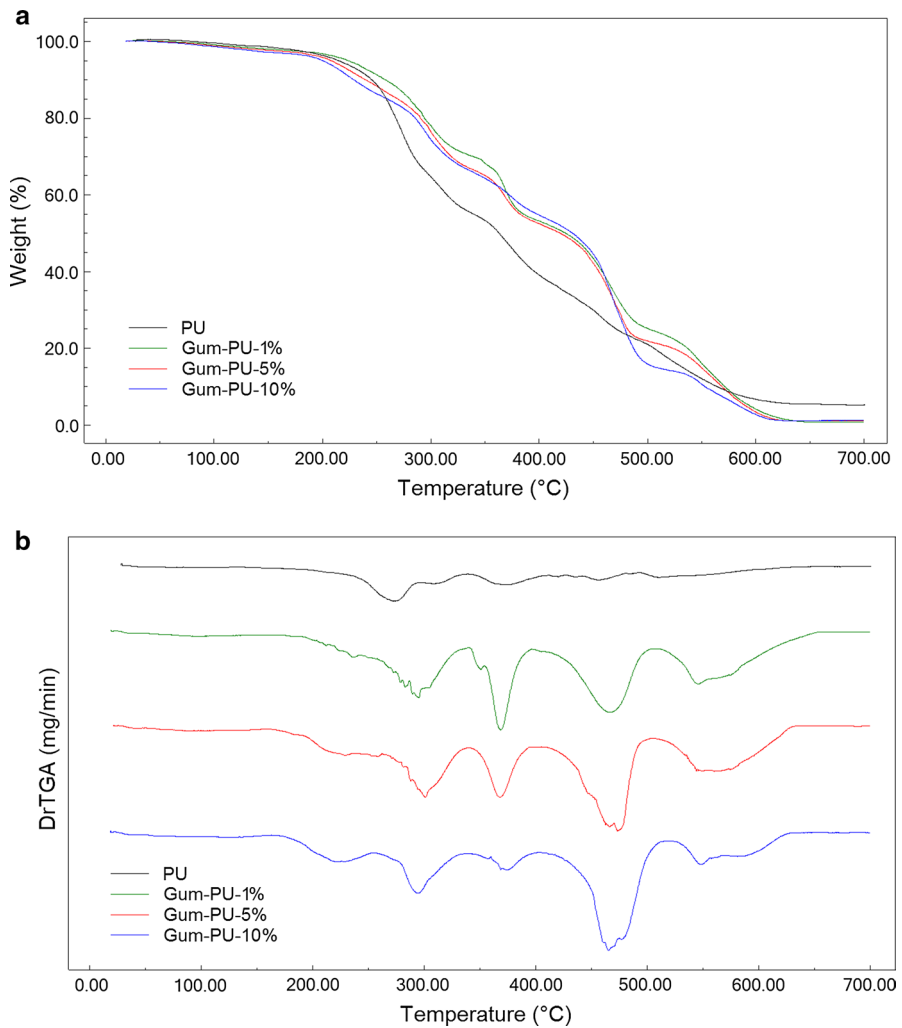


Fig. 3 TGA (a) and DTG (b) thermograms of pure PU and Gum-Arabic-based polyurethane structures

3% is observed due to structural moisture around 100 °C. The amount of this weight loss increases from the structure containing 1% Gum-Arabic to the structure containing 10% Gum-Arabic. The reason for this is that the surface hydrophilicity of the polymer increases due to the Gum-Arabic groups entering in the polymer structure and the polymer structure binds more moisture.

According to TGA thermograms, thermal degradation of the polyurethane structure starts around 200 °C. Gum-Arabic-based polyurethane structures are structurally degraded with a four-stage. Initial weight loss was observed between about 200 and 300 °C. In this weight loss, which is about 25%, there is degradation of the soft segments in the polyurethane structure. The second weight loss is about 15%, which

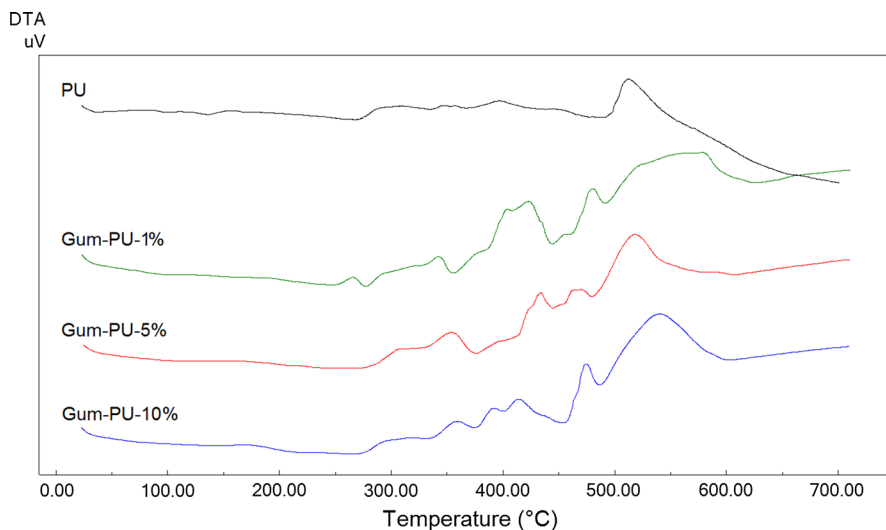


Fig. 4 DTA thermograms of pure PU and Gum-Arabic-based polyurethane structures

starts at 300 °C and ends at about 380 °C. This is weight loss has been observed in all Gum-Arabic-based polymers. This situation is caused by the degradation of the aliphatic isocyanate groups. The third weight loss is caused by the degradation of the hard segments in the polyurethane structure; it starts at 380 °C and continues up to 500 °C. This degradation has been observed around 35% for Gum-PU-1% structure, around 40% for Gum-PU-5% structure, and around 45% for Gum-PU-10% structure. As a result, thermo-oxidative degradation of the polymeric structure was observed in the last thermal degradation observed. At this stage, carbonization of the polyurethane structure occurs. All these findings are seen in accordance with the literature [28, 30, 31].

DTG graphs are given in Fig. 3b to compare the TGA results of pure polyurethane without Gum-Arabic structure and Gum-Arabic-based polyurethanes. While three main mass peaks were observed in the DTG curve of the pure polyurethane, four basic decomposition steps were observed in Gum-Arabic-based polyurethane structures. The first decomposition between 180 and 320 °C in TGA and DTG thermograms of the pure polyurethane structure is the degradation of the urethane bond structure and the second decomposition between 320 and 410 °C stems from degradation of soft segments in polymer structure [32, 33]. The final decomposition between 410 and 550 °C is seen as a C–C bond cleavage [32, 33]. In the thermograms of the Gum-Arabic-based polyurethane structures, in addition to these degradations, a weight loss due to the degradation of the Gum-Arabic units in the polymer structure was observed between 250 and 350 °C.

DTA thermograms of the synthesized pure PU and Gum-Arabic-based polyurethane structures are given in Fig. 4. It is seen that the polyurethane obtained for Gum-PU-1% structure is thermally stable up to 247.9 °C. In Gum-PU-5% structure, we see that this stability value increases up to 270 °C. Similar stability increase is

observed within the structure of Gum-PU-10%. The reason stem from the increase in the Gum-Arabic ratio of polyurethane structures, the amount of cross-linking increases and with this system, which gains structural stability. In the thermograms continuing up to 700 °C, four basic exotherms are seen for each polymer compatible with the TGA thermogram. The first exotherm is between about 270 °C and 355 °C. The second exotherm value is seen between 350 and 445 °C, and the third exotherm continues up to approximately 495 °C. The last exotherm, which is quite wide, starts at about 490 °C and completes at 630 °C. It shows the carbonization and thermal degradation of the polymeric structure. The last carbonization peak observed in Gum-PU-5% and Gum-PU-10% structures narrows temperature area. This change is due to Gum molecules entering the structure. This ultimately proves that Gum-Arabic groups are included in the polymer chain structures after synthesis.

The Gum-PU-1% structure started to decompose at approximately 250 °C and the decay of the small hexose units in the Gum structure firstly until 500 °C, and then the degradation of the soft segment and aliphatic groups. In Gum-PU-5% and Gum-PU-10% structures, it was clearly seen that these degradation peaks expanded and the amount of Gum entering the structure naturally increased. All these characterization findings show that thermally stable polyurethane structures have been obtained, which degrade between approximately 200 °C. These thermal analysis results show that structure-modified electrodes with these polyurethane structures can be used and stored safely up to approximately 180 °C. Polyurethane structures are suitable and useful materials as electrode modification materials for reading many biological fluids. All characterization data showed that the synthesized Gum-Arabic-based polyurethane structures are stable and durable. Thus, electrodes modified with these polymers can be used in a wide temperature range and can be easily sterilized after use.

Characterization of the Gum-Arabic-based polyurethane-modified electrodes

Determination of the polymer type to be used in the determination of L-phenylalanine

In the determination of phenylalanine, it is necessary to design polymeric structures that are not permeable to other electroactive and non-electroactive species, especially molecularly permeable to Phe structure. For this reason, Gum-Arabic-based polyurethanes have been preferred as a polymer structure that is strongly polar and will collect the L-Phe molecules on the electrode surface. The Gum-Arabic structure provides both the formation of a porous structure in the polymer microchain structure and increases the polarity. In addition, polyurethane structures are frequently preferred in sensor applications due to their flexible, durable and high adhesion properties [34–36]. Thus, Gum-Arabic-based polyurethane structures were preferred for electrode modification. Three different Gum-Arabic ratios have been preferred. Polyurethanes containing 1%, 5% and 10% Gum-Arabic were obtained and used for Pt electrode modification. L-Phe responses of prepared modified electrodes were investigated by DPV technique. For the 700 µM L-Phe, DPV responses of the

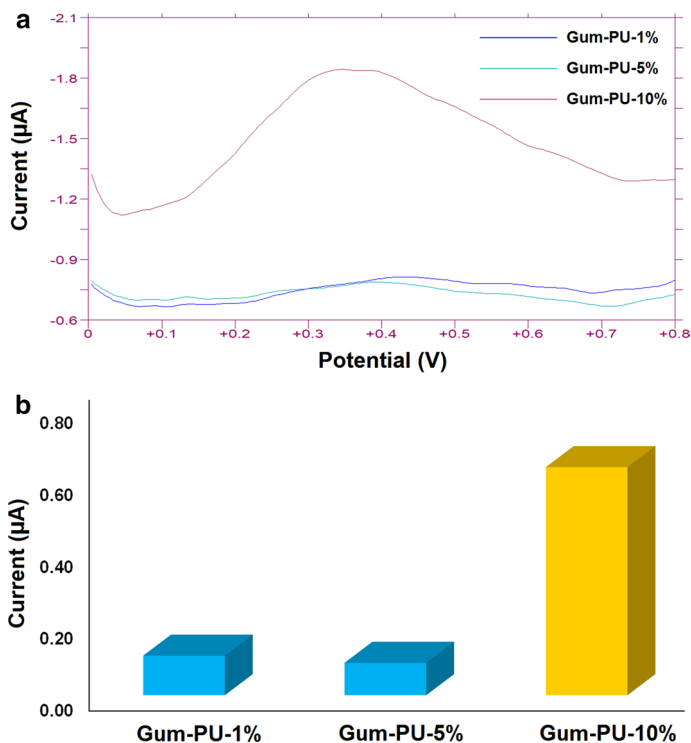


Fig. 5 **a** For L-Phe (700 μM) in citrate buffer (pH 8.0) DPV responses of the electrodes modified with Gum-PU-1%, Gum-PU-5% and Gum-PU-10% structures. **b** The L-Phe oxidation peak current amounts obtained from the electrodes modified with PU containing Gum-Arabic in different proportions

electrodes modified with Gum-PU-1%, Gum-PU-5% and Gum-PU-10% structures are shown in Fig. 5. According to these voltammograms, L-Phe responses of the electrodes modified with Gum-PU-10% structures are very high (Fig. 5a). For this reason, this type of polymer was preferred for advanced sensor optimizations.

Figure 5b also shows the L-Phe peak current amounts obtained from the electrodes modified with PU containing Gum-Arabic in different proportions. When these results are examined; the highest peak current is obtained with Gum-PU-10%-coated electrode. Thus, it has been chosen with Gum-PU-10% structure as an ideal polymer membrane in the design of the sensor for L-Phe determination.

The effect of the polyurethane film thickness on L-Phe response of modified electrode

In order to determine the effect of the film thickness of the Gum-PU-10% polymer on the peak current, the electrode was modified with four different film thicknesses. L-Phe voltammograms obtained from the modified electrodes prepared with

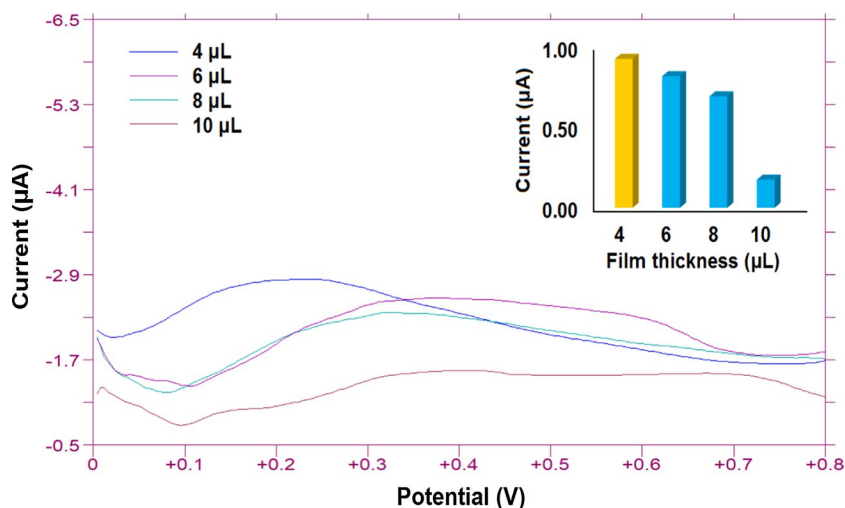


Fig. 6 DPV results of 700 μM L-Phe in citrate buffer (pH 8.0) on Gum-PU-10%-coated electrodes in different thicknesses. (Inset graph: The effect of film thickness on the L-Phe oxidation current.)

polymer solution in different volumes (4 μL , 6 μL , 8 μL , 10 μL) are shown in Fig. 6. As the thickness of the polyurethane film on the electrode surface increased, the peak current value for L-Phe decreased significantly. According to the inset bar graph in Fig. 6 drawn with these results, the highest peak current was seen at 4 μL film thickness. Thus, 4 μL polymer solution volume was chosen as optimum volume for the electrode modification.

Effect of electrolyte type on L-Phe response of modified electrode

1 mM L-Phe solutions were prepared with pH: 7.0 citrate (CB), phosphate (PB), phosphate-buffered saline (PBS) and Brinton–Robinson (B-R) buffer solutions. For 1 mM L-Phe, the voltammograms were taken by using these buffer solutions with the Gum-PU-10%-coated electrode by the DPV method. Obtained DPV results in different electrolytes are given in Fig. 7. According to these voltammograms, PBS buffer was chosen as optimum electrolyte type, since the highest peak current was seen in the PBS buffer (Fig. 7).

The effect of the electrolyte pH on L-Phe response of modified electrode

The pH value of the PBS buffer selected as the support electrolyte was adjusted to 4, 5, 6, 7, 8, and 1 mM L-Phe was measured by used these solutions with the Gum-PU-10%-coated electrode by DPV method. When the voltammograms in Fig. 8 are examined, it was observed that the peak current changed with the pH value of the PBS buffer. Since

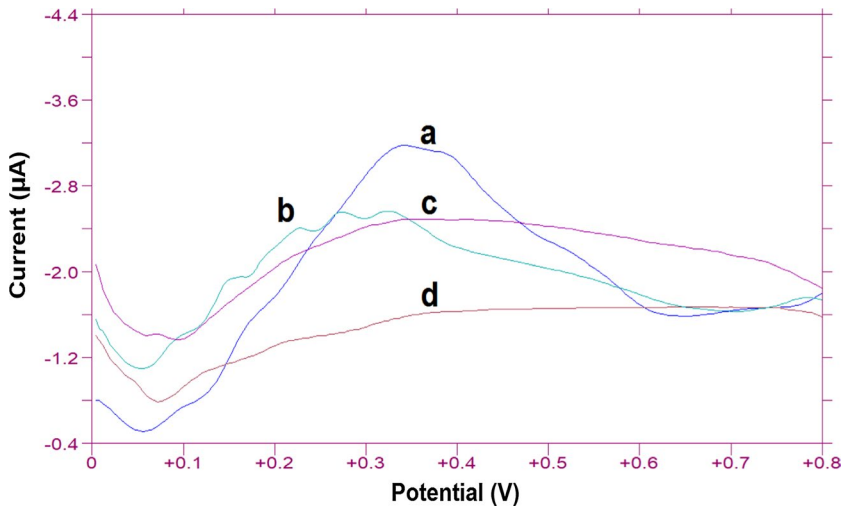


Fig. 7 DPV results for 1 mM L-Phe in different electrolytes (pH 7.0) with 4 μ L Gum-PU-10%-coated electrodes. (a: PBS buffer, b: PB, c: CB, d: B-R buffer)

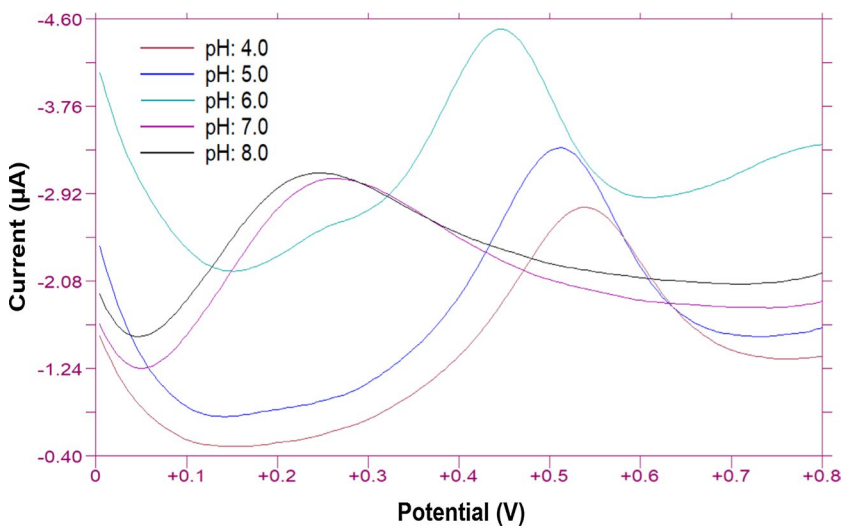


Fig. 8 DPV results of 4 μ L Gum-PU-10%-coated electrodes for 1 mM L-Phe in PBS buffer at different pH

the peak current shows the highest value at pH: 5.0, it has been decided to work with pH: 5.0 PBS buffer. The effect of the electrolyte pH on the oxidation current is shown in Fig. 9a, and the effect of the support electrolyte pH on the L-Phe potential is also given in Fig. 9b.

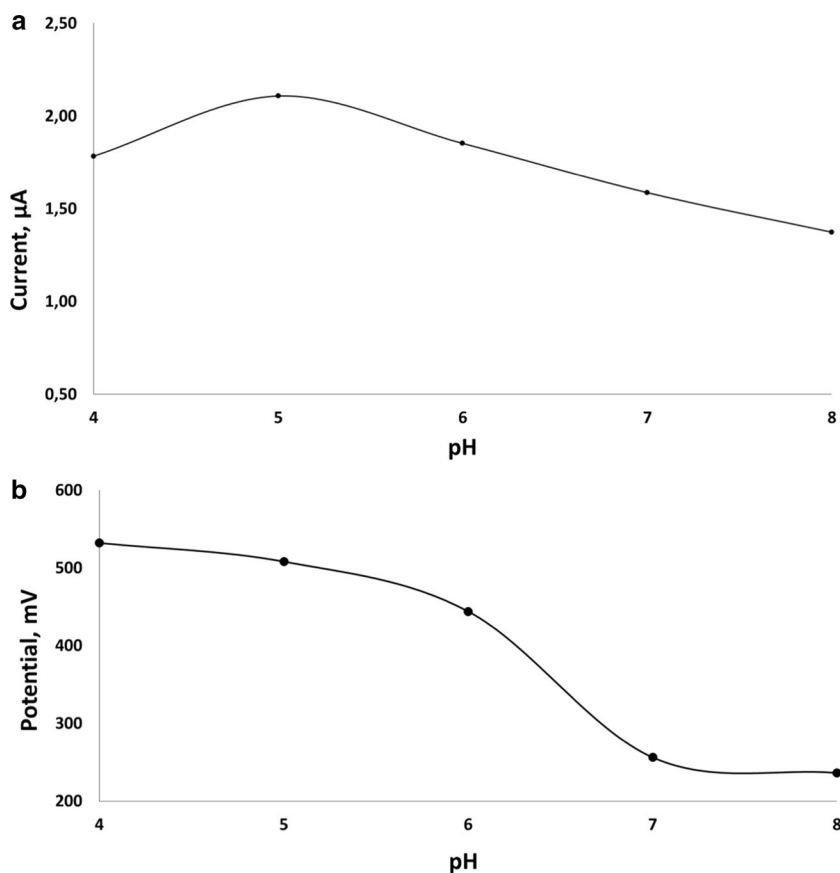


Fig. 9 **a** Effect of electrolyte pH on L-Phe oxidation current and **b** effect of electrolyte pH on L-Phe potential

Scanning speed effect on the electrochemical behavior of the prepared modified electrode

Voltammograms taken at 20, 30, 40, 45 and 50 mV/s scanning speeds in order to determine the effect of scanning speed of DPV method on the electrochemical behavior of 1 mM L-Phe of the prepared modified electrode are indicated in Fig. 10.

To determine the effect of scanning speed in DPV study, speeds of 20, 30, 40 and 50 mV/s, respectively, were studied. The peak current showed the greatest value at 40 mV/s, since it decreased at 50 mV/s. L-Phe (1 mM) response of 4 μL Gum-PU-10%-coated electrodes in PBS buffer was also made at 45 mV/s. According to the obtained current peak values at different scan speeds, 40 mV/s as optimum scanning speed was chosen.

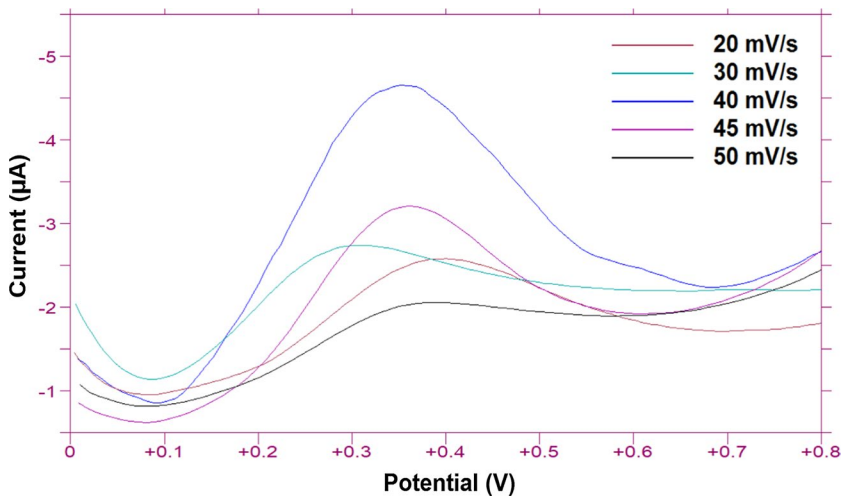


Fig. 10 L-Phe (1 mM) responses of 4 μ L Gum-PU-10%-coated electrodes in PBS buffer at different scan speeds

Interference effect and analytical parameters of the prepared modified electrode

In order to determine the interferences effect on L-Phe response of the prepared modified electrode, electroactive interferences such as ascorbic acid (100 mM), uric acid (100 mM) and non-electroactive species such as lactose (100 mM), sucrose (100 mM), glucose (100 mM), maltose (100 mM) are added into the analyte solution. Under optimum conditions for these interference molecules, measurements with Gum-PU-10%-coated electrode did not show a significant peak current. According to this figure, it has been determined that the modified electrode is only sensitive to L-Phe. Then, the 200, 300, 400, 500, 600, 700, 800, 900 and 1000 μ M L-Phe were measured in the presence of interferences (AA, UA, LC, SC, GL and ML: 100 mM). For the increased L-Phe concentrations, obtained DPV responses of the modified electrode are shown in Fig. 11.

In order to determine the linear range of the Gum-Arabic-modified Pt electrode, a calibration curve was built plotting the L-Phe response current with the increased L-Phe concentrations (200, 300, 400, 500, 600, 700, 800, 900 and 1000 μ M). This calibration curve is given in Fig. 12. The linearity equation and correlation coefficient were calculated as $I_{pc} = 0.002C + 0.0373$ and $R^2 = 0.9972$ (Fig. 12). The limit of detection (LOD) and the limit of quantification (LOQ) of the L-Phe electrode prepared with chemical modification were calculated as 48.01 μ M and 144.02 μ M, respectively.

Determining the stability of the modified electrode

To determine the stability of the L-Phe selective Gum-Arabic-based polyurethane-modified electrodes, DPV voltammograms were taken 10 times consecutively with

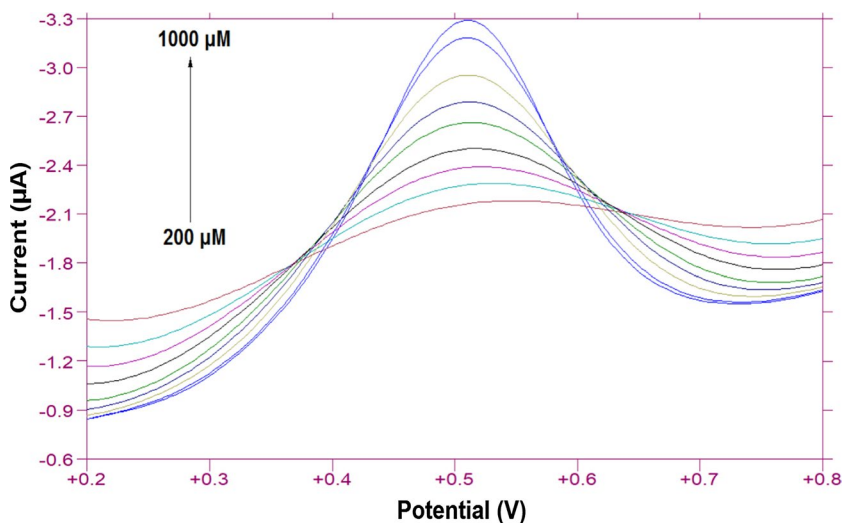


Fig. 11 For the increased L-Phe concentrations (200, 300, 400, 500, 600, 700 800, 900 and 1000 μM), DPV responses of the modified electrode in the presence of interferences (AA, UA, LC, SC, GL and ML: 100 mM)

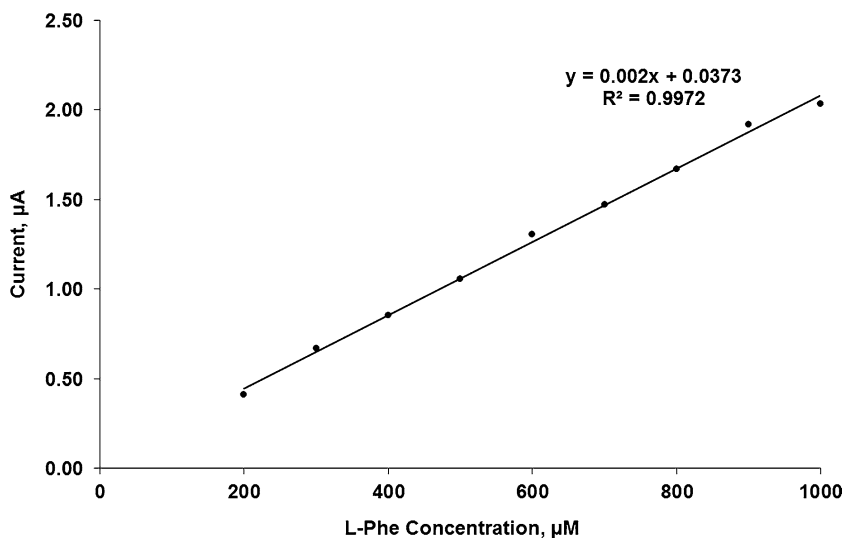


Fig. 12 For the increased L-Phe (200, 300, 400, 500, 600, 700, 800, 900 and 1000 μM) calibration curve (in the presence of interferences (AA, UA, LC, SC, GL and ML: 100 mM))

same optimum modified electrode for 1 mM L-Phe. The obtained DVP voltammograms are given in Fig. 13. Ten consecutive measurements were made with the obtained L-Phe selective electrode, and it was observed that 98.8% stable results

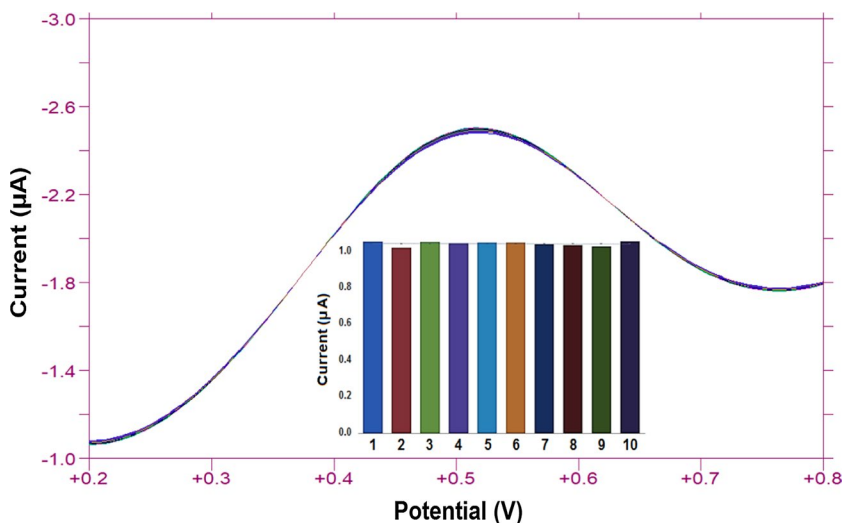


Fig. 13 For the 1 mM L-Phe, stability of modified electrode in the presence of interferences (AA, UA, LC, SC, GL and ML: 100 mM)

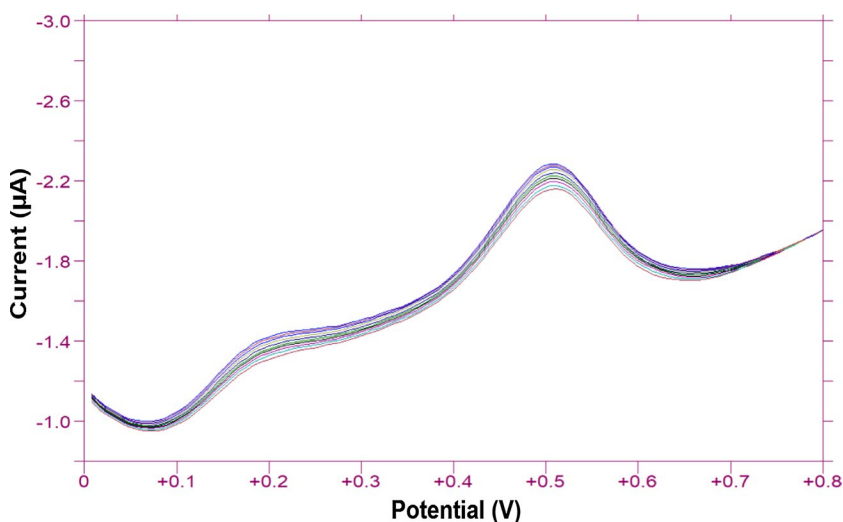


Fig. 14 Reproducibility results of modified electrode in the presence of interferences (AA, UA, LC, SC, GL and ML: 100 mM) (for 1 mM L-Phe)

were obtained between the first electrode and the tenth electrode and the % RSD value was 1.21% (Fig. 13).

Reproducibility results of modified electrode in the presence of interferences (AA, UA, LC, SC, GL and ML: 100 mM) for the 1 mM L-Phe are given in Fig. 14. This figure shows that the reproducibility of the modified electrode is very high.

Table 1 Comparison of techniques used in the detection of phenylalanine

Detection method	Linear range, M	LOD, M	R^2	Reference
NIR spectroscopy	$0\text{--}30 \times 10^{-3}$	1.16×10^{-4}	0.9203	[13]
DPV (MIP/ β -CD-MWNTs/PAN/CE)	$5.0 \times 10^{-7}\text{--}1.0 \times 10^{-4}$	1.0×10^{-9}	0.971	[14]
Amperometry	$0\text{--}6 \times 10^{-3}$	—	0.993	[15]
Chemiluminescence sensor (MIP-CL)	$1.3 \times 10^{-6}\text{--}5.44 \times 10^{-4}$	6.23×10^{-7}	0.9948	[16]
Linear Scan Voltammetry	$100\text{--}1000 \times 10^{-9}$	14.5×10^{-9}	0.9657	[17]
Liquid chromatography	$100\text{--}16,000 \times 10^{-6}$	—	0.999	[18]
Flow-injection spectrophotometry	$5 \times 10^{-6}\text{--}600 \times 10^{-6}$	4×10^{-6}	0.991	[19]
Tandem mass	$1 \times 10^{-6}\text{--}3 \times 10^{-3}$	1×10^{-6}	0.999	[20]
Liquid chromatography	$12 \times 10^{-5}\text{--}12 \times 10^{-3}$	1×10^{-7}	0.996	[21]
Cyclic voltammetry	$20 \times 10^{-6}\text{--}3.0 \times 10^{-3}$	15×10^{-6}	0.999	[22]
Cyclic voltammetry	$0.5 \times 10^{-3}\text{--}6 \times 10^{-3}$	0.5×10^{-6}	0.995	[23]
Amperometry	$50 \times 10^{-6}\text{--}9.1 \times 10^{-3}$	25×10^{-6}	0.997	[24]
MIP-AuNPs sensor	$1.0 \times 10^{-8}\text{--}1.0 \times 10^{-4}$	1.0×10^{-9}	0.975	[37]
Fluorescence	$0\text{--}2 \times 10^{-5}$	—	0.9917	[38]
Raman scattering	$0\text{--}500 \times 10^{-6}$	2.35×10^{-6}	0.997	[39]
DPV (Gum-PU-10% modified electrode)	$200\text{--}1000 \times 10^{-6}$	48.0×10^{-6}	0.9972	This study

NIR Near infrared, MIP/ β -CD-MWNTs/PAN/CE: polyaniline-modified carbon electrode based on β -cyclodextrin incorporated carbon nanotube composite material, *MIP-CL* molecularly imprinted polymer-chemiluminescence, *MIP-AuNPs* Molecularly imprinted film coated gold nanoparticles

In the literature, near infrared (NIR) spectroscopy, UV-visible spectroscopy, gas chromatography, chemiluminescence detection, amperometry, voltammetry, high performance liquid chromatography techniques and biosensors are used for Phe determination [13–19]. Some important results of these commonly used techniques are shown in Table 1. In this table, Cristina Quintelas et al. using the NIR technique made the Phe determination with a value of 0.9203 R^2 [13]. For the Phe determination, Leman Tarhan and colleagues used flow injection spectrophotometry technique and performed Phe determination with LOD value of 4×10^{-6} and R^2 value of 0.991 [19]. Phe determinations have also been successfully performed using amperometry, voltammetry, DPV, and CV techniques [15, 22–24]. When all these techniques are evaluated, it is seen that the Phe sensor obtained with the Gum-Arabic-based polyurethane-modified electrodes prepared within the scope of this study gives very successful results and is compatible with the literature and even has advantages over some techniques.

As a result, Gum-Arabic-based sensors are very advantageous in terms of repeatability and reproducibility. It is also very advantageous with its resistance to sterilization temperature, easy preparation, easy cleaning and practical applicability.

Conclusions

In this study, Gum-Arabic-based polyurethane membrane-modified electrodes as voltammetric sensor were prepared for the detection of L-phenylalanine. Firstly, Gum-Arabic-based polyurethane structures were synthesized as selective membranes to increase the cumulation of L-Phe molecule on the electrode surface. The polyurethane membrane structures have been diversified by using the Gum-Arabic structure at 1%, 5% and 10% molar ratios. Then, synthesized polymer structures were coated on the Pt electrode surface and their effects on L-Phe responses were examined. According to the voltammograms obtained, the highest peak current for L-Phe was obtained with Gum-PU-10% polymer-coated electrode. As a result of the optimization of this electrode, R^2 0.9972 value was obtained in the 200–1000 μM L-Phe concentration range. The limit of detection (LOD) of the L-Phe sensor prepared by selective chemical modification was calculated as 48.01 μM and the limit of quantitation (LOQ) as 144.02 μM . Ten consecutive measurements were made with the obtained L-Phe selective electrode, and it was observed that 98.8% stable result was obtained between the first and tenth electrodes and the % RSD value was 1.21%. According to these results, when the results of the sensors prepared with the literature are compared, very high linearity, high R^2 value and repeatability were achieved. A very wide range has been obtained as a linear measurement range. During PKU, the level of Phe in the blood rises above its normal level, (1.0×10^{-10} – 14×10^{-4} M). The prepared Gum-Arabic-based polyurethane-modified sensors may measure these values as a measuring range with very high accuracy and sensitivity. As a result, L-Phe sensors with high accuracy, sensitivity and repeatability were successfully prepared by modification of the Gum-Arabic-based polyurethane.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to declare.

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