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Preparation of Molecularly Imprinted Electrochemical L-Phenylalanine Sensor with *p*-Toluene Sulfonic Acid Modified Pt Electrode

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L-Phenylalanine (L-Phe) sensors were prepared with two steps by modifying the Pt electrode using the molecular imprinting technique. In first step, polymeric films based on p-toluene sulfonic acid (PTSA) were electropolymerized on the Pt electrode in the presence of L-Phe as template molecule. In the second step, L-Phe, the template molecule, was desorbed from the film structure coated on the electrode surface using acid. The surface properties and morphologies of the prepared modified electrodes were checked by SEM analysis technique. These modified electrodes were used for determination of L-Phe by differential pulse voltammetry (DPV). Electrodes obtained by molecular imprinting method showed $R^2 = 0.9997$ in the concentration range of 2–2000 μM L-Phe. The limit of detection (LOD) of L-Phe selective electrodes was found as 0.59 μM and the limit of quantification (LOQ) was 1.77 μM. The first three repetition result with the same modified electrode was observed as **96.83%** and the RSD value was **14.96%** for ten replicates. For twenty modified electrodes, reproducibility was obtained as **97.67%** stable between first electrode and twentieth electrode, and the RSD value was observed to be **2.33%**. According to these results, prepared molecular imprinted electrodes have high repeatability, stability and selectivity for the determination of L-Phe.
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L-Phe is an important essential amino acid found in the structure of all the proteins necessary for the body, and it is only taken from food.[1,2] L-Phe is converted into tyrosine in the body with the phenylalanine hydroxylase enzyme. It may also turn into DOPA, dopamine, norepinephrine and epinephrine.[3] As a neurotransmitter, these hormones are very important for our body and hormone metabolism. Therefore, L-Phe is especially important for physiological systems. High levels of phenylalanine in the human body can cause nerve damage.[4] In addition, high phenylalanine level is a symptom of Phenylketonuria (PKU) disease especially in new-borns.[5] PKU is a genetic disorder that results from the enzyme becoming dysfunctional as a result of mutations in the gene of the phenylalanine hydroxylase (PAH) enzyme.[5,6] Lack of activity of PAH enzyme in the liver prevents the conversion of phenylalanine to tyrosine. This causes an increase in the level of Phe and its metabolites in the blood.[7] The most reliable indicator for the diagnosis of phenylketonuria is the concentration of phenylalanine in the blood or urine.[6] In healthy human, the amount of phenylalanine in the blood is below 2 mg dl^{-1}.[8] In the neonatal screening test, the amount of phenylalanine in the blood is between 2 and 10 mg dl^{-1}, hyperphenilalaninemia, those between 10 and 20 mg dl^{-1} are moderate phenylketonuria, and those over 20 mg dl^{-1} are classical phenylketonuria.[8]

Capillary electrophoresis, fluorometry, chromatography, ion exchange column chromatography, mass spectrometry and spectrophotometry,[7] gas chromatography and electrophoresis, isotope dilution liquid chromatography/tandem mass spectrometry are the most common methods for detecting PKU.[9] In order to prevent the progression of mental and developmental disorders in children with PKU, PKU should be diagnosed rapidly in infants.[5,10,11] When this disease is diagnosed early, PKU is treated by following the low phenylalanine diet. In this way, the patient's normal development continues, but the level of L-Phe should be monitored frequently and throughout their lifetime. The determination of the L-Phe level during pregnancy is also very important in terms of determining the phenylketonuria disease.[12] In literature, many instrumental methods have been developed for the detection of L-Phe.[13] In these tests, phenylalanine can be determined in blood and urine samples. In human serum, these methods often require time. The preparation of the samples is quite complicated and should be done by skilled technicians.[14] Since the concentration of phenylalanine in urine is higher, urine analyses are preferred in determining phenylalanine.

There are few methods for the sensitive determination of phenylalanine, such as Gutrie test, fluorometric and HPLC-Tandem Mass Spectrometry.[15–18] Access to these modern mass spectrometric or chromatographic methods is not available in almost all health centres in many countries, and therefore the determination of phenylketonuria requires the sample to be sent to central laboratories.[10] This process is expensive and it takes days for the sample to arrive in the laboratories and return the results.[10] Therefore, there is a need for a method that is simple enough to be performed in all health centres/birth units and has also analytical properties suitable for sensitivity, selectivity and reproducibility. Therefore, electrochemical sensors are an important alternative. Biosensors in determining many important analyte types; used as fast, selective and advanced analytical tools.[9,19] In particular, electrochemical sensors are fast, practical and sensitive techniques. In addition, the need for efficient and expert personnel is very low and sample preparation steps are techniques that are not intensive. Polymeric structures are used effectively in the preparation or modification of electrochemical sensors. In particular, polymers with conductive, porous and selective properties are very important. Molecular imprinting technique is an important technique in the synthesis or increasing the selectivity of such polymers.

Molecularly imprinted polymers are versatile materials capable of molecular recognition.[10,20] Structurally, molecularly imprinted polymers consist of cross linked polymers synthesized in the presence of a template molecule.[21] These cross-linked polymers can be prepared by an electropolymerization or by conventional polymerization in the environment of the template. With the removal of the template molecule, selective gaps for template molecules are formed in the polymer structure. Thus, molecular imprinted structures have attracted the intense interest of scientists trying to develop sensors.[22–27] However, there are limited studies in the literature about the design of potentiometric sensors based on molecular imprinting technology. In the molecular imprinting method and polymer modification and electrode preparation processes, the polymer structures to be used are very important for the selectivity of the analyte.[28,29] By changing polymer structures, important electrochemical sensors can be prepared for detection of different analyte and biomarker molecules.[29–31]

The aim of this study is to prepare highly accurate, practical and reusable L-Phe electrodes suitable for clinical use. In this way, early

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detection of PKU disease will be possible. For the preparation of electrodes, electropolymerization of p-toluenesulfonic acid phenylalanine was performed on the surface of a Pt electrode using cyclic voltammetric sweep in the potential range from −1.0 V to +1.0 V. A conductive poly(p-toluene sulfonic acid) film was formed on the surface of the bare electrode. This film structure contains phenylalanine molecules. By leaching the L-Phe molecules, a conductive polymeric membrane structure is obtained which contains spaces in the molecular volume of the phenylalanine. Then, these modified electrodes prepared were used for determination of L-Phe samples. After the modification process, the increased electrode surface area led to an increase in L-Phe responses. That is, interference effect was not observed. Therefore, the developed voltammetric method is very simple, highly selective and sensitive for the determination of physiological fluids.

Experimental

Materials.—L-Phenylalanine (L-Phe) was supplied from Sigma-Aldrich. p-toluene sulfonic acid (PTSA) was also purchased from Sigma-Aldrich. The other chemicals were bought from Merck (Darmstadt, Germany) and they were of reagent grade. All electrolyte solutions were prepared at 100 mM concentration and then adjusted to pH with NaOH and suitable acid solutions.

Instrumentation.—For SEM analysis of synthesized bare Pt electrode, modified electrode and molecular imprinted electrode, the LEO-EVO SEM instrument was used. SEM samples were coated with Baltech sputter Au/Pd before analysis.

All electrochemical L-Phe sensor measurements were performed using the Bioanalytical Systems Electrochemical Analyzer (BAS 100B, Bioanalytical Systems, Inc.). Three electrode analysis cells were used for these measurements. In the electrochemical measuring cell, measurements were made with a platinum working electrode, a Pt wire auxiliary electrode and Ag/AgCl (3 M KCl) reference electrode. In order to determine the effect of electrode type used in electrochemical measurements, Au and Pt electrodes were used. Prior to measurements, Pt electrodes were cleaned and polished according to the standard procedure. Fine diamond polishing compounds and aqueous alumina slurry (Johnson Matthey Catalog Comp., United States) were used in this process. After each polishing process, the electrodes were washed with distilled water and sonicated in an ultrasonic bath (Branson model 2200) for 2–3 min. The effect of the electrolyte type used in DPV measurements was examined for various supporting electrolytes such as citrate (SitB), acetate (AstB), phosphate (PBS), Britton-Robinson (BRB) and sodium borate buffer (SBB), Na_2HPO_4, LiCl, KNO_3, KCl, and $NaClO_4$.

The pH measurements and adjustments of prepared solutions were made with Hanna HI 8314 pH-meter. Ultra-pure water was obtained from the Millipore brand Elix 20 model water system.

Preparation of L-phenylalanine imprinting electrochemical sensor.—Preparation steps of L-Phe imprinting electrochemical sensor are shown in Fig. 1. The monomer solution was prepared with a molar ratio of 1:3 (L-Phe:PTSA). The solution was then mixed in a magnetic stirrer for 5 min to allow the functional molecule and functional monomer to interact. Electrodes were immersed in the cell containing 5 ml of monomer solution and electro-polymerization was performed in 5 segment film thickness in the potential range from −1000 mV to +1000 mV by the alternating voltammetry method.

Template molecule desorption.—In order to remove the template molecule L-Phe from the polymeric structure, it is necessary to use a desorption agent that can break the interactions mentioned above. For this purpose, 0.1 M HCl solution was used as desorption agent. L-Phe imprinting gold and platinum electrodes were kept in 200 ml of 0.1 M HCl solution for 24 h. During this process, the template molecule has been desorbed, and the electrode surface area has increased by forming cavities on the electrode surface. Whether desorption process was performed efficiently was checked by DPV method.

Electrochemical optimizations for L-Phe determination.—After the preparation of L-Phe imprinting electrochemical sensor, optimization studies were started. These studies were carried out with L-Phe solutions prepared between pH: 7.4 PBS buffer and between 200–700 μM concentrations.

Results and Discussion

For selective and sensitive detection of L-Phe, a voltammetric sensor was prepared using p-toluene sulfonic acid with molecular imprinting electrode modification technique. In the study, firstly, the selection of the working electrode type was made. Pt electrode was chosen as the working electrode according to the results given in the supporting data (Figs. S1–S3 is available online at stacks.iop.org/JES/167/167508/mmedia). Electrode modification was performed in two steps. In the first step, PTSA were polymerized in the range from −1000 mV to +1000 mV, at 100 mV s^{-1} scan rate and five segments in the presence of L-Phe on the electrode surface with CV method. For this purpose, polymerization solutions were prepared to get a ratio of 1:1, 1:2, 1:3, 1:4 mM (Phe:PTSA). In the second step, PTSA coated electrodes were treated with HCl at different times in order to leach L-Phe molecules (Fig. S4). Then the electrode was washed with ultrapure water and transferred into citrate buffer for electrochemical measurements. Phe responses of modified electrodes were obtained by using DPV method. Scan rate (at CV and DPV), electrolyte type, pH, pulse amplitude, pulse width and pulse period parameters was optimised for prepared modified electrodes. Then, the electrochemical properties of the L-phenylalanine structure were determined with prepared these electrodes. After molecular printing on the Pt electrode, sensor properties such as film thickness, interference effect, stability, sensitivity, reproducibility and repeatability were also determined.

Optimizations of the MIP based L-phe electrode.—After the preparation of L-Phe imprinting electrochemical electrode, optimization studies were performed. These studies were carried out with L-Phe solutions prepared at pH: 7.4 PBS buffer between 200–-700 μM concentrations. On the responses of L-Phe selective electrodes, important parameters such as Mix ratio effect of template molecule: functional monomer, film thickness, scanning speed, pH, and support electrolyte type were optimized. Firstly, Mix ratio effect of template molecule (L-Phe) and functional monomer (PTSA) were investigated (Fig. S5). When the effect of L-Phe:PTSA molar ratio on the Phe measurements were examined, it was decided to perform electropolymerization with a monomer solution of 1:3 mole ratio.

Film thickness effect.—The effect of the film thickness of the MIP polymer prepared with the determined ratio of monomer solution was examined by changing the number of segments (3, 5, 7 and 9) in the CV method. The effect of film thickness on oxidation current of 700 μM L-Phe can be seen in Fig. 2. When the measurements made with increasing concentrations of L-Phe in the range of 200–700 μM were examined, it was decided to perform electropolymerization with a film thickness of 5 segments. In Fig. 2, it is clearly seen that the film thickness of 5 segments is optimum. For this reason, it has been decided to use electrodes coated with 5 segment film thickness in future studies.

Electropolymerization of PTSA was performed at 50, 100, 150 and 200 mV s^{-1} scan rate in the potential range from −1.000 V to +1.000 V at the determined monomer solution ratio (1:3; L-Phe:PTSA). After Phe desorption of the coated electrodes with 5 segment film thickness prepared at different scanning rates, prepared electrodes were used with DPV method in the range from −0.2 V to +0.8 V for detection of 700 μM L-Phe. Obtained DPV results are

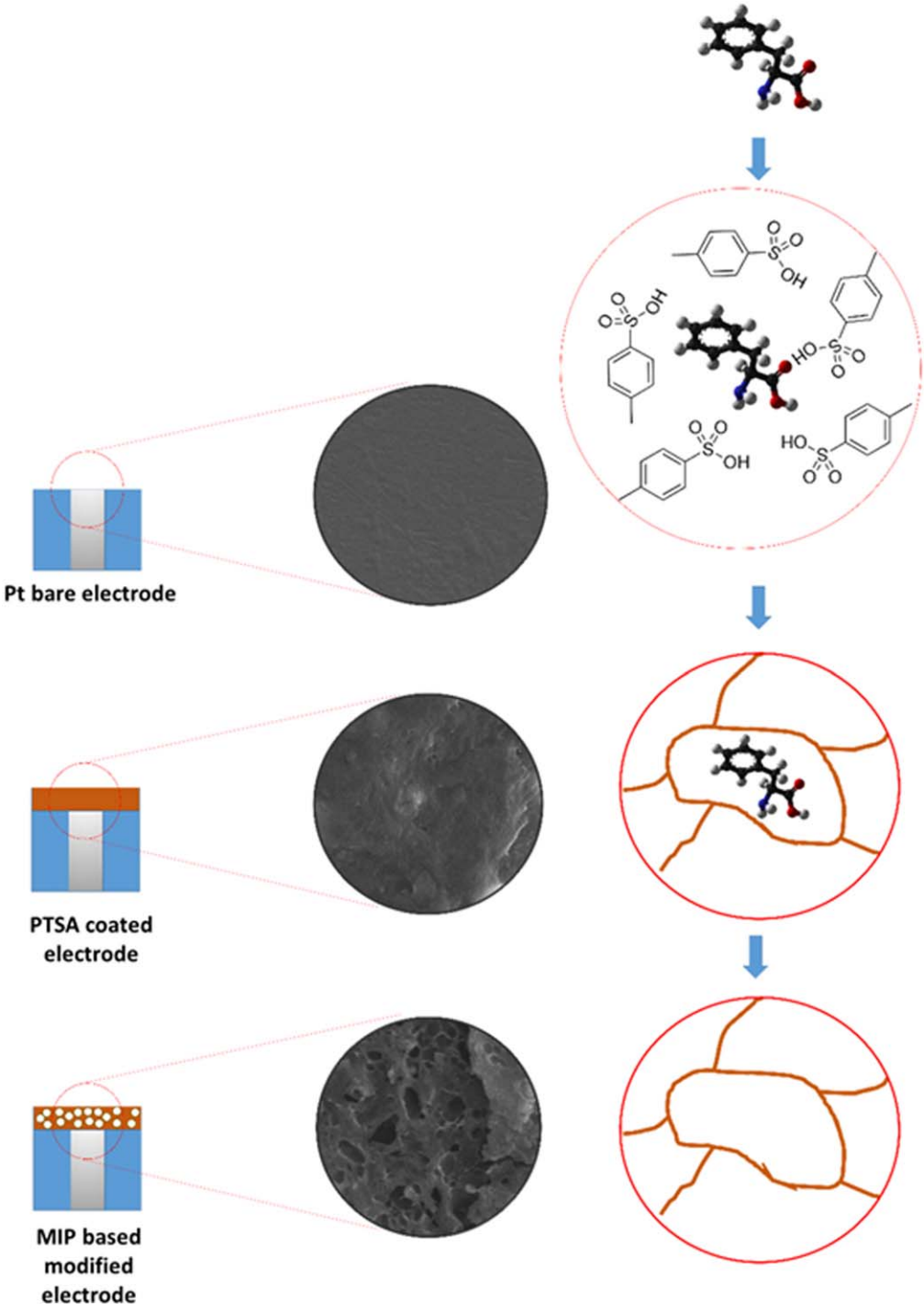


Figure 1. Preparation of L-Phe sensor by molecular imprinting method.

shown in Fig. 3. As seen from the inset graph in Fig. 3, it is seen that the scan rate of 150 mV s^{-1} is optimum.

Scan rate effect on the electropolymerization of PTSA.—

Investigation of the electrochemical behaviour of L-phe on the modified electrode.—Within the scope of the study, after determining the preparation parameters of the MIP-based electrode to determine the L-Phe concentration in physiological fluids, the electrode measurement parameters were optimized the electrochemical behaviour of L-Phe on the modified electrode was determined. After the relevant optimizations such as support electrolyte type, pH, scan rate, pulse amplitude, pulse width, pulse period and interference effect were made, the calibration curve was obtained for the increased concentration of phenylalanine. The stability and reusability of the MIP based modified electrode was then determined. The results of these optimization studies are given below.

Support electrolyte type effect.—To examine the type of support electrolyte on L-Phe responses, citrate (SitB), acetate (AstB), phosphate (PBS), Britton-Robinson (BRB) and sodium borate buffer (SBB) were used and electrode responses were received for 700 μM L-Phe solution at pH 7. When the differential pulse voltammograms obtained by using MIP based modified electrode are examined, it

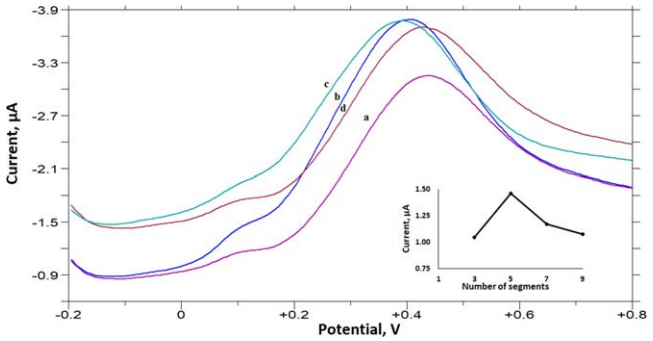


Figure 2. 700 μM L-Phe DPV responses of coated electrodes in different thickness (a: 3, b: 5 c: 7, d: 9 segments). The inset graph shows effect of film thickness on oxidation current of 700 μM L-Phe.

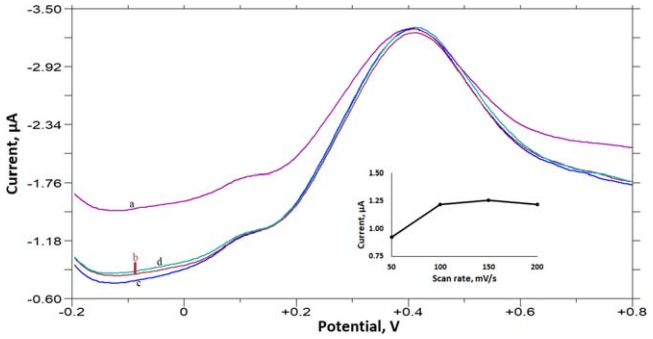


Figure 3. The effect of scan rate on the responses of 700 μM L-Phe. (a: 50 mV s^{-1}, b: 100 mV s^{-1}, c: 150 mV s^{-1} and d: 200 mV s^{-1}). The inset graph shows effect of scan rate on 700 μM L-Phe oxidation current.

was decided to work with citrate buffer, since the highest peak flow was observed in citrate buffer (Fig. 4).

Effect of the supporting electrolyte pH.—The pH of the citrate buffer selected as the support electrolyte was adjusted to 2, 4, 6, 7, 8, and 9 and 700 μM L-Phe were measured in these solutions with MIP based modified electrode by DPV method.

When the voltammograms obtained were examined, it was observed that the peak intensity increased in basic direction. As seen in Fig. 5, the oxidation peak intensity of L-Phe showed the greatest value at pH: 8.0. Since this oxidation peak intensity decreased at pH 9.0, taking into account the possibility of giving a higher peak in the range of 8–9, L-Phe (700 μM) response of MIP based electrode was also measured at pH 8.5. But, since the Phe peak current decreased at pH 8.5, pH 8.0 as optimum pH value of citrate buffer was chosen.

Scan rate effect on L-phe response of MIP based electrode in DPV measurements.—Scan rate effect on L-Phe response of MIP based electrode in DPV measurements were investigated at 10, 15, 18 and 20 mV s^{-1} scan rates. (L-Phe 700 μM) DPV responses and peak current values with different scan rates were given in Fig. 6. When L-Phe peak current values obtained in these voltammograms are examined, the ideal scan rate was chosen as 20 mV s^{-1}. To detect the effect of the scan rate; L-Phe measurements were made with prepared MIP based electrode at scan rates range from 20 mV s^{-1} to 40 mV s^{-1}. Obtained scan rate L-Phe oxidation current graph was given in Fig. 6. Since the peak current decreases at 40 mV s^{-1}, more intensive measurements were taken at low scan rate values. When the peak was observed to shrink; It was decided to study intermediate values between 20 mV s^{-1} and 10 mV s^{-1}. Worked at speeds of 15 mV s^{-1} and 18 mV s^{-1}. It was decided to work at this speed since the highest peak current was seen at a speed of 18 mV s^{-1}.

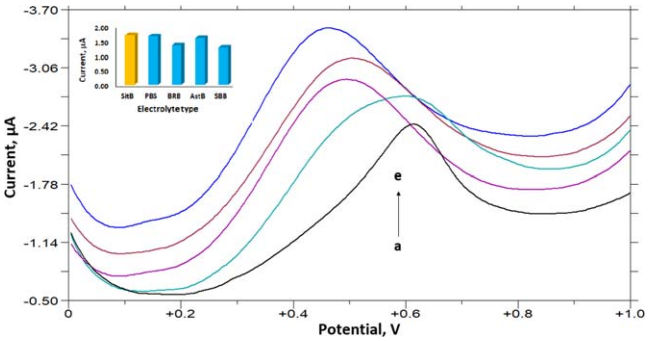


Figure 4. The effect of the support electrolyte type on 700 μM L-Phe responses. (a: SBB, b: BRB, c: PBS, d: Acetate and e: Citrate buffer). The effect of the support electrolyte type on the oxidation current of L-Phe (inset graph).

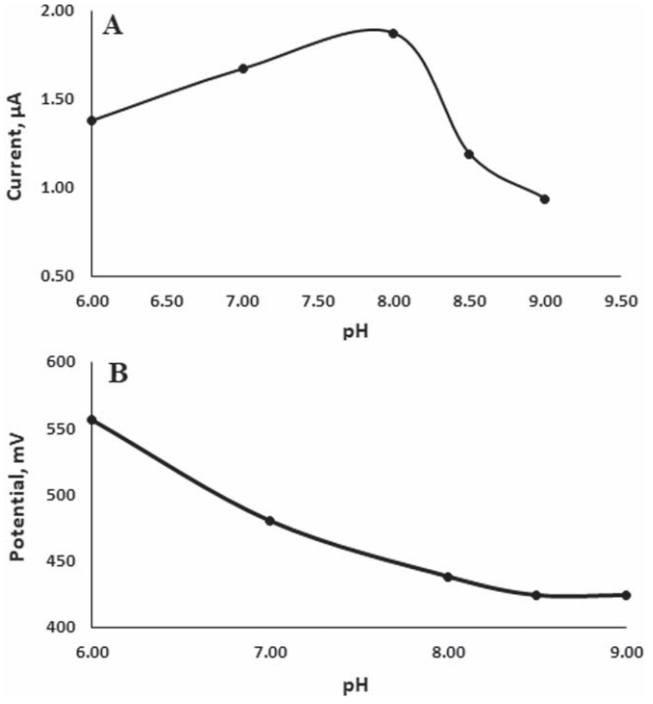


Figure 5. The effect of the support electrolyte pH on the oxidation current of L-Phe (A). The effect of support electrolyte pH on L-Phe potential (B).

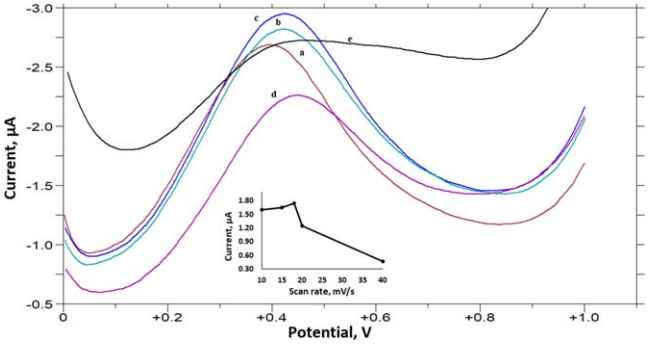


Figure 6. The effect of scan rate on 700 μM L-Phe DPV responses (a: 10 mV s^{-1}, b: 15 mV s^{-1}, c: 18 mV s^{-1}, d: 20 mV s^{-1} and e: 40 mV s^{-1}). The inset graph shows effect of DPV scan rate on L-Phe oxidation current.

Pulse amplitude effect.—DPV measurements at 40, 50, 80 and 100 mV were performed to determine the effect of pulse amplitude to the electrochemical behaviour of the prepared modified electrode

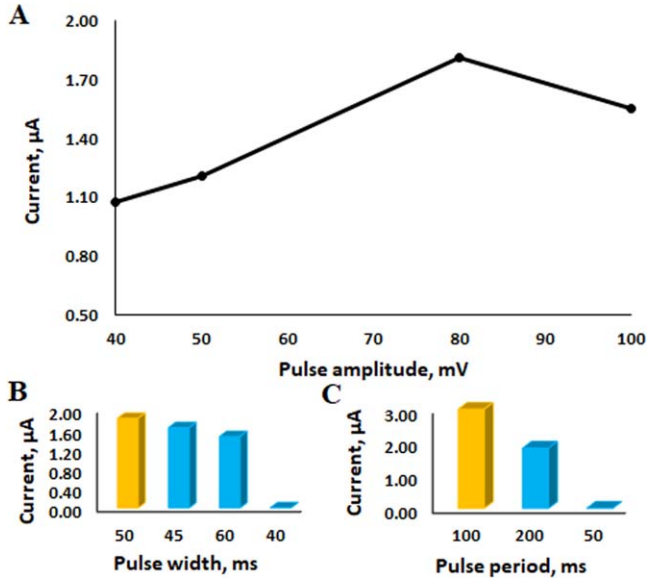


Figure 7. Effect of pulse amplitude on L-Phe oxidation current (A). Pulse width effect on L-Phe (700 μM) responses (B). Pulse period effect on the oxidation currents of L-Phe (C).

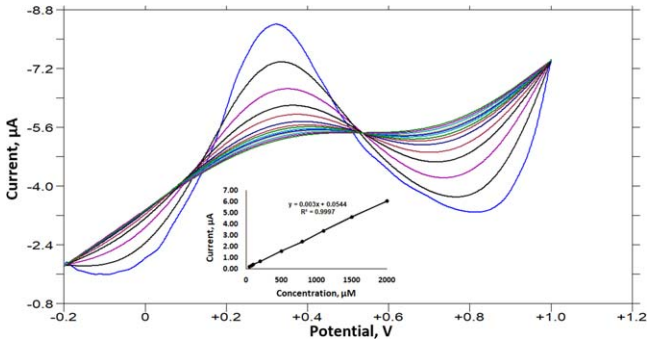


Figure 8. DPV responses of the modified electrode for L-Phe increased concentrations (2, 5, 10, 20, 30, 50, 80, 100, 200, 500, 800, 1100, 1500 and 2000 μM) in the presence of interferences; (interferences: ascorbic acid, uric acid, lactose, sucrose, glucose and maltose: 100 mM). The inset graph shows calibration curve of L-Phe.

for L-Phe sample (700 μM) (Fig. 7). Since the pulse current decreases when the Pulse Amplitude is reduced from 50 mV to 40 mV, it was studied at 80 mV and 100 mV. It was decided that optimum pulse amplitude values was 80 mV for L-Phe analysis.

Pulse width effect.—In order to determine the pulse width effect on the current peaks of the prepared modified electrode for 700 μM L-Phe, DPV measurements were made at 40, 45, 50 and 60 ms pulse width values (Fig. 7B). Since the pulse width was saturated when it was reduced from 50 ms to 40 ms, it was studied at 45, 60 ms values, and it was decided that 50 ms pulse width was optimum values for L-Phe detection.

Pulse period effect on the oxidation currents of L-phe.—L-Phe (700 μM) measurements of the prepared modified electrode were performed at 50, 100 and 200 ms values in order to determine the pulse period effect. The oxidation currents results of these measurements were given in Fig. 7C. An increase in peak current was seen when it was reduced from 200 ms to 100 ms, and it was decided to work with 100 ms because it gave saturation when it was reduced to 50 ms.

Interference effect and calibration graph.—In biomedical applications, pH determination is generally performed in blood or urine

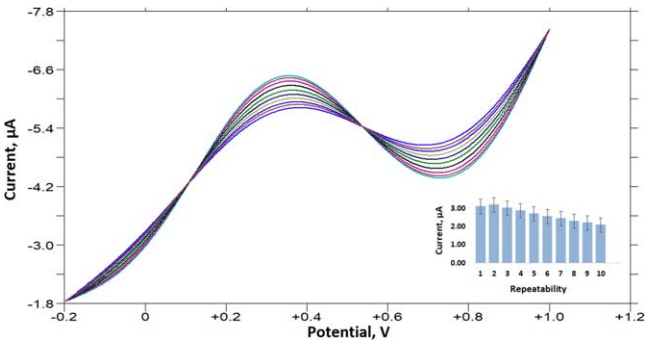


Figure 9. Repeatability of MIP based modified electrode for L-Phe (1 mM) in the presence of interferences (lactose, sucrose, glucose, maltose, ascorbic acid and uric acid: 100 mM). The inset graph shows repeatability results of MIP based modified electrode for the oxidation currents of 1 mM L-Phe in the presence of interferences.

samples. These samples can contain very important electroactive or non-electroactive interferant species. In particular, electroactive species can affect peak intensity by reducing or oxidizing at the same peak potential with the analyte. Non-electroactive species may contaminate the electrode surface and affect L-Phe responses. Thus, the effect of electroactive interferants such as ascorbic acid (100 mM), uric acid (100 mM), and non-electroactive species such as 100 mM lactose, sucrose, glucose, maltose were investigated. In addition, calibration graph of L-Phe detection were conducted on the MIP based modified electrode for 2, 5, 10, 20, 30, 50, 80, 100, 200, 500, 800, 1100, 1500 and 2000 μM L-Phe in the presence of electroactive and non-electroactive interferants. Voltammograms obtained as a result of these measurements are given in Fig. 8. As can be seen from these voltammograms, the imprinting electrode responded only to phenylalanine in the presence of both electroactive and non-electroactive species. That is, the prepared electrode is sensitive to phenylalanine and is also selective for phenylalanine. The calibration graph for the peak currents of the peaks obtained as a result of increased L-Phe concentration is shown in Fig. 8. It has been determined that the molecularly imprinted modified electrode is only sensitive to L-Phe.

The equation ($y = 0.003x + 0.0544$) and linearity $R^2 = 0.9997$ were calculated based on the data obtained in the range of 2–2000 μM L-Phe concentration. The detection limit (LOD, limit of detection) of the L-Phe selective molecularly imprinting sensor was found as 0.59 μM and the quantitation detection limit (LOQ, limit of quantitation) as 1.77 μM.

Determination of the stability of the MIP modified electrode.—To determine the stability of the L-Phe selector sensor; 1 mM L-Phe voltammograms were taken 10 times consecutively with the modified electrode using the DPV method. The results of these voltammograms are given in Fig. 9. After the desorption process of L-Phe molecules, stem from large amount of HCl, voids and pores were seen in the MIP based electrode surface. Thus, during this process, the template molecule has been desorbed, and the electrode surface area has increased by forming cavities, voids and pores on the electrode surface. After the electrode preparation process, the film on the electrode surface remains stable since L-Phe measurements are carried out at pH 7.4. The repeatability data of the prepared electrodes proves these results. The peak currents obtained for these voltammograms are shown in Fig. 9 depending on the number of measurements. 10 consecutive measurements were made with the same modified electrode, and it was observed that the first three repetitions showed **96.83%** stability and the RSD value was **14.96%** in ten repeats.

To test the reproducibility of the MIP based modified electrode, 20 electrodes have been modified with same preparation procedure and used for L-Phe (1 mM) detection individually, and it has been

Table I. The comparison of the properties of some L-Phe sensing electrode in the literature.

Detection Method	Linear Range, M	LOD, M	R^2	Reference
NIR	—	1.16×10^{-4}	0.9203	32
DPV (MIP/β-CD-MWNTs/PAN/CE)	5.0×10^{-7}–1.0×10^{-4}	1.0×10^{-9}	0.971	33
Amperometry	0–6×10^{-3}	—	0.993	34
MIP-CL	1.3×10^{-6}–5.44×10^{-4}	6.23×10^{-7}		35
Linear Scan Voltammetry	100–1000 $\times 10^{-9}$	14.5×10^{-9}	0.9657	36
Amperometry	50×10^{-6}–9.1×10^{-3}	25×10^{-6}	0.997	37
Cyclic Voltammetry	20×10^{-6}–3.0×10^{-3}	15×10^{-6}	0.999	38
Flow-Injection spectrophotometry	5×10^{-6}–600×10^{-6}	4×10^{-6}	0.991	39
Cyclic Voltammetry	0.5×10^{-3}–6×10^{-3}	0.5×10^{-6}	0.995	40
HPLC	100–16000 $\times 10^{-6}$	—	0.999	41
MIP- DPV	2–2000 $\times 10^{-6}$	0.59×10^{-6}	0,9997	This Study

NIR: Near infrared spectroscopy.
β-CD–MWNTs/PAN/CE: β-cyclodextrin and multi-walled carbon nanotube containing PAN/CE electrode.
MIP-CL: molecularly imprinted polymer-chemiluminescence.

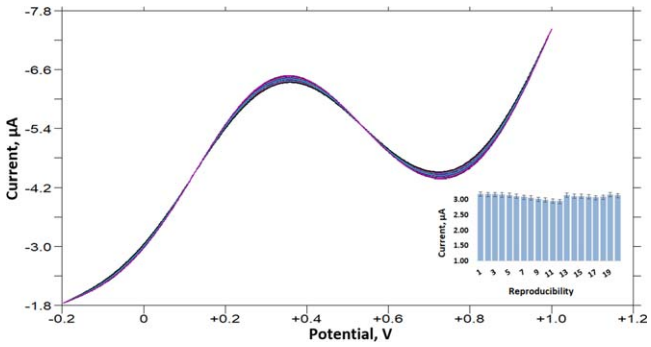


Figure 10. Reproducibility of MIP based modified electrode for L-Phe (1 mM) in the presence of interferences (lactose, sucrose, glucose, maltose, ascorbic acid and uric acid: 100 mM). The inset graph shows reproducibility results of MIP based modified electrode for the oxidation currents of 1 mM L-Phe in the presence of interferences.

observed that a **97.67%** stable result is obtained between the first electrode and the twentieth electrode and the RSD value is **2.33%** (Fig. 10).

Studies for the determination of L-Phe are very important for the detection of phenyl ketonuria disease. Therefore, studies are carried out to develop more sensitive and practical measurements. NIR, chemiluminescence, spectroscopy, amperometry, voltammetry and HPLC techniques are used in the literature for the determination of L-Phe.[32–41] Some important results of these commonly used techniques are shown in Table I. In addition, for the determination of L-Phe, Amperometry has been successfully performed with voltammetry, DPV and CV techniques.[33–38,40] When all these techniques are evaluated, it is seen that the L-Phe sensor obtained by the molecular suppression method prepared in this study gives very successful results and is compatible with the literature and even has advantages over some techniques.

Conclusions

Phe is a very important amino acid and biomarker molecule during the diagnosis and treatment of phenylketonuria (PKU), an important genetic disease. Especially, in the detection of this disease in new-borns, it plays a critical role in starting treatment early. Therefore, In this study, for determination of L-Phe in some physiological fluids, selective, sensitive and reliable PTSA based modified electrode and electrochemical sensor have been developed based on molecular imprinting technique. Modified electrodes were prepared by a two-step procedure. In the first step, PTSA films were coated in the presence of L-Phe in the potential range from −1000 mV to +1000 mV, at a scanning speed of 150 mV s^{-1} in 5 ml (pH: 7.4) PBS buffer on the bare electrode surface with CV technique. In the second step, the template molecule (L-Phe) in this film structure was leached and suitable pores were created for L-Phe molecules. The surface properties and morphologies of the prepared electrodes were checked by SEM analysis technique. While uncoated Pt electrode surface is quite smooth, PTSA coated electrodes have rough and non-porous surface. After L-Phe desorption, porosity and very high surface area were obtained on the surface. Modified electrode; Monomer solution is prepared by CV method. Support electrolyte type and pH, scan rate, pulse amplitude, pulse width, pulse period and interference effect are optimized for the MIP based modified electrodes prepared by making electropolymerization with 5 segmented film thickness. The oxidation peak current of L-Phe was increased linearly with increasing of L-Phe concentration in the presence of non-electroactive interference such as glucose, sucrose, maltose, lactose and electroactive interferences such as ascorbic acid, uric acid. As a result, PBST coated MIP based electrodes prepared in the study can be used as a sensor in the selective and sensitive detection of L-Phe according to its linear range and LOD values.

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