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To cite this article: Merzak Doulache *et al* 2020 *J. Electrochem. Soc.* **167** 027511

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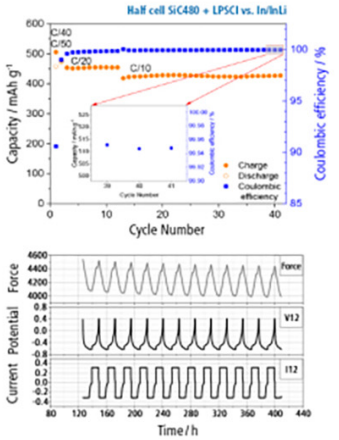
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Journal of The Electrochemical Society, 2020 **167** 027511
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Highly Sensitive and Selective Electrochemical Sensor Based on Polyglycine Modified Glassy Carbon Electrode for Simultaneous Determination of Amlodipine and Ramipril from Biological Samples

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In this study, the electrochemical behavior of the antihypertensive drugs amlodipine (AML) and ramipril (RAM) was investigated on polyglycine modified glassy carbon electrode (Poly(Gly)/GCE) as simultaneously. A simple and highly sensitive differential pulse adsorptive stripping voltammetric (DPAdSV) technique was developed for the simultaneous electrochemical determination of AML and RAM in 0.1 M H_2SO_4 solution (pH 1.0). The oxidation process of both analytes was found to be irreversible and exhibited mixed diffusion under adsorption-controlled process. The oxidation mechanism of drugs was proposed using the obtained data and discussed. The Poly(Gly)/GCE showed an enhancement effects on the oxidation current of AML and RAM with the significant peak potential differences of 0.52 V by AdSDPV technique. At the optimum conditions, the proposed method exhibited linear responses in concentration ranges 0.5–25 μM for AML and RAM with the detection limits of 0.08 and 0.130 μM, respectively. Finally, the proposed sensor was successfully applied for the sensitive detection and simultaneous determination of AML and RAM in human serum and human urine samples.
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Manuscript submitted October 28, 2019; revised manuscript received December 16, 2019. Published January 17, 2020.

Amlodipine (AML) is a long-acting dihydropyridine calcium channel blocker (CCB).[1] It inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle to protect the target organs. It is prescribed for the treatment of hypertension and angina.[2–4] But, an overdose of AML can lead to profound hypotension and slow the heart rate.[5,6]

Ramipril (RAM) is an angiotensin-converting enzyme inhibitor (ACEI), which is widely used to lowering high blood pressure (hypertension), congestive cardiac failure and kidney problems.[7] RAM is prodrug converted in the liver by de-esterification into its active form ramiprilat, which inhibits ACE, thereby blocking the conversion of angiotensin I to angiotensin II.[8,9] However, an overdose of RAM my cause hypotension, bradycardia and other side effects.[10,11]

Combination of ACEI and CCB has been successfully used in the antihypertensive therapy, since both groups of drugs act as vasodilators and have therapeutic synergy in blood pressure reduction. Furthermore, the combined antihypertensive therapy can decrease the damage of heart, brain, kidney, etc.[12–14] Thus, a fixed dose combination of AML/RAM is an effective antihypertensive drug with a good tolerability, simplicity, convenience, and cost effectiveness, as well as the compliance.[15]

There are a few reported analytical methods including RP-HPLC[16–19] and UV spectrophotometric[20] for the determination of the assay of this combination in pharmaceutical and biological samples. However, the mentioned methods are expensive, usually laborious, complex to be operated and need prolonged time. Therefore, a rapid, simple, low cost, accurate and sensitive assay is required for routine simultaneous determination of AML and RAM due to the therapeutic importance.

Electroanalytical techniques are very elegant methods due to their instrumental simplicity, high sensitivity, short analysis time and low cost.[21–24] However, using ordinary electrodes (Pt, Au, carbon paste, and graphite) bare electrodes to electrochemical detection of many molecules entail sometimes restrictions namely instable and weak electrochemical signal, high oxidation overpotential, and overlap the responses signal of analytes.[25] Nevertheless, the possibility of modifying the surface of electrochemical-based sensors with new conductive materials can significantly improve the sensitivity of the response signal and promote the electron transfer kinetics of analyte in terms of improved current response and reduced overpotential with respect to the bare electrode.[26–29]

So, polymer film modified electrodes (PMEs) have received much attention due to their high stability, sensitivity and selectivity towards analytes, strong adherence to electrode surface, ability to provide larger surface area by forming homogeneous film, and ability to promote electron transfer rates.[30–32] The electropolymerization of the electrode by poly-glycine causes the desired properties of low cost, increased sensitivity and selectivity, and enhanced electrochemical response because of increased electrode active surface area.[33–36]

Based on the above advantages of poly-glycine film, economical, simple and sensitive electrochemical sensor was developed for selective and sensitive detection of AML and RAM. Up to our knowledge, an electrochemical determination of these two drugs has not been previously described in the literature. Thus, the present work provides a novel method for simultaneous trace analysis of AML and RAM, which has a significant attraction in biological and chemical fields.

Experimental

Reagents and apparatus.—AML and RAM were obtained from Kocak Pharm. Company (Istanbul, Turkey), D.L-glycine (>99.0) was purchased from Shanghai Biochemical Institute (Germany). 1×10^{-3} M standard stock solutions of AML and RAM were prepared in distilled water and they put in refrigerator to keep fresh. H_2SO_4 (0.1 and 0.5 M) and Britton-Robinson buffer (0.04 M) solutions were used for the preparation of buffer solutions at pH range 0.3–8.0. The pH values were adjusted to the related pH by 0.1 M sodium hydroxide (Fluka) under the pH-meter. The other chemicals were analytical grade. All solutions were prepared using exclusively deionized water.

All electrochemical measurements were realized using a three-electrode cell at ambient temperature; the data was recorded with a potentiostat/galvanostat pocket STAT (IVIUM Technologies) coupled with a PC-computer. A three electrode system was used

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for all measurements; Ag/AgCl (saturated KCl) as the reference electrode, Pt wire as the counter electrode and GCE (Ø = 3 mm) and Poly(Gly)/GCE as the working electrodes. The pH was controlled with a pH meter Model 538 (WTW, Austria) instrument.

For the analytical application, differential pulse voltammetry (DPV) technique was used and all DPV parameters were optimized. The optimum values obtained for these parameters were given as follows; step potential: 20 mV; modulation amplitude: 230 mV; modulation time: 0.01 s; scan rate: 50 mV s^{-1}. Optimum differential pulse adsorptive stripping voltammetry (DPAdSV) conditions were; accumulation potential (E_{acc}): 0.1 V and accumulation time (t_{acc}): 30 s.

Preparation of Poly(Gly)/GCE electrode.—Before the modification procedure, the GCE was cleaned mechanically on wet fine emery paper with alumina slurry, thoroughly rinsed with distilled water and successively sonicated in ethanol and distilled water. The electropolymerization of glycine at GCE was done in aqueous solution containing 0.02 M glycine and 0.05 M H_3BO_3 (pH 5.25) using multipulse amperometry method. A potential of 1.8 V was applied to the electrode for 10 s, then this was switched to 0 V for 1 s. This protocol was repeated for an overall duration of 40 s. The obtained electrode was electrochemically treated in H_2SO_4 (0.1 M, pH 1.0) by scanning potential in the range from −0.25 V to 1.60 V for several cycles until reaching a very stable background current.

Quantification of AML and RAM in serum and urine samples.—In order to analyze AML and RAM in synthetic serum sample, 3.6 ml synthetic serum sample was mixed with 5.4 mL of acetonitrile and 1 ml of 1×10^{-3} M mixed AML/RAM standard solution. The sample was transported to ultrasonic bath and agitated for 10 min and subsequently centrifuged (3000 rpm, 10 min) for the precipitation of protein residues, and then the supernatant was taken carefully. Appropriate volumes of the supernatant were diluted using the selected supporting electrolyte. The concentration of mixture AML/RAM was varied in the range 0.5–25 μM in human serum samples. Quantifications were carried out by means of the calibration curve method from the related calibration equations. For humane urine study, 10 ml urine sample was collected from healthy voluntary and the same prepared procedure as when used for serum preparation was made, to build the calibration curve of mixture AML/RAM in urine samples.

Results and Discussion

Electrochemical behavior of AML and RAM on Poly(Gly)/GCE.—Figures 1a, 1b shows the cyclic voltammograms of 0.1 mM AML and RAM at the Poly(Gly)/GCE. As can be seen, anodic peaks appear at 0.75 and 1.34 V for AML and RAM, respectively. For both drugs, no cathodic peaks were observed on the reverse scan within the investigated potential range, indicating totally irreversible electron-transfer kinetics.

The voltammetric behavior of binary mixture AML and RAM (0.1 mM) in 0.1 M H_2SO_4 solution (pH 1.0) on GCE and PGly/GCE electrodes was illustrated in Fig. 1c. At the bare GCE, the AML and RAM gave two small anodic peaks at 0.82 V and 1.41 V, respectively and the cyclic voltammogram showed weak and insignificant current responses. Compared with a bare electrode, two well-defined peaks negatively shifted with peak current increase significantly at Poly(Gly)/GCE.

In addition, the DPV results shown in Fig. 1d for the bare GCE provided small current responses to AML and RAM. However, after modification of the GCE with the Poly(Gly) film, the oxidation peak current increased by a factors of 2 and 3 for AML and RAM, respectively. This indicates that Poly(Gly)/GCE has a good electrocatalytic ability to the electrochemical reaction of AML and RAM. The peak-to-peak separation for AML and RAM was about 0.5 V. As a consequence, the simultaneous determination of AML and RAM is suitable at Poly(Gly)/GCE surface without any interference.

Effect of electropolymerization time and Gly concentration.—The effect of the thickness of the Poly(Gly) film on the anodic peak

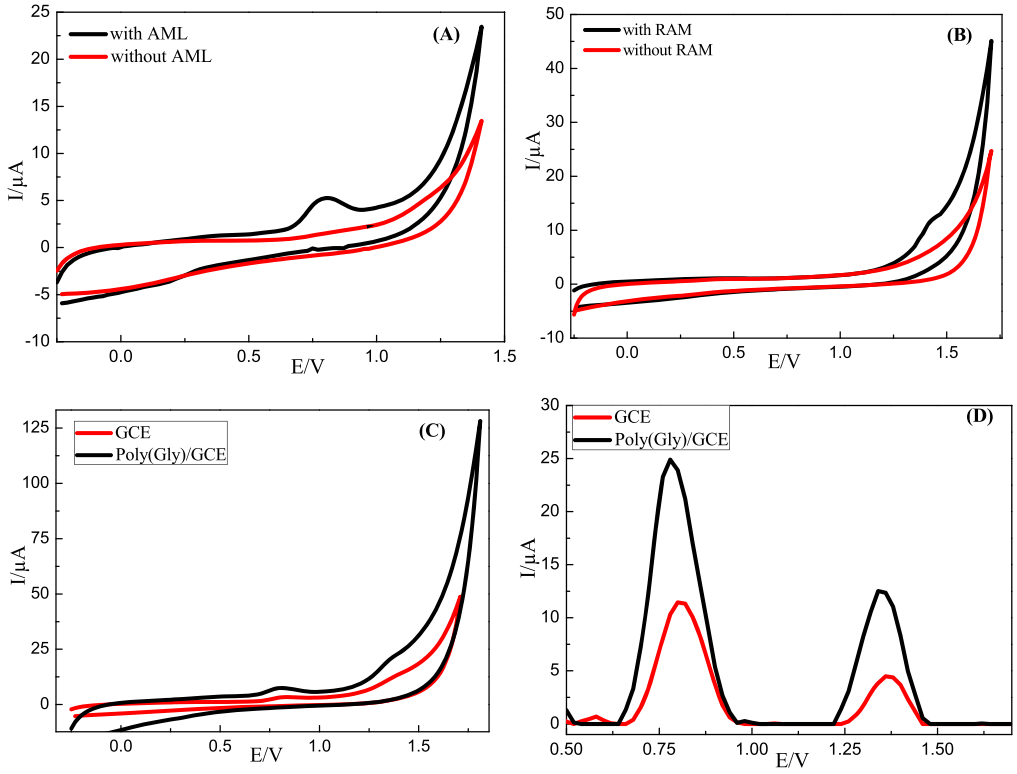


Figure 1. Cyclic voltammograms for (A) 0.1 mM AML and for (B) 0.1 mM RAM in 0.1 M H_2SO_4 using a Poly(Gly)/GCE electrode (C) CVs and (D) DPVs of 0.1 mM AML and RAM using the bare GCE and Poly(Gly)/GCE in 0.1 M H_2SO_4 (pH 1.0) solution.

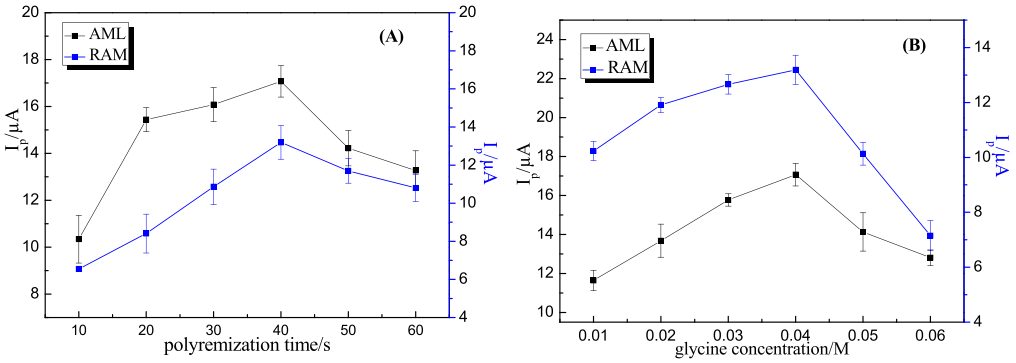


Figure 2. Effect of polymerization time (A) and concentration of glycine monomer (B) on the peak response of 0.1 mM AML and RAM in 0.1 M H_2SO_4. Error bars represent the standard deviation for three independent measurements.

current of AML and RAM was evaluated. It is well-known that film thickness can be easily controlled by the time of electropolymerization. As shown in Fig. 2a, the current responses of AML and RAM increased with increasing deposition time. The anodic currents of both drugs reached a maximum value at 40 s, and then decreased with further increase of the deposition time. This may be explained by the fact that very thin films do not possess sufficient active sites, whereas very thick films tend to block the electron transfer. Therefore, the optimal deposition time as 40 s was adopted to fabricate the Poly(Gly)/GCE.

Figure 2b shows the plot of the anodic peak currents against the glycine monomer concentration, the anodic peak currents of AML and RAM mixture enhanced by increasing the concentration of monomer from 0.01 to 0.04 M; above 0.04 M the peak currents of AML and RAM started to decline slightly. Hence, 0.04 M of monomer was chosen as the optimum concentration in this study.

The influence of pH.—The influence of pH on the oxidation process of 10 μM AML and RAM was investigated by DPVs in the pH range from 0.3 to 8 (Fig. 3a). As the pH increases, oxidation peak currents of both AML (Fig. 3b) and RAM (Fig. 3c) increased until pH 1.0 and then decreased at higher pH values. Hence, pH 1.0 was selected as the optimum pH value for the simultaneous determination of both drugs. It is also observed that, following the pH from 0.3 to 8.0, the peak potentials of AML and RAM shifted negatively, indicating the involvement of protons in the electrode reaction. The linear equation for AML was;

$$E_{pa}(V) = -0.020\ pH + 0.824 (R^2 = 0.978) \quad [1]$$

and for RAM;

$$E_{pa}(V) = -0.044\ pH + 1.406 (R^2 = 0.955) \quad [2]$$

The slopes of the respective equations were −44.5 and −22.5 mV pH^{-1}, implying that the equal number of electrons and protons participate in the RAM oxidation reaction and two-electrons and one-proton transfer to form AML cation.

The effect of scan rate.—The effect of scan rate on the peak currents of AML and RAM at the Poly(Gly)/GCE was characterized

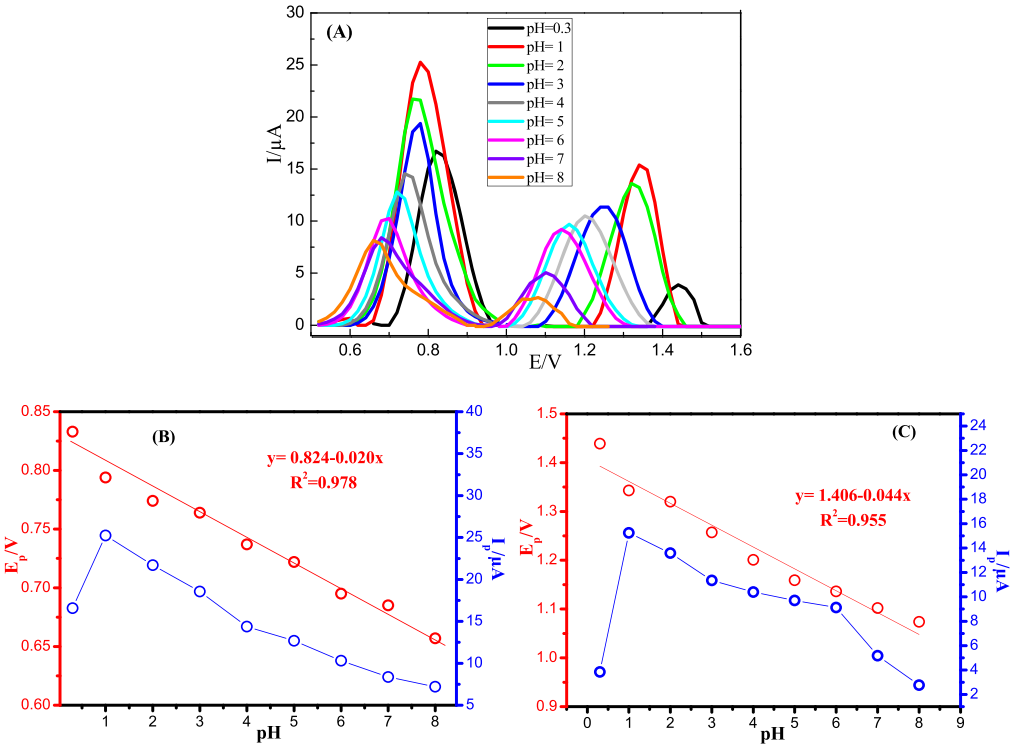


Figure 3. DPVs of 0.1 mM AML and RAM on Poly(Gly)/GCE at different pH values (A); (B) and (C) plots of peak current and peak potential of AML and RAM against of pH.

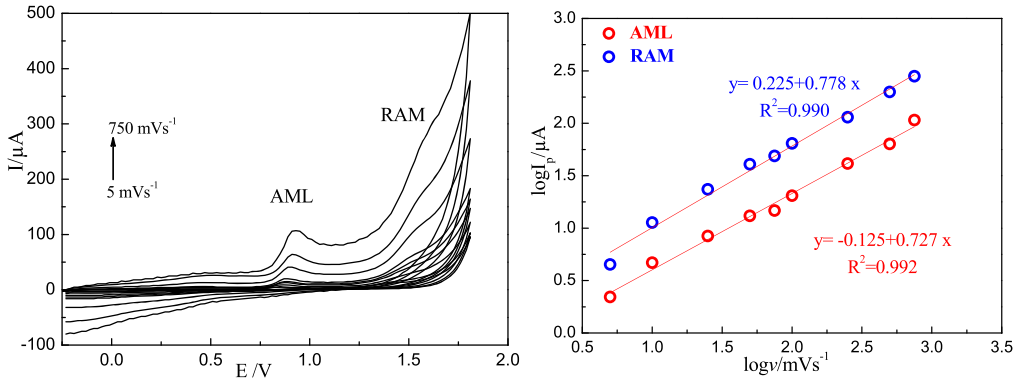


Figure 4. (A) CVs of poly-Gly/GCE in 0.1 M H_2SO_4 (pH 1.0) with 0.1 mM AML and RAM at different scan rates, 5–750 mV s^{-1}. (B) Logarithmic dependence of I_p on the logarithmic scan rate values.

by cyclic voltammetry, As shown in Fig. 4a, with increasing potential scan rate from 5 to 750 mV s^{-1} was verified a linear increase in the peak current for AML and RAM. The log I_p vs log v plots for each process are presented in Fig. 4b. The linear relationships with slope values 0.72 for AML and 0.77 for RAM were obtained. These slope values are between the theoretical values of 0.5, typical for diffusion-controlled process, and 1.0, reported for adsorption-controlled process.[37]

So, to determine which of these processes dominated the electron transfer of both drugs, we constructed the plots of peak current vs potential scan rate (Fig. 5a) and peak current vs square root of the scan rate (Fig. 5b). As can be seen, an excellent linear dependence between the I_p and v with a correlation coefficient of 0.990 for AML and 0.991 for RAM were obtained, which suggested that the adsorption process was mostly dominated in electrochemical reaction of AML and RAM on Poly(Gly)/GCE.

As well a linear dependence between the oxidation peak potential and the logarithm of scan rate were exposed as (Fig. 5c):

$$E_p = 1.605 + 0.087 \log v (R^2 = 0.970) \text{ for AML} \quad [3]$$

$$E_p = 0.949 + 0.050 \log v (R^2 = 0.977) \text{ for RAM} \quad [4]$$

In case of irreversible electrode process, the electron transfer number in electrochemical reaction can be calculated using Laviron equation:[38]

$$E_{pa} = E^o + 2.303\, RT/\alpha nF \log v \quad [5]$$

Where, E° is formal potential, n is number of electrons transferred, α is charge transfer coefficient, and. Other factors, such as F is Faraday's constant, T is absolute temperature, and R is ideal gas constant. Considering α equal to 0.5 for irreversible reactions and based on the slope of Eqs. 3 and 4, the number of electrons transferred in the oxidation of AML and RAM were calculated to be 2.31 (≈2) and 1.35 (≈1), respectively, which was consistent with previous studies.[7,39]

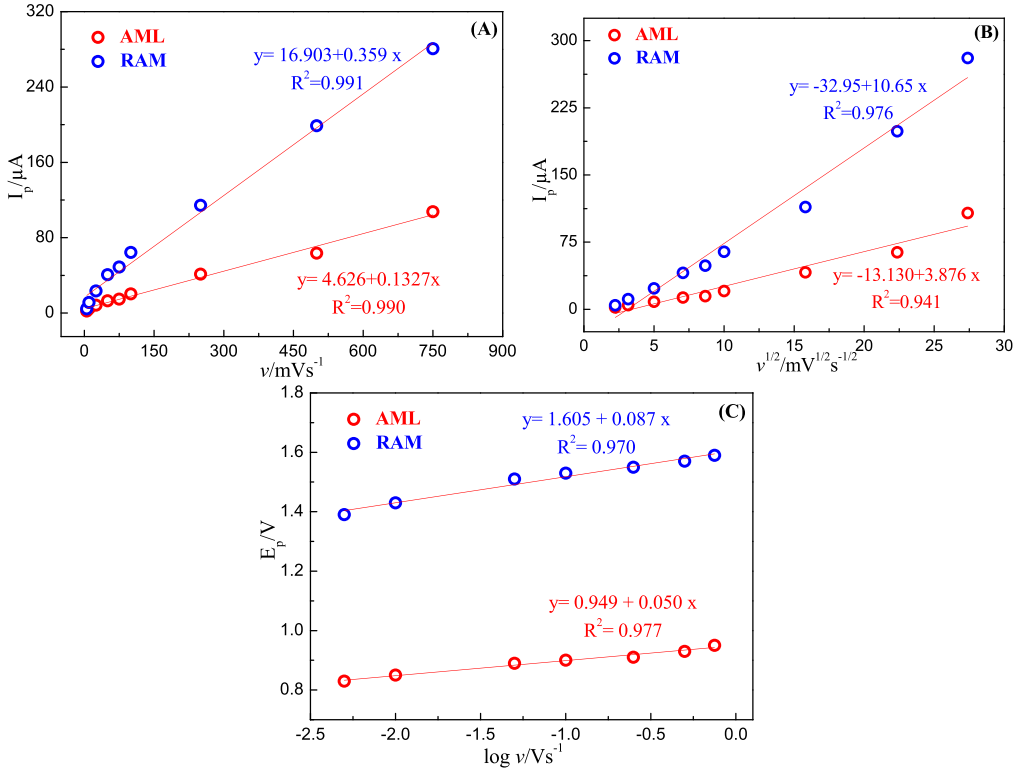
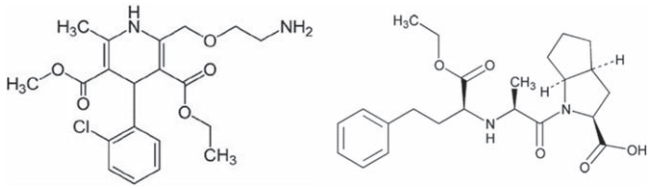


Figure 5. Plots of (A) I_p vs v; (B) I_p vs $v^{1/2}$ and (C) log I_p vs log v.



AML **RAM**

Scheme 1. Structural formulae of amlodipine (AML) and Ramipril (RAM).

Considering this set of results and according to previously studies for both drugs, the electrode reaction of AML is probably to be oxidized to form pyridine cation, with contribution of two-electrons and one-proton. For RAM mechanism reaction, the RAM molecules involving the oxidation of the secondary amine group with the loss of one electron (Scheme 1). The proposed mechanisms of the electrochemical reactions of both antihypertensives drugs may be proposed as following pathways in Scheme 2.

The effects of accumulation time and accumulation potential.—The effect of accumulation time and accumulation potential were studied in 0.1 M H_2SO_4 solution (pH 1.0) containing 50 μM AML and RAM. In Fig. 6a, at the accumulation potential of 100 mV, the anodic current of AML and RAM increased progressively with an increase in accumulation time and achieved a maximum value when the accumulation time was 30 s, decreasing afterwards. Therefore, the accumulation time of 30 s was chosen as an appropriate accumulation time for AML and RAM in subsequent studies. Moreover, we studied the effect of accumulation potential on the oxidation peak currents of AML and RAM using the optimized accumulation time. In Fig. 6b, the anodic current increased until 100 mV, after which the oxidation peak currents gradually decreased. Therefore, the optimal pre-concentration potential for determination of AML and RAM was 100 mV.

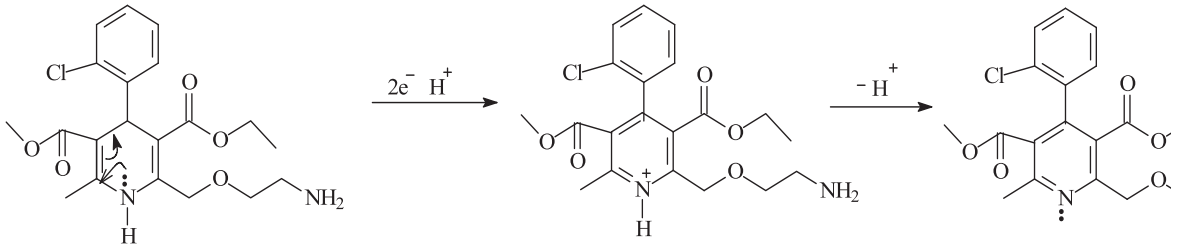
The results showed that after accumulation step, the DPV response of AML and RAM was about 3.7 and 2.7 times higher than the peak responses obtained without accumulation step, respectively (Fig. 7a). Compared with bare GCE, Poly(Gly)/GCE provided the highest AML and RAM oxidation peaks (Fig. 7b).

Besides, the enhancement in the oxidation peak currents of drugs at the proposed surface may be related to the high electronic conductivity of Poly(Gly) film and the enhancement in the electroactive surface area which can be elucidated by CV method. The electrochemistry of 5.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl (supporting electrolyte) was investigated at the bare and Poly(Gly)/GCE electrode surfaces (Figure not shown). Using the Randles-Sevcik equation for reversible redox processes controlled by diffusion[40] and the anodic and cathodic peak currents registered at 50 mV s^{-1}, the surface areas of the bare and poly-Gly/GCE were calculated to be 0.085 and 0.165 cm^2, respectively. These results indicated that after GCE surface modification by Poly(Gly) film, the electroactive area is approximately two times that of the bare GCE. This confirms the change in surface property of the modified electrode. Hence, the fabricated Poly(Gly)/GCE has stable and sensitive electrochemical behavior.

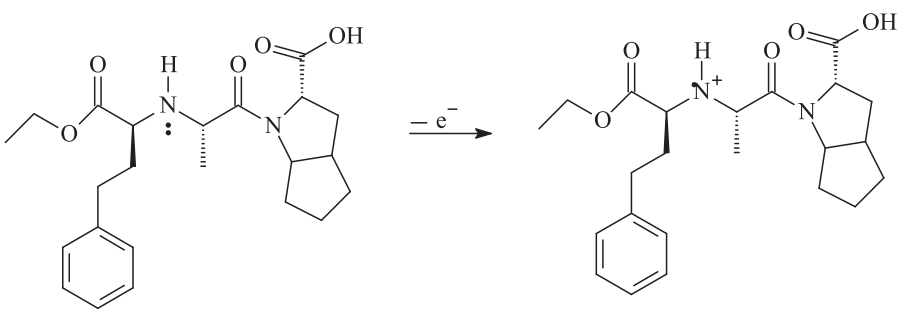
Simultaneous determination of AML and RAM on Poly(Gly)/GCE.—Firstly, in order to check the influence of each drug in the simultaneous determination of its binary mixture, we studied one in the presence of the other; so that the concentration of RAM was keep up constant at 2.5 μM and the AML concentration was varied from 0.5 to 50 μM.

As observed in Fig. 8a, the current response of AML increased linearly with its concentration, while the current response of RAM remained constant. Similarly, when the concentration of AML was fixed at 2.5 μM RAM was varied in the range from 0.5 to 50 μM (Fig. 8b), the current response of RAM increased regularly with its concentration, while the current response of AML remained constant.

It can be concluded that the change in concentration of one studied drug had no significant influence on the peak current and peak potential of another drug. It is very important to note that the oxidation processes of AML and RAM on Poly(Gly)/GCE are independent.



AML



RAM

Scheme 2. Proposed electro-oxidation mechanisms of AML and RAM.

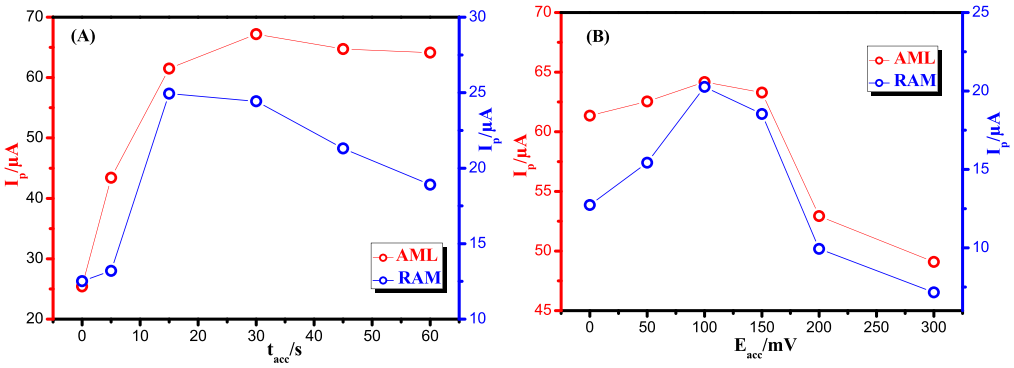


Figure 6. Effect of accumulation time (A) and accumulation potential (B) on the oxidation response of 50 μM of AML and RAM.

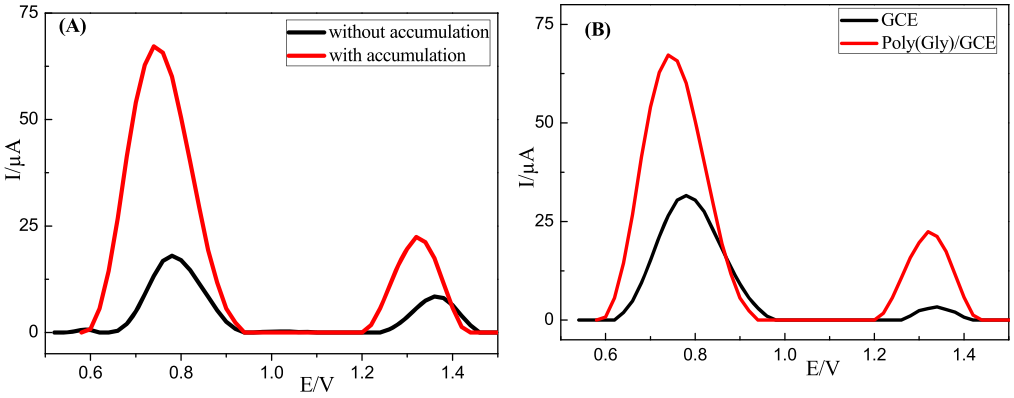


Figure 7. (A) DP voltammograms for 50 μM AML and RAM in 0.1 M H_2SO_4 (pH 1.0) at Poly(Gly)/GCE; without accumulation and with accumulation for 30 s at 0.1 V. (B) DPAdS voltammograms of the bare GCE and Poly(Gly)/GCE in 0.1 M H_2SO_4 (pH 1.0) with 5×10^{-5} M AML and RAM.

Secondly, the equal concentrations of AML and RAM changed synchronously from 0.5 μM to 50.0 μM and the obtained DPAdSV responses were displayed in Fig. 8c. The analytical curves obtained were linear for the concentration ranges from 0.5 to 25.0 μM for AML and RAM. The equations associated to these analytical curves were:

$$I_p(\mu A) = -1.412 + 2.530\, C_{AML}(\mu M)(r^2 = 0.997) \text{ for AML} \quad [6]$$

and

$$I_p(\mu A) = -0.503 + 1.493\, C_{RAM}(\mu M)(r^2 = 0.994) \text{ for RAM} \quad [7]$$

Both LOD and LQD values were calculated using the relations ks/m, where s is the standard deviation of the 5 repeated peak currents of the lowest concentration of the range, m is the slope of the linearity curve, k = 3 for LOD and 10 for LOQ.[41] The necessary

Table I. Regression data of the calibration curve for quantitative determination of AML and RAM by DPAdSV method in supporting electrolyte.

	0.1 M H_2SO_4 (pH 1.0)	
	AML	RAM
Peak potential (V)	0.77	1.28
Linearity range (μM)	0.5–25	0.5–25
Slope (μA μM^{-1})	2.530	1.493
SE of slope	0.051	0.040
Intercept (μA)	−1.412	−0.503
SE of intercept	0.136	0.221
Correlation coefficient (r)	0.998	0.997
LOD (μM)	0.085	0.130
LOQ (μM)	0.284	0.435
Within-day of the peak current (RSD%)[a)]	1.47	1.10
Between-day of the peak current (RSD%)[a)]	2.70	1.56
Within-day of the peak potential (RSD%)[a)]	1.82	0.88
Between-day of the peak potential (RSD%)[a)]	1.53	0.89

a) Obtained from average of five experiments.

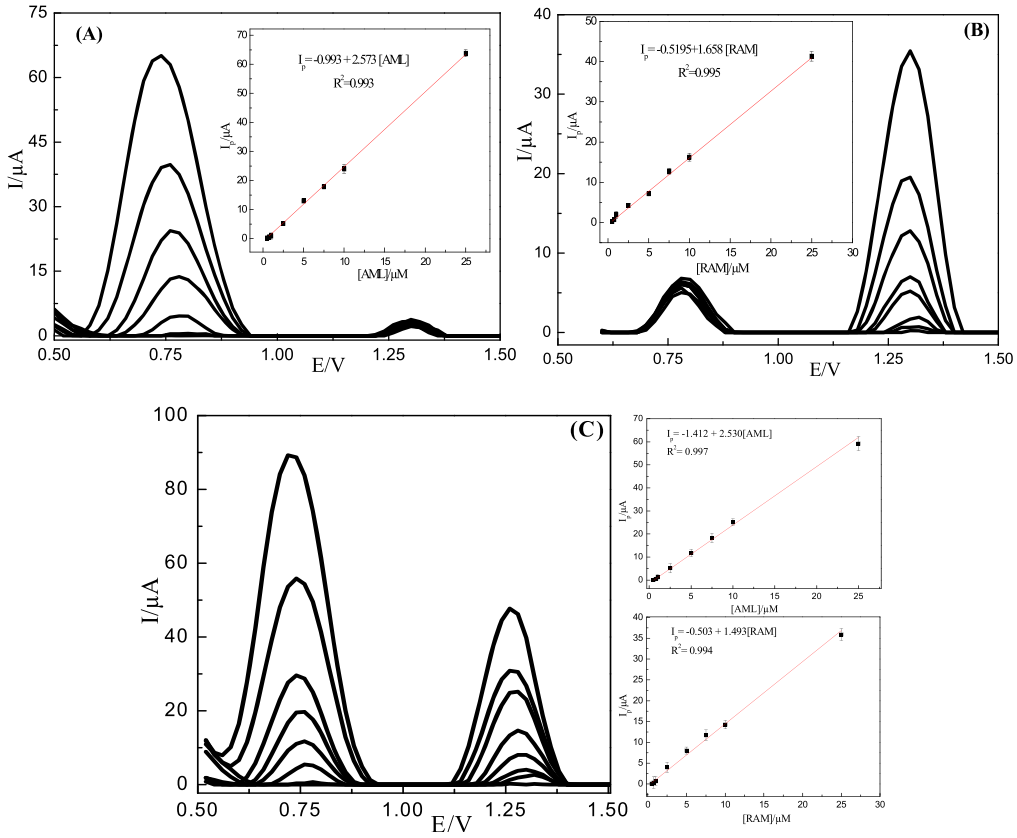


Figure 8. (A) DPAdSV responses of 2.5 μM of RAM and varying concentrations of AML (0.5–25 μM); (B) DPAdSV responses of 2.5 μM of AML and varying concentrations of RAM (0.5–25.0 μM); (C) DPAdSV responses for simultaneous addition of AML and RAM from 0.5 to 25.0 μM. Experimental conditions: 0.1 M H_2SO_4 (pH = 1.0) at Poly(Gly)/GCE. The insets display the calibration curves for peak current vs concentration.

Table II. Comparison of the analytical parameters obtained using different techniques for the determination of AML and RAM.

	Concentration range (μM)		LOD (μM)		
Technique	AML	RAM	AML	RAM	References
UV	12–61	36–84	—	—	20
RP-HPLC	9.8–29	9.6–29	1.22	1.20	16
RP-HPLC	24.4–220	12.0–108	11.0	10.7	19
RP-HPLC	34–88	24–600	0.049	0.17	17
RP-HPLC	0.5–7.8	2.4–38	0.08	0.36	18
DPAdSV	0.5–25	0.5–25	0.08	0.13	This work

validation steps and calculated analytical parameters for both antihypertensive drugs such as LOD, LOQ, precision, sensitivity, etc listed as details in Table I.

Table II shows a comparison between analytical results of the proposed method and some other methods involved in the simultaneous determination of AML and RAM. The analytical performance of the present method is quite comparable or even better than the published analytical method. Moreover, the proposed method did not require any sample pretreatments or time-consuming extractions steps, other than precipitation of the serum proteins with acetonitrile and a dilution step with the selected supporting electrolyte. Once again, it should be mentioned that there is no relating report on electrochemical methods for the simultaneous determination of AML and RAM until this article was undertaken.

Assay of AML and RAM in biological samples.—The applicability of the proposed technique to the human serum and urine samples was also investigated. The calibration voltammograms of binary mixture AML/RAM in serum and urine samples were given in Figs. 9 and 10, respectively. The obtained calibration equations of AML/RAM in spiked biological samples, and related validation parameters are shown in Table III as details. The best analytical characteristics and calibration data were obtained, confirming the reliability of this developed sensor for the simultaneous analysis of AML and RAM in biological samples.

Interference study.—The aim of the interference study is to investigate the effect of some co-exiting species on binary antihypertensive compounds assay. Therefore, some inorganic substances and possible biologic molecules were chosen for this study such as K^+, Na^+, Cl^-, NO_3^-, ascorbic acid and dopamine. 1×10^{-5} M AML and RAM was used as fixed concentration for interference experiments. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±10% relative error in the determination.[42] The results indicated that the same concentrations of K^+, Na^+, Cl^-,

Table III. Regression data of the calibration curve for quantitative determination of AML and RAM by DP AdSV method in serum and urine samples.

	Serum sample		Urine sample	
	AML	RAM	AML	RAM
Peak potential (V)	0.78	1.28	0.78	1.29
Linearity range (μM)	0.5–7.5	0.5–5.0	0.5–7.5	0.5–7.5
Slope (μA μM^{-1})	2.816	1.950	2.853	2.097
SE of slope	0.062	0.054	0.089	0.059
Intercept (μA)	−1.412	−1.099	−0.614	−0.438
SE of intercept	0.167	0.142	0.212	0.175
Correlation coefficient (r)	0.998	0.998	0.997	0.998
LOD (μM)	0.139	0.04	0.022	0.025
LOQ (μM)	0.456	0.130	0.074	0.083
Repeatability of the peak current (RSD%)[a)]	1.87	1.64	0.54	1.45
Reproducibity of the peak current (RSD%)[a)]	3.61	2.38	2.75	2.04
Repeatability of the peak potential (RSD%)[a)]	0.32	0.41	0.65	0.33
Reproducibity of the peak potential (RSD%)[a)]	0.55	0.65	1.49	0.89

a) Obtained from average of five experiments.

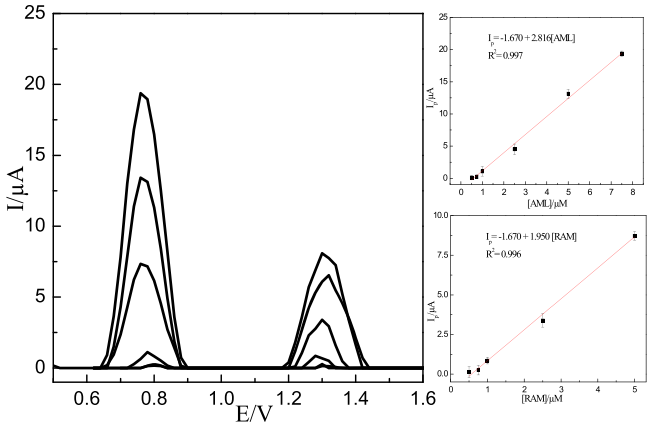


Figure 9. DPAdS voltammograms recorded at Poly(Gly)/GCE for simultaneous addition of AML and RAM from 0.5 to 7.0 μM in serum samples, the insets display the calibration curves for peak current vs concentration.

NO_3^-, and same concentrations of dopamine and ascorbic acid with studied drugs have not significant effect on current responses of studied drugs. On the other hand, the interference effect on the current responses of AML and RAM observed in the 100-fold and

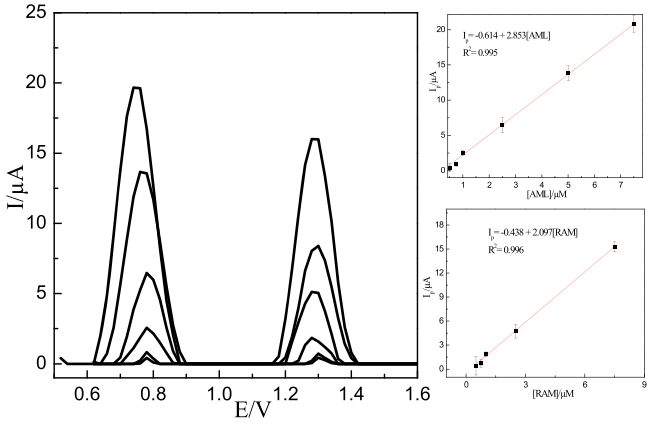


Figure 10. (B) AdSDP voltammograms recorded at Poly(Gly)/GCE for simultaneous addition of AML and RAM from 0.5 to 7.0 μM in urine samples. The insets display the calibration curves for peak current vs concentration.

Table IV. Tolerance of interferences on the determination of 2.5 μM AML and RAM.

		±% Error	
	Tolerance level ratio of (Foreign substance: AML/RAM)	AML	RAM
K^+	1:1	6.29	4.33
	10:1	1.34	1.61
	10:1	6.17	4.09
	1000:1	6.64	3.98
Na^+	1:1	3.77	1.59
	10:1	0.22	0.79
	100:1	8.84	2.39
	1000:1	8.70	1.58
Cl^-	1:1	5.02	4.00
	10:1	0.78	1.2
	100:1	5.84	3.80
	1000:1	7.67	2.78
NO_3^-	1:1	4.70	4.43
	10:1	1.23	1.59
	100:1	5.55	3.60
	1000:1	6.27	2.50
Dopamine	1:1	1.59	5.09
	10:1	5.87	3.72
	100: 1	4.13	28.35
	1000: 1	11.4	24.74
Ascorbic acid	1:1	1.84	5.67
	10:1	1.56	2.29
	100:1	5.89	8.59
	1000:1	9.87	15.55

1000-fold excess of dopamine and in 1000-times higher concentration of ascorbic acid. The tolerated concentration of organic and inorganic molecules shown in Table IV.

Conclusions

In this work, a fast and sensitive electrochemical sensor (Poly (Gly)/GCE) for the simultaneous determination of binary mixture of amlodipine and ramipril is elaborated. The Poly(Gly) film provides a high specific surface area and improves the conductivity of the modified electrode. The behavior of both drugs at Poly(Gly)/GCE indicates that this electrode might be used for analytical purposes, particularly as a sensor. Electro-analytical measurements were applied with AdSDPV technique. This technique could provide a

favorable perspective in the fabrication of electrochemical platforms based on electrodes modified with poly-glycine film for simultaneous detection at least two antihypertensive drugs. In comparison to published methods, the proposed sensor is highly sensitive, speedy and inexpensive for drugs sensing from biological samples without any time-consuming sample extraction, evaporation or filtration steps. Consequently, this method has the potential for being an attractive alternative for simultaneous determination of AML and RAM in biological fluids.

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