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**Abstract**

A novel efficient differential pulse voltammetric (DPV) method for determination gallic acid (GA) was developed by using an electrochemical sensor based on [Cu<sub>2</sub>tpmc](ClO<sub>4</sub>)<sub>4</sub> immobilized in PVC matrix and coated on graphite (CGE) or classy carbon rod (CGCE). The proposed method is based on the gallic acid oxidation process at formed [Cu<sub>2</sub>tpmcGA]<sup>3+</sup> complex at the electrode surface. The complexation was explored by molecular modeling and DFT calculations. Voltammograms for both sensors, recorded in a HNO<sub>3</sub> as a supporting electrolyte at pH 2 and measured in 2.5 × 10<sup>-7</sup> to 1.0 × 10<sup>-4</sup> M of GA, resulted with two linear calibration curves (for higher and lower GA concentration range). The detection limit at CGE was 1.48 × 10<sup>-7</sup> M, while at CGCE was 4.6 × 10<sup>-6</sup> M. CGE was successfully applied for the determination of the antioxidant capacity based on GA equivalents for white, rosé and red wine samples.

**Keywords:** gallic acid, dinuclear copper(II) octaazamacrocyclic complex, PVC matrix, voltammetric sensor

## 1. Introduction

Gallic acid, a type of phenolic acid, is occurring in plants in the form of free acids, esters, catechin derivatives and hydrolysable tannins [1]. As one of the most biologically-active phenolic compounds of plant origin, gallic acid is commonly used in the pharmaceutical and food industry. Plant polyphenols are multifunctional and can be reducing agents, hydrogen donating antioxidants, singlet oxygen quenchers and in some cases metal chelating agents [2]. Gallic acid is used as a standard for determining the phenol content of various analytes by the Folin-Ciocalteu spectrophotometric method and the results are reported in gallic acid equivalents. The measurement of “total phenols” is a good indication of the level of antioxidants present in the sample [3].

The importance of the phenolic compounds has led to the development of various methods for their determination in various types of samples, including chemiluminescence [4] spectrophotometry [5] and capillary electrophoresis [6,7] as well as chromatography [8]. Electrochemical methods were also employed for this purpose. Gallic acid and other phenolic compounds were determined in wine samples and plant extracts at a glassy carbon electrode [9-13], at hanging mercury drop electrode [14,15] and at carbon paste electrode modified with nanotubes [16,17]. Electrodes with polyphenols at carbon-polyvinyl chloride [18], polyepinephrine modified glassy carbon electrode [19] and a natural bentonite, modified with different quaternary ammonium cations [20] have also been described and successfully analytically applied. Cyclic, differential pulse and adsorptive stripping voltammetric techniques were mostly used in those reported works [21]. Recently, a photoelectrochemical sensor applied to sense gallic acid has been designed with polyaniline–reduced graphene oxide–titanium dioxide [22], while a voltammetric electronic tongue system (ET) made from an array of

modified graphite-epoxy composites plus a gold microelectrode was used in qualitative and quantitative analysis of polyphenols found in wine [23].

Bearing in mind the most of past research in electrochemical determination of GA and other phenols, the main disadvantage of GCE was polymerization and adsorption of investigated and other species on the electrode itself. Therefore, GCE should be polished to provide new active surface before any electrochemical measurement. Inability for determination of more samples in a row with the same electrode without previous preparation and better electrode sensitivity were the reasons for the taken electrode modifications [16-20]. Polymer modified electrodes however, have many advantages such as improved electro catalysis, absence of surface fouling and prevention of undesirable reactions competing kinetically with the desired electrode process. On the other hand, DPV technique has many advantages compared with conventionally used CV, e.g. a better sensitivity, selectivity and response [24].

It is important to mention biosensors as powerful specific analytical devices that also have been used for the detection of phenolic compounds. Biosensors based on several types of copper-containing enzymes which catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen, such as tyrosinase [25,26] and laccase [27,28] were coupled to electrochemical or optical techniques for this purpose.

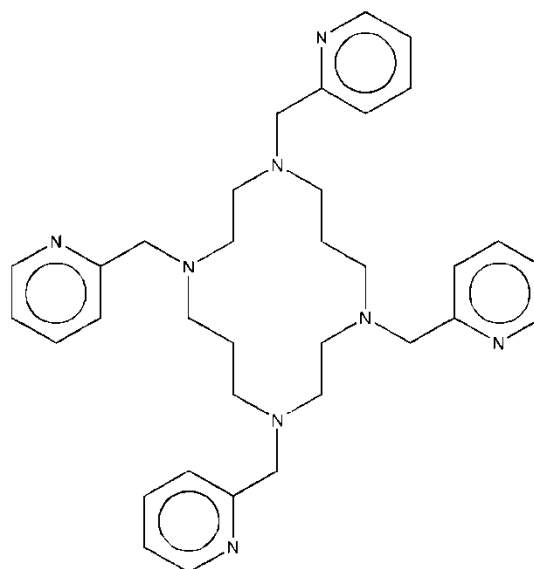
The general idea of this work was to examine application possibility of simple and efficient sensor for rapid differential pulse voltammetric determination of GA at low concentrations, based on previously synthesized  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex immobilized in a PVC matrix. To the best of our knowledge, there is no report which relates to the application of complexation agent immobilized in PVC matrix as a modifier for graphite or classy carbon rod and the determination of gallic acid or antioxidant capacity of real samples. Similarly as in the

case of copper containing enzymes, it could be very useful and interesting to examine the possibility of polyphenols oxidation in a presence of synthetic copper macrocyclic complex. Also, modification of the electrode surface should prevent polymerization on the electrode itself. Furthermore, the probability of formation of GA sensitive reversible  $[\text{Cu}_2\text{tpmcGA}]^{3+}$  complex could be investigated by molecular modeling and Differential Function Theory (DFT) calculations. The determination of structural parameters obtained by molecular modeling is a powerful tool to add chemical and physical information to those obtained by other techniques.

## 2. Experimental

### 2.1. Chemical and reagents

The initial macrocyclic ligand  $\text{N,N}',\text{N}'',\text{N}'''$ -tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane (*tpmc*) (Scheme 1) and the next synthesized  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex which was electroactive sensor material, were prepared by the procedure described in literature [29,30]. 1,4.8.11-tetraaza-cyclotetradecane 98%, as a starting compound, was used as received from Aldrich Chemical Company. The purity of synthesized ligand and complex were tested by elemental analyses performed by standard micromethods (Anal. Calcd. for  $(\text{C}_{34}\text{H}_{44}\text{N}_8)$  (%): C, 72.30; H, 7.85; N, 19.84. Found: C, 72.48; H, 7.89; N, 19.68). Poly(vinyl chloride), PVC, was used as Pevicon made by Fostatbolaget, Sweden. Dibutyl phthalate (DBP) and gallic acid (GA) were used as received from Fluka. Nitrite and sulfuric acid, potassium nitrate (all p.a.) and appropriate mixture of acetic acid and sodium hydroxide for acetate buffer, all purchased from Merck and Sigma-Aldrich, were used in supporting electrolyte investigations. Tetrahydrofuran (THF) was available from Merck and distilled before use and high purity water (Millipore, 18  $\text{M}\Omega$  cm resistivity) were used as solvents.



Scheme 1.  $N,N',N'',N'''$ -tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane (*tpmc*).

## 2.2. GA standard

Gallic acid (p.a) was used for preparation test solutions and as standard for measurement of the antioxidant capacity. The stock solution of gallic acid ( $1 \times 10^{-3}$  M) was prepared in methanol (HPLC grade). Once prepared standard was protected from light with aluminum foil and kept in refrigerator. It was stable for at least 1 month. The working standards were prepared freshly from the stock solutions for each new measurement.

## 2.3. Electrode preparation

The PVC membrane contained 5 % (w/w)  $[Cu_2tpmc](ClO_4)_4$ , 38 % PVC and 57 % dibutyl phthalate. A total of 0.270 g of these reagents was mixed with 2.5 mL THF. A glassy carbon (GC) rod ( $\phi$  3 mm) (Sigardur-Sigri Electrographite, GmbH, Germany) was directly coated by dipping two times in the mixture. The same procedure was used for coating graphite rod ( $\phi$  6 mm) made by Sigma Aldrich. The formed coating bead on the GC and graphite was allowed to dry in air over night and then, electrode was kept in  $10^{-4}$  M GA solution for about 24 h. This modified GC and graphite electrodes were finally used as voltammetric sensors for determination of GA. The voltammetric responses of the electrodes were measured in the series of standard GA solutions within the range of  $2.5 \times 10^{-7}$  to  $1.0 \times 10^{-4}$  M in a  $10^{-2}$  M solution of  $HNO_3$  as a supporting electrolyte.



#### 2.4. Electrochemical measurements

Cyclic and differential pulse voltammetric measurements were recorded on a Metrohm 797 VA Computance instrument (Herisau, Switzerland). The triple-electrode system consisted of a working electrode, GC or graphite electrode modified with PVC membrane with activated dinuclear Cu(II)/*tpmc* complex as sensor component, a reference Ag/AgCl, KCl (3 M) (Model 6.0728.010) and an auxiliary separate platinum rod electrode (Model 6.0340.000). The cyclic and the differential pulse voltammograms of standard GA solutions were recorded in the potential range from 0.0 to 1.0 V, using a scan rate of 100 mV/s (for CV) and 10 mV/s (for DPV). A modulation time was varied from 20 to 80 ms, and pulse amplitude was changed in range of 10 to 90 mV. The measurements were performed in unaerated solutions. The pH measurements were carried out using a Hanna Instruments pH-meter with a Hanna Instruments combined pH reference electrode. All of the experiments were done at room temperature (25<sup>0</sup>C).

#### 2.5. Sample preparation

Determination antioxidant capacity presented as mg/L of GA in white, rosé and red wine samples were done using an extremely simple procedure involving the direct addition of a sample aliquot in the electrochemical cell without any sample pretreatment. The wine samples in triplicate, commercially available in Serbia were analyzed by the proposed voltammetric method used as a standard for determination of antioxidant capacity. An aliquot of 1mL of wine was added to 9 mL of 10<sup>-2</sup> M HNO<sub>3</sub> solution in electrolytic cell and homogenized with a magnetic stirrer before voltammetric measures described in previous section.

#### 2.6. Spectrophotometric determination of total phenolic content

The total phenolic content (TP) of the wines was determined using the Folin –Ciocalteu micro method (F–C) as adapted for wine analysis [31]. Gallic acid was used to obtain the calibration curve and results were expressed as mg gallic acid equivalents per litre of wine (mg/L of GA). Red and rosé wine samples required dilution.

### 2.7. Computation details

Based on Cambridge Structural Database search published in our previous paper [32] starting structure for optimization copper complexes with *tpmc* ligand were chosen. Geometries of all structures were fully optimized with DFT (Differential Function Theory) method, according to procedure described before [32]. Reaction energies were calculated as a difference between ZPE corrected electronic energies of reaction products and reactants [33].

## 3. Results and Discussion

For better understanding the general idea of this work, the attention should be drawn to several issues regarding the construction and operation of working electrode. Polymer modified electrodes are often prepared by casting solution droplet containing dissolved polymer onto the surface and allowing the solvent to evaporate, by dip or spin coatings, or via electropolymerization in the presence of the dissolved monomer. The purpose of polymer film, from the chemical point of view is to immobilize sensor component, promote electron transfer and to prevent electrode surface contamination by other species presented in the sample [34]. This electrode was prepared by simple coating technique using PVC matrix as carrier of active component. The more conductive polymer nafion was not compatible with  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex, so less conductive but more durable polymer matrix consisted from PVC and DBP as

plasticizer was used instead. Membrane was coated on a GCE and graphite for comparison.  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex immobilized in PVC matrix is activated by GA solution. The probable operation mechanism of the sensor involved complexation between dinuclear Cu(II)/tpmc complex immobilized in membrane and GA determined in the solution, and two step electrochemical oxidation of captured GA, probably in the complex ion  $[\text{Cu}_2\text{tpmcGA}]^{3+}$ .

A number of dinuclear metal(II) complexes with *tpmc* and additional bidentate ligand are known [35,36], but there is no evidence of dinuclear copper *tpmc* complex with GA. Assuming that reversible formation of this complex on the surface of the membrane is sensitive to different concentration of GA in solution, the next step was to investigate formation possibility of proposed dinuclear copper *tpmc* complex with GA.

### 3.1 Structure of $[\text{Cu}_2\text{tpmcGA}]^{3+}$ complex ion and DFT calculations

Based on the results of the extensive CSD search [32] and molecular modeling, the structure of optimized  $[\text{Cu}_2\text{tpmcGA}]^{3+}$  complex ion was obtained (Figure 1). Details are given in supplementary materials. This structure is in agreement with other structures found in CSD [30, 37- 39].

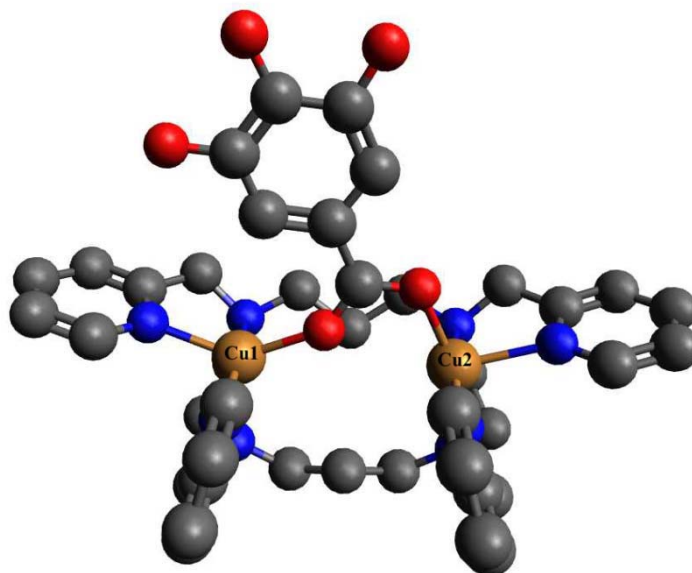


Figure 1. Optimized structure of  $[\text{Cu}_2\text{tpmcGA}]^{3+}$  complex ion. Colors: yellow-copper; red-oxygen; blue-nitrogen; grey-carbon. Hydrogen atoms are omitted for clarity.

Complex stability was examined by calculation of value for the energy of reaction (please see supplementary material) which is 10.4 kcal/mol in favor of  $[\text{Cu}_2\text{tpmcGA}]^{3+}$  complex. The results of energy calculations clearly indicated that gallic acid would have high preference for binding to  $[\text{Cu}_2\text{tpmc}]^{4+}$  complex in aqueous solution, and that the  $[\text{Cu}_2\text{tpmcGA}]^{3+}$  complex will be formed even in the case of very low concentration of gallic acid.

### 3.2. Electrochemical investigation

Electrochemical behavior of GA on the surface of bare and coated glassy carbon and graphite electrode was investigated by CV technique. Cyclic voltammograms of  $10^{-4}\text{M}$  GA in  $\text{HNO}_3$  solution as supporting electrolyte (pH about 2) at graphite a) and glassy carbon electrode b) (GE and GCE) are presented in Fig. 2. CV at modified both electrodes with activated  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex immobilized in PVC matrix (CGE and CGCE) (Fig. 3) were recorded

under the same conditions. Voltammograms exhibited two anodic oxidation peaks on both bare electrodes (both parts of Fig 2). The first peak of GA appeared in the region of 0.5 - 0.6 V and it originated primarily from the oxidation of the ortho-hydroquinone group in the ring of the molecule. The second oxidation peak about 0.9 V was caused by the oxidation of the third –OH group of GA [40]. On the basis on analysis of the voltammograms it could be noticed that the peak current decreased in every next cycle due to absorption and polymerization of GA on the bare electrodes surfaces. On the other hand, coating of the membrane over the GCE and GE decreased a current density on the electrode, but prevent polymerization Fig.3. In both coated electrodes cycling barely changed the shape of the cyclovoltammograms. The most significant changes were observed between the first and second cycles. Further cycling led to stabilization of the electrodes, which achieved steady state already after 2-3 cycles.

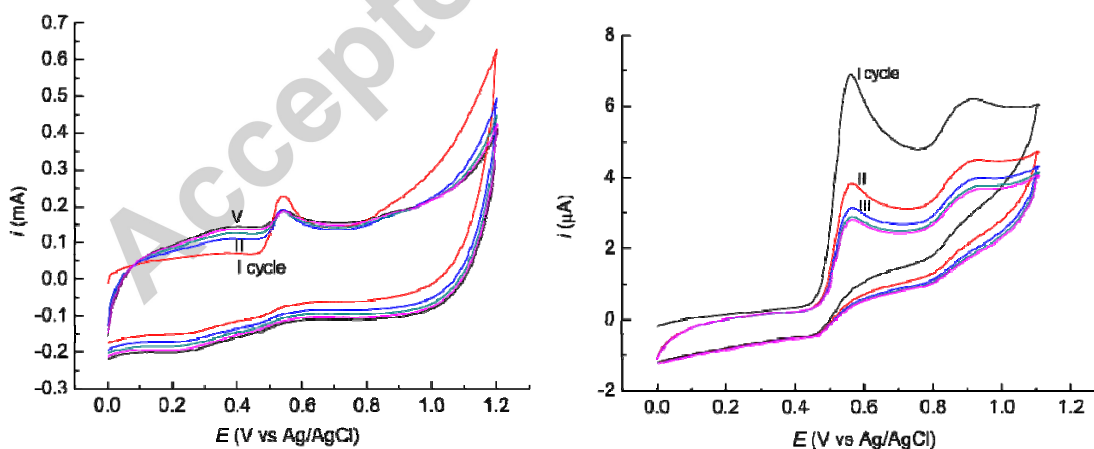


Figure 2. Cyclic voltammetric profiles for the electrochemical oxidation of  $10^{-4}$  M gallic acid in  $\text{HNO}_3$  solution (pH about 2) at bare GE (a) and GCE (b); scan rate of 100 mV/s

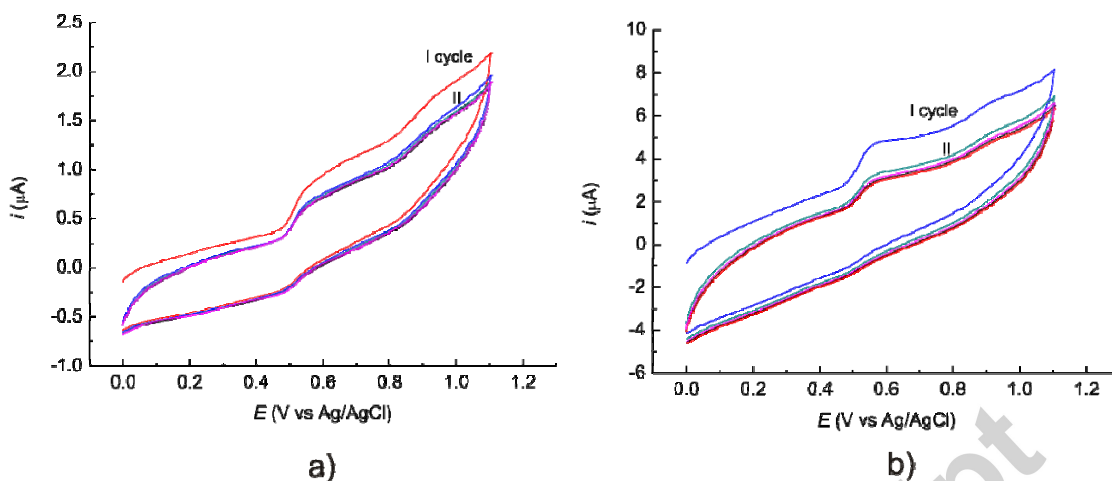


Figure 3. Cyclic voltammetric profiles for the electrochemical oxidation of  $10^{-4}$  M gallic acid in  $\text{HNO}_3$  solution (pH about 2) at CGE (a) and CGCE (b); scan rate of 100 mV/s

Differential pulse voltammetry (DPV) was selected for determination of GA as a suitable electroanalytical technique due to its low background currents and low detection limits. Working conditions were studied by variation of modulation time and modulation amplitude in order to provide lowest detection limit. After variation of modulation time from 20 to 80 ms, and changing pulse amplitude in range of 10 to 90 mV, the optimal modulation time of 40 ms and pulse amplitude of 50 mV were chosen taking into account the distortion and height of the peak of quantification, and also forego peak.

### 3.3. Selecting of supporting electrolyte and optimal pH

Bearing in mind that the sensory component, dinuclear  $\text{Cu}/\text{tpmc}$  complex could be blocked at higher pH values where the smaller  $\text{OH}^-$  ion could easily bridge two copper centers in

macrocyclic complex and form  $[\text{Cu}_2\text{OH}(\text{tpmc})]^{3+}$  ion [30], the acidic environment was selected for GA determination.

Different supporting electrolytes were tested:  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$  and acetate buffer. All voltammetric features exhibited small shoulder before the first oxidation peak of GA (Fig. 4 (a)) and the origin of forego peak is described to oxidation of obtained nitrate, sulfate or acetate bridged dinuclear copper complex itself, obtained by complexation of dinuclear copper/*tpmc* complex and anion from supporting electrolyte. These and similar structures were already reported in literature [30,37,38]. The highest and well pronounced signal for GA was recorded in  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ , with better baseline and smaller forego peak in  $\text{HNO}_3$ . Therefore, the nitric acid was chosen for supporting electrolyte in following investigations. Differential pulse voltammograms in  $\text{HNO}_3$  solution with different pH values were analyzed by construction of diagram of the influence of the pH on peak potential and peak current (Fig. 4 (b)). The previous assumption that GA oxidation became difficult with an increase in pH was confirmed by those results. The value of  $\text{pH} = 2$  was chosen for further studies.

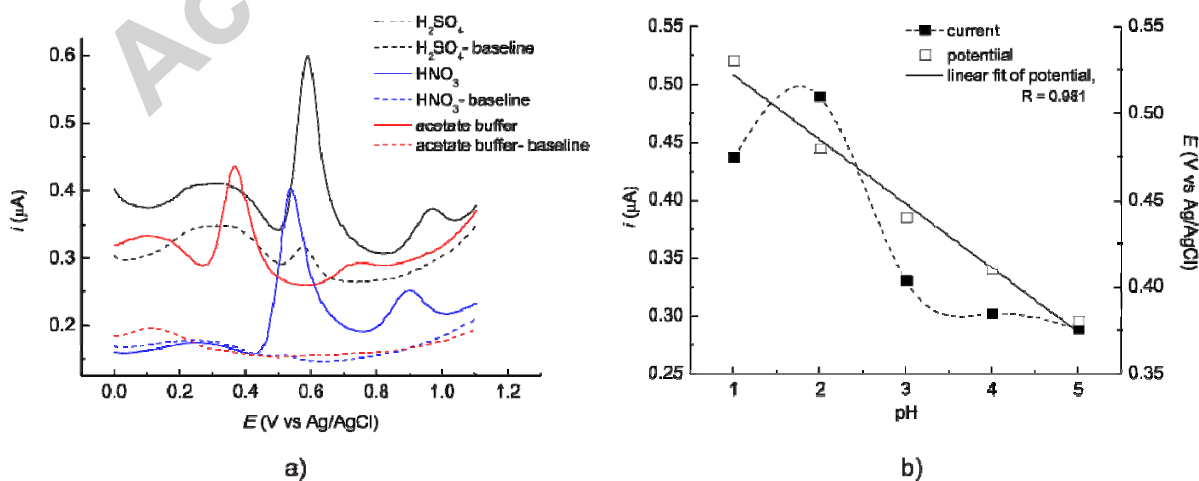


Figure 4. Differential pulse voltammograms of  $10^{-5}$  M GA in  $10^{-2}$  M  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$  and 0.1 M acetate buffer as supporting electrolyte at CGE (a); The influence of the pH on peak potential and peak current obtained from differential pulse voltammograms of  $10^{-6}$  M GA in aqueous solution of different concentrations of  $\text{HNO}_3$  (b).

### 3.4. DPV study with different electrodes

In order to investigate impact of PVC matrix and different electrode material on voltammetric response, DPV was recorded at coated graphite and GC with and without complex in the polymer matrix (Fig. 5). It was obvious that supporting electrolyte in absence of GA gave a good baseline and electrode coated with PVC matrix without sensory component -  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  respond barely even to large amounts of GA in the standard solution. In such way, DPV response of the CGE and also CGCE came only from the dinuclear copper/*tpmc* complex immobilized in a polymer matrix. The peaks were now well-defined (Fig. 5). The origin of forego peak as a consequence of oxidation of obtained nitrate bridged dinuclear copper complex itself, made by complexation of  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  and nitrate from supporting electrolyte, is confirmed due to absence of this peak in voltammogram of CGE coated only with PVC matrix. The first oxidation peak of GA on both electrodes was recorded on 0.50 V, while second less expressive oxidation peak appeared on 0.87, but it was lost for GA concentrations smaller than  $10^{-6}$  M for CGE and  $10^{-5}$  M for CGCE. Although CGCE exhibited higher peak current, it was found that faster loses its response to GA concentrations (limit was about  $10^{-6}$  M), while with CGE it was possible to detect concentrations of  $10^{-7}$  M order of magnitude. Larger surface and porosity of the graphite electrode could be a possible reason for that behavior.



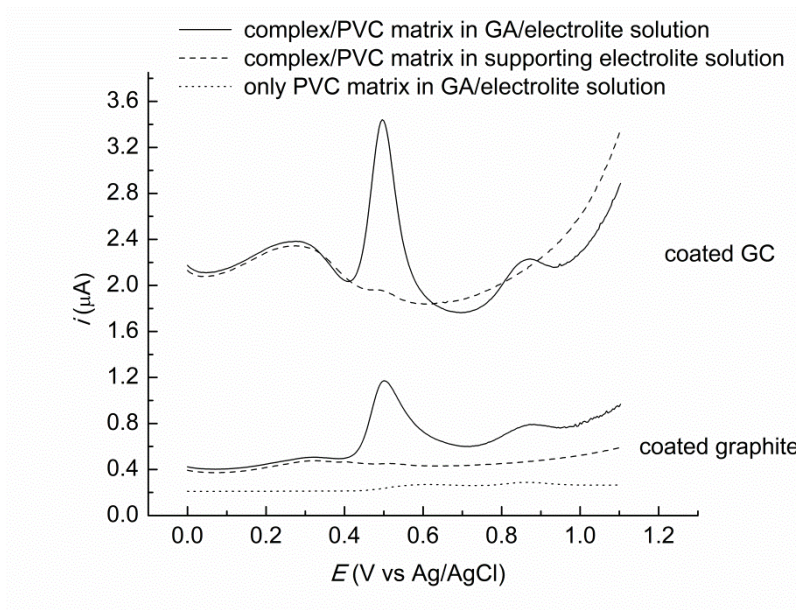


Figure 5. . Comparison of voltammetric responses provided by GC and graphite electrodes modified with/without copper complex:  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex incorporated in polymer matrix, recorded in  $10^{-4}$  M GA and supporting electrolyte (solid line);  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex incorporated in polymer matrix, recorded only in a  $\text{HNO}_3$  as a supporting electrolyte, pH about 2 (dash line); polymer matrix without  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  complex, recorded in  $10^{-4}$  M GA (dotted line); scan rate, 10 mV/s.

### 3.5. Construction of the calibration curve

Differential pulse voltammograms of various concentrations of GA from  $2.5 \times 10^{-7}$  to  $1.0 \times 10^{-4}$  M recorded on more sensitive CGE (compared with CGCE) were shown in Fig.6 (a). The first anodic peak of GA was chosen for quantification of GA due to its reversibility, very good reproducibility and linear dependence on current density of this peak vs. the concentration of gallic acid. The calibration curve was constructed by plotting the current density of first anodic peak vs. the concentration of GA. There were two resulting calibration linear plots (Fig.6, (b)). The higher concentration range is described by the regression equation:  $I_p (10^{-7} \text{ A}) = 5.06 \times 10^4 \times C_{\text{GA}} + 6.8209$ , where  $I_p$  is the current density of first anodic peak and  $C_{\text{GA}}$  is the concentration of gallic acid expressed in mol/L. The lower concentration range is expressed by the relationship:  $I_p (10^{-7} \text{ A}) = 3.895 \times C_{\text{GA}} + 1.14 \times 10^6$  ( $R = 0.9857$ ), and with detection limit (LOD) of GA determination of  $1.48 \times 10^{-7}$  M. Detection limit was estimated from three times the standard

deviation. The limiting point,  $2.63 \times 10^{-6}$  M vs.  $6.9310^{-7}$  A, obtained by extrapolating of the linear plots, determined which of these two mentioned equations should be used. Standard deviations of  $0.025 \mu\text{A}$  in the oxidation peak current and  $0.006$  V in the peak potential for three repeated detections of GA concentration of  $5 \times 10^{-5}$  M as well as the decrease in peak current to 23.5% of the initial current after three months, suggests good reproducibility and stability of results when the modified electrode was used.

Two regression equations were obtained from voltammograms for CGCE:  $I_p (\mu\text{A}) = 4.4 \times 10^3 \times C_{\text{GA}} + 0.93$  for higher concentration range, and  $I_p (\mu\text{A}) = 2.4 \times 10^5 \times C_{\text{GA}} + 0.03$  for lower concentration range, with LOD of  $4.6 \times 10^{-6}$  M of GA.

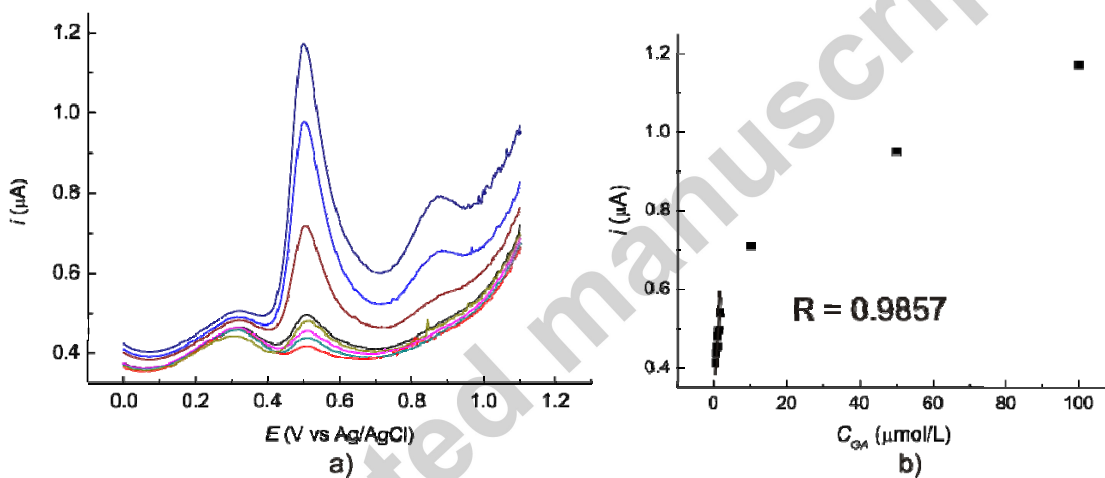


Figure 6. DPV of CGE with GA added in concentration of  $2.5 \times 10^{-7} - 1 \times 10^{-4}$  M, pH = 2 (a); calibration curves for GA (b).

For comparative purposes, relevant characteristics of some of the modified electrodes [16-19] were listed with data of the proposed electrode for GA determination (Table 1). The proposed CGE is comparable in LOD and in linear range with reported ones. In some cases it had better characteristics.

Table 1. Some response characteristics of different modified electrodes for GA determination

Electrode	Technique	Supporting electrolytes and working pH	Linear range	Detection limit (LOD)	Ref
CPE modified with carbon nanotubes	DPV	phosphate buffer solution, pH 2.5	15–0.5 $\mu\text{M}$	0.3 $\mu\text{M}$	[16]
CPE modified with multi-walled carbon nanotubes	DPV	Britton-Robinson buffer solution, pH 2	1.00–6.25 $\mu\text{M}$ ,	0.27 nM	[17]
Carbon – Poly(vinyl chloride) composite electrode	DPV	phosphate buffer solutions, pH 5.0	29 $\mu\text{g/L}$ –0.45 mg/L	0.16 $\mu\text{g/L}$	[18]
polyepinephrine/GCE	AdSV	phosphate buffer solution, pH 1.88	1.0–20.0 $\mu\text{M}$	0.663 $\mu\text{M}$	[19]
Graphite modified with [Cu <sub>2</sub> tpmc](ClO <sub>4</sub> ) <sub>4</sub> immobilized in PVC matrix	DPV	Nitric acid, pH 2	0.25–1 $\mu\text{M}$ 5 $\mu\text{M}$ –0.1 mM	0.148 $\mu\text{M}$	Proposed electrode

### 3.6. Interference study

The potential interfering agents for electrochemical determination of gallic acid are phenolic acids, flavonoids and other organic molecules in minor amounts such as ascorbic acid. The studied compounds (ascorbic acid-AS, naringin-NA, p-coumaric acid-COU, vanillin-VA, caffeic acid-CAF and quercetin-QUE) were chosen due to differences and complexity in structure. The influence of possible interfering compound was examined by comparing peak current produced by GA and peak current of GA and studied compound mixture (1:1, concentrations of  $10^{-5}$  M). Higher interferences were obtained in presence of caffeic acid and quercetin while the other substances (vanillin, naringin, ascorbic and p-coumaric acid) produced

smaller change of the signal (Fig.7). According to the literature [3,10], the first anodic peak could be ascribed to the oxidation of different polyphenolic compounds that had a structure with orthodiphenol (catechol) groups at B-ring, like catechin, gallic, caffeic, quercetin and some others.

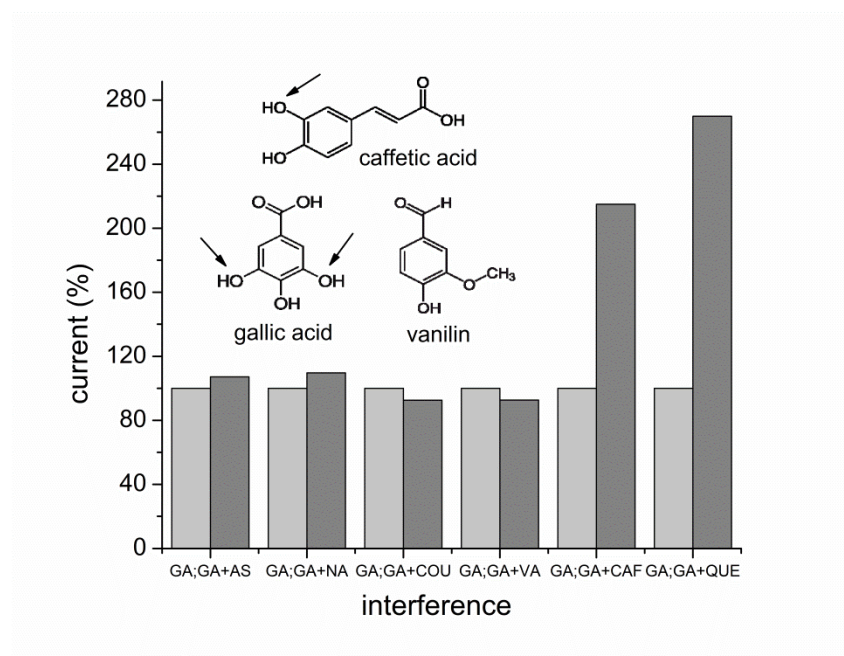


Figure 7. Histogram of the influence of interfering compounds on current obtained for determination of GA under optimized experimental conditions. Some molecule structures are with and without electroactive OH<sup>-</sup> groups.

### 3.7. Determination of the antioxidant capacity of the wine samples

In the present study, the estimation of the antioxidant capacity of the wine samples was based on gallic acid current oxidation using DPV technique. As the first anodic peak could be ascribed to the oxidation of different polyphenolic compounds present in wines that had a structure with orthodiphenol groups at B-ring, thus, these compounds were the major contributors to the antioxidant capacity in wine determined by electrochemical method. Five

different wine samples were analyzed with proposed CGE. The results of antioxidant properties of the wine samples were presented as mg/L of GA calculated from calibration curve and presented in Table 2. The same wine samples were determined at a bare GCE by CV technique. The surface of GC was refreshed before each experiment because surface polymerization, while the same CGE electrode was used for all determinations. The total phenols were determined by Folin–Ciocalteu method and results were not comparable with those obtained by the electrochemical methods. The reason is detection of all phenols by spectrophotometric method (not only polyphenols with orthodiphenol groups at B-ring). Furthermore, F–C reagent may react with many non-phenolic organic substances (sugars, proteins, ascorbic acid, etc.) and inorganic substances (Fe(II), metabisulfite, sodium phosphite, etc.), giving an overestimation of the phenolic content [41].

Table 2. The antioxidant capacity of wine samples determined by electrochemical and spectrophotometric methods

Samples	DPV on CGE (mg/L of GA)	CV on GCE (mg/L of GA)	F–C (mg/L of GA)
White wine	406.58	437.38	748.34
Rosé wine	406.58	445.20	671.75
Red wine 1	420.17	448.37	534.19
Red wine 2	365.76	368.99	578.88
Red wine 3	581.81	611.41	701.42

## Conclusion

The application of complexation agent  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  immobilized in PVC matrix as a modifier for coating graphite or classy carbon rod and the determination of gallic acid and antioxidative capacity of wine samples was presented in this paper. Electrode with synthesized macrocyclic complex showed the same effect on oxidation of polyphenols as a biosensors based

on natural macrocycles in copper-containing enzymes. Electroactive  $[\text{Cu}_2\text{tpmcGA}]^{3+}$  was used as a sensor material for determination of low concentrations of GA at simple coated CGE and CGCE by sensitive DPV technique. The practical usefulness of the CGE was demonstrated by the estimation of the antioxidant capacity, expressed as gallic acid equivalents, in white, rosé and red wine samples, using an extremely simple procedure without any sample pretreatment.

The proposed electrode had advantages such as simple preparation, good reproducibility and electrochemical surface stability. Its detection limit is quite comparable with reported modified and unmodified electrodes for determination of GA. CGE was chosen for real sample analysis due to better voltammetric response and lower LOD, which is  $1.48 \times 10^{-7}$  M. Still, we rightly assumed that application of more conductive polymer matrix coated on transducer surface could provide lower LOD of the electrode. Hence, the next task would be to find suitable and compatible CP for better adopting of complexation agent  $[\text{Cu}_2\text{tpmc}](\text{ClO}_4)_4$  and to provide electrode which might have better analytical characteristics.

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**Highlights:**

- Synthesized copper/macrocylic complex is sensitive to detect gallic acid
- Voltammetric sensor based on copper/macrocylic complex is developed
- The lower detection limit is recorded at modified graphite then glassy carbon electrode
- Sensor was applied for the determination of the antioxidative capacity of wine samples
- Copper macrocylic complex is explored by molecular modeling and DFT calculations

\*Graphical Abstract (for review)

