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# Voltammetric sensor based on electropolymerized poly(Neutral Red) and pillar[3]arene[2]hydroquinone ammonium derivative for dopamine and ascorbic acid determination

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## Abstract

The approaches to the synthesis of newly obtained pillar[3]arene[2]hydroquinone and its derivative with functional ammonium substituents (P[3]A[2]HQae) are suggested. The structures of the macrocycles synthesized were confirmed and characterized by a complex of physical methods. P[3]A[2]HQae has found its application as an electron transfer mediator for the determination of dopamine and ascorbic acid with the voltammetric sensor developed. For a successful sensor assembly the glassy carbon electrode (GCE) was modified with carbon black (CB), P[3]A[2]HQae and electropolymerized form of Neutral red (polyNR). P[3]A[2]HQae demonstrated better mediator properties compared to unsubstituted pillar[5]arene due to the 5–6-fold increase in the redox currents of Neutral red (NR) during its electropolymerization. Under optimal conditions the sensor allowed performing the determination of dopamine and ascorbic acid in the ranges from 10 nM to 1.0 mM and from 1 nM to 1 mM, respectively. The limits of detection were of 10 nM for dopamine and and 1 nM for ascorbic acid. The polyNR peaks positions after its interaction with the analytes provided the opportunity to discriminate the sensor response towards dopamine and ascorbic acid. The possibility of dopamine and ascorbic acid determination in real drug samples was demonstrated.

## Key findings

- Electrochemical sensor based on carbon black, pillar[3]arene[2]hydroquinone ammonium derivative and poly(Neutral red) for dopamine and ascorbic acid determination was developed.
- Pillar[3]arene[2]hydroquinone derivative with functional ammonium substituents have better mediator properties compared to unsubstituted pillar[5]arene.
- The developed sensor provides the opportunity of discrimination of the sensor responses towards dopamine and ascorbic acid.



## Accompanying information

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**Supplementary information**
Supplementary materials: READ
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**Sustainable Development Goals**


## 1. Introduction

Nowadays lots of investigations are devoted to electrochemical sensors development owing to their numerous advantages against the conventional methods of analysis. They allow getting the information about the chemical composition of the object studied more quickly [1]. This feature can enlarge their using in industry, foodstuff quality and safety control [2], eco-monitoring [3], medical diagnostics [4] and other application areas. The advantages of the electrochemical sensors are their relative simplicity and compactness that allow creating the miniaturized devices for *in situ* analysis and «point-of-care» testing mode [5]. They make it possible to carry out the rapid analysis and do not

require any expensive analytical equipment or highly qualified staff which is unavailable for conventional methods of chromatography and mass spectrometry. Besides, the electrochemical sensors have low cost and user-friendly interface [6] that makes them appealing to a wide range of users.

Pillar[5]arenes (P5A) are the class of macrocyclic compounds consisting of hydroquinones or its derivatives bounded with methylene (-$CH_2$-) bridges at 2,5 – positions. P5A were synthesized in 2008 by T. Ogoshi et al. [7] and up to date it is one of the most demanded paracyclophanes group. High demand in this macrocycle class can be explained by such factors as stable symmetrical structure, rigid frame, macrocycle synthesis simplicity and ease of functionalization. All of this makes them the attractive objects for the establishment of novel molecules capable of "host-guest" interactions [8].

The ability of P5A to form complexes and play the role of electron transfer mediator has a great significance for sensor devices development [9]. P5A structure provides an opportunity of easy introduction of functional groups which can be useful for sensors and biosensors development based on functionalized P5A derivatives [10]. There are several sensors based on such compounds for the determination of hydrogen peroxide [11], bisphenol A [12], propanolol [13], caffeic acid [14], organophosphorus and carbamate pesticides [15], troponin I [16], paraquat [17], serotonin [18], and tyramine [19]. The development of novel methods for synthesis of substituted P5A allows creating the compounds with pre-known physical and chemical properties [20]. Therefore, the modern opportunities of functionalized P5A synthesis are interesting for the further searching for approaches with enhanced analytical response of macrocyclic structures.

Previously in our research group the implementation of 1,4-benzoquinone in P5A structure was proposed to enhance the redox activity of P5A [21]. Pillar[n]arene[m]quinones and their derivatives have the same advantages as P5A, such as a frame structure, but the replacement of the electron donor alkyl groups with the electron acceptor oxygen atoms led both to the electron density redistribution to upper and lower rims of the macrocycle and changes in their complexation and electrochemical properties [22].

In this work, it is proposed to use the novel pillar[3]arene[2]hyd-roquinone functionalized with ammonia substituents (P[3]A[2]HQae) as an electron transfer mediator. P5A and hydroquinone have similar electrochemical behavior, as was shown by Ogoshi in 2011 [23]. Also, the possibility of reversible redox conversion of quinone and hydroquinone moieties in partially substituted P5A was reported [24]. This suggests using the pillar[3]arene[2]hydroquinone macrocycles as effective electron transfer mediators in electrochemical sensors.

Phenazine dye Neutral red (NR) was used as the sensitive coating of the developed electrochemical sensor. NR exhibits redox activity, and its electropolymerized form is widely used in electrochemical sensors and biosensors development as the electroactive polymeric matrix [25]. The sensors based on poly(Neutral Red) (polyNR) were used for NADH reduction [26], determination of cholesterol [27], catechol, hydroquinone [28], heavy metals [29], paracetamol [30], hydrogen peroxide [31] and antioxidant activity of dietary supplements assessment [32].

Dopamine is a crucial neurotransmitter, which belongs to the class of catecholamines specifically interacting with the postsynaptic dopamine receptors and plays an important role in human cognitive activity performance. The abnormalities in dopamine metabolism weakens the cognitive processes in the organism. Such violations of methabolism are typical for Parkinson's disease [33], depressive psychological disorders [34] and other diseases caused by problems with dopaminergic system [35]. Catecholamines level determination is necessary for differential diagnostics of hypertensive states [36].

Ascorbic acid is a powerful antioxidant that prevents the diseases caused by free radicals. [37]. Ascorbic acid is an ideal free radical scavenger because it interacts with reactive oxygen species quickly to form rather inactive radicals [38]. For this reason, ascorbic acid is able to protect phenolic and aromatic compounds from oxidation and widely used as antioxidant in foodstuff and beverages [39].

Based on the foregoing, dopamine and ascorbic acid determination is necessary in the areas of medical diagnostics and food industry. However, to date their determination is provided by conventional methods such as high-performance liquid chromatography [40, 41], mass spectrometry [42, 43], colorimetry [44, 45], electrophoresis [46, 47], and other spectroscopic methods [48, 49], so the development of simple compact portable sensors is an urgent task.

In this work, an electrochemical sensor based on carbon black (CB), pillar[3]arene[2]hydroquinone functionalized with ammonia substituents, and polyNR was developed for sensitive determination of dopamine and ascorbic acid. It should be noted the redox potentials of dopamine and ascorbic acid on bare electrodes are approximately equal [50], hindering the discrimination of analytes responses. The application of voltammetric sensor presented made it possible to use the redox peak potential separation for dopamine and ascorbic acid voltammetric detection in the range from 10 nM to 1 mM and 1 nM to 1 mM, respectively. The sensor was successfully tested for analytes determination in the samples of pharmaceutical preparations of ascorbic acid and dopamine.

## 2. Materials and methods

### 2.1. Reagents

Dopamine hydrochloride, Neutral red (N2,N2,7-Trimethylphenazine-2,8-diamine—hydrogen chloride), N-hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) were purchased from Sigma-Aldrich

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(Saint Louis, MO, United States). Carbon black was purchased from IMERYS (Willebroek, Belgium). Ascorbic acid was bought from Tatchimproduct (Kazan, Russia).

All other reagents were of analytical grade and used without any further purification. Deionized water Millipore-Q (Simplicity®, Merck-Millipore, Mosheim, France) was utilized for the working solutions preparation.

Mixture of 1 mg of CB and 1 mL of dimethylformamide (DMF) was ultrasonicated for 2 hours to obtain uniform suspension.

The structures of P5A derivatives studied (compounds 1–4) were synthesized at the Organic and Medical Chemistry Department of Kazan Federal University (Figure 1). Hexa-[ammoniyethylthioethoxy]-pillar[3]arene[2]hydroquinone hexatrifluoroacetate (P[3]A[2]HQae, macrocycle 4) was used as an electron transfer mediator in the electrochemical sensor content. The structure of macrocycles 2 and 4 was characterized with physical and chemical approaches: IR, $^{1}H$, $^{13}C$ NMR spectroscopy, MALDI and ESI MS (Figures S1–S8). The description of macrocycles investigations and the equipment used is described in the Supplementary Materials file.

### 2.2. Apparatus

Voltammetric measurements were performed with potentiostat-galvanostat CHI660E (CH Instruments, Inc., USA). The 5 mL electrochemical cell was equipped with a glassy carbon electrode (GCE) as a working electrode made of a SU2000 rod with a diameter of 2 mm from NII Graphite (Moscow, Russia) in a teflon pattern with a steel contact, platinum wire as a counter electrode (CHI 129, CH Instruments, Inc., USA), and an Ag/AgCl silver chloride electrode as a reference electrode (CHI 128, CH Instruments, Inc., USA). Data processing was performed using the CHI660E software.

### 2.3. Preliminary electrode modification with CB and P[3]A[2]HQae

The GCE was polished, rinsed with ethanol and further purified by cycling its potential in the range of -1.0 to +1.0 V in 0.2 M sulfuric acid until the currents stabilization. Then, 1 μL of CB suspension in DMF was twice drop-casted onto the GCE surface and each layer was dried in the oven at 80°C. After that, a mixture of 5 μL of 100 mM EDC and 5 μL of 400 mM NHS were used for further modification of the GCE/CB surface. The electrodes were incubated for an hour, then rinsed with distilled water and 5 μL of an aqueous solution of 0.1 mM P[3]A[2]HQae were drop casted on the surface and dried in the air at the room temperature.

### 2.4. NR electropolymerization

NR electropolymerization was carried out on GCE/CB/P[3]A[2]HQae by cycling potential in the range of -0.8 to 0.8 V from 0.1 M HEPES (pH 6.0) containing 0.1 M $NaNO_3$ and 0.4 mM NR. After the electropolymerization the electrode was transferred to a working buffer solution, and 10 cycles of potential scanning were recorded in absence of the monomer to remove unbound dye molecules from the polymeric film.

### 2.5. Dopamine and ascorbic acid determination

The sensor was incubated in 20 μL of aqueous dopamine solution with appropriate concentrations or in the samples of commercially available drugs for 20 minutes. The polyNR oxidation peak current played a role of analytical response recorded in 0.1 M HEPES containing 0.1 M $NaNO_3$, pH 6.0.

The samples of commercially available dopamine and ascorbic acid medical preparations were purchased from a local pharmaceutical network.

## 3. Results and discussion

### 3.1. P[3]A[2]HQae synthesis

For further electrochemical sensor development, a pillar[3]arene[2]hydroquinone with cationic groups at the ends of the substituents was required. Pillar[3]arene[2]quinone was used as a starting compound to obtain the desired P[3]A[2]HQae. In order to avoid any further side reactions, it was decided to convert the starting compound into the form of pillar[3]arene[2]hydroquinone (Scheme S1).

Sodium dithionite widely using in the chemical industry was chosen as a reducing agent. This salt is highly soluble in water and almost insoluble in an organic solvent, whereas pillar[5-m]arene[m]quinones have the opposite properties. Therefore, in order to achieve the maximum yields, it was decided to carry out the reaction in tetrahydrofuran/water mixture with the ratio 5:1 respectively, so that the reaction system was homogeneous.

The reaction was monitored by thin-layer chromatography, and the reaction product formation was confirmed by $^{1}H$ and $^{13}C$ NMR, IR spectroscopy and mass spectrometry. The signals shift to the region of strong fields is observed on $^{1}H$ NMR spectrum (Figure S1) which was obtained in the region of protons of aryl units resonation. This shift proves the completeness of the reduction of quinone units to hydroquinone ones.

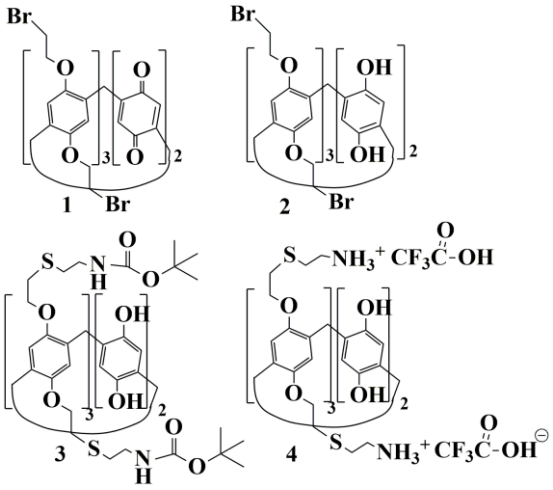


**Figure 1** The structures of P5A derivatives presented in this work.

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There were no carbon atom signals from the carbonyl groups of the quinone moiety in the $^{13}C$ NMR spectrum (Figure S2) in the region of 186-188 ppm. Also, there were some changes in the IR spectrum (Figure S4). The band at 1649 $cm^{-1}$ corresponding to the quinone moiety was no longer observed, however the band at 3100-3200 $cm^{-1}$ has appeared confirming the OH group formation.

Thus, in this work quinone moieties of P[5]A were reduced to obtain pillar[3]arene[2]hydroquinone with a yield of 85%.

To obtain the macrocycle containing primary amino groups, the step-by-step synthesis of the aminoethanethiol fragments introduction was chosen (Scheme S2). So, at the first stage, macrocycle 2 (pillar[3]arene[2]hydroquinone) reacted with *tert*-butyl(2-mercaptoethyl)carbamate in dehydrated DMF in the presence of potassium carbonate for 24 hours at the room temperature. Macrocycle 2 was further *in situ* involved into the BOC-protection remove reaction. As a result, macrocycle 3 was obtained with 91% yield.

Removal of *tert*-butoxycarbonyl protection was accomplished by the reaction between macrocycle 3 and trifluoroacetic acid. The reaction was carried out for 2 hours in dichloromethane. As a result, target macrocycle 4 (P[3]A[2]HQae) was obtained in a yield of 70.9%.

The structure of P[3]A[2]HQae was confirmed and characterized by a complex of physical methods, i.e., $^{1}H$ and $^{13}C$ NMR, IR spectroscopy, MALDI and ESI mass spectrometry, elemental analysis data. Figure S5 shows the $^{1}H$ NMR spectrum of P[3]A[2]HQae. The multiplet in the area of strong fields 2.45–2.63 ppm is attributed to the proton signal of methylene fragments in the thioether group. The multiplet in the 3.14 ppm area can be attributed to the methylene moiety at the ether group. The multiplet in the range of 3.51–3.58 ppm refers to methylene bridges of a macrocyclic platform. This can be explained by a violation of the system symmetry as well as the difference between methylene bridges providing the interaction between both of substituted fragments or interaction between the substituted ones with unsubstituted fragments. The multiplet in the range of 3.71–3.83 ppm can be referred to the methylene moiety of the primary amino group. The singlets set in the range of 6.43–6.69 ppm was attributed to ten protons of aryl fragments.

It was suggested that the protons of hydroquinone fragments are closer to strong fields and those of the substituted fragments – to the weak ones. Chemical shifts and multiplicity of signals in $^{1}H$ NMR spectrum of P[3]A[2]HQae correspond to the proposed structure. Also, a molecular peak of a single-charged ion was found in the ESI mass spectrum of P[3]A[2]HQae (Figure S7) that coincides with the molecular weight of the reaction product suggested.

## 3.2. Electrochemical sensor development

The formation of modifying layer on the base of CB, P[3]A[2]HQae and polyNR as well as its electrochemical behavior were further investigated. Previously, it was shown that the integration of P5A as an electron transfer mediator into the polyNR layer results in the improvement of the redox reaction conditions and the response sensitivity [51]. The enhancement of this effect was assumed for the synthesized macrocycle P[3]A[2]HQae bearing cationic substituents.

### 3.2.1. Immobilization of P[3]A[2]HQae

Since the immobilization of P[5]A onto bare GCE led to the passivation of electrode surface caused by chemisorption of intermediate oxidation products [52], the usage of CB is required. The same behavior was previously registered for pillar[3]arene[2]quinone [21, 53].

Firstly, GCE modification with CB was performed by drop casting method to obtain GCE/CB. Carbodiimide cross-linking reaction was than carried out to immobilize the macrocycle resulting in GCE/CB/P[3]A[2]HQae formation. The 0.1 mM stock solution of P[3]A[2]HQae was used in electrode preparation procedure. The typical cyclic voltammogram obtained with GCE/CB/P[3]A[2]HQae is presented in Figure 2.

A pair of peaks in the voltammogram was attributed to the quasi-reversible redox conversion of macrocycle synthesized.

Ten consecutive cyclic voltammograms were recorded with intermediary stirring to assess the reproducibility of the signals obtained (Figure 3). High stability and low level of deviation (±S.D.) have been observed for the modifying layers obtained. Better stability of voltammetric parameters of P[3]A[2]HQae compared to P5A can be caused by the absence of intramolecular hydrogen bonds [21].

To determine the optimal macrocycle quantity within the layer, the concentration of P[3]A[2]HQae solution was varied at the electrode modification step. Different concentrations of P[3]A[2]HQae equal to 0.05, 0.1, 0.5 and 1 mM were considered (Figure 4).

Almost 50% growth of peak currents was revealed after increasing the P[3]A[2]HQae concentration from 0.05 mM to 0.1 mM. Further increase resulted in the slow decrease in the signals accompanied by the changes of the curve shapes.

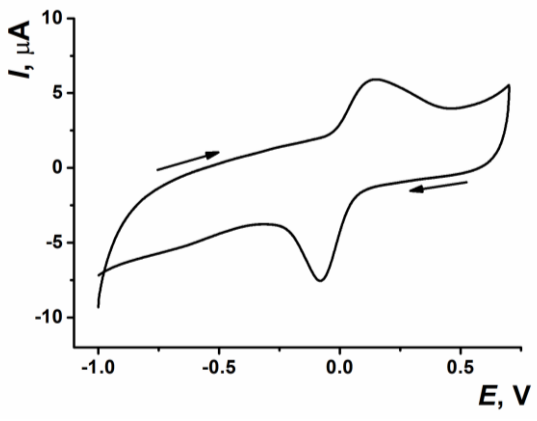


**Figure 2** Cyclic voltammogram recorded on GCE/CB/P[3]A[2]HQae, 0.1 M HEPES + 0.1 M $NaNO_3$, pH 6.0, 0.1 V/s.

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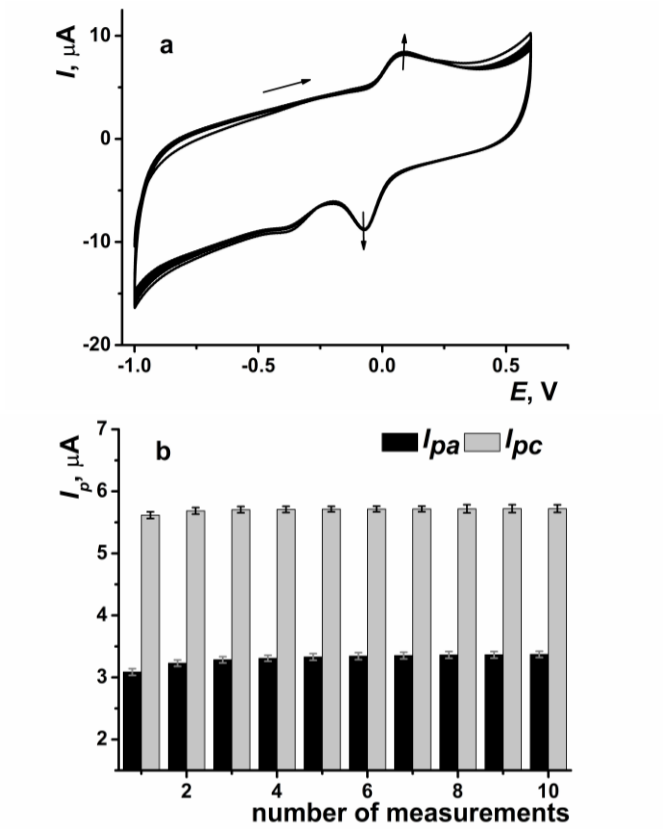


**Figure 3** Ten consecutive cyclic voltammograms recorded on GCE/CB/P[3]A[2]HQae, 0.1 M HEPES + 0.1 M $NaNO_3$, pH 6.0, 0.1 V/s (a); Anodic and cathodic peak currents stability assessment (b). Average ± S.D. for six individual sensors.

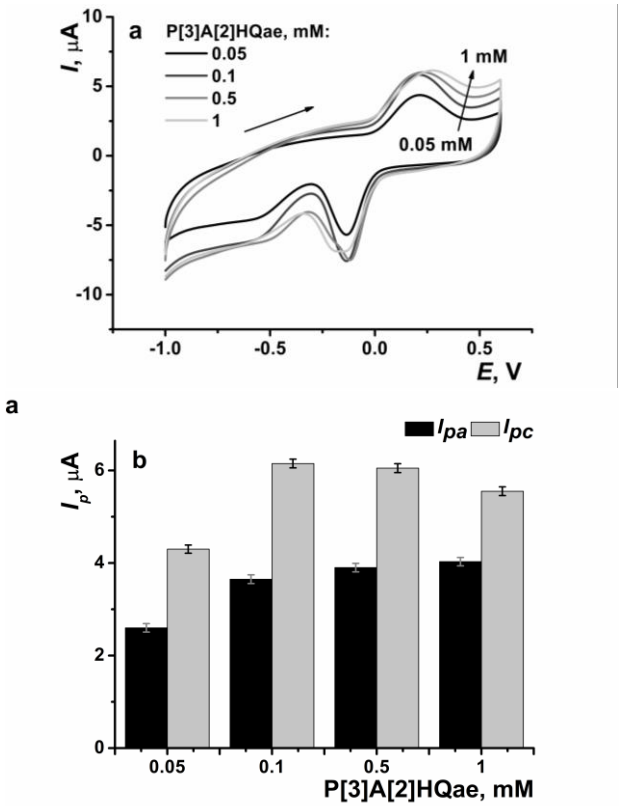


**Figure 4** Cyclic voltammograms recorded on GCE/CB/P[3]A[2]HQae with different macrocycle concentrations, 0.1 M HEPES+ 0.1 M $NaNO_3$, pH 6.0, 0.1 V/s (a); Anodic and cathodic peak currents dependence on GCE/CB/P[3]A[2]HQae concentration (b). Average ± S.D. for six individual sensors.

The peaks recorded became wider indicating the saturation of surface layer followed by the macrocycle aggregation, as it was previously reported for P[5]A [54].

### 3.2.2. PolyNR electropolymerization

In order to accumulate the electropolymerization products of NR on the GCE/CB/P[3]A[2]HQae surface, multiple potential scanning was performed in 0.4 mM monomer solution. In the previous studies [51, 53], it was shown that pH=6.0 is optimal for polymer layer formation on the electrode and polyNR signal measurements. The macrocycle concentration affected the electropolymerization curves morphology (Figure 5). Cyclic voltammograms obtained are typical for redox active films and contain the anodic peak of cation-radical formation (at about 0.7 V). In case of NR the polymeric product peaks potentials coincide with those for monomer (at about –0.5 V).

PolyNR peak current values recorded during the electropolymerization in the presence of different concentrations of P[3]A[2]HQae depended on the macrocycle concentration and allowed determining its optimal loading in the layer. The initial increase of P[3]A[2]HQae concentration led to significant growth of polyNR peak currents. However, with macrocycle concentration a dramatic signals decrease was obtained. This was due to macrocycle molecules aggregation mentioned above, which was a possible reason of the partial passivation of electroactive surface area. Thus, P[3]A[2]HQae concentration of 0.1 mM was chosen for further experiments.

As it was reported previously, the NR electropolymerization on the GCE modified with CB and unsubstituted P[5]A resulted in 5 times lower peak currents [51]. Thus, the functionalization of P[5]A core with terminal ammonium groups had positive influence on the quantity of redox active material accumulated.

## 3.3. Determination of ascorbic acid and dopamine

Low-molecular-weight compounds can be involved in electrostatic or coordination interactions with active sites within the polymer film. When polyNR interacts with such compounds on the modifying layer–solution interface it resulted in the changes of charge distribution and in the increase or decrease of the polymer redox peak currents [55].

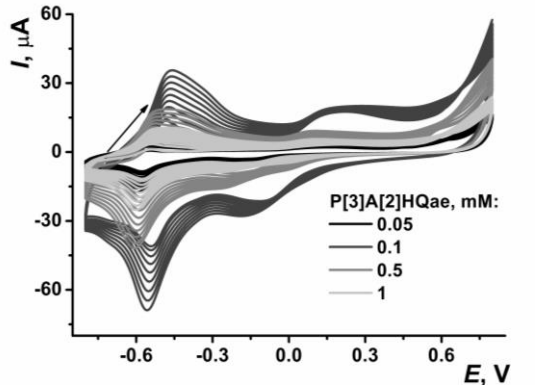


**Figure 5** The NR electropolymerization voltammograms dependence on macrocycle concentration recorded on GCE/CB/P[3]A[2]HQae, 0.1 M HEPES + 0.1 M $NaNO_3$, pH 6.0, 0.05 V/s.

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This enabled using the sensor based on CB/P[3]A[2]HQae/polyNR coating for ascorbic acid and dopamine determination, as they can interact with NR and its polymeric form [56].

Figure 6a shows the cyclic voltammograms obtained with the GCE/ CB/P[3]A[2]HQae/polyNR after its incubation in the solutions with different concentrations of ascorbic acid. Increasing of the ascorbic acid concentration led to the decrease in the polyNR peak currents. Based on the data, the calibration curve was obtained, showing the relationship between the change in oxidation peak currents and the logarithm of ascorbic acid concentration (Figure 6b).

The polyNR peak oxidation currents dependence on the logarithm of ascorbic acid concentration was linear in the range from 1.0 nM to 1.0 mM with a LOD of 1 nM, calculated for S/N = 3 criteria. The linear regression equation for the ascorbic acid calibration curve is:

$$I_{pa},\mu A = (7.5\pm0.1) - (2.40\pm0.02)\cdot(\log c,M), \quad n = 6,\ R^2 = 0.9995. \tag{1}$$

Similarly, dopamine was determined on the GCE/CB/P[3]A[2]HQae/polyNR in the concentration range from 10 nM to 1 mM. The LOD was equal to 10 nM, calculated for $S/N$ = 3 criteria. The cyclic voltammograms obtained after the sensor interaction with dopamine (Figure 7a), and the appropriate calibration curve (Figure 7b) is presented below. The linear regression equation for the calibration curve is:

$$I_{pa},\mu A = (8.76\pm0.07) - (1.89\pm0.01)\cdot(\log c,M), \quad n = 6,\ R^2 = 0.9998. \tag{2}$$

The possibility of simultaneous analytes detection with the voltammetric sensor proposed was evaluated. The cyclic voltammograms of polyNR redox conversion (Figure 8) were recorded after modified electrode incubation in 0.1 mM dopamine and 0.1 mM ascorbic acid separately or in their mixture. In presence of dopamine the polyNR redox peak potentials were shifted towards cathodic potential values, while in presence of ascorbic acid they shifted towards the anodic ones. This shift allows discriminating between the analytes without using other methods.

The shift in redox peak potentials occurred at dopamine concentrations above 1 μM and ascorbic acid concentrations above 0.1 μM (Figure S9). At lower concentrations this effect was not observed, probably, due to minimal changes in electron exchange conditions.

## 3.4. Real samples analysis

The electrochemical sensor based on GCE/CB/P[3]A[2]HQae/polyNR was tested for dopamine and ascorbic acid determination in three real pharmaceutical samples. These samples contained such additives and stabilizers as sodium disulfite and hydrochloric acid in the dopamine injection solution, flavoring agents and sucrose in the powdered ascorbic acid, and glucose, potato starch, and calcium stearate in the tablet form of ascorbic acid.

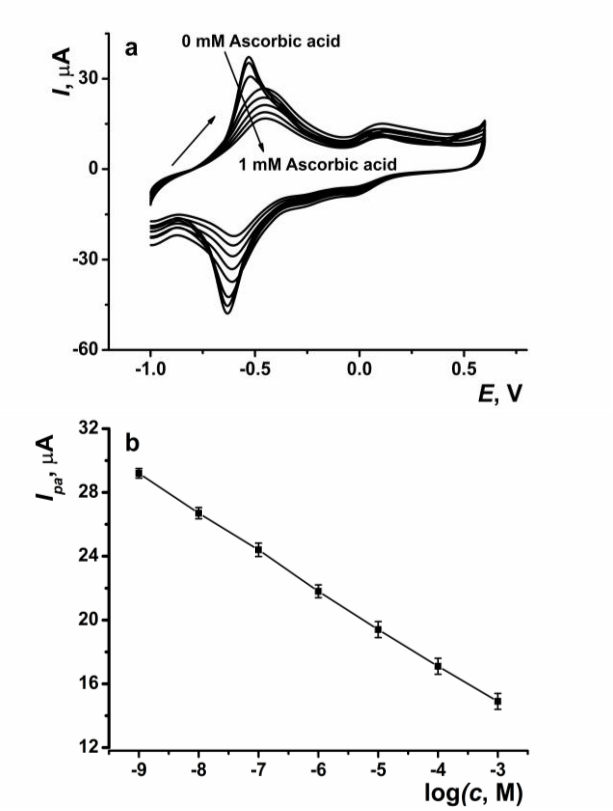


**Figure 6** Cyclic voltammograms recorded on GCE/CB/P[3]A[2]HQae/polyNR in presence of ascorbic acid in the range from 1.0 nM to 1.0 mM (a); Calibration curve for ascorbic acid determination with GCE/CB/P[3]A[2]HQae/polyNR, 0.1 M HEPES+ 0.1 M $NaNO_3$, pH 6.0, 0.1 V/s (b). Average ± S.D. for six individual sensors.

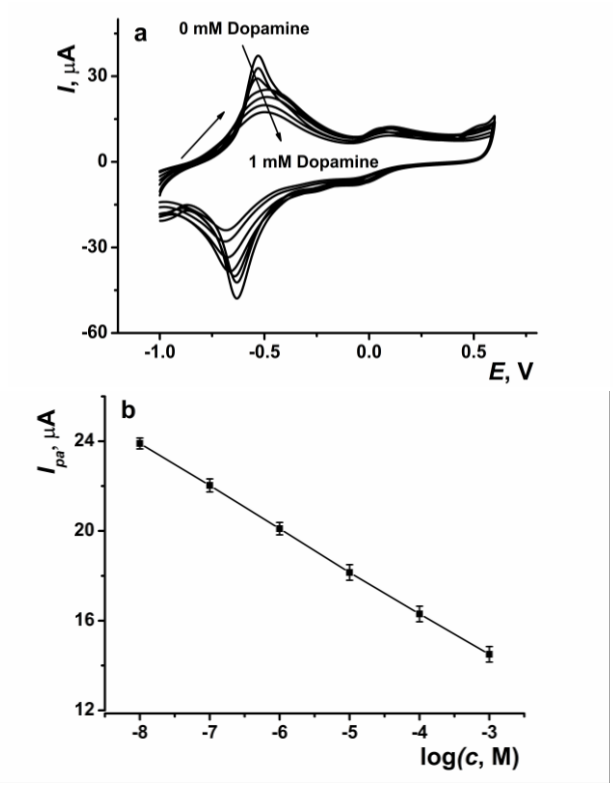


**Figure 7** a) Cyclic voltammograms recorded on GCE/CB/P[3]A[2]HQae/polyNR in presence of dopamine in the range from 10 nM to 1.0 mM, b) Calibration curve for dopamine determination with GCE/CB/P[3]A[2]HQae/polyNR, 0.1 M HEPES+ 0.1 M $NaNO_3$, pH 6.0, 0.1 V/s. Average ± S.D. for six individual sensors.

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The samples were used to prepare the stock solutions with the analyte concentration of 10 μM. The results of the voltammetric determination of dopamine and ascorbic acid in the pharmaceutical samples are presented in Table 1.

The analytical characteristics of the sensor developed for the ascorbic acid and dopamine determination were comparable to or better than those for the previously reported electrochemical sensors (Table 2–3). It should be noted that the levels of target analytes in biological liquids (30–35 μg/mL of dopamine in urine and 10–20 μg/mL of ascorbic acid in blood and urine) can provide the possibility of dopamine and ascorbic acid determination. The medicines based on dopamine and ascorbic acid contain much higher substance concentration compared with the biological samples.

**Table 1** Voltammetric determination of dopamine and ascorbic acid in pharmaceutical samples. Average ± S.D. for six individual sensors is presented.

| Sample | Labeled amount, mg | Allowable range, mg | Found |
|---|---|---|---|
| Dopamine (Injection Solution) | 40 | 36÷44 | 43±1 |
| Ascorbic Acid (Powdered Form) | 900 | 810÷990 | 921±25 |
| Ascorbic Acid (Tablet Form) | 100 | 90÷110 | 93±3 |

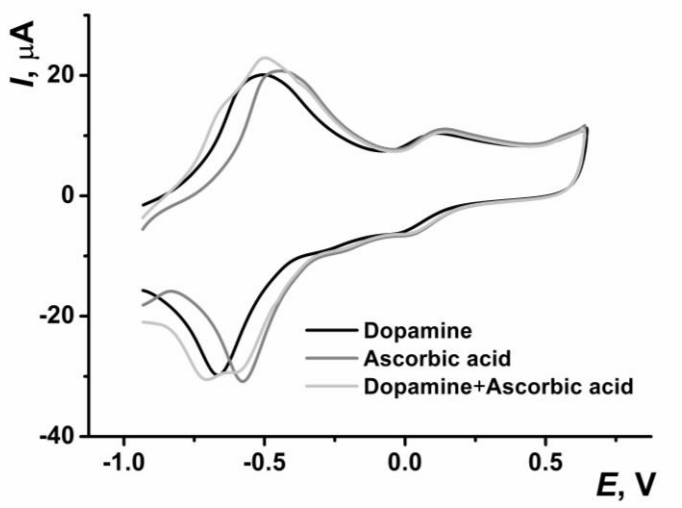


**Figure 8** Cyclic voltammograms obtained with GCE/CB/P[3]A[2]HQae/polyNR in presence of 0.1 mM dopamine, 0.1 mM ascorbic acid and their mixture, 0.1 M HEPES+ 0.1 M $NaNO_3$, pH 6.0, 0.1 V/s.

Low LOD levels obtained with the sensor developed allow diluting the complex matrices and minimizing the effects from interfering compounds and medicine stabilizers.

## 4. Limitations

There are no specific limitations in our study.

**Table 2** Comparison of the electrochemical characteristics of the sensors developed with other electrochemical sensors for ascorbic acid determination described in literature.

| Sensor composition | Determination method | Concentration range, M | Detection limit, M | Reference |
|---|---|---|---|---|
| Acetylene black paste electrode/Polyvinylpyrrolidone/Graphene Composite | LSV [a] | $6.0\cdot10^{-6}$–$1.0\cdot10^{-3}$ | $1.0\cdot10^{-6}$ | [57] |
| Platinum electrode/polypyrrole doped with potassium hexacyanoferrate | CV[b] | $1.0\cdot10^{-6}$–$1.0\cdot10^{-4}$ | $2.5\cdot10^{-7}$ | [58] |
| GCE/Ag nanoparticles/copper monoaminophthalocyanine acrylate | DPV [c] | $2.5\cdot10^{-8}$–$1.0\cdot10^{-4}$ | $5.0\cdot10^{-9}$ | [59] |
| Graphite electrode/cellulose nano-fibers/Ag nanoparticles | DPV | $2.0\cdot10^{-5}$–$4.5\cdot10^{-4}$ | $2.9\cdot10^{-8}$ | [60] |
| GCE/CB/P[3]A[2]HQae/polyNR | CV | **$1.0\cdot10^{-9}$–$1.0\cdot10^{-3}$** | **$1\cdot10^{-9}$** | This work |

[a] LSV – linear sweep voltammetry;
[a] CV – cyclic voltammetry;
[a] LSV – differential pulse voltammetry.

**Table 3** Comparison of the electrochemical characteristics of the sensors developed with other electrochemical sensors for dopamine determination described in literature.

| Sensor composition | Determination method | Concentration range, M | Detection limit, M | Reference |
|---|---|---|---|---|
| Gold electrode/graphene oxide/PEDOT:PSS [a] | DPV | $1.0\cdot10^{-8}$–$7.0\cdot10^{-7}$ | $8.0\cdot10^{-9}$ | [61] |
| Printed graphite electrode/block copolymer poly(DMAEMA [b] -b-styrene)/reduced graphene oxide | DPV | $1.0\cdot10^{-7}$–$3.0\cdot10^{-4}$ | $1.34\cdot10^{-7}$ | [62] |
| Electrochemically activated GCE with oxygen-containing surface functional groups | DPV | $2.0\cdot10^{-8}$–$1.0\cdot10^{-4}$ | $1.0\cdot10^{-8}$ | [63] |
| GCE/α-$K_7P_2VW_{17}O_{62}\cdot18H_2O$/carbon nanotubes/Au-Co nanoparticles | DPV | $1.25\cdot10^{-6}$–$2.81\cdot10^{-4}$ | $1.5\cdot10^{-7}$ | [64] |
| $Ti_{NCs}/G_m$-$Cu_{foil}$ [c] electrode | DPV | $1.0\cdot10^{-5}$–$4.0\cdot10^{-4}$ | $6.63\cdot10^{-6}$ | [65] |
| GCE/CB/P[3]A[2]HQae/polyNR | CV | **$1.0\cdot10^{-8}$–$1\cdot10^{-3}$** | **$1.0\cdot10^{-8}$** | This work |

[a] PEDOT:PSS – poly(3,4-ethylenedioxythiophene) polystyrene sulfonate;
[b] DMAEMA – 2-(Dimethylamino)ethyl methacrylate;
[c] $Ti_{NCs}/G_m$-$Cu_{foil}$ – Ti-nanocolumnar arrays/graphene monolayer-Cu foil.

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## 5. Conclusion

Thus, a method for the synthesis of a new cationic P5A derivative – P[3]A[2]HQae containing six ammonium groups was developed. The structure of the compound obtained was proved by a complex of physical methods. The resulting compound demonstrated quasi-reversible electrochemical behavior and had better mediator properties compared to unsubstituted P5A. These characteristics can be explained by the presence of terminal ammonium groups in the macrocycle substituents. Such properties of a new pillar[3]arene[2]quinone derivative provided its using instead of P5A to increase the efficiency of Neutral red phenazine dye electropolymerization on the GCE modified with CB and macrocycle. The best characteristics were observed with 0.1 mM P[3]A[2]HQae concentration both in case of the intrinsic electrochemical signal of the macrocycle registration and of the following Neutral red electropolymerization.

A novel voltammetric sensor based on GCE modified with CB, P[3]A[2]HQae and polyNR was developed for dopamine and ascorbic acid determination in aqueous solutions and in the real drug samples. This sensor demonstrated the sensitivity of the peak currents obtained and the promising opportunity of discrimination of the sensor responses towards dopamine and ascorbic acid. Such discrimination became possible on the suggested modifying coating whereas there were no separate peaks of the analytes obtained on bare GCE. The sensor based on the coating developed can be applied in the design of commercial sensor devices and serve as a platform for medical preparations, food additives and clinically significant analytes determination.

### Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page.

### Data availability statement

The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study.

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### Author contributions

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### Conflict of interest

The authors declare no conflict of interest.

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