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A Simple Over-Oxidized Molecularly Imprinted Polypyrrole for the Sensitive Detection of Dopamine in Human Serum

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Abstract

A simple electrochemical sensor for dopamine detection, is based on molecularly imprinted and electropolymerized over-oxidized polypyrrole (OPPy). The MIP-based electrode is obtained by electrocopolymerization of pyrrole (0.1 M) in the presence of the template molecular (dopamine, DA) (10^{-3} M). The square wave voltammetry (SWV) is used for the detection of dopamine in buffer solution. The current peak obtained at the MIP electrode was proportional to the logarithm of the DA concentration in the range of 10^{-11} to 5×10^{-8} M with a detection limit of 10^{-11} M. The proposed sensor was used for the detection of DA in spiked blood serum, satisfactory results were obtained.

Keywords

Dopamine, Molecularly Imprinted Polymers, Over-Oxidized Polypyrrole, Square Wave Voltammetry

1. Introduction

Dopamine (DA) whose formula is presented in **Figure 1**, is a neurotransmitter of the catecholamine family [1] that plays role of importance in the central nervous system of mammals [2] [3]. Low concentrations of dopamine in the central nervous system cause several neurological diseases [4], such as schizophrenia and Parkinson's disease [5] [6]. Therefore, detecting and determining the DA concentration in the biological medium with a sensitive method is quite important for diagnosis [7] [8]. Various methods were applied for the DA detection, such as spectrophotometry [9], liquid chromatography coupled with electrochemical detection (LC-ECD) [10], colorimetric [11], fluorescence [12] and

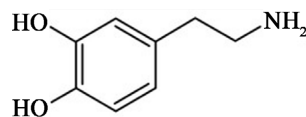


Figure 1. Formula of dopamine.

electrochemical sensors [13].

Despite the reliability of these methods, they require bulky and expensive instrumentation and the analysis time is long [14]. Scientific research is therefore directed towards the development of electrochemical biosensors that are able to produce a rapid, sensitive and low-cost response [15] [16]. Dopamine has a strong electrochemical activity, which makes its detection easy by electrochemical techniques. Generally, in body fluids [17] [18], the electrochemical detection of dopamine could be hindered by the presence of high levels of ascorbic acid (AA) and uric acid (UA) [15] [19]. For this reason, electrochemical sensors for measuring DA in biological systems must possess sensitivity and a high degree of selectivity in order to obtain a clear separation of the electrochemical signals of these three compounds [3] [20]. A molecularly imprinted polymer (MIP) is formed in the presence of a target molecule and after extraction, a complementary cavity is formed, with a chemical affinity for the target molecule which favours its adsorption [21] [22]. The high affinity is indeed based on the shape of the imprint as well as of the presence of functional groups with specific interactions with the target molecule. The modification of electrodes by molecular imprinting polymers (MIP) for the recognition of biomolecules is a promising method and it offers advantages such as thermal stability, physical robustness, average cost and easy preparation [23]. Many works are devoted to molecular imprinted composites for DA detection. Among them, an electrochemical sensor based on MIP was manufactured by electropolymerization of pyrrole in the presence of DA, on a carbon aerogel surface (CA); the obtained detection limit is 0.0004 μM [24]. Recently, molecularly imprinted polymer membranes of polypyrrole, including graphene oxide, were immobilized on the surface of micropipette tip carbon paste electrode (GO-MIP-PTE) for dopamine detection, a detection limit of the order of 10^{-8} M was obtained [8].

Polypyrrole (PPy) is a good candidate for the electrochemical detection, thanks to its ease of manufacturing, its high conductivity. Due to its good biocompatibility and to the presence of an amine group on the pyrrole cycle [25], it is a good candidate for DA imprinting. The overoxidation of polypyrrole (PPy) makes it possible to create electronegative groups (COOH, C=O) on the dorsal skeleton of PPy, which can attract the electropositive groups of DA and can repel anionic molecules such as AA and AU [1] [2] and [21]. Therefore, the electro-polymerization of over-oxidized pyrrole combined with MIP has been proposed to obtain a sensitive electrochemical sensor [21], with a detection limit of 4.5 nM.

In this study, a simple, low cost molecularly imprinted overoxidized polypyrrole for dopamine detection was obtained by electro-polymerizing pyrrole on a

glassy carbon electrode (GCE) surface. The prepared sensor was characterized by scanning electron microscopy (SEM), cyclic voltammetry (CV). After optimization of the working conditions, the sensor exhibited a high sensitivity for DA detection.

2. Experimental

2.1. Chemicals and Reagents

Potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6 \cdot 3H_2O]$), potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$), dopamine, alumina powder (Al_2O_3) and potassium chloride (KCl) were purchased from Sigma-Aldrich. Phosphate buffer solution (0.1 M, formed by mixing NaH_2PO_4 with Na_2HPO_4 solutions) was used as supporting electrolyte solution. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) solutions were used to adjust the proper pH value.

Pyrrrole monomer (Sigma Aldrich) was purified by double distillation, stored at low temperature and protected from light. The human serum was collected and stored at 4°C by Laboratoire d'analyses Medicales Dr Nabli Naoufel, Sousse, Tunisia. Double distilled water was used throughout the experiments and all voltammetry measurements are carried out under pure nitrogen bubbling at 25°C.

2.2. Apparatus and Methods

High magnification microstructural images were performed using high resolution ESEM Thermo-Fisher FEI Q250 with resolution better than 7 nm at low operating voltages of 5 - 10 kV. As in our case the overoxidized polypyrrole films are not sufficiently conductive, the charging problem and improvement of the image quality was obtained by coating the surface of the samples with a very thin layer of gold by vapor deposition.

All electrochemical measurements were carried out using a mini potentiostat model DY2000 (DIGI-IVY company, Germany) connected by an USB cable with a laptop. All electrochemical measurements were carried out in a conventional three electrode system, comprising a platinum wire as a counter electrode, an Ag/AgCl reference electrode with a saturated KCl solution and the working electrode being a glassy carbon disc of 3 mm in diameter modified by the molecularly imprinted overoxidized polypyrrole.

2.3. Preparation of NIP-OPPy and MIP-OPPy and Electrochemical Measurements

Before the electropolymerization, the surface of the glassy carbon electrode was polished by a gentle polishing with an aluminum powder of 0.05 μm in diameter in order to obtain a smooth and shiny surface. Then the electrode surface was immersed in ethanol for 5 min, sonicated in distilled water and then dried under nitrogen flow.

The modification of the glassy carbon electrode by the template-free polypyr-

role (NIP-OPPy) was prepared by cyclic voltammetry in the potential range (-0.6 to 1.2 V), during ten cycles of a 10^{-1} M pyrrole aqueous solution containing 10^{-1} M of potassium chloride, as supporting electrolyte. The same protocol was used for the electrodeposition of molecularly imprinted polymer (MIP-OPPy), but in the presence of 10^{-3} M of dopamine. After electropolymerization, the extraction of dopamine was carried out by incubating the electrodes in an NaOH solution (0.5 M) with gentle agitation and low scanning speed in a potential range of (-1 to 1 V) until the disappearance of the dopamine oxidation peak.

After dopamine extraction, the modified overoxidized polypyrrole electrode was incubated in the dopamine solution (0.1 M PBS solution, pH 6) for 20 min. then it was washed with distilled water and then immersed in a 0.1 M PBS solution (pH = 6) before electroanalysis. The same procedure was adopted for NIP-OPPy and MIP-OPPy. Square wave voltammetry (SWV) was used with the sweep frequency of 10 Hz, the amplitude of 40 mV and a step potential of 4 mV.

3. Results and Discussion

3.1. Electrochemical Polymerisation of Pyrrole

Figure 2(a) and **Figure 2(b)** show the cyclic voltammograms recorded during the electrochemical polymerization of pyrrole in the absence and in the presence of dopamine. From the first cycle the oxidation peak was observed at around 0.78 V, attributed to the oxidation of the monomer to a radical cation. Then the formation of a conductive polymer film on the glassy carbon electrode surface is observed by the increase in the intensity of the oxidation and reduction peaks. From the second cycle, a large anodic peak appears at 0.26 V corresponding to the oxidation of the formed polypyrrole and then, increases and shifts to a more anodic potential with the increase in the number of cycles [26] [27]. The charge passed during the formation of NIP is higher than of that in the formation of MIP, indicating that the template is becoming part of the polymeric film [28]. During the electrodeposition of the polypyrrole layer, DA molecules diffuse toward the electrode surface and are entrapped in the polymer film and interact with the pyrrole during its electropolymerization.

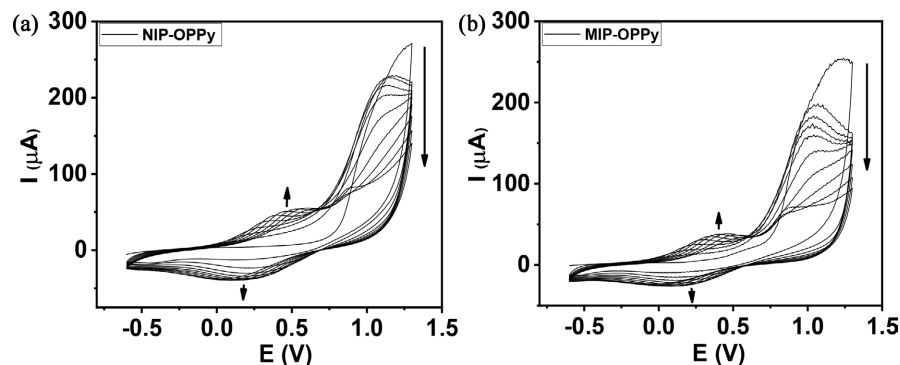


Figure 2. Cyclic voltammogram of 0.1 M Py in 0.1 M KCl solution on GCE at the scan rate 100 mV/s (a), in the presence of 10^{-3} M dopamine (b).

3.2. Overoxidation of the Polypyrrole Film

The overoxidation polypyrrole film was obtained electrochemically by cycling between -1 V and 1 V, in a sodium hydroxide solution (0.5 M), for five cycles. **Figure 3** shows that the high oxidation peak is observed at 0.3 V. We notice that the voltammograms recorded after the third cycle are almost confused, which proves that the overoxidation is complete. During the over-oxidation of the OPPy film, the formation of carbonyl and carboxylic groups could provide a better permselectivity for DA with the electrostatic force [2].

3.3. Electrochemical Characterizations

The electrochemical behavior of electrodes modified with MIP-OPPy was studied by cyclic voltammetry. **Figure 4** shows the electrochemical behavior of naked and modified GCE in 1 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ as redox probe. An almost reversible redox system was obtained with bare GCE ($\text{DE} \sim 100$ mV).

No redox peak was observed on the surface of (GCE/MIP-OPPy) indicating that the OPPy layer blocked the transport of electrons. After removal of the template molecules DA in 95% ethanol and DI water for 60 min, the current increased, which is due to the creation of cavities following the removal of the template [29].

3.4. SEM Characterization

SEM images of MIP-OPPy before and after extraction of the DA template are

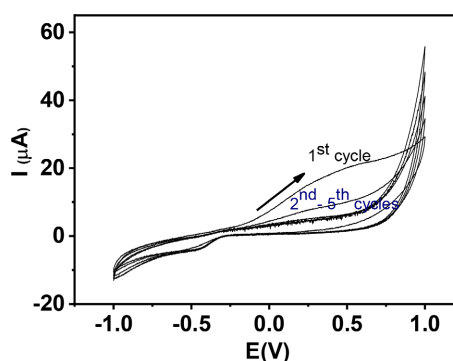


Figure 3. Overoxidation of the imprinted PPY in 0.5 M NaOH.

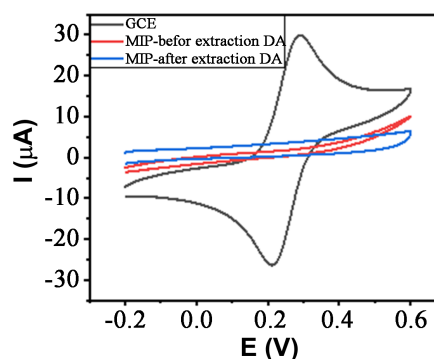


Figure 4. Cyclic voltammograms of 10^{-3} M $\text{Fe}(\text{CN})_6^{4-/3-}$ in 10^{-1} M KCl for bare GCE and modified electrode. Scan rate: 100 mV/s.

shown in **Figure 5**. After of MIP-OPPy, a spherical granular structure was obtained due to the formation of a polymer film (**Figure 5(a)**, **Figure 5(b)**). After removal of DA, a regular porous structure appeared on the surface of the MIP-OPPy electrode as shown (**Figure 5(c)**, **Figure 5(d)**), which is due to the produced cavities.

3.5. Optimization of the DA Electrochemical Detection

3.5.1. Effect of the Immersion Time

The effect of DA immersion time on the detection peak intensity is firstly studied. The results presented in **Figure 6** (determined from the measurements carried out with the SWV technique for a 10^{-5} M concentration of dopamine, at pH 6). From the voltammograms obtained it can be seen that the intensity of the current increases with the incubation time and that it remains constant beyond 20 min. A period of 20 min is therefore sufficient to saturate all the recognition

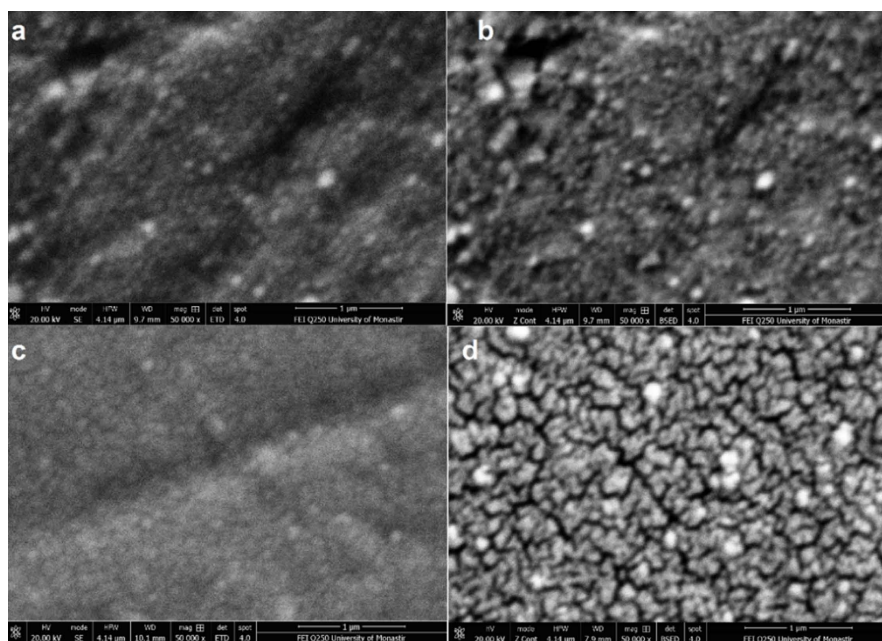


Figure 5. SEM images of the MIP-OPPy/GCE before extraction of DA ((a), (b)) and the MIP-OPPy/GCE after extraction of DA ((c), (d)).

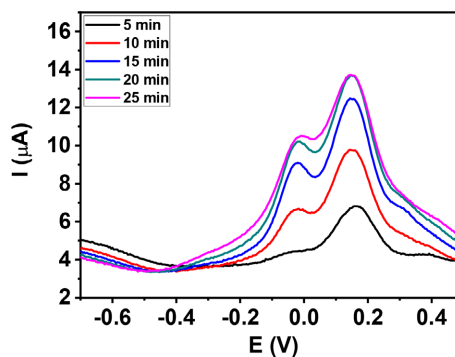


Figure 6. SWV recorded at different incubation time for 10^{-5} M DA in 0.1 M PBS, pH 6.

sites with the DA molecules. Based on these results, we choose 20 min for the DA incubation time during the next measurements.

3.5.2. Effect of pH Value

The pH of the carrier electrolyte influences the oxidation of DA at modified electrodes by affecting both peak currents and peak potentials [29]. **Figure 7** shows the effect of pH value on the peak position and intensity obtained by SWV on MIP-OPPy/GCE. The peak current increased with increasing pH value of the PBS solution until it reached 6, this value was chosen for DA detection. The oxidation potential decreases when pH value increases, showing that the oxidation reaction becomes easier, which is in agreement with proton release during the oxidation reaction of dopamine (**Figure 8**).

3.5.3. Effect of Scan Rate

Cyclic voltammograms of MIP-OPPy/GCE in 0.1 M PBS pH 6 containing DA were obtained at different scan rates and are shown in **Figure 9**. The values of

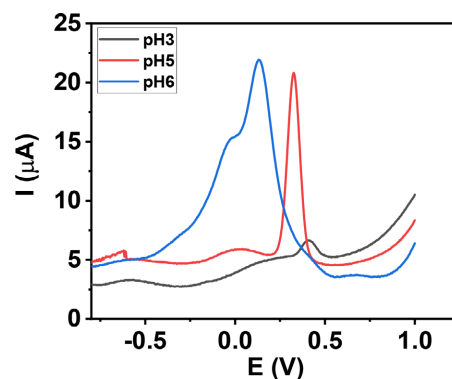


Figure 7. Dependence of SWVs on the value of pH for the MIP-OPPy electrode in 0.1 M PBS with 10 μM DA.

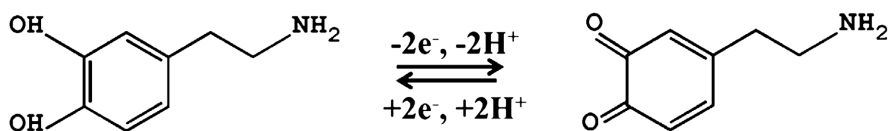


Figure 8. The mechanism for the DA redox reaction.

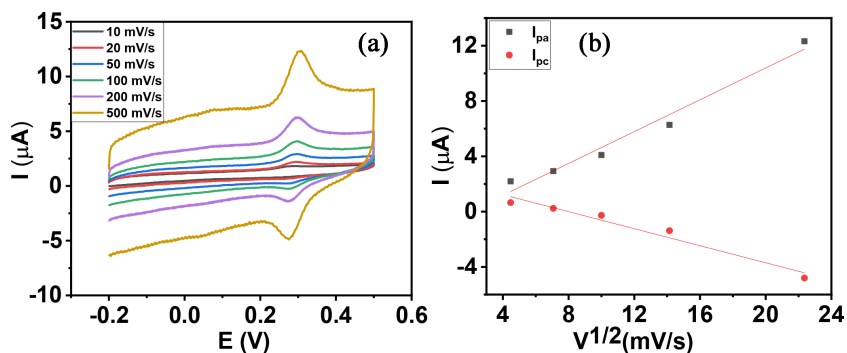


Figure 9. Electrochemical response of 10 μM DA in 0.1 M PBS at MIP/GCE with different scan rates.

the anodic peak current (I_{pa}) and the cathodic peak current (I_{pc}) show a linear relationship with the scan rate over the range of 10 to 500 mV/s. These results show that the electrocatalytic reaction is controlled by diffusion and reversible [2] [7].

3.6. Analytical Performance of the DA Sensor

The quantitative detection of DA was obtained by SWV in a 0.1 M PBS solution, at pH 6. **Figure 10(a)** shows the voltammograms obtained by SWV of different concentrations of DA on the modified electrode MIP-OPPy. The peak currents attributed to the oxidation of DA show a linear response with increasing concentration of DA in the range of 0.01 to 50 nM.

A good linear region with logarithm of DA concentration between 5×10^{-11} M and 5×10^{-8} M is shown in **Figure 10(b)**, black points. The linear regression equation was expressed as follows: $I_{pa} (\mu A) = 6.32 + 1.328 \log C_{DA} (M)$ with a correlation coefficient of $R^2 = 0.977$. The detection limit is 10^{-11} M corresponding to three times the noise of the background divided by the sensitivity.

The slope of the calibration plot for DA at the electrode modified by OPpy-MIP is 1.328, which is higher than that obtained for OPpy-NIP (slope: 0.66, $R^2 = 0.968$, **Figure 9(b)**, red points). The imprinting factor of the overoxidized polypyrrole is 2.

The relative standard deviation for the described sensor, over five determinations and removal of DA, is 5%. The reproducibility was studied, using 5 different dopamine sensors. The obtained relative standard deviation ranges from 10 % for all concentrations.

Different electrochemical sensors for the determination of DA some examples of previous results are summarized in **Table 1**. By comparing, our sensor with the others presented we notice a lower range detection limit, the linear range being limited in the higher range of concentrations.

Real Sample Analysis

Human serum was obtained in tubes by a Medical Analysis Laboratory, Sousse,

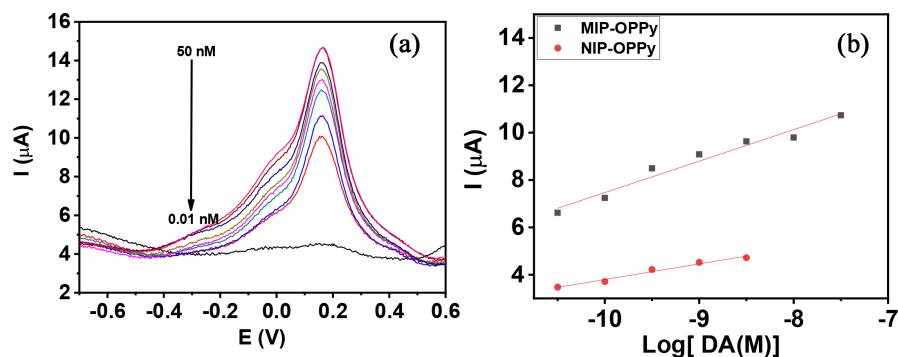


Figure 10. (a) SWV measured using the MIP/OPPy electrode in 0.1 M PBS buffer (pH 6) containing various concentrations of DA (0.01 nM - 50 nM); (b) Plot of oxidation peak currents versus logarithm of DA concentrations for MIP/OPPy electrode and NIP/OPPy electrode.

Table 1. Comparison with other published electrochemical sensor for the determination of DA.

Modified materials	Method	Linear range (μM)	LOD (nM)	References
MIP/AuNP/Gr/OPPy/GCE	DPV	0.5 - 8	100	[2]
MIP/o-aminophenol/gold electrode	DPV	0.02 - 0.2	1.98	[17]
MIP/OPPy/platinum	DNPV	10^{-2} - 0.1	4.5	[21]
MIP/PPy/CNTs	DPV	5×10^{-5} - 5	0.01	[23]
MIPPy/CA	DPV	0.007 - 35	0.4	[24]
MIP/PPy	SWV	10^{-5} - 1	0.0057	[27]
MIP/MWCNTs/GAs/GCE	DPV	0.005 - 20	1.67	[29]
MIP	DPV	0.5 - 40	130	[30]
MIP/p-aminothiophenol/gold	SWV	0.05 - 0.2	18	[31]
MIPs/MWNTs/GCE	DPV	0.625 - 100	60	[32]
MIP/OPPy/GCE	SWV	5×10^{-5} - 5×10^{-2}	0.01	This work

Table 2. Application of the sensor to determine DA in spiked human serum.

Samples	Added ($\mu\text{mole/L}$)	Found ($\mu\text{mole/L}$)	Recovery (%)
Serum 1	---	---	---
	4.524×10^{-2}	4.842×10^{-2}	107 ± 2
	1.345×10^{-1}	1.388×10^{-1}	103 ± 2
Serum 2	---	---	---
	4.524×10^{-2}	4.64×10^{-2}	102 ± 2
	1.345×10^{-1}	1.354×10^{-1}	100 ± 2

All measurements were taken, in five replicates.

Tunisia. After centrifugation for 10 min at 1000 r/min, the serum samples were diluted 1:10 in 0.1 M PBS. Concentrations of dopamine were added in the diluted serum samples and the recovery rates were calculated. The results are shown in **Table 2**. Recovery rates are in the range of 100%, showing that no interfering agent was detected in human serum.

4. Conclusion

In this work we succeeded in immobilizing in a single step an ultrathin film of OPpy imprinted by dopamine molecules. The extraction of the template (DA) is confirmed by electrochemical measurements. The obtained imprinting factor is 2. MIP-OPpy has demonstrated high sensitivity compared to published dopamine electrochemical sensors based on polypyrrole. This sensor can be easily manufactured at low cost and can be applied for the determination of dopamine

in human serum.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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