

Synthesis and Electrochemical Evaluation of Some Organic Molecules as an Antioxidant Agents

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Abstract

A series of new benzamides were synthesized. The chemicals structures were confirmed by elemental analyses ¹H NMR and ¹³C studies. The antioxidant activity of the synthesized compounds was evaluated by square wave voltammetry. A new approach, for antioxidant capacity determination was proposed. It is based on the using of the xanthine-xanthine oxidase system coupled with H₂O₂ electrochemical sensor.

Keywords: Voltammetry; Antioxidant capacity; Xanthine; Xanthine oxidase

Introduction

Reactive oxygen species (ROS) including superoxide anion (O₂^{•-}) hydrogen peroxide, and hydroxyl radical (OH[•]), are generated naturally *in vivo* during metabolic processes and keeps in a balance level in normal living organisms [1]. However, when a body is subject to the environmental or behavioral stressors (pollution, sunlight exposure, cigarette smoking, excessive alcohol consumption, etc.), excess ROS are generated [2]. If the excess ROS cannot be scavenged in time, they would attack and induce DNA, proteins and lipids damage, and impede normal cell functions [3]. Therefore, overproduction of ROS is associated with numerous diseases like cancer and Alzheimer's disease, as well as aging. In living systems, the deleterious effects of ROS can be neutralized by the endogenous and exogenous antioxidant systems [4]. Antioxidants are synthetic or natural substances that prevent or delay the oxidative damage by scavenging the free radicals. Fruits and vegetables are good sources of high amounts of known antioxidants.

The aims of this study are to synthesize derivatives of benzamides, from the arylamines and salicylic acid under the action of thionyl chloride, and tested as antioxidants.

The antioxidant capacity was evaluated, by coupling an amperometric sensor for H₂O₂ detection, obtained by modification of paste carbon graphite electrode with copper, with xanthine oxidase (XOD) immobilized at silice-xanthine (XA) enzymatic system, as generator of O₂^{•-} radicals. The advantages of this strategy consist to [5-16]:

- It works at low applied potential, allowing a significant decrease of the risk of electrochemical interferences;
- The antioxidant capacity evaluation, requiring the monitoring of H₂O₂ concentration in presence of antioxidant sample as well as in its absence, will be a global estimation of the free radicals (O₂^{•-}) and nonradical reactive species, (H₂O₂) interactions with the investigated antioxidant (AOX) (Reaction 1).

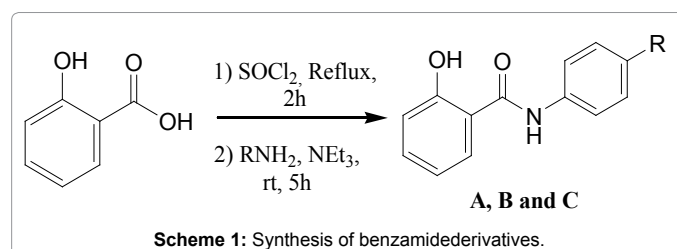
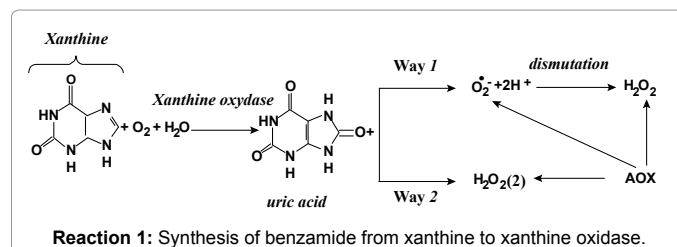
Experimental

Synthesis of some benzamides derivatives

Access to these amides requires the preparation of the acid chloride from salicylic acid by the action of thionyl chloride followed by nucleophilic attack of arylamines (Scheme 1). The different molecules

(A, B and C) shown in Scheme 2, are obtained and purified by chromatography on silica gel and characterized by ¹H and ¹³C NMR spectroscopy.

Experimental section: Melting points were taking for samples in capillary tubes with an electro-thermal apparatus and are uncorrected. ¹H NMR and ¹³C NMR were recorded on a Bruker Avance DPX250 spectrometre (300 MHz ¹H, 75 MHz ¹³C) using trimethylsilane as the internal standard, chemical shifts were reported in parts per million (ppm, δ units). Coupling constants were reported in units of hertz (Hz). Flash chromatography was performed on silica gel 60 (40–63 mesh). Thin layer chromatography (TLC) was carried out on Merck silica gel

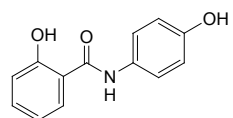


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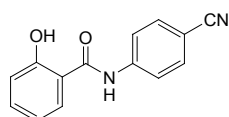
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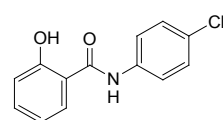
2-hydroxy-N-(4-hydroxyphenyl)benzamide

Molecule A



N-(4-cyanophenyl)-2-hydroxybenzamide

Molecule B



N-(4-chlorophenyl)-2-hydroxybenzamide

Molecule C

Scheme 2: The different molecules (A, B and C) obtained from benzamide derivatives, purified by chromatography on silica gel.

60F254 precoated plates. Visualization was made with ultraviolet light. All organic solvents were distilled immediately prior to use.

General procedure for the synthesis of compounds A, B and C: Salicylic acid (2 g, 0.013 mol) was dissolved in thionyl chloride (16.07 g, 0.13 mol). The mixture was stirred at reflux for 2 hours. After evaporation of the SOCl_2 , the residue obtained is dissolved in CH_2Cl_2 (20 mL) and phenylhydrazine (1.75 g, 0.016 mol) and triethylamine (1.36 g, 0.013 mol) were added. The reaction mixture is stirred at room temperature for 5 hours. After hydrolysis with a solution of NaOH (1N), the mixture was extracted with CH_2Cl_2 . The organic phases are combined, dried over MgSO_4 , filtered and evaporated under reduced pressure. The crude product thus obtained was purified by silica gel chromatography (eluent: ethyl acetate/hexane: 4/6) leading to the desired compound in good yield.

2-hydroxy-N-(4-hydroxyphenyl)benzamide A: This compound was obtained as a white solid (70%). Mp: 155-157°C. ^1H NMR (300 MHz, DMSO) δ 11.75 (s, 1H, NH), 10.45 (s, 1H, OH), 10.27 (s, 1H, OH), 7.78 (d, $J=8.7\text{Hz}$, 2H, ArH), 7.30 (d, $J=8.7\text{Hz}$, 2H, ArH), 7.10-6.95 (m, 2H, ArH), 6.85 (t, $J=16.8\text{Hz}$, 1H, ArH), 7.50 (t, $J=13.6\text{Hz}$, 1H, ArH); ^{13}C NMR (75 MHz, DMSO): δ 167.8, 159.4, 154.1, 137.2, 131.8, 123.1, 121.5, 120.1, 118.1, 118.0.

N-(4-cyanophenyl)-2-hydroxybenzamide B: This compound was obtained as a (marron Claire) solid (73%). Mp: 164-166°C. ^1H NMR (300 MHz, DMSO) δ 11.41 (s, 1H, NH), 10.70 (s, 1H, OH), 6.9-7.9 (m, 8H, ArH); ^{13}C NMR (75 MHz, DMSO): δ 164.8, 159.4, 140.2, 133.6, 132.4, 128.9, 122.3, 121.5, 119.9, 116.0, 115.8, 108.2.

N-(4-chlorophenyl)-2-hydroxybenzamide C: This compound was obtained as a white solid (67%). Mp: 185-187°C. ^1H NMR (300 MHz, DMSO) δ 11.58 (s, 1H, NH), 10.13 (s, 1H, OH), 6.9-8.2 (m, 8H, ArH); ^{13}C NMR (75 MHz, DMSO): δ 164.8, 159.4, 134.0, 133.6, 129.9, 129.1, 128.9, 123.0, 121.5, 119.9, 116.0.

Apparatus

Electrochemical experiments were performed using a voltalab potentiostat (model PGSTAT 100, Eco Chemie B. V., Utrecht, The Netherlands) driven by the general purpose electrochemical systems data processing software (voltalab master 4 software).

All the electrochemical experiments were performed in a standard one-compartment three-electrode cell. The reference electrode was SCE and the counter electrode was platinum. All electrode potentials were referred to this reference electrode. The working electrode was copper modified carbon paste electrode (Cu-CPE).

Reagents and solutions

All chemicals were of the highest quality. Graphite powder (spectroscopic grade RWB, Ringsdorf-Werke GmbH, Bonn-Bad Godesberg, Germany) was obtained from Aldrich and was used without further purification. CuSO_4 was obtained from Merck chemicals. Deionised water was used to prepare all solution.

Preparation of the electrochemical sensor

The carbon paste unmodified was prepared by adding paraffin oil to carbon powder and thoroughly hand-mixing in a mortar and pestle. The resulting paste was packed into the electrode and the surface was smoothed. The electrochemical sensor was developed by depositing the copper at fixed potential (0.1 V for 1 hour) onto the carbon paste electrode surface.

Procedure

The device constructed for the measurement of the antioxidant capacity is given in Figure 1. The free radical was generated in column following the reaction 1 and the graph plot, giving reduction H_2O_2 current density versus $[\text{H}_2\text{O}_2]$, was carried out. In the second time, the investigated antioxidant associated to xanthine solution were pouring in column and response behaviour was recorded.

Results and Discussion

Electrochemical behaviour of the studied molecules

The cyclic voltammograms (CVs) recorded at Cu-CPE in the supporting electrolyte containing or not molecule are presented in Figure 2. In presence of molecule A in medium, we note, higher current densities of the anodic side and the occurrence of a reduction peak in the cathodic scanning.

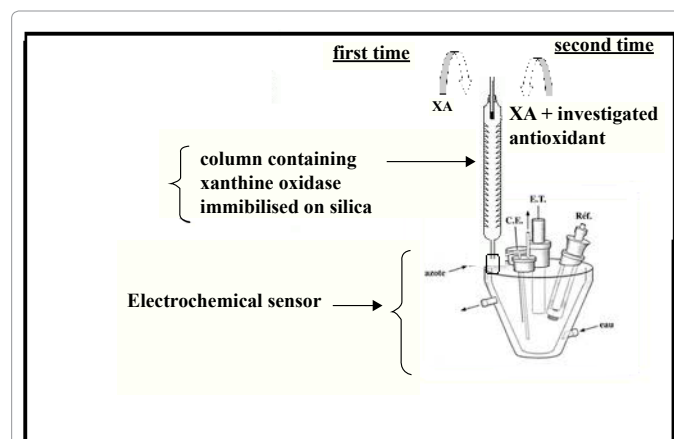


Figure 1: Scheme of the device constructed for the measurement antioxidant capacity.

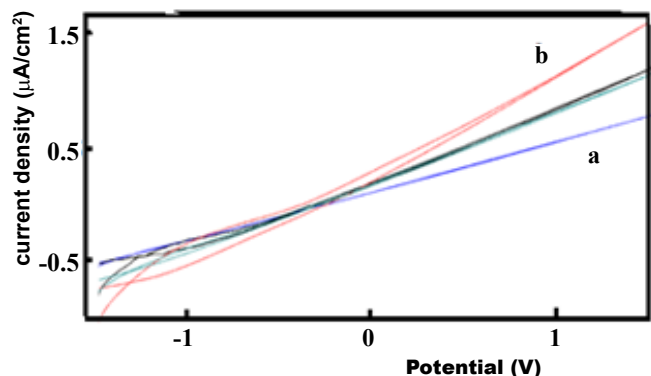


Figure 2: Cyclic voltammograms recorded at Cu-CPE in a-buffer solution, b-buffer solution containing molecule A.

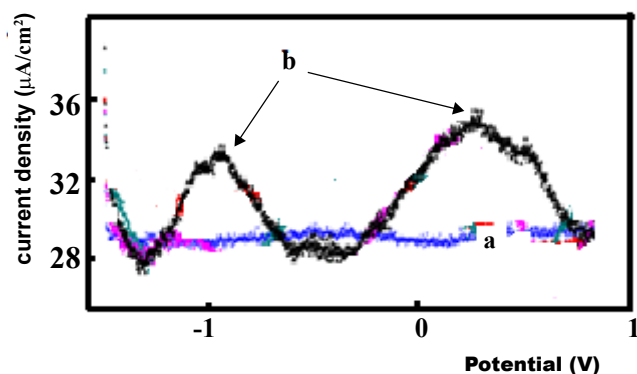


Figure 3: Square wave voltammogram recorded at Cu-CPE in a-buffer solution and b- buffer solution containing molecule A.

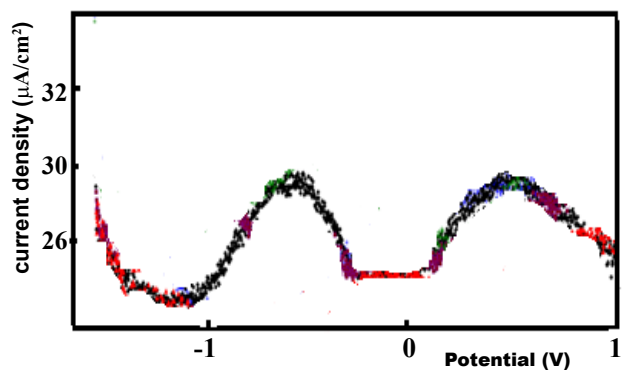


Figure 4: Square wave voltammogram recorded at Cu-CPE in a-buffer solution and b-buffer solution containing molecule B.

The square wave (SQW) voltammograms obtained, respectively, in buffer solution (curve a) and buffer solution containing molecule A (curve b) are shown in Figure 3. In the presence of the molecule A in the buffer solution causes the appearance of two redox peaks in the SQW voltammogram. The first one around -1.0 V and the second at 0.4V versus SCE. Peaks can be attributed to redox responses of molecule A.

The same behavior was observed in the presence of the molecule B in the electrolytic solution. The two redox peaks are shifted by about 0.5 V (Figure 4).

A compared square wave voltammograms corresponding to studied molecules shows that only the molecules A and B gives rise to two well-defined redox peaks (Figure 5).

Antioxidant capacity evaluation

Molecule A: The anti-oxidant properties of the studied molecules were evaluated, investigating square wave voltammetry, by comparing the reduction of H_2O_2 in the presence and absence of the considered molecule.

We can see from Figure 6 that, the progressive addition of the solution containing molecule A, leads to the decrease of the H_2O_2 reduction current density (in absolute value). The complete inhibition of the reduction reaction of hydrogen peroxide requires 100 μL /100 mL(buffered solution) (Figure 7).

The inhibition of the oxidative capacity of molecule A by H_2O_2 was investigated in Figure 8. The hydrogen peroxide formed in the silica column is added, to a phosphate buffer solution containing the molecule A, in the electrochemical cell. We note that the antioxidant molecule A power down depending on the amount of H_2O_2 paid.

The same experiments were conducted for molecules B and C. The same behavior is observed (Figures 9 and 10).

Conclusion

In this work, the antioxidant capacities of three synthesized

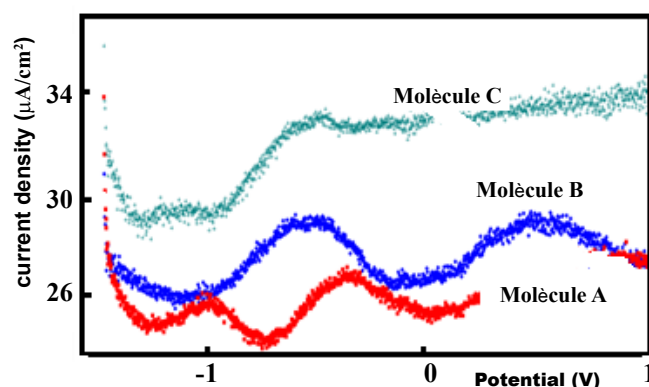


Figure 5: SQW voltammograms recorded in buffer solution containing, respectively, the studied molecule.

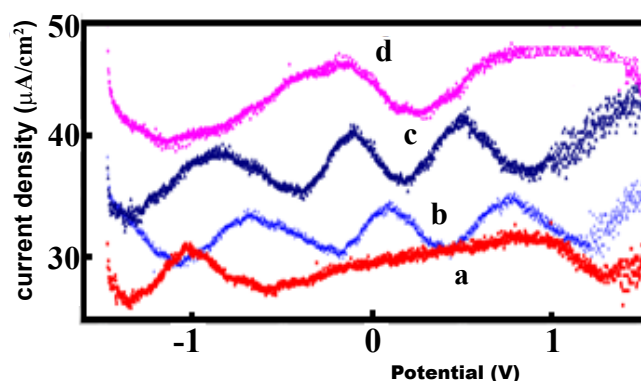


Figure 6: SQW voltammograms enregistered at Cu-CPE, in buffer solution containing, d-100 μL of H_2O_2 , c-addition of 8 μL of molecule A, b-addition of 12 μL of molecule A and a- addition of 14 μL of molecule A.

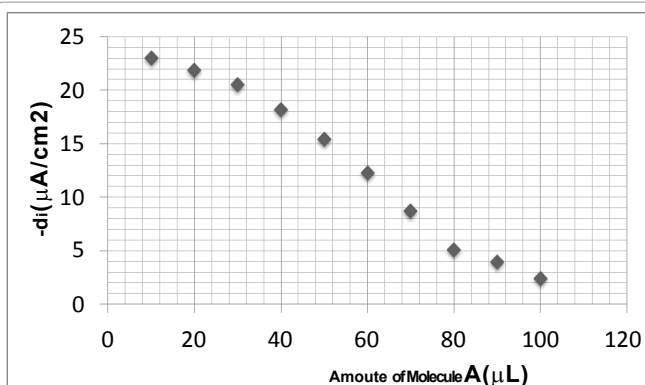


Figure 7: Evolution of the H₂O₂ reduction current density with the concentration of molecule A.

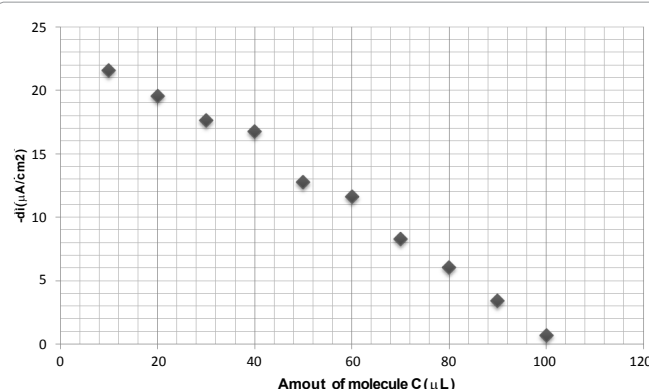


Figure 10: Evolution of the H₂O₂ reduction current density with the concentration of molecule C.

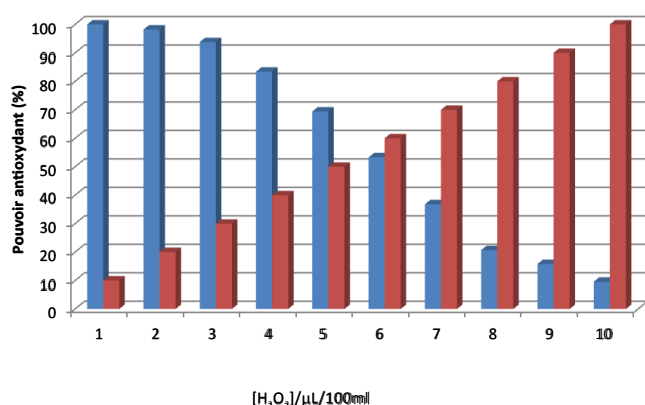


Figure 8: Evolution of antioxidant capacity of molecule A depending on the H₂O₂ concentration.

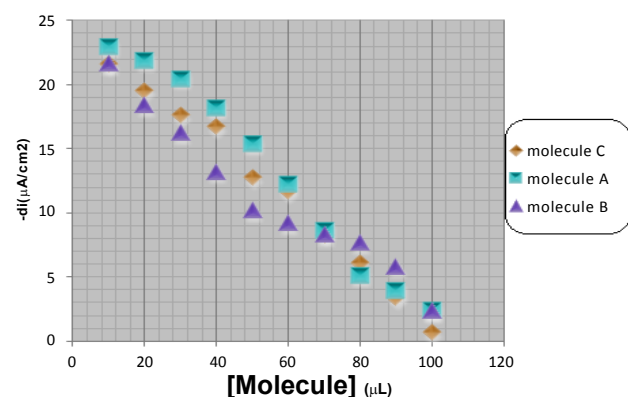


Figure 11: Comparison of Antioxidant capacity of the studied molecules.

Antioxidant capacity (AOC) of the studied molecules varies in the following sense (Figure 11):

- For concentrations ≤ 60 μL/100mL

AOC (molecule B) ≥ AOC (molecule C) ≥ AOC (molecule A)

- For concentrations ≥ 60 μL/100mL

AOC (molecule C) ≥ AOC (molecule A) ≥ AOC (molecule B)

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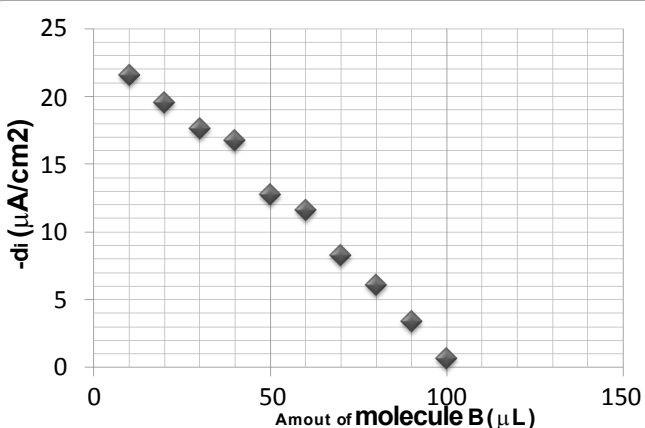


Figure 9: Evolution of the H₂O₂ reduction current density with the concentration of molecule B.

molecules were investigated based on an electrochemical sensor using copper modified carbon paste electrode as work electrode. The study of SQW voltammograms showed that this sensor had good properties in detection of H₂O₂ in electrochemical cell. H₂O₂ is generated in silica column based on xanthine/xanthine oxidase system. The experiment procedure exhibited good stability and reproducibility.

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