

*Dedicated to prof. dr. I. C. Popescu  
on the occasion of his 70<sup>th</sup> anniversary*

## **ELECTROCHEMICAL CHARACTERIZATION OF TWO OLEAMIDE ANALOGUES ANTI-OBESITY AGENTS**

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**ABSTRACT.** Electrochemical characterization of two oleamide analogues was performed by cyclic voltammetry, differential pulse voltammetry, and rotating disk electrode voltammetry. Modified electrodes have been obtained by scanning the potential in the domain of the first oxidation peaks.

**Keywords:** *oleamide analogues, cyclic voltammetry, differential pulse voltammetry, rotating disk electrode voltammetry, modified electrodes*

### **INTRODUCTION**

Many researches are nowadays dealing with elucidating of obesity molecular mechanism and developing new potent therapeutic agents with less secondary effects. Oleic acid amide (oleamide) and some of its structural analogues are amides of the naturally present in food fatty acids. These compounds occur also endogenously as signaling molecules with various biological effects depending on tissue type. The most studied biological activities of oleamides are the sleeping modulator effect, decreasing of pain perception, decreasing of body temperature, and regulator effects on cardiovascular system and lipid metabolism.

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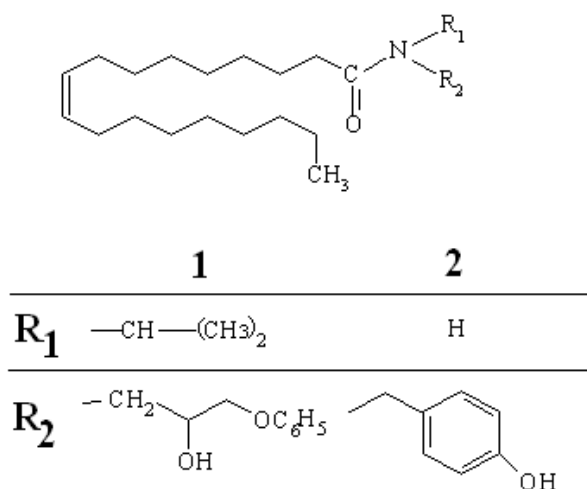
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N-(2-hydroxyethyl)oleamide (OEA) is an important analogue of oleamide; it is a fatty acid amide biosynthesized from oleic acid and phosphatidylethanol-amine mainly in brain, liver, adipocytes and small intestine [1, 2]. OEA acts on peroxisome proliferator-activated receptor alpha (PPAR- $\alpha$ ) and less on cannabinoid receptors [3, 4]. Both endogenous and oral/parenteral administration of OEA induced a satiety signal leading to the decrease of food intake and body weight loss [3, 5]. This activity is opposed to that induced by the anandamide, (5Z,8Z,11Z,14Z)-N-(2-hydroxyethyl) icoso-5,8,11,14-tetraenamide, which is a similar amide that contains 4 double bonds and 3 double allylic positions. This antagonist induces a motivation for feeding and pleasure, activating mainly the cannabinoid receptors [6]. This difference could be also due to the gap between the oxidation potentials of the two amides, OEA possessing only one isolated double bond, while anandamide is much easier oxidized at the double allylic positions, making impossible the generation of hydrogen peroxide by PPAR- $\alpha$ . Therefore, we examined two compounds **1** and **2** (Fig. 1) with similar structures that have different oxidation capacities.

In this paper we present the results obtained in the study of the electrochemical profiles of compounds **1** and **2**, which were synthesized as promising oleamide analogues [7], similar to the natural active compounds useful for anti-obesity therapy.



**Figure 1.** Structures of the investigated compounds

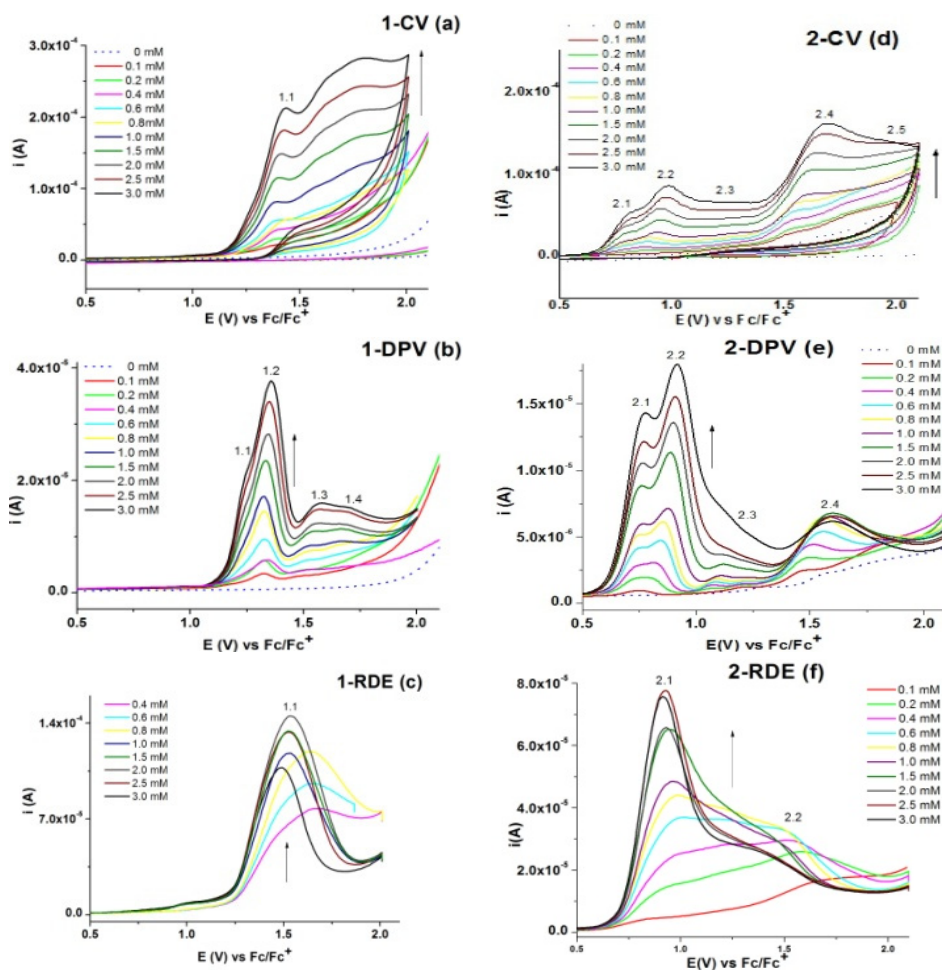
## RESULTS AND DISCUSSION

The compounds **1** and **2** were synthesized from oleic acid and 1,1'-carbonyldiimidazole to give the oleyl-imidazole intermediate, which reacted with the corresponding amines according to the procedure previously described [7]. The characterization by IR, <sup>1</sup>H- and <sup>13</sup>C-NMR spectra confirmed the compounds structure.

The electrochemical behavior of these two oleamide analogues was studied in acetonitrile (CH<sub>3</sub>CN) containing tetrabutylammonium perchlorate (TBAP) as supporting electrolyte, using stationary or rotating glassy carbon electrodes. The results of electrochemical experiments carried out in anodic scans by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and rotating disk electrode voltammetry (RDE) are shown in Fig. 2. All curves were recorded individually, starting from the stationary potential in cathodic or anodic scans. No cathodic processes have been evidenced.

The anodic processes are denoted in the order in which they appear in the voltammograms (eg with 1.1 – 1.4 or with 2.1 – 2.5 for compounds **1** or **2**, respectively). Two main anodic processes (1.1 and 1.2 and 2.1 and 2.2, respectively) are noticed for all kind of curves: CV, DPV and RDE (Figure 2). They can be attributed to the specific oxidation processes of each oleamide.

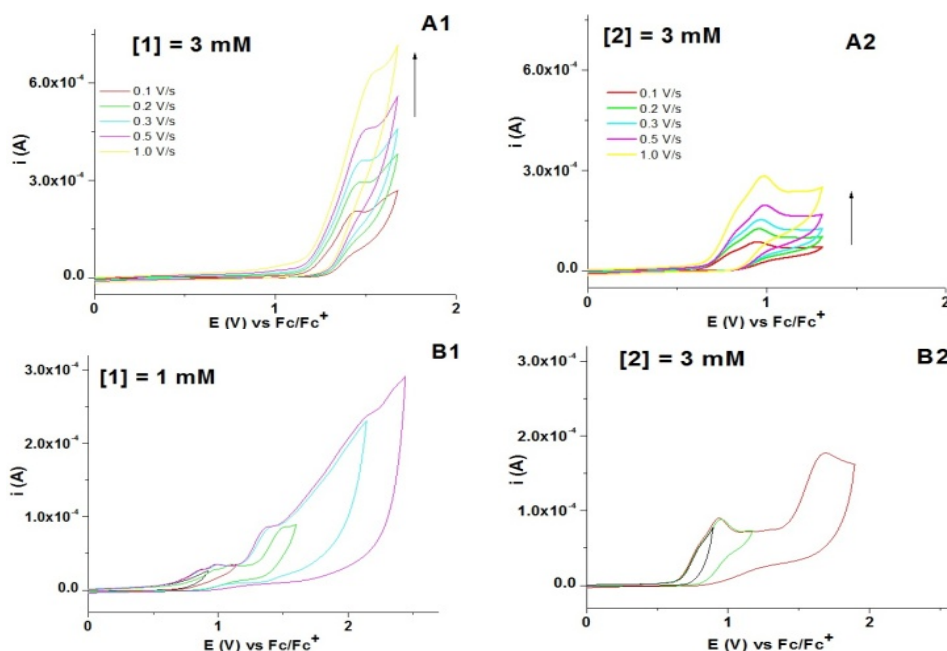
The reaction mechanism which explains these peaks was previously studied when determining the phenols capacity to be antioxidants or promoters of lipide peroxidation. This behavior could make the difference between the treatment and the worsening of cancer [8]. The phenols possessing low oxidation potentials can easily quench the generated radical cations on the lipid double bonds; natural caffeic acid, but also alkylated phenols (if they do not contain electro withdrawing groups (EWGs) on the molecule) are effective. Unfortunately, the phenols substituted with EWGs or the anisoles have much higher oxidation potentials, and they behave even as catalyst in the electron transfer from the oleic double bond to the electrode. A better understanding of these processes could be achieved if the experiments are conducted using a buffer, which prevents the inherent solvent acidification by the TBAP oxidation [9]. At biological pH-s, the compound **2** would have had oxidation potentials lower than **1**, and compound **1** would not be affected by pH. This difference is induced by the capacity of the phenolic system to form relative stable semiquinone and then quinone (by hydration) forms [10].



**Figure 2.** CV (a,d), DPV (b,e) and RDE (c,f) curves for **1** (a, b, c) and **2** (d, e, f) obtained for different concentrations of each compound

Analyzing the curves obtained in Figure 2 it can be concluded that oxidation of **2** occurs more readily than that of compound **1**. The oxidation processes are, respectively: phenol oxidation to *o*-semiquinone and then to *o*-quinone in compound **2**, and alkene and anisole oxidation to cation radicals (occurring at the same area of potentials) in compound **1**.

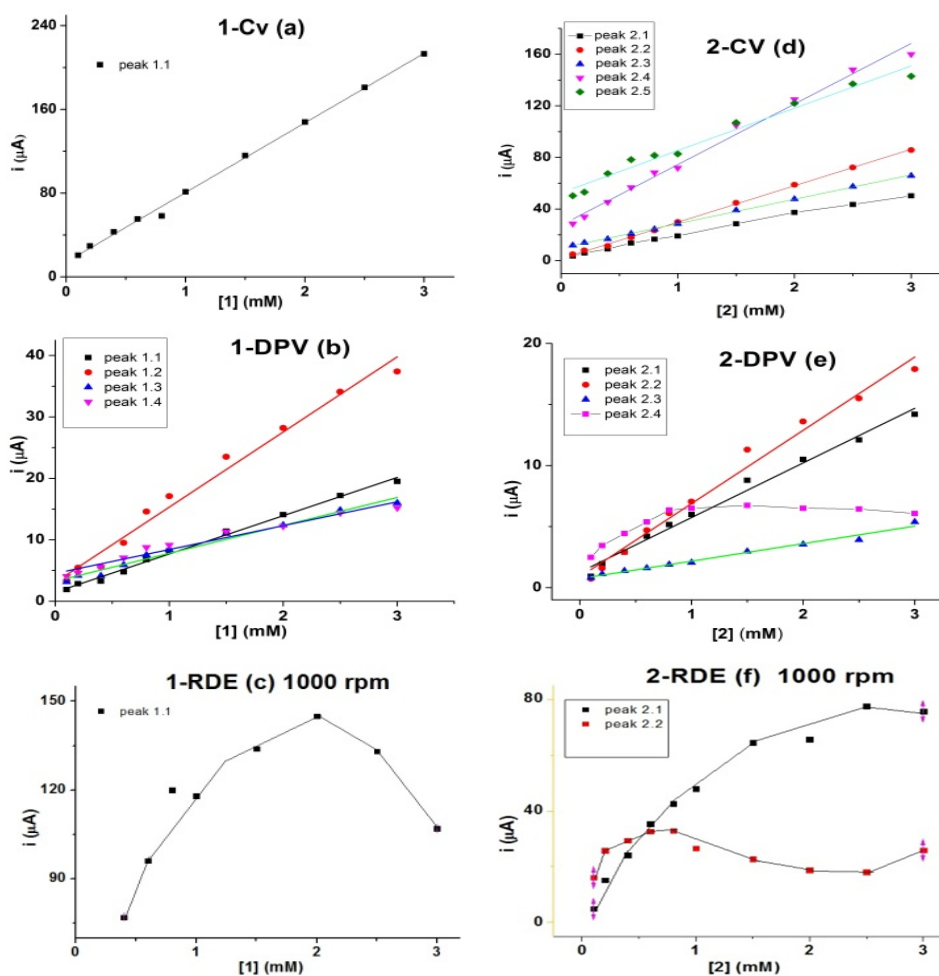
The influences of the scan rate and scan domain on the CV curves was also studied for the two compounds, and the obtained curves showed that all processes are irreversible (Figure 3).



**Figure 3.** CV curves at different scan rates (A1, A2) and various of scan domains (B1, B2) in the potential range of the first anodic peak for **1** and **2** in 0.1 M TBAP,  $\text{CH}_3\text{CN}$

It can be seen from Figure 4 that dependences of peak currents on oleamide concentrations obtained from all methods are linear for both compounds in CV and DPV (Table 1), but parabolic or sigmoid, respectively, in RDE for compounds **1** or **2**. The last shapes are connected to the formation of insoluble products or films on the electrode surface from both oleamide analogues. It can be seen from Table 1 that correlation coefficients for the linear dependences of the peak currents on concentration of compound **1** or **2**, [**1**] or [**2**], respectively, are good.

It can be seen from figure 5 that by scanning the potential in the range of the first anodic peaks in solutions of **1** or **2** the currents decrease drastically in successive cycles, showing the electrode blocking. Modified electrodes have been obtained by cycling the potential between 0 and the potential of the first two peaks. The transfers of these modified electrodes in ferrocene solution have shown that the reversible ferrocene signal is deeply changed on modified electrodes being smaller in intensity and very flat, confirming the electrode blocking with an insulating layer.



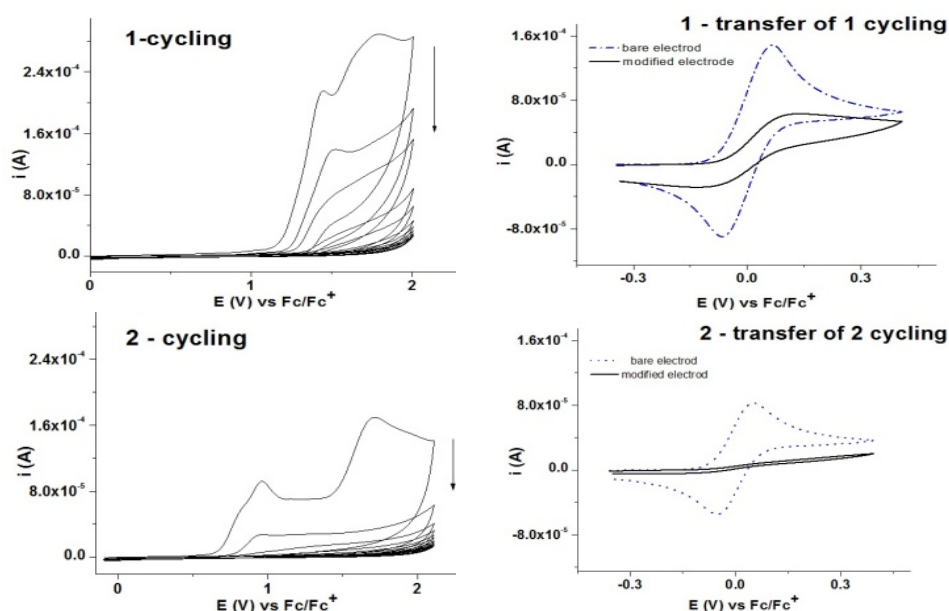
**Figure 4.** Dependences on concentration of the currents in CV, DPV and RDE

**Table 1.** Equations for the 1.1-1.4 and 2.1-2.3 peak currents (A) vs the corresponding oleamide concentration, [1] or [2] in mole/L

Compound	Method	Equation	Correlation coefficient
1	CV	$i_{\text{peak 1.1}} = 14.19 + 66.47 \cdot [1]$	0.997
	DPV	$i_{\text{peak 1.1}} = 1.46 + 6.23 \cdot [1]$	0.994
		$i_{\text{peak 1.2}} = 3.07 + 12.23 \cdot [1]$	0.980
		$i_{\text{peak 1.3}} = 3.25 + 4.54 \cdot [1]$	0.978
		$i_{\text{peak 1.4}} = 4.52 + 3.88 \cdot [1]$	0.958

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Compound	Method	Equation	Correlation coefficient
2	CV	$i_{\text{peak } 2.1} = 9.33 + 7.34 \cdot [2]$	0.995
		$i_{\text{peak } 2.2} = 1.48 + 28.28 \cdot [2]$	0.999
		$i_{\text{peak } 2.3} = 9.77 + 18.89 \cdot [2]$	0.999
		$i_{\text{peak } 2.4} = 27.43 + 46.97 \cdot [2]$	0.989
		$i_{\text{peak } 2.5} = 52.55 + 32.79 \cdot [2]$	0.973
	DPV	$i_{\text{peak } 2.1} = 1.26 + 4.47 \cdot [2]$	0.988
		$i_{\text{peak } 2.2} = 0.87 + 6.0 \cdot [2]$	0.984
		$i_{\text{peak } 2.3} = 0.73 + 1.44 \cdot [2]$	0.981



**Figure 5.** CV curves during successive cycles in millimolar solutions of **1** or **2** (**1-cycling** and **2-cycling**, respectively) in 0.1M TBAP, CH<sub>3</sub>CN (left side), and the corresponding CV curves of ferrocene in 0.1M TBAP, CH<sub>3</sub>CN solution on bare (dashed lines) and modified (full lines) electrodes (right side), obtained after the transfers of the electrodes prepared by scanning the potentials (20 cycles in solutions of **1** or **2** in 0.1M TBAP, CH<sub>3</sub>CN, respectively)

Modified electrodes can be obtained either by scanning the electrode potential between 0 and the potential of the second main oxidation peaks, or by controlled potential electrolysis at potentials in the domain of the second main oxidation peaks.

Electrochemical characterization of oleamides **1** and **2** led to concordant results with those obtained for another oleamide having a similar structure, which has been investigated previously [11]. These investigated compounds show different electroactivities, evidenced by different potentials for the anodic peaks. This behavior is explained by the differences in their structures. In the present study, all electrochemical curves agree that compound **2** is oxidized easier than **1**, due to its phenolic structure.

## CONCLUSIONS

The electrochemical behavior of two oleamide analogues was investigated in acetonitrile containing tetrabutylammonium perchlorate as supporting electrolyte. Two main anodic irreversible processes were identified by cyclic and differential pulse voltammetry. Scanning the potential in the range of the first anodic peak led to the coverage of the electrode with an insulating layer. This coverage was confirmed by rotating disk electrode curves. Modified electrodes can be easily obtained either by potential scans or by controlled potential electrolysis in the domain of the first two oxidation peaks.

The electrochemical studies of the two compounds performed by cyclic, differential pulse and rotating disk electrode voltammetries allowed highlighting the different features of the electrochemical behaviour of these two oleamide analogues in view of their potential anti-obesity action. The compound **1** has a higher oxidation potential, and it is prone to activate mainly the peroxisome proliferator-activated receptor alpha, while the compound **2** can act more as an activator of cannabinoid receptors, inducing hunger and pleasure. At the same time, if the concentration of hydrogen peroxide generated by peroxisome is too high, compound **1** seems not to have anymore antioxidant capacity, but even worse, it catalyses the oleoyl oxidation. The compound **2** can protect the organic tissue against the formation of radical cations in surplus. In this way, both compounds can act as potential anti-obesity agents. The corresponding tests are in progress.

## EXPERIMENTAL SECTION

Oleamides **1** and **2** (Figure 1) were synthesized according to the procedure described in [6]. For electrochemical investigations acetonitrile and tetrabutylammonium perchlorate (TBAP) from Fluka were used (as received) as solvent and supporting electrolyte, respectively.



The electrochemical experiments were carried out using a PGSTAT 12 AUTOLAB potentiostat coupled to a three-compartment cell. The CV curves were generally recorded at 0.1V/s or at various rates (0.1 - 1V/s) when studying the influence of the scan rate. DPV curves were recorded at 0.01V/s with a pulse height of 0.025V and a step time of 0.2 s. RDE experiments were performed using Autolab RDE-2 rotating disk electrode at a scan rate of 0.01V/s, with rotating rates between 200 and 2000 rpm. The working electrodes were glassy carbon disks (diameter of 3 mm). Their active surface was polished before each determination with diamond paste (0.25  $\mu\text{m}$ ). Ag/10 mM AgNO<sub>3</sub> in 0.1M TBAP, CH<sub>3</sub>CN was used as reference electrode. The potential was referred to the potential of the ferrocene/ferricinium redox couple (Fc/Fc<sup>+</sup>) which in our experimental conditions was +0.07V. A platinum wire was used as auxiliary electrode.

The electrochemical experiments were performed at 25°C under argon atmosphere.

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