Electrochemical Evidence in Mechanism of Toxicity of Mefenamic Acid Overdose in the Presence of Glutathione and *N*-Acetyl-*L*-Cysteine

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Electrochemical Evidence in Mechanism of Toxicity of Mefenamic Acid Overdose in the Presence of Glutathione and *N*-Acetyl-*L*-Cysteine

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In this study, the electrochemical oxidation of mefenamic acid was investigated in the presence of glutathione and *N*-acetyl-*L*-cysteine. The results revealed that the mefenamic acid was involved in a catalytic reaction with glutathione and *N*-acetyl-*L*-cysteine. This investigation presents some electrochemical evidence for the mechanism of action of these compounds in mefenamic acid poisoning.
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Mefenamic acid (MFA) belongs to the *N*-aryl-anthranilic acid comprising a class of non-steroidal anti-inflammatory drugs (NSAIDs), which were licensed as prescription-only medicines in the UK and Europe in the early 1960s and today are widely used in the treatment of pain, arthritis, and dysmenorrheal.[1,2] The MFA has been applied to hepatotoxicity, nephrotoxicity, gastrointestinal toxicity, and the hypersensitivity reactions that are caused by the formation of reactive metabolites such as carboxyl-group-containing NSAIDs and protein-reactive acylating metabolites by phase II metabolism.[3] There have not been reported any side effects for the rocemmended doses of the mefenamic acid. However, the mefenamic acid overdose carries a significantly higher risk of dose-related central nervous system (CNS) toxicity compared with other commonly used NSAIDs and a substantially higher risk of convulsion.[3,4] Our literature survey indicated that in the oxidative bioactivation of the MFA by human liver microsomes (HLM) and recombinant human P450 enzymes in the presence of GSH; three GSH conjugates are produced, which result from the GSH conjugation of the two quinine imines formed by further oxidation of 4′-hydroxy-MFA and 5-hydroxy-MFA (Fig. 1).[4–6]

In the normal conditions, the reaction between the glutathione (**3**) and the MFA (**1**) immediately occurs, and the MFA was rapidly detoxified. However; in an overdose of the MFA with the depletion of cellular **3**; free MFAs bind to the cellular macromolecules; and consequently; hepatotoxicity, nephrotoxicity, gastrointestinal toxicity, and cell death happen.[6]

Due to the similarity between the electrochemical oxidation and the cytochrome P450-catalyzed oxidation in liver microsomes,[7] it would be of special interest to investigate the anodic oxidation of **1** in the presence of glutathione in different conditions. In this work, we have shown a novel pathway in the electrochemical oxidation of **1** in the presence of **3**.

Moreover, *N*-acetyl-L-cysteine is a sulfhydryl compound, which is expected to be an auxiliary compound in the MFA poisoning. It acts as an alternative substrate in the reaction with MFA. It is also found to act as a facilitator in the synthesis of glutathione.[8–10]

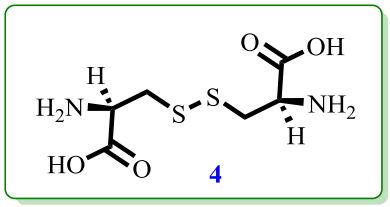
The results in this study showed that the glutathione stored in the human liver was consumed by the product obtained from the oxidation of **1**. Besides, *N*-acetyl-*L*-cysteine could be useful in the treatment of the MFA overdose. However, in comparison, the rate of catalytic oxidation of *N*-acetyl-*L*-cysteine (**3′**) was more than that of glutathione.[8,9]

Experimental

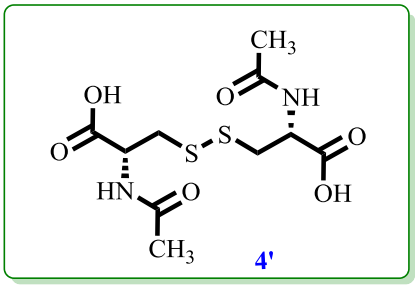
Apparatus and reagents.—Cyclic voltammetry and controlled-potential coulometry were performed using an Autolab model PGSTAT 20 potentiostat/galvanostat. A glassy carbon disk was applied as the working electrode (1.8 mm in diameter) in the voltammetry, whereas a platinum wire was used as the counter electrode. The working electrode used in controlled-potential coulometry was an assembly of four carbon rods (6 mm in diameter and 4 cm in length) and a large platinum gauze constituted the counter electrode. The working electrode potentials were measured vs an Ag/AgCl reference electrode (all electrodes were from the AZAR electrode). All chemicals (mefenamic acid, glutathione, and *N*-acetyl-*L*-cysteine) were reagent-grade materials from Sigma-Aldrich Co., and phosphate salts and other inorganic salts were of pro-analysis grade from Merck & Co. These chemicals were employed without any further purification.

Synthesis of compounds 4 and 4′; Electroorganic synthesis of 4 and 4′.—A mixture of a phosphate buffer (ca. 50 ml; c = 0.2 M, pH = 7.0) in water/ethanol (30:70 v/v) solution, containing mefenamic acid (**1**) (0.02 mmol) and glutathione (0.3 mmol) (**3**) or *N*-acetyl-L-cysteine (**3′**) was electrolyzed in a divided cell at 0.6 V vs Ag/AgCl. The electrolysis was terminated when the current decreased by more than 95%. After having finished the electrolysis, the precipitated solid was collected by filtration and washed several times with water. The products were characterized by infrared spectroscopy,[7] mass spectroscopy, and melting point measurements.

Characterization of product 4.—Mp > 238 °C (Dec.). $IR_{(KBr)}$: 3030, 2917, 1622, 1586, 1488, 1382, 1297, 1194, 1091, 847, 778, and 675 cm^{-1}. MS (EI): *m/z* (relativeintensity): 241 ($M^{+\cdot}$, 8.1.1), 169.1 (32.4), and 147.2 (100).



Characterization of product 4′.—Mp > 70 °C (Dec.). $IR_{(KBr)}$: 3443, 2924, 2853, 2431, 1653, 1387, 1298, 1131, 958, and 511 cm^{-1}.



Results and Discussion

Voltammetric study.—The electrochemical study of the 0.4 mM solution of mefenamic acid (**1**) in a phosphate buffer solution

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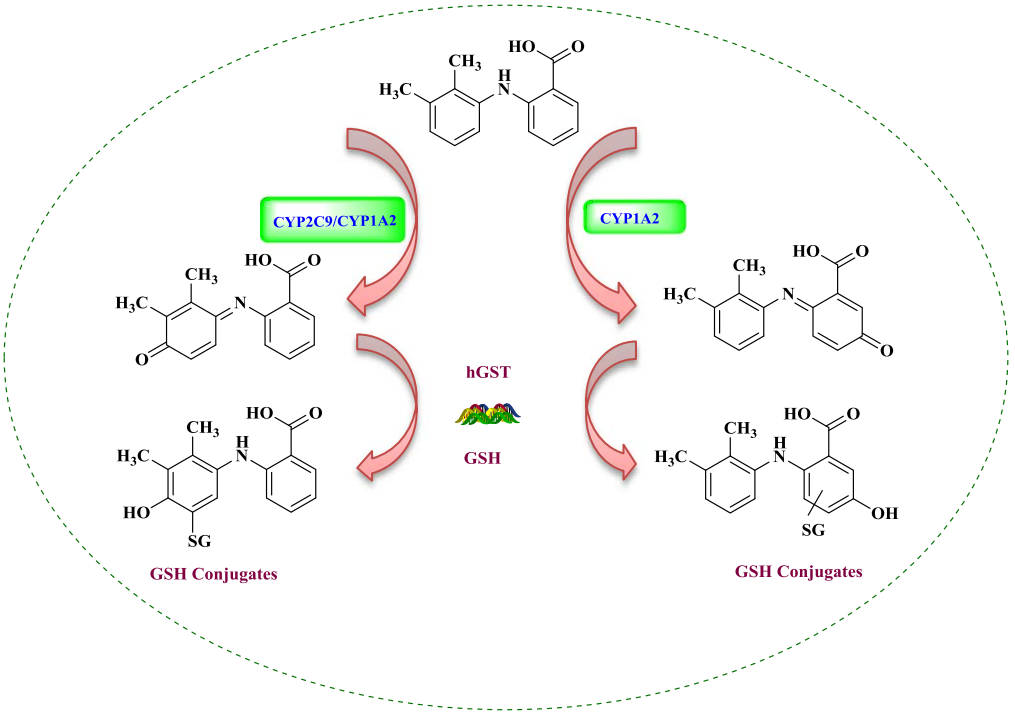


Figure 1. All of the pathways in oxidative bioactivation of the MFA using the human liver microsomes (HLM) and the recombinant human P450 enzymes [5].

(c = 0.2 M, pH 7.0), was carried out on a glassy carbon electrode using cyclic voltammetry (Fig. 2, I). This voltammogram shows one anodic peak (A_1) in the positive-going scan at 0.63 vs Ag/AgCl, which corresponds to the oxidation of **1** to radical cation (**2**). This radical cation is very unstable in the chemical reactions and produces some electro-inactive components in the studied potential range, leading to the irreversible electrochemical oxidation of **1**.[11,12]

The electrochemical oxidation of mefenamic acid (**1**) was studied at various scan rates to obtain further information. The normalized cyclic voltammograms of **1** in different scan rates are shown in Fig. 2.II. The current on the square-root of the scan rate ($I/v^{1/2}$ μA $s^{1/2}$ $mV^{-1/2}$) was divided to carry out the normalization. As can be seen in Fig. 2, II, proportional to the augmentation of potential sweep rate, the peak potential of peak A_1 (E_p^{A1}) shifted to the

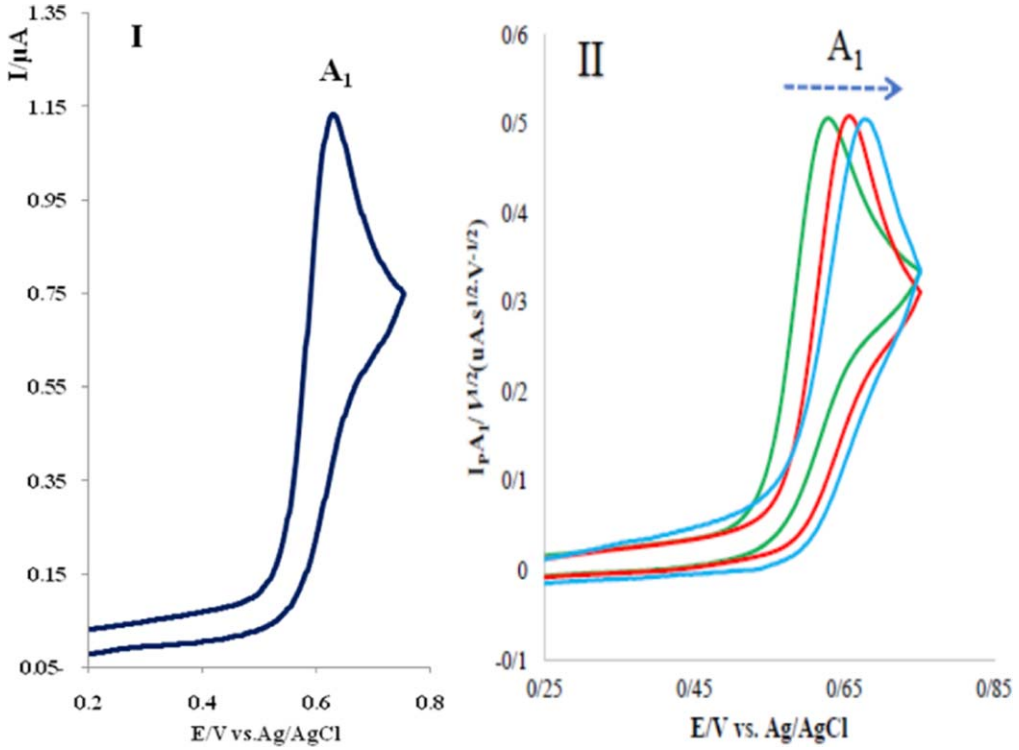


Figure 2. I: Cyclic voltammogram of the 0.4 mM mefenamic acid in the scan rate 5 mV s^{-1}. II: Normalized cyclic voltammograms of 0.4 mM mefenamic acid in the scan rate (a) 5 mV s^{-1} (b) 25 mV s^{-1} (c) 100 mV s^{-1}, on a glassy carbon electrode, in a phosphate buffer solution (c = 0.2 M, pH 7.0), t = 25 ± 1 °C.

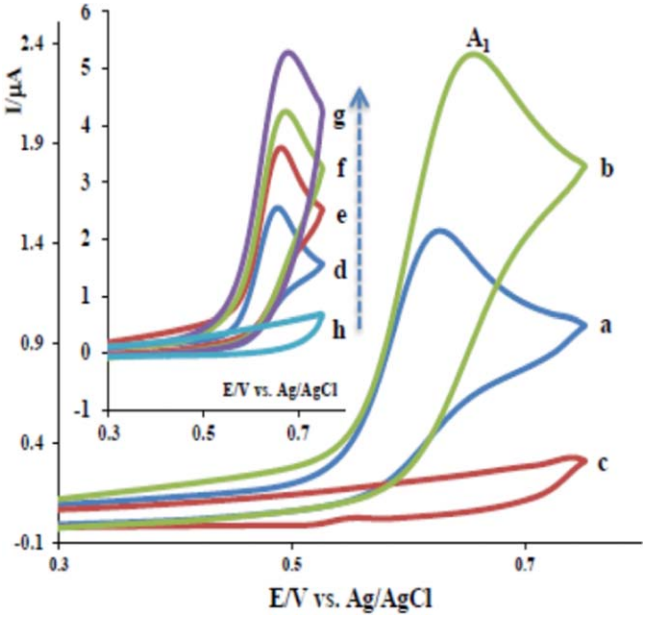


Figure 3. Cyclic voltammograms of the 0.4 mM mefenamic acid: (a) in the absence; (b) in the presence of 1.2 mM glutathione, and (c) cyclic voltammograms of the 0.4 mM glutathione in the absence of **1** in scan rate 10 mV S^{-1}, Inset: Typical cyclic voltammograms of the 0.4 mM mefenamic acid in the presence of various concentrations of the glutathione. The concentrations of glutathione from(d) to (g) are: 0, 0.4, 1.2, and 2 mM, respectively, (h) the 0.4 mM glutathione in the absence of **1** on a glassy carbon electrode, in a phosphate buffer solution (c = 0.2 M, pH 7.0); scan rate: 25 mV s^{-1}; t = 25 ± 1 °C.

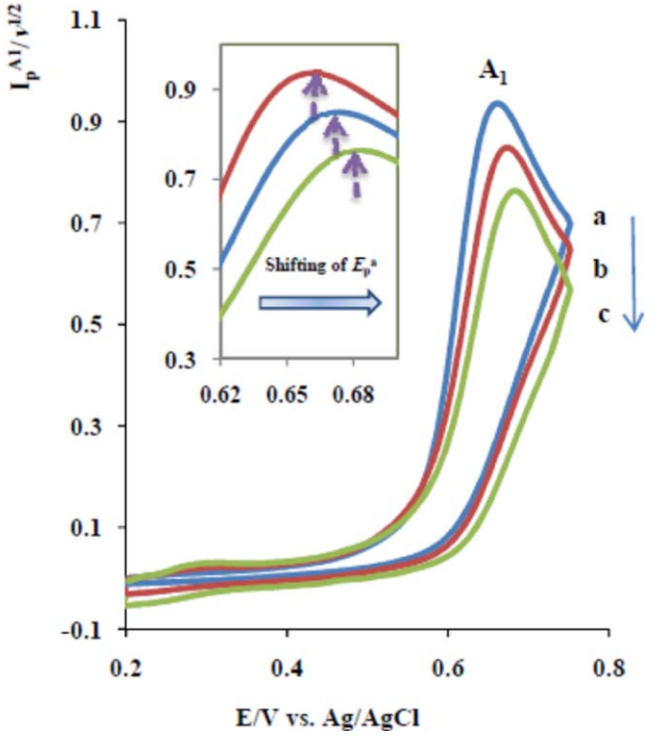


Figure 4. Normalized cyclic voltammograms ($I_p^{A1}/v^{1/2}$) of the 0.4 mM mefenamic acid in the presence of 1.2 mM glutathione. Scan rates from (a) to (c): 5, 25, and 100 mV s^{-1} on a glassy carbon electrode, in a phosphate buffer solution (c = 0.2 M, pH 7.0), t = 25 + 1 °C.

positive potential by scan rate. The increase in E_p^a indicates that the electrochemical oxidation of mefenamic acid (**1**) in these conditions was an irreversible reaction.[11,12]

The electrochemical oxidation of a 0.4 mM solution of **1** in the presence of 1.2 mM glutathione (**3**) in a phosphate buffer solution (c = 0.2 M, pH 7.0) was investigated on a glassy carbon electrode in some detail (Fig. 3. curve b). Under these conditions, the anodic peak current (A_1) increased, and the peak potential (E_p^{A1}) shifted to the positive potential.

Numerous studies were conducted by varying the concentration of the MFA during the cyclic voltammetric experiments of the MFA in the presence of **3**. As shown in Fig. 3, inset, the current of peak A1 (I_p^{A1}) sharply increased with the concentration of **3** increasing.

The potential scan rate is a useful experimental parameter in the cyclic voltammetric method and can be applied to control τ, a measure of the period during which a stable electroactive species can interact with the electrode. Indeed, with increasing the scan rate, this factor (τ) is limited compared to t, where t is the characteristic lifetime of a coupled chemical reaction. This manner was completely confirmed by plotting the variation $I_p^A/v^{1/2}$ vs the potential scan rate (Fig. 4). Under the above conditions, the plot of $I_p^A/v^{1/2}$ vs the potential scan rate is a characteristic shape typical of an $E_rC'_I$ mechanism established by Nicholson and Shain.[13]

Furthermore, several controlled-potential coulometry experiments were performed in an aqueous solution containing 0.02 mmol of **1** in the presence of a different amount of **3** 0.02, 0.1, 0.2, 0.3 and 0.4 mmol at 0.6 V vs Ag/AgCl to investigate the mechanism of the electrooxidation of the MFA in the presence of **3**. The cyclic voltammetric analysis was carried out to monitor the electrolysis progress (Fig. 5, I).

The results show that proportional to the progress of coulometry, the anodic peak (A_1) decreased and disappeared when the charge consumption was about 24, 33, 44, 52 and 66 coulombs respectively. The calculated apparent number of electrons (n_{app}) consumed per molecule of the MFA indicated a linear relationship between it and the mmols of **3** (Fig. 5, II). As can be seen in Fig. 5 II, the amount of the consumed electrons increased with the increasing of **3** values.

On the other hand, the number of n_{app} for **1** was calculated to be equal to 11 electrons at zero concentration of **3** (Fig. 5, II). The difference in the time scales of the cyclic voltammetry and coulometry methods is considered to be the reason for the inequality of consumed electrons in these two techniques. In fact, under coulometric conditions, there is enough time to produce side reactions with low rates. The results of the coulometry experiments revealed that in these conditions the catalytic mechanism was predominant over the Michael addition mechanism. Finally, according to the data obtained from the cyclic voltammetry and coulometry experiments and the IR spectrum[7] and melting points[7] obtained from the electrolysis product; an EC' (catalytic) mechanism between the produced intermediates in the oxidation of **1** and **3** was proposed for the final product (Scheme 1).

Based on the results, the glutathione (**3**) reacted with the electrochemically generated radical cation of the MFA. This reaction converted **3** into cystine (cy-cy)[14] (4) (Scheme 2). However, when comparing our results to those of older studies, which proposed the formation of a GH-mefenamic acid adduct as the main product in the oxidation of mefenamic acid (**1**) in the presence of glutathione (3), it must be pointed out that the catalytic reaction between electrochemically generated compounds of **1** and **3** is the primary and dominant reaction.

Next, the electrochemical oxidation of 1 in the presence of N-acetyl-*L*-cysteine (3′) was investigated. *N*-acetyl-*L*-cysteine (3′) is a sulfhydryl compound that acts as an alternative substrate for the reaction with the toxic metabolite.[8–10] Thus, it can be used as an antidote of choice for the overdose toxicity of the MFA. Figure 3I shows a typical voltammetric curve for the electrooxidation of the

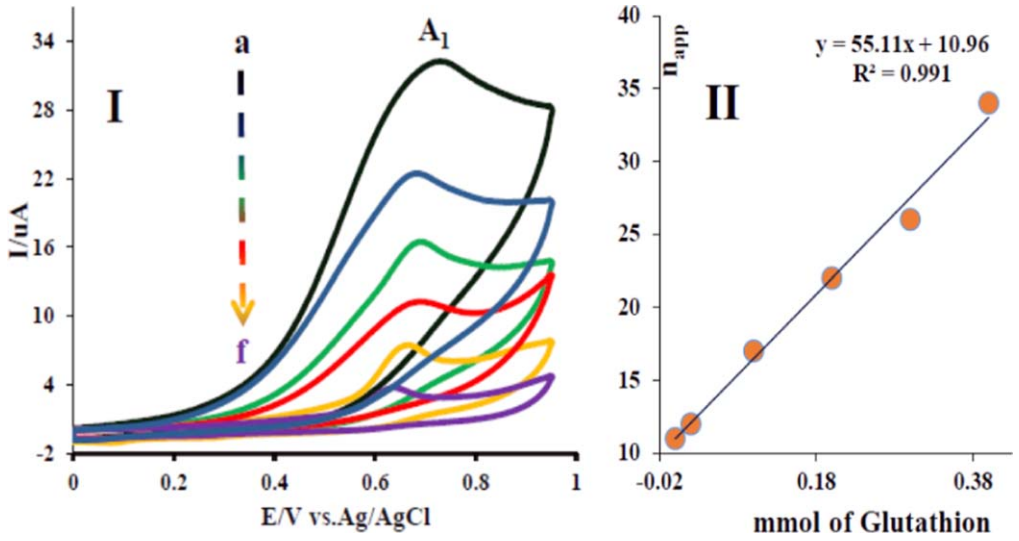
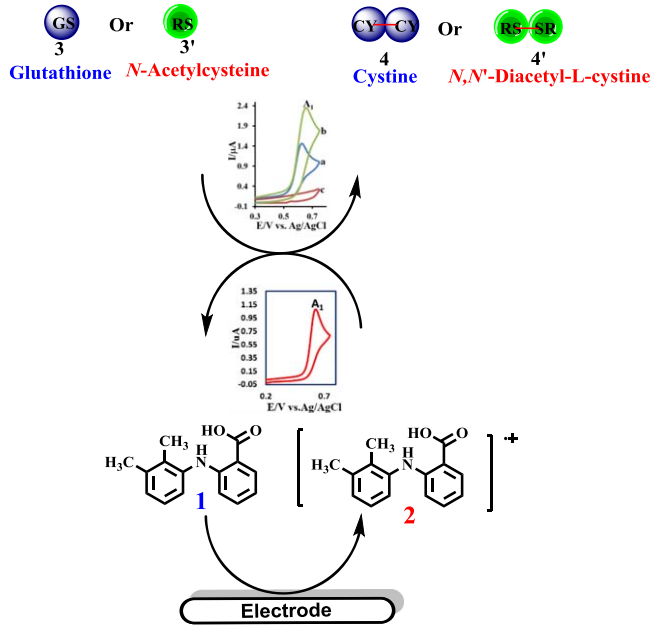


Figure 5. (I) Cyclic voltammograms of 0.02 mmol **1** in the presence of 0.3 mmol **3**, during controlled-potential coulometry at 0.6 V vs Ag/AgCl. After consumption of: (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, and, (f) 50 C. Scan rate 100 mV s^{-1}. (II) Variation of apparent number of electrons (n_{app}) vs the mmol of the glutathione during the controlled-potential coulometry of 0.02 mmol of **1** in the presence of **3** (0.02, 0.1, 0.2, 0.3, and 0.4 mmol).

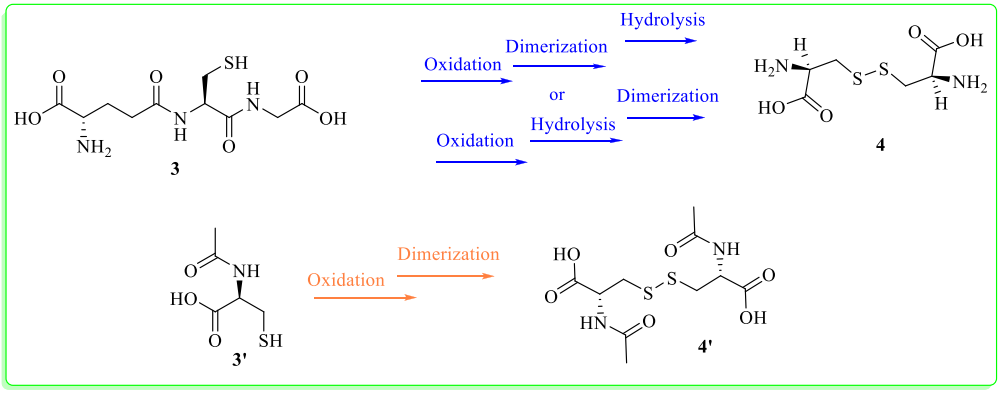


Scheme 1. Proposed mechanism for the electrochemical oxidation of the mefenamic acid in the presence of the glutathione and the *N*-Acetyl-L-Cysteine.

0.4 mM solution of **1** in the absence and presence of 0.4 mM *N*-acetyl-*L*-cysteine (**3′**). In these conditions, all of the observations such as increasing the IpA1 in the presence of *N*-acetyl-*L*-cysteine (**3′**) and the dependency of the I_p^{A1} on the concentration of **3′** (Fig. 3, II) were similar to those in the electrooxidation of **1** in the presence of **3**.

Having analyzed the results from the electrochemistry (cyclic voltammetry and controlled-potential coulometry), spectroscopy (the FT-IR data of the final product), and the melting point of the final product demonstrated that the overall reaction mechanism of the electrooxidation of **1** in the presence of **3′** was the electrocatalytic mechanism (*EC′*) (Scheme 1). Accordingly, the electrochemical oxidation of **1** in the presence of *N*-acetylcysteine (RSH) (**3′**) with a catalytic reaction resulted in the formation of *N,N'*-Diacetyl-*L*-cystine (RSSR) (**4′**). The catalytic oxidation of **3′** and the formation of RSSR applied the other redox systems that have been previously reported.[14] The comparison of the current of the peak A_1 (I_p^{A1}) in the presence of *N*-acetyl-*L*-cysteine (**3′**) (Fig. 6, curve b) with that of the glutathione (**3**) (Fig. 6, curve c) shows an increase in the current of peak A_1 (I_p^{A1}) in the presence of glutathione (**3**), which indicates that the electrochemical reaction rate of **3′** with **1** is more than that of **3**.

The existence of an *EC′* mechanism in the electrooxidation of **1** in the presence of **3** and **3′** is supported by the following evidence: (a) The dependency of the anodic peak current (I_p^{A1}) on the glutathione and *N*-Acetyl-L-Cysteine concentrations in the cyclic



Scheme 2. Proposed mechanism for the electrochemical oxidation of the glutathione and the *N*-Acetyl-L-Cysteine.

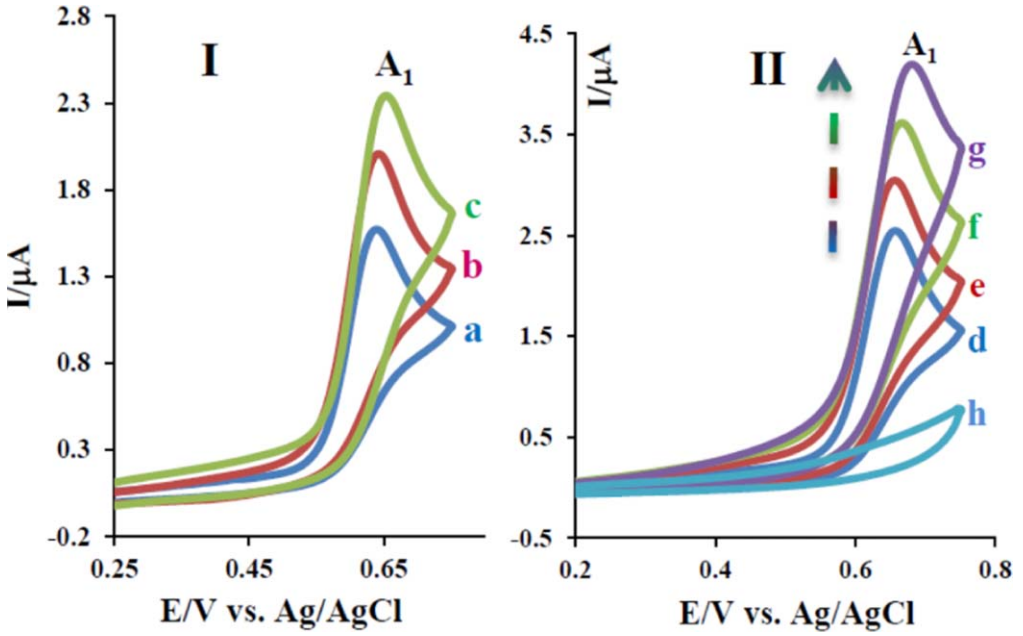


Figure 6. (I) Cyclic voltammograms of the 0.4 mM mefenamic acid: (a) in the absence; (b) in the presence of the 0.4 mM *N*-Acetyl-*L*-Cysteine and (c) in the presence of the 0.4 mM glutathione, scan rate: 10 mV S^{-1}. (II) Cyclic voltammograms of the 0.4 mM mefenamic acid in the presence of different concentrations of the *N*-Acetyl-L-Cysteine. The concentrations of (d) to (g) are: 0, 0.4, 1.2, and 2 mM, respectively and (h) the 0.4 mM *N*-Acetyl-L-Cysteine in the absence of the mefenamic acid on a glassy carbon electrode, in a phosphate buffer solution (c = 0.2 M, pH 7.0); scan rate: 25 mV S^{-1}.

voltammetric experiments of **1** in the presence of **3** and **3′**. (b) The dependency of n_{app} on the glutathione and *N*-acetyl-L-cysteine concentrations in the controlled-potential coulometric experiments of **1** in the presence of **3** and **3′**.

Conclusions

In an earlier paper, the oxidative bioactivation of mefenamic acid (**1**) using the human liver microsomes (HLM) and the recombinant human P450 enzymes was performed, and a pathway for the Michael-type reaction of these toxic compounds with the glutathione was reported.[5] Nevertheless, the results in this work indicate that these toxic compounds participate in a catalytic reaction with **3** and **3′**. In other words, the electrocatalytic mechanism (*EC′*) between electrochemically generated compounds of **1** and **3** is the main and dominant reaction.

Acknowledgments

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References

1. L. Bjarnason and A. J. Macpherson, *Pharmacol. Ther.*, **62**, 145 (1994).
2. A. Kamour, S. Crichton, G. Cooper, D. J. Lupton, M. Eddleston, J. A. Vale, J. P. Thompson, and S. H. L. Thomas, *Br. J. Clin. Pharmacol.*, **83**, 855 (2017).
3. I. Bjarnason, J. Hayllar, A. J. MacPherson, and A. S. Russell, *Gastroenterology.*, **104**, 1832 (1993).
4. M. P. Grillo, M. T. Lohr, and J. C. M. Wait, *Drug Metab. Dispos.*, **40**, 1515 (2012).
5. H. Venkataraman, M. W. den Braver, N. P. E. Vermeulen, and J. N. M. Commandeur, *Chem. Res. Toxicol.*, **27**, 2071 (2014).
6. S. Muraoka and T. Miura, *Life Sci.*, **72**, 1897 (2003).
7. H. Shayani-Jam and D. Nematollahi, *Chem. Commun.*, **46**, 409 (2010).
8. M. Ramen T, *Med. Chem.*, **5**, 037 (2015).
9. S. Whillier, J. E. Raftos, B. Chapman, and P. W. Kuchel, *Redox Rep.*, **14**, 115 (2009).
10. S. C. Lu, *Biochim. Biophys. Acta*, **1830**, 3143 (2013).
11. J. Bard and L. R. Faulkner, *Electrochemical Methods* (Wiley, New York) 2nd ed., p. 523 and p. 50 (2001).
12. F. Varmaghani and D. Nematollahi, *J. Phys. Org. Chem.*, **25**, 511 (2012).
13. R. S. Nicholson and I. Shain, *Anal. Chem.*, **37**, 178 (1965).
14. B. Särnstrand, A.-H. Jansson, G. Matuseviciene, A. Scheynius, S. Pierrou, and H. Bergstrand, *J. Pharmacol. Exp. Ther.*, **288**, 1174 (1999), http://jpet.aspetjournals.org/content/288/3/1174.short.