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## THE QUALITATIVE DETERMINATION OF OSELTAMIVIR PHOSPHATE IN TAMIFLU<sup>®</sup> CAPSULE BY CYCLIC VOLTAMMETRY

*A gold electrode was used in the voltammetric determination of oseltamivir phosphate standard in 0.05 M NaHCO<sub>3</sub>. Oseltamivir phosphate as a standard and as a component of Tamiflu<sup>®</sup> capsule exhibited the identical cyclic voltammogram. The peaks originating from excipients in the capsule did not appear under the applied electrochemical conditions. An electrochemical method for the qualitative determination of oseltamivir phosphate in Tamiflu<sup>®</sup> capsules by cyclic voltammetry was developed. The presence of oseltamivir phosphate as standard and as a content of Tamiflu<sup>®</sup> capsule in electrolyte as well as their concentrations were simultaneously checked by HPLC. The lack of the current/concentration dependency was established. The non-pretreated glassy carbon electrode cannot be used for the determination of oseltamivir phosphate under identical experimental conditions presented for the gold electrode.*

It is known that antivirals are valuable supplementation to vaccines for the control and prevention of influenza [1], and are likely to be active against a new pandemic variant [2]. Oseltamivir phosphate (Figure 1) is the best known orally active neuraminidase inhibitor antiviral drug [3,4] that slows the spread of influenza virus between cells in the body by stopping the virus from chemically cutting ties with its host-cell median time to symptom alleviation is reduced by 0.5–1 days [5]. The neuraminidase inhibitors are effective against both influenza A and B and are considered less toxic and less likely to promote development of drug-resistant influenza than adamantanes [6].

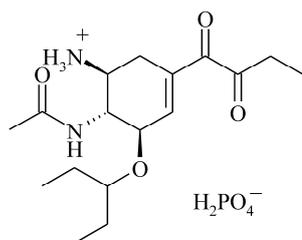


Figure 1. Structural formula of oseltamivir phosphate.

Oseltamivir phosphate can be identified by thin-layer chromatography, specific optical rotation, infrared spectrophotometry and tests characteristic for orthophosphates [7]. Determination, by International Pharmacopeia, can be done by high-performance liquid chromatography [7,8] or by titration with perchloric acid [7].

Other methods include capillary electrophoresis [9], spectrofluorometry [10], micellar electrokinetic chromatography [11], colorimetric [12] and liquid chromatographic methods [12,13] as well as LC–MS–MS [14]. The voltammetric determination and electrochemical behavior of oseltamivir phosphate as well as its determination in Tamiflu<sup>®</sup> capsules have not been published.

The aim of the present study was the qualitative voltammetric determination of oseltamivir phosphate as standard and in Tamiflu<sup>®</sup> capsules. Gold and glassy carbon electrodes were tested as working electrodes under identical experimental conditions using 0.05 M NaHCO<sub>3</sub> (pH 8.4) as a supporting electrolyte. During the voltammetric investigation of oseltamivir phosphate standard and oseltamivir phosphate in the Tamiflu<sup>®</sup> capsules, the presence of oseltamivir phosphate was confirmed by the simultaneous HPLC determination.

### EXPERIMENTAL

#### Materials

Oseltamivir phosphate has not been officially approved for use according to pharmacopeia and consequently, it is not possible to order the reference EDQM standard. Oseltamivir phosphate (Roche), kindly provided by the Medicines and Medical Devices Agency of Serbia (Belgrade, Serbia) was used as a pure substance without further purification. A comparative experiment was performed with capsulated oseltamivir phosphate (30 mg) marketed by Roche as Tamiflu<sup>®</sup>, which, in addition to oseltamivir contained the following excipients: talk, povidon, sodium stearyl fumarate, corn starch and sodium croscarmellose. We dissolved 25 mg of oseltamivir phosphate in 10 cm<sup>3</sup> of 0.05 M NaHCO<sub>3</sub> (stock solution) and added into electrolyte in equal aliquots starting from 1 cm<sup>3</sup>. 25 mg of Tamiflu<sup>®</sup> was dissolved in

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solution containing  $9 \text{ cm}^3$  of  $0.05 \text{ M NaHCO}_3$  and  $1 \text{ cm}^3$   $\text{CH}_3\text{OH}$  (stock solution) and added into electrolyte in equal aliquots starting from  $1 \text{ cm}^3$ .  $\text{CH}_3\text{OH}$  and  $\text{NaHCO}_3$  were of analytical grade (Merck). The supporting electrolyte,  $0.05 \text{ M NaHCO}_3$  was prepared with  $18 \text{ M}\Omega$  deionised water.

### Apparatus

Standard equipment was used for the cyclic voltammetry measurements using a three electrode electrochemical cell, as previously described in detail [15–17]. Polycrystalline gold (surface area  $0.500 \text{ cm}^2$ ) and glassy carbon Sigmadur GMBH Germany (surface area  $0.29 \text{ cm}^2$ ) which served as the working electrodes were prepared as earlier [15–17]. A gold wire was used as the counter electrode and a saturated calomel electrode as the reference electrode. All the potentials are given *versus* SCE. The electrolyte was deoxygenated by purging with nitrogen.

The characteristics of the HPLC instrument are already described in detail [15–17] and the HPLC method performed during the simultaneous electrochemical experiment for oseltamivir determination has been published by Lindegardh *et al.* [14].

### RESULTS AND DISCUSSION

In our study of electrochemical behavior of oseltamivir phosphate and development of the appropriate method, a solution of oseltamivir phosphate standard was prepared as described in the Experimental part and used in the cyclic voltammetry measurements. In the first part of the work the gold electrode was used. The cyclic voltammogram of the clean gold electrode of oseltamivir phosphate ( $0.025 \text{ mg cm}^{-3}$ ) is presented in Figure 2. Simultaneous HPLC analysis of the bulk of electrolyte confirmed that  $0.025 \text{ mg cm}^{-3}$  oseltamivir phosphate is presented in the electrolyte. Starting from  $-1.2 \text{ V}$  one can observe an apparent reversible oxidative/reductive reaction between  $-0.5$  and  $-0.7 \text{ V}$ . In the anodic direction, the anodic current increases from  $0.4 \text{ V}$  and reaches the maximum starting from the area of the oxides formation. In the entire region of oxides formation at the gold electrode, the oxidative activity of oseltamivir phosphate is obvious. The lowering of the oxides reduction currents can also be noticed from the cyclic voltammogram. Starting from  $-0.8 \text{ V}$  the exact same reactions were observed as it was obtained starting from  $-1.2 \text{ V}$ , and as it is presented in Figure 3. The potential was cycled continuously for two hours and the cyclic voltammogram was quite stable in both cases. Holding the potential for  $10 \text{ min}$  at the peak potentials and in the area of oxide formation did not affect the voltammogram even in the first sweep. The cyclic voltammogram of oseltamivir phosphate at the gold electrode in  $0.05 \text{ M NaHCO}_3$  (Figures 2 and 3) is quite reproducible.

With the addition of the next two aliquotes of stock solution of oseltamivir phosphate containing  $2.5 \text{ mg cm}^{-3}$ , the linearity of the current–concentration dependency was not observed, and even the voltammogram remained the same. With the simultaneous HPLC analysis of the bulk of electrolyte, the presence of the added concentrations was confirmed.

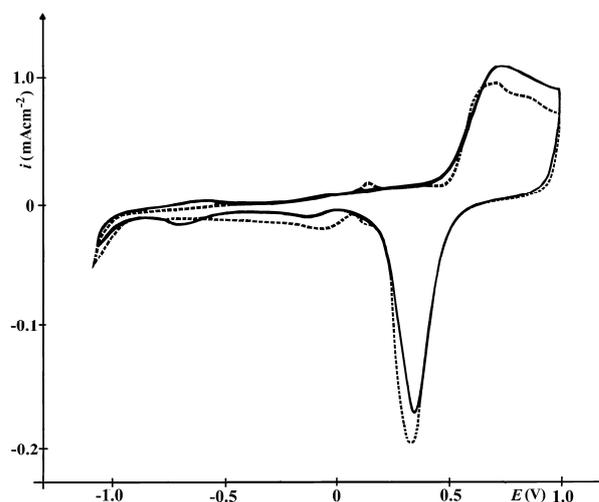


Figure 2. Cyclic voltammogram of the clean gold electrode in  $0.05 \text{ M NaHCO}_3$  (dash line) and in the presence of  $0.025 \text{ mg cm}^{-3}$  oseltamivir phosphate (full line) in the area of the potential from  $-1.2$  to  $1.0 \text{ V}$ , sweep rate  $50 \text{ mV/s}$ .

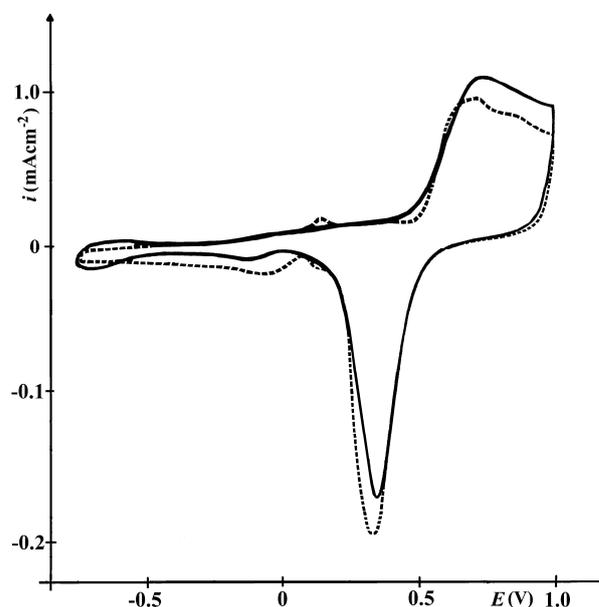


Figure 3. Cyclic voltammogram of the clean gold electrode in  $0.05 \text{ M NaHCO}_3$  (dash line) and in a presence of  $0.025 \text{ mg cm}^{-3}$  oseltamivir phosphate (full line) in the area of the potential from  $-0.8$  to  $1.0 \text{ V}$ , sweep rate  $50 \text{ mV/s}$ .

Additionally, the glassy carbon electrode was tested as a working electrode in the same manner of the electrode cleaning and under identical experimental condi-

tions as it was presented for the gold electrode. Starting from  $-1.2$  V as well as from  $-0.8$  V in the presence of  $0.025$  mg cm<sup>-3</sup> oseltamivir phosphate the cyclic voltammogram of glassy carbon electrode was the same as without it. The non-pretreated glassy carbon electrode cannot be used as the working electrode.

In addition, the determination of oseltamivir phosphate in Tamiflu<sup>®</sup> capsules was performed at the gold electrode. The cyclic voltammograms of the gold electrode in a presence of  $0.025$  mg cm<sup>-3</sup> Tamiflu<sup>®</sup> were surprisingly the same as given for  $0.025$  mg cm<sup>-3</sup> of oseltamivir phosphate standard (Figures 2 and 3). The cyclic voltammogram of Tamiflu<sup>®</sup> also remain stable during the cycling. With the addition of the next two aliquots of stock solution of Tamiflu<sup>®</sup> the voltammogram remains the same and with the simultaneous HPLC analysis of the bulk of electrolyte, the presence of the added concentrations was confirmed.

The cyclic voltammograms of Tamiflu<sup>®</sup> are quite reproducible. According to identical cyclic voltammograms of oseltamivir phosphate standard (Figures 2 and 3) and Tamiflu<sup>®</sup> capsule content it is evident that oseltamivir phosphate can be qualitatively determined in capsule Tamiflu<sup>®</sup> at gold electrode in  $0.05$  M NaHCO<sub>3</sub>.

The excipients presented in Tamiflu<sup>®</sup> capsules: talk, sodium stearil fumarate, corn starch and sodium croscarmellose are insoluble in water [18]. Besides oseltamivir phosphate, only povidon is soluble in water, but it is obvious that it is not electroactive under the conditions presented in Figure 2. The excipients were separately tested in our previous investigations [15–17,19] concerning commercial pharmaceutical compounds. The present excipients did not affect the electroactivity of oseltamivir phosphate in Tamiflu<sup>®</sup> capsule. It is qualitatively determined as the component of the capsule Tamiflu<sup>®</sup> Roche in the same way as the pure standard. The qualitative determination of oseltamivir phosphate as standard and in Tamiflu<sup>®</sup> capsule in any case should not be compared with our previously published results concerning qualitative determination of macrolide antibiotics [15–17] obtained under the same experimental conditions. Azithromycin, clarithromycin and erythromycin are strongly dependent on the starting potential  $-1.2$  V and with the starting potential  $-0.8$  V, they are completely inactive on the gold electrode. The peak potentials, peak height and peak reversibility are quite different for each antibiotic standard as well as for oseltamivir phosphate standard. Antibiotics in capsules and tablets in the presence of the excipients exhibit significantly different electrochemical behavior and consequently the different cyclic voltammograms comparing to their standards. Only oseltamivir phosphate exhibits the quite same cyclic voltammogram as a standard and as a content of Tamiflu<sup>®</sup> capsule.

## CONCLUSION

In conclusion, it should be pointed out that a simple and fast voltammetric method for the qualitative determination of oseltamivir phosphate was developed and applied for the qualitative determination of oseltamivir phosphate in Tamiflu<sup>®</sup> capsules. Starting from  $-1.2$  V as well as from  $-0.8$  V, an apparent reversible oxidative/reductive reaction between  $-0.5$  and  $-0.7$  V occurs. In the anodic direction, the anodic current increases from  $0.4$  V and reaches a maximum starting from the area of the oxides formation. In the entire region of the oxides formation at the gold electrode this maximum oxidative currents of oseltamivir phosphate remain unchanged. The lowering of the oxides reduction currents is also noticed. The identical cyclic voltammograms of oseltamivir phosphate standard and of Tamiflu<sup>®</sup> capsule content showed that oseltamivir phosphate is qualitatively determined in Tamiflu<sup>®</sup> capsule content at the gold electrode in  $0.05$  M NaHCO<sub>3</sub>. The glassy carbon electrode cannot be used as working electrode and did not exhibited any affinity to the oxidative/reductive reactions of oseltamivir phosphate starting from  $-1.2$  V as well as from  $-0.8$  V.

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**IZVOD****KVALITATIVNO ODREĐIVANJE OSELTAMIVIR-FOSFATA U TAMIFLU<sup>®</sup> KAPSULAMA POMOĆU CIKLIČNE VOLTAMETRIJE**Milka L. Avramov Ivić<sup>1</sup>, Slobodan D. Petrović<sup>2,3</sup>, Dušan Ž. Mijin<sup>2</sup>, Katica M. Drljević-Đurić<sup>4</sup><sup>1</sup>IHTM – Centar za Elektrohemiju, Univerzitet u Beogradu, Beograd<sup>2</sup>Tehnološko-metalurški fakultet, Univerzitet u Beogradu, Beograd<sup>3</sup>Hemofarm Stada, Farmaceutsko hemijska industrija, Vršac<sup>4</sup>Agencija za lekove i medicinska sredstva Srbije, Beograd

(Naučni rad)

Aktuelna ispitivanja lekova zahtevaju razvoj brzih metoda za prepoznavanje standarda lekova u komercijalnim kapsulama i tabletama. U opsegu potencijala od  $-1,2$  V kao i od  $-0,8$  V do  $1,0$  V oseltamivir-fosfat kao standard i sastojak Tamiflu<sup>®</sup> kapsule podleže oksidativno-reduktivnoj reakciji između  $-0,5$  i  $-0,7$  V kao i anodnoj reakciji počevši od  $0,4$  V, sa platomom struja uočenih u celom opsegu potencijala formiranja oksida na elektrodi od zlata. Oseltamivir-fosfat kao standard i kao komponenta Tamiflu<sup>®</sup> kapsule snižava struje redukcije oksida na zlatu. Držanje potencijala na vrednostima pojave pikova ne utiče na promenu cikličnog voltamograma, tj. na navedene reakcije. Cikliziranje potencijala u toku više časova ne dovodi do promene cikličnog voltamograma u oba opsega potencijala. Ciklični voltamogram za koncentraciju oseltamivir-fosfata kao standarda i kao sastojka Tamiflu<sup>®</sup> kapsule od  $0,025$  mg cm<sup>-3</sup> bio je identičan i po dodatku naredne dve koncentracije pa se na elektrodi od zlata ne može ispitivati i koncentraciona zavisnost u  $0,05$  M NaHCO<sub>3</sub>. Koncentracije leka u elektrolitu su potvrđene simultanom HPLC analizom prema aktuelnoj farmakopeji. Ekscipijenti prisutni u Tamiflu<sup>®</sup> kapsuli nisu ni na koji način uticali na ponašanje oseltamivir-fosfata kao standarda, tj. neaktivni su pod primenjenim eksperimentalnim uslovima. Elektroda od staklastog ugljenika je takođe testirana u oba opsega potencijala i pod identičnim eksperimentalnim uslovima je potpuno neaktivna, za razliku od elektrode od zlata, u prisustvu oseltamivir-fosfata kao standarda i kao sastojka Tamiflu<sup>®</sup> kapsule. Elektroda od zlata se u navedenim opsezima potencijala koristeći  $0,05$  M NaHCO<sub>3</sub> kao elektrolit i pod navedenim elektrohemijskim uslovima može efikasno koristiti za kvalitativno određivanje oseltamivir-fosfata u Tamiflu kapsuli.

Ključne reči: Oseltamivir-fosfat • Tamiflu<sup>®</sup> • Kapsula • Ciklična voltametrija • HPLC

Key words: Oseltamivir phosphate • Tamiflu<sup>®</sup> • Capsule • Cyclic voltammetry • HPLC