ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2017, *62*(6-7), 579-586

DIFFERENTIAL PULSE VOLTAMMETRIC DETERMINATION OF ROSUVASTATIN *VIA* GLASSY CARBON ELECTRODE

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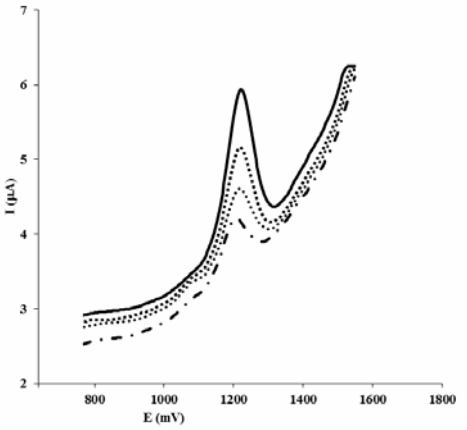
Received November 18, 2016

In this work, the electrochemical behavior of Rosuvastatin which is a member of the drug class of statins used to treat high cholesterol and related conditions, and to prevent cardiovascular disease, was investigated with in a wide pH range (pH 0.3-7.0) using differential pulse voltammetric technique at glassy carbon electrode. All the parameters effecting the differential pulse, were optimized, and with differential pulse voltammetric techniques, the maximum peak current was observed in the 0.1 M sulfuric acid medium. Scan rate studies were also conducted using cyclic voltammetric technique, in the 0.1 M sulfuric acid medium, and the electrochemical reaction of rosuvastatin was found as diffusion-controlled mechanism. Linear relation range specified in the range of 0.005-0.1 mM and limit of detection calculated as 1.33 nM. In optimized conditions, the proposed method was applied for the determination of rosuvastatin from pharmaceutical dosage forms named, ROSUCOR® and REAKT®.



INTRODUCTION

Rosuvastatin, (ROS, bis((E)-7-(4-(4-flurophenyl)-6-1sopropyl-2-(methyl (methylsulfonyl) amino) pyrmidin-5yl) (3R,5S) -3,5-dihydroxyhept-6-enoic acid, figure 1) calcium salt), is a cholesterol lowering active pharmaceutical ingredient of statin group. Statins are the most effective cholesterol lowering drug group. By blocking the production of HMG - CoA enzyme used to make the body's cholesterol, statins reduce the synthesis of cholesterol in the liver. ROS is one of the most effective drugs in the statin group of drugs. ROS has structural resemblances with most other statins, such as atorvastatin, cerivastatin and pitavastatin. ROS lowers total cholesterol, LDL, triglycerides and apolipoptoein B, while raising HDL. ROS in the prevention of atherosclerosis, hypercholesterolemia, hyperproteinemia, hypertriglyceridemia and uses of myocardial infarction prophylaxis and stroke prophylaxis.[1-7]

Electroanalytical methods emerge with the interplay between electricity and chemistry; in other words, they were used to measure electrical quantities, such as current, potential, or charge and their relationship with the chemical parameters. These methods are widely used in fields like environmental monitoring, industrial quality control or biomedical analysis.[8-12] Another field in which electrochemical methods are extensively used is drug analysis and these methods have proved to be highly sensitive due to the straight forwardness, low cost and relatively short analysis time.[12-15]

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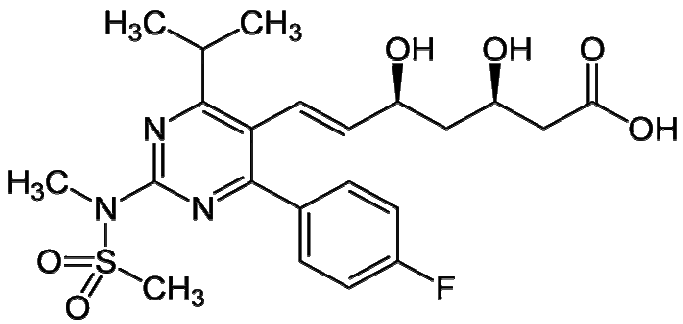

Fig. 1 – Chemical Structure of Rosuvastatin.

Carbon-based working electrodes suggest a much wider potential range than metal electrodes.[13, 16-17] Through carbon-based working electrodes glassy carbon electrode (GCE), the hardest non-diamond carbon electrode material base, is the most common carbon-based electrode because of its excellent mechanical and electrical properties, wide potential range, chemically inert nature and impermeability to gases.[13, 16-17] They found place in many different applications areas, such as direct electron transfer studies,[18] behavior of nanomaterials,[19,20] especially in drug analyses[21-24] since their performances are relatively reproducible.[16-23]

In the literature there exist some studies related to rosuvastatin determination using spectrophotometric methods,[25-27] chromatographic methods,[28-30] electrochemical method with different electrodes and materials such as dropping mercury electrode,[31] vertically aligned carbon nanotubes,[32] cathodically pretreated boron-doped diamond electrode,[33] In this work, the electrochemical behavior of Rosuvastatin was investigated using differential pulse voltammetric technique at glassy carbon electrode. In a wide pH range (pH 0.3-7.0), E_p *vs* pH studies were performed and all the parameters effecting the differential pulse were optimized. In optimized conditions, the proposed method was successfully applied for the determination of rosuvastatin from pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

Voltammetric measurements were obtained using a BAS 100 W (Bioanalytical System, USA) Electrochemical Analyzer. The three-electrode system consisted of working counter and reference electrode was used. In the system different working electrodes such as a glassy carbon (GC, BAS; 3mm, diameter) electrode, a 3 mm diameter boron doped diamond electrode (BDDE) (Windsor Scientific Ltd; West Berkshire UK) and an edge plane pyrolytic graphite (EPPG) electrode (BAS; 3 mm) were used. Also, a platinum wire counter electrode and an Ag□AgCl saturated KCl reference electrode was used. The GC electrode was polished manually with aqueous slurry of alumina powder (Φ, 0.01μm) on a damp smooth polishing cloth (BAS velvet polishing pad) just before each experiments. The pH was measured using a pH meter Model 538 (WTW, Weilheim, Germany) using a combined electrode (glassy electrode-reference electrode) with an accuracy of ±0.05 pH. Operating conditions for DPV were: pulse amplitude, 50 mV; pulse with, 50 ms; scan rate, 20 $mV.s^{-1}$; for DPV were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV.

Reagents

ROS and its pharmaceutical dosage forms were kindly supplied from pharmaceutical companies Salutis for ROSUCOR® (Lot.01239) and Aset for REAKT® (Lot.10162015) in Turkey. Stock solution of ROS ($1x10^{-3}$ M) was prepared in methanol (CH_3OH) and working solutions of ROS for the voltammetric experiments were prepared from stock solution by dilution of the selected supporting electrolyte and contained a constant amount of CH_3OH (20%, v:v). Different supporting electrolytes of H_2SO_4 solutions (0.1 and 0.5 M), acetate (1.0 M CH_3COOH; pH 3.7–5.7), and phosphate (0.2 M H_3PO_4; 0.2 M $NaH_2PO_4{\cdot}2H_2O$; pH 2.0–8.0) were prepared for electrochemical measurements. Analytical curves were obtained by the addition of aliquots of the previously prepared ROS standard solutions into the measurement cell containing 10.0 mL of the 0.1 M H_2SO_4. DPV voltammograms were obtained after each aliquot addition. All reagents were in analytical grade, and were prepared by doubled distilled water. All experiments were realized at room temperature, all solutions were protected from light and used within 24 h to avoid decomposition.

Validation of the analytical method

In order to validate the proposed electrochemical method, precision, accuracy, linear range, limit of detection and quantification values were obtained, reported and discussed according to ICH Guidelines and USP criteria. Precision of the proposed method was achieved by within day repeatability and between day repeatability results of the 0.05 M ROS in terms of RSD % and bias % values. Accuracy of the proposed method was realized by recovery studies. Know amount of ROS was added to the pharmaceutical dosage forms and recovery studies were reported in terms of RSD % and recovery %.

Analyses of Rosuvastatin from Dosage forms

Ten tablets of ROSUCOR® containing 10 mg ROS and ten tablets of REAKT® containing 10 mg ROS were prepared separately. The tablets were weighted, crushed and powdered. From the powder, the required amount of powder, equivalent to a stock solution of $1x10^{-3}$ M ROS was weighed. The tablet solution was prepared in 50 mL with CH_3OH and sonicated for 10 min. The solution is then filtered and the clear solution was diluted with the selected supporting electrolyte containing a constant amount of CH_3OH (20%, v:v). Using the validated calibration regression equations, the content of the tablet amounts were calculated.

RESULTS AND DISCUSSION

Prior to the experiments, different electrodes of GC, BDD and EPPG electrodes were examined to find the optimum transducer for electrochemical determination of Rosuvastatin using differential pulse voltammetry. As can be seen from figure 2, EPPG electrode reached over potential more easily than other electrodes in 0.1 M H_2SO_4 solution containing 20% ACN and 1×10^{-4} M ROS. BDDE also gave smaller response than GCE towards 1×10^{-4} M ROS, therefore further experiments were conducted using GCE.

Cyclic Voltammetric Behavior of ROS

The electrochemical behavior of ROS on GCE was investigated using cyclic voltammetry. As shown in figure 3, the cyclic voltammetric profile of ROS at 1×10^{-4} M in a 0.1 M H_2SO_4 solution at the GCE, was irreversible. ROS exhibit only one well-defined oxidation peak at 1.2 V without the presence of any cathodic peak on the reverse scan.

Influence of the pH

Within a wide pH range (pH 0.3-7.0) using differential pulse voltammetric technique at glassy carbon electrode, the peak current of the ROS was followed. It was observed that, the peak current was decreased with increase the pH values (Figure 4).

The influence of pH on the peak potential was followed by the following equation.

$$Ep\ (\text{mV}) = 1288 - 11.69\ \text{pH}\quad r = 0.997$$

The peak potential of ROS decreased in small values while the buffer solution pH was increasing. With differential pulse voltammetric techniques, maximum current was observed in the pH 0.3 sulfuric acid medium and for further studies this medium was used (Figure 4).

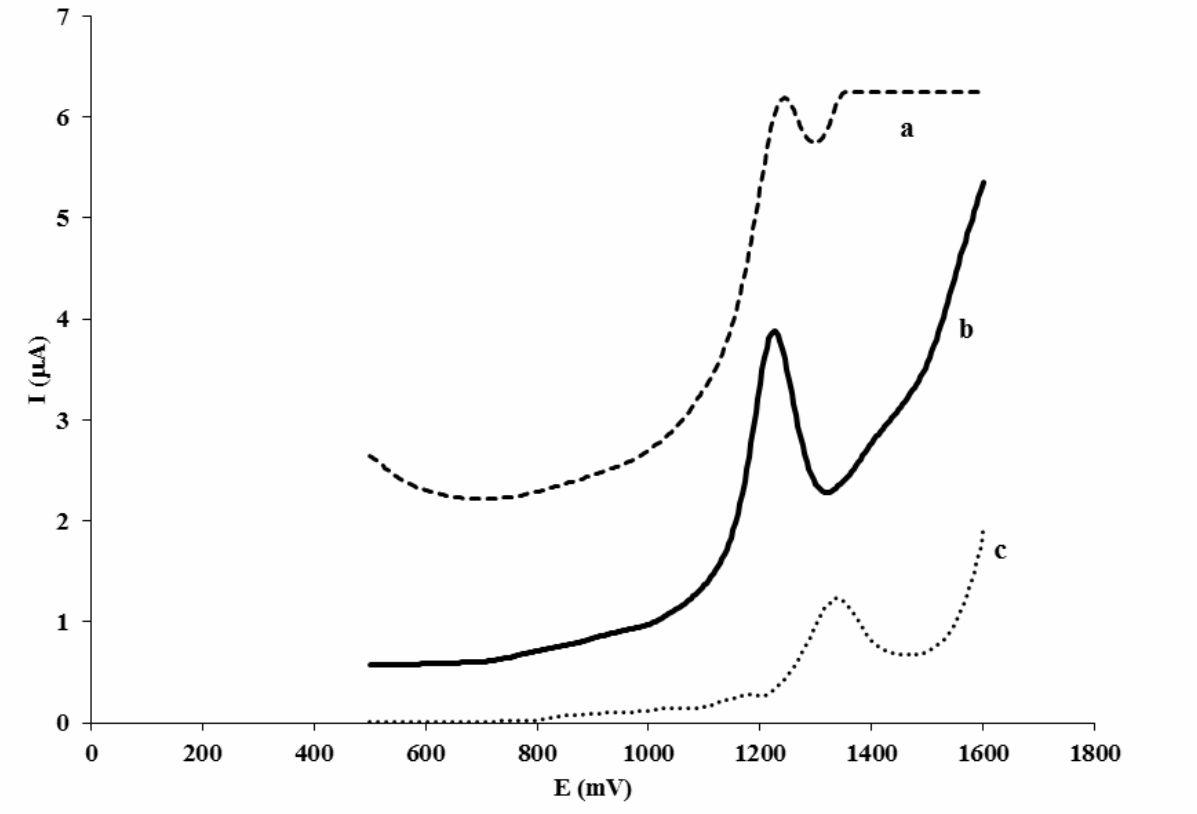

Fig. 2 – Differential Pulse Voltammograms of 1×10^{-4} M ROS solution in 0.1 M H_2SO_4 using a) edge plane pyrolytic graphite b) glassy carbon c) boron doped diamond electrode.

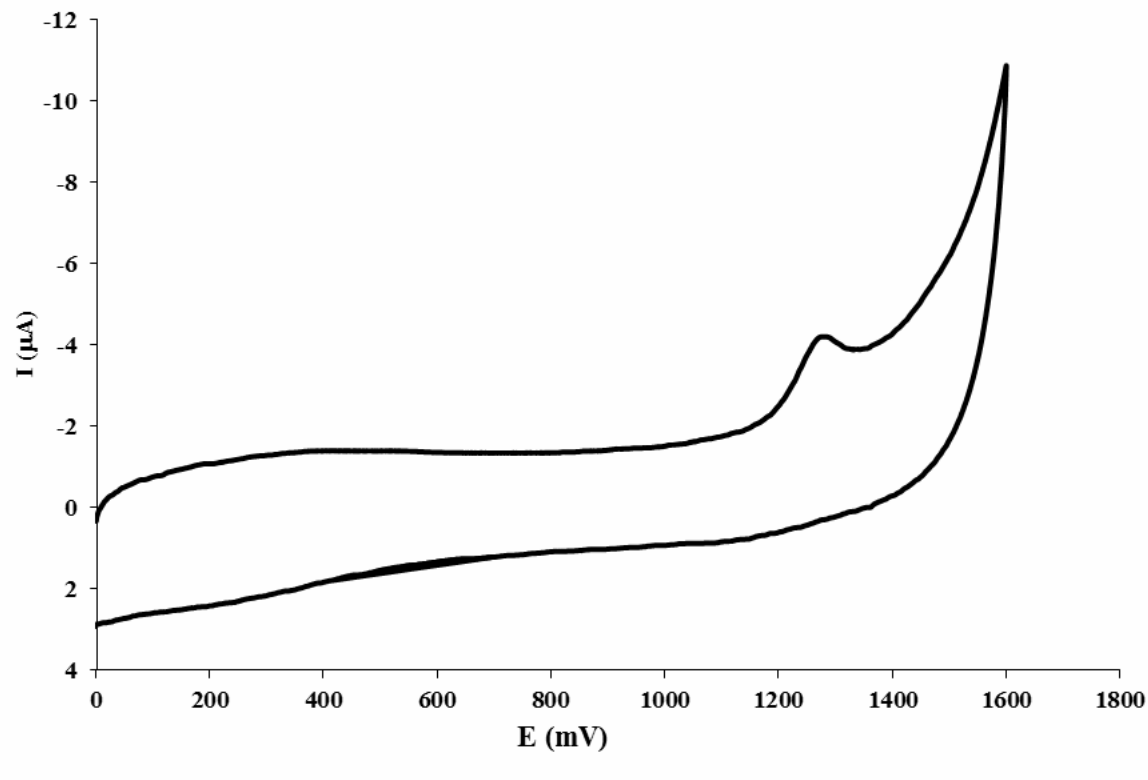

Fig. 3 – The cyclic voltammogram of 1×10^{-4} M ROS with a scan rate of 100 mV s^{-1} in 0.1 M H_2SO_4.

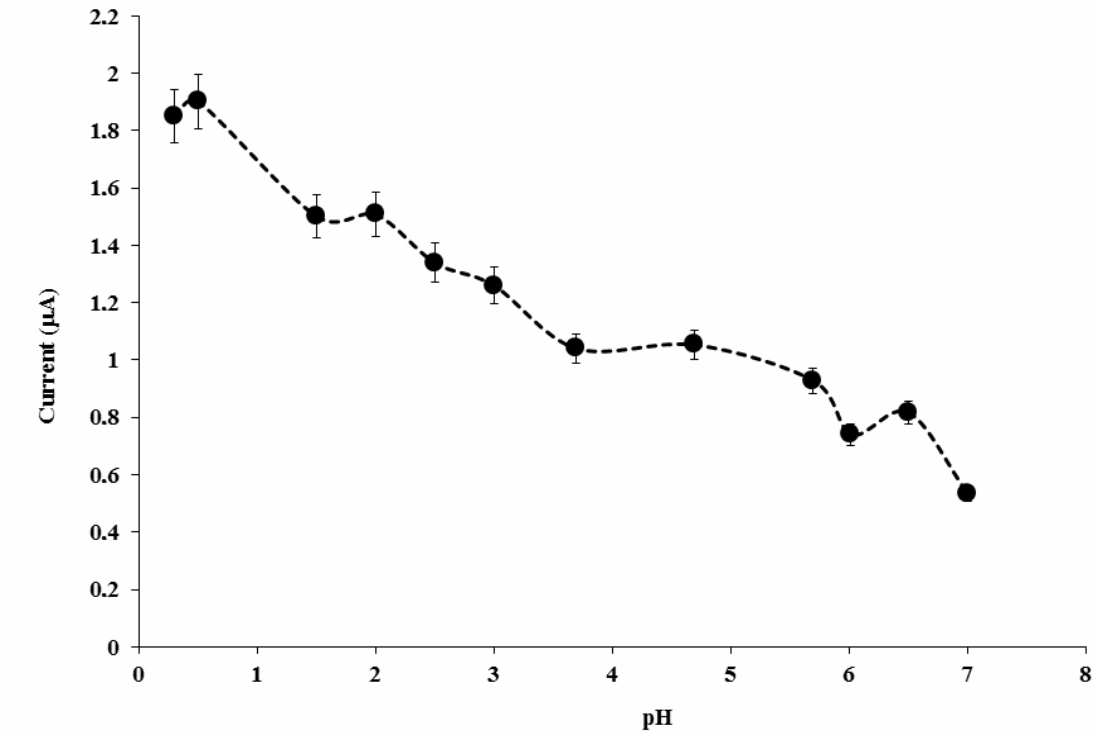

Fig. 4 – Plot of I_p vs pH of 1×10^{-4} M ROS solution using DPV.

Influence of scan rate

Mechanistic studies in pharmaceutical analyses can be conducted by using scan rate studies. These studies can give information about how much electron is transferred in the electrochemical reaction or how the electrochemical oxidation/reduction mechanisms. Therefore, the electrochemical oxidation behavior of ROS was studied in different scan rates from 5-1000 mV s^{-1}.

The linearity between I_p *vs* $v^{1/2}$ suggesting that a diffusion controlled mechanism is followed in oxidation of rosuvastatin.

$$I_p\,(\mu A) = 0.19\ v^{1/2}\ (mV.\ s^{-1}) - 0.33\ (r = 0.993)$$

Moreover, the slope of the equation of log (I_p) *vs* log (v) suggest also diffusion controlled mechanism. The slope of this equation is close 0.5, proves diffusion controlled process.[34]

$$\log I_p\,(\mu A) = 0.58 \log v\ (mV/s) - 1.01\ (r = 0.992)$$

As a result of scan rate studies, in the pH 0.3 sulfuric acid medium, the reaction was found diffusion-controlled mechanism.

The plot of E_p vs. log v was linear; this behavior is consistent with the EC nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step.

According to Laviron,[36] E_p can be defined by the following equation; where α is the transfer coefficient of the oxidation of ROS, k^0 is standard heterogeneous rate constant, v is scan rate, E^0 is formal potential, R is gas constant, T is temperature and F is Faraday constant, n is the number of electrons that are involved in electro oxidation of rosuvastatin.[35] Generally, α is used as 0.5 for irreversible processes.[36] E_p vs. log v graph, was found as E_p =0,05log v + 1.31 and n was calculated as 2.38 from this equation.[37, 38]

For an irreversible process, αn can also be calculated from the difference between the E_p and half wave potential ($E_{p/2}$) where α is 0.5 according to the following equation; [39]

$$\Delta E_p = E_p - E_{p/2} = (47.7/\alpha n)\ mV$$

N value was found as 1.98 (~2), as results these two calculations verified each other.

Analytical characterization and validation

Analytical characterization rosuvastatin determination using glassy carbon electrode was achieved in the supporting electrode of 0.1 M H_2SO_4 where the maximum peak current was obtained. Increasing concentration of Rosuvastatin was added to the medium and the plot of I_p *vs* concentration showed a linearity between 0.005-0.1 mM Rosuvastatin with an equation of I_p (μA) = 17.99 C + 0.14 (r=0.995) (Figure 5). Limit of detection and limit of quantification values were calculated form the equations LOD=3.3 s/m, LOQ=10s/m where s is the standard deviation of the response and m is the slope of the calibration curve.[13, 40-42]

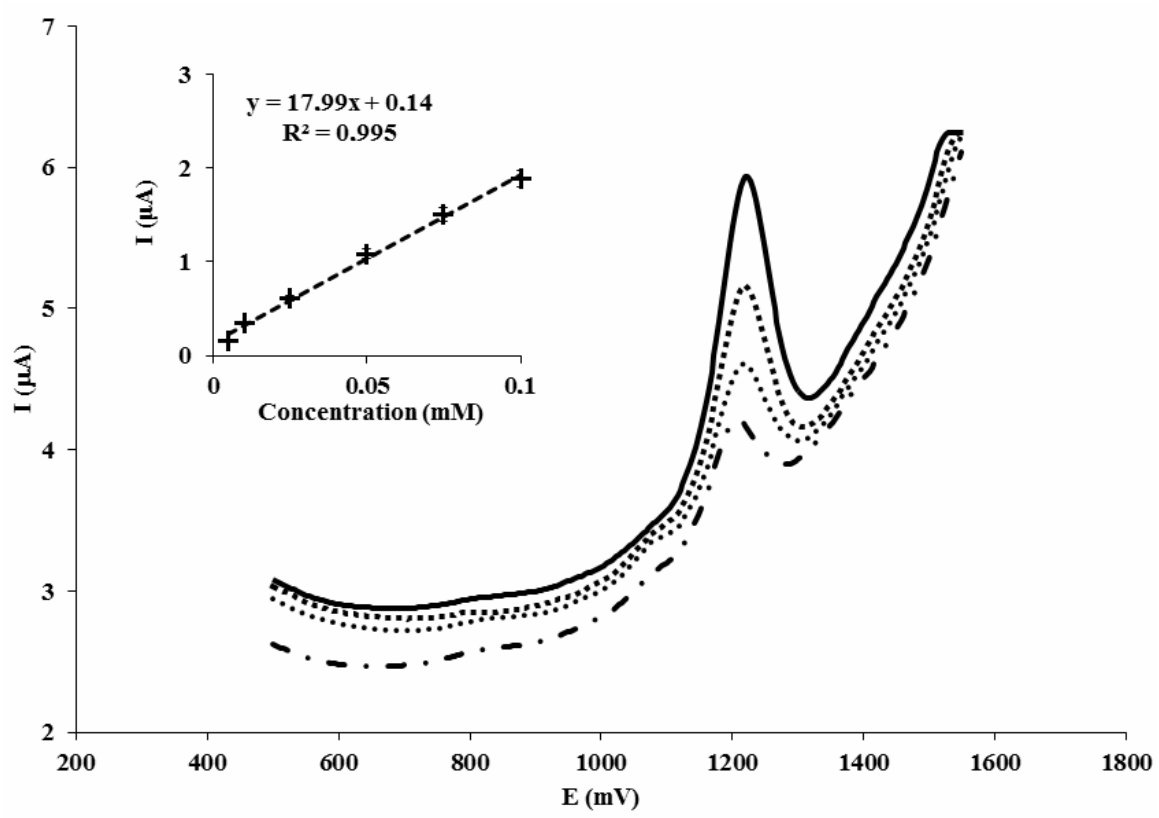

Fig. 5 – Differential Pulse voltammogram of increasing concentrations of ROS in 0.1 M H_2SO_4.

Table 1

Regression data of the calibration graphs for ROS in 0.1 M H_2SO_4

Measured potential (mV)	1265
Linearity range (mM)	0.005-0.1
Slope (μA mM^{-1})	17.99
Intercept (μA)	0.14
Correlation coefficient (*r*)	0.995
LOD (nM)	1.32
LOQ (nM)	4.02
Repeatability of peak current (RSD %)[a]	1.23
Repeatability of peak potential (RSD %)[a]	0.38
Reproducibility of peak current (RSD %)[a]	1.94
Reproducibility of peak potential (RSD %)[a]	0.96

[a] Results from five experiments for the 0.05mM of ROS in 0.1 M H_2SO_4.

Table 2

Recovery results for the ROSUCOR® and REAKT®

	ROSUCOR®	**REAKT®**
Labeled claim (mg)	10	10
Amount found[a] (mg)	10.06	10.02
RSD %	0.75	0.69
Bias %	-0.60	-0.20
Added (mg)	5.00	5.00
Found[a] (mg)	5.04	5.02
Average recovered %	100.85	100.40
RSD % of recovery	1.12	1.15
Bias %	-0.85	-0.40

[a] Results from five experiments for the 0.05mM of ROS in 0.1 M H_2SO_4.

Between day and within day repeatability studies were also performed to show precision of the method. The values of RSD% and other statistical evaluation of the validation parameters are shared in Table 1.

Application to Pharmaceutical Dosage Forms

In optimized conditions, determination of Rosuvastatin was achieved in Rosuvastatin containing pharmaceuticals named ROSUCOR®

and REAKT®. Using proposed method, 10 mg Rosuvastatin was found in ROSUCOR® and REAKT® as 10.06 mg and 10.02 mg, respectively. By adding pure substance on pharmaceuticals, recovery studies were performed and it was recovered as %100.85 and %100.40 respectively as summarized in table 2.

CONCLUSION

Electrochemical behavior of Rosuvastatin which is a member of the drug class of statins used to treat high cholesterol and related conditions, and to prevent cardiovascular disease, was investigated in 0.1 M H_2SO_4 at glassy carbon electrode. The electrooxidation mechanism was also studied by conducting cyclic voltammetric studies in different pH values as a wide pH range (pH 0.3-7.0). Scan rate studies were assisted to understand the mechanism, and found as diffusion controlled where 2 electrons are involved in the reaction. Linear relation range specified in the range of 0.005-0.1 mM and limit of detection calculated as 1.33 nM limit of quantitation was calculated as 4.02 nM. In optimized conditions, the proposed method was successfully applied for the determination of rosuvastatin from pharmaceutical dosage forms named, ROSUCOR® and REAKT®. As a result of recovery results, the suggested method is found as free from interferences coming from excipients in the tablet dosage forms.

REFERENCES

1. R. Zhang, Y. Li, X. Jiang and L. Wang, *Curr. Ther. Res.*, **2009**, *70*, 392–404. 2.
2. M. J. Chapman and F. McTaggart, *Atherosclerosis Supp.*, **2002**, *2,* 33-37.
3. S. G. Simonson, A. Raza, P. D. Martin, P. D. Mitchell, J. A. Jarcho, C. D. Brown and D. W. Schneck, *Clin. Pharmacol. Ther.*, **2004**, *76,* 167-177.
4. M. Igel, T. Sudhop and K. Bergmann, *J. Clin. Pharma.,* **2002**, *42,* 835-845.
5. M. H. Davidson, *Expert Opin Invest Drugs*, **2002**, *11*, 125-141.
6. F. McTaggart, L. Buckett, R. Davidson, G. Holdgate, A. McCormick, D. Schneck, and M. Warwick, *Am. J. Cardiol.*, **2001**, *87*, 28-32.
7. F. McTaggart, *Atherosclerosis Supp.*, **2003**, *4*, 9-14.
8. J. Bockris and S.U.M. Khan, "Surface Electrochemistry: A Molecular Level Approach", Plenum Press, New York and London, 1993.
9. C. M. A. Brett and A. M. Oliveira-Brett (Eds.), "Electrochemistry, principles, methods and applications", Oxford University Press, New York, 1993.
10. C. M. A. Brett and A. M. C. F. Oliveira-Brett, "Electroanalysis", Oxford University Press, 1998.
11. R. Greef, R. Peat L. M. Peter, D. Pletcher and J. Robinson, "Instrumental Methods in Electrochemistry", Ellis Horwood, England, 1990.
12. A. J. Bard and L. R. Faulkner, "Electrochemical Methods, Fundamentals and Applications", 2nd edition, John Wiley and Sons, New York, 2001.
13. S. A. Ozkan, "Electroanalytical Methods in Pharmaceutical Analysis and Their Validation", HNB Publishing, New York, 2012.
14. S. A. Ozkan, B. Uslu and H. Y. Aboul-Enein, *Crit. Rev. Anal. Chem.*, **2003**, *33*, 155-181.
15. S. A. Ozkan and B Uslu. *J. Pharma. Biomed. Anal.*, **2016**, *130*, 126–140.
16. C. E. Banks and R. G. Compton, *Analyst*, **2006**, *131*, 15-21.
17. P. Fanjul-Bolado, D. Hernández-Santos, P. J. Lamas-Ardisana, A. Martín-Pernía and A. Costa-García, *Electrochim. Acta.*, **2008**, *53*, 3635-3642.
18. J. Wang, M. Li, Z. Shi, N. Li and Z. Gu, *Anal. Chem.*, **2002**, *74*, 1993-1997.
19. H. Luo, Z. Shi, N. Li, Z. Gu Q. Zhuang, *Anal. Chem.*, **2001**, *73*, 915-920.
20. L. Chen, Y. Tang, K. Wang, C. Liu and S. Luo, *Electrochem. Commun.*, **2011**, *13*, 133-137.
21. J. Wang, M. Li, Z. Shi, N. Li and Z. Gu, *Electroanalysis*, **2002**, *14*, 225-230.
22. K. Wu, J. Fei and S. Hu. *Anal. Biochem.,* **2003**, *318*, 100-106.
23. B. Uslu, B. Doğan and S. A. Özkan, *Anal. Chim. Acta,* **2005,** *537*, 307-313.
24. M. Emami, M. Shamsipur and R. Saber, *J. Solid State Chem.,* **2014**, *18*, 985-992.
25. A. A. Ramadan, H. A. S. N. A. Mandil and N. O. O. R. Alshelhawi., *Int J Pharm Pharm Sci*, **2014,** *6*, 579-85.
26. G. V. Rao, M., Shaiba, P. Bhargavi, T. D. Kumar and C. H. B. Swethapriya, *Orient J. Chem.*, **2010**, *3*, 1215-7.
27. A. Gupta, P. Mishra and K. Shah, *J. Chem.*, **2009,** *6*, 89-92.
28. H. O. Kaila, M. A. Ambasana, R. S. Thakkar, H. T. Saravaia, and A. K. Shah, *Indian J Pharm Sci* **2010**, *72*, 592.
29. C. K. Hull, P. D. Martin, M. J. Warwick and E. Thomas, *J. Pharmaceut. Biomed. Anal.*, **2004**, *35*, 609-614.
30. T. R. Kumar, N. R. Shitut, P. K. Kumar, M. C. Vinu, V. V. P. Kumar, R. Mullangi and N. R. Srinivas, *Biomed. Chromatog.,* **2006,** *20*, 881-887.
31. A. A. Ramadan, H. Mandil and N. Ghazal, *Int J Pharm Pharm Sci*, **2014**, *7*, 389-396.
32. T. A. Silva, H. Zanin, F. C. Vicentini, E. J. Corat and O. Fatibello-Filho, *Sensor Actuat. B*: *Chem.,* **2015**, *218*, 51-59.
33. T. A. Silva, G. F. Pereira, O. Fatibello-Filho, K. I. Eguiluz and G. R. Salazar-Banda, *Diam. Relat. Mater.,* **2015**, *58*, 103-109.
34. D. K. Gosser, "Cyclic voltammetry: simulation and analysis of reaction mechanism", VCH. Publishers, Inc., USA, 1993.
35. H. Karimi-Maleh, F. Tahernejad-Javazmi, A. A. Ensafi, R. Moradi, S. Mallakpour and H. Beitollahi, *Biosens. Bioelectron.,* **2014**, *60*, 1–7.
36. E. Laviron, *J. Electroanal. Chem.,* **1979**, *101*, 19–28.
37. H. Lund and O. Hammerich (Eds.), "Organic Electrochemistry, Revised and Expanded", fourth edition, Marcel Dekker Inc. Pub., 2001.

38. J. Grimshaw, "Electrochemical Reactions and Mechanism in Organic Chemistry", first edition, Elsevier Science Publication, 2000.
39. R. Greef, R. Peat, L.M. Peter, D. Pletcher and J. Robinson, "Instrumental Methods in Electrochemistry", Ellis Horwood, England, 1990.
40. C. M. Riley and T. W. Rosanske, "Development and Validation of Analytical Methods", Elsevier, Amsterdam, The Netherlands, 1996.
41. J. Ermer and J. H. Miller, "Method Validation in Pharmaceutical Analysis", Wiley-VCH, Weinheim, 2005.
42. M. E. Swartz and I. S. Krull, "Analytical Method Development and Validation", Marcel Dekker, New York, 1997.

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Title: DIFFERENTIAL PULSE VOLTAMMETRIC DETERMINATION OF ROSUVASTATIN VIA GLASSY CARBON ELECTRODE
Subject:
Author: leylakaradurmuş
Keywords:
Comments:
Creation Date: 7/19/2017 12:24:00 PM
Change Number: 4
Last Saved On: 7/19/2017 12:30:00 PM
Last Saved By: LUIZA
Total Editing Time: 6 Minutes
Last Printed On: 7/19/2017 12:30:00 PM
As of Last Complete Printing
Number of Pages: 9
Number of Words: 2,895 (approx.)
Number of Characters: 16,504 (approx.)