Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 197–202
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

# Voltammetric assay of azithromycin in pharmaceutical dosage forms

Biljana Nigović [a,*], Branimir Šimunić [b]

[a] *Faculty of Pharmacy and Biochemistry, University of Zagreb, 10000 Zagreb, Croatia*
[b] *R&D, PLIVA, Pharm. Ind. Inc., Prilaz b Filipovica 25, 10000 Zagreb, Croatia*

Received 25 November 2002; received in revised form 30 January 2003; accepted 30 January 2003

**Abstract**

The oxidative behaviour of azithromycin was studied at s glassy carbon electrode in different buffer system using cyclic, linear sweep and differential pulse voltammetry. The oxidation process was shown to be irreversible over the entire pH range studied (5–11) and was diffusion–adsorption controlled. Analytical method with adequate precision and accuracy was developed for the determination of azithromycin in phosphate buffer at pH 7 as supporting electrolyte containing 10% methanol and 0.05 M ammonium acetate. The peak current varied linearly with azithromycin concentration in the range 1–15 μg/ml. The procedure was successfully applied for assay of the drug in the pharmaceutical dosage forms. The relative standard deviation ($n = 5$) of 2.18% was obtained.


*Keywords:* Azithromycin; Differential pulse voltammetry; Electrochemical oxidation; Pharmaceutical analysis

## 1. Introduction

Azithromycin is a novel macrolide antibiotic and semisynthetic erytromycin derivative [1]. It exhibits a more extensive spectrum of activity, grater acid stability, better oral biovailability and more favourable pharamcokinetic behaviour than erytromycin [2]. Its unique pharamcokinetic properties include extensive tissue distribution and high drug concentrations within cells. The most innovative feature is the efficacy and safety of a 3-day oral regimen [3]. Azithromycin plays a leading role in the treatment of respiratory tract infections, toxoplasmosis, nonclassical pathogens such as *Helicobacter Pylori*, pediatric infections and opportunistic infections in AIDS.

Azithromycin has been analyzed in biological samples by microbiological method [4] and high performance liquid chromatography using electrochemical [5–7] and fluorescence [8] detector. However, there is noticeable shortage of methods described in the literature for its determination in pharmaceutical dosage forms. Chromatographic methods developed for azithromycin quantitation [9–11] demand expensive equipment and could not be available in many laboratories. Spectrophotometric [12] and fluorimetric [13] reported methods require a prior derivatization of the drug. Only one

* Corresponding author. Fax: +385-1-4856-201.
*E-mail address:* biljana@pharma.hr (B. Nigović).

0731-7085/03/$ - see front matter 
doi:10.1016/S0731-7085(03)00060-8

electrochemical method has been described in literature [14], but it is based on reduction of azithromycin in strongly basic media at mercury electrode. The oxidative behaviour and determination of azithromycin at a glassy carbon electrode has been not reported.

The aim of the present study has been to examine the oxidative properties and assay of azithromycin at a glassy carbon electrode using cyclic, linear sweep and differential pulse voltammetry. An electroanalytical procedure for the determination of azithromycin in its pharmaceutical formulations was optimized. The official high performance liquid chromatographic (HPLC) method [1] was chosen as the comparative method in evaluating the technique.

## 2. Experimental

### 2.1. Materials and reagents

Azithromycin dihydrate, kindly provided by Pliva (Zagreb, Croatia), was used without further purification. Stock solution (100 μg/ml; $1.32 \times 10^{-3}$ M) was prepared in methanol–water (50:50, v/v), and stored in a refrigerator at 4 °C. It remained stable for at least 4 weeks [6].

Standard solutions were prepared daily by diluting of the stock solution with a selected supporting electrolyte containing 10% methanol and appropriate salt for the ionic strength of the medium. The supporting electrolytes solutions, 0.05 M acetate buffer, 0.1 M phosphate buffer and Britton–Robinson buffer (0.04 M in acetic, phosphoric and boric acid) were used for the voltammetric studies. Buffer solutions were adjusted by adding the necessary amount of NaOH in order to obtain the appropriate pH. Salts used for the ionic media (ammonium acetate, sodium perchlorate), and the other chemicals were of analytical grade (Merck and Sigma). All solutions were prepared using doubly distilled water.

### 2.2. Apparatus

The voltammetry experiments were performed using an EG&G Princeton Applied Research Model 273A potentiostat controlled by the Model 270/250 Research Electrochemistry Software v. 4.30. A three-electrode system was composed of a glassy carbon working electrode ($\varnothing = 2$ mm, EG&G/PAR), Ag/AgCl reference electrode and a platinum auxiliary electrode.

To provide a reproducible active surface and improve the sensitivity and resolution of the voltammetric peaks, the working electrode was polished with 0.5 μm alumina powder on a polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper. The electrode cleaning procedures require only 2 min. All the solutions examined by electrochemical techniques were purged for 10 min with water-saturated nitrogen, after which a continuous stream of nitrogen was passed over the solutions during the measurements. All measurements were carried out at room temperature.

The pH of buffered solution was measured with a Radiometer PHM 85 digital pH-meter (Radiometer, Copenhagen, Denmark) using combined glass electrode (Radiometer PHC 2406L).

### 2.3. Analysis of pharmaceutical dosage forms

Sumamed® capsules and tablets were obtained from Pliva (Zagreb, Croatia) as a gift. The amounts declared of azithromycin are 250 mg per capsule and 500 mg per tablet. Excipients, such as microcrystalline cellulose, hydroxypropylmethylcellulose, lactose and titanium dioxide are added to dosage forms. Twenty tablets were weighted accurately and crushed to a fine powder. In the case of capsules, the contents of ten capsules were completely removed from shells. The accurately weighted quantities of these powders equivalent to 250 mg of azithromycin were transferred to different 50-ml volumetric flasks. About 25 ml of methanol (99.8%) was added to dissolve the active material. After sonicating and shaking the mixture for 10 min, it was completed to volume with the same solvent, mixed and passed through a 0.5 μm mash filter. An aliquot of the filtrate was then transferred into a calibrated flask and a series of dilutions was made with phosphate buffer at pH

7 as supporting electrolyte containing 10% methanol and 0.05 M ammonium acetate. The content of the drug in pharmaceutical preparations was determined referring to the regression equation.

## 3. Results and discussion

### 3.1. Voltammetric behaviour

Successive cyclic voltammograms of azithromycin obtained in phosphate buffer of pH 7 having 10% methanol at scan rate of 100 mV/s are shown in Fig. 1. In cyclic voltammograms one well-defined anodic peak was observed. The fact that no peak was observed in the reverse scan suggests that the oxidation process is an irreversible one. The peak currents decrease with succeeding potential scans suggesting an adsorbed species formation on the electrode surface.

The effect of potential scans rate, $v$, on the peak current and the peak potential of azithromycin was evaluated. Inset in Fig. 1 shows the influence of the square root of the scan rate on the peak current. A linear relationship was observed between 10 and 200 mV/s ($r = 0.9993$) which is typical of diffusion-controlled currents [15]. As the scan rate was increased from 0.5 to 1 V/s the currents were adsorption controlled. The variation of $\log i_p$ vs. $\log v$ is linear over the scan range 10–500 mV/s, and corresponds to equation:

$$\log i_p \ (\text{nA}) = -0.47 + 0.65 \log v \quad (v \text{ in mV/s})$$

$$r = 0.9973.$$

The slope of 0.65 is close to the theoretically expected value of 0.5 for a diffusive process.

The peak potential was shifted to less positive values on increasing the scan rate, which confirms the irreversible nature of the oxidation process. The reversibility of the process was studied at the glassy carbon electrode using cyclic voltammetry. The value of $\alpha n_a$, product of transfer coefficient and number of electrons transferred in the rate determining step, was determined from Tafel treatment ($\log i$ vs. $E$) of the voltammetric curves [16]. The $\alpha n_a$ value obtained (0.37) show the total irreversibility of the electron transfer process. It was also demonstrated by the linear relationship obtained between the peak potential $E_p$ and the logarithm of scan rate in the range 10–500 mV/s.

The electrooxidation of azithromycin was also studied over pH range 3–11 in buffer media. The wave was well developed at pH > 5. The pH dependence of the peak potential obtained when differential pulse voltammetry was used is shown in Fig. 2. The potential of anodic peak of azithromycin is shifted linearly towards less positive values with increasing the pH between 5 and

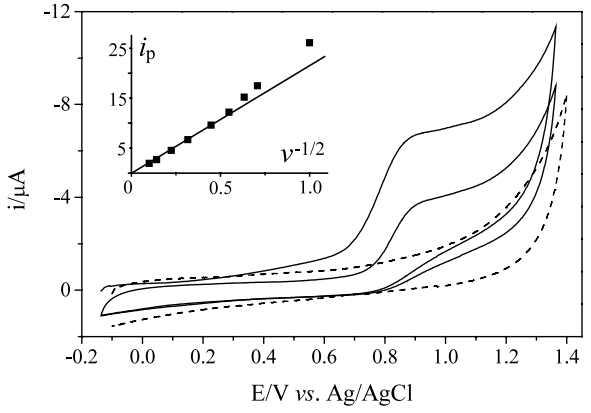


Fig. 1. Successive cyclic voltammograms of azithromycin ($2.64 \times 10^{-4}$ M) in phosphate buffer of pH 7, containing 10% methanol and 0.05 M ammonium acetate, at a scan rate of 100 mV $s^{-1}$; dashed line represents blank solution. Inset: variation of peak current, $i_p$ (μA), with scan rate, $v^{1/2}$ (V $s^{-1}$).

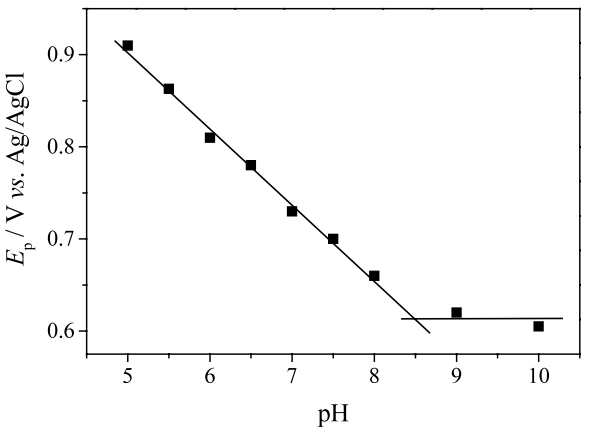


Fig. 2. Effect of pH on peak potentials for $1.98 \times 10^{-5}$ M azithromycin solutions in phosphate buffer by means of differential pulse voltammetry at a glassy carbon electrode.

8.5 by 0.075 V/pH. At pH > 9, the $E_p$ is pH-independent. The linear regions of the $E_p$–pH plot intersect at about 8.5 which correspond to the p$K_a$ values of the dimethylamino group on the sugar moiety of reduced form of azithromycin.

Of the different functional groups of azithromycin, the amine group is the most easily oxidizable. Dialkylamines are oxidized forming a radical cation by loss of one electron [17]. The similar voltammetric behaviour of structurally analogous drug, erithromycin, indicates that the mechanism proposed for the anodic oxidation of azithromycin is initiated by one-electron transfer to form the cation radical at nitrogen on the desosamine sugar residue [18]. Namely, erithromycin has no nitrogen atom in macrocyclic lactone ring. At pH < p$K_a$ the anodic oxidation process involves the deprotonation of cation radical formed. This result is in accordance with the plot $E_p$ vs. pH (Fig. 2).

### 3.2. Optimization of measurement conditions

The influence of several electrolytes (acetate, phosphate, Brriton–Robinson) on the analytical signal was studied using different electroanalytical techniques. Considerable improved sensitivity can be achieved by application of differential pulse voltammetry for the determination of azithromycin. Its adsorption on the electrode surface is not strong enough for the significant adsorptive accumulation and hence, it is not analytically useful. The best curve and the highest current were obtained in phosphate buffer of pH 7 containing 10% methanol (v/v) and 0.05 M ammonium acetate (Fig. 3). A study of the influence of the ionic strength of the medium on the definition of the voltammetric peak revealed that the use of ammonium acetate achieves minimal background current. The peak current increase in the pH range 6–8 and then it is decreased up to alkaline pH conditions (Fig. 4). This indicates that the mechanism of the oxidation is different at neutral and basic conditions. Anodic peak was not observed below pH 4.5.

The optimum instrumental conditions were chosen from a study of the variation of the peak current with pulse amplitude, pulse width and scan rate. With increasing pulse amplitude from 25 to

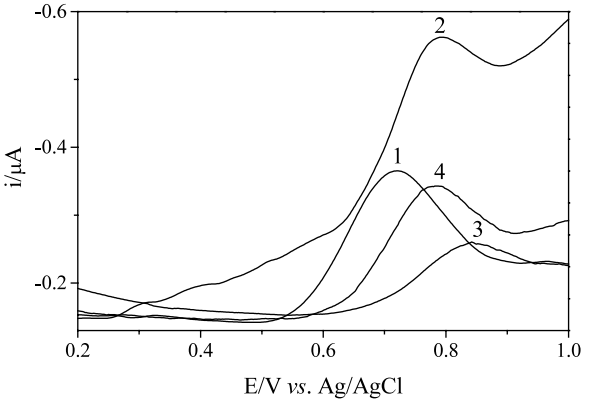


Fig. 3. Differential pulse voltammograms obtained for $1.98 \times 10^{-5}$ M azithromycin solutions in different electrolytes having 10% methanol: (1) phosphate buffer pH 7 and 0.05 M ammonimu acetate; (2) phosphate buffer pH 7 and 0.1 M sodium perchlorate; (3) Britton–Robinson buffer pH 6.2; (4) acetate buffer pH 6.6; scan rate: 20 mV $s^{-1}$; pulse amplitude: 50 mV; pulse width: 30 ms.

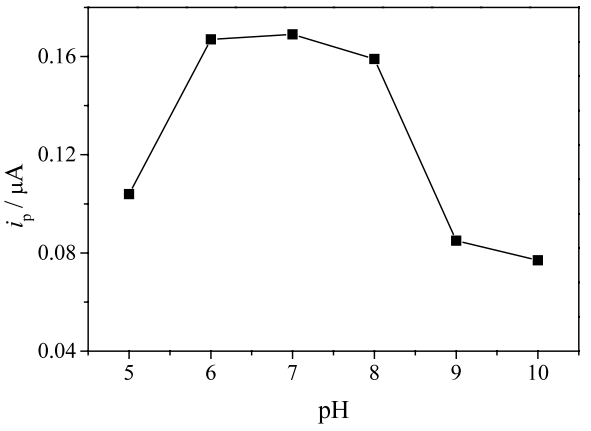


Fig. 4. Influence of pH on peak current for azithromycin ($1.98 \times 10^{-5}$) in phosphate buffer containing 10% methanol and 0.05 M ammonium acetate.

80, the peak current increased also but the peak became less sharp and ill defined. However, the peak current decreased as the pulse width increased from 30 to 90 ms. The peak current increased linearly with the scan increment up to 20 mV. Thus, the best peak definition was recorded when using 50 mV pulse amplitude, 30 ms pulse width and 20 mV/s scan rate.

### 3.3. Quantitative aspects

On the basis of the electrochemical oxidation of azithromycin at glassy carbon electrode, analytical method was developed involving differential pulse voltammetry for the determination of the drug. A linear relation between peak current and azithromycin concentration was found in the range 1–15 μg/ml ($1.32 \times 10^{-6}$–$1.98 \times 10^{-5}$ M). However, the peak current function exhibits a decrease with increase of concentration. This reveals the blocking effect due to adsorption of the substrate on the electrode surface. The calibration plot is described by the following regression curve:

$$i_{pa}\ (\mu A) = 7.896 \times 10^{3} C\ (M) + 0.019 \quad r = 0.9982.$$

Standard deviations for the slope and intercept of the calibration curve were $2.74 \times 10^{2}$ and 0.004, respectively. Based on the signal-to-noise ratio of 3, the detection limit was calculated to be 0.7 μg/ml ($9.24 \times 10^{-7}$ M).

The inter-day reproducibility of the method was evaluated for seven independent determinations of $1.32 \times 10^{-5}$, $6.6 \times 10^{-6}$ and $1.32 \times 10^{-6}$ M solutions, yielding relative standard deviations of 1.75, 2.28 and 4.71%, respectively. The RSD value for intra-day assay reproducibility at $1.32 \times 10^{-5}$ M solution ($n = 5$) was found to be 0.87% indicating good repeatability and accuracy of the method.

### 3.4. Azithromycin assay in pharmaceutical formulations

The applicability of the proposed voltammetric method for the assay of a simple dosage form was examined by analysing capsules and tablets. In order to validate and to obtain the precision and accuracy of the developed method a recovery studies have been carried out at different concentration levels. The amount of azithromycin present in corresponding solution was calculated from the calibration equation. The analysis of azithromycin in its pharmaceutical formulations exhibited the mean recovery of 98.83% and the relative standard deviation of 2.18%, indicating adequate precision and accuracy of the proposed method. The effect of excipients on the voltammetric response of azithromycin was studied using the above process. For the recovery studies, known amounts of pure drug were added to the different pre-analysed formulations of azithromycin, and the mixtures were studied by the proposed analysis method. Comparing the azithromycin recoveries, it was found that microcrystalline cellulose, hydroxypropylmethylcellulose, lactose and titanium dioxide did not interfere with assay. Therefore, the proposed method can be used as a selective method.

The results obtained with the described method for the analysis of azithromycin in pharmaceutical formulations were compared with those obtained by reported HPLC method [11]. The chromatographic method with electrochemical detection resulted in an average value of 99.23% with a relative standard deviation of 1.99%. These results indicate that no significant difference exists between the performances of two methods as regards the accuracy and precision. Moreover, the proposed method is more simple, rapid and inexpensive.

## 4. Conclusions

The proposed differential pulse voltammetry procedure can be used successfully to determine azithromycin in pharamceutical dosage forms. It compares reasonably well with reported HPLC method and can be a good alternative for the analytical determination of azithromycin because it is simple, fast and low cost, it has sufficient precision, accuracy and sensitivity.

## References

[1] The United States Pharmacopoeia, 25th rev., Easton, Rand McNally, Taunton, MA, 2002, Fourth Supplement, p. 189.
[2] H. Lode, K. Borner, P. Koeppe, T. Schaberg, J. Antimicrobial. Chemother. 37 (1996) 1–8.
[3] H.J. Dunn, L.B. Barradell, Drugs 51 (1996) 483–505.
[4] T. Turcinov, S. Pepeljnjak, J. Pharm. Biomed. Anal. 17 (1998) 903–910.
[5] K.D. Riedel, A. Wildfeuer, H. Laufen, T. Zimmermann, J. Chromatogr. Biomed. Appl. 114 (1992) 358–362.
[6] S. Kees, S. Spangler, M. Wellenhofer, J. Chromatogr. A. 812 (1998) 287–293.

[7] C. Taninaka, H. Ohtani, E. Hanada, H. Kotaki, H. Sato, T. Iga, J. Chromatogr. Biomed. Appl. 738 (2000) 405–411.
[8] J. Sastre-Torano, H.J. Guchelaar, J. Chromatogr. Biomed. Appl. 720 (1998) 89–97.
[9] I. Kanfer, M.F. Skinner, R.B. Walker, J. Chromatogr. A. 812 (1998) 255–286.
[10] N. Kovacic-Bosnjak, J. Marincel, N. Lopotar, G. Kobrehel, Chromatographia 25 (1988) 999–1003.
[11] R. Gandhi, C.L. Kaul, R. Panchagnula, J. Pharm. Biomed. Anal. 23 (2000) 1073–1079.
[12] B.S. Kuchekar, D.B. Shinde, Indian-Drugs 38 (2001) 442–443.
[13] P.Y. Kashaba, J. Pharm. Biomed. Anal. 27 (2002) 923–932.
[14] G.R. Zhang, X.L. Wang, Z.X. Liu, Q.Y. Zhuang, Fenxi-Huaxue 28 (2000) 22–25.
[15] R. Greef, R. Peat, L.M. Peter, D. Pletcher, J. Robinson, Instrumental Methods in Electrochemistry, Ellis Horwood, Chichester, 1985, pp. 185–189.
[16] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, Wiley, New York, 1980, pp. 213–242.
[17] J. Grimshow, Electrochemical Reactions and Mechanisms in Organic Chemistry, Elsevier, Amsterdam, 2000, pp. 276–281.
[18] H. Wang, A. Zhang, H. Cui, D. Liu, R. Liu, Microchem. J. 64 (2000) 67–71.