JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Doping Agent Naphazoline Hydrochloride: Development of Simple and Fast Voltammetric Method for Its Determination in Human Serum

Tuğçe Çetinkol[1], Funda Öztürk[1*], Pınar Esra Erden[2]

[1]Department of Chemistry, Faculty of Science and Arts, Tekirdağ Namık Kemal University, Tekirdağ, Turkey
[2]Department of Chemistry, Polatlı Faculty of Science and Arts, Ankara Hacı Bayram Veli University, Ankara, Turkey

Abstract: Electrochemical behavior of naphazoline hydrochloride on a carbon paste electrode that was modified with aluminium oxide nanoparticles (Al_2O_3NPs) was investigated in a Britton-Robinson (BR) buffer (pH 7.0) using various voltammetric techniques. The results support the presence of an irreversible and diffusion-controlled electrochemical oxidation signal of naphazoline hydrochloride which is approximately at 0.9 V *vs.* Ag/AgCl. A selective, accurate, and simple square-wave anodic adsorptive stripping voltammetric method was proposed for naphazoline hydrochloride detection. The linear response was within the range of 5.0×10^{-8} – 3.0×10^{-5} mol L^{-1} with a detection limit of 2.6×10^{-9} mol L^{-1} (0.642 µg L^{-1}). In addition, the proposed method was also utilized for naphazoline hydrochloride determination in human serum sample.

Keywords: Aluminum oxide nanoparticles, doping agent, electrochemical techniques, naphazoline, human serum

Submitted: August 03, 2018. **Accepted:** February 08, 2019.

Cite this: Çetinkol T, Öztürk F, Erden P. Doping Agent Naphazoline Hydrochloride: Development of Simple and Fast Voltammetric Method for Its Determination in Human Serum. JOTCSA. 2019;6(1):79–88.

DOI: https://dx.doi.org/10.18596/jotcsa.450793.

***Corresponding author. E-mail**: fozturk@nku.edu.tr.

INTRODUCTION

Naphazoline, 2-(1-naphthylmethyl)-2-imidazoline hydrochloride (NZ HCl) (Figure 1), is a relatively long-lasting vasoconstrictor, which realizes its action by interacting with the alpha receptors of vascular smooth muscle. It is used as an important ingredient of various pharmaceutical preparations especially in nasal and ophthalmic solutions (1,2). Its empirical formula is $C_{14}H_{15}ClN_2$. NZ HCl is a white crystalline powder and it is soluble in water and ethanol. NZ HCl belongs to the group of α-sympatomimetics that are agonists of α-adrenergic receptors (3). When NZ HCl is found in a serum sample from an athlete, the athlete is charged for doping, therefore, determination of it in serum samples is crucial.

Different analytical techniques including spectrophotometry (4-9), fluorimetry (10), high performance liquid chromatography (11,12), gas chromatography (13), potentiometry (3,14,15) and liquid chromatography-mass spectrometry (16) were used for the determination of NZ HCl separately or in the presence of other drugs. However, all these methods were generally used for nasal and eyes drops and not for serum samples in the determination of NZ HCl. Only one report was found that designates NZ HCl in serum (16).

The electrochemical method has many advantages, such as instrumental simplicity, high sensitivity and selectivity, short analysis time, and moderate cost, hence various methods such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) (17,18) were proposed for the analysis of drugs and drug related molecules in pharmaceutical dosage forms and biological fluids. Oliveira et al. developed, a batch-injection analysis technique with square-wave voltammetric detection for the simultaneous

determination of zinc and naphazoline species. In this study boron-doped diamond electrode was employed as the working electrode. The linear working range and detection limit of the method for naphazoline were reported as 3.0-21.0 µmol L^{-1} and 0.04 µmol L^{-1}, respectively. Analytical application of this method was investigated in pharmaceutical samples (19).

Carbon paste electrodes (CPEs) have the advantage of quick and easy preparation and their surface is easily renewable and reproducible. They also offer a low residual current. Therefore, these electrodes are widely used for the voltammetric analysis in many electrochemical studies (20). Metal oxide nanoparticles (MONPs) have gained much attention in the fabrication of modified electrodes due to their unique properties (21-23). As electrode modification material MONPs present large surface-to-volume ratio, high surface reaction activity and good catalytic efficiency (21). Therefore, incorportion of MONPs into carbon paste matrix can be a good approach to improve the analytical characteristics of the resulting electrode. In this study Al_2O_3NPs were used for the modification of carbon paste electrode and the resulting electrode was applied to the electrochemical analysis of NZ HCl. To the best of our knowledge, there is no method reported for the voltammetric analysis of NZ HCl in biological fluids. This study introduces a simple, rapid, reliable and sensitive, square wave anodic adsorptive stripping voltammetric (SWAAdSV) method which can be used in determination of NZ HCl in human serum samples using an aluminium oxide nanoparticles modified carbon paste electrode (Al_2O_3-NPs-CPE).

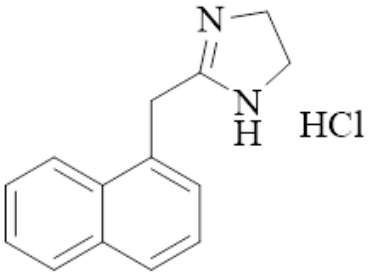

Figure 1. Structure of naphazoline hydrochloride.

EXPERIMENTAL SECTION

Materials

Al_2O_3-NPs (2-4 nm×2800 nm), and paraffin oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaOH, methanol, H_3BO_3, H_3PO_4, and CH_3COOH were supplied from Merck (Darmstadt, Germany). Graphite powder (<20 µm) was acquired from Fluka. (Britton-Robinson (BR) buffer solution was prepared by mixing 0.04 mol L^{-1} of H_3PO_4–CH_3COOH–H_3BO_3 solution and adding 0.2 mol L^{-1} of NaOH to the required pH value. NZ HCl stock solution was prepared at a concentration of 1.5×10^{-3} mol L^{-1} in water. Standard working solutions of NZ HCl were obtained by diluting the stock solution with BR buffer and pH values of the diluted solutions were adjusted to the desired values (such as 2.0, 4.0, 6.0, 7.0, 8.0, 9.0 and 10.0) for pH studies with 0.2 mol L^{-1} NaOH. All aqueous solutions were prepared using double-distilled water.

Apparatus and measurements

CV, SWV and SWAAdSV experiments were performed on a Drop Sens µstat 400 electrochemical analyzer (Lianera, Spain). A computerized IVIUM electrochemical analyzer (Ivium Technologies, Netherlands) was used for the electrochemical impedance spectroscopy (EIS) experiments. A modified carbon paste electrode, a platinum wire and Ag/AgCl electrode were employed as working, counter and reference electrodes, respectively. Thermo Scientific Orion Model 720A Benchtop pH meter with an Orion combination pH electrode (Waltham, USA) was used for the pH measurements. EIS experiments were performed in the frequency range from 10^5 Hz–0.05 Hz with 10 mV as the amplitude in 0.1 M KCl with 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1). All experiments were performed under ambient conditions.

10.0 mL of NZ HCl solutions in BR buffer were used in all voltammetric studies. The test solution was purged with high purity nitrogen (99.99%) for 60 s before the first run and 30 s between all individual successive runs. Voltammograms were obtained by applying a positive-going scan (frequency (Hz): 10; E_{step} (V): 0.005; $E_{amplitude}$ (V): 0.005) after 3 s equilibration time.

Preparation of unmodified and modified electrodes

Al_2O_3-NPs-CPE was constructed by mixing the graphite powder (12.0 mg) with the Al_2O_3-NPs (3.0 mg) and then adding paraffin oil (10 µL). The resulting mixture was homogenized in a mortar for 30 minutes. CPE without modification (UCPE) was constructed by mixing 15.0 mg graphite powder with 10 µL paraffin oil. The homogenized paste was inserted into the electrode and the CPE surface was smoothed. The CPEs were washed with double distilled water and BR buffer between experiments.

Analysis of real samples

Serum samples, supplied from healthy individuals, were analyzed to investigate the practical use of the proposed method. 1.0 mL of serum sample was transferred to the electrochemical cell containing 10.0 mL of BR buffer solution and then aliquots from stock NZ HCl solution were added. Calibration curve method was utilized to determine the NZ HCl concentration in serum samples.

RESULTS AND DISCUSSION

Electrochemical behavior of NZ HCl on CPE modified with Al_2O_3-NPs

CV and SWV techniques were used to investigate the electrochemical behavior of NZ HCl. Figure 2 depicts the CVs of NZ HCl at pH 7.0 at UCPE and Al_2O_3-NP-CPE. A single well-defined oxidation peak was obtained at a potential of 0.93 V and 0.94 V at UCPE and Al_2O_3-NPs-CPE at pH 7.0, respectively. As expected, no peak was observed in blank BR buffer scanned under the same operational conditions. No cathodic peak was observed at the reverse scan indicating a totally irreversible electrode reaction (Figure 2). The oxidation peak of NZ HCl was higher at Al_2O_3-NPs-CPE compared with that of the peak at UCPE. The increase in peak current confirms a higher electron transfer rate for NZ HCl at Al_2O_3-NPs-CPE. It can be concluded that Al_2O_3 nanoparticles are suitable modifiers for the electrochemical determination of NZ HCl.

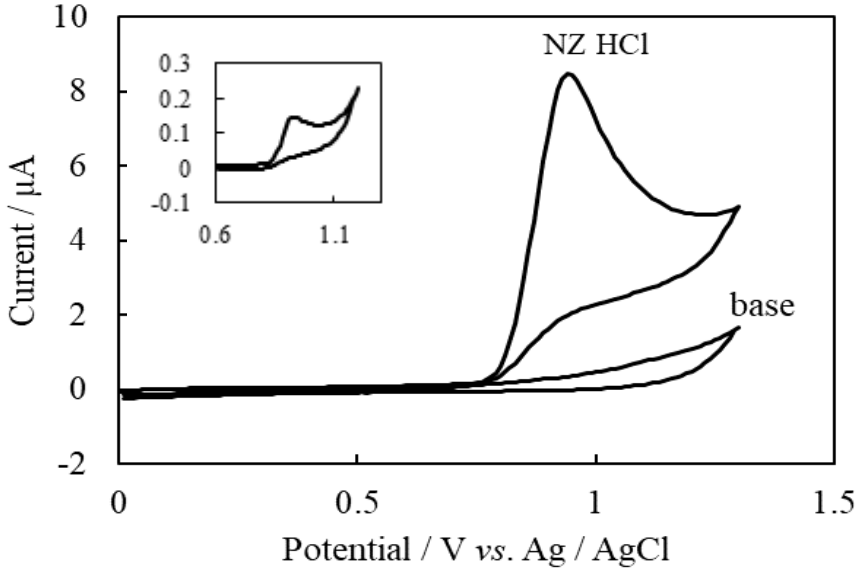

Figure 2: Cyclic voltammograms of 1.5×10^{-3} mol L^{-1} NZ HCl solutions at Al_2O_3-NPs-CPE in BR buffer solution pH 7.0 and scan rate 0.1 V s^{-1} (inset: cyclic voltammograms of NZ at bare CPE at the same condition).

More detailed experimental studies were also done to reveal the characteristic of oxidation. First of all, the effect of scan rate (*v*) on the anodic peak current (*ip,a*) was studied by CV (Figure 3). The peak potential shifts to more positive potential values with the increasing scan rate from 0.01 to 0.225 V s^{-1} which indicates that the electrooxidation step is not reversible.

The plot of the peak current versus square root of scan rate (Figure 3; inset A) reveals a linear relationship (R^2 = 0.9802). This relationship indicates a diffusion-controlled process for the electrochemical reaction mechanism.

Linear relationships of logarithm of peak current ip,a versus logarithm of scan rate (V s^{-1}) following the regression equation with R^2= 0.9796 were obtained (Figure 3; inset B). In this equation the value of the slope was found to be very close to the theoretical value of 0.5 for diffusion species (24). The result further confirms that the diffusion phenomenon is dominant.

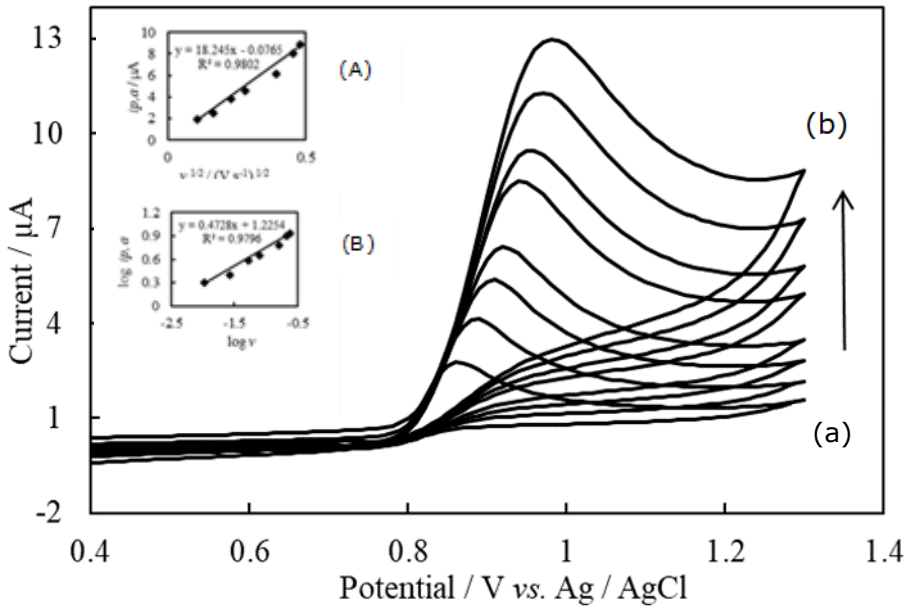

Figure 3: Effect of potential scan rate on both anodic peak current and anodic peak potential of 1.5×10^{-3} mol L^{-1} NZ HCl at pH 7.0 (inset: (A) Curve of peak current versus square root of scan rate, (B) curve of logarithm of peak current versus logarithm of scan rate (a: 0.01 V s^{-1}, b: 0.025 V s^{-1}, c: 0.05 V s^{-1}, d: 0.075 V s^{-1}, e: 0.1 V s^{-1}, f: 0.175 V s^{-1}, g: 0.2 V s^{-1}, h: 0.225 V s^{-1})

EIS studies of UCPE and Al_2O_3-NPs-CPE were conducted in 0.1M KCl solution with 5.0 mM $[Fe(CN)_6]^{3-/4-}$ in the frequency range, $0.05–10^5$ Hz with 10 mV as the amplitude (Figure 4). In the Nyquist plot of impedance spectra the semicircle part represents the electron transfer limited process and its diameter is equal to the electron transfer resistance, R_{ct} (25). The R_{ct} for Al_2O_3-NPs-CPEs (curve B) is smaller than that of UCPE (curve A) indicating that incorporation of Al_2O_3 nanoparticles into carbon paste matrix facilates electron transfer.

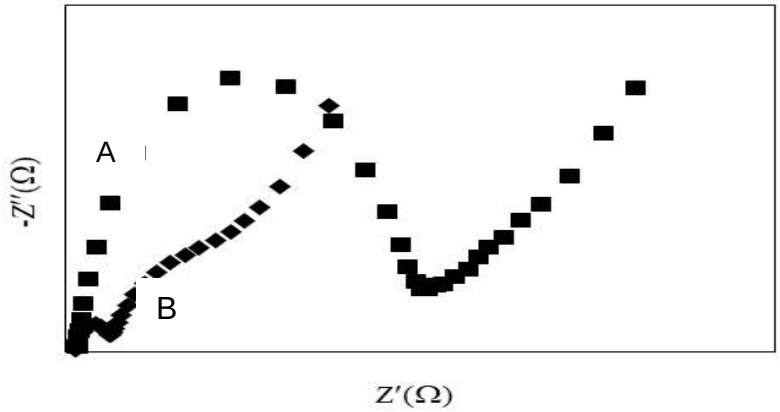

Figure 4: The Nyquist curves of (A) UCPE and (B) Al_2O_3-NP-CPE in 0.1 M KCl solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$.

Influence of pH

pH of the test solution is an important parameter that has a significant influence on the electrochemical behaviors of molecules. Thus, the influence of pH on the electrochemical behavior of NZ HCl was investigated in the pH range of 2.0–10.0 (Figure 5). Two different trends can be observed in the SW voltammograms as the pH varies between 2.0–7.0 and 7.0–10.0. The first trend is that the anodic peak potential shifts to more cathodic values and peak current increases as pH varies from 2.0 to 7.0. Conversely, as the second trend, the peak potential shifts to more anodic values when pH varies from 7.0 to 10.0, and the peak current decreases. The greatest peak current was obtained at pH 7.0 (data not shown), and it was chosen as the optimum pH.

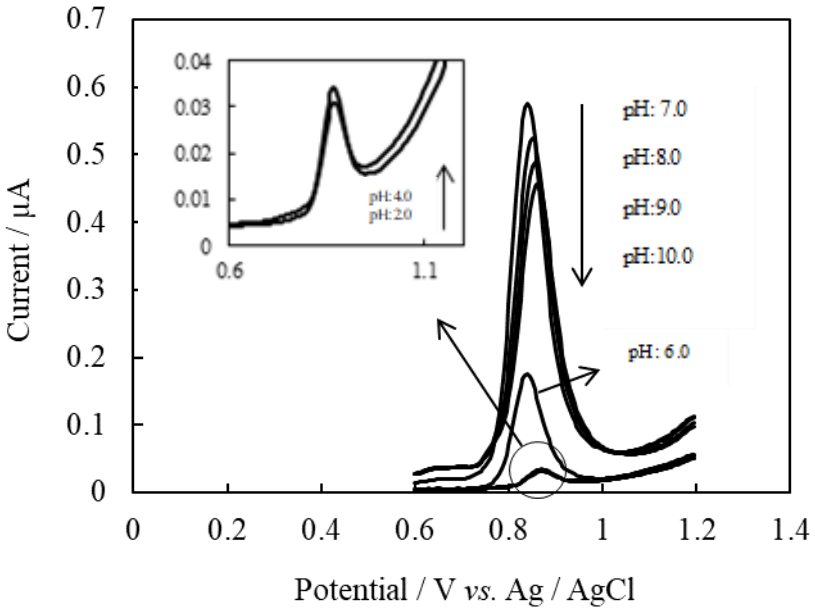

Figure 5: Effect of pH on SWVs of 1.0×10^{-5} mol L^{-1} NZ HCl.Inset:SWVs obtained at pH 2 to 4.

Optimization of the experimental variables

In order to specify the optimum working conditions, instrumental parameters and experimental variables were investigated and optimized. Various instrumental parameters including frequency (f), scan increment (ΔE_i) and pulse amplitude (ΔE_a) were optimized for 1.0×10^{-6} mol L^{-1} NZ HCl in a BR solution at pH 7.0 and found to be f: 10 Hz, ΔE_i: 5 mV, ΔE_a: 5 mV.

The effect of the deposition potential on the SWAAdSV signal was evaluated in the range of 0.0 V – 0.8 V for a 1.0×10^{-6} mol L^{-1} NZ HCl solution. The maximum peak current was obtained at 0.6 V (data not shown). The effect of deposition time on peak current was also studied in the range from 15 to 150 s for 1.0×10^{-6} mol L^{-1} NZ HCl and 90 s was found as the optimum deposition time (data not shown).

Linear working range and detection limit

In this study, electrochemical determination of NZ HCl with adsorptive techniques was achieved and a lower limit of detection than studies reported in the literature was obtained.

After the operating conditions were optimized, SWAAdSV was conducted in the BR buffer solution (pH 7.0) containing different NZ HCl concentrations in order to obtain the analytical curve. The obtained values for the peak current gave a linear relationship with the NZ HCl concentrations in the range from 5.0×10^{-8} mol L^{-1} (0.0123 mg L^{-1}) to 3.0×10^{-5} mol L^{-1} (7.40 mg L^{-1}) (Figure 6). The calibration plots are represented by the equation $I_{p,a}$ (µA) = 0.0667 [NZ HCl] (µmolL^{-1}) + 0.0298 with a correlation coefficient of 0.9949 (Figure 7). The high value of the correlation coefficient indicates a good linearity, confirming the validity of the SWAAdSV method for NZ HCl determination. Table 1 depicts the characteristics of the calibration plot.

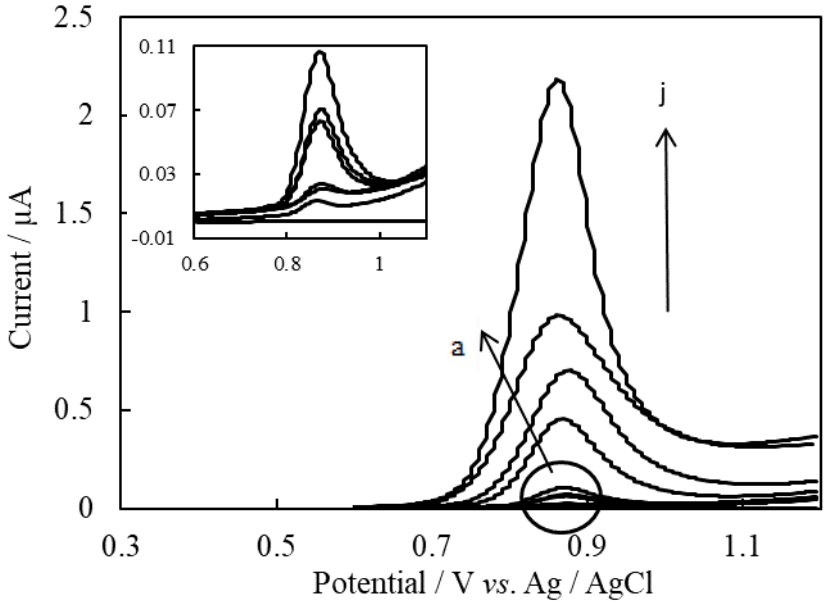

Figure 6: SWAAdS voltammograms of NZ HCl at different concentrations (in µmol L^{-1}) a: 0.05, b: 0.08, c: 0.1, d: 0.5, e: 0.8, f: 1.0, g. 5.0, h: 8.0, i: 10.0, j: 30. Inset: SWAAdS voltammograms from a to f.

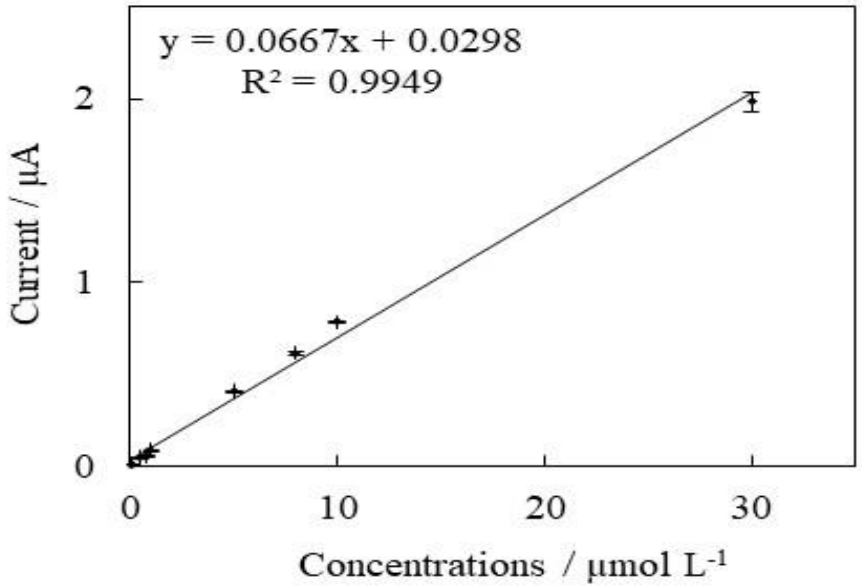

Figure 7: Calibration curve for NZ HCl solutions in BR buffer of pH 7.0 ($t_{deposition}$: 90.0 s, $E_{deposition}$: 0.6 V). Error bars show the standard deviation of three measurements.

Limit of detection (LOD) and limit of quantification (LOQ) values were estimated according to the following equations: $LOD = 3\frac{s}{m}$ $LOQ = 10\frac{s}{m}$ (18) where *s* refers to the standard deviation of intercept of calibration curve and *m* refers to the slope of the related calibration curve. *LOD* and *LOQ* values were calculated as 2.6×10^{-9} mol L^{-1} (0.642 µg L^{-1}) and 8.7×10^{-9} mol L^{-1} (2.147 µg L^{-1}), respectively (Table 1). These low LOD and LOQ values indicates that the presented method can be utilized to analyze the NZ HCl concentration of highly diluted samples. It is also important to highlight that the LOD obtained in our study was lower than the detection limits previously obtained for potentiometric methods (3,14,15,19).

Stability of the proposed method

The stability of NZ HCl in the BR buffer at pH 7.0 was investigated under the optimum working conditions by observing the changes in both the anodic peak potential and the anodic peak current of standard NZ HCl solution. Relative standard deviation (RSD) of peak current and peak potentials obtained after three series of measurements were calculated as 1.1% and 0.1%, respectively (Table 1). It can be concluded that the absence of notable change in peak potential and peak current indicates the excellent stability of the method. Moreover, the stability of standard stock solution was investigated for 2 weeks. The standard stock solution of NZ HCl was

kept in dark at +4 ˚C. No changes were observed in the peak potential or peak current of NZ HCl and it was found to be stable during this period.

Table 1: Regression data of the calibration curve for assay of NZ HCl by SWAAdSV.

Parameter	Value
Linear working range / mol L^{-1}	$5.0 \times 10^{-8} - 3.0 \times 10^{-5}$
Slope (m) / (μA L mol^{-1})	0.0667
Intercept / A	3.0×10^{-8}
Standard deviation of calibration (s_r) / A	4.74×10^{-8}
Standard deviation of slope / (μA L mol^{-1})	0.0017
Standard deviation of intercept (s) / A	3.53×10^{-8}
LOD / mol L^{-1}	2.6×10^{-9}
LOQ / mol L^{-1}	8.7×10^{-9}
Regression coefficient (R^2)	0.994
Repeatability of peak potential (RSD)[a] %	0.1
Repeatability of peak current (RSD)[a] %	1.12

[a]Calculated for 3 replicate measurements.

Analytical application of the presented method

NZ HCl was determined in spiked human serum samples to investigate the applicability of the presented method. Table 2 shows the results of the analysis of serum samples. The accuracy of the method was evaluated by its recovery values. The mean recoveries are in the range of 98.25 – 104.41% (Table 2). The recovery values are very close to 100% indicating that the method shows high accuracy. The reproducibility of the method was estimated from three replicate measurements of electrochemical signal of different NZ HCl solutions. The RSD of recovery values are within the range 0.85 – 3.81% (Table 2). It can be concluded that the precision of the method is very satisfactory for the analysis of serum samples. Hence, this method can be used safely in determination of NZ HCl content in human serum samples. t_{test} was also used to evaluate the performance of the method. The results presented in Table 2 shows that the t_{exp} values does not exceed the $t_{(critical)}$ values confirming that the results of the proposed method and the spiked amount show no difference at a confidence level of 95%.

Table 2: Results of NZ HCl amounts in human serum spiked by standard NZ HCl determined using the presented SWAAdSV method.

Sample	Spiked amount, μg	Found amount, μg	Recovery value, %[a]	RSD, %[b]	$t_{exp.}$
Standard in serum I	2.47	2.45; 2.34; 2.49	98.25 ± 7.75	3.18	0.024
Standard in serum II	12.34	12.88;12.99; 12.77	104.41 ± 2.21	0.85	1.06

[a]Results of recovery values are given as mean ± $\frac{ts}{\sqrt{N}}$ at 95% confidence level); [b]RSD Relative standard deviation; $t_{critical}$ value for 95% confidence level is 4.30 for two degrees of freedom.

Table 3 compares the characteristics of potentiometric and voltammetric methods reported for the analysis of NZ HCl with the proposed method. It is clear from the table that the method exhibits a low detection limit, wide linear range and good recovery. Among electrochemical methods for NZ HCl proposed method exhibits the best analytical characteristics.

Table 3: Comparison of electrochemical methods reported for the determination of NZ

Method	Working electrode	Linear range mol L^{-1}	Detection limit mol L^{-1}	Recovery pharmaceutical preparation %	Recovery serum %	Ref.
Potentiometric	ISPE ICPE	7.0×10^{-7}-1.0×10^{-2}	5.6×10^{-7} 5.9×10^{-7}	98.6-101.3 96.4-103.8	-	(3)
Potentiometric	NPZ-TPB ion pair (conventional) NPZ-TPB ion pair (Graphite coated)	1.0×10^{-5}-5.0×10^{-2} 5.0×10^{-6}-5.0×10^{-2}	4.0×10^{-6} 2.5×10^{-6}	97.9-101.8	-	(14)
Potentiometric	SPE CPE	1.0×10^{-6}-1.0×10^{-2}	3.5×10^{-6} 1.5×10^{-6}	98.3-99.0 97.20-97.30	-	(15)
BIA-SWV	BDD electrode	3.0×10^{-6}-2.1×10^{-5}	4.0×10^{-8}	-	-	(19)
SWAAdSV	Al_2O_3-NP-CPE	5.0×10^{-8}-3.0×10^{-5}	2.6×10^{-9}	-	98.2-104.4	This study

ISPE:*in situ* modified screen printed electrode; ICPE: *in situ* modified carbon paste electrode; NPZ-TPB: naphazoline tetraphenylborate; SPE: screen printed electrode; CPE: Carbon paste electrode; Al_2O_3-NP-CPE: aluminium oxide nanoparticles modified carbon paste electrode; BIA- SWV: batch-injection analysis system with square wave voltammetry; BDD: boron- doped diamond.

CONCLUSION

Electrochemical determination of NZ HCl in human serum was achieved with SWAAdSV method for the first time. Al_2O_3-NPs-CPE electrode enhanced the analytical performance of the proposed method in terms of LOD, LOQ and linear working range. The proposed method offers a simple, sensitive, rapid and low-cost approach to the analysis of NZ HCl in human serum samples. Moreover, the method was successfully applied to serum samples, with the advantage of no requirement of time consuming extraction step.

ACKNOWLEDGEMENTS

This work was funded by Namık Kemal University Research Fund. Project No. NKU BAP. 00.10.YL.14.03. The authors would like to thank to Prof. Esma Kılıç at Ankara University for helpful discussions.

REFERENCES

1. Meloun M, Syrový T, Vrána A. The thermodynamic dissociation constants of ambroxol, antazoline, naphazoline, oxymetazoline and ranitidine by the regression analysis of spectrophotometric data. Talanta. 2004 62(3):511-22.

2. Manzoori J, Amjadi, M. Spectrofluorimetric and cyclodextrin-enhanced spectrofluorimetric methods for the determination of naphazoline in nasal and eye drops. Indian Journal of Chemistry. 2003 42A:2988-92.

3. Mohamed GG, El-Dien FN, Frag EY, Mohamed MEB. In situ modified screen printed and carbon paste ion selective electrodes for potentiometric determination of naphazoline hydrochloride in its formulation. Journal of Pharmaceutical Analysis *J. Pharm. Analysis*. 2013 *5*:367-75.

4. Souri E, Amanlou M, Farsam H, Afshari A. A rapid derivative spectrophotometric method for simultaneous determination of naphazoline and antazoline in eye drops. Chemical and Pharmaceutical Bulletin. 2006 54(1):119-22.

5. Goicoechea HC, Collado MS, Satuf ML, Olivieri AC. Complementary use of partial least-squares and artificial neural networks for the non-linear spectrophotometric analysis of pharmaceutical samples. Analytical and Bioanalytical Chemistry. 2002 374(3):460-5.

6. Joseph-Charles J, Bertucat M. Simultaneous determination of naphazoline nitrate and tetramethylthionine base in eye drops by first-derivative UV spectrophotometry. Analytical Letters. 1999 32:373-82.

7. Zhu S, Liu Y. Spectroscopic analyses on interaction of Naphazoline hydrochloride with bovine serum albumin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012 98:142-7.

8. El deen Sayed N, Hegazy M, Abdelkawy M, Abdelfatah R. Spectrophotometric, chemometric and chromatographic determination of naphazoline hydrochloride and chlorpheniramine maleate in the presence of naphazoline

hydrochloride alkaline degradation product. Bulletin of Faculty of Pharmacy. Cairo University, 2013 51(1):57-68.

9. Ali NW, Hegazy MA, Abdelkawy M, Abdelfatah, RM. Simultaneous determination of naphazoline hydrochloride, chlorpheniramine maleate and methylene blue in their ternary mixture. Pakistan Journal of Pharmaceutical Sciences. 2013 26:641-8.

10. Díaz BC, Terrones SC, Carretero AS, Fernández JMC, Gutiérrez AF. Comparison of three different phosphorescent methodologies in solution for the analysis of naphazoline in pharmaceutical preparations. Analytical and Bioanalytical Chemistry. 2004 379:30-4.

11. Huang T, Chen N, Wang D, Lai Y, Cao Z. A validated stability-indicating HPLC method for the simultaneous determination of pheniramine maleate and naphazoline hydrochloride in pharmaceutical formulations. Chemistry Central Journal. 2014, 8, 7: 1-9.

12. Korodi T, Dulavová M, Urban E, Kopelent-Frank H, Lachmann B. A Stability-Indicating HPLC method for the determination of naphazoline and its degradation product and methyl parahydroxybenzoate in pharmaceutical preparations. Journal of Liquid Chromatography & Related Technologies. 2014 37:1321-33.

13. Massaccesi M. Gas chromatographic determination of some imidazolines in pharmaceutical preparations using the FFAP in stationary phase. Pharmaceutica Acta Helvetiae. 1987 62(10-11):302-5.

14. Ghoreishi SM, Behpour M, Nabi M. A novel naphazoline-selective membrane sensor and its pharmaceutical applications. Sensors and Actuators B: Chemical. 2006 113(2):963-9.

15. Frag EY, Mohamed GG, El-Dien FN, Mohamed ME. Construction and performance characterization of screen printed and carbon paste ion selective electrodes for potentiometric determination of naphazoline hydrochloride in pharmaceutical preparations. Analyst. 2011 *136*:332-9.

16. Saito T, Morita S, Kishiyama I, Miyazaki S, Nakamoto A, Nishida M, Namera, A, Nagao M, Inokuchi S. Simultaneous determination of dibucaine and naphazoline in human serum by monolithic silica spin column extraction and liquid chromatography–mass spectrometry. Journal of Chromatography B. 2008 872(1-2):186-90.

17. Öztürk F, Küçükkolbaşı S, Kaçar C, Kılıç E. Electrochemical studies of olmesartan medoxomil and its detection in pharmaceutical dosage forms and biological fluids by cathodic adsorptive stripping voltammetric method. Journal of the Brazilian Chemical Society. 2014 25(5):920-7.

18. Öztürk F, Taşdemir IH, Durmuş Z, Kiliç E. Electrochemical behavior of disopyramide and its adsorptive stripping determination in pharmaceutical dosage forms and biological fluids. Collection of Czechoslovak Chemical Communications. 2010 75(6):685-702.

19. Thiagoda CO, Jhonys MF, Rodrigo A, Abarza M, Eduardo MR. A batch injection analysis system with square-wave voltammetric detection for fast and simultaneous determination of naphazoline and zinc. Talanta. 2016 152:308-13.

20. Uslu B, Ozkan SA. Electroanalytical application of carbon based electrodes to the pharmaceuticals. Analytical Letters. 2007 40(5):817-53.

21. Shi X, Gu W, Li B, Chen N, Zhao K, Xian Y. Enzymatic biosensors based on the use of metal oxide nanoparticles. Microchimica Acta. 2014 181(1-2):1-22.

22. Öztürk FÖ, Erden PE, Kacar C, Kilic E. Amperometric biosensor for xanthine determination based on Fe_3O_4 nanoparticles. Acta Chimica Slovenica. 2014 61(1):19-26.

23. Karimi-Maleh H, Ahanjan K, Taghavi M, Ghaemy M. A novel voltammetric sensor employing zinc oxide nanoparticles and a new ferrocene-derivative modified carbon paste electrode for determination of captopril in drug samples. Analytical Methods. 2016 8(8)1:1780-8.

24. J. Wang, Analytical electrochemistry, 2 nd edition, Wiley-VCH, New York 2000.

25. Chang BY, Park SM. Electrochemical impedance spectroscopy. Annual Review of Analytical Chemistry. 2010 3: 207-9.