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Ultra-trace Determination of Preconcentrationed Naltrexone using Electromembrane Extraction followed by FFT Square Wave Admittance Voltammetry in Urine Samples

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Abstract- In the present paper, naltrexone determined at ultra-trace scale was performed by combining fast Fourier transform square wave admittance voltammetry with electromembrane extraction in urine samples. On this approach, a constructed three-microelectrode setup positioned at the upper end of the hollow fiber in a micro-pipette tip, which was then applied for the extracted analyte determination. The employed membrane was consisted 85% of 2-nitrophenyl octyl ether and 15% di-(2-ethylhexyl) phosphate immobilized in the pores of a hollow fiber. A DC potential of 150 V within the time of 25 min was applied, followed by the analytes migration from 1 mM HCl as the sample solution, through the supported liquid membrane into an acidic acceptor solution with pH 3.0 placed in the lumen of hollow fiber. Based on the obtained results, the introduced method exhibited one the linear range 5-1000 ng/mL (R^2=0.993). Besides, the detection limit of 0.5 ng/mL was attained. Since the preconcentration factor was found to be 80 in the urine sample after the performed process, it can be concluded that the introduced method could be classified among qualified techniques for naltrexone detection in some complex samples.

Keywords- Naltrexone, Fast Fourier transform square wave voltammetry, Electroanalytical detection, Blood sample

1. INTRODUCTION

Naltrexone HCl (NAL.HCl) is a drug which chemical structure is shown as Fig. 1, is usually prescribed in the cases of treating addiction to specific drugs or drug abuse. In addition, it is chemically related to naltrexone which is used originally for the treatment of opioid overdoses and sickly dependences such as alcoholism [1]. Due to its clinical high importance, probable side effects and the risks that its overdoses can cause to human health, various methods have been developed to analyze NAL HCl in body fluids and formulations. It has been quantified using methods such as gas chromatography (GC) [2,3], high performance liquid chromatography (HPLC) [4,5], potentiometry [6] and electrochemical methods [7]. Although high sensitivity is not hard to achieve using some of mentioned techniques such as GC, HPLC, they confront some obstacles like tedious sample pretreatment and high cost procedures. As a solution, electrochemical techniques can be adequate options as they are being more concentrated due to their remarkable advantages such as high sensitivity, simplicity, selectivity, and relatively low cost, compared with other analytical methods [8,9]. From another aspect, random noises form unpredictable sources could cause serious errors specifically when the current magnitude is in the ranges of nano and pico ampere. Consequently, the application of fast Fourier transform (FFT) filtrate besides the conventional electrochemical techniques can lead to a more sensitive systems [10]. In FFT based electroanalytical processes, the electrochemical signal initially would be transformed into the frequency domain and could be then simply filtered [11,12]. Among the FFT voltammetric techniques, Fast Fourier Transform Square Wave Voltammetry (FFTSWV), is classified as sufficiently selective, cost-effective and quick methods. This paper introduces a basically new approach to SWV, namely FFT square wave admittance voltammetry (FFTSWAV) [13,14].

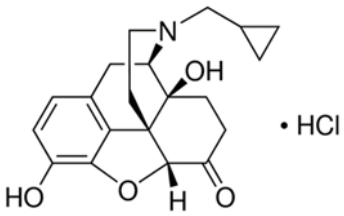

Fig. 1. The chemical structure of naltrexone HCl

Using FFT assisted systems for analytical purposes leaded to lower detection limits. In concise, the analyte signal would be obtained using the admittance fluctuations linked to the occurred variations in the electrical double layer [15]. From another point of view, since the analyte can usually be found at trace and even ultra-trace amounts in complicated matrices. However, it is a critical challenging task to have an efficient sample clean up technique before the detection step; this can lead to an effective detection. On this purpose, sample preparation

techniques such as liquid phase microextraction (LPME) could be a reasonable pretreatment which includes laws of liquid-liquid extraction and miniaturized nature of solid phase extraction as well. Hence, the advantages of both of them could be reached [16]. In this classification, electromembrane extraction (EME) is a linking technique between LPME and electrophoresis, which was introduced by Pedersen-Bjergaard et al. in 2006 for the first time. In EME, the charged species are exposed to an external electric field from the aqueous sample solutions through a supported liquid membrane (SLM), and then into the acceptor solution. It can be considered as a layer of a water immiscible organic solvent on a supportive fixing component [17]. Besides, not only EME can be used for miniaturized and quick extraction, but also it triggered great deal of attention as a good replacement of conventional treatment methods and has presented broad applications for pharmaceuticals extraction from different complex samples [17]. Herein, EME and FFTASWAV were coupled together to propose a novel technique trace determination of NAL. Since in FFTASWAV the admittance of a square wave signals were achieved based on the application of discrete FFT thus, the sensitivity of the determination step enhanced considerably. A three-microelectrode measurement setup was designed and exposed to the sample solution through a micropipette tip.

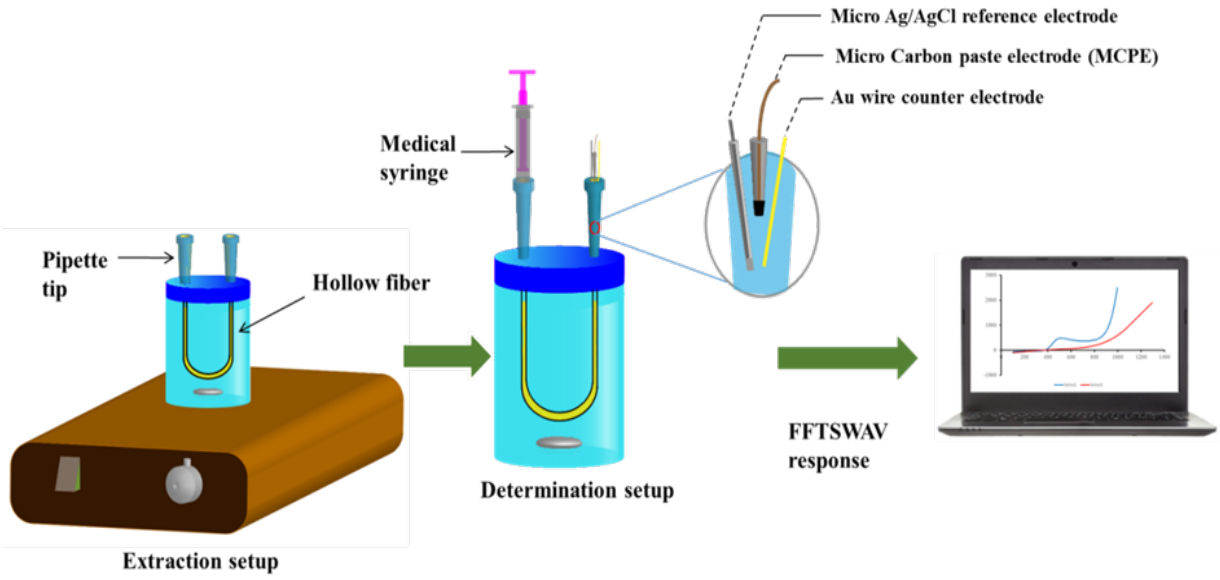

Fig. 2. The designed extraction setup for the determination of NAL using EME-FFTSWAV

Some determinative parameters which had the potential to affect extraction recovery of NAL were investigated and optimized. At last, the introduced technique capability for direct spike of urine as the real sample was examined.

2. EXPERIMENTAL

2.1. Chemical materials

The used hollow fiber was a PP Q3/2 polypropylene made in Germany with wall thickness of 200 μm, i.d. 1200 μm, and 0.2 μm of pores. Naltrexone HCl ingredient was provided from an Iranian pharmaceutical company 2-nitrophenyl octyl ether (NPOE-99%) and Bis(2-ethylhexyl(phthalate (DEHP) at analytical grade was obtained from Aldrich. Also, hydrochloric acid (37%), sodium hydroxide (98%) and ethanol (96%) were purchased from Merck. For real sample analysis, urine samples were gained from three patients under treatment.

2.2. Instrumental construction

A three-electrode configuration was used for the electrochemical measurements. A micro carbon paste electrode i.d. 800 um, gold wire i.d. 500 μm and an Ag/AgCl handmade microelectrode were used as the working, the counter, and the reference electrode, respectively. In order to make the CPE, graphite powder and paraffin oil at (20% w/w) ratio was mixed and intensively compressed into a plastic syringe. The electric contact was provided through a copper wire placed at the end of the tube. The electrode surface was renewed on a soft clean paper, and after each step. A homemade Ag/AgCl microelectrode was produced based on literature and utilized as the reference electrode [18]. The reference electrode has been made with an Ag wire with inner diameter of 0.5 mm coated with a thin film of AgCl which was then immersed into a saturated KCl solution. The handmade reference electrode, showed good stability and reproducibility during the electrochemical analyses. As Fig. 2 depicts, all of the three electrodes were set in desired distances towards each other and placed into the channel of a micropipette tip.

2.3. The setup construction

The employed potentiostat for the FFTSWA voltammetric measurements was designed and constructed at center of excellence in electrochemistry at university of Tehran, Tehran, Iran. The potentiostat was controlled by designed software in our lab, by which all of the procedures including; applying potential waveform, acquiring current, data processing and plotting were accomplished.

A special potential waveform was programmed for FFTSWAV measurement, for reaching the highest possible analysis sensitivity. It contains two sections; a potential step for accumulation and a potential ramp for the test. Initially, the accumulation potential (E_s) of 700 mV was applied to the working electrode, after 5 seconds, the waveform containing multiple SW pulses (amplitude of E_{sw} and frequency of f_o) in combination with FFT was performed which superimposes on a staircase potential. The number of sampled currents at SW pulse

cycle was 8, and N_c is the number of sampled current. Cyclic voltammetry (CV) and FFTSWAV were the methods which were used through their specific advantages [19,20].

2.4. The employed separation cell

The setup used for the EME measurements included a donor phase, a polypropylene hollow fiber, and an acceptor phase. The membrane was immersed in the organic solution for 30 s in order to soak the membrane pores with the organic solvent. The donor and acceptor phase volumes were 15 mL and 80 µL in all experiments, respectively. Two Platinum wires were employed as electrodes in the donor and acceptor solutions. In the next step, SLM and acceptor solution were implanted in the sample reservoir. After that, two gold electrodes and another electrode as the anode i.d. 200 µm as the cathodes and the anode respectively were set in the donor solution at the center of the U-shaped hollow fiber. The electrodes were then linked to a homemade power supply and a predetermined voltage was applied while the solution was being stirred at 500 rpm. Next, the analyte, which was extracted into the acceptor solution, was moved into the micropipette tip (Fig. 2). Finally, the placed miniaturized microelectrode set up into the micropipette was ready to use for the electrochemical measurements.

3. RESULT AND DISCUSSION

3.1. The electroanalytical studies

The cyclic voltammetric response of the prepared CPE was investigated initially in the blank solution; PBS (0.1 M) pH=3, and it is shown in Fig. 3.

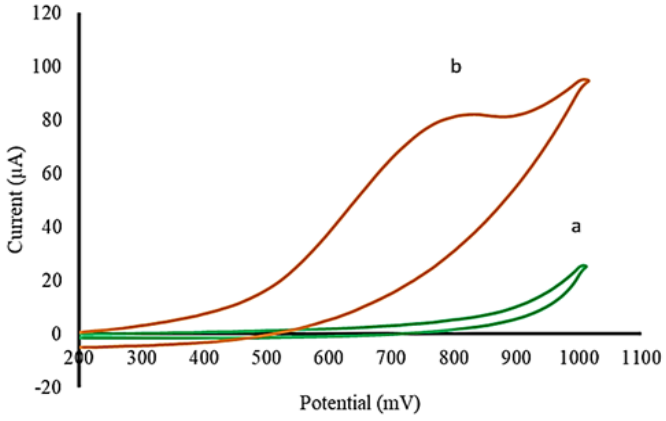

Fig. 3. The voltammetric response of the CPE in (a) PBS pH=3, (b) 20 µg/mL NAL in 0.1 M PBS pH=3 at scan rate of 0.5 $V.s^{-1}$

As it can be observed, a sharp oxidation peak was appeared at about 750 mV. In the next step, before the electrochemical measurements in the separation cell, the FFTSWAV technique was applied to the CPE. In order to reach the highest efficiency for NAL determination, some effective parameters affecting FFTSWAV, including frequency, amplitude and pH of the acceptor phase were optimized.

3.2. The accumulation parameters

The signal produced by the analyte has high possibility to be influenced by the parameters such as accumulation time and potential. Thus, the accumulation time 0-10 s and the potential -1000-1000 were varied. The sample solution contained 20 µg/mL NAL in PBS pH=3. The highest response towards the analyte was achieved at the accumulation potential of 700 mV and the time of 5 s. The reason could be analyte species positive charge of NAL in the bulk solution, which could have quicken the mass transfer towards the electrode surface containing potentials at negative values. The reason could be analyte species positive charge of NAL in the bulk solution, which could have quicken the mass transfer towards the electrode surface containing potentials at negative values.

3.3. FFTSWAV method parameters

The proposed method included two variables; frequency (Hz) and amplitude (mV), which both would have the scan rate role in cyclic voltammetry measurement [21], following the received signal would be highly connected to these variables value, thus it was decided to optimize the parameters to reach the best performance of the electrode. On this purpose, the frequency from 88 to 2830 Hz, the amplitude from 10 to 150 mV and 8 cycles were considered for NAL solution 20 µg/mL in PBS pH=3.

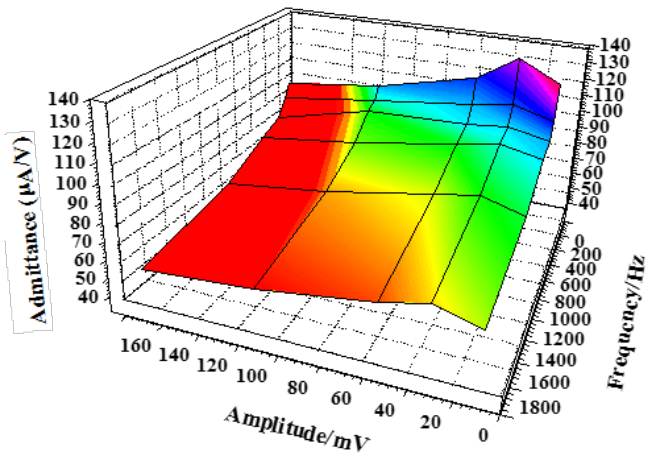

Fig. 4. The frequency and amplitude optimization for CPE in NAL 20 µg/mL; PBS 0.1 M pH=3

The result has been shown in Fig. 4. As it is depicted, the maximum signal was reached for the frequency 88 Hz and amplitude of 30 mV. Moreover, it is shown that as the frequency increased, the signal enhanced as well. It could have happened because of the stated role for the frequency consequently, by increase of the SW frequencies, the signal sensitivity of SWAV could be limited due to some reasons such as; R_s, electrode surface area. Besides, it is also seen that the obtained signal enhanced as the amplitude rose up to 30 mV and the reason could be allotted to higher rates of analyte diffusion. As the results showed, frequency 88 Hz, amplitude of 30 mV and 8 cycles were chosen as the optimized values for further steps.

3.4. The acceptor phase pH effect

Another significant factor in the FFTSWAV response of NAL is the pH of acceptor solution. At this stage, pH of the acceptor phase containing 20 µg/mL NAL was varied in the range of 1–5 values.

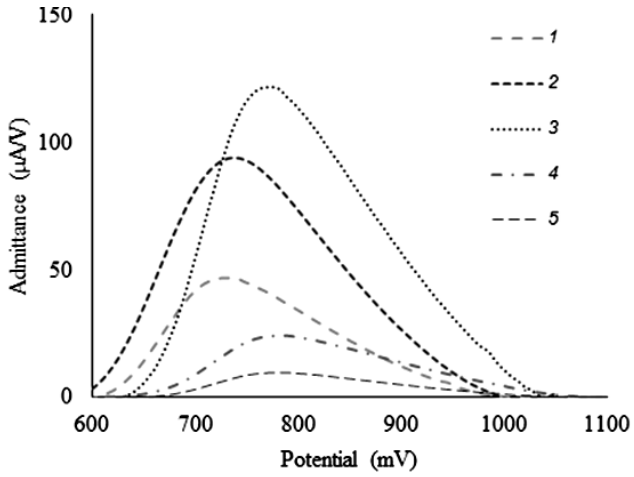

Fig. 5. The pH effect on the CPE response in NAL 20 µg/mL; 0.1 M PBS pH=3, stripping potential of 700 mV, stripping time of 10 s, frequency of 88 Hz, cycle of 8, amplitude of 3 mV

The results presented in Figure 5 depicted that as the pH solution increased from 1 to 5, the shift of peak potential to more positive values was observed, indicating the participation of protons in the electrode process and vice versa. The Maximum FFTSWAV signal of NAL was obtained when the ample solution pH was adjusted at 3, therefore, pH=3 was chosen as the optimum pH for the electrochemical determination of NAL.

3.5. The EME parameters optimization

Since the developed method is combination of electroanalytical methods and membrane technology, other various factors such as the organic solvent type, pH value for the donor and acceptor solutions, the applied voltage and extraction time. Among stated variables, the pH value for the acceptor phase (pH=3) was optimized as expressed in previous sections. Other

potential determinative factors were examined through a face centered central composite design (FCCD) and response surface methodology (RSM). To reduce test numbers and more simplicity, the SLM composition was noticed individually for each single variable. Other conditions were considered as mentioned.

3.6. The organic solvent type

The solvent nature used in SLM, has a noteworthy role in reaching to the adequate results in an EME process [21,22]. In such extractions, addition of an ion-pair reagent could assist the process. At this stage, 5%, 10%, 15%, 20% and 30% percentages of DEHP were added separately to NPOE as the carrier. The obtained results are shown in Fig. 6. Based on the result, DEHP plays a significant role in the analyte transfer. In conclusion, the DEHP concentration could have severely affect a hydrophilic analyte extraction such as NAL as an ion-pair reagent. As it is obvious in Fig. 6, DEHP enrichment from 5 to 15% enhanced the efficiency factor.

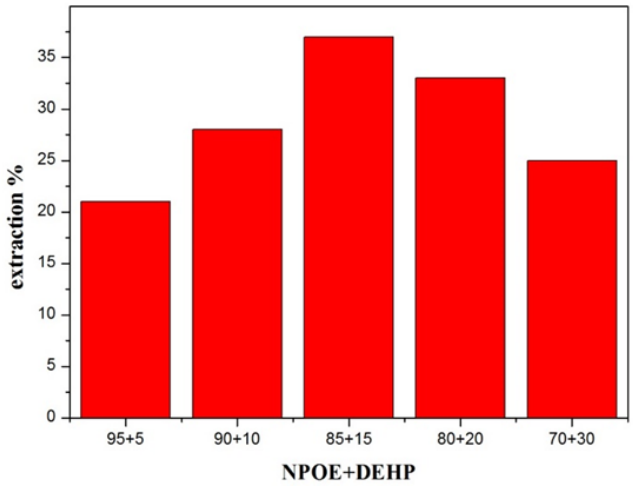

Fig. 6. The effect of solvent type in EME in NAL 20 µg/mL, extraction time: 25 min, applied voltage: 150 V, feed pH=2.5 and receive source pH=1.0

It is also clear that the carrier at higher concentrations (>15%) in the membrane caused the signal to be decreased considerably. The SLM electrical resistance could be the reason for heightening of the signal values. Through this occurrence bubbles could be created around the fiber which could affect the system stability, subsequently, extraction recoveries diminished. As the result NPOE 85% beside DEHP 15% were selected as optimized composition for the SLM.

3.7. The effective parameters on the NAL extraction recovery

As mentioned before, the RSM including CCD was used for reaching the optimum value for some determinative EME variables. The reasonable optimized values for chosen parameters

would be reached through considering almost all the possible interactions between the variables. On this basis, the produced signal could be forecasted at any desirable point in the factor limitation whether it is possible in the real experiment. To have a general conception of the model following equation could be assumed;

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j \quad (1)$$

where Y, X_i and X_j are the dependent and the independent variables, respectively. Also, β_i, β_{ii} and β_{ij} show the regression coefficients for the linear, quadratic and interaction effects of the model and β_0 is the deviation between the observed and predicted responses at the considered stage. Thus, the total number of required points, which were demanded (N) could be considered as:

$$N = 2^f + 2f + C_p \quad (2)$$

where the number of significant factors is shown as f, number of the factorial points, axial points and center points is depicted as 2^f, 2f and Cp. As the plan, [16] the tests with three central points were considered in a random order. The each run signal was selected as the objective response in this process. The archived data were evaluated using analysis of variance (ANOVA) and shown in Table. 1. As it is obvious, there was a reasonable correlation between the experimental and the three experimental factors (R^2=0.998). The model can be assumed as follow:

$$Current = 97.34 + 0.1902V + 4.706t - 9.607\,pH + 0.1134Vt - 0.0683VpH + 0.0311tpH + 0.0159V^2 + 0.1947\,pH^2 \quad (3)$$

The relationship between the explanatory and currents in a three dimensional representation of the response surface is displayed in Fig. 7 (A, C, E). At this stage, the variables while being kept at the central level were fluctuated within the experimental range. The achieved RSM plots implied to the interactions existence among the examined variables. Also, Fig. 7 (B, D, F) depicts two-dimensional contour plot leaded from the considered model. During all of the experiment the concentration of 20 µg/mL of NAL was used. The SLM nature is highly determinative for the amount of time and the applied to the EME. Due to NPOE having high electrical resistance, high voltages in the long periods of time could be used in order to have the maximum extraction efficiency. Based on the obtained statistical data (Fig. 7A, B), the chosen values for the voltage of and extraction time were 150 V and 25 min, respectively.

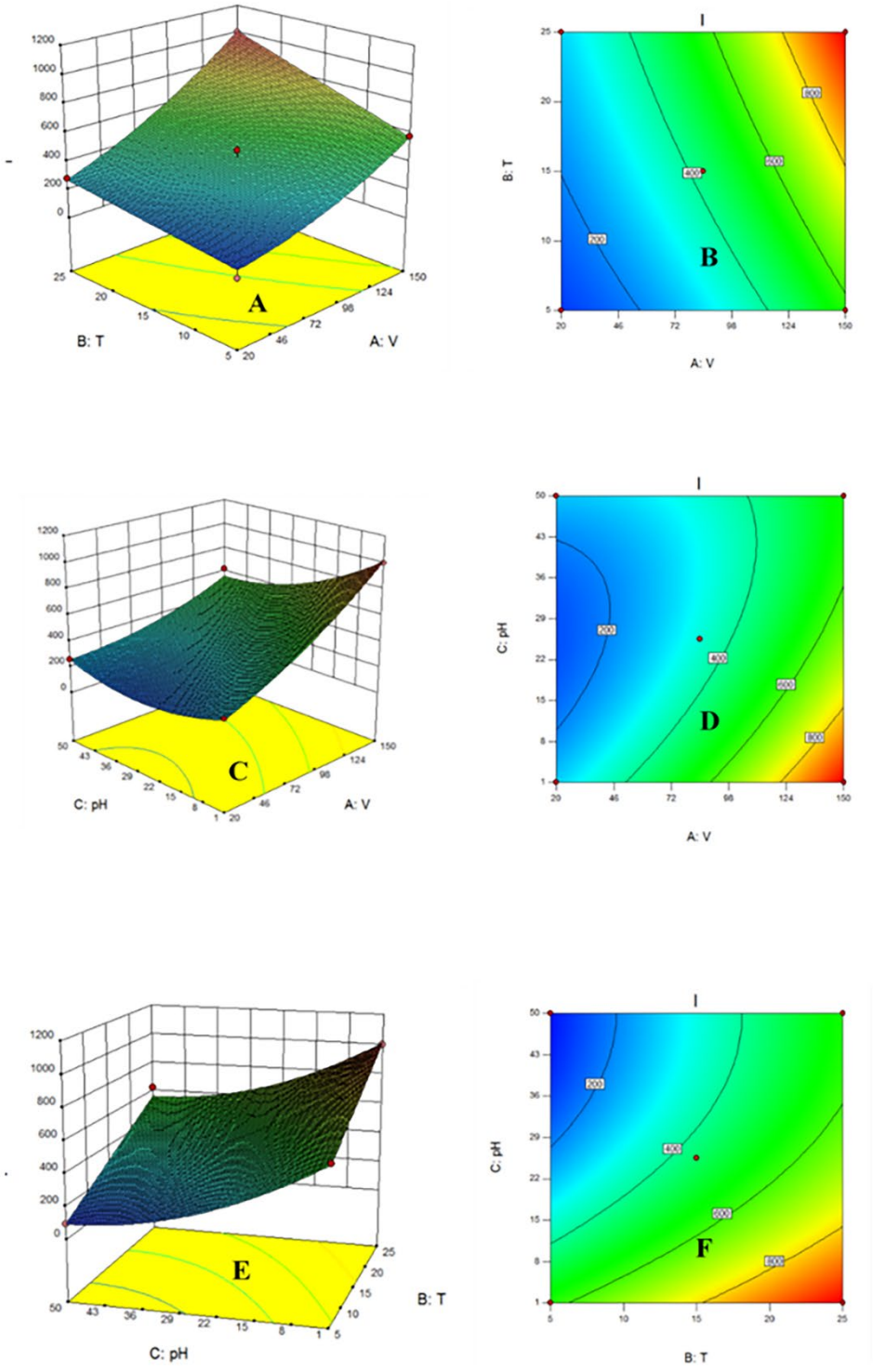

Fig. 7. (A and B) three-dimensional recovery percentage surfaces and contour plots against: (C and D) voltage and time: voltage and pH of donor phase; time and pH of donor phase of NAL 20 µg/mL

Table 1. Experimental factors, levels and analysis of variance (ANOVA) table for response surface of the quadratic model

Factors		**Levels**			
		-1	0	**1**	
Voltage (V)	A	20	85	**150**	
Extraction time (min)	B	5	15	**25**	
pH; Donor phase	C	1	2	**7**	
Source	**Sum of squares**	**df**	**Mean square**	**F-Value**	**P-Value Prob>F**	
Model	9.75×10^{5}	8	1.22×10^{5}	3.65×10^{1}	1.67×10^{-5}	**Significant**
A-voltage	3.46×10^{5}	1	3.46×10^{5}	1.04×10^{2}	7.43×10^{-6}	
B-Time	2.30×10^{5}	1	2.30×10^{5}	6.88×10^{1}	3.37×10^{-5}	
C-Sample pH	1.51×10^{5}	1	1.51×10^{5}	4.53×10^{1}	1.48×10^{-4}	
AB	4.37×10^{4}	1	4.37×10^{4}	1.31×10^{1}	6.84×10^{-3}	
AC	9.48×10^{4}	1	9.48×10^{4}	2.84×10^{1}	7.05×10^{-4}	
BC	4.65×10^{2}	1	4.65×10^{2}	1.39×10^{-1}	7.19×10^{-1}	
A^2	1.38×10^{4}	1	1.38×10^{4}	4.12	7.70×10^{-2}	
C^2	4.14×10^{4}	1	4.14×10^{4}	1.24×10^{1}	7.86×10^{-3}	
Residual	2.67×10^{4}	8	3.34×10^{3}			
Lack of fit	2.57×10^{4}	2	4.28×10^{3}	8.30	1.11×10^{-1}	**Not significant**
Pure Error	1.03×10^{3}	6	5.16×10^{2}			
Cor Total	1.00×10^{6}	16				

The electrical force to derive the extraction of NAL was provided by the applied voltage source. The extraction efficiency factor was improved as the applied voltage enhanced. From another aspect, considering different values of pH in the donor solution can affect the extraction efficiency. Transforming the analyte to their ionized form, could strongly help the analyte passage through the electrical field. Hence, for NAL, the transformation would cause the sample solution to contain the analytes with positive charges [23]. According the RSM plots resented in (Fig. 7 C-F), it was observed that as the pH in sample solution heighten up from 1.3 to 2.5, the extraction recoveries increased as well, so the pH of 2.5 was selected as the optimum value for the donor phase In the next step, the optimized conditions were employed

to evaluate the applicability of the proposed method for the extraction and quantification of NAL. The limit of detection (LOD) and limit of quantification (LOQ), the linearity, the extraction recovery ER(%), the preconcentration factor (PF), and precision of EME-FFTSWAV were also studied in NAL various concentration levels spiked in some urine samples. The extraction recovery (ER%) was calculated as below:

$$ER\% = n_f / n_i \times 100 \tag{4}$$

where n_f is the percentage of the number of moles of analyte in the acceptor phase and is the originally present in the sample solution. Also, the reconcentration factor (PF), the ratio between the final desired species concentration in the acceptor phase ($C_{f,a}$) and the initial concentration of analyte ($C_{i,s}$) in the sample solution, was considered as:

$$PF = \frac{C_{f,a}}{C_{i,s}} \tag{5}$$

where was the calculated from a calibration graph obtained from direct electrochemical determination of NAL standard solutions. Also, all of the fixed analytical parameters are presented in Table 2.

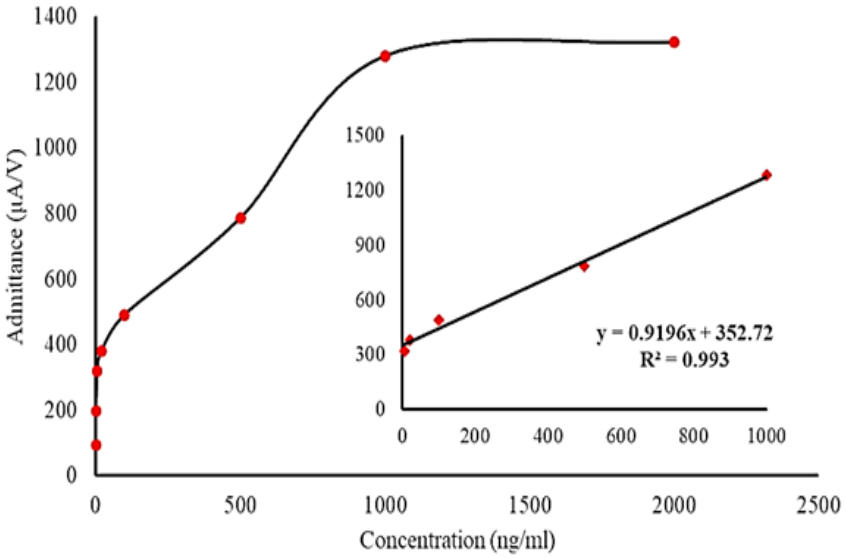

Fig. 8. The calibration curve of NAL in urine samples under the optimized conditions; stripping potential: 700 mV, stripping time: 5 s, frequency: 88 Hz, cycle: 8, amplitude: 30 and PBS pH=3. EME conditions: time: 25 min, applied voltage: 150 V, feed and receive pH=3 and 1

The LOD value was provided using the practical experiment data; where the signals-to-noise ratio was 3, the applied concentration was assumed as LOD and when it was 10 the

concentration was considered as the LOQ value which was also the first point of the calibration curve. As shown in the calibration curve voltammogram, Fig. 8, a wide linear range was observed. Table. 2 presents the analytical characteristics. PF was defined as the final analyte concentration in the acceptor phase to the initial concentration of analyte in the sample solution ratio. Repeatability was assessed by extracting three replicates of NAL 20 μg/mL and reproducibility was considered through the sample extraction under the same concentration for three consecutive days.

Table 2. Figure of merited for determination of NAL by EME-FFTSWAV in urine samples[a]

Evaluation parameter[b]		**Value**
Limit of detection (LOD) (ng/mL)		0.5
Limit of quantification (LOQ) (ng/mL)		5
Dynamic linear range (DLR) and Determination coefficient (R^2)	Linear range 1	5-1000 (0.993)
Preconcentration factor (PF)		61
Extraction recovery % (ER %)		27
RSD% (n=3)	Intra-day	4.5
	Inter-day	7.5

[b]Preconcentration factor, extraction recovery and RSD% were calculated based on extraction of 20 μg/mL of NAL from whole blood samples

Table 3. Comparison of EME- FFTSWAV with some other extraction methods used for extraction and determination of NAL

Method[a]	Sample	LOD (ng/mL)	DLR (ng/mL)	R^2	Ref.
EME–HPLC–UV	urine	10	20-1000	0.998	[1]
SPE–HPLC–DAD	plasma	10-500	1-240	0.998	[24]
LC–MS/MS	urine	0.2	2-1000	0.994	[25]
SPE–GC–MS	plasma	1	2-60	0.995	[26]
PPY/CNT/GCE	human blood serum	4	13-3400	0.9968	[27]
EME- FFTSWAV	urine	0.5	5-1000	0.993	This work

[a]SPE: solid phase extraction, HPLC: high performance liquid chromatography, MS: Mass Spectroscopy, GCE: Glassy Carbon Electrode

Moreover, a comparison between the present work and some recent related records is shown in Table. 3. The figures of merit are comparable or even better than the previously reported measurement assays for NAL. As it is expressed, lower LOD and a wider linear range were achieved by the proposed method. Besides, this developed method, showed high potential

for online, selective, cost effective and sensitive determination of NAL. Also, the method included simplicity and ease of procedure.

4. CONCLUSION

In the present study, FFTSWAV in combination with EME was used as a new sensitive setup for extraction and ultra-trace determination of NAL in urine samples. On the basis of this novel detection method, the sensitivity of the electrochemical determination step increased considerably. From another point of view, using EME before the FFTSWAV analysis caused the sensitivity of the detection to be improved greatly, due to the preconcentrating of the analyte in the acceptor phase. Moreover, EME capability of high clean up, the electrode life time improved as the surface passivation decreased. Different variables affecting the EME extraction efficiency were optimized by the FCCD. Furthermore, an anionic ion-pair reagent had the potential to support the extraction relatively polar substances. On this aim, a mixture of NPOE and DEHP was considered as the organic membrane. Finally, the proposed method was successfully used for the determination of NAL in urine samples without any pretreatment in very short analysis time.

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