The Natural Diatomite from Çaldıran-Van (Turkey): Electroanalytical Application to Antimigraine Compound Naratriptan at Modified Carbon Paste Electrode[§]

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Abstract: This paper is the first report describing the characterization of local diatomite of Çaldıran-Van region (Eastern Anatolia, Turkey). Special attention was paid to the ability of its electroanalytical performance at modified electrodes and to the potential application of diatomite-modified electrode. For this purpose, the determination of Naratriptan which is a novel oral triptan (5-hydroxytryptamine receptor agonist) in migraine treatment, by means of a carbon paste electrode modified with 10% (w/w) of diatomite was studied using cyclic and square-wave voltammetry. The experimental conditions that affect the electrode reaction process were studied in terms of pH of the supporting electrolyte, scan rate, accumulation variables, modifier composition and square-wave parameters. Using square-wave stripping mode, the drug yielded a well-defined voltammetric response in Britton-Robinson buffer, pH 4.0 at 0.84 V (*vs* Ag/AgCl) (a pre-concentration step being carried out with an open circuit at 120 s). The process could be used to determine Naratriptan concentrations in the range $5x10^{-7}$–$9x10^{-7}$ M, with a detection limit of $1.25x10^{-7}$ M (46.5 μg L^{-1}). The applicability of the method to spiked human urine samples was illustrated.

Keywords: Diatomite, characterization, modified carbon paste electrode, cyclic voltammetry, square-wave stripping voltammetry, naratriptan, urine.

1. INTRODUCTION

The use of silica and silica-containing matrices in the field of modified electrode was not widespread in the beginning of the 90's. However, it became popular in the mid 90's [1, 2] and successful applications have increased exponentially during the past decade [3-11]. Since the pure silica does not display any electroactivity, the electrodes modified with this modifier are only used to accumulate the electroactive analytes on the electrode surface prior to their detection by a suitable electrochemical technique [12-16]. The accumulation process has been attributed to the adsorption or ion exchange properties of silica, *via* weakly acid silanol groups (pK_a around 7) on its surface [14]. Despite good characteristics of pure silica-based electrodes in comparison with unmodified electrodes, their use remains uncommon [3].

Diatomite, also called diatomaceous earth or kieselguhr, is a siliceous, sedimentary rock consisting principally of the skeletal remains of diatoms; unicellular aquatic plants related to the algae lived in the ocean or lake [17]. Apart from being made of fossil skeletons, diatomite is essentially opal or hydrous silica in composition ($SiO_2.nH_2O$). In addition to bound water, siliceous skeleton may also contain small amounts of associated in organic components-alumina, principally-and lesser amounts of iron, alkaline earth, alkali metals, and other minor constituents. Active centers of diatomite having active hydroxyl groups on its silica surface responsible for adsorption may be characterized as the following: (*i*) single silanols (≡SiOH), (*ii*) geminal silanols or silanediols (=$Si(OH)_2$), (*iii*) H-bonded single silanols, H-bonded geminals, and their H-bonded combinations. In addition, silica surface consists of siloxane groups or ≡Si-O-Si≡ bridges with oxygen atoms, which are usually regarded as hydrophobic [18, 19]. The chemical composition and the physical structure of diatomite make it of great scientific and commercial value for a wide spectrum of uses. Its extremely porous structure with strong adsorbability, low bulk density, high surface area, chemical inertness, low thermal conductivity, high purity and inexpensiveness are attributes responsible for its utility as a filter aid, adsorbent, functional filler in paint, paper and rubber, thermal insulator, carrier for active ingredient and diluents, and aggregate [20-31]. It seems therefore to be a promising material for modification of electrodes. However, in spite of these attractive properties, the use of diatomite in connection with the voltammetric analysis has not been attempted and no reference is mentioned in literature.

Naratriptan as hydrochloride (Fig. **1**) is one of a novel class of drugs (triptans) for the acute treatment of migraine [32]. It is an orally active serotonin, 5-hydroxytryptamine (5-HT) receptor agonist that potently and selectively binds to 5-$HT_{1B/1D}$ subtypes.

Compound has pKa values, with the tertiary nitrogen on the piperidine ring at pK_{a1} = 9.7 and the sulphonamide group at pK_{a2} > 12. Naratriptan tablets are well absorbed, with about 70% oral bioavailability. Following administration, the peak concentrations are obtained in 2 to 3 hours. Drug is predominantly eliminated in urine, with 50% of dose

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recovered unchanged and 30% as inactive metabolites. The elimination half-life is approximately 6 hours [33, 34]. A survey of the literature revealed that only three methods have been reported for the determination of Naratriptan. These methods are not specific for the drug and some require extensive sample manipulation. Liquid chromatographic/ electrospray tandem mass spectrometric assays from rabbit plasma [35] and human serum [36] have been reported for understanding the pharmacokinetics of Naratriptan and other antimigraine drugs. A micellar electrokinetic capillary chromatography method has been developed and validated to allow the analysis of a wide range of pharmaceuticals and excipient materials, including also Naratriptan [37]. Recently, the USA Pharmacopeia [38] recommended a high-performance liquid chromatographic method for the determination of the compound in tablet formulation. However, to the best of our knowledge, no literature data were found on the electrochemical behaviors of Naratriptan and other triptans of pharmaceutical interest, except in an earlier work of Sagar *et al.* dealing with a differential pulse voltammetric determination of Sumatriptan, the first of triptans, in a tablet dosage form using glassy carbon electrode [39]. First attempt was made to study the electrooxidation of Naratriptan at glassy carbon electrode by cyclic and differential pulse voltammetry in a very recent work of our group [40].

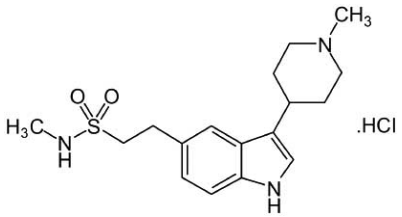

Fig. (1). Molecular structure of Naratriptan.

This paper is the first report describing the characterization of local diatomite of Çaldıran-Van region in the east of Turkey. In this work, special attention will be given to the potential use of diatomite as a modifier incorporated into a carbon paste electrode, and to the application of diatomite-modified electrode for the analysis of Naratriptan in spiked human urine samples.

2. EXPERIMENTAL

2.1. Materials and Reagents

The raw diatomite used in this study was collected as a natural resource from Çaldıran region of Lake Van Basin in East Anatolia (Turkey). Samples were ground and sieved to a particle size of 230 mesh. Samples were then washed with distilled water to remove fines and other adhered impurities, dried at 100℃, desiccated and stored in tightly stoppered polyethylene bags for further use.

Naratriptan was kindly provided by GlaxoSmithKline Pharm. Ind. (İstanbul, Turkey) and used without further purification. Naratriptan stock solutions, renewed daily, were prepared by direct dissolution in doubly distilled water. The solutions under voltammetric study were prepared by serial dilution of the stock solution with Britton-Robinson (BR) buffer (pH 2.0-10.0, 0.04 M).

All solutions were prepared from Analar-grade reagents (Merck and Sigma) in doubly distilled water, and kept in the dark in a refrigerator. When necessary, desoxygenation was accomplished by passing purified nitrogen through the cell.

2.2. Apparatus and Procedures

2.2.1. Sample Characterization

During characterization of diatomite samples, X-Ray Fluorescence Spectrometer (XRF, Philips 2400) was used for chemical analysis. Thermal analysis (TG-DTA) was carried out using Rigaku 2.22E1 Thermal Analyzer under the following operational conditions: heating from 10 to 1099℃ at a rate of 20℃ min^{-1} in atmospheric air. Fourier-transform infrared (FT-IR) measurement was mounted on a Bio-Rad Win-IR spectrometer at a resolution of 2 cm^{-1} in KBr pellet at room temperature. For mineralogical analysis, X-Ray Diffraction (XRD) pattern of diatomite were recorded with a Philips PW 1830-40 X-ray diffractometer with a Cu-K_α radiation.

The surface area of the diatomite was determined, according to Sears' method [41], by weighing 1.5 g of diatomite, and acidifying with dilute hydrochloric acid to pH of 3–3.5. Sodium chloride (30 g) was then added with stirring, and the mixture was made up to a total volume of 150 mL with distilled water. The solution was titrated with 0.10 M sodium hydroxide and the pH measured throughout the titration process. The volume, *V*, required to increase the pH from 4 to 9 was recorded. The surface area (*S*) was estimated from the equation [42]: $S\ (m^2\ g^{-1}) = 32\ V - 25$.

Moreover, thin cross-sectional analysis was applied on the diatomite rock collected from Çaldıran-Van region.

The characterization of electrode surfaces was performed with a DeltaNu ExamineR Raman microscopy system with a 785 nm laser source.

2.2.2. Electrochemical Experiments

Cyclic voltammetric measurements were carried with a Bioanalytical System BAS 100B/W (USA). Square-wave stripping voltammetric (SWSV) measurements were performed on an IviumStat (The Netherlands) electrochemical analyzer. Before evaluation, the stripping curve data were treated using the baseline correction. A standard one-compartment three electrode cell of 10 mL capacity was used in all experiments. Working electrodes were home-made unmodified and diatomite modified carbon paste electrodes, the counter electrode was a platinum wire and an Ag/AgCl electrode (NaCl, 3 M, BAS) served as reference.

Unmodified carbon paste (CPE) was prepared by thoroughly mixing spectroscopic-grade graphite powder (Merck, 0.600 g) and mineral oil (nujol, Sigma, 0.300 g) until obtaining a uniformly wetted paste. A diatomite-modified carbon paste (DMCPE) was prepared in the same way, except that the appropriate amount of diatomite (0.100 g) was added to the mixture. Both unmodified and modified pastes were then packed into the end of a home-made glass cylindrical tube (surface area, 7 mm^2) equipped with a copper wire providing an inner electrical contact. Appropriate packing was achieved by pushing the electrode

surface against a sheet of paper in order to discharge the excess of mineral oil. Smoothing of the electrode surface was made by hand-polishing on a weighing paper. When necessary (in order to avoid memory effects), a new surface was obtained by pushing an excess of paste out of the tube and polishing it again on a weighing paper.

For stripping studies, appropriate amounts of Naratriptan working solutions were placed in a cell containing a buffer solution. The electrode was placed in the cell, and the solution was stirred at 700 rpm at a chosen accumulation potential throughout the selected accumulation period. The stirring was then stopped after a 10 s rest period, and an anodic sweep was carried out towards more positive potentials using the selected voltammetric technique. The measurements were made at room temperature.

For the determination of Naratriptan in spiked urine samples, the pre-concentration / voltammetry scheme was adopted. Blank urine samples were obtained from a healthy person (male, age 30 years). Urine samples were spiked with varying volumes of stock Naratriptan solution, treated with 0.7 mL acetonitrile as urine protein precipitating agent, and then the volumes were completed to 2 mL with the same urine samples. After vortexing for 1 min, the mixture was then centrifuged for 10 min at 4000 rpm for getting rid of protein residues and supernatant was discarded. The analysis was carried out by adding 10 µL of clear Naratriptan-urine solutions to the voltammetric cell containing 10 mL of BR buffer, pH 4.0 (in 1:1000 urine dilutions). The square-wave stripping voltammograms were recorded under optimized conditions. Quantification was performed by means of calibration curve method.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Çaldıran-Van Diatomite

Quantitative chemical analysis of diatomite obtained by XRF technique revealed that the Çaldıran-Van diatomite consists mainly of SiO_2 (69.70%), and it has 11.50% Al_2O_3, 4.40% Fe_2O_3, 0.65% TiO_2, 0.80% Na_2O, 1.40% K_2O and 11.55% loss on ignition.

The sample is composed of plaque and circular-shape diatom particles with sizes of 0.005-0.025 mm in clay matrix according to the thin cross-sectional analysis.

The surface area of diatomite was estimated by using simple and rapid Sears' method. Even though this method was developed mainly for pure silica, it can also be applied for samples containing high silica content. According to this method, the estimation of surface area depends mainly on the hydroxyl groups present on the surface. The surface area of diatomite used in this study was calculated as 48 $m^2 g^{-1}$.

FT-IR analysis was carried out in the range 400-4000 cm^{-1}, in order to investigate the surface characteristics of diatomite. The FT-IR spectrums of diatomite show major adsorption bands at 3436, 1625, 1094 and 797 cm^{-1}, as depicted in Fig. (**2**). The band at 3436 cm^{-1} is due to the free silanol group (SiO-H) on the surface, the band at 1625 cm^{-1} represents H-O-H bending vibration of water, the band at 1094 cm^{-1} may be attributed to siloxane (-Si-O-Si-) group stretching and the peak at 797 cm^{-1} may correspond to SiO-H vibration [26, 43].

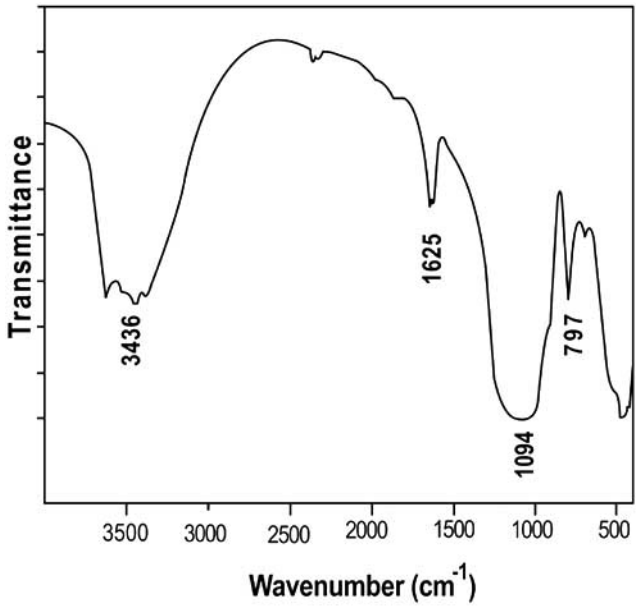

Fig. (2). FT-IR spectrum of diatomite sample.

In order to determine the heating process, a thermogravimetric-differential thermal analysis (TG-DTA) curve was obtained. A typical TG-DTA curve for the diatomite is shown in Fig. (**3**). The TG curve exhibits two distinct weight loss steps and the DTA curve showed two endothermic peaks and one exothermic peak. In the first step, diatomite shows weight loss of about 8.9% up to 522°C, which is accompanied by two endothermic peaks at around 100 and 500 °C in the DTA curve, probably due to dehydration and dehydroxylation, respectively. The second weight loss step of 2.4% above 550°C corresponds to falling down of crystal structure, which is accompanied by one exothermic peak at around 900°C [31, 44].

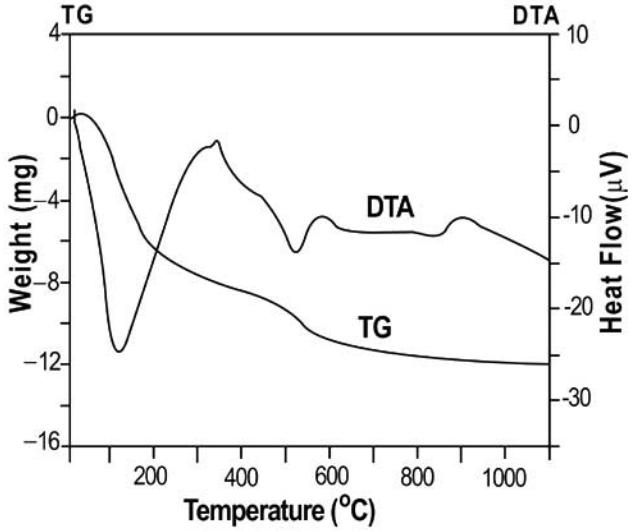

Fig. (3). TG-DTA curve of diatomite sample.

XRD analysis on oriented thin film (not shown) indicated that diatomite has an extensively amorphous matter and it exists in the structure of smectite, calcite, quartz, feldspar.

3.2. Characterization of the Diatomite-Modified Carbon Paste Electrode

Raman spectra of carbon paste and carbon paste modified with diatomite are shown in Fig. (**4**). In the carbon paste spectrum, peaks from the mixed forms of sp^2 and sp^3 carbon were observed. The small and sharp peak at 1260 cm^{-1} corresponds to sp^3 (diamond like structure) and the peaks at 1350 and 1574 cm^{-1} are associated with the G and D bands of sp^2 graphite, respectively [45, 46]. When the spectrum of carbon paste/diatomite composite is considered the G band of graphite (1574 cm^{-1}) broadened and centered at 1560 cm^{-1} is associated with bond disordering of the graphite. It can be found that Raman spectra of carbon paste and carbon paste/diatomite composite are different at 1500-1650 cm^{-1} region. These differences demonstrate that there is an interaction at the interface of carbon paste and diatomite.

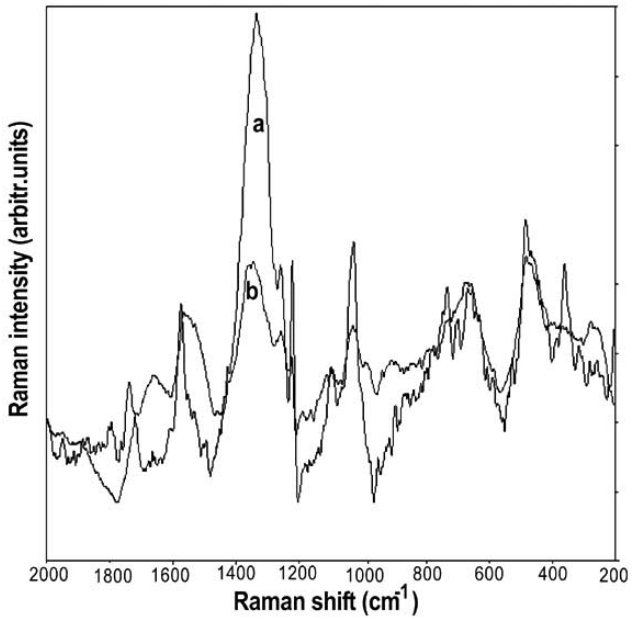

Fig. (4). Raman spectra of carbon paste (**a**) and diatomite-modified carbon paste (**b**) electrodes.

3.3. Electroanalytical Performance of Diatomite-Modified Carbon Paste Electrode

3.3.1. Cyclic Voltammetry of Naratriptan

In our earlier work, glassy carbon electrode was employed in a broad pH range (2.0-10.0) in order to demonstrate its usefulness for studying the electrochemical behavior of Naratriptan [40]. Our results showed that in the potential window 0 V and +1.8 V, the drug was irreversibly oxidized in three or four oxidation steps depending on the pH and supporting electrolyte nature.

A similar study was carried out to investigate the electrochemical behavior of Naratriptan at CPE and DMCPE over the potential range 0 to +1.5 V. The drug exhibited one distinct and well-defined oxidation peak (peak I) and one broad peak (peak II) at more positive potential (Fig. **5A**). No corresponding reduction peaks were observed on scan reversal, indicating irreversibility of the processes. In case of DMCPE, the oxidation steps were not well separated from each other, their shapes being sigmoidal (Fig. **5B**).

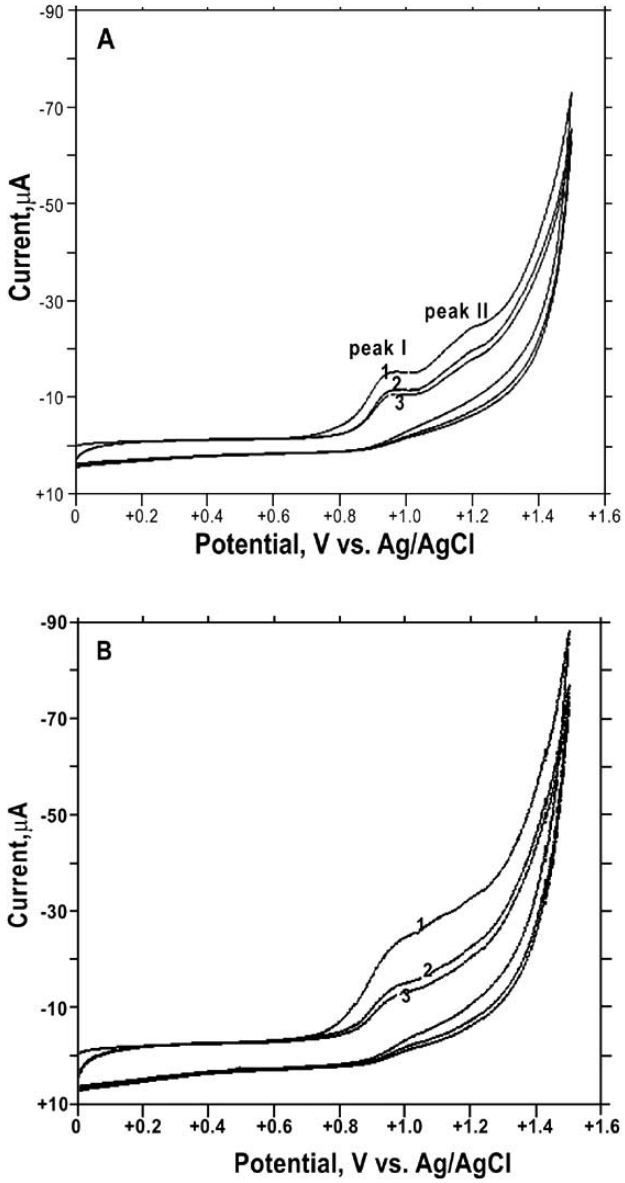

Fig. (5). Multisweep cyclic voltammograms of $2x10^{-4}$ M Naratriptan in BR buffer, pH 4.0 at CPE (**A**) and DMCPE (**B**). Scan rate, 100 mV s^{-1}. The numbers indicate the number of scans.

3.3.2. Stripping Analysis of Naratriptan

In further investigation, we focused our efforts on the pre-concentration of Naratriptan at CPE and DMCPE.

The influence of the scan rate on the accumulation behavior of Naratriptan at CPE and DMCPE by means of lineer sweep stripping voltammetry was evaluated in order to establish the nature of the electrode process. Various stripping voltammograms of $2x10^{-4}$ M Naratriptan solution in a BR buffer of pH 4.0 within the range 5-1000 mV s^{-1} were run with accumulation time of 120s under open circuit condition. When the scan rate (v) increased, the peak potentials shifted in the positive direction which confirms the irreversibility of the electrode process. The stripping peak current (i_p) showed increasing trend with the scan rate. The plots of logarithm of peak current versus logarithm of scan rate gave straight lines and the corresponding equations:

for CPE log i_p (μA) = 0.48 log v (mV s^{-1}) + 0.02 (r = 0.996)

for DMCPE log i_p (μA) = 0.43 log v (mV s^{-1}) + 0.16 (r = 0.995)

had a slope close to the 0.5, expected for an ideal reaction of solution species [47]. The peak current versus square root of the scan rate also showed straight lines according to the following equation [48]:

for CPE i_p (μA) = 0.98√v (mV s^{-1}) – 0.24 (r = 0.998)

for DMCPE i_p (μA) = 0. 83√v (mV s^{-1}) + 1.84 (r = 0.981)

These facts suggest diffusion of Naratriptan on the pores of the electrode and the accumulation processes are diffusion-controlled.

3.3.3. Optimization of Square-Wave Stripping Response at Diatomite-Modified Carbon Paste Electrode

Although linear scan and cyclic voltammetry are attractive techniques for the electrochemical characterization of analytes, they are not sensitive for the determination of low contents of compounds. In order to check the analytical performance of DMCPE, square wave voltammetry was applied.

The systematic studies of various experimental and instrumental parameters that affect the stripping voltammogram response were carried out to establish the optimum conditions.

Electrode composition is one of the most important aspects to be considered. To improve the analytical characteristics of the bare CPE, initial work involved a study of the percentage of diatomite in the paste. Different modified carbon pastes (5-15%) were studied for their voltammetric signals. As the content was increased, peak current increased and then decreased. Furthermore, the background current always increased with the diatomite content. When the content increased, DMCPE offered more reactive sites and a much higher adsorbability for Naratriptan. Since diatomite is an insulator and can lower the DMCPE conductivity, peak current decreased when diatomite content was too high. Maximum current was obtained when the diatomite composition in the paste was 10%. In order to show special effects of diatomite on the Naratriptan oxidation, the electrochemical responses at CPE and DMCPE were compared by SWSV (Fig **6**). If drug undergoes 120-s open circuit accumulation during the potential sweep from +0.4 to +1.40 V at DMCPE, the peak current is enhanced about 2.5 times for 10^{-5} M Naratriptan solution, compared with the peak current obtained by means of the bare CPE, the peak potential being almost the same in each case. The increase in the peak currents at DMCPE is due to a higher amount of Naratriptan entrapped in the porous volume of diatomite.

Further optimization of the analytical signal focused on studying the effect of pre-concentration/stripping conditions, such as accumulation potential and time. The influence of the accumulation potential (E_{acc}) either at open circuit potential or at a potential range from +0.2 V to +0.6 V on the square-wave stripping signal was studied with accumulation time of 120 s at DMCPE for 1x10^{-4} M Naratriptan solution. For pH 4, the i_p versus E_{acc} plot (Fig. **7A**) showed a

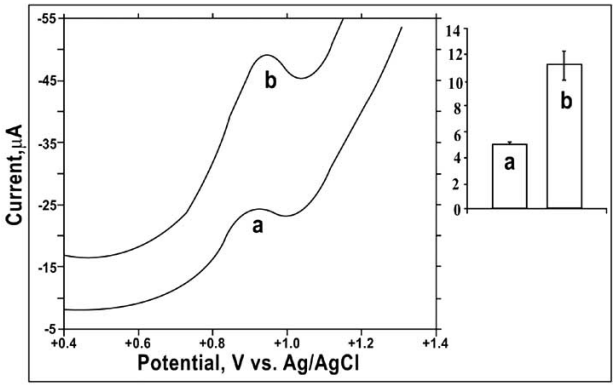

Fig. (6). Square-wave stripping voltammograms of 1x10^{-5} M Naratriptan in BR buffer, pH 4.0 at CPE (**a**) and DMCPE (**b**). Inset: the results were also demonstrated with bar charts. Pre-concentration period, 120 s at open circuit condition; SWV conditions: pulse amplitude, 25 mV; frequency, 25 Hz; step potential, 12 mV.

maximum value for the stripping peak current (ca. 25 μA) at +0.4 V; the value was lower than the value (ca. 27.5 μA) obtained at open circuit voltage. That is the reason of an increased accumulation rate, due to more favorable alignment of the molecule by the electric field at the electrode-solution interface. For further analytical studies, the deposition stage was carried out at open circuit potential. After fixing the accumulation potential, the deposition time was varied between 0 and 480s (Fig. **7B**). Sharp increasing peak current was obtained up to 120, indicating an enhancement of Naratriptan ion concentration at the surface of the electrode. For longer times above 120 s, the peak current became almost constant. Hence for all subsequent measurements, a pre-concentration time of 120s was employed.

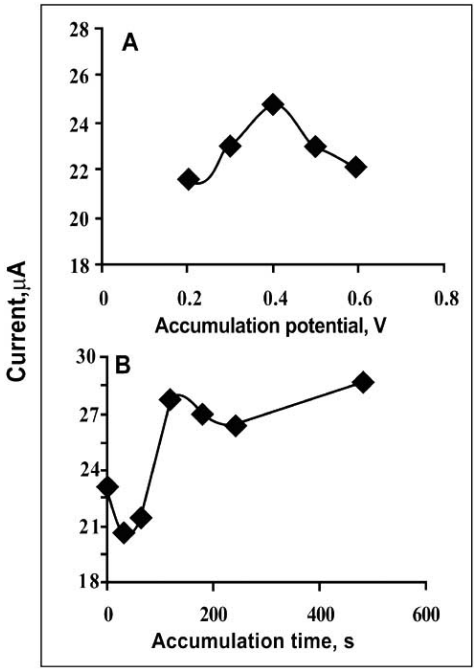

Fig. (7). The effect of accumulation variables on the stripping signals for 1x10^{-4} M Naratriptan in BR buffer, pH 4.0. Electrode, DMCPE; SWV conditions as in Fig. (**6**).

The attention was next turned to the effect of solution condition such as pH, which is a critical factor affecting both the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. The effect of varying pH of the pre-concentration solution on the stripping response of DMCPE at constant Naratriptan concentration ($1x10^{-4}$ M) was performed under these selected conditions. It can be observed from Fig. (**8A**) that increasing the pH from 2.0 to 4.0 resulted in a slow decrease in the peak potential then decreased linearly between pH 4.0 and 10.0, with a negative slope of 56.4 mV per pH unit (r = 0.996), indicating the involvement of same number of protons and electrons in the oxidation. It is known that in acidic solution, there are more proton ions competing with Naratriptan on the adsorption onto diatomite. The peak potentials seem to be affected by the concentration of H^+, suggesting the presence of protonation step in the oxidation mechanism.

Analyzing the evolution of peak current (Fig. **8B**), it is possible to observe that this parameter was affected by the pH value, reaching a maximum at pH 4.0. The i_p versus pH plot indicates that the proton transfer is slower than the electrochemical step [49]. The more well-defined signal with a reasonably higher sensitivity was obtained with a BR buffer, comparing to those obtained in phosphate buffer. Hence BR buffer pH 4.0 was used as a favorable buffer throughout this study.

Final optimization of the stripping signal centered upon varying the several instrumental parameters, which affects directly the voltammetric response, such as frequency, scan increment, pulse amplitude, stirring rate and rest period (data not shown). The effect of frequency was studied in the range 20 to 150 Hz. The peak heights increased with square-wave frequency due to the increase in the effective scan rate but the peak shape was distorted at frequencies higher than 80 Hz. This is attributed to the greater contribution of the capacitive current at higher frequencies. The influence of scan increment was investigated between 5 and 20 mV. The peak height increased up to 12 mV because the effective scan rate was increased, but at higher values of step potential, the peak heights decreased. The analytical signal is dependent on the pulse amplitude even if this parameter seems to be less important than the frequency. Pulse amplitudes were varied between 25 and 75 mV. Peak heights increased upon increase of the pulse amplitude. However, the peak shape became wider at higher than 25 mV. Modification of the stirring rates did not affect the response of the electrode. The working conditions decided upon were a stirring rate of 700 rpm and rest period of 10 s. All the experimental conditions are summarized in Table **1**. These conditions were used to study the effect of concentration.

Table 1. Optimum Conditions for the Determination of Naratriptan by SWSV at DMCPE

Diatomite composition	10%, w/w
pH	4.0
Electrolyte	BR buffer
Accumulation potential	Open circuit
Accumulation time	120 s
Rest time	10 s
Stirring rate	700 rpm
Pulse amplitude	25 mV
Frequency	80 Hz
Step potential	12 mV

3.3.4. Electroanalytical Application

Square-wave stripping voltammograms at different concentrations of Naratriptan were recorded under the maximum current experimental conditions. The use of Naratriptan oxidation at +0.84 V as the analytical peak allowed quantification in the range of 5 x 10^{-7} M to 9 x 10^{-7} M with a regression equation of $i_p(\mu A) = 1x10^7$ C (M) – 4.85 (correlation coefficient, 0.995). The limit of detection (LOD) and limit of quantification (LOQ), defined as 3.3 *s*/*m* and 10 *s*/*m* (*s* is the standard deviation of the intercept and *m* is the slope of the calibration plot), were 1.25 x 10^{-7} M (46.5 μg L^{-1}) and 3.80 x 10^{-7} M (141.4 μg L^{-1}), respectively. The repeatability of the stripping signal was realized in terms of relative standard

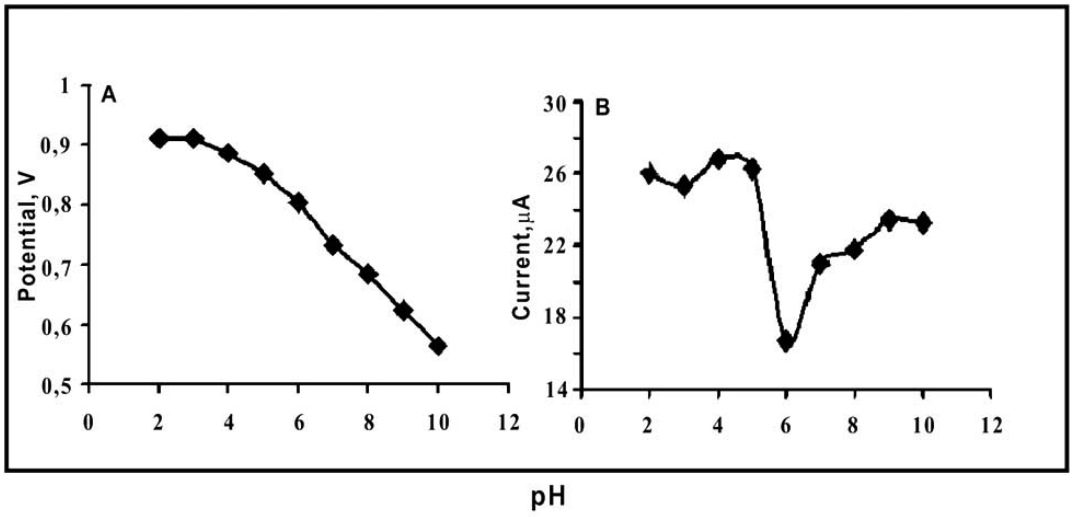

Fig. (8). The effect of pH on the stripping signals of $1x10^{-4}$ M Naratriptan. Electrode, DMCPE; pre-concentration period; 120 s at open circuit condition; SWV conditions as in Fig. (**6**).

deviation for three identical measurements carried out at a concentration level of 5 x 10^{-7} M drug concentration. The relative standard deviation (R.S.D.) was found to be 4.82%.

The selectivity of such determination could be limited by interactions of structurally similar compounds with Naratriptan. However, this is not a case of samples with one class of compounds or one analyte present.

To check the stability of Naratriptan, the voltammograms of stock solutions stored in the dark and in refrigerator were recorded after two-week periods. When the voltammograms were compared with those of samples freshly prepared they did not show any appreciable change, demonstrating that Naratriptan solutions were stable for this period.

In view of the sensitivity (extremely low detection limit) of the proposed method, DMCPE was applied to the determination of Naratriptan in simulated human urine.

The presence of proteins, major components of the sample matrix which could be adsorbed on the electrode surface together with analyte is an important drawback of adsorptive voltammetric techniques. In case of our study, when the voltammograms were recorded in 1:1000 blank urine dilutions smooth curves were not obtained in the potential range from 0 V to +1.4 V comparing to those of the background electrolyte. To obviate this problem, the extraction with acetonitrile followed by centrifugation protocol, that is quick and easy, was applied. For determination of drug in spiked urine samples, three replicate samples at levels of 6 x 10^{-7}, 7 x 10^{-7} and 9 x 10^{-7} M of Naratriptan were run through the procedure. Fig. (**9**) shows typical stripping curves of Naratriptan in the urine sample. As can be seen from the figure, a peak appeared in the baseline—due to the existence of the potentially interfering substances in a diluted urine sample – at less positive potential (ca. +0.5 V), was well separated from the analytical peak (ca. +0.85 V). The percentage recovery of Naratriptan in urine was determined by comparing the peak currents of known amount of drug concentrations in serum with their equivalents in related calibration curve. The results of these analyses are summarized in Table **2**. Good recoveries of Naratriptan were achieved from this type of matrices. There was no degradation of the drug in solution during the experiments.

3.3.5. Proposed Mechanism of Naratriptan Oxidation

Though the elucidating the oxidation mechanism of Naratriptan is beyond the aim of this study, a short comment can be made. Bearing in mind that the studies of indole chemistry have shown the several preferred electroactive centers on the ring [50], we may assume that the oxidation of Naratriptan proceeds through two distinct pathways. The first is oxidation of tertiary nitrogen on the piperidine ring in the molecule. This mechanism is in agreement with reported data concerning the Zolmitriptan N-oxide formed on the dimethylamino ethyl group at the same position with piperidine ring, which is one of the major metabolites of Zolmitriptan (one of triptans) [51]. Taking into account the studies based on oxidation chemistry of structurally related indolic compounds [52, 53], in the second pathway, several oxidation processes may be located on the indole ring, leading to a mixture of products.

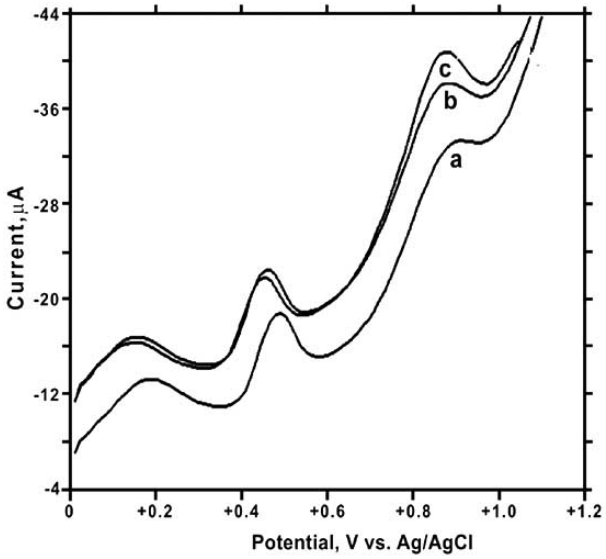

Fig. (9). Square-wave stripping voltammograms obtained for the determination of Naratriptan in spiked urine. Urine spiked at a Naratriptan level of 6 x 10^{-7} M (**a**), 7 x 10^{-7} M (**b**) and 9 x 10^{-7} M (**c**) For operating conditions, see Table **1**.

Table 2. Recoveries of Naratriptan in Spiked Urine Samples by SWSV at DMCPE

Amount Spiked (M)	*Amount Recovered [a] (M)*	*Recovery (%) ± RSD (%)*
6 x 10^{-7}	5.8 x 10^{-7}	97.2 ± 1.32
7 x 10^{-7}	6.9 x 10^{-7}	98.4 ± 1.82
9 x 10^{-7}	8.7 x 10^{-7}	96.5 ± 1.67

[a] Calculated using the calibration curve. Values reported are the average of three independent analysis of the same sample.

4. CONCLUSIONS

This paper presents an initial study of the possibility of use of diatomite for the development of a new specific electrode for electroanalysis. Diatomite incorporated on carbon paste electrode has been found to allow the investigation of the redox properties of Naratriptan. Using the diatomite-modified carbon paste electrode by means of SWSV, a detection limit of 1.25 x 10^{-7} M (46.5 μg L^{-1}) was found, which is forty times better than that (4.95x10^{-6} M) obtained at glassy carbon electrode by differential pulse voltammetry in our previous work [40]. The detection limit found for Naratriptan is low enough to reach the levels expected in urine after applying therapeutic doses. The procedure is simple, rapid and low cost; it does not require time consuming extraction or pretreatment steps other than the centrifugal separation of the precipitated protein in urine samples.

This process could be important in the development of an analytical technique for the determination of Naratriptan in real samples, either as an analytical technique itself or as a detection system for HPLC. The electrochemistry of the process can be improved by modification of diatomite with

inorganic films or organic moieties. Further work is being done in this direction.

This paper also presents an attempt to fill an important gap in industry for using local diatomite of Çaldıran- Van region in Turkey, as an industrial raw material.

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