CEA-1

A Green Method for the Determination of Mebeverine in Its Pharmaceutical Products and Human Serum Based On Diatom Modified Carbon Paste Electrode Using Square Wave Adsorptive Anodic Stripping Voltammetry (SW-AdASV)

Fared M. El-badawy[a,b], Hanaa S. El-Desoky[a] and Mohamed M. Ghoneim[a]

Abstract

The first use of a green method of analysis using diatom for trace determination of mebeverine in its pharmaceutical formulation and human serum based on its great properties such as high surface area, hydrophilicity, and adsorption. in addition to diatom requires no chemical modification. Characterization of Diatom using FTIR showed bands corresponding to AlMgOH, Si-O-Al, SiOH, and OH, while XRD showed that diatom is amorphous containing silica of quartz crystal shape, SEM revealed that diatom has porous surface. Cyclic Voltammetry of the modified electrode revealed that the modified electrode has higher surface area and greater adsorption. Mebeverine hydrochloride (MV.HCl) was determined based on 5% diatom modified carbon paste electrode 5% (w/w)DA/CPE as a sensor with LOD and LOQ of 1.80×10^{-9} and 6.00×10^{-9} respectively. Then, the modified electrode was successfully applied for the trace determination of MV.HCl in different pharmaceutical preparations; "Coloverin A", "Colona" and "Colofac" tablets without interferences from excipients and co-formulated drugs. The achieved detection limit in human serum was 8.0×10^{-9} M. The described adsorptive anodic stripping voltammetric method is reliable, simple, and sensitive enough for assay of MV.HCl in real plasma samples, pharmacokinetic studies, quality control and assurance of the drug in industry and in clinical laboratories.

Keywords:-
Square wave anodic stripping voltammetry; SW-AdASV; diatom; Mebeverine

_____
[a] Analytical and Electrochemistry Research Unit, Department of Chemistry, Faculty of Science, Tanta University, 31527 Tanta, Egypt
[b] Egyptian Ministry of Industry, Chemistry Administration, Cairo, Egypt

1. Introduction

Mebeverine hydrochloride (MV.HCl) has the IUPAC name 3,4-dimethoxybenzoic acid 4-[ethyl-(2-[4-methoxyphenyl] -1-methylethyl) -amino] butyl ester (Fig. 1) potent direct antispasmodic agent used mainly for the smooth muscles of the gastrointestinal tract and particularly effective against the colonic spasm [1] It is used in the treatment of abdominal pain and spasm associated with gastrointestinal disorders such as mucous colitis [2]

Due to the vital importance of MV.HCl, there are many reported methods for its determination in its pharmaceutical formulations and in biological fluids. Among these methods are HPLC[3-7], spectrophotometry [8-11], GC-MS [12], and electrochemistry [13-16].

On one side, most of the reported chromatographic is expensive method of analysis required a high maintenance cost, high acquisition cost, time-consuming procedures, senetive equilibration, it also may requires derivatization treatment prior to analysis and expensive grades of reagents, eluents, and equipment [3-7, 12],

On other side, there are some reported electrochemical methods based on potentiometry [13], square-wave anodic stripping voltammetry (SW-ASV) [14, 16] and differential pulse voltammetry (DPV) [15] listed in (Table 1). It is noticed that Potentiometry and SW-AdASV onto bare Carbon paste electrode CPE did not offer a sufficient sensitive quantitation limit of MV.HCl that can cover pharmacokinetic studies and therapeutic drug monitoring. Duo to the MV.HCl conc in plasma was reported to be less than 10 ng. mL^{-1} (< 2.15×10^{-8} M) after a single oral dose of 405 mg of mebeverine hydrochloride [6] or in the range of 25 to 0.73 ng mL^{-1} (5.36×10^{-8} M to 1.57×10^{-9} M) after oral administration of two Colona® tablets (100 mg Mebeverine + 25 mg Sulpiride)[7]. The other SW-AdASV using gold nanoparticles/silica gel [15] indicated narrow linear segment variations of the (i_p) with MV.HCl conc. in addition to hard procedures of preparation and expensive chemicals, moreover, the reported SW-AdASV on functionalized multiwall carbon nanotube modified carbon paste electrode dependes on in expensive materials, which was further functionalized.

Since the modification of the carbon paste electrode depend on modifying the properties which help in those modifications. First, the chemical composition of diatom contains hydrous silica compositions ($SiO_2.nH_2O$), in which water is bounded within a siliceous skeleton. It may also contains small amounts of associated inorganic components like alumina, principally or lesser amounts of iron, alkaline earth or alkali metals, and/or other minor constituents [17] So, it is mainly hydrophilic with many functional groups responsible for adsorption such as [≡SiOH], [=Si(OH)2] ,[≡Si-O-Si≡] and other hydrogen bond forming groups [18, 19]. In addition to the morphological structure of the diatom and its

3

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9th International Conference
on
Chemical & Environmental
Engineering**
3-5 April 2018

porous surface that contribute in increasing the surface area and hence increasing the accumulating amount of the analyte in the preconcentration step,

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed with Princeton Applied Research (PAR, Princeton, Oak Ridge, TN, U.S.A.) Potentiostats Models 273 A and 263 A. A voltammetric cell consisting of a C-2 stand with a carbon paste working electrode (BAS Model MF-2010, 3 mm in diameter and 1 mm in depth), an Ag/AgCl/KCl$_s$ reference electrode (BAS Model MF-2063) and a platinum wire counter electrode (BAS Model MW-1032) were used.
Fourier transform infrared (FTIR) spectra were measured with Thermo scientific nicolet iS50 FTIR, USA.
JSM-6510 LV, JEOL-Japan (with Gold coating unit: SPI-Module sputter coater) scanning electron microscope (SEM) was used to observe the surface morphology of the carbon paste (CP) and the modified CP with various ratios of diatom.

2.2. Materials

Diatom was locally prepared, and Mebeverine hydrochloride (MV.HCl) stander and (Colona tablets$^{®}$ each tablet contains 100 mg mebeverine + 25 mg sulpiride) was kindly provided by Bayer Pharmaceuticals (Middle East) Rameda Company, Egypt.
Other pharmaceutical samples examined in this work: Colofac$^{®}$ tablets (BGP Products, Egypt, each tablet contains 135 mg mebeverine), and Coloverin A$^{®}$ tablets (Chemi Pharm Company, Egypt, each tablet contains 135 mg mebeverine + 5mg chloridazepoxide).

2.3. Preparation of mebeverine hydrochloride and drug product solutions

A standard stock solution of 1.0×10^{-3} M MV.HCl was prepared in methanol (Merck) and stored in the dark at 4^{0}C. A more dilute solutions of (1.0×10^{-8} to 1.0×10^{-4} M) MV.HCl were prepared daily in methanol just before use.
Ten tablets of each of the pharmaceutical products of MV.HCl were weighed and the average mass per tablet was determined, and then grounded to a fine powder. A weighed portion of the homogeneous powder equivalent to 1.0×10^{-3} M MV.HCl was accurately transferred into a 100-mL volume calibrated flask containing 70 mL methanol. The content of the flask was sonicated for about 10 min and then filled up with methanol. Convenient more-dilute working solutions were then obtained by accurate dilution with methanol.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9ᵗʰ International Conference
on
Chemical & Environmental
Engineering**
3-5 April 2018

2.4. Serum sample preparation

Six serum samples of three healthy volunteers were stored frozen until assay. 1 mL of each sample were fortified with various concentrations (1.0×10^{-8} to 1.0×10^{-3} M) of MV.HCl in small centrifugation tubes then completed to 2 mL with methanol to denature and precipitate proteins. After vortexing each of the serum samples for 2 min, the precipitated proteins were separated by centrifugation for 3 min at 14,000 rpm. The clear supernatant layers were filtered through 0.45 μm Milli-pore filters to obtain protein-free spiked human serum samples.

2.5. Preparation of the Carbon Paste Electrodes (CPEs)

The working electrode was a Teflon rod with end cavity (3 mm diameter and 5 mm deep) bored at one end for paste filling (BASi-MF-2010) and contact was made with a copper wire through the centre of the rod. The carbon paste was prepared by thoroughly hand mixing 5 g of graphite powder (Sigma-Aldrich, Milwaukee, WI, US 1-2 μm) with 1.8 mL of paraffin oil (Sigma-Aldrich, US, d=0.84 g mL^{-1}) in an agate mortar with pestle. The paraffin oil–based paste was packed into the well of the electrode and its surface was manually smoothed by polishing on clean paper. Several sweeps of cyclic voltammetry within range of (−0.4 to 1.4 V) were applied to the electrode immersed in the supporting electrolyte to obtain a low background current. For electrode surface re-generation, after recording each voltammogram, the CPE was transferred into a blank electrolyte solution and series of cyclic scans were performed until a voltammogram corresponding to the residual current was obtained.

2.6. Preparation of the modified carbon paste electrode

An amount (4.5 g) of graphite powder and 0.5 g of diatom were mixed uniformly by milling in a small agate mortar. Then 1.8 mL paraffin oil was added and milled again to give a homogenous 10% (w/w)DA/CP. Various modified carbon pastes containing different mass percentages of diatom (3, 5, 7,10, and 15% w/w) were similarly prepared.

3. Results and discussion

3.1. Diatom and DA/CPE characterization

The FTIR spectrum of Diatom shows a broad band in the range 2900 to 3800 cm-1 (Fig. 2A) with its specific peak at 3447 cm^{-1}, which is attributed to the stretching vibration of the OH group [20]. As water-bearing mineral, the most distinct feature evident within the Diatom spectra is the bending vibration of the OH group at 1636 cm^{-1} [21]. The most intense band at 1105 cm^{-1} is related to the Si-O stretching vibrations. The band at 795

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

9th International Conference
on
Chemical & Environmental
Engineering
3-5 April 2018

cm^{-1} is attributed to AlMgOH bending vibration [20]. The Si-O-Al and Si-O-Si deformation vibrations in Diatom spectrum were observed at 467 cm^{-1} [20]. The FTIR spectrum revealed that no any function groups are associated with graphite structure, (curve II). It also revealed many characteristic functional Diatom (curve I) compared to graphite (curve II).

X ray diffraction was recorded for diatom (Fig. 2B) The broad band in the range 15 to 35 confirm the amorphous structure of the diatom[22] while the sharp band at 26.75[23] is characteristic for quartz so some of the silica in the diatom has quartz morphology structure

Continued to show the improved electrochemical properties of the modified electrodes, the microscopic areas of the bare and diatom modified carbon past electrode were calculated using 1.0×10^{-3} M $K_4Fe(CN)_6$ as an electrochemical probe[24] by recording cyclic voltammograms at different scan rates (Fig. 2C). The magnitude of the redox peak currents at the same scanning rate was found to increase obviously after modification, which can be attributed to higher surface area and excellent solution-electrode interact as a result of the decreased hydrophobicity. The following Randles–Sevcik equation (1) For a reversible process can be used[24];

$$i_p = (2.69 \times 10^5)\, n^{2/3} A\, D^{o1/2}\, v^{1/2}\, C^o \qquad (1)$$

Where, i_p refers to the peak current in ampere, A is the surface area of the electrode in cm^2, n is the electron transfer number, D^o is diffusion coefficient, C^o is the concentration of $K_4Fe(CN)_6$ in M and v is the scan rate in V.s^{-1}. Linear dependence of the anodic peak currents (i_{pa}) to the square root of scan rate ($v^{1/2}$) was achieved for bare CPE and 5%(w/w) DA/CP/CPE with correlation coefficient(r^2=0.999) (Fig. 2C inside)

For 1.0×10^{-3} M $K_4Fe(CN)_6$, n = 1, $D^o = 7.63 \times 10^{-6}$ cm^2 s^{-1}[25], and from the slope values for the i_{pa}- $v^{1/2}$ relation are (33.22 and 40.89) for bare CPE and DA/CPE respectively, the microscopic surface areas for bare CPE and DA/CPE were found to be 0.045, and 0.055 cm^2, respectively. So, it was remarkable that the active surface area of DA/CPE was greater than that for bare CPE by about 22.2%.

Scanning electron microscope was used to investigate the used modifier (Fig. 2D) and Shows an amorphous porous pattern of a high surface area also the surface of diatom is porous increasing surface area too. Furthermore, the investigation of both CPE and DA/CPE using SEM showed a rough surface of the modified electrode that leads to a higher surface area of DA/CPE than CPE (Fig. 3), this is due to the rough surface of modified electrode.

Due to the high surface area and hydrophplicity of DA/CPE compared to the bare CPE, MV.HCl was determined using this relevant sensor in the present analytical study.

3.2. Analytical study

3.2.1. Optimization of the modifier ratio

Square wave voltammograms was recorded 5×10^{-7} MV.HCl in B-R universal buffer of pH 5 and accumulation parameters (E_{acc}= 0.0 V and t_{acc}= 50 s) onto the modified carbon paste electrode with different ratios of diatom (Fig. 4). It was found that the well-defined peak with the highest peak current is observed at 5%(w/w) DA/CPE so it was used for the rest analytical study.

3.2.2. Effect of pH of the medium

Based on the strong adsorption behavior of MV.HCl onto DA/CPE, voltammograms of 5.0×10^{-7} M MV.HCl were recorded in B-R universal buffer of various pH values following its preconcentration onto the DA/CPE at 0.0 V for 50 s (Fig. 5) it was observed that the peak current was increased with pH increasing till pH 5 then decreased dramatically, then the best developed peak current was at pH 5 so it was used for the rest optimizations.

3.2.3. Effect of pulse parameters

It is clearly known that The pulse parameters such as frequency (f), pulse height (a) and scan increment ($\varDelta E_s$) have a great effect on the response obtained by SW-AdASV, the peak current magnitude was investigated by the variation of f (10 - 140 Hz), $\varDelta E$s (2-12mV) and a (5- 35mV) on the SW-AdASV onto DA/CPE at pH 5 for determination of 5.0×10^{-7} M MV.HCl prior to preconcentration for 50s at accumulation potential 0.0 V; The best developed and symmetrical voltammetric peaks were obtained at f = 100 Hz and $\varDelta E_s$= 10 mV. However, the pulse height had no influence on i_p. So, 25 mV was used in rest of study.

3.2.4. Effect of accumulation parameters

The electrode potential may affect the electric field at the electrode-solution interface, and hence the interfacial tension which is certain to have influence upon the adsorption occurring at this interface [26]. So, the effect of E_{acc} on the peak current magnitude was tested over the potential range of −0.2 to +0.6 V. The best enhanced peak current magnitude was achieved at the potential range of 0.0 to +0.2 V (Fig. 6). This could be attributed to the increase of the accumulation rate and the favorable alignment of the analyte molecules by the action of electric field at the electrode-solution interface (which may be due to electrostatic attraction between the negative nature of electrode at this potential range and the positive charged (protonated) MV.HCl molecules)[27]; but the better peak was observed at 0.0 V so it was used as the optimum accumulation potential.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9th International Conference
on
Chemical & Environmental
Engineering**
3-5 April 2018

Effect of the preconcentration time (t_{acc}) on SW-AdASV peak current magnitude was then investigated at accumulation potential 0.0 V. It was found that the response for 5.0×10^{-7} and 2.0×10^{-7} M MV.HCl was linear up to 60 and 100 s respectively then leveled off (Fig. 7).This indicated that the adsorptive equilibrium onto DA/CPE surface was achieved i.e, full surface coverage was approached. In the present analytical study, t_{acc} of 90 was applied.

3.3. Validation of the analytical method

Voltammograms of various concentrations of MV.HCl were recorded under the optimized operational conditions of the developed SW-AdASV method. Linear variation of i_p (µA) with concentrations C (nM) of bulk MV.HCl was obtained over a wide concentration range (Fig. 8A). Limits of detection (LOD) and quantitation (LOQ) were estimated using the expressions(2,3) [28]:

$$LOD = 3 \text{ S.D}/ b \qquad (2)$$
$$LOQ = 10 \text{ S.D}/ b, \qquad (3)$$

Where, S.D is the standard deviation of the intercept of the calibration curves (or the blank) and b is the slope of the calibration curve. The obtained results and the corresponding regression equations were listed in (Table 2).These results indicated the extreme sensitivity and reliability of the proposed SW-AdASV method for quantification of bulk MV.HCl.

Repeatability, reproducibility, precision and accuracy[29] of the proposed SW-AdASV methods were evaluated by performing five replicate measurements for various concentrations of MV.HCl, (5×10^{-8} 1×10^{-7} 1.2×10^{-7}) M [over 1 day (intra-day assay) and for 3 days (inter-day assay)] using DA/CPE, (Table 3). Satisfactory mean recoveries (%R ± RSD%) (98.40 ±1.01 to 101.10 ± 0.65) and bias% were achieved indicating the repeatability, reproducibility, precision and accuracy of the described method.

The robustness[29] of the proposed method was examined by studying the effect of small variation of some effective operational conditions such as pH (4.5 to 5.5), accumulation potential (-0.05 to 0.05), and accumulation time (85 to 95 s) on %R and RSD% (Table 4). Since, the obtained %R and %RSD values (103.83 ±0.91 to 97.52 ± 1.13) were insignificantly affected within the studied range of variation of the operational conditions, the developed SW-AdASV method could be considered robust.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9ᵗʰ International Conference
on
Chemical & Environmental
Engineering**
3-5 April 2018

4. Application

The developed SW-AdASV was successfully applied in determination of MV.HCl in serum by recording voltammograms of various concentrations of MV.HCl in serum under the optimized operational conditions of the developed SW-AdASV method. Linear variation of i_p (µA) with concentrations C (nM) of bulk MV.HCl was obtained over a wide concentration range $5 \times 10^{-9} - 1.6 \times 10^{-7}$ (Fig. 8b), the charactaristics of the callipratipn are in (Table 2).

MV.HCl was determined in its different pharmaceutical formulations Colofac® tablets (135 mg mebeverine) also as a binary as binary mixture with Sulpiride (SPR) drug in Colona tablets® (100 mg mebeverine + 25 mg sulpiride) and with chloridazepoxide (CDP) drug in Coloverin A® tablets (135 mg mebeverine + 5 mg chloridazepoxide). Furthermore, the performance of the DP-AdASV method was also assessed by the calculation of Student t -test and F-values[30] compared with the SW-AdASV. (Table 5) showed a good agreement with the results of the HPLC and that the calculated t- and F-values at 95% confidence limit for five degrees of freedom did not exceed the theoretical values[30]. This indicated that both the developed SW-AdASV and RP-HPLC methods did not differ significantly with respect to accuracy and precision. Hence, the developed SW-AdASV method was found to be sensitive, accurate, precise, repeatable and reproducible and can be successfully used for the routine analysis of MV.HCl in its formulations.

5. Conclusion

Mebeverine hydrochloride was easily determined in its pharmaceutical formulation and biological fluid utilizing 5%(w/w)DA/CPE, the modifier used is green as t needs no chemical modification and has no hazard. The utilized method is fast, reliable, precise, and robust in addition to the validity of the method that were investigated using F-test and t -test for determination of MV.HCl in different pharmaceutical formulations with different conc and different additives reflecting selectivity too.

6. Refrances

[1] Zayed, S.I.M., Simultaneous determination of mebeverine hydrochloride and sulpiride using the first derivatives of ratio spectra and chemometric methods. Analytical sciences, 2005. **21**(8): p. 985-989.

[2] Reme, S.E., et al., Mediators of change in cognitive behaviour therapy and mebeverine for irritable bowel syndrome. Psychological medicine, 2011. **41**(12): p. 2669-2679.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9th International Conference
on
Chemical & Environmental
Engineering**
3-5 April 2018

[3]   Haggag, R.S., R.A. Shaalan, and T.S. Belal, Validated HPLC determination of the two fixed dose combinations (chlordiazepoxide hydrochloride and mebeverine hydrochloride; carvedilol and hydrochlorothiazide) in their tablets. Journal of AOAC International, 2010. **93**(4): p. 1192-1200.

[4]   Swetha, A.V., et al., Development and validation of new analytical methods for the assay of Mebeverine hydrochloride in tablet dosage form. International Journal of Advances in Pharmaceutical Research Oct, 2015. **6**(10): p. 320-331.

[5]   Arayne, M.S., N. Sultana, and F.A. Siddiqui, A new RP-HPLC method for analysis of mebeverine hydrochloride in raw materials and tablets. Pakistan journal of pharmaceutical sciences, 2005. **18**(2): p. 11-14.

[6]   Stockis, A., P.J.M. Guelen, and D. De Vos, Identification of mebeverine acid as the main circulating metabolite of mebeverine in man. Journal of pharmaceutical and Biomedical Analysis, 2002. **29**(1): p. 335-340.

[7]   Walash, M.I., et al., Simultaneous determination of sulpiride and mebeverine by HPLC method using fluorescence detection: application to real human plasma. Chemistry Central Journal, 2012. **6**(1): p. 1-12.

[8]   Siddiqui, F.A., et al., Spectrophotometric Multivariate Calibration Approach: Application in Quantitative Determination of Mebeverine in Bulk Drug and Pharmaceutical Formulations. World Applied Sciences Journal, 2014. **32**(7): p. 1418-1422.

[9]   Naveed, S., et al., Method Development of Mebeverine Hydrochloride by Using UV Spectrophotometric Method. International Journal of Applied Science-Research and Review, 2015. **2**(1): p. 001-005.

[10]   El-Didamony, A.M., Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2008. **69**(3): p. 770-775.

[11]   Lotfy, H.M., et al., Simultaneous determination of mebeverine hydrochloride and chlordiazepoxide in their binary mixture using novel univariate spectrophotometric methods via different manipulation pathways. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2016. **155**: p. 11-20.

[12]   Tulich, L.J., et al., Determination of two mebeverine metabolites, mebeverine alcohol and desmethylmebeverine alcohol, in human plasma by a dual stable isotope-based gas chromatographic-mass spectrometric method. Journal of Chromatography B: Biomedical Sciences and Applications, 1996. **682**(2): p. 273-281.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9ᵗʰ International Conference
on
Chemical & Environmental
Engineering**
3-5 April 2018

[13] Ali, T.A., et al., Improved Determination of Mebeverine Hydrochloride in Urine, Serum and Pharmaceutical Preparations Utilizing a Modified Carbon Paste Electrode. Int. J. Electrochem. Sci, 2015. **10**: p. 2439-2454.

[14] Naggar A. H., Kotb A., Gahlan A. A. and El–Sayed A. Y., Square wave anodic stripping voltammetric determination of Mebeverin hydrochloride in tablet and urine at carbon paste electrode. Applied Chemistry, 2014. 77: p. 29391-29394.

[15] Salama, N.N., et al., Utility of gold nanoparticles/silica modified electrode for rapid selective determination of mebeverine in micellar medium: comparative discussion and application in human serum. Ionics, 2016. **22**(6): p. 957-966.

[16] Ghoneim, M.M., H.S. El-Desoky, and F.M. El-badawy, Carbon Nanotubes Modified Electrode for Enhanced Voltammetric Sensing of Mebeverine Hydrochloride in Formulations and Human Serum Samples Journal of The Electrochemical Society, 2017. **164**(6): p. B212-B222.

[17] Caliskan, N., et al., The natural diatomite from caldiran-van (Turkey): electroanalytical application to antimigraine compound naratriptan at modified carbon paste electrode. Combinatorial Chemistry & High Throughput Screening, 2010. **13**(8): p. 703-711.

[18] Zhuravlev, L.T., The surface chemistry of amorphous silica. Zhuravlev model. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2000. **173**(1): p. 1-38.

[19] Al-Ghouti, M.A., et al., The removal of dyes from textile wastewater: a study of the physical characteristics and adsorption mechanisms of diatomaceous earth. Journal of Environmental Management, 2003. **69**(3): p. 229-238.

[20] Farmer, V.C., Infrared spectra of minerals Mineralogical society. 1974.

[21] Yitagesu, F.A., et al., Spectral characteristics of clay minerals in the 2.5-14μm wavelength region. Applied clay science, 2011. **53**(4): p. 581-591.

[22] Rajan, A.S., S. Sampath, and A.K. Shukla, An in situ carbon-grafted alkaline iron electrode for iron-based accumulators. Energy & Environmental Science, 2014. **7**(3): p. 1110-1116.

[23] Siddiqui, I., M.T. Shah, and I. Ahmed, X-RAY Diffraction (XRD) Analyses OF Thar, Sonda And Meting-Jhimpir Coalfields, SINDH. Sindh University Research Journal-SURJ (Science Series), 2009. **41**(1).

[24] Allen, et al., Electrochemical methods: fundamentals and applications. University of Texas at Austin, John Wiley & Sons, Inc, 2001: p. 156-176.

[25] McCreery, R.L., Carbon electrodes: structural effects on electron transfer kinetics. Electroanalytical chemistry, 1991. **17**: p. 221-374.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

9ᵗʰ International Conference on
Chemical & Environmental Engineering
3-5 April 2018

[26] Ghoneim, M.M., et al., Nano Determination of Kinase Inhibitor Sorafenib Drug in Pharmaceutical Formulation and Human Blood at a Modified Carbon Paste Electrode. Journal of The Electrochemical Society, 2016. 163(6): p. B215-B226.

[27] Manisankar, P., G. Selvanathan, and C. Vedhi, Utilization of sodium montmorillonite clay-modified electrode for the determination of isoproturon and carbendazim in soil and water samples. Applied clay science, 2005. **29**(3): p. 249-257.

[28] Miller, J.N., Basic statistical methods for analytical chemistry. Part 2. Calibration and regression methods. A review. Analyst, 1991. **116**(1): p. 3-14.

[29] Validation of analytical procedures: text and methodology Q2(R1), International Conference On Harmonization Of Technical Requirements For Registration Of Pharmaceuticals For Human Use Guideline, Geneva, Switzerland, 2005, 1-13.

[30] Harvey, D., Modern analytical chemistry McGraw-Hill New York, 2000: p. 727-728.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

9th International Conference on
Chemical & Environmental Engineering
3-5 April 2018

Table 1 Comparison of the quantitation limits of the reported electrochemical methods to that of the described SW-AdASV method for Mebeverin hydrochloride determination at carbon past electrode (CPE) or the modified ones.

Method	Electrode	Linear range\ M	LOQ\ M	Ref.
Potentiometry Bulk form	Tricresylphosphate/CPE *o*-nitrophenyloctylether/CPE	$3.0\times10^{-7} – 1.0\times10^{-2}$ $1.0\times10^{-7} – 1.0\times10^{-2}$	1.00×10^{-6} 3.33×10^{-7}	[13]
SW-AdASV Bulk form Human urine	CPE	$4.9\times10^{-7} –9.0\times10^{-6}$ $7.5\times10^{-7}–9.0\times10^{-6}$	2.72×10^{-6} 7.87×10^{-7}	[14]
DPV Bulk form Human serum	Gold nanoparticles / silica gel / CPE	$4.0\times10^{-8} – 1.0\times10^{-5}$ $2.0\times10^{-7} – 9.0\times10^{-6}$	5.0×10^{-9} 6.7×10^{-8}	[15]
SW-AdASV Bulk form Human serum	functionalized multiwall carbon nanotube /CPE	$5.0\times10^{-10} – 3.5\times10^{-8}$ $8.0\times10^{-10} – 2.0\times10^{-8}$	4.4×10^{-10} 6.7×10^{-10}	[16]
SW-AdASV Bulk form Human serum	diatom /CPE	$5.0\times10^{-9} –2.0\times10^{-7}$ $8.0\times10^{-10} – 2.0\times10^{-8}$	6.00×10^{-9}	**This work**

Table 2: Characteristics of the calibration curves of SW-AdASV determination of bulk (MV.HCL) in a B-R universal buffer of pH 5; under the optimal operational conditions.

	Bulk	Serum
Linearity range/ (M)	$5.0\times10^{-9} –2\times10^{-7}$	$5\times10^{-9} – 1.6\times10^{-7}$
Slope / (µA/nM)	0.035	0.107
S.D	0.011	2.16×10^{-2}
S.E	0.0055	8.82×10^{-2}
Intercept / (µA)	0.64	0.113
S.D	0.021	8.91×10^{-2}
SE	0.0105	3.64×10^{-2}
Correlation coefficient (*r*)	0.996 ± 0.002	0.997 ± 0.002
Number of points (*N*)	11	13
Mean *LOQ* / (M)	6.00×10^{-9}	2.50×10^{-9}
Mean *LOD* / (M)	1.80×10^{-9}	8.33×10^{-9}

N=4

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

9th International Conference on Chemical & Environmental Engineering
3-5 April 2018

Table3. Results of intra-day and inter-day assays of various concentrations of bulk (MV.HCL) by the optimized SW-AdASV method utilizing using DA/CPE

Day	[Taken] / M	Mean[Found] M	%R	% Bias	Precision RSD %
			Intra-day		
	1×10^{-8}	0.994×10^{-8}	99.36	−0.64	0.73
	1×10^{-7}	1.004×10^{-7}	100.4	−0.4	0.62
	1.2×10^{-7}	1.199×10^{-7}	99.94	0.12	0.49
			Inter-day		
	5×10^{-8}	4.921×10^{-8}	98.40	−1.58	1.01
1	1×10^{-7}	0.998×10^{-7}	99.81	−0.19	0.81
	1.2×10^{-7}	1.195×10^{-7}	99.58	−0.42	0.73
	5×10^{-8}	5.001×10^{-8}	100.02	0.02	0.32
2	1×10^{-7}	1.002×10^{-7}	100.22	0.22	0.17
	1.2×10^{-7}	1.202×10^{-7}	100.17	0.17	0.25
	5×10^{-8}	4.982×10^{-8}	99.64	−0.36	0.92
3	1×10^{-7}	1.011×10^{-7}	101.10	1.10	0.65
	1.2×10^{-7}	1.201×10^{-7}	100.08	0.08	0.51

Table4: Validation studies for determination of 2×10^{-8} M bulk (MV.HCL) by means of the described SW-AdASV method onto DA/CPE.

Variables	Operational Conditions	R ± RSD.% (n = 3)
pH		
4.5	E_{acc} = 0.0 V	98.87± 0.92
5.0		99.55± 0.98
5.5		99.21± 0.52
Preconcentration potential (E_{acc})		
-0.05	pH = 5	99.39± 0.77
0.0		99.55± 0.98
0.05		99.99± 0.63
Preconcentration time (t_{acc})		
85	pH = 5,	97.52± 1.13
90	E_{acc} = −0.20 V	99.55± 0.98
95		103.83± 0.91

Table 5: Assay of (MV.HCl) in its pharmaceutical formulations by means of the described stripping voltammetric method in comparison with f-MWCNT/CPE method

Method	[Taken] (mg/tablet)	Mean [Found] / (mg/tablet)	%R	% Bias	Precision RSD %
Colofac®					
DA/CPE	135	134.45	99.59	0.41	0.55
7%f-MWCNT/CPE	135	133.64	98.99	1.01	0.45
F-value	1.49				
t-test	1.89				
Colona ®					
DA/CPE	100	99.21	99.21	0.79	0.63
7%f-MWCNT/CPE	100	99.85	99.85	0.15	0.66
F-value	1.10				
t-test	1.57				
Coloverin A®					
DA/CPE	135	134.77	99.83	0.17	0.67
f-MWCNT/CPE	135	134.01	99.27	0.73	0.58
F-value	1.33				
t-test	1.41				

Theoretical *F*-value , ,F(0.05, 4, 4)= 6.388 and *t-test* ,t(0.05,7)= 2.36 at 95% confidence limit for $n_1 = 5$ and $n_2 = 5$.

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9th International Conference
on
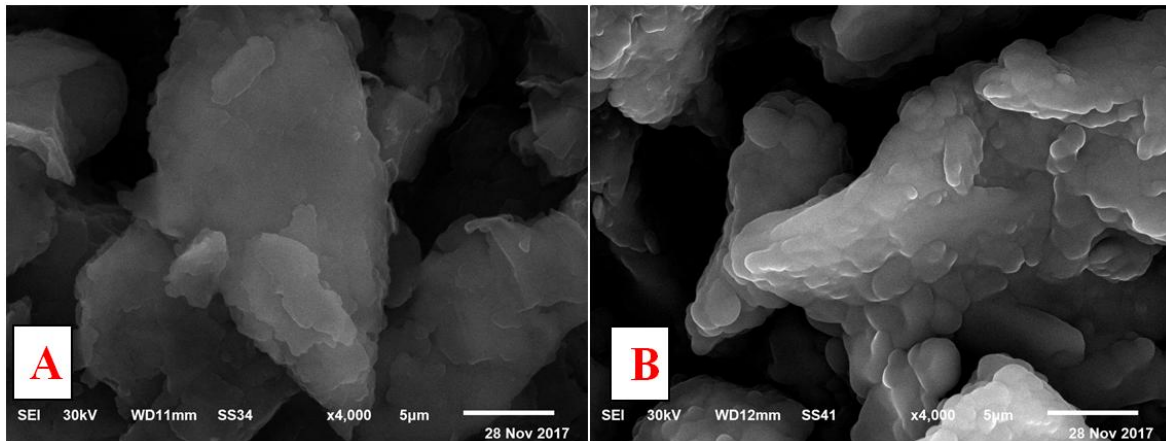Chemical & Environmental
Engineering**
3-5 April 2018
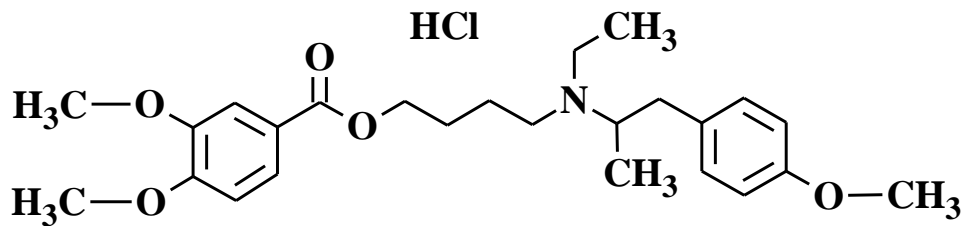
Fig.1. Chemical structure of Mebeverine hydrochloride (MV.HCl)
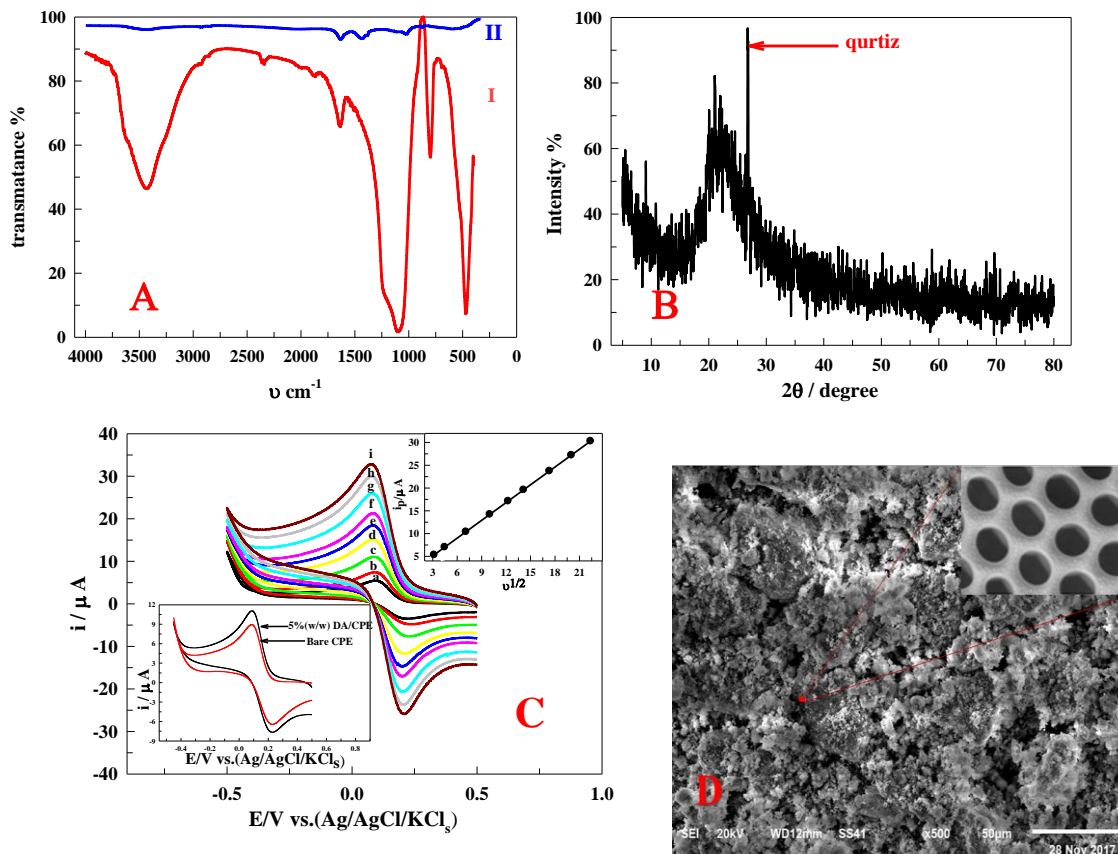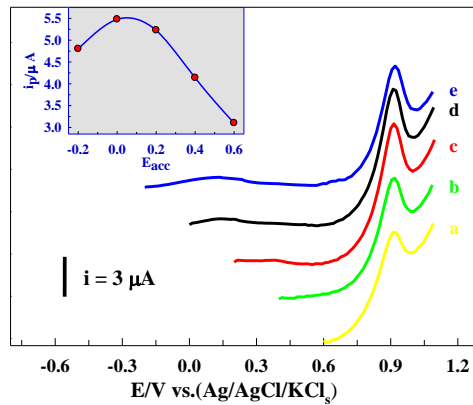
Fig. 2: A) FTIR of (I) for diatom (II) graphite and, B) XRD for diatom, C) cyclic voltammetry for 1.0×10^{-3} M $K_4Fe(CN)_6$ onto 5% (w/w)DA/CPE and D) SEM of diatom.

Fig. 3: SEM image for A) CPE and B) 5% (w/w)DA/CPE
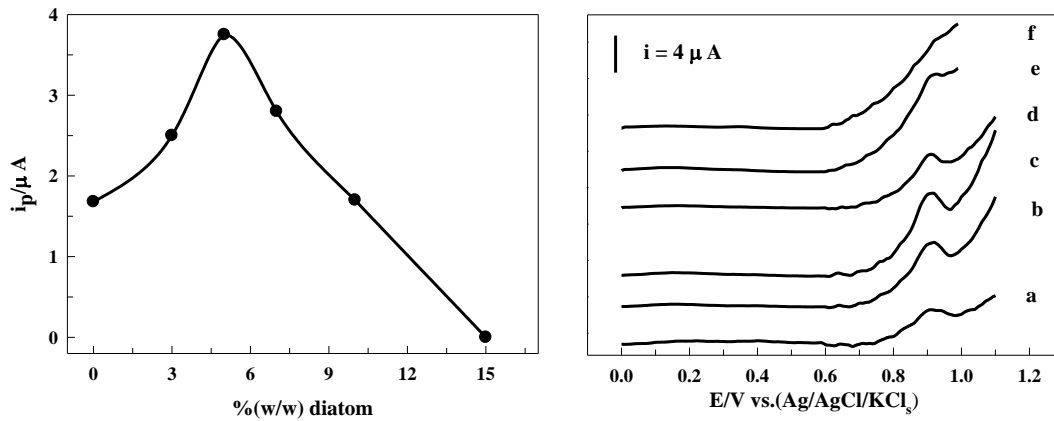
**Fig. 4: SW-AdASV chromatogram of 5×10^{-7} M MV.HCl in B-R universal buffer of
pH 5 onto CPE modified by different ratios of diatoms.**

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

9th International Conference
on
Chemical & Environmental
Engineering
3-5 April 2018
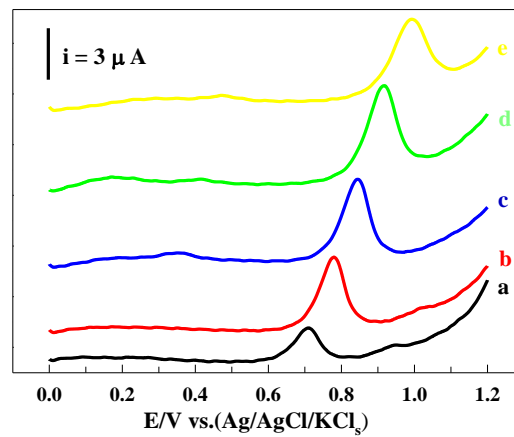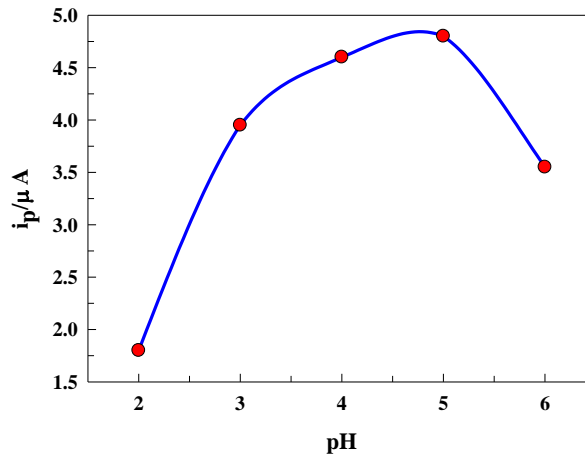
Fig. 5: Voltammograms and its corresponding plot for the effect of pH on the SW-AdASV peak current intensity for $5x10^{-7}$ M of (MV.HCl)

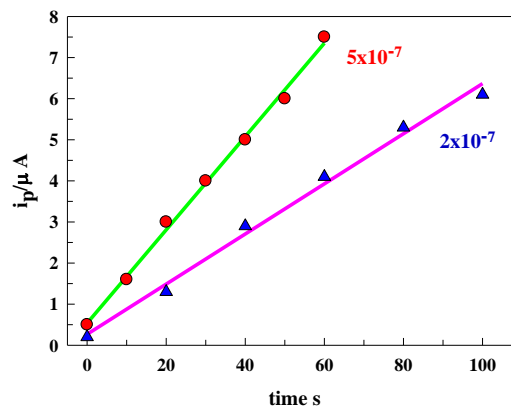**Fig. 6: Voltammograms and its corresponding plots for the effect of accumulation potential on the SW-AdASV peak current intensity for 5×10^{-7} M of (MV.HCl) in the B-R universal buffer solution of pH5**
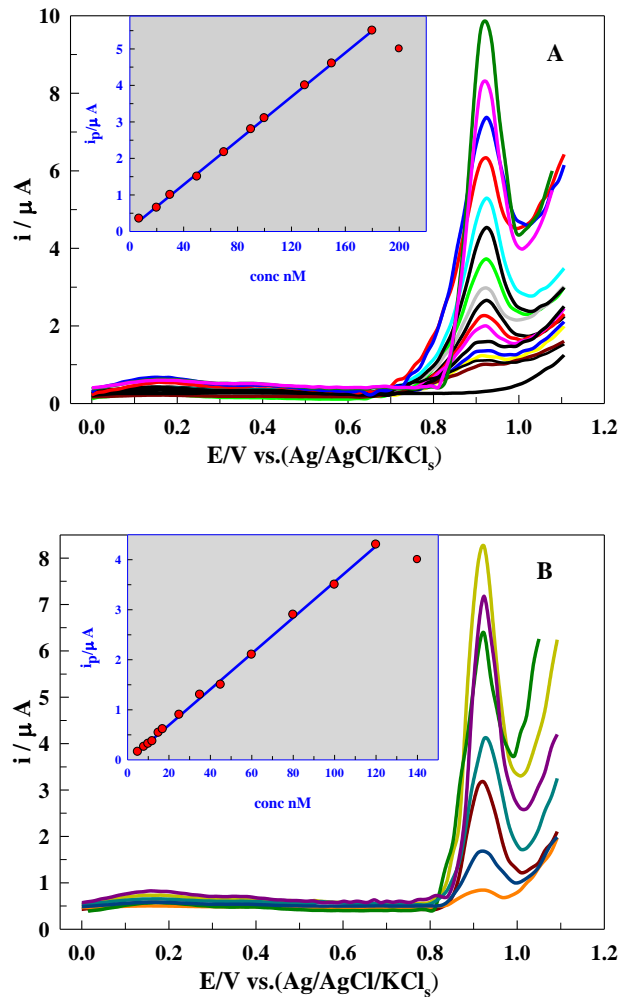

Fig. 7: Voltammograms and its corresponding plots for the effect of accumulation time on the SW-AdASV peak current intensity for 5×10^{-7} and 2×10^{-7} M of (MV.HCl) in the B-R universal buffer solution of pH5

Military Technical College
Kobry El-Kobbah,
Cairo, Egypt

**9th International Conference
on
Chemical & Environmental
Engineering**
3-5 April 2018

**Fig. 8: SW-AdAS voltammograms recorded in the B-R buffer of pH 5 for various
concentrations of (MV.HCl) A) in bulk and B) in serum at DA/CPE after
preconcentration for 100.**