Talanta 84 (2011) 223–234

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Stripping voltammetric determination of silymarin in formulations and human blood utilizing bare and modified carbon paste electrodes

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ARTICLE INFO

Article history:
Received 16 December 2010
Received in revised form 8 January 2011
Accepted 13 January 2011
Available online 21 January 2011

Keywords:
Silymarin
Carbon paste electrode
Montmorillonite clay
Stripping voltammetry
Determination

ABSTRACT

Silymarin is one of the most powerful natural substances that have the ability to protect and rebuild the liver cells damaged by alcohol and other toxic substances. Silymarin showed two irreversible anodic peaks in buffered solutions (pH 2.5–8.0) at either the bare carbon paste electrode or the montmorillonite-Ca modified carbon paste one. These two peaks have been attributed to oxidation of the two phenolic OH groups at positions C-20 and C-7 of silymarin molecule. A square-wave adsorptive anodic stripping voltammetry method was optimized for determination of silymarin utilizing the bare and the modified carbon paste electrodes. The method was fully validated and successfully applied for the determination of silymarin in commercial formulations and human serum without prior extraction utilizing both carbon paste electrodes. Limits of quantitation of 1×10^{-7} and 7×10^{-9} mol L^{-1} silymarin have been achieved in bulk form or in formulations while 2×10^{-7} and 8×10^{-9} mol L^{-1} silymarin were achieved in spiked human serum utilizing the bare carbon paste electrode and the modified one, respectively. The two electrodes exhibited excellent selectivity towards silymarin even in the presence of $10^2$to 10^3-fold excess of its co-formulated drugs, common excipients, and common metal ions. The pharmacokinetic parameters of silymarin in plasma of healthy human volunteers were estimated following the administration of a single oral dose of 120 mg silymarin utilizing the modified carbon paste electrode. The estimated pharmacokinetic parameters were favorably compared with those reported in literature.

1. Introduction

Silymarin (SMR) is a mixed extract of polyphenolic flavonoids isolated from the dried seeds of milk thistle plant [1,2]. It is a complex mixture of four flavonolignan isomers (silybin, isosilybin, silydianin and silychristin) with the empirical formula $C_{25}H_{22}O_{10}$ (Scheme 1). Among the isomers, silybin is the major and most biologically active component and represents about 60–70%, followed by silychristin (20%), silydianin (10%), and isosilybin (5%) [1–3].

SMR has been proven to be one of the most powerful natural substances that have the ability to protect and rebuild the liver cells damaged by alcohol and other toxic substances by stimulating protein synthesis [3–6]. The cytoprotective effects of SMR are mainly attributable to its antioxidant and free radical scavenging properties. These reduce the chance of liver cells damaged by free radicals, inhibit production of superoxide radicals and nitric oxide, inhibit lipid peroxidation and oxidative enzymes and protect liver cell membrane [5]. SMR is metabolized in human to its conjugated form, consisting mainly of sulfates and glucuronides and excreted into bile and urine. Its half-life ($t_{1/2}$) of elimination is 3–4 h. The major sites for glucuronidation were the phenolic OH groups at C-20 and C-7 of SMR molecule [6–8]. Pharmacokinetic study of SMR in healthy human volunteers after the administration of a single dose of 120–200 mg SMR [9,10] indicated that SMR was rapidly absorbed with a t_{max} of 0.875–2.1 h, and C_{max} of 1.13–6.04 μg mL^{-1}.

Various analytical methods for assay of SMR in biological fluids and/or in formulations have been reported. These include spectrophotometry [11–13], colorimetry [14], LC–MS [15,16], HPTLC [17], HPLC [6,18,19] and differential-pulse voltammetry [20] methods. The reported spectrophotometric methods for assay of SMR in its formulations [11–13] require formation of colored species prior to the analysis. One of these methods was based on oxidation of SMR with $K_2S_2O_3$ in alkaline medium and further coupling of the product with 3-methyl-2-benzothiazolinone hydrazone hydrochloride forming intensely colored species [11]. The second method was based on oxidation of SMR with $KMnO_4$ at pH 7.0 [12], while the third one was based on formation of blood red colored complex with $FeCl_3$ and 1,10-phenanthroline or blue colored complex with Folin–cioCalteu reagent in alkaline medium [13]. These methods are not sensitive enough for assay of SMR in biological fluids since their achieved limits of quantitation (LOQ) were 5.18×10^{-6} to 2.07×10^{-5} mol L^{-1} SMR (2.5–18 μg mL^{-1} SMR). Although the reported chromatographic methods [6,15–19] are sensitive enough for assay of SMR in biological fluids, they are

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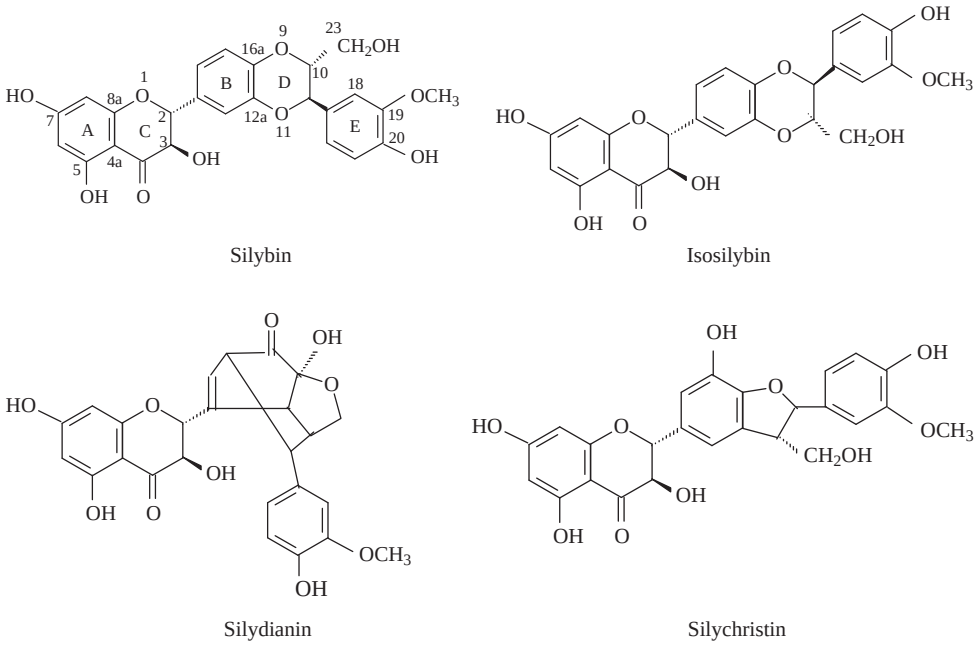
doi:10.1016/j.talanta.2011.01.027



Scheme 1. Structure of main silymarin components.

complicated, expensive, require sample pretreatment and time-consuming extraction steps prior to the analysis. On the other side, the reported voltammetric method for assay of SMR in its formulations using a glassy carbon electrode [20] is selective towards SMR in the presence of vitamin E, however the method was not optimized for quantitation of SMR in biological fluids.

To our knowledge, adsorptive stripping voltammetry technique utilizing either the bare carbon paste or the modified carbon paste electrodes has not been reported for assay of SMR. The bare carbon paste electrode (CPE) is a mixture of an electrically conducting graphite powder and a pasting liquid (usually Nujol oil). It has been widely used in electro-analytical chemistry as a working electrode for determination of various organic and inorganic species. However, the sensitivity of the bare CPE is relatively poor for trace assay of these species. In order to improve its sensitivity, a fascinating and effective way is to modify it by mixing with some other unique substances. Because montmorillonite natural clay has high chemical and mechanical stability, well-layered structure and strong adsorptive properties attributed to the expandability of its internal layers, it has been used successfully in this laboratory as a modifier for carbon paste electrode to assay of some biologically active compounds [21,22].

In this work, the electro-oxidation of SMR in buffered solutions has been studied and discussed. Besides, a comparative electro-analytical study utilizing the bare carbon paste electrode and a modified one with montmorillonite-Ca natural clay coupled with an optimized square-wave adsorptive anodic stripping voltammetry method has been carried out for trace determination of SMR in bulk form, commercial formulations and human blood.

2. Experimental

2.1. Materials and solutions

Silymarin (SMR) (Sigma Chemical Co., St. Louis, MO, USA) and its various commercial formulations were the substances of interest in the present investigation. The analyzed formulations were: (1) "Legalon capsules®" labeling to contain140 mg SMR per capsule (Chemical Industries Development Co., Egypt), (2) "Hepaticum capsules®" labeling to contain 140 mg SMR per capsule (Medical Union Phramaceuticals Co., Egypt), (3) "Seralon-E capsules®" labeling to contain 120 mg SMR + 40 mg vitamin E (as α-tocopherol acetate) per capsule (Tiba Pharmaceuticals, Egypt), (4) "Mepacure capsules®" labeling to contain 50 mg SMR + 30 mg dimethyl dimethoxy biphenyl dicarboxylate (DDB) per capsule (Mepaco, Egypt) and (5) "Silymarin plus capsules®" labeling to contain 140 mg SMR + 200 mg acetylcysteine + 100 mg vitamin E (as α-tocopherol) + 150 mg vitamin C + 25 μg selenium + 10 mg Zn + 300 i.u. vitamin A (as β-carotene) per capsule (Sedico Pharmaceutical Co., Egypt).

Standard stock solution (1×10^{-3} mol L^{-1}) of each of bulk SMR, vitamins A, C and E, DDB, and acetylcysteine were prepared in methanol (Merck) and stored at 4 °C. The desired solutions (1×10^{-6} to 1×10^{-4} mol L^{-1}) were prepared by appropriate dilutions of the standard stock solutions with methanol.

The drug content of twenty capsules of each of the commercial formulations under investigation was individually mixed, weighed and the average mass per capsule was determined. A weighed portion of each of the mixed contents equivalent to 1×10^{-3} mol L^{-1} SMR was accurately transferred into a 100-mL volume calibrated flask containing 70 mL methanol (Merck). The content of flasks were sonicated for about 15 min and then filled up with methanol. The solutions were then filtrated through 0.45 μm milli-pore filters (Gelman, Germany). The desired concentrations of SMR were obtained by accurate dilution with methanol.

2.2. Spiked human serum

Twelve serum samples of four healthy volunteers (three samples from each volunteer) were stored frozen until assay. Samples of the human serum (each of 1 mL) were fortified with various concentrations (1×10^{-6} to 1×10^{-4} mol L^{-1}) of SMR in small centrifugation tubes (3 mL polypropylene micro-centrifuge tubes). Each of these samples was 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.

2.3. Supporting electrolytes

Britton–Robinson (B–R) universal (pH 2–11) and acetate (pH 3.75–5.75) buffers were prepared in de-ionized water and were used as supporting electrolytes.

2.4. Preparation of the bare and modified carbon paste electrodes

The carbon paste was prepared by mixing an amount (5 g) of graphite powder (1–2 μm, Aldrich, Milwaukee, WI, USA) and 1.8 mL Nujol oil (Sigma, $d = 0.84\,g\,mL^{-1}$) uniformly by milling in a small agate mortar. Whereas 10% (w/w) montmorillonite-Ca modified carbon paste (MMT-Ca modified CP) was prepared by mixing an amount (4.5 g) of graphite powder and 0.5 g of montmorillonite-Ca natural clay (Fine powder < 5 μm, ECC America Inc., Southern Clay Products Subsidiary, Gonzales, TX, USA) uniformly by milling in a small agate mortar. Then 1.8 mL Nujol oil was added and milled again to give a homogenous 10% (w/w) MMT-Ca-modified CP. Various modified carbon pastes containing different mass percentages of MMT-Ca clay (5 and 15%, w/w) were similarly prepared. The body of the electrode was a Teflon rod with end cavity (BASi Model MF-2010, 3 mm diameter and 1 mm deep) bored at one end for paste filling. Contact was made with a copper wire through the centre of the Teflon rod. An amount of the prepared bare CP or MMT-Ca-modified CP was pressed into the end cavity of the electrode body and leveled off with a spatula. Surface of the constructed bare CPE or MMT-Ca modified CPE was manually smoothed by polishing on clean paper before use.

2.5. Apparatus

Computer-controlled Potentiostats Models 263A and 394-PAR (Princeton Applied Research, Oak Ridge, TN, USA) with the software 270/250-PAR were used for the voltammetric measurements. A micro-electrolysis cell consisting of C-2 stand with electrode body (BASi Model MF-2010), an Ag/AgCl/3 M KCl reference electrode (BASi Model MF-2079), and a platinum wire counter electrode was used. A magnetic stirrer with a Teflon-coated magnet was used to provide the convective transport during the preconcentration step. An Eppendorf centrifuge (Model 5417 C, Hamburg, Germany) was used for separation of precipitated proteins from human serum samples prior to the assay. A micropipette (Eppendorf-Multipette® plus) was used for transferring the analyte solutions throughout the experimental work. De-ionized water was used through out the present work.

2.6. Procedure

10 mL volume of the supporting electrolyte was introduced into the micro-electrolysis cell and a smoothed bare CPE or MMT-Ca-modified CPE was then immersed in the electrolyte. Several cyclic sweeps were then applied until reaching low background current. An aliquot of the analyte solution was then introduced into the electrolysis cell, and a preconcentration potential was applied to the working electrode for a selected time while the solution was stirred at 400 rpm. At the end of the preconcentration time, stirring was stopped and a 5 s rest period was allowed for the solution to become quiescent. The voltammograms were then recorded using the bare CPE or the modified one by scanning the potential towards the positive direction using square-wave potential waveform. After each measurement, the used carbon paste or the modified one was carefully removed, the cavity of the electrode was cleaned with de-ionized water and dried with a tissue and a new CPE or MMT-Ca-modified CPE was constructed as described in Section 2.4.

For determination of SMR in human serum samples, medium exchange treatment for the working electrode was performed after the preconcentration step by immersing it in the blank electrolyte [23]. This is to avoid interferences from low molecular weight proteins which may remain after centrifugation.

The mean percentage recovery (%R) for the found concentrations was calculated as a percent of the nominal concentrations in the standard solutions. Accuracy; was expressed as a relative error ($RE\% = ([C_{found}/C_{taken}] - 1) \times 100$) while precision was assessed from the relative standard deviation in percentage (RSD%) of the mean recovery.

2.7. Pharmacokinetic study

Four healthy male volunteers (aged 45–50 years) were anticipated in this study. Pharmacy Ethical Committee of the Faculty of Pharmacy at Tanta University, Egypt approved (2/F-March 2010) the study protocol for the human study of SMR. Written informed consent was obtained from each volunteer prior to anticipating in the study. Subjects were divided into two groups; each includes two subjects. Each subject received an oral single dose of 120 mg SMR (one Seralon-E capsule®) in the morning, after an overnight fast. The subjects were housed at the Ramadam Specialist Hospital (at Tanta City, Egypt) throughout period of blood sampling. Blood samples (about 3 mL each) were obtained at 0 (pre-dose), 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 6.0, 8.0 and 12.0 h after the oral administration. The blood samples were centrifuged immediately at 3000 rpm for 15 min and the plasma fractions were then rapidly separated and stored in coded polypropylene tubes at −20 °C until the assay. Following separation of proteins by methanol and centrifugation, the plasma samples were analyzed by means of the optimized SW-AdASV method utilizing the montmorillonite-Ca modified CPE.

3. Results and discussion

3.1. Cyclic voltammetric studies

Cyclic voltammograms of SMR in the B–R universal buffer (pH 2.5–8.0) showed two irreversible anodic peaks (Fig. 1). The peak current (i_p) of the first one was almost pH independent over the entire pH range (Fig. 1, inset: curve a) while that of the 2nd peak decreased upon the increase of pH of the medium higher than 6 in a dissociation curve shape (Z-shaped curve) indicating the deprotonation of an electro-active OH group of SMR molecule (Fig. 1, inset: curve b). The pH value corresponding to the half-height of the i_p versus pH plot of the 2nd peak (Fig. 1, inset: curve b) was found to be 6.5. This value ($pH = pk_a = 6.5$) agrees with those reported in the literature for pK_{a1} of SMR (7.2–7.4) [24] and its isomers, silybin (6.9–7.0), silychristin (6.5–6.6) and silydianin (6.6–7.1) [25] which is due to dissociation of phenolic OH group at C-7 of ring A of SMR [25].

The peak potentials E_p of the two anodic peaks shifted towards less positive potentials with the increase of pH of the medium indicating the involvement of protons in the electrode process. Rectilinear plots of the peak potentials E_p versus pH for the 1st and 2nd anodic peaks were obtained; their corresponding regression equations were:

(1st peak) $E_p\,(V) = 1.009 - 0.067pH \quad (r = 0.996 \text{ and } n = 7)$
(2nd peak) $E_p\,(V) = 1.459 - 0.076pH \quad (r = 0.997 \text{ and } n = 7)$

On the other side, as the scan rate was increased, the peak potentials of the two anodic peaks shifted towards more positive potentials as expected for irreversible oxidation processes [26,27].

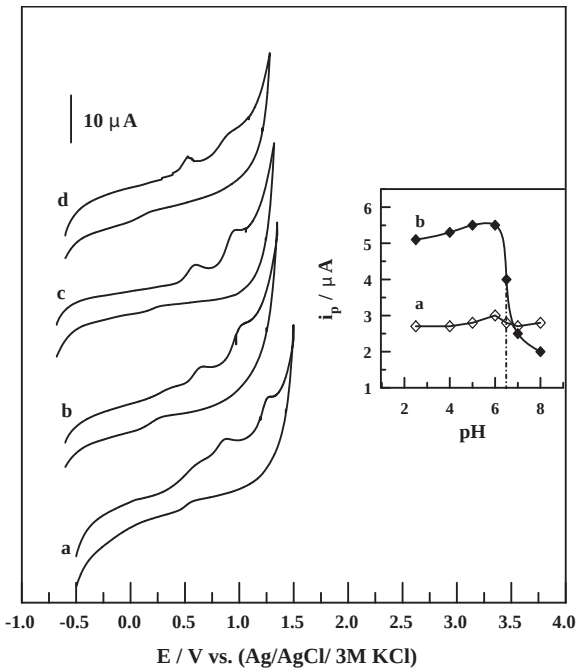


Fig. 1. Cyclic voltammograms of 1×10^{-4} mol L^{-1} SMR recorded at the bare CPE in the B–R universal buffer of different pH values: (a) pH 2.5, (b) 4, (c) 6 and (d) 7; scan rate = 300 mV s^{-1}. *Inset*: Plots of i_p versus pH of the 1st peak (curve a) and 2nd peak (curve b).

Plots of peak potentials E_p of the two anodic peaks at different pH values versus logarithm of scan rate ν were linear; their corresponding regression equations were:

$$\text{(1st peak)} \quad E_p\,(V) = (0.047 - 0.060)\log(\nu(\text{mV s}^{-1})) + (0.570 - 0.386) \quad (r = 0.996 \text{ and } n = 6)$$

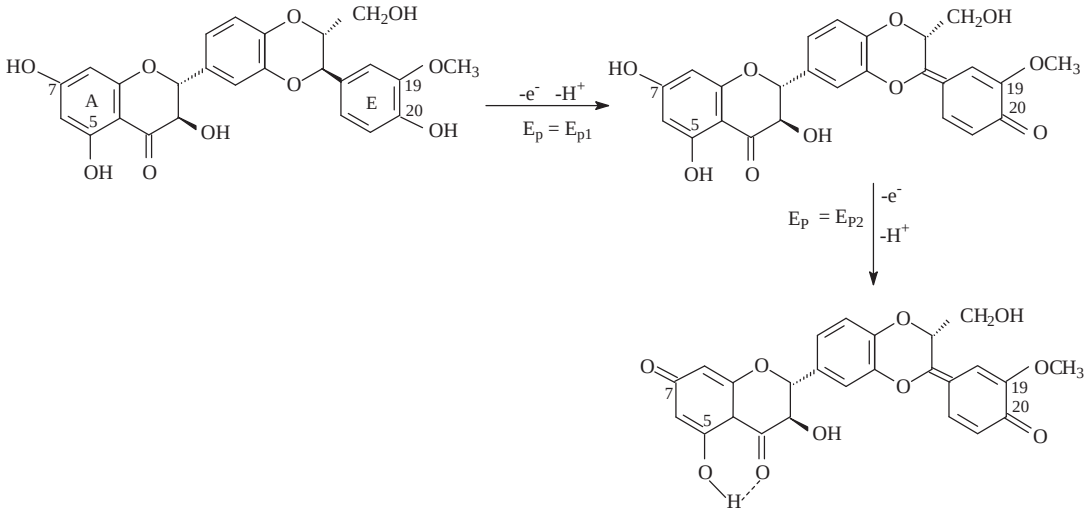
$$\text{(2nd peak)} \quad E_p\,(V) = (0.041 - 0.058)\log(\nu(\text{mV s}^{-1})) + (0.795 - 0.908) \quad (r = 0.996 \text{ and } n = 6)$$

According to Nicholson and Greef [26,28], values of αn_a (product of symmetry transfer coefficient α and number of electrons n_a transferred in the rate-determining step) of 0.49–0.63 (1st peak) and 0.51–0.72 (2nd peak) were estimated from slope values of the obtained E_p versus log ν plots $\{(\Delta E_p\ (V))/\Delta\log(\nu\ (\text{mV s}^{-1})) = 0.059/2\alpha n_a\}$. The number of protons (p) involved in the rate-determining step was estimated from slope values of the E_p–pH plots of the two anodic peaks using the relation $(\Delta E_p\ (V))/\Delta\text{pH} = (0.0591/\alpha n_a)\,p$ [29] and was found to equal one ($p = 1$).

According to the results reported in the literature [30–33] and those obtained in this study, the first oxidation peak of SMR at various pH values ($E_{p1} = 0.510$–0.850 V) may be attributed to oxidation of o-methoxy-phenolic moiety (C-19, C-20) of the E ring of SMR molecule (Scheme 1). Whereas, the second oxidation peak ($E_{p2} = 0.940$–1.280 V) may be attributed to oxidation of resorcinol group (C-5, C-7) of ring A [30,33,34]. The OH group at position C-7 is probably the most reactive phenolic OH one that easily undergoes deprotonation process ($pK_{a1} = 6.5$–7.0) [24,25]. While the phenolic OH group at position C-5 in the same ring is partly deactivated by an internal H-bond formation with the adjacent keto group forming a more stable 6 membered ring [25]. So, the second oxidation peak of SMR may be due to oxidation of OH group at position C-7 [30,34,35] via the transfer of one electron and one proton. Accordingly, the electro-oxidation reaction of SMR at the CP electrodes can be expressed as shown in Scheme 2. The ease of oxidation of the phenolic groups of SMR is of importance for its effectiveness as an antioxidant [36,37].

The affinity of SMR to be absorbed onto the bare CP and MMT-Ca-modified CP electrodes was designated by recording cyclic voltammograms of 5×10^{-6} mol L^{-1} SMR in the B–R universal buffer (pH 2.5–8.0) and in acetate buffer (pH 3.75–5.75) following preconcentration onto the working electrode surface at open circuit conditions (e.g., Fig. 2A and B, curves a) and then by adsorptive accumulation at +0.10 V (versus Ag/AgCl/3 M KCl) for 200 s {e.g., Fig. 2A and B, curves (b) 1st cycle and (c) 2nd cycle}. Following preconcentration, a broad small oxidation peak was observed at the bare CPE (Fig. 2A, curve b), while a better developed peak was observed at the modified CPE (Fig. 2B, curve b) especially in acetate buffer of pH 4, indicating a better adsorption of SMR onto the MMT-Ca-modified CPE even at open circuit conditions (Fig. 2B, curves a). Moreover, a substantial decrease of the monitored voltammetric peak current was observed in the 2nd cycle (Fig. 2A and B, curves c) indicating desorption of SMR from the electrode surface.

Cyclic voltammograms of 5×10^{-6} mol L^{-1} SMR were recorded at different scan rates ν (50–500 mV s^{-1}) following preconcentration of SMR by adsorptive accumulation onto the working electrode surface at +0.10 V for 200 s. Linear Randles–Seveik plots (i_p (μA) versus ν (mV s^{-1})) were obtained using both the bare CP and the MMT-Ca-modified CP electrodes with slope values of 0.007 and 0.019 μA mV^{-1} s ($r = 0.998 \pm 0.001$ and $n = 6$), respectively, indicat-



Scheme 2. Electrode reaction of silymarin.

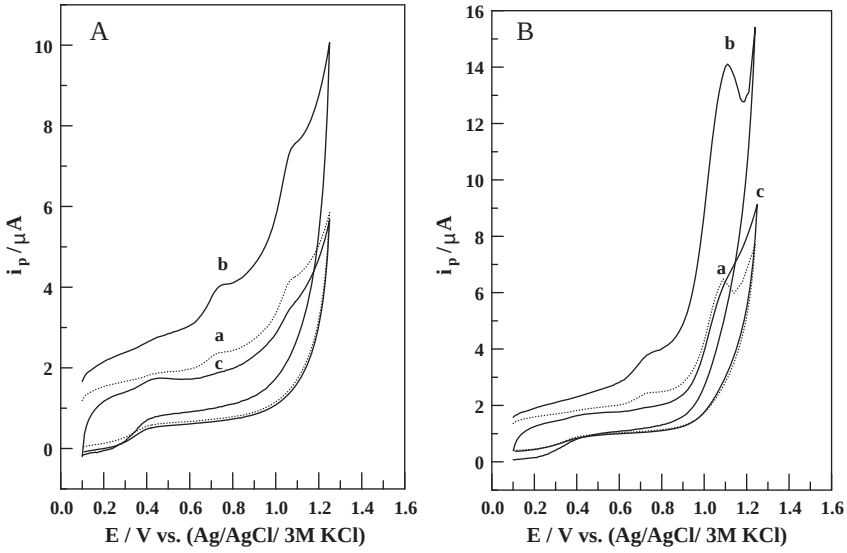


Fig. 2. Cyclic voltammograms of 5×10^{-6} mol L^{-1} SMR in acetate buffer of pH 4 recorded at the bare CPE (A) and at the MMT-Ca-modified CPE (B) following preconcentration at open circuit conditions (curve a) and then by adsorptive accumulation at E_{acc} = +0.1 V for 200 s onto the electrode surface (b, 1st cycle and c, 2nd cycle); scan rate = 300 mV s^{-1}.

ing that the oxidation process of SMR at both electrode is controlled by adsorption [38]. Moreover, linear plots of log (i_p (μA)) versus log (ν (mV s^{-1})) were obtained using both the bare CP and the MMT-Ca-modified CP electrodes; their corresponding regression equations were:

(Bare CPE) $\log(i_p\,(\mu A)) = 0.77 \log(\nu(\text{mV s}^{-1})) - 1.49$
($r = 0.998$ and $n = 6$)
(Modified CPE) $\log(i_p\,(\mu A)) = 0.95 \log(\nu(\text{mV s}^{-1})) - 1.82$
quad($r = 0.999$ and $n = 6$)

The slope values of 0.77–0.95/(μA mV^{-1} s) are close to the expected theoretical value (1.0) for an ideal reaction of surface species [39]. These results indicated again the adsorptive behavior of SMR especially onto surface of the developed MMT-Ca-modified CPE.

3.2. Square-wave stripping voltammetry studies

3.2.1. Composition and stability of the modified CPE

SW-AdAS voltammograms of 1×10^{-6} mol L^{-1} SMR in the acetate buffer of pH 4 at CPE modified with various mass percentages (% w/w) of MMT-Ca natural clay were recorded following preconcentration by adsorptive accumulation at $E_{acc} = -0.5$ V (versus Ag/AgCl/3 M KCl) for 200 s. The peak current magnitude of the 2nd anodic peak increased upon the increase of percentage (% w/w) of MMT-Ca natural clay in the modified CPE up to 10% (w/w). Such enhancement of stripping peak current was expected due to the strong adsorptive properties of MMT-Ca natural clay. At higher percentages (w/w) of MMT-Ca natural clay in the modified CPE the peak current decreased due to the decrease of conductivity of the modified CPE, which leads to hinder the electron transfer process and increase the background current. Therefore, 10% (w/w) MMT-Ca modified CP electrode was used in the rest of the present analytical study. On the other side, insignificant difference of peak current or its standard deviation (0.20–0.24) was noticed over a week utilizing the fabricated MMT-Ca modified CP electrode confirming that this electrode was stable and efficient towards the trace determination of SMR.

3.2.2. Optimization of square-wave operational parameters

3.2.2.1. Effect of pH of the medium. SW-AdAS voltammograms of 1×10^{-6} mol L^{-1} SMR were recorded following preconcentration by adsorptive accumulation for 200 s at −0.5 V (versus Ag/AgCl/3 M KCl) in acetate buffer of various pH values (3.75–5.75) using both the bare CP and the 10% (w/w) MMT-Ca-modified CP electrodes. As shown in Fig. 3 (curve b), MMT-Ca-modified CPE exhibited significant enhancement of the peak current in comparison to that of the bare CPE (Fig. 3, curve a). Moreover, the peak current magnitude of the 2nd peak was better developed than that of the 1st one upon using both the bare CPE and the modified one especially at pH 4 (Fig. 3, inset). Hence, all subsequent work was based on measurements of the 2nd peak current in acetate buffer of pH 4.

3.2.2.2. Square-wave pulse parameters. Voltammograms of 1×10^{-6} mol L^{-1} SMR in acetate buffer of pH 4 following pre-

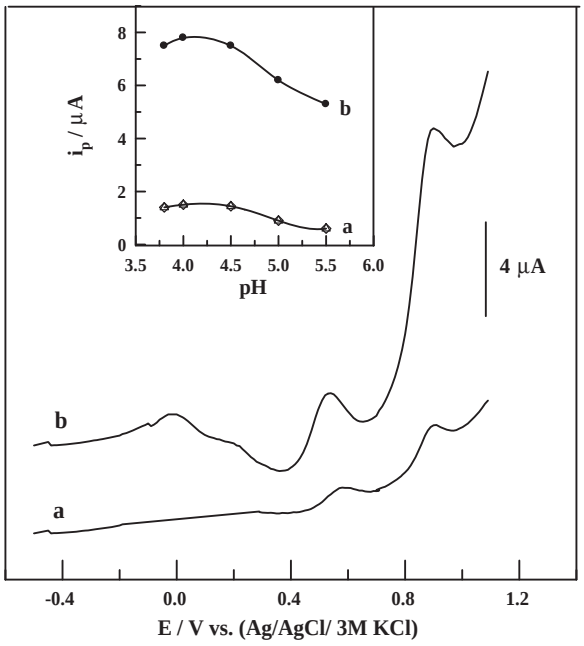


Fig. 3. SW-AdAS voltammograms of 1×10^{-6} mol L^{-1} SMR in acetate buffer of pH 4 recorded following preconcentration by adsorptive accumulation at $E_{acc} = -0.5$ V for 200 s onto the bare CPE (a) and onto the MMT-Ca-modified CPE (b). *Inset*: Plots of i_p versus pH of the 2nd peak for the bare CPE (a) and the MMT-Ca-modified CPE (b); f = 80 Hz, ΔE_s = 10 mV and E_a = 25 mV.

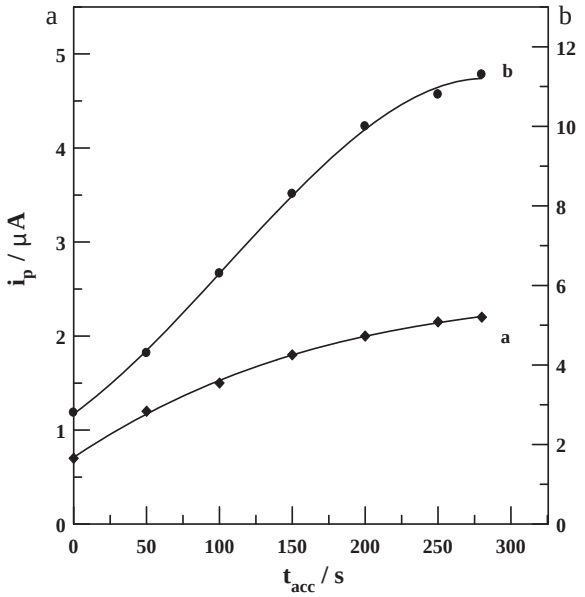


Fig. 4. Effect of the preconcentration time (t_{acc}) on the SW-AdASV peak current (i_p) of 1×10^{-6} mol L^{-1} SMR in acetate buffer of pH 4 following preconcentration onto the bare CPE (a) and the MMT-Ca-modified CPE (b) by adsorptive accumulation at +0.1 V; f = 80 Hz, ΔE_s = 10 mV, and E_a = 25 mV.

concentration onto the bare CPE or the modified one at −0.5 V for 200 s were recorded at various pulse parameters (frequency f = 10–80 Hz, scan increment ΔE_s = 2–10 mV, and pulse-amplitude E_a = 5–30 mV). Although the SW-AdAS voltammetry peak current magnitude of SMR was almost directly proportional to each of f, ΔE_s, and E_a, however a better developed voltammetric peak was obtained under the following pulse-parameters: f = 80 Hz, ΔE_s = 10 mV and, E_a = 25 mV using both the bare CPE and the modified one.

3.2.2.3. Preconcentration parameters. Effect of varying the preconcentration potential E_{acc} (−0.5 to +0.4 V) on the 2nd peak current magnitude of the SW-AdAS voltammogram of 1×10^{-6} mol L^{-1} SMR in acetate buffer of pH 4 was evaluated following preconcentration for 200 s onto the bare CPE or the modified one. A better enhanced peak current was achieved within the potential range of 0.0 to +0.2 V using both electrodes. At more positive potentials the peak current gradually decreased. Therefore, a preconcentration potential of +0.10 V (versus Ag/AgCl/3 M KCl) was applied throughout the present analytical studies.

On the other side, SW-AdAS voltammograms of 1×10^{-6} mol L^{-1} SMR were recorded at increasing preconcentration time (t_{acc}) under the foregoing optimal operational conditions using both the bare CP and the MMT-Ca-modified CP electrodes. The response was linear up to 200 s (Fig. 4). Therefore, a preconcentration time of 200 s was applied in the rest of the present study.

According to the foregoing results the optimum operational conditions for determination SMR with SW-AdASV method based on measurement of the peak current of its 2nd oxidation peak (E_{p2} = +1.10 V versus Ag/AgCl/3 M KCl) utilizing both the bare CP and the MMT-Ca modified CP electrodes in acetate buffer of pH 4 were: E_{acc} = +0.10 V, t_{acc} = 200 s, f = 80 Hz, ΔE_s = 10 mV and, E_a = 25 mV.

3.2.3. Method validation

3.2.3.1. Linearity. Linear dynamic ranges of 1×10^{-7} to 4×10^{-6} mol L^{-1} and 7×10^{-9} to 1.5×10^{-6} mol L^{-1} SMR were obtained in acetate buffer of pH 4 following preconcentration by adsorptive accumulation at +0.10 V for 200 s onto the bare CP and the MMT-Ca-modified CP electrodes, respectively. Their corresponding regression equations were:

$$\text{(Bare CPE)}: i_p\,(\mu\text{A}) = 2.45 \pm 0.003C\,(\mu\text{mol L}^{-1}) - 0.02 \pm 2.45 \times 10^{-2} \quad (r = 0.996 \text{ and } n = 11)$$

$$\text{(Modified CPE)}: i_p\,(\mu\text{A}) = 10.50 \pm 0.017C\,(\mu\text{mol L}^{-1}) + 1.30 \pm 7.35 \times 10^{-3} \quad (r = 0.998 \text{ and } n = 14)$$

Limits of detection (LOD) and quantitation (LOQ) of bulk SMR were estimated using the expressions [40]:

$$\text{LOD} = \frac{3\text{S.D.}}{b} \quad \text{and} \quad \text{LOQ} = \frac{10\text{S.D.}}{b}$$

where S.D. is the standard deviation of the replicate blank responses (or the intercept of the calibration plot) and b is the slope of the regression equation. LOD of 3×10^{-8} mol L^{-1} and 2.1×10^{-9} mol L^{-1} and LOQ of 1×10^{-7} mol L^{-1} and 7×10^{-9} mol L^{-1} SRM were achieved by the optimized SW-AdASV method utilizing the bare CP and the MMT-Ca modified CP electrodes, respectively. The results indicated the reliability of the optimized SW-AdASV method for trace assay of bulk SMR utilizing both electrodes.

3.2.3.2. Selectivity. The selectivity [41] of the optimized SW-AdASV method utilizing both the bare and MMT-Ca modified CP electrodes was examined by recording voltammograms of various concentrations of standard solutions of bulk SMR and of the tested commercial formulations containing excipients and/or co-formulated drugs (vitamins A, C, and E, acetylcysteine, DDB, Se^{4+}, and Zn^{2+}). The voltammograms of all the tested solutions were similar and showed no any voltammetric peaks due to any of the excipients and/or co-formulated drugs over the applied potential range (+0.1 to +1.3 V versus Ag/AgCl/3 M KCl). Moreover, insignificant differences in the percentage recoveries and relative standard deviations were achieved in the absence (98.89 ± 1.04 to 99.85 ± 0.68) and in the presence of excipients and co-formulated drugs (97.09 ± 0.52 to 98.03 ± 1.32). Furthermore, although some of the published works [20,42,43] exhibited that vitamins C and E are electro-active under definite experimental conditions at different kinds of working electrodes, in the present work they were found electro-inactive within the applied potential range (e.g., Fig. 5A and B). The later behavior agrees with that reported in some other published works [44,45]. It indicated also that these excipients and vitamins did not interfere with SMR determination. This is because these compounds did not deposit on the electrode surface under the experimental conditions of the applied electrochemical method [44–46] and/or oxidation of SMR occurred at a very positive potential than their oxidation potentials [47]. Consequently, all the obtained results demonstrated that the optimized SW-AdASV method utilizing both electrodes is selective towards SMR under the optimized experimental conditions.

3.2.3.3. Accuracy and precision. Accuracy and precision [41] of the optimized SW-AdASV method were evaluated by performing three replicate measurements for various concentrations of bulk SMR (2×10^{-7}, 5×10^{-7} and 1×10^{-6} mol L^{-1}) through intra-day and inter-day assays following preconcentration by adsorptive accumulation onto both the bare and the MMT-Ca-modified CP electrodes at +0.10 V for 200 s. Furthermore, accuracy and precision of the optimized SW-AdASV method were statistically compared with those obtained using a reference voltammetric method [20]. Satisfactory results were achieved (Table 1) utilizing both CP electrodes.

3.2.3.4. Robustness and inter-laboratory precision. The robustness [41] of the optimized SW-AdASV method for assay of SMR utilizing both CP electrodes was examined by evaluating the influence of

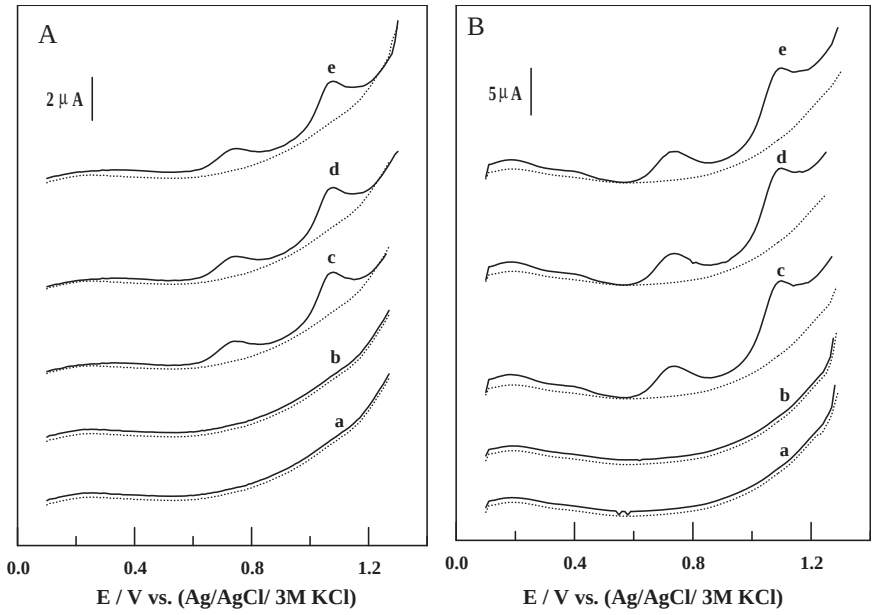


Fig. 5. SW-AdAS voltammograms recorded in the acetate buffer of pH 4 following preconcentration by adsorptive accumulation at +0.1 V for 200 s at the bare CPE (A) or MMT-Ca modified CPE (B) for: (a) a solution of 10^{-4} mol L^{-1} of each of Cu^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+} and Se^{4+}, (b) a solution of 10^{-4} mol L^{-1} of each of vitamins (A, C and E), acetylcysteine and DDB, (c) a solution of 8×10^{-7} mol L^{-1} bulk SMR, (d) a solution of the investigated "Silymarin plus capsules®" formulation containing 8×10^{-7} mol L^{-1} SMR, and (e) a solution of 8×10^{-7} mol L^{-1} bulk SMR + 10^{-4} mol L^{-1} of each of vitamins (A, C and E), acetylcysteine, Zn^{2+} and Se^{4+}; dotted line is the background.

small variations in some of the most important operational conditions (pH 3.8–4.2), accumulation potential E_{acc} (0.0 to +0.2 V) and preconcentration time (190–210 s). The obtained percentage recoveries and relative standard deviations (98.33 ± 0.74 to 100.23 ± 1.12) indicated insignificant effect within the studied range of variation of the optimum operational conditions, and consequently the optimized SW-AdASV method was considered reliable for assay of bulk SMR utilizing the bare CP and the MMT-Ca-modified CP electrodes and it could be considered robust.

The inter-laboratory precision [41] of the optimized SW-AdASV method for assay of SMR utilizing both electrodes was identified using two PAR Potentiostats, 263A (Lab. 1) and 273A (Lab. 2) at different elapsed times. The results obtained due to Lab. (1)-to-Lab. (2) (97.43 ± 0.88 to 99.25 ± 1.64) and even day-to-day (98.52 ± 1.43 to 104.22 ± 0.92) were found reproducible, since insignificant difference in the recoveries and relative standard deviations were noticed.

3.2.4. Applications

3.2.4.1. Assay of SMR in commercial formulations. Analysis of solutions of the various commercial formulations of SMR (those indicated in Section 2.1) using the optimized SW-AdASV method was conducted (using calibration curve method) without the necessity for formation of colored species [11–13], sample pretreatment, or time-consuming extraction steps [6,15–19] prior to analysis (Table 2). The validity of the optimized SW-AdASV method was further assessed by applying the standard addition method [48] for three different standard SMR solutions added to a pre-analyzed

Table 1
Results of intra-day and inter-day assays of various concentrations of bulk SMR by the optimized SW-AdASV method utilizing the bare CP and the 10% (w/w) MMT-Ca modified CP electrodes ($n = 3$) compared to those obtained by a reference voltammetric method [20].

Day	[Taken] × 10^7 (mol L^{-1})	Mean [Found] × 10^7 (mol L^{-1}) ± S.D.			%R ± RSD			RE%		
		Proposed method		Reference method	Proposed method		Reference method	Proposed method		Reference method
		CPE	Modified CPE		CPE	Modified CPE		CPE	Modified CPE	
Intra-day										
1	2	1.997 ± 0.005	1.998 ± 0.006	–	99.85 ± 0.25	99.90 ± 0.30	–	−0.15	−0.10	–
	5	4.989 ± 0.012	4.995 ± 0.005	4.979 ± 0.022	99.78 ± 0.24	99.90 ± 0.10	99.58 ± 0.44	−0.22	−0.10	−0.42
	10	10.002 ± 0.011	10.010 ± 0.007	10.030 ± 0.018	100.20 ± 0.11	100.10 ± 0.07	100.30 ± 0.18	0.02	0.10	0.30
Inter-day										
1	2	1.989 ± 0.012	1.999 ± 0.033	–	99.45 ± 0.60	99.95 ± 1.65	–	−0.55	−0.05	–
2		1.978 ± 0.027	1.988 ± 0.032	–	98.90 ± 1.37	99.40 ± 1.61	–	−0.01	−0.60	–
3		1.954 ± 0.014	1.969 ± 0.015	–	97.70 ± 0.72	98.45 ± 0.76	–	−2.30	−1.55	–
1	5	4.917 ± 0.022	4.983 ± 0.055	4.977 ± 0.042	98.34 ± 0.44	99.66 ± 1.10	99.54 ± 0.84	−1.66	−0.34	−0.46
2		4.943 ± 0.050	5.006 ± 0.030	4.898 ± 0.014	98.86 ± 1.01	100.12 ± 0.60	97.96 ± 0.29	−1.14	0.12	−2.04
3		4.950 ± 0.062	5.016 ± 0.120	4.955 ± 0.044	99.03 ± 1.24	100.32 ± 2.33	99.10 ± 0.89	−1.00	0.40	−0.90
1	10	9.890 ± 0.080	9.990 ± 0.014	9.890 ± 0.032	98.90 ± 0.81	99.90 ± 1.40	98.90 ± 0.32	−1.10	−0.10	−1.10
2		9.880 ± 0.015	9.962 ± 0.018	9.888 ± 0.050	98.80 ± 1.52	99.62 ± 0.18	98.88 ± 0.51	−1.20	−0.38	−1.12
3		9.780 ± 0.012	9.840 ± 0.022	9.857 ± 0.072	97.80 ± 1.23	98.40 ± 2.23	98.57 ± 0.73	−2.20	−1.60	−1.43

n: number of the replicated measurements; S.D.: standard deviation, %R: recovery, RSD%: relative standard deviation and RE%: relative error.

Table 2
Results of analysis of 5×10^{-7} mol L^{-1} SMR in commercial formulations by the optimized SW-AdASV method utilizing the CP and the 10% (w/w) MMT-Ca-modified CP electrodes (using calibration curve method) compared to those obtained by a reference voltammetric method; ($n = 4$) [20].

	Legalon capsules®		Hepaticum capsules®		Seralon-E capsules®		Mepacure capsules®		Silymarin plus capsules®	
	Proposed method	Reference method	Proposed method	Reference method	Proposed method	Reference method	Proposed method	Reference method	Proposed method	Reference method
Bare CPE										
[Found] $\times 10^7$ (mol L^{-1})	4.98	4.97	5.05	4.94	4.99	4.75	5.05	4.85	5.03	4.96
	4.97	5.01	5.03	4.99	4.98	4.87	5.03	4.98	5.06	5.06
	4.98	4.94	5.01	5.00	4.75	4.98	4.98	5.02	5.07	5.04
	5.00	4.97	5.02	5.02	4.88	4.96	5.06	5.00	5.01	4.98
Mean [Found] $\times 10^7$ (mol L^{-1}) ± S.D.	4.98 ± 0.013	4.97 ± 0.029	5.03 ± 0.017	4.89 ± 0.029	4.90 ± 0.112	4.89 ± 0.105	5.03 ± 0.030	4.96 ± 0.077	5.04 ± 0.028	5.01 ± 0.048
Mean %recovery ± RSD	99.65 ± 0.25	99.45 ± 0.57	100.55 ± 0.34	99.75 ± 0.64	98.00 ± 2.23	97.82 ± 2.10	100.60 ± 0.71	99.25 ± 1.54	100.85 ± 0.55	100.2 ± 0.95
F-value	5.19		3.54		1.13		4.70		2.98	
t-test	0.64		2.21		0.12		1.59		1.18	
MMT-Ca-modified CPE										
[Found] $\times 10^7$ (mol L^{-1})	5.00	4.97	5.03	4.94	4.98	4.75	4.97	4.85	4.96	4.96
	5.02	5.01	4.90	4.99	4.94	4.87	5.10	4.98	4.94	5.06
	4.99	4.94	5.02	5.00	4.85	4.98	5.07	5.02	5.07	5.04
	4.94	4.97	5.02	5.02	4.99	4.96	5.08	5.00	5.12	4.98
Mean [Found] $\times 10^7$ (mol L^{-1}) ± S.D.	4.99 ± 0.034	4.97 ± 0.029	4.99 ± 0.062	4.89 ± 0.029	4.94 ± 0.064	4.89 ± 0.105	5.06 ± 0.058	4.96 ± 0.077	5.02 ± 0.086	5.01 ± 0.048
Mean %recovery ± RSD	99.75 ± 0.68	99.45 ± 0.57	99.85 ± 1.24	99.75 ± 0.64	98.8 ± 1.28	97.82 ± 2.10	101.10 ± 1.11	99.25 ± 1.54	100.45 ± 1.73	100.2 ± 0.95
F-value	1.42		3.75		2.70		1.92		3.32	
t-test	0.68		0.14		0.80		1.95		0.25	

Theoretical *F*-value = 6.6 and *t*-test = 2.45 at 95% confidence limit for $n_1 = 4$ and $n_2 = 4$.

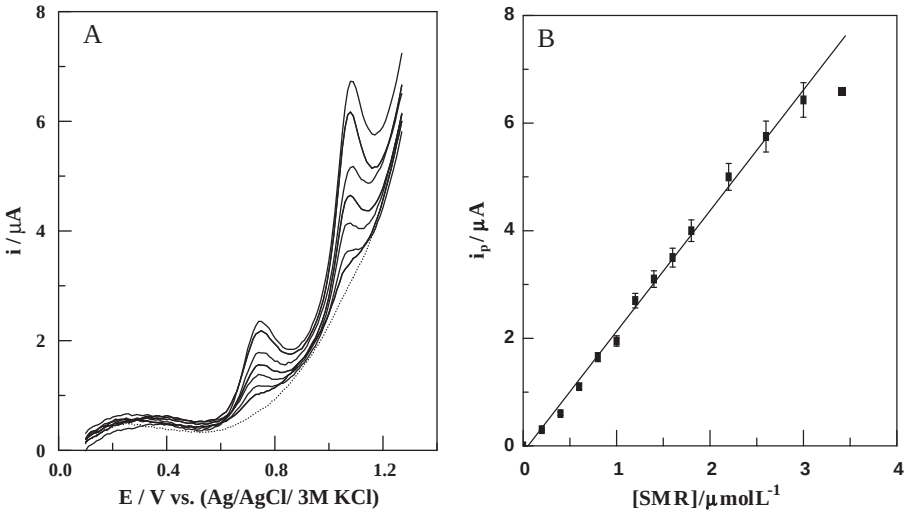


Fig. 6. (A) Representative SW-AdAS voltammograms for spiked human serum sample with various concentrations of SMR: 2×10^{-7}, 4×10^{-7}, 6×10^{-7}, 8×10^{-7}, 1×10^{-6}, 1.4×10^{-6} and 1.6×10^{-6} mol L^{-1} (from down to up) recorded in the acetate buffer of pH 4 following preconcentration onto the bare CPE by adsorptive accumulation at +0.1 V for 200 s. Dotted line is the background. (B) Plot of peak current (i_p/μA) versus [SMR/μmol L^{-1}] spiked in human serum.

one of each of the investigated commercial formulations. Insignificant differences between the concentrations taken and found were achieved using both calibration curve and standard addition methods. The obtained results were statistically compared with those obtained by a reference voltammetric method [20]. Since the calculated *F*-value did not exceed the theoretical one (Table 2), there was insignificant difference between the optimized SW-AdASV method and the reference one [20] with respect to reproducibility [49]. Also, insignificant difference was noticed between the two methods regarding accuracy and precision as revealed by *t*-test value [49] (Table 2). The results demonstrated that the optimized SW-AdASV method utilizing both the bare and the MMT-Ca-modified CP electrodes was quite reliable and sensitive enough for determination of SMR in commercial formulations even in the presence of its co-formulated drugs.

3.2.4.2. Assay of SMR in spiked human serum. The optimized SW-AdASV method utilizing both the bare and the MMT-Ca-modified CP electrodes was also successfully applied for assay of SMR spiked in twelve human serum samples of four healthy volunteers without prior extraction, taking into consideration the medium exchange method described in Section 2. SW-AdAS voltammograms of various concentrations of SMR spiked in human serum samples were recorded under the optimum operational conditions utilizing the bare (e.g., Fig. 6A) and the MMT-Ca modified CP (e.g., Fig. 7A) electrodes.

As shown in Figs. 6A and 7A, no interfering peaks from endogenous human serum constituents were appeared in blank human serum within the studied potential range. Variations of the peak currents (i_p (μA)) versus concentration of SMR ([SMR] (μmol L^{-1})) in each of the spiked human serum samples utilizing the bare CP (e.g., Fig. 6B) and the modified CP electrodes (e.g., Fig. 7B) were linear. Characteristics and the mean regression data of the obtained calibration plots of three samples of each of four volunteers are reported in Table 3. Average limits of quantitation (LOQ) of 2×10^{-7} and 8×10^{-9} mol L^{-1} and limits of detection (LOD) of 6×10^{-8} and 2.4×10^{-9} mol L^{-1} SMR in spiked human serum were achieved by the optimized SW-AdASV method utilizing the bare CP and MMT-Ca modified CP electrodes, respectively. Moreover, satisfactory mean percentage recoveries (%*R*), accuracy (%RE) and

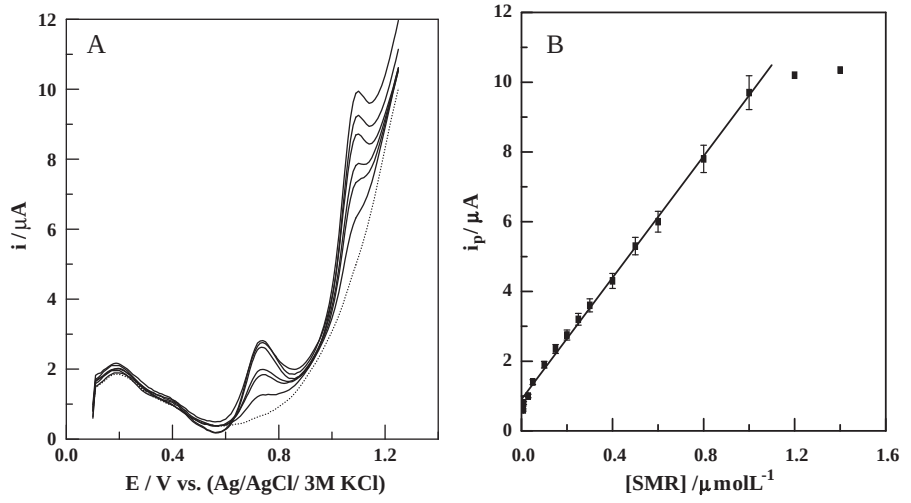


Fig. 7. (A) Representative SW-AdAS voltammograms for spiked human serum sample with various concentrations of SMR: 5×10^{-8}, 1.5×10^{-7}, 2×10^{-7}, 3×10^{-7}, 4×10^{-7} and 5×10^{-7} mol L^{-1} (from down to up) recorded in the acetate buffer of pH 4 following preconcentration onto the MMT-Ca modified CPE by adsorptive accumulation at +0.1 V for 200 s. Dotted line is the background. (B) Plot of peak current (i_p (μA)) versus [SMR (μmol L^{-1})] spiked in human serum.

Table 3
Mean regression data of calibration curves for quantitative determination of SMR by the optimized SW-AdASV method utilizing both the bare CP and the 10% (w/w) MMT-Ca-modified CP electrodes in spiking human serum samples of four volunteers (three samples of each volunteer were analyzed).

	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4
Bare CPE				
Linearity range ($mol\,L^{-1}$)	2×10^{-7} to 3×10^{-6}	2×10^{-7} to 3.5×10^{-6}	2.5×10^{-7} to 3.0×10^{-6}	1.5×10^{-7} to 3×10^{-6}
Slope ($\mu A/\mu mol\,L^{-1}$) ± S.D.	2.28 ± 0.002	2.22 ± 0.003	2.30 ± 0.001	2.25 ± 0.002
Intercept (μA) ± S.D.	−0.20 ± 0.0456	−0.19 ± 0.0456	−0.22 ± 0.0575	−0.25 ± 0.0337
Correlation coefficient (r)	0.998 ± 0.001	0.997± 0.002	0.998 ± 0.001	0.996 ± 0.002
LOD ($mol\,L^{-1}$)	6.0×10^{-8}	6.0×10^{-8}	7.5×10^{-8}	4.5×10^{-8}
LOQ ($mol\,L^{-1}$)	2.0×10^{-7}	2.0×10^{-7}	2.5×10^{-7}	1.5×10^{-7}
%R[a]	98.50	98.70	100.20	98.50
%RE[a]	−1.50	−1.30	0.20	−1.50
RSD%[a]	1.05	1.22	1.02	1.55
%R[b]	98.71	98.58	100.11	97.55
%RE[b]	−1.29	−1.42	0.11	−2.45
RSD%[b]	1.53	1.24	0.80	0.90
Modified CPE				
Linearity range ($mol\,L^{-1}$)	8×10^{-9} to 1.0×10^{-6}	8.5×10^{-9} to 1.0×10^{-6}	8×10^{-9} to 1.5×10^{-6}	7.5×10^{-9} to 1×10^{-6}
Slope ($\mu A/\mu mol\,L^{-1}$) ± S.D.	9.87 ± 0.002	10.10 ± 0.001	9.25 ± 0.003	8.00 ± 0.002
Intercept (μA) ± S.D.	$0.97\pm7.89\times10^{-3}$	$1.02\pm8.58\times10^{-3}$	$0.99\pm7.89\times10^{-3}$	$1.04\pm6.00\times10^{-3}$
Correlation coefficient (r)	0.998 ± 0.001	0.996 ± 0.001	0.996 ± 0.002	0.994 ± 0.003
LOD ($mol\,L^{-1}$)	2.4×10^{-9}	2.55×10^{-9}	2.4×10^{-9}	2.25×10^{-9}
LOQ ($mol\,L^{-1}$)	8.0×10^{-9}	8.5×10^{-9}	8.0×10^{-9}	7.50×10^{-9}
%R[a]	100.20	99.40	99.70	99.20
%RE[a]	0.20	−0.60	−0.30	−0.80
RSD%[a]	1.35	0.99	1.84	0.87
%R[b]	99.32	99.24	100.82	102.53
%RE[b]	−0.68	−0.76	0.82	2.53
RSD%[b]	1.42	1.32	2.02	1.24

[a] For assay of 5×10^{-7} $mol\,L^{-1}$ SMR ($n=5$).
[b] For assay of 1×10^{-6} $mol\,L^{-1}$ SMR ($n=5$).

precision (RSD%) of SMR were obtained for various concentrations of SMR (2×10^{-7}, $5\times10^{-7}$and 1×10^{-6} $mol\,L^{-1}$ SMR) in spiked human serum samples (e.g., Table 3) indicating insignificant differences between the spiked and the detected amounts of SMR in human serum samples and consequently no interference from endogenous human serum constituents. The results indicated the reliability of the optimized SW-AdASV method for the trace assay of SMR in spiked human serum. However the MMT-Ca modified CPE is much sensitive for assay SMR.

Moreover, effects of various foreign species that are likely to be in biological samples on analysis of SMR spiked in human serum were evaluated. This was performed by analysis of standard solutions of 8×10^{-7} $mol\,L^{-1}$ SMR spiked with various excess amounts of the foreign species (common metal ions, excipients, amino acids, co-formulated and co-administrated drugs) under the optimum operational conditions (Table 4). The tolerance limit for foreign species was taken as the largest amount yielding a signal error of 5% for determination of SMR. It was found that the interferences from common metal ions to the determination of SMR under the optimized experimental conditions were negligible even in the presence of about 10^2 to 10^3-fold excess of these common metal ions. This is because in the positive potential range, metal ions are electro-inactive and will not co-deposit with SMR during the deposition step [44] (e.g., Fig. 5A and B). Also trace determination of SMR (e.g., 8×10^{-7} $mol\,L^{-1}$) was successfully performed by means of the optimized SW-AdASV method utilizing the bare and modified CP electrodes with insignificant interferences even in the presence of about 10^2-fold excess of common excipients (e.g., starch, gelatin and lactose) [46], amino acids, co-formulated drugs (vitamins A, C and E [45], acetylcysteine, and DDB), or co-administrated drugs (e.g., aspirin, ketoprofen, ketorolac, ibuprofen and gabapentin). All of these substances were electro-inactive under the optimized procedural conditions within the applied potential range. The results reported in Table 4 clearly demonstrate again the reliability of the optimized SW-AdASV method for assay of SMR in biological fluids.

Table 4
Interferences from foreign species on analysis of 8×10^{-7} $mol\,L^{-1}$ SMR by the optimized SW-AdASV method utilizing both the bare CP and the 10% (w/w) MMT-Ca-modified CP electrodes.

Foreign species	[a]Tolerance level ($mol\,L^{-1}$)	
	Bare CPE	Modified CPE
Cu^{2+} and Fe^{3+}	1.5×10^{-5}	1.0×10^{-5}
Ca^{2+}, Mg^{2+}, Zn^{2+}, $Al^{3+}$and Se^{4+}	1.0×10^{-4}	1.0×10^{-4}
Na^{+} and K^{+}	3.0×10^{-3}	2.0×10^{-3}
Cl^{-}, SO_4^{-2}, PO_4^{-3} and Ac^{-}	5.0×10^{-4}	5.0×10^{-4}
Valine, phenylalanine, serine, histidine and threonine	2.5×10^{-4}	2.0×10^{-4}
Oxalic acid, uric acid, glucose, sucrose, starch, gelatin and lactose	3.0×10^{-4}	2.0×10^{-4}
Aspirin, ketoprofen, Ketorolac, ibuprofen and gabapentin (Co-administrated drugs)	8.0×10^{-4}	5.0×10^{-4}
Vitamins (A, C and E) acetylcysteine, and DDB (co-formulated drugs)	1.0×10^{-4}	1.0×10^{-4}

[a] For 5% signal error.

3.2.4.3. Pharmacokinetic analysis. A pharmacokinetic study was performed on the plasma samples of four healthy volunteers by the optimized SW-AdASV method utilizing the MMT-Ca modified CPE following the administration of an oral single dose of 120 mg SMR (one Seralon-E capsule® labeling to contain 120 mg SMR + 40 mg vitamin E). The metabolites of SMR (which are consisting mainly of sulfates and glucuronides at the phenolic OH groups at carbon atoms 7 and 20 [6–8]) are not oxidizable. Hence, it does not give any oxidation peaks during the assay of plasma samples indicating again the selectivity of the optimized stripping voltammetry method towards SMR. The obtained mean plasma concentration–time profiles for two groups of volunteers are shown in Fig. 8.

The pharmacokinetic parameters: the area under the plasma concentration–time profile from time zero to the last measurable

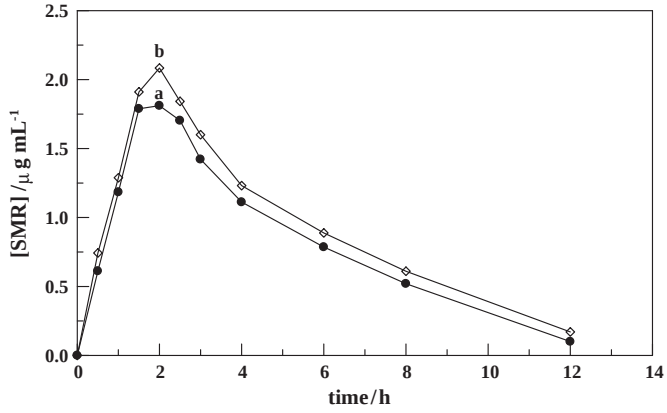


Fig. 8. Mean plasma concentration–time profiles obtained for two groups of healthy subjects {each subject administrated an oral single dose of 120 mg SMR (one Seralon-E capsule®)} by means of the optimized SW-AdASV method utilizing the 10% (w/w) MMT-Ca-modified CPE.

Table 5
Main pharmacokinetic parameters estimated for two groups (each includes two subjects) of healthy male volunteers following an oral administration of 120 mg SMR (a single Seralon-E capsule®).

Parameter (unit)	Estimated values[a]	
	Group (1)[b]	Group (2)[b]
C_{max} (μg ml^{-1})	1.811	2.084
t_{max} (h)	2.0	2.0
AUC_{0-12} (μg h ml^{-1})	9.997	11.385
$AUC_{0-\infty}$ (μg h ml^{-1})	11.002	12.985
K_{el} (h^{-1})	0.21	0.18
$t_{1/2}$ (h)	3.30	3.85

[a] Average of two measurements.
[b] Mean value of two subjects.

sample time (AUC_{0-12}) and to infinity ($AUC_{0-\infty}$); the maximum plasma concentration (C_{max}); time of the maximum plasma concentration (t_{max}); terminal rate constant (K_{el}) and terminal half-life time ($t_{1/2}$) were estimated. C_{max} and t_{max} were estimated directly from the concentration–time profile. The area under the plasma concentration–time profile from time zero (pre-dose) to time of last quantifiable concentration (AUC_{0-12}) was estimated using the linear trapezoidal method [50]. The terminal rate constant (K_{el}) was estimated by applying a simple linear regression of log concentration on time to at least the last three time points. The terminal half-life time ($t_{1/2}$) was estimated as [$\ln 2/K_{el}$]. The area under the plasma concentration–time profile from time zero to infinity ($AUC_{0-\infty}$) was estimated as [$AUC_{0-12} + (C_{12}/K_{el})$] (Table 5). The obtained mean plasma concentration–time profiles (Fig. 8) and the estimated mean pharmacokinetic parameters (Table 5) were favorably compared with those reported in literature [9,10] confirming the reliability of the optimized SW-AdASV method for assay of SMR in human blood utilizing the MMT-Ca modified CPE.

4. Conclusions

The optimized square-wave adsorptive anodic stripping voltammetric method utilizing both the bare carbon paste and the montmorillonite-Ca modified carbon paste electrodes is reliable for trace determination of SMR in commercial formulations and in spiked human serum; however the modified electrode is much sensitive. The optimized method possesses many advantages for assay of SMR such as free from prior extraction or formation of colored species, simple and fast compared to the reported spectrophotometric and chromatographic methods [11–13,15–19]. The optimized method utilizing the montmorillonite-Ca modified carbon paste electrode has achieved lower quantitation limit (LOQ = 7×10^{-9} mol L^{-1} SMR) compared to that of the reported voltammetric method (LOQ = 1.87×10^{-7} mol L^{-1}) using a glassy carbon electrode [20]. So, it was successfully applied for assay of SMR in real human plasma. The optimized square-wave adsorptive anodic stripping voltammetric method coupled with the bare CPE or the modified one could be recommended for use in quality control and clinical laboratories.

Acknowledgements

The authors express their gratitude to Ramadan Specialized Hospital's staff (Tanta City, Egypt), for the kind care and housing of the volunteers and for providing all the facilities for collecting and treatments of the plasma samples required for the present pharmacokinetic analysis. Deep thanks are also to the Alexander von Humboldt Foundation (Bonn, Germany) for donating the Electrochemical Analyzer (263A-PAR) used in the present study to one of the authors (M.M. Ghoneim).

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