

Clay-Protein Nanocomposite Based Electrochemical Sensor for the Determination of Ascorbic Acid

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Abstract: This paper describes sensitive ascorbic acid (AA) determination on montmorillonite clay and silk protein sericin nanocomposite (MMT-Ser NC) modified pencil graphite electrode (PGE) for the first time. Nanocomposite structures were synthesized by desolvation technique and cross-linked with glutaraldehyde (GA). The synthesized MMT-Ser NC was evaluated by various methods. As a result of the characterization studies, it was determined that the sericin was successfully converged with the montmorillonite structure, and that the MMT-Ser NCs were homogeneous and uniform, with the size of 150 nm and zeta potentials of approximately -27.6 mV. MMT-Ser NC modified electrodes were evaluated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM). Under the optimized conditions, working linear range for the modified sensor was found as 10-1000 µM with square wave voltammetry and the limit of detection (LOD) was found as 8 µM. The relative standard deviation for reproducibility was calculated as % 4.82 (n=6). Real sample analysis was performed with drug samples and the recovery values ranged from 94.64% to 111.2%.

Askorbik Asit Tayini için Kil-Protein Nanokompozit Bazlı Elektrokimyasal Sensör

Anahtar Kelimeler

Askorbik asit,
Elektrokimyasal sensör,
Montmorillonit-serisin nanokompozit,
Kalem grafit elektrot.

Özet: Bu makalede, montmorillonit-serisin nanokompozit (MMT-Ser NC) ile modifiye edilmiş kalem grafit elektrot (PGE) ile askorbik asit (AA) tayini ilk defa ifade edilmiştir. Nanokompozit yapılar desolvasyon tekniği ile sentezlenmiş ve glutaraldehit (GA) ile çapraz bağlanmıştır. Sentezlenen MMT-Ser NC çeşitli metotlar ile değerlendirilmiştir. Karakterizasyon çalışmaları sonucunda serisinin montmorillonit yapısı ile başarılı bir şekilde bir araya geldiği, MMT-Ser NC'lerin homojen ve eş dağılımlı olup boyutlarının 150 nm ve zeta potansiyellerinin de yaklaşık olarak -27,6 mV olduğu tespit edilmiştir. MMT-Ser NC ile modifiye edilmiş elektrot yüzeyleri dönüşümlü voltametri (CV), elektrokimyasal empedans spektroskopisi (EIS) ve taramalı elektron mikroskopisi (SEM) ile karakterize edilmiştir. Modifiye sensörün optimize koşullar altında kare dalga voltametri (SWV) ile doğrusal çalışma aralığı 10-1000 µM ve gözlenebilirlik sınırı (LOD) 8 µM olarak bulunmuştur. Tekrar üretilebilirlik için bağıl standart sapma ise % 4,82 (n=6) olarak hesaplanmıştır. İlaç örneği ile gerçek örnek analizi gerçekleştirilmiş ve geri kazanım değerleri % 94,64 ile % 111,2 aralığında elde edilmiştir.

1. Introduction

Vitamin C, is an essential water-soluble nutritious vitamin which plays indispensable roles in various chemical and biological systems as a powerful antioxidant and free radical scavenger [1]. L-Ascorbic

acid, (AA) represents the biochemically active form of vitamin C, with readily two dissociable protons and physiologically appears as an ascorbate anion [2]. AA performs specific metabolic activities by maintaining natural barrier against oxidative stress due to its reductive characteristics by reacting with free

radicals and reactive oxygen species thus involvement in the defense system, as well as biosynthesis of various enzymes, aminoacids etc. [3,4]. These nutritional and physiologically essential compounds are widely distributed in plant cells and cannot be synthesized in human body, therefore, the best dietary sources of this vitamin are fruits and vegetables from nature and recommended by physicians as tablet forms to support health. [1,5,6]. Deficiency of AA can associate with many diseases in human body such as cardiovascular disease, type II diabetes mellitus, atherosclerosis, stroke, cancer, scurvy [1]. Given the considerable health prominence as an antioxidant, AA analysis is particularly significant in biological matrix especially in serum, blood or urine. AA is also employed for medical purposes in pharmaceutical formulations due to its beneficial antioxidant effects [7]. Moreover, AA is widely used in commercial foodstuff as stabilizers and for prolonging the shelf-life of the products, so, they are well-known indicators for food quality processes [8]. Due to all these critical prominence in both biochemistry and industry, reliable monitoring of AA content in biological media, drugs and foods still presents as attractive research area.

Various techniques have been documented for accurate and specific detection of ascorbic acid which include titrimetry with an oxidizing agent [9], capillary electrophoresis [10], spectrophotometry [11], fluorimetry [12], chromatography [13,14], electrochemistry [2,15] which were commonly focused on the analysis of AA in pharmaceuticals and food products. Amongst, electrochemical techniques have extensively been preferred due to its simplicity with respect to time consuming sample pre-treatments involving techniques, and their low-cost instrumentation, portability, rapid response, high sensitivity and suitability for *in-situ* detection in real samples [2]. Nevertheless, accurate sensing of AA using bare electrodes has been hindered by the high over-potentials required or by the interference reasoned by coexisting biomolecules and the electrode fouling problems by side products. Therefore, it is necessary to minimize these challenges by modification of working electrode surfaces with active materials or implying various composites [16]. Up to now various modifications based on polymers, nanomaterials and their composites have been employed for AA detection [17–27], but there is still a need for new sensitive attempts.

Naturally occurring clay minerals have attracted great attention for the electrode modifications as they offer advantages such as wide availability and low-cost as well as formation of controllable surface structures with high chemical stability, high ion exchange capacity and large surface area. Mousty [28] summarized clearly the electrodes modified with clay for biosensing applications in a review and electrochemical sensors based on the electrodes with

clay minerals modification have extensively been developed at present. Montmorillonite (MMT), is a hydrophilic smectite 2:1 phyllosilicates clay, with unique structural and physicochemical characteristics such as high cation exchange capacity, good sorption and catalytic activity, as well as, intercalation, swelling, porosity, non-toxic properties [29,30]. They can function as matrices for electro-active ions for surface modification applications by easily manipulating the properties by forming stable and high surface area structures on the electrodes.

Sericin is a natural watersoluble protein with molecular mass between 10kDa to 300 kDa, which is derived from silkworm (*Bombyxmori*) [31]. Sericin, as a silk protein, has huge area in biomedical applications due to its superb oxygen permeability, biocompatibility, cell-protective and antioxidant effect, moisture-modulating, anticancer and anticoagulant properties. It is also well known that amine, hydroxyl, carbonyl, aromatic or sulfhydryl of the protein provides biofunctionality to be readily bonded to the surfaces [32,33].

Considering the excellent characteristics presented by montmorillonite clay and sericin protein, in the proposed work we intended to synthesize and explore the use of their combination in a nanocomposite structure on a pencil graphite electrode (PGE) for the application of sensitive AA detection. The nanostructured MMT-sericin nanocomposite modification on the sensor has led to high physical and mechanical strength and the proposed sensor was shown to be potentially useful for sensitive AA detection between 10-1000 μM with square wave voltammetry (SWV) with a limit of detection (LOD) of 8 μM . The sensor has advantageous and was applicable for pharmaceutical vitamin C specimen.

2. Material and Method

2.1. Chemicals and Reagents

Montmorillonite (MMT-K10), gluteraldehyde, ascorbic acid, potassium chloride (KCl), ethanol, L-ascorbic acid (>99.5%), dopamine ($\geq 98.5\%$), uric acid (>99.5%) and phosphate buffer saline were collected from Sigma-Aldrich. Stock solution of ascorbic acid was prepared freshly in phosphate buffer solution (PBS) of pH 7.0 before the measurements. Bevitin C (Abfar Drug) was purchased from a local pharmacy and dissolved in PBS of pH 7.0. The aforementioned materials were of analytical class and employed as collected with no purification process.

2.2. Apparatus

Electrochemical analyses were recorded using CH Instruments CHI660C model potentiostat/galvanostat and Gamry Interface 1000 model potentiostat with three electrode system. A

pencil graphite electrode (PGE) in which a pencil was employed as a bearer for graphite leads (Tombo, HB, 0.5 mm diameter, Japan) and the electrical contact with the lead was received by soldering a metallic wire to the metallic part was employed as the working electrode. A platinum (Pt) wire was used as the auxiliary electrode and a silver/silver chloride (Ag/AgCl, 3 M KCl) as the reference electrode.

The obtained MMT-Ser NCs were evaluated by attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR), dynamic light scattering (DLS), zeta measurement and scanning electron microscopy (SEM). The structural and chemical characterization and differentiation of MMT, sericin and MMT-Ser NCs were performed by ATR-FTIR analysis with Nicolet TM ISTM 50 spectrometer (Thermo Fisher Scientific, USA). The SEM images were used to evaluate in terms of shape and size distribution. Therefore, the morphology of nanocomposites and modified electrodes was examined by FIB-SEM (Tescan GAIA 3, Czech Republic). The specimens were manufactured by dropping 5 μL of composite nanostructure suspension on plates and desiccated under nitrogen gas. It is then covered with gold using the Precision Surface Abrasion and Coating System (Leica EM ACE600, Germany). DLS analyses of the nanocomposites were recorded by a Zetasizer Nano ZS instrument (Malvern Instruments, UK). For this, the electrophoretic mobility of the nanocomposite solution was analyzed, and the obtained data was modified to ζ -potential and size values with the Helmholtz-Smoluchowski equation.

2.3. Electroanalytical measurements

Electrochemical measurements were gathered in a three electrode cell system at room temperature and the solutions were treated with nitrogen for 5 min before the analyses. Cyclic voltammograms of electrodes in 5.0 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ /0.1 M KCl solution were run between - 0.4 V and 0.9 V using scan rate as 100 mVs^{-1} and electrochemical impedance was applied at 0.2 V frequency range between 100,000-0.1 Hz vs. Ag/AgCl. Square wave voltammetric (SWV) measurements were performed in the potential range between -0.30 V and 0.6 V in phosphate buffer at pH 7.0 following instrumental parameters as: frequency: 50 Hz; Puls width: 50 mV; Amplitude: 4 mV.

2.4. Synthesis of MMT-Ser Nanocomposite

The MMT suspension was prepared at a concentration of 3%, followed by an *ex-situ* nanocomposite and subsequent desolvation method respectively for obtaining montmorillonite-sericin nanocomposite (MMT-Ser NC). For this purpose, 1 mL of MMT suspension was mixed with 30 mg mL^{-1} sericin solution. Afterwards, 4 mL of ethanol was added to the suspension to convert it to composite structures, until the solution appeared in white

cloudy color. Next, 8 % glutaraldehyde (GA) solution was added to crosslink the composite nanostructures under constant stirring (500 rpm) for 4 hours at room temperature and then washed by centrifugation and incubated at 4 ° C for further studies.

2.5. Preparation of MMT-Ser NCs modified PGEs

PGEs were dipped in the MMT-Ser NCs solution for several time durations between 10-120 minutes and the nanocomposites were accumulated on PGE by passive adsorption method. The modified graphite electrodes were incubated for drying at room temperature (30 min.) and gently washed with double distilled water to get rid of the loosely bonded NCs. The obtained modified electrodes were named as MMT-Ser NC/PGE.

2.6. Pharmaceutical vitamin C sample preparation

The proposed nanocomposite based biosensor was applied in commercial pharmaceutical vitamin C ampules containing 100 mg mL^{-1} of AA. For this purpose, known amount of vitamin C were added into the 0.1 M PBS (pH 7.0) and % recoveries were estimated for two different additions of the sample.

3. Results

3.1. Characterization of MTT-Ser NCs

The protein-clay nanocomposites may be constructed by straight adding of two solutions together comprising the protein and the clay suspension. The emerged nanostructure is named as *ex-situ* composite nanostructure or nanocomposite because the majority of the protein is located in external ranges of the clay [34]. Herein, Sericin doped MMT nanocomposites (MMT-Ser NCs) were prepared by the addition of glutaraldehyde (GA) as crosslinking agent for the first time on the literature. The GA acts on the amine group of proteins at neutral pH, is a linear dialdehyde. The aldehyde fragments of GA are bonded with the amine ends of proteins through the formation of Schiff base ($-\text{C} = \text{N}-$) which made the nanostructure stable under cross-linking conditions [35,36].

As a result of the characterization studies, the particle size of the composite nanostructures were approximately 150 ± 15.20 nm with a good polydispersity index (PDI) about 0.083 ± 0.014 (Figure 1.B). The zeta potential of the NCs was obtained as -27.60 ± -3.02 mV (Figure 1.C). The DLS results were supported by SEM analyses by displaying a very homogeneous and uniform structure (Figure 1.D).

ATR-FTIR analyses were demonstrated in the Figure 1.A. According to the results, Sericin NP spectra exhibited characteristic absorption at 1680 cm^{-1} and

1500 cm^{-1} , for the C-O in the amide-I and for N-H bending of the amide-II of the proteins, respectively. In the spectrum of MMT which was also seen in MMT-Ser NCs with a slight shift at 1030 cm^{-1} of the Si-O-Si due to GA crosslinking supported the presence of MMT. The characteristic peak of the sericin was also seen in the same spectrum which proved the nanocomposite construction [37,38].

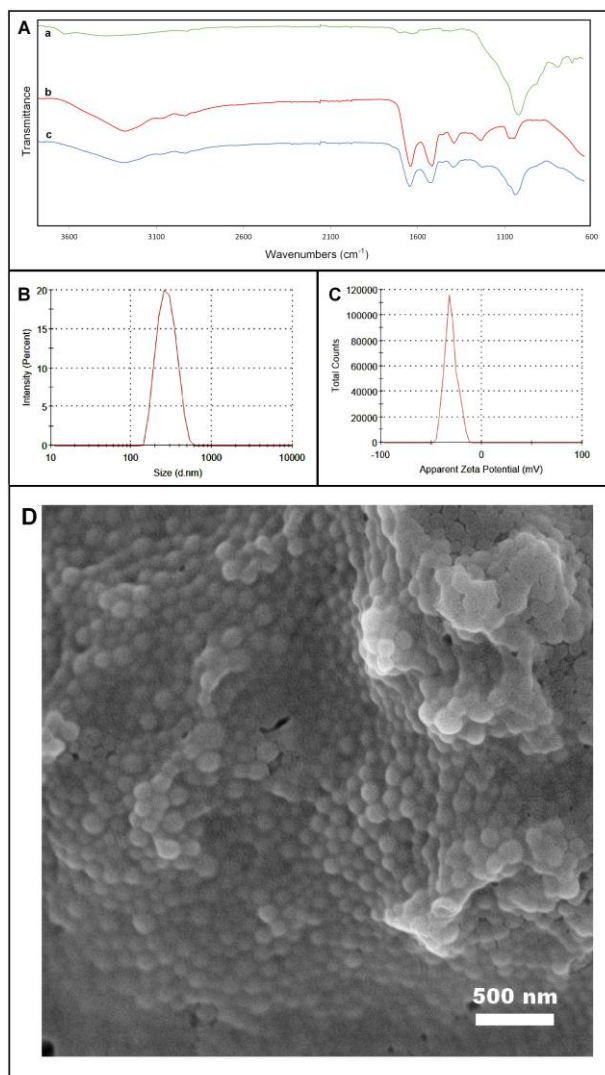


Figure 1. A) ATR-FTIR spectrum of a) MMT, b) Sericin NP, c) MMT-Ser NCs, B) The size distribution of MMT-Ser NCs, C) The zeta potential of MMT-Ser NCs, D) SEM image of MMT-Ser NCs, scale bar: 500 nm

3.2. Surface Morphology analysis of MMT-Ser NC/PGE

After it was shown that the synthesis of uniform MMT-Ser NCs were obtained successfully, the nanocomposites were further transferred on PGE surfaces for use as electrochemical sensor. The change in the topography and the microscopic structure of PGEs before and after the modification with MMT-Ser NCs were examined by using SEM for low and high magnifications, respectively (Figure 2). Bare PGE displayed fragmental graphite flakes piled up untedly (Figure 2.a and 2.b). After MMT-Ser NCs

modification on PGE via physical passive adsorption, a coverage of homogeneous layer and agglomeration of globular nanocomposite were observed. Also, the nanocomposites preserved its original spherical shape with a size of approximately 170 nm (Figure 2.c and 2.d).

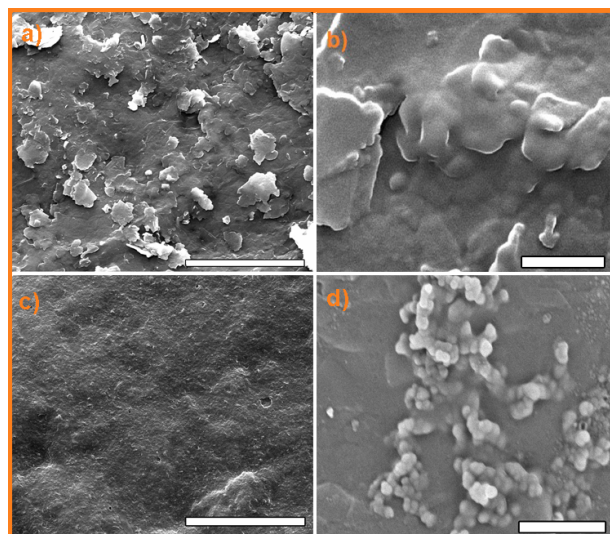


Figure 2. SEM images before and after modification; a) Bare PGE (10 μM), b) BarePGE (1 μM), c) MMT-Ser NC modified PGE (10 μM) ve d) MMT-Ser NC modified PGE (1 μM).

3.3. Electrochemical Studies of MMT-Ser NC/PGE

The effect of MMT-Ser NC modification on the electrochemical feedback of PGE and the electron transfer process was examined by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements in 0.1 M KCl containing 5.0 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ redox pair (Figure 3). Figure 3A showed that MMT-Ser NC modification on the sensor (curve b) has led to a raise in the cathodic and anodic peak currents and a decline in peak to peak separation of redox pair (ΔE_p) indicating easier electron separation than bare PGE (curve a) because of better electron pathway between the electrode and the solution. The anodic and cathodic peak currents, peak potentials and peak potential separation values obtained for $\text{Fe}(\text{CN})_6^{4-/3-}$ system were summarized in Table 1. It can also be considered that the porous nanocomposite structure exhibited higher electroactive surface area than PGE according to the Randles-Sevcik equation [39].

The interfacial feature of the bare and modified PGE by EIS was shown in Figure 3.B a and b, respectively. Nyquist plots exhibited a semicircle described by the charge transfer resistance (R_{ct}) equal to the semicircle diameter of the plot at high frequencies and the linear portion corresponding to the diffusion process at low frequencies. It was observed that the R_{ct} value of the electrode/solution interface (R_{ct} : 450 Ω) decreased after modification of PGE (R_{ct} : 280 Ω) by nanocomposite as an indication of the increase of the electron transfer rate at the electrode/solution

interface. This result was also consistent with CV results.

Table 1. Behavior of bare and modified PGE in redox probe solution.

Electrode	PGE	MMT-Ser NC/PGE
I _{pa} (μA)	191.5±33.14	233.6±9.06
E _{pa} (V)	0.120±0.219	0.327±0.013
I _{pc} (μA)	178.4±33.05	245.3±1.95
E _{pc} (V)	0.329±0.22	0.150±0.002
ΔE _p (V)	0.209±0.115	0.177±0.013

N=3, %95 confidence level.

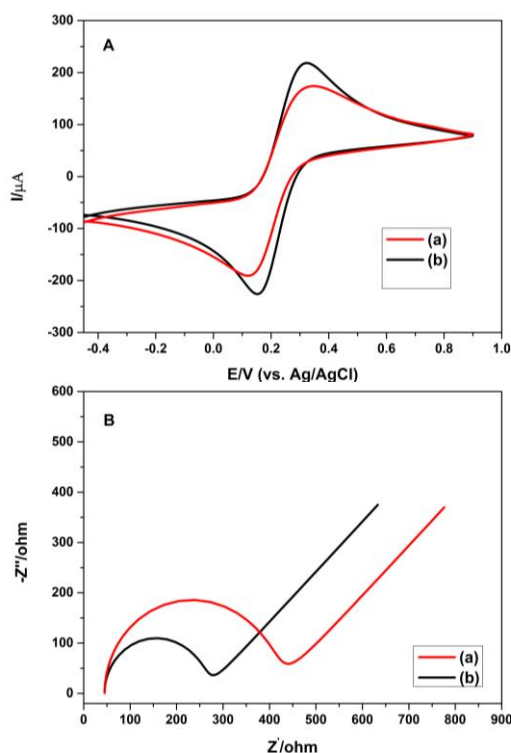


Figure 3. A) Cyclic voltammograms of a) PGE, b) MMT-Ser NC/PGE in 5.0 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ in 0.1 M KCl. (E_i : -0.4 V, E_f : 0.9 V vs. Ag/AgCl). Scan rate: 100 mVs^{-1} , B) Nyquist plots (E : 0.2 V, Frequency range: 100,000-0.1 Hz).

3.4. Investigation of electrochemical behavior of AA

To assess the effect of electron transfer kinetics of AA on the electrode surface properties of the sensor, cyclic voltammetry was performed to examine the electrochemical potential of AA on bare (Figure 4.A) and the modified PGE (Figure 4.B) in the N_2 saturated 0.1 M pH 6.0 PBS in the absence (curves a) and presence of 0.5 mM L-AA (curves b). CVs for each electrode showed a single oxidation peak at about +0.3 V vs Ag/AgCl and no reduction peak was observed in the reverse scan as an indicator of the irreversible character of the electrode reaction, and this result is consistent with the literature [20]. This irreversible oxidation of AA was proved to proceed by the release of two electrons and two protons, leading to formation of dehydroascorbic acid [26]. According to the results, coating of MMT-Ser NCs on PGE improved the adsorption of AA to the electrode surface and increased the peak current values significantly (about

3.5 times). This was an indication that the obtained sensor surface could be successfully used for the electrochemical analysis of AA. Compared to the voltammogram obtained with bare electrode, the modification showed accelerated electrooxidation process of AA on the electrode. Thus, MMT-Ser NC modified PGE was used as the working electrode in all subsequent experiments. In addition, 4 cycles of alternating voltammograms with the modified surface demonstrated that the oxidation peak of AA decreased with the increase in the number of cycles indicated the adsorption of AA on the electrode surface (Figure 4.C). Furthermore, scan rate experiment was conducted in order to prove that the oxidation mechanism of AA is adsorption controlled.

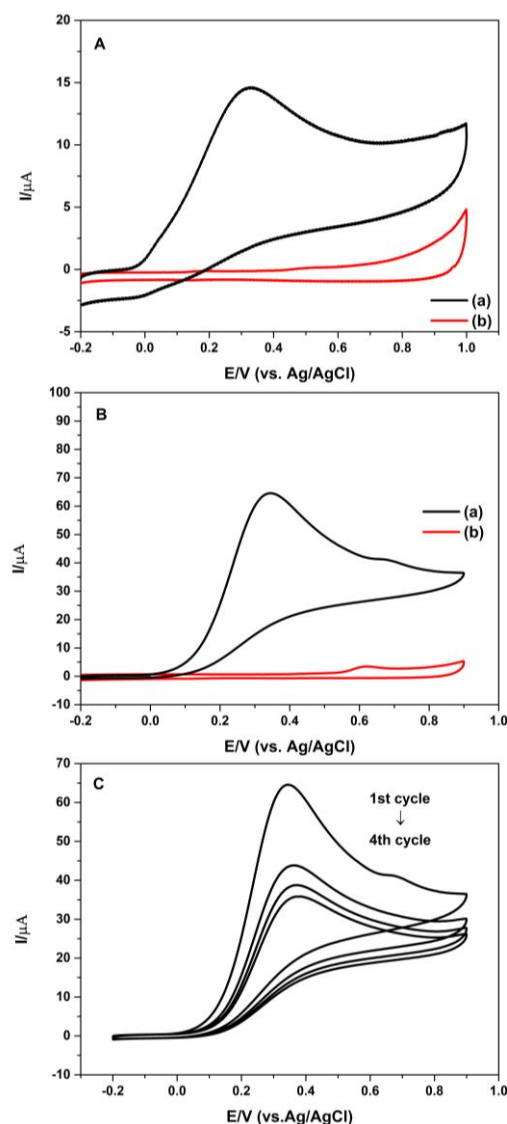


Figure 4. Cyclic voltammograms of A) Bare PGE, B) MMT-Ser NC modified PGE; a) in the absence, b) in the presence of 0.5 mM of AA in PBS pH 6.0. (scan rate: 100 mVs^{-1}), C) MMT-Ser NC modified PGE, 4 alternating cycles in 0.5 mM of AA in PBS pH 6.0.

3.5. Scan rate study

Scan rate experiment provides useful information about the oxidation/reduction mechanism of

electroactive species in electrochemical sensor development studies so that oxidation mechanism of L-AA on MMT-Ser NC/PGE in pH 6.0 PBS was studied in the range of 10-100 mVs⁻¹. The effect of the scan rate (SR) on the peak current (I_p) of L-AA was plotted as illustrated in Figure 5. Anodic peak currents increased linearly as the scan rate increased showing that L-AA oxidation was adsorption-controlled on the sensor. This typical behavior of L-AA was found to be consistent with the literature [40].

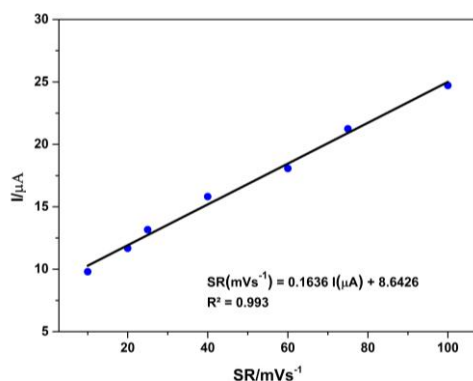


Figure 5. Scan rate study for MMT-Ser NC/PGE. The linear graph corresponding peak currents vs. scan rates between 10-100 mVs⁻¹ in pH 6.0 PBS containing 0.5 mM AA.

3.6. Optimization Studies

The analytical performance of the MMT-Ser NC modified sensor was analyzed by using a sensitive technique, square wave voltammetry (SWV) and optimization of the parameters were summarized in Table 2.

Table 2. The results for optimization of SWV parameters.

Parameter	Studied Range	Optimum Value
Frequency (Hz)	10-70	50
Amplitude (mV)	10-70	50
Puls width (mV)	3-7	4

As the nanocomposite modification of the surface was carried out by physical adsorption, the most important parameter in this method was the adsorption time in the NC solution. The PGEs were

immersed in MMT-Ser NC solution for different durations (15, 30, 45, 60, 90 minutes) and the maximum peak currents were observed for 45 minutes (Figure 6.A). When the adsorption time was increased, the peak current started to decrease due to saturation of the surface. For this reason, the physical adsorption time of the nanocomposite structure was determined as 45 min in all the experimental studies.

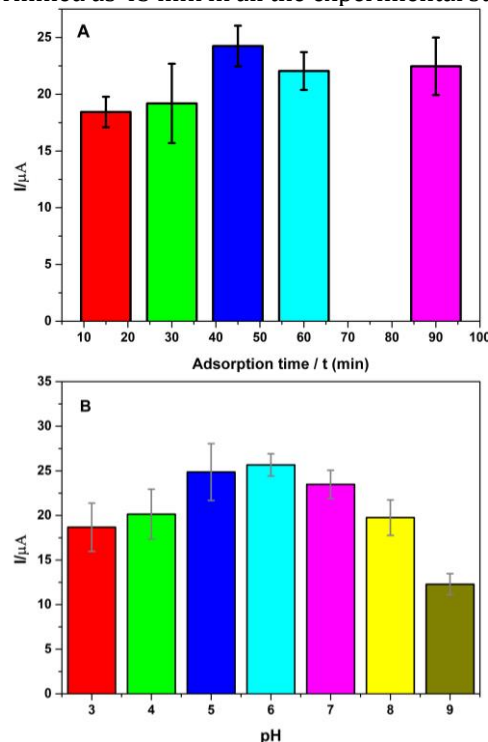


Figure 6. A) The effect of physical adsorption time of MMT-Ser NC on PGE surface on AA response. **B)** The effect of supporting electrolyte pH on the oxidation of AA.

The effect of the supporting electrolyte pH on the oxidation peak of AA was examined and the maximum peak current was obtained at pH 6.0 solution, also this pH was the most convenient medium for the adsorption of the AA on MMT-Ser NC/PGE (Figure 6.B). According to the literature, it was also reported that the electrooxidation of AA is widely accepted below pH 8 [2]. Further experiments were employed under optimized conditions by SWV.

Table 3. Analytical performance of different electrochemical AA sensors based on nanomaterials/composites.

Modified electrode	Linear Range / μM	LOD / μM	Reference
AuNPs/P(PDA)-GO/GCE	6.0-2400	1.764	[17]
RGO-Ag/PANI/GCE	50-1000	0.5	[18]
MoS ₂ /PEDOT/GCE	20-140	5.83	[41]
AuNPs@MoS ₂ /GCE	1000-70000	100	[20]
Au/RGO/GCE	240-1500	0.002	[21]
RGO-ZnO/GCE	50-2350	3.71	[22]
GF@NiCo ₂ O ₄	200-750	50	[23]
3D-MoS ₂ /rGO/Au@GCE	2-5400	0.93	[24]
PANI/HNTs hierarchical nanotubes	5 - 5500	0.21	[25]
Zn-NiAl LDH/rGO superlattice	0.5-11	13.5	[42]
MWCNTs/Cu ₂ O/Ag ₂ O	0.05-1628.5	0.011	[43]
AgNPs-PSi	20-60	0.83	[44]
ZnNi NPs@f-MWCNT	300-1100	0.51	[19]
MMT-Ser NC/PGE	10-1000	8	This work

AuNP: Gold nanoparticle, P(PDA): Poly(2,6-pyridinedicarboxylic acid), GO: Graphene oxide, GCE: Glassy carbon electrode, RGO: Reduced graphene oxide, PANI: polyaniline, MoS₂: Molybdenum disulphide, PEDOT: poly(3,4-ethylenedioxythiophene), ZnO: Zinc oxide, GF: graphene fiber, NiCo₂O₄: Nickel cobaltite, Au: Gold, HNT: Halloysite nanotubler, Ag: silver, LDH: Double layer hydroxide, AgNR: Silver nanorod, P(Arg): poli(l-arginine), MWCNTs: Multiwalled carbon nanotubes, Cu₂O: Copper dioxide, Ag₂O: Silverdioxide, AgNPs-Psi: Silver nanoparticle decorated stain-etched mesoporous silicon. ZnNi NPs@f-MWCNT: Zinc Nickel nanoparticles-multiwalled carbon nanotubes.

3.7. The analytical performance of MMT-Ser NC/PGE for AA detection

The sensitivity for the determination of AA with MMT-Ser NC/PGE nano-biosensor was analysed by SWV to examine peak current dependence on the concentration of AA. As can be seen in Figure 7.A, after successive additions of AA, the oxidation peak currents increased linearly with increase of the concentration between 10 μM and 1000 μM of AA. From the calibration graph (Figure 7.B), the relationship between the oxidation peak currents and the concentration of AA was estimated given by the equation;

$$I(\mu\text{A}) = 0.0612 C(\mu\text{M}) - 0.4697 \quad R^2 = 0.993 \quad (1)$$

The limit of detection, LOD, (based on $S/N=3$) was found to be 8 μM . The wide linear range and the moderately low LOD values of the proposed sensor were compared to other electrochemical AA sensors comprising various modified sensors (Table 3). MMT-Ser NC/PGE sensor system has not been previously used to detect any analyte in the literature. Considering the other studies in the literature, it has been observed that the sensor system developed has a competitive performance with other sensor systems.

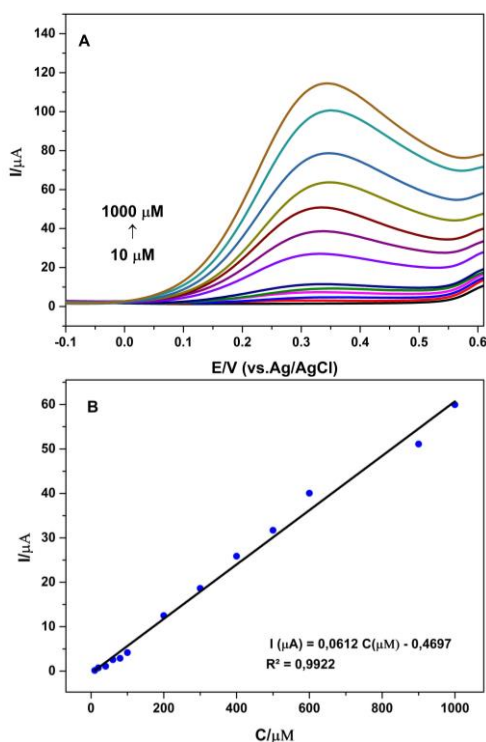


Figure 7. A) Linear detection range by SWVs recorded at MMT-Ser NC/PGE in pH 6.0 PBS between 10-1000 mM (from bottom to top) of AA (Ei: -0.1 V, Ef: 0.6 V, Frequency: 50 Hz; Puls width: 50 mV; Amplitude: 4 mV, equilibrium time: 10 s). **B)** Calibration graph of peak current vs. the AA concentration.

3.8. Reproducibility, selectivity investigation and AA assay in real sample

Reproducibility investigation of MMT-Ser NC/PGE sensors prepared independently under the same applicability of the sensor was tested for the determination of AA in Vitamin C tablets by the standard addition method with known concentrations of the analyte. The recoveries were between 94.6% and 111.2% for two different additions of samples which proved that the electrochemical sensor can practically be applied for vitamin analysis (Table 4).

Table 4. Results for detecting of AA in pharmaceutical vitamin C sample using MMT-Ser NC/PGE sensor.

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%) n=3
Pharmaceutical Vitamin C	200.0	189.3	94.6	4.54
Pharmaceutical Vitamin C	150.0	166.0	111.2	5.10

4. Discussion and Conclusion

Nanocomposite materials based on clay and silk protein were used together in the presented study in which superior properties of the two different materials were blended to provide a functional material for the modification of transducer surface. The montmorillonite-sericin nanocomposite were synthesized by desolvation method following glutaraldehyde crosslinking by acting amine groups of sericin to make the nanocomposites stable. The results of the characterization studies revealed that the synthesis of MMT-Ser composite nanostructures were prepared successfully; homogeneous and uniform nanocomposites were obtained and supported with both morphological and chemical analysis results. The synthesized montmorillonite-sericin composite nanostructures were characterized and investigated as electrode modifier as they can act as matrixes for electro-active ions as well as their versatility, ease of manipulation, high adsorption ability, biocompatibility and low cost of production. MMT-Ser NC were first time applied for the nanostructuring of electrode surfaces and showed good sorption properties for the substrate and the analyte as well as sensitive electroanalysis of ascorbic acid in standards and pharmaceuticals with satisfactory results in comparison with the other electrochemical nanostructured sensors reported in the literature. The sensor also can be potentially useful for biomedical applications and determination of other bioanalytes.

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