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Construction of nanoparticles composite sensor for atorvastatin and its determination in pharmaceutical and urine samples

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ABSTRACT

In the present work, first time a composite electrode based on nano-silica and zinc oxide nanoparticles were investigated for electrochemical determination of atorvastatin. The electrochemical behavior of atorvastatin at carbon paste electrode, initially modified with nano-silica was investigated. Further, the effect of addition of ZnO nanoparticles toward the electrochemical behavior of atorvastatin was studied and both were compared. The cyclic voltammetric peak current had a significant enhancement at nano-silica modified electrode as compared with the bare electrode. With addition of ZnO nanoparticles, it was observed that there was a large enhancement in the peak current as compared to that of nano-silica modified electrode. Parameters such as number of electrons transferred, diffusion coefficient, and heterogeneous rate constant were calculated. Limit of detection and quantification was calculated under the optimized conditions. For the analytical applications, pharmaceutical dose form and human urine sample analysis were performed. Various interferents were used to investigate the interference in the analytical application and was found that the proposed method would be well adopted for clinical trials and real sample analysis.

1. Introduction

Hyperlipidemia is a condition where abnormal elevated level of lipids occurs in blood. The most effective class of drugs used in the treatment is statins. Atorvastatin (ATR), belongs to the group statins and is chemically known as (3R, 5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid. In addition, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a key enzyme for cholesterol biosynthesis [1,2] and which has influenced on reducing triglycerides as well cholesterol [3–5]. ATR as anti-inflammatory is predominantly used in treatment of heart attack. In addition, heterozygous familial hypercholesterolemia in pediatric patients [6] is also treated with ATR. In 1985, Dr. Bruce D. Roth with Parke-Davis synthesized ATR and it is popularly marketed under various brand names in combination or single generic. The manufactures evaluate the quality of ATR using TLC or HPLC. Further, various reported methods are present for the drug determination, which involves use of LC/MS [7,8], LC–ESI–MS/MS [9], HPLC–ES–MS/MS [10] and various spectrophotometry methods [11–13], adopted RP-HPLC [14,15], and other HPLC methods [16]. These proposed methods have low sensitivity, slow and expensive. Hence, it is essential to develop a method for the determination of ATR, which is less time consuming, simplified, and less expensive. Electrochemical methods with these improved properties would be a good alternative source. A few electrochemical methods reported involved the use of adsorptive stripping voltammetry using glassy carbon electrode (GCE) [17,18] cyclic and differential pulse voltammetry (DPV) at a carbon paste electrode (CPE) in the presence of an enhancing agent, cetyltrimethyl ammoniumbromide (CTAB) [1] and DPV using boron-doped diamond electrode and GCE [19].

In the area of chemically modified electrodes, carbon paste electrodes (CPEs) have attracted a wide range of analyst in recent years. CPEs have unique advantages such as easiness in the preparation, various ligands depending on the application can be easily mixed during the paste preparation, cheaper, and low back ground current interferences during analysis [20,21]. These electrodes also promote open circuit accumulation of species proceeding to its voltammetric quantification. Organic polymers, ligands and inorganic ion exchangers such as clays or zeolite can be used as modifiers [22–24]. CPEs can easily prepared by mixing carbon

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(graphite) powder and a binder such as paraffin oil. Further, in the preparation of chemically modified carbon paste electrodes, suitable modifiers can be used and in this case, the mechanism of operation of these electrodes is known to be depending on the properties of modifier used to achieve sensitivity and selectivity toward the analyte.

Nano-silica materials are robust inorganic solids, which have high specific area, and three-dimensional structure consisting highly open spaces connected to each other [25,26]. A key factor in preparing a sensor, with improved selectivity would be high diffusion of preferred species to numerous available binding sites, which is a major advantage of using nano-silica [27]. In addition, combination of silica and carbon paste can produce an electrode with different response and the mechanical properties [28,29].

In this work, we have constructed a new carbon paste composite electrode comprising of silica nanoparticles and zinc oxide nanoparticles. The electrode was utilized for the electro-oxidation and determination of ATR. The key important observation has been shown as the nano-silica and nano zinc oxide composite increased the sensitivity of the electrode largely. The voltammetric determination with modified electrode had a low detection limit. The electrode was successfully used in the pH range of 3.0–11.2 and in the presence of different interferents. The differential pulse voltammetric technique was used to investigate the limit of detection and quantification. The method was also applied for the real sample analysis such as human urine and pharmaceutical dose forms. It was observed that, low limit of detection, easiness in fabrication of electrode, low cost material used in electrode and application of the electrode even in the presence of various interferents makes this electrode material suitable for determination of analyte in trace amount.

2. Experimental

2.1. Materials and apparatus

ATR, and all other chemical used were purchased from Sigma-Aldrich and used without further purification. Throughout the experiment, double distilled water was used. Phosphate buffer solution (range: 3.0–11.2 pH) was prepared with Na_2HPO_4, KH_2PO_4 and Na_3PO_4 ($I = 0.2$ M) [30]. Solutions were stored at 4° C prior to use. Elico pH meter with combine glass electrode was used to adjust the pH of the buffer solution. A three-electrode system was used which comprises of carbon paste electrode with modifiers as a working electrode, Ag/AgCl as a reference electrode and platinum wire as a counter electrode. The electrodes were connected to CHI Company, Austin, USA (Model D630) electrochemical analyzer controlled by computer with CHI version 12.16.

2.2. Synthesis of nano-silica

Precipitation method was used to prepare silica-wet slurry by adding hydrochloric acid drop wise to aqueous sodium silicate at temperature 55° C with constant stirring at 600–700 rpm. Initially HCl was added to silicate solution to get semi-gelatinous point at pH 9.0–9.5. The gel formation was avoided by stirring the solution for additional 10 min without addition of HCl. Further, more HCl was added until the slurry reached pH 3.0–3.5. After aging for 24 h at 50° C the slurry was heated on heater until all the water evaporated. A fine powder was obtained by crushing the crystals with glass rod. The powder was washed several times to remove chloride ions and then dried completely. Then it was crushed to get nano-silica particles.

2.3. Synthesis of ZnO nanoparticles

Zinc oxide nanoparticles were synthesized by controlled precipitation method using zinc nitrate and sodium hydroxide. In this experiment, 100 ml of 0.1 M of zinc nitrate ($Zn(NO_3)_2{\cdot}4H_2O$) solution was mixed with 10 mg/l of sodium dodecyl sulfate and kept under constant stirring using magnetic stirrer to completely dissolve the zinc nitrate for 1 h. After complete dissolution of zinc nitrate, 100 ml of 0.2 M NaOH aqueous solution was added to Zinc nitrate solution with vigorous stirring (2000 rpm), drop by drop (slowly for 45 min). The reaction is allowed to proceed for 2 h after complete addition of sodium hydroxide. The beaker was sealed at this condition and kept for 2 h. After the completion of reaction, the solution was allowed to settle for overnight and further, the supernatant solution was separated carefully. The remaining solution was centrifuged for 10 min, and the precipitate was removed. Thus, precipitated ZnO nano particles were washed repeatedly with deionized water and ethanol to remove the by-products which were bound with the nanoparticles and then air dried at 60° C for 4 h. The obtained product was calcined at 500° C for 3 h using a muffle furnace. During drying, $Zn(OH)_2$ was completely converted in to ZnO.

2.4. Preparation of CPE and modified CPE

CPE was prepared by hand mixing graphite powder and paraffin oil as a binder in a ratio 7:3 (*w*/*w*) using mortar and pestle for 30 min to get a homogenized paste. Further, the paste was filled in a hollow electrode purchased from CHI Company connected with copper wire [31]. The nano-silica modified carbon paste electrode (NS/CPE) and ZnO nanoparticles modified carbon paste electrode (ZnO/CPE) was prepared by addition of nano-silica and zinc oxide nanoparticles to the mortar during paste preparation, respectively.

The electrode surface area was analyzed using cyclic voltammetric technique with 1.0 mM $K_3Fe(CN)_6$ as a probe at different scan rates in 0.1 M KCl as supporting electrolyte. For an irreversible electrode process and at temperature (T) = 298 K the equation is as follows:

$$I_p = \left(2.69 \times 10^5 n^{3/2} A_0 \sqrt{D_R}\sqrt{\upsilon} C_0\right) \quad (1)$$

where Ip: anodic peak current, n: number of electron transferred during the electrode reaction = 1, A_0: surface area of the electrode, D_R: diffusion coefficient = 7.6×10^{-6} cm^2 s^{-1}, υ: scan rate and C_0: concentration of $K_3Fe(CN)_6$. Using slope for the plot of Ip versus $\upsilon^{1/2}$ area of the electrode was calculated [32].

2.5. Sample preparation

Ten Atorva 20 tablets were ground to fine powder in a mortar. A stock solution of 1.0 mM was prepared by transferring an accurately weighed amount to a 100 ml volumetric flask and dissolved in ethanol. Appropriate solutions were prepared by taking suitable aliquots from stock solution and diluting them with phosphate buffer solutions. Transferring each solution in voltammetric cell the analysis was performed by standard addition method [33]. Differential pulse voltammetric technique was used between 0.6 to 1.2 V after accumulation time 60 s.

3. Results and discussion

3.1. SEM, TEM and XRD analysis

The SEM analysis for nano-silica and ZnO nanoparticles was performed to get the fine surface topography and composition (Fig. 1A1). The SEM image for zinc oxide nanoparticles showed