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Electrochemical design of a new nanosensor based on cobalt nanoparticles, chitosan and MWCNT for the determination of daclatasvir: a hepatitis C antiviral drug

Shereen M. Azab^{*a} and Amany M. Fekry^b

Daclatasvir (DAC) is listed on the World Health Organization's list of essential medicines needed in a basic health system, therefore, electrochemical and impedance spectroscopic methods are necessary to obtain information about its mechanism using a carbon paste electrode (CPE) modified with chitosan (Cs)/multi-walled carbon nanotubes (CNT) and cobalt nanoparticles (CoNPs). The simultaneous determination of DAC with the hepatitis B antiviral drug entecavir (ENT) was also investigated by differential pulse voltammetry in universal buffer pH 2.0. The experimental results specify a linear relation between the DAC peak current and its concentration in the range from 1.0 nM to 12 μM, leading to a detection limit of 8.82×10^{-10} M. Finally, this novel sensor was successfully used to determine DAC in human biological fluids such as urine, blood serum and in pharmaceutical formulations.

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1. Introduction

The introduction of the direct-acting antiviral drug; daclatasvir (DAC) is altering the treatment selections for hepatitis C virus (HCV). DAC is used in the treatment of chronic hepatitis C genotype 3 infections, it hinders the HCV nonstructural protein NS5A.^{1,2} Modern research proposes that it targets two steps of the viral replication route, allowing fast decline of HCV RNA.¹ Chitosan (Cs) is a natural cationic biopolymer of β-(1→4) linked 2-amino-deoxy-D-glucopyranose that has both hydroxyl groups which might be oxidized to -CHO or -COOH, and amino groups that provides lone pair electrons for metal ions. Cs can be modified easily possessing adhesion properties,³ so it improves hydrophilicity and biocompatibility *via* covalent grafting techniques. Cs has been widely used in sensor preparations since it has affinity toward metal ions. Chitosan films can be functionalized with other substances such as polymers, nanostructures, biomolecules, or dyes to improve the film's mechanical properties. Chitosan has high adsorption properties in its matrix.²⁴ Chitosan modified electrodes were used for the determination of heavy metals.⁴⁻⁶ Conducting polymer sensors have been used successfully for drug determination to improve selectivity of analysis.^{7,8} CNTs are porous materials that offer high effective surface area, mass transfer and catalysis due to their mechanical strength, electrical conductivity and

chemical stability. A decrease in over-potential can be detected with CNTs producing a more reversible voltammetry.^{9,10} Thus, CNTs can powerfully enhance electron transfer processes. Combining chitosan with magnetic nanoparticles is an alternative approach to reduce the adverse effects of nanoparticles.¹¹

Recently, cobalt nanoparticles have been used in medical applications because of their good magnetic properties and their unlimited properties on proton relaxation.¹² They can enter the human cells very rapidly,¹³ which has drawn the interest of researchers toward it. The co-existence of chitosan, CNTs and Co nanoparticles creates a negative charge which is favorable for the further immobilization of biomolecules that are positively charged. Up to date, only few articles concerning the determination of DAC using liquid chromatographic methods were reported.¹⁴⁻¹⁷ To our knowledge, there no electrochemical method fabricated for the determination DAC either alone or in the presence of interfering substances. In the present article, a novel modified electrode was developed for the first time by electro-polymerization of cobalt nanoparticles on the surface of CPE mixed with chitosan/CNT to be used for the electrochemical determination of daclatasvir either in commercial pharmaceutical samples or in real samples.

2. Experimental

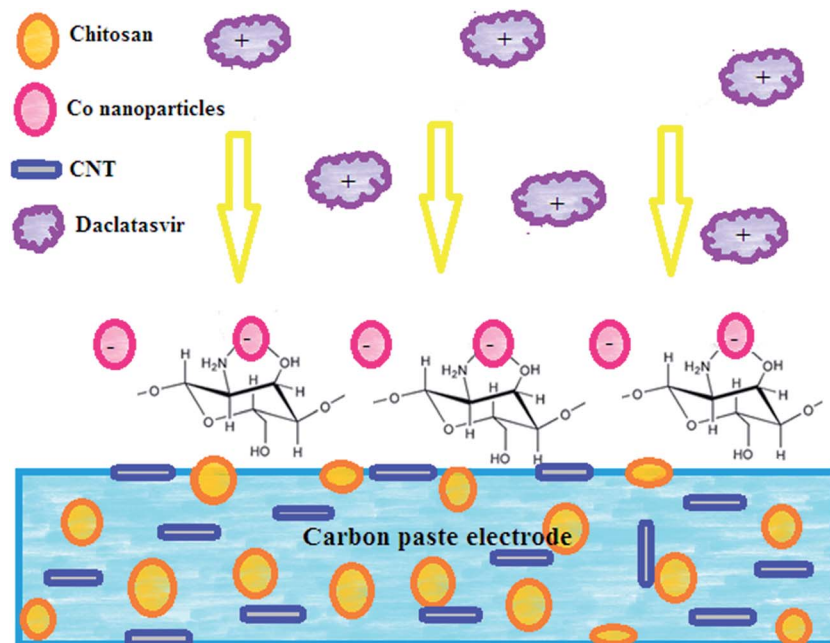
2.1. Materials and equipment

Daclatasvir (DAC) and Daktavira tablets were provided from the European Egyptian Pharmaceutical Industries, Egypt, chitosan was purchased from sigma and the multiwalled carbon nanotubes was purchased from sigma and was immersed in

^aPharmaceutical Chemistry Dept., National Organization for Drug Control and Research [NODCAR], 6 Abu Hazem Street, Pyramids Ave, P. O. Box 29, Giza, Egypt. E-mail: sheryspecial@yahoo.com; Tel: +20 2 01 225591629

^bChemistry Department, Faculty of Science, Cairo University, Giza-12613, Egypt





Scheme 2

3.1.2. Surface characterization

3.1.2.1. SEM. The surface morphology of (A) bare CPE, (B) CPE/Cs/CNT and (C) CPE/Cs/CNT–Co were represented by SEM respectively in Fig. 2. Bare CPE appears like a dark and smooth surface, while CPE/Cs/CNT shows brittle, rough chitosan bead which act as conductive cores that facilitate the transfer of electrons, and CNTs appears as unbundled, aligned, randomly

distributed tubes (inset) providing more porosity, causing an enhancement in the effective surface area of CPE/Cs/CNT, so it could accumulate more CoNPs on its surface. The SEM of CPE/Cs/CNT–Co appears to be light, cloudy and spherical nanoparticles with diameters ranging from 40–80 nm.

3.1.2.2. TEM. The TEM image of (A) CPE/Cs/CNT and (B) CPE/Cs/CNT–Co was presented in Fig. 3. CPE/Cs/CNT shows CNT as a curly, bamboo like structure with diameter ≈ 25 nm, while CPE/Cs/CNT–Co shows Co nanoparticles with average diameter ≈ 35 nm.

3.1.2.3. EDX and elemental mapping. EDX data of CPE/Cs/CNT–Co (Fig. 4) indicated the presence of Co, carbon and oxygen peaks. Also the elemental analysis confirmed that about 92% of CPE/Cs/CNT–Co is coated by CoNPs. The elemental mapping confirms that O, C and Co are uniformly distributed across the whole mapped area on the sensor's surface.

3.1.3. Effect of pH. The amount of current of 1.0 mM DAC in B–R buffer with different pH values was detected using cyclic voltammetry on CPE/Cs/CNT–Co surface (Fig. 5A). The best result was obtained at pH 2.0. The comparison of the influence of pH on the anodic peak current of DAC upon using bare CPE and CPE/Cs/CNT–Co was shown in Fig. 5B, the results show that in acidic pH values (<6.0), higher currents appear due to the pK_a value of DAC which is 6.09. It was also detected that the peak potentials shifted to less positive values with increasing pH, leading to a linear regression equation expressed as $E_p = -0.0578pH - 1.197$ ($R = 0.9993$). The slope of 57 mV pH^{-1} indicates that the system obeys the Nernst equation. Also the Nyquist plots for CPE/Cs/CNT–Co at different pH values were shown in Fig. 5C with semicircles and lines, each corresponds to a charge transfer resistance and a diffusion process at both high and low frequencies, respectively. It is clear from Nyquist plot that the mechanism depends on diffusion and charge

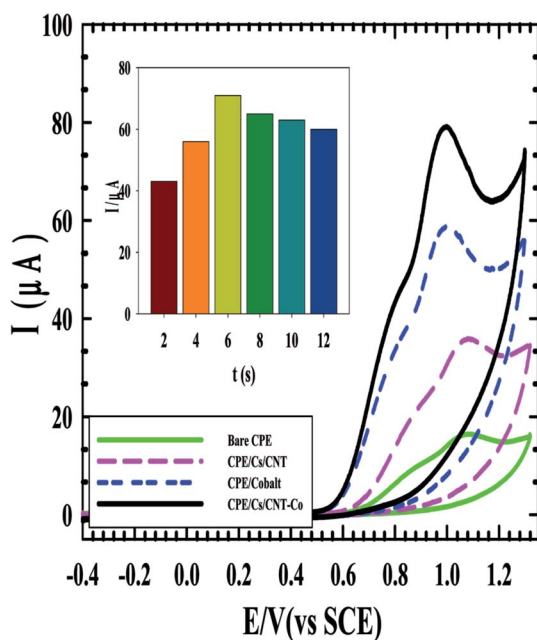


Fig. 1 Electrochemical oxidation responses of DAC in B–R buffer pH 2.0 using different electrodes surfaces. The inset: represents the effect of changing the electrodeposition time of Co nanoparticles on CPE/Cs/CNT.



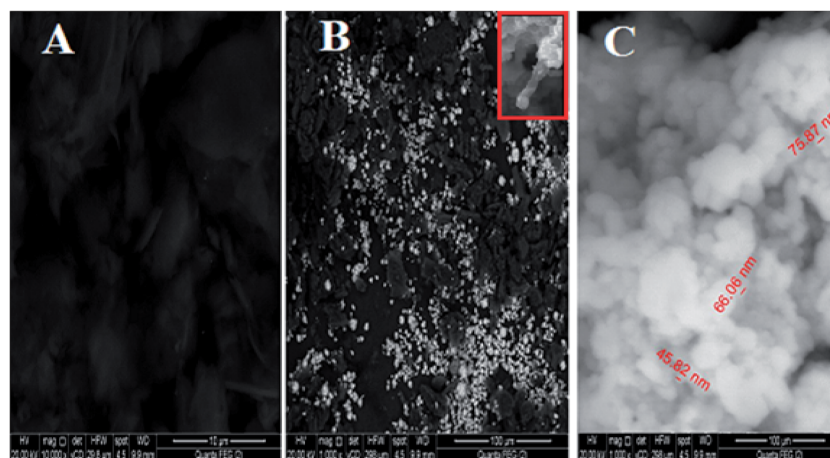


Fig. 2 The SEM of (A) bare CPE, (B) CPE/Cs/CNT and (C) CPE/Cs/CNT-Co.

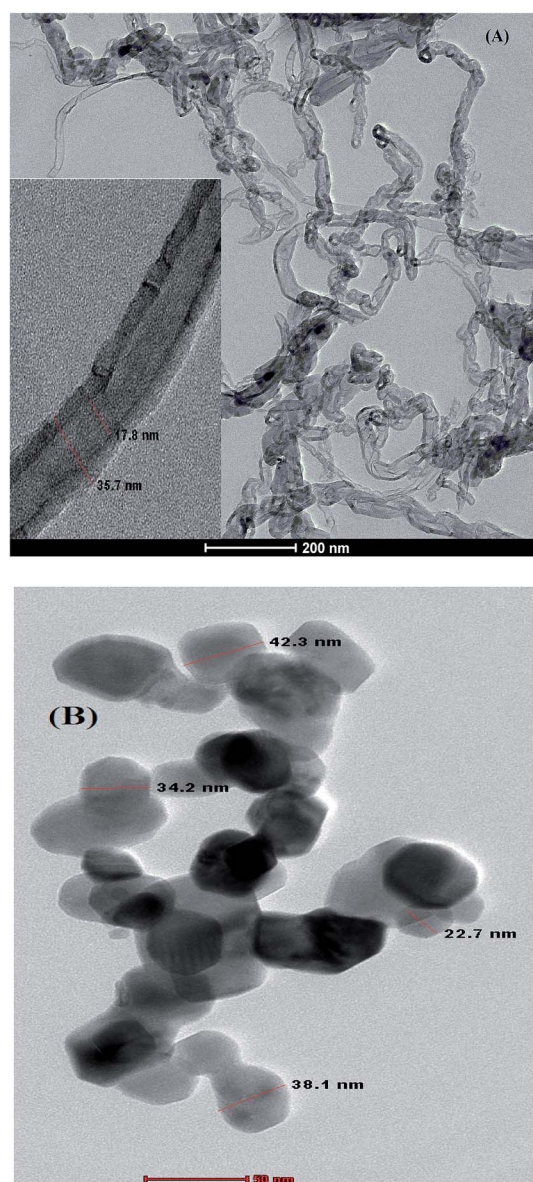


Fig. 3 TEM image of (A) CPE/Cs/CNT and (B) CPE/Cs/CNT-Co.

transfer mechanism. The semicircle diameter and the impedance values increase with increasing pH values indicating a decrease in the conductivity^{21,22} and giving the same order of the CVs results.²² The curves are simulated with one time constant model (Scheme 3). R_s is the solution resistance, R is the charge transfer resistance and W is Warburg impedance for diffusion.^{23,24} The Constant-Phase Element (CPE) of the capacitance is used instead of ideal capacitance owing to surface heterogeneity.²⁵ Thus, the impedance value at bare electrode is 243 k Ω cm² compared to 111 k Ω cm² for the modified CPE/Cs/CNT-Co electrode at pH 2.0.

3.1.4. Effect of scan rate. The influence of changing the scan rate over the range of 10–300 mV s⁻¹ was studied to detect the diffusion and adsorption properties of 1.0 mM DAC at CPE/Cs/CNT-Co (Fig. 6). The positive shift in the peak potential approves that the oxidation process of DAC is irreversible. The plot of peak current *versus* square root of scan rate was constructed (inset A) and a straight line was obtained fulfilling the equation I_p (μ A) = 9.872 $\sqrt{\nu}$ - 14.48; $R = 0.9916$. This relationship indicates that diffusion mechanisms are involved in the electrochemical reaction. Also a plot of log peak current *versus* log scan rate was performed giving a linear relationship with a slope of 0.572 (inset B), suggesting that DAC oxidation on CPE/Cs/CNT-Co is primarily controlled by diffusion with some adsorption support, as observed from impedance data. Thus, CV data ensure well EIS data.

The electro-active surface area of bare CPE and CPE/Cs/CNT-Co were achieved by CV technique, using 1.0 mM K₃Fe(CN)₆ based on the Randles-Sevcik equation:^{26,27}

$$I_p = 2.69 \times 10^5 n^{3/2} AD^{1/2} \nu^{1/2} C \quad (1)$$

For K₃Fe(CN)₆, $n = 1$ and $D = 7.6 \times 10^{-6}$ cm² s⁻¹, the area were evaluated to be 0.072 cm² and 0.217, for bare CPE and CPE/Cs/CNT-Co, respectively. Thus after modification the surface area of the electrodes was considerably increased. These areas were used to calculate the diffusion coefficient of 1.0 mM DAC at 100 mV s⁻¹ which increases from 9.37×10^{-8} cm² s⁻¹ in case of using bare CPE to 2.24×10^{-7} cm² s⁻¹ after the functionalization of CPE/Cs/CNT surface with Co nanoparticles.



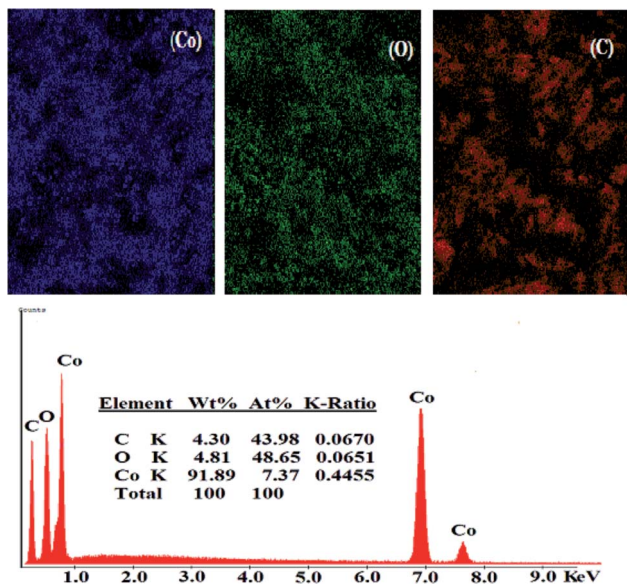


Fig. 4 The elemental mapping and the EDX with the elemental analysis data of CPE/Cs/CNT–Co surface.

To find out the surface coverage of the adsorbed molecule; eqn (2) was used.²⁸

$$I_p = (n^2 F^2 A \Gamma \nu / 4RT) \quad (2)$$

The surface coverage of the adsorbed molecule (Γ) is a very important parameter that indicates the behavior of the sensor, and they were found to be $1.35 \times 10^{-10} \text{ mol cm}^{-2}$ and $2.278 \times 10^{-10} \text{ mol cm}^{-2}$ for bare CPE and CPE/Cs/CNT–Co, respectively. The results show that the catalytic activity of DAC increases in the presence of Cs/CNT and Co nanoparticles. Also the determination of the sensor dynamics parameters (α and n) is essential to determine the proposed mechanism of DAC. The relationship between E_p and $\ln \nu$ (inset C) is given by Laviron equation:¹⁴

$$E_p = E_0 + [RT/(\alpha n F)] \ln[(RT k_s)/(\alpha n F)] + [RT/(\alpha n F)] \ln \nu \quad (3)$$

where α is the electron transfer coefficient, k_s is the standard rate constant of the surface reaction and E_0 is the formal potential obtained from the relation between E_p and ν by extrapolating to the vertical axis at $\nu = 0$ and it was found to be 0.965 V (inset D), αn can be calculated from the slope of E_p vs. $\ln \nu$. In this system, the slope is 0.048, αn was calculated to be 1.87. Since for a totally irreversible electron transfer, α assumed as 0.5, then n was calculated to be 3.74 which indicated that four electrons were involved in the oxidation of DAC. The value of k_s can be calculated from the intercept of Laviron equation and was found to be $2.44 \times 10^3 \text{ s}^{-1}$.

3.2. Chronoamperometry measurements

Chronoamperometry data measured for DAC at a constant potential of +990 vs. SCE are shown in Fig. 7. The calibration curve has been derived at early times of 2 seconds which gives

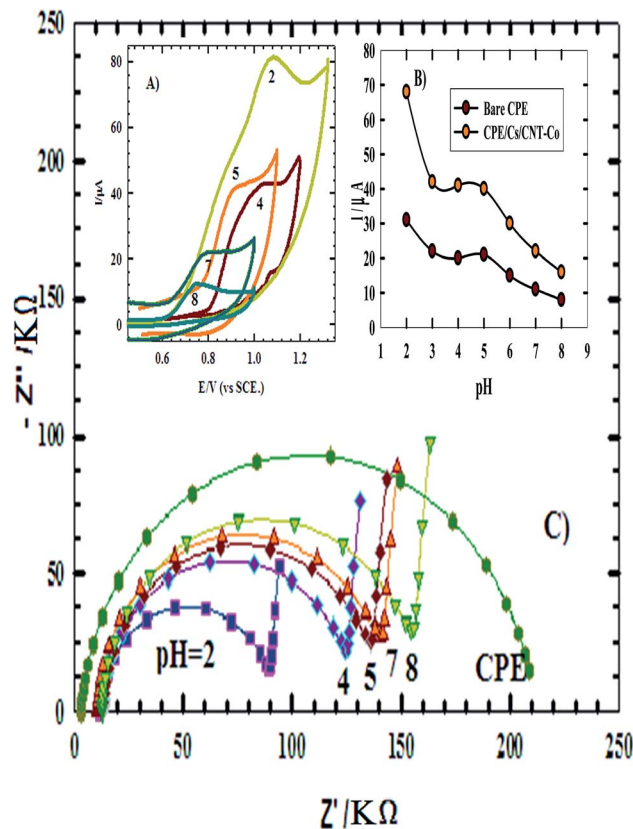
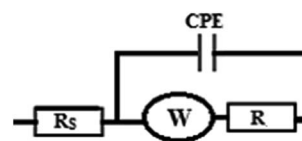


Fig. 5 (A) Cyclic voltammograms of 1.0 mM DAC in B–R buffer at different pH values, with scan rate 100 mV s^{-1} recorded at CPE/Cs/CNT–Co. (B) Graph of current response of DAC at different pH values using bare CPE and CPE/Cs/CNT–Co. (C) Nyquist plots for DAC using bare CPE and CPE/Cs/CNT–Co in B–R buffer with different pH values.



Scheme 3

the best correlation coefficient of 0.997.^{29,30} The linear equation is $I_p (\mu\text{A}) = 35C + 59$. Thus, the response time is 2 seconds and the recovery is 17 seconds at which the current becomes stable. This response time is lower than that observed by Arora *et al.*³¹ (60 s), Ali and Mohamed³² (5 s). Du *et al.*³³ with response time of 7 s and Tang *et al.*³⁴ biosensor with a response time of less than 15 s.

3.3. Characterization and linearity of CPE/Cs/CNT–Co

Quantitative determination based on the linear relationship between the oxidation current and the concentration of DAC from $1.0 \times 10^{-9} \text{ M}$ to $1.2 \times 10^{-5} \text{ M}$ (Fig. 8) at CPE/Cs/CNT–Co was studied using DPV at scan rate 10 mV s^{-1} (inset A). The parameters of the calibration plots are summarized in Table 1. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following equations: $\text{LOD} = 3S/$



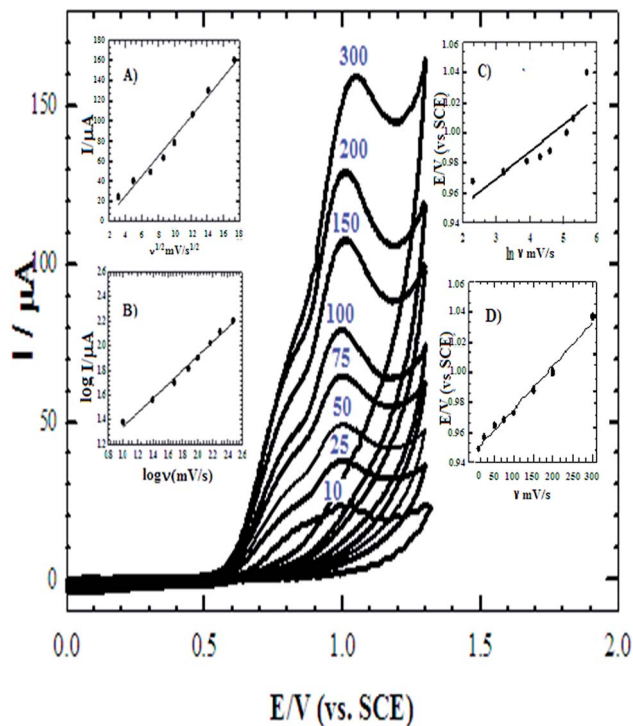


Fig. 6 Cyclic voltammograms (CV) of 1.0 mM DAC at CPE/Cs/CNT-Co in B-R buffer pH 2.0 at: 10, 25, 50, 75, 100, 150, 200 and 300 mV s^{-1} . Inset (A) plot of the anodic peak current values versus square root of scan rate. Inset (B) plot of log the anodic peak current versus log the scan rate. Inset (C) plot of the anodic peak potential values versus ln scan rate. Inset (D) plot of the anodic peak potential values versus scan rate.

m and $\text{LOQ} = 10S/m$.³⁵ Where S is the standard deviation and m is the slope of the calibration curve and was found to be 8.82×10^{-10} M and 2.94×10^{-9} M, respectively. Also, the effect of concentration was studied using impedance spectroscopy (inset B). The results showed that as the concentration increases, impedance value decreases, where the ionic/electronic charge transfer resistance shows noticeable decrease in values indicating less electronic resistance and more conductivity. The optimized procedure has also been well applied for the determination of DAC in spiked serum and urine samples. The obtained validation data are given in Table 1 and compared with that obtained from the raw material.

3.4. Effect of some biological interferences and co-administered drugs

Ascorbic acid (AA) and uric acid (UA) are the most important interferences that coexist in the extracellular fluid of the central nervous system. To check the sensitivity of the method, the electrocatalytic oxidation of DAC in the presence of 1.0 mM AA and 1.0 mM UA was investigated in urine sample at the surface of bare CPE and CPE/Cs/Co using DPV. Fig. 9A shows that weak oxidation responses were detected at bare CPE with some overlapping between AA and UA while upon using CPE/Cs/Co the oxidation peaks of DAC (at 800 mV), AA (at 390 mV) and UA (266 mV) were higher, sharper and well separated. DAC may also interact with anticonvulsants, antimycobacterial agents

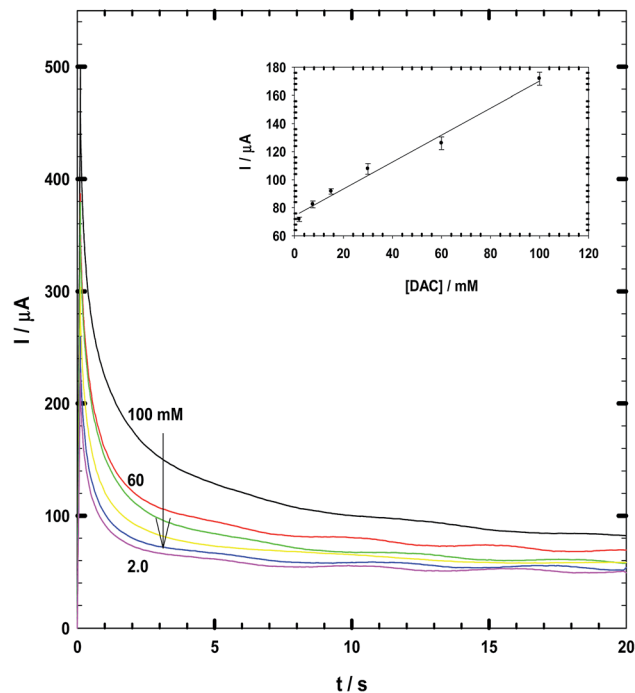


Fig. 7 Chronoamperometry diagram for 1 mM DAC in B-R buffer pH 2.

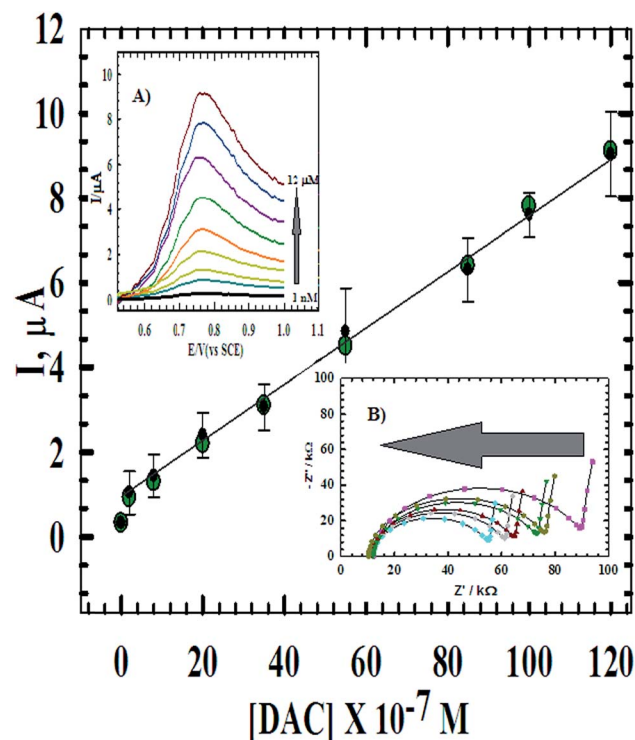


Fig. 8 The calibration curve of DAC at CPE/Cs/CNT-Co. Inset (A) DPV of the successive additions of DAC in B-R buffer and 10 mV s^{-1} . Inset (B) the Nyquist plot of the successive additions of DAC.

and other antiviral drugs. To check the sensitivity of the method, the electrocatalytic oxidation of DAC in the presence of entecavir (ENT) (an antiviral drug used in the treatment of



Table 1 Regression data of the linear ranges by DPV using CPE/Cs/CNT-Co for quantitative determination of DAC in raw material, urine sample and serum sample

Parameters	Raw material	Urine sample	Serum sample
Linearity (M)	1.0×10^{-9} to 1.2×10^{-5}	6×10^{-9} to 1.1×10^{-5}	9×10^{-9} to 1.1×10^{-5}
LOD (M)	8.82×10^{-10}	1.52×10^{-9}	7.07×10^{-9}
LOQ (M)	2.93×10^{-10}	5.06×10^{-9}	2.35×10^{-8}
Slope	0.0704	0.0841	0.0611
SE of slope	0.0088	0.0038	0.0055
Intercept	0.6320	0.9110	1.0131
SE of intercept	0.0017	0.0744	0.0624
Correlation coefficient (<i>r</i>)	0.9997	0.9993	0.9990

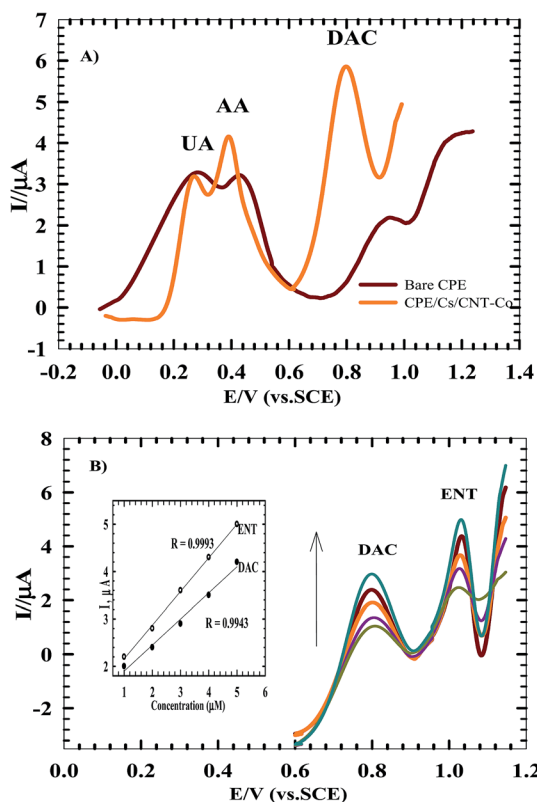


Fig. 9 (A) DPV of B-R buffer solution (pH 2.0) containing DAC with AA and UA monitored at bare CPE and CPE/Cs/CNT-Co. (B) DPV of B-R buffer solution (pH 2.0) containing DAC and ENT by varying their concentrations from 1 to 5 μM at CPE/Cs/CNT-Co. Inset: electrocatalytic peak current versus DAC and ENT concentration.

chronic hepatitis B in adults with compensated liver disease of active viral replication), is an important issue to be studied since both drugs are co administered by patients who suffer from virus B and C. The simultaneous determination studied in B-R buffer using CPE/Cs/CNT-Co exhibits two anodic peaks for DAC and ENT at 0.79 V and 1.12 V, respectively with good peak separation of 0.33 V. The current response increased linearly with increasing of concentrations of both compounds (Fig. 9B) obeying the following equations:

$$I_p (\mu\text{A}) = 0.550C (\mu\text{M}) - 1.350, \text{ for DAC}$$

$$I_p (\mu\text{A}) = 0.710C (\mu\text{M}) - 1.450, \text{ for ENT}$$

These results show the high selectivity of the proposed method for the determination of DAC.

3.5. Stability of repeated CVs

Fig. 10 A shows the repeated CVs for 1 mM DAC in B-R buffer (pH = 2.0) at CPE/Cs/CNT-Co for 50 cycles. The decrease of the peak current from the first to the last cycle was about 10%. Also an impedance spectroscopy experiment was performed at the first cycle and after 50 cycles (Fig. 10B). It was found that the curve shape doesn't change. This ensures that the mechanism is the same which depends on both charge transfer and diffusion processes. Impedance value from the first to 50 cycles increases with a small value indicating a low decrease in current value. FT-IR analyses were performed for both CPE/Cs/CNT-Co and CPE/Cs/CNT electrodes in absence and presence of DAC after 50 cycles. For CPE/Cs/CNT, MWCNTs absorption band at 1615 cm^{-1} is due to the stretching of C=C bonds and the peak at 2885 cm^{-1} is due to the stretching of C-H bonds. Cs shows absorption peaks at 3427 cm^{-1} for -OH group and 2885 cm^{-1} for CH_3 groups. The interactions between DAC with MWCNT and chitosan occurs *via* hydrogen bonds formation between the -OH of Cs and C=C in CNT with -NH in DAC, allowing the formation of electrostatic interactions (Fig. 10C). A strong IR changes were also observed in FT-IR charts in case of CPE/Cs/CNT-Co due to the presence of Co nanoparticles that appears as doublet at 567 cm^{-1} and 646 cm^{-1} indicating a very strong interaction between the electrode surfaces and DAC (Fig. 10D).

3.6. Analytical application and robustness

For the purpose of the practical applicability of the proposed method and the modified sensor, DPV method was effectively applied for the investigation of DAC in five Daktavira tablets without any sample extraction and after dilution to obtain 1.0 mM DAC solution. The validity of the proposed method was evaluated by the standard addition technique and the accuracy has been determined giving satisfactory recoveries from 98.40 to 101.6% and relative standard deviation from 0.03 to 0.43% revealing that the excipient presented in tablet does not interfere with the active ingredient. The results suggested that CPE/Cs/CNT-Co has high reproducibility and would be useful sensor for quantitative analysis of DAC in pharmaceutical



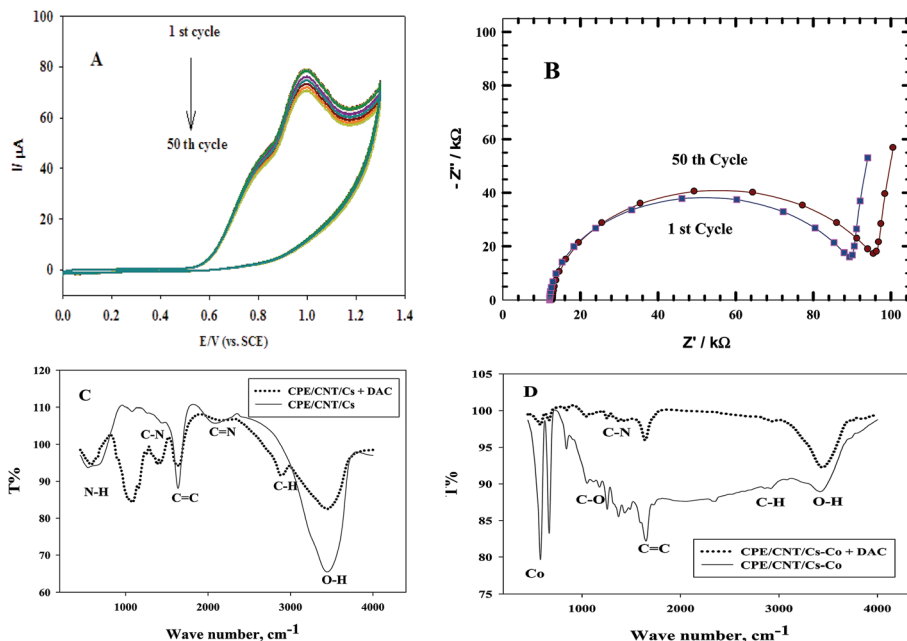


Fig. 10 (A) Repeated CVs of 1 mM DAC in B–R buffer (pH = 2.0) at CPE/Cs/CNT–Co for 50 times. (B) Impedance spectroscopy of the first and the 50th cycles of 1 mM DAC in B–R buffer (pH = 2.0) at CPE/Cs/CNT–Co (C) FT-IR spectrum of CPE/Cs/CNT in the presence and absence of DAC (D) FT-IR spectrum of CPE/Cs/CNT–Co in the presence and absence of DAC.

formulations. The robustness was examined by evaluating the variation of pH = 2.5 instead of pH = 2.0. The obtained results indicated that the proposed procedures for DAC assay can be considered robust.

4. Conclusion

This work establishes the ability of a new CPE sensor for the electrochemical determination of the antiviral drug DAC in pharmaceutical formulations, biological samples and in the presence of the co-administered drug ENT. Some parameters such as the diffusion coefficient, surface coverage and the number of electrons involved in the oxidation reaction were calculated. The results showed that the method was sensitive, precise and selective with no significant effects of the excipients background. Experimental results specify a linear relation between DAC peak current and its concentration in the range from 1.0 nM to 12 μM, leading to a detection limit of 8.82×10^{-10} M. Finally, this novel sensor was successfully used to determine DAC in human biological fluids such as urine and blood serum and in pharmaceutical formulations. So this sensor exhibited attractive specific capacitance and long life time, which will supply a new insight for various applications in the future.

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