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Portable Nanoparticle-Enhanced Sensor for Tigecycline Determination in Biological Fluids and in the Presence of Its Degradation Products

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Tigecycline (TGC) is a novel potent antibiotic with recently proven anticancer activity against leukemia, glioma, and lung cancer. In-line TGC potentiometric sensors are fabricated for monitoring TGC in its pure form, pharmaceutical formulation, presence of its degradation products, and spiked human plasma. In-line sensors act as greener, portable, and economical alternatives to the classical off-line separation-based techniques. Classical and advanced liquid-contact (LC) and solid-contact (SC) sensors were fabricated, where the best performance was observed with the modified SC sensor (sensor VI) with potassium tetrakis (4-chlorophenyl) borate as ionic exchanger, β-cyclodextrin ionophore and cobalt oxide nanoparticles, showing a Nernstian response of 30 mV decade^{-1} in the linear range of 10^{-2}–10^{-6} M. Statistical comparison was carried out for the results obtained from proposed SC sensors and the official method on TGC pure form. Additionally, method greenness was evaluated using a semi-quantitative analytical eco-scale, scoring approximately 95 points, which was the highest greenness achievement score when compared to the proposed LC sensors or *British Pharmacopeial* chromatographic method.
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A globally disturbing trend has emerged, emphasizing the critical importance of developing new antimicrobial agents for the coming Era, due to the presence of developed resistance mechanisms displayed primarily by multidrug-resistant bacteria against existing antibiotics. As a result, newly developed antimicrobials and their evaluation against resistant pathogens became a highly focused interest.[1]

In June 2005, the United States Food and Drug Association (US-FDA) approved the use of tigecycline (TGC) against a wide range of bacterial infections owing to its broad-spectrum activity in vitro and in vivo. TGC was found to be highly active against gram-positive anaerobic pathogens and nearly all gram-negative bacteria, except for *Pseudomonas aeruginosa* and *Proteus species*.[2] TGC is chemically known as (4S,4aS,5aR,12aS)−9-(2-tert-butylaminoacetylamino)−4,7-bis-dimethylamino-3,10,12,12a-tetrahydroxy-1,11-dioxo1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxamide.[3] It is the first novel intravenous drug in the glycylcycline class, which is considered a modified tetracycline analog (Fig. 1).[1] It inhibits bacterial protein translation by binding to the 30 S ribosomal subunit and interfering with the entrance of amino-acyl tRNA molecules into the ribosome A-site.[4] TGC was considered the first-line therapy for emergency cases of severe systemic infections as well as for treating complicated intra-abdominal infections, skin infections, and community-acquired pneumonia.[2]

Since cancer patients are highly susceptible to different pathogenic infections, promising antibiotic anticancer strategies have proven effective against different types of cancers.[2] Up-to-date studies have revealed that TGC has direct antitumor effects against certain types of cancers, including acute myeloid leukemia, glioma, and non-small cell lung cancer, by activating signaling pathways and selectively destroying mitochondrial function in cancer cells.[5]

Consequently, TGC concentrations should be crucially monitored and quantified in plasma, especially when administered to patients as an anticancer drug, to avoid any severe side effects that might arise, such as gastrointestinal intolerance and renal and liver toxicity.[5] Therefore, there is a necessary demand for the development of a rapid, accurate, and sensitive method for the TGC analysis.

However, few TGC monitoring methods have been reported. These methods include spectrophotometry,[4,6] spectrofluorimetric,[7,8] and chromatography methods.[9–19] Although potentiometric technologies are highly suggested as green, economical, and portable technologies that may be used for in-line monitoring of small samples, no potentiometric methods for TGC detection have been reported.

Initial ion-selective electrodes (ISEs) were developed using conventional liquid contact electrodes based on internal solutions, which had an unstable response and a short lifespan. All-solid-state electrodes have been developed to address these inadequacies.[20]

Electroanalysis technology has been developedby modifying the surface of sensors and improving the electron transfer rate. Nanoparticles have been frequently used in electrochemical sensors owing to their special physicochemical properties, such as large surface area to volume ratio, outstanding mechanical strength, high electrocatalytic activity, and conductance.[21–23]

Specifically, cobalt oxide nanoparticles (CoO-NPs) are considered an advanced promising material that is used in various sensors' applications, due to its' chemical/thermal stability, highly catalytic active properties, and prominent theoretical capacity.[24]

According to the United States Environmental Protection Agency, green chemistry is the focus of scientists' efforts to develop the most innovative techniques of analysis thereby encouraging waste reduction, energy conservation, and the use of less hazardous compounds. Thus, the greenness of the proposed approach was assessed using a semi-quantitative analytical eco-scale.[25]

In this study, a novel, sensitive, and green potentiometric method for the determination of TGC was developed based on the fabrication of liquid contact ion-selective electrodes (LC-ISEs) and solid contact ion-selective electrodes (SC-ISEs) as potentiometric sensors. Consequently, the sensor that gave the best selectivity and linearity was applied to determine the TGC in its pharmaceutical dosage form (Tygacil® vials), in the presence of its degradation products, and in spiked human plasma.

Materials and Methods

Chemicals and reagents.—Tigecycline standard was purchased from AmBeed Company (Arlinton Hts, USA); its' purity was found to be 100.48% ± 1.03 according to *British Pharmacopeia* method.[3]

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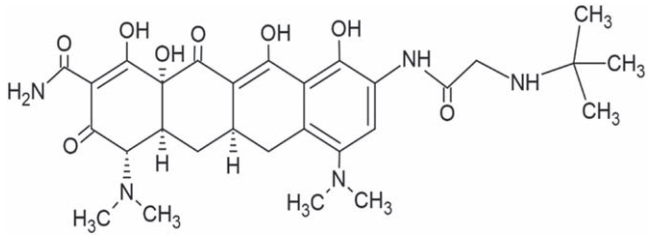

Figure 1. Chemical structure of Tigecycline.

Absolute ethanol (100% purity) was obtained from Chem-Lab (De Arend, Belgium). Graphite fine powder was purchased from Central Drug House, India. Potassium tetrakis (4-chlorophenyl) borate, polyvinyl Chloride (PVC), tetrahydrofuran (THF), cobalt (II, III) oxide nanoparticles (CoO-NPs), nitrobenzene, and β-cyclodextrin (β-CD) were purchased from Sigma Aldrich (Missouri, USA). Hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), potassium chloride (KCl), potassium dihydrogen phosphate, and paraffin Oil (Analytical Grades) were obtained from El-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt). Distilled water; purified by GFL distillatory Type 2012 (Germany); was used in all solutions' preparations. Human plasma was purchased from VACSERA Company, Egypt. The Dosage form; Tygacil® vials; product of Pfizer pharmaceutical Company (Batch Number: ALVE14), was purchased from local market.

Equipment.—The Shimadzu electronic balance (model number: ATY224), (Japan). Jenway digital ion analyzer model 3505 (Jenway, UK). Ag/AgCl double junction reference electrode was purchased from Sigma Aldrich (Missouri, USA). Jenway pH glass electrode (Jenway, UK) was used. Transmission electron microscopy (TEM), (JEM-2100 TEM) (Tokyo, Japan) was operated at an accelerating voltage 80–200 kV. TEM was used for characterization of CoO-NPs.

Standard solutions.—TGC stock standard solution (1.0×10^{-2} M) was prepared in 25 ml volumetric flask by weighing 146.40 mg and dissolved in phosphate buffer solution pH 5 (buffer was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in 1000 ml of distilled water, and the pH was adjusted to 5 with 1 M potassium hydroxide).

TGC working standard solutions were prepared with the same solvent through serial dilutions from the stock standard solution, in a descending order of concentrations in the range from (1.0×10^{-7} M) to (1.0×10^{-3} M).

Degradation products.— *Acidic andalkaline degradation.—* Degradation products of TGC were prepared by refluxing 10.00 mg of pure drug with 20 ml of 0.1 M HCl or NaOH at 70 ° C for 5 h. The solutions were cooled, neutralized to pH about 7 by using drops of 0.1 M NaOH or HCl, respectively and then placed under vacuum for evaporation till dryness. The residues were separately extracted, each with 50 ml ethanol, then filtered into 50 ml volumetric flasks and diluted to volume by using the same solvent, obtaining a solution demanded to contain degradation products equivalent to 0.2 mg ml^{-1} intact drug.

Thermal degradation.—TGC powder (292.80 mg) was placed in a thermostatically controlled oven at 40 °C for 8 h. Then, it is constituted using 50 ml phosphate buffer, obtaining a solution of a final concentration of 1.0×10^{-2} M.

Procedures.— *Liquid contact (LC) sensors fabrication.*—Three "Liquid Membranes" were separately prepared in a glassy petri dish (5 cm diameter). First, for sensor (I) preparation, 350 mg of nitrobenzene as a plasticizer was mixed with 190 mg of PVC and 10 mg of potassium tetrakis (4-chlorophenyl) borate, and then mixed thoroughly with 5 ml of THF until homogeneity was achieved. For sensor (II) preparation, the same components as sensor (I) were used, but with the addition of 40 mg β-CD as an ionophore. However, for the sensor (III) preparation, an extra 50 mg of CoO-NPs was added to the same constituents used in Senor (II). All ingredients were mixed and dispersed well. Finally, all the membranes were left to solidify for 24 h at room temperature before constructing their corresponding sensors.

Solid contact (SC) sensors fabrication.—Three "solid sensors" were prepared in a glassy mortar using the ratio of total solid powder to liquid paraffin (80:20 v/v). Traditional sensor (IV) was prepared using the ion pair potassium tetrakis (4-chlorophenyl) borate, graphite powder, and paraffin oil in ratios of 5%, 65%, and 30% respectively. For sensor (V) preparation, β-CD was added to the same constituents used in Senor (IV). Finally, the advanced sensor (VI) was prepared by incorporating CoO-NPs into all previously mentioned constituents in sensor (V). All the components were well dispersed homogeneously by continuous mixing for at least 15 min before constructing their corresponding sensors.

Assembly of LC-IS working sensor.—A disk (10 mm in diameter) was cut using a cork borer from the LC membrane and adhered on 1.5 cm micro plastic tips by THF, forming the LC-ISE. An internal solution composed of equimolar concentrations of KCl and TGC (1.0×10^{-3} M). LC-ISE should be preconditioned by soaking it overnight (24 h) in a 1.0×10^{-3} M TGC solution. The electrical connection was accomplished through a crocodile cable, which joined the silver wire inside the internal solution with the copper wire inside the potentiometer.

Assembly of SC-IS working sensor.—The homogenous constituents formed a creamy paste, where a portion taken from each membrane was separately and compactly packed (without any gap formation) in a plastic insulin syringe with dimensions 2.0 mm in diameter and 3.0 mm in depth. SC-ISE was preconditioned by soaking overnight (24 h) in a 1.0×10^{-3} M TGC solution. The SC-ISE surface was polished using soft sandpaper before being used for the measurements. An electrical connection was achieved through copper wire insertion into the syringe hole for the composite linkage. Regarding sensor renewal, manual pressure was applied through the syringe piston, and it was then re-polished.

Construction of calibration curves.—All measurements were performed by dipping the constructed sensors, along with Ag/AgCl as a reference electrode, in 100 ml beakers containing 50 ml of various molar concentrations of TGC ($1.0 \times 10^{-7} - 1.0 \times 10^{-2}$ M) ranging from low to high concentrations. Continuous stirring was used to achieve reading equilibration. Calibration curves were created by graphing potential values (mV) against the concentration logarithm.

Effect of pH.—The pH effect was studied at two different concentration levels (1.0×10^{-3} and 1.0×10^{-4} M) of TGC solutions, adjusted with phosphate buffer at pH values ranging from 2.0 to 10.0 at 1 pH unit interval. The pH was adjusted to get the appropriate values using either HCl or KOH. Finally, the measured potential at each pH value was recorded.

Selectivity of sensors.—Using the separate solutions approach, the potentiometric selectivity coefficient Log K^{pot}_{AB} (primary ion, interfering ion) of several inorganic and organic species was evaluated. The potentials were separately measured for standard TGC solution and aqueous solutions of interfering ions at equimolar concentrations (10^{-3} M). The following equation was used to calculate the potentiometric selectivity coefficients: Log K^{pot}_{AB} = (EB–EA)/S, where K^{pot}_{AB} is the potentiometric selectivity coefficient, EA and EB are the EMF reading of 10^{-3} M solutions of each drug and its interferents, respectively, and S is the slope (mV/decade) of the calibration plot.

Potentiometric determination of TGC in Tygacil® vials.—Different portions of Tygacil® lyophilized powder (29.30 mg & 2.93 mg) were transferred into two 50 ml volumetric flasks and each was filled to the mark with phosphate buffer at pH 5 to prepare samples with conc. of 1.0×10^{-3} and 1.0×10^{-4} M respectively. Potentiometric measurements were applied using the advanced CoO-NP SC sensor (sensor VI) in conjunction with an Ag/AgCl reference electrode. The potential results were then compared with those of the calibration plots.

Potentiometric determination of TGC in spiked human plasma.—Into two stoppered shaking tubes, plasma samples (4.5 ml) were placed, and then 0.5 ml of 1.0×10^{-4} and 1.0×10^{-5} mol l^{-1} TGC solutions were added and shaken separately. The advanced CoO-NP SC sensor (sensor VI) in conjunction with the Ag/AgCl reference electrode was dipped directly into the prepared solutions, excluding any matrix pretreatment. The resultant potentials were recorded, and the corresponding regression equations calculated the respective concentrations.

Potentiometric determination of TGC in the presence of its degradation products.—Into a series of 10 ml volumetric flasks, different volumes of the intact drug were transferred and mixed with its degradation products. Then, volumes were completed with phosphate buffer to the mark. Potentiometric measurements were carried out in different prepared mixtures containing a fixed concentration of TGC (1.0×10^{-4} M) while varying concentrations of either its acidic, alkaline, or thermal degradation products in phosphate buffer (pH 5.0). The potential of each mixture was separately recorded with the advanced CoO-NPs sensor (sensor VI) and the concentration of TGC was calculated from the corresponding regression equations.

Results and Discussion

Surface morphology of SC sensors by TEM.—High-resolution TEM was used to characterize graphite powder, CoO-NPs, and their mixtures. The results showed that the CoO-NP particles were in the nano-range (≈22 nm) and uniformly dispersed in the graphite powder (Fig. 2). This contributed to the conductance enhancement and provided better sensitivity when used, as discussed in a later section.

Sensors optimization.—TGC is an organic compound containing amino groups (Fig. 1) with cationic exchange capacity. The cationic drug was transferred across the membrane using a PVC membrane loaded with tetrakis (4-chlorophenyl) borate as an anionic salt. The two phases should be mostly electro-neutral, and the lipophilic cationic exchanger in the membrane greatly reduces co-ion extraction, resulting in a typical Nernstian reaction.

Regarding LC-ISEs, the PVC matrix serves as a constant support and traps ion-association complexes. Thus, a nitrobenzene plasticizer is needed as a solvent regulator, aiding in the membrane's malleability, modifying the ion-distribution exchanger's constant, and helping to adjust the membrane permittivity. This affords high selectivity and sensitivity. In SC-ISEs, graphite powder is used as a carbon source and paraffin oil is used as a plasticizer, with tetrakis (4-chlorophenyl) borate as an ion exchanger.

Performance characteristics of the proposed sensors.—Sensor (I), the traditional LC-ISE sensor with a tetrakis (4-chlorophenyl) borate cationic exchanger, showed a narrow linearity range (1.0×10^{-4} M − 1.0×10^{-2} M). Regarding sensor (II), β-CD as an ionophore was added as a trial to improve the linearity range, as reported previously.[26] However, no improvement was observed, which may be attributed to the high molecular weight of TGC and its inability to fit into the β-CD cavity. Concerning senor (III), CoO-NPs, a reported modifier, were used to increase the selectivity and sensitivity of the sensors, as reported in the literature;[27] however, no further improvement took place.

Because the three LC-ISEs showed a narrow linearity window (Fig. 3a), shifting to the SC design was a good, processed choice. Consequently, the results improved as the linearity ranges were (1.0×10^{-5} M − 1.0×10^{-2} M) for sensors (IV) and (V) and (1.0×10^{-6} M − 1.0×10^{-2} M) for sensor (VI).

Sensor (IV), the traditional SC-ISE sensor, showed good linearity as graphite powder has a large surface area, high electron transfer capability, superior hydrophobicity, and stable chemical characteristics, which may aid in enhancing the paste homogeneity. Accordingly, the electrochemical properties of the sensors were improved. Moreover, there is no inner filling solution, as in the LC design, and thus there is no inner interface. Therefore, the ion-exchange process occurs near this junction, and the potential difference is measured. The ion-exchange process takes place around this interface, and the potential difference is measured solely across this interface, ignoring the effect of diffusion potential.[28,29]

The addition of β-CD to graphite in sensor (V) did not show any enhancement to its response. On the contrary, the incorporation of CoO-NPs in sensor (VI) increased its sensitivity 10 folds, due to the great surface area and mechanical strength of the selected NPs, as well as their super-lightweight, diverse electrical characteristics, thermal stability, and outstanding chemical properties.[30]

The electrochemical performance characteristics of sensors (IV), (V), and (VI) were investigated, as shown in Table I, according to IUPAC standards.[31] The three studied sensors showed constant potential values daily for four weeks and the calibration slopes changed by 2 mV per decade. The slopes of sensors (IV), (V), and (VI) are 28, 32.5, and 30 mV per decade, respectively. Figure 3b shows the calibration curves for the three sensors. Their detection limits were calculated in accordance with IUPAC criteria.[31]

Validation of the method was carried out in accordance with ICH criteria.[32] The proposed method (SC-ISE) was linear in the concentration range of (1.0×10^{-5} M–1.0×10^{-2} M) for sensors (IV) and (V); however, (1.0×10^{-6} M–1.0×10^{-2} M) for sensor (VI) of TGC standard solutions in triplicate. The correlation coefficients were close to unity, indicating high linearity. The accuracy of the results was verified using SC-ISE sensors by determining five different concentrations of pure TGC. Recovery percentages were determined from the corresponding regression equations and were found to be 100.73 ± 0.610, 99.62 ± 0.773, and 99.27 ± 0.802 for sensors (IV), (V) and (VI) respectively (Table I). Precision results were obtained by using the proposed SC sensors (IV, V, and VI) to calculate three concentrations of TGC three times within their linearity ranges (intra-day/repeatability) and on three successive days (inter-day/intermediate precision). Robustness was tested by making small pH changes, as stated in Table I.

Effect of pH.—A pH study ranging from 2 to 10 was carried out on (at 1.0×10^{-3} M and 1.0×10^{-4} M) of TGC to determine the optimal pH values required for SC-sensor activation. TGC (Fig. 1) has been reported to possess five ionizable groups (two acidic and three basic).[33] The reported pKa values of TGC are variable because of the presence of basic and acidic ionizable groups (2.8, 4.4, 7.4, 8.9, and 9.5).[34] The pKa value of 9.5 (strongest basic) and 2.8 (strongest acidic). Therefore, at pH $>$ 8, the TGC started to exist in the deprotonated form, and a remarkable drop in the readings occurred (Fig. 4). At pH $<$ 3, the TGC would be in protonated form with a small amount of the zwitterionic form. In addition, at pH $<$ 2, the ion pair reacts with the protons found in highly acidic media instead of reacting with the drug. The results showed that the sensor responses were stable over a pH range of 4–8, and the optimum working pH range of the sensors was adjusted to 5.

Sensors selectivity.—Selectivity is a critical feature of ISEs. It represents the ability of the sensor to detect target ions in complicated matrices. The selectivity of TGC over interfering ions is influenced by the ion exchange process at the sensor/sample

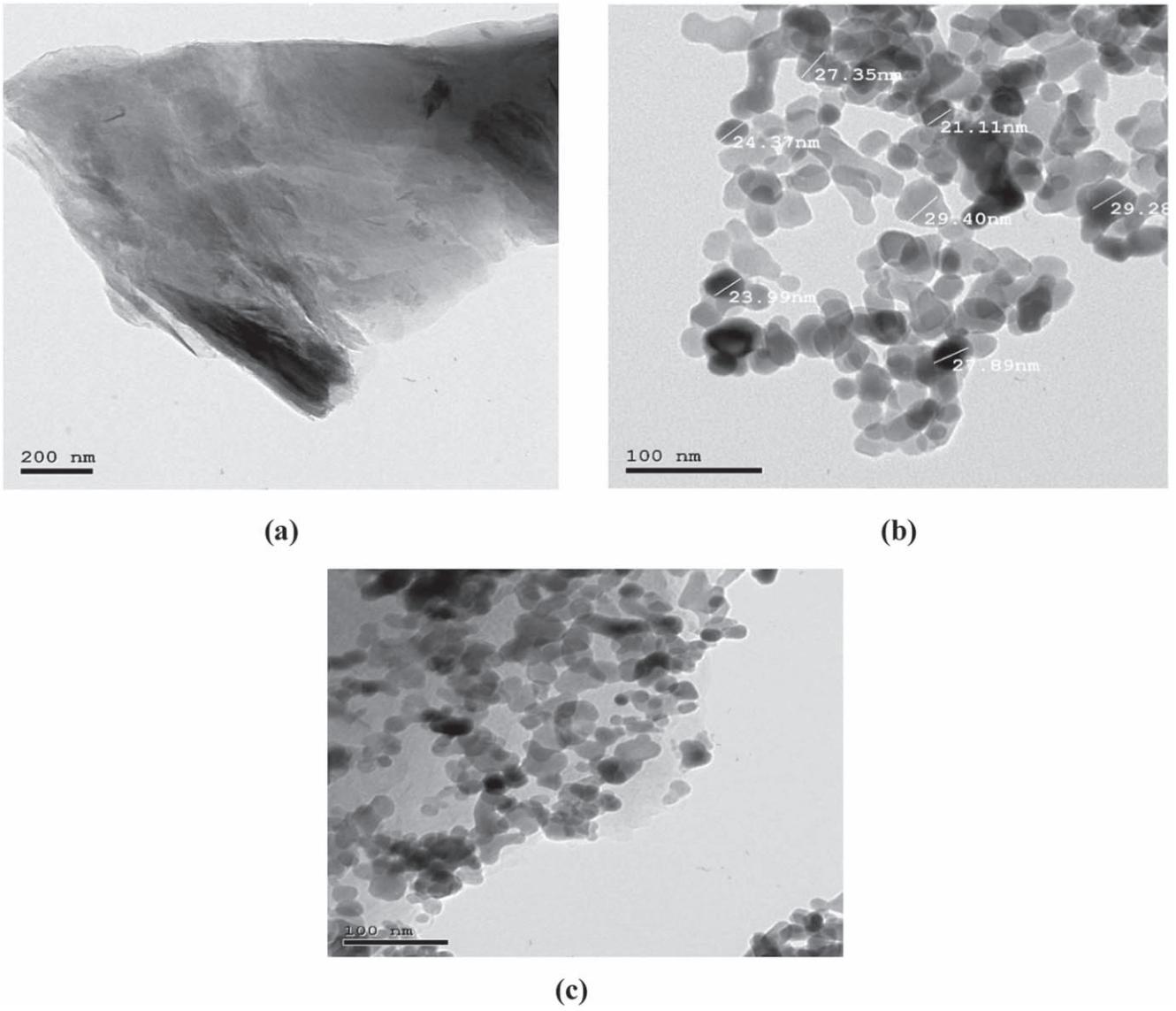

Figure 2. Transmission electron microscopy images of materials used in sensors: (a) Graphite powder only, (b) CoO-NPs only, and (c) Graphite powder mixed with CoO-NPs.

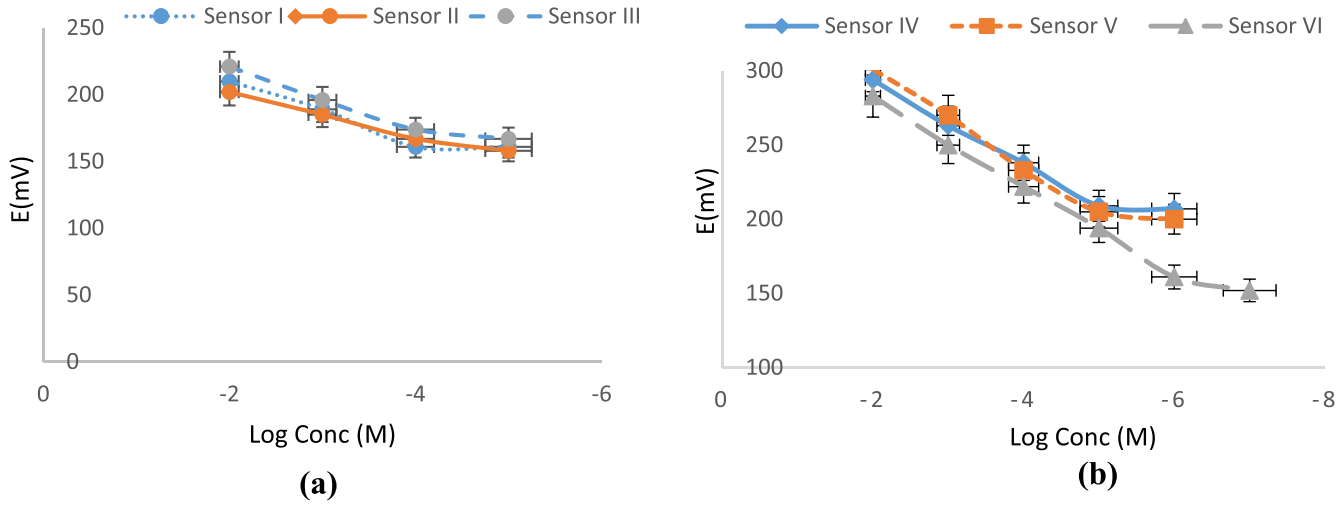

Figure 3. Profile of the potential in mV to log concentration of Tigecycline using: (a) liquid contact sensors and (b) solid contact sensors.

Table I. Electrochemical response characteristics of the investigated sensors.

Parameters	Traditional SC-sensor (sensor IV)	β-CD SC-sensor (sensor V)	Advanced SC-sensor (sensor VI)
Slope (mV/decade)[a)]	28	32.5	30
Intercept (mV)	349	366	342
LOD (mol. L^{-1})[b)]	1.1×10^{-5}	1.5×10^{-5}	1.6×10^{-6}
Response time (s)	45	45	45
Working pH range	4–8	4–8	4–8
Concentration range (M)	10^{-5} M – 10^{-2} M	10^{-5} M – 10^{-2} M	10^{-6} M – 10^{-2} M
Stability (weeks)	4 weeks	4 weeks	4 weeks
Correlation coefficient (r)	0.9985	0.9975	0.9989
Accuracy[c)]Mean ± S.D.	100.73 ± 0.610	99.62 ± 0.773	99.27 ± 0.802
Precision (RSD%)	0.863	0.994	0.747
Repeatability[c)] Inter-day precision[c)]	1.890	1.420	1.511
Robustness[d)]	1.435	1.231	1.850

a) Result of five determinations. b) Limit of detection (measured by interception of the extrapolated arms of calibration plots). c) Average of nine determinations of 3 concentrations of each drug (10^{-2}, 10^{-3} and 10^{-4} M). d) Average of nine determinations of 3 concentrations of each drug (10^{-2}, 10^{-3} and 10^{-4} M) at different pH levels (±0.5).

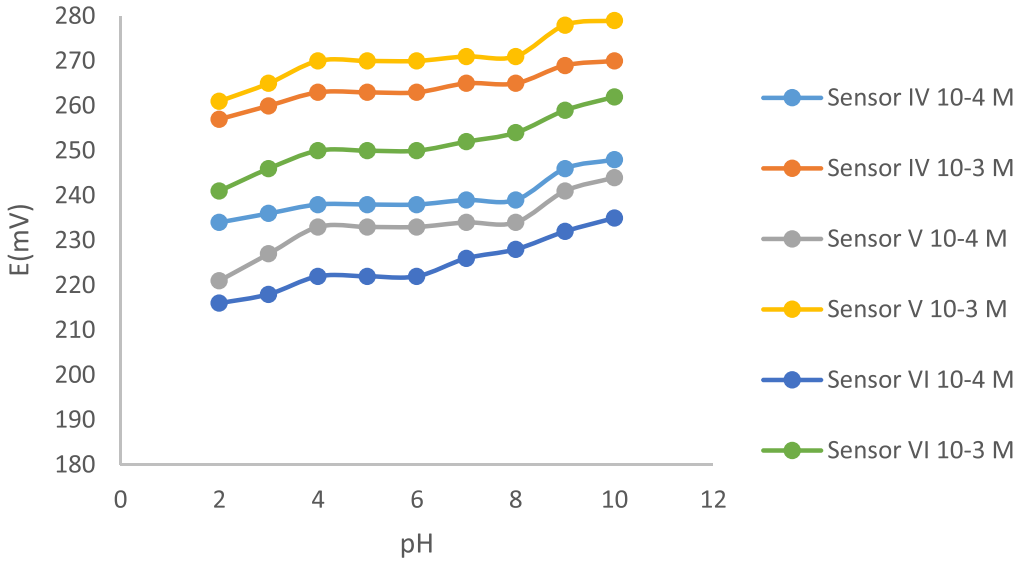

Figure 4. Effect of pH on the response of the proposed sensors at concentrations 10^{-3} and 10^{-4} M.

interface, ion mobility at the interface, and hydrophobic contacts between the ions of the analyte and the sensor surface.

A separate approach was used to assess the sensors when numerous common interfering substances were present.[31] The suggested sensors were tested for selectivity in the presence of TGC degradation products, various inorganic species (K^+, Na^+, NH_4^+, Mg^{2+}, and Ca^{2+}), and organic species found in pharmaceutical additives (lactose monohydrate) or plasma. The three SC-sensors were found to have greater selectivity for the determination of the drug in the presence of its degradation products, organic and inorganic interferents, as the selectivity coefficients (K) are low as shown in Table II, none of the studied species interfere. Inorganic species have greater mobility and permeability due to differences in ionic size, mobility, and permeability in comparison with TGC. In addition, when the selectivity of the suggested sensors was tested in the presence of a structurally related compound (tetracycline), the selectivity coefficients obtained for all sensors were of the order of 10^{-2}, showing that they did not significantly disrupt the functioning of TGC-ISE. This could be explained by the fact that TGC ions are easily exchanged into the organic membrane phase, and tetracycline has a lower lipophilic character, which affects its partition coefficient into the membrane. The addition of β-CD either alone (sensor V) or in combination with CoO-NPs (sensor VI) did not further improve the selectivity.

Response time.—The response time is a significant component of the ISE's analytical application. In this work, it was measured by soaking the sensor in multiple TGC concentrations (1.0×10^{-5} M–1.0×10^{-2} M) and recording the time taken to attain values within 2 mV of the ultimate stabilized potential. The three SC sensors had a response time of less than one minute around 45 s. The absence of a water film in the graphite-based sensors explains the quick response time, especially at low concentrations. As a result, the suggested method saves time and is appropriate for TGC applications.

Potentiometric determination of TGC in Tygacil® vials.—An advanced CoO-NP SC sensor (sensor VI) was successfully used for the direct determination of TGC in Tygacil® vials without any pretreatment steps (Table III). The lactose excipient had no effect on the results, and the found % was within the accepted limits.

Potentiometric determination of TGC in spiked human plasma.—The TGC determination in spiked human plasma results revealed that the advanced CoO-NP SC-sensor (sensor VI) can determine a wide concentration range of the drug with good accuracy, without the need for protein precipitation as a treatment process. The plasma was spiked with two concentrations (1.0×10^{-5} M) above the reported C_{max} and one concentration ($1.0 \times$

Table II. Potentiometric selectivity coefficients (Log K^{pot}_{AB}) of the three proposed sensors were calculated by separate selectivity method.

Interferent[a)]	Selectivity coefficient[b)]		
	Traditional SC-sensor (sensor IV)	β-CD SC-sensor (sensor V)	Advanced SC-sensor (sensor VI)
Tetracycline	23×10^{-2}	19.94×10^{-2}	32.9×10^{-2}
Sodium	3.10×10^{-2}	3.54×10^{-2}	3.35×10^{-2}
Potassium	2.17×10^{-2}	4.13×10^{-2}	2.91×10^{-2}
Calcium	4.47×10^{-2}	2.73×10^{-2}	2.36×10^{-2}
Magnesium	4.07×10^{-2}	2.34×10^{-3}	1.66×10^{-2}
Cobalt	2.26×10^{-2}	2.11×10^{-3}	1.59×10^{-2}
Zinc	1.90×10^{-2}	2.54×10^{-2}	1.15×10^{-2}
Glucose	3.98×10^{-3}	2.07×10^{-3}	1.56×10^{-3}
Lactose	2.17×10^{-3}	2.39×10^{-3}	1.15×10^{-3}
Alanine	9.40×10^{-3}	1.13×10^{-3}	4.87×10^{-3}
Lysine	8.60×10^{-3}	1.68×10^{-3}	5.10×10^{-3}
Acidic degradation product	6.24×10^{-2}	4.56×10^{-2}	4.32×10^{-2}
Alkaline degradation product	4.58×10^{-2}	2.98×10^{-2}	1.78×10^{-2}

a) Aqueous solutions of 1.0×10^{-3} M were used. b) Each value is the average of three determinations.

Table III. Analysis of pharmaceutical dosage forms and spiked human plasma samples using the advanced CoO-NP solid contact sensor.

Tygacil® vials	TGC Found%
10^{-3} M	102.15 ± 1.23
10^{-4} M	99.63 ± 0.65
Spiked human plasma	**Recovery%**
10^{-3} M	97.66 ± 1.56
10^{-4} M	98.14 ± 1.86
10^{-6} M	99.64 ± 2.08

*The values represent mean recovery percentages ± standard deviations of six readings.

10^{-6} M) below the reported C_{max} of TGC in human plasma,[12] where the results obtained were accurate and within the accepted range as shown in Table III. The pH of the plasma sample was 7 ± 0.5, which was within the pH range of the proposed sensor. Between measurements, the sensing component was rinsed with bi-distilled water to prevent it from sticking to the surface of some of the matrix components. The proposed sensors can be successfully used in in vitro experiments and clinical applications.

Potentiometric determination of TGC in presence of its degradation products.—The acidic/alkaline hydrolysis of TGC leads to amidic linkage cleavage on both sides, resulting in compounds (A) and (B). The suggested degradation pathway is illustrated in Fig. 5.

Laboratory prepared mixtures were formulated to contain aliquots of the intact drug together with its degradation product, varying percent ratios from 100:0 to 10:90 respectively, for example the ratio drug: degradation product (90:10) contains 4.5 ml drug and 0.5 ml degradation product. The advanced CoO-NP SC sensor was effective for the selective determination of intact drugs in the presence of up to 60%, 70%, and 80% acidic, alkaline, and thermally induced degradation products, respectively (Table IV).

Statistical analysis.—SC-ISE sensor data were statistically compared to those obtained using the *British Pharmacopeial* chromatographic method for TGC pure sample determination[3] using Student's t-test and F-value, where no significant difference was observed (Table V).

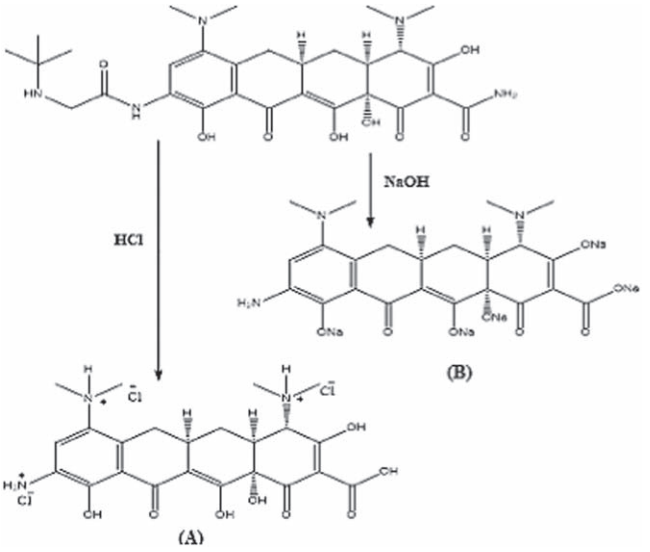

Figure 5. Suggested degradation pathway for (A) acidic and (B) alkaline degradation products.

Green assessment profile.—Pharmaceutical analysis is still looking for greener ways to reduce the amount of organic waste produced and to move away from traditional analytical separation technologies that generate roughly 50 ml of trash for each analytical data point. A semi-quantitative analytical eco-scale was used to determine the greenness of the proposed method.[25] The analytical eco-scale was calculated by deducting the penalty points from 100 for each component of the analytical technique. The ideal green technique recorded an eco-scale value of 100, the outstanding green method recorded an eco-scale value of >75, and the acceptable green method recorded an eco-scale value of >50 according to its standards. If the procedure yields a value of 50 or less on the eco-scale, it is regarded as insufficiently green.

The penalty points for the proposed electrochemical method using SC-ISE, LC-ISE, and the official method for determining the TGC[3] were calculated. Electrochemical methods using SC sensors are the greenest, with 95 points when compared with the constructed liquid contact sensors or the official chromatographic method (Table VI).

Table IV. Determination of Tigecycline in laboratory prepared mixtures containing different ratios of Tigecycline and its induced degradation products by the advanced CoO-NP SC-sensor.

Ratio drug: degradation product	Drug recovery (%) ± S.D [a]		
	Acidic degradation	Alkaline degradation	Thermal degradation
100:0	100.13 ± 1.143	100.13 ± 1.143	100.23 ± 1.13
90:10	99.87 ± 1.47	99.87 ± 1.47	99.87 ± 1.47
80:20	99.11 ± 0.87	99.11 ± 0.87	99.11 ± 1.17
70:30	101.49 ± 0.97	100.09 ± 1.07	100.29 ± 1.27
60:40	101.72 ± 1.25	100.41 ± 0.64	100.78 ± 1.63
50:50	103.4 ± 1.37	100.68 ± 1.09	100.48 ± 1.17
40:60	105.4 ± 1.63	101.36 ± 0.92	101.26 ±0.71
30:70	113.3 ± 1.77 [b]	103.68 ± 1.39	101.88 ± 1.74
20:80		107.18 ± 1.48 [b]	104.78 ± 1.47
10:90			109.39 ± 1.94 [b]

a) Average of three determinations. b) Rejected.

Table V. Statistical comparison of the results obtained by the proposed sensors and the official method on pure form.

Items	TGC-official method [a]	Traditional SC-sensor (sensor IV)	β-CD SC-sensor (sensor V)	Advanced SC-sensor (sensor VI)
Mean	100.48	100.34	99.96	100.83
SD[c]	1.03	1.68	1.69	1.54
Variance	1.06	2.82	2.85	2.37
n	5	4	4	5
SEM[d]	0.462	0.839	0.843	0.690
Student's		0.150	0.567	0.426
t-test		(2.364)[b]	(2.364)[b]	(2.306)[b]
F value		2.64	2.67	2.23
		(6.59)[b]	(6.59)[b]	(6.39)[b]

a) *British Pharmacopeial* method. b) Figures between parentheses represent the corresponding tabulated values of t and F at P = 0.05. c) Standard deviation. d) Standard error of the mean.

Table VI. Penalty points for greenness assessment of the proposed potentiometric method using liquid contact and solid contact sensors compared to the official method.

Hazard reagents	Penalty points		
	LC-ISE	SC-ISC	Official method [a]
Tetrahydrofuran (5 mls)	6	—	—
Phosphate buffer	—	—	0
Phosphoric acid (1 ml)	—	—	2
Acetonitrile (14 mls)	—	—	8
Paraffin oil (1 ml)	—	2	—
Instruments			
Energy	0 < 0.1 kWh per sample	0 < 0.1 kWh per sample	1 ⩽ 1.5 kWh per sample
Occupational Hazard	0	0	0
Waste	6	3	7
Total Penalty Points	Σ 12	Σ 5	Σ 18
Analytical Eco-Scale Total Score	**88**	**95**	**82**

[a] *British Pharmacopeial* method.

Conclusions

A novel, innovative, and green electrochemical method for determining the TGC was developed. A comparative study proved that SC sensors were a superior choice than LC sensors, as they showed higher sensitivity and stability. In addition, this study demonstrated the importance of cobalt oxide nanoparticle incorporation in sensor fabrication, as it showed high sensitivity (linearity from 1.0×10^{-6} M to 1.0×10^{-2} M) with a Nernstian slope of 30 mV decade^{-1}. Also, this advanced SC sensor was characterized using TEM, which ensured optimal homogeneity, uniformity, good dispersion, small particle diameter, and greater surface area, all of which contribute to better electrocatalytic linearity. This advanced SC sensor (sensor VI) was applied for the determination of the drug in its pharmaceutical dosage form, spiked human plasma, and in the presence of its acidic/alkaline and thermally induced degradation products. Finally, both types of sensors (LC and SC) were evaluated using a semiquantitative analytical eco-scale. SC sensors scored around 95 points, which was the highest greenness achievement score when compared to the LC sensors or even the *British Pharmacopeial* method.

Availability of data and material

The data are available from the corresponding author upon sensible request.

Competing Interest

The authors declare that there is no conflict of interest.

Ethics Approval and Consent to Participate

Ethics Committee approval from Cairo University number **AC 2754** was obtained. The study conducted in compliance with the International Guidelines for Research Ethics.

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Authors' Contributions

Conceptualization: Ghada M. El-Sayed, Heba T. Elbalkiny; **Methodology:** Nourhan A. Abd El-Fatah, Heba T. Elbalkiny; **Formal analysis and investigation:** Maha A. Hegazy, Manal Mohammed Fouad; **Writing - original draft preparation:** Nourhan A. Abd El-Fatah, Heba T. Elbalkiny; **Writing - review and editing:** Ghada M. El-Sayed, Maha A. Hegazy, Manal Mohammed Fouad; **Funding acquisition:** Not applicable; **Resources:** Nourhan A. Abd El-Fatah, Ghada M. El-Sayed, Heba T. Elbalkiny; **Supervision:** Maha A. Hegazy, Manal Mohammed Fouad; All authors have revised and approved the manuscript.

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