Adsorptive stripping voltammetric determination of Tetracycline in pharmaceutical capsule formulation using Poly(Malachite green) modified glassy carbon electrode

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\textbf{ABSTRACT}

A selective and sensitive electrochemical method based on glassy carbon electrode modified with poly(malachite green) was developed for determination of tetracycline in pharmaceutical capsule formulation. Cyclic voltammetry and electrochemical impedance spectroscopy using \([\text{Fe(CN)}_6]^{3-/4-}\) as a probe were used to characterize the potentiodynamically deposited poly(malachite green) on the surface of glassy carbon electrode. In contrast to the unmodified glassy carbon electrode, the fabricated poly(malachite green) modified glassy carbon electrode showed catalytic property towards two steps irreversible oxidation of tetracycline. Better correlation of the oxidative peak current with the scan rate than with the square root of scan rate indicated that the oxidation reaction of tetracycline at the modified electrode was predominantly controlled by electron exchange step at the solution polymer interface. Under optimized solution pH, and accumulation parameters, the square wave adsorptive anodic striping peak current response of the modified electrode showed linear dependence on concentration of tetracycline in the range 5–100 \(\mu\)M with determination coefficient, method detection limit, and quantification limit of 0.99588, 1.6 \(\mu\)M, and 5.3 \(\mu\)M, respectively. The tetracycline content of a capsule sample claimed to have 250 mg/capsule was found to be 250.53 mg/capsule with 0.21% deviation. Excellent spike recovery result of 99.80%, and 98.49–99.78% recovery of tetracycline in capsule sample in the presence of 50–200% of UA, AA, and ampicillin validated the applicability of the method for determination of tetracycline in real samples with complex matrix like capsule formulations.

1. Introduction

Tetracyclines constitute a family of well-known broad-spectrum antibiotics routinely used for prevention and control of diseases in human and veterinary medicine. They inhibit bacteria’s protein synthesis by blocking ribosome action against the linking of aminoacil-tRNA’s and destroy bacterial cellular membrane [1]. Moreover, they also find wide applications as a feed additive for cattle breeding; as an alternative drug for people allergic to penicillin; and in the control of plant diseases [1]. In human medicine, tetracycline antibiotics also have been used for the treatment of different types of infections, including respiratory tract infections, urethritis and severe acne [2]. In the past decade, tetracyclines have been the drug of choice for treatment of malaria due plasmodium falciparum [3]. Because of their board spectrum activity and low production cost; the four tetracyclines (tetracycline, chlortetracycline, oxytetracycline, and doxycycline) are commonly used in food protection animals including honeybee [4].

Tetracycline (TC) can adsorb strongly onto environmental materials, where it still keeps its activity [5]. TC (Figure 1), which has significant effect on gram-positive and gram-negative bacteria, is widely used as an important antibiotic to control bacterial infections in both animal models and clinical studies [6, 7]. This antibiotic molecule is found in some food products, such as milk [8] and egg [9]. In spite of its use for prevention and control of many bacterial diseases, tetracycline has gastrointestinal side effects, nausea, vomiting, and diarrhea [10]. It is known as a hepatotoxic C agent in pregnant women leading to serious threat to human health [11, 12]. Various studies indicated that even low-level dose of TC for long period could show bacteria resistance [13] necessitating...
continuous monitoring of its level in different samples including pharmaceutical formulations.

Microbiological method [14], high–performance liquid chromatographic method (HPLC) with different detectors [15, 16, 17, 18], have been reported for determination of tetracycline in real samples. Although these methods are known of their sensitivity and accuracy, their high cost and complicated operation procedure limited their extensive application [19]. Therefore, the need to develop a sensitive, accurate, less expensive and easy operation method for determination of TC in different samples is still important.

Electrochemical methods, which are simple, fast, sensitive, inexpensive, environmentally friendly, have been known as potential methods for tetracycline determination [19]. Voltammetric methods using carbon-based electrodes modified with various materials [5, 20, 21, 22, 23, 24, 25, 26] have been reported for determination of TC in real samples. Most of the reported electrode surface modifiers are not readily available, are expensive, and the modification seem to be time taking. Thus, development of an alternative sensitive, selective and accurate electrochemical method using easily available surface modifiers is crucial.

Recently, numerous conjugated polymers (intrinsically conducting polymers) including malachite green have been electrochemically synthesized for chemical and biochemical sensors applications [27, 29]. Poly(malachite green) modified electrochemical sensors have been reported to exhibit interesting enhancement in the electrocatalytic activity towards the oxidation or reduction of selected electroactive species [29, 30, 31].

To the best of our knowledge, poly(malachite green) modified glassy carbon electrode has not been reported for determination of TC in pharmaceutical capsule sample. The aim of this work was thus to develop a sensitive, selective, and accurate stripping voltammetric method based on poly(malachite green) modified glassy carbon electrode (PMG/GCE) for determination of tetracycline in pharmaceutical capsule formulation.

2. Experimental part

2.1. Chemicals and reagents

Tetracycline (99.81%, sigma Aldrich, USA), malachite green (Hime- dia Laboratories Pvt. Ltd., ethanol (97%, Fine Chemical General Trading, Addis Ababa, Ethiopia), sulfuric acid (98%) and hydrochloric acid (35.4%) both from Loba Chemie Pvt.Ltd, disodium hydrogen phosphate (99%) and sodium dihydrogen phosphate (97%) both from Sisco Research Laboratories Pvt.Ltd, ascorbic acid (99%) and sodium hydroxide (97%) both from Blulux Laboratories Ltd were used. All chemicals were of analytical grade that they were used without further purification. Distilled water was used throughout the experiment.

2.2. Apparatus

Electrochemical experiments were conducted using CHI760E electrochemical workstation (CH Instruments, Austin, USA) with a conventional three-electrode system at room temperature. PMG/GCE or bare glassy carbon electrode, platinum coil, and Ag/AgCl (3 M KCl) were employed as working, auxiliary, and reference electrode, respectively. The pH meter (Adwa model AD800), electronic balance (Denver Instrument), and refrigerator were used to adjust the pH of solution, measure mass, and preserve samples, respectively.

2.3. Fabrication of PMG/GCE

PMG film was deposited on the surface of glassy carbon electrode following reported procedure with minor modification [28]. Briefly: prior to electropolymerization, the surface of glassy carbon electrode was polished consecutively with alumina slurries of different course sizes (1.0, 0.3, and 0.05 μm) to a mirror like surface. Electropolymerization of malachite green on the surface of the polished GCE was conducted by scanning the potential of the GCE in 10 mM malachite green monomer dissolved in pH 6 PBS containing 0.5 M NaNO3 between -1.4 to 1.8 V at a scan rate of 0.1 V s⁻¹ for 10 cycles. After rinsing the electrode copiously with distilled water, and scanning in monomer free 0.5 M sulfuric acid between -0.8 to +0.8 V until steady voltammogram was obtained, the fabricated PMG/GCE was ready for use.

2.4. Standard preparation

The phosphate buffer solution (PBS) was prepared by mixing equi-
molar (0.1 M) of NaH2PO4 and Na2HPO4 the pH of which was adjusted by adding 0.1 M NaOH or HCl. Tetracycline stock solution (1 mM) was prepared by dissolving 0.024 g in 50 mL of volumetric flask with ethanol. The working solution (0.1 mM) and the various calibration standard solutions of TC were prepared from the stock solution through serial dilution with PBS of the appropriate pH.

2.5. Real sample preparation

Tetracycline capsule sample was prepared following the reported procedure [1] with minor modification. Briefly: TC capsules (EPHARM brand) with a nominal content of 250 mg/capsule were purchased from a pharmacy in Bahir Dar city, Ethiopia. The powder collected from five randomly selected capsules was weighed to acquire the mean mass and then homogenized using a mortar and pestle. Capsule stock solution was prepared by dissolving an appropriate amount of the powder (about 30 mg) in ethanol to a final volume of 100 mL. After filtering for removing insoluble particles, nominal 56.98 μM capsule sample solution was prepared from the capsule stock solution using PBS pH 7.5 for determination, recovery and interference studies.

3. Results and Discussion

3.1. Fabrication and characterization of PMG/GCE

Cyclic voltammograms of glassy carbon electrode in pH 6.0 PBS containing malachite green are presented in Figure 2. As can be seen from the figure, the peak current of the cathodic (peak-1) and anodic (peak-2) peaks that appeared at -0.5, and 0.9 V, respectively increased with increasing scan cycles indicating polymer film growth.

Inset of Figure 2 (curves a & b) depicts the CVs of bare GCE (a) and PMG/GCE (b) in a monomer free 0.5 M of H2SO4 solution. While peak-1 (curve a) at -0.6 V and peak-1’ (curve b) at -0.4 V are assigned to reduction of molecular oxygen at the unmodified and modified GCE, respectively, couple of redox peaks (peak-2 & peak-2’), which are distinct to the PMG/GCE, indicated deposition of a redox active polymer film at the surface of the GCE. The observed lower oxygen reduction potential (-0.4 V) at the PMG/GCE relative to its value at the unmodified GCE (-0.6 V) is clear indication of catalytic behavior of the modified surface of the electrode and hence PMG polymer film.
3.2. Characterization of PMG/GCE

The modified electrode was characterized by two techniques; cyclic voltammetry and electrochemical impedance spectroscopy using [Fe(CN)6]3-/4- as a probe.

3.2.1. Cyclic voltammetric characterization

Cyclic voltammetry using [Fe(CN)6]3-/4- as a probe was used to characterize the poly(malachite green) modified glassy carbon electrode (Figure 3). Appearance of couple of redox peaks in opposite scan directions at both electrodes with comparable peak current but with lower peak-peak separation at the PMG/GCE (ΔE 170 mV) than at the unmodified electrode (ΔE 337 mV) indicated catalytic property of the PMG/GCE towards the Fe3+ ⇔ Fe2+ reaction and hence confirming the surface modification of the electrode.

3.2.2. EIS characterization

Electrochemical impedance spectroscopy (EIS) data enables to evaluate the electrical nature of the electrode surface including the double layer capacitance, charge transfer resistance, solution resistance and hence nature of the modifier. Nyquist plots of the unmodified (a) and modified (b) glassy carbon electrodes are presented in Figure 4. As can be seen from the figure, both electrodes exhibited combination of semicircle of different diameters at high frequency region and linear line at low frequencies. The semicircle portion at higher frequencies corresponds to the electron transfer limited process, and the linear portion at lower frequencies corresponds to the diffusion process [32].

3.3. Cyclic voltammetric investigation of TC at PMG/GCE

3.3.1. Electrochemical behavior of TC at PMG/GCE

Figure 5 shows cyclic voltammograms of bare GCE and PMG/GCE in the absence and presence of 0.1 mM TC in pH 7.0 PBS. While two extremely weak and broad oxidative peaks (a & b) are observed at the unmodified GCE (curve A of Inset), appearance of two well resolved oxidative peaks (a’ & b’) with significantly enhanced peak current at the PMG/GCE (curve B of Inset) showed catalytic property of the polymer modifier towards oxidation of TC. In contrast to the broad peaks (a and b of curve A) centered at 786 and 1023 mV, respectively at the unmodified electrode, appearance of the same peaks (a’ and b’ of curve B) at the modified electrode at much reduced potentials (730 and 924 mV, respectively) could also be taken as supporting evidence for the catalytic property of the modifier towards oxidation of TC.

3.3.2. Effect of scan rate on peak potential and peak current

Cyclic voltammograms of PMG/GCE in pH 7.0 PBS containing TC at various scan rates (20–300 mV s−1) are presented in Figure 6. While observed oxidative peak potential shift in the positive direction for both peaks (a & b) with increasing scan rate confirmed the irreversibility of the oxidation reactions, better correlation of peak current on scan rate (R2 0.9978) (Figure 7A) than on square root of scan rate (R2 0.9616) (Inset of Figure 7A) indicated participation of both adsorption and diffusion mass transport with predominantly the adsorption controlled mechanism, which is supplemented by slope of 0.60 (Figure 7B) for plot of log ip vs log scan rate.
3.4. SWV investigation of TC at PMG/GCE

The electrochemical oxidation of TC in pH 7.0 PBS was further investigated using two pulse techniques: square wave voltammetry and differential pulse voltammetry. The peak current response of the two techniques for 0.1 mM of TC in pH 7.0 PBS was almost the same (Inset of Figure 8). Thus, for purpose of shortening analysis time, square wave voltammetry was selected for quantification of TC in capsule formulation.

Figure 8 presents corrected for blank square wave voltammograms of 0.1 mM TC in pH 7.0 PBS at unmodified GCE (curve A), and PMG/GCE (curve B). Two oxidative peaks still appear at both electrodes, with nearly threefold enhanced current response at the PMG/GCE showing the sensitivity of the method for TC determination.

3.4.1. Effect of solution pH on peak current and peak potential

Investigation of the effect of pH on the peak parameters (current and potential) is important to predict the possible reaction pathways. Figure 9 shows square wave voltammograms of TC in PBS of pH in the range 5.0—9.0. As can be seen from the figure, both peaks (a & b) showed potential shift in the negative direction with increasing pH indicating participation of protons in the oxidation reactions. The potential shift with pH value for both peaks showed linear dependence on pH in the studied range with slope of 0.059 (peak-a) (curve b of Figure 10) and 0.051 (peak-b) (curve d of Figure 10) showing participation of protons and electrons in exactly 1:1 ratio [2]. In agreement to previously reported works, an oxidation reaction mechanism of TC involving two electrons and two protons (Scheme 1) through the phenolic moiety with the subsequent addition of hydroxyl group was proposed [23].

Table 1. Summary of the calculated RC-circuit elements.

| Electrode Type | $R_s$ (Ω) | $R_{ct}$ (kΩ) | $C_{dl}$ (F) |
|----------------|-----------|--------------|-------------|
| Bare GCE       | 70        | 1.0          | 4.0 $\times$ 10$^{-6}$ |
| PMG/GCE        | 70        | 13           | 1.2 $\times$ 10$^{-6}$ |

Figure 5. CVs of unmodified GCE (a & b) and PMG/GCE (c & d) in pH 7.0 PBS containing no (a & c) and 0.1 mM TC (b & d) at a scan rate of 0.1 V s$^{-1}$. Inset: corrected for background current CVs of unmodified GCE (A) and PMG/GCE (B).

Figure 6. CVs of PMG/GCE in pH 7.0 PBS containing 0.1 mM TC at different scan rates (a–k: 20, 40, 60, 80,100, 125, 150, 200, 250, and 300 mV s$^{-1}$, respectively).

Figure 7. Plot of (A) Ipa versus $\nu$, (B) log(Ipa) versus log($\nu$) of 0.1 mM TC in pH 7.0 PBS at PMG/GCE. Inset: plot of oxidative peak current versus square root of scan rate.

Figure 8. Corrected for blank square wave voltammograms of (A) bare GCE, and (B) PMG/GCE in pH 7.0 PBS containing 0.1 mM TC. Inset: voltammograms of DPV (curve 1) and SWV (curve 2) of PMG/GCE in pH 7.0 PBS containing 0.1 mM of TC.
Although is accompanied with peak shape distortion, peak current for both peaks (curves a & c of Figure 10) increased with pH from 5.0 to 7.5 and then decreased at values beyond 7.5. Comparing the two characteristic peaks of TC, peak (a) that appears at a potential region where capacitive current response of the electrode is minimum was chosen for quantification. Thus, pH 7.5 that gives a well defined peak with the highest current was selected for further analysis.

The observed current response trend of the PMG/GCE for TC could be explained considering the pKa of TC (pKa 3.3, 7.7 and 9.5) [33] and pKa of malachite green (pKa 6.9 & 10.3) [34]. As can be seen from curves (a & c of Figure 10), while the increasing trend with pH from pH 5.0 to 7.5 might be ascribed to the electrostatic attraction between the positive charged TC and increasing negative charge density of malachite green, the decreasing current beyond pH 7.5 might be ascribed to repulsive forces between the two.

3.5. Optimization of accumulation parameters

As the rate determining step is found to be predominantly adsorption controlled, optimization of the accumulation potential (Eacc) and accumulation time (tacc) is crucial.

3.5.1. Eacc

Figure 11 shows the effect of varying the accumulation potential from 450 to 650 mV on the anodic peak current of the two oxidative peaks keeping accumulation time 10 s. As can be observed from the inset, the oxidative current response increased with increasing Eacc from 450 to 600 mV after which the resulting peak current was declined. Therefore, Eacc of 600 mV was chosen for further experiments.

3.5.2. tacc

Figure 12 presents square wave voltammograms of TC at Eacc of 600 mV applied for various length of time. As can been observed from the inset of the figure, the peak current for both peaks increased with increasing the tacc although with different sensitivity. As a compromise between the peak current increment and analysis time, tacc of 20 s was chosen as the optimum value.

3.6. Analytical performance of the method

3.6.1. Variation of peak current with TC concentration

Under the optimized conditions, square wave adsorptive anodic stripping voltammograms (SWAdASVs) were recorded for series of standard solutions of TC (Figure 13). The oxidative peak current response of PMG/GCE for TC showed linear dependence on concentration over the range 5–100 μM (Figure 14) with regression equation, limit of detection (LoD = 3SDm/n; n 7) of Iρ (μA) = -0.252-0.014C (μM), (R² = 0.994), 1.6 μM and 2.1 μM, respectively for peak (a) and Iρ (μA) = -0.300-0.014C (μM), (R² = 0.996), 5.4 μM, and 7.02 μM, respectively for peak (b). The same slope for the two regression equations indicated that the sensitivity of the developed method for TC is the same whether peak (a) or peak (b) is considered.

3.7. Determination of level of TC in capsule formulation

The proposed SWAdASV method was used to determine the TC level in EPHARM brand capsule formulation. Peak (a), whose peak shape looks independent of pH and exhibited lower limit of detection, was used in the determination of the level of TC in capsule sample, spike recovery and interference recovery analysis.
Figure 15 presents the SWAdASVs of PMG/GCE in pH 7.5 PBS containing no TP (curve a), the studied capsule sample claimed to have 56.98 μM of TC (curve b), and the same sample solution spiked with 40 μM standard TC (curve c). Inset of Figure 15 presents the voltammograms for the unspiked and spiked capsule sample corrected for the blank. The detected level of TC in the capsule sample solution compared with the theoretical level (according to the label), and spike recovery results as calculated using the regression equation for peak (a) are summarized in Table 2. Detection of 57.10 μM of TC in capsule sample claimed to contain 56.98 μM, which is with an error of only 0.21%, indicated the extent of accuracy of the developed method and the capsule preparation.

To validate the reliability of the results, spike recovery and interference recovery analysis were conducted. While spike recovery was conducted for a capsule sample spiked with 40 μM of standard TC (curve 2 of Inset of Figure 15), interference recovery was investigated at 50, 100, and 200% of selected potential interferents (uric acid, ascorbic acid and ampicillin) (Figure 16). As can be seen from Table 2, detection of TC in capsule sample with a spike recovery result of 99.80%, and interference recovery results in the range 98.49 (for 200% of Amp) to 99.78% (for 50% of UA) indicated the reliability of the results and hence validated the applicability of the method for determination of TC in a real capsule formulation sample with a complex matrix.

3.8. Comparison of the present method with reported methods

The performance of the present method was compared with recently reported methods for determination of TC in terms of their linear dynamic range, and limit of detection parameters (Table 3). As can be seen from the table, the linear dynamic range of the present method (5–100 μM) is the second widest following the method using MWNT/COOH-GO/CPE [21], and 200% of selected potential interferents (uric acid, ascorbic acid and ampicillin) (Figure 16). As can be seen from Table 2, detection of TC in capsule sample with a spike recovery result of 99.80%, and interference recovery results in the range 98.49 (for 200% of Amp) to 99.78% (for 50% of UA) indicated the reliability of the results and hence validated the applicability of the method for determination of TC in a real capsule formulation sample with a complex matrix.
method which of course needs tedious electrode preparation. The method
detection limit of the method is also the fourth lowest value following the
methods that use carcinogenic Pb(II) [25], and expensive gold nanoparticle
[23], and MWNT/GO [21]. Thus, the present method is an excellent
candidate for determination of TC in real samples using a relatively cheap
material following a one-step electrode modification procedure.

4. Conclusion

In this study, potentiodynamically fabricated poly(malachite green)
modified glassy carbon electrode was characterized using cyclic vol-
tammetry and electrochemical impedance spectroscopy using
[Fe(CN)6]3-/4- as a probe. Tetracycline showed two successive oxidative
peaks with no peak in the reduction scan direction indicating irrevers-
ibility of the oxidation of tetracycline at the poly(malachite green)
modified glassy carbon electrode. In contrast to the unmodified glassy
carbon electrode, oxidative peaks with about threefold enhanced peak
current at the poly(malachite green) modified glassy carbon electrode
showed catalytic effect of the modifier towards tetracycline oxidation.
While the observed peak potential shift with scan rate con-
fi rmed the irreversibility of the reaction, better correlation of the anodic peak cur-
rent with scan rate than with square root of scan rate added to slope of
0.60 for plot of log peak current versus log scan rate con-
fi rmed that the reaction of tetracycline at the polymer modified electrode is predomi-
nantly adsorption controlled. The effect of experimental parameters such
as accumulation potential, accumulation time and pH on the

Table 2. Summary of detected level of TC in the capsule sample solution, percent spike recovery, and interference recovery results of the method based on PMG/GCE.

| Purpose of analysis | Sample analyzed | Spiked TC (μM) | Added interferent (μM) | Detected TC (μM) | Detected/Recovery (%) |
|---------------------|-----------------|---------------|------------------------|------------------|-----------------------|
|                     | Capsule sample* | 0             | 0                      | 0                | 57.10                 | 100.21 |
| TC in capsule sample|                 | 40            | 0                      | 0                | 96.79                 | 99.80  |
| Spike recovery      | Capsule sample* | 0             | 28                     | 0                | 56.86                 | 99.78  |
| Interference study  | Capsule sample* | 0             | 57                     | 0                | 56.66                 | 99.44  |
|                     | Capsule sample* | 0             | 114                    | 0                | 56.48                 | 99.12  |
|                     | Capsule sample* | 0             | 0                      | 28               | 56.75                 | 99.60  |
|                     | Capsule sample* | 0             | 0                      | 57               | 56.71                 | 99.53  |
|                     | Capsule sample* | 0             | 0                      | 114              | 56.66                 | 99.44  |
|                     | Capsule sample* | 0             | 0                      | 0                | 56.71                 | 99.53  |
|                     | Capsule sample* | 0             | 0                      | 57               | 56.66                 | 99.44  |
|                     | Capsule sample* | 0             | 0                      | 114              | 56.12                 | 98.49  |

* capsule sample claimed 56.98 μM (according to labeled value); AA ascorbic acid; UA uric acid; Amp ampicillin.

Table 3. Comparison of the proposed method with selected recently reported methods for TC determination.

| Electrode used            | Method   | Linear range (μM) | Detection limit (μM) | Ref.   |
|---------------------------|----------|-------------------|----------------------|--------|
| MWNT/COOH-GO/CPE          | DPSV     | 20–310            | 0.36                 | [20]   |
| Graphite-polyurethane composite | DPV     | 3.8–38.0       | 2.6                  | [21]   |
| AuNP/MWNT/GCE             | DPV      | 11.25–315         | 0.09                 | [22]   |
| Poly(Urethane)/CPE        | DPV      | 3.8–19            | 2.6                  | [21]   |
| PtNPs/C/GCE               | DPV      | 9.99–44.01        | 4.28                 | [23]   |
| Pb/poly(AP)/GCE           | DP/SV    | 0.05–10           | 0.004                | [24]   |
| Graphite-polyurethane composite | DPV   | 4.00–40.0         | 2.80                 | [25]   |
| p-Mel@ERGO/GC             | DPV      | 10–80             | 5.0                  | [26]   |
| PMG/GCE                   | SWV      | 5–100             | 1.6                  | This work |
electrochemical behavior of TC at PMG/GCE were evaluated. Detection of TC in capsule sample with an accuracy of 100.21% as claimed to be, spike recovery of 99.80%, and interference recovery in the range 98.49–99.78% in the presence of 50–200% of UA, AA, and Amp validated the reliability of the method. Compared to most of the recently reported methods for determination of TC, low limit of detection, and wide range of linear dynamic range of the present method make it an excellent potential candidate for determination of trace level of TC in real samples.

Declarations

Author contribution statement

Mahlet Turbale: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Amsalu Moges, Mulgeta Dawit: Contributed reagents, materials, analysis tools or data.

Meareg Amare: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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