Determination of Clomipramine using eco-friendly solid-contact ionophore-doped potentiometric sensor

Adel M. Michael[1], Amr M. Mahmoud[2*] and Nesma M. Fahmy[1]

Abstract

Introduction Clomipramine is a tricyclic antidepressant acting as a serotonin reuptake inhibitor. Its maximum plasma concentration (C_{max}) is 13–310 ng/mL, the therapeutic range is 220–500 ng/mL and its toxic effect appears in doses above 900 ng/mL.

Objectives The fabrication of eco-friendly solid-contact ion-selective electrodes to evaluate the concentration of Clomipramine in different matrices based on disposable screen-printed carbon electrode.

Methods Disposable screen-printed carbon electrode was utilized as a substrate to fabricate the proposed sensors. The sensors were optimized to determine Clomipramine using calix[4]arene as an ionophore into PVC polymeric membrane to enhance selectivity towards the target analyte. The solid-contact sensor potential stability was improved by the incorporation of graphene nanoparticles transducer layer.

Results The sensors were assessed as per the IUPAC recommendations. The linearity range was 1×10^{-2} to $1 \times 10^{-5.3}$ M. The sensors were successfully applied to determine CLM in the pharmaceutical formulation. Furthermore, the ion selective electrodes were applied for Clompiramine assay in spiked plasma for the purpose of Point-of-Care testing to be a diagnostic tool for therapeutic monitoring of the cited central nervous system agent. The findings were statistically compared to the reported method showing no statistically significant difference.

Conclusion This work was concerned with developing a green analytical method for the determination of Clomipramine. The proposed SC-ISE was mixed with graphene nanocomposite transducer interlayer. The graphene layer succeeded in preventing the formation of an aqueous layer so resulted in a stable, reproducible standard potential besides the rapid response time.

Keywords Clomipramine, Point-of-care, Green chemistry, Supramolecular chemistry, Calix[4]arene, Graphene nanoparticles, Solid-contact ion-selective electrodes

*Correspondence:
Amr M. Mahmoud
amr.bekhet@pharma.cu.edu.eg
[1]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ahram Canadian University, October city, Egypt
[2]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Introduction

Nowadays health and environmental protection is of increasing importance. Green Chemistry was launched to provide safer methods for health and the environment. This was obtained by using safe and low risk potential solvents [1]. And also controlling the produced waste products or carrying out waste treatment [2]. Several techniques were developed to evaluate the greenness of the method as analytical Eco-Scale [3], Green Analytical Procedure Index which is abbreviated GAPI [4], Analytical greenness calculator; AGREE [5, 6]. Electrochemical methods are considered inherently green techniques through the use of water and buffers as the main solvents. Moreover, potentiometric measurements are time and cost-effective, besides being simple with very few sample pre-treatment steps [7–10].

Clomipramine HCl is a tricyclic antidepressant acting as a serotonin reuptake inhibitor [11]. Its chemical name is 3-(3-Chloro-10,11-dihydro-5 H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine hydrochloride and the chemical structure is shown in Fig. 1. It is used in adults to treat symptoms of obsessive-compulsive disorder (OCD), depression, anxiety, and some eating disorders. It can be used as an adjunct for neuropathic pain as well as the treatment of premature ejaculation [12]. It has been approved for treating children above ten years old with OCD [13], attention deficit hyperactivity disorder (ADHD) and paediatric nocturnal enuresis [14]. Its maximum plasma concentration (C_{max}) is 13–310 ng/mL [15]. The therapeutic range is 220–500 ng/mL and its toxic effect appears in doses above 900 ng/mL. Detecting low concentrations of CLM in biological fluids is crucial as to avoid toxicity or severe side effects such as blurred vision, urinary retention, and cardiac problems [14].

The literature revealed that CLM has been determined by spectrofluorimetry [16], and spectrophotometry [17]. Moreover, it was determined in biological fluids by immunoassays in urine [14], and by chromatography in serum [14, 18]. Electrochemical methods of analysis include potentiometric titration stated in the British pharmacopoeia (BP) [11], direct potentiometric assay by carbon paste electrodes [19, 20] and voltammetry [21].

The developed sensors have many advantages over the methods in the literature. Firstly, to improve the selectivity and sensitivity of ISE; the PVC membrane was doped with an ionophore [22]. The ionophore binds the target ion or form a complex with it to assure the transfer of the ion under investigation form the aqueous sample solution to the lipophilic membrane. This is obtained by decreasing the Gibbs free energy of transfer and thus results in increasing the sensitivity [23]. ISE widely uses ionophores as (2-hydroxypropyl)-β-cyclodextrin (2-HP-β-CD) and calixarenes (CX) as they have a 3D basket, cup or bucket structure [24]. The lipophilic core in their structure results in inclusion complex with selective attachment to the target ion [25]. They are utilized in ion-selective electrodes, selective membranes, as well as stationary phases [26] and have been widely used in electrochemical sensing [27, 28]. Secondly, Graphene (GR) is a 2D layer of graphite showing high hydrophobicity, chemical stability and superior electronic and electrochemical properties [29]. It is widely used in fabricating biological and electrochemical sensors [30, 31]. The inclusion of graphene inside polymers as a nanocomposite increases their electrical conductance [32, 33]. It can be prepared by solution casting where graphene is dispersed in organic solvent and then mixed with the polymer [34, 35]. Last but not least, the proposed method was proven to be eco-friendly. The method's greenness was evaluated by Analytical greenness calculator; AGREE and GAPI approach.

The aim the current work was the fabrication of a green solid-contact ion-selective electrode (SC-ISE) with high accuracy and sensitivity to quantify CLM in the dosage form and plasma. To achieve that calix[4]arene was impregnated in the PVC polymeric membrane as the ionophore, and graphene was used as interlayer between the ion sensing membrane and the screen-printed carbon electrode as capacitive ion-to-electron transducer. The fabricated sensor was characterized and used to determine CLM in pharmaceutical formulation and spiked human plasma.

Materials and methods

Chemicals and materials

Clomipramine pure powder was supplied by Novartis Pharmaceutical Company, Cairo, Egypt, with purity 99.55±0.89%. Anafranil® tablets manufactured by Novartis pharmaceutical company (Egypt) were obtained from the Egyptian market claimed to include 25 mg Clomipramine HCl per tablet. Potassium tetrakis(4-chlorophenyl)borate (KTpClPB), calix[4]arene, and *o*-nitrophenyl octyl ether (*o*-NPOE) were bought from Sigma Aldrich
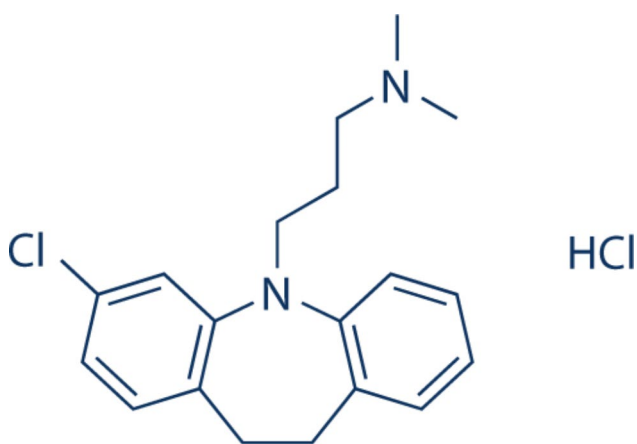

Fig. 1 Chemical structure of Clomipramine HCl

Michael *et al. BMC Chemistry*   (2023) 17:27

Page 3 of 9

(Missouri, United States). Polyvinyl chloride (PVC) was bought from Fluka (Steinheim, Germany). Graphene nano-platelets (6–8 nm thick × 5 microns wide) were obtained from Strem Chemicals INC. (Newburyport, USA). Britton – Robinson (BR) buffer was prepared by mixing phosphoric acid (0.04 M), acetic acid (0.04 M) and boric acid (0.04 M). Buffer solutions of different pH values were adjusted by the addition of 0.2 M NaOH. Screen-printed carbon electrodes (C-SPE) showing a diameter of 3 mm was obtained from CH Instruments, Inc., (Texas, USA).

Instrument
Potential measurements were conducted using Jenway digital pH / mV meter - model 3505 (mad in UK) in combination with an Ag/AgCl double junction reference electrode (made in Germany).

Standard solutions
Stock standard solution of CLM
Clomipramine solution (1×10^{-2} M) was prepared by transferring 90 mg of the standard CLM into a 25- mL volumetric flask followed by adding BRB buffer pH 3.0 to dissolve the powder, then the volume was completed to the mark using the same buffer.

Working standard solutions of CLM
Different working standard solutions of CLM with concentrations $1 \times 10^{-5.3}$ to 1×10^{-2} M were freshly prepared in a series of 25- mL volumetric flasks and each time the volume was completed to the mark by BRB buffer pH 3.0.

Human plasma
The human plasma samples were obtained from VAC-SERA, Cairo, Egypt.

Fabrication of sensor
Preparation of the Ion Selective membrane (ISM)
The ISM was formed by mixing 33.17 wt% PVC with 0.23 wt% KTpClPB, and 0.42 wt% of CX-4 and then dissolving them in 66.60 wt % of *o*-NPOE, followed by mixing with 6 mL of THF till getting a fully homogenous solution, specified as ISM(CX4).

Assembly of sensor modified with graphene nanocomposite
Graphene nanocomposite (Gr-NC) has been prepared as stated in the literature [29, 36]. The dispersion was used to form the graphene nanoparticles where 10 mg graphene nano-platelets were dispersed into 1.0 mL xylene and sonicated for 5 min. 95 mg PVC were mixed with 3 mL of THF and 0.2 mL *o*-NPOE plasticizer. The THF solution was added to the graphene dispersion and sonicated for 10 min in order to get the graphene nanocomposite [29]. To form the screen-printed carbon

sensor, 10.0 μL Gr-NC dispersion was drop-casted onto the screen-printed carbon electrode and left to evaporate overnight at room temperature. Then, 10.0 μL of the ionophore-doped ion sensing membrane ISM(CX4) was drop-casted onto the C-SPE/Gr-NC and left to evaporate overnight to obtain sensor I: C-SPE/Gr-NC/ISM(CX4). To evaluate the role of Gr-NC on sensor performance, a control sensor (sensor II) was fabricated, with the exclusion of Gr-NC layer, sensor II: C-SPE/ISM(CX4).

Sensors characterization
Sensors calibration
Each sensor separately was conjugated with the Ag/AgCl double-junction reference electrode. Then immersed the sensor into CLM drug solutions ($1 \times 10^{-5.3}$–1×10^{-2} M). The electromotive forces (*emf*) readings were allowed to equilibrate to within ±1 mV then the reading was recorded. Calibration curves were constructed by plotting the recorded *emf* obtained from the sensor versus the corresponding − log concentrations of CLM. Then, the regression equations corresponding to each sensor were computed.

Estimation of the slope, response time and selectivity of the proposed sensors
The slope, response time and selectivity of the proposed sensors were evaluated in as per the IUPAC guidelines [37].

Application to the dosage form
Ten tablets were weighed and crushed and 0.075 g (equivalent to one tablet) were transferred to 50- mL volumetric flask and the volume was completed to the mark with BRB buffer pH 3. 25 mL of the prepared solution was transferred to a 50- mL volumetric flask and the volume was completed with the buffer to get a claimed concentration 8.8×10^{-4} M Clomipramine HCl (equivalent to 8×10^{-4} M Clomipramine free base).

Spiked plasma
A volume 0.5 mL blank plasma was placed in a falcon tube, spiked with fixed amount of CLM and a solutions equivalent to 1×10^{-4} and 1×10^{-5} CLM were mixed with plasma, pH adjusted to 3.0 using BRB buffer and spiked samples were analysed.

Results and discussion
The combination of graphene and PVC nanocomposite provided a hydrophobic transducer layer on SC-ISE. The addition of calix[4]arene as an ionophore provided stable potential, and improved the stability, reproducibility, and selectivity of the fabricated electrodes. Solid-state transduction-based electrochemical sensor allows direct quantitation of the ions under investigation with minimal

Table 1 Electrochemical response characteristics of the fabricated sensors

Parameter	C-SPE/ISM(CX4)	C-SPE/Gr-NC/ISM(CX4)
Linear range	1×10^{-2} to $1 \times 10^{-5.3}$ M	1×10^{-2} to $1 \times 10^{-5.3}$ M
Slope (mV/decade)	-54.06	-55.42
Response time (s)	15	10
Working pH range	2–6	2–6
Stability (weeks)	4	4
LOD	$1 \times 10^{-5.39}$ M	$1 \times 10^{-5.52}$ M

samples preparation. It is easy to handle and maintain in comparison with conventional liquid contact ion selective electrodes (LC-ISEs).

Method development and optimization
Membrane composition
The ISM is primarily formed of a polymer matrix which acts as the mechanical support to the membrane. A plasticizer which dissolves the ion exchanger and plasticizes the membrane, in addition to modifying the lipophilicity of the membrane. An ion exchanger which is a hydrophobic ion with a charge opposite to that of the target ion, and an ionophore which selectively binds to the target ion. Furthermore, the plasticizer modifies the distribution coefficient (K_d) of different species, so affects the performance characteristics of the electrode [38]. The ionophore calix[4]arene has higher binding strength to CLM, so it lowers the free energy of transfer

of CLM from the aqueous phase to the ISM and leads to a selective signal of the electrode towards CLM. The composition of both sensors is presented in **Table S-1** (Supporting Information).

Potentiometric performance
As shown in Table 1, fabricated sensors showed Nernstian response towards CLM in the range of 1×10^{-2} to $1 \times 10^{-5.3}$ M CLM. The response time is a crucial aspect for analytical procedures. The time required for the sensors to get to the final stable potential ± 1 mV was found to be 10 and 15 seconds for C-SPE/Gr-NC/ISM(CX4) and C-SPE/ISM(CX4), respectively as shown in Figure S-1 (Supporting Information) and Table 1.

pH effect on sensors performance
Optimum experimental conditions were studied for the quantitative applications of the fabricated potentiometric sensors. The potentiometric response has been reported by measuring *emf* of each sensor at different pH values in the range (3.0–9.0) using BRB buffer as shown in Fig. 2. It can be observed from the figure that the sensors have pH range 2.0–6.0, at this pH, CLM has positive charge (pK_a 9.42) [39] and can be exchanged at the membrane interface and produce *emf* changes.
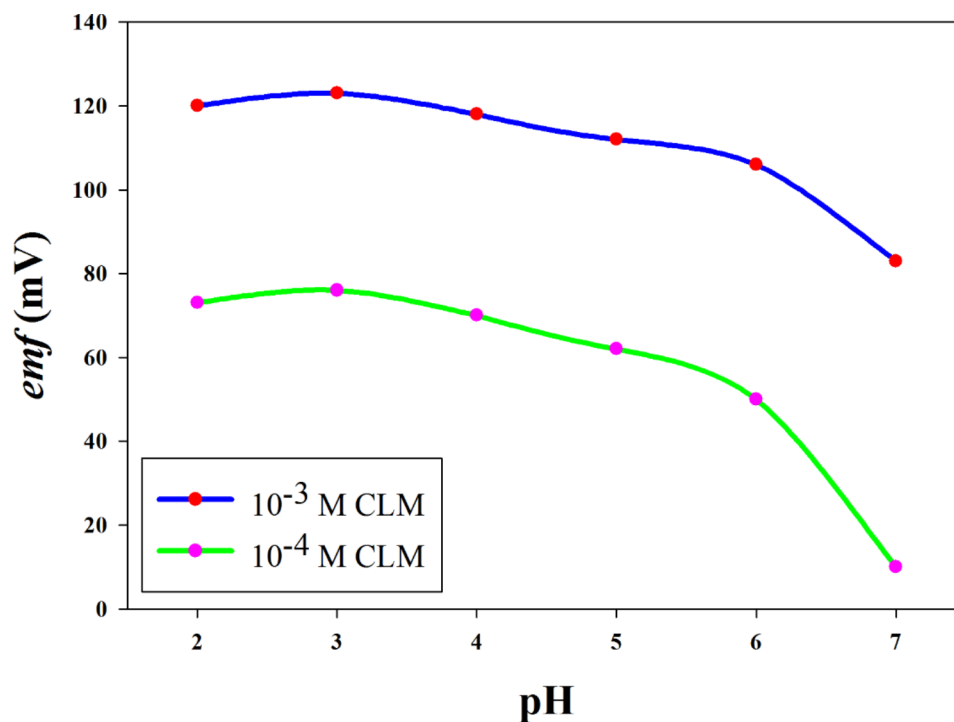

Fig. 2 The effect of pH on the developed sensors

Michael *et al. BMC Chemistry*   (2023) 17:27

Page 5 of 9

Table 2 Potentiometric selectivity coefficients (-log K) of the proposed sensors using the separate solutions method

Interfering ion (10^{-3} M)	C-SPE/ISM(CX4)	C-SPE/Gr-NC/ISM(CX4)
Sodium chloride	-2.84	-3.44
Magnesium sulphate	-3.86	-4.09
Potassium chloride	-2.77	-3.35
Ammonium sulphate	-2.75	-3.38
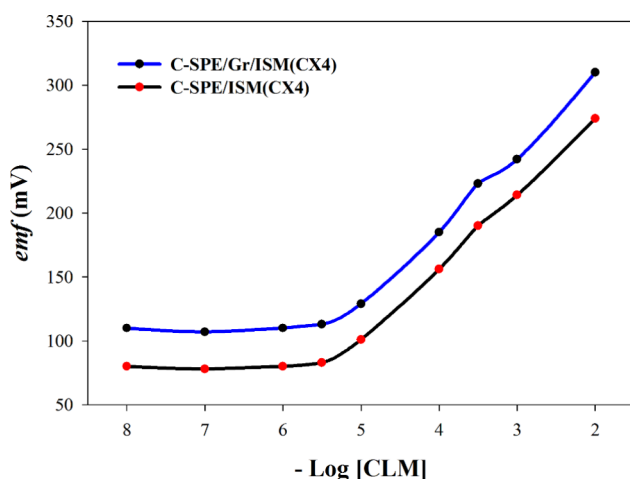
Fig. 3 Profile of the potential in *emf* versus -log concentrations of CLM (M) obtained with the fabricated sensors

Selectivity of the sensor

Selectivity coefficients of the fabricated sensors were computed using separate solution method and the following equation [30]:

$$-\log(K^{pot}{}_{A,B}) = \frac{E_1 - E_2}{2.303RT/Z_AF} + \left(1 - \frac{Z_A}{Z_B}\right)\log a_A$$

where $K^{pot}{}_{A,B}$ is the potentiometric selectivity coefficient, E_1 is the potential measured in 10^{-4} M CLM solution, E_2 is the potential measured for 10^{-3} M of the interfering ion. Z_A and Z_B are the charges of CLM and interfering ion, respectively, a_A is the activity of the drug and $2.303RT/Z_AF$ represent the slope of the investigated sensor (mV/concentration decade). The selectivity coefficients are presented in Table 2. The good selectivity of the sensors is due to the optimized combination of lipophilic cation exchanger, *o*-NPOE as a plasticizer with high dielectric constant, in addition to incorporation of the calixarene as supramolecular ionophore.

Method validation

The potentiometric performance of the fabricated sensors was evaluated in the concentration range of 1×10^{-2} to $1\times10^{-5.3}$ M. The electrodes revealed a good Nernstian response as shown in Fig. 3. Accuracy of the methods

Table 3 Validation parameters of the developed electrochemical methods

Parameter	C-SPE/ISM(CX4)	C-SPE/Gr-NC/ISM(CX4)
Linearity		
Slope[a]	-54.06	-55.42
Intercept	411.23	382.11
R^2	0.9932	0.9936
Range	$1\times10^{-2} - 1\times10^{-5.3}$ M	
Accuracy[b]	100.60 ± 1.75	101.05 ± 1.10
Precision	0.668	0.822
Repeatability[c]	1.862	1.516
Intermediate precision[c]		

[a] Average of three determinations

[b] Accuracy results were done for (5×10^{-5}, 5×10^{-4} and $5\times10^{-3.5}$ M) of CLM.

[c] RSD for repeatability and intermediate precision were done for (10^{-5}, 10^{-4} and 10^{-3} M) of CLM.

Table 4 Determination of CLM in pharmaceutical formulation and spiked plasma

Application	C-SPE/ISM(CX4)	C-SPE/Gr-NC/ISM(CX4)
Pharmaceutical Formulation (Anafronil tablets)® B.N: Y0021	99.48 ± 1.16[*]	102.10 ± 1.27[*]
Spiked plasma	102.65 ± 1.72[*]	100.26 ± 2.10[*]

[*] Average of triplicate measurements

was assessed by covering three concentration levels in triplicates showing accepted percent recovery. Precision was examined and the results from applying repeatability and intermediated precision showed accepted RSD. The LOD and LOQ were calculated and a full validation sheet is presented in Table 3.

Pharmaceutical applications
Potentiometric assay of CLM in dosage form

> *The two sensors were successfully applied for the assay of CLM in Anafranil tablets® showing no interference of excipients as represented on Table 4.*

#### Potentiometric determination of CLM in spiked plasma

Plasma was spiked with 10^{-4} and 10^{-5} M CLM, and the sensors provided reliable results with acceptable accuracy and precision as shown in Table 4.

Statistical comparison

The results obtained upon using the proposed sensors were compared to the results obtained upon applying the reported method [11]. The calculated student's t-test and F test values indicated that there was no significant difference as shown in Table 5.

Michael *et al. BMC Chemistry*　　　(2023) 17:27

Page 6 of 9

Table 5 Statistical analysis of the results obtained by the proposed sensors and those obtained by the reported method for the analysis of CLM in its pure powdered form

Parameter	C-SPE/ISM(CX4)	C-SPE/Gr-NC/ISM(CX4)	Reported method
Mean (Recovery %)	99.98	99.97	99.55
Variance	3.39	2.78	0.89
n	5	5	5
Student's t-test (2.36)	0.46	0.49	----
F-test (6.38)	3.80	3.12	----
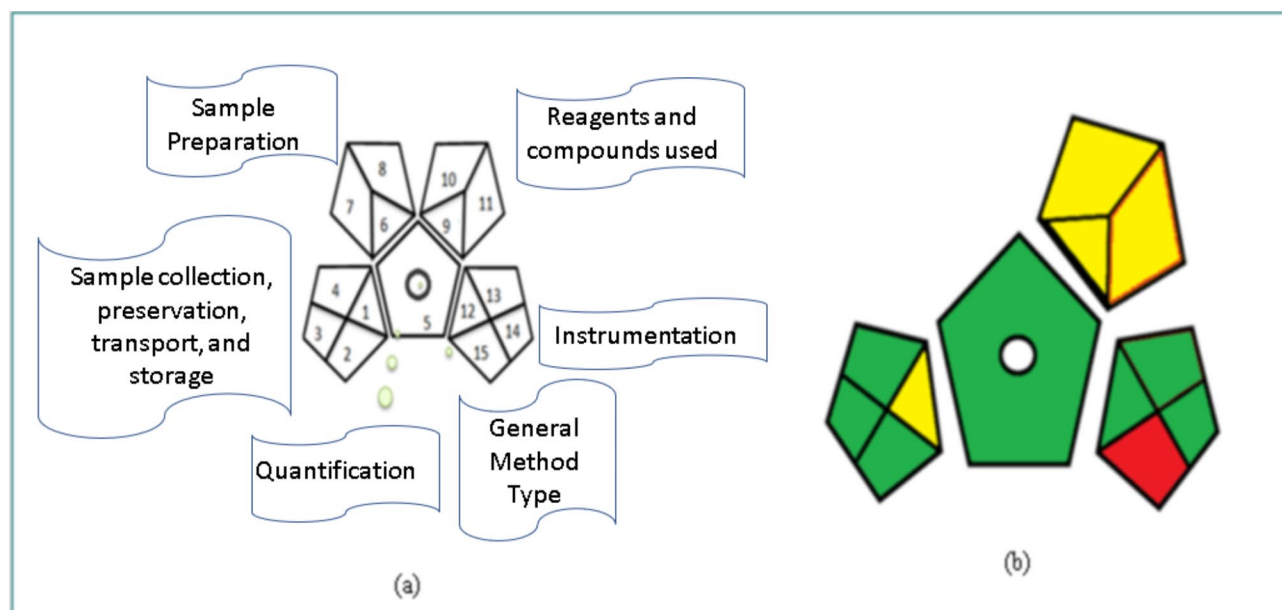
Fig. 4 (a) GAPI original pentagram (b) GAPI pentagram for the ISE procedure

Greenness assessment

GAPI tool is a visual display of data composed of five pentagrams. These pentagrams assess the environmental impact of the method's main stages as sample collection, preservation, transport and storage, general method type, sample preparation, reagents used and instrumentation. They are marked by red, yellow and green colours representing high, medium and low hazardous effect, respectively. There is no sample preparation step in the current method, so its representing pentagram is eliminated. The obtained GAPI pentagrams shown in Fig. 4 have a dominance of green colour suggesting low hazardous effect on the environment. However, the pentagram shows red colour representing that there was no waste treatment, and yellow colour equivalent to low reagents safety used; as BRB buffer and THF were incorporated in membrane fabrication have some health hazards.

The greenness analytical calculator; AGREE is the most recent greenness assessment tools. It is a user-friendly free software for assessing analytical operations. Assessment is based on twelve criteria, and alternative weights are applied to each one of them. Each one of the twelve input variables is converted to a numerical scale ranging from 0 to 1. The sum of the assessment results for each variable indicates the final greenness score. The result is a circular graph, showing the overall score in the centre. Values near 1 have dark green colour indicating that the tested procedure is more environmental friendly. The proposed ISE has an AGREE score of 0.82 and a middle green colour, Fig. 5, proving its low environmental impact and declaring that it is a green method.

Water layer test

The test was conducted by subjecting the electrode to a high concentration of the interfering ion (mebeverine) and then estimate the drift in potential following testing a sample of lower concentration of the drug under investigation. The test was performed by measuring the potential of GR/ISM in 10^{-3} M CLM solution then changing to 10^{-2} M mebeverine solution then back to CLM solution. If a water layer was created under the membrane, its ionic structure would be changed due to ion fluxes and a
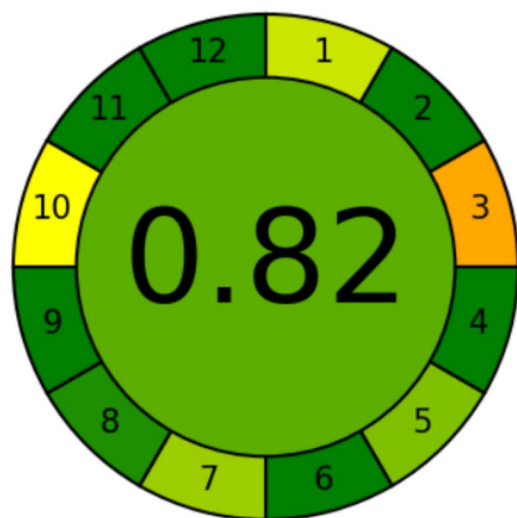
Michael *et al. BMC Chemistry*   (2023) 17:27

Page 7 of 9

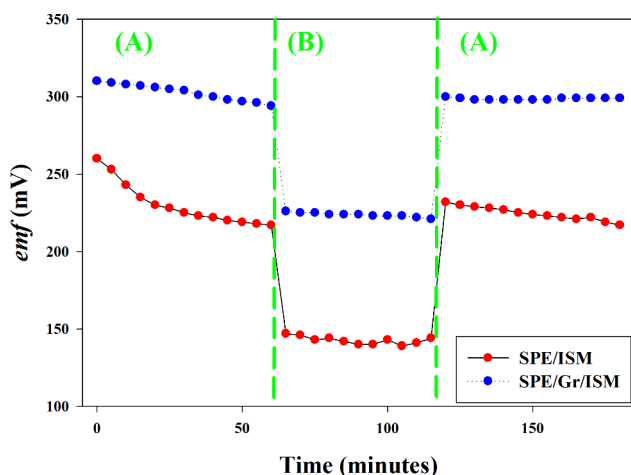**Fig. 5** AGREE Greenness assessment score of the ISE procedures

Fig. 6 Potentiometric water layer test, the potential was recorded in solution (A) 10^{-3} M CLM, solution (B) 10^{-2} M Mebeverine and solution (A) 10^{-3} M CLM again using SPE/ISM and SPE/Gr/ISM.

potential drift would be noticed. The fabricated sensors showed a significant potential drift indicating the presence of an aqueous layer below the membrane, while GR/ISM sensor showed a more stable potential over time indicating that there was no water layer formed beneath the membrane due to the hydrophobicity of graphene as well as the conductivity and electrical capacity of the formed nanocomposite as shown in Fig. 6.

Conclusion

This work was concerned with developing a green analytical method for the determination of CLM. The proposed SC-ISE was mixed with graphene nanocomposite transducer interlayer. The graphene layer succeeded in

preventing the formation of an aqueous layer so resulted in a stable, reproducible standard potential besides the rapid response time. The reproducibility and stability of the proposed graphene-based sensor allows its application in CLM analysis in QC labs or in biological fluids to personalize the patient's dosage.

List of Abbreviations

AGREE	Analytical GREEnness Metric Approach
ADHD	Attention deficit hyperactivity disorder
BP	British pharmacopoeia
BRB	Britton – Robinson buffer
C_{max}	Maximum drug plasma concentration
C-SPE	Screen-printed carbon electrode
CX	Calixarene
CLM	Clomipramine
K_d	Distribution coefficient
Emf	Electromotive forces
GAPI	Green Analytical Procedure Index
Gr-NC	Graphene nanocomposite
2-HP-β-CD	(2-hydroxypropyl)-β-cyclodextrin
ISM	Ion sensing membrane
IUPAC	International Union of Pure and Applied Chemistry
LC-ISEs	Liquid contact ion selective electrodes
LOD	Limit of detection
OCD	Obsessive-compulsive disorder
o-NPOE	*o*-nitrophenyl octyl ether
PVC	Polyvinyl chloride
KTpClPB	Potassium tetrakis(4-chlorophenyl)borate
QC	Quality control
RSD	Relative standard deviation
SC-ISEs	Solid-contact ion-selective electrodes
THF	Tetrahydrofuran

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13065-023-00938-x.

Supplementary Material 1

Michael *et al. BMC Chemistry*   (2023) 17:27

Page 8 of 9

Data Availability
(ADM)
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
All authors have no competing interests.

References

1. Elhassan MM, Mahmoud AM, Hegazy MA, Mowaka S. Univariate and Multivariate Determination of Dapagliflozin and Saxagliptin in bulk and Dosage Form.Journal of AOAC International 2022.
2. Horvath IT, Anastas PT. Innovations and green chemistry. Chem Rev. 2007;107(6):2169–73.
3. Van Aken K, Strekowski L, Patiny L. EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters. Beilstein J Org Chem. 2006;2(1):3.
4. Płotka-Wasylka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta. 2018;181:204–9.
5. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Anal Chem. 2020;92(14):10076–82.
6. Fahmy NM, Abdullatif HA, Michael AM, Ayad MF, Trabik YA. Smart Eco-Friendly Spectrophotometric Methods Resolving Highly Overlapping Spectra: Application to Veterinary Antibiotic Injections.Journal of AOAC International2022.
7. Elhassan MM, Mahmoud AM, Hegazy MA, Mowaka S. In-line monitoring of sitagliptin dissolution profile from tablets utilizing an eco-friendly potentiometric sensor. Chem Pap. 2021;75(8):4165–76.
8. Lotfy HM, Awad AM, Shehata MA. Novel ion selective electrode for determination of pregabalin in pharmaceutical dosage form and plasma. Anal Bioanal Electrochem. 2012;4(5):507–17.
9. Soliman SS, Mahmoud AM, Elghobashy MR, Zaazaa HE, Sedik GA. Point-of-care electrochemical sensor for selective determination of date rape drug "ketamine" based on core-shell molecularly imprinted polymer. Talanta. 2023;254:124151.
10. Arafa RM, Mahmoud AM, Eltanany BM, Galal MM. Voltammetric determination of Oxybutynin Hydrochloride utilizing Pencil Graphite Electrode decorated with gold nanoparticles. Electroanalysis; 2022.
11. Pharmacopoeia BH. The British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare Products Regulatory Agency (MHRA). *Publisher: TSO (The Stationery Office)* 2019.
12. Kim SW, Choi JB, Kim SJ, Kim KS, Kim CM, Lee DH, Choi WS. Tolerability and adequate therapeutic dosage of oral clomipramine for the treatment of premature ejaculation: a randomized, double-blind, placebo-controlled, fixed-dose, parallel-grouped clinical study. Int J Impot Res. 2018;30(2):65–70.
13. Wilson M, Tripp J. Clomipramine. StatPearls [Internet].edn.: StatPearls Publishing; 2021.
14. Melanson SE, Lewandrowski EL, Griggs DA, Flood JG. Interpreting tricyclic antidepressant measurements in urine in an emergency department setting: comparison of two qualitative point-of-care urine tricyclic antidepressant drug immunoassays with quantitative serum chromatographic analysis. J Anal Toxicol. 2007;31(5):270–5.
15. Hewson C, Conlon P, Luescher U, Ball R. The pharmacokinetics of clomipramine and desmethylclomipramine in dogs: parameter estimates following a single oral dose and 28 consecutive daily oral doses of clomipramine. J Vet Pharmacol Ther. 1998;21(3):214–22.
16. Rahman N, Afaq N. Optimization and validation of spectrofluorimetric method for the determination of clomipramine hydrochloride via ion-pair complexation with alizarin red S. Anal Methods. 2010;2(5):513–8.
17. Abdelmageed OH, Khashaba PY. Spectrophotometric determination of clotrimazole in bulk drug and dosage forms. Talanta. 1993;40(8):1289–94.
18. Mostafavi SA, Tahvilian R, Poudeh MD, Rafeepour Z. A simple Sample Preparation with HPLC-UV Method for Estimation of Clomipramine from plasma. Iran J Pharm research: IJPR. 2010;9(3):243.
19. Elashery SEA, Attia NF, Omar MM, Tayea HMI. Cost-effective and green synthesized electroactive nanocomposite for high selective potentiometric determination of clomipramine hydrochloride. Microchem J. 2019;151:104222.
20. Faridbod F. Clomipramine determination by potentiometric PVC membrane and carbon paste sensors. Anal Bioanal Electrochem. 2012;4(3):315–26.
21. UĞURLU E, YAĞMUR S, YILMAZ S. Determination of clomipramine hydrochloride from its commercial drug form by voltammetry. J Sci Perspect. 2018;2(1):1–8.
22. Abd El-Rahman MK, Zaazaa HE, ElDin NB, Moustafa AA. Just-dip-it (potentiometric ion-selective electrode): an innovative way of greening analytical chemistry. ACS Sustain Chem Eng. 2016;4(6):3122–32.
23. Niu X, Mo Z, Yang X, Sun M, Zhao P, Li Z, Ouyang M, Liu Z, Gao H, Guo R. Advances in the use of functional composites of β-cyclodextrin in electrochemical sensors. Microchim Acta. 2018;185(7):1–17.
24. Ersoz M. Transport of mercury through liquid membranes containing calixarene carriers. Adv Colloid Interface Sci. 2007;134:96–104.
25. Eddaif L, Shaban A, Telegdi J. Sensitive detection of heavy metals ions based on the calixarene derivatives-modified piezoelectric resonators: a review. Int J Environ Anal Chem. 2019;99(9):824–53.
26. Sayin S, Ozbek C, Okur S, Yilmaz M. Preparation of the ferrocene-substituted 1, 3-distal p-tert-butylcalix [4] arene based QCM sensors array and utilization of its gas-sensing affinities. J Organomet Chem. 2014;771:9–13.
27. Palanisamy S, Thirumalraj B, Chen S-M. A novel amperometric nitrite sensor based on screen printed carbon electrode modified with graphite/β-cyclodextrin composite. J Electroanal Chem. 2016;760:97–104.
28. Aree T. Supramolecular complexes of β-cyclodextrin with clomipramine and doxepin: Effect of the ring substituent and component of drugs on their inclusion topologies and structural flexibilities. Pharmaceuticals. 2020;13(10):278.
29. Moaaz EM, Mahmoud AM, Fayed AS, Rezk MR, Abdel-Moety EM. Determination of tedizolid phosphate using graphene nanocomposite based solid contact ion selective electrode; green profile assessment by eco‑scale and GAPI approach. Electroanalysis. 2021;33(8):1895–901.
30. Hernández R, Riu J, Bobacka J, Vallés C, Jiménez P, Benito AM, Maser WK, Rius FX. Reduced graphene oxide films as solid transducers in potentiometric all-solid-state ion-selective electrodes. J Phys Chem C. 2012;116(42):22570–8.
31. Wang Y, Li Y, Tang L, Lu J, Li J. Application of graphene-modified electrode for selective detection of dopamine. Electrochem Commun. 2009;11(4):889–92.
32. Chee W, Lim H, Huang N, Harrison I. Nanocomposites of graphene/polymers: a review. RSC Adv. 2015;5(83):68014–51.
33. Zhang H-B, Zheng W-G, Yan Q, Yang Y, Wang J-W, Lu Z-H, Ji G-Y, Yu Z-Z. Electrically conductive polyethylene terephthalate/graphene nanocomposites prepared by melt compounding. *polymer* 2010, 51(5):1191–1196.
34. Li X, Xiao Y, Bergeret A, Longerey M, Che J. Preparation of polylactide/graphene composites from liquid-phase exfoliated graphite sheets. Polym Compos. 2014;35(2):396–403.
35. Zhang X, Liu X, Zheng W, Zhu J. Regenerated cellulose/graphene nanocomposite films prepared in DMAC/LiCl solution. Carbohydr Polym. 2012;88(1):26–30.

36. Mahmoud AM, Moaaz EM, Rezk MR, Abdel-Moety EM, Fayed AS. Microfabricated Solid‑contact potentiometric sensor for determination of Tedizolid phosphate, application to Content Uniformity Testing. Electroanalysis. 2023;35(1):175–83.
37. Lindner E, Umezawa Y. Performance evaluation criteria for preparation and measurement of macro-and microfabricated ion-selective electrodes (IUPAC Technical Report). *Pure and Applied Chemistry* 2008, 80(1):85–104.
38. Diamond D. Principles of chemical and biological sensors. Wiley; 1998.
39. Li M, Zhang H, Chen B, Wu Y, Guan L. Prediction of pKa values for neutral and basic drugs based on hybrid artificial intelligence methods. Sci Rep. 2018;8(1):1–13.

Publisher's Note