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Resolution of opiate illicit drugs signals in the presence of some cutting agents with use of a voltammetric sensor array and machine learning strategies

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ABSTRACT

In the present work, the resolution and quantification of mixtures of different opiate compounds in the presence of common cutting agents using an electronic tongue (ET) is evaluated. More specifically, ternary mixtures of heroin, morphine and codeine were resolved in the presence of caffeine and paracetamol. To this aim, an array of three carbon screen-printed electrodes were modified with different ink-like solutions of graphite, cobalt (II) phthalocyanine and palladium, and their responses towards the different drugs were characterized by means of square wave voltammetry (SWV). Developed sensors showed a good performance with good linearity at the μM level, LODs between 1.8 and 5.3 μM for the 3 actual drugs, and relative standard deviation (RSD) ca. 2% for over 50 consecutive measurements. Next, a quantitative model that allowed the identification and quantification of the individual substances from the overlapped voltammograms was built using partial least squares regression (PLS) as the modeling tool. With this approach, quantification of the different drugs was achieved at the μM level, with a total normalized root mean square error (NRMSE) of 0.084 for the test subset.

1. Introduction

The consumption and trafficking of illicit drugs have increased significantly over the last years, creating a negative impact in people's health and in the economy, while contributing to an increase of criminality [1]. The increase in consumption and trafficking has also enriched the illicit drug markets, which are powerful systems of production and distribution that generate large amount of unwanted activities [2]. In this direction, the rapid detection of illicit drugs to disarticulate such markets and safeguard the public still remains a challenge for authorities.

The main drawbacks posed by currently used on-site methods for the detection of illicit drugs and their precursors are the low accuracy of color tests, or the high cost and low portability of spectroscopic tests. In the light of the pressing need for better drug test systems at border controls, BorderSens project [3] aims to establish the basis for the development of a portable device capable to quickly test for different drugs, precursors and cutting agents, with outstanding accuracy and reduced false positives and false negatives. However, given the challenge that such a task represents, the quantitative analysis of opiates mixtures is investigated herein as a proof-of-concept of what can be achieved.

In the USA, the Controlled Substances Act (CSA) defines five classes of drugs: narcotics, depressants, stimulants, hallucinogens and anabolic steroids [4]. Among those, narcotics (also known as "opioids") represent one of the biggest health and economic burden, accounting 63% of deaths by drug overdose in the USA in 2015 and nearly 70% in 2018 [5,6]. A particular class of opioids are opiates, which originate or are derived from naturally occurring alkaloids found in certain poppy species, specifically *Papaver somniferum* [7]. Common opiates include opium, heroin, morphine and codeine.

Several alternate methods for the individual and simultaneous determination of opiates have been reported in the literature. Those are based on common analytical techniques such as chromatography [8,9], capillary electrophoresis [10,11], chemiluminescence [12,13], diffuse reflectance near-infrared spectroscopy [14] or surface plasmon resonance (SPR) based immunosensors [15]. Despite being powerful, these techniques present some disadvantages that hinder their application for the on-site drug monitoring, like requiring a sample pre-treatment step and/or laboratory facilities (low portability), being time-consuming and

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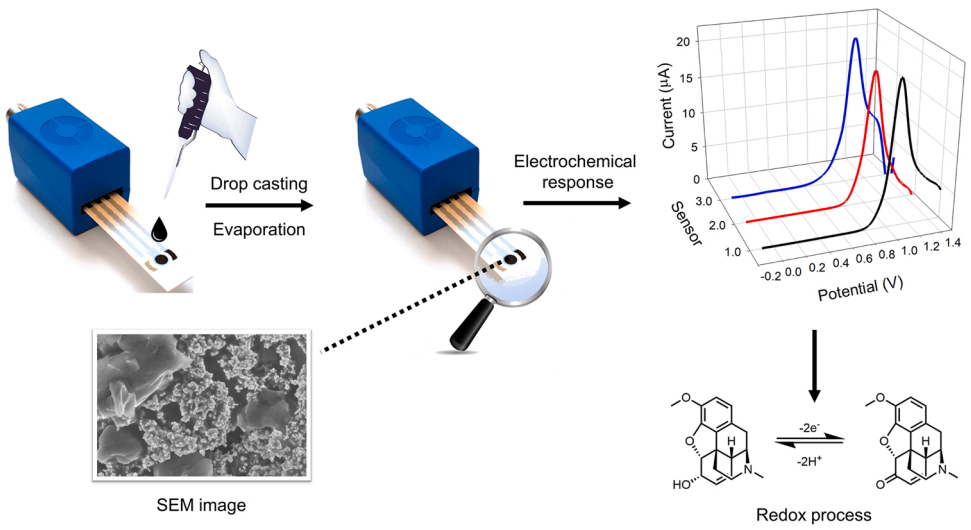

Fig. 1. Scheme of the experimental procedure for the electrode surface modification. Firstly, an ink-like solution was prepared incorporating the corresponding modifier. Then, 1 μL was dropped on the surface and dried at 40 °C.

quite costly (both from the equipment and reagents side).

In this regard, the use of electrochemical sensors represents a promising alternative which may allow to overcome these limitations. These devices can be used as powerful analytical tools for the analysis of illicit substances from street and biological samples as they offer low-cost measurement systems with rapid response, simple usage and high portability; all of them required characteristics for point-of-use forensic applications. Despite the advantages that electrochemical methods may offer, the simultaneous determination of the aforementioned compounds can be challenging given their similar electrochemical response and the complexity of the samples [16]. Precisely, the main drawback is that, when using bare electrodes, there might be some overlapping between peaks, or even peak suppression [17]. Actually, the most complicated situation is the discrimination between heroin and morphine, since both molecules present the same functional groups in their skeleton and consequently, their corresponding fingerprint is quite similar. To overcome this difficulty, modification of the electrochemical sensors may be required, but it might also be necessary to couple these techniques with other strategies such as the use of chemometrics; a combination that is known as electronic tongue (ET) [18,19].

According to the IUPAC [20], an ET is defined as "a multisensor system, which consists of a number of low selective sensors and uses advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate data analysis". These biomimetic systems, in opposition to classical approaches, are based on the combination of low selective and/or cross-responsive sensors to obtain rich and complementary analytical information. Next, the coupling with chemometric tools to analyse the data allows to deconvolute complex overlapping electrochemical responses and achieve the simultaneous quantitative determination of several analytes. Thus, such an approach allows to extract meaningful chemical information from these complex data.

In the present work, the capabilities of ET-based systems in forensic applications will be demonstrated by attempting the simultaneous determination of three opiates (heroin, morphine and codeine) in the presence of two common cutting agents (paracetamol and caffeine). The chosen voltammetric sensor array consisted of three screen-printed carbon electrodes (SPCE) modified with graphite, cobalt (II) phthalocyanine and palladium inks, and square wave voltammetry (SWV) was the measuring technique. Firstly, the behavior of the sensors towards each of the compounds was evaluated individually, characterizing its analytical response . Secondly, a partial least square regression (PLS) model for their simultaneous quantification at the μM level was built from the measured voltammograms.

2. Experimental

2.1. Reagents and samples

Standards of heroin hydrochloride and codeine were purchased from Chiron Chemicals, Australia. Morphine hydrochloride, potassium monophosphate, potassium chloride and potassium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cobalt(II) phthalocyanine (CoPc) and palladium powder (< 1 μm, ≥ 99.9%; Pd), which were used as modifiers, as well as mesitylene and polystyrene, which were used for the preparation of the ink composite, were also obtained from Sigma-Aldrich (St. Louis, MO, USA). Graphite powder (particle size < 50 μm) was received from BDH (BDH Laboratory Supplies, Poole, UK).

Samples were prepared in 20 mM phosphate buffer (PBS) at pH 7.0 containing 100 mM KCl as supporting electrolyte for the electrochemical measurements. All aqueous solutions were prepared using MilliQ water (ρ > 18.2 MΩ cm). All reagents were of analytical grade and used without further purification. Fresh stock solutions were prepared daily in order to prevent its degradation.

2.2. Apparatus and voltammetric measurements

SWV measurements were performed using a multi-channel potentiostat/galvanostat/impedance analyzer MultiPalmSens4 (PalmSens, Houten, The Netherlands) controlled by MultiTrace software. ItalSens screen-printed carbon electrodes (SPCE) containing a graphite working electrode (3 mm diameter), a carbon counter electrode and a pseudo-silver reference electrode (PalmSens, The Netherlands) were used for the measurements. SWV measurements were performed by placing 50 μL of the sample onto the SPCE. The single scan SWV parameters were as follows: potential range from −0.2 to 1.5 V, step potential of 5 mV, amplitude of 25 mV and frequency of 10 Hz.

2.3. Modification of the electrode surface

SPCE were modified with standard catalysts employed in electroanalysis, as are CoPc and Pd [21,22], incorporated using a self-formulated graphite-polystyrene ink. More in detail, the mixture contained the following mass fractions: 58% of graphite, 32% of powdered polystyrene and 10% of modifier, in this case graphite, cobalt

(II) phthalocyanine or palladium.

The corresponding modifier, graphite and polystyrene were thoroughly mixed with 250 μL of mesitylene for 2 h. After that, the mixture was sonicated for 2 min in order to obtain a medium thick solution. Finally, 1 μL of the ink-like composite was dropped onto the working electrode surface of a SPCE and dried at 40 °C for at least 1 h in order to remove the solvent (Fig. 1). After that, the electrodes were ready to use, not requiring the usage of any activation step.

2.4. Characterization of the electrode by scanning electron microscopy

The morphological characterization of the modified SPCE electrodes was performed by Field Emission Gun-Scanning Electron Microscope (FEG-SEM) of Zeiss, model MERLIN SM0087 and Energy Dispersive X-Ray Analysis (EDX). Imaging was performed based on secondary, back-scattered electrons.

2.5. Samples under study

In the present work, two different scenarios were evaluated with the proposed ET. Firstly, the analysis of ternary mixtures of heroin, codeine and morphine was considered to assess the potential of the ET to achieve their individual quantification. Secondly, the quantification of the same drugs in the presence of two common cutting agents (viz. paracetamol and caffeine) was attempted to assess the potential of the ET in a more realistic scenario.

To this aim, two different sets of samples were prepared (one for each of the above-mentioned scenarios). Each set of samples consisted of a train subset, which is used to build the model, and a test subset, which is used to assess its performance. The concentrations of the compounds mixtures of the train subset were defined by an experimental design, while for the test subset, some extra samples with concentrations for each of the compounds randomly distributed along the experimental domain were also prepared. Besides, in order to keep the number of samples required down to a reasonable level, two different experimental designs were employed as the number of samples required increases exponentially with the number of compounds considered.

In the first case (mixtures of the three drugs), samples for the train subset were prepared based on a tilted factorial experimental design 3^3 (27 samples) [23]. With this approach it is possible to get a better distribution of the samples that avoids the repetition of concentrations as per the selected levels. The concentrations ranges considered for each of the compounds were in the range 0–750 μM, with 15 extra samples forming the test subset.

In the second case (mixtures of the three drugs plus the two cutting agents), samples for the train subset were prepared based on a central composite face-centered (CCF) experimental design with 3 levels of concentration (27 samples). As already stated, this was preferred as the number of samples required to complete a full factorial design would be just too high ($3^5 = 243$ samples). In this case the concentrations for each of the compounds ranged from 0 to 750 μM, with 17 extra samples forming the test subset.

As an extra precaution to control drifts or periodic trends as the same sensing units were used for the whole series, all this samples were analysed in random order and alternating their measurement with the measurement of a blank solution (PBS), which served as cleaning stage of the electrode surfaces, but also as control.

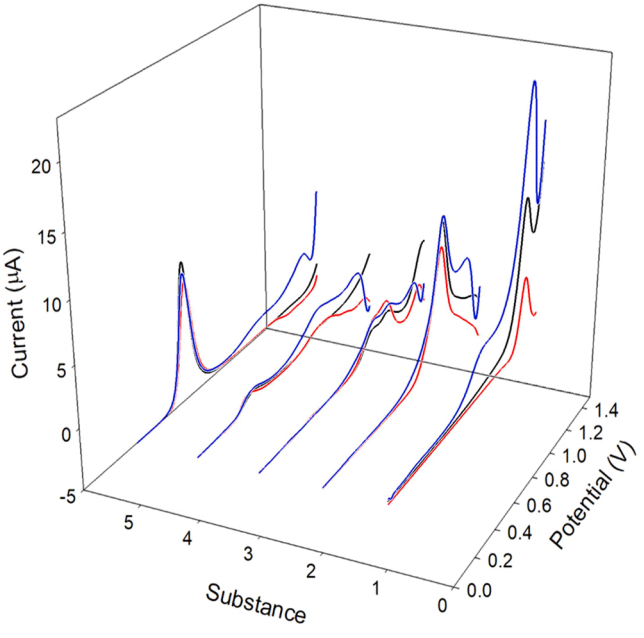

Fig. 2. Electrochemical fingerprint of 300 μM solutions of the five substances under study: 1) caffeine, 2) codeine, 3) heroin, 4) morphine and 5) paracetamol with the proposed array in this work: Carbon (red), CoPc (black) and Pd (blue).

2.6. Data analysis

Initial analysis of the voltammetric signals was done with MultiTrace software (PalmSens, Houten, The Netherlands), which allowed to calculate the peak heights and areas for the different stock samples. From those, the calibration plots were build using the data of the replicate measurements (n = 4) with the aid of Sigmaplot (Systat Software Inc., San Jose, CA, USA), and analytical parameters such as sensitivity, linear range, LOD, etc. were calculated.

Chemometric analysis was done in Matlab R2018b (MathWorks, Natick, MA, USA), making use of its Statistics and Machine Learning Toolbox, by specific routines written by the authors. Briefly, upon measurement of the set of samples described in Section 2.5 with the sensor array, voltammetric responses of the three electrodes were combined into a single vector. Genetic algorithms (GAs) were then used as feature selection tool to reduce the number of inputs to be fed to the chemometric model given the large dimensionality of the voltammetric data [24]. Next, quantitative models to individually quantify each of the analytes were built by partial least squares regression (PLS-1) [25], and their performance was then assessed towards the samples that formed the test subset to obtain more realistic performance indicators.

3. Results and discussion

3.1. Characterization of the sensor array

Modified electrodes can be prepared by several different techniques [23,26]. In the present work, the approach used for the modification of the electrodes is based on the use of a composite material (containing the different modifiers) through the formation of an ink-like paste, which is then casted onto the electrode, generating a new surface highly suitable to carry out electrochemical measurements. Besides, the preparation and modification with these inks is extremely easy and has a very low-cost, making this methodology an interesting approach for the fabrication of chemically-modified transducers.

In our case, after an initial screening of different electrochemical modifiers considered in prior studies involving ETs [27–31], an array of three electrodes was prepared using bare graphite, Pd and CoPc as the modifiers. The selection was based on the suitability of the different electrodes to obtain the discrimination of the different drugs (data not shown) [32]. The inclusion of graphite provided somehow a control point that allowed to actually evaluate the advantages derived from the incorporation of the other modifiers. Pd is well-known for its good (electro)catalytic activity towards a wide range of reactions and compounds, while the usage of nanoparticles has demonstrated to be an attractive alternative to the respective bulk metals given its higher

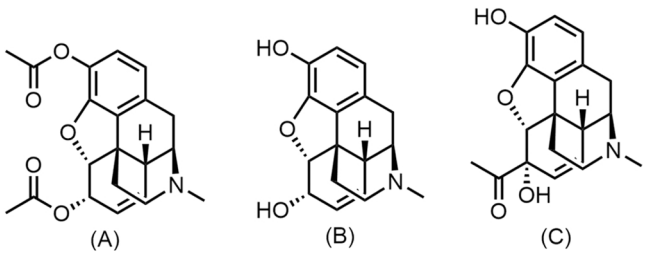

Fig. 3. Chemical structure of (A) heroin, (B) morphine and (C) 6-MAM (a heroin metabolite).

surface/mass ratio and improved electrochemical properties. Lastly, phthalocyanines are reported to be efficient electrocatalysts in the determination of many important inorganic, organic, or biological compounds.

Before tackling the resolution of the mixtures with their corresponding cutting agents, which is the main goal of this work, the modified electrodes were first physically and electrochemically characterized.

Upon modification of the SPCE as described in Section 2.3, those were characterized by SEM imaging. Microscopy studies show that the two modifiers are distributed quasi-homogeneously along the graphite layers (Fig. S1, supplementary material), and more importantly, confirm the presence of the modifiers inside the ink-like composite.

Next, the evaluation of the voltammetric responses of each of the modified sensors towards each of the compounds individually was carried out to assure that distinguished signals are generated . That is, to ensure that the electrodes respond to the different analytes, and that differentiated responses are also obtained between them. To carry out the measurements, SWV was chosen given its high sensitivity and fast scan rates, which in combination with the compact low-power instrumentation required for electrochemical measurements, offers particular promise for decentralized security screening applications [33,34].

As can be observed in Fig. 2, different overlapping peaks can be remarked. In the case of heroin, an irreversible oxidation split peak is shown around 0.97 V, corresponding to the oxidation of the tertiary amine group (Fig. 3A), resulting in a secondary amine which is then further oxidized (Figs. 3 and S2) [35]. In addition, a smaller extra peak appeared at a lower potential (ca. 0.40 V, which is more evident in Fig. 4), corresponding to the oxidation of the phenol group of 6-monoacetylmorphine (6-MAM, Fig. 3C) present in a 3% w/t in the sample. 6-MAM is an impurity, commonly found in heroin synthesis, that comes both from the incomplete acetylation of morphine as well as from the hydrolysis of heroin (as it is a product of its hydrolysis).

Similarly, morphine also shows the oxidation peak ca. 0.40 V

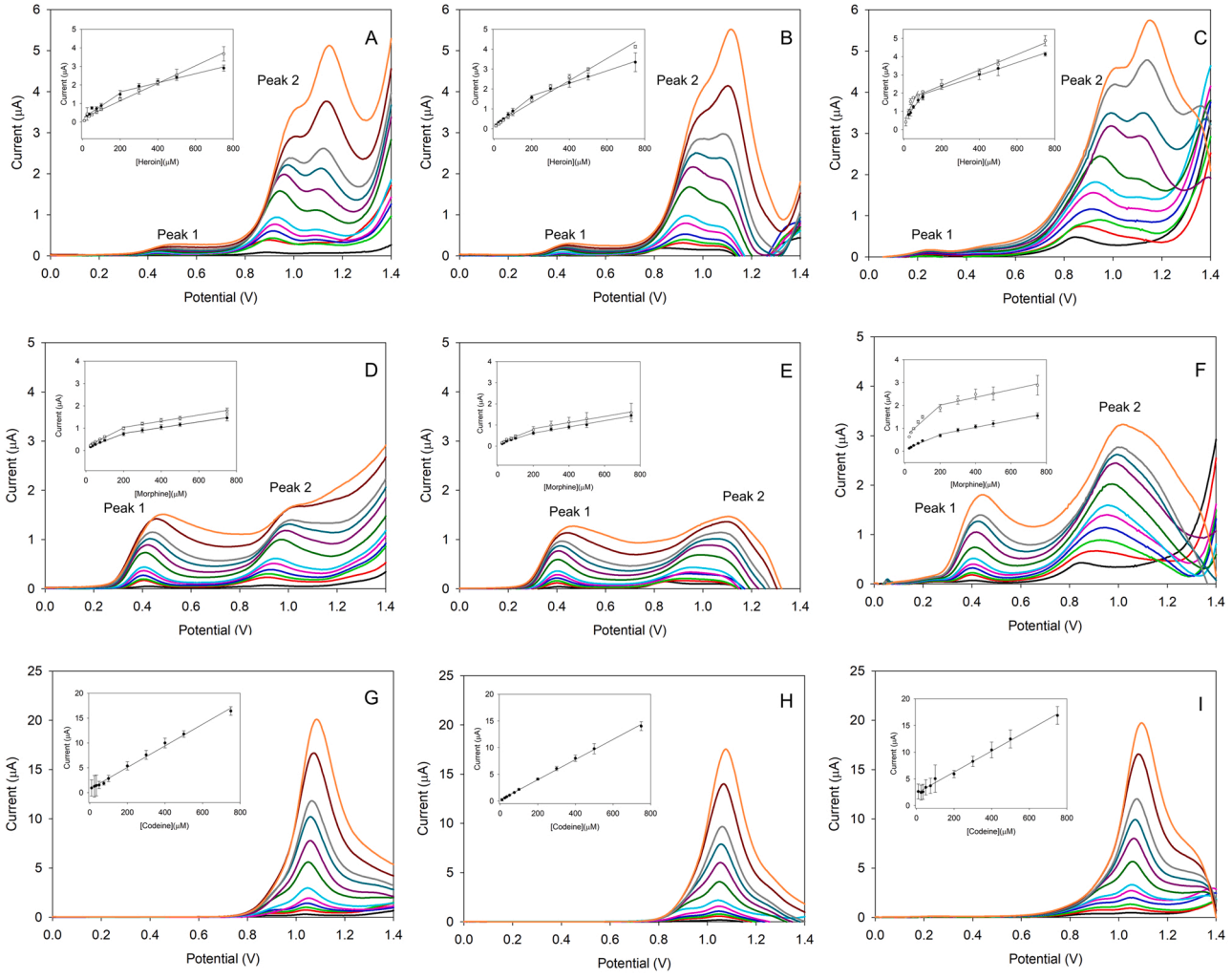

Fig. 4. Square wave voltammograms obtained for (A-C; top row) heroin, (D-F; middle row) morphine and (G-I; bottom row) codeine using (A,D,G; left column) graphite, (B,E,H; middle column) CoPc and (C,F,I; right column) Pd, respectively. Series of plots correspond to increasing concentrations from 10 to 1000 μM. Insets correspond to the linear regressions of peak height (at the observed potential maximum) vs. concentration, excluding the point 1000 μM as saturation of the voltammetric signal was reached for certain compounds.

Table 1
Calibration data (y vs. x) for the separate determination of heroin, morphine, codeine, paracetamol and caffeine employing the proposed sensor array.

Compound	Potential (V)	Sensitivity (nA μM^{-1})	Intercept (μM)	r	LOD (μM)	Linear Range (μM)
Sensor 1: Carbon						
Heroin	0.49	6.1	0.28	0.986	3.33	25–200
		2.5	1.13	0.985		200–750
	1.16	4.7	0.21	0.997	31.8	25–750
Morphine	0.43	3.3	0.10	0.996	5.33	25–200
		1.3	0.51	0.997		200–750
	0.99	4.2	0.16	0.995	8.65	25–200
		1.4	0.76	0.994		200–750
Codeine	1.11	21.7	0.72	0.998	1.80	25–750
Paracetamol	0.39	22.3	0.12	0.999	0.82	25–750
Caffeine	1.33	15.6	4.02	0.993	44.0	50–750
Sensor 2: CoPc						
Heroin	0.43	7.2	0.15	0.999	3.95	25–200
		3.1	1.03	0.996		200–750
	1.14	5.5	0.22	0.994	83.3	25–750
Morphine	0.40	2.8	0.071	0.994	2.88	25–200
		1.5	0.32	0.995		200–750
	1.11	3.4	0.11	0.994	96.6	25–200
		1.5	0.53	0.991		200–750
Codeine	1.09	18.9	0.21	0.999	4.29	25–750
Paracetamol	0.40	17.4	-0.16	0.999	0.75	25–750
Caffeine	1.36	10.8	0.0069	0.998	65.0	50–750
Sensor 3: Pd						
Heroin	0.27	14.3	0.41	0.988	5.31	25–200
		3.5	1.59	0.991		200–750
	1.01	34.8	0.12	0.975	14.8	25–200
		4.2	1.57	0.966		200–750
Morphine	0.46	3.2	0.086	0.985	25.9	25–200
		1.5	0.44	0.993		200–750
	1.01	6.9	0.62	0.954	60.7	25–200
		1.7	1.68	0.961		200–750
Codeine	1.11	19.8	2.31	0.997	11.7	25–750
Paracetamol	0.38	19.0	-0.11	0.999	3.33	25–750
Caffeine	1.35	11.6	17.2	0.983	104	200–750

corresponding to the phenol group of 6-MAM [36], but being more prominent in this case. The second peak corresponds to the oxidation of the tertiary amine group (Fig. 3B), which in this case is not further oxidized (Fig. S3). In the case of codeine, only one broad peak can be observed, which again corresponds to the oxidation of the tertiary amine (Fig. S4), but also showing a small shoulder, which is almost superimposed and that is attributed to the oxidation of the 6-hydroxy groups [37]. Finally, in the case of paracetamol, only a well-defined Gaussian peak corresponding to the oxidation of the amide group (Fig. S5) is observed ca. 0.40 V [38,39], while in the case of caffeine, also a single peak (Fig. S6) is obtained, which corresponds to the oxidation of the C-8 to N-9 bond to give the substituted uric acid [39,40]. In this direction, the proposed mechanisms for the electrochemical oxidation of the different evaluated substances are provided in Figs. S7 and Figs. S2 to S6.

It can also be seen that the oxidation of the phenol group is overlapped with the oxidation peak of paracetamol. Similarly, the same situation occurs with the second oxidation peak of heroin and morphine, which also will overlap with the one from codeine. However, this is not an issue given distinguishable voltammetric profiles are obtained; i.e. different peak shapes and sensitivities are still obtained for each of the compounds. Lastly, in the case of caffeine, a single oxidation peak at higher potential is obtained (ca. 1.33 V).

After this first general overview of the voltammetric responses, the calibration curves for the five compounds under study were constructed by measuring solutions of increasing concentration from 25 to 750 μM. This step is relevant for further quantification models as it is important to identify the proper working ranges. All of the electrochemical measurements were performed in PBS at pH 7.0. The selection of this neutral pH is due to the fact that heroin and morphine suffer hydrolysis reactions at basic pH, as is described in the literature [41,42].

In all cases, the peak height which corresponds to the maximum of the oxidation signal was taken. This characterization is essential not only to evaluate the response of the sensors, but also to determine the concentration ranges which they can operate and that will be used to do the analysis with the electronic tongue approach. As can be observed in Fig. 4s and S7, the responses obtained were linear for the vast majority of cases in the studied ranges ($r > 0.99$), showing one peak or two depending on the compound evaluated.

In the case of heroin, two peaks were shown. C and CoPc modified inks presented two linear ranges for the peak 1 (black), corresponding to the oxidation of the phenol group of 6-MAM. The lower linear range goes from 25 to 200 μM and the high range from 200 to 750 μM. For peak 2 (red), one linear range is presented (from 25 to 750 μM), corresponding to the oxidation of the amine group (Fig. 4AB). Pd modified ink showed a different performance displaying two linear ranges for peak 1 and peak 2 (Fig. 4C).

The next compound analysed was morphine. In this occasion, a similar response was given for the three sensors of the array. Two peaks were observed, with two linear ranges for each of them (Fig. 4D–F). The low range goes from 25 to 200 μM and the high from 200 to 750 μM, similar to heroin as could be expected.

Codeine and paracetamol showed a single peak with good linearity over the whole range (from 25 to 750 μM) with the three modifiers tested (Fig. 4G–I and Fig. S7J–L). Lastly also a single peak is obtained for caffeine, but with different linear range based on the electrode considered. Employing C and CoP, the linear range goes from 50 to 750 μM, whereas with Pd modified ink, the linear range narrows from 200–750 μM (Fig. S7M–O).

Based on the previous results, for the multi-determination of the drugs mixtures, the concentration working ranges were streamlined from 0 to 750 μM for heroin, morphine, codeine, paracetamol and caffeine. The analytical parameters derived from the calibration curves for each sensor are summarized in Table 1.

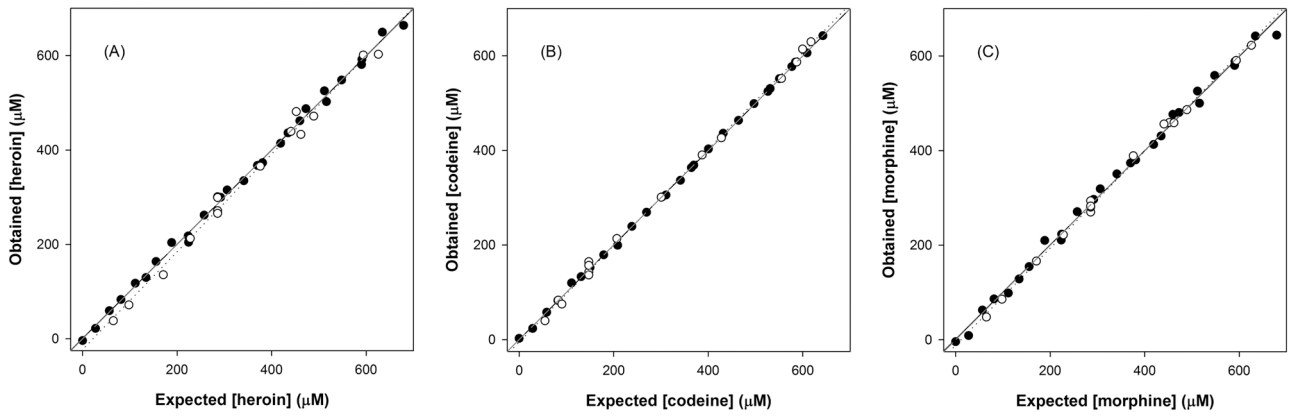

Fig. 5. Modeling ability of the optimized GA-PLS model for the 3 compounds case. Comparison graphs of obtained vs. expected concentrations for (A) heroin, (B) morphine and (C) codeine, for both the train (•, solid line) and test subsets (○, dotted line). The dashed line corresponds to the ideal comparison line (y = x).

3.2. Repeatability and reproducibility studies

Upon completing the calibration, a stability study was done in order to demonstrate that the sensors were capable to withstand the large number of measurements necessary when developing ET applications.

To this aim, codeine was selected as the substance to evaluate the variation on its voltammetric response upon successive measurements, assuming a similar behavior for the rest of the drugs. More specifically, a stock solution of codeine of 450 μM was measured for 25 consecutive times employing the same electrodes and measuring also a blank (PBS solution) in between each measurement to evaluate the repeatability of the sensors. Thus, each sensor was used for 50 consecutive measurements; a significant number given the disposable nature of SPEs.

Voltammetric data were collected and analyzed, from which the relative standard deviation (% RSD) was calculated. The values for carbon, CoPc and Pd modified ink-sensors were 1.84%, 2.01% and 2.14%, respectively. Significantly, the three modified sensors presented better stability than the unmodified sensor, which showed a RSD value of 9.87%. Thus, from this study it was concluded that no fouling or drift effects are observed with the proposed sensor array. This, in essence, means that any possible adsorption of the oxidized forms of the studied compounds did not affect appreciably the electrodes' performance or stability, given no differences were observed along consecutive measurements; equivalently, the same could be said for the applied potentials, if there is any doubt on the relatively high values used.

Complementary to the previous study, the reproducibility of construction of the ink-modified SPCE was also assessed. The experiment was done preparing each modified ink by triplicate (n = 3) and measuring consecutively with a heroin stock solution. The results for each sensor present a good construction reproducibility with RSD values of 3.97%, 6.95% and 5.67% for carbon, CoPc and Pd inks, respectively.

3.3. Quantitative analysis of drug mixtures using PLS regression

Despite different voltammetric profiles are obtained for each of the compounds when analysed individually (Fig. 4), even with different response for the different considered electrodes, it is clear that there will be an overlap on the voltammetric responses when mixtures of those are to be analysed at pH 7 (Figs. S8 and S9). Thus, in order to achieve the individual quantification of each of the compounds, the use of chemometric methods is required as such quantification cannot be achieved via univariate regression (taking either the peak height or area). In this direction, ET approach relies on the combination of an array of sensors that show complimentary responses towards the compounds of interest, with a multivariate calibration method that allows to build a model that relates the responses of the different sensors with the concentration of each of these compounds [19,43].

In the previous section, the sensitivities of each of the electrodes towards each of the compounds have been shown different (Table 1), a situation highly desirable when developing an ET application. Thus, the next step prior to build the quantitative model that allows to detetermine the individual substances from the overlapped voltammograms was the selection of the chemometric tool to be used. In this case, given the ultimate goal of this research project is developinga device to detect different illicit drugs, PLS-1 was chosen as the modeling tool given it is one of the simplest (e.g. in comparison to artificial neural networks, ANNs) and widely used multivariate calibration techniques to choose [44].

Lastly, although the use of a pre-processing stage to reduce the number of input variables is not required when PLS is being used, it has demonstrated that even in such cases this data reduction stage improves the model's prediction and generalization ability [43]. Again, as the aim is to develop the simplest model possible, the use of GAs as feature selection tool was chosen given upon identification of the most relevant features, no further computing processing will be required for each new measurement that is being performed. Thus, in this manner, the most relevant features from each of the voltammograms were selected with the aid of GAs and used as input into the PLS model. The outcome of GAs optimization is shown in Fig. S10, where the raw voltammetric responses for 300 μM solutions of each of the considered compounds is plotted with cross marks underneath corresponding to the selected features. On the contrary to the straighforward idea that the algorithm will select essentially the points corresponding to the peaks' maxima, it finally uses regions with less overlap and where the differences between signals are more pronounced. (that is, the points corresponding to the front and back of the peaks).

As already stated in Section 2.5, two different sets of samples were prepared: the first one in which ternary mixtures of the three considered drugs were considered, while in the second one also the presence of two different cutting agents was examined. The aim of the first set was to confirm the potential of the proposed ET to carry out the individual quantification of the opiates, whereas the second one aims to confirm that the ET is able to counterbalance the interferences of the cutting agents and successfully carry out the quantification of the drugs. In both cases, GA-PLS models were built using the data for the train subset, and its performance assessed towards the samples of the test subset, selecting the number of latent variables (LVs) that lead to the lowest root mean square error (RMSE).

3.3.1. Mixtures of the three drugs

From the raw voltammetric responses of the three electrodes (318 current values x 3 sensors), a total of 103 features were selected by use of GAs and used to build the PLS-1 models (Fig. S10). The selected number of LVs were 7 for heroin, 15 for codeine and 8 for morphine. Next, the

Table 2
Fitted regression lines for the comparison between obtained vs. expected values for the different sets of samples and the three considered APIs.

Compound	Slope	Intercept (μM)	r	RMSE (μM)	NRMSE	Total NRMSE
train subset (n = 27)						
Heroin	0.998 ± 0.019	0.7 ± 7.5	0.999	9.39	0.014	0.014
Codeine	1.000 ± 0.007	0.1 ± 2.7	1.000	3.35	0.005	
Morphine	0.996 ± 0.026	1.3 ± 10.0	0.998	12.5	0.019	
test subset (n = 15)						
Heroin	1.035 ± 0.064	-22.5 ± 24.3	0.995	20.6	0.030	0.026
Codeine	1.020 ± 0.026	-5.1 ± 9.4	0.999	10.1	0.016	
Morphine	1.026 ± 0.030	-11.3 ± 11.5	0.999	11.1	0.016	

Intervals are calculated at the 95% confidence level. RMSE: root mean square error; NRMSE: normalized root mean square error.

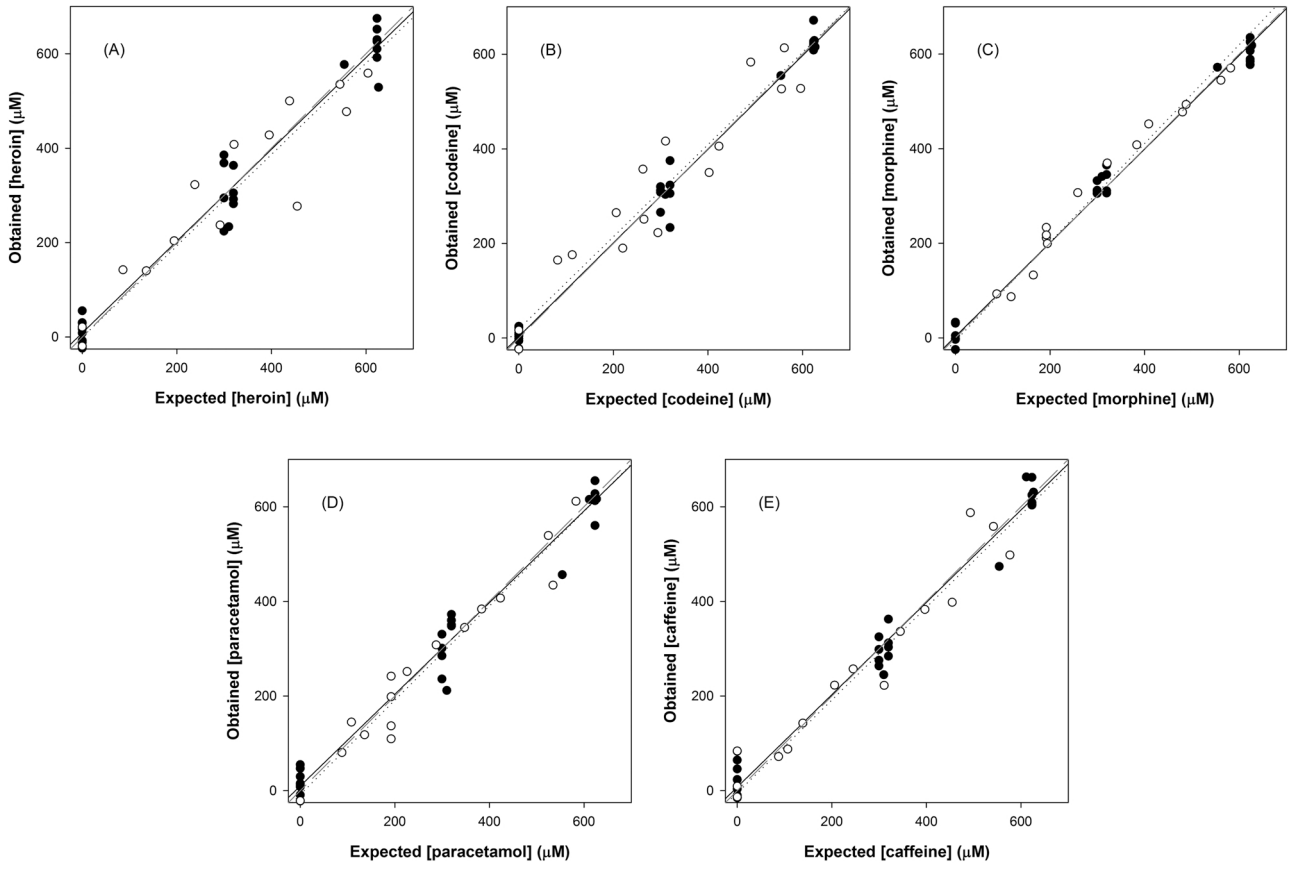

Fig. 6. Modeling ability of the optimized GA-PLS model for the 5 Compounds case. Comparison graphs of obtained vs. expected concentrations for (A) heroin, (B) morphine, (C) codeine, (D) paracetamol and (E) caffeine, for both the train (•, solid line) and test subsets (○, dotted line). The dashed line corresponds to the ideal comparison line (y = x).

Table 3
Fitted regression lines for the comparison between obtained vs. expected values for the different sets of samples and the five considered compounds.

Compound	Slope	Intercept (μM)	r	RMSE (μM)	NRMSE	Total NRMSE
train subset (n = 27)						
Heroin	0.972 ± 0.068	8.7 ± 27.3	0.986	43.5	0.061	0.053
Codeine	0.990 ± 0.041	3.1 ± 16.6	0.995	26.2	0.037	
Morphine	0.990 ± 0.040	3.0 ± 16.2	0.995	25.5	0.036	
Paracetamol	0.969 ± 0.071	9.7 ± 28.8	0.984	45.9	0.065	
Caffeine	0.976 ± 0.063	7.5 ± 25.5	0.987	40.6	0.057	
test subset (n = 17)						
Heroin	0.968 ± 0.169	-0.7 ± 69.3	0.953	76.3	0.107	0.077
Codeine	0.974 ± 0.169	19.2 ± 58.0	0.954	63.3	0.089	
Morphine	1.042 ± 0.087	-5.9 ± 28.5	0.989	30.1	0.043	
Paracetamol	0.997 ± 0.124	-8.4 ± 38.8	0.975	41.8	0.059	
Caffeine	0.981 ± 0.130	-4.5 ± 39.7	0.972	50.1	0.070	

Intervals are calculated at the 95% confidence level. RMSE: root mean square error; NRMSE: normalized root mean square error.

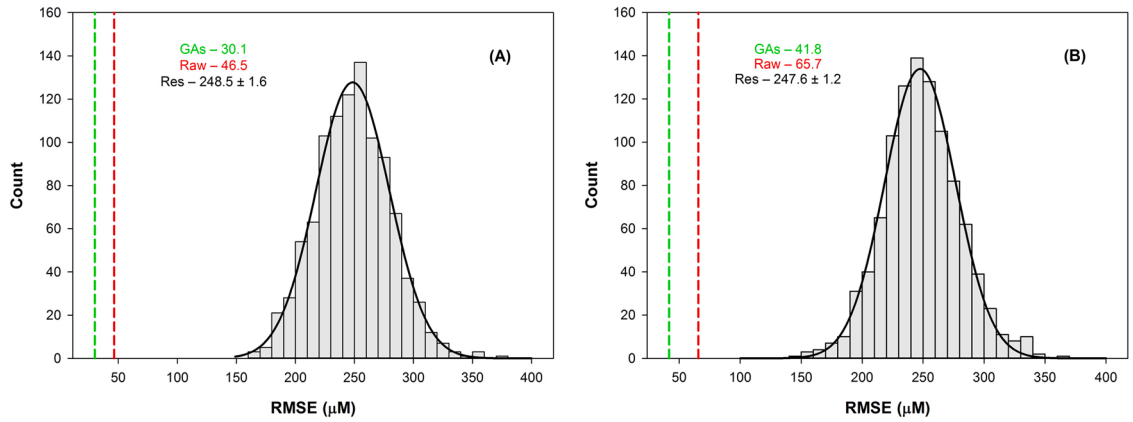

Fig. 7. Histogram comparing the success of PLS models with raw data (red) and GAs-PLS (green) to that of shuffled models (1000 iterations) for (A) codeine and (B) paracetamol, for the set of samples corresponding to mixtures of the 5 compounds. For the shuffled models, the data was fitted to a 3 parameter Gaussian curve and the RMSE values compared to the former. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

comparison graphs of predicted vs. expected concentrations were built (Fig. 5) and the linear regressions of the comparison lines fitted (Table 2) for the three drugs. As can be seen from the plot, a satisfactory trend is obtained in all the cases, with regression lines close to the ideal one (y = x). Moreover, from the regression parameters in the table we see these are very close to the ideal ones (i.e. 1 slope and correlation coefficient, and 0 intercept), being all of them within the intervals calculated at the 95% confidence level. Fig. S11 displays how obtained values are within the expected joint confidence intervals for all cases.

Despite the satisfactory trend, it has to be reckoned that slightly better performance is obtained for the train subset, but this is due to the fact that the data of the test subset is not used at all during the modeling stage, and thus it provides a more realistic metric of the model performance. However, if comparing the RMSE values obtained for both subsets, it can be seen how the differences are actually not that high, which confirms the goodness of the model and the capability of the proposed ET to achieve the simultaneous quantification of the three drugs. Thus, the next step was to confirm whether using the same approach we are able not to only quantify mixtures of the pure drugs, but also to detect and quantify the cutting agents considered, which would provide a more realistic use of the developed strategy.

3.3.2. *Mixtures of the three drugs and the two cutting agents*

Under the same conditions as above, but making use of a different experimental design given the larger number of analytes to be considered, a new set of samples was prepared as described in Section 2.5 and measured employing the sensor array. As before, GA-PLS models were built to determine each analyte in the mixture, but in this case selecting a total of 114 features from the raw voltammetric responses (Fig. S10). The selected number of LVs were 13 for heroin, 18 for codeine, 10 for morphine, 6 for paracetamol and 12 for caffeine. Again, the comparison graphs of predicted vs. expected concentrations were built for each of the analytes (Fig. 6), and the linear regression parameters calculated (Table 3). Again, a satisfactory trend is obtained for both subsets, with slightly better behavior for the train subset as already discussed, but with RMSE values of the same order of magnitude.

Although the model performance metrics are slightly worst for the 5 compounds case than for the 3 compounds one (total NRMSE for the three drugs of 0.084 vs. 0.026 for the test subset), it has to be considered the higher complexity of the case. That is, on the one side, the use of a fractional experimental design (as is the CCF) to keep the number of samples required to build the model reasonable. On the other side, and as already reported, the presence of certain cutting agents can influence the voltammetric response, up to the point that the observed peak for the pure compound might not be seen in the presence of the adulterant [16,17].

In regards to the latter, herein we have demonstrated how making use of a proper set of samples, the model is able to correctly quantify both the drugs and cutting agents. Thus, the same approach can be applied to the identification and quantification of others mixtures. Moreover, if multi-way processing methods are to be used instead of two-way PLS models, the correction of the presence of an interfering species, even if not initially considered in the response model, might be possible thanks to their "second order advantage" [45,46].

Therefore, taking all this into account, it's clear that the use of the herein proposed voltammetric ET shows huge potential to carry the identification and quantification of seized drug samples, either those being pure or already mixed with other drugs and/or cutting agents.

Lastly, despite already taking the precaution of using a separate validation subset of data (the test subset), a permutation test or "target shuffling process" was carried out to demonstrate that neither the high dimensionality of the data nor the use of GAs and PLS-1 is resulting in over-fitted models. Such test allows the identification of incorrectly perceived cause-and-effect relationships in modeling ("chance correlation") by taking as null hypothesis that samples labels are exchangeable. Briefly, this test involves repeatedly and randomly reordering of the responses variables (Y), followed by the building of a new model upon shuffling of the data labels. In other words, a new model is built upon assignment of an "incorrect" y-value to each sample corresponding to the one from another sample. This process is repeated several times to ensure that the statistics calculated are significant (up to 1000 times in our case). For each of the permutations, the different performance metrics were calculated and compared to the actual model with the proper labels. As an example, a histogram summarizing the RMSE values of the different models for the three drugs plus codeine and paracetamol are shown in Fig. 7, from which the significance of the obtained results is evident.

4. Conclusions

The potential of ETs for the simultaneous determination and quantification of different opiates in the presence of common cutting agents has been demonstrated. More specifically, a voltammetric sensor array consisting in three SPCE modified with graphite, cobalt (II) phthalocyanine and palladium inks were employed to extract the electrochemical fingerprints of heroin, morphine, codeine, caffeine and paracetamol by means of SWV. Despite the advantages that electrochemical methods may offer, the simultaneous determination of the aforementioned compounds can also be challenging given their similar electrochemical response; especially when attempting the discrimination between heroin and morphine, since both molecules present the same functional groups in their skeleton. Thus, a partial least square regression (PLS) model for

the quantification of heroin, morphine, codeine, caffeine and paracetamol mixtures at the μM level was built employing a central composite face-centered (CCF) experimental design. A very satisfactory performance was obtained, demonstrating that the use of the herein proposed voltammetric ET shows huge potential to carry the identification and quantification of seized drug street samples, either those being pure or already mixed with other drugs and/or cutting agents.

Overall, the advantages of ETs to deconvolute complex overlapping electrochemical responses and achieve the simultaneous quantitative determination of several analytes have been shown. Moreover, the use of a properly formulated graphite-polystyrene ink has been demonstrated as a simple approach to obtain an array of modified electrodes with different responses towards the compounds under study.

As for the data processing, GAs allowed to reduce the number of inputs fed to the model through the identification of its most relevant features, what in turn reduced its complexity , and at the same time improved its performance. Lastly, by conducting a permutation test or "target shuffling process", it was also demonstrated that neither the high dimensionality of the data or the use of GAs and PLS resulted in overfitted models. In fact, this verification provided a high significance for the obtained RMSE values, higher than 99.99%, both for train and test subsets (P value lower than $3{\cdot}10^{-5}$), as illustrated in Fig. 7.

In conclusion, the results presented herein suggest the potential of these devices to be used as analytical tools for the detection of illicit substances from street samples offering low-cost measurement systems with rapid response, simple usage and high portability; all of them ideal characteristics for point-of-use forensic or law-enforcement applications.

CRediT authorship contribution statement

Dionisia Ortiz-Aguayo: Methodology, Investigation, Writing – original draft. **Ceto Xavier Cetó:** Methodology, Software, Writing – original draft. **Karolien De Wael:** Resources, Writing – review & editing, Funding acquisition. **Manel del Valle:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2021.131345.

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