Artificial Neural Networks for the Resolution of Dopamine and Serotonin Complex Mixtures Using a Graphene-Modified Electrode

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Abstract. This work explores an electrode modified with electrochemically reduced graphene oxide (ERGO) for the voltammetric resolution of mixtures of neurotransmitters and its most common interferents. This enhanced sensitivity device coupled with advanced chemometric tools, such as artificial neural networks (ANNs), is able to resolve and quantify complex mixtures with overlapped signals. In this case, it has been developed with dopamine (DA), serotonin (5-hydroxytryptamine, 5-HT) and its main physiologic interferents, ascorbic acid (AA) and uric acid (UA), which play a relevant role in human body. The results obtained for individual analysis make evident a twice sensitivity increase versus the unmodified electrode. Furthermore, it has been attained an ANN model with good correlation ability allowing the separation and quantification of each compound with comparison slope of predicted vs. Expected concentrations with correlation better than 0.974. In short, the developed ERGO-modified sensor not only improved the signal but also it permitted to resolve and quantify each compound in complex mixtures when the proper chemometric treatment is used.

Keywords: Graphene, dopamine, serotonin, ascorbic acid, uric acid.

1. Introduction

From graphite, which is the most common and thermodynamically stable form of carbon, it is obtained graphene, a novel an interesting nanomaterial for electrochemical use. A large number of approaches have been employed to synthetize graphene, the most common procedure is the chemical oxidation of graphite [1–3], where graphene oxide (GO) is obtained as product.

GO is considered an individual sheet of graphite with more structural defects than graphene and rich in oxygen functional groups [4]. It is demonstrated that the amount of oxygen in the graphene surface is associated with different properties, for example, graphene and reduced graphene oxide show high conductivity, against GO which has insulating properties. The non-conductive properties of GO lead to the adoption of reduction methodologies to revert to graphene properties again. The reduction step can be carried out via chemical, thermal or electrochemical reduction [5–7]. In this work, the used nanomaterial was electrochemically reduced graphene oxide (ERGO), which was obtained by applying a certain potential on the GO deposited onto the electrode. The use of electrochemical techniques for the reduction of the material have as advantage the control of oxygen functionalities on the material surface [8].

Other graphene interesting properties, like its stunning mechanical, thermal and electronic properties, its huge active area, the fast-heterogeneous electronic transfer and its exceptional conductivity, together with the ability to be modified by anchorage of biomolecules, makes of it an outstanding material for the developing of sensors and biosensors.

Dopamine (DA) and serotonin (or 5-hydroxytryptamine, 5-HT) are two monoamine neurotransmitters that play a relevant role in human's central nervous system. DA is a catecholamine that controls a large variety of functions such as locomotion, cognition or non-voluntary actions. On

the other side, serotonin plays an important role in several processes including temperature regulation, movement control, mood regulation, sleep or appetite [9–12]. Alterations in their usual concentration levels are linked with neurological disorders, as anxiety, schizophrenia, Parkinson's or Alzheimer's diseases [13–15]. The normal concentrations in human blood samples are around 10 μM for DA and 2 μM for 5-HT [16].

The early detection of abnormal concentration levels of these neurotransmitters may help diagnosing major diseases. Generally, DA and 5-HT are analyzed in urine and blood samples by chromatographic procedures, for example high pressure liquid chromatography (HPLC) or gas chromatography (GC) [17–20]. Since DA and 5-HT are electrochemically active, this work reports an electroanalytical method for their detection, which is more selective and sensitive than chromatographic techniques. Against these advantages, the detection of DA and 5-HT trough electrochemical methods has as inconvenient the common physiologic interference of ascorbic acid (AA) and uric acid (UA), which display similar electrochemical response. Nevertheless, the signal overlapping problem could be overcame by data processing using advanced chemometric techniques.

Some of the most used chemometric tools for the quantification analysis are partial least squares (PLS) and artificial neural networks (ANNs) [21].

PLS is a mathematical method which decomposes at the same time the responses and the target values in a set of variables that maximize the covariance between them. Afterwards, a linear model that maximizes the relation among new variables is built [22,23]. On the other hand, ANNs is a method that mimics the function of human brain, it is made up of different processing units (neurons) linked together. ANNs is more flexible method as it can use linear or non-linear functions to build the model. This fact makes ANN especially useful in complex trials or non-lineal responses [24,25]. Despite the advantages of ANN, the data recorded with voltammetric sensors is of high complexity, rendering difficult the modelling. One of the solutions proposed in the bibliography is the use of data pretreatment to compress the information; some remarkable implementations for this purpose are principal component analysis (PCA), discrete wavelet transform (DWT) and fast Fourier transform (FFT) [26].

Checking the literature there are different electroanalytical methods for the detection of neurotransmitters in complex mixtures; in these the most common strategies to deal with the overlapping of the signals is the use of biocomposites [27–29] with or without nanomaterials [30–34] to improve sensor performance. Although papers about the selective detection of DA, 5-HT, AA and UA have often been reported, they have mostly tackled the issue as a sample with one important analyte and 2 or 3 interfering compounds, what makes easier its quantification. Herein, it is presented also a resolution of a complex mixture, but in this instance, we attempted the resolution of a 4 compound-mixture with two primary analytes and two interfering species, using an easy, cheap and reproducible method which could be easy scalable.

2. Experimental

2.1. Reagents and chemicals

All reagents were of analytical quality grade; all solutions were prepared using deionized water from a Mili-Q system (Millipore, Billerica, MA, USA). All samples were prepared on phosphate buffer (HK_2PO_4, H_2KPO_4 and KCl) all were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Dopamine hydrochloride and uric acid were from Sigma Aldrich, ascorbic acid were from Panreac Química (Panreac Química SLU, Barcelona, Spain) and serotonin were purchased from Acros Organics (Thermo Fisher Scientific, Geel, Belgium). Resineco epoxy kit resin was supplied from Resineco Green Composites (Barcelona, Spain) and graphite powder (particle size < 50 μm) was received from BDH (BDH Laboratory Supplies, Poole, UK). Graphene oxide were purchased for Nanoinnova Tech. (Madrid, Spain).

2.2. Sensor building and modification

A graphite-epoxy composite handmade electrode (GEC electrode), as previously established in our research group, was used as the unmodified working electrode (WE) [35]. The first step to prepare the composite was to mix the epoxy resin with its corresponding hardener and add to the previously prepared mixture the graphite powder before its hardening. Afterwards, the mixture was manually homogenized for 60 minutes and then hardened during two days at 40 ^{0}C. Finally, the electrode was polished until obtain a homogeneous surface with sandpapers.

GO was immobilized on the WE surface by physical adsorption via drop casting method. For the modification, 40 µL of the GO placed dispersion (1 mg·mL^{-1} in ultrapure water), firstly sonicated 1 hour, was casted on each electrode. Then, it was dried at 40 ^{0}C in the oven. ERGO was achieved by electrochemical reduction of the GO placed on the electrode surface [36]. The protocol for the electro-reduction of GO consisted of applying 10 cyclic voltammetry (CV) cycles in a potential range from +1.90 V to -2.30 V at 0.1 V·s^{-1}. Next, a more focused range is applied to ensure the complete reduction, this range goes from +1.00 V to -0.70 V at a scan rate of 0.1 V·s^{-1}. In this way, it is attained a blank signal which it is not interfering with sample response, making simpler the final analysis. For its proper characterization the electrode surface was analyzed electrochemically and by scanning electron microscopy (SEM) by using a scanning electron microscope EVO®MA10 (Zeiss, Germany) operated at 20 kV.

2.3. Electrochemical measurement

All CV measurements were performed on Bas-Zahner IM6e (Kronach, Germany) potentiostat controlled by Thales software at room temperature and without stirring. In the measurements it was used a typical three electrodes circuit, formed by a combined reference-counter electrode of Ag/AgCl (0.1 M KCl) and Pt, respectively, and, as a working electrode, a handmade GEC electrode. The CV curves were carried out in electrolyte solution (phosphate buffer 50 mM at pH=7.4 with 0.1 M KCl) in a range from +0.70 V to -0.20 V at a 10 mV·s^{-1} scanning rate. EIS experiments were performed on AUTOLAB PGSTAT30 (Ecochemie, Netherlands) employing the same assembly as in CV measurement.

2.4. Experimental design

A set of standards containing the 4 analytes was used to build the ANN response model. The design chosen for defining the experimental layout was a fractional factorial design with 4 factors, 3 levels and one central point (L36), obtaining then, the training subset. The model performance was evaluated with an external set of samples, which was defined randomly along the training subset concentration range, this group is labeled as test subset. Finally, the model was built with a total of 45 samples (see table S. 1 on supplementary information). These 45 samples were measured randomly to avoid trends and reduce the error sources.

In order to obtain reproducible voltammetric signals, an electrochemical cleaning stage was implemented between each measurement, specially to minimize dopamine fouling process on the electrode surface. For the cleaning step, a +1.20 V static potential was applied during 40 s in a cell containing 20 mL of HCl 0.1 M.

2.5. Data processing

Chemometric analyses were performed using MATLAB 2016b (MathWorks, Natick, MA, USA) and its Neural Network and Wavelet Toolboxes. The data was processed via artificial neural networks.

To preprocess the departure data, it was used discrete wavelet transform (DWT), with a *Daubechies* mother function and a second level of compression.

3. Results and discussions

3.1. Characterization of the electrode surface

In order to characterize the electrode surface, there were carried out different studies by electrochemical and microscopic techniques.

On the one hand, SEM microscopy was used to characterize the changes on the surface electrode when it is modified with the different graphene derivates. Fig. 1A depicts the GEC electrode bare surface; as the picture shows, the composite is evenly distributed with no obvious particle distribution. After the deposition of GO on the surface, and its successive electroreduction (Fig. 1B and 1C), it is possible to identify new white zones that were assumed as ERGO; amplifying the image of these white parts, it proved that it was a tissue-like material, which corresponds with typical characteristics of graphene. Contrasting the images, it can be claimed the correct deposition of ERGO on the electrode surface. On the other hand, electrochemical techniques such as CV or EIS were used to study the electroreduction of GO to ERGO and their related responses. In the bibliography one may find a wide range of methods to reduce GO [3,6]; in this work we followed an already established protocol [36], where GO is reduced by applying several voltammetric cycles with the CV technique (described previously at sensor building and modification section). To ensure the correct reduction of GO was accomplished, several assays were done using a common standard, in this case the redox couple $[Fe(CN)_6]^{3-/4-}$. The first trial (depicted in Fig. 1D) compares the CV response between a bare GEC electrode and an ERGO-GEC electrode, showing a reversible redox peak and great correlation between the two voltammograms and a better response for the modified electrode, due to the ERGO excellent conducting properties. Additionally, changes on the surface area where followed via EIS; with this technique it could be observed the oxidation states of graphene, checking by this way the previous results. In Fig. 1E, Nyquist plots, carried out between 0.5 MHz and 0.05 Hz with a sinusoidal voltage perturbation of 10 mV amplitude were recorded comparing: a bare GEC electrode in blue, the same electrode modified via drop casting with GO, in black, and finally, ERGO, once the electroreduction is fulfilled, in red. As expected GO shows larger charge transfer resistance than GEC electrode and ERGO-GEC electrode, what means that the order of conductivity is ERGO-GEC as the most conductive material and GO as the least conductive. It could be described GO as insulator by one fact, the quantity of oxygenated functionalities. On one hand, the existence of functional groups disrupts the sp2 configuration and its aromaticity, making the charge transfer harder. On the other hand, carboxylic groups are deprotonated at pH 7.2 producing negative charges on the electrode surface, which hinders the electron transfer repelling electrostatically the redox couple $[Fe(CN)_6]^{3-/4-}$ [5,8].

Finally, the active surface area was characterized according to the Randles–Sevcik equation (eq 1), whereby n is the number of electrons involved in the redox reaction, F the Faraday's constant (96485 $C \cdot mol^{-1}$, c the concentration of electroactive substance ($mol \cdot cm^{-3}$), A the electrode active area in cm^2, v the scan rate ($V \cdot s^{-1}$), R the gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$), T the temperature in K and D the analyte diffusion coefficient ($cm^2 \cdot s^{-1}$). Applying 5 different scan rates (25, 50, 100, 200 and 500 $mV \cdot s^{-1}$) it could be calculated the active area of WE from the slope of the fitted line of $v^{1/2}$ *vs.* $Ip \cdot c^{-1}$ graph. The calculated active areas were, 33.90 mm^2 for GEC electrode, 46.90 mm^2 for the ERGO-modified electrode, whereas the geometric area was 28.27 mm^2 (Ø=6 mm). These results confirm the correct modification of the electrode, as adding graphene on the surface causes wrinkles, as it is also perceptible in SEM images; these wrinkles increase the active area of the electrode, a fact observed in the obtained results.

$$Ip = 0.446 \cdot n \cdot F \cdot c \cdot A \cdot \sqrt{v} \cdot \left(\frac{nDF}{RT} \right)^{\frac{1}{2}} \qquad (1)$$

In summary, the characterization confirms the correct modification of the electrode via drop casting relating the appearance of a tissue-like material with the increase of the surface area of the electrode and the CV signal improvement. Furthermore, electrochemical tests confirm the electroreduction of GO.

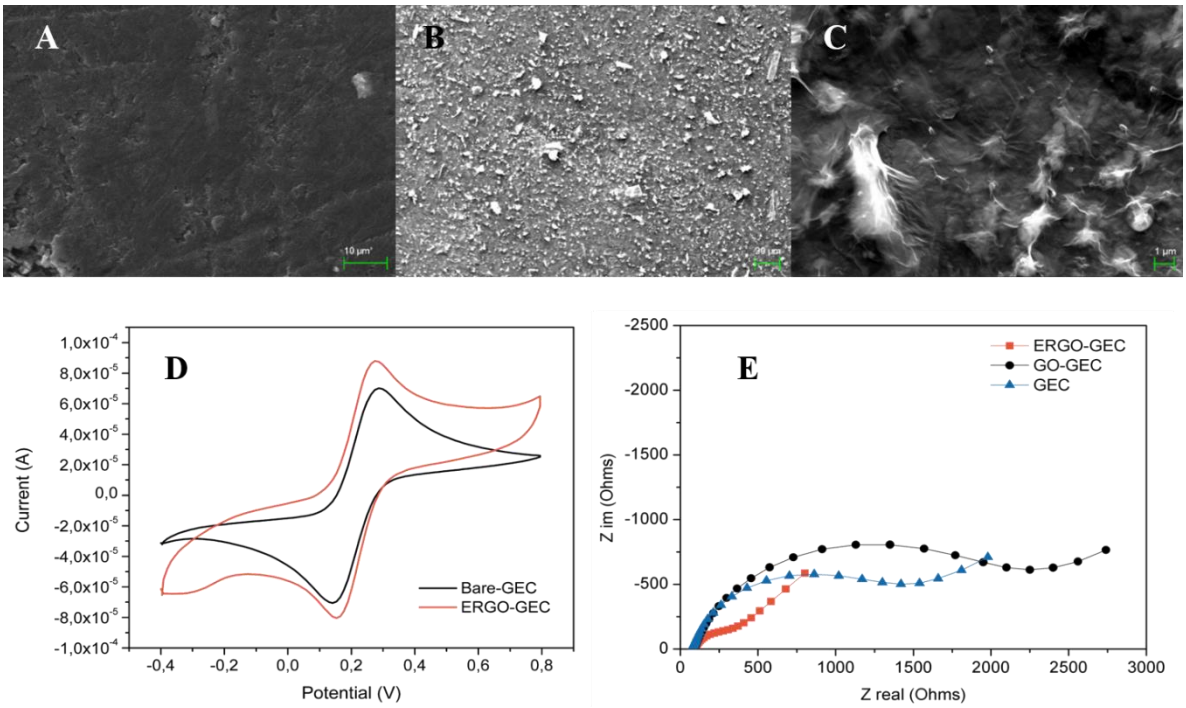

Fig. 1. Characterization of the surface electrode via SEM images of GEC electrode, A, and ERGO modified electrode, B and C. D, comparison of CV bare-GEC and a modified ERGO-GEC electrodes. E, impedimetric measurements of the modification process. Measurements in 50 mM phosphate buffer + 0.1 M KCl containing 5mM $[Fe(CN)_6]^{3-/4-}$ pH=7.4.

3.2. Individual responses and calibration curves

Ascorbic acid, uric acid, dopamine and serotonin signals where firstly studied individually in order to assign peaks and characterize them. It is well known that these compounds have close oxidation potentials, what makes difficult the analysis of their complex mixture samples. Fig. 2, depicts a CV voltammogram where it is not possible to fully distinguish the four compounds; in black it is represented a mixture of AA, DA, 5-HT and UA (following peak order) and in red the same mixture without UA, allowing the identification of AA and 5-HT peaks. Finally, it could be assigned the average oxidation potentials for AA, UA, DA and 5-HT as 0.00 V, 0.30 V, 0.17 V and 0.32 V, respectively.

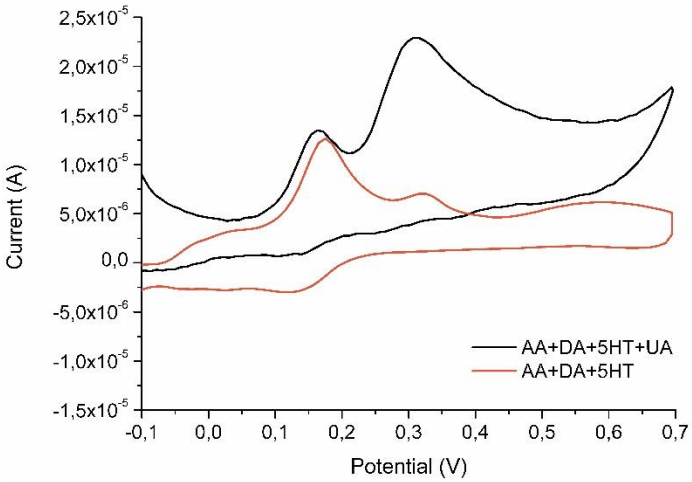

Fig. 2. ERGO modified electrode CV signals for dopamine, DA (20 µM), serotonin, 5-HT (5 µM), ascorbic acid, AA (75 µM) and uric acid, UA (50 µM) mixture. Measure in 50 mM phophate buffer + 0.1 M KCl.

Table 1. Calibration curves information for the four substances considered in the study.

		AA	UA	DA	5HT
ERGO-GEC	Slope[a]	$3.49\cdot10^{-8}\pm3.24\cdot10^{-9}$	$1.57\cdot10^{-7}\pm2.05\cdot10^{-9}$	$2.55\cdot10^{-7}\pm5.59\cdot10^{-9}$	$3.70\cdot10^{-7}\pm5.81\cdot10^{-9}$
	Intercept (A)	$3.02\cdot10^{-6}\pm8.03\cdot10^{-8}$	$1.28\cdot10^{-6}\pm3.60\cdot10^{-7}$	$-2.01\cdot10^{-6}\pm3.17\cdot10^{-7}$	$-2.95\cdot10^{-7}\pm1.72\cdot10^{-7}$
	R	0.9953	0.9867	0.9902	0.9991
	LOD (µM)	21.5	1.62	2.53	2.87
BARE-GEC	Slope[a]	$2.78\cdot10^{-8}\pm1.1\cdot10^{-9}$	$1.00\cdot10^{-7}\pm6.6\cdot10^{-9}$	$1.03\cdot10^{-7}\pm2.3\cdot10^{-10}$	$1.22\cdot10^{-7}\pm4.3\cdot10^{-9}$
	Intercept (A)	$7.55\cdot10^{-8}\pm1.74\cdot10^{-8}$	$1.96\cdot10^{-7}\pm3.45\cdot10^{-7}$	$2.81\cdot10^{-7}\pm2.37\cdot10^{-9}$	$4.90\cdot10^{-7}\pm4.81\cdot10^{-8}$
	R	0.9952	0.9953	0.9999	0.9141
	LOD (µM)	48.4	97.7	3.05	11.7

[a] in $(A\cdot\mu mol^{-1}\cdot mol^{-1})$

Calibration curves (see Fig. S.2 in supplementary information) for the 4 compounds were carried out considering the concentration range in human body, where AA and UA concentrations are higher than those of DA and 5-HT [37,38]. The working ranges for each compound were between 11-575 µM for AA, 11-410 µM for UA, 5-115 µM for DA and 1.5-30 µM for 5-HT. In all calibration curves, it is shown that for the same concentration, bare-GEC electrodes have lower currents than ERGO-GEC electrodes. Another remarkable fact is noted on ERGO-GEC 5-HT calibration curve, where a better linearity was achieved as the electrode saturation was avoided (see Fig. S.2 in supplementary information). Moreover, in correspondence of lower slope values for bare-GEC electrodes (information on table 1), a clear gain in sensitivity was observed for the modified electrodes. This gain is also evidenced with lower LOD values attained with ERGO-GEC electrodes (2.15 µM toward 48.4 µM for AA, 1.62 µM against 97.7 µM for UA, 2.53 µM against 3.05 µM for DA and 2.87 µM versus 11.7 µM for 5-HT).

3.3. Electrode study and validation of the method

Since for the quantification study experiment numerous consecutive measurements were required, the stability of the electrodes was an important issue. Concretely, it was studied the response of the modified electrode after 15 consecutive measurements and the better cleaning procedure. In this assay it was analyzed a DA and AA mixture, given that dopamine causes fouling problems which lead to

analyzing difficulties and AA is its direct electrochemical interference. Electrochemical and chemical cleanings were evaluated. The chemical cleaning part included: NaOH 0.1 M, HCl 0.1 M, EtOH:H_2O (1:3) and MeOH:H_2O (1:3); in all cases, the electrode was submerged in the dissolution for 3 minutes in constant stirring. The electrochemical cleaning procedure consisted in rinsing the electrodes in HCl 0.1 M or in phosphate buffer 50 mM + 0.1 M of KCl at pH=7.4 during 40 s at a constant potential, 1.2 V. In table 2 it can be observed that the electrochemical methods provided lower variation coefficient (% RSD) results than the chemical ones. Given the obtained results, it was chosen as the best cleaning method the electrochemical cleaning with HCl 0.1 M because its low % RSD and its confidence interval which included the average of measurements (see Fig. S.3 in supplementary information).

Table 2. Electrochemical cleaning results.

	Average[a] (A)	SD (A)	%RSD	Slope (A)	Confidence Interval[b] (A)
HCl (0.1M)	$1.78 \cdot 10^{-5}$	$9.08 \cdot 10^{-7}$	5.10	$-7.43 \cdot 10^{-8}$	$\pm 7.9 \cdot 10^{-8}$
PBSC (1mM+0.1MKCl)	$1.65 \cdot 10^{-5}$	$7.61 \cdot 10^{-7}$	4.62	$-9.08 \cdot 10^{-8}$	$\pm 8.9 \cdot 10^{-8}$

[a] n=10
[b] α=0.05

Since the previous assay was developed for 15 measurements, in order to ensure the stability of the electrodes in the whole training experiment, it was decided the usage of 3 replicats of the modified electrodes (15 sample for each electrode) to perform the whole experiment. For this reason, it was mandatory to develop a precision study of the whole electrodes collection, where have been calculated the repeatability or inter-assay (5 different electrodes under the same operating conditions over a short period of time, n=5) and the intermediate precision or intra-assay (5 different days under the same operating conditions, n=6). The results show a great repeatability of 2.84 % and an acceptable intermediate precision of 2.96 %, concluding with an acceptable confidence, that there are no differences between electrodes.

3.4. Simultaneous quantification study

For the quantification and resolution of the different compounds considered it was mandatory the use of complex chemometric tools due to, as already mentioned, the difficulty in discriminating among the individual peaks in the voltammograms. For this purpose, it was decided to use ANNs.

The building of the model was based on L36 fractional design with 3 levels and 4 factors, giving a model formed by a group of 35 samples in a concentration range of $2 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ M for AA and AU and $4 \cdot 10^{-6}$ to $5 \cdot 10^{-5}$ M for DA and 5-HT, named training subset. For the cross-validation of the generated model, 9 additional samples were distributed randomly throughout the previous used concentration ranges, forming in this way the external test subset.

Though ANNs were used, a compression prior step was needed. This preprocessing allows to reduce the complexity of input data, preserving the relevant information among the highly dimensional data. Making easier the input information, the better will perform the ANN and its generalization ability.

Based on previous works [39], the compression model was optimized. Finally the was used as compression approach the discrete wavelet transform (DWT) employing the mother function *Daubechies* 3 and a second decomposition level [39,40]. The DWT pretreatment allowed compressing the initial data set information (206 currents per sample) up to 73.3%.

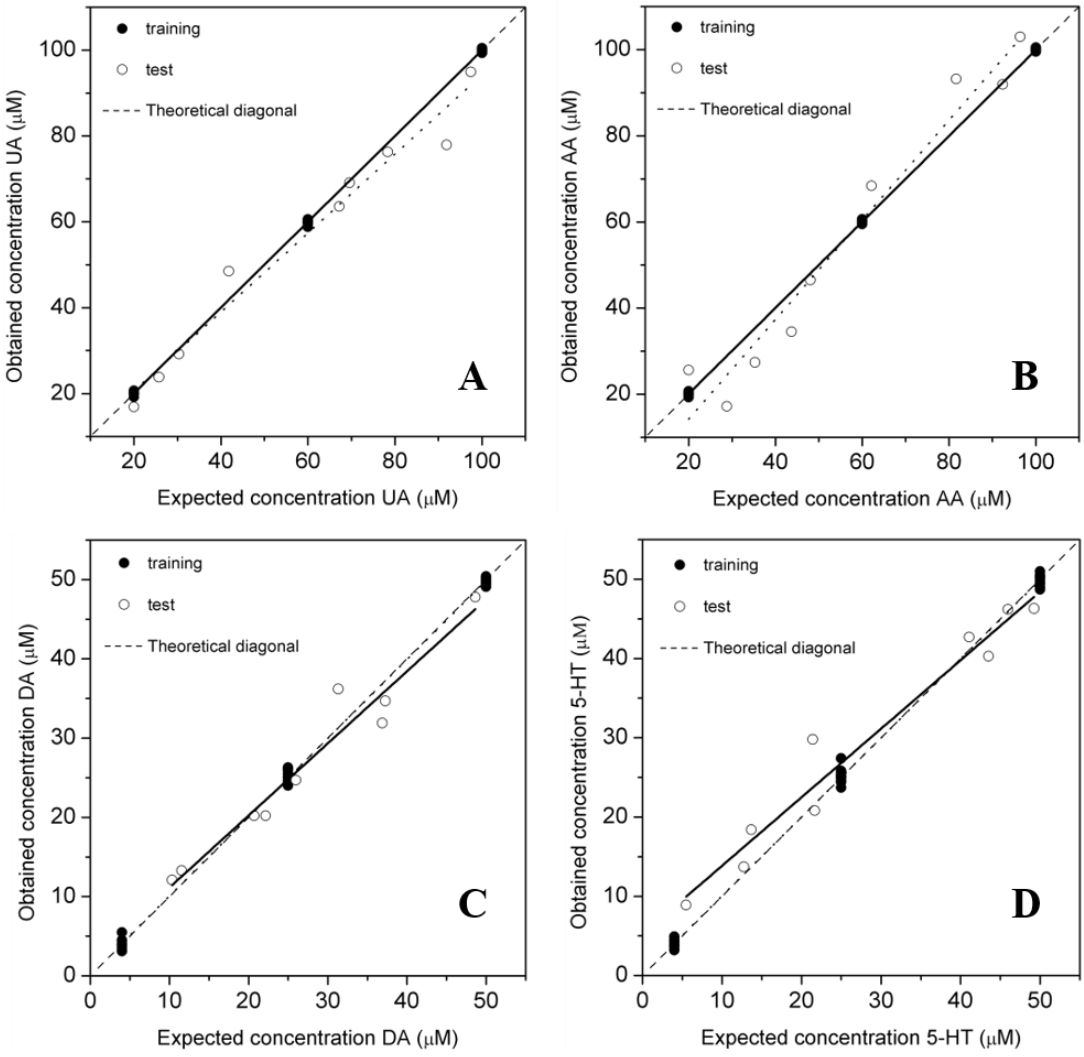

Fig. 3. ANNs expected vs. predicted compartion graphs obtained fot the four studied substances, UA (A), AA (B), DA (C) and 5-HT (D).

Once the data was ready to be modelled, it was proceeded to evaluate systematically the final ANN configuration, whereas the lower the RMSE, the better the model (indicating that the difference between the predicted values by the ANN and the real ones is minimum). Eventually, the better ANN had 55 neurons in the input layer (corresponding to the 55 wavelet approximation coefficients obtained after the compression step), 9 neurons and *tansig* (hyperbolic tangent sigmoid) transfer function in the hidden layer and 4 output neurons and *purelin* (linear transfer function) function in the output layer (corresponding to the 4 studied compounds).

To visualize how good is the predictive performance of the developed model, comparison graphs of predicted vs. expected concentrations were prepared for each compound, displayed in Fig 3. All the graphs acquired from DWT-ANN show satisfactory trends, with lower dispersion and great linearity (correlation coefficients greater than 0.974), having usually better results for the training subset than for the test one, because of it was used to build the prediction model.

Table 3 presents the obtained parameters in the fitted comparison lines for each compound. As evident from the predicted vs. expected graphs, great linearity parameters were achieved. As pointed out above, correlation values were greater than 0.974, moreover, slopes near to 1 and intercepts around 0 were obtained (all values within the confidence interval, α=0.05), indicating that the test and train subsets had linearities similar to the ideal prediction model (x=y function). Apart, the calculated RMSEs demonstrates the model high efficiency, obtaining a total NRMSE of 0.088. The present results lead to consider the developed DWT-ANN as a satisfactory model able to quantify the four considered analytes.

Finally, the limit of detection (LOD) for each compound, was estimated (Table 4) from the corresponding expected vs. predicted graph, obtaining with it an approach of the curve fitting. Considering in this way the LOD proportional to typical error [41] of the regression line, we added this extra information to the fitted model.

Table 3. ANN quantification results.

TRAIN SUBSET							
	Correlation	Slope	Intercept (µM)	RMSE (µM)	Total RMSE (µM)	NRMSE	Total NRMSE
DA	0.9994	0.994±0.012	0.13±0.37	0.374	0.55	0.0047	0.0098
5-HT	0.9993	0.996±0.013	0.09±0.41	0.417		0.0052	
AA	0.9999	0.998±0.004	0.13±0.26	0.627		0.0014	
UA	0.9999	0.998±0.004	0.11±0.30	0.724		0.0157	
TEST SUBSET							
DA	0.9744	0.91±0.19	2.0±5.6	0.036	5.37	0.1072	0.088
5-HT	0.9794	0.86±0.16	5.2±5.5	0.044		0.0763	
AA	0.9784	1.16±0.22	-8.9±14	0.051		0.0761	
UA	0.9847	0.92±0.14	2.2±9.3	0.036		0.0913	

Table 4. Obtained limits of detection from expected vs. Obtained fitting curves.

	AA	UA	DA	5-HT
TRAIN SUBSET				
Standard Error	0.37	0.42	0.63	0.73
LOD (µM)	2.08	1.38	2.08	2.42
TEST SUBSET				
Standard Error	7.3	5.1	2.8	3.2
LOD (µM)	8.08	18.24	10.3	12.1

4. Conclusions

The presented work sets out to a graphene-modified sensor capable to enhance detection properties of interesting analytes in electroanalysis. Thanks to the use of powerful chemometric techniques, such as ANN, it was possible the resolution of complex samples, in this case a mixture of 4 close-responding compounds.

To sum up, the performed assays demonstrated the achieving of electrode modification, with an easy and precise method, illustrating thus the interesting applications of graphene in the sensor and biosensor domain. The developed sensor allows to improve the results obtained for a bare electrode making feasible the opportunity to work with advanced data treatment to reach splendid results. Obtained CV voltammograms were finally preprocessed employing DWT, to reduce the complexity of signals, the compression was followed for the applying of the ANN model, which had a NRMSE equal to 0.0876, an acceptable value.

The overall proposed work has been successfully applied in the resolution of a high interesting mixture of 4 electrochemically similar compounds. The general approach resulted in a potential quantification tool for complex samples, which can be easy adapted to other family compounds.

Glossary

5-HT Serotonin
AA Ascorbic Acid
ANN Artificial Neural Networks
CRGO Chemically Reduced Graphene Oxide
CV Cyclic Voltammetry
DA Dopamine
DWT Discret Wavelet Transform
ERGO Electrochemically Reduced Graphene Oxide
FFT Fast Fourier Transform
GEC Graphite Epoxy Composite
GO Graphene Oxide
PLS Partial Least Squares
TRGO Thermally Reduced Graphene Oxide
UA Uric Acid
WE Working Electrode

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