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Simultaneous quantification of four principal NSAIDs through voltammetry and artificial neural networks using a modified carbon paste electrode in pharmaceutical Samples

Dr. Guadalupe Yoselin Aguilar Lira

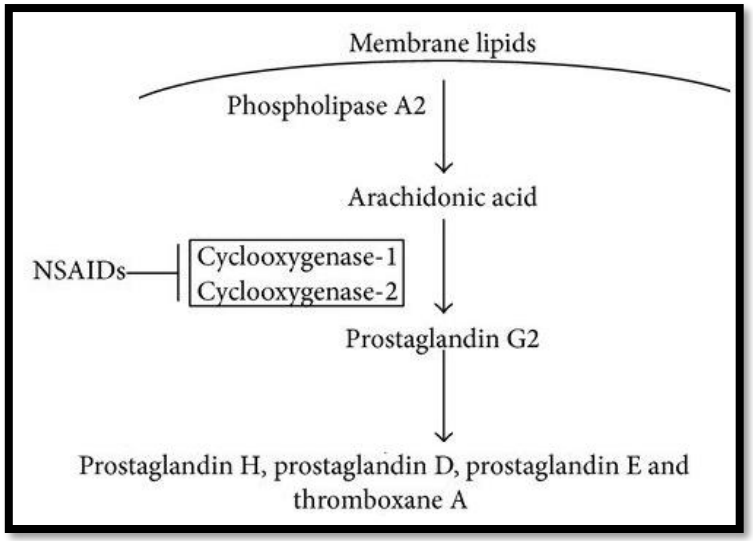
Co-authors:
Dr. Prisciliano Hernández Martínez, Dr. Giaan Arturo Álvarez Romero, Dr. Juan Manuel Gutiérrez Salgado.

Introduction

Nonsteroidal anti-inflammatory drugs **(NSAIDs)**

Relieve pain, reduce high temperature and reduce inflammation.

NSAIDs mechanism of action [1]



Side effects

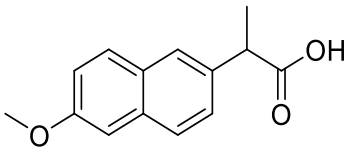
Peptic ulceration, digestive disorders, temporary deafness, recent studies mention that they may be related to heart attacks.

[1] Katzung, B.G. Trevor, A.J. (2015). *Basic & clinical pharmacology*: Thirteenth edition, Mc Gram Hill Education.

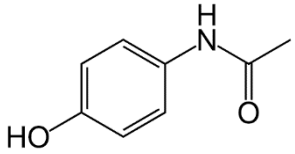
Introduction

NSAIDs

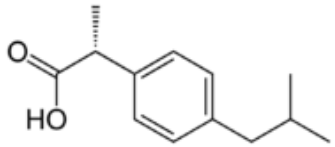
Naproxen



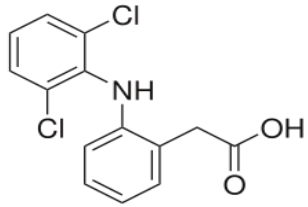
Paracetamol



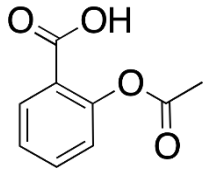
Ibuprofen



Diclofenac



Aspirin



If their use is constant

Pharmaceutical industry (HPLC) [1]

Disadvantages:
Such as the need for sample preparation, long analysis times, high cost associated with the use and maintenance of the equipment.

[1] Aguilar-Arteaga, K., Rodríguez, J., Miranda, J.M., Medina, J., Barrado. E. (2010). *Talanta,* **80**,1152–1157.

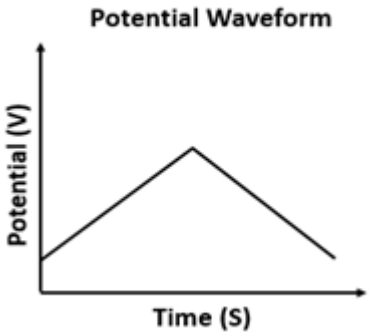
Introduction

Electrochemical methodos

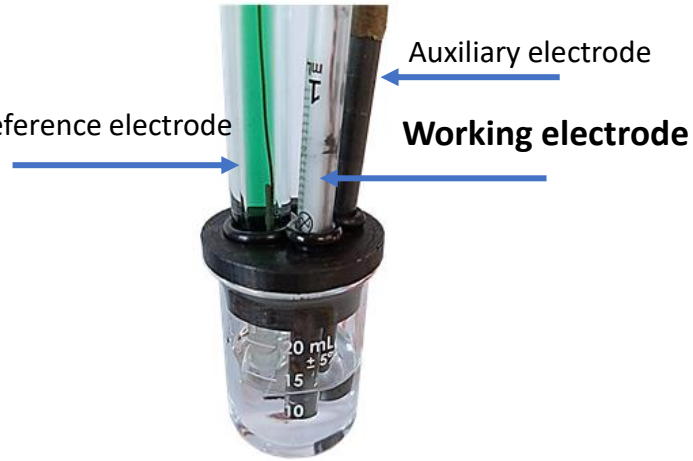
Voltammetry

- They do not require sample treatment and / or are not destructive
- Good sensitivity and reproducibility
- Short times for analysis
- Economical and miniaturizable

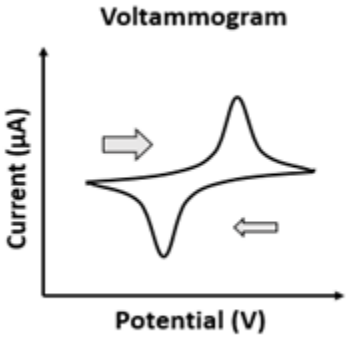
Input



Potentiostat



Output



Working Electrode:
Carbon and / or modified, silk-screened, metallic: platinum, gold, etc.

Introduction

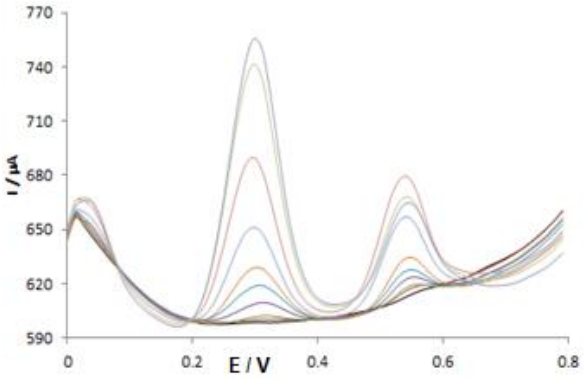
Working electrode:
Carbon paste electrode
(CPE)

- Wide potential window
- The surface can be quickly renewed
- Easy preparation
- Miniature scale
- Incorporation of other materials

Carbon paste electrode modified with multi-wall carbon nanotubes
(MWCNT-CPE)

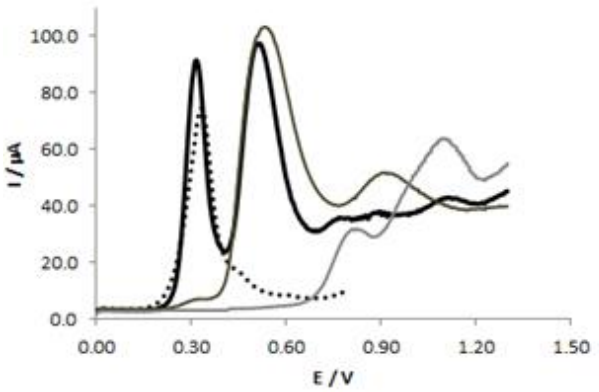
- High thermal stability
- Large contact surface area
- Fast electron transfer
- High sensitivity

Voltammetry



Problematical:

- Analyze more than one analyte
- Solve the overlap of voltammetric signals



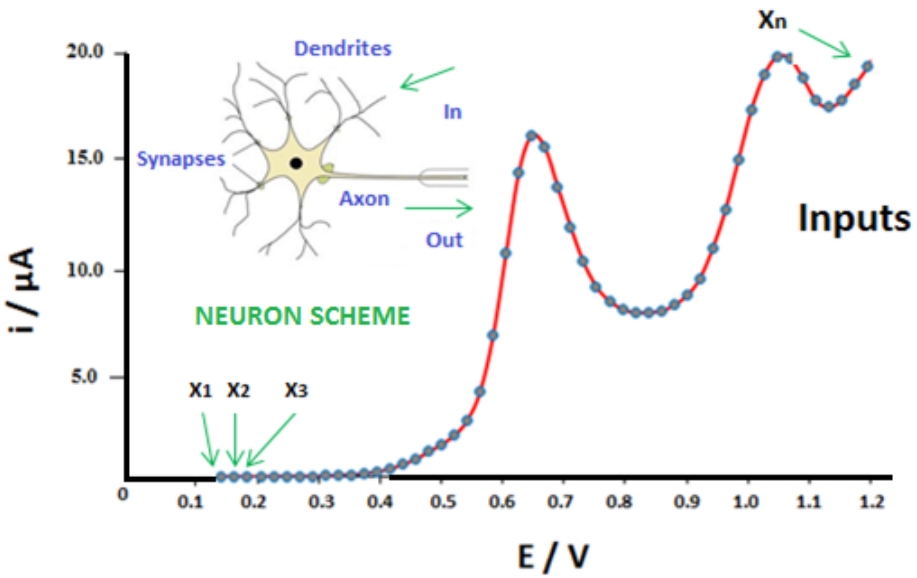
Introduction

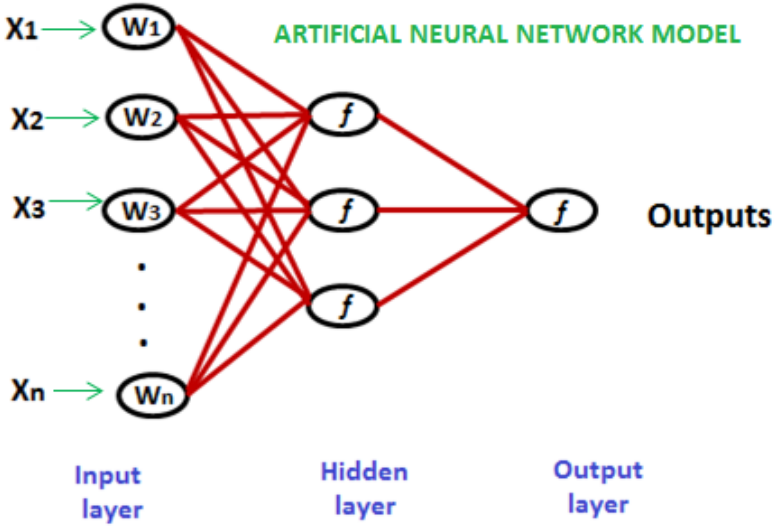
Chemometric Methods

- Principal Component Analysis (PCA) [1,2]
- Partial least squares (PLS) [3]
- **Artificial Neural Networks (ANN) [4,5]**

ANN

Artificial neurons simulate the basic functions of biological neurons: Input, processing, output, and passing information to other neurons. Each input is given a weight to signify how important it is compared to other input.





[1] Simeon, V.; Pavkovic, D.; Branica-Jurkovic, G. *Anal. Chim. Acta 1992,* ***263****,* 37-42.
[2] Díaz-Cruz, J. M.; Tauler, R.; Grabaric, B. S.; Esteban, M.; Casassas, E. *J. Electroanal. Chem. Interfacial Electrochem. 1995****, 393,*** *7-16.*
[3] Cabanillas, A. G.; Diaz, T. G.; Espinosa-Mansilla, A.; Lopez, F. S. *Talanta* 1994***, 41****, 1821-1832****.***
[4] Chan, H.; Butler, A.; Falck, D. M.; Freund, M. S. *Anal. Chem. 1997,* ***69,*** 2373-2378.
[5] Cladera, A.; Alpı´zar, J.; Estela, J. M.; Cerda`, V.; Catasu´ s, M.; Lastres, E.; Garcı´a, L. *Anal. Chim. Acta 1997,* ***350****, 163-169.*

Objective

Propose a methodology based on voltammetric methods together with ANNs as a modeling and calibration tool to quantify NSAIDs simultaneously.

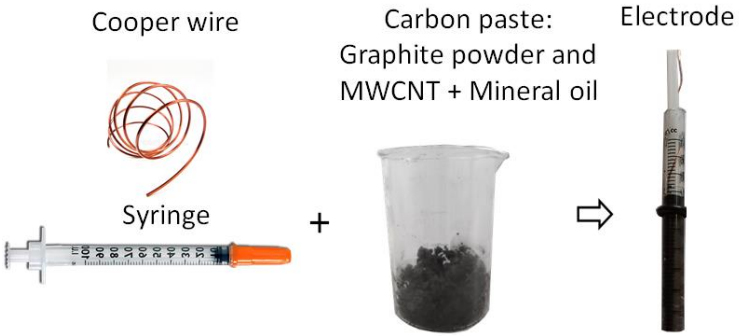
Particular objectives:

- Build the proposed working electrode.
- Study the electrochemistry of NSAIDs with the proposed working electrode.
- Optimize instrumental variables for the quantification of NSAIDs with the Box Behnken design.
- Build artificial neural network models for the simultaneous quantification of NSAIDs.

Methodology

Electrochemical characterization

Electrode Preparation



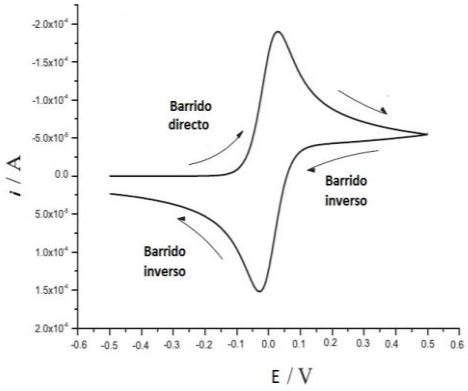
MWCNT-CPE

- 30 % Graphite powder
- 10 % MWCNT
- 60 % Mineral oil

Analysis of the electrochemical system of NSAIDs by CV with the proposed working electrode.

Optimization of differential pulse voltammetry (DPV) for the quantification of NSAIDs.

- Reference electrode Ag/AgCl saturated.
- A Britton Robinson (BR) 0.1 mol·L^{-1} buffer solution (7.0-11.0).



Box Behnken design [1]

$$Y = \beta_0 + \sum_{i=1}^{K} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j + \varepsilon,$$

Optimize: the maximum anode current intensity.

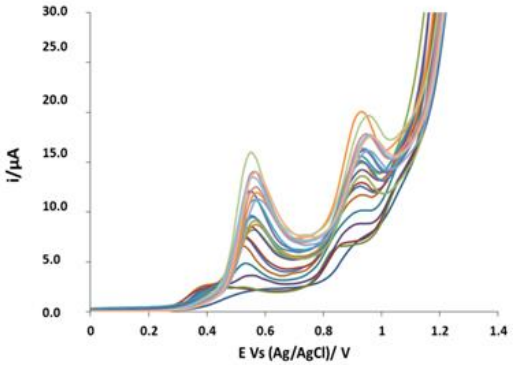
[1] Montgomery, D. C. Design and analysis of experiments, 8th ed.; Publisher: Wiley, USA, 2012.

Methodology

Quantification of NSAIDs by ANN

Data processing

Generation of 27 voltammograms (using a 3^{5-2} fractional factorial design).



A matrix of peak intensities of dimensions [189 x 27] (intensities x number of samples).

The matrix of concentrations of dimension [4 x 27] (i.e., paracetamol, diclofenac, naproxen, and ibuprofen), aspirin was not quantified in the model as it was considered only as an interferer.

The pretreatment of the data

Discrete Wavelet Transform (DWT) using the 4th level wavelet decomposition of a Daubechies function (db4) [1].

- All data sets were normalized in the interval of [-1,1].
- The final input matrix to feed the ANN model has a dimension of [18 x 27].

[1] Aguilar-Lira, G.Y.; Gutiérrez-Salgado, J.M.; Rojas-Hernández, A.; Rodríguez-Ávila, J.A.; Páez-Hernández, M.E.; Álvarez-Romero, G.A. *J. Electroanal. Chem.* *2017*, 801, 527–535.

Methodology

Quantification of NSAIDs by ANN

ANN Model

- The described data set of 27 samples, were selected for the training set.
- The testing set was conformed using an external set of 10 additional samples randomly generated within the concentration range described above.
- The hidden layers were established through a trial-and-error process, modifying the number of neurons in the layers until an appropriate number of neurons were found that favored obtaining a satisfactory linear regression coefficient.
- The final MLP model was 18x10x8x4 (18 input neurons, 10 neurons in the first hidden layer, 8 neurons in the second hidden layer and 4 output neurons).
- The activation functions established were: purelin for the input layer, tansing for the two hidden layers, and purelin for the output layer.
- The chosen training algorithm was Bayesian regularization, with a training error set at a value of 0.001, together with a learning rate of 0.01.

MATLAB® R2021a (MathWorks, Natick, MA, USA) platform using the Deep Learning and Wavelet Toolboxes.

Results and discussion

Electrochemical characterization

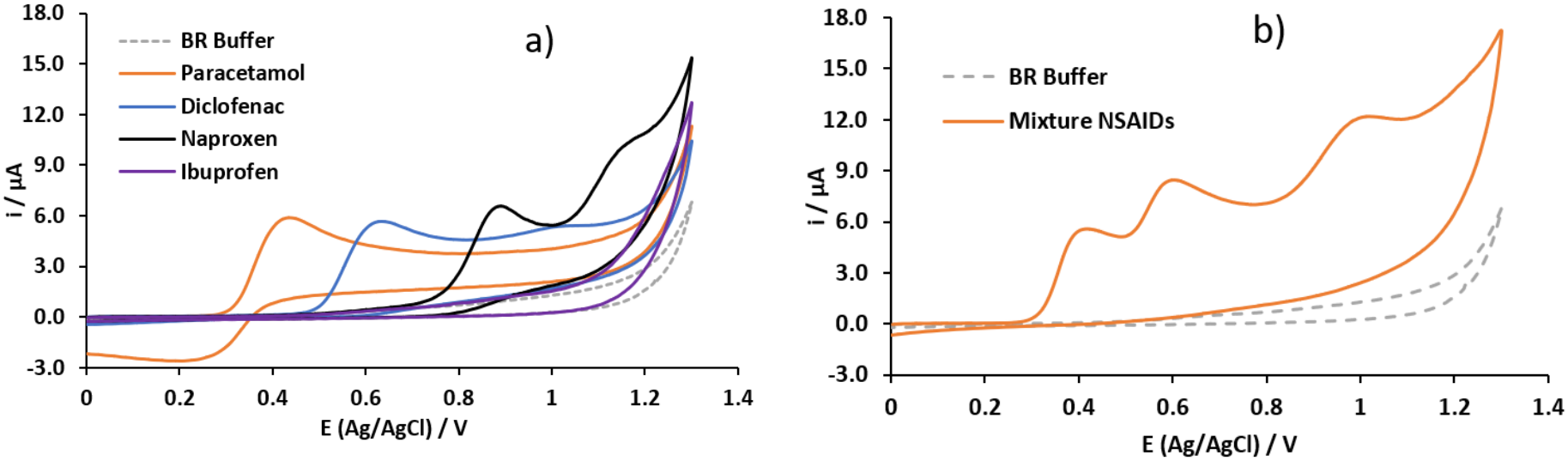
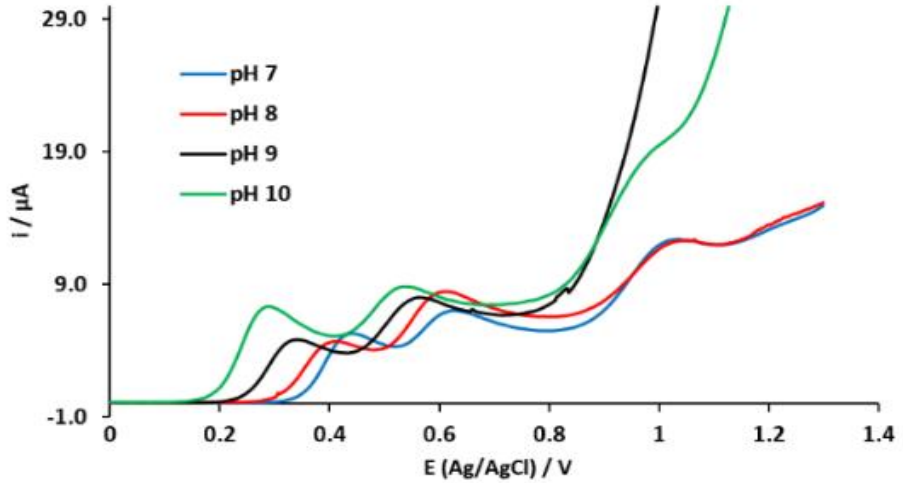


Figure 1. CVs obtained for the systems containing the NSAIDs and the supporting electrolyte (pH 7 BR buffer 0.1 mol·L^{-1}), using the proposed working electrode and in presence of aspirin. Potential window from 0 to 1.3 V and at a scan rate of 100 mV·s^{-1}; **(a)** individual for each NSAIDs, (**b**) mixture of the four NSAIDs.

Results and discussion

Electrochemical characterization

pH study



The highest current intensity is obtained at pH 10 for the mixture of drugs.

Figure 2. CVs obtained for the NSAIDs mixture at different pHs (range of 7-10) in a 0.1 mol·L^{-1} BR buffer, using a potential window of 0 to 1.3 V and a scan rate of 100 mV·s^{-1}.

Results and discussion

Electrochemical characterization

Different CV scan rates were studied, and the maximum anodic peak current was plotted vs the square root of the scan rate for each NSAID.

A correlation coefficient greater than 0.99 was obtained after the proper statistical analysis, which suggests that the diffusion of the electroactive species to the surface of the electrode governs the oxidation processes.

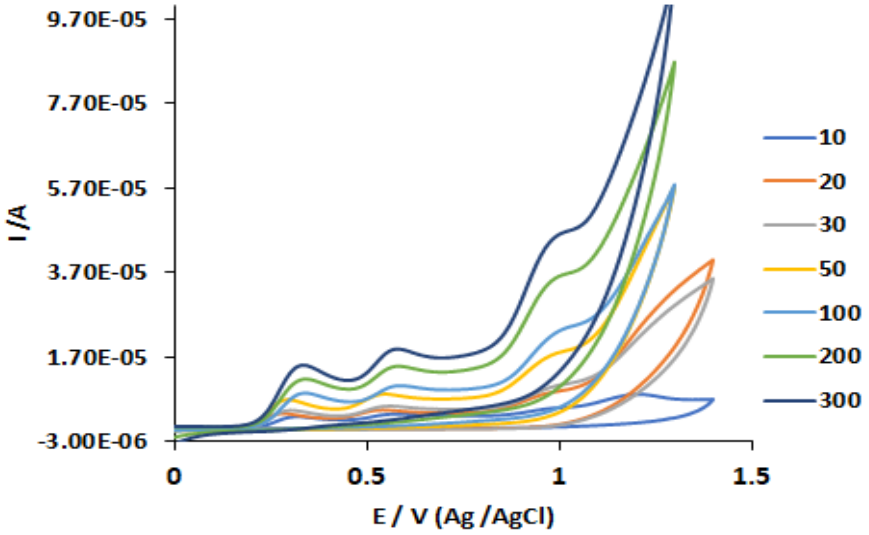


Figure 3. CVs obtained for the NSAIDs mixture at different scan rates (10 to 300 $mV \cdot s^{-1}$) , using a potential window of 0 to 1.3 V.

Results and discussion

Electrochemical characterization

Optimization of differential pulse voltammetry (DPV) for the quantification of NSAIDs

Box Behnken design (BBD)

- A BBD with three levels was used for the optimization of the four variables related to the DPV.
- 27 experiments were carried out, generating the corresponding voltammograms of NSAIDs.

The proposed second-order model regression that correlates the current response and the DPV factors is shown in equation (2).

$$Y = 1.747 + 0.22X_1 + 0.352X_2 - 0.935X_3 + 1.082X_4 - 0.476X_2^2 - 0.431X_2^2 - 0.308X_3^2 - 0.012X_4^2 - 0.309X_1X_2 + 0.211X_1X_3 + 0.209X_1X_4 + 0.297X_2X_3 + 0.66X_2X_4 + 1.507X_3X_4 \quad (2)$$

Table 1. Optimal DPV parameters found with the Box Behnken design

X_1 (V)	X_2 (s)	X_3 (s)	X_4 (V)	Y (µA)
0.00585	0.75	0.05	0.05	5.24

Minitab® V.18 software.

Results and discussion

Quantification of NSAIDs using ANN

- Using the optimal parameters of the DPV to analyze the 27 samples considering different concentrations of the NSAIDs. using a 3^{5-2} fractional factorial design.

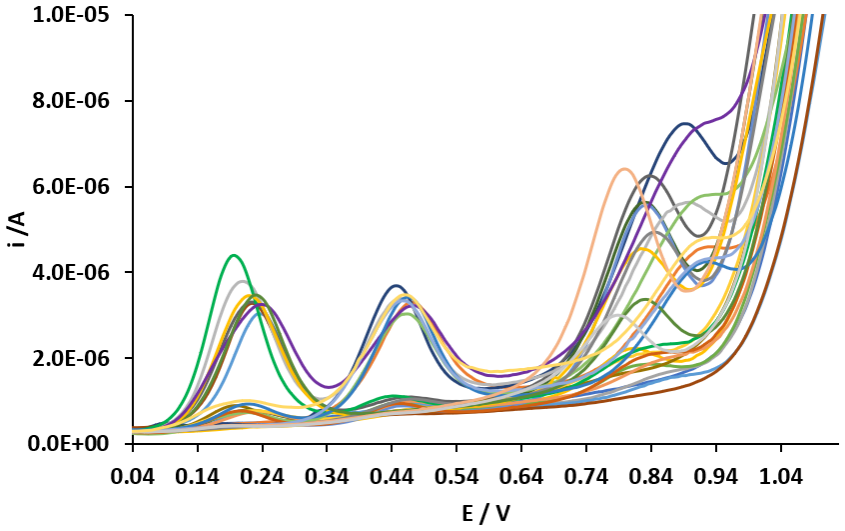


Figure 4. 27 DPV was obtained at different concentrations of the NSAIDs, (ranging from 5x10^{-7} to 7x10-5 mol·L^{-1}). using a potential window of 0 to 1.3 V.

- The testing set was conformed using an external set of 10 additional samples randomly generated within the concentration range described above.

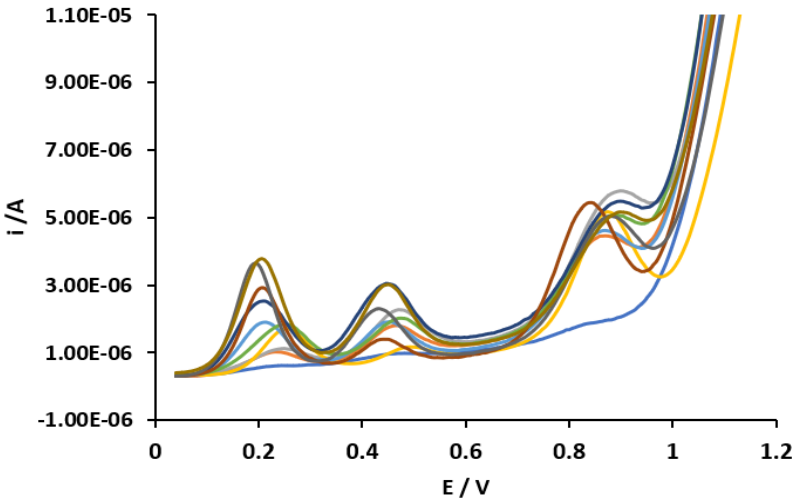


Figure 5. 10 DPV was obtained at different concentrations of the NSAIDs, (ranging from 5x10^{-7} to 7x10-5 mol·L^{-1}). using a potential window of 0 to 1.3 V.

Results and discussion

Quantification of NSAIDs using ANN

ANN modeling

- The final MLP model was 18x10x8x4 (18 input neurons, 10 neurons in the first hidden layer, 8 neurons in the second hidden layer and 4 output neurons).
- The relationship between the concentrations obtained and those expected was evaluated, both for the training and test phases.
- In this sense, the linear regression obtained from the comparison was a measure of the model's goodness.
- Given ideal conditions, the line must have a slope equal to 1 and its intersection equal to 0.
- The comparative graphs between the real concentrations of paracetamol, diclofenac, naproxen, and ibuprofen and those predicted with the MLP model for the training and test data set are shown in Figures 6 and 7, respectively.

Results and discussions

Quantification of NSAIDs using ANN

ANN modeling

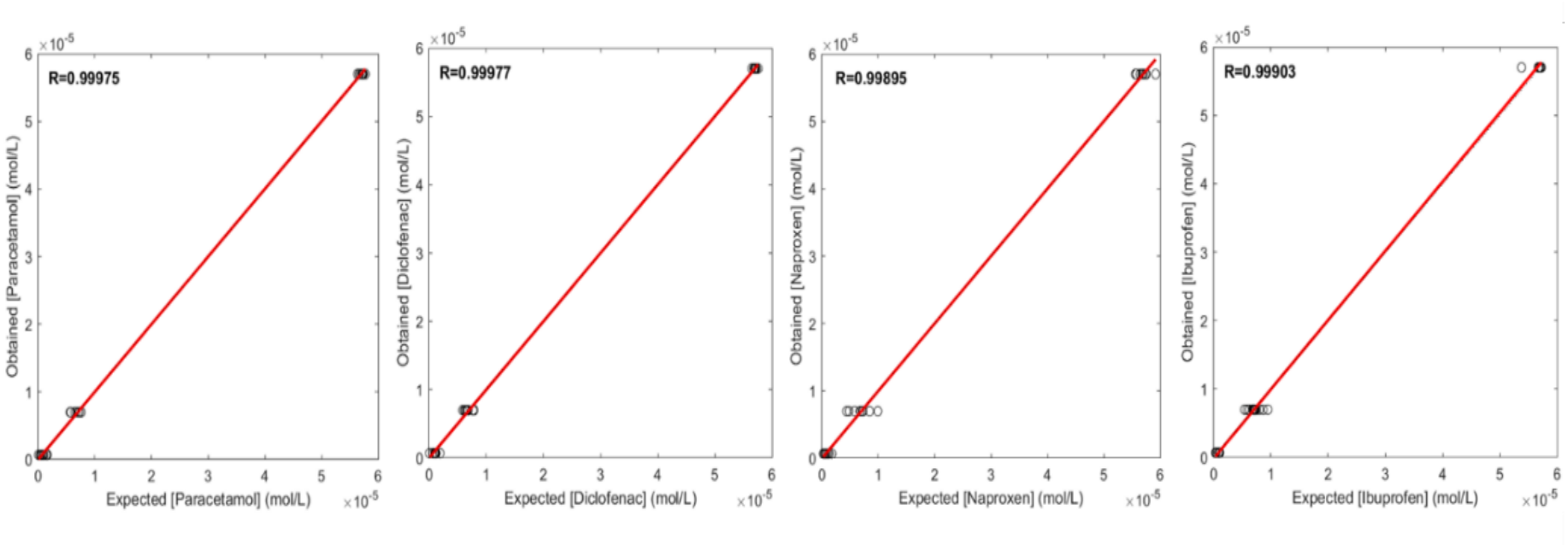


Figure 6. Comparison between the expected NSAIDs concentration and those obtained after MLP training phase.

Results and discussion

Quantification of NSAIDs using ANN

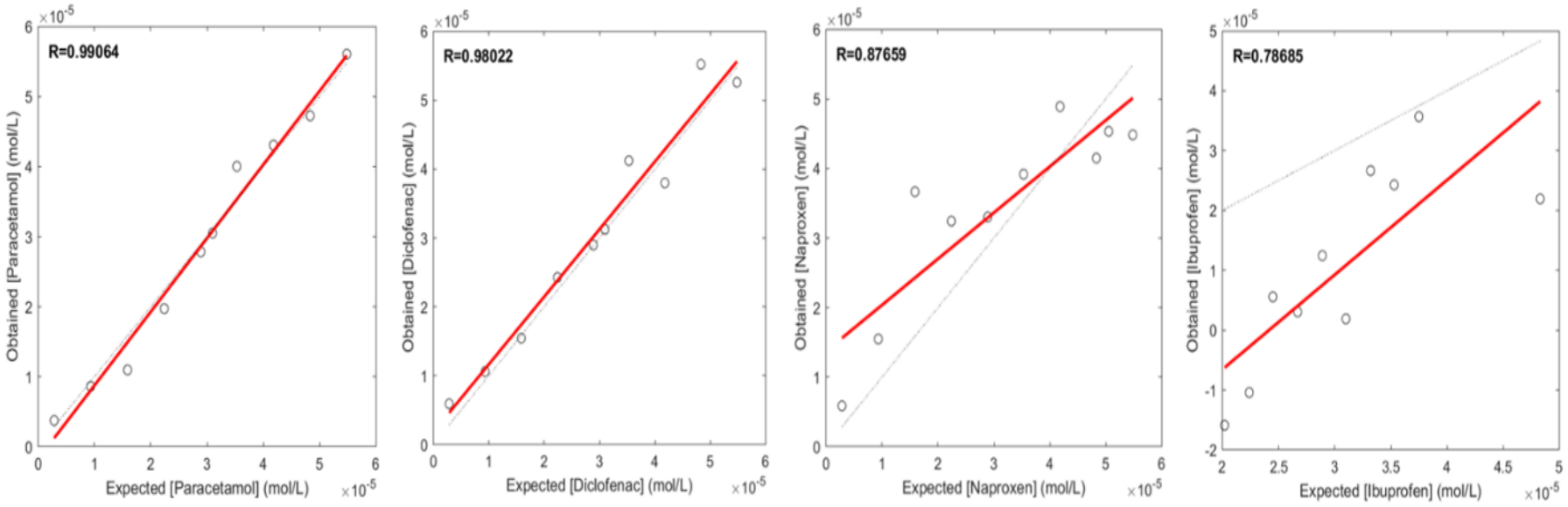


Figure 7. Comparison between the expected NSAIDs concentration and those obtained during MLP test phase.

The high level of linearity allows having a linear regression coefficient of the data obtained very close to one (R=0.98) for paracetamol and diclofenac, while for the naproxen and ibuprofen, the correlation value was 0.87 and 0.78 respectively, aspirin was present as an interfering agent in the mixture.

Conclusions

- In this work, a potential tool for voltammetric determinations is presented. A combination of DPV and DWT-ANN allowed us to obtain satisfactory results for quantifying paracetamol, diclofenac, naproxen, and ibuprofen in the presence of aspirin.

- The use of DWT is helpful to compact voltammograms preserving the analytical information of the original records. Multivariable models created with ANN correctly describe the complexity in voltammograms caused by overlapping peaks without needing a pretreatment step on the samples.

- Finally, carbon paste electrodes with nanotubes are low-cost and easy-to-make devices that allow us to determine the drugs in the order of microgram per liter.

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Thank for your attention