published by Ural Federal University
eISSN 2411-1414
chimicatechnoacta.ru

ARTICLE
2025, vol. 12(2), No. 12204
DOI: 10.15826/chimtech.2025.12.2.04

Voltammetric electronic tongue for identification the pharmaceutical preparations of naproxen

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Abstract

A multisensory system of the "electronic tongue" type was developed based on glassy carbon electrodes modified with PEC@GO, PEC@SWCNT, PEC@CB and PEC@CP composites for the identification of pharmaceutical preparations of naproxen using differential pulse voltammetry. To improve the reliability of voltammetric recognition of pharmaceutical preparations of naproxen, chemometric data processing using PCA and SIMCA was proposed. It was shown that the multisensory system of the "electronic tongue" type correctly distinguishes 100% of the samples. To achieve such a degree of recognition, the multisensory system should include at least 4 sensors. The accuracy of recognition was tested on 3 samples of commercially available naproxen pharmaceuticals: "Nalgesin", "Teraliv", and "Nexemezin", produced by different manufacturers.

Key findings

- Various low-dispersed forms of carbon are a good modifier for obtaining voltammetric sensors with cross-sensitivity to naproxen.
- Voltammetric curve processing by chemometric procedures allow identifying naproxen drug forms and determining their manufacturers.
- A reliable multisensory system for identifying dosage forms of naproxen should consist of at least four different sensors.

Accompanying information

Article history
Received: 29.11.24
Revised: 16.12.24
Accepted: 26.12.24
Available online: 15.01.25

Keywords
multisensory systems, voltammetry, chemometrics, pharmaceutical analysis, naproxen, chitosan polyelectrolyte complex

Funding
This study was funded by the Russian Science Foundation Grant No. 23-23-00340, https://rscf.ru/project/23-23-00340/.

Supplementary information
Supplementary materials: READ
Transparent peer review: READ

Sustainable Development Goals

1. Introduction

The growth of the pharmaceutical industry, the increase in production volumes and product range observed in recent decades have led to the aggravation of a number of problems associated with the quality of both foreign and domestic pharmaceutical products. According to the World Health Organization, the share of counterfeit drugs in the drug market in some countries can reach 30%, while the share of counterfeit drugs in the domestic pharmaceutical market is estimated at 12% [1]. It is known that the most informative methods for identifying counterfeit drugs are IR spectroscopy [2], UV spectrophotometry [3], chromatographic methods [4], and NMR [5]; however, they require complex expensive equipment, complex sample preparation, highly qualified personnel, and are not suitable for on-site analysis. Therefore, the development of express, simple and cheap methods for identifying and monitoring the quality of pharmaceuticals is very important. Multisensory systems such as the "electronic tongue" [6–12] meet these requirements, allowing the identification of several components of the analyzed solution at once, i.e., having cross-sensitivity [13]. To increase the sensitivity of the sensors, it seems promising to use composite modifiers based on conductive polymers [14] and various carbon modifiers [15]. Examples of polymer composites with carbon-containing sorbents (CarboblackC) [16], graphene oxide and carbon nanotubes [17] are known. Varying the size, shape and structure of modifier particles allows achieving cross-sensitivity of the sensors to the components being determined and excipients present in the analyzed preparations. A significant expansion of the capabilities of such multisensory systems is the use of chemometric processing of the received signal by the principal component analysis (PCA) [18] and soft independent modeling by class analogy (SIMCA) [19]. This combination, multisensory systems, based on differences in the shape of the entire voltammogram, arising from the unique

composition of minor components in preparations manufactured by different companies, allow identifying the manufacturers of the pharmaceutical preparations. PCA is one of the most widely used chemometric methods in the field of multisensory systems. PCA allows one to study the data structure in general and understand the relationship between samples and variables, such as the presence of groups of similar samples, the presence of outliers, the presence of correlated variables carrying similar information, etc. [20]. However, PCA is only a way of presenting data and analyzing the structure of variance. Classification based on PCA is possible, but requires the introduction of additional metrics, such as the distance between classes and between samples, which this is implemented in the SIMCA algorithm. In addition, any classification model implies a check of classification ability as an integral attribute, but in PCA as such these capabilities are not built in. Therefore, based on the PCA score graph for samples from a limited set, one cannot draw conclusions about how well a multisensory system is able to classify samples. Such conclusions require mandatory verification with an independent test set of samples. The SIMCA method constructs not a single PCA model common to all the samples, but separate PCA models for the samples of each class. As a result of the algorithm, the distance from a new unknown sample to known classes is calculated and, according to the principle of the smallest distance (in the coordinates of the principal components), the class affiliation of the new sample is determined [21, 22].

In this paper, we present for the first time a multisensory system of the “electronic tongue” type consisting of four composite sensors based on a glassy carbon electrode (GCE) modified with a polyelectrolyte complex of chitosan and N-succinyl chitosan (PEC) [23, 24] with various carbon particles, such as single-walled carbon nanotubes (SWCNT), graphene oxide (GO), CarboblackC (CB) and Carbopack (CP) for the identification of pharmaceuticals with naproxen as the active substance. The test set included drugs from three manufacturers: Nalgesin, Teraliv and Nexemezin. The relevance of naproxen is due to its prevalence in therapeutic practice [25, 26] and its use in clinical trials as a comparison drug in the development and testing of new non-steroidal anti-inflammatory drugs. The proposed approach, in our opinion, significantly expands the possibilities of voltammetric determination of naproxen content in pharmaceutical preparations [27–32], since it allows us to distinguish generics from original drugs and identify the manufacturer.

2. Experimental

2.1. Reagents and solutions

All the chemical reagents were of analytical grade and were used without additional treatment. High-purity deionized water (specific conductivity 0.1 μS) was used to prepare the solutions. PEC was prepared by mixing chitosan hydrochloride (polycation), obtained from a solution of chitosan in hydrochloric acid by dissolving 0.25 g of chitosan (molecular weight 30 kDa) with a degree of deacetylation of 75% in 50 ml of 1% HCl and N-succinyl chitosan (polyanion) (JSC Bioprogress, Russia) followed by air drying. PEC composites with carbon particles SWCNT (diameter 0.7–1.1 nm) (Sigma-Aldrich, USA), GO (powder: 15–20 sheets, 4–10% oxidation at the edges) (Sigma-Aldrich, USA), CB (specific surface area 10 m^2/g, particle size 60–80 mesh (0.18–0.25 mm)) (Restek, USA), and CP (specific surface area 100 m^2/g, particle size 60–80 mesh (0.18–0.25 mm)) (Sigma–Aldrich, USA) were prepared according to the method described previously [33]. The commercially available pharmaceutical preparations of naproxen were selected as the objects of analysis: Nalgesin (P1) (JSC Krka, d.d. Novo mesto, Slovenia), Teraliv275 (P2) (JSC Bayer, Germany), Nexemezin (P3) (JSC Pharmasyntez, Russia) purchased from a retail pharmacy network (Table 1). Solutions of pharmaceutical preparations based on naproxen were prepared by dissolving one tablet containing 275 mg of the active substance in 50 ml of phosphate buffer solution, followed by keeping it in an ultrasonic bath for 30 min and filtering on a black ribbon filter. The tablets were preliminarily regrinded in a mortar. Solutions with smaller concentrations were obtained by dilution of the initial solutions.

2.2. Fabrication of sensors with cross sensitivity

The selection of the optimal composition of composites based on PEC and carbon nano- and microparticles was made on the basis of previously conducted studies described in [33]. To modify GCE, composites prepared by mixing 2 mg CB or CP with 1 ml PEC and 3 mg GO or SWCNT with 1 ml PEC, followed by keeping in an ultrasonic bath for 60 min, were used.

The electrode surface was modified with the drop casting method. Using an automatic dispenser, 0.3 μl of the prepared PEC@CB, PEC@CP, PEC@GO and PEC@SWCNT composite solution was taken and applied to the pre-polished GCE surface, followed by drying the electrode for 6 min under an infrared lamp (250 W) at 80 °C and cooling at room temperature for 3 min.

Table 1 Composition of pharmaceutical preparations with active substance naproxen, 275 mg.

Pharmaceuticals	Nalgesin	Teraliv275	Nexemezin
Manufacturer	JSC Krka, d.d. Novo mesto, Slovenia	JSC Bayer, Germany	JSC Pharmasyntez, Russia
Party	464180 DA8512	86940302 BT19281	40721 10423
Excipients	Povidone K30, *sucrose, talc,* magnesium stearate, purified water	*sucrose*, Povidone K30, *talc,* magnesium stearate	Copovidone, lactose monohydrate, *sucrose*, silicon dioxide, *talc*

 DOI: 10.15826/chimtech.2025.12.2.04

Before each measurement, the GCE surface was carefully polished for 1 min using a deagglomerated suspension based on Al_2O_3 (0.3 μm) and polishing material (Allied High Tech Products Inc., USA), after which the electrode was rinsed with deionized water, dried in air at room temperature and modified again.

2.3. Electrochemical measurements and data analysis

Differential pulse voltammograms (DPV) were recorded on a portable potentiostat/galvanostat Corrtest CS100 (Wuhan Corrtest Instruments Corp., Ltd, China) in a three-electrode cell thermostatted at 25 °C and consisting of indicator electrode (GCE) (2 mm diameter), a silver chloride reference electrode Ag/AgCl with saturated KCl, and an auxiliary electrode in the form of a platinum plate, at a potential scan rate of 20 mV/s, with a potential range from 0 to 1.8 V. An array of voltammetric data for each sample was formed from 10 parallel measurements, including 250 values of instantaneous currents at different potentials. Chemometric data processing using PCA and SIMCA methods was performed using The Unscrambler X software (CAMO Software, Oslo, Norway).

3. Result and Discussion

The active substance of the studied pharmaceutical preparations, naproxen, (*S*)-6-methoxy-α-methyl-2-naphthaleneacetic acid, is oxidized on PEC composite-modified electrodes in a neutral environment in the potential range of 0.9÷1.25 V with the formation of two peaks on the voltammograms (Figure 1), which is consistent with the electrochemical behavior of naproxen described earlier in [28, 29]. The proposed scheme of electrooxidation of naproxen is shown in Figure 2. The composition of pharmaceutical preparations of naproxen from three manufacturers with the same amount of active substance and different auxiliary components are presented in Table 1.

It should be noted that the values of instantaneous currents and the shape of the entire voltammetric curve depend differently on the nature of the modifier due to a change in not only the Faraday, but also the capacitive components of the current (Figure 1). Apparently, this is due to the nature of the modifiers. This ensures the condition of cross-sensitivity of the electrodes, which is necessary for the functioning of voltammetric systems of the "electronic tongue" type [13].

To establish the similarities and differences between the drugs of different manufacturers, chemometric processing of the recorded voltammograms (Figure 2) PCA [18] was used. It reduces multivariate data without losing important information, using a set of new orthogonal variables called principal components (PC), which are linear combinations of the original ones, while each voltammogram is transformed into a point on the PC plane, and by the mutual arrangement of the points one can judge the similarities and differences of the studied samples. Sets of adjacent points (parallel measurements) correspond to pharmaceutical preparations of a particular manufacturer (Figure 3). The optimal number of principal components was chosen so that the sum of the explained variance was not less than 90%.

Score plots of PCA models in the PC1-PC2 coordinates (Figure 3) show that when using one composite sensor (GCE/PEC@CB, GCE/PEC@CP, GCE/PEC@GO or GCE/PEC@SWCNT), clusters of different naproxen preparations (data from parallel measurements) intersect, which does not allow them to be reliably identified and attributed to a particular manufacturer. To increase the probability of recognition, various variants of multisensory systems with two, three and four indicator electrodes modified with PEC composites with CB, CP, GO, SWCNT were used. The main advantage of PCA is the ability to summarize the information contained in large data sets (for example, four voltammograms (Figure 4) recorded using different sensors for each sample) to a two- or three-coordinate point on the counting graph, where all the samples under consideration are also presented.

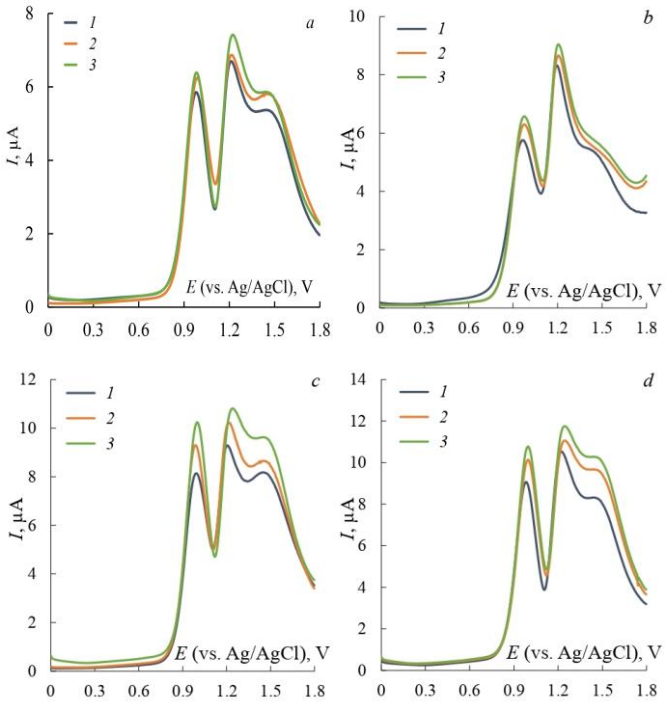

Figure 1 DPV of 1 mM solutions of naproxen drugs from three manufacturers: (1) – Nalgesin, (2) – Teraliv, (3) – Nexemezin, registered on sensors (a) GCE/PEC@CB, (b) GCE/PEC@CP, (c) GCE/PEC@GO, (d) GCE/PEC@SWCNT against the background of a phosphate buffer solution pH = 6.86, *v* = 20 mV/s.

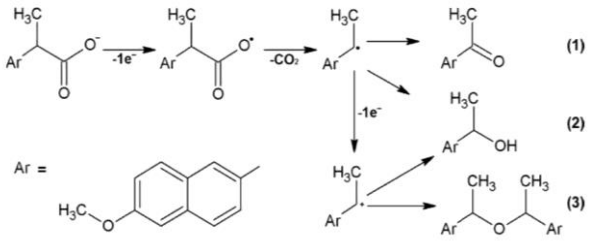

Figure 2 Scheme of electrooxidation of naproxen [28].

 DOI: 10.15826/chimtech.2025.12.2.04

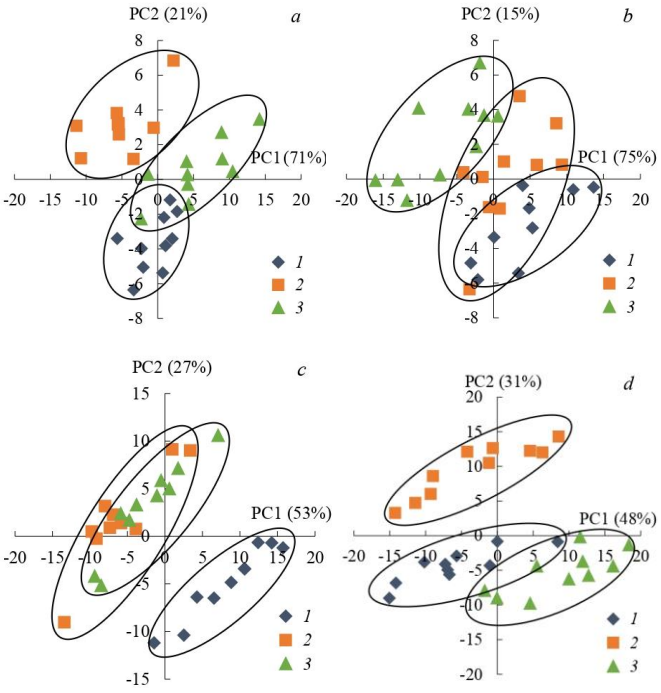

Figure 3 Score plots of PCA model for DPV of 1 mM solutions of naproxen from different manufacturers (1) – Nalgesin, (2) – Teraliv, (3) – Nexemezin for single-sensor systems: (a) GCE/PEC@CB, (b) GCE/PEC@CP, (c) GCE/PEC@GO, (d) GCE/PEC@SWCNT.

PCA models of two- and three-sensor systems, which are given in Supplementary 1 (Figures S1, S2), did not allow distinguishing between the samples under study. The score plots for the four-sensor system shown in Figure 5 indicate the formation of non-overlapping clusters of naproxen preparations from different manufacturers.

However, PCA is only a way of projecting experimental data onto the PC system and analyzing their variance. The SIMCA was used to attribute naproxen pharmaceutical preparations to the manufacturers. The SIMCA classification [19] was applied to calculate the proportions of test samples of preparations classified as the corresponding reference samples. Solutions of reference samples (RS) and test samples (TS) were prepared independently from the preparations of different production batches. The obtained data are presented in Table 2, which shows that for single-sensor systems the errors of the second kind (false acceptance) in recognizing naproxen preparations reach 100%. The use of two- and three-sensor systems also does not solve the identification problem (for more details, see Tables S1 and S2 in the supplementary materials). The results of SIMCA classification of naproxen preparations using a four-sensor system indicate their unambiguous identification (Table 2). For all the studied samples, the results of SIMCA classification using the four-sensor system give 100% matches within the cluster, i.e., 100% of the voltammetric data of the studied naproxen drug (transformed into a point) were included in the clusters of the calibration model of the same drug, and the voltammetric data of the studied drug were not included in the clusters of the calibration models of the other naproxen drugs (0%).

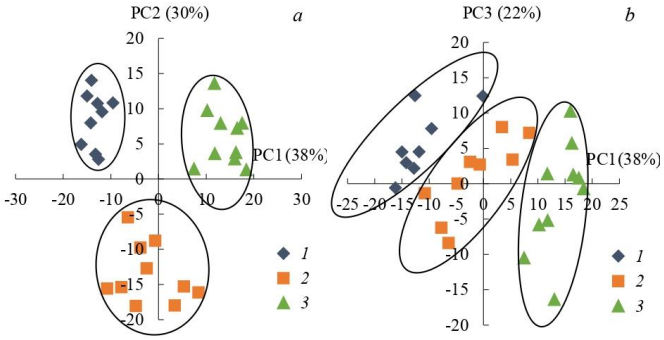

Figure 5 Score plots of PCA model for DPV of 1 mM solutions of naproxen from different manufacturers (1) – Nalgesin, (2) – Teraliv, (3) – Nexemezin for four-sensor system: GCE/PEC@CB+GCE/PEC@CP+GCE/PEC@GO+GCE/PEC@SWCNT.

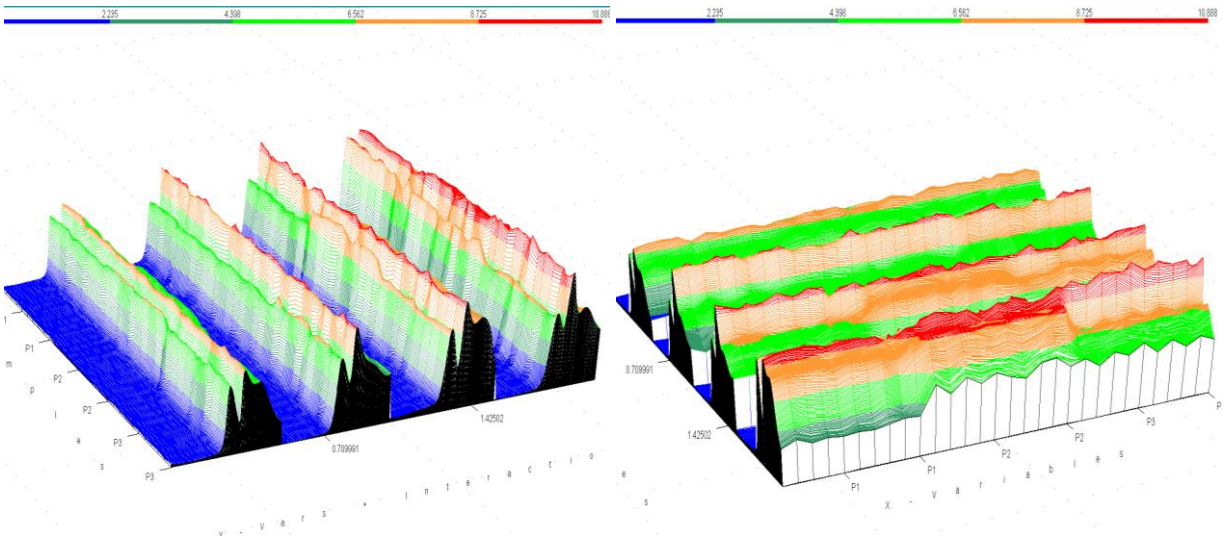

Figure 4 Array of DPV of 1 mM solutions of naproxen from different manufacturers (1) – Nalgesin, (2) – Teraliv, (3) – Nexemezine sequentially registered on: GCE/PEC@CB, GCE/PEC@CP, GCE/PEC@GO, GCE/PEC@SWCNT against the background of a phosphate buffer solution pH = 6.86, v = 20 mV/s.

 DOI: 10.15826/chimtech.2025.12.2.04

Table 2 Results of SIMCA classification of naproxen drugs from different manufacturers at individual GCE modified with PEC@CB, PEC@CP, PEC@GO and PEC@SWCNT and using the four-sensor system (DPV of 1 mM naproxen solution against a phosphate buffer solution pH = 6.86, n = 10, P = 0.95).

Sensor	GCE/PEC@CB			GCE/PEC@CP			GCE/PEC@GO			GCE/PEC@SWCNT			four-sensor system		
RS* / TS**	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
P1	100	20	0	100	30	0	100	0	30	100	0	30	100	0	0
P2	0	100	20	50	100	20	0	100	0	0	100	0	0	100	0
P3	0	100	100	0	50	100	50	0	100	50	0	100	0	0	100

* Reference samples; ** test samples.

4. Limitations

The main limitations of this proposed electronic language for identifying the drugs, manufacturer and monitoring their quality is the use of GCE and a classic three-electrode cell. In the future, it is planned to create a portable devices [34] on screen-printed electrodes [35] for work "in the field".

5. Conclusions

The voltammograms of naproxen show two clearly defined oxidation peaks, the maximum currents and shape of which vary depending on the nature of the composite modifier. Thus, varying the composite modifier allows for cross-sensitivity of sensors to be achieved, and it is quite easy to form the required set of sensors of various types to build multisensory systems for recognizing naproxen. It was shown that multisensory systems with fewer than four sensors, even in combination with chemometric data processing (PCA and SIMCA classification), do not provide reliable drug recognition with a probability close to 100%. For reliable recognition of naproxen pharmaceutical preparations, the multisensory system must have at least four sensors of various types. In this case, "Nalgesin", "Teraliv" and "Nexemezin" were successfully classified without errors of the first and second kind. The sensitivity and selectivity of the sensors in the multisensory system to the active substance and the qualitative and quantitative composition of excipients allow the developed system to be used not only to identify the manufacturers of the naproxen preparations, but also to detect counterfeits and expired preparations.

Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page.

Data availability statement

We collected and shared the data in the article.

Acknowledgments

None.

Author contributions

Conceptualization: Z.R.A., B.E.O., T.Yu.B.
Data curation: Z.R.A., B.E.O., T.Yu.B.
Formal Analysis: V.A.A., I.G.I., M.G.R.
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Methodology: Z.R.A.
Project administration: Z.R.A.
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Visualization: B.E.O., M.G.R.
Writing – original draft: Z.R.A., B.E.O.
Writing – review & editing: Z.R.A.

Conflict of interest

The authors declare no conflict of interest.

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