

Proceeding Paper

Simultaneous Sensing of Codeine and Diclofenac in Water Samples Using an Electrochemical Bi-MIP Sensor and a Voltammetric Electronic Tongue [†]

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[†] Presented at the 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, 1–15 July 2021; Available online: <https://csac2021.sciforum.net/>.

Abstract: Codeine and diclofenac overdoses have been widely reported. Here, a biomimetic sensor (bi-MIP) was devised, and an electronic tongue was used to analyze water samples simultaneously containing both these drugs. The bi-MIP sensor limits of detection for diclofenac and codeine taken individually were 0.01 µg/mL and 0.16 µg/mL, respectively. Due to a cross-reactivity effect when using the bi-MIP sensor, the electronic tongue was shown to differentiate samples containing both analytes. The results confirm the feasibility of simultaneous detection of two target analytes via a bi-MIP sensor. Additionally, they demonstrate the ability of a multi-sensor to classify different water samples.

Keywords: drug analysis; molecularly imprinted polymer sensor; nanoparticles; electrochemical multi-sensor; chemometrics; water



Citation: El Youbi, H.; Diouf, A.; Bouchikhi, B.; El Bari, N. Simultaneous Sensing of Codeine and Diclofenac in Water Samples Using an Electrochemical Bi-MIP Sensor and a Voltammetric Electronic Tongue. *Chem. Proc.* **2021**, *5*, 63. <https://doi.org/10.3390/CSAC2021-10483>

Academic Editor: Nicole Jaffrezic-Renault

Published: 30 June 2021

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1. Introduction

Diclofenac (DCF) and codeine (COD) are drugs administered to treat certain human health problems. Here the focus is first on DCF, which is a non-steroidal anti-inflammatory drug (NSAID), widely prescribed for the treatment of a wide variety of conditions. It reduces the need for morphine after surgery and is effective against menstrual pain and endometriosis. Although DCF has outstanding medical features, it is sometimes misused and can, as a result, easily move into the synovial fluid. This unfortunately leads to a reduction in the secretion of prostaglandins [1]. In consequence, the consumer can experience many health problems [2].

The second study focus is on COD, which is an opiate used clinically for its analgesic, antitussive and antidiarrheal properties. However, it is said to be addictive and can cause psychological damage to the patient if abused. Extreme consumption of COD can even cause death [3]. For these reasons, the World Health Organization (WHO), the US Food and Drug Administration (FDA), and the European Medicines Agency (EMA), among other international organizations, have issued strict warnings about the adverse effects of COD [4].

Electrochemical methods are very good candidates for drug analysis [5]. This is attributed to their low cost, lower detection limits, wide range of potential windows, and ease of surface renewal.

Firstly, electrochemical devices based on molecularly imprinted polymers (MIPs) can be considered as good alternatives to conventional techniques. However, according to

our literature research, the MIP strategy has not yet been exploited for the simultaneous detection of these two analytes. Currently, the immobilization of MIPs, as a sensing element on portable electrochemical transducers, such as screen-printed electrodes (SPEs), offers an interesting approach. A study has been reported for the detection of dopamine and uric acid using MIP technology.

Secondly, as drugs are usually released in wastewater, and wastewater treatment plants are not totally efficient, this work focuses on the analysis of mineral water samples with different concentrations of the drugs in question. When multiple targets are to be detected, it is appropriate to use various electrical interfaces, such as multi-sensor systems.

The following content of this study is devoted to the qualitative analysis of drugs in mineral water samples using a voltammetric electron tongue (VE-Tongue) combined with chemometric methods. When using the bi-MIP sensor, a cross-reactivity effect due to the presence of several compounds was encountered. To avoid it, qualitative analysis via VE-Tongue can help to classify/discriminate drug samples with different concentrations of the drugs in question.

Taking all these points into consideration, the primary objective of this paper was to report on the development of an electrochemical sensor based on molecularly imprinted polymers for the simultaneous detection of DCF and COD. Electrochemical techniques, such as electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), and cyclic voltammetry (CV), were used to investigate the electrochemical behavior of the electrodes during the different steps of the bi-MIP sensor fabrication. Principal components analysis (PCA) was used to process the database from the VE-Tongue sensor array for the purpose of discriminating between water samples containing DCF and COD.

2. Materials and Methods

2.1. Samples

Five sets of mineral water samples were prepared for the electrochemical analysis:

Set 1: Mineral water sample used as reference sample which was not spiked;

Set 2: Mineral water samples spiked with different concentration of diclofenac (0.001, 0.01, 0.1, 1, 10, 100, 300, 500 $\mu\text{g/mL}$);

Set 3: Mineral water samples spiked with codeine at the same concentrations as described above;

Set 4: Mineral water samples spiked with diclofenac at the same concentrations as described above, but each containing 300 $\mu\text{g/mL}$ codeine;

Set 5: Mineral water samples spiked with codeine at the same concentrations as described above, but each containing 300 $\mu\text{g/mL}$ diclofenac.

2.2. Instrumentation and Electrochemical Techniques

Figure 1 shows the experimental setup used in this study. The five sets described above were studied using both detection systems (i.e., bi-MIP sensor and VE-Tongue).

The bi-MIP sensor was designed on a screen-printed gold electrode (Au-SPE).

The voltammetric electronic tongue (VE-Tongue) consisted of an array of 5 working electrodes made of gold, copper, glassy carbon, platinum, and palladium. A silver/silver chloride (Ag/AgCl) reference electrode and a platinum counter electrode completed the three-electrode configuration.

A computer interfaced to a potentiostat device was used for data acquisition. Using the potentiostat, electrochemical characterization techniques, including CV, DPV and EIS, were run.

These three established techniques were used for the electrochemical measurements. The CV was operated from -0.4 to 0.6 V at a scan rate of 30 mV/s. To investigate the surface properties of the bi-MIP sensor, the EIS was performed in an open circuit at a low AC potential of 10 mV amplitude and a frequency range of 0.1 to $50,000$ Hz. The retention properties of the bi-MIP sensor were investigated using DPV over a potential range of

−0.2 to 0.3 V and a slew rate of 50 mV/s. All measurements were performed at room temperature (25 °C).

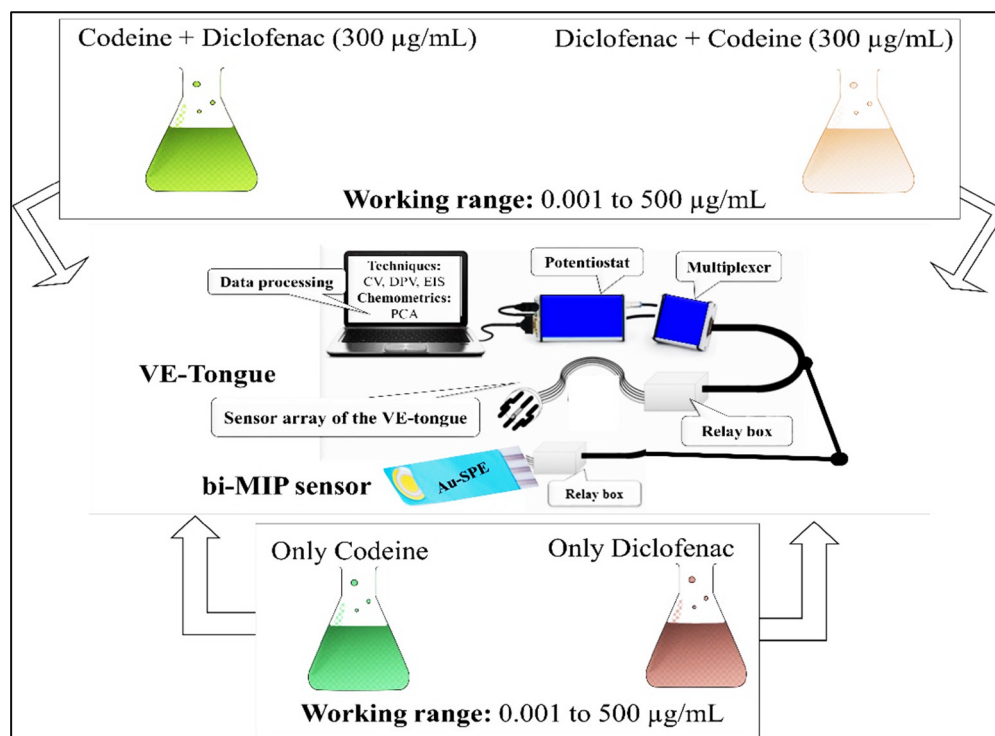


Figure 1. Graphical overview of the experimental setup.

2.3. Bi-MIP Sensor Preparation

Figure 2 illustrates the procedures for the bi-MIP sensor elaboration. Briefly, a layer of polyvinyl carboxylic chloride (PVC-COOH) was first assembled to modify the bare Au-SPE. Then, after activation of -COOH groups by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS), a solution (1 mg/mL), containing simultaneously DCF and COD, was deposited on the modified electrode. After DCF and COD binding, a solution containing methacrylic acid, as the functional monomer, and silver nanoparticles (AgNPs) was immobilized. An extraction stage of template molecules followed to complete the fabrication of the bi-MIP sensor.

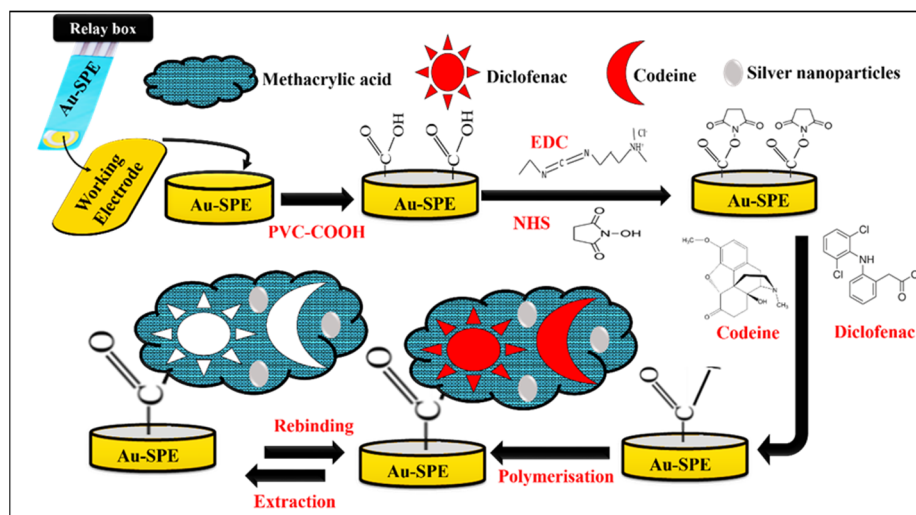


Figure 2. The development stages of the bi-MIP sensor.

2.4. Data Analysis

The multivariate responses of the VE-Tongue were processed by a known unsupervised method called PCA. This statistical technique reduces the dimensionality of the multivariate data while retaining maximum information on new variables called principal components (PCs) [6,7]. This allows for better visualization of the data and better interpretation of the analyzed samples.

3. Results and Discussion

3.1. Biomimetic Receptor Assembly

During the development of the biomimetic sensor, several immobilization procedures to form the sensitive layer were performed. After each step, the electrochemical behavior of the electrode was observed using a supporting electrolyte (PBS pH 7.4) containing electroactive species ($[\text{Fe}(\text{CN})_6]^{4-/3-}$). For this purpose, the CV and EIS techniques were run in PsTRACE software. The results of these characterizations are presented in Figure 3. At each step of the sensor development, the electrochemical behavior of the electrode changed compared to the bare electrode. Moreover, the CV and EIS results were in good agreement.

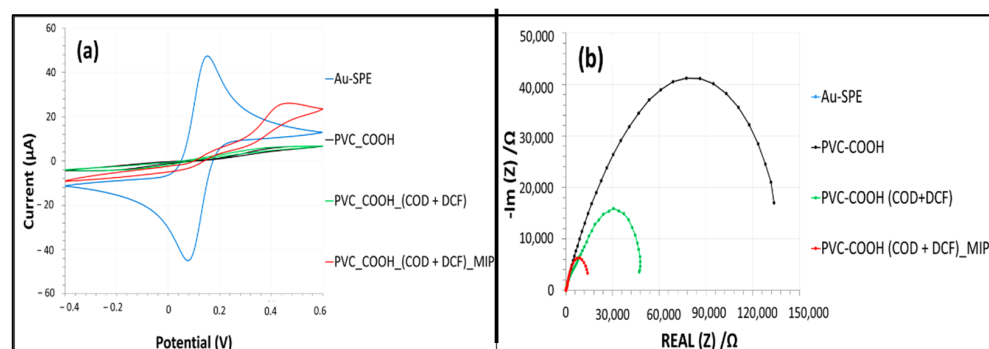


Figure 3. Electrochemical signals corresponding to the development stages of the bi-MIP sensor: (a) cyclic voltammograms, (b) Nyquist diagrams.

3.2. Bi-MIP Sensor Responses

In the first step, the analysis of DCF alone (set 2), at different concentrations on the bi-MIP sensor, was performed using the differential pulse voltammetry (DPV) technique. The calibration curves related to these responses are shown in Figure 4. A clear decrease in the amplitude of the voltammograms was observed as the concentration of DCF increased, expressed in the linear regression equation shown in Figure 4a. The equation is $y = -0.083 \text{Log}(C) - 0.355$ with a determination coefficient $R^2 = 0.93$. The calculated detection limit was $0.01 \mu\text{g/mL}$ using the formula described by DIOUF et al. [8].

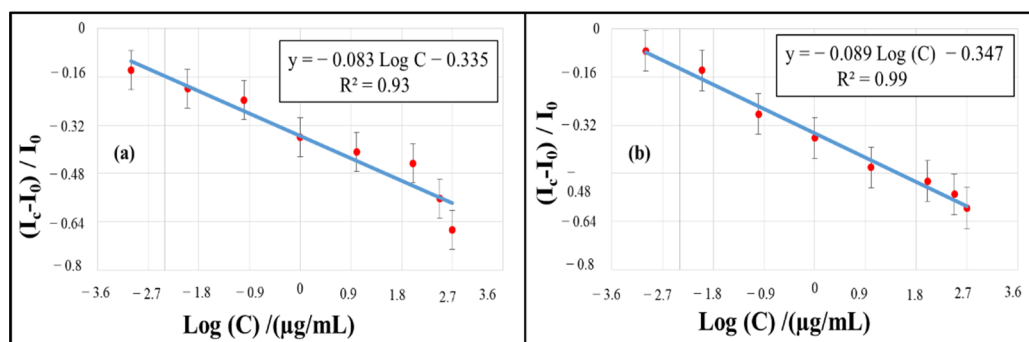


Figure 4. Calibration curves of the bi-MIP sensor with increasing concentrations of: (a) diclofenac (b) codeine, from 0.001 to $500 \mu\text{g/mL}$.

Secondly, COD alone (set 3) was analyzed under the same conditions. The corresponding equation of the bi-MIP sensor responses (voltammograms) is shown in Figure 4b. Here, a similar trend to that of the DCF was obtained with a calibration equation of $y = -0.089\text{Log}(C) - 0.347$ with $R^2 = 0.98$. The limit of detection was $0.16 \mu\text{g/mL}$.

When detecting the two analytes individually, it was found that the bi-MIP sensor had almost equivalent sensitivity. However, because of cross-reactivity, the results for the simultaneous detection of both analytes by the bi-MIP were not satisfactory. An electronic tongue was used to explore a potential strategy to address this.

3.3. PCA Analysis of the VE-Tongue Dataset

Due to cross-reactivity and limitations encountered with the bi-MIP sensor, measurement of samples containing both target analytes simultaneously was performed using the VE-Tongue. After data pre-processing, principal components analysis (PCA) was used to classify the samples from all sets. The results are presented in Figure 5, which shows the projections of the experimental results onto a two-dimensional (2D) space formed by the first two principal components; 78.90% of the total variance of the data was explained by the first two PCs indicating significant pattern separation.

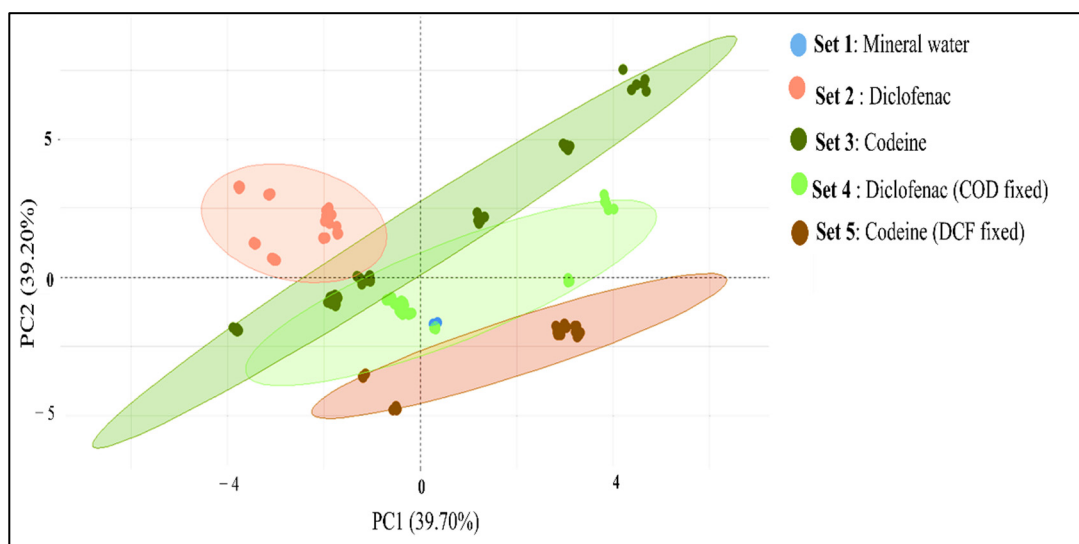


Figure 5. PCA plot showing the discrimination of the different sets using ΔI and Area as features. ΔI is the difference between the maximum current of the oxidation wave and the reduction wave. Area is the area of the VE-Tongue response (voltammogramme) using the trapezoidal method.

PCA was also applied to data after analysis of samples from set 4 and set 5, according to their concentrations.

Set 4 contained water samples with varying concentrations of DCF and a fixed concentration of COD ($300 \mu\text{g/mL}$) for each. As shown in Figure 6a, all samples in set 4 were well separated with only 85.87% of the total variance expressed by PC1 and PC2. In addition, the samples containing low and high concentrations of DCF clustered in the top right and bottom of the graph, respectively.

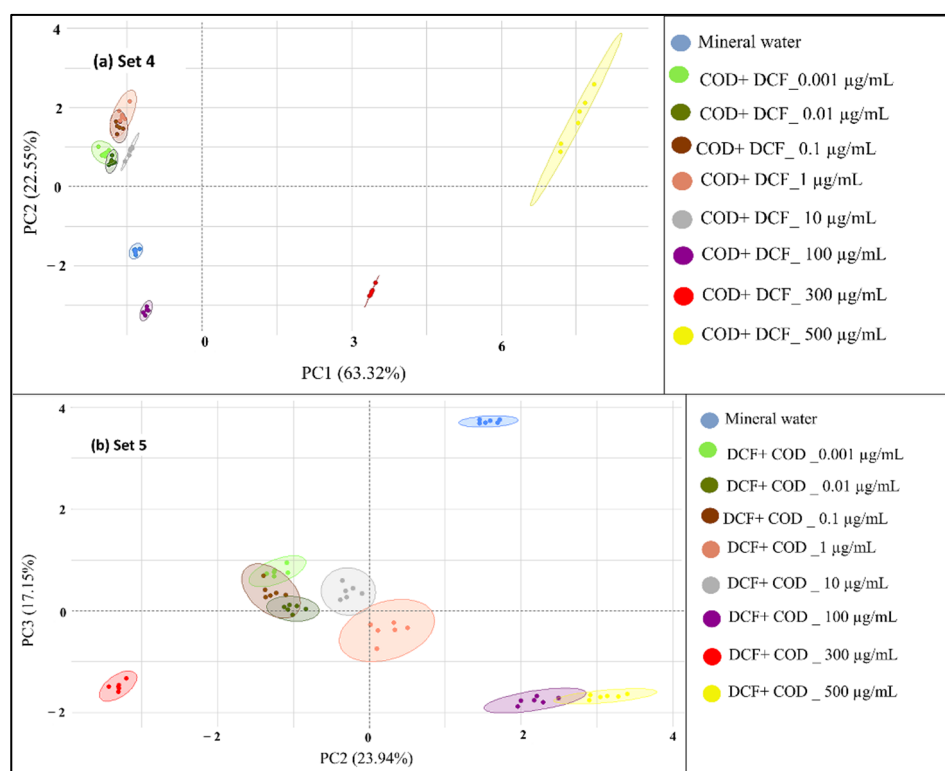


Figure 6. PCA plot showing the discrimination between set 1 and water samples of (a) set 4 and (b) set 5 at different concentrations using DI and Area as features.

In Figure 6b, the same trend is also observed for the analysis of samples in set 5. In this set, COD was varied but the concentration of DCF was maintained at 300 µg/mL. In the graph, the clean water sample and the spiked samples are well separated, with a score of 41.1% of the total variance, expressed as PC2 and PC3.

These results clearly show that the VE-Tongue was able to discriminate water samples containing several compounds at different concentrations.

4. Conclusions

In this study, a new bi-MIP-sensor-based electrochemical detection system for the detection of diclofenac and codeine was proposed. The principle of simultaneous detection was highlighted by using an electronic tongue combined with pattern recognition methods. The proposed analytical tools represent a breakthrough in water analysis.

Author Contributions: Conceptualization, N.E.B.; methodology, H.E.Y., N.E.B.; Software, B.B.; validation, N.E.B.; formal analysis, H.E.Y., N.E.B. investigation, N.E.B., H.E.Y.; resources, N.E.B.; data curation, N.E.B., H.E.Y.; writing—original draft preparation, H.E.Y., N.E.B., A.D.; writing—review and editing, H.E.Y., N.E.B.; visualization, supervision, N.E.B.; project administration, N.E.B.; funding acquisition, N.E.B.; Software: B.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Authors gratefully acknowledge Moulay Ismaïl University of Meknes for financial support of the project “Research support”.

Conflicts of Interest: The authors declare no conflict of interest.

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