

# Simultaneous Sensing of Codeine and Diclofenac in Water Samples using an Electrochemical Bi-MIP Sensor and a Voltammetric Electronic Tongue<sup>†</sup>

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**Abstract:** Codeine and diclofenac overdoses have been widely reported. Here, a biomimetic sensor (bi-MIP) is devised, and an electronic tongue is used to analyze water samples containing simultaneously these drugs. The bi-MIP sensor limits of detection for diclofenac and codeine taken individually were of 0.01 µg/mL and 0.16 µg/mL, respectively. Due to cross reactivity effect when using the bi-MIP sensor, the electronic tongue has proven to differentiate samples containing both analytes. The results reflect 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.

## 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 aspects, it is sometimes misused and can therefore easily move into the synovial fluid. This unfortunately leads to a reduction in the secretion of prostaglandins [1]. As a result, the consumer finds himself in many health problems [2].

The second study focus 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.

First in this study, electrochemical devices based on molecularly imprinted polymers (MIPs) can be considered as good alternatives to conventional techniques. However, ac-

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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 efficient enough, 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 makes sense to use various electrical interfaces, such as multi-sensor systems.

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

Taking all these points into consideration, the objective of this paper was first to report on the development of an electrochemical sensor based on molecularly imprinted polymer 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 fabrication. Principal component 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 and 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  $\mu\text{g}/\text{mL}$ );

**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  $\mu\text{g}/\text{mL}$  codeine;

**Set 5:** Mineral water samples spiked with codeine at the same concentrations as described above, but each containing 300  $\mu\text{g}/\text{mL}$  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 (bi-MIP sensor and VE tongue).

The bi-MIP sensor is designed on 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, such as CV, DPV and EIS were run.

Indeed, three known 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).

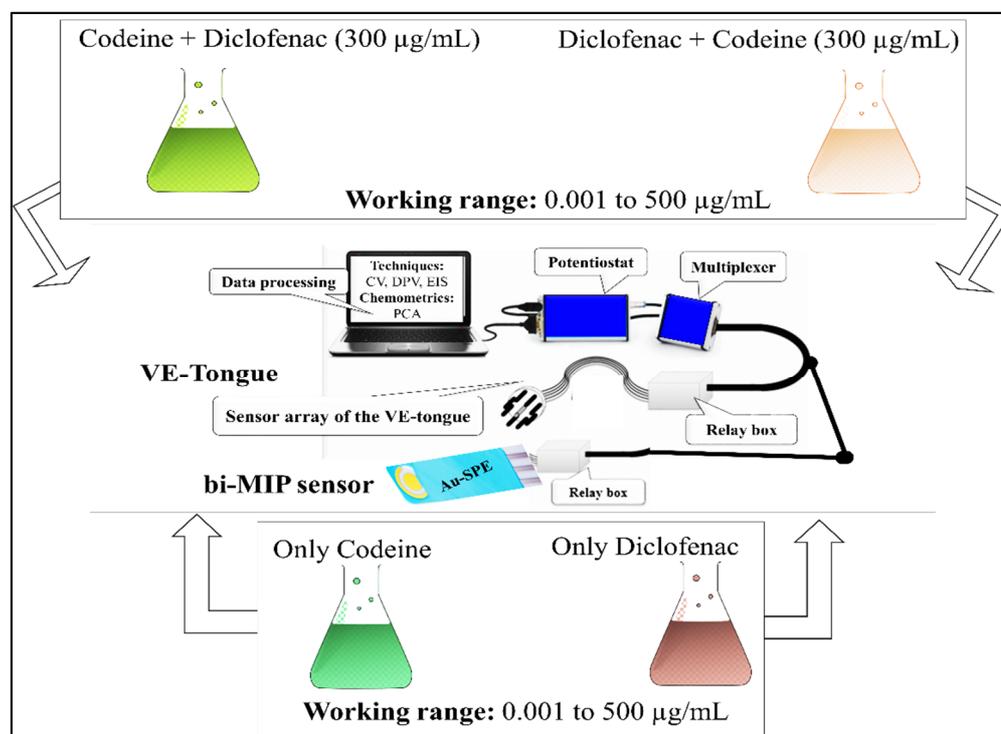


Figure 1. Graphical overview of the experimental set-up.

### 2.3. Bi-MIP Sensor Preparation

Figure 2 illustrates the procedures of 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.

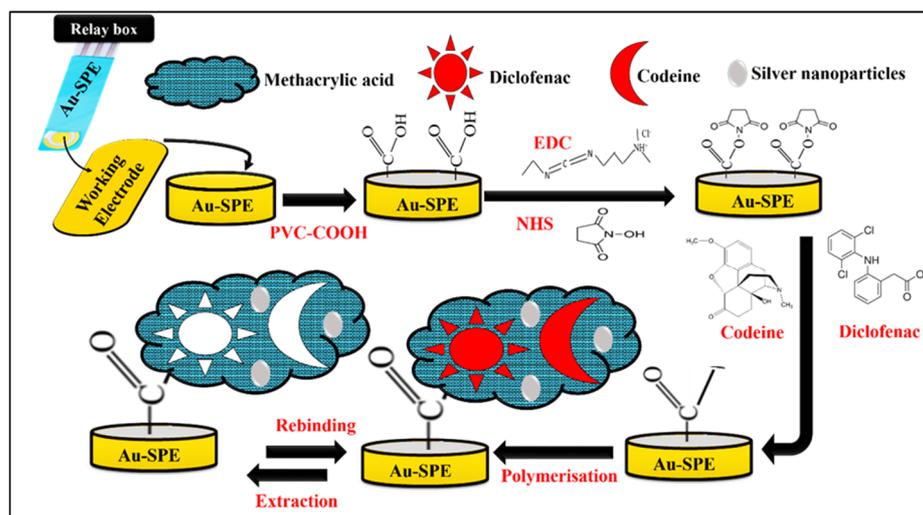


Figure 2. The development stages of the bi-MIP sensor.

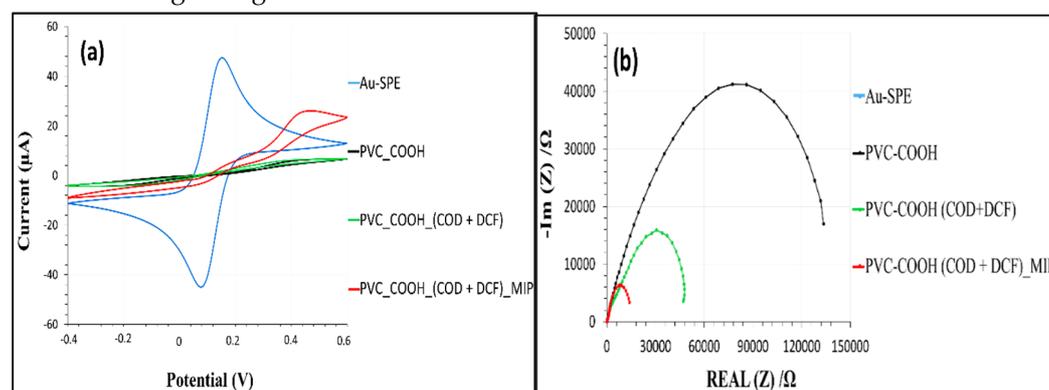
## 2.2. Data Analysis

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 immobilisation procedures to form the sensitive layer were performed. After each step, the electrochemical behaviour 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 a PsTRACE software. The results of these characterisations were presented in Figure 3. It can be seen that at each step of the sensor development, the electrochemical behavior of the electrode changes compared to the bare electrode. Moreover, CV and EIS results are in good agreement.



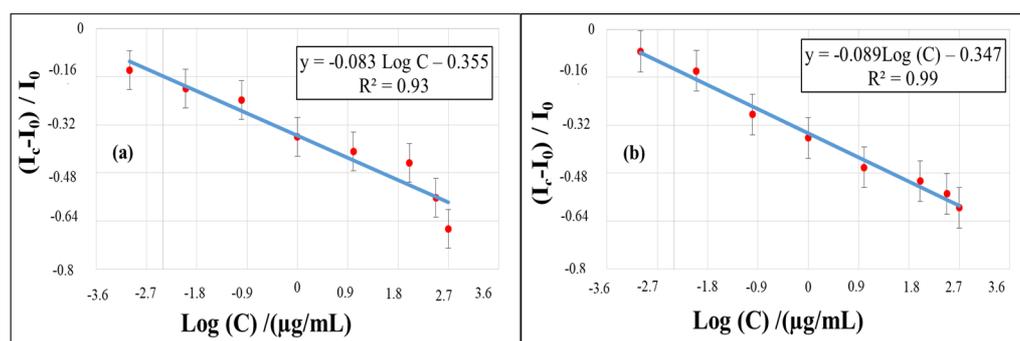
**Figure 3.** Electrochemical signals corresponding to the development stages of the bi-MIP sensor: **(a)** Cyclic voltammograms, **(b)** Nyquist diagrams.

### 3.2. Bi-MIP Sensor Responses

In a first step, the analysis of DCF alone (set 2), at different concentrations on the bi-MIP sensor, is 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 increases resulting in a 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 is 0.01 µg/mL using the formula described by *DIUIF et al.* [8].

Secondly, COD alone (set 3) is analysed 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 is 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 µg/mL.

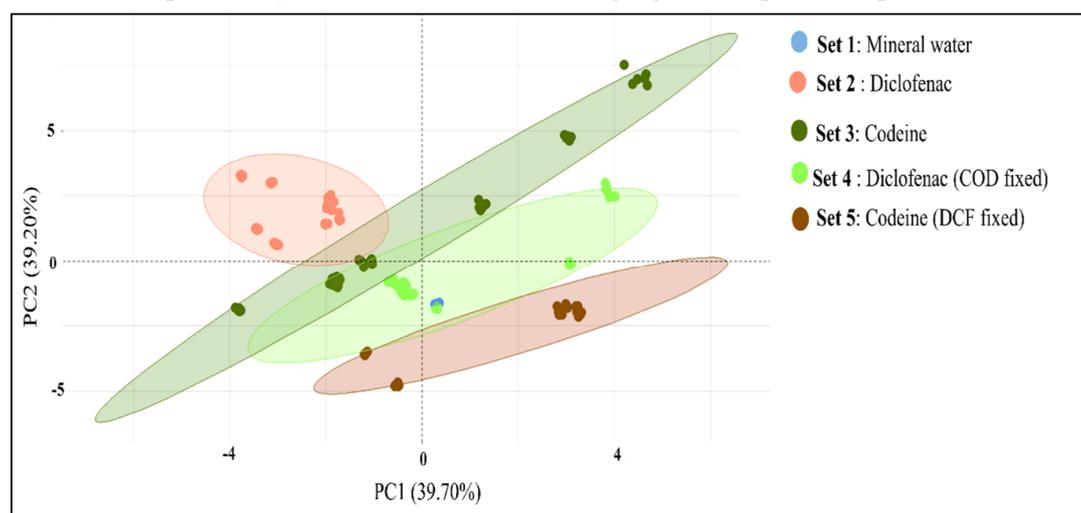
When detecting the two analytes individually, it was found that the bi-MIP sensor had almost similar sensitivity. However, due to the effect of cross-reactivity, the results of simultaneous detection of both analytes by the bi-MIP were not satisfactory. An electronic tongue was used to explore this strategy.



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

### 3.3. PCA analysis of the VE-Tongue dataset

Due to cross reactivity and limitation encountered with the bi-MIP sensor; the measurement of samples simultaneously containing both target analytes was performed using the VE-Tongue. After data pre-processing, Principal Component 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. A score of 78.90% of the total variance of the data is explained by the first two PCs indicating significant pattern separation.



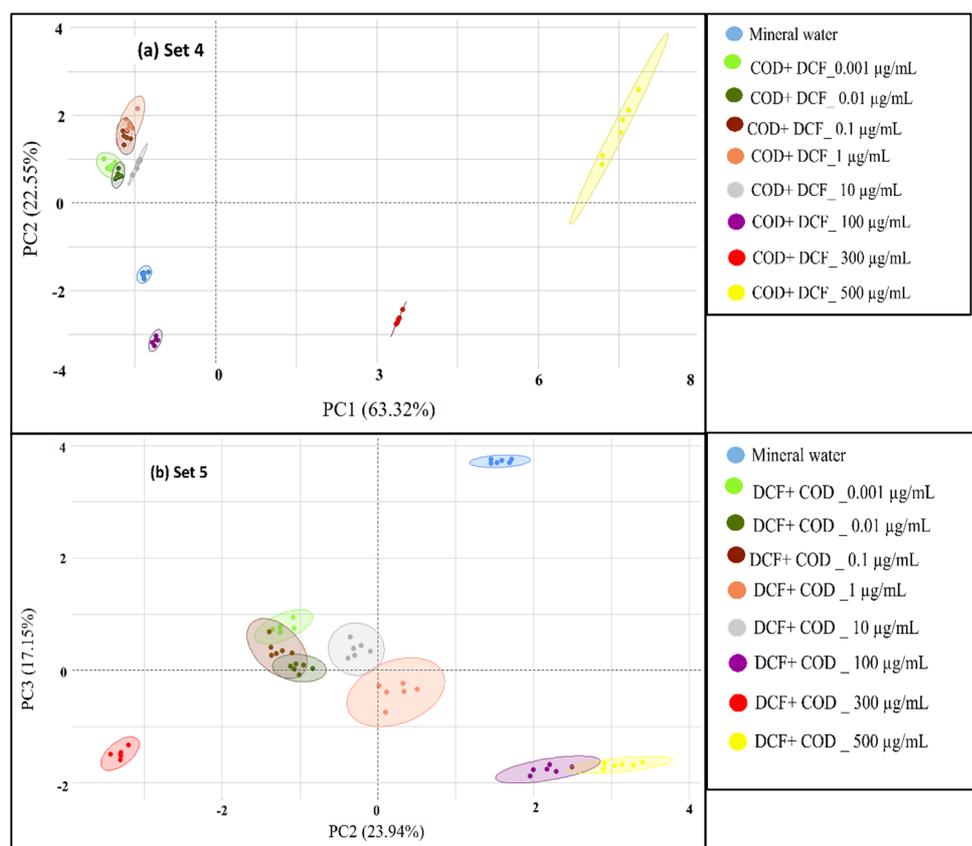
**Figure 5.** PCA plot showing the discrimination of the different sets using  $\Delta I$  and Area as features.  $\Delta I$  is the difference between the maximum current of the oxidation wave and the reduction wave. Area is the area of the VE-Tongue response (Voltammogramme) using the trapezoidal method.

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

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

In Figure 6b, the same trend is also observed for the analysis of samples in set 5. In this set, COD is varied but the concentration of DCF is maintained at 300  $\mu\text{g/mL}$ . 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.



**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  $\Delta I$  and Area as features.

#### 4. Conclusions

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

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

#### References

1. Kormosh, Z.; Hunka, I.; Bazel, Y.; Laganovsky, A.; Mazurenko, I.; Kormosh, N. Determination of diclofenac in pharmaceuticals and urine samples using a membrane sensor based on the ion associate of diclofenac with Rhodamine B. *Open Chem.* **2007**, *5*, 813–823.
2. Elbalkiny, H.T.; Yehia, A.M.; Safa'a, M.R.; Elsharty, Y.S. Potentiometric diclofenac detection in wastewater using functionalized nanoparticles. *Microchem. J.* **2019**, *145*, 90–95.
3. Häkkinen, M.; Launiainen, T.; Vuori, E.; Ojanperä, I. Comparison of fatal poisonings by prescription opioids. *Forensic Sci. Int.* **2012**, *222*, 327–331.
4. Tobias, J.D.; Green, T.P.; Côté, C.J. Codeine: Time to say “no”. *Pediatrics* **2016**, *138*.
5. Gimenes, D.T.; Cunha, R.R.; de Carvalho Ribeiro, M.M.A.; Pereira, P.F.; Muñoz, R.A.A.; Richter, E.M. Two new electrochemical methods for fast and simultaneous determination of codeine and diclofenac. *Talanta* **2013**, *116*, 1026–1032.
6. Haddi, Z., Conception et développement d’un système multicapteurs en gaz et en liquide pour la sécurité alimentaire, Thèse d’Université, Université Claude Bernard - Lyon I, **2013**.
7. Motia, S.; Bouchikhi, B.; El Bari, N. An electrochemical sensor based on molecularly imprinted polymer conjointly with a voltammetric electronic tongue for quantitative diphenyl phosphate detection in urine samples from cosmetic product users. *Sensor Actuat. B-Chem.* **2021**, *332*, 129449.

8. Diouf, A.; Bouchikhi, B.; El Bari, N. A nonenzymatic electrochemical glucose sensor based on molecularly imprinted polymer and its application in measuring saliva glucose. *Mat. Sci. Eng. C* **2019**, *98*, 1196–1209.