

A sensor array based on molecularly imprinted polymers and machine learning for the analysis of fluoroquinolone antibiotics

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ABSTRACT: Fluoroquinolones (FQs) are one of the most important types of antibiotics in the clinical, poultry and aquaculture industries, and their monitoring is required as the abuse has led to severe issues, such as antibiotic residues and antimicrobial resistance. In this work, we report a voltammetric electronic tongue (ET) for the simultaneous determination of ciprofloxacin, levofloxacin and moxifloxacin in both pharmaceutical and biological samples. The ET comprises four sensors modified with three different customized molecularly imprinted polymers (MIPs) and a non-imprinted polymer (NIP) incorporated with Au nanoparticles decorated multi-wall carbon nanotubes (Au-fMWCNTs). MWCNTs were first functionalized to serve as supporting substrate, while anchored Au nanoparticles act as catalyst. Subsequently, MIP films were obtained by electropolymerization in the presence of the different target FQs. The sensors' morphology was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), while the modification process was followed electrochemically step by step employing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probe. Under the optimal conditions, the MIP(FQs)@Au-fMWCNTs sensors exhibited differentiated responses, limits of detection (LOD) ca. $0.1\ \mu\text{M}$ and a wide detection range up to $300\ \mu\text{M}$ for the three FQs. Lastly, the developed ET presents satisfactory agreement between expected and obtained values when used for the simultaneous determination of the three FQs in the mixtures ($R^2 \geq 0.960$), that was successfully applied to the analysis of FQs in commercial pharmaceuticals and spiked human urine samples.

Introduction

Fluoroquinolones (FQs), one of the most important family of antibiotics, are used individually or in combination against a broad spectrum of Gram-positive and Gram-negative bacteria in clinical and animal husbandry^{1,2}. Among them, ciprofloxacin (CFX), levofloxacin (LFX) and moxifloxacin (MFX) are the most common in current clinical practice. LFX and MFX are considered as respiratory drugs that have good bactericidal activity against most of respiratory pathogens, while CFX is used to treat the infections of the urinary tract and intestine. Structurally, FQs are similar as they are all derived from nalidixic acid (NA), sharing the same

quinoline ring, but with different substitutions. Although FQs usage is approved worldwide and their significant curative effects have been demonstrated, consequences resulting from their misuse such as antibiotic residues (mainly related to food safety and environmental contamination) or antimicrobial resistance (essentially requiring therapeutic drug monitoring) cannot be neglected³⁻⁵. Thus, FQs monitoring is required in different stages, such as in pharmaceutical factories when produced, in hospitals during its administration to the patients or in the environment for the treatment of wastewater. To date, the detection of FQs using electrochemical sensors has been proposed as a cheaper, time- and laborsaving alternative to complex detection methods such as high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS)⁶.

Nevertheless, most reported electrochemical sensors target only one specific FQ, and cannot achieve the simultaneous multi-determination in practical applications, or deal with the interferences that may arise from certain complicated matrices^{7,8}. This can be rather thorny in the case of FQs family given the common-shared structure and similar natures.

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Compared with the conventional approach, by coupling a sensor array providing responses to various analytes with appropriate data processing, electronic tongues (ETs) allow the discrimination and simultaneous quantification of multiple analytes^{9,10}, while they might also be able to solve other problems related to sensors such as drifts, interferences, cross-sensitivity and/or matrix effects.

To further improve the performance of ETs, one of trending strategies is using a proper set of biorecognition elements such as enzymes peptides, etc¹¹. Recently, the feasibility of incorporating molecularly imprinted polymers (MIPs) into ETs has been investigated for the simultaneous determination of structurally similar analytes in various fields^{12,13}. As artificial customized receptors, MIPs not only can afford equivalent specificity to a bioreceptor, but offer higher physical and chemical stability than biological recognition elements^{14,15}. More importantly, the key-lock recognition mechanism between MIPs and the target compounds defined by the size and structure of the template molecules offers a simple and straightforward procedure for developing a series of MIP based sensors to be combined into a sensor array for the development of ETs. However, despite MIPs' many merits, there is still space for the improvement of their integration into sensors, especially in regards to sensitivity¹⁶.

Compared with other polymerization methods, electropolymerization can generate controllable MIPs thin films directly onto the sensors, representing a simpler, and highly reproducible approach for the integration of MIPs with electrochemical sensors^{17,18}. Besides, the lower sensitivity derived from MIPs' intrinsically inferior conductivity and electrocatalytic activity could be improved by introducing nanomaterials¹⁹, e.g., carbon nanotubes (CNTs)²⁰ or metallic nanoparticles (NPs)²¹.

To this end, herein we explore the electropolymerization of pyrrole onto multi-wall CNTs (MWCNTs) as supporting substrate that can lead to MIPs with a larger surface area, higher conductivity and amplified signal. In addition, the incorporation of metallic gold NPs (Au-NPs) can also act as a catalyst for the electrochemical reactions and further enhancing sensitivity. In this manner, MIPs integrated with these nanomaterials were synthesized towards ciprofloxacin, levofloxacin, and moxifloxacin. Next, the obtained sensors were characterized both morphologically and electrochemically, and finally combined into a sensor array, which was successfully employed to develop a MIP based ET for the analysis of FQ antibiotics in commercial drugs and spiked human urine samples.

Experimental section

Materials and reagents.

Graphite powder (particle size <50 μm) and epoxy resin kit for fabricating graphite-epoxy composite (GEC) electrodes were supplied by Resinco (Barcelona, Spain). Pristine MWCNTs (outer diameter $\sim 10\text{-}30\text{nm}$) were obtained from SES Research Inc. (Houston, TX, USA). Pyrrole (Py) and anhydrous trisodium citrate were purchased from Alfa-Aesar (Ward Hill, MA, USA). Gold (III) chloride solution in hydrochloric acid (30 wt.% HAuCl_4), perfluorinated resin solution containing Nafion, ciprofloxacin (CFX), levofloxacin (LFX), moxifloxacin (MXF), flumequine (FLQ) and nalidixic acid (NA) were purchased from Aldrich (St. Louis, MO, USA), while sodium borohydride, potassium hexacyanoferrate (II) trihydrate and potassium hexacyanoferrate (III) from Panreac (Barcelona, Spain). Ethanol (EtOH, 96 vol. %) and NaOH were bought from Scharlau (Barcelona, Spain).

All solutions employed in the experiments were prepared using deionized water from a Milli-Q System (Millipore, Billerica, MA, USA). Various buffer solutions were used in this work for different purposes. Concretely, 50 mM phosphate buffer (PBS) at pH 7.0 containing 100 mM potassium chloride was used for the electrochemical characterization, while 100 mM PBS at pH 10.0 was used for the electrochemical extraction and cleaning. 100 mM acetate buffer at pH 3.5 was used for the electropolymerization and the preparation of the stock solutions', while 100 mM acetate buffer at pH 4.5 was employed for the analysis of FQs.

Apparatus.

An EVO MA10 (Zeiss, Oberkochen, Germany) and a JEOL 1210 (Peabody, MA, USA) were used for scanning and transmission electron microscopy (SEM/TEM), respectively. An Autolab PGSTAT30 (Ecochemie, Utrecht, Netherlands) with multichannel configuration controlled by GPES and FRA software packages was used for the electrochemical measurements.

Fabrication of MIP(FQs)@Au-fMWCNTs sensor.

Hand-crafted GEC electrodes, that served as sensor platforms for the construction of the MIP-based sensor array, were prepared according to the previous reports from our group²². Briefly, the paste was prepared by mixing 58 % of graphite powder and 42 % of the epoxy

resin. Next, this paste was placed into a PVC tube, in which a copper disc soldered to a connector had previously been inserted, and cured at 40 °C for 2 days. Finally, the electrodes were polished and ready to use. Pristine MWCNTs were pre-treated with strong acids to obtain carboxylic functionalized MWCNTs (fMWCNTs). First of all, MWCNTs were dispersed in an acid mixture of concentrated H₂SO₄/HNO₃ (v/v 3:1) by ultrasonication for 6h at 40 °C. Then, washed with Milli-Q water until neutralized, and dried in the oven for further using. The decoration of fMWCNTs with AuNPs was performed according to the previously reported procedures²³. Specifically, 500 µL of 10 mM HAuCl₄, 500 µL of 10 mM trisodium citrate and 18.4 mL Milli-Q water were mixed in a round-bottom flask, to which 20 mg of fMWCNTs and 10 mL of anhydrous ethanol were added. After sonicating for 10 mins, 600 µL of a 100 mM freshly prepared cold NaBH₄ lasted for 10 hours under continuous stirring. The obtained product (denoted as Au-fMWCNTs) was thoroughly washed with Milli-Q water and dried in the oven at 40 °C overnight.

For the modification of the sensors, 2.0 mg of the dried Au-fMWCNTs powder was dispersed in 1 mL of N, N-dimethylformamide (DMF), into which 100 µL of Nafion were added to improve the stability of Au-fMWCNTs on the surface of the electrodes upon drop casting. Finally, 4 µL of Au-fMWCNTs dispersion in DMF was evenly dropped onto the surface of the GEC and dried in the oven.

The synthesis of the MIP(FQs) film on the surface of Au-fMWCNTs was performed by the electropolymerization method. The solution containing 0.05 M pyrrole as the functional monomer, 0.01M FQ (either CFX, LFX, or MFX) as the template molecule and 20 mL of acetate buffer (pH 3.5) as the supporting electrolyte was purged with nitrogen for 10 mins before using for the synthesis of the MIP(FQ) film. The electropolymerization was conducted by cycling the potential from 0.0 V to +1.3 V vs. Ag/AgCl at a scan rate of 50 mV·s⁻¹ (Figure S1). Subsequently, extraction of the template molecules from the polymer film was done in a two steps procedure. First, cycling the potential from 0.0 V to +1.5 V vs. Ag/AgCl at a scan rate of 100 mV·s⁻¹ in PBS (pH 10.0) (Figure S1), followed by the immersion of the electrodes into a mixture of EtOH/0.1 M NaOH (1:1 v/v) for 30 mins.

Similarly, the same procedures as above-mentioned were used for the synthesis of the non-imprinted polymer on Au-fMWCNTs (NIP@Au-fMWCNTs), except that no FQ was added during the electropolymerization. The purpose of the NIP is to be used as control. Electrochemical measurements. All electrochemical measurements were performed at room temperature in a standard three electrode cell made up of an Ag/AgCl (3 M KCl) reference electrode, a Pt wire as auxiliary electrode and the MIP(FQs)@Au-fMWCNTs or NIP@Au-fMWCNTs modified GECs as the working electrodes. Both cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) for the electrochemical characterization were conducted in a 5 mM K₃[Fe (CN)₆]/K₄[Fe (CN)₆] solution in PBS (pH 7.0). For CV measurements, the potential was scanned in the range from -0.2 V to +0.7 V at a scan rate of 50 mV·s⁻¹, while for EIS, a frequency range between 100 kHz to 100 mHz with a fixed AC amplitude of 10 mV and an applied potential of +0.24 V was considered.

For the analysis of FQs, differential pulse voltammetry (DPV) measurements were performed by scanning the potential from +0.6 V to +1.5 V with a step potential of 5 mV and a pulse amplitude of 50 mV without stirring. Prior to each measurement, the electrodes were incubated in the solution to be analyzed for 300s under stirring to enhance the enrichment of the analytes into the material. To regenerate the electrodes, a fix potential of +1.2 V for 90 s was applied in PBS (pH 10.0) after each measurement. Samples preparation. Given the

different solubility of the different FQs, the stock solutions of CFX, LFX, MFX and NA were prepared in acetate buffer (pH 3.5), whilst the stock of FLQ was prepared in 0.1M NaOH solution. In order to characterize the analytical response of the developed sensors towards each of the analytes, the individual calibration curves were constructed by measuring solutions of increasing concentrations prepared by proper dilution of the FQs' stock solutions in buffer (pH 4.5).

For the simultaneous quantification of CFX, LFX and MFX, two subsets of samples were prepared and measured under identical conditions in randomized order. On the one side, the training subset was composed by 27 samples based on a 33 tilted factorial design²⁴, in which the concentrations of FQs were specified in the range from 2 to 300 μ M for CFX, LFX and MFX. On the other side, to evaluate the performance of the proposed model, the testing subset was composed by 10 samples that were randomly distributed in the concentration domain defined by the factorial design.

To demonstrate its applicability, the MIP-based ET was applied to the determination of the FQs under study both in commercial pharmaceuticals and biological fluids. On the one hand, commercial antibiotic drugs were bought from the local drug stores with the doctor's prescription. Specifically, four different FQ antibiotic drugs were evaluated: Cetraxal plus (3 mg·ml⁻¹ ciprofloxacin and 0.25 mg·ml⁻¹ fluocinolone acetonide; Laboratorios SALVAT, S.A., Barcelona, Spain), Ciprofloxacin Normon (250 mg ciprofloxacin; Normon Laboratories, S.A., Madrid, Spain), Levofloxacin Aurovitas (500 mg levofloxacin; APL Swift Services Limited, Birzebbugia, Malta) and Moxifloxacin Qualigen (400 mg moxifloxacin; Pharmathen, S.A., Attiki, Greece). For the analysis, drugs were dissolved or diluted in acetate buffer (pH 4.5) directly to make the expected concentrations fit in the experimental domain of the built model. Moreover, as there might be the cases in the clinical where multiple antibiotics are used in combination, or for quality control purposed during drugs production, the above antibiotic drugs were analyzed both individually or in mixtures. On the other hand, considering the urine excretion rate of FQ antibiotics²⁵, the analysis of FQs in human urine was also attempted. Urine samples were collected in sterile bottles from volunteers, diluted 40 times with acetate buffer (pH 4.5), and then spiked with the FQ stocks. Again, the dilution of urine samples was required so as to ensure the concentration falls within the experimental domain of the model. All real samples were measured under the same conditions as already described, without any further pre-treatment.

Data Processing.

Multivariate analysis was carried out in MATLAB 7.1 (MathWorks, Natick, MA, USA) by means of specific routines developed by the authors employing its Statistics and Neural Network toolboxes. The voltammetric responses of the sensor array were combined and compressed employing discrete cosine transform (DCT), which allowed to reduce the dimensionality of the input signals, while preserving the relevant information²⁶. Next, the obtained coefficients were either submitted to principal component analysis (PCA) or artificial neural networks (ANNs).

Results and discussion

Design of MIP(FQs)@Au-fMWCNTs based ET.

The main goal of this work is the development of a sensitive ET for the simultaneous identification and quantification of FQs (Figure S2). In this regard, the fabrication of MIP based electrochemical sensors towards FQs (MIP(FQs)) is based on the electropolymerization approach. To further improve the performance of the MIP-based sensors, fMWCNTs that have high conductivity and AuNPs that have electrocatalytic properties were incorporated before the electropolymerization of MIP(FQs) films, as depicted in Scheme S1a. Upon synthesis of the different MIPs, those sensors were combined into a sensor array to achieve the simultaneous determination of the three FQs in the mixtures. (Scheme S1b).

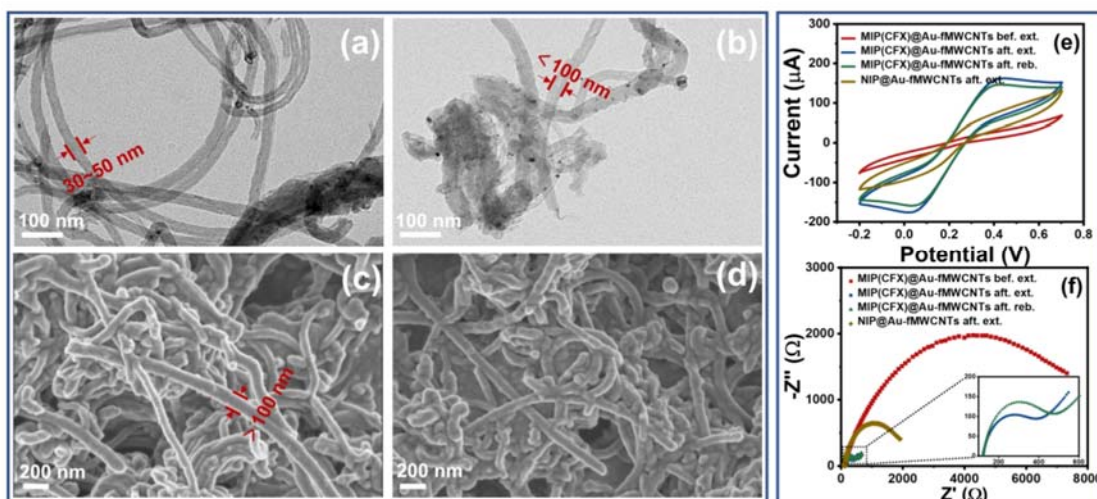


Figure 1. TEM images of (a) fMWCNTs and (b) Au-fMWCNTs. SEM images of MIP(CFX)@Au-fMWCNTs on GEC (c) before and (d) after the extraction of the template. Electrochemical characterization of the different steps involved from the preparation of the sensors to the actual sensing by means of (e) CV and (f) EIS employing a 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution in PBS: (from top to bottom) synthesis of the MIP towards CFX, before and after extraction of the template; MIP towards CFX upon rebinding; and NIP after extraction process

One of the key parameters for MIP-based sensors is the thickness of the MIP films, which is critical for the mass and electron transport. This was optimized by adjusting the number of cycles during the electropolymerization in the range from 5 to 35 cycles. As shown in Figure S3a, the current peak of MIP(CFX)@Au-fMWCNTs is higher when 10 cycles were selected, thick film cannot ensure an efficient electron and mass transport. Moreover, a sufficient number of specific sites also plays an important role in the specific recognition of the template by the MIP.

While a low concentration of the template might lead to a poor imprinting, a too large amount of the template is not good for the formation of the polymers. To this end, the molar ratio of Py to CFX in the electropolymerization solution was optimized (Figure S3b), from which a ratio of 5:1 was selected as the optimal.

Morphological characterization of MIP(FQs)@Au-fMWCNTs sensors.

Both SEM and TEM were used to characterize the morphology of the fabricated nanomaterials. Figure S4a corresponds to the SEM top view of the bare GEC electrode, showing a smooth and uniform surface, which is in contrast to the images after Au-fMWCNTs casting (Figure S4b) and electropolymerization (Figure S4c), undoubtedly confirming the modification of the electrode. As mentioned earlier, the combination of MWCNTs and AuNPs with MIP films allows to obtain a synergistic effect that results in an improvement of the sensor's performance. Figure 1a-b show the TEM images of fMWCNTs before and after its decoration with AuNPs, from which it can be seen that the diameter of the CNTs in both cases is certainly smaller than 100 nm. Furthermore, the surface of the CNTs seen in Figure 1a is smooth and without particles on them, while the ones in Figure 1b are clearly decorated with small particles on the surface, corresponding to the AuNPs.

Since different MIPs were obtained by introducing different FQs molecules during the electropolymerization process, the MIP(CFX) film grown onto Au-fMWCNTs will be presented as an example. The morphology of the MIP(CFX)@Au-fMWCNTs before and after the extraction of the template molecule was characterized by SEM, as shown in Figure 1c-d, respectively. From Figure 1c, it can be seen how the diameter of Au-fMWCNTs increased significantly due to the growth of the MIP film on the surface, as this is now larger than 100 nm. It is noteworthy that a thin and uniform MIP film grew along the surface of the Au-fMWCNTs instead of integrally aggregating as a top layer that covers the whole. A peculiar morphology is attributed to the electropolymerization method employed, which not only allows a better control over the distribution and thickness of the MIP film, but also facilitates the surface imprinting and the formation of a core-shell structure²⁷. Finally, from the comparison of Figure 1c-d, it can be seen how there are no significant morphological changes in the MIP(CFX)@Au-fMWCNTs structure after the extraction of the template and immersion in the EtOHNaOH mixture, confirming the stability of the developed nanocomposite material. Moreover, the SEM image of the NIP@Au-fMWCNTs (Figure S4c) also shows a similar morphology, confirming the expected similarities between the different synthesized MIP and NIP films.

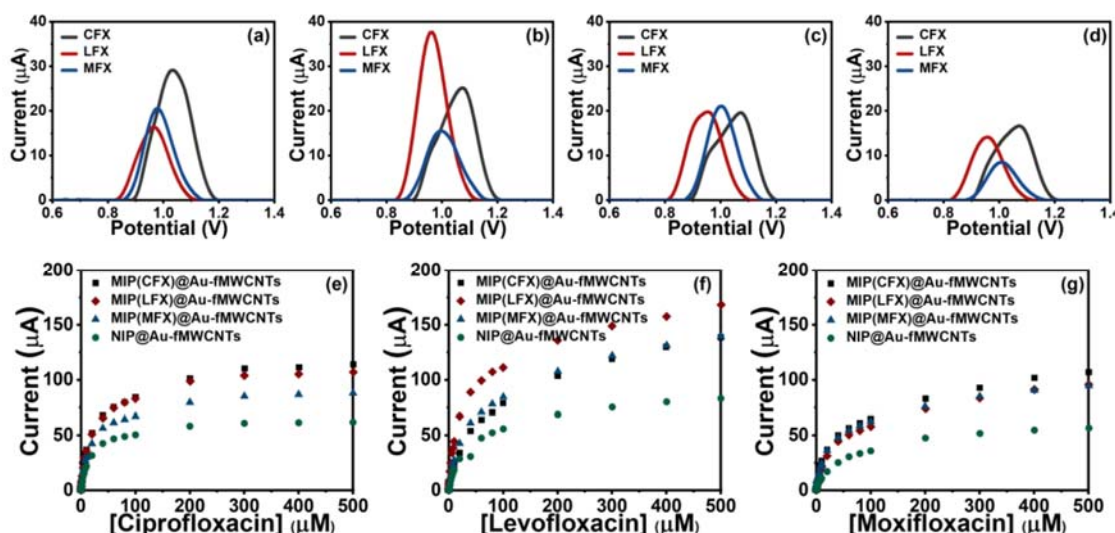


Figure 2. (a-d) Baseline corrected DPV curves of (a) MIP(CFX)@Au-fMWCNTs, (b) MIP(LFX)@Au-fMWCNTs, (c) MIP(MFX)@Au-fMWCNTs and (d) NIP@Au-fMWCNTs towards 10 μM solutions of: (black) CFX, (red) LFX and (blue) MFX. (e-g) Calibration plots of the three target FQs (e) CFX, (f) LFX and (g) MFX with the MIPs- and NIP-based sensors.

Electrochemical characterization of MIP(FQs)@Au-fMWCNTs sensors.

The electrochemical behavior of the sensors was investigated by CV and EIS in a 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ solution. Firstly, the response of the bare GEC was evaluated (Figure S5a), from which the reversible redox peaks of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ were observed. Next, this was compared to fMWCNTs and Au-fMWCNTs modified GEC, demonstrating that loading of fMWCNTs with AuNPs enhanced the electron transfer, which is reflected in more intense peaks in CV and a much lower charge transfer resistance in EIS. Subsequently, analogous comparison was conducted between the bare MIP and that incorporating Au-fMWCNTs (Figure S5c-d). As it can be seen, the MIP(CFX)@Au-fMWCNTs provides a better electrochemical performance than the MIP(CFX), but worse than Au-fMWCNTs (Figure S5c-d). As it can be seen, the MIP(CFX)@Au-fMWCNTs provides a better electrochemical performance than the MIP(CFX), but worse than Au-fMWCNTs (Figure 2 and S5) due to the poor electrical conductivity and blocked electron transport contributed by polymeric matrix. After the extraction of the template, we can observe a significant improvement of the electrochemical behavior both in the CV and EIS for the MIP because the sites created during the extraction process facilitate the electron and mass transport, while the NIP still showed a quite resistive behavior. Lastly, rebinding of these sites in the MIP film with the template was evaluated, and the sluggish diffusion of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ due to the "gate-controlled effect" is clearly reflected in the graph. Consequently, taking the above results from CV and EIS, the suitability of the developed MIP-based electrochemical sensor has been confirmed.

Optimization of the measuring conditions.

In order to enhance the voltammetric responses of the sensors, the measuring conditions of FQs were optimized. As previous, the response of the MIP(CFX)@Au-fMWCNTs sensor is shown as an example, taking the peak height from the DPV measurement towards a stock solution of CFX as the parameter to maximize. Firstly, the influence of the pH of the buffer was investigated in the range from 3.5 to 5.5 (Figure S6), obtaining that pH 4.5 is the most suitable.

Secondly, the incubation time was also optimized as an essential factor for the binding of the target analytes into the MIP film sites. Figure S7a shows how the response of the MIP-based sensor increases with the incubation time, until reaching a maximum at 300 s, indicating that the saturation has been reached. In addition, the time of the electrochemical treatment required for the sensor's regeneration after the sensing was also studied, observing that for the measurement of a 10 μ M CFX solution, 30 s is sufficient to recover the baseline (Figure S7b). However, to ensure that proper regeneration is achieved even when higher target concentrations are tested, 90 s is chosen as the regeneration time for further experiments.

Calibration of MIP(FQs)@Au-fMWCNTs to FQs.

One of the fundamental steps when developing sensors is the characterization of their response in terms of linearity, sensitivity, limit of detection (LOD), reproducibility, etc. Furthermore, when developing an ET, it is also important to assess the cross-responses towards the different target analytes. To this aim, solutions with increasing concentrations of different FQs were analyzed using the different sensors by DPV under the optimized conditions. An extract of the voltammetric responses is provided in Figure 2a-d (extracted from Figure S8a-d), where it can be seen how the three different MIP-based sensors show a voltammetric response to the three FQs (CFX, LFX and MFX respectively), and so does the NIP-based sensor. However, the differences in the peaks' position and height should be noted. As anticipated, the NIP@Au-fMWCNTs based sensor shows the lowest response to the three FQs compared with the other three MIP-based sensors due to the lack of recognition sites towards the FQs. Furthermore, when comparing the responses of specific MIP(FQ)@Au-fMWCNTs based sensor to the different FQs, it can be found that each MIP shows the highest response to its specific template (Figure 2). That is, MIP(CFX)@Au-fMWCNTs clearly shows the highest peak to CFX, while MIP(LFX)@Au-fMWCNTs does to LFX, confirming the specific recognition of the different MIP(FQ) films. The fact that MIP(MFX)@Au-fMWCNTs response to MFX is not as significantly higher, is attributed to the intrinsically higher electrochemical response of the other FQs, as is seen for the NIP. In this regard, it can be deduced that the similar structure and nature of FQs, all based on different substitutions made to the quinoline ring, hinders the training of highly specific MIP-based sensors, but leading to a class-selective material rather than a highly specific one; a situation, which is well suited for ET approaches, as the chemometrics can assist in improving its specificity. After preliminary assessing the responses of the different sensors to the different FQs, the proper calibration of each MIP(FQs)@Au-fMWCNTs and NIP@Au-fMWCNTs was carried out. From Figure S9, it can be seen how the peak currents increase steadily with increasing FQs' concentration. A hyperbolic response was observed as evidenced in Figure 2e-g, from which the dissociation constant (KD) and the

maximum binding response at saturation (B_{max}) were calculated, by fitting those to a one site saturation ligand binding model (Langmuir model). Furthermore, a logarithmic relationship between the concentrations and the corresponding peak heights was found for low concentrations (from which the limit of detection could be calculated). In all the cases, a good linear relationship for the three FQs is obtained, with R^2 values close to 1.

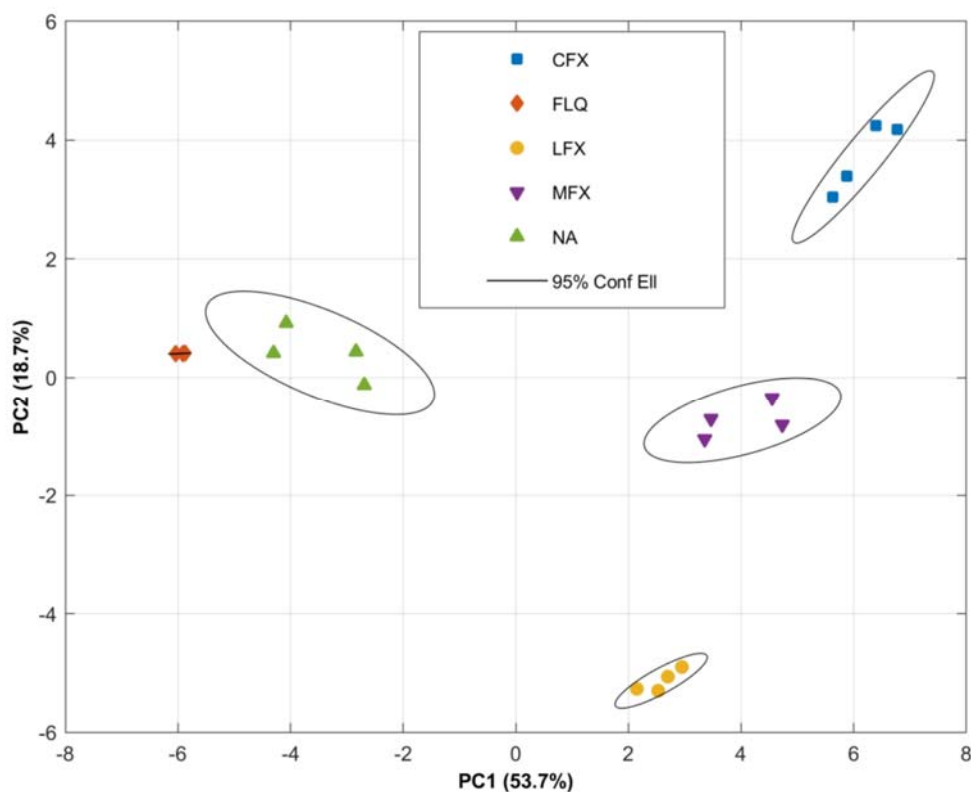


Figure 3. Scores plot obtained from the DCT-PCA of the voltametric responses of the MIP-based ET towards different FQs. Ellipses plotted correspond to 95% confidence limits for each of the clusters.

Qualitative analysis of FQs.

Despite MIP-based sensors are meant to be highly specific, those might still show certain cross-response when analogous molecules are analyzed; for instance, when analyzing the various FQs. Generally speaking, one of optional solution to the issue of deficient selectivity may be seeking to synthesize more advanced MIP-based materials. For example, in this case, deficient selectivity for FQs may be partially attributed to the use of electropolymerized Py instead of a more sophisticated polymerization method employing carefully screened monomers and cross-linkers. However, the truth is that similar behavior has also been reported with other polymerization strategies^{28,29}. In this direction, herein we propose the combination of chemometrics with MIPs as a straightforward solution to further improve its selectivity. Such an approach allows to shift the complexity from the chemistry to the data

processing side, while in turn, further advantages might also be obtained; e.g., being able to work with non-linear systems or to counterbalance possible matrix effects^{9,30}.

In order to demonstrate such statement, the analysis of stock solutions of four different FQs was carried out employing the three developed MIP- and the NIP-based sensors under the optimized conditions. Next, the voltammetric responses were compressed by means of DCT, from 114 points down to 12 coefficients with high fidelity as evidenced from Figure S10, and submitted to PCA (Figure 3). Firstly, it should be noted that ca. 72.4% of the original data variance is now represented by only those two first principal components (PCs), providing a better representation of samples' measurements that allows an easier assessment of its (dis)similarities. Secondly, we can see how different clusters corresponding to each of the FQs are, and how these clusters separate from each other. Consequently, the superb ability of ET to discriminate FQs being well denoted.

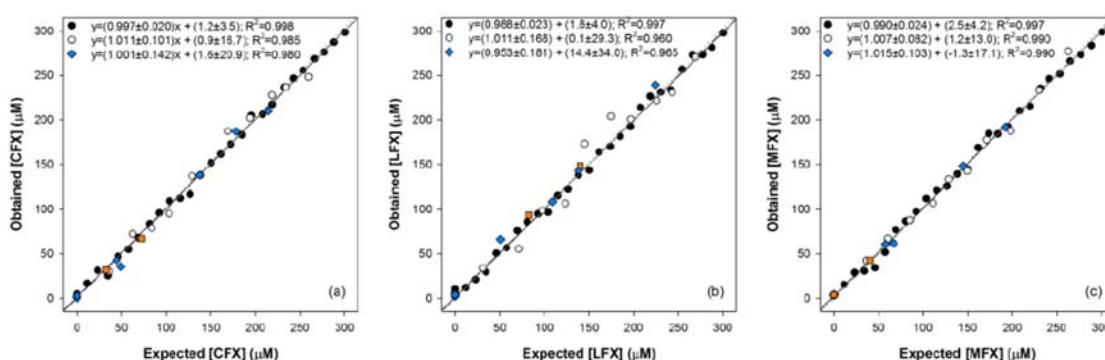


Figure 4. Modeling ability of the DCT-ANN. Comparison graphs of obtained vs. expected concentrations for (a) CFX, (b) LFX and (c) MFX, for both the training (●, solid line) and testing subsets (○, dotted line). Dashed line corresponds to the ideal comparison line ($y=x$). The results of the analysis of the pharmaceutical samples (◆) and spiked human urine samples (■) are also plotted.

Quantification of FQs.

In view of the above results, the quantification of mixtures of different FQs was attempted; concretely, mixtures of CFX, LFX and MFX. To this aim, the set of samples described in experimental section were analyzed, and as before, the obtained responses were compressed by means of DCT. In this case, ANNs were chosen for the data modelling, making use of the samples of the training subset to optimize the topology of the neural network, while those of the test subset were used to assess the model performance. In this manner, ensuring a more realistic assessment of the model performance as different data subsets are used for each step. After an iterative trial-and-error process, in which the different neural network parameters were tuned, the final architecture had 48 inputs neurons (12 coeffs. x 4 sensors) in the input layer, 7 neurons and logsig transfer function in the hidden layer and 3 neurons and tansig transfer function in the output layer (one for each of the FQs under study).

Subsequently, comparison graphs of predicted vs. expected concentrations were built, both for the training and testing subsets, to easily assess its performance (Figure 4). Moreover, the regression parameters were also calculated to numerically assess the goodness of the modelling. As can be observed from Figure 4, for each FQ, a good trend was obtained with regression lines close to the ideal ones ($y=x$, slope, intercept and correlation coefficient values of 1, 0 and 1, respectively). Consequently, confirming the potential of the approach not only to discriminate between different FQs (as shown previously with the PCA), but also to quantify them individually even in the mixtures. Finally, to further demonstrate the applicability of the proposed MIP-based ET, some pharmaceutical and human urine samples were analyzed. For both of them, no other sample pretreatment than its proper dilution to fit within the evaluated range of concentrations was carried out. As before, the corresponding readings of the sensors were compressed with DCT and interpolated into the built ANN model. Those are also plotted in Figure 4, where a good agreement between the expected and predicted values is also observed despite the relatively complex matrixes of the pharmaceutical tablets (excipients, fillers, disintegrants, binders, etc.) and urine (urea, inorganic salts, creatinine, uric acid, proteins, vitamin, etc.). Again, confirming the suitability and advantages derived from the combination of MIPs and ETs, as not only the lack of selectivity of MIPs has been addressed, but those were successfully applied to the quantification of FQs mixtures in real samples.

Conclusions

Herein, a sensitive ET based on different MIPs incorporating Au-NPs decorated MWCNTs has been developed for the analysis of FQs. In contrast to the conventional methods for MIPs' synthesis, in this work, MIPs films were electropolymerized directly on the surface of Au-fMWCNTs. The use of electropolymerization facilitates the integration of MIPs with voltammetric sensors, while the combination of CNTs large surface/mass ratio and high conductivity and the catalytic effect of AuNPs with the specific recognition of MIPs, leads to a synergistic effect that leads to an improved performance of the developed sensors.

The developed sensors were morphologically and electrochemically characterized to confirm the modification of the electrodes and assess their analytical responses. Despite the good performance obtained, the similar structure of the different FQs, hinders the obtaining of highly specific MIPs, leading to a class-selective material rather than a highly-specific one. In this direction, to improve its specificity, developed MIP-based sensors were combined into a sensor array to develop a MIP-based ET.

Thanks to the use of chemometrics, it was possible not only to discriminate between different FQs, but also to achieve the simultaneous quantification of ciprofloxacin, levofloxacin and moxifloxacin. Finally, the developed ET was applied to the analysis of pharmaceutical and biological samples, with satisfactory recovery values obtained, confirming the lack of matrix effects. Overall, the proposed approach represents an appealing and promising tool for the determination of FQs, but more importantly, paves the way for the development of similar applications. On the one side, electropolymerization represents a facile approach for the fabrication of MIP based sensors, while the integration of nanomaterials leads to an improved electrochemical behavior. On the other side, chemometric methods provide a straightforward approach to tackle MIPs cross-response.

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