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Application of spectroelectroanalysis for the quantitative determination of mixtures of compounds with highly overlapping signals

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Abstract

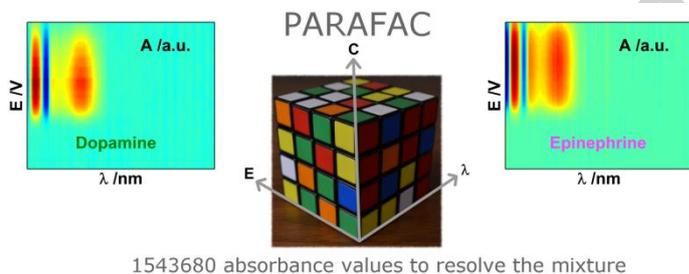
The amount of qualitative and quantitative information provided by a UV–vis absorption spectroelectrochemistry (SEC) experiment is sometimes wasted. However, almost all electrochemical and spectroscopic data can provide valuable information. In this spirit, the main objective proposed in this work is the quantitative resolution of catechol/dopamine (CAT/DA) and dopamine/epinephrine (DA/EP) mixtures, using spectroelectrochemical sensors in long optical path length arrangement based on bare optical fibers in parallel configuration with respect to carbon nanotubes or screen-

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printed electrodes. These compounds show extremely similar electrochemical and spectroscopic responses at high acidic pH, being impossible to determine their concentrations in the mixtures just using univariate regression models. To our knowledge, the SEC ability to resolve complex mixtures has never been demonstrated before with signals with this degree of overlapping. The quantitative analysis of these mixtures is possible using multivariate regression analysis of a set of time-resolved spectroelectrochemical data with a powerful statistical tool such as parallel factor analysis (PARAFAC). PARAFAC enables us to extract all the information from the experiments, allowing us to quantify the different analytes in mixtures of varying concentrations with excellent results. This milestone for spectroelectroanalysis illustrates the expected capabilities of SEC and demonstrates experimentally the potential of this technique for sensing of biomolecules.

Graphical abstract



Keywords: spectroelectrochemistry; carbon nanotubes; screen-printed electrodes; optical fibers; dopamine; epinephrine.

1. Introduction

Separately, electrochemistry and UV–vis absorption spectroscopy are not particularly selective techniques to identify the different organic compounds present in a complex mixture. Both electrochemical and spectroscopic signals commonly show broad peaks and bands, which often include information about more than one process or compound.

Nevertheless, it should be noted that finding two compounds that exhibit the same electrochemical behaviour and identical spectroscopic properties is practically impossible. For this reason, UV–vis absorption spectroelectrochemistry (SEC), the result of coupling electrochemistry and UV–vis absorption spectroscopy [1,2], should be a powerful hybrid technique for analysis.

UV–vis absorption SEC can be defined as a multi-response technique that enables the simultaneous monitoring of the electrochemical and the spectroscopic evolution of an electron-transfer process, all in a single experiment [3–8]. Therefore, signals of different nature are obtained at the same time, giving an overview about the changes that take place in solution, in the electrode surface, or in both of them, during the course of a reaction [9]. In accordance to the numerous advantages of UV–vis absorption SEC, the use of this technique, which has been established in multiple and diverse research fields [10], raises interest in the study of compounds of biological significance.

The use of UV–vis absorption SEC in the field of quantitative analysis is becoming increasingly important [11–21]. Anyway, when this technique is compared to other analytical techniques such as typical electrochemical, spectroscopic or chromatographic techniques, for example, there are very few papers dealing with quantitative analysis. Probably, SEC is not yet a benchmark reference in this field due to the difficulty of obtaining reproducible results with appropriate analytical quality until a few years ago. In the field of analytical chemistry, most researchers are developing new methods and applications for consolidated techniques. However, SEC still needs to demonstrate experimentally its extraordinary capabilities. Therefore, the development of novel techniques, devices and methodologies is necessary for its use in a near future for industrial, pharmaceutical or food analysis. Undoubtedly, the potential of the set of techniques on this topic could be much higher, especially if we take into account that the large amount of data obtained in a single UV–vis absorption SEC experiment is not always exploited. It is noteworthy that the electrochemical signal can be recorded almost continuously and that a full spectrum is often registered every tens or hundreds of milliseconds. Moreover, the spectra can be obtained in different optical configurations depending on the analytical problem. As the system evolves during the experiment, the high amount of electrochemical and spectroscopic data contains essential information, not only to explain the electron-transfer processes but also to determine the amount of the compounds present in a complex mixture. Thus, the

recording of both electrochemical and spectroscopic responses during an experiment, and the use of multivariate analysis such as partial least squares regression (PLSR) or parallel factor analysis (PARAFAC), can provide us with valuable information for quantitative purposes.

PARAFAC is a generalization of Principal Component Analysis (PCA) to higher order arrays. As for bilinear PCA, the outcome of a PARAFAC model can be used as input to other models, often for regression. PARAFAC has an important property not possessed by the two-way model: if the latent factors show adequately distinct patterns of three-way variation, the model is fully identified [22,23]. Herein, the quantitative resolution of mixtures by UV–vis absorption SEC using PARAFAC is based on the fact that SEC contains the trilinear character required [16,24].

The SEC devices used in the present work have been previously developed by our group [11,25]. They consist of two bare optical fibers, fixed in parallel configuration, that sample the solution adjacent to a single-walled carbon nanotubes (SWCNTs) electrode or a commercial carbon screen-printed electrode, allowing us to work with a high temporal/potential and spectral resolution and in semi-infinite diffusion regime. The use of optical fibers in SEC attracts great attention due to their multiple advantages to guide the light beam [17,26,27]. In the same way, carbon nanotubes present excellent properties, particularly interesting for electrochemistry [28,29] and SEC [3]. Meanwhile, SEC measurements are greatly simplified by using screen-printed electrodes.

As is known, catechol (CAT), dopamine (DA) and epinephrine (EP, also called adrenaline) are three compounds of high biological interest. CAT, and its dihydroxybenzene isomers resorcinol and hydroquinone, are commonly used in cosmetics, pesticides, flavouring agents or medicines; however, according to their toxicity and low degradability, they are considered pollutants [30]. Meanwhile, DA is a catecholamine neurotransmitter with key roles in cognition, attention, behaviour, movement and motivation [31]. Finally, EP is a neurotransmitter that can regulate smooth muscle contraction and relaxation in skin, lungs, and heart, and vasoconstriction in blood vessels, among other functions [32].

The study and determination of mixtures of catecholamines, such as DA and EP [33–35], and CAT [36–38], is interesting for the scientific community. Although their

biological importance is very high, the novelty of this paper is not just the use of these analytes. As shown below, they constitute model compounds to experimentally demonstrate the usefulness of SEC for quantitative analysis. It should be noted that the parallel configuration selected for this work is related to a higher sensitivity for soluble compounds than the normal arrangement due to the longer optical path length (in this case, the distance between the bare optical fibers). After the initial quantification of DA using univariate analysis, the main purpose of this work is the resolution of complex mixtures of compounds of biological interest, CAT/DA and DA/EP, without fixing the concentration of any of them and using PARAFAC as chemometric tool. The experimental acidic conditions have been intentionally selected for this work to illustrate and demonstrate experimentally that SEC can resolve quantitatively even mixtures that show an almost full overlap of both electrochemical and spectroscopic signals, greatly increasing the difficulty in the resolution of these mixtures and thus requiring a creative solution. To the best of our knowledge, UV–vis absorption SEC in long optical path length arrangement combined with PARAFAC is employed here for the first time, enabling researchers to resolve these complex mixtures without the need to perform any previous separation step.

2. Experimental

2.1. Reagents and materials

SWCNTs (Sigma-Aldrich), 1,2-dichloroethane (DCE, 99.8% for HPLC, Acros Organics), polytetrafluoroethylene membranes (filter pore size 0.1 μm , Millipore Omnipore), polyethylene terephthalate (PET, 175 μm thick, HiFi Industrial Film), silver conductive paint (Electrolube), transparent polish (Essence), and two bare optical fibers (100 μm in diameter, Ocean Optics, Inc.) were used to fabricate the UV–vis absorption SEC device [11] employed for the study of DA and for the resolution of the CAT/DA mixture. A commercial carbon screen-printed electrode (DRP-110, Metrohm DropSens), two bare optical fibers (100 μm in diameter, Ocean Optics, Inc.), and transparent polish (Essence) were used to make the device [25] employed for the resolution of the DA/EP mixture. Catechol (CAT, 99+%, Acros Organics), dopamine

(DA, dopamine hydrochloride, 99%, Acros Organics), epinephrine (EP, L(-)-Epinephrine, 99%, Acros Organics), and HCl (37%, VWR) were used to prepare the solutions.

All reagents were used as received without further purification. All chemicals were of analytical grade. Solutions were prepared using ultrapure water (18.2 M Ω cm resistivity at 25 °C, Milli-Q, Millipore). All handling and processing were performed carefully, particularly when DCE was used.

2.2. Instrumentation

For the study of DA and for the resolution of the CAT/DA mixture, the SEC setup included a potentiostat/galvanostat (PGSTAT302N, Metrohm Autolab, Utrecht, The Netherlands), a halogen-deuterium light source (AvaLight-DH-S-BAL, Avantes BV, Apeldoorn, The Netherlands), and a spectrometer (QE65000 198–1006 nm, Ocean Optics, Inc., Florida, USA). SWCNTs were dispersed in DCE using a tip-sonicator (CY-500, Optic ivymen System, Spain). Stencils were made of polymethyl methacrylate (Maniplastic, S.L., Burgos, Spain). A laboratory hydraulic press (SpectroPress, Chemplex Industries, Inc., Florida, USA) was also used to transfer the SWCNTs film on the PET support. For the DA/EP mixture, SEC was performed using a customized SPELEC instrument controlled by DropView SPELEC software (Metrohm DropSens, S.L., Llanera, Spain).

2.3. Fabrication of the UV–vis absorption SEC devices and experimental setup

The SEC sensor based on SWCNTs and bare optical fibers employed for the DA and for the resolution of the CAT/DA mixture was developed and validated in one of our previous works [11]. It presents a working and a counter electrode made of SWCNTs and a silver reference electrode, all of them flat on the PET support where the bare optical fibers are fixed (Fig. S1a). For the DA/EP mixture, and in order to demonstrate the availability of our methodology for any laboratory (Fig. S1b), the two bare optical fibers were directly attached with polish to a commercial carbon screen-printed electrode in parallel configuration [25]. In the two cases, the UV–vis light beam passes

parallel to the working electrode surface through the first 100 μm of the solution adjacent to the working electrode surface, collecting the spectral changes that take place in this part of the solution during the electrochemical reaction and allowing us to have a good reproducibility and to achieve successful SEC results in the field of quantitative resolution of complex mixtures.

In order to work in a semi-infinite diffusion regime, a solution drop of 200 μL (in the case of the SWCNTs electrode) or of 100 μL (in the case of the commercial carbon screen-printed electrode) was placed on the cell, covering the three electrodes and the ends of the bare optical fibers. The integration time for the spectrometer was 200 ms. The initial solution was taken as reference spectrum for every measurement.

Data analysis was performed using MATLAB and R. We must emphasize that, in order to resolve the mixtures, the measurements should be performed with a high temporal and spectral resolution, data features that are critical in achieving this objective. As will be seen below, in the examples selected to illustrate the performance of SEC in parallel configuration, the voltammograms are separated less than 10 mV and the spectra less than 5 nm. In most studies, spectra are registered at different pulse or fixed potentials, employing long times, or recording only one wavelength, so the optical evolution of the electrochemical process is not obtained with a high temporal and spectral resolution. Under those conditions, the resolution of the mixtures should be extremely difficult, almost impossible. Therefore, the spectroelectrochemical setup used in this work plays a key role to meet the objective proposed herein. In this way, it should be highlighted the importance of the screen-printed electrodes and the SPELEC instrument that make it as easy as possible the resolution of this type of mixtures for researchers not necessarily experts in the field of SEC.

More information about the fabrication of the SEC devices and the experimental setup can be found in Section S1.

3. Results and discussion

To the best of our knowledge, this work shows, for the first time, calibration curves, limits of detection [39–41], prediction of the concentration of the test samples and so on for the quantification of DA in these conditions of pH and SEC configuration (section

3.1). In sections 3.2 and 3.3, we demonstrate that, when high quality trilinear responses are obtained, random mixtures of the analytes (without fixing the concentration of any of them) can be easily resolved even when both the electrochemical and the spectroscopic signals are completely overlapped.

3.1. Determination of dopamine by potentiodynamic UV–vis absorption SEC

The oxidation of DA, as other catecholamines, is related to a complex two-electron two-proton electrochemical reaction; however, the oxidation of DA in this acidic medium (all experiments are performed in 1 M HCl) is much simpler because only dopaminequinone is electrogenerated [42,43]. A SEC experiment of 7.5×10^{-4} M DA in 1 M HCl between +0.30 and +0.80 V at 0.01 V s^{-1} is shown in Fig. 1. Fig. 1a represents the cyclic voltammogram, where the anodic and the cathodic peaks can be observed at +0.664 and +0.445 V, respectively. The 3D plot of the spectra evolution with time/potential recorded during the cyclic voltammetry is displayed in Fig. 1b, showing the evolution of three bands at 250, 283, and 391 nm. These bands are associated to the electrochemical oxidation of DA to generate dopaminequinone [7,27], particularly the band at 391 nm which is only related to this last compound [43,44]. Finally, the evolution of these three bands is shown in Fig. 1c. The corresponding cyclic voltabsorptograms indicate that, according to the cyclic voltammogram, the oxidation of DA takes place from +0.50 V onwards.

Besides information about the electrochemical reaction mechanism, SEC allows us to perform quantitative analysis. A set of calibration samples between 1×10^{-5} and 1×10^{-3} M was analysed. Cyclic voltammograms and cyclic voltabsorptograms at 250 nm are plotted in Fig. S2a and Fig. S2b, respectively. As can be observed, the higher the DA concentration, the higher the current intensity and the higher the absorbance. Two univariate calibration curves were obtained using ordinary least squares (OLS) models, one for the current intensity at the anodic peak potential and the other for the absorbance at 250 nm at +0.80 V, versus DA concentration (Fig. S2c and Fig. S2d, respectively). These parameters were selected with the aim of obtaining good sensitivities for these methods. Taking into account the concentration values equal or less than 1×10^{-4} M, the limits of detection obtained were 6 μM and 19 μM for the electrochemical and the spectroscopic calibration curves, respectively. The

autovalidated character of SEC was demonstrated by plotting the predicted concentrations obtained with the electrochemical calibration curve versus the predicted concentrations obtained with the spectroscopic calibration curve. A slope of 1.00 ± 0.02 and an intercept of $[-0.03 \pm 9.59] \times 10^{-6}$ were obtained, demonstrating that both responses determine the DA concentration without distinction. Finally, the DA concentration in a test sample 2.5×10^{-4} M was estimated using both calibration curves. The figures of merit obtained are shown in Table 1.

3.2. Quantitative resolution of mixtures of catechol and dopamine by SEC

As has been stated, extremely adverse conditions have been selected to show the potential of SEC for the quantitative resolution of mixtures. Fig. 2 illustrates the difficulty of resolving the mixture of CAT and DA in acidic media. This figure is related to solutions 5×10^{-4} M CAT or 5×10^{-4} M DA in 1 M HCl. First, Fig. 2a shows the cyclic voltammograms between +0.30 and +0.80 V at 0.01 V s^{-1} , where it can be observed that the difference between the anodic peak potentials of both compounds is only 0.008 V. Secondly, Fig. 2b represents the initial spectra (taking a 1 M HCl solution as reference spectrum), where the difference in the position of the spectral band is 4 nm, a really small value for UV-vis absorption spectroscopy. In third place, Fig. 2c displays the spectra (taking the initial solution as reference spectrum) at +0.80 V during the SEC experiments between +0.30 and +0.80 V at 0.01 V s^{-1} , where it can be observed that the maximum separation between the spectral bands is only 6 nm. Therefore, the overlap between the electrochemical and the spectroscopic responses of CAT and DA is remarkably high. In fact, Fig. S3 is analogous to Fig. 2 but using a solution mixture of 5×10^{-4} M CAT 5×10^{-4} M DA in 1 M HCl. As can be noticed, there seems to be a single compound. In conclusion, electrochemistry, spectroscopy, and SEC at fixed potentials are not able to resolve this mixture for themselves, even less using univariate regression. Thus, time-resolved UV-vis absorption SEC and powerful multivariate analytical tools are required to solve this issue. A first resolution of the CAT/DA mixture at $\text{pH} = 7$ was previously performed using a reflection probe in normal arrangement to obtain the optical response [16]; however, the overlap between the spectroscopic responses of CAT and DA at $\text{pH} = 7$ is much less pronounced than in the present case (pH around 0). Moreover, in the previous work a product was deposited on

the electrode surface and in the present case the oxidation product is soluble. Therefore, in that work, a new electrode was needed for each sample calibration, instead of the same electrode for the whole calibration procedure that is only required in the present work using, moreover, the long optical path length configuration which is the most sensitive optical arrangement for soluble compounds. These facts are important advantages of the present work with respect to the previous one.

Two major reasons led us to perform this work: (i) some analyses need to be performed in acidic media and (ii) the resolution of this mixture in acid solution is much more complicated due to the high overlap between the electrochemical and the spectroscopic responses of CAT and DA, allowing us to demonstrate all the advantages offered by UV-vis absorption SEC for solving complex problems like this one. It should be noted that the most remarkable feature of this technique is its ability to observe a physicochemical process simultaneously from two different points of view, recording kinetic information that can be analysed using multivariate statistical tools to obtain not only quantitative information but also a whole picture of the electrode process.

In this work, a full spectrum between 198 and 1006 nm is recorded every 200 ms during the whole electrochemical experiment, resulting in a huge amount of data. Thus, the selection of about 10 values to construct univariate calibration curves is enough for relatively simple problems, but, for complex systems such as the mixture of CAT and DA, it makes more sense to use the large amount of data obtained by SEC with the aim of addressing the problem and providing a solution. As shown below, multivariate analysis using PARAFAC gives good results.

For this purpose, a three-dimensional data matrix containing the absorbance values between +0.50 and +0.80 V and between 225 and 600 nm for the 16 experiments with different concentrations of CAT and DA was constructed to develop the PARAFAC model. Table S1 lists the experiments conducted to accomplish this goal. As can be seen, the set of calibration samples includes concentrations between 1×10^{-5} and 1×10^{-3} M for both CAT and DA. Potentiodynamic measurements were selected, instead of potentiostatic experiments, attempting to obtain a better resolution in terms of potential/time dependency. Therefore, SEC experiments were carried out in 1 M HCl between +0.30 and +0.80 V at 0.01 V s^{-1} . For PARAFAC analysis, data in a limited potential window (absorbance values between +0.50 and +0.80 V) and in a limited

range of wavelengths (absorbance values between 225 and 600 nm) were selected according to the regions where the significant changes of absorbance take place, avoiding potential and wavelength regions without analytical information that would only introduce noise to the mathematical model. It should be highlighted that the matrix dimensions (151 potentials \times 473 wavelengths \times 16 concentrations) indicate that up to 1142768 values of absorbance were considered to build the PARAFAC model, selecting two components according to the two compounds present in the mixture (CAT and DA), a convergence criterion of 1×10^{-25} , no constraint and no scaling. The outputs of the PARAFAC model were the three factors (dimensions of 151×2 , 473×2 , and 16×2) shown in Fig. 3, obtained after 299 iterations and with a corcondia value of 100%.

Loadings and scores of CAT and DA obtained from PARAFAC are plotted in Fig. 3. Fig. 3a represents the loadings with respect to potential. As expected, the loadings with respect to the wavelengths (Fig. 3b) show a very similar behaviour with respect to the spectra displayed in Fig. 2c. The most striking results are shown in Fig. 3c and Fig. 3d, where the scores of CAT and DA corresponding to the concentration of CAT and DA, respectively, are plotted. Clearly, the higher the CAT and the DA concentrations, the higher the scores obtained from PARAFAC for each molecule. The coefficients of determination of the scores versus CAT and DA concentrations obtained from the raw data using OLS models (see Fig. S4) demonstrate the complete deconvolution of the mixture with respect to each analyte.

At first sight, it seems that UV-vis absorption SEC enables us to perform quantitative analysis using the scores obtained from PARAFAC. In order to construct the calibration curves of CAT and DA, two test samples (see Table S1), which were obviously included to build the PARAFAC model to obtain their corresponding scores, were excluded. Afterwards, detection of outliers was performed with least median of squares (LMS) regression. Fig. S5 represents the two calibration curves of scores of CAT and DA versus CAT and DA concentration, respectively, obtained using OLS regression models after removal of outliers. The detection limits obtained were 46 μM and 41 μM for the determination of CAT and DA, respectively. Obviously, this limit of detection is higher than the obtained for the DA alone due to the complexity of the mixture that makes more difficult to achieve a lower value. The last step was the evaluation of the prediction capability of these calibration curves. For this purpose, the concentrations of CAT and DA of two very different test samples of 2.2×10^{-4} M CAT 3.4×10^{-4} M DA

and 1.2×10^{-4} M CAT 9×10^{-5} M DA were estimated, obtaining satisfactory values for all the predicted concentrations. All data are listed in Table 2, where the figures of merit obtained can be observed.

3.3. Quantitative resolution of mixtures of dopamine and epinephrine by SEC

The successful procedure shown in the CAT/DA system was applied in a similar way to the resolution of the DA/EP mixture. To demonstrate that these experiments can be easily done in any laboratory, the device based on a carbon screen-printed electrode was used, with an optical path length around 0.13 cm. Table S2 indicates the experiments carried out. For a good understanding of the resolution of the DA/EP mixture, Figures S6 to S10 are analogous to Fig. 2, Fig. S3, Fig. 3, Fig. S4 and Fig. S5, respectively. The set of calibration samples includes concentrations between 1×10^{-5} and 1×10^{-3} M for both DA and EP. SEC measurements were carried out in 1 M HCl between +0.30 and +0.80 V at 0.01 V s^{-1} . For PARAFAC analysis, a three-dimensional data matrix was constructed containing the absorbance values in a limited potential window (between +0.50 V of the forward scan and +0.70 V of the backward) and in a limited range of wavelengths (between 225 and 600 nm) for the 16 experiments with different concentrations of DA and EP. The matrix dimensions ($201 \text{ potentials} \times 480 \text{ wavelengths} \times 16 \text{ concentrations}$) indicate that 1543680 absorbance values were considered to build the PARAFAC model, selecting two components according to the two compounds present in the mixture (DA and EP), a convergence criterion of 1×10^{-25} , no constraint and no scaling. The outputs of the PARAFAC model were the three factors (dimensions of 201×2 , 480×2 , and 16×2) obtained after 291 iterations and with a corcondia value of 100%. Limits of detection for determination of DA and EP were 121 μM and 216 μM , respectively. To evaluate the prediction capability, the concentrations of DA and EP of a test sample of 2.2×10^{-4} M DA 3.4×10^{-4} M EP were estimated. All data are listed in Table 3, summarizing the figures of merit obtained.

It should be noted that, although there are many works related to the quantification of an analyte in presence of interfering species (usually at constant concentration when a separation technique is not used), our work shows the importance of SEC to simultaneously determine the concentration of all compounds (even of the interfering

species) present in mixtures of varying concentrations, bringing quantitative analysis a step closer to reality.

4. Conclusions and future perspectives

SEC is very useful if compounds (i) are oxidized at different potentials and/or (ii) absorb electromagnetic radiation at different wavelengths, but also when all signals are highly overlapped, as is demonstrated experimentally in this work. The capabilities of resolving mixtures using SEC have been mentioned many times; however, to the best of our knowledge, they have never been experimentally demonstrated before with signals with this high degree of overlapping where the highest reproducibility of the measurements is required. Therefore, the new and most important concept that we show in this paper is the combined use of PARAFAC and UV–vis absorption SEC in parallel arrangement to quantitatively determine the concentration of different analytes in mixtures (CAT/DA and DA/EP) of varying concentrations of both molecules and at acidic pH, which is related to a high overlap of the electrical and optical signals.

Our spectroelectrochemical sensors provide the high-quality and reproducible responses required. Bare optical fibers in long optical path length configuration with SWCNTs electrodes or carbon screen-printed electrodes offer an excellent potential/temporal and spectral resolution, allowing disposability, versatility, fast analysis times, low cost and simple measurements and requiring a very small volume of solution. Although obtaining low detection limits was not the objective of the present work, we are aware that the values obtained here are still not comparable to others reported in literature using other techniques. However, this is just due to technical reasons and should be solved in the future with the development and use of better light sources, monochromators and detectors and the related improvement of the signal-to-noise ratio. Detection limits are expected to be improved.

In summary, this paper constitutes the basis for future works. It is devoted to showing the methodology, the path for other researchers to develop real spectroelectroanalysis methods. Moreover, it experimentally demonstrates the advantages of UV–vis absorption SEC and the promising possibilities of obtaining not only the understanding of electrochemical processes but also quantitative information for the analysis of

complex mixtures without the need to separate their components. In our opinion, it represents definitively a breakthrough for the SEC to receive the attention it deserves in quantitative analysis. This work, accompanied by the fact that companies have begun to commercialize SEC instruments during the last years, opens interesting perspectives for spectroelectroanalysis.

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Appendix A. Supplementary information

Supplementary information associated with this article is available.

Competing interests statement

The authors have no competing interests to declare.

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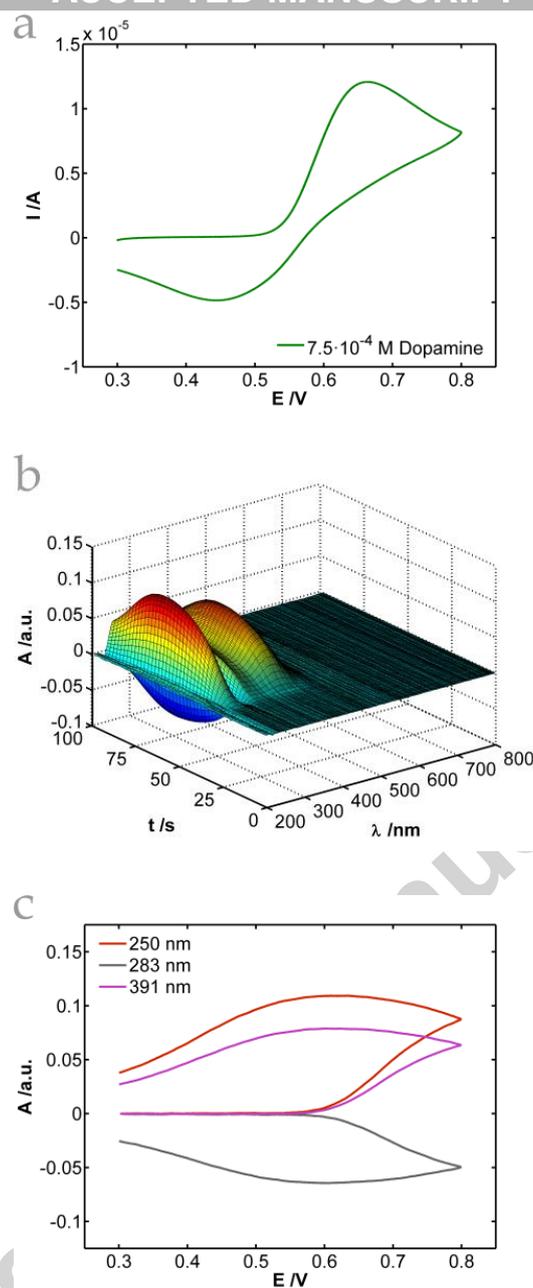


Fig. 1. SEC experiment of 7.5×10^{-4} M DA in 1 M HCl between +0.30 and +0.80 V at a potential scan rate of 0.010 V s^{-1} . (a) Cyclic voltammogram, (b) 3D plot of the spectra evolution with time/potential recorded concomitantly with the cyclic voltammogram, and (c) cyclic voltabsorptograms at 250, 283, and 391 nm.

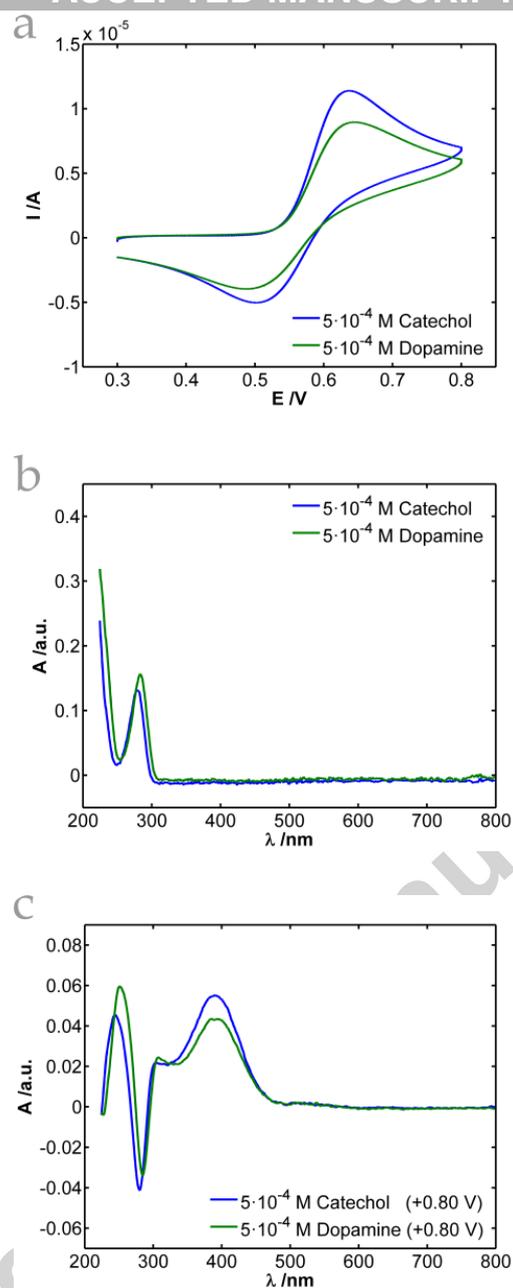


Fig. 2. This figure is related to solutions 5×10^{-4} M CAT or 5×10^{-4} M DA in 1 M HCl. (a) Cyclic voltammograms between +0.30 and +0.80 V at 0.01 V s^{-1} . (b) Initial spectra (taking a 1 M HCl solution as reference spectrum). (c) Spectra (taking the initial solution as reference spectrum) at +0.80 V during the SEC experiments between +0.30 and +0.80 V at 0.01 V s^{-1} .

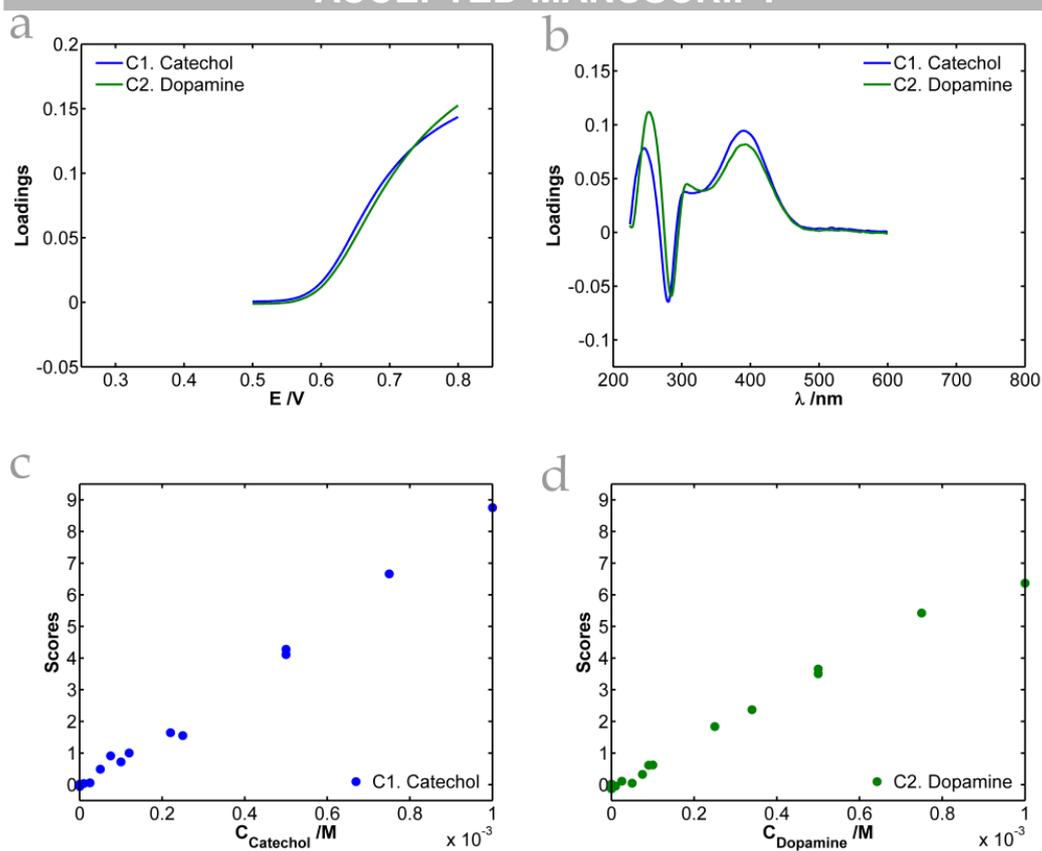


Fig. 3. (a) Loadings of CAT and DA with respect to potential. (b) Loadings of CAT and DA with respect to the wavelengths. Raw scores of (c) CAT and (d) DA with respect to the concentration of CAT and DA, respectively.

Table 1. Regression parameters obtained for the determination of DA in 1 M HCl in the $1 \times 10^{-5} - 1 \times 10^{-3}$ M concentration range and concentration estimated from univariate (OLS) calibration curves for a test sample of DA.

$C_{\text{Dopamine}} = 2.5 \times 10^{-4}$ M				
Analysis Method	R^2	S_{yx}	CI (M)	%RSD
I_{ap}	0.99996	3.9×10^{-8}	$[2.52 \pm 0.06] \times 10^{-4}$	1.03
$A_{250 \text{ nm}, +0.80 \text{ V}}$	0.9992	1.4×10^{-3}	$[2.56 \pm 0.29] \times 10^{-4}$	4.81

Analysis methods correlate current intensity at the anodic peak (I_{ap}) and absorbance at 250 nm and at +0.80 V ($A_{250 \text{ nm}, +0.80 \text{ V}}$) for potentiodynamic SEC measurements, versus DA concentration. R^2 , coefficient of determination; S_{yx} , residual standard deviation; C_{Dopamine} , concentration of dopamine in the test sample; CI, confidence interval; and %RSD, relative standard deviation ($n = 9$, $\alpha = 0.05$).

Table 2. Regression parameters of the calibration curves constructed after PARAFAC analysis (Fig. S5) for the determination of CAT and DA in 1 M HCl by UV-vis absorption SEC in the concentration ranges of $1 \times 10^{-5} - 1 \times 10^{-3}$ M and $1 \times 10^{-5} - 7.5 \times 10^{-4}$ M, respectively, and estimated concentrations for two test samples.

		$C_{\text{Catechol}} = 2.2 \times 10^{-4}$ M			$C_{\text{Catechol}} = 1.2 \times 10^{-4}$ M	
		$C_{\text{Dopamine}} = 3.4 \times 10^{-4}$ M			$C_{\text{Dopamine}} = 9 \times 10^{-5}$ M	
C	R ²	S _{yx}	CI (M)	%RSD	CI (M)	%RSD
C1	0.9989	1.1×10^{-1}	$[1.94 \pm 0.33] \times 10^{-4}$	6.88	$[1.21 \pm 0.33] \times 10^{-4}$	11.12
C2	0.9985	7.8×10^{-2}	$[3.34 \pm 0.26] \times 10^{-4}$	3.22	$[9.4 \pm 2.6] \times 10^{-5}$	11.39

C1, related to catechol, and C2, related to dopamine, correlate the scores of catechol and dopamine versus CAT and DA concentration, respectively. C, component; R², coefficient of determination; S_{yx}, residual standard deviation; C_{Catechol}, concentration of catechol in the test sample; C_{Dopamine}, concentration of dopamine in the test sample; CI, confidence interval; and %RSD, relative standard deviation (n = 12, α = 0.05).

Table 3. Regression parameters of the calibration curves constructed after PARAFAC analysis (Fig. S10) for the determination of DA and EP in 1 M HCl by UV-vis absorption SEC in the concentration range of $1 \times 10^{-5} - 1 \times 10^{-3}$ M and estimated concentrations for a test sample.

$C_{\text{Dopamine}} = 2.2 \times 10^{-4}$ M				
$C_{\text{Epinephrine}} = 3.4 \times 10^{-4}$ M				
C	R ²	S _{yx}	CI (M)	%RSD
C1	0.9906	3.0×10^{-1}	$[2.16 \pm 0.71] \times 10^{-4}$	13.94
C2	0.9735	3.4×10^{-1}	$[2.98 \pm 1.01] \times 10^{-4}$	14.64

C1, related to dopamine, and C2, related to epinephrine, correlate the scores of dopamine and epinephrine versus DA and EP concentration, respectively. C, component; R², coefficient of determination; S_{yx}, residual standard deviation; C_{Dopamine}, concentration of dopamine in the test sample; C_{Epinephrine}, concentration of epinephrine in the test sample; CI, confidence interval; and %RSD, relative standard deviation (n = 12 (Dopamine), n = 13 (Epinephrine), $\alpha = 0.05$).

Highlights

- Homemade carbon nanotube electrodes or commercial screen-printed electrodes are used.
- Bare optical fibers in parallel configuration offer excellent quantitative results.
- Catechol/dopamine and dopamine/epinephrine mixtures in varying amounts are resolved.
- These compounds show very similar electrochemical and optical signals at acidic pH.
- Spectroelectrochemistry combined with PARAFAC is very useful for analysis.

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