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Impact of normalization, standardization and pre-fit on the success rate of fitting in electrochemical impedance spectroscopy

Abstract: Fitting the data of an electrochemical impedance spectroscopy (EIS) typically requires manual estimation of the initial values before regression algorithms such as complex nonlinear least squares (CNLS) can be applied. This makes the success rate of the fitting dependent on the user input. Furthermore, the Randles circuit consists of parameters with substantially differing magnitudes (e.g. capacitors and resistors), which can also strongly affect the success rate of the fitting due to numerical effects. The aim of this work is to investigate methods addressing the described limitations of fitting. Therefore, a Python implementation performing a fit for EIS is benchmarked with an equivalent open source library. The examined implementation optionally includes the normalization of the parameter values, the standardization of the impedances and a pre-fit. Applying the same equivalent circuit without additional signal processing steps and with fixed initial values defined by midpoints of the value ranges, both implementations were able to fit 46.50% of the simulated database with different spectra. Applying the normalization of the parameter values (76.25%) or the same method with additional pre-fit (97.75%) lead to a significant improvement of the success rate. The standardization of impedances did not affect the success rate.

Keywords: Electrochemical Impedance Spectroscopy, EIS, Complex Nonlinear Least Squares, CNLS, Pre-fit, Randles circuit, fitting

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1 Introduction

Electrochemical impedance spectroscopy (EIS) is a method allowing chemical and physical phenomena on the biosensor surface to be examined separately in the context of biorecognition processes. One major advantage of EIS is the potential of label-free detection, avoiding the need of modifying biomolecules as part of a sample preparation. For the analysis of EIS data and the characterization of the biosensing surface, the acquired spectra are usually fitted using equivalent circuits like the Randles circuit. For analytical studies, certain fitted parameter values (resistors or capacitors) can be used as a function of a solution composition in order to determine its concentration [1]. For example, the charge transfer resistance R_{ct} is utilized for binding processes on functionalized sensor surfaces and thus for the determination of analyte concentrations [2]. Before fitting, initial estimations for the parameters to be fitted must be typically provided manually before applying a complex nonlinear least squares (CNLS) algorithm. This makes the success rate of the fitting dependent on the user input, since there is the possibility to get stuck in a local optimum. In contrast, fixed starting values may lead to limited fitting performance. Furthermore, the input of manually determined start values is unsuitable for systems with automatic evaluation of the EIS data, if a wide range of parameter values can occur. Another peculiarity is that the parameters of the equivalent circuit have enormous differences in magnitude, since resistors and capacitors are used. This in turn can lead to numerical effects that influence the success rate of a fit.

The fitting of the EIS data can in principle be performed by commercial software, but these are usually not freely available and can only be integrated into own implementations (hardware control, interpretation of the data, etc.) to a limited extent, since they are proprietary and do not provide an application programming interface (API). In addition, there are open-source community-driven solutions, such as the

Python package *impedance.py* [3], which do not suffer from the stated problems.

This work addresses the impact of additional signal processing steps to achieve higher success rates in the application of fitting algorithms. Here, the normalization of the parameter values, the standardization of the impedances and the pre-fit algorithm are investigated. These three methods are part of a custom implemented Python library called *EIS:CRéME* (Electrochemical Impedance Spectroscopy: Complex nonlinear least squares Regression for Modelling Equivalent circuits), which was compared with the Python package *impedance.py*. For this purpose, 400 spectra with different parameterizations of the Randles circuit representative for biosensors were simulated and evaluated with the mentioned approaches.

2 Materials and Methods

The following chapter describes the fitting methods implemented by *impedance.py* and *EIS:CRéME*, the simulation of the EIS data set and the evaluation methods.

2.1 Fitting methods

Figure 1: Basic procedure of a complete fitting method by *EIS:CRéME*. If individual methods are not used (standardization, normalization, pre-fit), the corresponding blocks are deleted.

The open source Python package *impedance.py* (release 1.3.0) ensures the analyzation of EIS data [3] by using CNLS regression. Here, any equivalent circuit can be used to fit data points via the Levenberg-Marquardt algorithm, which is provided by the function *scipy.optimize.curve_fit* [4]. The package *impedance.py* is used to benchmark the implemented environment *EIS:CRéME*. Therefore, optional Basin-hopping was not applied as part of *impedance.py*.

EIS:CRéME is also based on *scipy.optimize.curve_fit*, but allows additional methods to increase the success rate of fitting, especially for the use of the Randles circuit for electrochemical biosensors. These methods include normalization of parameter values, standardization of impedances and pre-fit. Further details to the described methods can be found in [5]. The procedure of a fit using *EIS:CRéME* with all methods enabled is shown in Figure 1.

When normalizing the parameter values, the parameters $\beta = \gamma \cdot 10^\varepsilon$ of the equivalent circuit are divided into a factor γ and an exponent ε in order to align the magnitudes of the parameter values. Subsequently, $x = \gamma$ or ε can be scaled to the same range of values $\{x_{scaled} | t_1 \leq x_{scaled} \leq t_2\}$ using

$$x_{scaled} = t_1 + \frac{x - \min(x)}{\max(x) - \min(x)} \cdot (t_2 - t_1) \quad (1),$$

where $\max(x)$ and $\min(x)$ are the assumed limits of the fitting, respectively (see Table 1). When standardizing impedances, both the real and imaginary parts of the impedance are used. Due to the standardization by means of z-score, real and imaginary parts have the same influence on the error calculation.

The basic idea of the pre-fit is to perform a fit with a RC network as an equivalent circuit before the actual fit is executed. Subsequently, the individual RC elements can be assigned to the components of the Randles circuit based on their time constant in order to perform an initial value estimation.

In the following, the index is used to indicate which methods were included for *EIS:CRéME*: N – normalization of parameters, S – standardization of impedances and P – pre-fit.

2.2 Simulation of EIS data

To evaluate and compare the different approaches, 400 artificial spectra based on Randles Circuit were simulated. Therefore, the following components were applied: the electrolyte resistance R_s , the charge transfer resistance R_{ct} , the constant phase element (CPE) and the Warburg impedance Z_W . The impedance Z_{CPE} of the CPE and Z_W are defined by

$$Z_{CPE}(f) = \frac{1}{Q(i2\pi f)^n} \quad (2)$$

$$Z_W(f) = \frac{1}{Q_W \sqrt{i2\pi f}} \quad (3).$$

Here, f is the frequency, Q is the pre-factor and n is the exponent of the CPE. Eq 3 can be assumed for theoretical electrodes with an infinitely large area and thus unlimited diffusion. Empirical conditions have been inserted which must be fulfilled in order to simulate the data. This is necessary

because otherwise cases can occur where Z_W dominates the spectrum and thus no meaningful spectra for the application of biosensors can be ensured. These conditions include that (I) $R_s < R_{ct}$, (II) the Nyquist impedance curve has geometrically two extrema and (III) $\lim_{f \rightarrow \infty} \text{Im}(z) \approx 0$. The resulting ranges of the parameters are shown in Table 1. In addition, Figure 2 shows the distribution of the parameter values.

Figure 2: Value distribution of the Randles circuit parameters by the simulation with specified conditions. The exponent of CPE n is not shown due to the limited representation with logarithmic y-axis scaling. The labeling of the y-axis varies for different violin plots.

Table 1: Ranges of simulated parameter values of the Randles circuit. A distinction is made between the target range of the individual parameters, the actual approximate range after compliance with the conditions and the limits of the fitting methods.

Parameter	Target	Actual	Fit limits
$R_s [\Omega]$	$[10^1:10^{10}]$	$[10^2:10^{10}]$	$[10^1:10^{11}]$
$R_{ct} [\Omega]$	$[10^1:10^{10}]$	$[10^3:10^{10}]$	$[10^1:10^{11}]$
$Q_w [S \cdot \sqrt{s}]$	$[10^{-14}:10^{-3}]$	$[10^{-12}:10^{-3}]$	$[10^{-13}:10^{-2}]$
$Q [S \cdot s^n]$	$[10^{-14}:10^{-4}]$	$[10^{-13}:10^{-4}]$	$[10^{-14}:10^{-3}]$
n	$[0:1]$	$[0:1]$	$[0:1]$

2.3 Evaluation of the success rate

All 400 spectra were fitted using the specified methods including *impedance.py* on the one hand and *EIS:CReme* with different combination of normalization, standardization and pre-fit on the other hand.

For each method, the success rate was determined, with an unsuccessful fit meeting at least one of the following characteristics: (I) Within $1E+05$ iterations, the tolerance of the cost function termination $f_{tol} = 1E-09$ cannot be undercut, which means that the difference of the cost function between two successive iterations is still high at the maximum number of iterations. (II) The calculated value for R_{ct} as an important parameter in the EIS has an error greater than 10% compared to the target value.

Furthermore, the median of the root mean square relative error (RMSRE), the Chi-squared test χ^2 and the number of required iterations was recorded for each method. Using R_{ct} as an example, the resulting distribution of relative errors (RE) with respect to the value determination of the parameters was investigated.

3 Results

The obtained results of the applied methods are shown in Table 2. It should be noted that RMSRE and χ^2 are very small, since only ideal spectra are examined. The results indicate that *impedance.py* and *EIS:CReme* give almost identical results, which is to be expected since both use the same Python method for fitting. Standardization of impedances shows no significant improvement in neither success rate, RMSRE nor χ^2 . In contrast, the normalization of the parameter values resulted in a significant improvement of the success rate, the RMSRE and χ^2 . However, this requires more complex calculations due to the increased number of parameters to be adjusted. Although the pre-fit approach can improve the success rate, it has no effect on the goodness of fit. A combination of normalization of the parameters and pre-fit lead to the best result. Here, a high accuracy is achieved by the normalization and, in addition, the success rate is significantly improved by the combination of both methods. In the present case just 2.25% of all spectra could not be fitted. However, the combined use of normalization and pre-fit leads to significant increase in computational effort, as indicated by the median of iterations required by a factor of 7 between *EIS:CReme* and *EIS:CReme_{NP}*. Also for the latter combination of methods, no improvement can be observed by adding the standardization of impedances.

Table 2: Results of the methods for fitting 400 simulated spectra. RMSRE and χ^2 are calculated for the successfully fitted spectra.

Parameter	Success rate [%]	Iterations	RMSRE	χ^2
impedance.py	46.50	80	1.2E-08	3.0E-16
EIS:CReme	46.50	80	1.2E-08	8.4E-16
EIS:CReme _S	45.75	78	1.2E-08	8.6E-15
EIS:CReme _N	76.25	171	1.6E-15	2.1E-23
EIS:CReme _P	65.50	157	2.3E-08	1.8E-16
EIS:CReme _{SN}	76.55	159	2.6E-13	1.1E-22
EIS:CReme _{NP}	97.75	556	2.0E-15	1.1E-23
EIS:CReme _{SNP}	97.75	526	1.6E-13	4.4E-23

To investigate the accuracies of the parameter values obtained from the fit, R_{ct} was used as an example to show the distribution of $RE_{R_{ct}}$ in Figure 3. For *impedance.py* and *EIS:CReme*, the median error was about $1E-10$ with a wide variance and outliers. By using parameter value normalization, the accuracy was significantly improved, resulting in a median of $1E-15$. The pre-fit has no effect on this result. The additional use of impedance standardization leads to a wider distribution of errors with a higher median ($1E-14$).

Figure 3: Relative error ER for the determination of the parameter R_{ct} using various methods.

4 Discussion

The present work investigates the influence of the normalization of the parameter values, the standardization of the impedances and the pre-fit on the success rate, the goodness of fitting and the error of the parameter calculation. For this purpose, the basic implementation of the fit of *EIS:CReme* was benchmarked with the open-source Python package *impedance.py*. Both implementations achieved similar results for all analyses.

The normalization of the parameters leads to a significant improvement of the success rate. Furthermore, RMSRE and χ^2 of the fit are also significantly improved as well as the relative errors $RE_{R_{ct}}$. As expected, the pre-fit improves the success rate, but has only little effect on the errors of the fit. The standardization of the impedances could not achieve any significant advantage, what is most likely due to too small differences between real and imaginary parts of the impedances for the investigated spectra. The most promising approach is the combination of normalization of parameter values and pre-fit. Here, a success rate of 97.75% could be achieved, with concurrent high accuracies ($\chi^2 = 1.1E-23$).

However, an increased computational effort must be accepted for this improvement. On the one hand, the normalization of the parameter values almost doubles the number of parameters (n is not split into γ and ϵ), making the fit more complex. On the other hand, the pre-fit is almost as computationally expensive as the fit itself.

To simulate the spectra, empiric conditions were used to obtain typical Randles circuits. The condition that geometrically two extrema must be present in the Nyquist plot prevents the Z_W from dominating the frequency response. However, this also has the disadvantage of discarding spectra where the Z_W initiates at the apex of the semicircle.

In addition, an ideal Z_W was assumed for all studies. For real systems, where Z_W with finite space or finite length can occur, other transfer functions have to be considered.

For further evaluation of the methods, real spectra must be used to investigate the influence of measurement errors, nonlinearities or drifts on the fitting. The methods presented have been studied only with respect to the Randles circuit, since it is commonly used for electrochemical biosensors. To address further applications of EIS, other equivalent circuits should be considered in future work. However, the pre-fit is not transferable to all equivalent circuits, e.g. once an inductance is included.

A tolerance of 10% was used for the determination of R_{ct} . This value was chosen exemplarily and must be adapted to specific applications. However, Figure 3 shows that the relative improvements of the success rate by the presented methods would be even higher when using a lower tolerance.

Author Statement

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