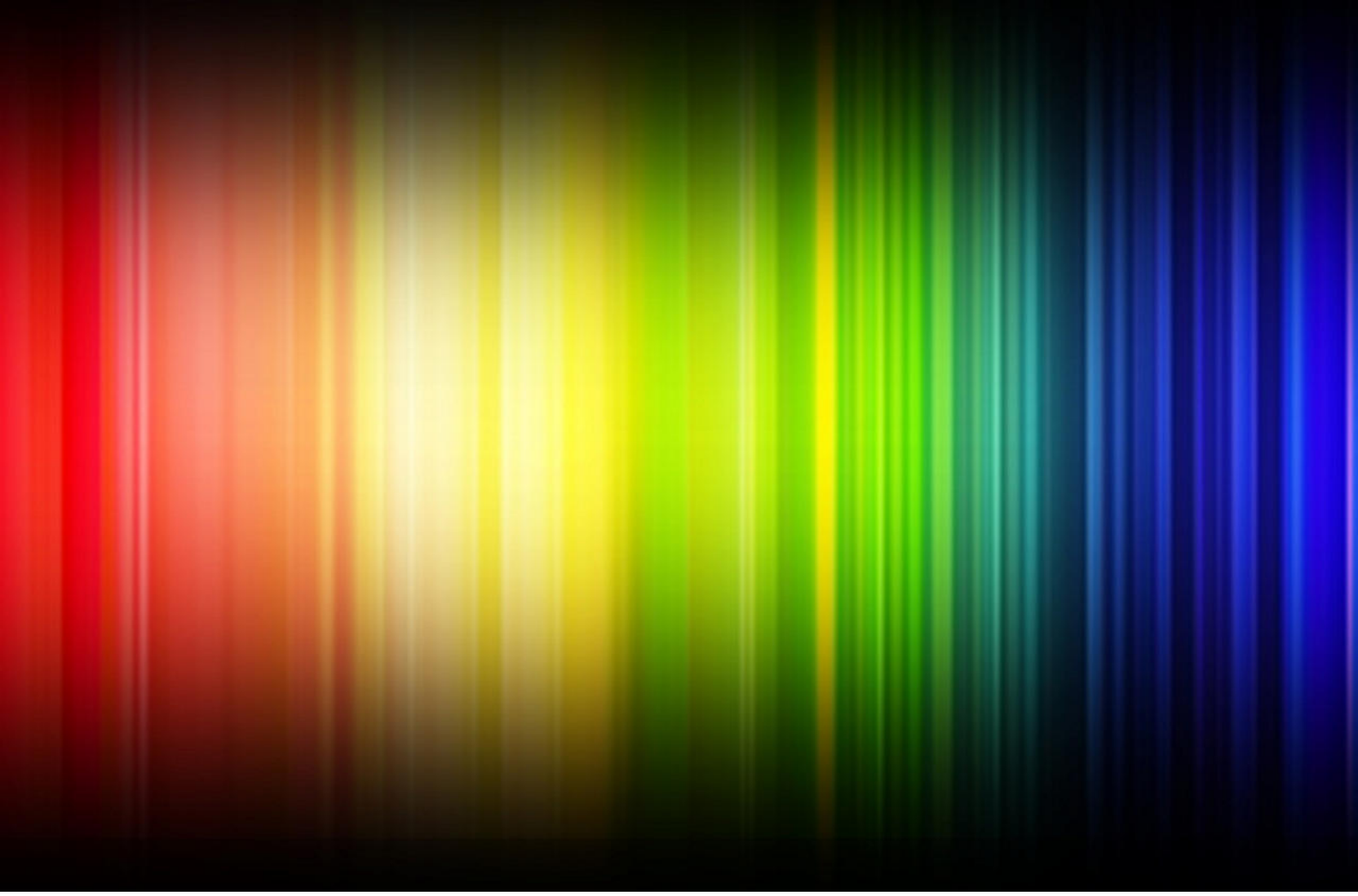


Application Note AN-SEC-001

Spectroelectrochemistry: an autovalidated analytical technique

Confirm results via two different routes in a single experiment

Spectroelectrochemical experiments not only provide outstanding qualitative information about samples, but also offer other quantitative data that can be considered when performing analyses.

A single set of experiments allows analysts to obtain two calibration curves: one with the electrochemical data and another with the spectroscopic information. The concentration of tested samples is calculated by using both curves, confirming the obtained results by two different routes.

In this Application Note, comparison between electrochemical and spectroscopic determinations demonstrates that the two methods measure uric acid (UA) indistinctively, with close agreement of the calculated values with empirical data.

INSTRUMENTATION AND SOFTWARE

Measurements taken in this study were obtained with 110 electrodes, REFLECELL, RPROBE-VISUV, and the SPELEC instrument (Figure 1) controlled with DropView SPELEC software. This setup allows analysts to obtain detailed, clear, and concise information about the electrochemical processes that take place on the electrode surface. DropView SPELEC is a dedicated software that provides spectroelectrochemical information and includes tools to perform an adequate treatment and analysis of the collected data.



Figure 1. SPELEC instrument used in this study.

APPLICATION: DETERMINATION OF URIC ACID

Aside from providing spectroelectrochemical qualitative data, this technique also allows the determination of quantitative information. In order to obtain both the electrochemical as well as the optical calibration curve, potentiostatic spectroelectrochemical experiments were performed at different concentrations of uric acid (UA) between 1×10^{-5} and 1×10^{-4} mol/L in 0.1 mol/L HCl. Amperometric detection was used with a potential of +0.80 V during 60 s. UV-Vis spectra were recorded in reflection configuration (400 ms integration time), then a series of 150 spectra were obtained using the amperometric detector within 60 s. Synchronization of the electrochemical and spectroscopic responses is completely assured by the SPELEC instrument.

RESULTS

Electrochemical profiles obtained with different concentrations of UA are plotted in Figure 2a showing that the current increases with increasing analyte concentration. In Figure 2b, UV-Vis spectra at 60 seconds show two absorption bands at 235 nm and 285 nm. Here, the absorbance (absolute value) increases with UA concentration, with the band at 285 nm being the most intense.

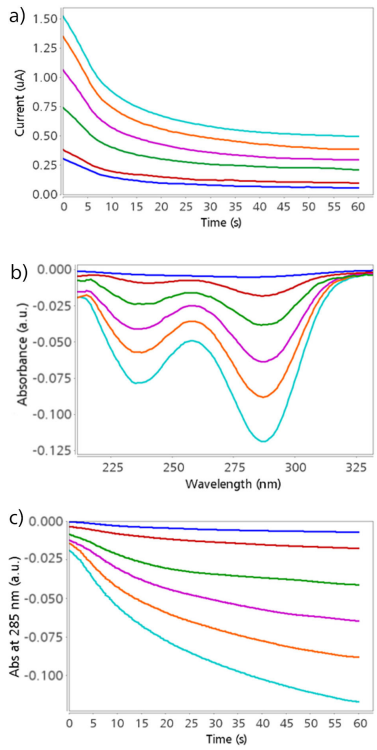


Figure 2. (a) Amperometric detection at +0.80 V and (b) UV-Vis spectra obtained at 60 s for different concentrations of UA in 0.1 mmol/L HCl. (c) Evolution of absorbance at 285 nm during 60 s obtained with "Spectra vs time" tool. UA concentration: 0.01 mmol/L (blue line), 0.02 mmol/L (red line), 0.04 mmol/L (green line), 0.06 mmol/L (pink line), 0.08 mmol/L (orange line) and 0.1 mmol/L (turquoise line).

In order to facilitate the understanding of the optical signal and ensure the accuracy of the selected data, evolution of the absorbance band at 285 nm vs time is shown in Figure 2c for each UA concentration assayed. This graph is obtained with the *"Spectra vs time"* tool implemented in DropView SPELEC software.

Two calibration curves were obtained with the current intensity and absorbance at 285 nm at 60 s versus UA concentration (Figure 3).

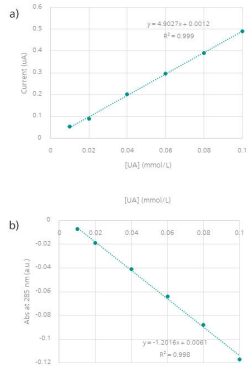


Figure 3. Calibration curves obtained from (a) electrochemical and (b) spectroscopic data.

The autovalidated character of spectroelectrochemistry was demonstrated by plotting the predicted concentrations with the electrochemical calibration curve versus the predicted concentrations with the spectroscopic calibration curve (Figure 4). This autovalidated character is related to the two independent responses obtained simultaneously for each sample, which is very useful for analytical purposes.

A slope of 0.999 and an intercept of 7×10^{-5} were obtained here. Consequently, with the slope being equal to unity and the intercept equal to zero, both electrochemical and spectroscopic signals determined UA without distinction, proving that UV-Vis spectroelectrochemistry is an autovalidated method for quantitative analysis.

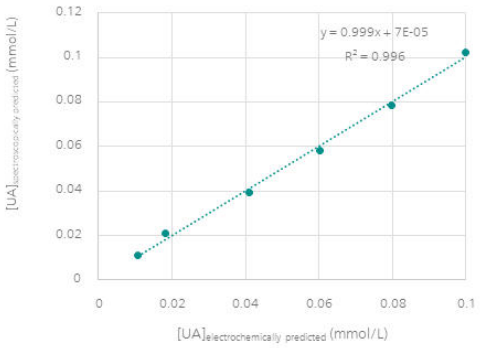


Figure 4. Predicted concentration with the electrochemical calibration curve versus the predicted concentrations with the spectroscopic calibration curve.

Finally, the UA concentration of two samples (Table 1) was estimated using both calibration curves. Spectroelectrochemical results show a current of 0.092 uA for 60 s and absorbance of -0.018 a.u. at 285 nm for sample 1, and a current of 0.297 uA and absorbance of -0.066 a.u. at 285 nm for sample 2. The agreement between the actual and predicted concentrations demonstrates the analytical features of this technique.

Table 1. Concentrations (in mmol/L) estimated from calibration curves constructed from electrochemical and spectroscopic data.

Sample	[UA]	$[UA]_{electrochemical}$	$[UA]_{spectroscopic}$
1	0.020	0.019	0.020
2	0.060	0.060	0.060

CONCLUSION

UV-Vis absorption spectroelectrochemistry is not only a very good technique to understand reaction mechanisms, but also an excellent technique for quantitative purposes. This study demonstrates the usefulness of this analytical technique with the determination of uric acid. In addition, the autovalidated character of spectroelectrochemistry has been demonstrated since electrochemical and spectroscopic calibration curves enable the determination of the concentration of uric acid without distinction, providing results that match exactly with the real concentration of the samples.

RELATED DOCUMENTS

Spectroelectrochemical instrument - SPELEC Instrument

Spectroelectrochemistry within everyone's reach - When combining two techniques became the perfect solution for your research

ACKNOWLEDGEMENTS

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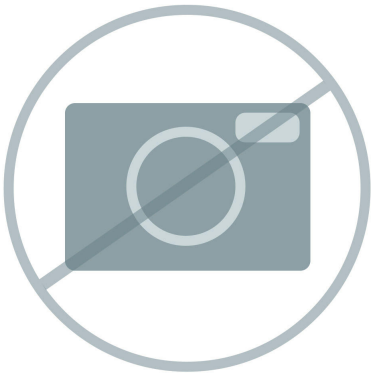
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SPELEC UV-VIS Instrument (200-900 nm)

SPELEC is an instrument for performing spectroelectrochemical measurements. It combines in only one box a Lightsource, a Bipotentiostat/Galvanostat and a Spectrometer (UV-VIS wavelength range: 200-900 nm) and includes a dedicated spectroelectrochemical software that allows optical and electrochemical experiments synchronization.



Reflection Cell for Screen-Printed Electrodes

Cell in Teflon suitable to perform reflection experiments with standard format Screen-Printed Electrodes with the electrochemical cell in the middle of the strip. Closing system with powerful magnets.



Reflection probe VIS-UV

Reflection probe VIS-UV designed to perform reflection experiments suitable to work with our Reflection Cell for our Screen-printed electrodes or with any conventional cell.



Screen-Printed Carbon Electrode

Screen-Printed Carbon Electrode (Aux.:C; Ref.:Ag). Suitable for working with microvolumes, for decentralized assays or to develop specific sensors.