A NEW METROLOGICAL TREATMENT OF THE CALIBRATION DATA OF THE CUMULATIVE STANDARD ADDITION METHOD

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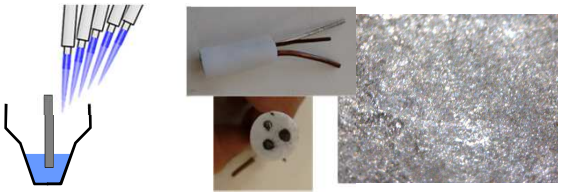
INTRODUCTION

This work presents methodologies for the development, optimization and validation of procedures for the electrochemical measurement of biochemical parameters in biological fluids. The developed methodology was applied to the voltammetric measurement of uric acid (UA) in human serum. The measurements were performed using a cumulative standard addition method (SAM-C), involving a new statistical treatment of the calibration data, which allows the calibration of the instrumentation in a small volume of serum. If the instrumental method of analysis does not consumes analysed item volume in the signal collection process, such as molecular spectroscopy, potentiometry and voltammetry, the need for a large volume of the analysed item can be overcome by performing consecutive additions of known quantities of the analyte to the same analytical portion as signal is being collected.

EXPERIMENTAL

Electrochemical cell configuration

- Nanocarbon Electrode (area = 0.5 cm^2) (25 % lignin, 60 % nanocarbon, 15 % mineral oil and a copper electrodeposit)
- Nanocarbon auxiliary electrode (area = 0.78 cm^2)
- Silver Reference Electrode
- Potentiostat/galvanostat MPQG-01 (Microchimica)



RESULTS

Electrode Calibration

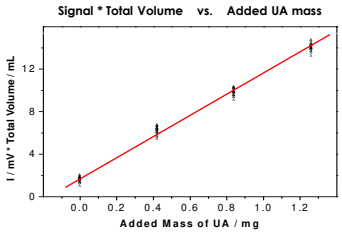


Figure 1: Calibration curve obtained for the analysis of human serum sample 01 using the nanocarbon electrode modified with copper and lignin using cyclic voltammetry with a scan rate of 500 mV s^{-1}. The standard additions were cumulative 250 µL addition of UA stock solution of 1.68 g L^{-1}.

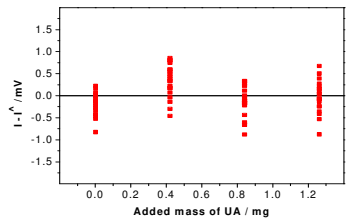
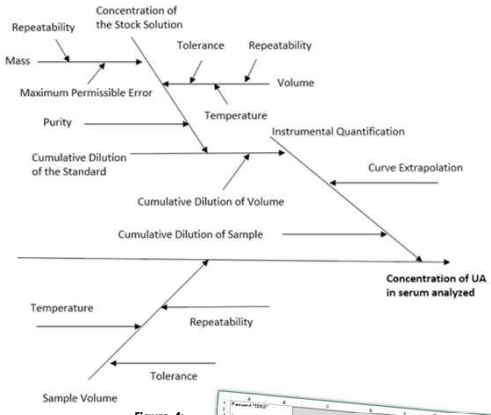


Figure 2: Regression residuals as a function of added uric acid mass for the calibration curve presented in Figure 4.

Figure 3: Cause-effect diagram with the uncertainty components.



Application of the electrode in human serum analysis

Table 1: Regression, linear correlation and, homoscedasticity and linearity tests parameters of performed standard addition calibrations.

Samples	Parameters*					
	a (mA*mL)	b (mA*mL/mg)	r	F	W	F_{LOF}
Serum 01	0.536	10.24	0.997	2.67	1.07	5.06
Serum 02	1.43	9.43	0.998	2.79	2.18	3.80
Serum 03	2.4	9.51	0.997	2.20	1.47	4.09
Serum 04	3.35	9.56	0.995	3.33	8.48	13.6
Serum 05	4.46	9.998	0.995	2.85	2.3	0.7
Human Serum 01	1.869	9.749	0.996	1.99	0.761	14.357
Human Serum 02	2.939	9.933	0.997	1.84	1.02	18.9

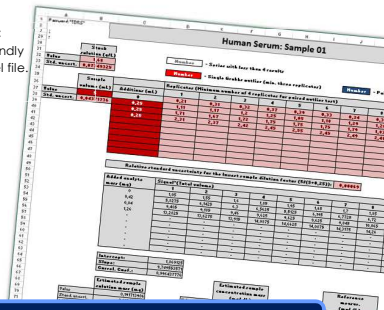
* a and b are the intercept and slope of the regression line, r is the Pearson's linear correlation coefficient, and F, W and F_{LOF} are the calculated parameters of the Fisher, Levene and ANOVA-LOF tests compared with critical values of 3.43, 4.05 and 4.896 respectively.

Table 2: Results of uric acid determination in spiked serum and human serum and respective reference values. The last column presents the absolute difference of estimated and reference values.

Samples	Estimated Mass concentration, γ_E Electrochemical Method (mg dL^{-1})	Reference mass concentration, γ_R (mg dL^{-1}) *	Compatibility Test (Absolute difference, $\lvert\gamma_E-\gamma_{Ref}\rvert$) (mg dL^{-1})
Spiked serum 01	1.03 ± 0.27	1.00 ± 0.02	0.04± 0.28
Spiked serum 02	3.03 ± 0.32	3.02 ± 0.06	0.01± 0.32
Spiked serum 03	5.06 ± 0.38	5.04 ± 0.09	0.02± 0.39
Spiked serum 04	7.01 ± 0.49	7.06 ± 0.13	0.05± 0.51
Spiked serum 05	8.93 ± 0.56	9.07 ± 0.17	0.10± 0.58
Human serum 01	3.83 ± 0.50	4.10 ± 0.12	0.30± 0.51
Human serum 02	5.92 ± 0.64	6.20 ± 0.12	0.30± 0.65

Reported expanded uncertainties were estimated for a confidence value of 95 %.
* For spiked serum samples, the reference values were estimated from the spiking procedure and for human serum samples the reference values were estimated by the spectroscopic and enzymatic standard method.

Figure 4: User-friendly MS-Excel file.



DISCUSSION AND CONCLUSIONS

Typically, analyte is added through a volume of analyte stock solution. However, since native analyte is diluted as a new volume of analyte stock solution is added, the construction of the calibration curve should take the added analyte mass, *m*, has the independent variable (i.e. the stock solution concentration times the added cumulative volume) and the total sample volume, *v*, (i.e. sample volume plus cumulative added stock solution volume) times the observed signal, *I*, (i.e. *v*I*) has the dependent variable.

The ratio between the intercept and the slope of the calibration curve (*v*I* vs. *m*) represents the estimated analyte mass in the item, m_S, and this value divided by the analytical portion volume, v_S, the analyte concentration, γ_S, in the item ($\gamma_S = m_S/v_S$). If the native analyte dilution as stock solution volume is added is not considered, measurements can be affected by a large error.

This error is larger if stock solution concentration used in standard additions is similar to native analyte concentration. The measurement uncertainty was estimated using developed *bottom-up* approaches and using pragmatic *top-down* approaches presented in the literature. The uncertainty components were combined using the Uncertainty Propagation Law or the numerical Kragten and Monte Carlo methods. The *bottom-up* assessments of measurements uncertainty involve the estimation of the extrapolation uncertainty from the regression model or using Monte Carlo simulations applicable to when the assumptions of the regression model are not valid.

The cumulative standard addition method was successfully applied to the analysis of human serum by adding 1.0, 3.0, 5.0, 7.0 and 9.0 mg dL^{-1} of AU to 5 mL of serum. The tools developed for the construction and optimization of working electrodes are applicable to the measurement of other analytes and matrices. The developed cumulative standard addition method and respective measurement models, are applicable to any kind of non-destructive chemical measurement of a solution.

Acknowledgments