Supplementary Material

Multi-laser-power calibration for quantitative determination of N and P in fertilizers by Raman spectroscopy

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General description of the MLPC and the comparative methods

Among the main univariate calibration methods used in spectrochemical techniques, the literature reports one-point calibration, two-point calibration, and multi-point calibration.

Calibration strategies with multiple points can be performed employing either (*i*) several standard solutions at increased analyte concentrations (while keeping instrumental parameters and other analytical conditions fixed), or (*ii*) a single standard solution and varying instrumental operating conditions or other analytical variables. The former includes methods such as external standard calibration (EC), while the latter presents a general class of calibration methods that can be referred to as multi-signal calibration (MSC). In the present work, MSC is used with Raman spectroscopy. The laser applied power was varied to generate several Raman signal intensities for a single calibration solution, generating several calibration points. The method described in this study is called multi-laser power calibration (MLPC).

MLPC-Raman. Samples and calibration solution (containing 5,000 mg L^{-1} N-nitrate, 5,000 mg L^{-1} N-urea, 5,000 mg L^{-1} P-phosphate, and 5,000 mg L^{-1} P-phosphite) were submitted to different laser applied powers in the 35.4 – 354 mW range (10 - 100% maximum power) to generate several Raman signal intensities. The MLPC curve was built with the integrated intensities recorded at the different laser applied powers for samples and the calibration solution (standard) on the *y* and *x* axis, respectively. The slope, *m*, of the MLPC curve equals C^{sam}/C^{std}, and the unknown analyte concentration in the sample, C^{sam}, can be determined using $C^{sam} = mC^{std}$ (where C^{std} is the analyte concentration in the calibration solution).

Raman spectroscopy with external standard calibration (EC-Raman). Calibration solutions with known analyte concentrations were prepared in the ranges of 500 - 5,000 mg L^{-1} N-urea, 500 - 5,000 mg L^{-1} N-nitrate, 1,000 - 20,000 mg L^{-1} P-phosphate, and 1,000 - 20,000 mg L^{-1} P- phosphite. Raman signal intensities associated with each of these solutions, as well as the blank, were obtained by submitting them to laser irradiation at 50% of the maximum laser power during 30 s (exposition time). Integrated intensities (*I*) in the regions of 850-920 cm^{-1}, 990-1020 cm^{-1}, 1024-1060 cm^{-1}, and 2340-2480 cm^{-1} were

plotted against the respective analyte concentrations ($C^{analyte}$) to generate external standard calibration (EC) curves for phosphate, urea, nitrate, and phosphite, respectively. The unknown analyte concentration in the samples was determined by substituting the blank-subtracted sample's response (I) into the calibration equation, which represents the linear relationship between analyte signal and analyte concentration, *i.e.*, $I = mC^{analyte} + b$ (where m and b represent the calibration curve slope and intercept, respectively).

Spectrophotometry with external standard calibration (EC-Spec). Similar to EC-Raman, calibration solutions with known analyte concentrations were prepared in the 10 - 100 mg L^{-1} P range, in the presence of NH_4VO_3 and $(NH_4)_6Mo_7O_{24}$. The spectrophotometer was set to the 465 nm wavelength (where the vanadomolybdophosphoric acid presents maximum absorption) and zeroed using the calibration blank. Molecular absorption signals (absorbance, A) were then obtained for the calibration solutions, and the EC curve was plot with A and $C^{analyte}$ on the y and x axis, respectively. Finally, the calibration equation ($A = mC^{analyte}$) was used to determine $C^{analyte}$ in the samples based on their respective A.

EC-HR-CS MAS. The same process used in EC-Raman and EC-Spec was used for EC-HR-CS MAS. In this case, calibration solutions containing 500-5000 mg L^{-1} N and 100-2000 mg L^{-1} P in 5% (v/v) H_2O_2 were aspirated into the sample introduction system of a ContrAA 300 Analytik Jena high-resolution continuum source flame atomic absorption spectrometer. Molecular absorption signals (A) were obtained by monitoring the $NO_{(g)}$ and $PO_{(g)}$ species (which were formed in the instrument's air-acetylene flame) at 215.360 and 247.620 nm, respectively. An air flow rate of 434 L h^{-1}, and acetylene flow rates of 45 L h^{-1} (NO) or 100 L h^{-1} (PO) were used to sustain the flame. The external standard calibration (EC) curve for total N and P was plot with A and $C^{analyte}$ values for each of the calibration solutions and the blank. The unknown concentrations of N and P in the samples ($C^{analyte}$) were calculated by substituting their blank-subtracted absorbances (A) into the respective calibrations equations ($I = mC^{analyte} + b$).

Kjeldahl method. For total N determination, 25 mL of the sample digest (see main text for sample preparation details) was transferred to Kjeldahl flasks, followed by the addition of about 50 mL of 40% (m/v) NaOH. After setting up the distillation apparatus, the solution was heated and distilled until one third of the original volume remained. The distillate was collected in a receiving flask containing 25 mL of 5% (m/v) H_3BO_3 plus 75 mL of water. A mixed indicator solution was separately prepared by dissolving 0.132 g of methyl red and 0.06 g of bromocresol green in 200 mL of ethanol. Four to six drops of the mixed indicator solution were added to the distillation receiving flask, and the solution was titrated with 0.05 mol L^{-1} H_2SO_4 until the titration endpoint was observed.