Analytical Methods

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Determination of sodium and calcium in powder milk using digital image-based flame emission spectrometry

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This paper proposes combining Multiple Linear Regression (MLR), and Digital Image-Based Flame Emission Spectrometry (DIB-FES) to overcome the inherent spectral interference in Flame Emission Spectrometry (FES) when sodium and calcium are present in the same sample. Metals in air-butane flame emit radiation which is revealed in the digital images captured by a webcam (detector), on the basis of 10 the Red-Green-Blue (RGB) color system. MLR calibration models for sodium and calcium were both developed and validated. In order to illustrate the feasibility of this proposal, the validated models were applied in the determination of sodium and calcium in powder milk. The results were compared with those obtained using Flame Atomic Absorption Spectrometry (FAAS) as the reference method. No statistically significant difference was observed between the results for both metals by using the paired t-test at a 95% confidence level. The accuracy of the models was also attested to by their recovery factors which fell between 97 and 103% for both metals. The results 15 confirm the model's discriminating power in isolating each analyte's information from a single digital image, even with spectral interference.

Introduction

Powder milk is obtained by dehydration of integral, skimmed, or partially skimmed cow milk. It is appropriate for human 20 consumption after being suitably processed.[1] Milk is chemically composed of triacylglycerols (especially from palmitic and oleic acids), vitamins (A, D, E and K), proteins (casein, albumins and globulins), enzymes (lipases, proteinases, oxidoreductases, phosphatases, catalase and peroxidase), lactose, and minerals 25 (chlorine, phosphorus, potassium, sodium, calcium, and magnesium in appreciable contents and iron, aluminum, bromine, zinc and manganese in low contents).[2] Among minerals it is important to monitor the potassium, sodium, calcium, and magnesium contents because they are directly related to 30 nutritional and health status of cows. When cows are affected by mastitis (inflammation of breast tissue) the content of potassium, calcium and magnesium decrease and the sodium content increases.[3]

According to Table 1 there are several analytical techniques for 35 determination of sodium and calcium depending on the application. Based on characteristics such as analytical frequency, linear range and limit of detection (LOD) the atomic spectrometric methods are often chosen as reference methods. However, it must be taken into account that they are not free of 40 interferences and instruments are expensive. An alternative is the use cheaper instruments such as flame photometers.

Flame Emission Spectrometry (FES) also known as Flame Photometry is the oldest technique of chemical analysis that does

not follow traditional chemical procedures.[4] Although it is a very simple and consolidated technique (similar to FAAS and ICP-50 OES), it is not free of interferences whether chemical, matrix, or spectral. A classic example of spectral interference occurs during the determination of sodium in the presence of calcium. The CaOH radical emission band intensity about the Na resonance line in air-butane flame is significant and produces positive 55 errors. In FES the problem currently has no solution, and analysis in FAAS is the most recommended alternative.

Digital Image-Based Flame Emission Spectrometry (DIB-FES) is a variant of traditional FES and was firstly proposed by Lyra and co-workers.[5,6] The main difference with respect to FES is how the 60 analytical signal is obtained. In DIB-FES a digital camera or video camera (webcam) is used along with digital image analysis to obtain the analytical signal. This new approach has produced excellent results for direct determinations of lithium in anti-depressives (tablets), sodium in Normal Saline (NS), and calcium 65 in waters, with similar results for indirect determinations of sodium diclofenac, sodium dipyrone, and calcium gluconate in injections (medicine). Since DIB-FES employs a simple webcam and no wavelength selector, the images (and analytical response) are susceptible to spectral interference in analysis. By applying 70 chemometric methods, especially inverse calibration which does not need explicit interference modeling, the DIB-FES has the potential to overcome this drawback. This paper proposes combining MLR and DIB-FES for determination of sodium and calcium in powder milk from a single digital image.

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Table 1 Available analytical techniques for determination of sodium and calcium.

Analytical technique	Sodium	Calcium	Reference
Gravimetry	yes	yes	7
Titrimetry	no	yes	7
Ion Exchange Chromatography (IEC)	yes	yes	7
Capillary Electrophoresis (CE)	yes	yes	8
Coulometry	no	yes	7
Potentiometry	yes	yes	7
Voltametry	no	yes	7
Ultraviolet-Visible spectrometry (UV-Vis)	yes	yes	9
Spectrofluorimetry	no	yes	7
Flame Emission Spectrometry (FES)	yes	yes	7
Flame Atomic Absorption Spectrometry (FAAS)	yes	yes	7
Inductively Coupled Plasma Optical Emission spectrometry (ICP-OES)	yes	yes	7
Inductively Coupled Plasma Mass spectrometry (ICP-MS)	yes	yes	10,11

Material and methods

Reagents, solutions, and samples

5 Distilled water was deionized by a Milli-Q (Millipore) system and then used throughout the work. Stock solutions of 100.0 mg L^{-1} Na, and of 100.0 mg L^{-1} Ca were prepared from their respective carbonate salts (dried at 120 ºC for 2 hours), by dissolution with 1% (v v^{-1}) HNO_3 aqueous solution.

10 The protecting agent was prepared as follow: 0.3 g of EDTA (free acid) was suspended in 750 mL of deionized water. By using a pH meter, concentrated NH_4OH was added until reach pH = 10. In a 1 liter volumetric flask the volume was made with deionized water.

15 The ionization buffer was a 10% (w v^{-1}) K^+ (prepared from KCl) aqueous solution.

The releasing agent was a 10% (w v^{-1}) Sr^{2+} (prepared from $Sr(NO_3)_2$) aqueous solution.

The working standard mixtures for the calibration and prediction
20 sets were prepared as follow: into a 100 mL volumetric flask, suitable aliquots of Na and Ca stock solution were added. Then 10 mL of the protecting agent were added and the volume was made with 1% (v v^{-1}) HNO_3. These mixtures were prepared within a complete factorial design[12] with calibration at (5^2), and
25 prediction at (4^2). The concentration levels are shown in Table 2. The calibration set consists of 25 standard working mixtures and prediction set consists of 16 standard working mixtures.

The concentration levels were scaled up based on previous studies[5,6] in order to guarantee a uniform image without
30 saturation (when a big and white spot appears) and maximum

Table 2 Concentration levels of calibration and prediction set mixtures.

Calibration	Analyte	Concentration levels (mg L^{-1})				
		-2	-1	0	1	2
	Na	0.28	0.84	1.40	1.96	2.52
	Ca	2.00	6.00	10.0	14.0	18.0
	Mixtures	Na				
		-2	-1	0	1	2
	Ca -2	CM 1	CM 6	CM 11	CM 16	CM 21
	-1	CM 2	CM 7	CM 12	CM 17	CM 22
	0	CM 3	CM 8	CM 13	CM 18	CM 23
	1	CM 4	CM 9	CM 14	CM 19	CM 24
	2	CM 5	CM 10	CM 15	CM 20	CM 25

Prediction	Analyte	Concentration levels (mg L^{-1})			
		-1.5	-0.5	0.5	1.5
	Na	0.56	1.12	1.68	2.24
	Ca	4.00	8.00	12.0	16.0
	Mixtures	Na			
		1	2	3	4
	Ca 1	PM 1	PM 5	PM 9	PM 13
	2	PM 2	PM 6	PM 10	PM 14
	3	PM 3	PM 7	PM 11	PM 15
	4	PM 4	PM 8	PM 12	PM 16

variation of the RGB components. Then, the limits of linearity of both analytes are the maximum concentration levels in the
35 factorial design.

Eight brands of powder milk were purchased from local supermarkets. Ashless filter paper (Whatman N° 40) was used to filter the samples after dissolution of the ashes after dry digestion.

Apparatus and softwares

40 Details of the digital image acquisition assembly are described elsewhere.[5,6] An Instant Creative webcam was used to capture the images, and was connected by a universal serial bus (USB) to a Pentium III 650MHz microcomputer (PC). The webcam captures 24-bit digital images (16.7 million colours) at a rate of 34 images
45 s^{-1}, with 352 x 288 pixels of spatial resolution. The images were recorded in software written in Delphi (version 3.0). The atomizer/emitter module of a flame emission photometer (model NK-2004 Digimed) was coupled to the webcam, and the combined system was used to generate and record the analytical
50 signal(s). The flame atomizer/emitter module was operated according to manufacturer's instructions for maximum sensitivity.[6]

The images were captured in the interconal area[13], which is localized approximately 2.5 cm (oxidant region of the flame) over
55 the photometer burner. The treatment of the digital images captured was made by means of additional software also written in Delphi (version 3.0). The software routine is described in details in the literature[5,6], and a fixed region of the images is exclusively used (25 x 25 pixels).
60 A Shimadzu model AA-3600 double beam flame atomic absorption spectrophotometer was used for reference measurements.

A Lavoisier muffle furnace (model 400C) was used for calcination of the powder milk samples.

Matrix equations (3-5) were solved-treated by using Matlab 2008®.

5 **Sampling, sample pre-treatment and reference method**

The sampling plan was carried out according to International Organization for Standardization (ISO) recommendations.[14]

In Brazil, the Instituto Adolfo Lutz (IAL) is the organization who recommends and describes the official methods of analysis in 10 foodstuff. According to IAL, for determination of metals in foodstuffs by using FAAS, two procedures for sample pre-treatment for determinations are described: wet and dry digestion.[15] In this work dry digestion was chosen.

The procedure for dry digestion was carried out as follow: the 15 amount of the sample was placed in a porcelain crucible and gently heated in Bünsen burner, avoiding burn the sample, until to obtain a light gray mass. Then the sample was calcinated at 550 ºC for 4 hours and a white mass was obtained (ashes). Because of differences in the linear ranges in the determination of 20 sodium and calcium by reference method, different amounts of sample were used in each determination. In the determination of sodium, the amount of sample was 10-20 mg and in the determination of calcium was 20-40 mg.

After wet digestion it is necessary to solubilize the sample. The 25 manner which this step is made depends on the analyte of interest. For analysis of most metal the ashes are solubilized in 1 mL of concentrated HNO_3 and the final volume is completed with deionized water in a volumetric flask. However for the determination of sodium and calcium the procedures of 30 solubilization of the samples are different as described below:

Determination of sodium: The ashes are dissolved in 1 mL of concentrated HNO_3. In order to minimize free sodium ionization in the flame during sodium measurements, it is necessary to add an ionization buffer to the blank, standard and sample solutions.

35 The ionization buffer consists of a metal solution with lower ionization energy than that of the analyte.[7] According to Le Châtelier principle's the ionization of this metal displaces the ionization equilibrium of the analyte avoiding losses in the analytical signal. The metals which are indicated for sodium 40 determinations are potassium, rubidium and cesium. As rubidium and cesium salts are much too expensive, potassium was used. Were added 10 mL the ionization buffer and the volume was made up to 100 mL in volumetric flask with deionized water. The blank was prepared in the same manner.

45 Determination of calcium: The ashes are dissolved in 1 mL of concentrated HNO_3. As in the determination of sodium it is necessary to add an ionization buffer to the blank, standard and sample solutions in order to minimize free calcium ionization in the flame during calcium measurements. The presence of 50 aluminates, phosphates, and sulfates in the samples causes the formation of refractory compounds in the flame. As a consequence, during calcium measurements in FAAS a loss of analytical signal is observed and is a serious drawback (chemical interference). It becomes necessary to add a releasing agent to the 55 blank, standard, and sample solutions. The releasing agent reacts with interfering agents to produce compounds which are more stable than their respective calcium compounds.[7] In calcium

determinations, the FAAS' spectrophotometer manufacturer recommends lanthanum and strontium as releasing agents. As 60 lanthanum salts are much too expensive, strontium was used. Were added 10 mL of the ionization buffer and 10 mL of the releasing agent. Then the volume was made up to 100 mL in volumetric flask with deionized water. The blank was prepared in the same manner.

65 After the pre-treatment, the samples were converted in solution and are ready for analysis by FAAS. The measurements of working standard solutions and samples were obtained at the same time in random sequence. The analyses of each metal were carried out in separate and experimental conditions were:

70 For sodium: current operation of the hollow cathode lamp = 10.0 mA, wavelength = 589.0 nm, slit width = 0.2 mm, burner height = 7.0 mm, burner angle = 0 degree, fuel = acetylene, fuel gas flow = 1.8 L min^{-1}, oxidant = air, background correction = self-reversing mode of the hollow cathode lamp and linear working 75 range = from 0.20 up to 1.00 mg L^{-1}.

For calcium: current operation of the hollow cathode lamp = 10.0 mA, wavelength = 422.7 nm, slit width = 0.5 mm, burner height = 7.0 mm, burner angle = 0 degree, fuel = acetylene, fuel gas flow = 2.0 L min^{-1}, oxidant = air, background correction = 80 Deuterium lamp (continuous source) and linear working range = from 1.00 up to 5.00 mg L^{-1}.

The analytical calibration curves were validated by means of Analysis of Variance (ANOVA)[12] and then used to estimate the concentrations of the analytes in the samples. These estimates 85 were made according to the following equation:

$$C_{analyte}(mg\ g^{-1}) = \frac{C_{analyte}(mg\ L^{-1}) \cdot V_{sample}(L)}{m_{sample}(g)} \quad (1)$$

where: $C_{analyte}$ is concentration of the analyte estimated by using linear regression with the ordinary least squares (OLS) method, V_{sample} is the volume of the volumetric flask and m_{sample} is the 90 mass of the sample.

Sampling, sample pre-treatment and proposed method

The sampling plan for the proposed method was also carried out according to ISO recommendations.[14]

For the proposed method, the dry digestion was also chosen and 95 carried out as for the reference method, but the amount of sample was 25-50 mg.

The ashes are dissolved in 1 mL of concentrated HNO_3 and 10 mL of the protecting agent was added. A white precipitate was obtained and filtered through N° 40 Whatman ashless filter paper. 100 Then the volume was made up to 100 mL in volumetric flask with deionized water. The blank was prepared in the same manner.

The concentrations of the analytes in the sample are also estimated by equation 1, but $C_{analyte}$ are estimated by using MLR.

105 **Recovery tests**

Recovery tests[16] were used in order to evaluate the accuracy, and any type of interference present in the proposed method. In this study, the referred evaluation was made by spiking. According to this strategy, the spiked sample is prepared by addition of the 110 analyte(s) after sampling and before all analytical procedure. The

analytical signal of the spiked sample must be within the linear response range of the method. The original sample is prepared by dilution of the same aliquot used in the spiked procedure to the same final volume. The recovery (or recovery factor) is calculated according to the following equation:

$$R_A = \frac{[X_A(O+S) - X_A(O)]}{X_A(S)} \qquad (2)$$

where $x_A(O+S)$ is the spiked concentration of the analyte in the sample, $x_A(O)$ is the original concentration of the analyte in the sample, and $x_A(S)$ is the spiked concentration of the analyte.

The spiked sample was prepared as follows: after sampling, 1.0 mL of the sodium stock solution and 5.0 mL of the calcium stock solution were added to the sample and then the proposed method was carried out. The spiked mass for sodium and calcium were 0.05 and 0.50 mg respectively.

Database

In inverse calibration methods the **y** vector corresponds to the matrix of the associated values for the property to be estimated. In this study the matrix corresponds to the sodium and calcium concentrations, that is, $\mathbf{y} = C_{Na}$ or C_{Ca}. The **X** matrix corresponds to the variables which are employed in the multivariate calibration models to estimate the values of the **y** vector. In DIB-FES the variables used were the values of the R, G, and B components of the images of the mixtures of sodium and calcium, that is, $\mathbf{X} = [R_{mix} \; G_{mix} \; B_{mix}]$.

Results and discussion

Choice between releasing and protecting agent

Normally, the manufacturers of atomic absorption spectrophotometers and flame photometers recommend lanthanum or strontium salts as releasing agents in the determination of calcium. However in DIB-FES these salts cannot be used because both emit visible radiation when excited in butane-air flame. In addition the salts are too much expensive. As an alternative, the literature describes the employment of protecting agents.[7] A protecting agent is a complexing substance which is able to react with the analyte to produce a complex which is more stable than the respective refractory compound of the analyte and is easily decomposed in the flame. For most of metals, EDTA is indicated. EDTA which is commercially available in di-hydrate disodium salt, and free acid forms. Being sodium one of the analytes, EDTA in free acid form was chosen. Because of its low solubility in water, it was dissolved by adding concentrated NH_4OH until reaching a pH of 10 in order to produce a buffer solution. The complexing between Ca^{2+} and $EDTA^{4-}$ ions occurs at pH $\geq$ 10.[7]

Digital images and REM emission phenomenon

In air-butane flames the emission bands that are found in the visible region are often due to the presence of CH and CN radicals and C_2 molecules. So, it is reasonable to admit the digital images will have two different contributions: the self-emission phenomenon of the flame (constant) and the emission of the

metal (variable). When two or more metals are present in the flame (if all are able to emit REM in visible region), the resulting color is a combination of each metal's individual color.

During the image treatment, in the digital images of standard working mixtures and samples, the software does not subtract the contribution of the blank in each RGB component. Then, the values of R_{mix}, G_{mix} and B_{mix} are not free of the contribution of the self-emission phenomenon of the flame (blank) and are used in modelling step.

Development and validation of MLR calibration models

An MLR model can be obtained using an **X** instrumental responses matrix with (m × k) dimensions. Where ''m'' represents the number of samples and ''k'' represents the number of variables (in this case, k = 3 for the RGB data components). The **y** column vector is for concentration values of the samples obtained by means of a reference method. The relationship between the **X** matrix and the **y** column vector is determined by application of the ordinary least squares method.[17]

Each dependent variable of **y** is expressed as a linear combination of the independent variables of the **X** matrix by using the expression:

$$\mathbf{y} = \mathbf{bX} \qquad (3)$$

where **b** is the column vector which has the regression coefficients, and is calculated by least squares from an **X** pseudo-inverse matrix:

$$\mathbf{b} = (\mathbf{X^T X})^{-1} \mathbf{X^T y} \qquad (4)$$

where the superscript indices (-1 and T) represent the inversion and transposition of the matrix.

With the model parameters determined, the new sample concentrations can be estimated from the following equation:

$$\mathbf{y_{pred}} = \mathbf{X_{pred} b} \qquad (5)$$

where $\mathbf{X_{pred}}$ represents the data matrix of the new samples.

The digital images of the calibration and prediction mixtures which provided the RGB values for developing the MLR models are shown in Figure 1.

Individual MLR models for each analyte are developed by using one variable (R_{mix}, G_{mix} or B_{mix}), two variables (R_{mix} and G_{mix}, R_{mix} and B_{mix}, and G_{mix} and B_{mix}), and three variables (R_{mix}, G_{mix} and B_{mix}). From all these models, the model with three variables does not show evidences of systematic error in the results. The discussion made concerns the model with three variables.

The evaluation of the MLR calibration models was made in terms of figures of merit: calibration correlation, prediction correlation, Root Mean Square Error of Calibration (RMSEC) and Root Mean Square Error of Prediction (RMSEP).

The correlation values between actual concentrations and predicted concentrations (for the calibration set) values were 0.9999 and 0.9998 for sodium and calcium respectively, and indicates a direct relationship between them. The Root Mean Square Error of Calibration (RMSEC) was then calculated according to the following equation:
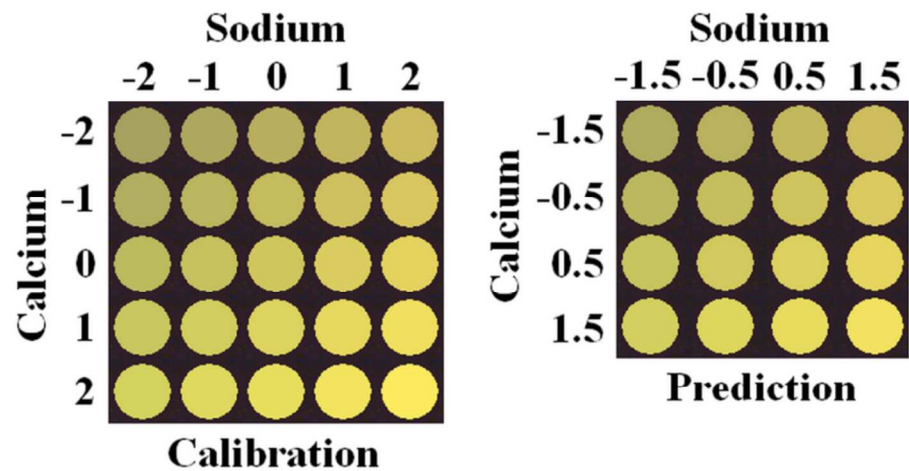
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Fig. 1 Digital images of the calibration and prediction sets. The position of the images of the standard working mixtures is according to Table 2.

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{N_{sample}}(c_{i,cal} - \hat{c}_{i,cal})^2}{N_{sample}}} \quad (6)$$

where $c_{i,cal}$ = actual concentration of the ith sample of the
5 calibration set, $\hat{c}_{i,cal}$ = predicted concentration of the ith sample of the calibration set and N_{sample} = number of samples of the calibration set.

The RMSEC values were 7.59 x 10^{-7} mg L^{-1} and 5.67 x 10^{-6} mg L^{-1} for sodium and calcium respectively. The values of calibration
10 correlation close to 1 and the low values of RMSEC obtained indicate that the calibration models are well fitted.

The MLR calibration models were applied to the prediction of 16 synthetic samples (prediction set). The correlation values between actual concentrations and predicted concentrations for this set
15 were 0.9998 and 0.9999 for sodium and calcium respectively, and as in the calibrations set indicates a direct relationship between them. Thereafter the Root Mean Square Error of Prediction (RMSEP) was calculated according to the following equation:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N_{sample}}(c_{i,pred} - \hat{c}_{i,pred})^2}{N_{sample}}} \quad (7)$$

20 where $c_{i,cal}$ = actual concentration of the ith sample of the prediction set, $\hat{c}_{i,cal}$ = predicted concentration of the ith sample of the prediction set, and N_{sample} = number of samples of the prediction set.

The values of RMSEP were 1.12 x 10^{-2} mg L^{-1} and 8.08 x 10^{-2} mg
25 L^{-1} for sodium and calcium respectively. Results of the prediction of the synthetic samples are shown in Table 3. In order to get further insight into the precision of the MLR models, linear regressions for the plots in Table 3 were applied. The estimated intercept and slope ($\hat{a}$ and $\hat{b}$ respectively) were compared with
30 their ideal values (0 and 1) using the Elliptical Joint Confidence Region methodology (EJCR).[18]

Table 3 Prediction set results (in mg L^{-1}).

| Prediction mixtures	Na		Ca	
	Actual	Predicted	Actual	Predicted
PM 1	0.56	0.56	4.00	3.89
PM 2	0.56	0.56	8.00	8.15
PM 3	0.56	0.55	12.0	11.9
PM 4	0.56	0.58	16.0	16.0
PM 5	1.12	1.10	4.00	4.08
PM 6	1.12	1.12	8.00	7.95
PM 7	1.12	1.11	12.0	11.9
PM 8	1.12	1.13	16.0	16.1
PM 9	1.68	1.70	4.00	4.00
PM 10	1.68	1.68	8.00	8.00
PM 11	1.68	1.69	12.0	12.1
PM 12	1.68	1.67	16.0	15.9
PM 13	2.24	2.25	4.00	3.95
PM 14	2.24	2.23	8.00	8.05
PM 15	2.24	2.22	12.0	12.0
PM 16	2.24	2.24	16.0	16.0
RMSEP	-	0.01	-	0.08
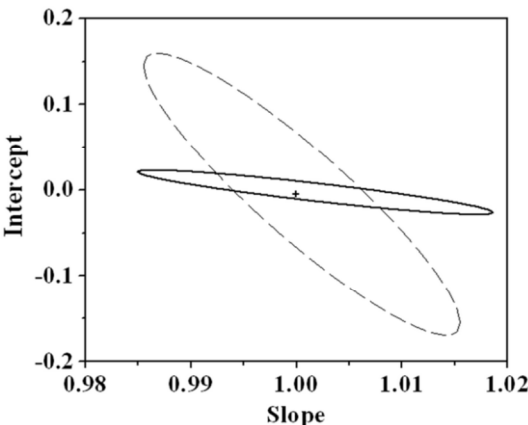
35 **Fig. 2** Elliptical joint confidence region for the intercept (a) and slope (b), corresponding to data in Table 3. Na (solid line), and Ca (dashed line). The cross marks the theoretical point (a = 0, b = 1).

In the two EJCR for the MLR models in Figure 2 the ellipses contain the theoretical point (a = 1, b = 0), thus indicating that proportional and constant errors were not present.[18] Because of values of prediction correlation close to 1, low values of RMSEP (when compared with the concentrations in the calibration set) and the presence of the theoretical point inside the EJCR the predictions from both MLR models could be considered validated.

Analytical determinations and validation of the results

The validated MLR models were applied in the determination of sodium and calcium in eight powder milk samples, and the results were compared to those obtained by the reference method (FAAS). Table 4 shows the results in terms of mg g^{-1} and shows good agreement. The application of the paired *t*-test, based on the hypothesis test, revealed no systematic difference between the results at a 95% (two sides) confidence level. In terms of analytical performance, the proposed system and method presented better results than the reference method, as revealed by the overall R.S.D. (n = 3) value.

As to accuracy and the presence of interferences, the MLR models were also evaluated by recovery tests, and these results are also shown in Table 4. Considering that most of the recovery factors were between 98 and 103% for both analytes, the DIB-FES method was suitable for the chosen samples.

Conclusions

In this study, was investigated the feasibility of using digital images captured with a webcam for determination of sodium and calcium in samples of powder milk using DIB-FES.

The self-emission phenomenon of the flame is present in all three variables (R_{mix}, G_{mix} and B_{mix}), therefore it becomes necessary to use all of them when modeling this phenomenon in order to avoid systematic error in the results.

Selection of a suitable flame region, allied with the linear concentration/RGB value relationship of the metals and the digital image components for the calibration mixtures was essential for the successful application of the MLR models. Not using radiation selectors is a significant simplification for the construction, and increases instrumentation lifetime for flame emission techniques. The well-known spectral problem of calcium interference over sodium was easily circumvented using multivariate calibration (with explicit modelling of the interfering calcium specimen). The addition of a protecting agent to chelate the calcium (and avoid emission due to the combustion of organic matter) was also responsible for the success of the proposed method. The results confirm the model's discriminating power in isolating each analyte's information from a single digital image, even with spectral interference.

The overall relative standard deviation obtained was smaller than that of the value set obtained from the reference method (FAAS) for sodium and calcium. The precision of the method can also be attested to by the low RMSEP values.

Using a webcam as the detector in the visible region of the electromagnetic spectrum, the multivariate DIB-FES method offers a simple and economically viable alternative to the

Table 4 Powder milk sample results (in mg g^{-1}) for both sodium and calcium determinations.

Samples	MLR	Recovery (%)	Reference
(1) Na	5.47 ± 0.04	101 ± 1	5.35 ± 0.11
(2) Na	3.02 ± 0.07	99 ± 2	3.08 ± 0.12
(3) Na	3.73 ± 0.05	101 ± 2	3.69 ± 0.12
(4) Na	3.30 ± 0.03	102 ± 3	3.35 ± 0.11
(5) Na	3.74 ± 0.06	98 ± 3	3.69 ± 0.12
(6) Na	3.65 ± 0.04	102 ± 2	3.70 ± 0.12
(7) Na	3.54 ± 0.07	99 ± 1	3.50 ± 0.11
(8) Na	4.54 ± 0.06	98 ± 4	4.60 ± 0.13
Overall RSD (%)	1.41	-	3.00
RMSEP	0.05	-	-
(1) Ca	15.0 ± 0.1	99 ± 1	15.1 ± 0.1
(2) Ca	9.18 ± 0.05	101 ± 1	9.08 ± 0.15
(3) Ca	9.06 ± 0.06	98 ± 2	9.12 ± 0.13
(4) Ca	8.93 ± 0.04	102 ± 1	8.88 ± 0.14
(5) Ca	9.04 ± 0.05	99 ± 3	9.12 ± 0.15
(6) Ca	9.22 ± 0.07	103 ± 2	9.14 ± 0.13
(7) Ca	8.56 ± 0.03	100 ± 2	8.65 ± 0.14
(8) Ca	10.1 ± 0.1	101 ± 1	10.0 ± 0.2
Overall RSD (%)	0.55	-	1.41
RMSEP	0.09	-	-

conventional FES solution for the problem treated in this work. Moreover the determination allowed to employ the same experimental condition and as a consequence, the reduction of time of analysis in at least two times when compared with FAAS, which is necessary to change the hollow cathode lamp between the analytes and experimental conditions.

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