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Direct determination of Calcium in milk by atomic absorption spectrometry using flow-injection analysis

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Abstract: A flow-injection system with sample and reagent addition by the synchronous merging zones approach for calcium determination in milk by flame AAS is proposed. Main parameters were optimized using a factorial design with central point. The optimum conditions were 2.5% (m/v) for La concentration, 8 mL min^{-1} for the carrier flow-rate, 20 cm for coiled reactor and 250 ìL for sample volume. Different sample preparation procedures were evaluated such as dilution in water or acid and microwave-assisted decomposition using concentrated or diluted acids. The optimized flow system was applied to determine Ca in eleven commercial milk samples and two standard reference materials diluted in water. Similar calcium levels were encountered comparing the results obtained by the proposed method (dilution in water) with those obtained using microwave-oven digestion. Results obtained in two standard reference materials were in agreement at 95% confidence level with those certified. Recoveries of spiked samples were in the 93% - 116% range. Relative standard deviation (n = 12) was < 5.4% and the sample throughput was 150 measurements per hour, corresponding to a consumption of 250 µL of sample and 6.25 mg La per determination.

Keywords: Calcium, milk, flame atomic absorption spectrometry, flow-injection analysis

Introduction

Milk contains a large variety of essential nutrients for the development and maintenance of a salutary life. This product contains a complex mixture of minerals including calcium, magnesium, sodium, potassium, chloride, sulfate, and phosphate [1]. Calcium in milk is distributed between the milk serum and the casein micelles [1]. Calcium is an essential macronutrient for humans, which represents approximately 2% of body weight in an adult person [2]. This element has mainly a structural function in bones and teeth, besides to regulate many vital biological functions. More recently, interest in calcium has centered on its role in preventing osteoporosis. It is known that the highest demands for this element are produced during the periods of maximum growth such as in childhood and adolescence, and also during lactation and in the elderly. The need recommended dietary allowance for calcium by Food and Nutrition Board is approximately 1300 mg per day for adolescents and 1100 mg per day for adults [2].

Several techniques have been used to quantify calcium in milk and derivates, mainly flame atomic absorption spectrometry (FAAS) [3-5], titrimetry [6-7], inductively couple plasma optical emission spectrometry (ICP OES) [8-10] and inductively couple plasma mass spectrometry (ICP-MS) [11,12]. FAAS and titrimetry techniques have been mostly used in routine analysis of milk due to their simplicity, high accuracy and precision and relative low cost compared with ICP OES and ICP-MS. The main drawback associated with manual determination of Ca in milk is the high amount of lanthanum used as modifier to avoid chemical interferences due to phosphates.

Nowadays, there is a high demand for chemical analysis of foods and agroindustrial materials with simplicity, low costs, generated residues and time, without loss of quality in results obtained. In this scenery, the association of the FAAS technique with flow-injection systems is a good option to eliminate or minimize the mentioned problems in conventional FAAS and titration methods. The flow-injection analysis (FIA) systems are excellent solution managers, once they allow several improvements, such as reduction in handling of samples and glassware, less reagents consumption and reduced chemical wastes [13,14]. Several methods can be found in literature associating flow-injection systems with spectrometric techniques in elemental inorganic determination in foods, mainly for pre-concentration purposes [15-17]. However, little attention has been given to the use of the FI-FAAS coupling for calcium determination in milk samples without sample preparation.

In this work a flow-injection system was developed for the direct determination of calcium in milk by flame atomic absorption spectrometry. The potentiality of a flow-injection system employing a simultaneous injection of sample and lanthanum in a merging zone approach was evaluated. Besides dilution in water, other sample pre-treatment procedures were studied for comparative purposes such as dilution in acid, and sample decomposition in a microwave oven system using diluted or concentrated acids.

Experimental

Apparatus or Instrumentation

The flow-injection system comprised an Ismatec (Zurich, Switzerland) IPC-8 multi-channel peristaltic pump equipped with Tygon™ pumping tubes, a Perkin Elmer (Shelton, CT, USA) AAnalyst 100 spectrometer with a deuterium lamp background corrector, a Kipp & Zonen (Delft, The Netherlands) BD 111 recorder, a manual injector-commutator, polyethylene tubing (i.d. 0.8 mm), coiled reactors and accessories. The monochromator was adjusted to 422.7 nm and the slit-width to 0.7 nm. Atomic signal was measured in peak height mode. A Perkin-Elmer Lumina™ multielementar hollow cathode lamp (P/N N305-0218) was used for the determination of Ca at lamp current of 30 mA and oxidizing air-acetylene flame.

The flow diagram of the system is shown in Figure 1. In the specified position in figure is illustrated the selection of 250 mL of analytical solutions or samples (A) and lanthanum (R) by the sampling loops L_1 and L_2, respectively. In the alternative position (indicated by the arrow), both A and R are simultaneously injected in the sample or reagent carrier (1% v/v HNO_3) streams C_1 and C_2, respectively. These carriers transport the content of L_1 and L_2 towards the confluent point x. The mixture is homogenized through the coiled reactor B where lanthanum displaces calcium from the refractory $Ca_3(PO_4)_2$ to be atomized and quantified by the spectrometer (FAAS). When the injector-commutator IC is switched back to initial position, a new cycle can be started.

An Anton Paar Multiwave microwave oven decomposition system (Graz, Austria) equipped with six pressure decomposition PFA vessels of 50-mL capacity was employed for the sample pretreatment.

Reagents, Analytical Solutions and Samples

High-purity water (resistivity 18.2 MW.cm) obtained by a Millipore Rios 5™ reverse osmosis and a Millipore Milli-Q academic™ deionizer system (Bedford, MA, USA) was used throughout the work. Nitric acid 65% v/v and hydrochloric acid 37% v/v (Merck, Darmstadt, Germany) were used throughout the work for preparing solutions and samples.

A 5% (m/v) La solution was prepared by dissolving 58.6 g La_2O_3 (Fluka, Switzerland) in 250 mL of concentrated hydrochloric acid (Merck, Darmstadt, Germany). The resultant solution was transferred to a 1000 mL volumetric flask and the volume was completed with deionized water. Diluted lanthanum solutions were prepared by appropriate dilution of the stock solution.

A 1000 mg L^{-1} calcium stock solution was prepared by the dissolution of 2.5 g $CaCO_3$ (Mallinckrodt, Phillipsburg, USA) in 50 mL of 0.1 mol L^{-1} HCl solution. This solution was transferred to a 1000 mL volumetric flask and the volume was completed with water. Analytical solutions in the 1.0 - 5.0 mg L^{-1} range were prepared daily by dilution of the stock solution.

Eleven commercial milk samples purchased at local markets in Araraquara city (São Paulo, Brazil) and two standard reference materials (SRM 8435 Whole Milk Powder; SRM 1549 Non Fat Milk Powder) from National Institute of Standards and Technology (Gaithersburg, MD, USA) were analyzed for calcium determination. Four different sample preparation procedures were evaluated, such as dilution (1:250, v/v or 1:3000 m/v) in water or 1% (v/v) HNO_3, decomposition in closed microwave oven digester system using 250 mg of sample plus 4 mL HNO_3 + 0.4 mL HCl concentrated acids or 4 mL of diluted (20% v/v) nitric acid. The heating program of the microwave oven used to decompose milk and determine calcium comprises the following time/power steps: 5 minutes/110-600 watts; 5 min/ 600–600 watts; 10 min/ 1000–1000 watts; 15 min/ 0–0 watts.

In addition, recovery tests were also investigated by adding 150–300 µL of 1000 mg L^{-1} Ca in 100 mL of diluted samples in triplicate. Statistical tests used in the data processing (mean, standard deviations and precision) were done using the Microcal Origin^{Ò} 7.0 program.

Optimization by factorial design

A factorial design with central point, three variables and three levels [low (-1); medium (0) and high (+1)] was done to check the influence of each studied variable and their simultaneous interactions in calcium determination in milk samples (Table 1). The preliminary study was done in analytical solutions containing phosphates at levels usually found in milk samples. The experimental variables studied were lanthanum concentration, length of the reaction coil B and flow-rate of the carriers C_1 and C_2. The generated matrix was evaluated by the Statistica version 6 software.

Results and discussion

Factorial design

The factorial design with central point (Table 1) comprised three variables and three levels [low (-1); medium (0) and high (+1)] furnished the Pareto's chart illustrated in Figure 2 [18]. This chart presented the estimated effects of the investigated factors (lanthanum concentration, reaction coil length and carrier flow-rate) and their interactions. This figure reveals that lanthanum concentration (factor A) and carrier flow-rate (factor C) are the main variables that significantly influence in the response (absorbance). The reaction coil length (factor B) and the interactions among the factors (AC, AB and BC) have no influence in the response. By a simple construction of a response surface graph (Figure 3) the parameters lanthanum concentration and carrier flow-rate could be defined as 1% (m/v) and 8 mL min^{-1}, respectively. For sample dilution in water, the minimum lanthanum concentration is 2.5% m/v, the selected concentration of R for further experiments. It should be stressed that this La concentration minimized / eliminated chemical interferences of phosphates. Despite of the coiled reactor did not influence significantly in the response, 20 cm was the length of the reactor chosen in order to help the dispersion of the sample zone from the injector to the nebulization chamber and to guarantee a certain time for calcium displacement from the $Ca_3(PO_4)_2$ refractory compound.

Sample treatment procedure and calcium determination in milk

The dissolution of milk in 1% (v/v) nitric or hydrochloric acid resulted in a slurry formation due casein flocculation which damaged sampling, aerosol formation and transportation in a repetitive mode, deteriorating the absorbance readings. According to Bernardi *et al.* the addition of acids in milk samples promotes the coagulation of the casein due to the increase of H^+ concentration, releasing calcium salts and decreasing the casein affinity in water. This phenomenon destabilizes the casein in the dispersant medium forming the flocculated substances [19,20]. This drawback was circumvented by using a simple dilution or dissolution of sample in water. The minimum dilution factors used were 1:250 v/v and 1:3000 m/v for liquid and powdered samples, respectively. For these dilutions, slurries were stable for a 4-hours working day. It's a good practice to manually homogenize the slurries in the flasks before sampling in the proposed FIA system.

Regarding sample decomposition in a microwave oven digester system, the use of diluted acids was not effective for a complete decomposition of some

Table 1. Factorial design matrix with central point

	Not codified variables			*Codified variables*		
Assays	**La**	**Coil length (cm)**	**Carrier flow-rate (mL min^{1})**	**Factor A**	**Factor B**	**Factor C**
	% (m/v)					
1	1	10	8	-1	-1	-1
2	5	10	8	1	-1	-1
3	1	20	8	-1	1	-1
4	5	20	8	1	1	-1
5	1	10	16	-1	-1	1
6	5	10	16	1	-1	1
7	1	20	16	-1	1	1
8	5	20	16	1	1	1
9	3	15	11	0	0	0
10	3	15	11	0	0	0
11	3	15	11	0	0	0
12	3	15	11	0	0	0

Table 2. Results for calcium determination (*n* = 3) in milk samples and SRMs (mean ± standard deviation) without pre-treatment (dilution in water) and after microwave-assisted digestion by the proposed FIA system.

Sample	**Dilution in water**	**Microwave oven decomposition**	**Labeled or certified values**
1 ^{a,*}	1200 ± 65	1325 ± 85	1200
2 ^{b,*}	1173 ± 64	1305 ± 83	1200
3 ^{c,*}	1173 ± 64	1246 ± 80	1200
4 ^{a,*}	1118 ± 61	1148 ± 73	1100
5 ^{b,*}	1122 ± 61	1148 ± 73	1100
6 ^{c,*}	1159 ± 63	1245 ± 80	1100
7 ^{a,*}	1216 ± 66	1130 ± 72	1200
8 ^{b,*}	1300 ± 71	1169 ± 75	1200
9 ^{c,*}	1154 ± 63	1130 ± 72	1200
10 ^{c,**}	15295 ± 833	16855 ± 1078	15400
11 ^{c,**}	8829 ± 481	9813 ± 628	9074
SRM 8435 ^{c,**}	9166 ± 494	9603 ± 614	9220 ± 490**
SRM 1549 ^{a,**}	12884 ± 208	12262 ± 785	13000 ± 500**

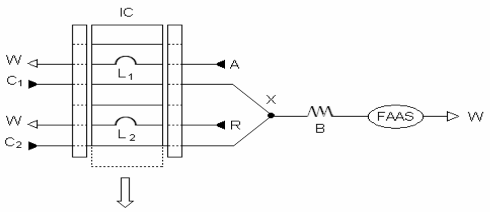

Figure 1. Flow diagram for calcium determination in milk. IC: injector-commutator; L_1,L_2: sampling loop (250 µL) for sample and lanthanum, respectively; A: analytical solutions or samples; R: 2.5% m/v La solution; C_1, C_2: carrier stream [1% (v/v) HNO_3] for sample or lanthanum, respectively; B: coiled reactor (20 cm); x: confluent point; FAAS: flame atomic absorption spectrometer (ë = 422.7 nm); W: wastes. Arrow indicates the next position of the IC.

powdered milk samples. The microwave-assisted decomposition using a mixture of concentrated nitric (4 mL) and hydrochloric (0.5 mL) acids was enough to produce clean digests solutions for different (whole, low-fat, non-fat) liquid and powdered milk samples.

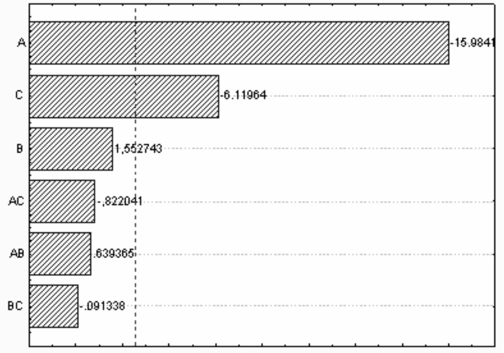

Figure 2. Pareto's chart related to influence of parameters investigated for 95% confidence level. A = lanthanum concentration; B = reaction coil length; C = carrier flow-rate; AC, AB and BC = interactions among parameters.

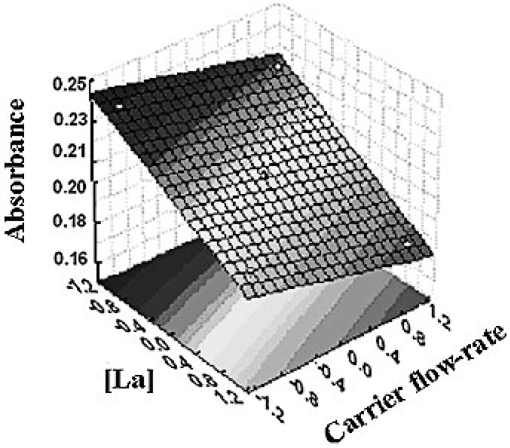

Figure 3. Response surface graph corresponding to the influence of lanthanum concentration and carrier flow-rate on absorbance.

Analytical curves with good linearity (R^2= 0.9997) were consistently obtained using 250 µL of analytical solutions in the 1.00 – 5.00 mg L^{-1} Ca range, 250 µL of 2.5% m/v lanthanum solution and fixing a flow-rate of 8.0 mL min^{-1} for carriers C_1 and C_2. The relative standard deviations (*n* = 12) was typically < 5.4% for a 4.00 mg L^{-1} Ca analytical solution. Calcium levels determined by the proposed method (dilution in water) in eleven commercial milk samples were compared with those obtained using microwave-oven digestion. Table 2 shows that similar results were obtained, mainly for liquid samples. It is important to point out too that for the studied commercial milk samples levels of calcium are close to the labeled values by manufacturer. The accuracy of the proposed method was checked after analysis of two standard reference materials (SRMs) from National Institute of Standards and Technology (SRM 8435 Whole Milk Powder; SRM 1549 Non Fat Milk Powder). A paired *t-test* showed that the obtained results were in agreement at 95% confidence level with those certified in the SRMs. Another observation considering Table 2 is that the method based on dilution of sample in water is more accurate than those using a microwave-oven system. Recoveries in the 93%-116% range were found for spiked milk samples. Limits of detection (LOD) and quantification (LOQ) calculated for Ca were 0.09 mg L^{-1} and 0.30 mg L^{-1}, respectively [21].

Conclusions

The proposed flow-injection system for direct determination of calcium in liquid and powdered milk samples is efficient, simple, relatively fast and it operates according the principles of Green Chemistry.

Milk samples could be prepared by simple dilution in water. As a consequence, analytical costs and reagents consumption are reduced, mainly the amount of lanthanum. The sample throughput was about 150 measurements per hour, corresponding to a consumption of 250 µL of sample and 6.25 mg of La per measurement. In a batch procedure, employing the same dilution factor used in the proposed method (1:250) a mass of 250 mg La is necessary.

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M.B. Petrovich, V. R. A. Filho, J. A. G. Neto , Determinação direta de cálcio em leite por espectrometria de absorção atômica empregando sistema de análise por injeção em fluxo

Resumo: Um sistema de injeção em fluxo envolvendo a adição de amostra e reagente por zonas coalescentes sincronizadas é proposto para a determinação de cálcio em leites por FAAS. Um planejamento fatorial com ponto central auxiliou na seleção dos principais parâmetros experimentais, sendo as condições ótimas estabelecidas em: solução de lantânio 2,5% (m/v), vazão do transportador 8 mL min^{-1}, comprimento da bobina de reação 20 cm e volume de amostra injetado 250 ìL. Quatro procedimentos de preparo de amostra foram avaliados: diluição em ácido nítrico diluído e em água, decomposição em forno de microondas empregando ácidos concentrados e diluídos. O sistema de análise por injeção em fluxo desenvolvido foi aplicado na determinação de cálcio em onze amostras de leite comerciais e em dois materiais de referência padrão diluídos em água. Teores similares de cálcio foram encontrados comparando-se os resultados obtidos pelo método proposto (diluição em água) com aqueles obtidos nos digeridos. As concentrações de cálcio determinadas nos dois materiais de referência padrão foram concordantes a um nível de 95% de confiança com seus valores certificados. Recuperações entre 93% a 116% foram obtidas. O desvio padrão relativo ($n = 12$) foi < 5,4% e a freqüência analítica equivalente a 150 medidas por hora, correspondendo a um consumo de 250 ìL de amostra e 6,25 mg de lantânio por determinação.

Palavras-chave: cálcio; leite;espectrometria de absorção atômica em chama; análise por injeção em fluxo.

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