Simultaneous Determination of Sodium, Potassium, Magnesium, and Calcium Ions in Milk Products by Indirect Photometric High-Performance Liquid Chromatography

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Indirect photometric chromatography (IPC) using a cerium(III) mobile phase is used to determine sodium, potassium, magnesium, and calcium ions in milk and infant formulas. Separation is complete within 17 min. No interference from the sample matrix is noted. Relative standard deviations were 5% for sodium and potassium and between 5 and 10% for magnesium and calcium. Good agreement between the IPC results and atomic absorption spectroscopy is found.

It is a common practice in quality-control laboratories of the dairy industry and the manufacturers of infant food formulas to determine the concentrations of sodium, potassium, magnesium, and calcium in their products. All four minerals are dietary essentials, and in the case of infant formulas, analysis is required by U.S. government regulations.

The determinations of these four metal ions in milk products are often carried out by ashing the organic material, dissolving the ash in concentrated acid, and, after dilution, quantitation through use of atomic absorption spectroscopy (AAS) (Murthy and Rhea, 1967). Other methods have shown that the problem of the complex matrix of milk can be overcome without the labor-intensive sample preparation process. These methods involve protein precipitation and separation (Maurer, 1977) or dilution followed by direct analysis (Rebmann and Hoth, 1971; Arpadjan and Stojanova, 1980; de la Guardia et al., 1986). However, these methods still require considerable time for the measurement step as each cation requires a different set of AAS instrumental conditions. The use of inductively coupled plasma atomic emission spectroscopy could alternatively be used. To date, no ion-exchange chromatographic methods have been shown that would enable all four cations in milk products to be determined in the same run with convenient detection.

Indirect photometric or "vacancy" chromatography (IPC) is a method by which analyte separation is effected through an ion-exchange process, following which analyte detection is achieved through a photometric process (Small and Miller, 1982). IPC uses photometrically active counterions in the mobile phase, with which nonabsorbent, injected sample ions compete for the ion-exchange sites. Upon elution from the column, the transparent sample ions substitute for the light-absorbing, displacing, counterions in the column effluent, causing a decreased absorbance at the detector and negative peaks to be recorded. Detection limits are related not only to the sharpness of the peak but also to the molar absorptivity of the mobile phase. The advantage of IPC is that it expands the capability of chromatographs equipped with either variable- or fixed-wavelength UV-vis detectors.

To date, a number of different eluents such as copper(II) (Small and Miller, 1982; Larson and Pfeiffer, 1983; Miyozaki et al., 1985), benzyltrimethylammonium ion (Larson and Pfeiffer, 1983; Iskandawani and Miller, 1985; McAleese, 1987), picolinic acid (Benson and Woo, 1984; Foley and Haddad, 1986), and benzylamine (Foley and Haddad, 1986) have been used for the separation of either inorganic or organic cations by IPC. All of these organic counterions are singly charged and would not be expected to be effective for the isocratic elution of both singly and multiply charged solute ions. The inorganic cation Cu(II) has a relatively low molar absorptivity (32 L/mol·cm) at 254 nm, and therefore determination of low levels of cations would be difficult. A Cu(II) mobile phase could be monitored at a wavelength such as 215 nm where the molar absorptivity is higher to partially alleviate this detection limit problem. However, if such a low wavelength was used, it is quite possible that components from the milk sample matrix could interfere with detection and thus quantitation of the desired cations.

Recently, we demonstrated how a cerium(III) mobile phase could be used to isocratically elute both alkali and alkaline-earth metals in a single sample by IPC (Sherman and Danielson, 1987). Detection limits at 254 nm using Ce(III) were substantially better than those found using Cu(II). We now extend the utility of this separation to the determination of sodium, potassium, magnesium, and calcium ions in milk products and infant formulas.

EXPERIMENTAL SECTION

Reagents. All solutions were prepared with doubly distilled, deionized water and were kept in Pyrex glassware. Cerium(III) mobile phase solutions were prepared from the perchlorate salt, while all standard solutions were prepared from the respective chloride salt. Lanthanum solutions were also prepared from the chloride salt. These salts were prepared from a variety of sources and were reagent grade or better in quality.

Preparation of Standard Solutions. A stock solution containing 534 ppm Na^+, 1734 ppm K^+, 120 Mg^{2+}, and 1749 ppm Ca^{2+} was prepared. A 1:100 dilution was made from this solution to produce a standard working solution. From this latter solution, five dilutions were made to prepare a series of standards in order to generate a standard response curve for the chromatographic analysis. In order to more accurately quantitate the relatively low level of magnesium ion, an additional 6 mL of a 6 ppm solution was added to these five standards. For the atomic absorption standards, another five dilutions of the standard working solution were made. To these standard solutions was added an additional 6 mL of a 6 ppm Mg^{2+} solution. Also, in order to minimize ionization of the sodium atoms in the air-acetylene flame, 10 mL of a 10 000 ppm Cs^+ solution was added. Likewise, 10 mL of a 10 000

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ppm La^{3+} solution was added to prevent the formation of calcium and magnesium phosphates.

Preparation of Milk Solutions. Skim milk, whole milk, powdered milk, and two types of infant formulas were obtained at a local grocery store. As recommended on the package, 5 level tablespoons (22.7 g) of the powdered milk was dissolved into 8 fluid oz. (237 mL) of water. Approximately 100 mL of the milk product was sonicated for 30 min. After sonication, a portion (between 0.5 and 2.0 g) of the product was weighed, quantitatively transferred to a 100-mL volumetric flask, and diluted to volume to produce a milk sample working solution. A 10-mL aliquot of the sample working solution was then placed in another 100-mL volumetric flask, along with 6 mL of a 6 ppm Mg^{2+} solution, and diluted to volume to produce the milk solution used for chromatographic analysis. Another 10-mL aliquot of the milk sample working solution was placed in a 100-mL volumetric flask, along with 6 mL of a 6 ppm Mg^{2+} solution, 10 mL of a 10 000 ppm Cs^{+} solution, and 10 mL of a 10 000 ppm La^{3+} solution, to produce the milk solution used for atomic absorption analysis. Four samples were made of each milk product for analysis by both IPC and AAS.

Chromatographic System. The chromatographic system consisted of a Waters Model 510 HPLC pump (Waters Asociates, Milford, MA), an IBM Model 9522 (254 nm) UV detector (Nicolet Instruments, Madison, WI), and a Spectra-Physics Model 4270 integrator (Spectra-Physics, San Jose, CA). The separations were effected with a 10 cm × 3.2 mm (i.d.) ION-210 transition-metals column (Interaction Chemicals, Mountain View, CA) at room temperature. The column was packed with 5-μm sulfonic acid derivatized poly(styrene-divinylbenzene) resin particles. The capacity of this strong cation exchanger was about 500 μequiv/g. The ion-exchange sites were converted from the sodium ion form to the cerium(III) form by passing approximately 2000 mL of a 0.01 M cerium(III) solution through the column at 1 mL/min. Upon equilibration of the column, the eluent was switched to a 0.05 mM cerium(III) solution in order to perform the separation.

Atomic Absorption Spectrophotometry. A Perkin-Elmer Model 560 atomic absorption spectrophotometer equipped with either a Na, K, Mg, or Ca hollow cathode lamp was used for all atomic absorption work. Conditions were as follows: sodium, 589-nm line, 0.7-nm slit width; potassium, 766-nm line, 2.0-nm slit width; magnesium, 285-nm line, 0.7-nm slit width; calcium, 423-nm line, 0.7-nm slit width. An air–actylene flame was used for all determinations.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of a separation of whole milk. A small amount of NH_4^+ ion could be detected as well as the four cations of interest. The separation is complete within 17 min with base-line resolution for each component. No interfering peaks were evident in the chromatogram. Other metals found in milk such as Cu, Fe, or Al would be expected to be at concentrations below 5 ppb after sample dilution. Although Zn^{2+} may be found at 0.1 ppm levels, all of these metals either had significantly different retention times and/or could not be detected under these chromatographic conditions. No significant difference was seen between two chromatograms representing an ashed milk sample and a nonashed sample, indicating interference by organic compounds in the sample matrix should not be a problem.

Each chromatographic standard solution was injected four times in order to generate standard response curves for each of the four ions of interest. The data for the response curves are given in Table I. The range of concentrations for each cation spans the anticipated amount to be found in the milk products. The average area of the

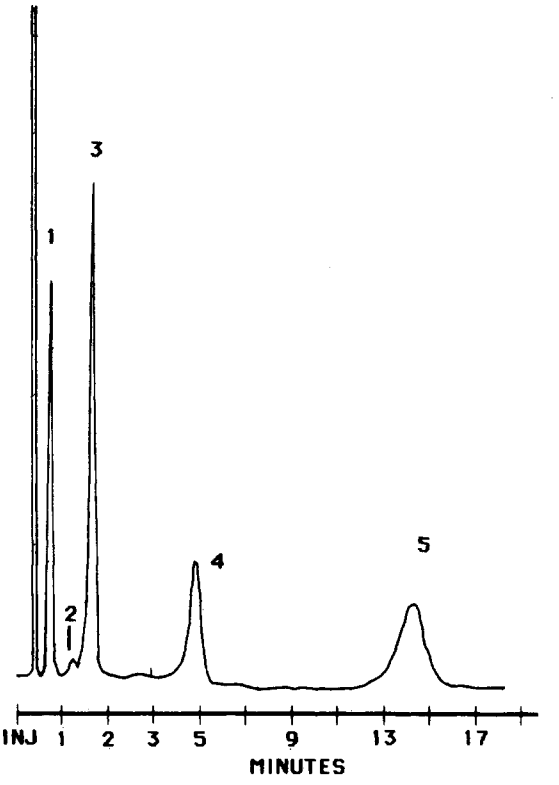

Figure 1. Separation of a whole milk sample: (1) sodium, (2) ammonium, (3) potassium, (4) magnesium, (5) calcium ions; eluent, 0.05 mM Ce(III); flow rate, 1.0 mL/min; sample volume, 20 μL. Peaks were changed positive by reversing detector output leads to the integrater. Chart speed of integrater was automatically switched from 1.0 to 0.5 cm/min at 3.0 min.

Table I. Peak Area vs Cation Concentration Standard Response Curve Data

concn, ppm	area	RSD, %	linear reg eq[a]	correln coeff[b]
			Sodium	
0.267	7.5	4.4	$y = (3.00 \times 10^4)x - 1781$	0.9964
0.534	13.8	2.7		
0.801	21.8	0.7		
1.07	29.8	0.2		
1.34	40.1	3.2		
			Potassium	
0.862	12.7	2.3	$y = (1.56 \times 10^4)x - 914$	0.9999
1.72	26.1	2.3		
2.60	39.8	1.9		
3.47	53.1	1.0		
4.34	67.3	1.9		
			Magnesium	
0.300	12.3	11	$y = (4.64 \times 10^4)x - 1903$	0.9953
0.360	15.0	7.3		
0.420	17.0	7.8		
0.480	21.7	9.8		
0.600	24.4	5.6		
0.900	40.4	4.0		
			Calcium	
0.874	17.9	8.9	$y = (2.30 \times 10^4)x - 3583$	0.9993
1.75	35.1	5.4		
2.62	56.4	2.8		
3.50	76.4	0.4		
4.37	97.8	1.5		

[a] Linear regression equation: y = peak area (arbitrary units), x = cation concentration (ppm). [b] Correlation coefficient.

Table II. Comparison of Cation Data for Milk Products Taken by Cerium(III) IPC and Atomic Absorption Spectroscopy

	IPC[a]	AAS[a]
skim milk		
Na^+	0.43 ± 0.01	0.47 ± 0.04
K^+	1.58 ± 0.01	1.75 ± 0.24
Mg^{2+}	0.14 ± 0.04	0.13 ± 0.02
Ca^{2+}	1.65 ± 0.43	1.13 ± 0.01
whole milk		
Na^+	0.45 ± 0.01	0.56 ± 0.04
K^+	1.49 ± 0.06	1.56 ± 0.02
Mg^{2+}	0.17 ± 0.02	0.12 ± 0.00
Ca^{2+}	1.24 ± 0.12	1.34 ± 0.03
powdered milk		
Na^+	0.49 ± 0.04	0.43 ± 0.01
K^+	1.58 ± 0.03	1.58 ± 0.01
Mg^{2+}	0.14 ± 0.01	0.12 ± 0.00
Ca^{2+}	1.90 ± 0.05	1.34 ± 0.02
infant formula A		
Na^+	0.55 ± 0.02	0.45 ± 0.01
K^+	1.43 ± 0.08	1.51 ± 0.03
Mg^{2+}	0.06 ± 0.00	0.06 ± 0.01
Ca^{2+}	1.29 ± 0.02	1.33 ± 0.22
infant formula B		
Na^+	0.41 ± 0.03	0.51 ± 0.00
K^+	1.54 ± 0.04	1.65 ± 0.04
Mg^{2+}	0.16 ± 0.03	0.17 ± 0.00
Ca^{2+}	1.20 ± 0.05	1.18 ± 0.01

[a] Concentrations given as milligrams of ion of interest per gram of sample ± standard deviation. $n = 4$ (multiple runs of the same preparation).

four injections for each cation concentration is given along with the relative standard deviation (RSD) for the series of injections. In general, the RSD values were less than 5% for sodium and potassium, due to the sharpness of the peaks. For magnesium and calcium, the RSD values were generally between 5 and 10%, because the peaks were broader and more difficult to integrate. The average RSD for the slopes of the linear regression equation was 5% or less. The correlation coefficients indicate a great deal of linearity in the response of each ion. Along with the correlation coefficient, the equation of the least-squares regression line is reported. Upon completion of the standard response curve, three types of milk and two infant formulas were chosen for analysis. The contents of sodium, potassium, magnesium, and calcium were determined in each sample by IPC and compared to the results obtained through the use of AAS (Table II). The correlation coefficient for a plot of the IPC values versus the AAS values was 0.9811. Reproducibility of the IPC method was generally less than 5% RSD for Na^+ and K^+ and less than 13% RSD for Ca^{2+} and Mg^{2+}. On the basis of the t-test, confidence limits at the 90% probability level approximate the standard deviation.

Comparison of the IPC data and typical mean values of these cations found in milk products are shown in Tables III and IV. Close agreement between our data and the typical mean values in Table III would not be expected since the latter data sets can have high standard derivations. Table IV shows the IPC data compared with the legally permitted values for sodium, potassium, magnesium, and calcium in infant formulas. The table shows that all but one magnesium determination fall within legally permitted limits.

After the chromatographic analysis of the five milk products was complete, the guard column–analytical column system was checked for degradation. Standard solutions were reinjected and their retention times compared with those of the original standard response curve. The change in retention times of the four ions from the first injection to the last injection was only 1% or less. Thus, after approximately 300 injections on this guard column–analytical column combination, essentially no degradation of performance was evidenced.

Table III. Comparison of IPC Data to Typical Mean Values for Whole Milk, Skim Milk, and Powdered Milk

value	IPC value, mg/8 fl oz.	typical mean value,[a] mg/8 fl oz.
whole milk		
Na^+	109	120
K^+	364	370
Mg^{2+}	33	28
Ca^{2+}	404	302
skim milk		
Na^+	106	126
K^+	388	406
Mg^{2+}	33	28
Ca^{2+}	404	302
powdered milk		
Na^+	100	128
K^+	387	430
Mg^{2+}	34	26
Ca^{2+}	465	301

[a] Pennington and Church, 1985.

Table IV. Comparison of U.S. Legally Permitted Values[a] for Sodium, Potassium, Magnesium, and Calcium in Infant Formulas to Values Found by IPC (mg/100 kcal)

	min	max	infant formula A	infant formula B
sodium	20	60	35	32
potassium	80	200	118	120
magnesium	6	*b*	4	13
calcium	50	*b*	104	94

[a] de la Guardia et al., 1986. [b] No maximum legal value.

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Registry No. Na, 7440-23-5; K, 7440-09-7; Mg, 7439-95-4; Ca, 7440-70-2.

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A New Method for Determination of Insoluble Cell Walls and Soluble Nonstarchy Polysaccharides from Plant Materials

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A two-step enzymatic procedure was established for accurate determination of insoluble plant cell walls (including polysaccharides, lignin, and wall proteins), the major component of "dietary fiber". Cytoplasmic proteins are efficiently eliminated by Pronase with presence of sodium lauryl sulfate and 2-mercaptoethanol, and starch is enzymatically removed by amyloglucosidase after solubilization in hot 90% DMSO and limited breakdown by Termamyl in boiling 30% DMSO. Cell walls of a good standard of purity are obtained with respect to their protein content (1–8%). The method allows the quantitative gravimetric determination of the plant cell walls with a good precision (cv = 5.6%), whatever the wall percent from very low contents (semolina, 1.4%, cv = 5.9%) to very high ones (wheat straw, 84.4%, cv = 0.9%). The technique is rapid and operates under mild conditions suitable for chemical preservation of wall polysaccharides. A separate determination of soluble nonstarchy polysaccharides by gas–liquid chromatography of alditol acetates from monosaccharides obtained after acid hydrolysis showed that they represent in most samples a very low proportion of total "dietary fiber" but in cereal ones (endospermic parts only) they constitute ~20–40%.

Since Trowell (1976) reported the beneficial action of so-called "dietary fiber" in man for prevention of intestinal diseases in developed countries, considerable literature has arisen reporting experiments on man and animal fed fiber-supplemented diets (Cummings et al., 1978; Bertrand et al., 1981; Nyman and Asp, 1982), food and feed composition tables (Englyst et al., 1982; Carré and Brillouet, 1986), and methodology of fiber determination (Schweizer and Würsch, 1979; Selvendran and DuPont, 1980; Englyst and Cummings, 1984; Asp et al., 1983; Prosky et al., 1984).

Although the biochemical significance of recently developed methods for the determination of dietary fiber has considerably improved as compared to the former crude fiber method, they are still proposed on nutritional grounds in accordance with Trowell's definition (1976) contrary to other plant nutrients (proteins, starches, ...) that are basically measured by chemical [e.g., DMSO–hydrochloric acid method for starches (Boehringer, 1976)] techniques prior to application of methods approximating their nutritional behavior [e.g., α-amylolysis for starches (Tollier et Guilbot, 1971)]. An excellent biochemical procedure has been developed by Selvendran (1975) for preparation of plant cell walls that was not initially designed for nutritional purposes; it provides a cell wall residue of optimum purity with respect to residual intracellular proteins and starch but could hardly be fitted to heavy-user requirements due to its length.

The aim of the present study was therefore to develop a rapid and accurate sequential enzymatic method for gravimetric determination of plant cell walls of general applicability to raw plant materials and processed food and feedstuffs. The related soluble nonstarchy polysaccharides will also be considered. Biochemical aspects of the technique (purity of residues, preservation of chemical structures, ...) will be emphasized.

EXPERIMENTAL SECTION

Samples. Description of the various products studied in the present work is presented in Tables I and II. These materials were ground in an IKA grinder with tap water refrigeration for not longer than 3 min; the ground material was sifted through a 0.5-mm screen, and coarser particles were reground until all the sample passed through a 0.5-mm sieve.

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