



Evaluation of Ca/P Ratios in Infant Milk Samples by CH₄-Ar Mixed Plasma-DRC-MS

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Methane mixed plasma dynamic reaction cell mass spectrometry was successfully used for accurate determination of Ca and P and Ca/P ratios in infant milk samples. The interfering $^{40}\text{Ar}^+$ at m/z 40 was reduced in intensity by up to five orders of magnitude using 1.1 mL min^{-1} CH_4 as reaction gas in a dynamic reaction cell. Polyatomic ion interferences ($^{14}\text{N}^{16}\text{O}^+\text{H}^+$, $^{15}\text{N}^{16}\text{O}^+$ and $^{14}\text{N}^{17}\text{O}^+$ etc.) at m/z 31 were eliminated by detecting P^+ as the product oxide ions $^{31}\text{P}^{16}\text{O}^+$ using O_2 as the cell gas. The poor sensitivity of $^{31}\text{P}^{16}\text{O}^+$ was improved to 3-fold by addition of 3 mL^{-1} methane to the plasma. An extending dynamic range technique based on adjusting the dynamic reaction cell bandpass parameters (RPa) was successfully employed to extend the linearity of $^{40}\text{Ca}^+$ from 1 mg L^{-1} up to 100 mg L^{-1} . LOD of Ca and P was 0.3 and 4 ng mL^{-1} , respectively. Results of three milk powder standard reference materials were in good agreement with the certified values. The proposed method was applied to determination of 160 milk samples collected from twenty different markets of China and the results of Ca/P ratio ranged from 1.3 to 1.9, which accorded with the official standards (1-2) of FAO/WHO.

Keywords: Ca/P ratios, CH₄-Ar mixed plasma-dynamic reaction cell mass spectrometry, Infant milk samples.

INTRODUCTION

Milk is the main basic foods for infants, because they contain essential nutrients (proteins, lipids, carbohydrates) and micronutrients (minerals, vitamins, enzymes)¹. The ratio of calcium to phosphorus (Ca/P ratio) in milk and dairy products is an important factor for the absorption of calcium. The absorption of calcium deteriorate when the Ca/P ratio is too low in the diet². According to the official standards (CODEX STAN 72-1981) of FAO/WHO, the ratio of Ca/P in infant formula should be in the range 1-2³.

Inductively coupled plasma mass spectrometry (ICP-MS), one of the most sensitive techniques, has been used extensively for determining trace elements in a wide range of foodstuffs⁴⁻⁸. Unfortunately, the determination of Ca or P by ICP-MS suffers from high background problems: $^{40}\text{Ca}^+$ (96.97 %) is interfered by $^{40}\text{Ar}^+$, $^{44}\text{Ca}^+$ (2.06 %) is interfered by $^{12}\text{C}^{16}\text{O}_2^+$ and $^{14}\text{N}_2^{16}\text{O}^+$, $^{42}\text{Ca}^+$ (0.64 %) is interfered by $^{40}\text{ArH}_2^+$ and $^{31}\text{P}^+$ (100 %) is interfered by $^{14}\text{N}^{16}\text{OH}^+$, $^{15}\text{N}^{16}\text{O}^+$ and $^{14}\text{N}^{17}\text{O}^+$ ^{9,10}. Some authors used the cool or cold plasma technique to alleviate the Ar-based interferences in ICP-MS analysis^{11,12}. However, it cannot eliminate the interfering N-oxide or hydroxides ions on P determination¹³.

Recently, it has been proved that the dynamic reaction cell (DRC) technique coupling with ICP-MS can alleviate effectively mass spectral interferences¹⁴⁻¹⁷. Chen *et al.*¹⁸

demonstrated CH_4 as the reaction gas for the determination of Ca, Fe and Zn in milk powder by DRC-ICP-MS. Bandura *et al.*¹³ successfully used O_2 as the reaction gas to oxidize S^+ and P^+ to allow their detection as SO^+ and PO^+ , this method reduced the effect of polyatomic isobaric interference at m/z 31 and 32 by detecting S^+ and P^+ as the product oxide ions that were less interfered. Yang *et al.*¹⁹ also approved the feasibility of the O_2 DRC-ICP-MS method for determination of $^{28}\text{Si}^+$, $^{31}\text{P}^+$ and $^{32}\text{S}^+$ as the oxide ion $^{28}\text{Si}^{16}\text{OH}^+$, $^{31}\text{P}^{16}\text{O}^+$ and $^{32}\text{S}^{16}\text{O}^+$ at m/z 45, 47 and 48, respectively. However, few papers take into account the simultaneous elimination of the complicate interferences for Ca and P determination in a single analysis²⁰, also no study of the improvement of poor PO sensitivity and suppression the excessively high sensitivity of Ca in ICP-DRC-MS has been reported so far.

The objective of this study was the development of a DRC-ICP-MS technique for the accurate determination of Ca and P and Ca/P in milk powder and liquid milk samples. This experiment has centered on the reduction of interferences on Ca and P using two strategies with ion-molecular reactions in a DRC. Also, the improvement of the $^{31}\text{P}^{16}\text{O}^+$ sensitivity by the methane-argon mixed plasma and on-line suppression the excessively high signal of $^{40}\text{Ca}^+$ were discussed. The optimization of the CH_4 -Ar mixed plasma-DRC-MS technique, its analytical performances and its applications to the determination of Ca and P and Ca/P ratios in selected samples were described in this paper.

EXPERIMENTAL

A Perkin-Elmer SCIEX ELAN DRC-e (dynamic reaction cell) ICP-MS instrument was described in detail elsewhere²¹. Methane (99.999 %, purity) gas was added to an additional port in the cyclonic spray chamber (PC³, Elemental Scientific, USA) via a calibrated mass-flow controller (32907, Cole-Parmer, USA), capable of regulating minute (measuring range 0-10 mL min⁻¹, precision 0.01 mL min⁻¹) flows of the gas. The operating parameters of the DRC-ICP-MS used for this work are summarized in Table-1. A CEM MARS X-press (CEM, Matthews, NC, USA) microwave apparatus equipped with Teflon vessels was used to digest the samples.

TABLE-1
INSTRUMENT OPERATING PARAMETERS

ICP-MS instrument	Perkin-Elmer Sciex Elan DRC-e
Sample introduction	PFA-400 MicroFlow nebulizer
Spray chamber	Cyclonic spray chamber (PC ³ Peltier Chiller)
Injector tube	1 mm id Quartz
RF power, W	1300
Plasma gas flow, L min ⁻¹	16
Auxiliary gas flow, L min ⁻¹	1.00
Nebulizer gas flow, L min ⁻¹	0.82 (Ar-plasma)
	0.74 (CH ₄ -Ar mixed plasma)
Optional gas (CH ₄), mL min ⁻¹	3.0
DRC gas	
CH ₄ (Channel A), mL min ⁻¹	1.1
O ₂ (Channel B), mL min ⁻¹	1.0
Rejection parameter, q	0.80 for ⁴⁰ Ca ⁺ , 0.70 for ³¹ P ¹⁶ O ⁺ , 0 for other ions
Rejection parameter, a	0.08 for ⁴⁰ Ca ⁺ , 0 for other ions
Autolens	on
Dwell time, ms	50
Sweeps	20
Readings	1
Replicate	3
Monitored ratios	⁴⁰ Ca ⁺ , ³¹ P ¹⁶ O ⁺ , ⁴⁷ Ti ⁺ and ⁴⁷ Ti ¹⁶ O ⁺

Reagents and standards: High purity water (18.2 MΩ cm⁻¹) used for the preparation of all blank, standards and sample solutions was obtained from a Millipore water purification system (Millipore, France). The stock solutions of Ca (1000 mg L⁻¹) and P (1000 mg L⁻¹) were purchased from National Center for Analysis and Testing of Steel Materials, China. Nitric acid (65-70 %, w/w, 99.9999 %) and hydrogen peroxide (35 %, w/w) were purchased from Alfa Aesar (Tianjing) Ltd. and Thermo Fisher Scientific Ltd. (Fisher scientific, USA), respectively. Two non-fat milk powder SRMs NIST 1549 (NIST, USA) and GBW08509a (NRCCRM, China) and a whole milk powder SRM GBW10017 (NRCCRM, China) were used to validate the proposed method.

Sample preparation: About 200 mg of milk sample or SRM was weighed into the Teflon vessel, to which 4 mL of HNO₃ and 2 mL of H₂O₂ were added. Samples were pre-digested 1 h at room temperature. Then digested using the following procedures: the temperature was ramped to 160 °C within 10 min at 800 W, holding 5 min and then ramped to 200 °C with 5 min at 1600 W, holding 0.5 h. After cooling, the final solution was made up to 50 mL by addition of ultra-pure water.

RESULTS AND DISCUSSION

Elimination of the interferences by dynamic reaction cell:

The operation of the dynamic reaction cell (DRC) could be mainly controlled by the cell gas type and flow rate of the reaction gas and values of the rejection parameter *q* (RPq) of the DRC system. Chen and Jiang¹⁸ found that a stable signal could be obtained in a shorter pressurized delay time when CH₄ was used as the reaction gas than NH₃. However, none of the reaction gases studied could react only with the ¹⁴N¹⁶OH⁺ and ¹⁵N¹⁶O⁺ while leaving the ³¹P⁺ free from interferences at its 'natural' isotope mass without suppressing its intensity significantly¹⁹. Another alternative is to use of the PO⁺ products of oxidation of P⁺ with O₂ in the DRC as analyte ion reduced the complicate interferences, because of the N-oxides or hydroxides cannot react with O₂²⁰. In this work, CH₄ was selected as the reaction gas to eliminate the ⁴⁰Ar⁺ interferences on ⁴⁰Ca⁺ determination and O₂ was used to reduce the N-oxides or hydroxides interferences by oxidation of ³¹P⁺ to ³¹P¹⁶O⁺ in a DRC. Fig. 1 shows the effects of the DRC gas flow rate on the signals of 20 ng mL⁻¹ Ca and 2 % HNO₃ blank solutions. The background equivalent concentration (BEC) was used as a criterion for DRC optimization. As shown in Fig. 1, at low CH₄ flow rate there was a rapid decrease of the signals of interfering species at *m/z* 40, which was mainly due to the reactions of ⁴⁰Ar⁺ with CH₄. With increasing CH₄ flow rate, the signal of ⁴⁰Ca⁺ was reached at a platform (Fig. 1) and the optimized CH₄ flow rate was selected at 1.1 mL min⁻¹ and the interference ions were reduced by up to 10⁵-fold. Fig. 2 shows the production profile of ³¹P¹⁶O⁺ by oxidizing reactions between ³¹P⁺ and O₂ in the DRC. There is a significant increase signal at *m/z* 47 which is mainly from ³¹P¹⁶O⁺ at a low O₂ cell gas flow rate, meanwhile the background signal at *m/z* 47 decreased gradually. The best BEC of 48 ng mL⁻¹ could be obtained for ³¹P¹⁶O⁺ when O₂ flow rate was set at 1 mL min⁻¹ (Fig. 2). The RPq values are important to filter out unwanted precursors of interfering species from the ion beam to eliminate interferences created in the cell by reaction gas. The optimized RPq value was 0.80 for ⁴⁰Ca⁺ and 0.70 for ³¹P¹⁶O⁺, the best BEC of ⁴⁰Ca⁺ was 0.09 ng and 48 ng mL⁻¹ for ³¹P¹⁶O⁺, respectively. The

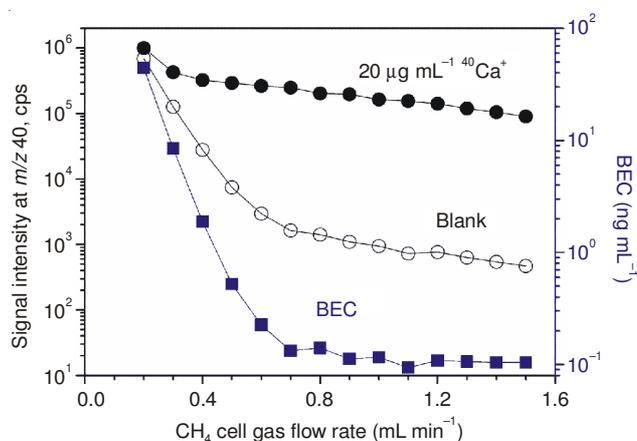


Fig. 1. Effects of CH₄ cell gas flow rate on ⁴⁰Ca⁺ signal intensity, matrix blank and background equivalent concentration (BEC). The concentration of Ca was 20 ng mL⁻¹. 2 % HNO₃ was treated as the matrix blank. RPq = 0.80

interferences of new m/z 47 from $^{47}\text{Ti}^+$ should be considered in this work. Fig. 3 shows the $^{47}\text{Ti}^+$ was effectively oxidized to $^{47}\text{Ti}^{16}\text{O}^+$ by O_2 in the DRC, which was similar with the results of Bandura *et al.*²². Thus, this method can be to a certain degree tolerant of the presence of Ti in food samples.

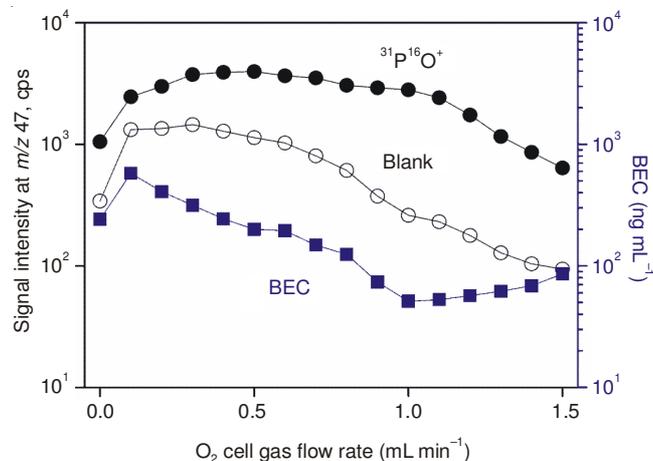


Fig. 2. Effects of O_2 cell gas flow rate on $^{31}\text{P}^{16}\text{O}^+$ signal intensity, matrix blank and background equivalent concentration (BEC). The concentration of P was 0.5 mg L^{-1} . 2% HNO_3 was treated as the matrix blank. $\text{RPq} = 0.70$

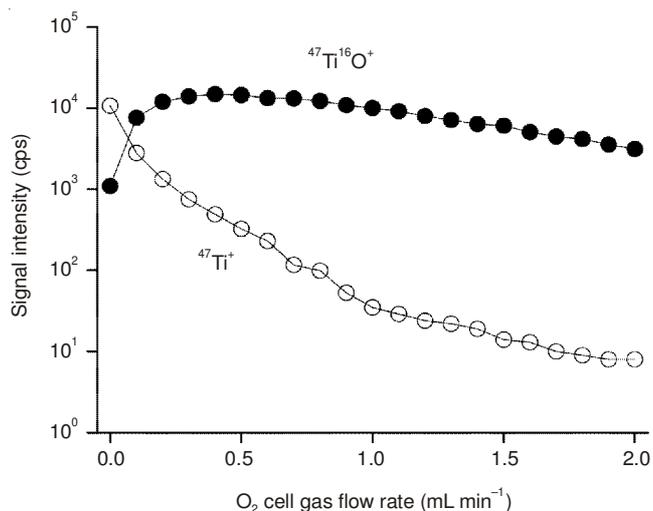


Fig. 3. Effects of O_2 cell gas flow rate on signals of $^{47}\text{Ti}^+$ and $^{47}\text{Ti}^{16}\text{O}^+$. The concentration of Ti was 10 ng mL^{-1} . $\text{RPq} = 0.70$

Signal improvement of PO by CH_4 -Ar mixed plasma:

The ionization potential for phosphorus is high (10.5 eV) which results in low ionization efficiency in the plasma and consequently, low sensitivity. Fortunately, some researchers reported that addition of organic compounds to the analyzed solution could increase signal intensity of the high ionization energy elements by ICP-MS detection²³⁻²⁸. In order to improve the poor signal intensity of $^{31}\text{P}^{16}\text{O}^+$, addition amounts of methane (3 mL min^{-1}) to the plasma was evaluated in this work. Fig. 4 shows the signal intensity changes as a function of the nebulizer gas flow rate in the conventional Ar-plasma-DRC-MS and CH_4 -Ar mixed plasma-DRC-MS. When the containing carbon compounds are introduced to the ICP, the maximum signal of $^{31}\text{P}^{16}\text{O}^+$ shifts to lower nebulizer flow rate (from 0.82 to 0.74

mL min^{-1}) due to changes in the plasma temperature and decreasing the nebulizer flow rate could improve matrix decomposition. As shown in Fig. 4, comparing to the conventional ICP-DRC-MS, the maximum signal intensity of $^{31}\text{P}^{16}\text{O}^+$ by CH_4 -Ar mixed plasma was increased 3-fold (from 4850 to 14 754 cps), meanwhile the blank signal for m/z 47 was decreased from 209 to 172 cps. In addition, the signal intensities of Ca and blank at m/z 40 were nearly not changes with or without methane addition. Therefore, the CH_4 -Ar mixed plasma was used to improve the sensitivity of PO instead of the conventional Ar-plasma.

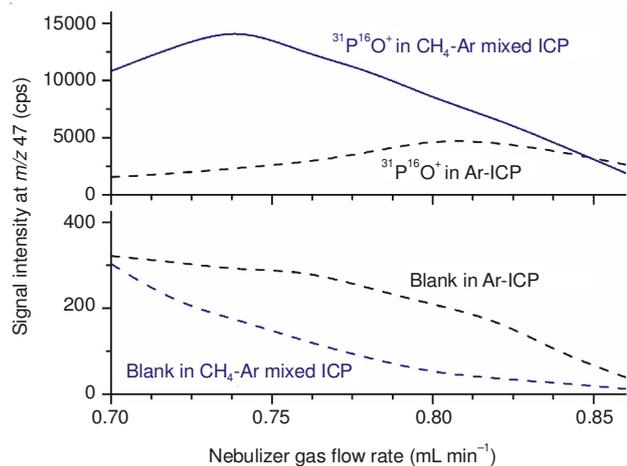


Fig. 4. Effects of the nebulizer gas flow rates on the signal intensity of PO and blank in conventional Ar-plasma-DRC-MS and CH_4 -Ar mixed plasma-DRC-MS. The CH_4 -Ar mixed plasma was defined as addition of 3 mL min^{-1} methane to the conventional Ar-plasma. The concentration of P was 1 mg L^{-1} . 2% HNO_3 was treated as the matrix blank. O_2 cell gas flow rate of DRC was 1 mL min^{-1} . $\text{RPq} = 0.70$

On-line dilution the high signal of $^{40}\text{Ca}^+$: In general, the Ca content of milk samples in analyzed solutions are ranged from 1 to 100 mg L^{-1} , which taking into account the dilution factor of 250. Further dilution of the sample is not always possible since $^{31}\text{P}^{16}\text{O}^+$ is of low sensitivity (4800 to $15\,000 \text{ cps/mg L}^{-1}$, Fig. 5C and D) comparing to that of $^{40}\text{Ca}^+$ ($7720\,000 \text{ cps/mg L}^{-1}$ in Fig. 5A). Although the detectors of ICP-MS are capable of measuring signals over nine orders of magnitude, there are still cases where very high concentrations of elements saturate the detector, such as 100 mg L^{-1} of Ca (Fig. 5C). In this work, we used a so-called extending the dynamic range (EDR) techniques²⁹ to suppress the high signal of Ca, which is accomplished by adjusting the DRC bandpass parameters (RPa) on a per-mass basis, sensitivity can be reduced on the selected isotopes without affecting other elements in a single analysis. Fig. 6 shows the effects of RPa value on the signal of Ca and background at m/z 40. As shown in Fig. 6, the extent of dilution factor of Ca can be selected by varying the RPa value. Meanwhile, the background signal at m/z 40 is also suppressed effectively with increasing of the RPa value. Taking into account the sensitivity and concentration of PO, 0.08 was selected as the RPa value in our work. Fig. 5 shows the calibration curves for Ca and PO with different methods: (A) Ca without EDR techniques; (B) Ca with EDR techniques; (C) PO with CH_4 -Ar mixed plasma; (D) PO with Ar-plasma.

The linearity ranges of Ca are extending up to 100 mg L⁻¹ (Fig. 5B), however, it only 1 mg L⁻¹ for Ca without EDR techniques (Fig. 5A).

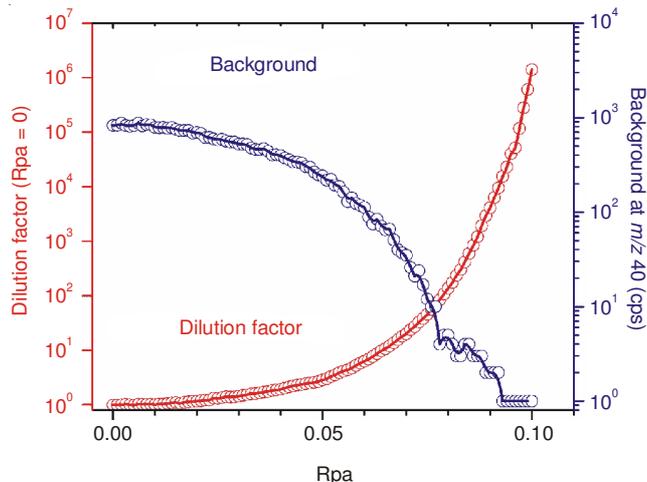


Fig. 5. Effects of RPa value on the dilution factors of ⁴⁰Ca⁺ signal and the background at *m/z* 40. The dilution factors were normalized to values obtained with RPa = 0. The concentration of Ca was 100 ng mL⁻¹. 2 % HNO₃ was treated as the matrix blank. The methane-argon mixed plasma-DRC-MS with the O₂ cell gas flow rate was 1.0 and RP_q = 0.70

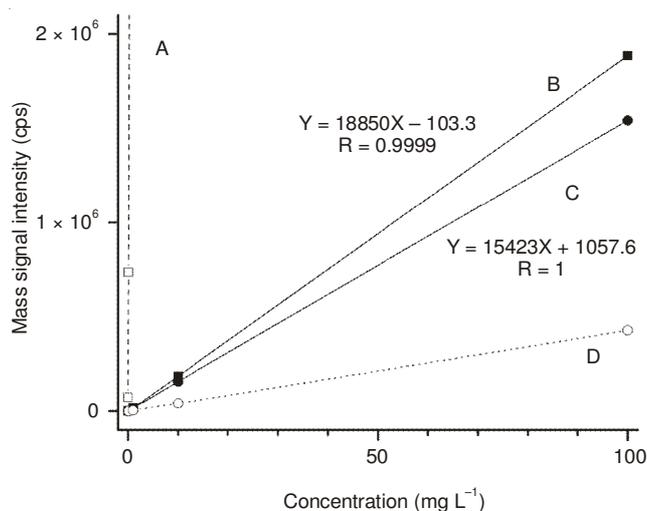


Fig. 6. Calibration curves for Ca and P by ICP-DRC-MS with different methods. (A) Ca without EDR techniques (RPa = 0); (B) Ca with EDR techniques (RPa = 0.08); (C) PO with CH₄-Ar mixed plasma; (D) PO with Ar-plasma

Analytical performances: The limit of detection (LOD, three times the standard deviation of produce blank) of 0.3 and 4 ng mL⁻¹ was obtained for Ca and P, respectively. To investigate the accuracy and precision of the proposed method, two non-fat milk powder reference materials (NIST SRM 1549

and GBW08509a) and a whole milk powder reference material (GBW10017) were analyzed (Table-2). The results of Ca and P and Ca/P ratios agreed well with the certified values. Reproducibility was calculated based on the triplicate sample digestions and analyses and was generally less than 5 % (RSD).

Ca and P and Ca/P ratios in infant milk products: In order to investigate the Ca, P and Ca/P ratios in infant milk products, eighty milk powder samples and eighty liquid milk samples were collected from 20 different markets during 2009 in China. For comparison, five breast milk samples came from five subscribers (breast-feeding period mothers). As shown in Fig. 7, the ratios of Ca/P were in the range of 1.3-1.9 in these infant foods, which were in agreement with the regulations of FAO/WHO (1-2). However, the Ca/P ratios of the infant milk powder samples (1.5-1.9) and the nature breast milk samples (1.7-1.8) are more agreement than the liquid milk samples (1.3-1.6). It may be demonstrated that the infant milk powder could be more conformable for baby who lacks breast milk feeding.

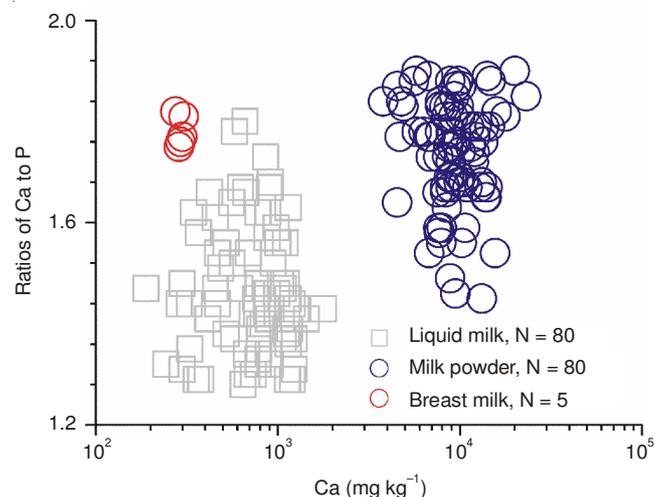


Fig. 7. Results of the Ca/P ratio as a function of the Ca concentrations in liquid milk, milk powder and the breast milk samples

Conclusion

A valid method has been developed for the determination of Ca and P and ratios of Ca/P in milk products by dynamic reaction cell ICP-MS. The significant interferences on ⁴⁰Ca⁺ and ³¹P⁺ determination were eliminated by CH₄ and O₂ as the reaction gas in a DRC, respectively. The methane-argon mixed plasma was found to be an excellent method for improving the poor signal intensity of ³¹P₁₆O⁺ (as the target ions for the ³¹P⁺ determination) to 3-fold. The EDR technique was used to suppress the high signal of Ca and extending the linearity up to 100 mg L⁻¹. The simple and accurate method has great

TABLE-2
RESULTS OF THE SRMS FOR Ca AND P AND Ca/P RATIO, N = 3

Sample	Ca (mg kg ⁻¹)		P (mg kg ⁻¹)		Ca/P ratio	
	Detected	Certified	Detected	Certified	Detected	Certified
NIST 1549	13100 ± 110	13000 ± 500	10600 ± 120	10600 ± 200	1.24	(1.23)
GBW08509a	12850 ± 120	12800 ± 200	10500 ± 100	10400 ± 300	1.22	(1.23)
GBW10017	9500 ± 110	9400 ± 300	7600 ± 100	7600 ± 300	1.25	(1.24)

potential for the investigation of Ca/P ratios in various food samples.

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