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ORIGINAL PAPER

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Determination of the ratio of calcium to phosphorus in foodstuffs by dynamic reaction cell inductively coupled plasma mass spectrometry

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Abstract An inductively coupled plasma mass spectrometer (ICP–MS) equipped with a dynamic reaction cell (DRC) was used for the determination of Ca and P in foodstuffs. In this study, two different reaction gases, CH_4 and O_2, were introduced successively through the different channels to alleviate different interferences in the same analysis run. The effect of the operating conditions of the DRC system was studied to get the best signal-to-noise ratio for each element. The interfering $^{40}Ar^+$ at *m/z* 40 was reduced in intensity by up to five orders of magnitude by using 1.0 mL min^{-1} CH_4 as reaction cell gas in the DRC. On the other hand, by using O_2 as the reaction gas, $^{31}P^+$ was converted to $^{31}P^{16}O^+$ that could be detected at *m/z* 47 where there was less interference. The limits of detection for Ca and P were 0.2 ng mL^{-1} and 0.3 ng mL^{-1}, respectively. This method was used to determine the concentrations of Ca and P and the ratio of Ca to P in NIST SRM 1549 non-fat milk powder and NIST RM 8345 whole milk powder reference materials and an infant milk powder and an infant cereal-rice sample purchased locally. The results for the reference samples agreed satisfactorily with the reference values. The accuracy of the determination was better than 4.1 and 0.9% for Ca and P, respectively. The results for infant milk powder and infant cereal were also found to be in good agreement with the value on the bottle label. Precision (RSD) between sample replicates was better than 4.8% for all the determinations.

Keywords Inductively coupled plasma mass spectrometry · Dynamic reaction cell · Calcium · Phosphorus · Foodstuffs

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Introduction

Calcium is essential to humans in order to maintain calcium homeostasis. Calcium serves many different purposes in the body [1, 2, 3, 4]. The ratio of calcium to phosphorus in foodstuffs is an important factor for the absorption of calcium. The absorption of calcium will deteriorate when the Ca to P ratio is too low in the diet. The ratio of Ca to P in foodstuff is particularly important for the infant. Milk and cereal are two important basic foods for infants, because they contain essential nutrients and micronutrients [5]. According to the regulations of Taiwan government, the ratio of Ca to P in infant milk powder should be in the range 1.2–2.0.

Inductively coupled plasma mass spectrometry (ICP–MS) is a powerful technique for trace multielement and isotopic analysis. It has been applied to a wide range of samples. However, it still has some limitations. The Ca and P contents of foodstuffs tend to be high. However, the determination of Ca and P by ICP–MS suffers from high background problems. Specifically, $^{40}Ca^+$ (96.97%) is interfered by $^{40}Ar^+$, $^{44}Ca^+$ (2.06%) is interfered by $^{12}C^{16}O_2^+$ and $^{14}N_2{}^{16}O^+$, $^{42}Ca^+$ (0.64%) is interfered by $^{40}ArH_2^+$, and $^{31}P^+$ (100%) is interfered by $^{14}N^{16}OH^+$ and $^{15}N^{16}O^+$. The analysis of phosphorus and calcium in foodstuffs will suffer from extra $^{14}N^{16}OH^+$ and $^{12}C^{16}O_2^+$ polyatomic ion interference caused by major constituents of foodstuffs. The determination of Ca and P in foodstuffs by ICP–MS is not an easy task. The cool or cold plasma technique has been successfully used to alleviate the interference caused by Ar^+ in ICP–MS analysis [6, 7]. The cool plasma technique cannot, however, be used for the removal of $^{14}N^{16}OH^+$ polyatomic ion interference in the determination of P. High-resolution ICP–MS has been successfully applied to the determination of trace elements in milk samples [5]. The reaction cell and/or collision cell technique have proved to be effective methods for alleviating spectroscopic interferences in ICP–MS analysis [8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. Vollkopf et al. demonstrated that NH_3 could be used as the reaction gas to alleviate carbon- and chloride-based spectral interference [9] and most argide inter-

ferences [17] in dynamic reaction cell (DRC) ICP–MS analysis. Chen et al. employed CH_4 as the reaction gas for the determination of Ca, Fe, and Zn in milk powder by DRC ICP–MS [14].

In the present work a DRC ICP–MS instrument and technique were employed for the determination of Ca and P in foodstuffs. In this study two different reaction gases were introduced into the DRC cell through different channels, successively, for determination of Ca and P in the same analytical run. The optimization of the DRC ICP–MS technique, its analytical figures of merit, and its application to the determination of Ca and P in selected foodstuffs are described in this paper.

Experimental

Instrumentation

An Elan 6100 DRC ICP–MS instrument (Perkin–Elmer Sciex, Concord, ON, Canada) was used. Samples were introduced with a concentric nebulizer with cyclonic spray chamber. ICP and DRC conditions were selected that maximized the ion signals of the elements studied while reducing the background to a minimum. Phosphorus standard solution was prepared from $H_2NaPO_4.H_2O$ (Sigma, St Louis, MO, USA). A mixture of 50 ng mL^{-1} Ca (AccuStandard, CT, USA) and 100 ng mL^{-1} P in 1% *v/v* HNO_3 (Tracepur, Merck, Germany) and 1% *v/v* HNO_3 (to be treated as the blank) were introduced into the nebulization system, successively, for optimization of Ca and P analysis. The aerosol generated was then transported to the ICP–MS for Ca and P determination. The DRC conditions were then selected to afford the best conditions for each element. Various gases used, including NH_3, CH_4, O_2, and H_2 were from Air Liquide, Taiwan (99.999% purity). The operating conditions of the DRC and ICP–MS used for this work are summarized in Table 1. Data acquisition parameters used for this study are also listed in Table 1.

A CEM MARS 5 (CEM, Matthews, NC, USA) microwave apparatus equipped with Teflon vessels was used to digest the foodstuffs.

Table 1 Equipment and operating conditions

ICP–MS instrument	Perkin–Elmer Sciex Elan 6100 DRC
Plasma conditions	
RF power	1300 W
Plasma gas flow	15 L min^{-1}
Auxiliary gas flow	1.10 L min^{-1}
Nebulizer gas flow	1.00 L min^{-1}
DRC parameters	
CH_4 reaction gas flow (channel A)	1.0 mL min^{-1}
O_2 reaction gas flow (channel B)	1.0 mL min^{-1}
Rejection parameter, *q*	0.86 for ^{40}Ca, 0.58 for $^{31}P^{16}O$
Rejection parameter, *a*	0.0
Autolens	On
Mass spectrometer settings	
Resolution	0.7 amu at 10% peak maximum
Dwell time	100 ms
Sweeps	3
Readings	1
Replicate	5
Mass-to-charge ratios monitored	^{40}Ca and ^{103}Rh, $^{31}P^{16}O$ and ^{103}Rh
Cell gas changes pause time	
Pressurize delay (from standard to DRC mode)	30 s
Exhaust delay (from DRC to standard mode)	30 s
Channel delay (gas channel change in DRC mode)	25 s

Sample preparation

The applicability of the method to real samples was demonstrated by the analysis of non-fat milk powder reference material NIST SRM 1549 and whole milk powder reference material NIST RM 8345 (National Institute of Standards and Technology, USA); an infant milk powder and an infant cereal-rice sample purchased from the local market. The sample dissolution procedure is described below. About 0.25 g of foodstuff was weighed into closed Teflon vessels. HNO_3 (70% *m/m*, 5 mL) was added to each vessel [14]. These mixtures were heated inside a CEM MARS 5 microwave digester to decompose the powder samples. After cooling, the digest was transferred to a 25-mL volumetric flask and diluted to the mark with pure water, followed by a 1:100 dilution after appropriate amounts of rhodium internal standard had been added to for ICP–MS analysis. Blank and standard solutions were prepared in 1% HNO_3. Rh (1 ng mL^{-1}) was added to all the standard and sample solutions to work as the internal standard. The final analyzing solutions contained about 0.01% *m/v* of powder samples. These solutions were then introduced into the DRC ICP–MS for the determination of Ca and P. The amounts of Ca and P present in these sample solutions were quantified by DRC ICP–MS with external calibration.

Results and discussion

Selection of DRC ICP–MS conditions

Several parameters affect the operation of the dynamic reaction cell (DRC). The type and flow rate of the reaction gas and values of the rejection parameter *q* (*Rpq*) of the DRC system were studied to get the best *S/N* value for ^{40}Ca and ^{31}P. Various gases, including NH_3, CH_4, O_2, and H_2, were tested as the reaction gas. After preliminary study we found that the $^{40}Ar^+$ background signal was reduced significantly when CH_4 or NH_3 was used as the reaction gas. Since a stable signal could be obtained in a shorter pressurize delay time when CH_4 was used as the reaction gas, CH_4 was selected in this work [14]. Fig. 1 shows the effect of the CH_4 flow rate on the signals of 50 ng mL^{-1} Ca and the blank at *m/z* 40. HNO_3 (1% *v/v*) was treated as the blank in this experiment. In this work, the values of the flow rate of different reaction gases have not been corrected for the different calibration factors of the mass flow controllers. As shown in Fig. 1, the blank signal at *m/z* 40 was suppressed significantly when CH_4 was used as the reaction gas while a *q* value of 0.8 was used. As shown in Fig. 1, a maximum *S/N* ratio could be obtained for ^{40}Ca when the CH_4 gas flow rate was about

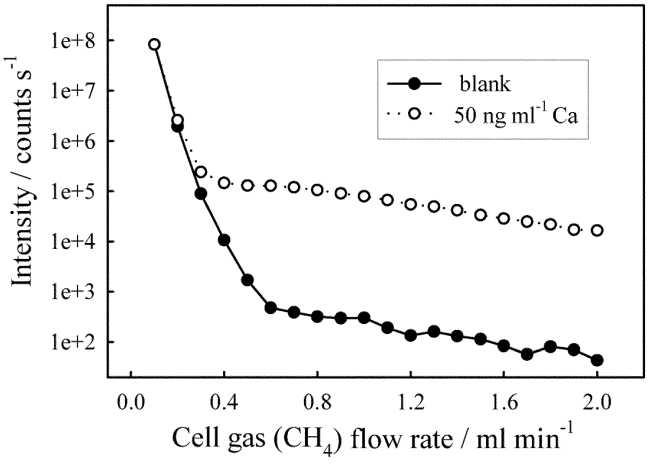

Fig. 1 Effect of the CH_4 reaction gas flow rate on signal intensity at *m/z* 40. Ca concentration was 50 ng mL^{-1} and 1% *v/v* HNO_3 was treated as the blank. *Rpq*=0.80; *Rpa*=0.0. The values of the flow rate have not been corrected for the calibration factor of the mass flow controller

1.0 mL min^{-1}. In the following experiments a CH_4 gas flow rate of 1.0 mL min^{-1} was selected.

However, we found that none of the reaction gases studied could react only with the $^{14}N^{16}OH^+$ and $^{15}N^{16}O^+$ while leaving the $^{31}P^+$ free from interferences at its 'natural' isotope mass without suppressing its intensity significantly. Another alternative is to find a specific reaction gas that can react with P^+ and produce a new polyatomic species at a new *m/z* that is free from interference by other species. As reported by Bandura et al. [18], O_2 is prone to having oxidation reaction with P; in the following experiments, O_2 was tested as the reaction gas for such purpose. Figure 2 shows the reaction profile of P^+ with O_2. As shown in Fig. 2, when the O_2 flow rate was less than 0.5 mL min^{-1}, the ion signal at *m/z* 47 increased with increasing O_2 gas flow rate when 100 ng mL^{-1} P solution was introduced

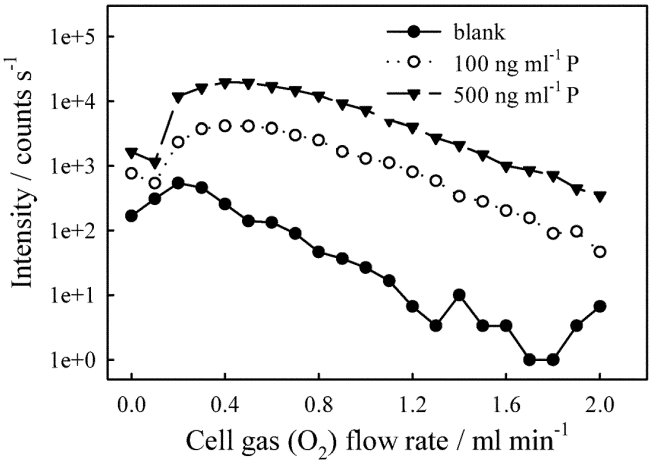

Fig. 2 Effect of the O_2 reaction gas flow rate on signal intensity at *m/z* 47; 1% *v/v* HNO_3 was treated as the blank. *Rpq*=0.58; *Rpa*=0.0. The values of the flow rate have not been corrected for the calibration factor of the mass flow controller

into the DRC ICP–MS. This could be due to the formation of the new species, $^{31}P^{16}O^+$, which was created in the cell by the reaction gas. In addition, the signal at *m/z* 47 for 500 ng mL^{-1} P was also monitored, which was about five times higher than that of 100 ng mL^{-1} P. This result further proved that the analyte signal at *m/z* of 47 was from the reaction of $^{31}P^+$ with the reaction gas O_2. A maximum *S/N* ratio could be obtained for $^{31}P^{16}O^+$ when the O_2 gas flow rate was about 1.0 mL min^{-1}. In the following experiments, an O_2 gas flow rate of 1.0 mL min^{-1} was selected.

Other important cell parameters of the DRC system are the rejection parameters *q* and *a*. Cell parameters can be controlled to filter out unwanted precursors of interfering species from the ion beam to eliminate interferences created in the cell by reaction gas. A higher operating point, *q*, increases the low-mass cutoff which could also decrease the transport efficiency of analyte ion. From the experiment we found that a maximum *S/N* value could be obtained for $^{31}P^{16}O^+$ when an *Rpq* value of 0.58 was used. In the following experiments, an *Rpq* value of 0.58 was adopted. In contrast, since the signal of Ca^+ was quite high compared to the $^{31}P^{16}O^+$ signal, a higher *Rpq* value of 0.86 was used for the determination of Ca in selected samples. This was done to effectively reduce the sensitivity of the ICP–MS instrument. Meanwhile, the rejection parameter *a* (*Rpa*) did not affect ion signals when the value was less than 0.1. The *Rpa* value was set at 0 in this study.

In order to determine these two elements in the same analysis, in this study CH_4 and O_2 were introduced into the DRC cell through channel A and channel B successively for determination of $^{40}Ca^+$ and $^{31}P^{16}O^+$, respectively.

CAUTION: The former reaction gas must be completely vented before introducing the other reaction gas. A channel delay time of 25 s was used between gas channel changes.

A CH_4 gas flow rate of 1.0 mL min^{-1} was used for ^{40}Ca determination and an O_2 gas flow rate of 1.0 mL min^{-1} was selected for $^{31}P^{16}O^+$ determination. The repeatability of the ion signals was determined by performing 20 consecutive determinations of Ca and P in a milk sample solution. We found that the repeatability of the signals of these 20 determinations was 2.9% and 4.0% for ^{40}Ca and $^{31}P^{16}O$, respectively. This experiment demonstrated that different reaction gases could be used sequentially to alleviate different interferences in the same analysis run without wasting experiment time. From the experimental result we found that the blank signals were only about 210 and 22 counts s^{-1} at *m/z* 40 and *m/z* 47, respectively, under the DRC ICP–MS conditions used in this work. A summary of the operating conditions of the DRC ICP–MS used in this work is given in Table 1.

In order to evaluate the significance of the $^{47}Ti^+$ isobaric interference in the determination of $^{31}P^{16}O^+$ a solution containing 10 ng mL^{-1} Ti was introduced into the ICP–MS with the DRC mode. *Rpq* was set at 0.58. Effects of the O_2 reaction gas flow rate on the $^{47}Ti^+$ and $^{47}Ti^{16}O^+$ signals were studied. Results are shown in Fig. 3. From the experiment, we found that Ti^+ was converted to TiO^+ effectively under these DRC conditions. Furthermore, as

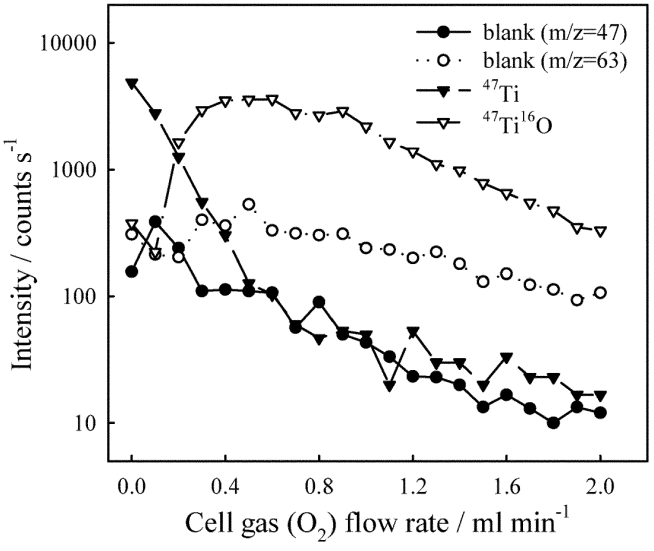

Fig. 3 Effect of the O_2 reaction gas flow rate on $^{47}Ti^+$ and $^{47}Ti^{16}O^+$ signals and on the blank signals at *m/z* 47 and 63

Table 2 Determination of Ca and P in foodstuffs by DRC ICP–MS[a] (*n*=3)

Sample and element	Concentration (μg g^{-1})		Recovery (%)	Ca/P ratio
	Found	Reference[b]		
NIST SRM 1549				
Ca	13200±130	13000±500		
P	10500±110	10600±200		
NIST RM 8345				
Ca	9600±370	9220±490		
P	7810±110	7800±490		
Infant milk powder				
Ca	3020±140	2900[c]	102	1.99
P	1520± 70	1550[c]	103	
Infant cereal-rice				
Ca	5010±240	3800[c]	96	1.57
P	3200±130	2800[c]	99	

[a]Values are means of three measurements±standard deviation
[b]Reference: NIST certified value
[c]Value labeled on the bottle

shown in Fig. 3, the signal of 10 ng mL^{-1} Ti^+ and the blank signal (1% HNO_3) at *m/z* 47 were equivalent when the O_2 gas flow rate was in the range of 0.8–1.2 mL min^{-1}. This experiment demonstrated that interference from $^{47}Ti^+$ on $^{31}P^{16}O^+$ determination was insignificant [18].

Determination of Ca and P in foodstuffs

In order to prove our system in authentic analyses, the NIST SRM 1549 non-fat milk powder and NIST RM 8345 whole milk powder reference materials were analyzed. The concentrations of Ca and P present in these samples were quantified by the external calibration method with Rh as internal standard – 1 ng mL^{-1} Rh was used as the internal standard for ^{40}Ca and $^{31}P^{16}O$ determination under different DRC settings. Calibration curves using six standard solutions of Ca and P were linear (r^2 better than 0.9999) in the range tested (0.005–1 μg mL^{-1}). The detection limits were estimated from these calibration curves and based on the concentration necessary to yield a net signal equal to three times the standard deviation of the blank (1% *v/v* HNO_3). The estimated detection limits were 0.2 ng mL^{-1} and 0.3 ng mL^{-1} for Ca and P, respectively. We believe that a lower detection limit could be obtained if the whole experiment was performed in a clean environment. The results obtained from analysis of the reference materials are listed in Table 2. As shown, the results agree satisfactorily with the certified values. The accuracy of the determination was better than 4.1 and 0.9% for Ca and P, respectively.

An infant milk powder sample and an infant cereal-rice sample purchased locally were also analyzed for the concentrations of Ca and P. Results are listed in Table 2. The results for infant milk powder and infant cereal samples were also found to be in good agreement with the values on the labels. Recovery was determined by spiking the sample solution with 250 ng mL^{-1} Ca and P and then determining the concentration by DRC ICP–MS. As listed in Table 2, recovery was in the range 96–103% for all determinations. The ratios of Ca to P were in the range of 1.2–2 in these infant foods. These experiments demonstrated that the concentrations of Ca and P in the food samples could be determined by DRC ICP–MS without significant spectroscopic interferences. Although the determination of Ca and P in food samples by ICP–MS has suffered from the severe spectroscopic interferences, the precision (RSD) between sample replicates was better than 4.8% for all the determinations.

Conclusion

The use of dynamic reaction cell ICP–MS provides a simple, rapid, and accurate technique to determine Ca and P routinely in food samples. The effectiveness of the DRC system for alleviation of the spectroscopic interferences was demonstrated. The use of different reaction gases in the same analysis run to alleviate different spectroscopic interferences should increase the flexibility of the DRC ICP–MS instrument and the analytical method. The proposed DRC ICP–MS method has the advantages of better sensitivity and speed of analysis over GFAAS and/or flame AAS. The method developed in this study could also be applied to the determination of Ca and P in other biological samples for various applications.

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