TRANSACTIONS OF THE CHINESE SOCIETY FOR AGRICULTURAL MACHINERY
Vol. 47, No. 1, 2016

doi:10.6041/j.issn.1000-1298.2016.01.026

Determination of Calcium Content in Milk Powder Based on Nonlinear Chemical Fingerprint Method

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Abstract: The inherent content of calcium in milk powder was rapidly determined by using nonlinear chemical fingerprint method. Firstly, inherent calcium content in three brands of milk powder was determined by using flame atomic absorption spectrometry as described by GB 5413.21 – 2010 (National Standards of PR China). Different content of calcium carbonate was added into the above three brands of milk powder, respectively. Using "$H^+ + Mn^{2+} + BrO_3^- +$ acetone + substrates in milk powder" as reaction system, nonlinear chemical fingerprints of milk powder with different calcium content were obtained, and there was a linear relationship between undulatory period and total content of calcium in milk powder, where total content of calcium consists of inherent calcium content in milk powder and the content of calcium added in milk powder. Then, inherent content of calcium in milk powder was calculated by the least square method. The results showed that total content of calcium in milk powder and undulatory period value had a good linear relationship within 0.022 7 ~ 0.032 3 g range of total content of calcium when the total amount of milk powder was 0.9g, the determination coefficient was 0.995 3 ~ 0.998 8, the recovery was 99.18% ~ 101.6%, the RSD was 0.47% ~ 1.63%, and the determination range of the inherent calcium content in milk powder was 0 ~ 0.032 3 g. The method developed has advantages of good accuracy and simple operation, and it is a practical and feasible method for determining the inherent calcium content in milk powder. In addition, the method can be as a reference in research on the methods for the determination of other components in other complex samples.

Key words: milk powder; calcium content; nonlinear chemical fingerprint; linear regression method

0 Introduction

With the rapid development of economy and technology, people's living standards are gradually improving, while people pay more attention to good health, especially for the healthy growth of their children, so they have higher consuming willingness[1]. Milk powder has been an important human nutrient source in people's daily life because the organic calcium content in milk powder is high and the organic calcium is beneficial to the absorption and utilization of human body. Therefore, milk powder is a kind of highly nutritious food, and it not only suits for infants but also for the old and infirm. At present, the methods for detecting inherent content of calcium in milk powder have chemical titration and flame atomic absorption spectrophotometry method. However, due to complicated operation, error of observation, low sensitivity and serious environmental pollution problems such as potassium cyanide solution used in experiments, chemical titration has been seldom used. Although flame atomic absorption spectrophotometry method for determining the calcium of milk powder has the advantages of simple operation and sensitivity, the equipment is expensive and needs specialized operation technology. Moreover, because there are many kinds of trace elements in milk powder, the interference is larger and brings a certain error for experimental

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Received date: 2015 – 05 – 20 Accepted date: 2015 – 09 – 15

Supported by National Science & Technology Program during the Twelfth Five-year Plan Period in Rural Areas of China (Grant No. 2012BAD12B07), Projects of Science and Technology Co-ordinating Innovative Engineering in Shaanxi Province of China (Grant No. 2011KTCQ03 – 08), Special Fund of Shaanxi Provincial Education Department to Serve Local Development (Grant No. 15JF009), and Science and Technology Bureau Technology Transfer Demonstration Project of Xi'an City in China (Grant No. CXY1513(7))

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results. Calcium ion selective electrode for determining calcium of milk powder and Raman spectroscopic quantification of calcium carbonate in spiked milk powder have been reported[2-3], however, the method also needs laborious process of pretreatment, and the operation is complicated. Therefore, it would be very important to develop the method of simple, low cost, fast and no pretreatment such as separation or purification for determining calcium of milk powder.

The discovery of Belousov-Zhabotinsky (B-Z) oscillatory chemical reaction promoted people to investigate the phenomenon of nonlinear chemical reaction[4]. Nonlinear chemical fingerprint was a kinetic fingerprint based on non-equilibrium chemical theory, and the phenomena of the reaction were complex and involved chemical oscillation, chemical turbulence, chemical pattern, chemical wave and so on. The reaction mechanism and applications of chemical oscillation had been investigated extensively and thoroughly by the domestic and foreign scholars, and many research results had been reported[5-17]. Due to the advantages of simple operation, good reproducibility and high precision of nonlinear chemical fingerprint method, the research on the nonlinear chemical reaction had aroused increasing attention for many scientists and become a hot topic in the analytical chemistry field. The applications of chemical oscillation in the analysis and detection of single component had been reported, such as trace metals ruthenium, manganese, silver plasma and so on[18-20]. However, the research on the application of nonlinear chemical reaction for the detection of calcium content in milk powder was rare. Thus, in this work, inherent content of calcium in milk powder was analyzed quantitatively by using "sulfuric acid - manganese sulfate - acetone - sodium bromate - composition of standard samples" as reaction system.

1 Materials and methods

1.1 Reagents and instruments

Sulfuric acid (1.00 mol/L), acetone (1.00 mol/L), sodium bromate (0.80 mol/L), manganese sulfate (0.08 mol/L), hydrochloric acid and calcium carbonate were used. All the chemicals used were of analytical reagent grade. Bi-distilled water was used throughout. The five brands of milk powder samples were provided by the Institute of Product Quality Supervision and Inspection in Shaanxi Province, and they were referred to as milk powder Ⅰ, milk powder Ⅱ, milk powder Ⅲ, milk powder Ⅳ and milk powder Ⅴ.

Preparation of standard milk powder samples: inherent calcium content in three brands of milk powder samples was determined as standard milk powder samples by flame atomic absorption spectrometry as described by GB 5413.21 - 2010 (National Standards of PR China).

A nonlinear chemical fingerprint instrument (Model MZ-1B-2) developed by Central South University and Xiangtan Ltd. for making whole set instruments and meters (Hunan, China) was used. The electrodes were 217 calomel and 213 platinum (Shanghai Precision & Scientific Instrument Co., China). Electronic balance (Model BS 224 S) and atomic absorption spectrophotometer were developed by Shanghai Precision & Scientific Instrument Co., China and American PE Company, respectively.

1.2 Experimental method

1.2.1 Measurement of quantitative information of standard samples and establishment of regression model

Four standard milk powder samples of a brand were spiked with known levels of calcium carbonate, namely, the content of calcium carbonate in standard milk powder samples was 0.0264, 0.0352, 0.0427, 0.0504 g, respectively. The total dosage of each mixed milk powder sample was 0.9 g. The following procedure was used in all experiments for the determination of standard milk powder samples. Nonlinear chemical reaction mixture was prepared by mixing of 25 mL of 1.0 mol/L sulfuric acid, 15 mL of 1.0 mol/L acetone, 15 mL of 0.08 mol/L manganese sulfate, 10 mL of doubly distilled water and 0.9 g of milk powder sample. All of the reaction mixture was added into the reactor. The reactor cover with two injection holes, the electrodes and a thermometer was closed. Then, the instrument was turned on, and the temperature in the reactor was adjusted to a constant temperature (50℃). The stir rate was adjusted to 800 r/min. When the constant rate stir just lasted for 3.0 min, 5.00 mL of the sodium bromate solutions was injected into the reactor. The menu was immediately dotted for plotting the relevant electric potential-time ($E-t$) curves until the potential oscillation disappeared. The undulatory period of nonlinear chemical fingerprint of milk powder was determined by Matlab software, and a linear regression model was established between undulatory period and total content of calcium in milk powder by using the least square method. In this work, total content of calcium in milk powder was composed of inherent content of calcium and the content of calcium added in milk powder.

1.2.2 Determination of unknown sample

The 0.9 g of milk powder sample of different batches of the same brand, the appropriate amount of calcium carbonate, 25 mL of 1.0 mol/L sulfuric acid, 15 mL of 1.0 mol/L acetone, 15 mL of 0.08 mol/L manganese sulfate and 10 mL of doubly distilled water were simultaneously added into the reactor. The reactor cover with two injection holes, the electrodes and a thermometer was closed. Then, the experimental method of Section 1.2.1 was used, and nonlinear chemical fingerprint of corresponding milk powder was determined. The undulatory period of nonlinear chemical fingerprint of milk powder was determined by Matlab software, and the content of calcium was calculated by using the established linear regression model.

2 Basic information of nonlinear chemical fingerprint of milk powder

In this study, the experimental method of Section 1.2.1 was used, and nonlinear chemical fingerprint was obtained by adding the 0.9 g of milk powder Ⅱ sample into the reactor. At this time, the content of calcium in milk powder Ⅱ only included inherent content of calcium. As shown in Fig. 1. The $e-f$ segment was named as inductive curve, and the $f-g$ segment was named as fluctuation curve. The e and h were the start point and end point of reaction, respectively. It was obvious from the Fig. 1 that basic characteristic information of nonlinear chemical fingerprint mainly included intuitive information and quantitative information. Inductive time (t_{ind}), undulatory period (τ_{und}), undulatory life (t_{und}), canyon potential (E_{can}), canyon time (t_{can}), peak top potential (E_{pet}), peak top time (t_{pet}), oscillatory start potential (E_{uns}), oscillatory end potential (E_{une}), oscillatory end time (t_{uns}), maximum amplitude ($\Delta E_{\max}$) and so on were defined as quantitative information. All of them were described in detail in the literatures[4,20].

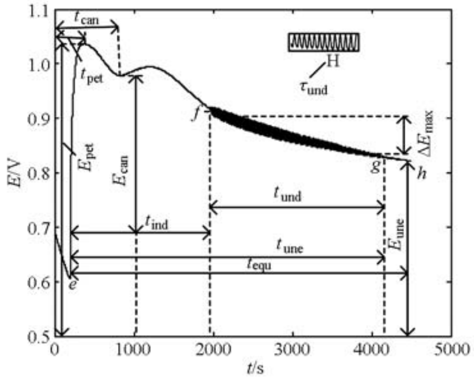


Fig. 1 Basic characteristic information of nonlinear chemical fingerprint of milk powder Ⅱ

3 Influence of species and their concentrations on nonlinear chemical fingerprint in the reaction system

Nonlinear chemical reaction involved many elementary reactions, and the reaction mechanism was complex. Moreover, nonlinear chemical reaction was highly sensitive to foreign substances, such as alkaloid, trace elements, organic acid, peptide, amino acids, vitamins, enzymes, ketones, hydroxyl, ether compounds and so on, and these substances had great influence on the shape and quantitative parameters of the $E-t$ curve of nonlinear chemical reaction system[4]. The substances of affecting characteristic information of nonlinear chemical fingerprint may be summed up as four kinds[21], namely, reaction substrate, reaction dissipation, coexisting substances (such as alkaloid, vitamins, enzymes and amino acids) and substances that existed in the system did not be involved in any chemical reactions. The fourth substances just affected the physical properties of the reaction system, which changed the Nernst relationship of non-equilibrium between the potential in working electrode course and the activities of reaction substances. The quantitative relationship between the potential in a spontaneous irreversible electrode course and the activities of the relevant substances can be described by Nernst equation[6]. In general case, any spontaneous oxidation-reduction reaction may be represented as follows

$$n_1O_1 + n_2R_2 + \cdots \rightarrow n_1R_1 + n_2O_2 + \cdots \qquad (1)$$

Where n_i is stoichiometric number. Let electron transfer number be $n = n_1n_2$, and the reaction can be divided into the oxidation and reduction half-reaction when the system is designed into the primary cell reaction. The reaction is as follows

The oxidation half-reaction

$$n_1R_1 - ne + \cdots \rightarrow n_1'O_1 + \cdots \qquad (2)$$

The reduction half-reaction

$$n_2O_2 + ne + \cdots \rightarrow n_2R_2 + \cdots \qquad (3)$$

The potential of corresponding electrode φ_O and φ_R in Eqs. (2) and (3) may be respectively expressed as

$$\varphi_O = \varphi_O^\circ + \frac{RT}{nF}\ln\frac{a_{O_1}^{n_1}\cdots}{a_{R_1}^{n_1}\cdots} \qquad (4)$$

$$\varphi_R = \varphi_R^\circ + \frac{RT}{nF}\ln\frac{a_{O_2}^{n_2}\cdots}{a_{R_2}^{n_2}\cdots} \qquad (5)$$

In terms of the electrode reactions of nonlinear-oxidation-reduction reaction, the activity extreme of the reactants and resultants will transform with the change of time t, and the activities are all nonlinear functions of time t. Thus, the potential is also the nonlinear function of time t, and the relationship between electric potential and time may be expressed as follows

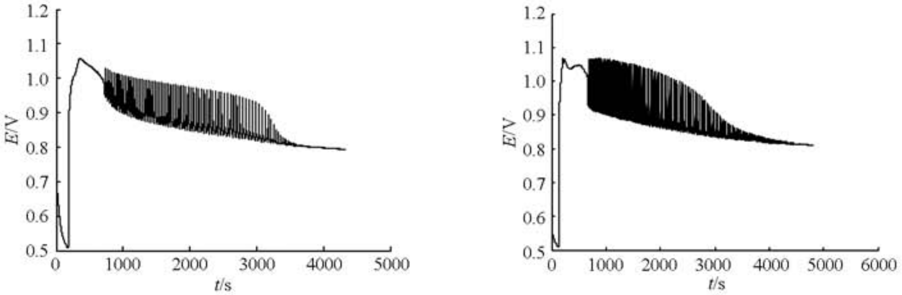
$$\varphi_R(t) = \varphi_R^\circ(t) + \frac{RT}{nF}\ln\frac{(a_{O_1}(t))^{n_1}\cdots}{(a_{R_1}(t))^{n_1}\cdots} \qquad (6)$$

It is obvious from the Eq. (6) that the changes of the reactants and their activities in the reaction system will result in the change of corresponding electrode potential, which will eventually lead to the change of the $E-t$ curve. These changes can be obviously seen from the changes of quantitative information and visual shape of nonlinear chemical fingerprint. For example, the fingerprint and quantitative information of different brands of milk powder Ⅳ and milk powder Ⅴ were determined by using "sulfuric acid – manganese sulfate – acetone – sodium bromate" oscillating system. As shown in Tab. 1 and Fig. 2.

Tab. 1 Effects of dosagesof reaction reagents in reaction system on quantitative information of nonlinear chemical fingerprint of milk powder Ⅴ

System No.	Sulfuric acid/ (mol·L^{-1})	Manganese sulfate/ (mol·L^{-1})	Sodium bromate/ (mol·L^{-1})	acetone/ (mol·L^{-1})	t_{ind}/ s	t/s	ΔE_{max}/V
1	0.75	0.08	0.80	0.50	729.498	5 599.698	0.130 6
2	1.15	0.08	0.80	0.50	713.598	3 621.000	0.107 1
3	0.75	0.08	0.80	1.50	931.602	6 609.402	0.132 5
4	1.00	0.06	1.00	1.00	856.998	5 616.198	0.118 8
5	1.00	0.06	0.80	1.50	1 054.20	3 188.400	0.070 2
6	1.00	0.08	0.80	1.00	736.002	2 874.798	0.074 0

It was obvious from Tab. 1 that concentration changes of the substances in the system had different effects on quantitative parameters of the fingerprint. Nonlinear chemical fingerprint of each sample is not the result of some kind of reaction substrate or dissipation substances, but the result of various degrees of all coexisting substances in the reaction system. Moreover, these coexisting substances have different effects on nonlinear chemical reaction among the substrates, as shown in Fig. 2. Therefore, characteristic information of nonlinear chemical fingerprint is the common-effect of different degrees of the substances in the system, which reflects throng characterization of each substrate in the sample. When the species and concentration of other coexisting substances in the system are invariable, the amount of



(a) Milk powder Ⅳ (b) Milk powder Ⅴ

Fig. 2 Nonlinear chemical fingerprints of milk powder Ⅳ and milk powder Ⅴ (detecting dosage: 0.900 5 g) the substance that has a significant effect on characteristic information of the fingerprint is only changed, and the change of characteristic information of nonlinear chemical fingerprint is the result of the effect of this substance. When function relationship exists between the change of quantitative information of the fingerprint and the concentration of the substance, quantitative analysis of the substance can be carried out by using this function relationship[21].

In this work, the effect of total content of calcium ($M_0+\Delta M$) in milk powder on undulatory period τ_{und} of nonlinear chemical fingerprint was shown in Fig. 3, where M_0 and ΔM were inherent content of calcium in milk powder and the content of calcium added in milk powder, respectively. τ_{und1}, τ_{und2}, τ_{und3} and τ_{und4} were undulatory period values that were determined when total content of calcium in milk powder was $M_0+\Delta M_1$, $M_0+\Delta M_2$, $M_0+\Delta M_3$ and $M_0+\Delta M_4$, respectively. When the content of calcium added ΔM in milk powder was known, inherent content of calcium M_0 in milk powder could be calculated by using the linear relationship between undulatory period and total content of calcium in milk powder, namely, the regression equation was $\tau_{\mathrm{und}}=k(M_0+\Delta M)+b$, where k and b were the slope and intercept of the regression equation, respectively. The results indicated that the effect of determined substance on undulatory period of nonlinear chemical fingerprint was greater when k value was larger. In theory, b was undulatory period of nonlinear chemical fingerprint when total content of calcium in milk powder was zero, and undulatory period value all depended on the effect of all the coexisting substances in addition to calcium of milk powder on nonlinear chemical fingerprint. Due to the differences of species and their content of coexisting substances in the different brands of milk powder, so k and b value in the regression equation also existed differences. In some cases, the effect of total content of very low or very high calcium on undulatory period of nonlinear chemical fingerprint may deviate from the linear relationship. It was obvious from the curve m and p or the curve l and q in Fig. 3. Therefore, in our study, total content of calcium ($M_0+\Delta M$) in milk powder needed to be in the range of 0.022 7 g to 0.032 3 g, and inherent content of calcium in milk powder was calculated by the least square method and the linear relationship between undulatory period and total content of calcium of corresponding milk powder. Inherent content of calcium in different batches of the same brand of milk powder can be calculated as long as undulatory period of corresponding milk powder was determined under the same experimental condition by using the linear regression equation. In this work, effects of other substances in milk powder on undulatory period of nonlinear chemical fingerprint have been basically considered in the intercept b of the linear regression equation, and interference experiment did not need to do.

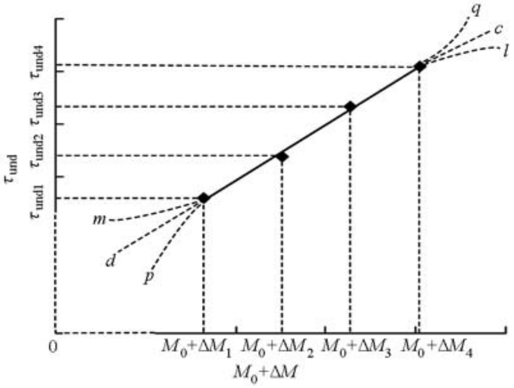


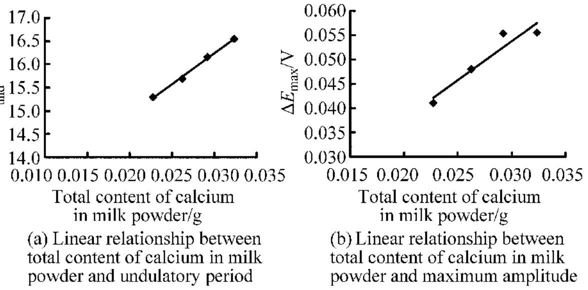
Fig. 3 Effects of total content of calcium in milk powder on undulatory period of nonlinear chemical fingerprint

4 Results and discussion

4.1 Effects of total content of calcium in milk powder on undulatory period of nonlinear chemical fingerprint

Due to the difference of the shape of the $E-t$ curve for different brands of milk powder, so corresponding characteristic parameters such as undulatory period, inductive time, maximum amplitude and so on were different. But there was often a linear relationship between one or more parameter of these characteristic parameters and the content of a substance in milk powder. For example, in certain range, there was a linear relationship between undulatory period or maximum amplitude of nonlinear chemical fingerprint in milk powder Ⅱ and total content of calcium in milk powder Ⅱ. As shown in Fig. 4.

Effects of total content of calcium on undulatory period of nonlinear chemical fingerprint of milk powder Ⅱ were shown in Fig. 5. Fig. 5b was local undulatory curve of corresponding nonlinear chemical fingerprint of milk powder Ⅱ in the range of 2 000 ~ 2 200 s.

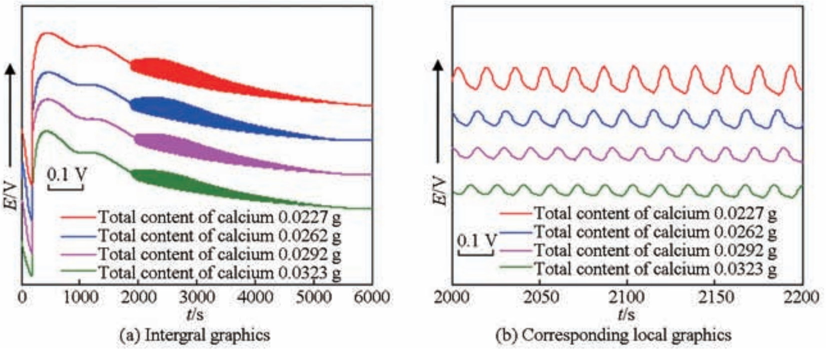


(a) Linear relationship between total content of calcium in milk powder and undulatory period

(b) Linear relationship between total content of calcium in milk powder and maximum amplitude

Fig. 4 Linear relationship between total content of calcium in milk powder Ⅱ and undulatory period and maximum amplitude of nonlinear chemical fingerprint

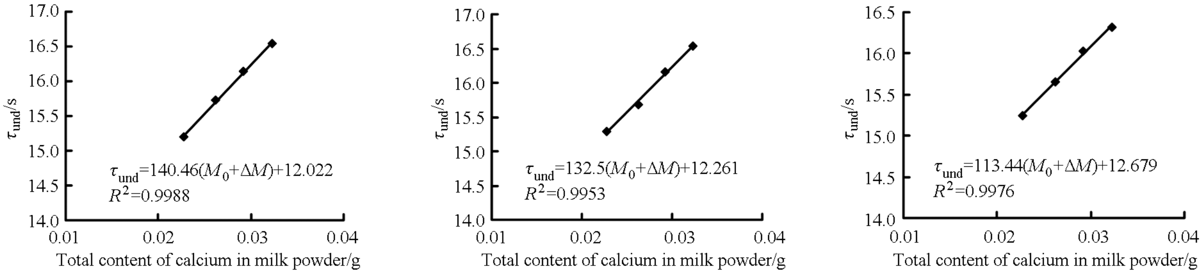
When total content of calcium in milk powder was in the range of 0.022 7 ~ 0.032 3 g, there was a linear relationship between total content of calcium in milk powder Ⅰ, milk powder Ⅱ or milk powder Ⅲ and undulatory period of nonlinear chemical fingerprint of corresponding milk powder. As shown in Fig. 6. The information provided necessary conditions for determining inherent content of calcium in all kinds of milk powder.



(a) Intergral graphics

(b) Corresponding local graphics

Fig. 5 Effects of total content of calcium on undulatory period of nonlinear chemical fingerprint of milk powder Ⅱ



(a) Milk powder Ⅰ

(b) Milk powder Ⅱ

(c) Milk powderⅢ

Fig. 6 Effects of total content of calcium in three bands of milk powder on undulatory period of nonlinear chemical fingerprint

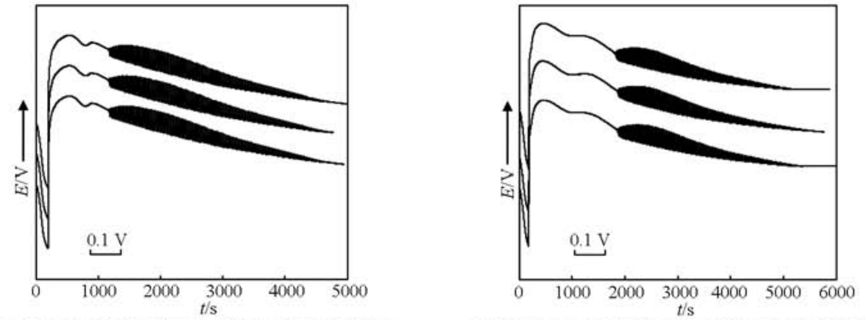
4.2 Determination inherent content of calcium in milk powder by nonlinear chemical fingerprint method

In this study, the experimental method of Section 1.2.1 was used, and three brands of milk powder were determined by nonlinear chemical fingerprint method. According to the linear relationship between undulatory period of nonlinear chemical fingerprint in milk powder Ⅰ, milk powder Ⅱ and milk powder Ⅲ and total content of calcium in corresponding milk powder, and inherent content of calcium of corresponding milk powder M_0 could be obtained by corresponding fitting equation in Fig. 6 when the amount of calcium ΔM in milk powder was known. Experimental results were shown in Tab. 2. With reference to Tab. 2 we could see that the relative standard deviation (RSD) of inherent content of calcium for various milk powder was less than or equal to 1.63%, and it indicated that the method had higher precision. The recovery rate was between 99.19% and 101.6%, and it indicated that the method had better accuracy. The experimental results were shown that nonlinear chemical fingerprint method was feasible for the quantitative analysis of inherent content of calcium in milk powder, and the developed method could provide the basis for the determination of the contents of other substances in a sample.

Tab. 2 Determination results of inherent calcium content in milk powder by nonlinear chemical fingerprint method

Samples No.	τ_{und}/s	Regression equation	Added/g	Found/g	GB Found/g	RSD/% ($n=3$)	Recovery/%
Milk powder Ⅰ	16.042	$\tau_{und}=140.46(M_0+\Delta M)+12.022$	0.016 1	0.012 5	0.012 3	0.56	101.6
Milk powder Ⅱ	15.759	$\tau_{und}=132.50(M_0+\Delta M)+12.261$	0.014 1	0.012 3	0.012 2	1.63	100.8
Milk powder Ⅲ	16.176	$\tau_{und}=113.44(M_0+\Delta M)+12.679$	0.018 5	0.012 3	0.012 4	0.94	99.19

To further verify and investigate the feasibility and reliability of the proposed method, different batches of milk powder Ⅰ and milk powder Ⅱ were spiked with calcium carbonate at different content (0.013 2 g and 0.015 6 g), respectively. The experimental method of Section 1.2.1 was used, and the experiments were carried out in triplicate. The nonlinear chemical fingerprints of two milk powder samples were shown in Fig. 7. The detection results were indicated in Tab. 3.



(a) Total content of calcium in milk powder Ⅰ :0.013 2 g　　(b) Total content of calcium in milk powder Ⅱ :0.015 6 g

Fig. 7　Effects of total content of calcium on nonlinear chemical fingerprints of milk powder Ⅰ and milk powder Ⅱ

Tab. 3　Determination results of inherent calcium content in milk powder Ⅰ and milk powder Ⅱ by nonlinear chemical fingerprint method

Samples No.	τ_{und}/s	Regression equation	Added/g	Found/g	GB Found/g	RSD/% ($n=3$)	Recovery/%
Milk powder Ⅰ	15.597	$\tau_{\mathrm{und}} = 140.46(M_0 + \Delta M) + 12.022$	0.013 2	0.012 3	0.012 3	0.47	100.0
Milk powder Ⅱ	15.935	$\tau_{\mathrm{und}} = 132.50(M_0 + \Delta M) + 12.261$	0.015 6	0.012 1	0.012 2	0.48	99.18

4.3　Detection range of inherent content of calcium in milk powder

In this study, total content of calcium in milk powder was in the range of 0.022 7 ~ 0.032 3 g by adding appropriate amount of calcium, and inherent content of calcium in milk powder was determined. Therefore, according to this research, when inherent content of calcium in milk powder was between 0 ~ 0.032 3 g, and inherent content of calcium in corresponding milk powder can be obtained by the proposed method. Moreover, the minimum limit of detection and the maximum limit of detection were 0 g and 0.032 3 g, respectively. Therefore, according to the above discussion, inherent content of calcium in milk powder was in the range of 0 ~ 0.032 3 g.

5　Conclusions

(1) Inherent calcium content in milk powder was determined and analyzed by nonlinear chemical fingerprint technique. According to the linear regression relationship between undulatory period and total content of calcium in milk powder, and inherent calcium content of milk powder was calculated.

(2) The results showed that total content of calcium in milk powder and undulatory period value had a good linear relationship within 0.022 7 ~ 0.032 3 g range of total content of calcium when total amount of milk powder was 0.9 g, the determination coefficient was 0.995 3 ~ 0.998 8, the recovery was 99.18% ~ 101.60%, the RSD was 0.47% ~ 1.63%, and the determination range of inherent calcium content in milk powder was 0 ~ 0.032 3 g.

(3) Therefore, the proposed method had high precision and accuracy. The method can not only be used to determine inherent calcium content in different batches of the same brand of milk powder, but also be used to determine the content of other substances in complex samples. Moreover, the method developed provided an approach for determining the content of trace metal elements in a sample. The method had the advantages of simple operation and low cost, and it had good application prospect.

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doi:10.6041/j.issn.1000-1298.2016.01.026

奶粉中钙含量的非线性化学指纹图谱法测定

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摘要： 采用非线性化学指纹图谱法对奶粉中固有钙含量进行定量分析。先用国标火焰原子吸收分光光度法对3份奶粉标准样品中固有的钙含量进行测定，分别向奶粉标样中加入不同量的碳酸钙，利用“硫酸-硫酸锰-丙酮-溴酸钠-标样成分”为反应体系，通过非线性化学指纹图谱法对含有不同量碳酸钙的奶粉进行测定，运用最小二乘法，拟合出其波动周期与奶粉中钙的总含量之间的一元线性关系，当奶粉中钙的加入量已知时，可求出奶粉中固有的钙含量。实验表明，当奶粉总量为0.9 g、奶粉中钙的总含量在0.022 7～0.032 3 g范围内时，钙的总含量与波动周期线性关系良好，决定系数 R^2 为0.995 3～0.998 8，样品加标回收率为99.18%～101.60%，RSD为0.47%～1.63%，奶粉中固有的钙含量检测范围为0～0.032 3 g。方法准确度高，操作简单，是一种切实可行的测定奶粉中固有钙含量的方法，也可作为复杂样本中其它成分定量分析借鉴的方法。

关键词： 奶粉；钙含量；非线性化学指纹图谱；线性回归法

中图分类号： TS252.7　　**文献标识码：** A　　**文章编号：** 1000-1298(2016)01-0202-07

Determination of Calcium Content in Milk Powder Based on Nonlinear Chemical Fingerprint Method

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Abstract: The inherent content of calcium in milk powder was rapidly determined by using the nonlinear chemical fingerprint method. Firstly, the inherent calcium content in three brands of milk powder was determined by using flame atomic absorption spectrometry as described by GB 5413.21—2010 (National Standards of PR China). Different contents of calcium carbonate were added into the above three brands of milk powder, respectively. Using "H^+ + Mn^{2+} + BrO_3^- + acetone + substrates in milk powder" as reaction system, the nonlinear chemical fingerprints of milk powder with different calcium contents were obtained, and there was a linear relationship between the undulatory period and the total content of calcium in milk powder, where the total content of calcium consisted of the inherent calcium content in milk powder and the calcium content of being added into milk powder. Then, the inherent content of calcium in milk powder was calculated by using the least square method. The results showed that the total content of calcium in milk powder and the undulatory period value had a good linear relationship within 0.022 7～0.032 3 g range of the total content of calcium when the total amount of milk powder was 0.9 g, the determination coefficient was 0.995 3～0.998 8, the recovery rate was 99.18%～101.60%, the RSD was 0.47%～1.63%, and the determination range of the inherent calcium content in milk

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收稿日期：2015-05-20　修回日期：2015-09-15

基金项目： “十二五”农村领域国家科技计划项目(2012BAD12B07)、陕西省科技统筹创新工程计划项目(2011KTCQ03-08)、陕西省教育厅服务地方专项(15JF009)和西安市科技局技术转移示范工程项目(CXY1513(7))

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powder was 0 ~ 0.032 3 g. The method developed has advantages of good accuracy and simple operation, and it is a practical and feasible method for determining the inherent calcium content in milk powder. In addition, the method can be used for reference in research on the methods for the determination of other components in other complex samples.

Key words: milk powder; calcium content; nonlinear chemical fingerprint; linear regression method

引言

随着经济与科技的快速发展，人们生活水平在逐渐提高的同时，对身体健康也日益重视，特别是对孩子的健康成长有着更高的消费意愿[1]。奶粉已成为人们日常生活中一种非常重要的营养食品，尤其是奶粉中有机钙含量较高，有利于人体吸收和利用，是婴幼儿及年老体弱者补钙的营养佳品。目前，对奶粉中固有钙含量的检测方法有化学滴定法和火焰原子吸收分光光度法。化学滴定法操作繁琐，终点观察误差大，灵敏度低，且滴定中使用的高浓度剧毒物如氰化钾溶液，对环境污染严重，已很少使用。应用火焰原子吸收法测定奶粉中的固有钙含量，虽然简便灵敏，但因仪器昂贵，需要专门的操作技术，同时由于奶粉中各种微量元素较多，测定时干扰较大，给实验结果带来了一定的误差。利用钙离子选择性电极[2]和拉曼光谱检测钙含量[3]也有报道，但是此方法同样需要复杂的前预处理工作，操作繁琐。为此，探索一种既简单、快速、低成本，又无需分离和提纯测定奶粉中固有钙含量的方法十分必要。

B - Z(Belousov & Zhabotinsky)振荡反应的发现促使人们开始对非线性化学反应现象进行研究[4]。非线性化学指纹图谱是一种建立在非线性化学反应基础之上的动力学指纹图谱，其反应包括化学振荡、化学湍流、化学斑图和化学波等许多化学混沌现象。对于非线性化学的反应机理[5-8]和应用[9-12]，国内外学者已经进行了较为广泛而深入的研究，并报道了其研究成果[13-17]。由于非线性化学指纹图谱技术操作简单、快速、重现性好、精密度高，已引起广大科学工作者的关注，成为当前分析化学领域的研究热点。利用化学振荡方法对单一成分如痕量金属钌、锰、银等离子进行分析检测已有研究[18-20]，但是利用非线性化学指纹图谱法对奶粉中固有钙含量的检测鲜有研究报道。因此，本文以硫酸-硫酸锰-丙酮-溴酸钠-标样成分为反应体系，采用非线性化学指纹图谱法对奶粉中固有的钙含量进行定量分析。

1　材料与方法

1.1　试剂和仪器

1.00 mol/L 硫酸、1.00 mol/L 丙酮、0.80 mol/L 溴酸钠、0.08 mol/L 硫酸锰溶液、盐酸和碳酸钙。以上试剂均为分析纯，水为二次蒸馏水。所用不同品牌奶粉(以下简称“奶粉”)由陕西省产品质量监督检验研究所提供。5 种奶粉样品分别编号为Ⅰ、Ⅱ、Ⅲ、Ⅳ、Ⅴ。

奶粉标准样品的配制：取不同奶粉样品 3 份，用国标火焰原子吸收分光光度法测定奶粉中固有的钙含量，作为研究用的奶粉标样。

MZ - 1B - 2 型非线性化学指纹图谱测定仪，湖南尚泰测控科技有限公司生产；217 型复合甘汞电极和 213 型金属铂电极，上海精密科学仪器有限公司；BS 224 S 型电子天平，上海精密科学仪器有限公司；原子吸收分光光度计，美国 PE 公司。

1.2　实验方法

1.2.1　标样定量信息测定与回归方程模型建立

取上述某一品牌的奶粉标样 4 份于反应器中，每份奶粉样品为 0.9 g，分别加入 0.026 4 、0.035 2 、0.042 7 、0.050 4 g 的碳酸钙试剂，再依次加入 25.00 mL 硫酸、15.00 mL 丙酮、15.00 mL 硫酸锰溶液和 10.00 mL 蒸馏水。盖好带注射孔和电极的反应器盖，打开恒温系统调节反应器，温度设为 50℃、恒速搅拌设定 800 r/min，点击菜单采集数据，搅拌到 3 min 时用注射器迅速加入 5.00 mL 溴酸钠溶液。记录 $E - t$ 曲线至电位 E 不再随时间 t 变化为止。利用 Matlab 软件确定所测奶粉指纹图谱的波动周期，采用最小二乘一元线性回归法建立波动周期与奶粉中钙的总含量的定量评价模型。本研究中，奶粉中钙的总含量为奶粉中固有的钙含量与奶粉中加入的钙量之和。

1.2.2　未知试样的测定

对于同品牌不同批次的奶粉，准确称取 0.9 g 奶粉样品和适量的碳酸钙于反应器中，再依次加入 25.00 mL 硫酸、15.00 mL 丙酮、15.00 mL 硫酸锰溶液和 10.00 mL 蒸馏水，盖好带注射孔和电极的反应器盖。然后采用 1.2.1 节的实验方法，测定指纹图谱，利用 Matlab 软件确定所测奶粉指纹图谱的波动周期，通过最小二乘一元线性回归法计算奶粉中固有的钙含量。

2　非线性化学指纹图谱基本信息

按照 1.2.1 节的实验方法，称取 0.9 g 的奶粉Ⅱ

样品于反应器中，此时奶粉中的钙含量只含奶粉中固有的钙含量，测得其非线性化学指纹图谱如图 1 所示。图中 $e \sim f$ 段为诱导曲线，$f \sim g$ 段为波动曲线，e 和 h 分别为非线性化学反应的起点和终点。由图可见，非线性化学指纹图谱的基本信息主要包括直观信息和可量化信息，其直观信息中包含诱导曲线、波动曲线、波峰形状、电位漂移曲线和停波曲线等；而可量化信息即特征参数包含峰谷电位 E_{can}、峰谷时间 t_{can}、峰顶电位 E_{pet}、峰顶时间 t_{pet}、诱导时间 t_{ind}、起波电位 E_{uns}、停波电位 E_{une}、停波时间 t_{une}、平衡电位 E_{equ}、平衡时间 t_{equ}、波动幅度 ΔE_{und}、最大波幅 $\Delta E_{\max}$、波动寿命 t_{und}、波动周期 τ_{und} 和波数 n_{wav} 等，各种特征参数的含义见文献[4,20]。

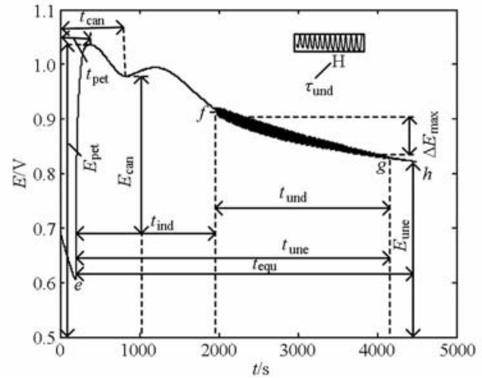


图 1　奶粉Ⅱ非线性化学指纹图谱的基本特征信息

Fig. 1　Basic characteristic information of nonlinear chemical fingerprint of milk powder Ⅱ

3　反应体系中各物质及其浓度对非线性化学指纹图谱的影响

非线性化学反应涉及众多基元反应，其反应机理较复杂，同时体系对外来化学物质的影响比较敏感，如化学物质生物碱、微量元素、有机酸、氨基酸、多肽、维生素、酶、酮类、羟基和醚类化合物等，这些物质对非线性化学反应体系 $E-t$ 曲线的形状和可量化参数均能产生很大影响[4]。归结起来，在体系中，影响非线性化学指纹图谱特征信息的物质主要有 4 类[21]，分别为反应底物、反应耗散物、共存于样品中的共存物质（如生物碱、维生素、酶和氨基酸等物质）以及存在于体系中但并不参与任何化学反应的物质，此类物质只是通过对反应体系物理性质的影响，来改变溶液工作电势与反应参与物活度之间的非平衡能斯特关系。利用 Nernst 方程可以对自发不可逆电极过程中电位和有关物质活度之间定量关系进行描述[6]。一般情况下，任何自发氧化还原反应可用通式表示为

$$n_1O_1 + n_2R_2 + \cdots \rightarrow n_1R_1 + n_2O_2 + \cdots \tag{1}$$

式中 n_i 为化学计量学数，设电子转移数 $n = n_1n_2$，当把其构建成原电池反应时，此反应可以拆分为氧化和还原半反应，反应式如下：

氧化半反应

$$n_1R_1 - n\mathrm{e} + \cdots \rightarrow n_1'O_1 + \cdots \tag{2}$$

还原半反应

$$n_2O_2 + n\mathrm{e} + \cdots \rightarrow n_2R_2 + \cdots \tag{3}$$

在式(2)、(3)中，相对应的电极电位 φ_O 和 φ_R 可以分别表示为

$$\varphi_O = \varphi_O^{\circ} + \frac{RT}{nF}\ln\frac{a_{O_1}^{n_1}\cdots}{a_{R_1}^{n_1}\cdots} \tag{4}$$

$$\varphi_R = \varphi_R^{\circ} + \frac{RT}{nF}\ln\frac{a_{O_2}^{n_2}\cdots}{a_{R_2}^{n_2}\cdots} \tag{5}$$

式中　φ_O°——标准电极电位

就非线性氧化还原反应的电极过程而言，反应物和生成物活度都随着时间 t 不断变化，是时间 t 的非线性函数，因此电位同样也是时间的非线性函数，其电位与时间关系式可表示为

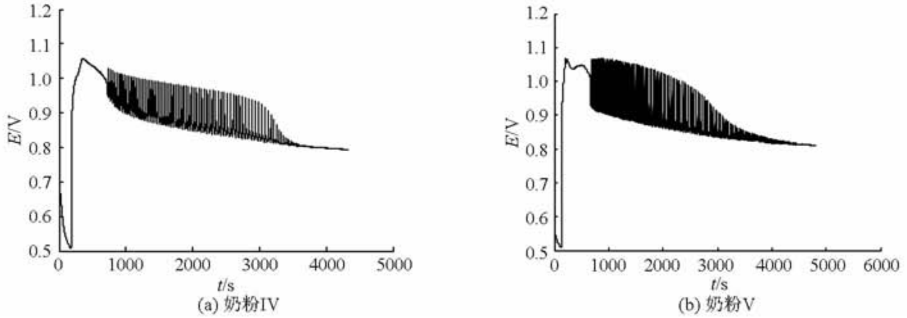
$$\varphi_R(t) = \varphi_R^{\circ}(t) + \frac{RT}{nF}\ln\frac{(a_{O_1}(t))^{n_1}\cdots}{(a_{R_1}(t))^{n_1}\cdots} \tag{6}$$

由式(6)可知，在非线性化学反应体系中各种反应物与其活度的改变，将导致相应时刻电极电位的改变，最终导致整个 $E-t$ 关系式发生改变，这些改变从非线性化学指纹图谱可量化信息和直观形状的变化中能明显看出来。如在“硫酸－丙酮－溴酸钠－硫酸锰”体系中，采用非线性化学指纹图谱测定不同品牌婴幼儿配方奶粉Ⅳ和Ⅴ的指纹图谱和可量化信息分别见表 1 和图 2。

表 1　体系中反应试剂的加入量对奶粉Ⅴ非线性化学指纹图谱定量信息的影响

Tab. 1　Effects of dosages of reaction reagents in reaction system on quantitative information of nonlinear chemical fingerprint of milk powder Ⅴ

体系编号	硫酸浓度 /(mol·L⁻¹)	硫酸锰浓度 /(mol·L⁻¹)	溴酸钠浓度 /(mol·L⁻¹)	丙酮浓度 /(mol·L⁻¹)	诱导时间 /s	停波时间 /s	最大波幅 /V
1	0.75	0.08	0.80	0.50	729.498	5 599.698	0.130 6
2	1.15	0.08	0.80	0.50	713.598	3 621.000	0.107 1
3	0.75	0.08	0.80	1.50	931.602	6 609.402	0.132 5
4	1.00	0.06	1.00	1.00	856.998	5 616.198	0.118 8
5	1.00	0.06	0.80	1.50	1 054.20	3 188.400	0.070 2
6	1.00	0.08	0.80	1.00	736.002	2 874.798	0.074 0



(a) 奶粉Ⅳ　(b) 奶粉Ⅴ

图 2　奶粉Ⅳ和奶粉Ⅴ的非线性化学指纹图谱(检测量:0.900 5 g)

Fig. 2　Nonlinear chemical fingerprints of milk powder Ⅳ and milk powder Ⅴ (detecting dosage: 0.900 5 g)

由表 1 可知,体系中各物质的浓度变化对指纹图谱可量化参数具有不同程度的影响。由图 2 可知,每种样本指纹图谱的形成并非只是由于某种或某些非线性化学反应底物(包括耗散物)的作用,而是体系中所有共存物质不同程度作用的结果,这些共存物质对由底物引起的非线性化学反应都有不同的影响。因此,非线性化学指纹图谱特征信息是体系中各物质不同程度共同作用的结果,反映的是样品中各物质的群集特征。当体系中其它所有共存物质的种类和浓度保持恒定不变时,而只改变某种对指纹图谱特征信息有明显作用的物质的用量时,非线性化学指纹图谱特征信息的变化就是该物质单独对指纹图谱作用的结果,当这种作用使指纹图谱定量信息产生的变化与该物质浓度之间存在某种确定的函数关系时,利用此关系就可对物质进行定量分析[21]。

在本研究中,奶粉中钙的总含量($M_0+\Delta M$)对指纹图谱波动周期 τ_{und} 的影响规律如图 3 所示,其中 M_0 和 ΔM 分别为奶粉中固有钙含量和奶粉中加入的钙量,τ_{und1}、τ_{und2}、τ_{und3}、τ_{und4} 为奶粉中钙的总含量分别为 $M_0+\Delta M_1$、$M_0+\Delta M_2$、$M_0+\Delta M_3$、$M_0+\Delta M_4$ 时测得的非线性化学指纹图谱波动周期值。利用波动周期和奶粉中钙的总含量之间的线性关系,即线性关系的回归方程 $\tau_{\mathrm{und}}=k(M_0+\Delta M)+b$,则当奶粉中钙的加入量 ΔM 已知时,通过此回归方程,就可求出奶粉中固有的钙含量 M_0,其中 k 和 b 分别为该线性回归方程的斜率和截距。通过实验可知,当 k 值越大时,表明被测物质对指纹图谱波动周期的影响越大,b 理论上是奶粉中钙的总含量为零时指纹图谱的波动周期,该波动周期值完全取决于奶粉中除钙外所有共存物质对指纹图谱的作用。由于不同品牌的奶粉,其共存物质的种类和含量不同,因此,其线性回归方程中 k 和 b 的值也存在差异。在某些情况下,很低或很高的钙的总含量对非线性化学指纹图谱的波动周期的影响可能偏离线性关系,如图 3 中曲线 m 和 p 或曲线 l 和 q 所示。因此,本研究考虑的问题需要奶粉中钙总含量($M_0+\Delta M$)在 0.022 7 ~ 0.032 3 g 范围内,运用最小二乘法,利用指纹图谱特征参数如波动周期与相应奶粉中钙的总含量之间的线性关系,可求出奶粉中固有的钙含量。对于同品牌不同批次的奶粉,利用这种线性回归方程,只要在相同条件下测得其指纹图谱的波动周期,同样可以求出奶粉中固有的钙含量。本研究奶粉中其它物质对指纹图谱波动周期的影响,在线性回归方程的截距 b 中已经基本考虑,无需做干扰实验。

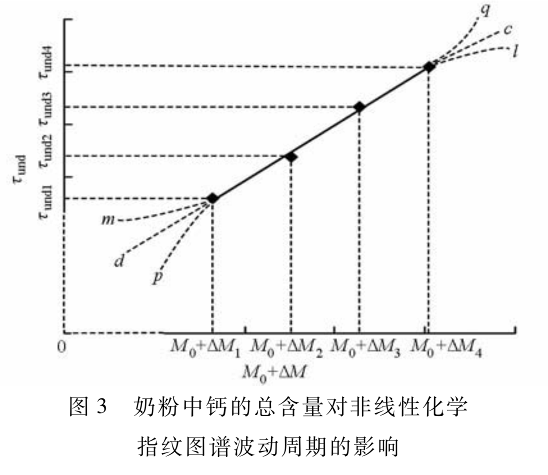


图 3　奶粉中钙的总含量对非线性化学指纹图谱波动周期的影响

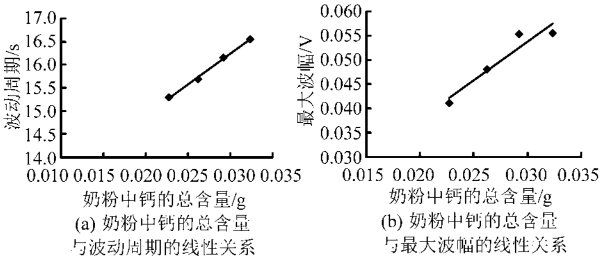
Fig. 3　Effects of total content of calcium in milk powder on undulatory period of nonlinear chemical fingerprint

4　实验结果分析

4.1　奶粉中钙的总含量对非线性化学指纹图谱波动周期的影响

不同奶粉样品非线性化学指纹图谱的 $E-t$ 曲线形状不同,所以其相应的特征参数如波动周期、诱导时间、最大波幅等也存在差异,但是这些特征参数中往往有一个或多个与奶粉中某个物质的含量呈线性关系。例如,在一定范围内,奶粉Ⅱ的非线性化学指纹图谱特征参数波动周期和最大波幅均与奶粉Ⅱ中钙的总含量呈一定的线性关系,如图 4 所示。

奶粉Ⅱ中钙的总含量对非线性化学指纹图谱波



(a) 奶粉中钙的总含量与波动周期的线性关系

(b) 奶粉中钙的总含量与最大波幅的线性关系

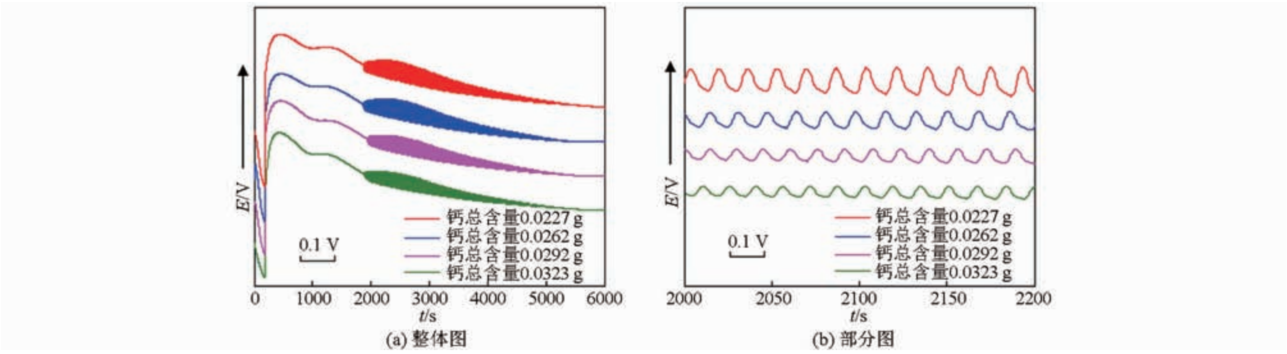
图4 奶粉Ⅱ中钙的总含量与非线性化学指纹图谱波动周期和最大波幅的线性关系

Fig. 4 Linear relationship between total content of calcium in milk powder Ⅱ and undulatory period and maximum amplitude of nonlinear chemical fingerprint

动周期的影响见图5，图5b的指纹图谱是在2 000～2 200 s时的部分波动曲线图。

如图6所示，当奶粉中钙的总含量在0.022 7～0.032 3 g范围时，奶粉Ⅰ、Ⅱ、Ⅲ的非线性化学指纹图谱的特征参数波动周期与相应奶粉中钙的总含量之间均呈线性关系，这些信息为测定各种奶粉中固有的钙含量提供了必要条件。

4.2 非线性化学指纹图谱法测定奶粉中固有的钙含量

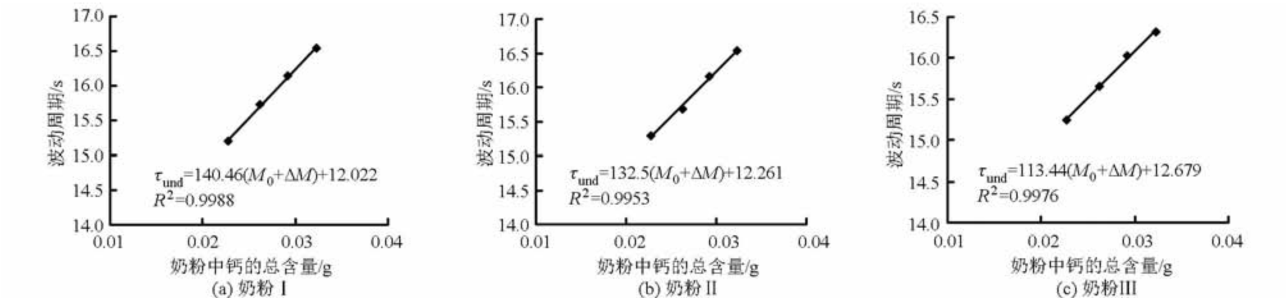


(a) 整体图

(b) 部分图

图5 钙的总含量对奶粉Ⅱ非线性化学指纹图谱波动周期的影响

Fig. 5 Effects of total content of calcium on undulatory period of nonlinear chemical fingerprint of milk powder Ⅱ



(a) 奶粉Ⅰ

(b) 奶粉Ⅱ

(c) 奶粉Ⅲ

图6 3种奶粉中钙的总含量对非线性化学指纹图谱波动周期的影响

Fig. 6 Effects of total content of calcium in three bands of milk powder on undulatory period of nonlinear chemical fingerprint

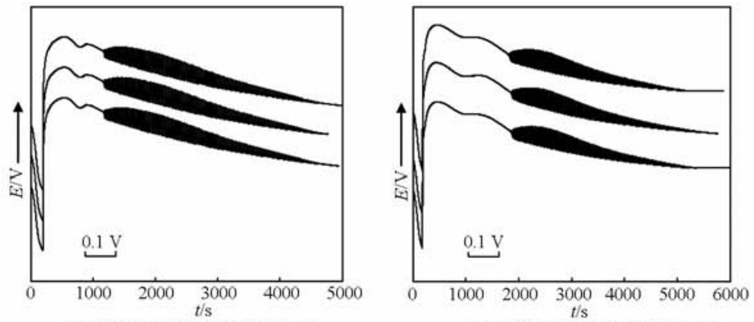
利用非线性化学指纹图谱法，按照1.2.1节的实验方法，测定3种奶粉，根据奶粉Ⅰ、Ⅱ、Ⅲ的非线性化学指纹图谱的特征参数波动周期与相应奶粉中钙的总含量之间所成线性关系，通过图6中相应的拟合方程，当奶粉中钙的加入量ΔM已知时，可求出相应奶粉中固有的钙含量M_0。实验结果见表2。由表2可知，各种奶粉中固有的钙含量测定结果的相对标准偏差（RSD）小于等于1.63%，说明该方法的精确度较好，回收率在99.19%～101.60%之间，说明该方法的准确度较高。实验结果表明，采用非线性化学指纹图谱法，对奶粉中固有的钙含量进行定量分析，其方法可行，同时还可以为样品中其它物质的含量测定提供依据。

为了进一步验证实验的可行性和可靠性，分别取样品与奶粉Ⅰ、Ⅱ同品牌不同批次的奶粉3份，按照1.2.1节的实验方法，用适量的碳酸钙标定，奶粉Ⅰ和奶粉Ⅱ中钙加入量分别为0.013 2 g和0.015 6 g，每个样品平行测定3次，2个奶粉的非线性化学指纹图谱见图7，其检测结果见表3。

表2 非线性化学指纹图谱法测定奶粉中固有的钙含量的结果

Tab. 2 Determination results of inherent calcium content in milk powder by nonlinear chemical fingerprint method

样品	波动周期/s	回归方程	R^2	钙加入量/g	检测值/g	国标检测值/g	RSD/%（$n=3$）	回收率/%
奶粉Ⅰ	16.042	$\tau_{und}=140.46(M_0+\Delta M)+12.022$	0.998 8	0.016 1	0.012 5	0.012 3	0.56	101.60
奶粉Ⅱ	15.759	$\tau_{und}=132.50(M_0+\Delta M)+12.261$	0.995 3	0.014 1	0.012 3	0.012 2	1.63	100.80
奶粉Ⅲ	16.176	$\tau_{und}=113.44(M_0+\Delta M)+12.679$	0.997 6	0.018 5	0.012 3	0.012 4	0.94	99.19



(a) 奶粉Ⅰ中钙加入量为0.013 2 g　　(b) 奶粉Ⅱ中钙加入量为0.015 6 g

图 7　钙的总含量对奶粉Ⅰ和奶粉Ⅱ非线性化学指纹图谱的影响

Fig. 7　Effects of total content of calcium on nonlinear chemical fingerprints of milk powder Ⅰ and milk powder Ⅱ

表 3　非线性化学指纹图谱法测定奶粉Ⅰ和奶粉Ⅱ中固有的钙含量的结果

Tab. 3　Determination results of inherent calcium content in milk powder Ⅰ and milk powder Ⅱ by nonlinear chemical fingerprint method

样品	波动周期 /s	回归方程	R^2	钙加入量 /g	检测值 /g	国标检测值 /g	RSD/% ($n=3$)	回收率 /%
奶粉Ⅰ	15.597	$\tau_{\text{und}} = 140.46(M_0 + \Delta M) + 12.022$	0.998 8	0.013 2	0.012 3	0.012 3	0.47	100
奶粉Ⅱ	15.935	$\tau_{\text{und}} = 132.50(M_0 + \Delta M) + 12.261$	0.995 3	0.015 6	0.012 1	0.012 2	0.48	99.18

4.3　奶粉中固有的钙含量的检测范围

本研究是通过加入适量的钙使得奶粉中钙的总含量在 0.022 7 ~ 0.032 3 g 范围内来测定奶粉中固有钙含量的。因此，针对本研究中的奶粉，当其中固有的钙含量在 0 ~ 0.032 3 g 之间时，通过此方法均能求出相应奶粉中固有的钙含量，所以对本研究中的奶粉，其最低和最高检测限分别为 0 g 和 0.032 3 g。基于此，奶粉中固有的钙含量的检测范围为 0 ~ 0.032 3 g。

5　结束语

利用非线性化学指纹图谱技术对奶粉中固有的钙含量进行定量分析，同时根据奶粉非线性化学指纹图谱波动周期与奶粉中钙的总含量之间的线性回归关系，测定奶粉中固有的钙含量。实验结果表明，当奶粉总量为 0.9 g、奶粉中钙总含量在 0.022 7 ~ 0.032 3 g 范围内时，钙总含量与波动周期值线性关系良好，决定系数R^2 为 0.995 3 ~ 0.998 8，样品加标回收率为 99.18% ~ 101.6%，RSD 为 0.47% ~ 1.63%，奶粉中固有钙含量检测范围为 0 ~ 0.032 3 g。因此，提出的非线性化学指纹图谱法具有较高的精密度和准确度，不仅可以用来测定同品牌不同批次的奶粉中固有的钙含量，而且也可以用于复杂样品中其它物质含量的测定，同时为样品中微量金属元素含量的测定提供了一种方法。该方法具有操作简单和分析成本低廉的优点，具有良好的应用前景。

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(上接第 201 页)

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