Simultaneous Determination of Iron, Copper and Cobalt in Food Samples by CCD-diode Array Detection-Flow Injection Analysis with Partial Least Squares Calibration Model

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Abstract A flow injection-CCD diode array detection spectrophotometry with partial least squares (PLS) program for simultaneous determination of iron, copper and cobalt in food samples has been established. The method was based on the chromogenic reaction of the three metal ions and 2- (5-Bromo-2-pyridylazo)-5-diethylaminophenol, 5-Br-PADAP in acetic acid - sodium acetate buffer solution (pH5) with Triton X-100 and ascorbic acid. The overlapped spectra of the colored complexes were collected by charge-coupled device (CCD) – diode array detector and the multi-wavelength absorbance data was processed using partial least squares (PLS) algorithm. Optimum reaction conditions and parameters of flow injection analysis were investigated. The samples of tea, sesame, laver, millet, cornmeal, mung bean and soybean powder were determined by the proposed method. The average recoveries of spiked samples were 91.80%~100.9% for Iron, 92.50%~108.0% for Copper, 93.00%~110.5% for Cobalt, respectively with relative standard deviation (R.S.D) of 1.1%~12.1%. The sampling rate is 45 samples h⁻¹. The determination results of the food samples were in good agreement between the proposed method and ICP-AES.

1. Introduction
Iron, Copper and Cobalt are all trace essential elements for human bodies. Lack of these essential elements can induce some diseases while it is harmful and deleterious for overtaken. Spectrophotometry is one of the most widely used methods in food analysis. However, it is not satisfying in selectivity for simultaneous determination multiple elements as their absorption spectra are overlapped seriously. Chemometrics can enlarge the application range of spectrophotometry. Partial least squares (PLS) is usually optimal multivariate calibration method, whose advantage is needless to ask for inverse matrix and avoiding the deviation brought in the process [1], therefore it is widely applied to the simultaneous spectrophotometric determination. Charge coupled device (CCD) as a new array detector can offer rapidly scan spectra of analytes in about 0.1sec.

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Applying CCD detector in flow injection analysis with PLS algorithm can more precisely and rapidly process overlapped spectral for multi-component analysis [2-4]. The main purpose of this work was to develop a new method for the simultaneous determination of Fe, Cu, Co based on their reaction with 5-Br-PADAP, using flow injection analysis with a CCD detector, the multi-wavelength data thus obtained can be processed by PLS algorithm. The proposed method was applied to the determination of these metals in food samples without previous separation.

2. Experimental

2.1 Reagents and apparatus
Standard stock solutions of 1000mg/l (in 0.05mol/L HNO₃) for Fe, Cu, Co; 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol (Fluka, Buchs, Switzerland); 5.0×10⁻³moll⁻¹ of 5-Br-PADAP solution was prepared with methanol. 1.0moll⁻¹ of sodium acetate buffer solution (pH 5.0) including 2% (v/v) Triton X-100 and 30gl⁻¹ Ascorbic acid was as the carrier. A spectrophotometric setup with flow injection -CCD-diode array detector (CCD-chip: TCD142D, TOSHIBA, Japan) and computer was coupled on-line forming a continuous spectrophotometric system [3,4]; Inductively Couple Plasma-Atomic Emission Spectroscopy (TRACE ACAN, TIA Company, USA) was use for comparison with the proposed method.

2.2 Procedure

2.2.1 Standard calibration set for PLS model
The standard calibration set containing 16 standard solutions was prepared in orthogonal-design of L₁₆ (4)³ with the concentrations of 0.50 ~ 5.00 µg/ml⁻¹ for Fe, 0.05~0.50µg/ml⁻¹ for Cu and 0~0.20µg/ml⁻¹ for Co. The standard solution of 200µl was injected automatically, then mixed with carrier at a flow rate of 2.3ml/min⁻¹ and reacted with chromogenic reagent at flow rate of 4.5ml/min⁻¹. Thereafter, the processed plug was passed through flow cell and the absorption spectra of three analytes were simultaneously collected by CCD-diode array detector over the range of 350~700nm. Absorbance matrix of standard calibration set was consisted of 200-selected wavelength spectra collected at an interval of two channels from 1100 to 1500 channel (519.9nm~580.8nm).

2.2.2 Preparation and analysis of samples
Food samples were weighed accurately and digested with nitric acid and perchloric acid for determination of Fe, Cu and Co. The residue was dissolved with 10ml double distilled water for determination. A portion of the digested sample solution was directly injected in the flow injection system. The absorbance matrix of analytes in the sample was established as the same procedure of the standard calibration set. According to the matrixes of concentration and absorbance for standard solutions, the contents of in the sample can be calculated by PLS calibration model with Matlab language. Both of the data of absorbance and concentration were standardized before processing with PLS.

3 Results and discussion

3.1 Absorption spectra of Fe, Cu and Co complexes
The maximum absorption wavelengths of the complexes of Fe, Cu, Co with 5-Br-PADAP are 557.2nm and 746nm for Fe(III) complex, 554.2nm for Cu(II) complex, 554.4nm and 588.0nm for Co(II) complex. The molar absorption coefficients of the analytes were determined in our experiments: \( \varepsilon_{557.2nm}^{Fe}=3.0\times10^4 \) l mol⁻¹
The absorption spectra of these complexes were overlapped severely and it is difficult to simultaneous determine the three metal ions using traditional spectrophotometry. However, a flow injection analytical technique and CCD multi-channel detector in combination with PLS calibration models allows the resolution of the three ion mixtures.

### 3.2. Influence of the variables

5-Br-PADAP is a kind of high sensitive chromogenic reagent and it can form colored complexes with many transition metal elements in acidic and neutral medium \(^5,^6\). The acidity of carrier was studied in the range of pH1.0~6.0 in our experiments. The result showed that the absorbencies of three complexes were highest and the most stable in the range of pH4.5~5.5. In this case, sodium acetate–acetic acid buffer solution of pH5.0 was selected as carries to control acidity of the reaction.

It has been reported that Cu( I), Fe (II) and Fe (III) can react with 5-Br-PADAP forming stable complexes. The Fe (II)-5-Br-PADAP complex is more stable than Fe (III)-5-Br-PADAP complex at pH 3-7 \(^7\). It is also observed in our experiments that the complex of Fe (II)- Br-PADAP is quickly and quantitatively formed at pH 3-6, especially in present of ascorbic acid. The concentration of the reducing agent in the carrier was investigated and the results showed that effect of ascorbic acid on absorbance of Cu is greater then that of Fe, and little effect on the absorbance of Co and ascorbic acid of 30gl\(^{-1}\) was chosen to add in the carrier.

In order to improve sensitivity and repeatability, surfactant of Triton X-100 was added in the reaction system. The experimental results indicated that the detection sensitivity and repeatability increase in a certain extent when Triton X-100 of 0.5~3.0% was added to the carrier. Therefore 2% of Triton X-100 was chosen to add in the carrier. Compared with the reaction system without the surfactant, the absorbances of Fe, Cu and Co complexes increased by 6.3%, 25.2% and 18.5%, respectively.

### 3.3 Optimizing of parameters of FIA

An orthogonal design of L\(_{16}(4)^3\) was used to optimize the parameters of FIA. Considering detection sensitivity, sampling rate and consuming of the reagent, the optimum parameters were selected as fellows: sample injection volume of 200\(\mu\)l, the flow rate of 2.3 mlmin\(^{-1}\) for carrier and 4.5 mlmin\(^{-1}\) for chromogenic reagent, respectively. Under the optimum conditions the absorption spectra of the mixed standard solutions with different concentration and tree-dimensional absorption spectra of three complexes collected by CCD detector-FI setup were showed in Figure 1 and Figure 2.

### 3.4 Calibration with PLS

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Fig.1. Absorption spectra of mixed standard solutions with different concentrations by FIA- diode array detector

Fig.2. Tree-dimensional absorption spectra of Fe, Cu and Co complex collected by FIA- CCD diode array detector
In order to resolve the severe overlapped spectra of the three complexes, multivariate calibration was performed with PLS algorithm by using an in-house software package written in Matlab for windows \[8\]. The effect of detected wavelength numbers of 50–250 wavelengths over the range of 1100–1500 channel was investigated in our experiment. The results indicated measured wavelength points of 200 were the most suitable for resolving the ternary system. In order to determine the optimum number of latent variables for the PLS calibration model, the cross-validation procedure of “Leave one sample out” was applied \[9\]. On the principle of the Haaland and Thomas criterion \[10\], the PLS model with 7 factors was used for the three analytes in the experiment. The standard error of prediction (SEP) were 0.0203–0.0446 for Fe, Cu and Co.

3.6. Application
Under the optimum conditions described above, the optimum linear ranges were 0.10–10µg/ml for Fe, 0.05–5.0µg/ml for Cu, 0.01–1.0µg/ml for Co, respectively. The limits of detection were 0.10 µg/ml for Fe, 0.05µg/ml for Cu and 0.01µg/ml for Co, respectively. The repeatability and precision of the method were satisfied with RSD in the range of 1.2%–12.1% with six replicate determinations of seven kinds samples. The effect of interfering species of 26 commonly found in food samples was investigated. The most interfering ions tested did not interfere the determination. Therefore, the three metal ions can be directly determined after digestion without any pretreatment by the proposed method.

Accuracy of the proposed method was validated using a certified reference material of tea and peach leaf (GBW07605 and GBW08501, Chinese Standard Material Center) determined by the proposed method and the determined values (n=6) of the certified reference material were within the given guarantee values. The spiked recoveries of samples were 91.80%–100.9% for Fe, 92.50%–108.0% for Cu, 93.00%–110.5% for Co. Accuracy of the method was also validated by means of comparison with ICP-AES method. The determination results of sesame, laver, soybean powder samples are in good agreement between the proposed method and ICP-AES and the relative error between the two methods was below 13.1%.

4 Conclusion
In this paper, a multi-components analysis with PLS program using Flow injection-CCD detector-spectrophotometry has been developed. The proposed method has been successfully applied to the simultaneous determination of Fe, Cu and Co in certified reference materials and food samples after digestion without further pretreatment. Compared with the traditional spectrophotometry, the proposed method provides good results for three analytes in terms of accuracy and precision and allows 45 determinations per hour for the digested food samples, and the results proved to be satisfied and met the criterion of food analysis.

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