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RESEARCH ARTICLE

FIRST DERIVATIVE SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF IRON AND CALCIUM USING BROMOPYROGALLOL RED.

Amin K. Qasim and Lazgin A. Jamil.

Faculty of Science, Department of Chemistry, University of Zakho, P.O. Box 12, Zakho-Duhok, Kurdistan Region, Iraq.

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Abstract

Bromopyrogallol Red (BPR) as analytical reagent has been used for simultaneous determination of iron and calcium using first-derivative spectrophotometry. The zero - crossing technique was employed using first-derivative for Fe-BPR and Ca-BPR at 600 nm and 558 nm respectively. The linear relationship for Fe and Ca determination obeys Beer's law from 0.2-1.2 µg/ml and 0.2-1.0 µg/ml respectively. The proposed method should be useful for accurate, precise, and rapid determination of iron and calcium in various samples with percentage recovery of about 101%.

The stability constants (mean of three values) were found to be $1.33 \times 10^8 \text{ L}^2 \cdot \text{mol}^{-2}$ and $9.13 \times 10^{11} \text{ L}^2 \cdot \text{mol}^{-2}$ for Fe-BPR and Ca-BPR complexes respectively which indicates that the complexes are stable.

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Introduction:-

Derivative spectrophotometry in the UV-Vis region is a useful technique in extracting qualitative and quantitative information from overlapping bands of the analyst and interference due to incompletely resolve peaks. A number of studies were reported showing the advantage of derivative spectrophotometry [1].

Derivative spectrophotometric methods have been used for the quantitative analysis of complex mixture because of their great sensitivity and selectivity as well as useful means of resolving two overlapping spectra and eliminating matrix interferences in the assay of two-component mixtures using the zero-crossing technique [2].

Iron and calcium are metals which appear together almost in several samples, both natural and artificial. In most cases, the characterization of these samples includes the determination of their metal ion content. The need for iron and calcium analysis in environmental and biochemical material has increased after reports on different roles of these metals in human health, diseases and industries [3]. Since the simultaneous presence of iron and calcium in many real samples, selective analytical techniques and methods have been put forward for simultaneous determination of both elements. Some simultaneous determination techniques and methods are derivative spectrophotometry, atomic absorption spectrometry, sequential flow injection technique, high-performance liquid chromatography, H-point standard addition and partial least squares methods. Among the proposed methods for simultaneous determination of species, derivative spectrophotometry has shown some advantages such as simplicity, speed, sensitivity and selectivity [4]. Derivative spectrophotometry has been used for the simultaneous determination of inorganic ions through the formation of their complexes with the same organic ligand. Some examples are mentioned in chapter one.

Corresponding Author:- Lazgin A. Jamil.

Address:- Faculty of Science, Department of Chemistry, University of Zakho, P.O. Box 12, Zakho-Duhok, Kurdistan Region, Iraq.

Experimental:-**Apparatus:-**

Spectral and absorbance measurements were carried out using UNICAM, HeλIOS β UV-Vis computerized single beam spectrophotometer Connected with hp laser Jet 1200 Series printer. The pH measurements were made by using both Cyber Scan 510 pc.PH meter with a combined glass electrode.

2.2. Reagents

All chemicals used were of the highest purity available. Double distilled deionizer water was used throughout this study and its dilution. Glass vessels were cleaned by double distilled water. And BPR reagent ($3.6 \times 10^{-4} \text{M}$) was prepared by dissolving 0.0207 gm of BPR (Hopkins and William/Ltd) in double distilled water and the volume was made to 100 ml in volumetric flask and kept in dark bottle.

2.2.1. Stock Iron (III) (100 ppm) solution

This solution was prepared by dissolving 0.723 gm of Ferric nitrate $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Fluka) in double distilled water and few drops of nitric acid (S.p.gr.1.41,65%)(Alpha α) were added to convert all Fe^{2+} to Fe^{3+} . The solution was completed to 1000 ml volumetric flask. The working iron (10 ppm) solution ($1.79 \times 10^{-4} \text{M}$) was prepared by diluting 10 ml of the above solution to 100 ml with double distilled water in a volumetric flask.

2.2.2. Stock calcium (II) (100 ppm) solution

This solution was prepared by dissolving 0.546 gm of calcium chloride hydrous $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Analar BDH) in double distilled water and a few drops of HCl were added to prevent “plating out”. The solution was completed to 1000 ml volumetric flask. The working calcium (10 ppm) solution ($2.492 \times 10^{-5} \text{M}$) was prepared by diluting 10 ml of the above solution to 100 ml with double distilled water in a volumetric flask.

2.2.3. Buffer (pH 10) solution (phosphate buffer)

The solution contained 50 ml 0.05 M Na_2HPO_4 with 2.2 ml of 0.1M NaOH. The solution was prepared with double distilled water and completed to 100ml in volumetric flask [5].

Recommended procedure:-

For the simultaneous determination of iron and calcium, a sample or standard solution containing 5-30 $\mu\text{g ml}^{-1}$ of iron and 5 - 25 $\mu\text{g ml}^{-1}$ of calcium was placed in a calibrated 25 ml flask. 5 ml of reagent solution and 2ml of sodium hydrogen phosphate was added to the mixture. Then the pH of the solution was adjusted to 10. Finally, the volume was made with 25 ml with deionize distilled water.

Fig. (1) show that Beer's law was obeyed in the concentration range 0.2-1.2 $\mu\text{g/ml}$ and 0.2-1.0 $\mu\text{g/ml}$ for determination of iron and calcium respectively.

2.3.1. First-derivative (ID) measurement

The first derivative absorption spectra of the Fe-BPR and Ca-BPR complexes in the basic media against a reagent blank were recorded. Zero-crossing wavelengths in the first-derivative spectra of Fe-BPR and Ca-BPR that can be used for their sensitive simultaneous determination were (600 nm) and (558 nm), respectively, and comparing the value with appropriate calibration graph in as Fig. (1). In the zero-crossing derivative method, it is necessary that zero-crossing wavelengths do not change with varying concentrations of the related species.

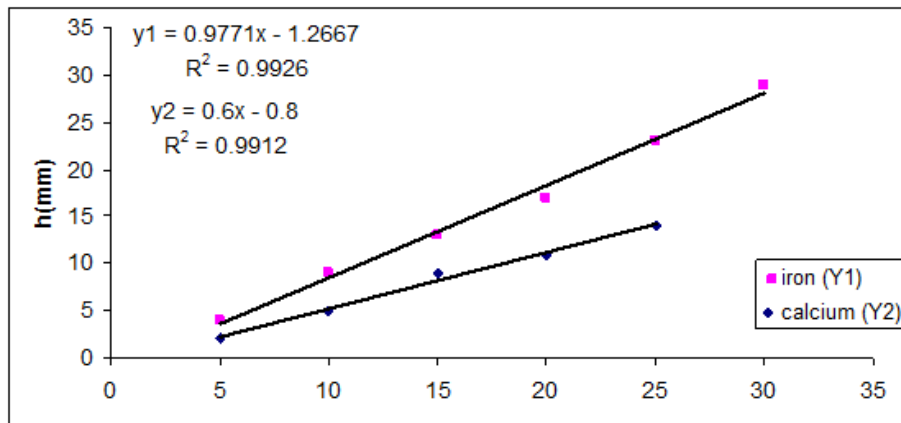


Fig. 1:- First-derivative signal height vs. concentration calibration graph for Fe and Ca complexes with BPR reagent.

Table 1:- The optimum condition and parameter of derivative spectra

Parameter	Value
Speed	1200 nm/min
Data interval	4.0 nm
Band width	2.0 nm
Order of addition	[M+B+R]
BPR reagent ($3.6 \times 10^{-4}\text{M}$)	5 ml
Buffer solution (pH10)	2 ml

Results and discussion:-

Effect of experimental variability:-

The effects of various parameters on the simultaneous determination of iron and calcium were investigated. One of the most important parameters was pH. Experiments in various pH show that the spectra of BPR, Fe-BPR and Ca-BPR complexes depended on the pH of the solution. The shape of the absorption spectra, maximum wavelengths and molar absorptivity's changed considerably when pH varied from 9 to 11 for iron and from 8 to 10.5 for calcium. The shape and maximum wavelengths of the spectra of iron and calcium complexes did not change at pH 10. Optimization of the other parameters such as BPR, Speed, Data interval, Band width and order of addition was performed spectrophotometrically for iron at 600 nm and for calcium at 558 nm.

Spectrophotometric measurement:-

Zero-order absorption spectra of iron and calcium showed certain overlapping that interfere with the direct simultaneous determination of this formulation[6]. The normal spectra of Fe (III)-BPR complex and Ca(II)-BPR complex in the presence of sodium phosphate buffer shows the absorbance maxima at 600 nm and 558 nm, respectively. The absorption spectrum of the mixture of iron and calcium was 548nm as shown in curve Fig. 2c. Since the spectral bands of complexes overlap, the determination of iron and calcium in their mixtures by zero-order (normal spectra) is frequently difficult. By using 1st derivative spectrophotometry, these samples can be analysed simultaneously.

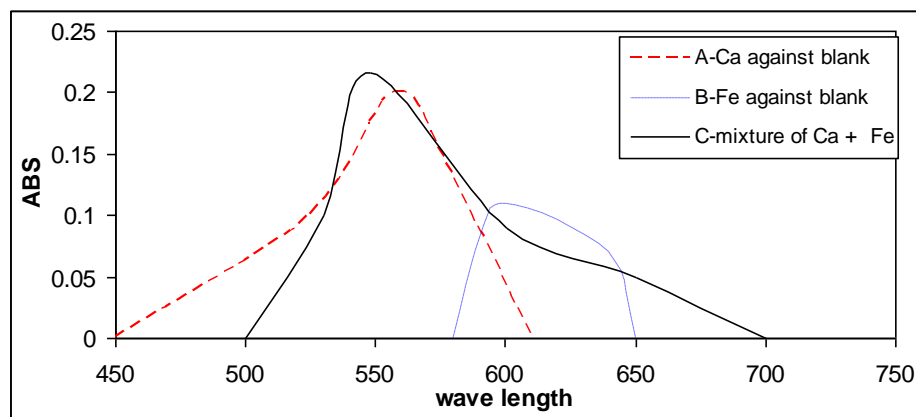


Fig. 2:- Absorption spectra of the BPR complexes of (A) Ca, (B) Fe and (C) mixture of Fe and Ca. [Fe]=20 μg of Fe (III)/25 ml and [Ca]=15 μg of Ca(II)/25 ml.

First-derivative spectrophotometry:-

The first derivative is a plot of the gradient $dA/d\lambda$ of the absorption envelope versus wavelength. Derivative spectra can be produced by processing the spectrophotometer output. The use of derivative spectra can increase the detection sensitivity of minor spectra features and reduce the error caused by the overlap of the analyte spectral band by interfering bands of other species in the sample[7]. Fig (3) and Fig (4) shows the 1st –derivative absorption spectra of the complexes of Fe and Ca with BPR and of a mixture of both complexes respectively.

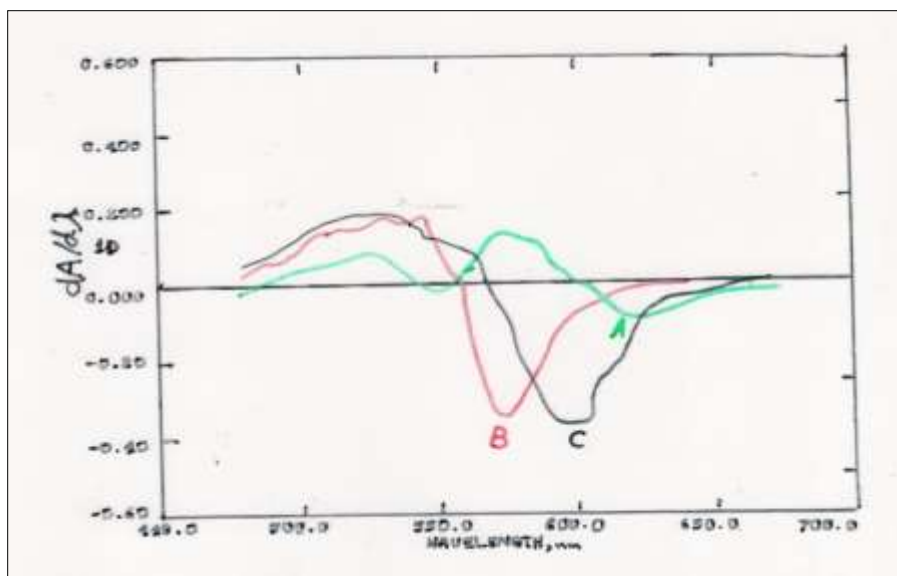


Fig. 3:- First-derivative signal height of the BPR complexes of (A)Fe, (B)Ca and (C)mixture of Ca and Fe.

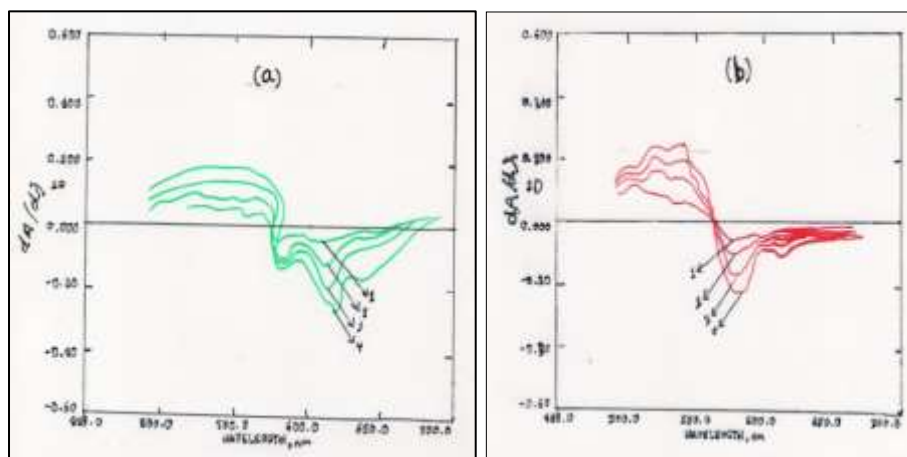


Fig. 4:- First derivative spectra of the solutions containing: a) fixed $10 \mu\text{g mL}^{-1}$ Calcium and 1: $10 \mu\text{g mL}^{-1}$, $15 \mu\text{g mL}^{-1}$, $20 \mu\text{g mL}^{-1}$ and $25 \mu\text{g mL}^{-1}$ iron. b) fixed $10 \mu\text{g mL}^{-1}$ iron and 1: $10 \mu\text{g mL}^{-1}$, $15 \mu\text{g mL}^{-1}$, $20 \mu\text{g mL}^{-1}$ and $25 \mu\text{g mL}^{-1}$ calcium Conditions: 25mL of solution containing 2 ml of buffer pH 10 and 5 ml of reagent ($3.6 \times 10^{-4}\text{M}$).

Selection of optimum instrumental conditions:-

Instrumental parameters such as $\Delta\lambda$, band width and scan speed were optimized to give a constant position of isodifferential/zero cross-over points. The optimum $\Delta\lambda$ for the first derivative was found to be 4.0 nm. A scan speed of 1200nm/min and band width of 2 nm was found suitable for simultaneous determination of iron and calcium. Response time was automatically selected by the spectrophotometer in accordance with the optical energy and speed of scan. And the noise level decreased with an increase in $\Delta\lambda$.

Analytical determination:-

Simultaneous determination of Fe and Ca by 1st-derivative spectrophotometry in the milk.

Milk products are a very important human nutrient since their consumption has increased in recent years. Milk contains a large variety of essential nutrients for the development and maintenance of a salutary life. This product contains a complex mixture of minerals, including calcium, magnesium, sodium, potassium, chloride, iron, and phosphate. Calcium in milk is distributed between the milk serum and the casein micelles. Calcium is an essential macronutrient for humans, which represents approximately 2% of body weight in an adult person. This element has mainly a structural function in bones and teeth, and also to regulate many vital biological functions[8]. The iron content of milk is of physiological interest. It is the only source of iron for the child for about 10 months[9].

Table 2:- Determination of Ca and Fe in the milk

Sample	Elements	Present method $\mu\text{g}/25\text{ ml}$	AAS
Milk	Ca	74.611	77.468
	Fe	0.399	0.429

Precision and accuracy:-

The accuracy and precision of the first- derivative simultaneous determination procedures are given in table (3). According to the results, the applicability of the method for the simultaneous determination of iron and calcium in their mixtures was clarified. The recovery and relative standard deviations are also shown in the table (4), indicating that the method was accurate and precise.

Table 3:- Statistical analysis of the determination of Fe and Ca in the mixtures by 1st-derivative spectrophotometry.

Element determined	Other element present		Slop	Intercept	Correlation coefficient
	Element	ppm			
Fe	Ca	0.4	0.97	1.266	0.992
Ca	Fe	0.4	0.6	0.8	0.991

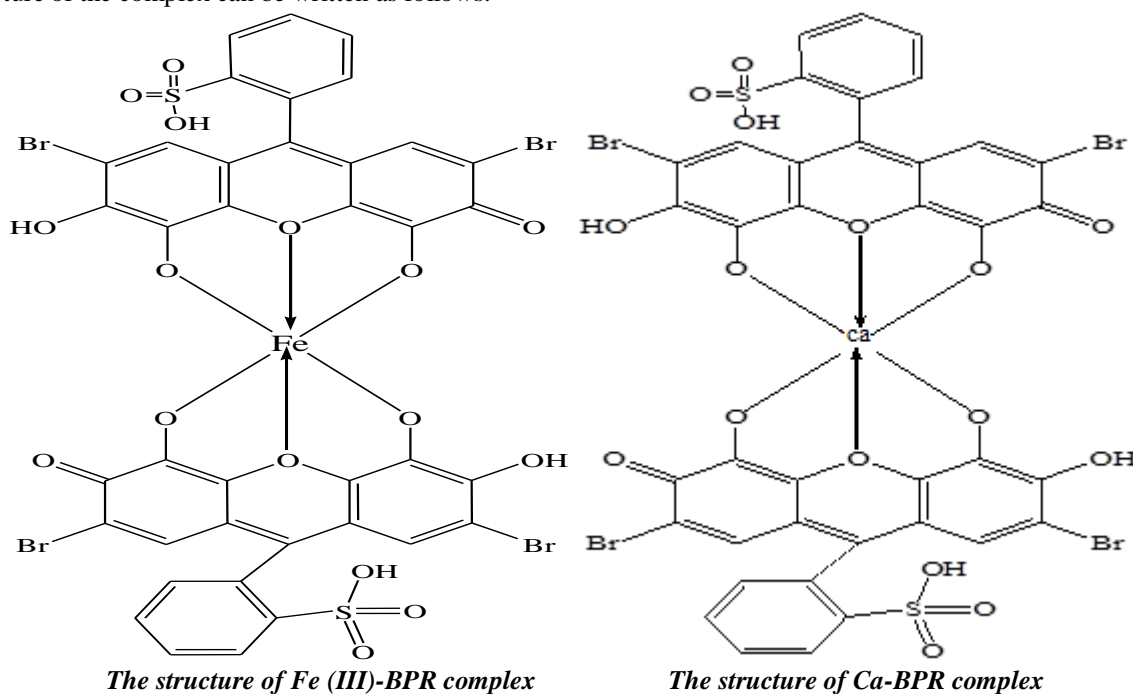
Table 4:- Precision and accuracy of the method

Fe ($\mu\text{g}/25\text{ml}$)		SD	RSD%	Recovery	Ca ($\mu\text{g}/25\text{ml}$)		SD	RSD	Recovery
Added	Found				Added	Found			
10	10.14	0.288	2.8402	101.4	10	10.25	0.375	3.65	102.5
15	15.12	0.2863	1.893	100.8	15	15.11	0.266	1.760	100.73
20	20.08	0.276	1.374	100.4	20	20.54	0.2356	1.147	102.7

Nature of the Complex:-

The iron trivalent and calcium were coupled with BPR reagent in basic medium. Job's continuous variation plot of spectrophotometric data from varying the BPR and metal ion concentration at pH=11 showed the existence of a 1 to 2 complexes, and the method of the mole-ratio for Fe (III) to BPR also was found to be 1:2. This is in agreement with ML_2 -type normal chelate of BPR with metal ions of coordination number 6 [10].

This shows that Fe(III) and Ca were coordinated to oxygen atoms of two BPR molecules. Thus, the suggested structure of the complex can be written as follows:



Conclusions:-

A sensitive and selective method was established for individual and simultaneous determination of iron and calcium using first derivative spectrophotometry with BPR in basic media. The proposed procedures were applied satisfactorily to assays of iron and calcium in a complex mixture in the range 0.4 – 1 ml of Fe and Ca, without the need for tedious and time-consuming separation procedures. The proposed method should also be useful for accurate, precise and rapid determination of iron and calcium in various samples. The stability constants (mean of three values) were found to be $1.33 \times 10^8 \text{ L}^2 \cdot \text{mol}^{-2}$ and $9.13 \times 10^{11} \text{ L}^2 \cdot \text{mol}^{-2}$ for Fe-BPR and Ca-BPR complexes respectively which indicates that the complexes are stable.

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