Chem. Educator **2000,** *5*, 27–30

Spectrophotometric Methods for the Simultaneous Determination of Metals in University Laboratories

Julia Pérez-Iglesias,* Hervilia M. Seco-Lago, Jesús M. Castro-Romero, José M. Fernández-Solís, and Victoria González-Rodríguez

Department of Analytical Chemistry, University School Polytechnic of Ferrol, University of A Coruña, Ctra. Aneiros s/n, Serantes, 15405-Ferrol. Spain, email:julia@udc.es

Abstract: Spectrophotometry is an analytical technique that is studied at the university level because of its ease of operation and low expense. Three different experimental methods of gradual difficulty are proposed for the application of this technique to the simultaneous determination of two metals. The techniques are applicable to the practice of all subdisciplines of experimental chemistry. These techniques are (1) absorption spectrophotometry, the determination of cobalt (II) and copper (II); (2) first-derivative spectrophotometry, the determination of chromium (III) and copper (II); and (3) first-derivative spectrophotometry using the zero-crossing method, the determination of copper (II) and mercury (II).

Introduction

This paper proposes three different experimental methods of graduated difficulty that apply spectrophotometry for the simultaneous determination of two metals. Spectrophotometry is an analytical technique that is applicable to all subdisciplines of experimental chemistry at the university level.

In spectrophotometry, the concentration of the analyte is deduced from the quantification of the solution's absorption, which obeys the Beer-Lambert's law, $A = \varepsilon bc = \log (I_0/I)$. A is the absorbance, ε the extinction coefficient, c the concentration of the solution, and b the path length of the cell; I and I_0 are, respectively, the radiation intensities transmitted by the sample and the reference [1].

These methods are applied to the determination of colored or colorless compounds. The most important characteristics of the spectrophotometric methods are a wide range of application, high sensitivity (the concentration range is from 10^{-4} to 10^{-6} M), good accuracy, facility, and simplicity.

The general procedure of a spectrophotometric analysis follows these steps:

1. The wavelength is selected. Measurements are taken at a wavelength of maximum absorbance because the change of the absorbance with each concentration is the largest at this point; so, the highest sensitivity is obtained.

2. The experimental conditions are optimized. The nature of the solvent, pH of the solution, temperature, time, and concentration of electrolyte and interfering substances are examined. The effects of these variables must be known and the analytical optimum conditions determined.

3. The linear absorbance–concentration relationship is tested. After the optimum conditions are established, calibration graphs are prepared by plotting the absolute values of absorbance against the corresponding concentrations for series of solutions with known standard concentrations of the ions. The linear intervals indicate the range of obedience of Beer's law.

4. The cleaning and calibration of the cells is important. The cells must be calibrated regularly to detect differences caused by scratches, corrosion, and wear. These cells have to be cleaned with wet paper moistened with methanol.

In these experiments the main application of spectrophotometric methods is for quantitative analysis in the determination of mixtures of multicomponents. Three of these methods are described: absorption spectrophotometry, first-derivative spectrophotometry, and first-derivative spectrophotometry using the zero-crossing method**.**

Absorption Spectrophotometry. In simple absorption spectrophotometry, quantitative determinations assume that only one absorbent substance that absorbs at a certain wavelength is in the solution. It is assumed that the effect of any other absorbents can be eliminated by use of a blank sample.

Using absorption spectrophotometry, a substance can be determined in the presence of others [2]. If the absorption spectra of the components of a mixture are not overlapped, each one can be determined independently; however, when the absorption bands are overlapped, we must consider that the absorbance results from an additive property. Therefore, at a certain wavelength, the absorbance of the mixture will be the sum of the absorbances of each component at that wavelength.

In order to apply these assumptions, it is necessary that: (a) no interaction occurs between the absorbent substances, (b) both substances obey Beer's law, (c) a wavelength where the absorptivity of both components are different must exist.

In this paper, this method is used for the simultaneous determination of Co(II) and Cu(II) with methylethylenediaminetetraacetic acid (MEDTA) as chelating agent. The experiments are based on the reaction between each metal with MEDTA to obtain the complexes, Co(II)-MEDTA and Cu(II)-MEDTA, respectively [3]. Another similar experiment has been described by Sawyer et al. [4].

First-Derivative Spectrophotometry. The first-order derivative considered is the rate of change of absorbance with respect to wavelength. Derivative spectrophotometry has been used for the determination of binary mixtures. Without previous separation, the absorption spectra may present near or overlapped maxima. The derivative (slope of the curve) is a function sensitive to quick changes, and it will amplify these

S1430-4171(00)01355-4, 10.1007/s00897990355a, 510027jp.pdf

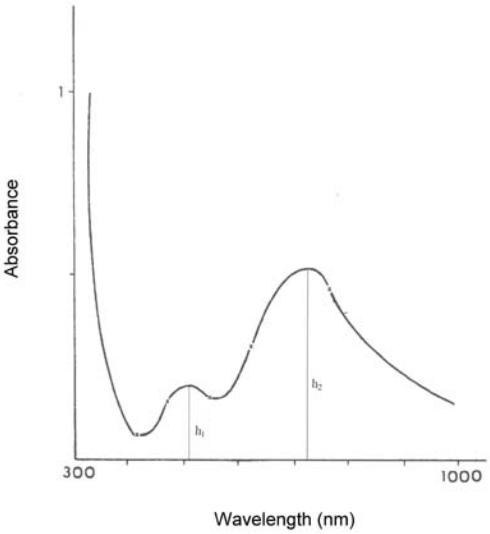

Figure 1. Absorption spectrum of a mixture of Co(II)-MEDTA and Cu(II)-MEDTA complexes, h_1, cobalt and h_2, copper.

regions of overlap. This method is not affected by some sources of error that affect the precision and accuracy of spectrophotometric measurements. Specifically, it decreases and eliminates base-line shifts, which improves the accuracy of quantification. Use of the first derivative also reduces the effects of scattered light produced by the thickness of the solution.

The derivative spectra have two characteristics [5]: (1) the absorption maximum of each spectrum corresponds to the point where the first derivative is zero; (2) the maximum and minimum of the first derivative correspond to inflexion points, points where the slope of the absorption spectrum is decreasing or increasing, respectively. The derivative measures the spectral slope, which is why a broad band in the absorption spectra is suppressed relative to a sharp band in the first derivative. If we suppose that Beer's law is obeyed in the absorption spectra, then, the lineal relationship between derivative and concentration is also obeyed.

The application of first-derivative spectrophotometry to the simultaneous determination of chromium(III) and copper(II) with MEDTA has been described [6].

First-Derivative Spectrophotometry Using the Zero-Crossing Method. In the quantitative analysis of mixtures of two components with highly overlapping spectra, the zero-crossing method, developed by O'Haver [7], applied to derivative spectrophotometry, is frequently used. This method is based on the measurement of the absolute value of the derivative spectrum of the mixture at an abscissa value (wavelength) where the intensity of one of the components of the mixture goes to zero. At this wavelength the intensity is directly proportional to the other component.

This method is not affected by systematic errors, but can be prone to accidental errors in cases where the interfering band of the zero crossing remains in an extreme region of the derivative.

In this paper, the zero-crossing method applied to first-derivative spectrophotometry is described for the simultaneous determination of copper(II) and mercury(II) with MEDTA [8].

Experimental

Apparatus and Reagents. A UV-vis SHIMADZU-160 spectrophotometer with 1-cm-path-length quartz cell was used. The first-derivative curves of the absorption spectrum were obtained by digital differentiation (convolution method). A TACUSSEL U8N model pH meter with combination standard electrode, saturated KCl solution as the reference electrolyte, and readability of ±0.1 pH unit was also used.

An aqueous solution of methylethylenediaminetetraacetic acid (MEDTA) 10% (m/v) was used. The standard copper(II) solution was prepared from copper(II) chloride dihydrate $CuCl_2{\bullet}2H_2O$ dissolved in distilled water. A stock solution of mercury(II) was prepared from mercury(II) chloride, $HgCl_2$, dissolved in distilled water. The standard chromium(III) solution was prepared in distilled water from chromium(III) nitrate nonahydrate, $Cr(NO_3)_3(H_2O)_9$. The standard cobalt(II) solution was prepared from cobalt(II) chloride hexahydrate, $CoCl_2{\bullet}6H_2O$, also dissolved in distilled water.

Procedure. *(a) The Simultaneous Determination of Co(II) and Cu(II) by Absorption Spectrophotometry.* An absorption spectrum was obtained by using a solution containing 2.00 mg mL^{-1} of Co(II), 0.50 mg mL^{-1} of Cu(II), and MEDTA for forming the complexes (Figure 1). The absorption spectrum of the solution was recorded in a wavelength range from 300 to 1000 nm. From spectra of the individual components, it can be deduced that the region of the spectrum between 400 and 550 nm corresponds to the Co(II)-MEDTA complex with λ_{max} = 515 nm and between 550 and 850 nm to the Cu(II)-MEDTA complex with λ_{max} = 730 nm. The maxima in each region (h_1 and h_2, respectively) are the parameters used to measure the concentrations of the ions. The spectrum was obtained using distilled water as blank.

The pH influence on the absorption spectra of Co(II)-MEDTA and Cu(II)-MEDTA mixtures was studied by using a series of solutions covering as wide as possible a pH range; the optimum working interval is from pH 3.0 to 5.0. A volume of 5.0 mL of 5% MEDTA solution was adequate to make complexes for 2.00 mg mL^{-1} of Co(II) and 0.50 mg mL^{-1} of Cu(II), but it was found that excess MEDTA solution did not affect the results. The formation of the mixture of Co(II)-MEDTA and Cu(II)-MEDTA complexes was instantaneous and remained stable to 100 ºC up to 24 hours after its formation. Heating time has no influence on complex formation.

The calibration curves for cobalt(II) and copper(II) were obtained by plotting the values of h_1 and h_2 against the corresponding concentrations for a series of solutions with different concentrations of the two ions. It was found that the determination of the h_1 and h_2 variations with the concentrations was not affected by the presence of the other ion. This findings supports the utility of the simultaneous determination of the two elements. In the absence of interfering ions, h_1 is linear with cobalt(II) concentration between the limits of 0.20 and 8.00 mg Co(II) mL^{-1} , and h_2 is linear with the copper(II) concentration between the limits 0.05 and 1.60 mg Cu(II) mL^{-1}.

Experimental runs with four different series of ten solutions each of Co(II)-MEDTA and Cu(II)-MEDTA were made in order to check the reproducibility and precision of the method.

(b) The Simultaneous Determination of Cr(III) and Cu(II) by First-Derivative Spectrophotometry. The absorption spectra were obtained by using a Cr(III)-MEDTA and Cu(II)-MEDTA solution containing 1.20 mg mL^{-1} of Cr(III) and 0.25 mg mL^{-1} of Cu(II) (Figure 2). As shown in the figure, the maxima interfere; therefore, it would not be possible to resolve the two components, chromium(III) and copper(II), by using absorption spectrophotometry with MEDTA; however, in the first-order derivative the presence of these two bands is clearly visible. The first derivative of the same solution was measured in a wavelength range from 430 to 1100 nm at 2400 nm min^{-1} with $\Delta\lambda$ = 32.0 nm (Figure 3). From Figure 3, it can be deduced that the region of the derivative spectrum between 490 and 660 nm corresponds to the Cr(III)-MEDTA complex and between 660 and 1020 nm to the Cu(II)-MEDTA complex. The differences between the

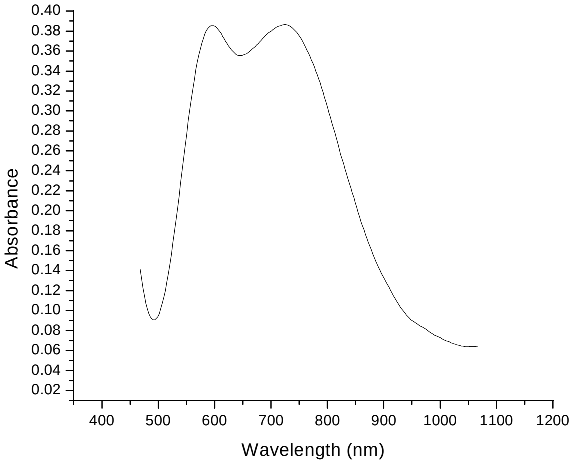

Figure 2. Absorption spectrum of a mixture of Cr(III)-MEDTA and Cu(II)-MEDTA complexes.

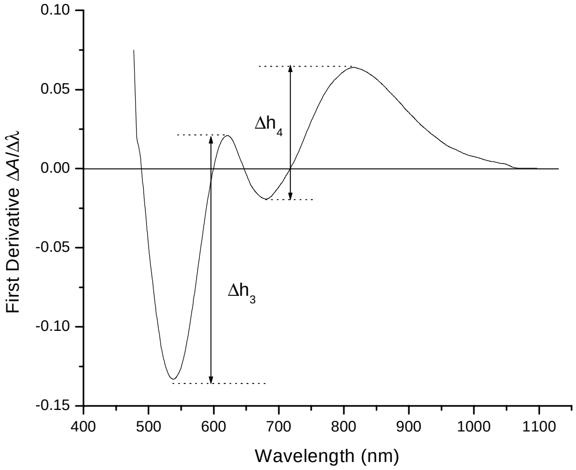

Figure 3. First-derivative absorption spectrum of a mixture of Cr(III)-MEDTA and Cu(II)-MEDTA complexes, Δh_3, chromium and Δh_4, copper.

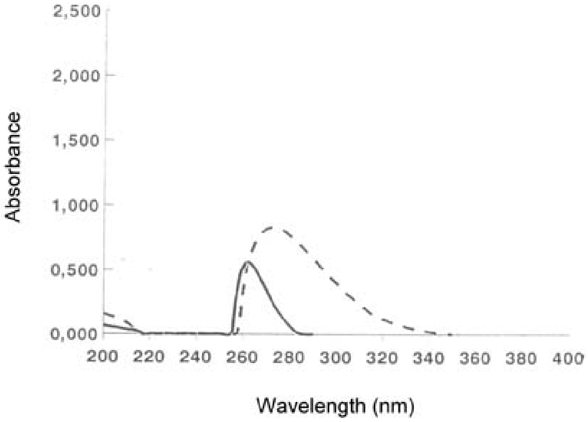

Figure 4. Absorption spectra of a mixture of Cu(II)-MEDTA and Hg(II)-MEDTA complexes; - - - represents Cu(II) and —— represents Hg(II).

minimum and the maximum in each region (Δh_3 and Δh_4, respectively) are the parameters used to measure the concentrations of the ions. The spectra and their first-derivative values were obtained against distilled water.

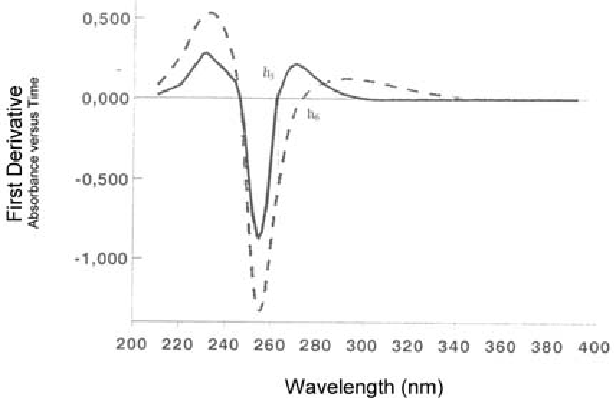

Figure 5. First-derivative absorption spectrum of a mixture of Cu(II)-MEDTA and Hg(II)-MEDTA complexes, h_5, copper and h_6, mercury; - - - represents Cu(II) and —— represents Hg(II).

The pH influence on the first-derivative spectra of Cr(III)-MEDTA and Cu(II)-MEDTA mixtures was studied by using a series of solutions in as wide as possible a pH range; the optimum working interval is from pH 2.0 to 4.0. A sediment in the solution is observed at pH 5.0; therefore, values in the derivative spectrum are not reliable and cannot be used. A volume of 0.4 mL of 5% MEDTA solution was adequate to make complexes (1.20 mg mL^{-1} of Cr(III) and 0.25 mg mL^{-1} of Cu(II)); excess MEDTA solution did not affect the results. Once the mixture of Cr(III)-MEDTA and Cu(II)-MEDTA complexes was formed, it was observed to be stable for less than 1 h. This system was also unstable at temperatures higher than 40 ºC and the first-derivative spectrum was not constant.

The calibration curves for chromium(III) and copper(II) were obtained by plotting the absolute values of Δh_3 and Δh_4 against the corresponding concentrations for a series of solutions with different concentrations of the two ions. It is found that the Δh_3 and Δh_4 variation with the concentrations of the respective ions was not affected by the presence of the other ion. This allows the simultaneous determination of the two elements without the necessity of solving simultaneous equations. In the absence of interfering ions Δh_3 is linear with the chromium(III) concentration between the limits 0.40 and 2.60 mg Cr(III) mL^{-1} and Δh_4 is linear with the copper(II) concentration between the limits 0.15 and 0.60 mg Cu(II) mL^{-1}.

Experiments with four different series of ten solutions each of Cr(III)-MEDTA and Cu(II)-MEDTA were run in order to check the reproducibility and accuracy of the method.

(c) The Simultaneous Determination of Cu(II) And Hg(II) by First-Derivative Spectrophotometry Using the Zero-Crossing Method. The absorption spectra were obtained by using Cu(II)-MEDTA and Hg(II)-MEDTA solutions prepared using 0.020 mg mL^{-1} of Cu(II) and 0.150 mg mL^{-1} of Hg(II), respectively. The absorption spectra for the analytes (Figure 4) exhibited maxima at 260 nm (Hg(II)-MEDTA) and 272 nm (Cu(II)-MEDTA). This overlap produced a useable maximum at 265 nm. The first derivative of the same solutions was measured in a wavelength range from 200 to 400 nm at 2400 nm min^{-1} with $\Delta\lambda$ = 6.4 nm (Figure 5). It can be seen that the derivative spectra are zero at 260 nm for Hg(II)-MEDTA and at 272 nm for Cu(II)-MEDTA. This should allow Cu(II)-MEDTA to be quantified at 260 nm; likewise, Hg(II)-MEDTA can be quantified at 272 nm where no effect from Cu(II)-MEDTA was observed. The absolute values of the first-derivative spectrum at each wavelength (h_5 and h_6, respectively) are the parameters used to measure the concentrations of the ions. Spectra and their first-derivative values were obtained against a blank prepared under the same conditions but containing none of the analytes.

The pH influence on the first-derivative spectra of Cu(II)-MEDTA and Hg(II)-MEDTA mixtures was studied by using a series of solutions prepared in as wide as possible a pH range. Spectra for the two components recorded over the pH range 5.0–9.0 showed no appreciable differences; therefore, pH 7.0 was chosen for subsequent experiments. A volume of 0.6 mL of 1% MEDTA solution was adequate to make complexes with 0.020 mg mL^{-1} of Cu and 0.150 mg mL^{-1} of Hg. Interference of excess MEDTA was investigated and it did not affect the results. Once the mixture of Cu(II)-MEDTA and Hg(II)-MEDTA complexes was formed, it was found to be stable up to 100 ºC and up to 24 hours after heating. The heating time had no influence on its formation.

The calibration curves for copper and mercury were obtained by plotting the absolute values of h_5 and h_6 against the respective concentrations for a series of solutions with increasing concentrations of the two ions. The h_5 and h_6 variation with the concentrations of the respective ions was not affected by the presence of the other ion. This supports the utility of this method for the simultaneous determination of the two elements without the necessity of solving simultaneous equations. In the absence of interfering ions, h_5 is linear with the copper(II) concentration between the limits 0.008 and 0.036 mg Cu(II) mL^{-1} and h_6 is linear with the mercury(II) concentration between the limits 0.025 and 0.300 mg Hg(II) mL^{-1}. Experiments with four different series of Cu(II)-MEDTA and Hg(II)-MEDTA solutions were performed in order to check the reproducibility and precision of the method.

References and Notes

1. Skoog, D. A.; West, D. M. *Análisis Instrumental,* 2nd ed.; Interamericana, México D. F., 1984.
2. Mason, W. B.; Henry, R. J. *Química Clínica: bases y técnicas;* JIMS: Barcelona, 1980; 1–47.
3. Castro-Romero, J. M.; Fernández-Solís, J. M.; Bollaín-Rodríguez, M. H.; Bermejo-Martínez, F. *Microch. J.* **1991,** *43,* 104–108.
4. Sawyer, D. T.; Heineman, W. R.; Beebe, J. M. *Chemistry Experiments for Instrumental Analysis*. Wiley & Sons: New York, 1984.
5. Merrick, M. F.; Pardue, H. L. *Clin. Chem*. **1986,** *32,* 598–602.
6. Seco-Lago, H.; Pérez-Iglesias, J.; Fernández-Solís, J. M.; Castro-Romero, J. M.; González-Rodríguez, V. *Fresenius J. Anal. Chem*. **1997,** *357,* 464–466.
7. O'Haver, T. C. *Clin. Chem.* **1979,** *25,* 1548–1553.
8. Seco-Lago, H. M.; Pérez-Iglesias, J.; Fernández-Solís, J. M.; Castro-Romero, J. M.; González-Rodríguez, V. *Anal. Let. 31,* **1998,** 2747–2756.