SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF CADMIUM, COPPER AND ZINC

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Summary—A method for the simultaneous spectrophotometric determination of cadmium, copper and zinc based on the formation of their complexes with 1,5-bis(di-2-pyridylmethylene)thiocarbonohydrazide is proposed. The absorption curves of these complexes overlap severely in the scanning range 380–480 nm. The analyte concentrations are calculated by a least squares fit of the pure spectra to the mixture spectra. A linear determination range of 0.1–1.7 µg/ml for cadmium, 0.1–1.3 µg/ml for copper and 0.2–1.2 µg/ml for zinc were obtained. The effect of interference was studied. The method has been applied to the determination of these metal ions in various type of materials.

Until recently, multicomponent determinations by spectrophotometry have been done by using a single wavelength for each component. Recently, the strategies available in analytical chemistry and, in particular, those using molecular spectroscopy have diversified. Thus, multicomponent determinations have been improved by using multiple wavelengths and different algorithms. Such algorithms include those of classical least-squares (CLS), the simplex interaction method, the inverse least-squares (ILS), the principal component regression (PCR), the partial least-squares (PLS) and other multivariate statistical methods.

Recently, Sala et al. applied a computer program, like MULTIC, to the resolution of a mixture of pharmaceuticals. Also, this program has been used by Gomez et al. for the simultaneous determination of calcium and magnesium in water. MULTIC is a multilinear regression program that involves fitting the calculated mixed spectrum to its experimental counterpart through the standard deviation, correlation coefficient and an independent term corresponding to the intercept on the absorbance axis. For simultaneous spectrophotometric determination of M components in a mixture to be achieved, each species must absorb according to Beer's law, and the absorbances must be additive. Both conditions (using 1 cm of the optical path-length) can be described by a mathematical model of the form:

\[ x = \sum S_A C_A + e \]  

where \( x \) is the measured (sum) spectrum, \( S_A \) is the pure spectrum of analyte \( A \), \( C_A \) is the concentration of analyte \( A \) in the mixture and \( e \) is the residual (difference between the observed response and the response calculated from the model). The equation (1) can be rewritten in shorthand matrix notation as:

\[ x = Sc + e. \]

Where \( S \) is the matrix containing the pure spectra as columns (size: \( p \times M \); where \( p > M \) is the number of wavelengths employed and \( M \) is the number of analytes) and \( c \) is a vector containing the coefficients/unknown concentrations (size: \( M \times 1 \)). When \( p > M \), the least squares solution to equation (2) is:

\[ c_{est} = (S'S)^{-1}S'x, \]

and the error vector is:

\[ e = x - Sc_{est}, \]

with the length:

\[ e = (e_1^2 + e_2^2 + e_3^2 + \ldots + e_p^2)^{0.5}. \]

This paper reports a method for the simultaneous spectrophotometric determination of cadmium, copper and zinc, based upon the formation of their respective complexes with 1,5-bis(di-2-pyridylmethylene)thiocarbonohydrazide (DPTH). The resolution of the mixtures was carried out by using the multiple linear regression mathematical algorithm included in the program MULTIC.
EXPERIMENTAL

Reagents

Analytical-reagent grade chemicals were used throughout.

Standard solutions of cadmium(II) (1039 mg/l), copper(II) (1042 mg/l) and zinc(II) (1047 mg/l) were prepared from their respective nitrates and standardized titrimetrically with EDTA. Working standard solutions were prepared by suitable dilution of the standard solutions.

A stock DPTH solution (2.3 × 10⁻³ M) in DMF was prepared by dissolving solid reagent samples prepared and purified by the authors. This solution was spectrophotometrically stable for at least a week.

Buffer solutions (pH 7.5) were prepared by mixing 8 ml of 0.2M NaH₂PO₄ with 42 ml of 0.2M Na₂HPO₄ and diluting to 100 ml with distilled water.

Apparatus and software

A Hewlett-Packard Model 8452A diode-array spectrophotometer was used for recording spectra. An H.P. Vectra QS/165 personal computer was used to control the spectrophotometer and collect data from it via an HP-IB interface. The programs used to obtain and process the spectra were supplied by the spectrophotometer manufacturer as bundled software. Mixtures were resolved using a multiple linear regression mathematical algorithm included in the program MULTIC.

Procedure

Preparation of standards. The program MULTIC requires the standardized spectrum of each component. With this purpose, four solutions for each component were prepared in 25 ml standard flasks with different concentrations of the metal ion (0.2, 0.4, 0.6 and 0.8 µg/ml), 3 ml of 0.1% m/v DPTH solution in DMF, 12 ml of DMF (to prevent precipitation of the reagent), 5 ml of buffer solution (pH 7.5) and dilution with distilled water to the mark. After a thorough mixing, the solutions were allowed to stand for 30 min. The absorbance spectra of these solutions were recorded over the wavelength range 390–480 nm at an integration time of 1 sec. Appropriate reagent blank was prepared, in triplicate, and was run in the same manner. The spectra of these three reagent solutions were recorded five times and the average spectrum was considered as the DPTH standard.

Each of the spectra of the Zn–DPTH, Cd–DPTH and Cu–DPTH complexes were corrected by subtracting the standard spectrum of the reagent and were standardized by dividing them into the Zn, Cd or Cu concentrations at which they were recorded; the average spectrum of each complex (corresponded to a 1 µg/ml) was used as standard for each ion. These standard spectra were stored in the corresponding computer subdirectory as a working model for subsequent determinations.

The average, corrected and standardized spectra were obtained using a subroutine of the spectrophotometer’s bundled software.

Sample preparation

The certified reference materials (CRMₙ) analysed to determine the accuracy of the proposed procedure were: Community Bureau of Reference (BCR), Certified Reference Materials (CRM钚) 186 Pig Kidney and 184 Bovine Muscle; National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMₙ) 1577a Bovine Liver. National Research Council Canada (NRCC) CRM, TORT-1 Lobster Hepatopancreas; Bureau of
Table 1. Tolerance to foreign ions in the determination of 0.8 µg/ml of Zn(II), Cd(II) and Cu(I) by the proposed method

<table>
<thead>
<tr>
<th>Tolerated ratio</th>
<th>Foreign ion without masking agent</th>
<th>Foreign ion with masking agent</th>
<th>Masking agent (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1000</td>
<td>SCN⁻, I⁻, C₂O₄²⁻, Ac⁻, SO₄²⁻, S₂O₃²⁻, S⁻, NO₃⁻, F⁻, Cl⁻, tartrate, citrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>Mo(VI), Sr(II), AsO₄³⁻, AsO₃⁵⁻, Pb(II), Ba(II), H₂O₂, WO₄³⁻, Li(I), Mg(II), Ca(II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Cr(IV), UO₂(II), VO₄³⁻, Y(III), Se(IV), Sb(III), Zr(IV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Mn(II)</td>
<td>Ag(I), Cr(III), S₂O₅ (400)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Fe(III), Al(III), F⁻ (800)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Hg(II), Hg(I)</td>
<td>S₂O₅ (400)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>EDTA</td>
<td>Ni(II)</td>
<td></td>
</tr>
</tbody>
</table>

Analysed Samples LTD 32a Aluminium Bronze; 20b Aluminium Alloy and 8e White Metal. These samples were prepared according to the following procedure:

(a) **Alloys.** Dissolve a known amount of accurately weighed sample (0.1 g) in about 30 ml of *aqua regia*; evaporate the resulting solution to dryness and add 1 ml of hydrochloric acid (1:1); then dilute with distilled water to 50–100 ml.

(b) **Biological materials.** The samples were first dried in accordance with the norms of the respective analysis certificates and mineralized as follows: in a reaction flask were placed 0.5–2.0 g of weighed sample and 10 ml of concentrated nitric acid, and the mixture was heated under reflux up to the disappearance of nitrous fumes. Then, 3 ml of hydrogen peroxide was added and the mixture was concentrated (this step was repeated until the solution was completely colourless). Next, the mixture was neutralized with NaOH and made to 25 ml with deionized water.

**Simultaneous determination of Zn, Cd and Cu**

In 25 ml standard flasks were placed aliquots of sample solutions to obtain Zn, Cd and Cu concentrations over their respective linear determination ranges (0.2–1.2 µg/ml for Zn, 0.1–1.7 µg/ml for Cd and 0.1–1.3 µg/ml for Cu), 3 ml of 0.1% m/v DPTH solution in DMF, 12 ml of DMF and 5 ml of buffer solution (in the case of aluminium alloys 1 ml of a 1.1M fluoride solution was added to prevent interference from Al(III)). The solutions were then diluted to the mark with water, after a thorough mixing, the

Table 2. Resolution of Zn(II), Cd(II) and Cu(II) mixtures over the wavelength range 430–480 nm

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zn (µg/ml)</th>
<th>Cd (µg/ml)</th>
<th>Cu (µg/ml)</th>
<th>Zn (µg/ml)</th>
<th>Cd (µg/ml)</th>
<th>Cu (µg/ml)</th>
<th>Residual (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.22 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>3.9766 × 10⁻⁴</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.80</td>
<td>0.60</td>
<td>0.18 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>0.59 ± 0.01</td>
<td>2.4562 × 10⁻⁴</td>
</tr>
<tr>
<td>3</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.69 ± 0.03</td>
<td>0.88 ± 0.03</td>
<td>0.87 ± 0.05</td>
<td>2.6614 × 10⁻³</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
<td>1.20</td>
<td>1.20</td>
<td>0.54 ± 0.04</td>
<td>0.97 ± 0.04</td>
<td>1.15 ± 0.06</td>
<td>4.1621 × 10⁻³</td>
</tr>
<tr>
<td>5</td>
<td>1.20</td>
<td>1.20</td>
<td>0.40</td>
<td>1.09 ± 0.03</td>
<td>1.08 ± 0.03</td>
<td>0.38 ± 0.05</td>
<td>3.6465 × 10⁻³</td>
</tr>
<tr>
<td>6</td>
<td>1.20</td>
<td>0.40</td>
<td>1.20</td>
<td>1.13 ± 0.04</td>
<td>0.27 ± 0.04</td>
<td>1.20 ± 0.06</td>
<td>4.8830 × 10⁻⁵</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.98 ± 0.09</td>
<td>0.85 ± 0.09</td>
<td>1.05 ± 0.14</td>
<td>2.6177 × 10⁻⁴</td>
</tr>
<tr>
<td>8</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.92 ± 0.02</td>
<td>1.09 ± 0.03</td>
<td>0.07 ± 0.03</td>
<td>1.6206 × 10⁻³</td>
</tr>
<tr>
<td>9</td>
<td>0.80</td>
<td>1.40</td>
<td>1.40</td>
<td>1.06 ± 0.08</td>
<td>0.90 ± 0.08</td>
<td>1.19 ± 0.13</td>
<td>2.2257 × 10⁻⁴</td>
</tr>
</tbody>
</table>

*The results correspond to the mean and standard deviation obtained from three consecutive determinations of each solution.*
solutions were allowed to stand for 30 min and their absorbance spectra were recorded and corrected by subtracting that of DPTH. To these absorption spectra was applied the MULTIC program for quantification of the components using the normalized spectra of Zn, Cd and Cu as standards (see equation (3)).

RESULTS AND DISCUSSION

The zinc(II) reacts with DPTH in the range pH 6.2–9.8 forming a soluble yellow complex with an absorption maximum at 415 nm and a molar absorptivity of 6.01 x 10^4 l/mol/cm; the stoichiometric ratio of this complex is 1:3 zinc:reagent. The complex is spectrophotometrically stable, after 30 min, for at least 24 hr. The cadmium(II) forms a complex with DPTH in the pH range 5.8–8.3 with an absorption maximum at 415 nm and a molar absorptivity of 5.64 x 10^4 l/mol/cm; the stoichiometric of this chelate is 1:3 cadmium:reagent and the complex solutions are stable for at least 24 hr. When dilute copper(II) solutions and DPTH solutions are mixed, two different complexes, one orange (λ_max = 420 nm), and the other red (λ_max = 500 nm), are formed according to the pH, thus, the red complex is formed in the pH range 13.2–13.6 with a molar absorptivity of 4.2 x 10^4 l/mol/cm and its stoichiometry is 1:2 copper:reagent. The graph of absorbance vs pH for the orange complex shows that the absorbance at 420 nm is maximum and constant in the alkaline region and also shows two possible optimum pH ranges of 1.8 4.4 and 7.1 8.4, the stoichiometry in both cases is 1:1 and the molar absorptivities are 2.8 x 10^4 l/mol/cm and 4.3 x 10^4 l/mol/cm, respectively. The orange copper complex is stable for at least 12 hr.

On the other hand, it is also well known that the quality of the results obtained in multicomponent analyses from extensively overlapping spectra depends on the wavelength range used. In order to study this factor different wavelength ranges (400–480, 415–480, 430–480, 450–480 and 465–480 nm) for recording the absorbance spectra of mixtures containing various concentrations of the three ions were applied. The best results (accuracy and precision) were obtained by recording the absorbance spectrum over the wavelength range 430–480 nm at an integration time of 1 sec and using the wavelength scan interval of the spectrophotometer (2 nm) except in the case of samples containing fluoride, tartrate, citrate, Sb(III), Cr(III), Bi(III), Al(III), Hg(I1), and Hg(I). In these instances the optimum wavelength range was 465–480 nm.

Once the optimum conditions had been established, the linear range for Zn, Cd and Cu was calculated. With this purpose, a series of solutions containing different concentrations of the metal ion were prepared for each analyte. The absorbance spectra of these solutions were recorded over the working wavelength range (430–480 nm). Then, the average absorbance of each spectrum was obtained and used to construct the univariate calibration graph. The curves obtained (Fig. 2) were linear over the ranges 0.2–1.2 μg/ml for Zn, 0.1–1.7 μg/ml for Cd and 0.1–1.3 μg/ml for Cu.

Finally, four pure standard solutions of each component of the mixture, with a concentration lying in the linear concentration range and giving absorbance readings lower than 1.0 were used to obtain the normalized spectrum for each component.

Under the optimum conditions and using the equation (3) for prediction of concentrations, 10 determinations of standard solutions containing 0.8 μg/ml of each metal ions gave a relative standard deviation of 6.4% for Zn, 5.1% for Cd and 3.5% for Cu.
The interference due to several cations and anions was studied in detail. For these studies different amounts of the ionic species were added to a mixture of Zn, Cd and Cu containing 0.8 μg/ml of each. The starting point was a 1000 fold m/m ratio of interferent to metal ions, and if interference occurred the ratio was progressively reduced until interference ceased. The tolerated limits were taken as those concentrations causing changes no greater than ±5% in the concentration of analytes.

The ions which interfere most strongly were Co(II), Ni(II), Fe(III), Hg(II) and Hg(I) because they form coloured complex with DPTH. While quantitatively significant, the interferences from these ions posed no insurmountable problems for the simultaneous determination of Cu–Zn–Cd because the interference can be overcome by determining the interfering metal together with the three analytes, which can be most readily accomplished by using the program MULTIC (this program allows the resolution of a maximum five components).

The tolerance limits are depicted in Table 1. The tolerance level for some metal ions can be increased by addition of thiosulphate, fluoride or tartrate as masking agents. Thus, 400 ppm of thiosulphate will mask 32 ppm of Ag(I) or Cr(III), 6 ppm of Hg(II) or Hg(I) and 4 ppm of Co(I); 800 ppm of fluoride will mask 32 ppm of Fe(II) or Al(III) and 4 ppm of Ni(I); and 800 ppm of tartrate will mask 32 ppm of Bi(III).

**Effects of foreign ions**

To verify the utility of the recommended procedure, the method was first used for the simultaneous determination of zinc, cadmium and copper in a set of synthetic samples containing different Zn, Cd, and Cu proportions. The results obtained by applying equation (3) to the resolution of the mixtures are summarized in Table 2, where the actual values are compared with the predicted concentrations. From these results (samples 1–8) we can conclude that the concentrations obtained for copper and zinc by the proposed method are in good agreement with those added, resulting in an RMSEP (root-mean-squared error of prediction) of 0.045 μg/ml for copper and 0.083 μg/ml for zinc. The amounts of cadmium found can be considered to be correct with the exception of those mixtures containing low concentrations of cadmium with regard to the other mixture com-
ponents; in these instances negative errors for cadmium were obtained. The RMSEP for cadmium was 0.122 µg/ml. On the other hand, in those instances where the analytes concentrations present in the mixture was higher than 3.1 µg/ml (e.g. sample 9) the method was not able to predict the true concentrations of metal ions with sufficient accuracy. This can be attributed to the high absorbance signals corresponding to these mixtures.

In order to test the applicability and matrix interferences of the proposed method to the analysis of real samples, the method was applied in a variety of situations. For this purpose, diverse spiked samples and reference materials were analysed. Thus, standard solutions containing different concentrations of Zn and Cd were added to 0.1 g of Al bronze 32a and Al Alloy 20b; standard solutions of Cd were added to 0.5 g of TORT-1-Lobster Hepatopancreas and Bovine Liver; standard solutions of Cu were added to 0.5 g of Bovine Muscle. These samples were prepared and analysed as described under the Experimental section. In the case of Aluminium Alloys, 1 ml of 1.1M fluoride solution were added to prevent interference from Al(III) and the absorption spectra of these solutions were recorded over the wavelength range 465–480 nm. The results obtained for the analysis of different aliquots of the prepared solutions containing known concentrations are shown in Table 3. These data indicate that the method would be effective for the analysis of samples of similar complexity.

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REFERENCES