1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid (DACT) is commonly used in the analysis of inorganic ions due to its capability of forming complexes with these ions. Depending on the pH value, DACT forms complexes with many metals, what was applied in titrimetric analysis of aluminium with the use of different indicators. At pH = 5, no interference was observed with the following ions: beryl, magnesium, calcium, strontium, barium; and at pH = 3 with mercury; whereas ions such as copper(II), iron(III), zinc, cadmium, cerium(III) and many other, interfered with the results (1).

This method was employed also in the quantitative analysis of manganese, chromium, and iron under very acidic conditions at pH from 1.5 to 3.5 (2), and antimony(III) at pH = 4.3 (3). Other published data provide information on the results of comparative analysis of various titrimetric methods in which DACT, EDTA or DTPA were employed (4, 5).

Complexes of some metals with DACT were determined also by electrophoresis (6).

In this paper, we report a new method for direct, simultaneous determination in one sample of ions such as: iron(III), lead(II) and bismuth(III) in complexes with DACT, using zero-order and derivative spectrophotometry.

The work was undertaken with an intention to develop a sensitive method for determination of these ions as drug impurities.

EXPERIMENTAL

Apparatus

Spectrophotometer: UV-Vis Cary 100 (Varian), 10 mm quartz cells; computer: PC Pentium MMX, 16 MB RAM, Hewlett-Packard LaserJet 6L printer and software (Microsoft Office 97, Statistica 5.1 edition 97).

Chemicals and reagents

All chemical and reagents were of analytical grade. 1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid (DACT) was supplied by Fluka Chemie AG; the solution was prepared by dissolving 0.3625 g DACT in 100.0 mL of sodium hydroxide (0.1 mol·L⁻¹). Prior to analysis, this solution was diluted 1:10 with water. Solutions (0.0001 mol·L⁻¹) of the salts of the ions studied were generally used. The salts: Pb(NO₃)₂, BiCl₃, FeCl₃ × 6H₂O, purchased
from POCh-Gliwice, were dissolved in water. 1.0 mL of hydrochloric acid (1.0 mol·L⁻¹) was added to the solutions of iron(III) and bismuth(III) salts.

**Preliminary analysis**

Evaluation of analytical quality of the obtained complexes of DACT with iron(III), lead(II) and bismuth(III) ions was carried out. By applying equimolar series method and spectrophotometric titration it was confirmed that the studied ions form complexes with DACT in a molar ratio 1:1.

These complexes have characteristic absorption spectra that differ in maxima; DACT – Fe has a maximum at $\lambda = 250$ nm ($A_1^{1\%} cm = 496$); DACT – Bi at $\lambda = 267$ nm ($A_1^{1\%} cm = 423$); DACT – Pb at $\lambda = 246$ nm ($A_1^{1\%} cm = 433$). The complexes were formed in basic solutions, at pH from 10 to 11. As the result of these preliminary investigations the conditions for the analysis, that conform to absorption rules ($A = f(c)$) were established.

**Procedure**

The ion solutions (10⁻⁴ mol·L⁻¹) at volumes from 2.0 to 12.0 mL were transferred to six 25.0 mL calibrated flasks. Next, 2.0 mL of DACT solution (10⁻³ mol·L⁻¹) was added to each flask. The samples were left for 15 min at room temperature and then diluted to volume with water. Absorbance was recorded at the maximum absorption wavelength selected for each complex. A solution prepared as described above but without any ion was used as a reference.

**RESULTS AND DISCUSSION**

Linearity was maintained within the following range: from 0.87 to 2.31 µg·mL⁻¹ for DACT – Fe; from 1.67 to 8.36 µg·mL⁻¹ for DACT – Bi; from 1.65 to 8.28 µg·mL⁻¹ for DACT – Pb. Absorbance is stable for 2 h what ensures repeatability of the results.

Comparable values of $\lambda_{max}$ and $A_1^{1\%}$ for the obtained complexes are a major obstacle that makes virtually impossible to simultaneously determine particular ions in multi-component mixtures by zero-order spectrophotometry. Therefore, attempt has been made to transform the spectra obtained into respective derivatives (10, 11). Figures 1-3 show the second order derivative spectra of the analyzed complexes.

Second derivatives of spectra for each concentration were calculated by “baseline-to-peak” technique. The curves recorded are characterized by a linear relationship between second order derivative and concentration with maxima at different wavelengths: for DACT – Fe $\lambda_{max} = 226$ nm; DACT – Pb $\lambda_{max} = 247$ nm; DACT – Bi $\lambda_{max} = 287$ nm.

**Fig. 1.** Second order derivative of absorption spectra for DACT-Fe solutions at different concentrations: $c_1 = 0.896$ µg·mL⁻¹, $c_2 = 1.344$ µg·mL⁻¹, $c_3 = 1.792$ µg·mL⁻¹, $c_4 = 2.240$ µg·mL⁻¹, $c_5 = 2.688$ µg·mL⁻¹.

**Fig. 2.** Second order derivative of absorption spectra for DACT-Pb solutions at different concentrations: $c_1 = 1.656$ µg·mL⁻¹, $c_2 = 3.312$ µg·mL⁻¹, $c_3 = 4.968$ µg·mL⁻¹, $c_4 = 6.624$ µg·mL⁻¹.

**Fig. 3.** Second order derivative of absorption spectra for DACT-Bi solutions at different concentrations: $c_1 = 1.672$ µg·mL⁻¹, $c_2 = 3.344$ µg·mL⁻¹, $c_3 = 5.016$ µg·mL⁻¹, $c_4 = 6.688$ µg·mL⁻¹.
Spectrophotometric determination of Pb(II), Fe(III) and Bi(III) in complexes with...

Next, the detection and determination limits (DL and QL, respectively), as well as recovery (%R) for particular ions were established [9]. Results are presented in Table 1.

Takin into consideration the fact that other ions may under these conditions also form complexes, their potential effect on the results obtained was investigated. To the solutions studied, containing iron(III), lead(II) or bismuth(III), the following ions were added successively: cobalt(II), cadmium(II), calcium(II), potassium(I), aluminium(III), nickel(II), zinc(II). Absorption spectra were recorded and compared with those for appropriate standard solutions. The obtained data indicate that ions such as cobalt(II), potassium(I), aluminium(III) and nickel(II) do not interfere with the results of iron(III), lead(II) and bismuth(III) determination, whereas calcium, magnesium and cadmium(II) affect these results.

Based on the above, experiments conditions were established for direct simultaneous determination of ions: iron(III), lead(II) and bismuth(III), in one sample, by zero-order or second order derivative spectrophotometry.

Simultaneous determination of iron (III), lead (II) and bismuth (III) ions.

Model solutions, the composition of which is presented in Table 2, were prepared. The complexes of ions studied, that is iron(III), lead(II) and bismuth(III), were prepared as described above. Zero-order absorption spectrum for a particular model solution was recorded in the range $\lambda = 200 – 400$ nm.

The resulting spectrum was transformed into a second derivative (Fig. 5) to obtain the spectrum with a shape and maximum at $\lambda = 287$ nm. This wavelength was characteristic of the second order derivative spectrum recorded for the standard solution of bismuth(III) ions. By applying “baseline-to-peak” technique (10, 11) the concentration of bismuth(III) was calculated, based on the comparison between the second derivative value for bismuth(III) in the analyzed model mixture and in the standard solution.

Next, a blank sample was prepared that contained bismuth(III) ions at the concentration exactly the same as was determined in the analyzed model mixture. Absorption spectrum of the analyzed mixture was recorded once again and compared with a spectrum recorded for the prepared blank sample.

The obtained spectrum was transformed into the second derivative (Fig. 6), with $\lambda_{\text{max}}$ at 247 nm and the concentration of lead(II) was calculated in the same manner as was described above. Under the applied experimental conditions, the interference of bismuth ions on the results calculated for lead(II) was eliminated. Iron(III) ions do not hamper lead determination. For quantification of iron(III) a new blank sample, containing bismuth and lead ions at concentrations thus determined, was prepared and used as a reference in registration of absorption for the analyzed sample. The content of iron(III) ions was calculated from the absorbance at $\lambda_{\text{max}}$ in zero-order spectrum, that is at 250 nm (Fig. 7).

The same procedure was applied for a model solution containing iron(III) and lead(II) ions. Concentration of lead(II) was calculated directly from the second order derivative and the concentration of iron by registration of a zero-order spectrum using a blank containing lead ions as a reference. Results obtained for model solutions are presented in Table 2.

Next, a blank sample was prepared that contained bismuth(III) ions at the concentration exactly the same as was determined in the analyzed model mixture. Absorption spectrum of the analyzed mixture was recorded once again and compared with a spectrum recorded for the prepared blank sample.

The obtained spectrum was transformed into the second derivative (Fig. 6), with $\lambda_{\text{max}}$ at 247 nm and the concentration of lead(II) was calculated in the same manner as was described above. Under the applied experimental conditions, the interference of bismuth ions on the results calculated for lead(II) was eliminated. Iron(III) ions do not hamper lead determination. For quantification of iron(III) a new blank sample, containing bismuth and lead ions at concentrations thus determined, was prepared and used as a reference in registration of absorption for the analyzed sample. The content of iron(III) ions was calculated from the absorbance at $\lambda_{\text{max}}$ in zero-order spectrum, that is at 250 nm (Fig. 7).

The same procedure was applied for a model solution containing iron(III) and lead(II) ions. Concentration of lead(II) was calculated directly from the second order derivative and the concentration of iron by registration of a zero-order spectrum using a blank containing lead ions as a reference. Results obtained for model solutions are presented in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Iron(III) ions</th>
<th>Lead(II) ions</th>
<th>Bismuth(III) ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL</td>
<td>$0.09 \mu g/mL$</td>
<td>$0.39 \mu g/mL$</td>
<td>$1.75 \mu g/mL$</td>
</tr>
<tr>
<td>QL</td>
<td>$0.26 \mu g/mL$</td>
<td>$1.21 \mu g/mL$</td>
<td>$5.32 \mu g/mL$</td>
</tr>
<tr>
<td>R [%]</td>
<td>96.08</td>
<td>96.6</td>
<td>97.81</td>
</tr>
<tr>
<td>RSD [%]</td>
<td>2.02</td>
<td>4.02</td>
<td>2.26</td>
</tr>
</tbody>
</table>

Table 1. Limits of detection (DL), quantitation (QL), recovery (%R) and precision (RSD [%]) for iron(III), lead(II) and bismuth(III) for $n=5$.  

Fig. 4. Zero-order absorption spectrum for a model solution of DACT-Fe + DACT-Pb + DACT-Bi; a blank was used as a reference.
Determination of iron (III), lead (II) in pharmaceutical substances

The developed method has been applied for the determination of lead(II) and iron(III) appearing as impurities in chosen pharmaceutical substances. Atomic absorption spectrometry was used as the comparative method.

Quantitative determination of lead(II) and iron(III) by derivative spectrophotometry

5 g of samples under determination were weighed to the nearest 0.1 mg, transferred into a 25 mL volumetric flask and dissolved in 15 mL of water. Then 2 mL of DACT solution at a concentration of 10^-3 mol/L were added and diluted to 25 mL with water. After 15 min. the absorbance of the solution was measured in the range from 200 to 400 nm against the blank solution prepared in the same way as for sample solution but without the examined substance.

The content of lead(II) in the examined substance was calculated based on the value of first derivative at $\lambda_{247}$. The content of iron(III) was calculated based on the absorbance measured at $\lambda_{250}$ against the blank solution containing the corresponding amount of lead (II).

The determination of lead(II) and iron(III) by ASA (7)

The measurements for sample solutions prepared as described above were carried out at $\lambda=283.3$ nm for lead(II) and $\lambda=248.3$ nm for iron(III), spectral slit width $s=0.4$ nm, current intensity of 6 mA and the time of measurement 3 s. The absorption versus concentration is linear within the range from 1.2 mg/mL to 8 mg/mL for lead(II) and from 0.5 mg/mL to 5.0 mg/mL for iron(III). The obtained results are presented in Table 3.

CONCLUSION

The experiments performed in this study have shown that iron(III), lead(II) and bismuth(III), similarly to calcium, magnesium and cadmium(II) ions, and unlike potassium, cobalt(II), aluminium or nickel, form complexes with DACT under alkaline conditions (pH = 10 – 11). As the aim of our work was to determine simultaneously iron(III), lead(II) and bismuth(III), the results refer to these ions only.

It was shown that iron(III), lead(II) and bismuth(III) ions form complexes at a molar ratio 1:1, and these complexes have characteristic absorption spectra with different maxima. Transformation of zero-order spectra into respective derivatives ensures better resolution and thus allows to directly determine the ions studied in one sample. Under the described conditions, it was possible to quantify bismuth(III) alongside with iron(III) and lead(II) ions based on the second order derivative spectrum at...
The use of a blank containing bismuth(III) at the concentration determined in the analyzed sample, guaranteed no interference from these ions while directly determining lead(II) using the second order derivative spectrum at $\lambda = 247$ nm.

Similarly, the effect of both bismuth and lead ions was eliminated in determination of iron(III) by zero-order spectrophotometry at $\lambda = 250$ nm.

The developed method demonstrates high sensitivity, the limit of detection for respective ions was within the range from 0.09 $\mu$g$\times$mL$^{-1}$ to 1.75 $\mu$g$\times$mL$^{-1}$, whereas the determination limit ranged from 0.26 $\mu$g$\times$mL$^{-1}$ to 5.32 $\mu$g$\times$mL$^{-1}$. The recovery was high as well, from 96.08% to 97.81%.

The results of quantification of the ions studied in model solutions and statistical data confirm high precision, repeatability and accuracy of the method (Table 2).

The determination results of limit concentration values of lead(II) and iron(III) in pharmacopoeial substances confirm the applicability of the developed method for the routine analysis. The results presented in Table 3 are within the allowable concentration range according to the Polish Table 2. Results of iron(III), lead(II) and bismuth(III) ions determination in complexes with DACT in model solutions.

<table>
<thead>
<tr>
<th>Model solution</th>
<th>Ion concentration [mg/mL]</th>
<th>Standard deviation $S_{\lambda}$</th>
<th>Confidence interval $t_{0.05}$</th>
<th>Relative standard deviation RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb(II)</td>
<td>6.87</td>
<td>6.90</td>
<td>0.5240</td>
<td>$\pm$ 0.6506</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>2.89</td>
<td>2.97</td>
<td>0.1587</td>
<td>$\pm$ 0.1970</td>
</tr>
<tr>
<td>Bi(II)</td>
<td>6.34</td>
<td>6.11</td>
<td>0.5087</td>
<td>$\pm$ 0.6317</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>8.59</td>
<td>9.32</td>
<td>0.3863</td>
<td>$\pm$ 0.4797</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>2.00</td>
<td>2.03</td>
<td>0.0624</td>
<td>$\pm$ 0.0775</td>
</tr>
<tr>
<td>Bi(II)</td>
<td>8.61</td>
<td>8.81</td>
<td>0.1043</td>
<td>$\pm$ 0.2365</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>4.29</td>
<td>4.26</td>
<td>0.0704</td>
<td>$\pm$ 0.0874</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>1.75</td>
<td>1.71</td>
<td>0.0269</td>
<td>$\pm$ 0.0335</td>
</tr>
<tr>
<td>Bi(II)</td>
<td>7.52</td>
<td>7.43</td>
<td>0.2378</td>
<td>$\pm$ 0.1254</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>3.43</td>
<td>3.45</td>
<td>0.1345</td>
<td>$\pm$ 0.1670</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>1.25</td>
<td>1.27</td>
<td>0.0912</td>
<td>$\pm$ 0.1133</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>6.01</td>
<td>6.04</td>
<td>0.0748</td>
<td>$\pm$ 0.0929</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>1.75</td>
<td>1.70</td>
<td>0.0626</td>
<td>$\pm$ 0.0778</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>4.29</td>
<td>4.28</td>
<td>0.0488</td>
<td>$\pm$ 0.0605</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>1.25</td>
<td>1.26</td>
<td>0.0575</td>
<td>$\pm$ 0.0714</td>
</tr>
</tbody>
</table>

Table 3. The content of Pb(II) and Fe(III) in pharmaceutical substances determined by SP and AAS with the statistical evaluation.

<table>
<thead>
<tr>
<th>Pharmaceutical substance (FP VI)</th>
<th>Allowable concentration of ions by FP VI $P_{\text{B(II)/Fe(III)}}$ [mg/g$^{-1}$]</th>
<th>Concentration of ions determined by SP $P_{\text{B(II)/Fe(III)}}$ [mg/g$^{-1}$]</th>
<th>Relative standard deviation RSD</th>
<th>Concentration of ions determined by AAS $P_{\text{B(II)/Fe(III)}}$ [mg/g$^{-1}$]</th>
<th>Relative standard deviation RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl$_2$</td>
<td>$10$</td>
<td>$8.07$</td>
<td>$1.92$</td>
<td>$8.59$</td>
<td>$1.58$</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>$10$</td>
<td>$8.31$</td>
<td>$1.28$</td>
<td>$9.16$</td>
<td>$1.54$</td>
</tr>
<tr>
<td>NaCl</td>
<td>$10$</td>
<td>$5.06$</td>
<td>$2.11$</td>
<td>$5.68$</td>
<td>$1.94$</td>
</tr>
<tr>
<td>KCl</td>
<td>$10$</td>
<td>$29.94$</td>
<td>$2.94$</td>
<td>$30.83$</td>
<td>$0.86$</td>
</tr>
<tr>
<td>ZnSO$_4$</td>
<td>$20$</td>
<td>$9.16$</td>
<td>$1.80$</td>
<td>$9.94$</td>
<td>$0.96$</td>
</tr>
</tbody>
</table>

$\lambda = 287$ nm. The use of a blank containing bismuth(III) at the concentration determined in the analyzed sample, guaranteed no interference from these ions while directly determining lead(II) using the second order derivative spectrum at $\lambda = 247$ nm. Similarly, the effect of both bismuth and lead ions was eliminated in determination of iron(III) by zero-order spectrophotometry at $\lambda = 250$ nm.

The developed method demonstrates high sensitivity, the limit of detection for respective ions was within the range from 0.09 $\mu$g$\times$mL$^{-1}$ to 1.75 $\mu$g$\times$mL$^{-1}$, whereas the determination limit ranged from 0.26 $\mu$g$\times$mL$^{-1}$ to 5.32 $\mu$g$\times$mL$^{-1}$. The recovery was high as well, from 96.08% to 97.81%.

The results of quantification of the ions studied in model solutions and statistical data confirm high precision, repeatability and accuracy of the method (Table 2).

The determination results of limit concentration values of lead(II) and iron(III) in pharmacopoeial substances confirm the applicability of the developed method for the routine analysis. The results presented in Table 3 are within the allowable concentration range according to the Polish
Pharmacopoeia VI (7) and the results obtained by AAS are comparative.

REFERENCES


Received: 28.06.2006