THE USE OF SURFACTANTS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF ZINC BASING ON COMPLEXES WITH AZO DYES.

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ABSTRACT. A simple and direct spectrophotometric methods for determination of zinc are based on reaction with azo dyes: 1-(2-pyridylazo)-2-naphthol (PAN) and Chromothrop 2B in the presence of benzyldimethyldodecylammonium bromide (BDDABr) have been developed. Optimum concentrations of PAN, Chromothrop 2B, BDDABr and pH ensuring maximum absorbance were defined. The effect of foreign ions was elucidated. The complex Zn-PAN-BDDABr shows maximum absorbance at 597 nm with the molar absorptivity value $8.55 \times 10^4 \text{L \ mol}^{-1} \text{cm}^{-1}$, and complex Zn-Chromothrop 2B-BDDABr has maximum absorbance at 595 nm with the molar absorptivity value $4.26 \times 10^4 \text{L \ mol}^{-1} \text{cm}^{-1}$. The Beer’s law is obeyed for zinc concentrations in the range $0.24-0.80 \mu \text{g mL}^{-1}$ for the complex Zn-Chromothrop 2B-BDDABr and $0.36-0.80 \mu \text{g mL}^{-1}$ for the complex Zn-PAN-BDDABr. The method basing on the complex Zn-PAN-BDDABr has been applied for the determination of zinc in milk, milk substituting preparations, insulin and blood serum.

INTRODUCTION. The very sensitive and selectivity methods for the spectrophotometric determination of many metals and also zinc are based on the reaction with azo-dyes [1-3]. The most popular of these chromogenic reagents are 1-(2-pyridylazo)-2-naphtol (PAN) and Chromothrop 2B. PAN belongs to group of pyridylazo dyes and Chromothrop 2B is example of o-hydroxyarylazo compounds. Metal-PAN complex does not dissolve in aqueous solution, so it is necessary to do a solvent extraction. This complex had been extracted with non-polar solvents such as chloroform or benzene, which was found to be carcinogenic. Application of surface active substances, which play the role of a protective colloids gives possibility to increase solubility of a Zn-PAN and Zn-Chromothrop 2B complexes and creates better conditions for determination [4-7]. The harmful organic solvents used in the extraction-spectrophotometric methods have been eliminated. An advantage of these methods is a direct spectrophotometric measurement of absorbance of analysed solution. Application of three component systems, and also more often use of four-component systems, causes the spectrophotometric methods to exhibit high sensitivity-molar absorptivity usually larger than $1 \times 10^5 \text{L \ mol}^{-1} \text{cm}^{-1}$ and can compete with other extraction-spectrophotometric and spectrophotometric methods [8-13].

EXPERIMENTAL. Reagents. All the reagents were of analytical grade and were used without further purification. Double-distilled water was used in all experiments. Zinc stock solution (1 mg mL$^{-1}$) was prepared by dissolving 0.43987 g of ZnSO$_4$·7H$_2$O (PPH”POCH” S.A.) in water with addition of 0.5 mL of concentrated H$_2$SO$_4$ and dilution up to 100 mL. Working solutions of 10 µg mL$^{-1}$ and 20 µg mL$^{-1}$ were obtained by diluting the stock solution. 1-(2-pyridylazo)-2-naphthol (PAN) solution in methanol
(1×10^{-3} \text{ mol L}^{-1}) \text{ was obtained by dissolving 0.0250 g of PAN (PPH”POCh” S.A.) in methanol and dilution up to 100 mL. Chromothrop 2B solution (1×10^{-3} \text{ mol L}^{-1}) \text{ was obtained by dissolving 0.12835 g of Chromothrop 2B (PPH”POCh” S.A.) in water and dilution up to 250 mL. Triton X-100 (1×10^{-2} \text{ mol L}^{-1}) \text{ was obtained by dissolving 3.2343 g of Triton X-100 (BDH) in water and dilution up to 500 mL. Benzylidimethyldecylammonium bromide (BDDABr) solution (1×10^{-1} \text{ mol L}^{-1}) \text{ was obtained by dissolving 3.8445 g of BDDABr (Fluka) in water and dilution up to 100 mL. Borate buffer pH=8 , pH=7.6 [14]. Control of blood serum Cormay HP and samples of human blood serum (Medical Laboratory). Insulin Actrapid, Mixtard (Polfa, Tarchomin, Poland)Milk Nan 1 (Nestle) and milk substituting preparations Isomil (Abbott Laboratories B.V.), Nutrison (Ovita Nutricia).}

**Apparatus.** An Jasco (Japan) model V-530 spectrophotometer UV-VIS was used for all absorbance measurements with a 10 mm glass cell. An Elwro (Poland) model N-517 pHmeter was used for pH measurements. The spectrometer ICP-OES made by Spectro Analytical Instruments (Germany) was used with the following parameters: 27.12 MHz, power 1.1 kW, nebulizer-concentric Meinhard. Mineralization was conducted in a Uni-Clever BM-1z microwave mineralizer, Plasmotronika (Poland).

**Procedure.**  

**Zn-PAN-BDDABr:** To the flask of 25 mL capacity were introduced 2mL of the BDDABr solution, 1.3 mL PAN solution, 3.5 mL borate buffer (pH=7.6) and 5-20 µg zinc. The flasks were filled up with water to the same volume. After 30 min, absorbance was measured at 597 nm in 10 mm cuvettes, against the blank and the calibration graph was plotted.

**Zn-Chromothrop 2B-BDDABr:** To the flask of 25 mL capacity were introduced 2.5 mL of the Chromothrop 2B solution, 4 mL borate buffer (pH=8), 2-20 µg zinc and 1mL BDDABr solution. The flasks were filled up with water to the same volume. After 15 min, absorbance was measured at 595 nm in 10 mm cuvettes, against the blank and the calibration graph was plotted.

**RESULTS AND DISCUSSION.** Absorption spectrum and effect of surfactants. Zinc reacts with PAN forming of an orange coloured water-insoluble neutral chelate. The application cationic surface active agent – benzylidimethyldecylammonium bromide or nonionic surfactant – Triton X-100 gives possibility to increase to solubility of the Zn-PAN complex and improves conditions for its determination.

Figure 1 presents the absorption spectra for the zinc chelate with PAN in the presence of BDDABr and Triton X-100. The complexes Zn-PAN-BDDABr and Zn-PAN-BDDABr Triton X-100 show the absorption maximum at the same wavelength \( \lambda_{\text{max}}=597 \text{ nm} \) (curve 1,2), and the ternary Zn-PAN-Triton X-100 system at 555 nm (curve 3). A large bathochromic effect of maximum absorption of studied system (curve 1) comparing with the complex Zn-PAN-Triton X-100 (curve 3, \( \Delta \lambda=42 \text{ nm} \)) was observed. In the presence BDDABr there is a significant hyperchromic effect. Introduction of Triton X-100 to Zn-PAN-BDDABr system distinctly lowers of absorbance. In consideration of more intensity of absorption of complex wavelength \( \lambda=597 \text{ nm} \) was choosen. Figure 2 presents the absorption spectra for the zinc complex with Chromothrop 2B in the presence of surfactants.
1. Zn-PAN-BDDABr complex measured against blank $\lambda=597\text{nm}$
2. Zn-PAN-BDDABr-Triton X-100 complex measured against blank $\lambda=597\text{nm}$
3. Zn-PAN-Triton X-100 complex measured against blank $\lambda=555\text{nm}$

1. Zn-Chromothrop 2B complex measured against blank $\lambda=515\text{nm}$, $\lambda=580\text{nm}$
2. Zn-Chromothrop 2B-BDDABr complex measured against blank $\lambda=595\text{nm}$
3. Zn-Chromothrop 2B-Triton X-100 complex measured against blank $\lambda=519\text{nm}$, $\lambda=585\text{nm}$
4. Zn-Chromothrop 2B-BDDABr-Triton X-100 complex measured against blank $\lambda=528\text{nm}$

**Media and the reaction time.** The complexation occurs in a weak alkaline medium with the optimum at pH=7.6 for the Zn-PAN-BDDABr complex and with the optimum at pH=8 for the Zn-Chromothrop 2B-BDDABr complex. In both cases in further experiments the borate buffer of these value pH was used. Absorbance of the complexes is constant after 30 min (Zn-PAN-BDDABr) and 15 min (Zn-Chromothrop 2B-BDDABr) at room temperature and remains constant for 1.5 h. Optimum concentrations of PAN, Chromothrop 2B, BDDABr and order of reagents addition were defined. The conditions required for the studied systems are given in Table 1.

**Table 1.** The conditions of determination of the studied complexes.

<table>
<thead>
<tr>
<th>System</th>
<th>$\lambda$ [nm]</th>
<th>pH</th>
<th>Time [min]</th>
<th>Order of reagent addition</th>
<th>Molar excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn-PAN-BDDABr</td>
<td>597</td>
<td>7.6</td>
<td>30</td>
<td>BDDABr-PAN-buffer-Zn</td>
<td>8</td>
</tr>
<tr>
<td>Zn-Chr2B-BDDABr</td>
<td>595</td>
<td>8.0</td>
<td>15</td>
<td>Chr.2B-buffer-Zn-BDDABr</td>
<td>-</td>
</tr>
</tbody>
</table>

- 130
- 31
Composition of the complexes. The continuous variation method was used to establish the stoichiometry of the investigated complexes. This method requires the measurement of the absorbance or other physical quantity in the series of solutions containing different amounts of two reacting components, yet the total sum of their moles is kept constant. It was found that in the presence BDDABr, PAN and Chromothrop 2B bound to Zn in the molar ratio 2:1.

Characteristic of the methods. Under the optimum conditions the calibration plot for zinc determination was obtained. The Beer’s law was obeyed over the Zn concentration range 0.36-0.80 µg mL\(^{-1}\) for the system with PAN in the presence BDDABr and 0.24-0.80 µg mL\(^{-1}\) for the system with Chromothrop 2B with BDDABr. Molar absorptivity for the complex Zn-PAN-BDDABr at 597 nm is \(8.55 \times 10^4\) L mol\(^{-1}\)cm\(^{-1}\) and the correlation coefficient \(r=0.9998\). For the complex Zn-Chromothrop 2B-BDDABr molar absorptivity at 595 nm is \(4.26 \times 10^4\) L mol\(^{-1}\)cm\(^{-1}\) and the correlation coefficient \(r=0.9967\).

Interference from coexisting ions. The studies of the effect of foreign ions on the determination of zinc with PAN in the presence BDDABr show that the selectivity of the method is rather poor. The cations Na\(^+\), NH\(_4\)\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\) and anions Cl\(^-\), Br\(^-\), NO\(_3\)\(^-\), SO\(_4\)\(^{2-}\), PO\(_4\)\(^{3-}\), CH\(_3\)COO\(^-\) (examined in the 1000-fold molar excess over zinc) do not affect the determination. All cations forming complexes with PAN have an interfering effect. It was found that ions F\(^-\), I\(^-\) may be present in 50-fold molar excess over Zn and Mn\(^{2+}\), Co\(^{2+}\) in 10-fold molar excess over Zn and Fe\(^{3+}\) 7-fold molar excess over Zn. Cation Pb\(^{2+}\) presents at the twenty times lower concentration than the Zn concentration and Mo\(^{5+}\), Ni\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\) present at the ten times lower concentration than the Zn negatively influenced the determination results. Ions CN\(^-\) and EDTA forming permanent complexes with zinc, interfered. Cation Al\(^{3+}\) also affects determination because hydrolyzes.

Analytical application. The most sensitive of the elaborated methods (Zn-PAN-BDDABr) was applied to the determination of zinc ions in blood serum samples, insulin samples as well as milk samples and milk substituting preparations. Before the determination was carried out, the samples of milk were mineralized in the microwave mineralized with concentrated HNO\(_3\).

Blood serum samples were subjected to the deproteinization process. A 1+1 mixture of 20% solutions of sulfuric and nitric acids was used [11]. It was also found that copper present in the serum was eliminated efficiently by complexation with tartrates.

Determination of zinc.

Milk samples and milk substituting preparations. In order to prepare the samples for analysis, 1 g of milk sample (NAN) and milk substituting preparations (ISOMIL, NUTRISON) were transferred to microwave mineralizer and mineralized using 6 mL of concentrated nitric acid. When mineralization was completed, the samples were quantitatively transferred to the flasks and volumes were made up to 25 mL with redistilled water. Then, to 5 mL aliquot of each sample the appropriate amount of the saturated NaOH solution was added to obtain pH of about 7.6. Next to 25 mL flasks the following reagents were added: 2 mL of BDDABr, 1.3 mL of PAN, 3.5 mL of borate buffer (pH=7.6) and prepared after mineralization of the milk solution. This was filled to 25 mL with redistilled water. Absorbance was measured after 30 min at 597 nm, relative to a blank test as standard solution. The zinc concentration was read from the standard curve obtained in the presence of all reagents used for the milk analysis.

Blood serum. In order to prepare the sample for analysis 1 mL of serum (standard serum HP and human blood serum) was poured into a test tube and centrifuged; then 1 mL of redistilled water and 0.5 mL of deproteinizing mixture (1+1 20% HNO\(_3\)+20%
H$_2$SO$_4$ [11]) were added. After mixing, the sample was heated in boiling water for 3-5 min, cooled down and centrifuged at 3000 turns min$^{-1}$. The sediment was filtered off and washed twice in 2 mL of redistilled water. Next to the solution an amount of saturated NaOH was added to final pH of 7.6. To a 25 mL flask was added: 2 mL of BDDABr, 1.3 mL of PAN, 3.5 mL of borate buffer (pH=7.6), prepared after deproteinization of the serum solution and 0.5 mL of sodium tartrate in order to mask copper present in the sample. This was filled to 25 mL with redistilled water. After 30 min equilibration time the absorbance of solutions was measured at 597 nm against the blank.

**Insulin.** Samples of 1.5 mL of insulin were transferred to a 10 mL standard flasks and dilute the samples with redistilled water to the mark. If MIXTARD insulin samples are analysed, before diluting the samples acidify them with hydrochloric acid (1·10$^{-3}$ mol L$^{-1}$) to obtain clear solution. In order to prepare the samples of MIXTARD insulin for analysis to the 5 mL of insulin solution an amount of saturated NaOH was added to pH 7.6. Zinc in ACTRAPID insulin was determination directly from sample. Than to each 25 mL flasks was added: 2 mL of BDDABr, 1.3 mL of PAN, 3.5 mL of borate buffer (pH=7.6) and 5 mL of insulin solution. This was filled to 25 mL with redistilled water. Absorbance was measured after 30 min at 597 nm against a blank solution.

The all results of Zn determination in the studied samples and statistical evaluation of the results are given in Table 2. In order to compare the results obtained with the use of the elaborated method the comparative ICP-OES method was also applied.

**Table 2.** Results of the determination of the content of zinc in milk samples, blood serum and insulin preparations by the developed method and the reference method ICP-OES.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Average result of determination [µg mL$^{-1}$]</th>
<th>Standard deviation SD n=3</th>
<th>µ$_{95}$=x±t×SD [µg mL$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISOMIL</td>
<td>BDDABr-PAN-Zn</td>
<td>1.871</td>
<td>0.02</td>
<td>1.871±0.08</td>
</tr>
<tr>
<td></td>
<td>ICP-OES</td>
<td>1.884</td>
<td>0.02</td>
<td>1.884±0.03</td>
</tr>
<tr>
<td>NUTRISON</td>
<td>BDDABr-PAN-Zn</td>
<td>1.342</td>
<td>0.04</td>
<td>1.342±0.05</td>
</tr>
<tr>
<td></td>
<td>ICP-OES</td>
<td>1.363</td>
<td>0.01</td>
<td>1.363±0.01</td>
</tr>
<tr>
<td>NAN</td>
<td>BDDABr-PAN-Zn</td>
<td>1.099</td>
<td>0.02</td>
<td>1.099±0.03</td>
</tr>
<tr>
<td></td>
<td>ICP-AES</td>
<td>1.130</td>
<td>0.04</td>
<td>1.130±0.05</td>
</tr>
<tr>
<td>SERUM CORMAY HP</td>
<td>BDDABr-PAN-Zn</td>
<td>0.159</td>
<td>0.02</td>
<td>0.159±0.05</td>
</tr>
<tr>
<td></td>
<td>ICP-OES</td>
<td>0.169</td>
<td>0.01</td>
<td>0.169±0.04</td>
</tr>
<tr>
<td>BLOOD SERUM 1</td>
<td>BDDABr-PAN-Zn</td>
<td>0.185</td>
<td>0.04</td>
<td>0.185±0.10</td>
</tr>
<tr>
<td></td>
<td>ICP-OES</td>
<td>0.188</td>
<td>0.03</td>
<td>0.188±0.08</td>
</tr>
<tr>
<td>BLOOD SERUM 2</td>
<td>BDDABr-PAN-Zn</td>
<td>0.167</td>
<td>0.04</td>
<td>0.167±0.09</td>
</tr>
<tr>
<td></td>
<td>ICP-OES</td>
<td>0.178</td>
<td>0.01</td>
<td>0.178±0.04</td>
</tr>
<tr>
<td>INSULIN ACTRAPID</td>
<td>BDDABr-PAN-Zn</td>
<td>2.760</td>
<td>0.05</td>
<td>2.760±0.13</td>
</tr>
<tr>
<td></td>
<td>ICP-OES</td>
<td>2.766</td>
<td>0.06</td>
<td>2.766±0.15</td>
</tr>
<tr>
<td>INSULIN MIXTARD</td>
<td>BDDABr-PAN-Zn</td>
<td>3.645</td>
<td>0.03</td>
<td>3.645±0.09</td>
</tr>
<tr>
<td></td>
<td>ICP-OES</td>
<td>3.676</td>
<td>0.05</td>
<td>3.676±0.13</td>
</tr>
</tbody>
</table>

**CONCLUSION.** The developed spectrophotometric methods for determination of zinc by means of PAN and Chromothroph 2B in the presence of benzylidimethyldodecylammonium bromide consist in the direct measurement of absorbance of the analysed solution. Therefore determined methods are simple, faster and safer compared to the know extraction-spectrophotometric methods. The harmful
organic solvents used in the extraction-spectrophotometric methods have been eliminated.
The application of surfactant, which acts as protective colloid, gives the possibility to increase the solubility of the Zn-PAN and Zn–Chromothrop 2B complexes in the analysed solutions and improves conditions for determination. The proposed method of determination of Zn in the form of its complex with PAN and BDDABr is precise and sensitive. The molar absorptivity of the complex is $8.55 \times 10^4 \text{L mol}^{-1}\text{cm}^{-1}$ and the Beer’s law is obeyed for zinc concentrations in the range 0.36 – 0.80 µg mL$^{-1}$. The studies carried out prove that method based on the complex Zn-PAN-BDDABr can be used for the determination of zinc in blood serum, insulin and various milk samples.

REFERENCES