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CHROMIUM(III) COMPLEXES WITH DL-METHIONINE AND L-CYSTEINE\*

Chromium(III) complexes with DL-methionine and L-cysteine have been investigated by pH-metric and spectrophotometric methods. In both investigated systems, three complexes of the composition  $M:L = 1:1$ ;  $1:2$ ;  $1:3$  have been found. Stability constants for the resultant complexes have been determined by the method of Bjerrum. Crystalline complexes with the investigated ligands of the type:  $[Cr(Met)_3] \cdot 2H_2O$  and  $Na[Cr(Cys)_2] \cdot 2H_2O$  were also obtained. The made combination of Cr(III) with the investigated aminoacid and structures of the complexes reported have been proposed on the basis of their spectra. A method for precipitation pure complexes from solutions has been devised using the ion-exchange process on the resin Sephadex A-25.

Introduction

The role of metal ions in the biochemical process with the contribution of aminoacids has not been fully understood as yet. The reactions between aminoacids containing sulphur and transition metals ions are of interest in consideration of still unutilized opportunities of the use of these compounds in therapy [1].

Information about Ni(II), Zn(II), Hg(II), Cd(II), Pb(II), Fe(II), Mn(II) and Co(II) complexes with some aminoacids containing sulphur is available in the literature [2-7]. But there are insufficient data on the Cr(III) complexes with methionine and cysteine. Scarce information [8-11] concerns solid complexes

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or studies on systems in the presence of quite a different ligand as e. g. violet R.

In the present study, systematic investigations of the systems: Cr(III) - DL-methionine -  $H_2O$  and Cr(III) - L-cysteine -  $H_2O$  are undertaken. They are aimed at thorough understanding of equilibrium reactions in solutions separation of solid complexes and determination of their structures.

### Experimental

#### Reagents and apparatus

The following reagents were used: DL-methionine (Reanal), L-cysteine hydrochloride monohydrate (Merck); sodium perchlorate monohydrate (Koch-Light), 70% perchloric acid (Hopkin and Williams), sodium hydroxide (POCH), chromium chloride hexahydrate (BDH), sodium chloride (POCH) and DEAE-Sephadex A25 (Pharmacia Fine). All the reagents were of analytical grade. The amino-acids were purified by crystallization from aqueous - ethanolic solutions. Chromium perchlorate was prepared according to Rasko and Salymsi [12]. The concentration of Cr(III) was measured by a redoxymetric method [13].

For pH measurements, a digital N517 pH-meter (Mera-Elwro) equipped with a glass OP-7171-1/A electrode and a calomel OP-830 (Radelkis) electrode, was used.

Absorption electronic spectra were recorded with a UV-VIS spectrophotometer (Carl Zeiss Jena). IR spectra were recorded on a Specord 71 IR spectrophotometer over range  $4600-700\text{ cm}^{-1}$  or a UR-10 spectrophotometer over the range  $700-400\text{ cm}^{-1}$ , using a KBr pellet.

### Procedure

The reactions in Cr(III) - L -  $H_2O$  systems (where L is methionine or cysteine) at room temperature proceed very slowly. The equilibrium is reached within a period of 1 month, and, therefore, an investigating procedure, as described by Naga-

ta and co-workers [14], was used. So, 40 samples of 50 cm<sup>3</sup> in volume were prepared. They contained various amount of the investigated aminoacid (from  $1 \cdot 10^{-3}$  to  $6 \cdot 10^{-2}$  mole/l), chromium (III) salt (from  $1 \cdot 10^{-4}$  to  $2,5 \cdot 10^{-5}$  mole/l) and NaOH. The ionic strength of all the solutions was constant and equal to 0.1 (NaClO<sub>4</sub>). The solutions were stored in an incubator at a temperature of  $25 \pm 0.1^\circ\text{C}$  for 1 month. Then, the pH values of the solutions were measured and used to calculate the stability constants. In spectrophotometric measurements, the solution containing Cr(III) and aminoacid were heated on a water bath for 2 h, and then their absorption spectra were recorded at room temperature. A solid complex of Cr(III) and L-cysteine was prepared in the following manner [8]. Aqueous solution of 50 cm<sup>3</sup> in volume containing 1 millimole of CrCl<sub>3</sub> and 3 millimoles of L-cysteine hydrochloride was heated in a water bath. When the colour of the solution had been changed from green to red-dish-violet, a solid NaOH was added. At pH about 7, dark bluish-red crystalls of the Cr(III) complex with L-cysteine were precipitated. The complex was filtered off, washed with ethanol and dried at 100°C. The crystalline complex of Cr(III) with DL-methionine was prepared according to Mizuchi and co-workers [15].

### Results and discussion

Stability constants of Cr(III) complexes with DL-methionine and L-cysteine.

The changes in the visible reflectance spectra of the investigated systems (Fig. 1) point to the formation of CrL<sub>n</sub> complexes. It was found, by the method of Job, that the complex of the composition M : L = 1 : 3 (Fig. 2) is a dominating one in both systems at a wavelength  $\lambda = 540$  nm. The values of stability constants calculated by the method of "corresponding solutions" [16] are given in Tab. 1. The Table contains also the values of dissociation constants of DL-methionine and L-cysteine [17].

The K<sub>n</sub> constants, found from the Bjerrum formation curves

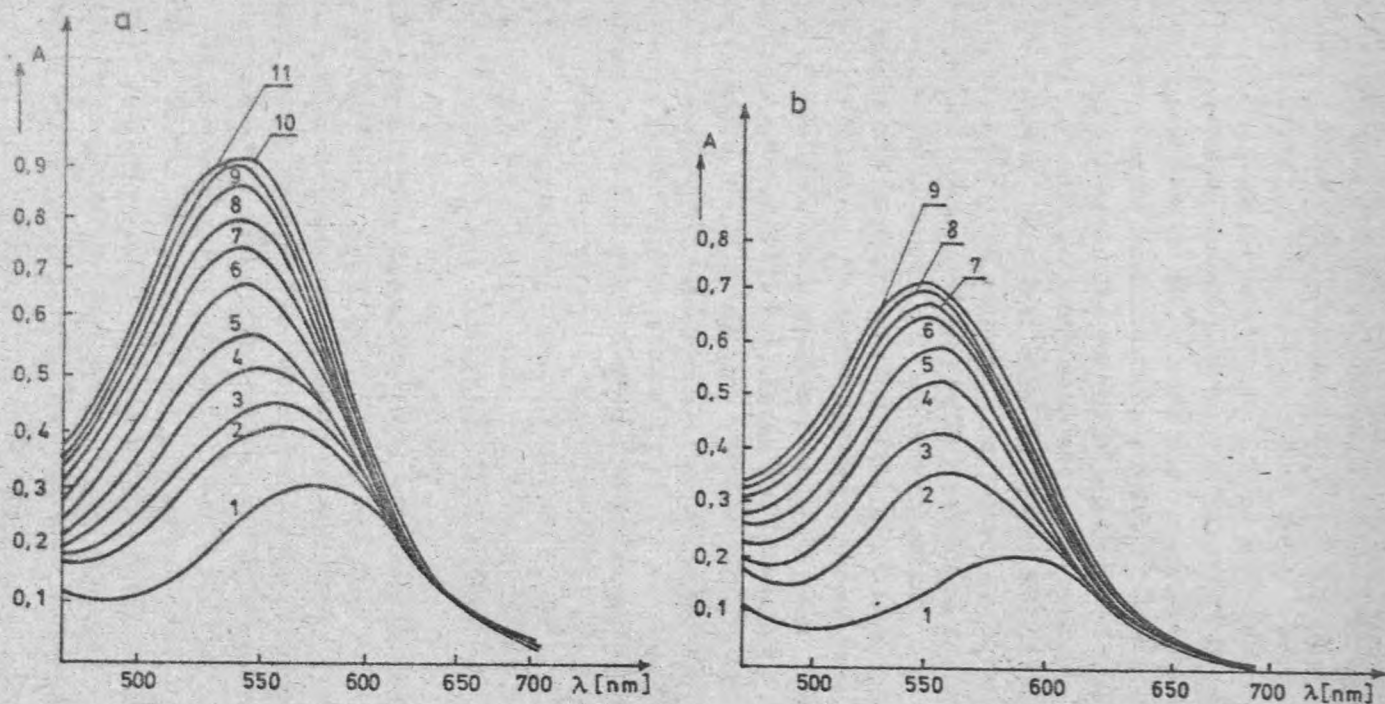


Fig. 1. Absorption spectra of a  $1 \cdot 10^{-2}$  M solution of  $(Cr ClO_4)_3$  with variable ligand concentrations (L): [1 - 0; 2 - 1.0; 3 - 2.0; 4 - 3.0; 5 - 4.0; 6 - 5.0; 7 - 6.0; 8 - 7.0; 9 - 8.0; 10 - 9.0; 11 - 10]  $10^{-2}$  mol/dm<sup>3</sup>; pH = 4.5; d = 2 cm. a - L = L-cysteine hydrochloride; b - L = DL-methionine



Table 1

Stability constants  $K_n$  of complexes Cr(III) with DL-methionine and L-cysteine found by spectrophotometric and potentiometric methods

| Aminoacid under investigation | Stability constants $\log K_n$ |                |                |                       |                |                | Dissociation constants $K_n$ of aminoacid |                |                 |
|-------------------------------|--------------------------------|----------------|----------------|-----------------------|----------------|----------------|---|----------------|-----------------|
|                               | spectrophotometric method      |                |                | potentiometric method |                |                | $pK_1$                                    | $pK_2$         | $pK_3$          |
|                               | $\log K_1$                     | $\log K_2$     | $\log K_3$     | $\log K_1$            | $\log K_2$     | $\log K_3$     |   |                |                 |
| DL-methionine                 | $7.45^{+0.30}$                 | $6.54^{+0.30}$ | $5.99^{+0.30}$ | $7.91^{+0.45}$        | $6.94^{+0.45}$ | $6.43^{+0.35}$ | $2.32^{+0.05}$                            | $9.15^{+0.05}$ |                 |
| L-cysteine                    | $8.05^{+0.30}$                 | $7.45^{+0.30}$ | $6.32^{+0.30}$ | $8.32^{+0.45}$        | $7.69^{+0.45}$ | $6.94^{+0.35}$ | $1.71^{+0.05}$                            | $8.35^{+0.05}$ | $10.36^{+0.05}$ |

(Fig. 3), show that DL-methionine and L-cysteine form with chromium(III), in aqueous solutions, complexes of high stability. The  $K_n$  constant values obtained by two methods are in agreement with each other.

Our values of the  $K_n$  constants of the Cr(III) complexes with DL-methionine and L-cysteine are between the values of the stability constants of Cr(III) complexes with ethylenediamine and those of Cr(III) complexes with oxalates (Tab. 2). It is likely to be associated with the fact that glycine, alanine, methionine and cysteine belong to the ligands co-ordinating metal ion through oxygen atom of carboxylic group and nitrogen atom of amine group. The values of  $K_n$  found show that the contribution of sulphur atom of thioether group of methionine and sulphhydryl group of cysteine with co-ordination with Cr(III) ion should be excluded. Should the co-ordination of Cr(III) ion through sulphur atom takes place, then the values of stability constants  $K_n$  would be higher than these for the Cr(III) complex with glycine or alanine. In accordance to the theory of Pearson [19], the Cr(III) ion belongs to ion class (a), and, therefore, it

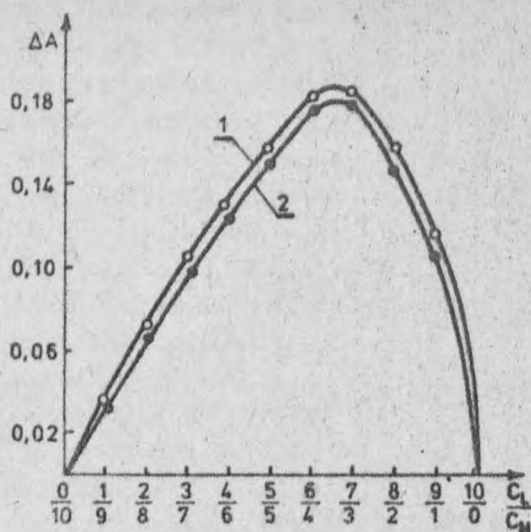


Fig. 2. Assignment of the complex composition by the method of continuous changes of Job. 1 - (Cr III) with L-cysteine; 2 - Cr(III) with DL-methionine, Concentration sum = 0.06 M,  $d = 1$  cm;  $\lambda = 540$  nm.  $A = A_m - A_{Cr}$ ;  $A_m$  = absorbance of the mixture,  $A_{Cr}$  = absorbance of the  $Cr(ClO_4)_3$

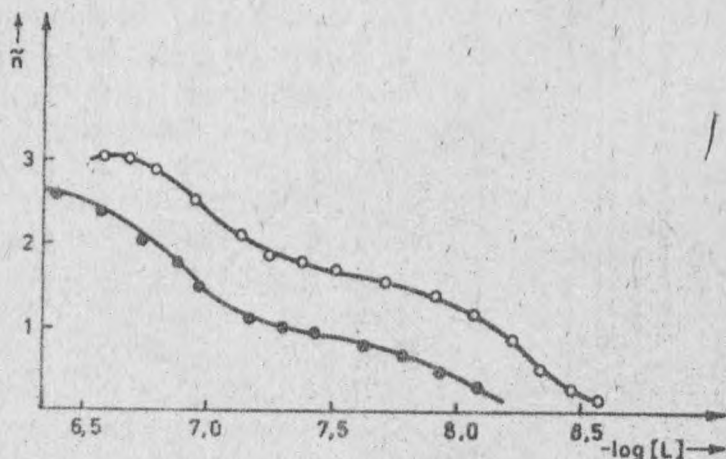


Fig. 3. The formation curves of Bjerrum for the systems: Cr(III) + DL-methionine;  $\circ$  - Cr(III) + L-cysteine

Table 2

Stability constants  $K_n$  of Cr(III) complexes  
with some ligands in an aqueous solution

| Ligand                  | $\log K_1$ | $\log K_2$ | $\log K_3$ | References |
|-------------------------|------------|------------|------------|------------|
| Ethylenediamine (en)    | 16.5       | 14         | -          | [18]       |
| Glycine (gli)           | 8.62       | 7.65       | -          | [18]       |
| $\alpha$ -alanine (ala) | 8.53       | 7.44       | -          | [18]       |
| Oxalate (ox)            | 5.34       | 5.17       | -          | [18]       |
| DL-methionine (met.)    | 7.45       | 6.54       | 5.99       | this study |
| L-cysteine (cys)        | 8.05       | 7.45       | 6.32       | this study |

forms complexes easier with ligands co-ordinating through nitrogen and oxygen atoms.

Preparation and properties of crystalline complexes  
of Cr(III) with DL-methionine and L-cysteine

A solid crystalline complex compound of Cr(III) with L-cysteine and a solid compound of Cr(III) with DL-methionine were prepared according to the above mentioned procedure. The molecular formulae of the two complex compounds were assigned on the basis of elemental analysis.

For  $\text{Na}[\text{Cr}(\text{L-cys})_2]2\text{H}_2\text{O}$ :

calculated (%) C 20.40; H 5.13; O 27.17; N 7.93; S 18.15; Cr 14.72  
found (%) C 20.51; H 5.33; O 27.31; N 7.87; S 18.02; Cr 14.65

For  $[\text{Cr}(\text{DL-meth})_3]2\text{H}_2\text{O}$ :

calculated (%) C 33.63; H 6.96; O 23.89; N 7.88; S 17.96; Cr 9.71  
found (%) C 33.50; H 7.02; O 24.00; N 7.89; S 17.80; Cr 9.58

To know structures of both solid complexes, their IR spectra (Tab. 3) and UV-VIS spectra of their solutions (Tab. 4) were made.

The shift of the band corresponding to stretching vibration  $\nu(\text{NH}_2)$  from  $2300\text{ cm}^{-1}$  to the range  $3220 - 3120\text{ cm}^{-1}$  in the complex  $[\text{Cr}(\text{Meth})_3] \cdot 2\text{H}_2\text{O}$  suggests the formation of a strong Cr-N

Table 3

Characteristic absorption bands in the IR spectra of the investigated aminoacids and their complexes with Cr(III)

| DL-Met | Band                  | $\text{Cr}(\text{Met})_3 \cdot 2\text{H}_2\text{O}$ | Band                  | L-Cys $\text{HCl} \cdot \text{H}_2\text{O}$ | Band            | $\text{NaCr}(\text{Cys})_2 \cdot 2\text{H}_2\text{O}$ | Band                   |
|--------|-----------------------|---|-----------------------|---|-----------------|---|------------------------|
| 3000 m | $\text{NH}_2$ V       | 3220 m  | $\text{NH}_2$ V       | 3050 m                                      | $\text{NH}_2$ V | 3120 m  | $\text{NH}_2$ V        |
| 2700 m | $\text{NH}_2$ $V_s$   | 3120 m  | $\text{NH}_2$ $V_s$   | 2565 m                                      | S-H V           | 1640 m  | C=O V                  |
| 1950 s | $\text{CO}_2^-$ $V_a$ | 1640 s  | $\text{CO}_2^-$ $V_a$ | 1740 s                                      | C=O V           | 1610 s  | $\text{CO}_2^-$ $V_a$  |
| 1400 s | $\text{CO}_2^-$ $V_s$ | 1420 s  | $\text{CO}_2^-$ $V_s$ | 1230 $V_s$                                  | C-O V           | 1560 m  | $\text{NH}_2$ $\delta$ |
| 1320 m | $\text{SCH}_3$ V      | 1320 m  | $\text{SCH}_3$ V      | 1210 $V_s$                                  | C-O V           | 1260  | C-O V                  |
| 780 m  | CS $V_a$              | 780 m   | CS $V_a$              | 690 m                                       | C-S V           | 670   | C-S V                  |
| 685    | CS $V_s$              | 685 m   | CS $V_s$              |   |                 |   |                        |
|        |                       | 450 m   | CrN V                 |   |                 |   |                        |

V - stretching vibration,  $V_s$  - symmetric stretching vibration,  $V_a$  - asymmetric stretching vibration,  $\delta$  - bending vibration, s - strong, m - medium, w - weak, v - very weak.



Table 4

Spectral data of the Cr(III) complexes with DL-methionine and L-cysteine

| Complex  | Band 1        |   | Band 2        |          | Band 3        |          | Band 4        |          |
|--|---------------|---|---------------|----------|---------------|----------|---------------|----------|
|  | max $\lambda$ | $\log E$<br>[ $\text{mol}^{-1}\text{cm}^{-1}$ ] | max $\lambda$ | $\log E$ | max $\lambda$ | $\log E$ | max $\lambda$ | $\log E$ |
| $\text{Cr}(\text{DL-Met})_3 \cdot 2\text{H}_2\text{O}^{\text{a}}$  | 416           | 1,71  |               |          | 571           | 1,79     |               |          |
| $\text{NaCr}(\text{L-Cys})_2 \cdot 2\text{H}_2\text{O}^{\text{b}}$ | 408           | 1,73  | 467           | 1,67     | 541           | 1,71     | 606           | 1,81     |

<sup>a</sup> Spectrum in 30%  $\text{HClO}_4$ .<sup>b</sup> Spectrum in  $\text{H}_2\text{O}$  pH = 7.0.

bond. Similarly, the shift of symmetric and asymmetric stretching vibrations of carboxylic group from  $1390\text{ cm}^{-1}$  and  $1400\text{ cm}^{-1}$  to  $1620\text{ cm}^{-1}$  and  $1420\text{ cm}^{-1}$ , respectively, points to the formation of a Cr-O bond. According to McAuliffe [20], the difference  $\Delta_r(\text{COO}^-)$  between  $V(\text{COO}^-_{\text{asym.}})$  and  $V(\text{COO}^-_{\text{sym.}})$  is a measure of the Cr-O bond strength ( $\Delta\nu_{200}$  confirms that the Cr-O bond is a strong one).

Since there is no change in the position of symmetric and asymmetric stretching vibrations of the C-S bond, it would suggest that sulphur in the thioether group is not combined with chromium. The bands in spectrum of the complex  $\text{Na}[\text{Cr}(\text{Cys})_2] \cdot 2\text{H}_2\text{O}$  were also clearly changed as compared with the cysteine spectrum. In the complex spectrum, there is no band corresponding to stretching vibration  $V(\text{S}-\text{H})$ , and the band of stretching vibration  $V(\text{C}-\text{S})$  is in the complex at  $670\text{ cm}^{-1}$ , which show that the sulphhydryl group can take part in the combination with metal. The characteristic band for the stretching vibration  $V(\text{C}-\text{O})$   $1740\text{ cm}^{-1}$  in the undissociated carboxylic group was shifted in the complex to  $1640\text{ cm}^{-1}$ , which show that the carboxylic group takes part in the combination with Cr(III). The possibility of such a link is supported by the shift of the stretching vibration  $V(\text{NH}_2)$  of amine group to  $3120\text{ cm}^{-1}$ .

also by the formation of the bending vibration  $\delta(\text{NH}_2)$  at  $1560 \text{ cm}^{-1}$ , which is a characteristic feature for the contribution of nitrogen of amine group in the bond of metal ion [21].

The electronic spectrum of the complex  $\text{Na}[\text{Cr}(\text{cys})_2] \cdot 2\text{H}_2\text{O}$  show 4 characteristic absorption bands 467, 408, 541, 606 nm (Tab. 4). Bands 467 and 541 nm result from the division of bands 408 and 606. Such a division of bands characteristic for  $\text{Cr}(\text{III})$  and  $\text{Co}(\text{III})$  complexes with aminoacids [22] which can form two geometric isomers mer (cis, trans) and fac(cis, cis) due to the position of bonds N-metal and O-metal. It has been found [22] that some sort of division in the bands of electronic spectrum takes place in the isomer mer(cis, trans) which is a complex of violet colour and is referred to as form  $\alpha$ . The isomer fac(cis, cis) is red in colour and presents form  $\beta$ . The  $\text{Cr}(\text{III})$  complex obtained with cysteine is violet in colour, being an  $\alpha$  form. The electronic spectrum of this complex shows a division of the bands. The solution of the complex  $\text{Na}[\text{Cr}(\text{Cys})_2] \cdot 2\text{H}_2\text{O}$ , in an acidic medium, changes its colour from violet to red and has only two bands 394 and 529 nm in its absorption spectrum. The spectrum of the complex solution following neutralisation shows also two bands 398 and 571 nm. This solution is violet in colour. The spectral analysis shows that the complex  $\text{Na}[\text{Cr}(\text{Cys})_2] \cdot 2\text{H}_2\text{O}$  in neutral and alkaline solutions assumes the form  $\alpha$ , but in acidic solution - the form  $\beta$ . The transition process from  $\alpha$  to  $\beta$  form is not a reversible one.

The reaction mixture, from which complex  $\text{Na}[\text{Cr}(\text{Cys})_2] \cdot 2\text{H}_2\text{O}$  was prepared, was separated in a chromatographic column filled with an ion-exchange resin (Sephadex A-25). By elution with 1-10% NaCl solutions, three fractions were separated: one of red colour which was not identified, and two of blue colour. The first blue fraction was identified as  $[\text{Cr}(\text{H}_2\text{O})_6]^{+3}$ , while the second one had a spectrum analogous to that of  $\text{Na}[\text{Cr}(\text{Cys})_2] \cdot 2\text{H}_2\text{O}$  in the  $\alpha$  form.

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# KOMPLEKSY Cr(III) Z DL-METIONINĄ I L-CYSTEINĄ

Kompleksy Cr(III) z DL-metioniną i L-cysteiną badano w roztworach wodnych metodami pH-metryczną i spektrofotometryczną. W obu badanych układach stwierdzono tworzenie się po 3 kompleksów o składzie Cr(III) : L = 1 : 1; 1 : 2; oraz 1 : 3. Metodą Bjerruma określono wartości liczbowe stałych trwałości wszystkich tworzących się kompleksów w roztworach. W stanie krystalicznym otrzymano kompleksy o składzie:  $[Cr(Met)_3] \cdot 2H_2O$  oraz  $Na[Cr(Cys)_2] \cdot 2H_2O$ . W oparciu o wykonane widma IR zaproponowano budowę strukturalną otrzymanych połączeń. Wykazano dużą przydatność metody wymiany jonowej na żywicy Sephadex A-25 do wydzielania w stanie czystym kompleksów chromu (III) z tioamino-kwasami.

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# КОМПЛЕКСЫ Cr(III) С DL-МЕТИОНИНОМ И L-ЦИСТЕИНОМ

Комплексы Cr(III) с DL-метионином и L-цистеином исследовано в водных растворах потенциометрическим и спектрофотометрическим методом. В исследованных системах наступает образование комплексов Cr(III) - лиганд, 1 : 1; 1 : 2; 1 : 3.

Методом Берума определено константы устойчивости всех комплексов в растворе. В кристаллическом виде мы оформили комплексы:  $[Cr(Met)_3] \cdot 2H_2O$  и  $Na[Cr(Cys)_2] \cdot 2H_2O$ . На основе элементарного анализа и спектров IR мы определили их формулу. Методом ионного обмена на смоле Sepadex A-25 мы разделили комплексы Cr(III) с тиаминокислотами.