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Biotechnology

Results of Physico-Chemical Study of Chelate-Type Compounds with Mixed Ligands

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ABSTRACT. The results of the study of physicochemical properties of chelate-type compounds with mixed ligands are presented. The formula for these compounds is $M \cdot gl \cdot L \cdot nH_2O$ (where: M = Mn, Zn, Fe, Co, Cu; gl is the glutamic acid molecule, L^{-2} citrate ion). Study of qualitative dissolubility of the above referred compounds in various solvents proved that these compounds are not soluble in water at ambient temperature, while at heating their dissolution capacity increases. In organic solvents (alcohol, acetone, dimethylformamide) they reveal low solubility indices. Thermographic study of the synthesized compounds show that these compounds are rather stable thermally and decomposition of water-containing compounds commences at a relatively low temperature (75-190^{0}C), while waterless compounds decompose at higher temperature (205-240^{0}C). Decomposition of these compounds proceeds gradually in three stages: I. detachment of water molecules; II. gradual oxidation of glutaminic acid; and III. citrate ion oxidation. The final product of thermolysis is metal oxide or a mix of metal oxide and coal. Study of IR absorption spectra of the compounds showed that neutral molecules of glutaminic acid coordinate with complexforming metals through carbonyl group oxygen atoms and amino group nitrogen atoms that lead to the formation of five-member metal-cycles. In case of the citric acid molecule, two of three carboxyl groups suffer deprotonation, and by means of oxygen atoms of this deprotonated carboxyl group they form bond with metal atoms. Premixes prepared on the base of a chelate-type compound with mixed ligands were tested in rabbit feeding and the relevant formulas were developed. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: microelement, chelate, mixed-ligand, solubility, thermographic study, citrate-ion, glutaminic acid, IR spectra

Microelements, the so-called “vital elements”, play substantial role in physiological and biological processes going on in poultry and animal organisms. One of the main reasons, determining low quantitative and qualitative indices of food products is deficiency of microelements in agricultural animals and poultry. The decisive role in the resolution of this problem is attributed to the provision of live organisms with optimal quantity and ratio of microelements. Since microelements fulfill their functions in living organisms in the form of chelate compounds, when the micronutrient deficiency is filled with chelated forms, the biological activity of animals and poultry increases dramatically [1-6].

The role of microelements, amino acids and hydroxy acids for normal growth and development of poultry and animals, as well as in metabolic processes occurring in their body is well-known [5-9]. Therefore, it is important to determine the methods of synthesis of chelate compounds which will contain such microelements, study of physical-chemical properties of the synthesized compounds and determination of efficiency of application of admixes for poultry and animal nutrition made on their base is most important.

Studies are in progress at the Laboratory of Problems of Agrarian Chemistry (Institute of Physical and Organic Chemistry, Tbilisi State University) for the creation of new generation premixes and their testing in experiments [7-13].

Applied Methods

The structure of chelate compounds synthesized by us was determined by microelement analysis. Identity was determined by their melting points and by X-Ray diffraction method.

Qualitative dissolubility of the synthesized compounds in various solvents was determined by trivial methods.

Thermal stability of synthesized chelate compounds was determined on the F.Paulik, J.Paulik, L.Erdey-derivatograph of Hungarian company, under atmospheric conditions

The nature of the citrate and glutamic acid ion in mixed-ligand chelate compounds, as well as the order of their binding to metals and the character of the coexistence of the two ligands, were studied on a Varian CARFY100 spectrophotometer,

Results and Discusion

In the framework of the above studies, we have synthesized $M\cdot gl\cdot L\cdot nH_2O$ type mixed-ligand chelate compounds and determined their composition by microelement analysis. The identity of these compounds was determined from their melting points and the X-ray diffraction method. Besides, their crystalline structure was determined and Roentgen-amorphous and iso-structural series were revealed. Premixes were made on the basis of the synthesized compounds and were tested in rabbit nutrition. Optimal composition formulation was drawn on the base of experimental data [11-12].

Physico-chemical studies of compounds of the type $M\cdot gl\cdot L\cdot nH_2O$ continued to determine their qualitative solubility in various solvents (water, alcohol, acetone, dimethylformamide). The conducted studies showed that they are dissolved in water at heating, while in other solvents they are characterized by low solubility or insolubility both at room temperature and at heating.

For the study of thermal stability of the synthesized chelate compounds and the progress of the process of thermolysis the thermographic investigation was performed in terms of TG=100 mg., T=7000C, DTA=DTG=1/5 sample heating speed 10 degree/min. (Table 1).

IR absorption spectra of synthesized chelate compounds - $Mn\cdot gl\cdot L$; $Zn\cdot gl\cdot L$; $Fe\cdot gl\cdot L\cdot 1.5\ H_2O$; $Cu\cdot gl\cdot L\cdot H_2O$; $Co\cdot gl\cdot L\cdot 1.5H_2O$ were recorded in the range of 400-4000cm-1. Samples for analysis were as a suspension of fine powder in liquid paraffin. The results of the measurement are given in Tables 2, 3.

As is seen from thermographic study all thermograms are characterized by some endo- and exo effects and corresponding effects on the curve (Table 1).

Analysis of thermogravigram of chelate compound $Mn\cdot gl\cdot L$ shows that detachment of 0.225 mole glutaminic acid (mass loss: practical 8.40%, theoretical 8.40%) corresponds to the first endoeffect (185^0C); at the next endoeffect (200^0C) 0.29 mole glutaminic

Table 1.

Results of thermographic studies of $M\cdot gl\cdot L\cdot nH_2O$ type mixed-ligand chelates

#	formula	T^0 C	mass loss, %		detached molecule, mole	solid decomposition product
			practical	theoretical		
1	$Mn\cdot gl\cdot L$	205	8.40	8.40	0.225 gI	$Mn\cdot gl_{0.775}\cdot L$
		200	9.10	8.39	0.29 gI	$Mn\cdot gl_{0.485}\cdot L$
		270	20.50	24.66	0.4656gI	$Mn\cdot gl_{0.0194}\cdot L$
		420	70.30	70.30	0.0194gI+L	Mn_3O_4.
2	$Zn\cdot gl\cdot L$	50	1.1	-	0.25 H_2O	$Zn\cdot gl\cdot L$
		240	16.91	16.91	0.46 gI	$Zn\cdot gl_{0.54}\cdot L$
		310	26.55	23.94	0.54gI	$Zn\cdot L$
		475	67,47	66.68	L	ZnO
3	$Fe\cdot gl\cdot L\cdot 1{,}5H_2O$	75	2.5	2.13	0.5 H_2O	$Fe\cdot gl\cdot L\cdot H_2O$
		120	4.1	4.36	H_2O	$Fe\cdot gl\cdot L$
		165	17.9	17.9	0.5gI	$Fe\cdot gl_{0.5}\cdot L$
		370	53.08	53.6	0.5gI+0.53L	$Fe\cdot L_{0.47}$
		475	42.42	41.36	0.47 L	Fe_3O_4+ C
4	$Cu\cdot gl\cdot L\cdot H_2O$	190	3.7	4..3	H_2O	$Cu\cdot gl\cdot L$
		260	3.9	3.9	0.11gI	$Cu\cdot gl_{0.89}\cdot L$
		350	57.9	58.89	0.89gI+L	CuO+ C
5	$Co\cdot gl\cdot L\cdot 1{,}5H_2O$	175	6.4	6.35	1,5 H_2O	$Co\cdot gl\cdot L$
		210	13.6	13.6	0.37gI	$Co\cdot gl_{0.63}\cdot L$
		280	22.1	27.04	0.63gI	$Co\cdot L$
		460	69.59	69.79	L	Co_3O_4

acid is detached (mass loss: practical 9.10%, theoretical 9.20%). Analysis shows that on the next endoeffect (270^0C) at the complete oxidation of the remaining 0.485 mole glutaminic acid the mass loss theoretically equals to 24.66%, while practical mass loss equals to 20.5%. Difference between the theoretical and practical data (4%) which is greater than the permitted one can be explained by the fact that at this temperature and at this endoeffect, glutaminic acid is not oxidized fully (0.0194 mole or 3.24g.) and remains unburnt. At 420^0C the complete oxidation of citrateion (mass loss: practical 70.30%, theoretical 70.92%) corresponds to the powerful exoeffect. The end product of thermolysis is metal oxide Mn_3O_4.

According to the analysis of $Zn\cdot gl\cdot L$ thermogram removal of moisture corresponds to the endoeffect at 50^0C (mass loss: practical 1.10%), which amounts to 0.25 mole H_2O. At the following endoeffect (200^0C) we observe partial oxidation of glutaminic acid (0.46mole) (mass loss: practical 16.91%, theoretical 16.95%), while practical mass loss of the endoeffect corresponding to 310^0C-temperature (26.55%) exceeds approximately by 2.61% of the theoretical loss (23.94%). This can be explained by the fact that complete oxidation of the remaining mass of glutaminic acid is accompanied by the commencement of citrate ion oxidation, which is completed at 475^0C, at the powerful exoeffect (mass loss: practical 67.47%, theoretical 66.68%) and the end product of thermolysis, as in the previous case is metal oxide ZnO.

Thermal decomposition of chelate compounds $Fe\cdot gl\cdot L\cdot 1{,}5H_2O$ and $Cu\cdot gl\cdot L\cdot H_2O$, as is seen from the Table proceeds gradually, by stages. At the endoeffects we observe gradual oxidation of water and glutaminic acid molecules, but at the powerful exoeffect we fix citrate-ion oxidation. The end product, in distinct from other compounds, is a mix of metal oxides and coal (Table 1).

Thermal decomposition of the chelate compound $Co\cdot gl\cdot L\cdot 1{,}5H_2O$ takes place analogously to the thermal decomposition of the (1) compound. In particular, the remaining 0.63 mole glutaminic acid can't manage to burn at the 210^0C at the endoeffect corresponding to the 280^0C, which is proved by the difference

Table 2.

Citrateion oscillation frequencies (cm^{-1}) found in IR adsorption spectra of $M\cdot gl\cdot L\cdot nH_2O$ type mixed ligand chelates

N	Compound	ν (OH) cm^{-1}	ν (C=O) cm^{-1}	$\nu(COO^-)cm^{-1}$	attribution
1	$Na_3C_6H_5OH$	3450 1240,1200,1105,1125, 1050,900,880,830		1580 1400-1320	ν (OH) δ(OH) ν_{as} (COO$^-$) ν_s (COO$^-$)
2	$Co\cdot gl\cdot L\cdot 1,5H_2O$	3479-3394 1257,1195,1157,1126, 1072,941,871,802	1735	1612	ν (OH) δ(OH) ν(COOH) ν_{as} (COO$^-$)
3	$Mn\cdot gl\cdot L$	3479-3394 1257,1195,1149,1126, 1072,941,887,802	1728	1589	ν (OH) δ(OH) ν(COOH) ν_{as} (COO$^-$)
4	$Zn\cdot gl\cdot L$	3394 1257,1218,1149,1126, 1079,1049,941,918 864,810	1705	1635	ν (OH) δ(OH) ν(COOH) ν_{as} (COO$^-$)
5	$Fe\cdot gl\cdot L\cdot 1,5H_2O$	3371 1226,1080,933,848, 802	1720	1589	ν (OH) δ(OH) ν(COOH) ν_{as} (COO$^-$)
6	$Cu\cdot gl\cdot L\cdot H_2O$	3571,3479,3394, 1257,1234,1195,1157 1126,1079,972,918,864	1735	1595	ν (OH) δ(OH) ν(COOH) ν_{as} (COO$^-$)

between the practical and theoretical (27.04%) mass losses at this endoeffect (22.10%) that exceeds the permissible value (5%). 0.0315 mole (4.65g.) glutaminic acid that is not burnt is oxidized at the powerful exoeffect (460^{0}C) together with the citrate ion and the end product of thermolysis in this case is the metal oxide Co_3O_4.

As to the spectrophotometric study of the synthesized compounds, the specter of citric acid (H_4L) show non-dissociated carboxyl group C=O valence oscillation bands in 1750-1720cm^{-1} zone, while non-dissociated oxy groups (OH) valence oscillation

Table 3.

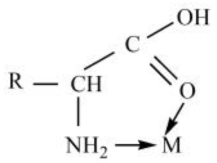
Oscillation frequencies (cm^{-1}) of glutaminic acid NH_2 and COOH groups found in IR spectra of M·gl·L·nH_2O type mixed-ligand chelates

gl	Co·gl·L·1,5H_2O	Mn·gl·L	Zn·gl·L	Fe·gl·L·1,5H_2O	Cu·gl·L·H_2O	attribution
3420	3395	3390	3394	3371	3360	ν_{as} (NH_2)
3320	3290	3250	3260	3230	3240	ν_s (NH_2)
1635	1612	1590	1560	1600	1610	δ(NH_2)
1560	1550	1520	1512	1550	1558	ν_{as} (COOH)
1420	1345	1345	1335	1340	1330	ν_s (CO)
1340	1257	1257	1257	1230	1234	δ(C=O)
-	570	520	560	550	570	ν(M-N)
-	440	470	480	460	440	ν(M-O)

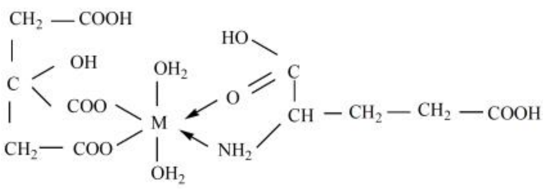
bands are observed in 3450cm^{-1} and other bands, which are associated with deformation oscillation of oxy groups (1290, 1205, 1165, 1125, 1050, 925, 880, 865, 825cm^{-1}) [14,15].

In the compounds the glutaminic acid (gl) can play the role of monodentant (through COO^- group oxygen) as well as bidentant ligand through the same oxygen and NH_2 group nitrogen atoms. It is also known that amino acids, depending on reaction conditions, forming zwitterions (NH_3^+-RCH-COO^-) tend to form linkage with metal through carboxyl group oxygen and NH_3^+ hydrogen (hydrogen bond) [16].

Tables 2 and 3 show attribution of some oscillation frequencies of IR spectra of the investigated compounds. For the purposes of comparison we offer spectral data of sodium citrate. We do not observe clearly expressed adsorption band characteristic of oxy group at 3450cm^{-1} in the IR absorption spectra of the compounds; instead, we fix appearance of wide absorption spectra in 3600-300cm^{-1} zone, which can be attributed to glutaminic acid S_{as} (NH_2) and S_s(NH_2) oscillations. Complex nature of the absorption band is conditioned by superimposition of the above mentioned absorption spectra of glutaminic acid, citrate ion and water and by the formation of hydrogen bonds between these ligands. Absorption bands in 1560-1610cm^{-1} zone are attributed to glutaminic acid ґ (NH_2). Absorption spectra at 520-570cm^{-1} and 440-480cm^{-1} can be attributed to valence oscillations of metal-nitrogen and metal-oxygen bonds. Therefore, we can suppose that glutaminic acid plays the role of a cyclic ligand and realizes this bondage to metal by means of amino group nitrogen atoms and carbonyl group C=O oxygen atoms by the formation of five- member metal-cycles, which schematically is expressed as follows:



On the basis of the analysis of IR absorption spectra we can suppose that citric acid in chelate compounds plays the role of a bibasic acid, fulfilling the function of a bidentant ligand by means of oxygen atoms of two deprotonated carboxyl groups, by the formation of octahedral configurations around metal atoms. Formation of the five-member metal-cycles and hydrogen bond systems refers to the stability of the structures of chelate compounds (Chugaev's "cycle rule"). Two water molecules are participating in filling up to the octahedral configuration. (possibly other geometrical configurations too). Possible octahedral coordination of M·gl·L·nH_2O type compounds can be presented schematically as follows:



Conclusion

The present study allows us to make the following conclusions:

- $M\cdot gl\cdot L\cdot nH_2O$ type mixed-ligand chelate compounds are dissolved in water at heating, while in other solvents (alcohol, acetone dimethyl formamide) they show low solubility or insolubility, both at the ambient temperature and at heating.
- Thermal decomposition of compounds proceeds in stages. In particular: I–water molecules are detached; II–glutaminic acid is gradually oxidized; III-citrate ion is oxidized. The end products of thermolysis are metal oxide or a mix of metal oxide and coal.
- In the synthesized compounds glutaminic acid molecules playing the role of bidentant ligand coordinate with complex-forming metals by means of carbonyl group oxygen and amino group nitrogen atoms by the formation of five-member metal-cycles.
- Citric acid plays the role of a bibasic ligand (at the expense of deprotonation of two carboxyl groups) and the formed citrate-ions (L^{-2}) coordinate with complex-forming metals through oxygen atoms of these groups.

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ბიოტექნოლოგია

შერეულლიგანდიანი ხელატური ტიპის ნაერთების ფიზიკურ-ქიმიური კვლევის შედეგები

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[*] ივანე ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი, პეტრე მელიქიშვილის ფიზიკური და ორგანული ქიმიის ინსტიტუტი თბილისი, საქართველო
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(წარმოდგენილია აკადემიის წევრის გ.კვესიტაძის მიერ)

შერეულლიგანდიანი ხელატური ტიპის ნერთების $M\cdot gl\cdot L\cdot nH_2O$ (სადაც: M=Mn,Zn,Fe,Co,Cu; gl-გლუტამინის მჟავას მოლეკულაა, L^{-2}-ციტრატ-იონი) საფუძველზე მომზადებული პრემიქსები გამოცდილია ბოცვრის კვებაში და შედგენილია რეცეპტები. მიღებული შედეგებიდან გამომდინარე, მიზანშეწონილად მივიჩნიეთ აღნიშნული შერეულლიგანდიანი ხელატური ნაერთების ფიზიკურ-ქიმიური თვისებების უფრო ღრმა კვლევა. კერძოდ, თვისობრივი ხსნადობის შესწავლით სხვადასხვა გამხსნელში დადგენილია, რომ ისინი წყალში ცივად არ იხსნებიან, ხოლო გაცხელებით ხსნადობა იზრდება. ორგანულ გამხსნელებში (სპირტი, აცეტონი, დიმეთილფორმამიდი) ხასიათდებიან მცირე

ხსნადობით. სინთეზირებული ნაერთების თერმოგრაფიული კვლევის საფუძველზე დადგენილია, რომ ისინი თერმულად საკმაოდ მდგრადები არიან და წყლის შემცველი ნაერთების დაშლა იწყება შედარებით დაბალ ტემპერატურაზე (75-190°C), ხოლო უწყლოსი - მაღალ ტემპერტურაზე (205-240°C). მათი დაშლა მიმდინარეობს თანდათანობით შემდეგი საფეხურების მიხედვით: I - წყდება წყლის მოლეკულები, II - ხდება გლუტამინის მჟავას თანდათანობითი დაჟანგვა და ბოლოს III-ადგილი აქვს ციტრატ-იონის დაჟანგვას. თერმოლიზის საბოლოო პროდუქტია ლითონის ოქსიდი ან ლითონის ოქსიდი და ნახშირის ნარევი. ნაერთების ი.წ. შთანთქმის სპექტრების შესწავლის საფუძველზე დადგენილია, რომ გლუტამინის მჟავას ნეიტრალური მოლეკულების კომპლექსწარმომქმნელ ლითონებთან კოორდინირება ხდება კარბონილის ჯგუფის ჟანგბადისა და ამინო-ჯგუფის აზოტის ატომების მეშვეობით ხუთწევრიანი ლითონო-ციკლების წარმოქმნით; ხოლო ლიმონის მჟავას მოლეკულაში სამი კარბოქსილის ჯგუფიდან დეპროტონირდება - ორი. ამ დეპროტონირებული კარბოქსილის ჯგუფის ჟანგბადატომების საშუალებით ისინი წარმოქმნიან ბმას ლითონის ატომებთან.

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