



(RESEARCH ARTICLE)



Synthesis and study of different-ligand chelates

Iamze Beshkenadze ¹, Nazibrola Klarjeishvili ^{1,*}, Nino Nonikashvili ¹, Dali Dzamashvili ² and Maia Gogaladze ^{1,3}

¹ *Petre Melikishvili Institute of Physical and Organic Chemistry, Iv. Javakhishvili Tbilisi State University, Tbilisi, Georgia.*

² *Rafiel Agladze Institute of Inorganic Chemistry and Electrochemistry, Iv. Javakhishvili Tbilisi State University, Tbilisi, Georgi.*

³ *Department of Food Technology, Georgian Technical University, Tbilisi, Georgia.*

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Abstract

Synthesis conditions have been established and different-ligand chelates have been synthesized with a general formula: [MArg]Hl·nH₂O, where M are bivalent metal ions M = Mn, Zn, Fe, Arg – amino acid arginine, Hl – citric acid bivalent anion, n – number of water molecules. The synthesized compounds have been studied using a number of physical and chemical methods:

composition – using trace element analysis, while individuality – by the methods of melting temperature measurement and X-ray-diffractometric study.

Chelate dissociation constants and determination coefficients have been measured by the conductometric research method. Qualitative solubility of chelates in different solvents has been established.

In order to study biological activity of the synthesized chelates, based on the experiments conducted on broilers it has been established that introduction of the mixture of microelement chelates into composition of broilers' combined feed premixes has had a positive effect on live weight gain and survival rate of birds.

Keywords: Chelate; Ligand; Microelement; Premix; Broiler Poultry

1. Introduction

Nowadays, population supply with a safe, high-quality agricultural production (poultry and animal meat) and improvement of environmental state is one of the urgent problems. Significant role in solution of this problem is devoted to so-called essential microelements, since the microelements take active part in normal growth and development of living organisms. That is why, their deficiency or abundancy in organism leads to disturbance of vital processes and a number of pathologies. Microelements' composition in the living organisms varies within limits of 10⁻³-10⁻⁵ %. All pathological processes taking place in the organisms of agricultural animals and poultry are underlaid by disturbance in metabolism, while metabolic disorder itself is mainly reasoned by undernutrition (malnutrition) of poultry and animals, in particular, by insufficient intake of proteins, fats, hydrocarbons, vitamins, micro- and macroelements with food. At that, it is known that indispensable microelements generally perform their functions in living organisms (including poultry and animals) in the form of coordination (chelate) compounds, concentration of which is strictly controlled and their deviance from a norm causes disturbance of physiological processes in the organism and a number of pathologies. Namely this factor predetermined the necessity of their introduction into premixes in chelate form. This is confirmed by the results of experiments carried-out by foreign and local scientists over the years [1-10]. Microelements perform diverse functions in the living organisms, in particular:

* Corresponding author: Nazibrola Klarjeishvili

- Participate in musculoskeletal system tissue building
- Participate in preservation of internal environment homeostasis
- Provide cellular membrane preservation
- Provide activation of biochemical reactions by an action on the enzyme system
- Have direct or indirect effect on the endocrine glands' function
- Act on the gastrointestinal tract symbiotic microflora

Manganese, zinc, and iron are selected among microelements as the study objects. Among amino acids, arginine is a basic amino acid, it represents nitrogen oxide donor, which promotes relaxation and elasticity of blood vessel system. This fact is of great importance for the treatment process of number of diseases (heart and blood vessel system, brain, immune and nervous system, atherosclerosis, genital system etc.). According to new studies, arginine consumption in excessive amounts by the immune cells (which protect brain) is a reason of contraction of Alzheimer disease [11-16].

Tribasic oxy-acid citric acid is selected as an acid ligand. By means of carboxyl and hydroxyl groups, it forms the stable homo- and heteronuclear coordination compounds with metals (citrates). Citric acid is an important product of metabolism in the living organisms. While participating in the tribasic acid and glyoxylate cycles, it is a key link of the system of cell respiration biochemical reactions. Addition of coordination (chelate) compounds of homo- and heteronuclear citrates obtained on the basis of citric acid to the poultry diet promotes increase in birds' productivity, food compensation, improvement of poultry meat and bone tissue quality, and enzyme activation [17-20].

Taking the above-mentioned factors into account, our scientific team has established the synthesis conditions and has synthesized arginine-containing manganese, zinc and iron citrates. Study of physical-chemical and biological activity of the synthesized compounds has been conducted.

2. Materials and used methods

Manganese acetate, Zinc oxide, Iron acetate, oxy-acid – Citric acid, and amino acid – Arginine have been used in the synthesis of chelate compounds. The mentioned reagents of Sigma-Aldrich brand have been purchased. Vitaminic and mineral premix and broiler chickens have been used for study of biological activity. The following methods have been applied:

- Trace element analysis – for establishment of chelate compounds' composition;
- Melting temperature measurement and x-ray-diffractometric study – for establishment of chelates' individuality;
- Solubility – in order to study the qualitative solubility of compounds in different solvents;
- Conductometric study – in order to determine dissociation constant of solutions containing chelate compounds;
- Weighing method – in order to establish broiler's weight gain;
- Count method – in order to establish broiler's survival rate;
- Method of recording of the consumed feed – in order to determine feed consumption and conversion.

3. Results and analysis

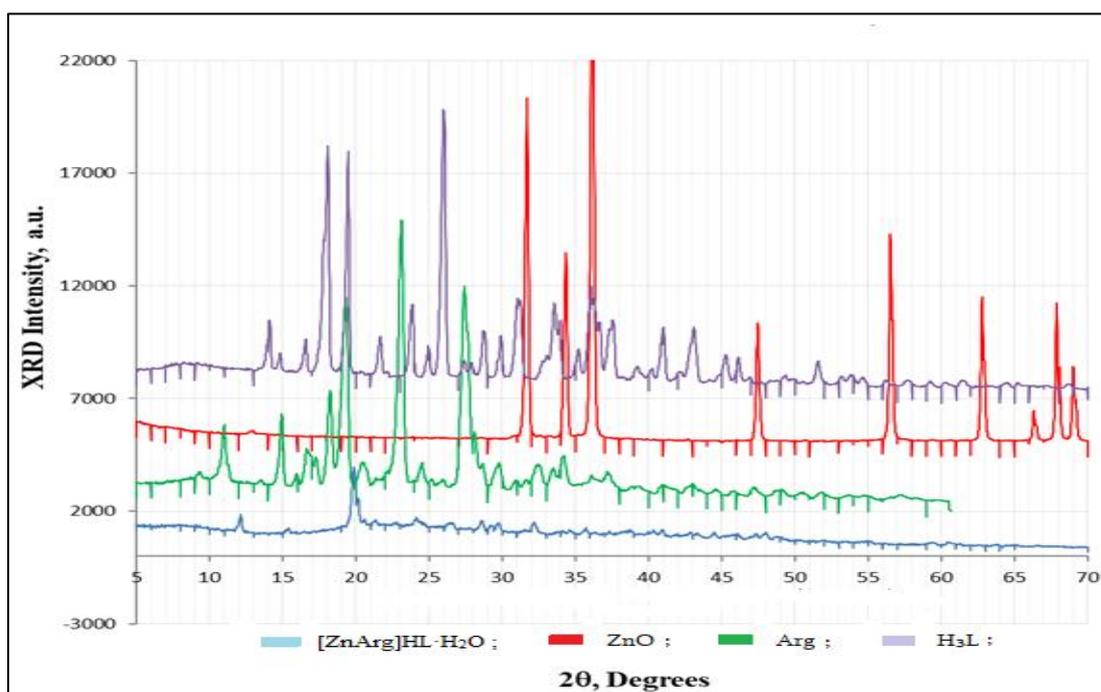
We have developed the synthesis method and have synthesized different-ligand chelates. Microelements (Mn, Zn, Fe), arginine, and citric acid in molar ratio 1:1:1 and 1:2:1, respectively, have been taken for synthesis. The reagents taken have been dissolved in the minimum water volume, in the neutral medium, under conditions of vigorous mixing and heating, through further concentration on the water bath. The individuality of synthesized chelates has been established via measurement of melting temperature using the apparatus – melting point /SMP10/. Chelates are easily fusible compounds and melt within temperature limits of 75-143°C. Qualitative solubility of compounds in different solvents has been determined as well, according to which they are distinguished by high solubility in water, and by poor solubility in alcohol, acetone and dimethylsulfoxide (Table 1).

Table 1 Some physico-chemical characteristics of metal citrates containing arginine

#	The formula of the Compound	Mol Mass	Melting t Oc	Humidity B (%)	Solubility				Conductometric Survey Results	
					Water	Alcohol	Acetone	Dmf*	R2	pKa
1	[MnArg]HL·2H ₂ O	455.5	130	1.49	+	sl. sol.	sl. sol.	sl. sol.	0.84	4.24
2	[MnArg ₂]HL·2H ₂ O	630.12	118	1.97	+	sl. sol.	sl. sol.	sl. sol.	0.92	4.43
3	[ZnArg]HL·H ₂ O	448.02	100	0.46	+	-	-	-	0.79	4.29
4	[ZnArg ₂]HL·H ₂ O	621.77	75	0.95	+	-	-	-	0.81	4.17
5	[FeArg]HL·4H ₂ O	492.67	143	0.65	+	-	-	-	0.73	3.61
6	[FeArg ₂]HL·4H ₂ O	667.15	116	0.89	+	-	-	-	0.75	3.51

+ Soluble, -Insoluble, sl. sol Slightly soluble

In order to determine the dissociation constant of arginine-containing chelate citrates, a conductometric study has been conducted using the device Ph and Conductivity Sensor LE703. For this purpose, the solutions of molar concentration with concentration limits from 0.0007 to 0.00002M have been prepared for compounds. Experiment has been conducted in the thermostat at 25°C. Experimental results are given in Table 1. R² – regression assessment indicator, which shows how the experimental data are close to the respective function of the graph, it varies within limits of 0.73-0,92. In our opinion, an increased numerical value of R² with 1:1 and 2:1 ratio of Arg:M is explained by formation of stable pentatomic (five-membered) cycles by one (or two) mole arginine atom around metal ions.

**Figure 1** Diffractogram of the compound [ZnArg]HL·H₂O

Humidity B has been determined on the analyzer AXIS ADGS50. It varies within the limits of 1.49-0.46%. In addition to melting temperature measurement, the individuality of compounds has been established using diffractometric method,

as well. X-ray-diffractometric study has been conducted using DPOH-4.07 with $\text{Cu}_{\text{K}\alpha}$ ($\lambda=0.154184$ nm) irradiation. During exposition, samples rotated in their own plane by means of the special device – ПП-13. For comparison, the diffractograms of initial compounds have been taken as well (Fig. 1-4).

As is seen from the figure, based on the diffractographic study of chelate compound $[\text{ZnArg}]\text{HL}\cdot\text{H}_2\text{O}$ (1), the compound doesn't include initial reacting substances (zinc oxide, arginine and citric acid). The diffractogram of the obtained compound is distinguished by diffraction maximums and intensities, peculiar to it, and differs from diffractographic pattern of initial reacting substances. From here, one can conclude that a new individual substance has been obtained.

The chelate compounds $[\text{MnArg}]\text{HL}\cdot 2\text{H}_2\text{O}$ (2), $[\text{FeArg}]\text{HL}\cdot 6\text{H}_2\text{O}$ (3) and $[\text{FeArg}_2]\text{HL}\cdot 4\text{H}_2\text{O}$ (4) are amorphous substances, while diffractograms of initial substances: manganese acetate, iron acetate, citric acid and arginine are characterized by diffraction maximums and intensities, peculiar to them. Thus, one can conclude that in all three cases, formation of new individual compounds takes place (Figure 2) (Figure 3) (Figure 4).

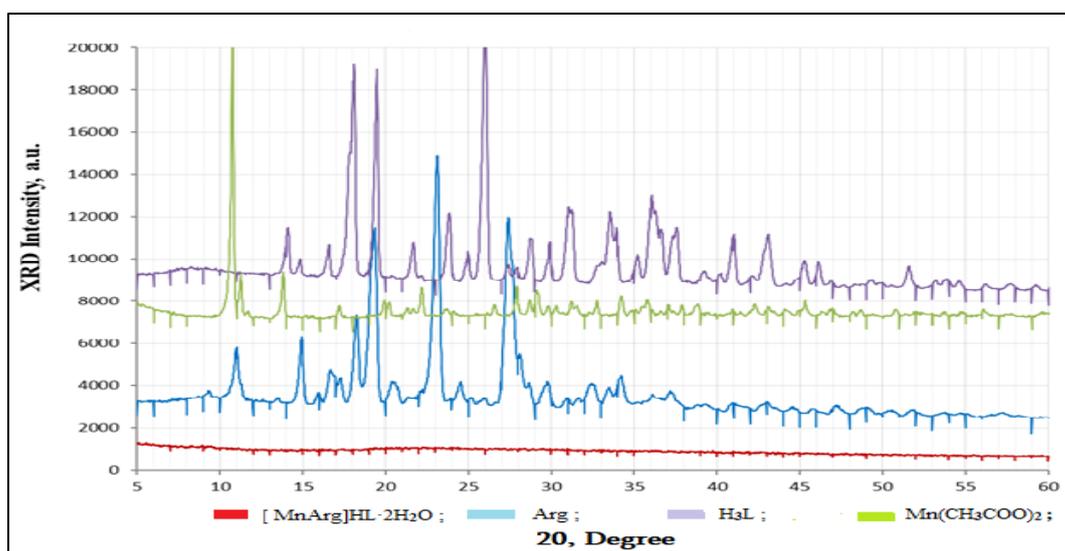


Figure 2 Diffractogram of the compound $[\text{MnArg}]\text{HL}\cdot 2\text{H}_2\text{O}$

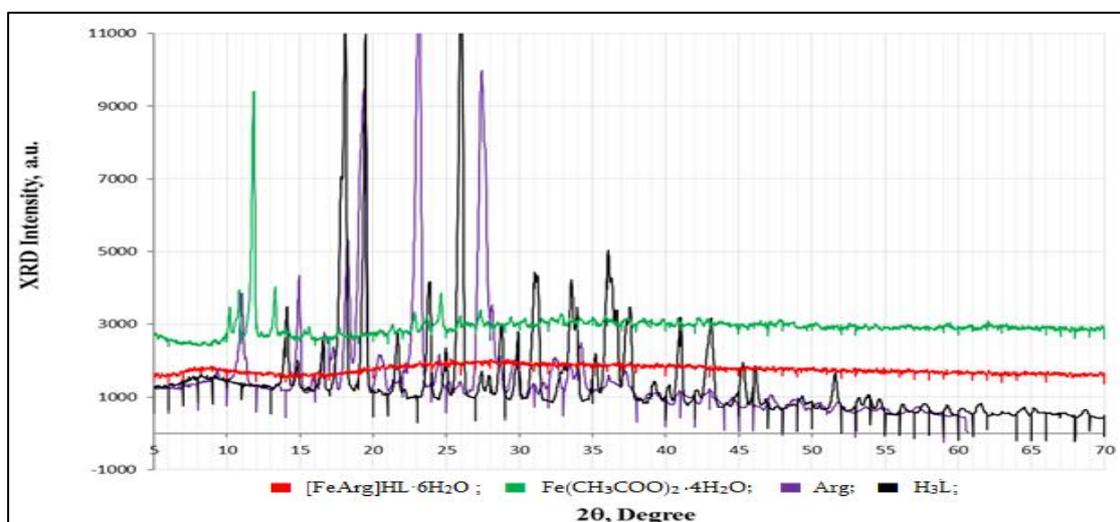


Figure 3 Diffractogram of the compound $[\text{FeArg}]\text{HL}\cdot 6\text{H}_2\text{O}$

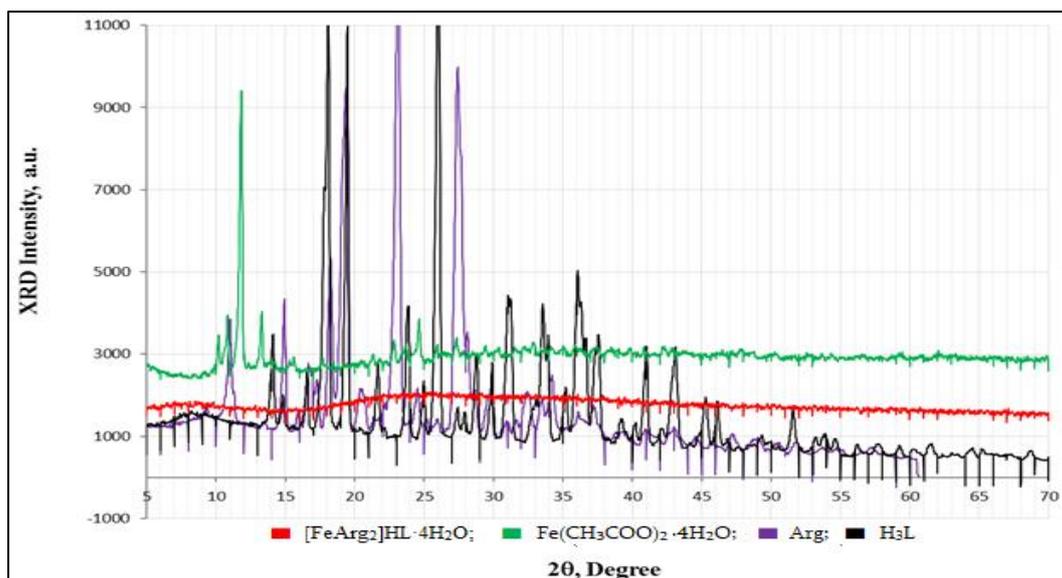


Figure 4 Diffractogram of the compound [FeArg₂]HL·4H₂O

With the purpose of studying biological activity of synthesized compounds, a pilot experiment on broilers has been conducted. Chelate mixtures for broiler's 100 gr combined feed premixes have been prepared (Table 2).

Table 2 Mixture composition

X Mixture	Xnorm(g)	Xmax(g)
[MnArg]HL·2H ₂ O	57.95	66.64
[ZnArg]H]·H ₂ O	37.91	43.60
[FeArg]·4H ₂ O	35.17	40.44

20 broilers each, for three groups: one control and two test have been selected for a test according to zootechnical analogy principle. Experiment was lasted for one month. During a test we have studied bird's live weight in the beginning and in the end of experiment, results of which are given in Table 3.

Table 3 Change in live weight during the experimental period

Groups	Changes in live weight (g)					
	Start the test			End of test		
	Mini	Max	M+m	Mini	Max	M+m
Control	1410	1570	1490±80	1425	1610	1517±92.5
Xnorm	1480	1640	1560±80	1540	1700	1620±80
Xmax	1510	1700	1595±95	1560	1770	1655±105

Bird's survival rate has been studied as well (Table 4), according to which this index is maximal in the test groups.

Table 4 Bird's survival rate during a test, %

Group	Number of birds,(wing)		Maintenance %
	Beginning of the experiment	End of experiment	
control	20	14	75
X norm	20	17	90
X max	20	20	100

4. Conclusions

Based on the results of carried-out experiment, the following conclusions can be made:

- Synthesis conditions have been established and arginine-containing manganese, zinc and iron citrates have been synthesized;
- The individuality, composition, solubility in different solvents, and dissociation constant have been determined;
- Results of experiments conducted in order to study the biological activity showed that an introduction of mixture of microelement chelates into composition of broiler's combined feed premixes has had a positive impact on live weight gain and bird's survival rate.

We are introducing for evaluation the manuscript of the research paper titled "The synthesis and study of different-ligand chelates" intended for publication in the International Journal of Science and Research Archive (IJSRA).

As is well known, production of ecologically safe, high-quality food products (mainly poultry and animal meat) is one of the most urgent problems. The significant role in solution of this problem is played by essential microelements, which can be explained by the important function performed by microelements in the living organisms. Based on this fact, the scientific team has synthesized different-ligand chelates of microelements: manganese, zinc and iron. Amino acid arginin has been selected as a ligand, while citric acid – as an acid ligand. Physical and chemical properties of the synthesized chelates have been studied. In order to study the biological activity, an experiment on broilers has been conducted.

Each author declares, that there is no conflict of interests regarding this paper (which is certified by authors' signatures and sent to you as an attached file). The described material is in the process of being published and is not being considered for publication elsewhere.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed. The authors of the manuscript no conflicts of interest have

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