



Some Cobalt (II) Amino acids Complexes Synthesis, Characterization, and Biological Activity

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Received: October 2018

Accepted: January 2019

Abstract:

Amino acid complexes of Co (II) with glycine, serine, arginine, and aspartic acid have been synthesized by mixing the salts with the amino acids in alkaline media, they were characterized by EDX, FTIR, UV/Vis, TGA, XRD, and electric conductivity measurements. The four ligands (Arg, Asp, Gly, Ser) were complexed with Co²⁺ ion and have been found to act as bidentate chelating agents coordinating through COO⁻ and NH₂. The antibacterial activity of amino acid complexes was evaluated against four bacteria strains, two kind of gram positive (*Staphylo coccus aureas*, *Entero coccus feacalis*), and two kind of gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*). The Co (II) complexes were found to have varied degree of inhibitory effect against bacteria.

المستخلص

تم تخليق اربعة معقدات احماض امينية من ايون الكوبالت ثنائي التكافؤ مع الارجنين وحمض الاسبارتيك والسيرين والجلالسين وتم التعرف عليهم بواسطة اجهزة التحليل المختلفة وهي جهاز مطيافية تشتت الطاقة بالاشعة السينية ومطيافية الاشعة تحت الحمراء ومطياف الاشعة المرئية وفوق البنفسجية والتحليل الوزني الحراري وحيود الاشعة السينية وقياس التوصيلية الكهربائية. وتم التوصل الي ان الاحماض الامينية الاربعة تعمل كعوامل مخلبية ثنائية التنسيق مع ايون الكوبالت . وتم تحديد النشاط الحيوي للمعقدات تجاه اربعة سلالات بكتيرية، نوعان من ايجابية الجرام ، ونوعان من سالبة الجرام ، وثبت انها لها القدرة على قتل البكتريا بدرجات متفاوتة.

Keywords: Cytotoxic, biotransfer, reoplastic.

Introduction

The microbial resistance represses challenging problem and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, it measures must be taken to reduce this problem, for example, by control of the use antibiotic, to conduct research attempt to oversee the genetic mechanisms of resistance, besides supporting research endeavors for synthesizing a isolating new drug from their natural sources, and to continue

studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and effective antimicrobial drugs to the patient. (Kabbani, *et al*, 2007) For centuries, people have used cobalt and other ions to inhibit the growth of harmful microbes. Coordination complexes of transition metals have been widely studied for their antibacterial, antifungal and potential cytotoxic chemotherapeutic agents. They have been evaluated against several

pathogenic fungi and bacteria with promising results. One of the approaches to enhance the efficacy of the drugs is based on their modification of their physical and chemical properties. In addition to strengthen ability to combat infection or neoplastic disease, These new drugs must exhibit selective toxicity towards microbes, chemical stability, and optimum rates of bio-transformation and elimination. (Johari, *et al*, 2009).

Experimental

Materials and methods

Chemicals and reagents:

All chemicals used in the synthesis are of analytical grad. They cobalt (II) chloride hexahydrate, sodium hydroxide, glycine, serine, arginine, aspartic acid, distilled water and deionized water, absolute ethanol, formic acid.

Instruments used: IR spectrometer (FTIR), UV/Vis- Spectrometer, Conduct meter, Thermo gravimetric analysis (TGA), X-Ray Diffraction (XRD), Energy dispersive X-ray (EDX).

Method

Synthesis of Cobalt amino acids complexes

For four amino acids complexes with cobalt (II) ions using as ligands arginine, aspartic acid, glycine, and serine (Co-Arg, Co-Asp, Co-Gly, and Co-Ser, respectively). The solid complexes, $ML_2.nH_2O$ type, were prepared following the procedure described in the literature, (Stanila, *et al*, 2007). 1:2 molar ratio (aqueous solution) of metal chlorides and ligands (glycine, serine, arginine, aspartic acid), the pH of solution was adjusted to about 8-10 using sodium hydroxide, the solutions were under stirring using magnetic stirrer about one hour at room temperature until precipitate was formed, filtered off, washed, dried and kept in a desiccator over anhydrous $CaCl_2$.

Characterization

IR spectra were recorded on Fourier-Transform (FT.IR) Spectrophotometer,

Tensor 27 Co.Brucker 2003 at a range (400-4000 cm^{-1}) using KBr discs, Electronic spectra were recorded on a U.V-Vis. Spectrophotometer (Shimadzu, UV-1650PC Spectrophotometer using formic acid as a solvent at room temperature. X-ray diffraction patterns were recorded on a Philips diffractometer with Cu-K α radiation. The scan range was between 3 and 80° 2 θ , TGA was carried out on a Perkin-Elmer model TGS-2 instrument, and EDX analysis were recorded by using LEOS430 scanning electron microscope coupled with energy dispersive X-ray analyzer model Oxford LINK ISIS.

Microbial strains

The amino acid complexes were tested against the following microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*. Antibacterial tests were then carried out by disc diffusion method. (Pal, *et al*, 2007), Bacterial strains were cultured overnight at 37°C in agar.

Result and Discussion:

Physical analysis:

The corresponding cobalt complexes, are soluble in formic acid but insoluble in THF, cyanomethane, benzene, dichloromethane, chloroform, DMSO, DMF+DMSO mixture and ethanol. Accordingly, the conductivity of the four complexes, were measured in 20% formic acid were nil and the results obtained, indicate their nonelectrolyte nature.

Energy dispersive x-ray spectra (PCEDX) study:

The PCEDX profile of Co.Gly, Co.Ser, Co.Asp, and Co.Arg complexes confirmed the presence of O, Co, C, N. The prominent nitrogen and oxygen clearly confirms the functional group of amino acid ligand and Co peak presence indicates formation of complexes. These results had been summarized in tables (1), (2), (3) and (4) for Co.Gly, Co.Ser, Co.Asp, and Co.Arg respectively.

Table (1) PCEDX reading of Co.Gly complex:

Element1	Result	Unit	δ3
O	39.18	%	0.215
Co	24.05	%	0.507
C	19.59	%	0.116
N	11.44	%	0.221
Trace element	5.584	%	0.320

Table (2) PCEDX reading of Co.Ser complex:

Element2	Result	Unit	δ3
O	39.50	%	0.265
C	23.96	%	0.264
Co	19.59	%	0.132
N	9.32	%	0.210
Trace element	4.519	%	0.130

Table (3) PCEDX reading of Co.Asp complex:

Element3	Result	Unit	δ3
O	44.62	%	0.283
C	26.77	%	0.109
Co	16.42	%	0.192
N	7.81	%	0.231
Trace element	4.378	%	0.145

Table (4) PCEDX reading of Co.Arg complex:

Element4	Result	Unit	δ3
C	33.86	%	0.292
N	26.341	%	0.108
O	22.58	%	0.211
Co	13.85	%	0.107
Trace element	3.369	%	0.110

Thermal Analysis:

Thermal Gravimetric Analysis (TGA) for Cobalt complexes were investigated and recorded weight loss per mg against the temperature. TGA curve for four complexes figure (1), (2), (3), and (4) displays three stages of mass loss within the temperature range of 100–500 °C. The first stage is at 120–180 °C, corresponding

to the dehydration of water of crystallization. The second stage occurs at around 200–320 °C, corresponding to the loss of the volatile gases from decomposition of amino acids ligand. The third stage occurs at the temperature above to 450 °C, notes the weight increases, that indicate the oxidation of cobalt ion to form cobalt oxide. These feature summarized in table (5).

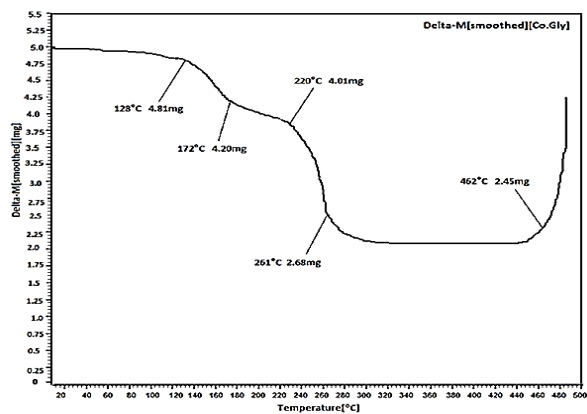


Figure (1) Thermal gravimetric curve of Co.Gly complex

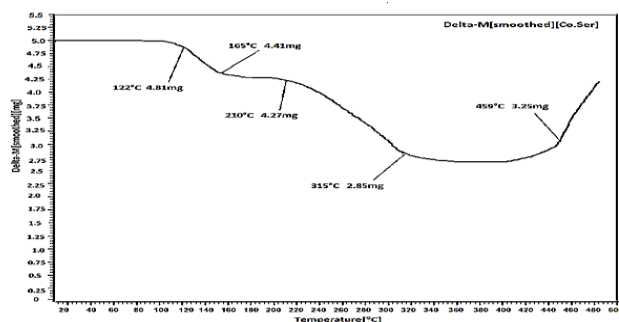


Figure (2) Thermal gravimetric curve of Co.Ser complex

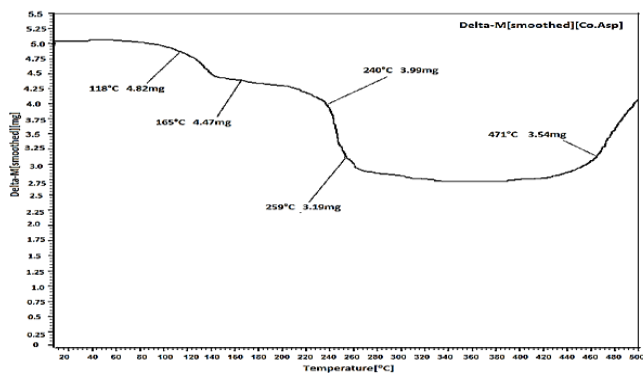


Figure (3) Thermal gravimetric curve of Co.Asp complex

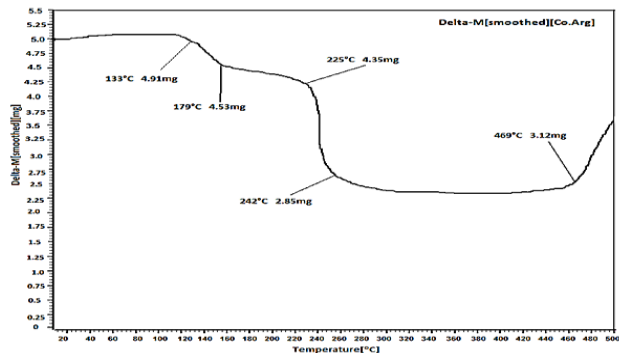


Figure (4) Thermal gravimetric curve of Co.Arg complex

Table (5) Weight loss per mg against to the temperature per (°C) reading of Co.Gly, Co.Ser, Co.Asp, and Co.Arg complexes:

Co.Gly		Co.Ser		Co.Asp		Co.Arg	
Temp(°C)	Weight loss(mg)	Temp(°C)	Weight loss(mg)	Temp(°C)	Weight loss(mg)	Temp(°C)	Weight loss(mg)
128	4.81	122	4.81	118	4.82	133	4.91
172	4.21	165	4.39	165	4.47	179	4.53
220	4.01	210	4.27	240	3.99	225	4.32
261	2.68	315	2.85	259	3.19	242	2.85
462	2.60	459	2.79	471	2.99	469	2.79

Infrared spectra

The IR spectra for Co-Gly, Co-Ser, Co-Asp, and Co-Arg complexes showed a difference between the vibrational frequencies $\nu_{as}(\text{Coo}^-)$ at 1600 cm^{-1} and $\nu_s(\text{Coo}^-)$ at 1400 cm^{-1} , generally increase from the theoretical values of free amino acid when the M-O bond strength depending on the carboxylate coordination, (Nagae, *et al*, 1994). Two very well resolved bands at 1500 cm^{-1} and 1620 cm^{-1} for ν_s and ν_{as} of bending vibration and broad peak at 3200 cm^{-1} for stretching vibration are an indication of the bond of the amino group to the metal ion, (Mejnikov *et al*, 2000). Infrared spectra of the complexes were also measured in the

region $400\text{--}700\text{ cm}^{-1}$ in order to identify frequencies related to M-O and M-N bands. The M-O frequencies were identified at rang ($600\text{--}800\text{ cm}^{-1}$), while M-N frequencies were identified at range ($400\text{--}600\text{ cm}^{-1}$). These results are in agreement with literature value, being similar to other metal complexes with amino acids, (Batiu, *et al*, 2005). The $\nu_{(\text{O-H})}$ stretching vibration do appear in the complexes at range ($3450\text{--}3750\text{ cm}^{-1}$) suggesting the presence of the crystal and coordinated water in these compounds. These feature is absolutely different in glycine, serine, aspartic acid, and arginine spectrum. Which were summarized in table (6).

Table (6) Important peaks appeared in the IR-Spectra of Glycine, Serine, Aspartic acid, and Arginine ligand and Co(Gly), Co(Ser), Co(Arg), and Co(Asp) complexes:

Compound	COO-		NH3			M-O	M-N	O-H
	Vasy	Vsy	Str(broad)	Bendasy	Bendsy			
Co.Gly	1647.28	1415.81	3271.41	-	1554.4	610	443.85	3564.6
Co.Ser	1604.84	1400.38	3430.00	-	1477.54	624.96	528.52	3465.02
Co.Arg	1631.85	1485.09	3568.46	-	1562.02	621.11	451.36	3637.90
Co.Asp	1616.42	1460.05	3418.01	-	1510.02	547.81	430.65	3640.00

UV/Vis spectra study:

The features of the electronic spectra of the cobalt (II) complexes are very similar to each other. The electronic structures of cobalt (II) complexes with different ligands have been presented in the literature, (Shishkin, *et al*, 2005), (Conarmondm, *et al*, 1982). Based on the simplest model, three spin-allowed crystal

field bands are expected. ${}^4A_2(F) \rightarrow {}^4T_2(F)$, ${}^4A_2(F) \rightarrow {}^4T_1(F)$, ${}^4A_2(F) \rightarrow {}^4T_1(P)$, and some absorption bands are observed, which are attributed to charge transfer from the non-bonding orbitals of the oxygen atoms in the ligand to the cobalt (II) d orbitals. The last absorption bands are assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$

transitions of the ligand (Nassirinia, *et al*, 2008), (Rodríguez, *et al*, 2009).

X-ray Diffraction (XRD) study:

X-ray diffraction patterns for the four cobalt-amino acid complexes (Co.Gly,

Co.Ser, Co.Asp, and Co.Arg) were recorded and calculated parameters are given in table (7) all complexes had a monoclinic crystal lattice.

Table (7) Data recorded and calculated from XRD study

		Co.Gly	Co.Ser	Co.Asp	Co.Arg
Molecular formula		C4H12CoN2O8	C6H16CoN2O8	C8H14CoN2O10	C12H34CoN8O6
Crystal system		monoclinic	monoclinic	monoclinic	monoclinic
Unit cell	a	9.200 A	9.5990 A	9.999 A	9.5080 A
	b	15.1230 A	15.3440 A	16.004 A	9.4280 A
	c	8.3210 A	8.9440 A	9.874 A	9.3090 A
	α	99.98°	100.47°	108.54°	78.86°
	β	111.78°	113.93°	118.32°	105.080°
	δ	79.56°	81.37°	89.76°	120.13°
Molecular weight		242.93	302.93	356.93	444.94
Density per g/cm ³		1.087	1.356	1.597	1.304

Biological activity tests:

The antibacterial activity of amino acids complexes was investigated against isolated gram positive strain (*Staphylo coccus aureas*, *Entero coccus feacalis*), and two kind of gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*). The Co (II) complexes of amino acid have inhibitory effect both against gram positive and gram negative.

The sensitivity of these complexes was determined the diameter of zone occurred by mean value (X') of four difference concentrations (5 mg/weight) of complexes. The sensitivity of complexes was evaluated according to chart (>10mm ≡ SS), (10-20mm ≡ MS), and (<20mm ≡ HS), this illustrated in table (8), (9), (10), and (11) for Co.Gly, Co.Ser, Co.Asp, Co.Arg, respectively.

Table (8) Diameter ± SD and sensitivity of 5mg for Co.Gly complex against four types of bacteria:

complex	Co(Gly)	sensetivity
Type of bacteria		
<i>Escherichia coli</i>	17.89 ± 0.23	MS
<i>Staphylo coccus aureas</i>	17.1785 ± 0.114	MS
<i>Pseudomonas aeruginosa</i>	21.5188 ± 0.00	HS
<i>Entero coccus feacalis</i>	15.5949 ± 0.17844	MS

Table (9) Diameter \pm SD and sensitivity of 5mg for Co.Ser complex against four types of bacteria:

complex Type of bacteria	Co(Ser)	sensetivity
<i>Escherichia coli</i>	16.17 \pm 0.19	MS
<i>Staphylo coccus aureas</i>	15.665 \pm 0.18754	MS
<i>Pseudomonas aeruginosa</i>	21.23603 \pm 0.16	HS
<i>Entero coccus feacalis</i>	14.98077 \pm 0.3279	MS

Table (10) Diameter \pm SD and sensitivity of 5mg for Co.Asp complex against four types of bacteria:

complex Type of bacteria	Co(Asp)	sensetivity
<i>Escherichia coli</i>	19.745 \pm 0.0032	MS
<i>Staphylo coccus aureas</i>	18.558 \pm 0.2961	MS
<i>Pseudomonas aeruginosa</i>	20.34923 \pm 0.20913	HS
<i>Entero coccus feacalis</i>	14.12177 \pm 0.17277	MS

Table (11) Diameter \pm SD and sensitivity of 5mg for Co.Arg complex against four types of bacteria:

complex Type of bacteria	Co(Arg)	sensetivity
<i>Escherichia coli</i>	16.515 \pm 0.38	MS
<i>Staphylo coccus aureas</i>	15.8443 \pm 0.216	MS
<i>Pseudomonas aeruginosa</i>	17.5012 \pm 0.2885	MS
<i>Entero coccus feacalis</i>	10.2165 \pm 0.25299	MS

Conclusion:

Four amino acid complexes with Co(II) ion were synthesized by 1:2 molar ratio of cobalt salt and glycine, serine, aspartic acid, and arginine as ligand in basic media, and characterized by PCEDX, TGA, FTIR, UV/Vis, XRD, and electrical conductivity measurement. Thermal gravimetric analysis of four complexes showed weight loss between 120-180°C, which equivalent to the loss water of crystalize that was in agreement with result obtained from IR, PCEDX, and XRD analysis.

The Electrical conductivity of the complexes can provide us with information

about the number of ions involved in the complexes in solution, which can give us a clear image about the structural geometry of the complexes. The electrical conductivity measurements were recorded of 2 mg/ml of complex solutions. The Co.Gly, Co.Ser, Co.Asp and Co.Arg complexes are non-electrolyte solution, (neutral complex) inductive of the coordination to Co through the two COO⁻ and two NH₂ groups, this suggestion was confirmed by the IR-spectra of the complexes. As result as the complexes form are [Co(gly)₂.(H₂O)₂] (a),

[Co(Ser)₂.(H₂O)₂] (b), [Co(Asp)₂.(H₂O)₂] (c), [Co(Arg)₂.(H₂O)₂] (d), respectively, which illustrated in figure (5):

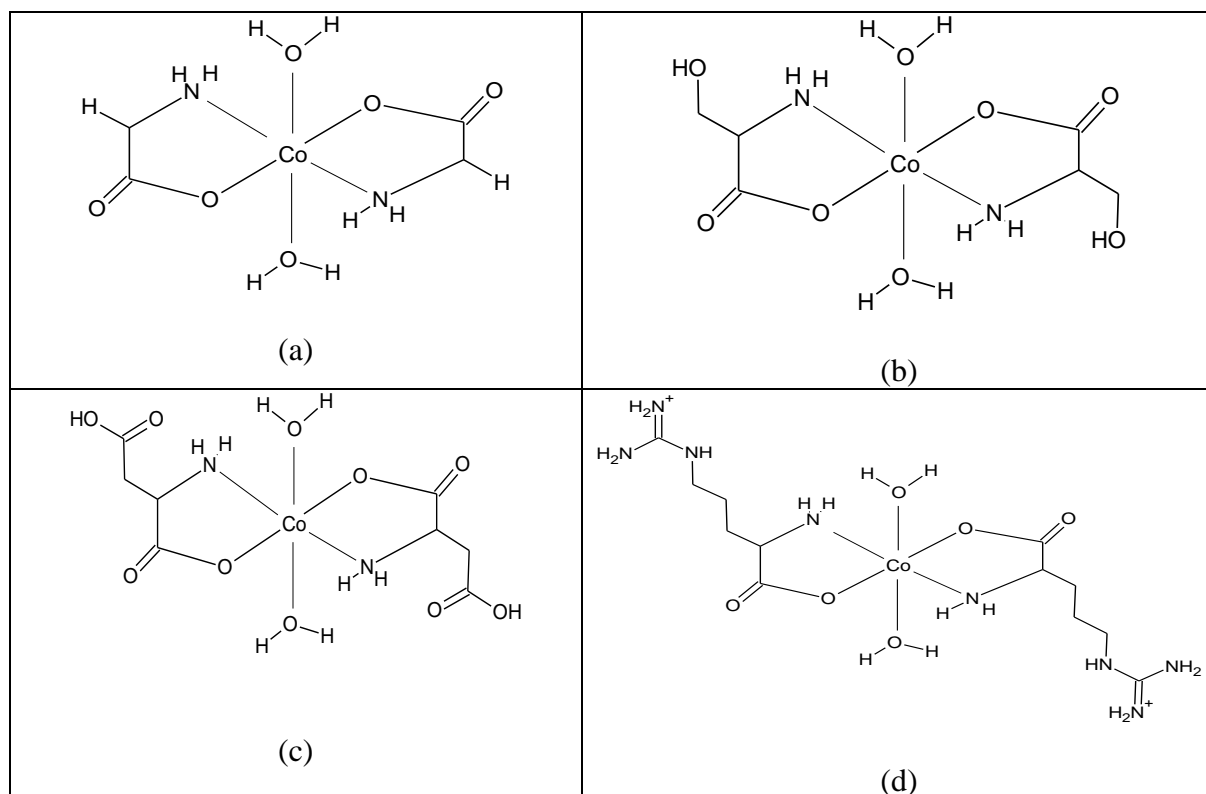


figure (5): Structures of [Co(Gly)₂.(H₂O)₂] (a), [Co(Ser)₂.(H₂O)₂] (b), [Co(Asp)₂.(H₂O)₂] (c), and [Co(Arg)₂.(H₂O)₂] (c) complexes.

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