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Complexation Equilibria of Vitamin B9, Glycine Oligopeptides with Di- and Trivalent Metal Ions

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UV-visible spectrophotometric technique was used to study the complexation equilibria involved during the formation of binary and ternary biocomplexes of some divalent metal ions [Cu(II), Co(II) and Ni(II)] and trivalent [Fe(III), Al(III) and Cr(III)], with folic acid and biologically relevant glycine oligopeptides [glycine (G), glycylglycine (GG), glycyl-L-phenylalanine (GP) and glyclglycylglycine (GGG)], to investigate the complexation behavior of these systems as these systems could mimic many biological interactions of the vitamins.

Keywords: Folic acid, Glycine oligopeptides, Complexes, UV-visible spectrophotometry.

INTRODUCTION

It is well known that group vitamins B complex form wide organic-compounds that cannot be synthesized by humans and necessary for the tropism of human beings and need to be part of our daily intake. It occurs in living cells as essential substances for growth[1]. Folic acid, one of important known vitamin B family known as vitamin B9 is structurally composed of pteroic acid and glutamic acid connected *via* an amide linkage[2]. It is essential for normal human cell division, cell growth, formation of red blood cells and energy production[3-5]. It also gives a definite effect in inhibiting the growth of tumors[6]. The use of folic acid in dietary supplements has been dramatically increasing in recent years because of folates established role in reducing the prevalence of neural tube defects and its role in reducing the risk of cardiovascular disease and anemia[7,8]. Chemically, the solubility of folic acid is crucial for drug delivery, absorption, transferring in the human body and crystallization in the manufacturing process[9]. Glycine oligopeptides such as glycylglycine, glycyl-L-phenylalanine and glyclglycylglycine are biological molecules that are made up of simplest amino acid glycine playing many important roles in various biochemical processes[10-15].

Considering the biological importance of folic acid (their possible application in medicine by combining the therapeutic properties of folic ligand and the other ligand in one compound and in chemical modeling of the transport and storage of some metal ions in living systems), the considerable attention has been paid in recent years on the study of the complexation equilibria of hydroxamates, phenolates, amino acids, non-protein amino acids and vitamins with metal ions[16-26]. The fact that, the ternary systems are somewhat better models for complicated biological systems, as the importance of ternary complexes in biochemical systems is beyond question. So, it is worthwhile to assemble information on their formation, stability and structure and on the mutual influence of two ligands bound to the same metal ion. In this study, a UV-visible spectrophotometric investigation of metal ion-vitamin B9-glycine peptide unit was performed to elucidate the complexation equilibria of these systems.

EXPERIMENTAL

All chemicals were of analytical grade and used without further purification. Folic acid (FA, **Scheme-I**)) was purchased from Aldrich (Germany) with 99 % purity. Glycine oligopeptides (glycine (G), glycylglycine (GG), glycyl-L-phenylalanine (GP) and glycylglycylglycine (GGG)) (**Scheme-I**) used was of analytical grade chemicals with purity 99 % and were produced by Sigma (Germany). Copper chloride dihydrate ($CuCl_2{\cdot}2H_2O$, 99.0 % purity) was a product of Kanto Chemical Co., Inc. (Japan). Nickel chloride hexahydrate ($NiCl_2{\cdot}6H_2O$, 97.0 % purity) and cobalt nitrate hexahydrate ($Co(NO_3)_3{\cdot}6H_2O$, 99.0 % purity) were obtained from Acros Organics, USA. Aluminum(III) chloride anhydrous ($AlCl_3$, 99.999 % purity), chromium(III) chloride anhydrous ($CrCl_3$, 99.99 % purity) and iron(III) chloride hexahydrate ($FeCl_3.6H_2O$, 97 % purity) salts were supplied from Sigma-Aldrich, UK. Sodium nitrate (99 %

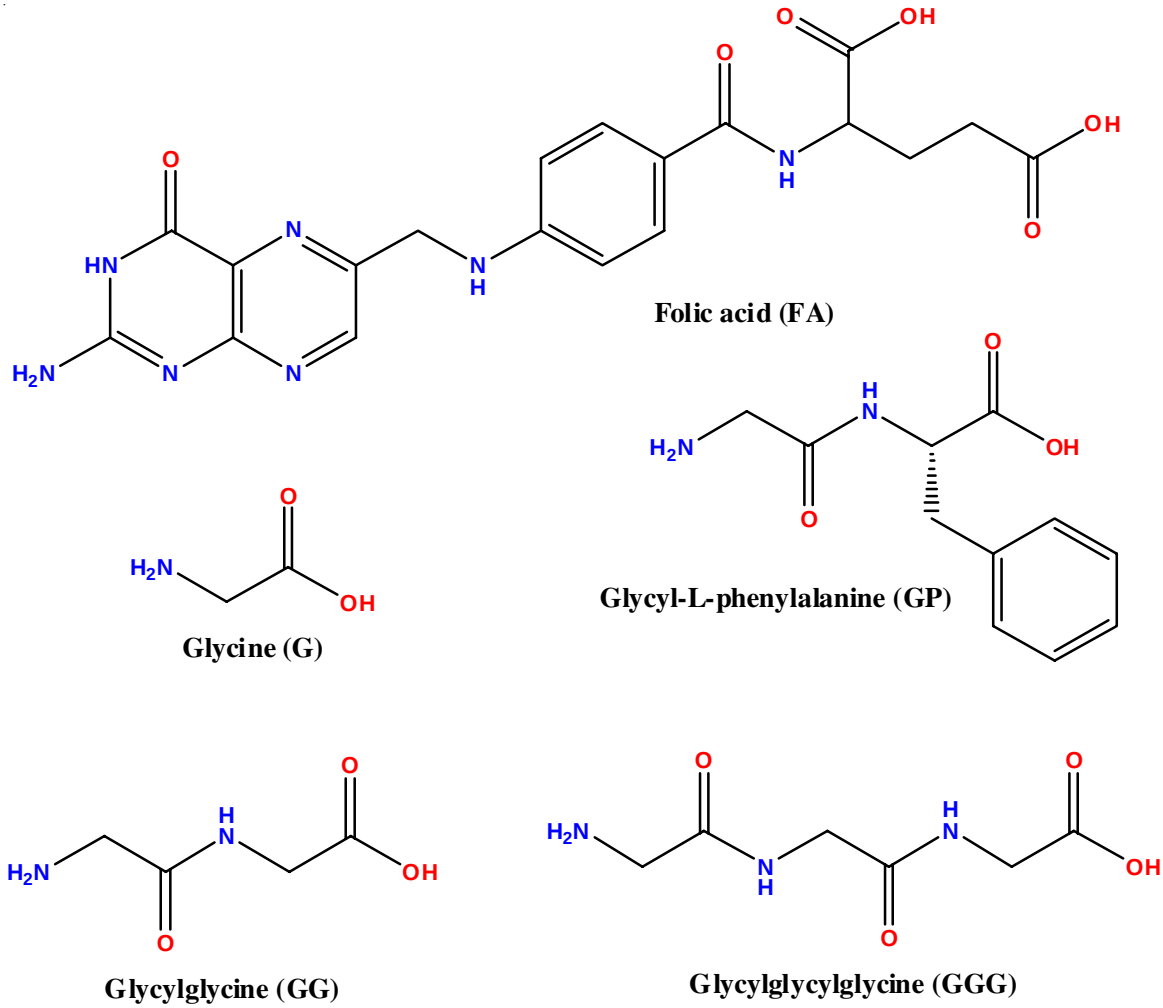
purity) from Acros Organics (USA) was used. Buffer, Hydrion® Tablets (pH values of 4.00 ± 0.02, 7.00 ± 0.02 and 9.00 ± 0.02) were purchased from Sigma-Aldrich, USA. All solutions were freshly prepared daily before measurements. Chemicals were accurately weighed then dissolved in ultra-pure water (NANO pure-Ultrapure water that deionized with 18.3 MΩ cm^{-1} resistance and distilled).

UV-Visible spectrophotometric measurements: Di- and trivalent metal ion complex species were spectrophotometrically studied in solutions at about 298 K using a compact, double-beam Jasco V-530 UV/Visible Scanning Spectrophotometer with standard 1 cm quartz cells working on its current supply (220V) device. UV/visible bandwidth, scan speed and data interval used are 0.1 nm, 100 nm/min and 1 nm, respectively. The solutions of different binary metal-ligand complex species stability determination were done at various concentration ratios [$T_L : T_M = 1 : 3$, L (vitamin B9) = FA (folic acid), M (metal ion) = Cu(II), Co(II), Ni(II), Fe(III), Cr(III) and Al(III) metal ions, P (peptide = G, GG, GP and GGG glycine oligopeptides], while measurements for various mixed ligand complex species were done at concentration ratios $T_L : T_M : T_P = 1:1:1$. Order of mixing of solutions of different reagents was maintained strictly throughout the work. The vitamin B9 solution was first added to the metal ion solution, then glycine oligopeptide was added to this binary solution and again kept for a few minutes to reach complete equilibrium. The pH adjustment was done using Techne pH meter (model 3540) with glass and calomel electrode assembly and checked frequently with buffer solutions of Buffer, Hydrion®Tablets (pH values of 4.00 ± 0.02, 7.00 ± 0.02 and 9.00 ± 0.02). The pH of desired solution was adjusted using hydrochloric acid (Panreac, Spain) and sodium hydroxide (Across Organics, USA) solution of suitable concentration. Each measurement was repeated at least four times.

RESULTS AND DISCUSSION

Ultraviolet-visible absorption spectroscopic measurements of vitamin B9 (folic acid, FA) in different pH medium (pH ≈ 3, 4, 5, 6, 7, 8 and 9) were shown below in Fig. 1. Based on the above UV-visible spectroscopic results, it is concluded that the protonation equilibria of folic acid was affected by the pH changes of the aqueous medium and due this equilibria could be explained based on the molecular structure of folic acid (**Scheme-I**).

In the present study, we followed the UV-visible absorption spectra of folic acid different pH media, in which the measurements indicates two different maximum absorption bands at two different wavelengths (λ = 313 and 376 nm) and the absorbance increases with the increment of the basic media of the folic acid solution. These transitions involves energies



Scheme-I: Molecular structure of folic acid and glycine oligopeptides

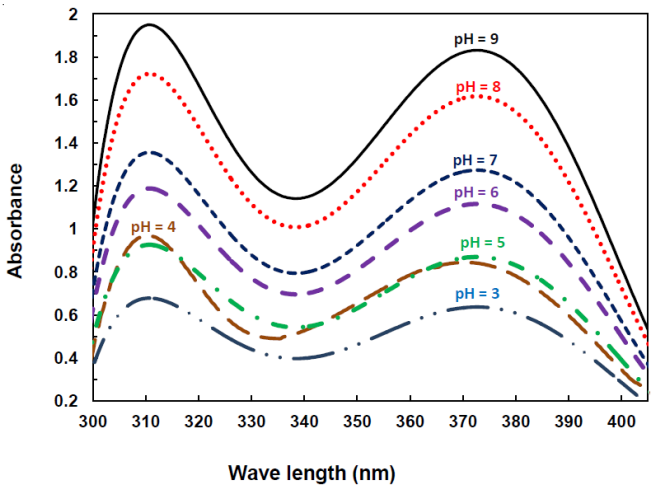


Fig. 1. UV-visible absorption spectra of 0.001 mol dm^{-3} folic acid (FA) in different pH medium solutions

of 31949 and 2666 cm^{-1} due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, respectively. Also, there are four isopiestic points were found at four different wavelengths (λ = 307, 315.5, 343 and 368 nm) for the protonated folate species around pH 4 and 5.By comparing the maximum absorption wavelength, the shapes of absorption curve of each metal, ligand, binary and mixed ligand complexes, the longer and shorter shifting peaks and/or the new peaks that occur in those graphs; it proves the complex species formations in each system[25]. From the present UV-visible spectrum measurements in Figs. 2-7, we can conclude the following investigations:

• The binary complex species of trivalent metal ions *i.e.*, iron(III), aluminum(III) and chromium(III) involving the glycine oligopeptides exhibited one absorption band at maximum wavelength (λ_{max}) at 304 nm with energy of 32895 cm^{-1} for FeG; 305 nm with energy of 33003 cm^{-1} for FeGG; 306 nm with energy of 33003 cm^{-1} for FeGGG; 306 nm with energy of 33111 cm^{-1} for FeGP; 306 nm with energy of 33111 cm^{-1} for AlG; 305 nm with energy of 33003 cm^{-1} for AlGG; 306 nm with energy of 33111 cm^{-1} for AlGGG; 307 nm with

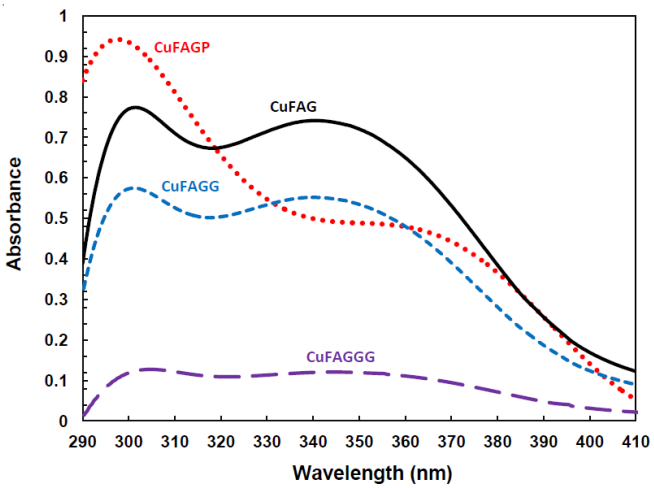


Fig. 2. UV-visible absorption spectra of ternary copper metal ion [Cu(II)] complexes involving folic acid (FA) and glycine oligopeptides; glycine (G), glycylglycine (GG), glyclglycylglycine (GGG) and glycyl-L-phenylalanine (GP); 0.001 mol dm^{-3} copper(II) + 0.001 mol dm^{-3} folic acid + 0.001 mol dm^{-3} glycine oligopeptides)

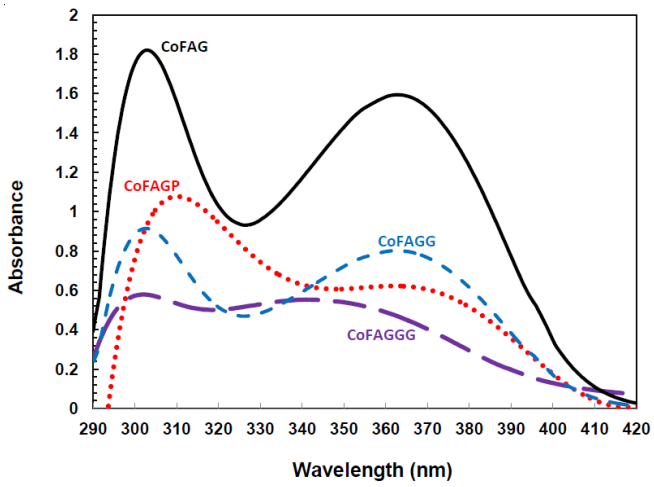


Fig. 3. UV-visible absorption spectra of ternary cobalt metal ion [Co(II)] complexes involving folic acid (FA) and glycine oligopeptides; glycine (G), glycylglycine (GG), glyclglycylglycine (GGG) and glycyl-L-phenylalanine (GP); 0.001 mol dm^{-3} cobalt(II) + 0.001 mol dm^{-3} folic acid + 0.001 mol dm^{-3} glycine oligopeptides)

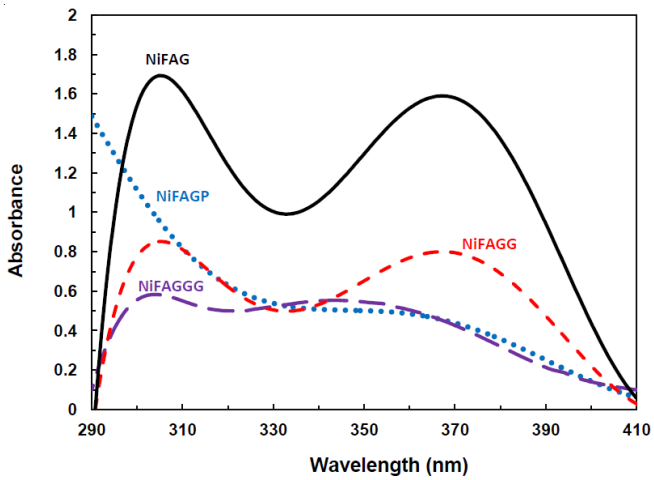


Fig. 4. UV-visible absorption spectra of ternary nickel metal ion [Ni(II)] complexes involving folic acid (FA) and glycine oligopeptides; glycine (G), glycylglycine (GG), glyclglycylglycine (GGG) and glycyl-L-phenylalanine (GP); 0.001 mol dm^{-3} nickel(II) + 0.001 mol dm^{-3} folic acid + 0.001 mol dm^{-3} glycine oligopeptides)

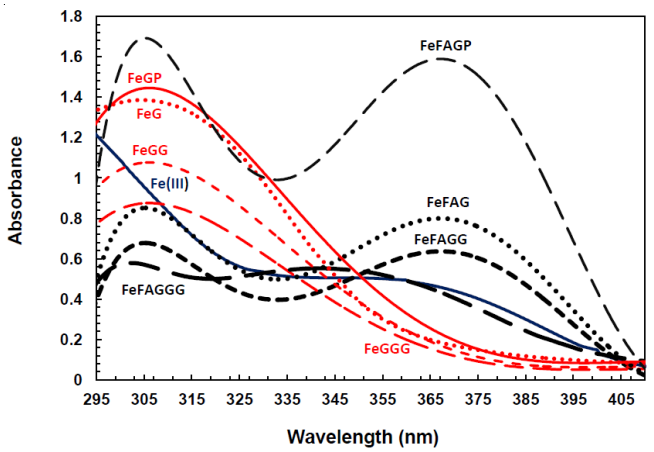


Fig. 5. UV-visible absorption spectra of ternary iron metal ion [Fe(III)] complexes involving folic acid (FA) and glycine oligopeptides; glycine (G), glycylglycine (GG), glyclglycylglycine (GGG) and glycyl-L-phenylalanine (GP); 0.001 mol dm^{-3} iron(III) + 0.001 mol dm^{-3} folic acid + 0.001 mol dm^{-3} glycine oligopeptides)

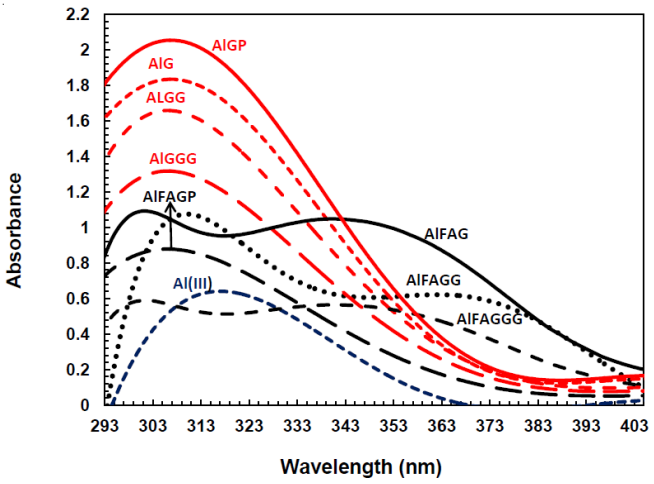


Fig. 6. UV-visible absorption spectra of ternary aluminum metal ion [Al(III)] complexes involving folic acid (FA) and glycine oligopeptides; glycine (G), glycylglycine (GG), glyclglycylglycine (GGG) and glycyl-L-phenylalanine (GP); 0.001 mol dm^{-3} aluminum(III) + 0.001 mol dm^{-3} folic acid + 0.001 mol dm^{-3} glycine oligopeptides)

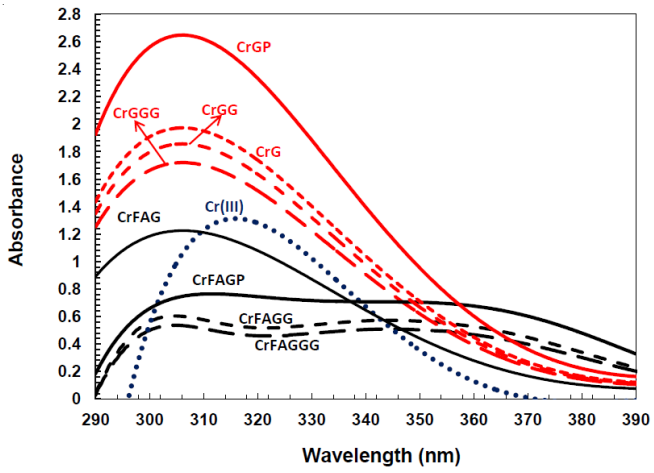


Fig. 7. UV-visible absorption spectra of ternary chromium metal ion [Cr(III)] complexes involving folic acid (FA) and glycine oligopeptides; glycine (G), glycylglycine (GG), glyclglycylglycine (GGG) and glycyl-L-phenylalanine (GP); 0.001 mol dm^{-3} chromium(III) + 0.001 mol dm^{-3} folic acid + 0.001 mol dm^{-3} glycine oligopeptides)

energy of 33220 cm^{-1} for AlGP; 306 nm with energy of 33111 cm^{-1} for CrG; 306 nm with energy of 33111 cm^{-1} for CrGG; 306 nm with energy of 33111 cm^{-1} for CrGGG; 307 nm with energy of 33220 cm^{-1} for CrGP complex species. As seen above, each iron(III), aluminum(III) and chromium(III) metal binary complex species involving glycine oligopeptides give an absorption band in which Fe(III), Al(III) and Cr(III) have d^5, P^6 and d^3 electronic configuration with spectroscopic ground term symbol of ^6S, ^1S and ^4F, respectively. These orbitals are non-degenerate states and cannot split by either an octahedral or tetrahedral fields[25,27], hence *d-d* transition should be expected for all of the above complexes. However, the single absorption band observed is assigned to $\pi \rightarrow \pi^*$ transition of the chromophoric groups in the complexes.

• The ternary systems involving copper(II)-folic acid-glycine oligopeptides complex species was found to display two absorption maximum wavelengths (λ_{max}) at 300, 341 nm with energies of 32463, 37372 cm^{-1}, respectively, for glycine; 300, 345 nm with energies of 32463, 37914 cm^{-1}, respectively, for glycylglycine; 304, 350 nm with energies of 32895, 37914 cm^{-1}, respectively, for glycylglycylglycine; and 298, 360 nm with energies of 32895, 38997 cm^{-1}, respectively, for glycyl-L-phenylalanine complex species. Copper(II) has a d^9 configuration with one unpaired electron. All the copper(II) complexes are either blue or green. In this case, copper(II) has a d^9 electronic configuration, which implies a spectroscopic ground state term symbol of ^2D. The ^2D orbitals splitted in a tetrahedral field into two sub-energy levels, namely $^2T_2$ and ^2E. Hence *d-d* transition is expected from $^2T_2 \rightarrow {}^2E$ transitions. The first UV-visible absorption band in each metal complex is assigned to metal ligand charge transfer, while the second band is assigned to $^2T_2 \rightarrow {}^2E$ transitions due to a tetrahedral geometry.

• The ternary complexes involving cobalt(II)-folic acid-glycine oligopeptides complex species exhibited two absorption maximum wavelengths (λ_{max}) at 304, 364 nm with energies of 32895, 39430 cm^{-1}, respectively, for glycine; 304, 364 nm with energies of 32895, 39430 cm^{-1}, respectively, for glycylglycine; 301, 340 nm with energies of 32895, 36830 cm^{-1}, respectively, for glycylglycylglycine; and 310, 364 nm with energies of 33581, 39430 cm^{-1}, respectively, for glycyl-L-phenylalanine complex species. The electronic configuration of cobalt is d^7 with a spectroscopic ground term ^4F. The Co(II) complex show two bands assigned to $^4A_2 \rightarrow {}^4T_2$ and $^4A_2 \rightarrow {}^4T_1$ and this assignments support the proposed tetrahedral geometry for the complex.

• The ternary systems involving divalent nickel metal ion-folic acid-glycine oligopeptides complex species were shown two absorption maximum wavelengths (λ_{max}) at 304, 368 nm with energies of 32895, 39864 cm^{-1}, respectively, for glycine; 305, 368 nm with energies of 33003, 39864 cm^{-1}, respectively, for glycylglycine; 303, 343 nm with energies of 32823, 37156 cm^{-1}, respectively, for glycylglycylglycine; and 349 nm with energy of 37810 cm^{-1} for glycyl-L-phenylalanine complex species. The electronic configuration of nickel(II) is d^8, with a spectroscopic ground term of ^3F. The absorption bands appeared was assigned to $^3A_2 \rightarrow {}^3T_2$ and $^3A_2 \rightarrow {}^3T_1$ transitions. The band at 349 nm is assigned to $n \rightarrow \pi^*$ transition.

• The ternary systems involving ferric-folic acid-glycine oligopeptides complex species was found to display two absorption maximum wavelengths (λ_{max}) at 305, 367 nm with energies of 33003, 39760 cm^{-1}, respectively, for glycine; 305, 367 nm with energies of 33003, 39760 cm^{-1}, respectively, for glycylglycine; 303, 343 nm with energies of 32823, 37156 cm^{-1}, respectively, for glycylglycylglycine; and 305, 367 nm with energies of 33003, 39760 cm^{-1}, respectively, for glycyl-L-phenylalanine complex species.

• The ternary systems involving aluminum(III)-folic acid-glycine oligopeptides complex species was found to display two absorption maximum wavelengths (λ_{max}) at 301, 340 nm with energies of 32610, 36831 cm^{-1}, respectively, for glycine; 311, 364 nm with energies of 33690, 39430 cm^{-1}, respectively, for glycylglycine; 303, 342 nm with energies of 32823, 37051 cm^{-1}, respectively, for glycylglycylglycine; and 306 nm with energy of 33111 cm^{-1} for glycyl-L-phenylalanine complex species.

The ternary systems involving chromium-folic acid-glycine oligopeptides complex species was found to display two absorption maximum wavelengths (λ_{max}) at 305 nm with

energy of 33003 cm^{-1} for glycine; 304, 347 nm with energies of 32895, 37591, cm^{-1}, respectively, for glycylglycine and glycylglycylglycine and 311, 354 nm with energies of 33710, 38350 cm^{-1}, respectively, for glycyl-L-phenylalanine complex species.

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REFERENCES

1. J. Yang, R. Han, B. Su, C. Lin, N. Wang and J. Hu, *J. Anal. Sci.*, **14**, 965 (1998).
2. J.G. Donnelly, *Crit. Rev. Clin. Lab. Sci.*, **38**, 183 (2001).
3. M. Lucock, *Mol. Genet. Metab.*, **71**, 121 (2000).
4. A. Brzezinska, P. Winska and M. Balinska, *Acta Biochim. Pol.*, **47**, 735 (2000).
5. T. Tamura and M.F. Picciano, *Am. J. Clin. Nutr.*, **83**, 993 (2006).
6. O. Stanger, *Curr. Drug Metab.*, **3**, 211 (2002).
7. M. McCarthy, *Lancet*, **347**, 677 (1996).
8. M. Eichholzer, O. Tonz and R. Zimmermann, *Lancet*, **367**, 1352 (2006).
9 Z. Wu, X. Li, C. Hou and Y. Qian, *J. Chem. Eng. Data*, **55**, 3958 (2010).
10. Meroz and D. Horn, *Proteins*, **72**, 606 (2008).
11. C.D. Fjell, J.A. Hiss, R.E. Hancock and G. Schneider, *Nat. Rev. Drug Discov.*, **11**, 37 (2012).
12. J.C. Reubi, *Endocr Rev.*, **24**, 389 (2013).
13. B. Murray and R. FitzGerald, *Curr. Pharm. Des.*, **13**, 773 (2007).
14. J.C. Hutton and K. Siddle, Peptide Hormone Secretion: A Practical Approach, IRL Press at Oxford University Press (1990).
15. J.C. Bulinski, *Int. Rev. Cytol.*, **103**, 281 (1986).
16. A.Y. Rajhi, Y.H. Ju, A. Angkawijaya and A.E. Fazary, *J. Solution Chem.*, **42**, 2409 (2013).
17. A.E. Fazary, *J. Chem. Eng. Data*, **58**, 2219 (2013).
18. A. Angkawijaya, A.E. Fazary, S. Ismadji and Y. H. Ju, *J. Chem. Eng. Data*, **57**, 3443 (2012).
19. A. Angkawijaya, A.E. Fazary, E. Hernowo, S. Ismadji and Y.H. Ju, *J. Solution Chem.*, **41**, 1156 (2012).
20. E. Hernowo, A. Angkawijaya, A.E. Fazary, S. Ismadji and Y.H. Ju, *J. Chem. Eng. Data*, **56**, 4549 (2011).
21. A.E. Fazary, E. Hernowo, A. Angkawijaya, T.C. Chou, C.H. Lin, M. Taha and Y.H. Ju, *J. Solution Chem.*, **40**, 1965 (2011).
22. A. Angkawijaya, A.E. Fazary, E. Hernowo, M. Taha and Y.H. Ju, *J. Chem. Eng. Data*, **56**, 532 (2011).
23. A.E. Fazary, M. Taha and Y.H. Ju, *J. Chem. Eng. Data*, **54**, 35 (2009).
24. M.M. Khalil and A.E. Fazary, *Monatsh. Chem.*, **135**, 1455 (2004).
25. A.E. Fazary, *Bull. Chem. Soc. Ethiopia*, **28**, 393 (2014).
26. A.E. Fazary and A.M. Ramadan, *TCME Complex Metals*, **1**, 139 (2014).
27. H.D. William and J. Fleming. Spectroscopic Methods in Organic Chemistry, McGraw-Hill Book Company Ltd., London, edn 4 (1980).