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Structural characterization of calcium glycinate, magnesium glycinate and zinc glycinate

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Metal glycinate chelates are formed by glycine and metal compounds through chemical reactions. Calcium glycinate, magnesium glycinate and zinc glycinate are kinds of new-type and ideal nutrient supplements, which have satisfactory physico-chemical properties and bioactivities. They are important for prophylaxis and treat metal deficiency. The structural characterization shows that the metal ion is bonded to the amino and carboxyl group to form two five-membered rings. This paper mainly studies the structure characterization of the metal chelated glycinates by their solubility, infrared spectrum, thermal analysis, mass spectrometry, polycrystal diffraction, the metal contents and glycine contents of calcium glycinate, magnesium glycinate and zinc glycinate.

Keywords: Calcium glycinate; magnesium glycinate; zinc glycinate; structure characterization.

1. Introduction

Metal glycinate chelates are formed by glycine and metal compounds through chemical reactions, and are kinds of new-type and ideal nutrient supplements, which have satisfactory physico-chemical properties and bioactivities. They are the raw materials of great quantity of health care products having large market in China. But they are restricted because their structures not detected. Calcium glycinate, magnesium glycinate and zinc glycinate are formed by 2 to 1 chelate, and the metal ion is bonded to the amino and carboxyl group to form two five-membered rings (Fig. 1).

Understanding the structures of these species require a couple of analytical tools for their

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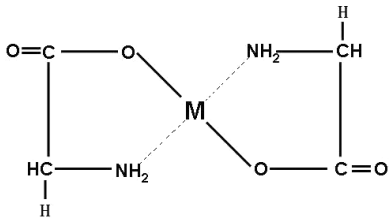


Fig. 1. The structure of chelated metal glycinate.

determination. Infrared spectroscopy and X-ray powder was applied to prove the reaction of iron and amino acid carboxyl group with one or more coordinate covalent bonds.[1] If iron ion reacts with more than one carboxyl functional groups, it can generate to a 1:2 or 1:3 metal amino acid chelate molecules. 1:2 zinc glycinate was studied by X-ray diffraction to show the two chelating five-membered rings.[2,3] The thermodynamic parameters for the formation of glycine complexes were detected.[4] The difference between the ^{13}C NMR spectra of magnesium glycinate and glycine was reported.[5] And a study was undertaken for the structures and fragmentation modes of the gasphase complexes of zinc attached to deprotonated amino acids in the gas phase by electrospray ionization.[6] But the above detections cannot readily assess the structures of the chelated metal glycinate. Here, we present the structural characterization of calcium glycinate, magnesium glycinate and zinc glycinate by their solubility, infrared spectrum, thermal analysis, mass spectrometry, polycrystal diffraction, the metal content and glycine content of them.

2. Materials and Methods

2.1. *Samples*

Calcium glycinate (Fan mei (Beijing) Biological Products Co., Ltd., batch: 20071103C, 20071105C), magnesium glycinate glycine chelated magnesium (Fan mei (Beijing) Biological Products Co., Ltd., batch: 20071111M, 20071113M), zinc glycinate (Fan mei (Beijing) Biological Products Co., Ltd., batch: 20071107Z, 20071109Z).

2.2. *Solubility*

Metal oxide and glycine were the two raw materials which were used in the making of chelated metal glycinate. The solubilities of metal oxides and chelated metal glycinate were investigated at 25°C. 200 mg of metal oxides (calcium oxide, zinc oxide, magnesium oxide) and the batches of chelated metal glycinates respectively, were dissolved in 20 mL water, and then added 1 mol/L hydrochloric acid in drops.

2.3. *Infrared spectrum*

All infrared spectrum of calcium glycinate, magnesium glycinate, and zinc glycinate were collected using a NEX us FT-IR spectrometer (Thermo Nicolet).[7] The mid Fourier transform infrared spectrum recorded at 300 K in the range of 400–4000 cm^{-1} following the KBr pellet technique. The functional groups were identified by the infrared spectrum.

2.4. *Mass spectrum*

High resolution mass spectrometry was used to determine the exact molecular weight of the compounds,[8] which was an important parameter for chelate determination. The experimental setup with micro Q-TOF mass spectrometer (BRUKER), which includes a sample manipulator and a time-of-flight mass spectrometer (TOF-MS), which are housed in an ultra-high vacuum chamber (UHV) with a base pressure of about 10^{-9} Torr.

2.5. *Thermal analysis*

The thermal stabilities of GZC were studied by gravitation thermal analysis (GTA) using TGA-Q500 TGA (U.S. TA company) between the temperatures 30°C and 1000°C at a heating rate of 15°C/min in nitrogen atmosphere, and differential thermal analysis (DTA) using DSC-Q200 (U.S. TA company) between the temperatures 40°C and 200°C at a heating rate of 10°C/min in nitrogen atmosphere.

2.6. *Polycrystal diffraction*

X-ray absorption spectra were recorded at the institute of Materia Medica,[9] Chinese Academy of Medical Sciences with the storage ring SPEAR operating at 3 GeV and ring currents of 50 ± 100 mA. Selenium K-edge spectra were recorded on beamline 7-3 using a Si (220) double crystal monochromator with an upstream vertical aperture of 1 mm.

Selenium K-edge X-ray absorption spectra were measured as the Ka fluorescence excitation spectra using a Canberra 13-element germanium detector. The spectrum of hexagonal Se was collected simultaneously with each data set for energy calibration, with the first inflection of its absorption edge taken to be 12,658.0 eV. Sulfur K-edge spectra were recorded on beamline 6-2 with a Si (111) double crystal monochromator. Sulfur fluorescence was collected using a Stern–Heald–Lytle detector. Spectra were calibrated with reference to a solid sodium thiosulfate standard measured periodically during the run, the lowest energy K-edge absorption peak of which was 2469.2 eV. Samples were at room temperature for the sulfur measurements. Background subtraction and normalization were carried out according to established procedures.

2.7. *The contents analysis of metal ions*

Ion chromatography method was a high sensitivity, good accuracy method for the content analysis of metal ions. We used ion chromatographic methods to determine the metal ion contents of calcium glycinate and magnesium glycinate.[10] But we could not determine zinc ion by the ion chromatography, so we used the inductively coupled plasma mass spectrometry (ICP-MS) to simultaneously determine the calcium, magnesium and zinc ion contents.[11]

The concentration of calcium and magnesium cations (Ca^{2+}, Mg^{2+}) was determined with a Dionex ICS-3000 dual system consisting of a dual pump (DP) module, an eluent generator (EG) module, a detector chromatography (DC) module (single temperature zone configuration), and an autosampler (AS). The eluent contained 60 mmol/L sodium hydroxide (NaOH), and the flow rate was 0.25 mL/min. Detection was the conductivity detector (CD) with integrated cell held at 35°C. The conductivity suppressor was CSRS ULTRA II (4 mm), the guard column was Ionpac CG12A (cations) (4 × 50 mm), and the column was Ionpac CS12A (cations) (4 × 250 mm).

The concentrations of zinc cations (Zn^{2+}) was carried out on an ICP-MS (ICP-MS X-7, Thermo Scientific), which was operated with the plasma screen plus sensitivity enhancement option fitted, Xt interface cones, and with Peltier cooling of the spray chamber. A standard quartz nebulizer was used, together with a standard quartz impact bead spray chamber and standard single piece,1.5 mm ID injector quartz torch. The instrument was operated using standard instrument operation. Plasma Lab software was applied to instrument control, data acquisition, and analysis. The instrumental and operating condition were optimized with the commentated tune solution. The operating parameters of ICP-MS instrument were as follows: RF power 1830 W, coolant gas flow 14.3 L/min, auxiliary gas flow 0.95 L/min, nebulizer gas flow 0.87 L/min, pump rate 1.0 mL/min, and peak jumping data acquisition mode: dwell time 10 s, duration time 60 s, and three replicates per sample. The isotope of SC (IS) was monitored at m/z 45, full validation according to the FDA guidelines was, as far as applicable for ICP-MS, performed for the assay.

2.8. *The contents analysis of glycine*

Ion exchange chromatography was the common method for determination of amino acid. The methods were applied to analyze the glycinate contents of calcium glycinate, magnesium glycinate and zinc glycinate.

The concentration of glycine was determined with a Dionex ICS-3000 dual system consisting of a DP module, an EG module, a DC module (single temperature zone configuration), and an AS. The eluent contained 25% 250 mmol/L sodium hydroxide (NaOH), and the flow rate was 0.25 mL/min. Detection was the amperometric detector, the guard column was Aminopac PA-10 Dionex (anions) (4 × 50 mm), and the column was Aminopac PA-10 Dionex (anions) (4 × 250 mm).

The ionic species was identified and quantified by interpolation on a proper calibration curve. All experiments were performed at room temperature and lasted approximately 10 min for each injected sample.

3. Results

3.1. *Results of solubility*

The chelated metal glycinate after oral administration was absorbed in the small intestine. We studied the solubility in the different pH solutions (pH 2–12). The solubilities of chelated metal glycinates were better than the corresponding metal oxides (calcium oxide, zinc oxide, magnesium oxide). The calcium

glycinate, magnesium glycinate and zinc glycinate were also clear in the acidic solution.

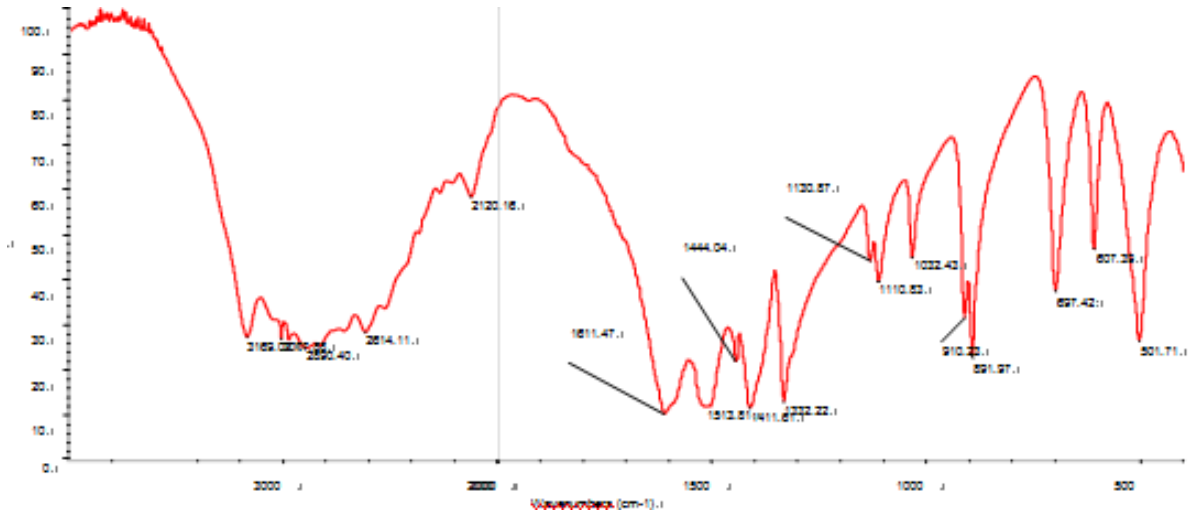
3.2. *Results of infrared spectrum*

We studied the infrared spectrum of chelated metal glycinate and glycine. The formation of NH_2–M bond and COO–M bond and the disappearance of NH_3-glycine bond and COO- bond were the indications of the formation of five-membered ring structure of chelated metal glycinates.

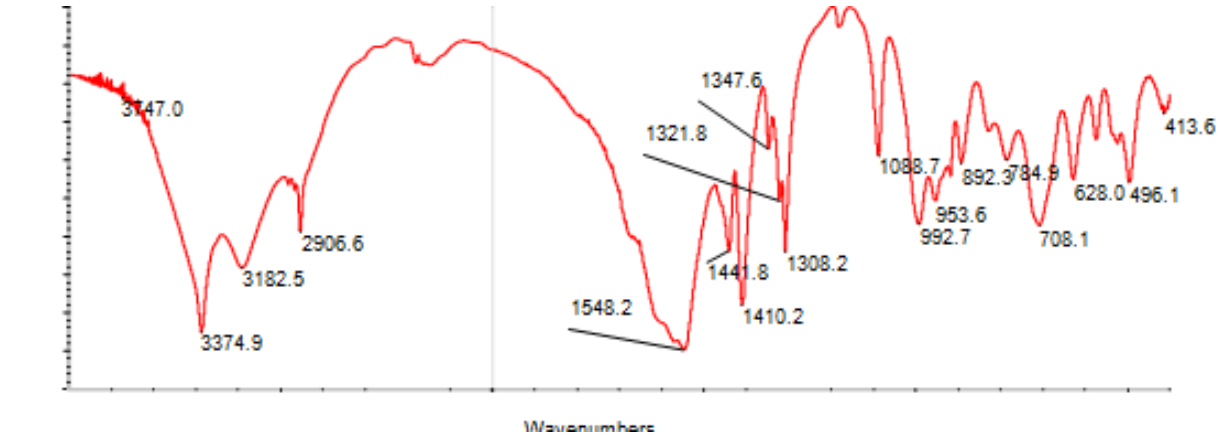
There were NH_3 and COO- groups in the molecules. In the infrared spectrum of chelated metal glycinate, NH_3 peaks (1111 cm^{-1}, 1131 cm^{-1}, 2120 cm^{-1}) and COO-characteristic peaks (502 cm^{-1}, 607 cm^{-1}, 697 cm^{-1}) all disappeared (Note COO- and other base groups combined). The NH_2 peaks (3342 cm^{-1}, 3450 cm^{-1}) also inferred that the three chelated metal glycinates have generated M–NH_2 group and COO–M groups (Fig. 2).

3.3. *Results of mass spectrum*

Calcium glycinate, magnesium glycinate and zinc glycinate were analyzed by high-resolution mass spectrometries. The results showed that all the three had the molecular ion peaks ([M + Gly2 + H]1+, M: metal ions, Gly: glycine) in the mass spectrum, which proved that molar ratio is 1:2 of M and glycines (Fig. 3).

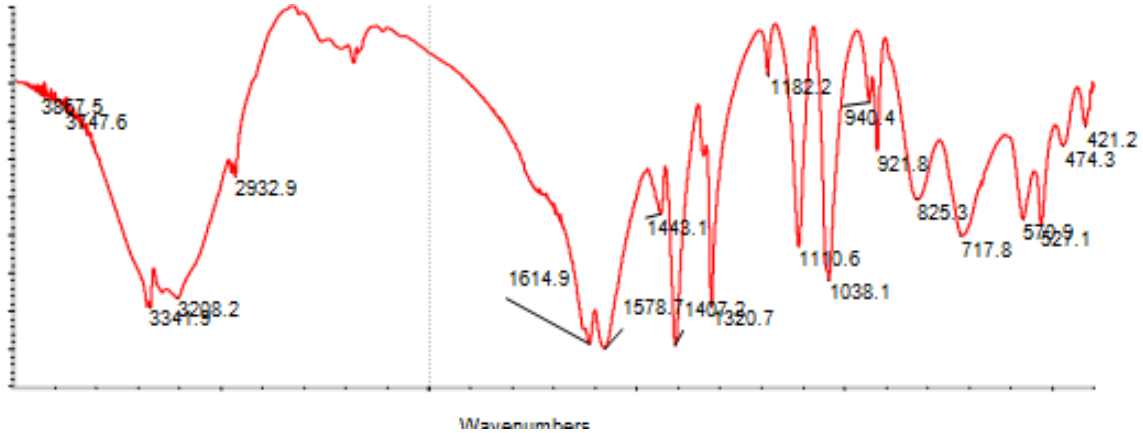


(a)

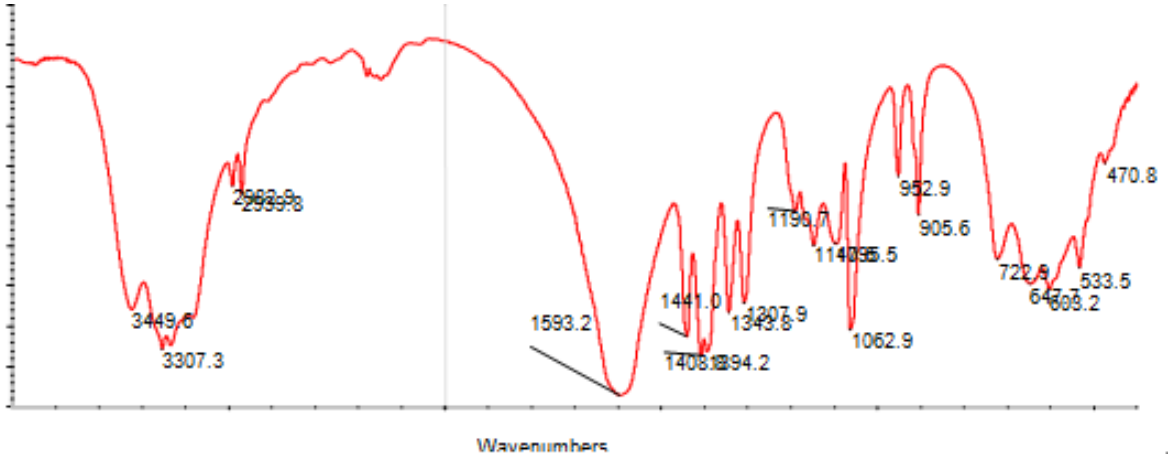


(b)

Fig. 2. The infrared spectrum of glycinate (a), calcium glycinate (b), magnesium glycinate (c), and zinc glycinate (d).

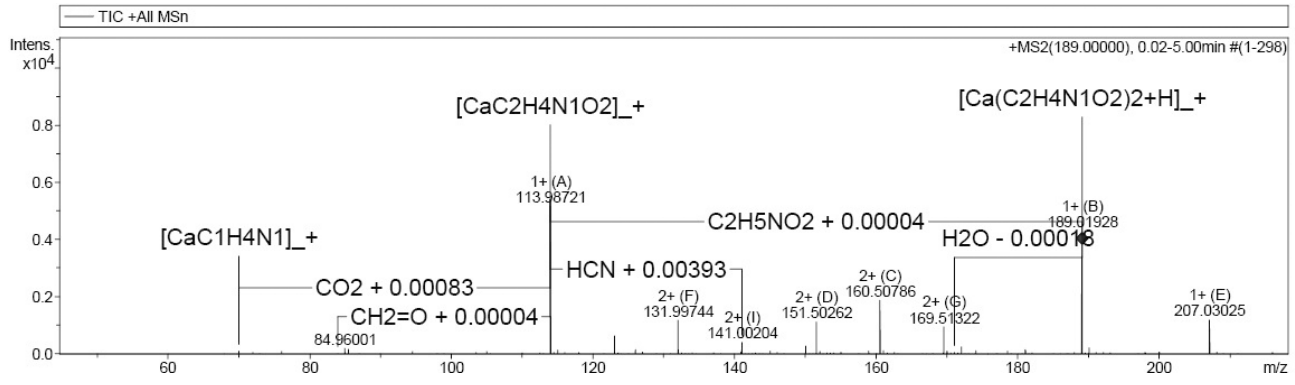


(c)



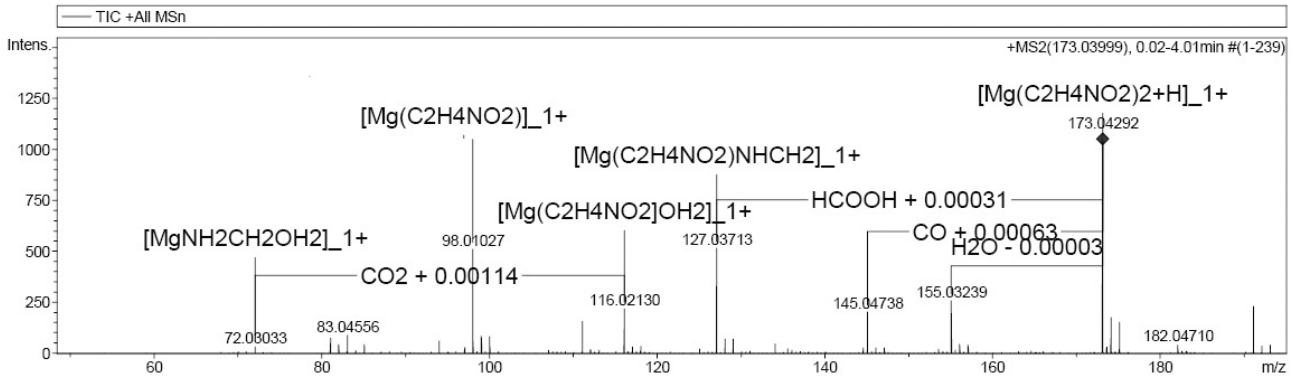
(d)

Fig. 2. (*Continued*)



(a)

Fig. 3. The mass–mass spectra of calcium glycinate (a), the mass–mass spectra of magnesium glycinate (b), the mass–mass spectra of zinc glycinate (c).



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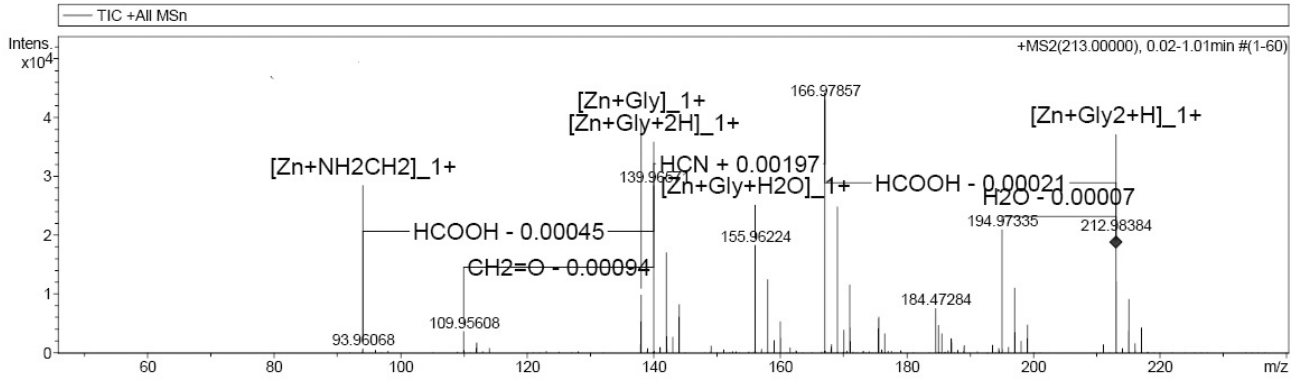
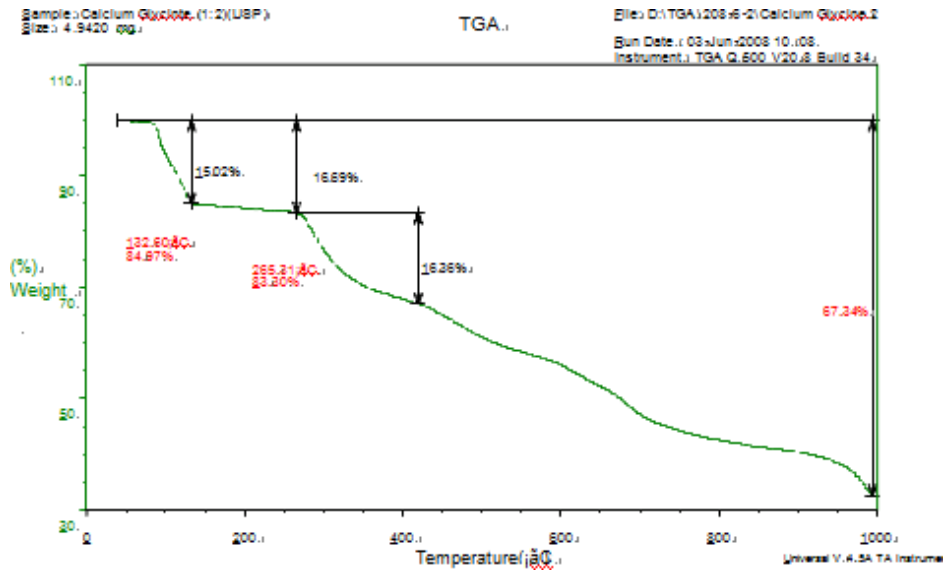


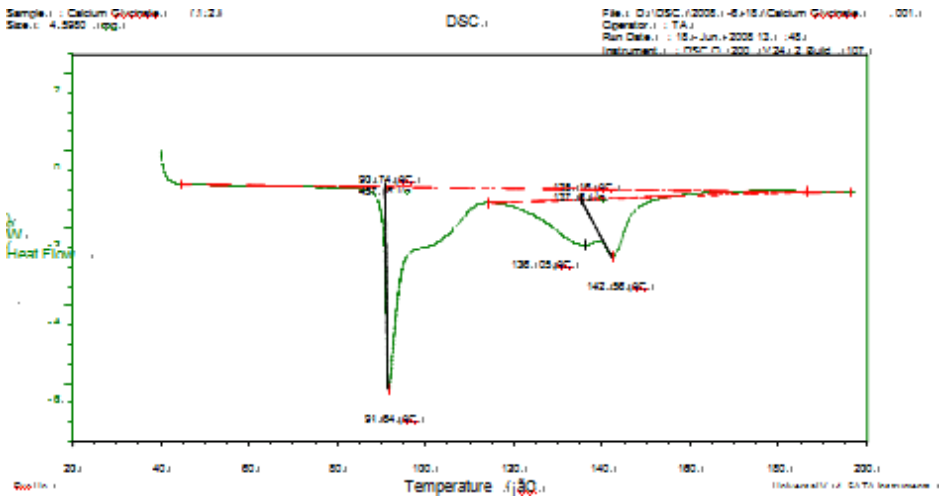
(c)

Fig. 3. (*Continued*)



(a)

Fig. 4. The GTA of calcium glycinate (a), the DTA of calcium glycinate (b), the GTA of magnesium glycinate (c), the DTA of magnesium glycinate (d), the GTA of zinc glycinate (e), the DTA of zinc glycinate (f).



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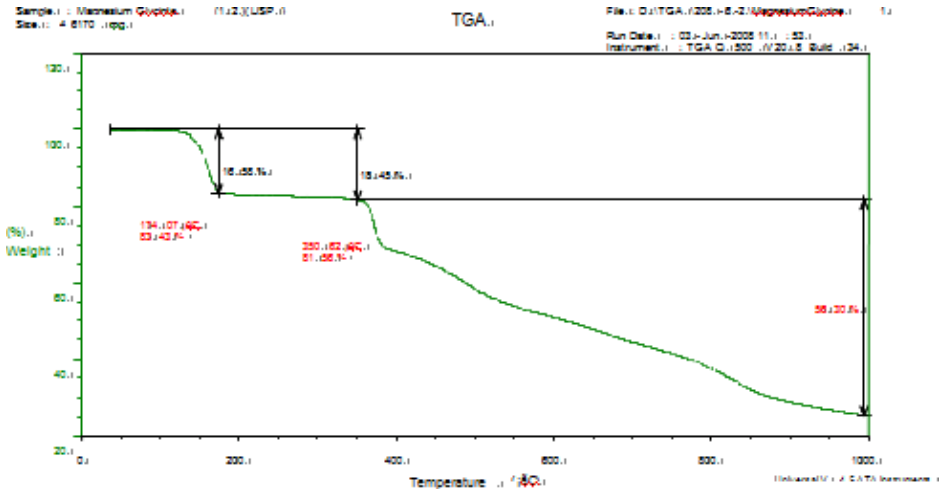

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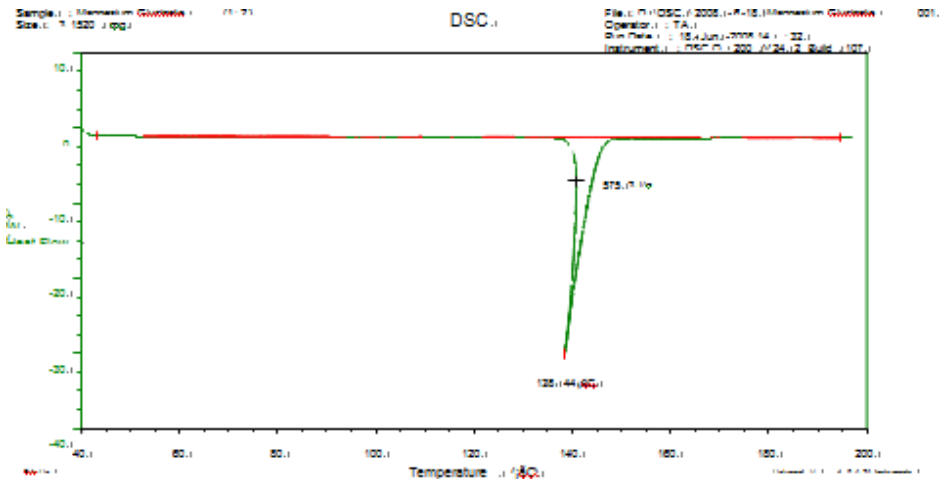
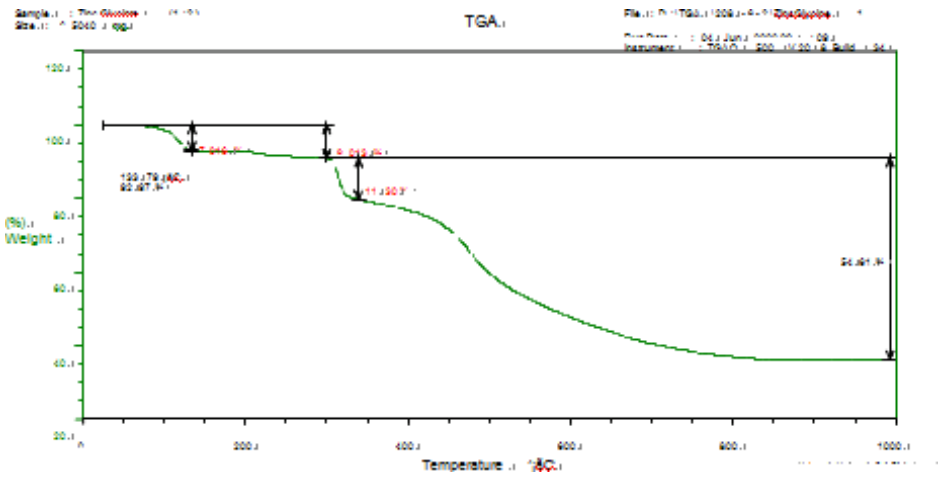
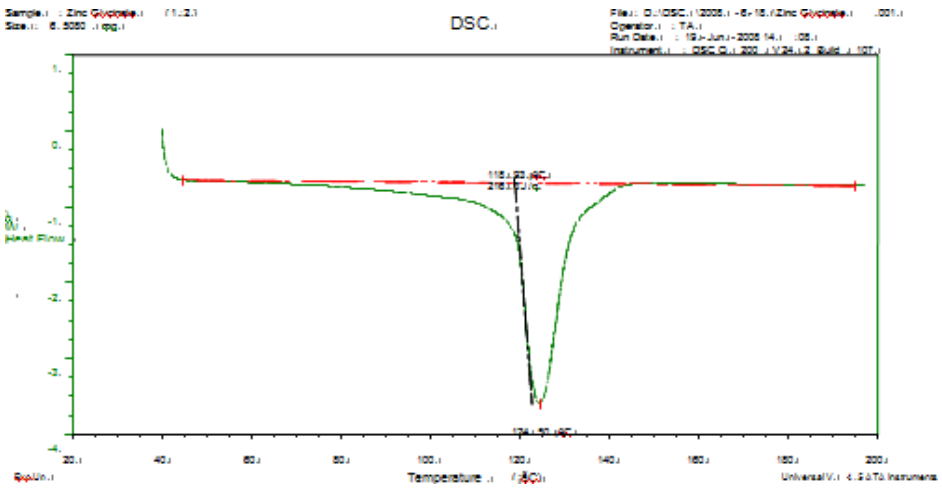


(d)

Fig. 4. (*Continued*)



(e)



(f)

Fig. 4. (*Continued*)

Mass spectrometry proved that M–O ionic bond and M...N coordinate covalent bond exists in molecules. They confirmed the structures of calcium glycinate, magnesium glycinate and zinc glycinate, the structures were shown in Fig. 1.

3.4. *Results of thermal analysis*

We studied calcium glycinate, magnesium glycinate and zinc glycinate for the GTA and differential scanning analysis. The scanning prints showed the

Table 1. Results of thermal analysis.

	Calcium glycinate	Magnesium glycinate	Zinc glycinate
The temperature range	90–180°C	130–180°C	60–180°C
The weight loss range	15.02°C–16.69%	16.58°C–18.45%	7.02°C–9.01%
The supposed molecular formula	$Gly_2Ca.2H_2O$	$Gly_2Mg.2H_2O$	$Gly_2Zn.H_2O$
The theoretical value of water content	16.06%	17.27%	7.78%
Conclusion	Contained two molecular crystal water	Contained two molecular crystal water	Contained one molecular crystal water

Table 2. The contents of calcium ion in calcium glycinate.

Batch	Calcium glycinate (%)
20071103C	14.2
20071105C	14.9

Table 3. The contents of magnesium ion in magnesium glycinate.

Batch	Magnesium glycinate (%)
20071111M	9.8
20071113M	10.1

GTA and DTA in Fig. 4. The results were summarized in Table 1.

3.5. *Results of polycrystal diffraction*

Polycrystal diffraction methods were used to study glycine and three chelated metal glycinates.

Table 4. The contents of zinc ion in zinc glycinate.

Batch	Zinc glycinate (%)
20071107Z	32.7
20071109Z	28.3

Table 5. The glycine contents of the three metal chelated glycinates.

Sample	Batch	The glycine content (%)
Calcium glycinate	20071103C	67.0
	20071105C	65.2
Zinc glycinate	20071107Z	66.9
	20071109Z	66.6
Magnesium glycinate	20071111M	71.0
	20071113M	71.6

The results showed that: the powders of glycine and three chelated metal glycinates were quite different. It proved that chemical reaction happened during the formation of chelated compounds.[12]

3.6. *Results of metal ion contents analysis*

The results were shown in Tables 2–4.

3.7. *Results of the contents analysis of glycine*

Ion exchange chromatography was commonly used for the determination of amino acid, in which we utilized it to analyze the glycine content of the three chelated metal glycinates. It could be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.[10] The method was used to determine the glycine contents of the three metal chelated glycinates, the glycine contents were shown in Table 5.

4. Discussion

The summary of the results of solubility, infrared spectroscopy, thermal analysis, mass spectrometry, polycrystal diffraction, metal content and glycine content are shown in Table 6.

With this result, we can determine that all the three chelated metal glycinates have five-membered ring structure (Fig. 1). Calcium glycinate and magnesium glycinate contained two crystalline water molecules and zinc glycinate contained one crystalline water molecule.

Table 6. Summary of the comprehensive analysis results.

Molecular formula	Mol.wt.	The theoretical value of water content (%)	The actual value of water content (%)	The theoretical value of glycine content (%)	The actual value of glycine content (%)	The theoretical value of metal ion content (%)	The actual value of metal ion content (%)
$(NH_2CH_2COO)_2Zn{\cdot}H_2O$	231.51	7.78	8.28	63.98	69.4	28.25	30.50
$(NH_2CH_2COO)_2Mg{\cdot}2H_2O$	208.43	17.27	16.95	71.06	71.1	11.66	10.00
$(NH_2CH_2COO)_2Ca{\cdot}2H_2O$	224.20	16.06	17.46	66.07	63.0	17.88	14.60

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