Chapter 152
In situ Preparation and Characteristics of a New Water-Soluble Heme Iron Via Hemin-Arginate Coacervation

Wenhang Wang, Ting Liu, Ying Zhang, Rujie Chen and Anjun Liu

Abstract A new water-soluble heme iron (WSHI) was prepared and characterized. Crystalline hemin and L-arginate in molar proportions of 1:3 were mixed in a acetone solution (acetone: water = 100:10, V: V), stirred at 25 °C, for 12 h, and then centrifuged, washed with acetone, freeze-dried, referred to as WSHI. No clear differences in UV spectrum, IR spectrum between hemin-arginate and hemin was founded, while a notable distinct of thermal sensitivity occurred by differential thermal analysis (DTA), which partly explained dissolution elevation of hemin-arginate. Hemin-arginate is stable and no coagulate occurred in Carbonate solution, phosphate solution, or tea water, respectively. So, Hemin-arginate can be used as a new heme iron supplement in food additives, functional foods, and pharmaceuticals.

Keywords Hemin · L-arginate · Coacervation · Dissolution · Stability

152.1 Introduction

Iron is the most abundant essential trace elements in the body, the main component of hemoglobin, myoglobin, and cytochrome enzymes, and involved in oxygen transshipment, carbon dioxide exchange, and tissue respiration process [1, 2]. In addition, iron participate in red blood cell formation and maturation, and associated with the catalytics of β-carotene into vitamin A, purine and collagen

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synthesis, antibody production, lipid transportation from the blood and drugs detoxification in liver [3].

Iron deficiency anemia is one of the world's highest incidence of nutritional deficiency diseases, which is storage iron in the body cannot meet the needs of normal erythropoiesis, due to insufficient iron intake, low absorption, increased demand, and losing too much [4, 5]. About 500 million people worldwide suffer from anemia, of which nearly half of the iron deficiency anemia. Iron deficiency anemia has become the most serious public health problem with the prevalence rate second only to tuberculosis. World Health Report (2002) of World Health Organization pointed that iron deficiency is one of ten top preventable health risk factors in world [6].

A lot of iron supplement have been on the market recently, but most is some nonheme iron supplement, such as ferrous chloride, ferrous sulfate, ferrous lactate, ferrous fumarate, and ferrous gluconate. Due to the impact of food phytic acid, oxalic acid, tannic acid, phosphate, carbonate, nonheme iron supplement has a low absorption rate for the body, which is generally 5–8 %. In addition, some side effects (prone to nausea, bloating or abdominal pain, digestive disorders, diarrhea, constipation, and other symptoms), and special metal taste of rust cause its difficulty of long-term consumption. Excessive intake of nonheme iron in body leads to iron overload in the body, resulting in iron accumulation poisoning [7–9].

Heme, also known as porphyrin iron, is a key assistance factor of many biologically active macromolecules, such as hemoglobin, myoglobin, cytochrome, and peroxidase. Heme is a natural class of porphyrin compounds, a combination with the organic molecules, widely present in the animal's blood, muscle, and some plant tissues [10]. The basic structure of the heme is composed of porphyrin and ferrous. Two N atoms of the porphyrin ring was covalently combined with the iron, the other two N atoms is contacted the iron with a Coordination bond, that is to say, the entire molecule of heme is in a resonance state. When heme combines with oxygen, the molecule of the heme iron is oxidized to trivalent heme namely as hemin; when the separation of heme with oxygen, the heme iron molecule is reduced to divalent, which is called heme [11]. Heme iron is a well-known, ideal antianemia drug, due to good promotion of bone marrow hematopoietic, treatment of animals hemolytic, and hemorrhagic anemia [12, 13].

Heme iron is usually extracted from pork or cow blood with acetone-HCl method. In fact, Probable formation of large insoluble heme polymers in digestion was another disadvantage for heme application although its good absorption, so some research efforts to improve the water-soluble porphyrin iron dispersion [14, 15].

This paper was focused on preparation WSHI via hemin-arginate coacervation, in order to improve the solubility of the heme iron. At the same time, UV, IR, and DTA of WSHI were characterized and its stability in different solutions (tea, phosphate, and carbonate) was studied.

152.2 Materials and Methods

152.2.1 Reagents and Materials

Fresh pork blood was obtained from Jingwu meat food Co., Ltd., added with 0.5 % sodium citrate for anticoagulation. Other reagents were of analytical grade.

152.2.2 Preparation of WSHI

Anticoagulated blood was centrifuged at 4,000 r/min, for 15 min. the upper was discarded and, the bottom red blood cells was Collected. Red blood cells were added with a final concentration of 0.2 % Na_2SO_3. After mixing, adding five times volume of acetone solution (acetone:6 mol/L HCl = 100:3, v:v), the reactive substances was disturbed for 2 h, and then filtrated. The filtered liquid was added with constantly dropping 1 mol/L NaOH solution until pH 4.6, and then precipitated by the sodium acetate solution with the final concentration of 1 %. The precipitation was separated with centrifugation, spurred by repeated washing with ethanol and distilled water, crystallization. The dried matter is hemin.

Crystalline hemin and L-arginate in molar proportions of 1:3 were mixed in an acetone solution (acetone: water = 100:10, V: V). the mixture was stirred vigorously to react, at 25 °C, for 12 h, and then was centrifuged at 10,000 g, for 5 min. The precipitates were collected, washed with acetone, and freeze-dried. The dried matter is WSHI [16].

152.2.3 Detection of Hemin Content

Hemin content of WSHI was detected with the Colorimetry method [17]. 20 mg hemin sample was completely dissolved at 100 mL 0.1 mol/L NaOH solution. 0.5, 1.0, 2.0, 4.0, 5.0 mL of the above solution was mixed with 100 mL 0.1 mol/L NaOH solution again, respectively. With Solvent blank as control, all absorbance values were measured at 392 nm, and the standard curve of hemin is drawn. 20 mg WSHI sample was detected by the same above procedure. According to standard curve of hemin, the hemin content of WSHI was calculated.

152.2.4 UV: Visible Absorption Spectroscopy

0.5 g WSIH and hemin were dissolved in 0.1 mol/L NaOH solution, fully stirred and centrifuged. The supernatant was scanned in 300–800 nm with 0.1 mol/L NaOH solution for blank sample.

152.2.5 IR Absorption Spectroscopy

1 mg WSIH and hemin was ground into about 2 μm particles, and mixed with 100 mg spectroscopically pure KBr powder, followed grinding again. The mixture was made into a thickness of 1 mm, diameter of 10 mm, transparent sheets under 10 MPa pressure. Absorption spectrums of the sheets were observed in 4,000–500 cm^{-1}.

152.2.6 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry analysis was carried out using a Polymer differential scanning calorimetry DSC141 (seraram corp., France). Temperature was raised from 50 to 200 °C, at a heating rate of 10 °C/min^{-1} [18].

152.2.7 Stability of WSIH

0.1 g of ferrous lactate, ferrous fumarate, ferrous gluconate, WSHI, ferrous chloride, and ferrous sulfate was weighed, divided in each test tube, and labeled. About 5 g green tea was boiled in 100 mL deionized water for 2 min and then cooled. The cold tea water was filtered for next detection. 10 ml tea water was added slowly and mixed completely. After 20 min standing, the change of the solution was observed. The stability of different iron supplement in the 0.2 mol/L sodium phosphate and 0.1 mol/L sodium carbonate was detected at the same procedure.

152.3 Results and Discussion

152.3.1 Hemin Content of WSHI

A new, water-soluble heme iron named WSHI was prepared via hemin-alrginate coacervation. WSHI was completely dissolved in distilled water.

The standard curve of hemin was showed in Fig. 152.1, with a linear relationship between absorbance and the hemin concentration in the range of 0 μg/mL–10 μg/mL. The regression equation was $Y = 0.0844\ X + 0.0112$, Y represented absorbance, X represented concentration. According to the formula in Fig. 152.1, the purity of WSHI prepared is 86.9 %.

152.3.2 UV, IR Spectrum

Absorption Spectroscopy of WSHI and hemin in the range of 300–800 nm were showed in Fig. 152.2. According to Fig. 152.2, the maximum absorption peak in visible spectrum of WSHI and hemin was found at the wave length of 392 nm. No other apparent differences were observed in absorption of WSHI and hemin.

The infrared absorption spectra of WSHI and hemin were shown in Fig. 152.3. The common characteristic absorption peak were determined at the wave number of 2919, 1717, 1272, 939, 841, and 719 cm^{-1}.

An absorption peak appeared at 2,919 cm^{-1} is attributed to carboxyl group stretching, while 1,272 cm^{-1} indicated C–N stretching, and 939 cm^{-1} and 841 cm^{-1} is attributed to C–H bending vibration of hydrocarbon compounds. In addition, 719 cm^{-1} indicated C–H bending vibration of benzene compounds.

From Fig. 152.3, A distinct absorption of at 3,422 cm^{-1} and 1626 cm^{-1} occurred in WSHI. 3,422 cm^{-1} is mainly caused by alcohols or phenols structure

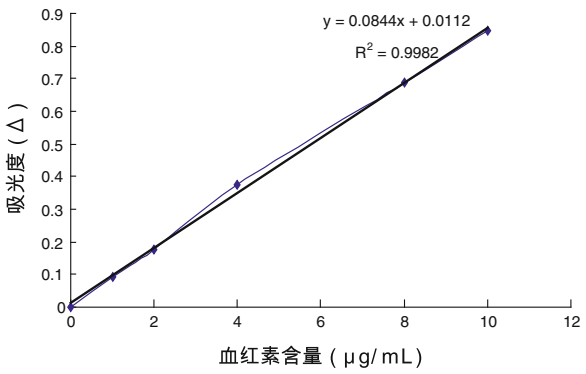

Fig. 152.1 Standard curve of hemin

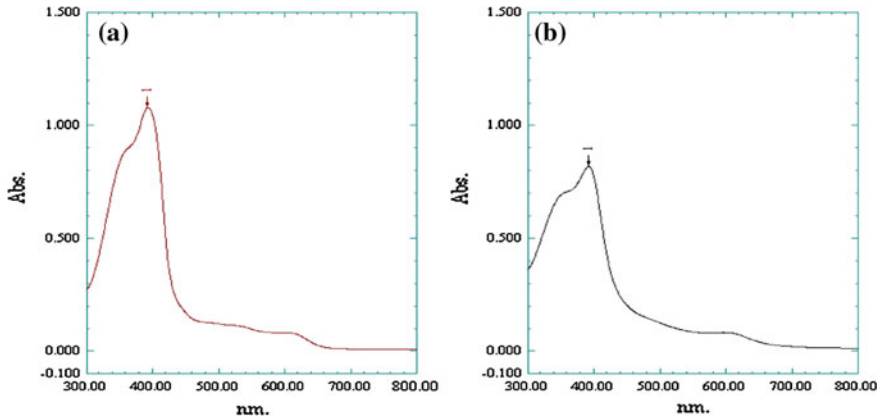

Fig. 152.2 UV spectrum of WSHI and hemin. **a** Hemin. **b** WSHI

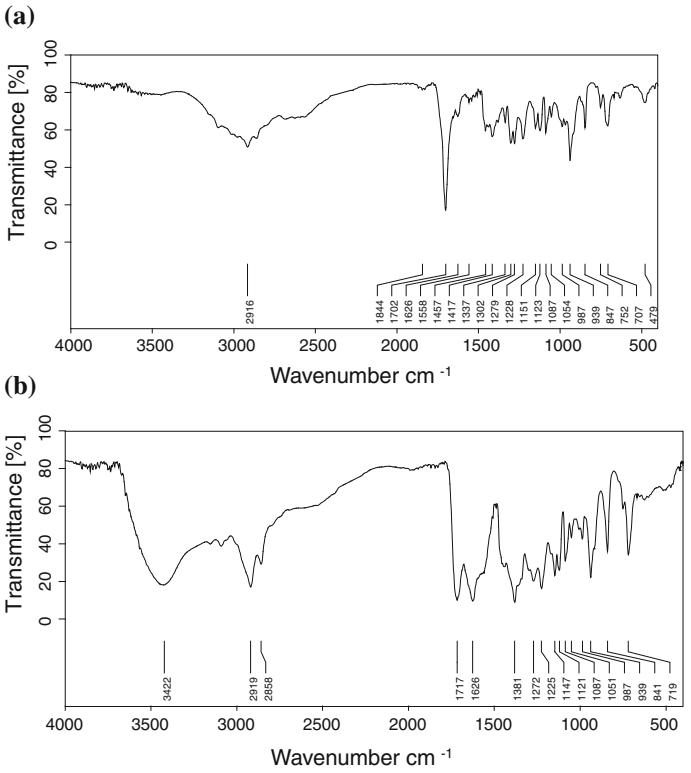

Fig. 152.3 IR spectrum of WSHI and hemin. **a** Hemin. **b** WSHI

in the OH stretching vibration, 1,626 cm^{-1}, amine compounds of NH bending vibration. It contributes to peptide of WSHI.

152.3.3 DSC Analysis

Figure 152.4 showed that hemin melted at 341.1 °C, but WSHI melted at 52.9 °C. It is obvious that both of them did not have fixed melting point.

From Fig. 152.4, hemin began to melt at 341.44 °C. There may be two melting range, 359.08 °C first endothermic peak, 380 °C second endothermic peak. WSHI began to melt at 52.92 °C, and along with the temperature gradually increasing, to generate other substances in the late degeneration due to thermal instability of it. It began to release heat at about 175 °C, and the maximum amount of heat release was obtained at 158 °C.

152.3.4 Stability of WSHI

Many green vegetables, such as seek, spinach, parsley all contain carbonate, and the ferrous lactate, ferrous chloride, ferrous sulfate iron in iron combine to form the insoluble precipitate, thus affecting the iron absorption. Patients with anemia in the iron at the same time, large-scale use of these substances would greatly reduce the absorption rate of iron precipitation can also cause stomach discomfort and indigestion [19, 20].

Stability of WSHI in different solutions was studied with other iron supplements for comparisons.

From Fig. 152.5a, except WSHI, other iron supplements rapidly produced a black precipitate after mixing with tea water. This is because tea contains large amounts of tannic acid, which being iron-binding agents reacted with many nonheme irons, such as Ferrous lactate, ferrous fumarate, ferrous gluconate, ferrous sulfate, ferrous chloride, to form insoluble tannin iron precipitation.

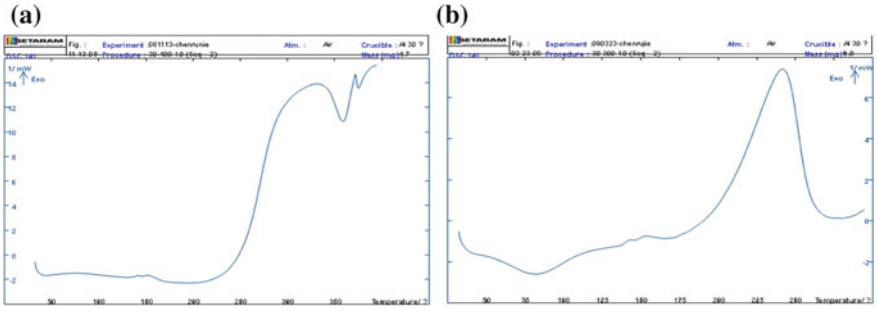

Fig. 152.4 DTA analysis of WSHI and hemin. **a** Hemin. **b** WSHI

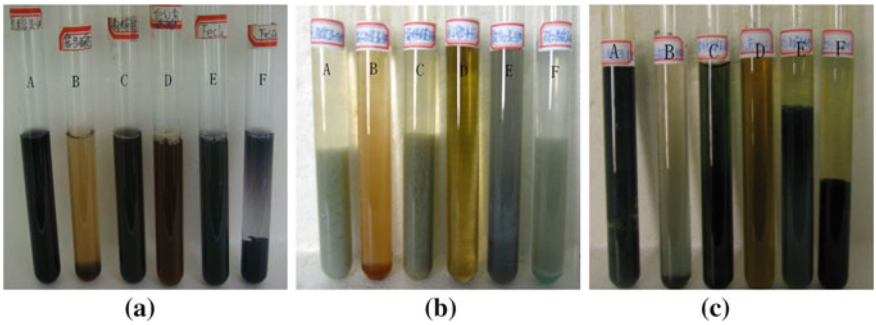

Fig. 152.5 stability of iron supplements in solutions. **a** Stability of irons in tea water. **b** Stability of irons in 0.2 % sodium phosphate solution. **c** Stability of irons in 0.1 % Sodium carbonate solution. A: Ferrous lactate; B: ferrous fumarate; C: ferrous gluconate; D: WSHI; E: ferrous sulfate; F: ferrous chloride

An insoluble precipitate formed in 0.2 % sodium Phosphate solutions, after all nonheme iron supplements added to while WSHI is an exception, as shown in Fig. 152.5b.

It can be seen from Fig. 152.5c, after mixed 0.1 % carbonate buffer solution with a variety of iron supplement, ferrous lactate, ferrous chloride, ferrous sulfate, a dark green flocculent precipitate occurred.

152.4 Conclusions

The UV spectrogram and Infrared absorption spectrum of WSHI was analogous to hemin, but differential thermal analysis was significantly different from hemin. WSHI was soluble and stable in distilled water and other solution, such as in Carbonate, phosphate, and tea water, which indicated its potential application as a new heme iron supplement in food additives, functional foods, and pharmaceuticals.

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