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Speciation analysis of iron in traditional Chinese medicine by flame atomic absorption spectrometry

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Abstract

In view of octanol, a long-chain alkanol, resembled as the configuration of carbohydrate and adipose in human body, the octanol-solubility and water-solubility were used to define the species of iron in medicine, to identify the lipophily and bioavailability of coordinated iron complex, and octanol–water system was adopted to study the distribution of iron in decoction of eight single medicines and compatibility of semen persicae and flos carthami in stomach and intestine. To study the effect of compatibility of medicines, the different acidity of stomach and intestine on the species of iron in phytomedicine decoction, the total concentration, octanol- and water-solubility concentration of iron in medicinal materials or decoctions under gastric and intestinal acidity, were determined, respectively, by flame atomic absorption spectrometry, analyzed and compared. The different acidity of digestive site, the different composition of medicine, and the compatibility of medicines, have greatly affected the species of iron, the pharmacological activity of coordinated iron complex in decoctions. Such factors, especially the concentration of octanol-solubility iron, could be the basis of the dosage to avoid iron overload.

Keywords: Speciation analysis; Iron; Medicinal plant; Flame atomic absorption spectrometry

1. Introduction

Being one of the most abundant metals in the human body, iron plays important roles in cellular processes such as the synthesis of DNA, RNA, and proteins; electron transport; ATP production, cellular respiration; cell proliferation and differentiation; and regulation of gene expression [1,2]. But iron deficiency anemia is a highly prevalent and seemingly intractable problem, particularly among women in developing countries. A report from the World Health Organization estimates that 46% of the world's 5–14 year-old children are anemic. In addition, 48% of the world's pregnant women are anemic. Iron deficiency affects more

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than 2 billion people [3]. Deficiency of iron will bring diseases such as hemopthisis and diabetes, depress the resistance of infection, affect thermoregulation and develop [4,5].

Though iron is essential for life, it is also potentially more toxic than other trace elements. Iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage, coronary heart disease, and cancer [6–9]. Biological damage may occur on several levels, including lipid peroxidation, protein peroxidation, and DNA crosslinkage, which may lead to cell death [10]. Disorders of iron metabolism are among the most common diseases of humans and encompass a broad spectrum of diseases with diverse clinical manifestations, ranging from anemia to iron overload and, possibly, to neurodegenerative diseases [1]. This is due to the lack of effective means to protect human cells against iron overload and to the role of iron in the generation of free radicals [11–13]. For example, iron has become a focus of interest in studying the pathophysiology of CNS degenerative diseases. One of the mechanisms by which iron cause degenerative damage is generation of excessive free radicals, which results in oxidative stress.

Since for the vital physiological function, iron should be the importance pharmacodynamic material basis related about iron deficiency. For example, the content of iron in herbs used traditionally for anemia treatment is high [14]. Since no the total content of iron are valid, which species of iron in medicine are effective and have pharmacodynamical activity? How about the influence of different acidity of digestive site, the composition of medicine, and compatibility of medicines on the species of iron? Since for the potential toxicity, how to design the dosage of iron in medicinal plant for avoiding the iron overload? All of these questions, we thought, should be given enough attention, during the clinical practice.

Effective chemical composition in medicine should be conformed with the morphology of chemical species as a core, be based upon the mutual function of organic composition and trace elements, for solving the position and substance of the effective chemical composition in medicine and correctly understanding the bioavailability of the compatible substance it forms [15]. Complexation in medicine has greatly affected the absorption, transport and metabolism of trace element [16]. So, pharmacodynamical activity of coordinated iron complex in medicine rests no on the total content, but on the quantification of specific species, especially which could be extracted by water, taken orally, and could be absorbed by digestive site. The study on the species of the trace element, the lipopily and bioavailability of iron is a key for exploring the pharmacodynamic material basis and functional mechanism in medicine. But the analysis of trace element in medicine was focused on the determination the total content [17] or just the concentration of inorganic species and organic species [18] or the species including the dissolved and particulate, exchangeable and nonexchangable, organic and inorganic [19]. The current methods for the assessment of iron bioavailability were divided into iron solubility and absorption studies; the disadvantages of iron solubility include the impossibility of measuring absorption or incorporation of iron, furthermore, only the solubility of nonheme iron, and not heme iron, can be studied; to study iron absorption, chemical balance in man has been a good, but laborious and expensive way, whole-body counting has the disadvantage of causing radiation exposure, the measurement of plasma iron response did not seem to be of great value, repletion bioassays using rats or other animals were of limited use because the accuracy of extrapolation to man is unknown [20]. We thought, the definition of iron bioavailability in medicine should include both solubility and absorption of iron, i.e. bioavailable iron is the specie of iron that could be dissolved in decoction, then taken orally, absorbed by organism.

Octanol is a long-chain alkanol, resembled as the configuration of carbohydrate and adipose in human body; pharmacology evaluates the lipopily and absorbability of medicine usually with the distribution coefficient of K_{ow}; K_{ow}, is the quotient of octanol- and water-solubility concentration [21]. The decoction is the most common use method to phytomedicine. Stomach and intestine is the main digestive and absorptive organ. So the octanol- and water-solubility were used to define

the species of iron in medicine, to identify the lipopily and bioavailability of coordinated iron complex, octanol–water system was adopted to study the distribution of iron in decoction of eight medicines and compatibility of Semen Persicae and Flos Carthami in stomach and intestine. The influence of difference digestive site acidity, difference compositions of single medicine, and the compatibility of medicines, on the species of iron, was studied.

2. Materials

2.1. Apparatus

AAS measurements were carried with a GBC 932AA spectrometer (GBC Co., Australia). Iron hollow cathode lamp (GBC Co.) was used as a light source. The pH values were measured with a model Mettler Toledo 320-S pH meter (Mettler Toledo Co., Shanghai, China) supplied with a combined electrode. A model HJ-3 magnetic stirrer (Jintan Medical Apparatus Co., Jiangsu, China), a model TD4 centrifugal machine (Hunan Centrifugal Machine Co., China) and Milli-Q-purified water apparatus (Millipore Co., Bedford, MA) were used for the test. The wavelength, spectral bandpass of monochromator and lamp current were set to 248.3, 0.2 nm and 15.0 mA, respectively. The flow velocity of air and acetylene was 0.7 and 1.8 l/min, respectively. All measurements were performed with D lamp background correction. All signals were processed in the peak area mode.

2.2. Materials and reagents

Flos carthami (A), semen persicae (B), radix pseudostellariae (C), fructus mori (D), vespertilio superans thomas faeces (E), flos chrysanthemi (F), atractylodes macrocephala koidz (G), and colla corii asini (H) were purchased from Zhangzhou Zhuantang drugstore, identified by apothecary, Professor You Kaihong. After picked out the clay and sundries, eight medicinal materials were rapidly cleaned twice with Milli-Q-purified water, roasted 4 h on 75–80 °C. Iron working standards 0.5, 1, 2, 3, 4, 5, 10, 50, 60 μg/ml, were prepared after serial dilution of the stock solutions (1000 mg/l, GBC Co.) with 0.2 mol/l HNO_3. All chemicals and reagents employed were of analytical reagent grade and Milli-Q-purified water was used throughout.

3. Methods

3.1. Medicinal materials pretreatment

Eight medicinal materials (2.000 g) were, respectively, weighed, levigated, kept in 20 ml mixed acid(HNO_3/$HClO_4$, 4/1) 24 h, heated on 80 °C until the liquid was close dry, added 2 ml HNO_3 (1/1) and 5 ml water, kept this solution lightly boiling until clarification, then added water until 50 ml. Above-mentioned solution was used to determine the total concentration of iron in medicine.

3.2. Decoction preparation

In order to follow the traditional decocted method, medicinal materials used for decoction were kept status in quo from drugstore. Decoctions were prepared as follows: single medicinal materials 18.000 g or compatibility of flos carthami (A, 9.000 g) and semen persicae (B, 9.000 g) was added, respectively, 180 ml water, heated to boil then kept lightly boiling for 1 h, filtrated, respectively, and the filter liquor was kept, added, respectively, another 180 ml water twice and manipulated as above. The filter liquors from three times filtration were collected together, divided into nine shares, added water until 50 ml. Three shares makes up of a group to parallel tests.

3.3. Different species of iron determination

The solution from Section 3.1 was used to determine the total concentration of iron in eight medicines. A group of decoctions from Section 3.2 were, respectively, operated as Section 3.1 then determined the total concentration of iron in decoction. Adjusted the acidity of decoction to gastric acidity (pH 1.3) [22] or intestinal acidity

Table 1
Analytical results of iron in phytomedicines and their decoctions (μg/g)

	A	B	C	D	E	F	G	H
Medicine	1452.5	177.5	525.0	685.0	1462.5	507.9	192.5	215.0
Decoction	51.2	23.3	110.5	70.0	25.5	37.8	62.3	79.5
Extract percent (%)	3.5	13.1	20.9	10.2	1.7	7.4	32.3	37.0

A, flos carthami; B, semen persicae; C, radix pseudostellariae; D, fructus mori; E, vespertilio superans thomas faeces; F, flos chrysanthemi; G, atractylodes macrocephala koidz; H, colla corii asini.

(pH 7.6) [22], respectively, six share decoctions (two group of decoctions) from Section 3.2 were kept 24 h, oscillated 2 h two times with 5 ml octanol each time, divided into water phase and octanol phase. Water phase and octanol phase were gathered together, respectively. Water phase solution was directly used to determine the concentration of water-solubility iron, octanol phase solution was, respectively, slaked with mixed acid, operated as Section 3.1, then used to determine the octanol-solubility concentration of iron.

The concentration of iron in medicine or decoction was determined with FAAS. The linear calibration was obtained by determination the iron working standards.

4. Results and discussion

4.1. Total concentration of iron in medicinal materials and decoctions

The total concentrations of iron in eight medicinal materials and their decoctions are shown in Table 1. The extract percent of iron in eight medicinal materials is the quotient of the total concentration of iron in decoction and in medicinal material. Both the concentration of iron in medicinal material/decoction and the extract percents of iron are quite different. The total concentration of iron in medicinal material or decoction, thomas faeces (E) or radix pseudostellariae (C) is far higher than others, respectively. There is not positive correlation between the total concentration of iron in medicinal material and that in decoction. The total concentration of iron in flos carthami (A) and thomas faeces (E) is close, but the total concentration of iron in decoction, flos carthami (A) is two times of thomas faeces (E). So, the total concentration of iron in decoctions, which is taken orally and actually entered into the digestive site, was dependent not only on the total concentration of iron in medicinal materials but also on the other organic and inorganic composition. In different medicinal material, the compositions are different, then the ligands of iron are different, the species of iron are also different, the soluble compositions of iron are different too. So, the extract percents of iron in medicinal materials rest with the other organic and inorganic composi-

Table 2
Analytical results of water-solubility iron and n-$C_8H_{17}OH$-solubility iron in decoctions under gastric and intestinal acidity (μg/g)

Decoctions		A	B	C	D	E	F	G	H
Gastric acidity (pH 1.3)	Water-solubility iron	47.5	21.7	102.5	45.0	22.5	35.0	32.5	47.5
	Octanol-solubility iron	3.7	1.6	8.0	25.0	3.0	2.8	29.8	32.0
	K_{ow1}	0.08	0.07	0.08	0.56	0.13	0.08	0.92	0.67
Intestinal acidity (pH 7.6)	Water-solubility iron	50.0	8.0	97.5	50.0	11.7	27.5	22.5	47.5
	Octanol-solubility iron	1.2	15.3	13.0	20.0	13.8	10.3	39.8	32.0
	K_{ow2}	0.02	1.91	0.13	0.40	1.18	0.37	1.77	0.67
K_{ow2}/K_{ow1}		0.25	27.29	1.63	0.71	9.08	4.63	1.92	1.00
Iron in decoction		51.2	23.3	110.5	70.0	25.5	37.8	62.3	79.5

Table 3
Effect of compatibility of Flos Carthami(A) and Semen Persicae (B) (1:1) on the extract percent (%)

	Medicine (μg/g)	Decoction (μg/g)	Extract percent (%)
Flos Carthami (A)	1452.5	51.2	3.52
Semen Persicae (B)	177.5	23.3	13.13
Average concentration of A and B	815.0	37.25	4.57
Compatibility of A and B	815.0	70.0	8.59

Table 4
Effect of compatibility of Flos Carthami (A) and Semen Persicae (B) (1:1) on the species of iron in decoction (μg/g)

Decoction	Decoction under gastric acidity		Decoction under intestinal acidity		Iron in decoction
	Water-solubility iron	Octanol-solubility iron	Water-solubility iron	Octanol-solubility iron	
A	47.5	3.7	50.0	1.2	51.2
B	21.7	1.6	8.0	15.3	23.3
C_1	34.60	2.65	29.00	8.25	37.25
C_2	20.0	50.0	22.5	47.5	70.0
C_2/C_1	0.58	18.87	0.78	5.76	1.88

C_1, average concentration of A and B; C_2, compatibility of A and B.

tion in medicine. It is not reasonable to estimate the pharmacodynamical activity of iron just based on the total content in medicine.

4.2. Effect of gastric and intestinal acidity on the species of iron

Octanol-solubility iron has higher pharmacodynamical activity than water-solubility iron for its lipopily and bioavailability [21]. So the content of octanol-solubility iron is the basis of estimation the pharmacodynamical activity of coordinated iron complex in medicines. K_{ow} reflects the distribution of two species of iron. Table 2 indicates the effect of gastric and intestinal acidity on the species of iron, and K_{ow} in eight decoctions of single medicine. It can be seen from K_{ow2}/K_{ow1} (the ratio of K_{ow2} to K_{ow1}) that the effect of the acidity of digestive site on the species and quantification of iron in decoctions are quite different for different decoctions. The effect is little for colla corii Asini (H), the species and quantification of iron in decoction are same under gastric and intestinal acidity, but the effect is great for semen persicae (B), K_{ow2}/K_{ow1} is 27.29, the content of octanol-solubility iron under intestinal acidity is 9.56 times of the same specie of iron under gastric acidity. In eight decoctions, the K_{ow2}/K_{ow1} of semen persicae (B), radix pseudostellariae (C), vespertilio superans thomas faeces (E), flos chrysanthemi (F) and atractylodes macrocephala koidz (G), exceeds 1, the K_{ow2}/K_{ow1} of flos carthami (A) and fructus mori (D) under 1. That is to say, compared with gastric acidity, the concentration of octanol-solubility iron, the lipopily and bioavailability of coordinated iron complex under intestinal acidity enhance in five decoctions, decrease in two decoctions. So the pharmacodynamical activity of coordinated iron complex in different decoction, under different acidity of digestive site, is quite different and it must be the basis of the dosage.

Under different digestive site acidity, the concentration of hydrogen and hydroxide is different. Hydrogen and hydroxide would affect the character and quantity of charge of coordinated iron complex, at the same time, hydroxide might act as a ligand of iron, bring competitive coordinated reaction. In different decoction, the ligand of iron and the stability of coordinated iron complex are

Table 5
Effect of compatibility of Flos Carthami (A) and Semen Persicae (B) (1:1) on K_{ow} in decoction

	K_{ow1} gastric acidity	K_{ow2} intestinal acidity	K_{ow2}/K_{ow1}
Flos Carthami (A)	0.08	0.02	0.25
Semen Persicae (B)	0.07	1.91	27.29
Aver. Concentration of A and B	0.08	0.28	3.5
Compatibility of A and B	2.50	2.11	0.84

different, so the effect of digestive site acidity on the species of iron, lipopily and bioavailability of coordinated iron complex, and K_{ow2}/K_{ow1} are different. The stability constant of coordinated iron complex in colla corii asini (H) is the highest, flos carthami (A) and semen persicae (B) is lower.

4.3. Effect of compatibility on the species of iron

On clinical practice, Flos Carthami (A) and Semen Persicae (B) have similar property and efficacy, are commonly used at the same time to invigorate the circulation of blood and remove blood stasis concurrently for enhancing the pharmacodynamical activity, have remarkable curative effect to cure the cardiovascular disease [23,24]. From our above experiment, in single medicine of Flos Carthami (A) and Semen Persicae (B), the different acidity of stomach and intestine has greatly affected the species, the lipopily and bioavailability of coordinated iron complex. The stability of coordinated iron complex in them is weaker and it would be avail to study the effect of compatibility on the species of iron.

Tables 3–5, respectively, shows the effect of compatibility of flos carthami (A) and semen persicae (B) on extract percent of iron, the species of iron, and K_{ow}. Compared the relevant analytical results with average value of flos carthami (A) and semen persicae (B), we can conclude that compatibility greatly increases the concentration of iron that could be dissolved in water in decoction 87.9%, the extract percent of iron 87.9%, the concentration of octanol-solubility iron 18.87% under gastric acidity and 57.6% under intestinal acidity. The concentration of water-solubility iron under gastric and intestinal acidity decrease 42, 22%, respectively. So, by means of compatibility, the content of octanol-solubility iron, the lipopily and bioavailability of coordinated iron complex in

Table 6
Analytical result of iron in decoction ($n = 10$)

Medicine	Added iron (μg/g)	Iron found (μg/g)	Recovery (%)	R.S.D. (%)
Flos Carthami (A)	0.0	51.2	–	0.44
	50.0	100.6	98.8	1.02
Semen Persicae (B)	0.0	23.3	–	0.39
	20.0	43.8	102.5	0.77
Radix Pseudostellariae (C)	0.0	110.5	–	1.18
	100.0	210.2	102.5	0.12
Fructus Mori (D)	0.0	70.0	–	0.34
	50.0	120.5	101.0	0.67
Superans Thomas Faeces (E)	0.0	25.5	–	0.40
	20.0	45.3	99.0	0.51
Flos Chrysanthemi (F)	0.0	37.8	–	0.76
	20.0	57.7	99.5	0.89
Atractylodes Macrocephala Koidz (G)	0.0	62.3	–	0.62
	50.0	111.5	98.4	0.39
Colla Corii Asini (H)	0.0	79.5	–	0.58
	50.0	129.1	99.2	1.23

decoction enhances greatly, the pharmacodynamical activity improves. This experiment proved the relevant compatibility law and clinical experience. Compatibility also weakens the effect of acidity on the species of iron and K_{ow}. Since during the decoct, all of compositions in flos carthami (A) and semen persicae (B), including the inorganic and organic substances, are taking chemical reaction or physical change within or between the single medicine. Compared with the single medicine, the ligands of iron change, so the species of iron changes, the content of soluble composition and octanol-solubility iron increases, then the stability, lipopily and bioavailability of coordinated iron complex enhance.

4.4. Recovery test

The precision and accuracy of pretreatment to medicinal materials, and the reliability of analytical method are tested by spiking the samples. The results in Table 6 showed that the recoveries were reasonable for the analysis of iron in medicines, in range of 98–103%. No systematic error could be seen in the added-found method for determination of iron with FAAS in samples of phytomedicine or its decoction.

5. Conclusion

There is not positive correlation between the total concentration of iron in medicines and that in decoctions. The solubility of iron in medicine rests with the other organic and inorganic composition in medicine. It is not reasonable to estimate the pharmacodynamical activity of iron just based on the total content in medicine.

Viewed on the difference of the lipopily and bioavailability, the octanol- and water-solubility could be used to define the species of iron in medicine, to estimate the lipopily and bioavailability of coordinated iron complex. The difference acidity of digestive site, difference compositions of single medicine, and the compatibility of medicines, have greatly affected on the species and quantification of iron in phytomedicine. Four factors, the extract percents of iron, the acidity of digestive site, the compatibility of medicines, especially the concentration of octanol-solubility iron, could be the basis of the dosage.

References

[1] T.L. Pauline, H. Marja, A.P. Per, Y. Young, Mol. Aspects Med. 22 (2001) 1.
[2] A.R. Tracey, Pediatric Neurol. 2 (2001) 130.
[3] H. Tapiero, L. Gate, K.D. Tew, Biomed. Pharmacother. 55 (2001) 324.
[4] H. De-Fang, K. Heng, Chin. J. Studies Trace Elements Health 2 (2001) 80.
[5] M. Jin-Feng, Chinese J. Studies Trace Elements Health 3 (1999) 72.
[6] L.W. Powell, Gut 11 (1970) 980.
[7] A. Reunanen, H. Takkunen, P. Knekt, R. Seppanen, A. Aromaa, J. Intern. Med. 238 (1995) 223.
[8] R.G. Stevens, B.I. Graubard, M.S. Micozzi, K. Neriishi, B.S. Blumberg, Int. J. Cancer 56 (1994) 364.
[9] J.I. Wurlzeman, A. Sliver, D.M. Schreinemachers, R.S. Sandler, R.B. Everson, Cancer Epidemiol. Biomarkers Prev. 5 (1996) 503.
[10] A.M.Y. Lin, Free Radic. Biol. Med. 3 (2001) 225.
[11] E. Mendez-Alvarez, R. Soto-Otero, A. Hermida-Ameijeiras, M.E. Lopez-Martin, J.L. Labandeira-Garicia, Free Radic. Biol. Med. 8 (2001) 986.
[12] S.Y. Qian, G.R. Buettner, Free Radic. Biol. Med. 11/12 (1999) 1447.
[13] F. Gavino, C. Guido, Coord. Chem. Rev. 184 (1999) 291.
[14] O.J. Omolo, S.C. Chhabra, G. Nyagah, J. Ethnopharmacol. 58 (2) (1997) 97.
[15] Z.-Q. Cao, Chinese J. Acta Universitatis Traditions Medicalis Sinensis Pharmacologiaeque Shanghai 1 (2000) 36.
[16] Z. Xu-Xiang, M. Han-Bin, Chin. Herbal Med. 6 (1997) 373.
[17] Z. Qi-Feng, P. Shan-Shan, Z. Ning, W. Jian-Hong, L. Li, H. Jian-Jun, Chin. J. Spectroscop. Spectr. Anal. 2 (2001) 237.
[18] L. Lei, X. Ming-Yong, S. Zhen-Hua, W. Xi-Hong, S. Da-Hai, W. Xiao-Ru, Chem. J. Chin. Univ. 5 (2000) 707.
[19] Y. Yu-Qiong, H. Rong, Chin. J. Spectroscop. Spectr. Anal. 2 (1994) 73.
[20] K.J.H. Wienk, J.J.M. Marx, A.C. Beynen, Eur. J. Nutr. 2 (1999) 51.
[21] Z. Wen-Sheng, L. An-Liang (Eds.), Medicinal Chemistry, Higher Education Press, Peking, China, 1999, p. 6.
[22] Z. Guo-Jiang (Ed.), Physiology, People Sanitation Press, Peking, China, 1998, p. 110.
[23] W. Ben-Xiang (Ed.), Modern Pharmacology of Traditional Chinese Medicines, Tianjin Science and Technology Press, Tianjin, China, 1997, p. 600.
[24] L. Sen-Lin, H. Jing, L. Jiang, Chinese J. Studies Trace Elements Health 2 (1997) 33.