

An Optimization Method for Experimental Conditions of Spectrophotometric Determination of Metal Complexes

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Abstract

Spectrophotometry is an attractive alternative to titration method and gravimetric method when determining the concentration of metal ions, but there are many options in chromogenic agent, pH and chromogenic time, we choose phenanthroline as chromogenic agent and apply orthogonal experimental design to optimize the combination. We then used and compared Job method with molar ratio method in the determination of the composition of Tris(1,10-phenanthroline) iron, we hold the opinion that molar ratio method is more general and more effective in this experiment. Under the optimized condition, we can plot a standard curve from measurement of ferric standard solutions of gradiently ascending concentration, with which we can reasonably predict the purity of our self-made iron(II) oxalate dihydrate; we get results very close to what is measured using titration method, while titration method behaves much worse in respect of meeting the requirements of green chemistry.

Introduction

Metal is a indispensable component of this colorful world and our life and usually exists as complexes. So we always need a green, effective and efficient method to determine composition and concentration of metal complex of ordinary, micro, trace quantity in samples such as minerals, blood plasma or serum^{1,2} and plants.^{3,4} As is widely accepted, titration method and gravimetry method behave very well when determining metal ions of ordinary quantity. However, they both fail to work if micro or trace analysis is required. Furthermore, neither redox titration nor coordination titration is a preferred choice for the green determination of iron; gravimetry method is complicated and finicky to operate, as well as not energy-saving. Spectrophotometry based on Beer-Lambert Law promise a larger potential as green and effective methods to determine metal ions of either ordinary or trace quantity.⁵⁻⁷ But practically there are so many options in chromogenic agent,⁸⁻¹⁰ and other factors to vary such as chromogenic time, properties of the determination system, etc.

As a highly representative metal, iron is the most used metal in the world, and is broadly distributed in nature and human bodies. It's cheap and often suitable for us to explore general determination method. So, to carry out our work and illustrate our ideas conveniently, we choose iron(II) together with phenanthroline to demonstrate the great role of orthogonal design in optimizing experimental conditions, namely, λ (wavelength), pH (amount of NaAc), T (chromogenic time) and V_{phen} (amount of phenanthroline). A noticeable problem is that the size of orthogonal table will explode as the number of level of even just one factor increases. Wavelength is exactly the very factor with lots of levels, but we can deal with this by varying wavelength as unifactor and get a narrower range to take care according to the changing trend of absorbance. To show advantages and disadvantages of two method for determining the composition of metal complexes, we choose complex of copper(II) and sulfosalicylic acid together with iron(II) complex mentioned above to form a bright contrast, and reveal the relationship between coordination number and the better method. After that, we'll determine the purity of some self-made $FeC_2O_4 \cdot 2H_2O$ solid by determining concentration of iron ion

using both titration method and spectrophotometry. While titration method produced a lot of waste, spectrophotometry tackles with the problem using a simple standard curve. And two results being in conformity will inform us that the improved spectrophotometry method behaves very well in determining concentration of ion and is undoubtedly the most direct approach to meet the requirements of green chemistry. The experiment outline is sketched below.

1. Find the optimization conditions using orthogonal design
 - (a) Narrow wavelength range
 - (b) Orthogonal experiment
2. Determine composition of metal complex
 - (a) Job method
 - (b) Molar ratio method
3. Measure purity of $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$
 - (a) Spectrophotometric method
 - (b) Titration method

Experimental

Narrow wavelength range

We first prepared 12 identical solution with phen, NaAc and hydrochloride inside without drawing attention to the concrete concentration. Then add 1.00cm^{-3} $1.00 \times 10^{-3}\text{mol}\cdot\text{dm}^{-3}$ ferric standard solution and dilute to 50mL. The absorbance were measured respectively after the same time interval, which are shown in Table 2. We can see that the optimal wavelength must lie in 490-520nm.

Orthogonal experiment

We studied four factors that have five levels each, and they are shown in Table 1 below.

Table 1: Factors

Index	λ /nm	V_{phen} /mL	V_{NaAc} /mL	T /min
1	488	0.5	1	4
2	498	1	3	6
3	508	2	5	8
4	518	3	7	10
5	528	4	9	12

We first prepared 0.15%phen solution, 1mol/L NaAc solution, 10% hydroxylamine hydrochloride solution, $100\mu\text{g}\cdot\text{cm}^{-3}$ and $20\mu\text{g}\cdot\text{cm}^{-3}$ ferric standard solution. The NaAc solution could be used to roughly adjust pH and hydroxylamine hydrochloride solution was used to prevent iron(II) from being oxidized. According to the chosen factors, we established a $L_{25}(5^6)$ orthogonal table, which is shown in Table 3, as well as the absorbance measured according to Table 3.

Composition determination

By molar ratio method First $1.00 \times 10^{-3} \text{mol}\cdot\text{dm}^{-3}$ ferric standard solution, 1mL hydroxylamine hydrochloride and 7mL NaAc were added into eight 50mL beakers in order, and then 0.00mL, 1.00mL, 1.50mL, 2.00mL, 2.50mL, 3.00mL, 3.50mL, 4.00mL, 4.50mL 0.15%phen were added to each beaker, and the solutions were accurately diluted to 50mL with volumetric flasks. The first solution without phen added served as reference solution. Adjusting the wavelength of spectrophotometer to 508nm, absorbance of each solution was measured accurately after 12min and shown in Table 6.

By Job method According to the Table 7 and Table 8, solutions were prepared and absorbance of which were measured after 12min. The results are also shown in Table 7 and Table 8.

Purity measurement of $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$

Synthesis of $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ 10.00g $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ was dissolved in 15mL deionized water, and the solution was acidized with 3mL 2mol/L H_2SO_4 ; the beaker was heated until $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ dissolved completely. Then 60mL 1mol/L $\text{H}_2\text{C}_2\text{O}_4$ was added into the beaker, and the solution was heated until it boiled, stirring continuously was necessary to prevent splashing. After standing precipitation for a while, the supernatant should be poured out. Then the precipitation was well washed by deionized water and collected by suction filtration. The product was washed by acetone twice and then dried and weighed for further use.

Spectrophotometric method

Plot standard curve According to the optimum combination deduced above, iron solutions of gradiently ascending concentration were prepared by adding 0.0mL, 1.0mL, 2.0mL, 3.0mL, 4.0mL, 5.0mL $20\mu\text{g} \cdot \text{mL}^{-1}$ ferric standard solution into six 50mL volumetric flasks, and then 1mL hydroxylamine hydrochloride, 7mL NaAc, 2mL 0.15%phen were added in order. Adjusting the wavelength of spectrophotometer to 508nm, absorbance of each solution was measured accurately after 12min and shown in Table 9.

Concentration determination 0.2g self-made $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ was dissolved by 15mL 2mol/L H_2SO_4 in beaker and diluted accurately to 250mL using a volumetric flask, and then diluted 100 times. And absorbance of which was measured and shown in Table 10.

Titration method 1.8g-2.0g $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ was dissolved by 25mL 2mol/L H_2SO_4 in beaker and heated to 40-50°C, then diluted accurately to 250mL using a volumetric flask. 25.00mL of the solutions were added into conical flask. And it was titrated by standard potassium permanganate solution, with a consumption of $V_1\text{mL}$. Then we used about 0.1g zinc powder to reduct Fe(III) ion, the solution was heated until it boiled for about 10min

to ensure the iron was reduced completely. And we added a small amount of H_2SO_4 to dissolve residual zinc powder. Then the solution was titrated again by standard potassium permanganate solution, with a consumption of $V_2\text{mL}$. The only available Xiao-Jie Zhou's results in our group are shown in Table 12 and Table 11.

Results and discussion

Find the optimization condition

Narrow wavelength range

Table 2: Unifactor λ

Index	λ/nm	<i>Absorbance</i>
01	450	0.305
02	460	0.333
03	470	0.362
04	480	0.378
05	490	0.385
06	500	0.399
07	510	0.409
08	520	0.374
09	530	0.292
10	540	0.196
11	550	0.112
12	560	0.062

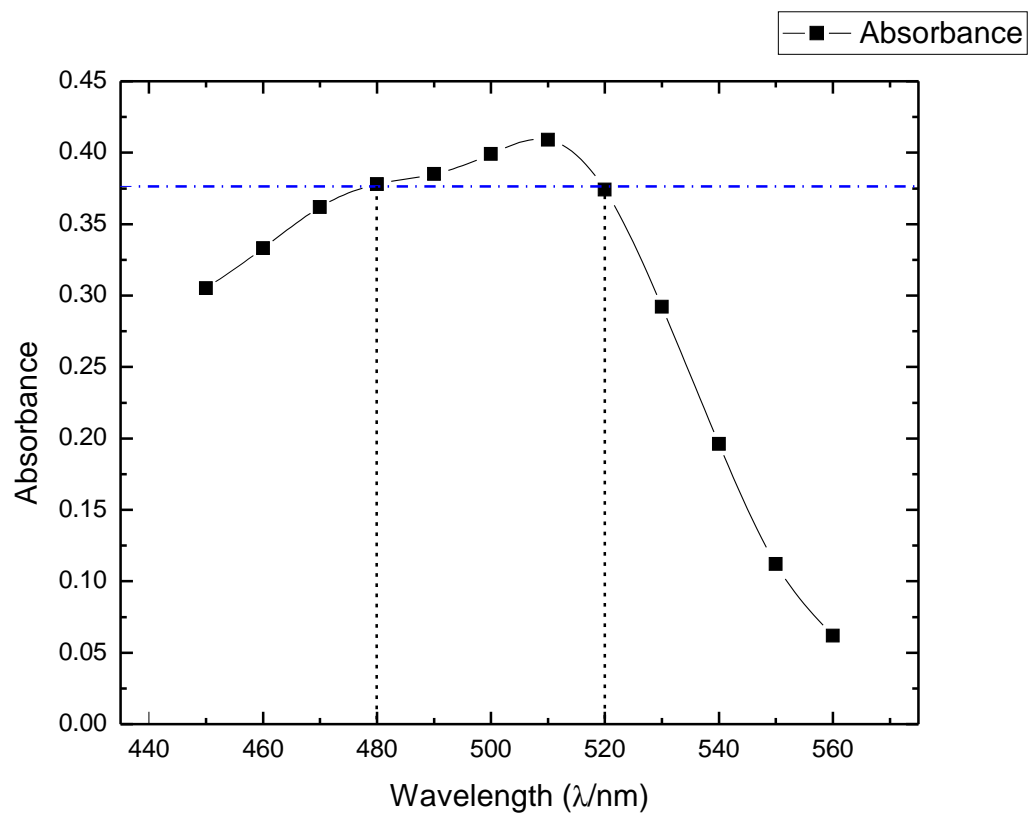


Figure 1: Molar ratio method

We can see intuitively from the Figure 1, the wavelength corresponding to the absorbance peak must lie in $480\text{-}520\text{nm}$.

Orthogonal experiment

Table 3: Orthogonal Table

Index	λ/nm	2	$V_{\text{phen}}/\text{mL}$	T/min	$V_{\text{NaAc}}/\text{mL}$	6	<i>Absorbance</i>
01	488	1	0.5	4	1	1	0.305
02	488	2	1	6	3	2	0.391
03	488	3	2	8	5	3	0.397
04	488	4	3	10	7	4	0.388
05	488	5	4	12	9	5	0.395
06	498	1	1	8	7	5	0.416
07	498	2	2	10	9	1	0.400
08	498	3	3	12	1	2	0.416
09	498	4	4	4	3	3	0.403
10	498	5	0.5	6	5	4	0.319
11	508	1	2	12	3	4	0.413
12	508	2	3	4	5	5	0.433
13	508	3	4	6	7	1	0.416
14	508	4	0.5	8	9	2	0.338
15	508	5	1	10	1	3	0.407
16	518	1	3	6	9	3	0.388
17	518	2	4	8	1	4	0.390
18	518	3	0.5	10	3	5	0.320
19	518	4	1	12	5	1	0.392
20	518	5	2	4	7	2	0.400
21	528	1	4	10	5	2	0.305
22	528	2	0.5	12	7	3	0.273
23	528	3	1	4	9	4	0.309
24	528	4	2	6	1	5	0.307
25	528	5	3	8	3	1	0.317

We process the data from Table 3 by range analysis. We use T_i and K_i to represent summary and average of absorbance corresponding to the i^{th} level referred in Table 1. The results are shown below in Table 4.

Table 4: Experimental data processing

	λ/nm	V_{phen}/mL	T/min	V_{NaAc}/mL
T_1	1.876	1.555	1.850	1.825
T_2	1.854	1.915	1.821	1.844
T_3	2.007	1.917	1.858	1.846
T_4	1.890	1.942	1.820	1.893
T_5	1.511	1.909	1.889	1.830
K_1	0.3752	0.3110	0.3700	0.3650
K_2	0.3708	0.3830	0.3642	0.3688
K_3	0.4014	0.3834	0.3716	0.3692
K_4	0.3780	0.3884	0.3640	0.3786
K_5	0.3022	0.3818	0.3778	0.3660
Range	0.0992	0.0774	0.0138	0.0136

We compare the range of each factor and draw the conclusion that wavelength and the amount of chromogenic agent have a significant impact on absorbance because their ranges are much greater, while the chromogenic time and pH have relatively weak influence. Meanwhile, Table 4 tells us the best combination of factors, on which all later absorbance measurement are based:

Table 5: Optimum combination of factors

	λ	V_{phen}	T	V_{NaAc}
Optimum Index	3(2.007)	4(1.942)	5(1.889)	4(1.893)
Concrete Data	508 nm	3 mL	12 min	7 mL

Determine composition of metal complex

Molar ratio method

The function of fitting line on the left is $y = 0.742x + 0.0264 (R = 0.9979)$, and the function of fitting line on the right line is $y = 0.254 (R = 1)$ which are shown below in Figure 2. According to the figure, we know coordination number measured by molar ratio method is 3.07.

Table 6: Molar ratio method

Index	1	2	3	4	5	6	7	8
V_{phen}/mL	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50
V_{Fe}/mL	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$\frac{V_{phen}}{V_{Fe}}$	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50
Absorbance	0.100	0.140	0.172	0.213	0.252	0.254	0.254	0.254

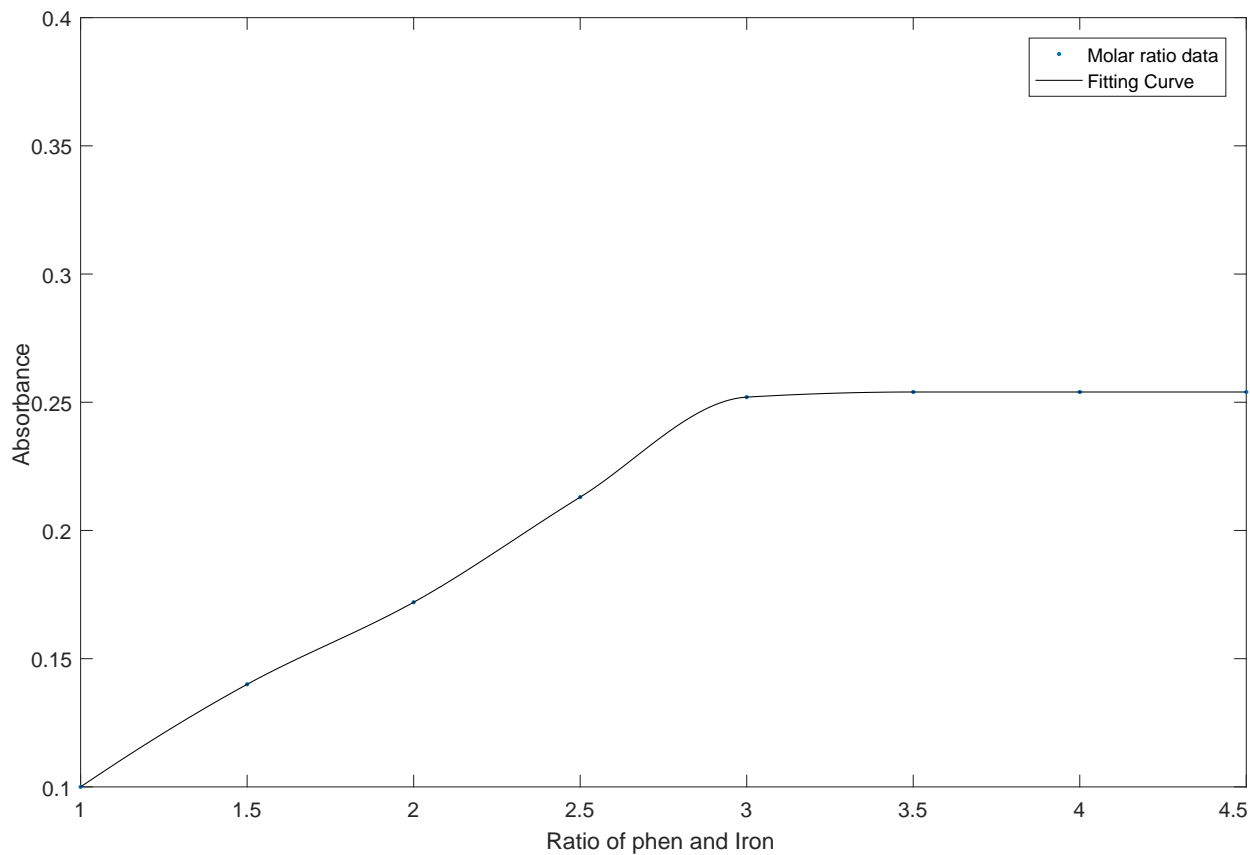


Figure 2: Molar ratio method

To some extent, the correlation coefficient reflects the superiority of the molar ratio method in the terms of accuracy. A reason why it is more accurate than Job method is that when $\frac{V_{phen}}{V_{Fe}} \geq 3$, the slope of the line is almost 0 in theory. So compared with Job method, the molar ratio methods correlation coefficient is closer to 1 which indicates better precision. In a word, as for studying more general coordination compound, typically whose coordination number is not 1, the molar ratio method behaves much better. According to

the figure, we know coordination number measured by Job method is 2.79.

By Job method

We use T_L to represent $\frac{V_{phen}}{V_{Fe}+V_{phen}}$. When $T_L < 0.75$, the fitting line is

$$y = 0.733x - 0.030 (R = 0.998)$$

, and when $T_L \geq 0.75$, the fitting line is

$$y = -1.908x + 1.904 (R = 0.994)$$

They are shown below in Figure 3.

Table 7: Job method of iron

Index	1	2	3	4	5	6	7	8	9
V_{phen}/mL	0.0	1.8	2.4	3.0	3.5	4.0	4.4	4.8	5.3
V_{Fe}/mL	8.0	6.2	5.6	5.0	4.5	4.0	3.6	3.2	2.7
T_L	0.00	0.23	0.30	0.38	0.44	0.50	0.55	0.60	0.67
<i>Absorbance</i>		0.141	0.185	0.252	0.292	0.330	0.377	0.410	0.451
Index	10	11	12	13	14	15	16	17	18
V_{phen}/mL	5.7	6.0	6.2	6.4	6.6	6.9	7.2	7.5	8.0
V_{Fe}/mL	2.3	2.0	1.8	1.6	1.4	1.1	0.8	0.5	0.0
T_L	0.71	0.75	0.78	0.80	0.83	0.86	0.90	0.94	1.00
<i>Absorbance</i>	0.490	0.490	0.405	0.368	0.320	0.258	0.186	0.117	

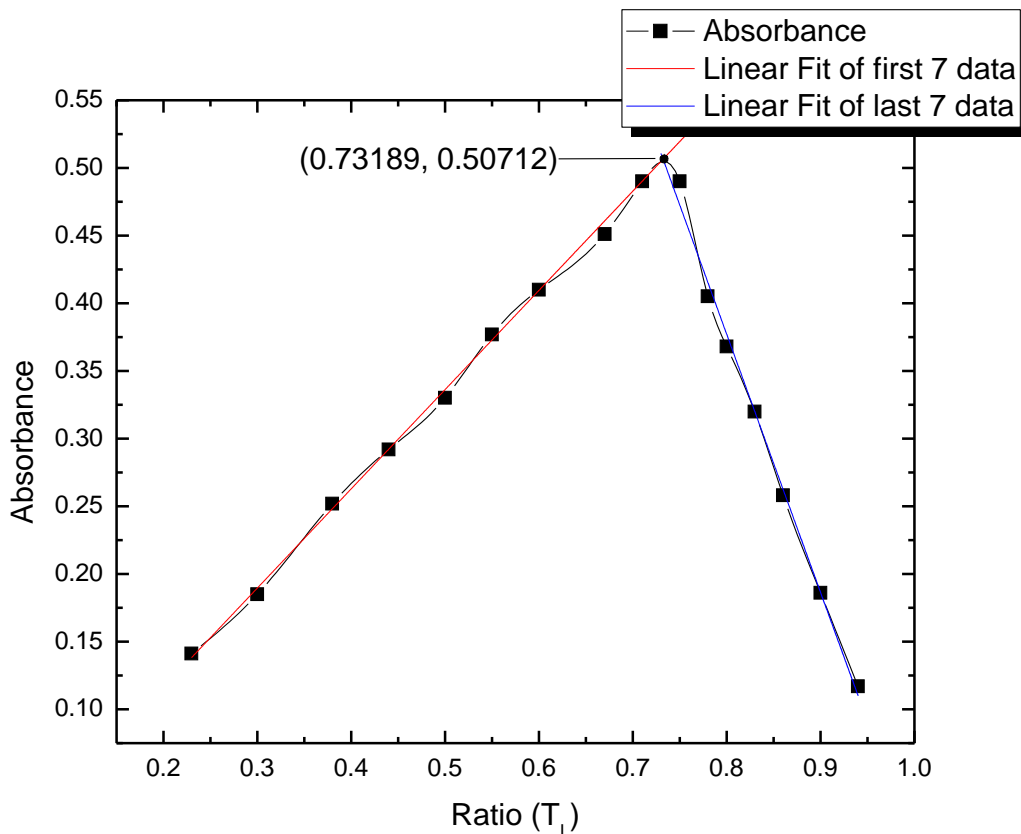


Figure 3: Job method of iron

So the measured ratio of phen and Fe n_{Fe} is calculated by

$$n_{Fe} = \frac{T_L}{1 - T_L} = 2.70$$

The reason why the measured ratio is smaller than theoretical number is when $T_L \geq 0.75$, the data points we used to fit the line is compressed in the X scale, which means great loss of accuracy. And this is unavoidable when the ratio of anion and cation isn't 1. Besides, the slope of the theoretical line on the right is so great, which indicates a large relative deviation. Specifically, let's see the results of determining composition of copper(II) and sulfosalicyclic acid complex whose ratio of anion and cation is 1 using Job method.

We use T_L to represent $\frac{V_{H_3SSR}}{V_{Cu(NO_3)_2} + V_{H_3SSR}}$. When $T_L < 0.5$, the fitting line is

$$y = 0.748x + 0.026 (R = 0.987)$$

and when $T_L \geq 0.5$, the fitting line is

$$y = -0.818x + 0.850 (R = 0.998)$$

They are shown below in Figure 4.

Table 8: Job method of copper

Index	1	2	3	4	5	6	7
V_{H_3SSR}/mL	0	2	4	6	8	10	12
$V_{Cu(NO_3)_2}/\text{mL}$	24	22	20	18	16	14	12
T_L	0	0.083	0.167	0.250	0.333	0.417	0.500
<i>Absorbance</i>	0.037	0.076	0.140	0.225	0.274	0.296	0.312
Index	8	9	10	11	12	13	
V_{H_3SSR}/mL	14	16	18	20	22	24	
$V_{Cu(NO_3)_2}/\text{mL}$	10	8	6	4	2	0	
T_L	0.583	0.667	0.750	0.833	0.917	1.000	
<i>Absorbance</i>	0.310	0.298	0.244	0.171	0.097	0.031	

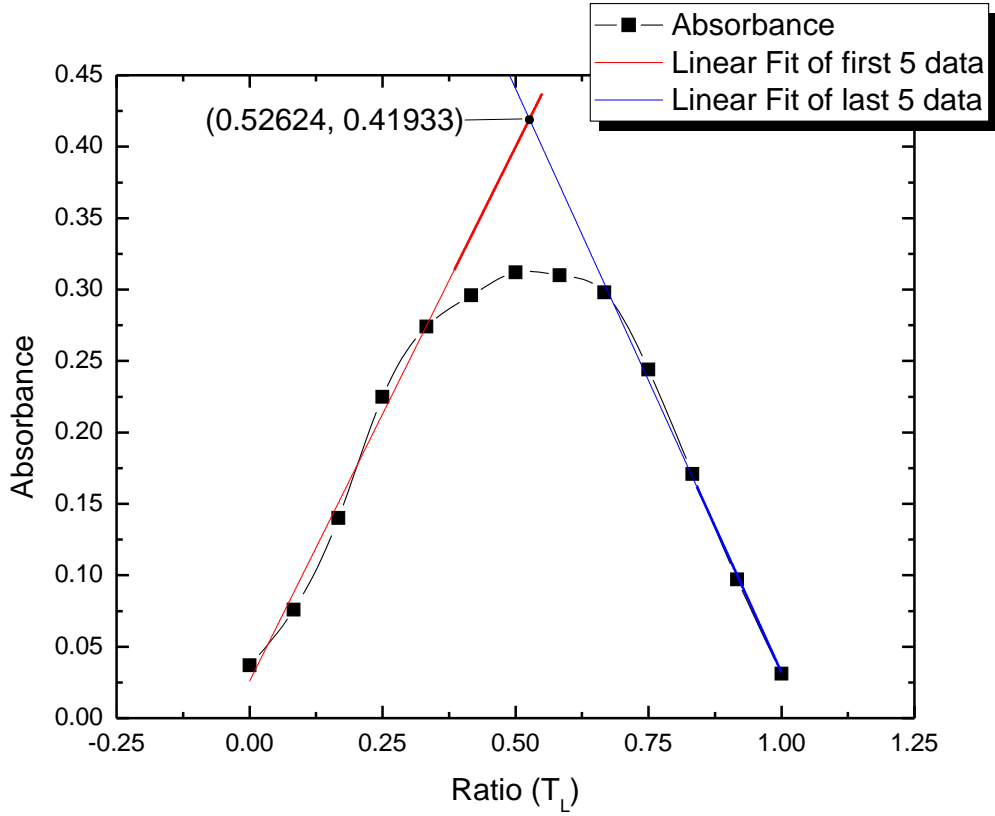


Figure 4: Job method of copper

So the measured ratio of H_3SSR and Cu n_{Cu} is calculated by

$$n_{Cu} = \frac{T_L}{1 - T_L} = 1.11$$

Notice that in this condition, we use much less data points to fit and get a better result.

So it's very clear that if the ratio of anion and cation or vice versa is 1 or close to 1, the position of absorbance peak is easy to tell and the line on the two sides are fitted more accurately with less point. So molar ratio method is always available in principle, while as for complexes with large ratio, such as $[Fe(CN)_6]^{3-}$, it's reasonable to predict that the absorbance peak will be pushed aside along with the axis, which is really difficult for us to tell it's accurate composition both technically and mathematically.

Purity measurement of $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$

Spectrophotometric method

Plot standard curve Using data from Table 9, we plot the standard curve which is shown in Figure 5, the function of fitting line is $y = 0.2026x + 0.00076$ ($R = 0.9995$), so it is reasonable to think it pass the origin accurately. Then we can use this curve to determine the concentration of iron in unknown sample as long as we dissolve it and measure its absorbance.

Table 9: standard curve data

V_{Fe}/mL	0.0	1.0	2.0	3.0	4.0	5.0
$c_{\text{Fe}}/\mu\text{g}\cdot\text{mL}^{-1}$	0.0	0.4	0.8	1.2	1.6	2.0
<i>Absorbance</i>	0.000	0.081	0.166	0.247	0.316	0.410

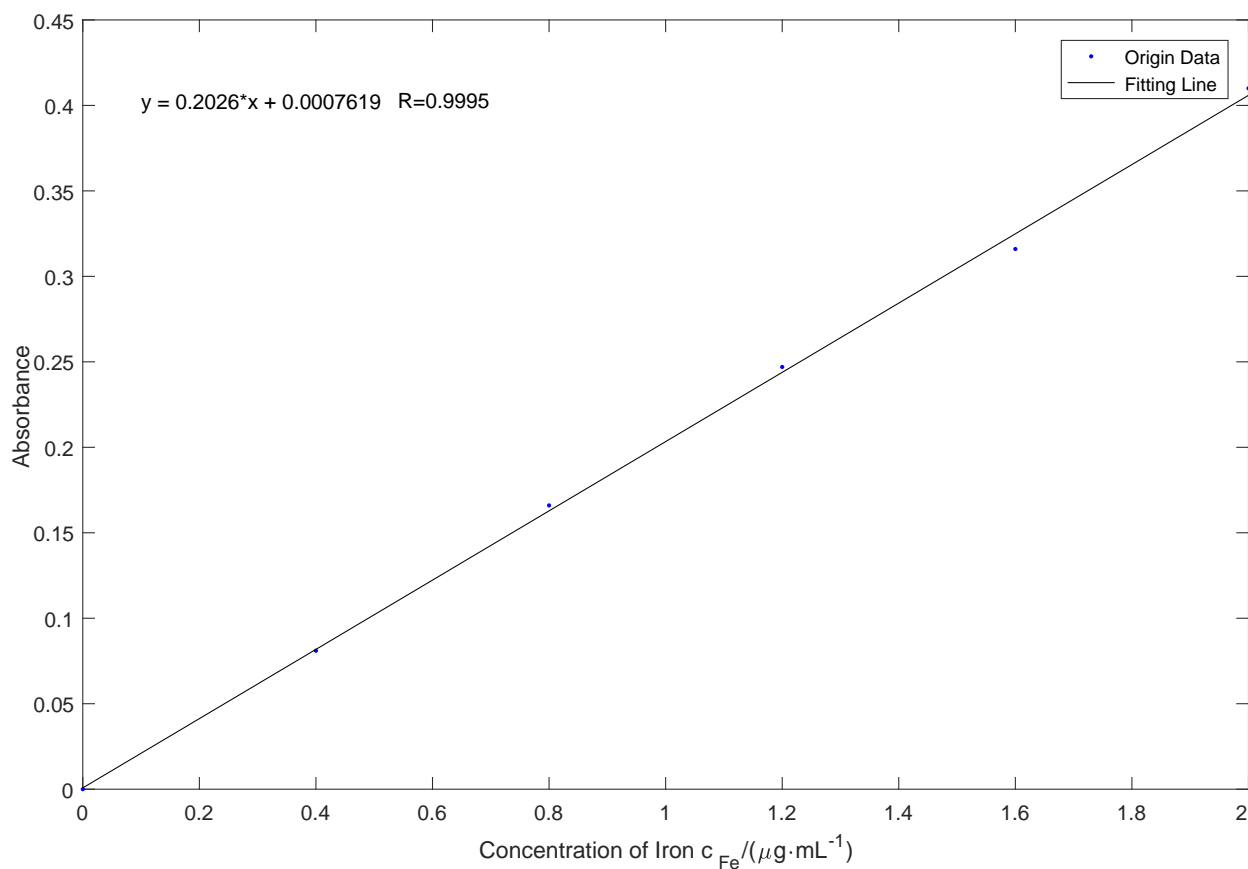


Figure 5: standard curve

Concentration determination We apply the standard curve to determine the concentration of iron in our self-made $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ sample, therefore getting purity easily by simply calculating:

$$\omega = \frac{c_{\text{Fe}} \times 250\text{mL} \times 179.9\text{g/mol}}{m_{\text{sample}}}$$

Table 10: Purity Measurement by standard curve

Sample Index	Mass/g	Absorbance	$c_{\text{Fe}}/\mu\text{g}\cdot\text{mL}^{-1}$	purity
1	0.2083	0.546	2.52	97.2%
2	0.2170	0.534	2.46	91.1%
3	0.2023	0.501	2.31	91.8%
4	0.2020	0.509	2.35	93.5%
5	0.2048	0.517	2.38	93.4%
6	0.2184	0.530	2.44	89.8%

By titration method

We can first get the concentration of standard potassium permanganate solution easily using data from Table 11 by calculating:

$$c_{\text{KMnO}_4} = \frac{2}{5} \times \frac{m_{\text{NaC}_2\text{O}_4}}{134.0\text{g/mol} \times V_{\text{average}}} = 0.01895\text{mol/L}$$

Table 11: standard of KMnO_4

Index ^a	V_0/mL	$V_{\text{average}}\text{mL}$
1	24.44	
2	24.42	24.43
3	24.42	

^a: Mass of sample $\text{NaC}_2\text{O}_4=6.2013\text{g}$

Table 12: Titration of $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$

Index ^a	V_{1start}/mL	V_{1end}/mL	$V_{1average}/\text{mL}$	V_{2start}/mL	V_{2end}/mL	$V_{2average}/\text{mL}$
1	0.10	30.60		0.00	9.85	
2	0.00	30.48	30.49	0.00	9.90	9.85
3	0.00	30.50		0.00	9.80	

^a:Mass of sample $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O} = 1.8012\text{g}$;

Then we can easily calculate the purity by calculating:

$$\omega = \frac{5}{2} \times \frac{V_{2average} \times c_{\text{KMnO}_4} \times 179.9\text{g/mol}}{m_{\text{sample}}} \times \frac{250\text{mL}}{25\text{mL}} = 93.2\%$$

Accuracy comparison

The comparison of purity measured between titration method and spectrophotometry is shown in Table 13.

Table 13: Purity comparison

	Titration	standard
Purity	93.2%	93.4%

We find the result of using standard curve is in conformity with that of using titration method, therefore validating the accuracy of spectrophotometry and illustrating that spectrophotometry really meets the requirement of green chemistry.

Conclusion

We report that using spectrophotometry to determine iron of micro even trace quantity is effective and meets the requirements of green chemistry. And we apply orthogonal experimental design to optimize the combination of some factors. Meanwhile, we knew the ratio of phen and iron in the complex is 3 : 1 and the ratio of H_3SSR and copper is 1 : 1, we analyzed the curve and drew a conclusion that molar ratio method is more general and effective in

determine composition of coordinary compound. Then we plotted a standard curve, with which we can predict the concentration of iron in unknown sample. The result is very accurate, which is validated by measuring the purity of $\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$. Most importantly, the chromoginice agent can be specifically chosen in various conditions so spectrophotometry can be widely applied to diverse ions of diverse quantity.

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