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Study the Effect of adding Ascorbic acid, Folic acid and Calcium on Stability Iron(II) in Physiological media

Th. Al-Ghabra[1]*, L. Mamoli[1]

[1]Department of Chemistry, Faculty of Sciences, University of Damascus, Syria

***Corres.author: ghabra2004@yahoo.com**

Telephone : 009633717687, Mobile Number : 00963955682040

Abstract: The aim of this research is a chemical study of the absorption Iron(II) in physiological media in the human body and to determine the optimum conditions for the absorption within the duodenum.The study has been carried on to pharmaceuticals that contain a high percentage of iron in laboratory conditions resemble physiological conditions within the duodenum including pH and temperature.Also to study the effect of adding some materials on stability of Iron II in physiological media to decide Whether this effect is positive or negative such as ascorbic acid , folic acid and calcium.We have also put optimum conditions of substances that increase the absorption of Iron (II) for substances that have a positive effect on the stability of Iron(II) such as ascorbic acid and folic acid and we have studied separation time between doses of iron and other materials that have a negative effect on the stability of Iron(II) in the duodenum such as calcium.

Key words: physiological media – duodenum- Stability iron (II) - folic acid - ascorbic acid - separation time.

Introduction:

Iron exists in the structure of many of the cells in the human body, such as hemoglobin (Hb), muscle tissue (meloglobin) responsible for storing oxygen in the muscles and the enzymes responsible for the oxidation of carbohydrates and fat within the body.And is one of the most important metal ions in the human body.

Intestine absorbs iron on duo/dual forms because it is the least availabe form and not constant.[1] We study the factors that increase the stability of duo iron that lead to increase the absorption process in the intestine and enable the human body to benefit from it optimallyto the most.

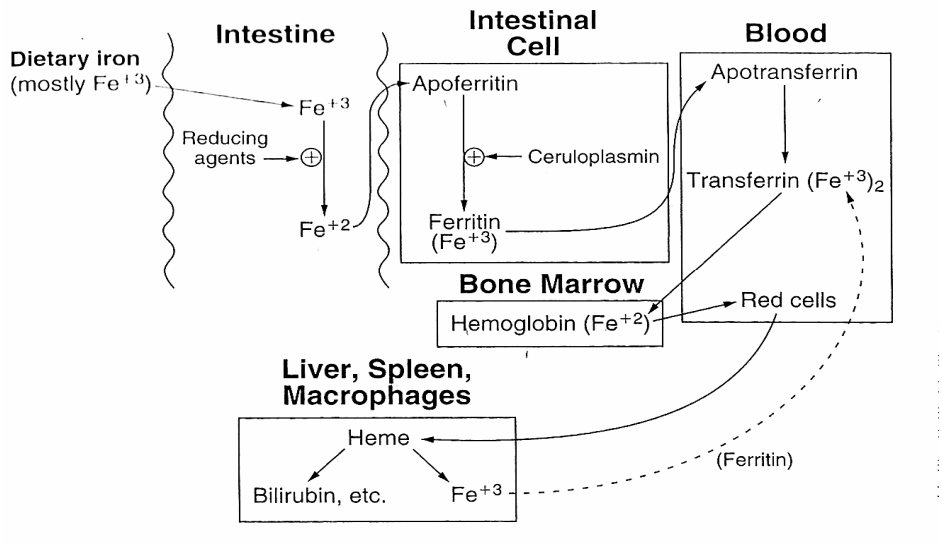
Mechanism of iron absorption in the human body:

Iron is absorbed in the upper part of the intestine by 5-10% of the iron exists in food in the presence of a factor returns such as vitamin C .The remaining iron becomes a burden on the colon.[2]

Iron is absorbed in the wall of the intestine in duo form, and if the proportion of duo forms is increased the absorption of iron will be increased within intestine, after the duo iron crossing the wall of intestine it is re-oxidized again to triple iron and associated with a protein called ferritin because it is not allowed to triple iron

to be free without link in the bloodstream and then travels through the blood to be used in the body such as the liver, bone marrow and other organs.

Upon its arrival to the centers of its use, triple iron liberates from ferritin to be used in the synthesis of red blood cells, or to be used in other uses.



Figure(1) absorption of duo iron in the human body.[1]

Study Reference :

(although I made a wide search ,I could not find a rsearch pertaining to this studyafter 2008)

A study is published in Human Nutrition Journal In 1986 :
As a result 299 studies presented in this area. Meals containing non hemi iron and knighted radioactive isotope of iron found that diets did not contain ascorbic acid, iron absorption was less absorbed than that contained ascorbic acid.[3]

A study published in the Journal of Clinic Nutrition in 2001:
Absorption of iron was studied in the presence of vitamin C on three periods:Long-term ,medium-term and short-term .
The absorption of iron Increased to 14% in long-term, the absorption of iron Increased to 14% in medium-term and increased to 12% in short-term .[4]

A study published in 1965 in the British medical journal
This study showed that there is a relationship between iron deficiency in pregnant women and folic acid found that giving doses of folic acid reduces the likelihood of anemia in pregnant women .[5]

A study was conducted by the University of Kansas in the United States in 1991:
Used 3 types of calcium salts-
Calcium phosphate calcium carbonate and, calcium citrate
The first reduced iron absorption about 62-65%,the second reduced iron absorption about 49-50% and the third reduced iron absorption about 20-25%.

Therefore proposed the use of calcium carbonate as a dietary supplement.[6]

A study was published in Food Chemistry in 2006:
It studied suitabl pH on the stability of duo iron that has been added to fruit juices .

It used reagent pyridine derivatives in the measurement method to determine the optimal conditions to realize the stabilization of the added iron and color consistency of the juice that contains citric acid.
the result of the study showed that the proper pH range is between 5-8 .[7]

A study was published in Meta Science in 2007:
It studied the effect of adding ascorbic acid and citric acid on the stability of beef coulor.

It showed that the color stability increases when ascorbic acid is added for meat.This stability was increased when the concentration of ascorbic acid was raised from 3% to 10% this result attributed to the reduction properties of this acid .
By adding the combination of ascorbic acid and citric acid the stability of chromatography of beef decreased because of the oxidizing properties of citric acid.[8]

Research Materials and Methods

First- Ferrous Sulfate Heptahydrate (FeSo4.7H2O)
SECOND-PAR Reagent: 4-(2-Pyridylazo)resorcinol monosodium salt mono hydrate indicator reagent.

We use sodium salt of PAR which has the outlined formula
C11H8N3NaO2 *H2O
that reacts with Duo Iron to make complex by the following equation:

4-(2-pyridylazo)resorcinol= H2L .
$MX2 + 2H2L \rightarrow M(HL)2+ 2HX$

where M = FeII، (X = Cl)

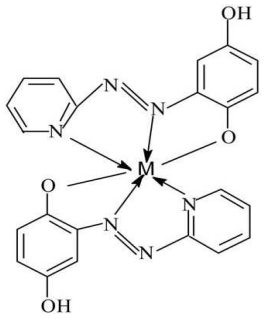


Figure (2) Chemical formula of PAR complex.[9]

Third- (borax 0.05 Molar):
Sodium salt of boric acid (tetra sodium borate) prepared by mixing sodium hydroxide with boric acid.
Na2B4O7.10H2O
Borax Mouka solution is used in the measurement method .
Fourth- folic acid 0.1 Molar C19H17N7O6
Fifth- ascorbic acid (0.1 Molar)

Outlined Formula: C6H8O6

Sixth- EDTA solution 0.1 Molar used in the method of measurement (masking reagent)

Methods of Measurement:

There are several ways to measure duo iron one of them:

1-The method uses ortho phenanthroline, that makes orange complex with duo iron .
2-The method uses reagent of pyridine derivatives which is more selective and welknown :

Highly selective and sensitive spectrophotometric determination of Iron(II)with 4-(2-Pyridylazo) resorcinol(PAR):
This method has been used in the measurement for duo iron to high selectivity , sensitivity and stability colorimetric with duo iron bar complexes.
This method is still being used to determinate duo iron in analytical laboratories in pharmaceutical plants.[10]

Results and Discussion:

Study the effect of ascorbic acid on the stability of duo iron in similar conditions of physiological media:

Standard series was prepared containing 10 ml of Ferrous Sulfate Heptahydrate 0.1 Molar where the degree of PH 3-4 then added a mixture of borax and sodium hydroxide as buffer solution to become PH 6.5 similar degree of biological media in the intestinal then added different concentrations of ascorbic acid and measured absorption at the wavelength of 520 nm using PAR Reagent 0.1 Molar at a temperature of 37°C, pH 6.5 and added the PAR after one minute of the mixing process.

Table (1) Shows the values of the absorption of duo iron with different concentrations of ascorbic acid

Volume (ml) ferrous sulphate 0.1 M	Added ascorbic acid concentration (mol / l)	Absorption reading A1	Absorption reading A2	Absorption reading A3	Absorption reading A (Average)
10	0	0.054	0.052	0.053	0.053
10	0.005	0.056	0.055	0.057	0.056
10	0.01	0.064	0.062	0.063	0.063
10	0.015	0.069	0.069	0.069	0.069
10	0.02	0.074	0.074	0.074	0.074
10	0.025	0.079	0.08	0.078	0.079
10	0.03	0.080	0.081	0.082	0.081
10	0.035	0.082	0.082	0.081	0.082
10	0.04	0.082	0.081	0.082	0.082

We noted from the table (1) when the concentration of ascorbic acid is increased the duo iron absorbance is increased as well.

This result is attributable to the reduction properties of ascorbic acid.We noticed by increasing the concentration of ascorbic acid to more than 0.03 mol / liter.

So it is in vain to increase ascorbic acid higher than its limit to increase the stability of duo iron because the duo iron absorbance does not increase.

Study time effect on different concentrations of ascorbic acid on duo iron absorption when the temperature and pH similar to the physiological media:

A series of standard containing 10 ml of Ferrous Sulfate Heptahydrate 0.1 Molar was prepared a mixture of borax and sodium hydroxide then was added to become the PH of the media similar to duodenum-about 6.5; then different concentrations of ascorbic acid added. We measured absorption at the wavelength of 520 nm using PAR reagent 0.1 Molar at a temperature of 37 ° C and pH 6.5, PAR was added after 15 minute , 30 minute , 45 minute , 60 minute and 90 minute .

Table (2) shows the values of the absorption of duo iron after mixing it with different concentrations of ascorbic acid and different time periods

Volume (ml) ferrous sulphate 0.1 M	Added ascorbic acid concentration (mol / l)	A After 15 minute	A After 30 minute	A After 45 minute	A After 60 minute	A After 90 minute
10	0	0.055	0.054	0.055	0.056	0.055
10	0.005	0.055	0.061	0.060	0.062	0.069
10	0.01	0.059	0.068	0.070	0.072	0.079
10	0.015	0.069	0.073	0,079	0.085	0.088
10	0.02	0.077	0.079	0.083	0.097	0.099
10	0.025	0.079	0.083	0.093	0.108	0.111

We can deduce from the table (2) that when you increase the stay time of ascorbic acid with iron sulfate the absorption of duo iron increases but when we increase the stay time more than an hour process becomes useless .

Study the effect of folic acid on the stability of duo iron in similar conditions of physiological media:

Standard series was prepared containing 10 ml of Ferrous Sulfate Heptahydrate 0.1 Molar where the degree of pH 3-4 then added a mixture of borax and sodium hydroxide as buffer solution to become pH 6.5 similar degree of biological media in the intestinal then added different concentrations of folic acid and measured absorption at the wavelength of 520 nm using PAR reagent Molar 0.1 at a temperature of 37 ° C , pH 6.5 and added the PAR after one minute of the mixing process.

Table (3) Shows the values of the absorption of duo iron with different concentrations of folic acid

Volume (ml) ferrous sulphate 0.1 M	Added Folic acid concentration (mol / l)	A1	A2	A3	A Average
10	0	0.055	0.054	0.056	0.055
10	0.005	0.067	0.067	0.066	0.067
10	0.01	0.071	0.074	0.077	0.074
10	0.015	0.081	0.083	0.079	0.081
10	0.02	0.085	0.085	0.086	0.085
10	0.025	0.088	0.090	0.092	0.090
10	0.03	0.092	0.092	0.092	0.092
10	0.035	0.091	0.092	0.093	0.092

We note from the table (3) above that the absorption of duo iron increased with increasing concentration of folic acid, but increased it more than 0.03 mol / liter become useless.This result is attributable to reduction properties of folic acid but emanated from a certain percentage become useless .

Study time effect on different concentrations of folic acid on duo iron absorption when the temperature and pH similar to the physiological media:

A series of standard containing 10 ml of Ferrous Sulfate Heptahydrate 0.1 Molar was prepared a mixture of borax and sodium hydroxide then was added to become the pH of the media similar to duodenum-about 6.5 ; then different concentrations of folic acid added. We measured absorption at the wavelength of 520 nm using PAR reagent 0.1 Molar at a temperature of 37°C and pH 6.5, PAR was added after 15 minute , 30 minute , 45 minute , 60 minute and 90 minute .

Table (4) shows the values of the absorption of duo iron after mixing it with different concentrations of folic acid and different time periods

Volume (ml) ferrous sulphate 0.1 M	Added folic acid concentration (mol / l)	A After 15 minute	A After 30 minute	A After 45 minute	A After 60 minute	A After 90 minute
10	0	0.056	0.055	0.056	0.057	0.059
10	0.005	0.059	0.062	0.065	0.071	0.073
10	0.01	0.064	0.073	0.071	0.073	0.075
10	0.015	0.071	0.078	0.074	0.075	0.077
10	0.02	0.076	0.079	0.079	0.080	0.083
10	0.025	0.079	0.081	0.087	0.094	0.097
10	0.03	0.080	0.090	0.093	0.102	0.105

We note from the table (4) useless of mixing duo iron sulfate solution 0.1 Molar with folic acid solution more than 30 minutes.

Study the effect of calcium gluconate on the stability of duo Iron in similar conditions of physiological media:

Standard series was prepared containing 10 ml of Ferrous Sulfate Heptahydrate 0.1 Molar where the degree of PH 3-4 then added a mixture of borax and sodium hydroxide as buffer solution to become PH 6.5 similar degree of biological media in the intestinal then added different concentrations of calcium gluconate and measured absorption at the wavelength of 520 nm using PAR Reagent 0.1 Molar at a temperature of 37 ° C , pH 6.5 and added the PAR after one minute of the mixing process. .

Table (5) Shows the values of the absorption of duo iron with different concentrations of gluconate calcium

Volume (ml) ferrous sulphate 0.1 M	Added calcium gluconate concentration (mol / l)	A1	A2	A3	A Average
10	0	0.005	0.009	0.007	0.007
10	0.005	- 0.004	- 0.002	- 0.006	- 0.004
10	0.01	- 0.001	- 0.001	- 0.001	- 0.001
10	0.015	0.006	0.005	0.004	0.005
10	0.02	0.010	0.012	0.011	0.011
10	0.025	0.015	0.015	0.015	0.015
10	0.03	0.016	0.018	0.017	0.017
10	0.035	0.018	0.018	0.018	0.018

We note from the table (5) there was positive absorption for iron duo when there is no calcium gluconate but when you add a 0.01 mol/l of calcium gluconate the absorption became negative suggesting a negative effect of ions of calcium on duo iron absorption in low concentrations but when the gradual increase of the concentration of calcium gluconate the absorption of duo iron increased.

That's a negative effect on calcium absorption of duo iron in low concentrations and this effect turns into a positive effect in high concentrations but it was immediately measured without leaving enough time to mix the two components (Duo iron sulfate 0.1 Molar with calcium gluconate 0.1 Molar).

Study time effect on different concentrations of calcium gluconate on duo iron absorption when the temperature and PH similar to the physiological media:

A series of standard containing 10 ml of Ferrous Sulfate Heptahydrate 0.1 Molar was prepared a mixture of borax and sodium hydroxide then was added to become the PH of the media similar to duodenum-about 6.5; then different concentrations of calcium gluconate added. We measured absorption at the wavelength of 520 nm using PAR reagent 0.1 Molar at a temperature of 37°C and pH 6.5, PAR was added after 15 minute , 30 minute, 45 minute, 60 minute and 90 minute .

Table (6) shows the values of the absorption of duo iron after mixing it with different concentrations of calcium gluconate and different time periods

Volume (ml) ferrous sulphate 0.1 M	Added calcium gluconate concentration (mol / l)	A After 15 minute	A After 30 minute	A After 45 minute	A After 60 minute	A After 90 minute
10	0	0.017	0.015	0.016	0.018	0.022
10	0.05	-0.152	0.154-	-0.162	- 0.167	- 0.169
10	0.01	-0.192	- 0.209	-0.202	-0.214	- 0.219
10	0.015	-0.201	- 0.220	-0.231	-0.233	- 0.234
10	0.02	-0.244	- 0.256	-0.260	-0.269	- 0.266
10	0.025	-0.256	- 0.263	-0.266	-0.273	- 0.278
10	0.03	-0.262	- 0.267	-0.271	-0.282	- 0.284

We note from the table (6) negative effect of duo iron absorption after periods of mixing it with different concentration of calcium gluconate.
By increasing the concentration of calcium gluconate the negative effect increases.
There was no remarkable difference after 30 minute, 60 minute and 90 minute. Hence,the calcium gluconate needs to stay with duo ferrous sulphate half an hour to give this negative effect on the absorption of duo iron.

Recommendations & Conclusions

First- Duo iron concentration increases by adding ascorbic acid .so the absorption of duo iron increases in the duodenum due to reduction ability of ascorbic acid .The continuous increase of the concentration of ascorbic acid is useless in the absorption of iron.

Second- to increase the time of stay of ascorbic acid with iron sulfate the absorption of duo iron increases. But to increase the time of stay more than an hour, the process becomes useless. so it is preferable to give vitamin C simultaneously with an iron supplement.

Third-Duo iron concentration increases by adding folic acid so the absorption of duo iron increases in the duodenum and continues to increase. The continuous increase of the concentration of folic acid is useless in the absorption of iron.

Fourth- increasing stay time of folic acid with iron sulfate increases the absorption of duo iron and stability in duodenum. Increasing the stay time to more than 30 minutes becomes useless. so it is advisable to give folic tablets simultaneously with an iron supplement.

Fifth- Calcium affects negatively on the stability of duo iron so its absorption in the duodenum decreases. It is recommended to separate the intake time of calcium supplement from iron supplement no less than two hours to avoid the occurrence of negative impact.

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