United States Patent Office 3,506,403 Patented Apr. 14, 1970

3,506,403

COLORIMETRIC METHOD FOR DETERMINING IRON IN FLUIDS

Rodney Ian Fryer, North Caldwell, and Bernard Zigmund Senkowski, Bloomfield, N.J., assignors to Hoffmann-La Roche Inc., Nutley, N.J., a corporation of New Jersey

No Drawing. Filed Dec. 19, 1967, Ser. No. 691,693
Int. Cl. G01n 31/22
U.S. Cl. 23—230 8 Claims

ABSTRACT OF THE DISCLOSURE

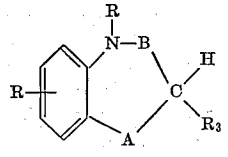
A colorimetric method for quantitatively determining the iron content of aqueous solutions prepared from iron-containing materials wherein the iron is present in its water-soluble ferrous state by treating the ferrous ions with 5 - (2 - pyridyl)-2H-1,4-benzodiazepines and water-soluble salts thereof to produce a brilliant purple colored solution which can be quantitated by standard colorimetric means.

BACKGROUND OF THE INVENTION

Techniques have been developed for quantitatively determining the iron content of various aqueous solutions by means of reacting the iron in either its ferric or ferrous state with various color forming reagents such as ammonium thiocyanate, bathophenanthroline, dipyridyl, etc. These techniques have been of importance in determining the iron content of various iron-containing materials such as ores, foods, beverages, such as wines, etc. However, this process has suffered from several disadvantage due to the fact that known color forming reagent are too sensitive to extraneous sources. In many cases, interfering bodies or contaminents do not produce with these color forming reagents, a sufficient color differentiation between the blank and the aqueous sample to be tested. This makes quantitative determination of the iron content in this sample difficult to carry out by standard colorimetric instruments such as a Beckman Spectrophotometer.

SUMMARY OF THE INVENTION

In accordance with this invention we have discovered that when a compound selected from the group consisting of compounds of the formula:



(I)

wherein A is selected from the group consisting of



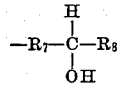
and



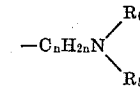
B is selected from the group consisting of



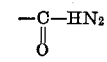
and $—CH_2—$; R is selected from the group consisting of halogen, hydrogen, trifluoromethyl, nitro and amino; R_2 is selected from the group consisting of



hydrogen, lower alkyl and



n is an integer from 2 to 7; R_3 is selected from the group consisting of hydrogen, hydroxy, lower alkyl, lower alkoxy and lower alkanoyloxy; R_4 is 2-pyridyl; R_5 is selected from the group consisting of lower alkyl and hydrogen; and R_6 is selected from the group consisting of lower alkyl, hydrogen



and —C≡N; and R_5 and R_6 where taken together with their attached nitrogen atom form a radical selected from the group consisting of piperazinyl, pyrrolidinyl, piperidinyl, lower alkyl substituted piperazinyl, lower alkyl substituted pyrrolidinyl, and lower alkyl substituted piperidinyl; R_7 is lower alkyl; and R_8 is selected from the group consisting of lower alkyl and hydrogen;

and water soluble salts thereof are added to an aqueous solution containing ferrous ions, a purple colored solution is obtained which can be quantitated as to its iron content by standard colorimetric means.

The color differentiation with different concentrations of ferrous ions produced by the compound of Formula I above, is such that the concentration of iron therein can be easily determined by standard colorimetric instruments. Furthermore, the compounds of Formula I above, are not sensitive to extraneous sources so as to include interferences from trace contaminants of iron. Therefore, the method of this invention provides a simple colorimetric means of quantitatively determining the iron content of iron-containing materials such as iron ores, wines, foodstuffs, etc.

DETAILED DESCRIPTION OF THE INVENTION

The term "lower alkyl" as used throughout this specification includes both straight and branched chain alkyl groups having from 1 to 7 carbon atoms such as methyl, ethyl, propyl, isopropyl and the like. The term "lower alkanoyloxy" refers to both straight chain and branched chain aliphatic carboxylic acid moieties, such as acetoxy, propionyloxy, butyrloxy and the like. The term "halogen" includes bromine, chlorine, fluorine and iodine.

Also included within the purview of the present invention are the water soluble acid addition salts of the compounds of Formula I above. Any conventional water soluble acid addition salts of the compounds of Formula I above, can be utilized in the process of this invention to quantitatively determine the iron content of aqueous solutions. Among the acid addition salts which may be utilized in accordance with this invention, includes salts of compounds of the Formula I with organic or inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, acetic acid, formic acid, succinic acid, maleic acid, p-toluene sulfonic acid and the like.

Examples of benzodiazepine compounds of Formula I above which are particularly suitable as the color forming reagent in the process of this invention include the following:

7 - bromo-1,3 - dihydro-1-(4-[4-methyl-1-piperazinyl]-butyl)-5(2-pyridyl)-2H-1,4-benzodiazepin-2one;

7-amino-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one;

1 - methyl - 1-[3-(7-bromo-5-(2-pyridyl)-1,3-dihydro-2-oxo-2H-1,4-benzodiazepin-yl)propyl] urea whose preparation is disclosed in U.S. patent application Ser. No. 677,092, filed Oct. 23, 1967 in the name of Earley et al.;

3-acetoxy-7,9-dibromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one;

7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepine;

7-amino-1,3-dihydro-1-methyl-5-(2-pyridyl)-2H-1,4-benzodiazepine;

7-bromo-1,3-dihydro-(3-dimethylaminopropyl)-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one;

7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one 4-oxide;

7-bromo-1,3-dihydro-1-(β-hydroxypropyl)-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one;

7-bromo-5-(2-pyridyl)-1,3-dihydro - 1 - [3 - (N - cyanomethylamino) propyl]-2H-1,4-benzodiazepin-2-one whose preparation is disclosed in U.S. patent application Ser. No. 677,092, filed Oct. 23, 1967, Earley.

The benzodiazepine compounds of Formula I above, can be utilized in quantitating the ferrous ion content by colorimetric means in any aqueous solution containing ferrous ions. Therefore, the process of this invention provides a means for quantitating the iron content of any iron containing material. Among the many iron containing materials whose iron content can be quantitated by the process of this invention are included foods, beverages such as wine, iron ores, etc.

In order to quantitate the iron in the iron containing materials by the process of this invention, it is first necessary to extract the iron from the sample of the iron-containing material in the ferrous state into an aqueous solution. Any conventional means for extracting the iron into an aqueous solution in a ferrous state can be utilized in accordance with this invention. The various methods of extracting iron in the ferrous state into an aqueous solution from various iron-containing materials such as ores, foods, beverages and body fluids are well known in the art and do not form part of this invention.

If the aqueous solution contains the free iron in the ferric state, the ferric ion can be treated with a chemical reducing agent to convert it to the desired ferrous state. In general, any known chemical reducing agent can be used in this step of the procedure. These include, for example, ascorbic acid, hydrazine sulfate, thioglycolic acid, sodium thioglycolate, sodium hydrosulfite, sodium metabisulfite, sodium bisulfite, sodium sulfite, hydroxylamine hydrochloride, hydroxylamine sulfate, hydroquinone, stannous chloride, etc. In the preferred embodiment of the invention, however, the chemical reduction of free iron from the ferric state to the ferrous state is carried out using thioglycolic acid or ascorbic acid as the reducing agent. The use of thioglycolic acid or ascorbic acid is advantageous since it permits the reduction of the free iron from the ferric state to the ferrous state to be accomplished at room temperature. The quantity of ascorbic acid or thioglycolic acid which is used in this step of the process is not particularly critical. A sufficient amount of the reducing agent should be used, however, to completely convert the free iron to the ferrous state. An excess of thioglycolic acid can be used, if desired, to insure complete conversion.

When the iron has been liberated from the sample to be quantitated in the water soluble ferrous state, the compound of Formula I above is added to an aqueous solution containing the extracted ferrous ions. Upon mixing, a complexing takes place between the ferrous ion and the compound of Formula I above. The formation of the complex is evidenced by the development of a brilliant purple color. The precise manner in which the compound of Formula I above is added to the ferrous ion solution does not limit the scope or practice of this invention. Generally, however, the compound of Formula I above is added to the system in the form of a relatively dilute aqeuous solution. In the preferred practice of the invention, there is used an aqueous solution containing from about 0.25 percent to about 0.75 percent by weight of the compound of Formula I above.

The quantity of the compound of Formula I which is added to the aqueous solution is variable. In all instances,

however, a sufficient quantity of the compound of Formula I above should be provided to react with all of the ferrous irons present in the aqueous solution. In order to insure, however, that all of the ferrous ions present in the mixture have been complexed, it is preferred to add a quantity of the color forming compound of Formula I above, which is in excess of that actually required to complex all of the ferrous ions which are available for complexing.

The desired purple coloration of the mixture will be noted immediately upon the addition of the compound of Formula I above to the system. The color deepens as the reaction proceeds to completion. After standing for awhile at room temperature, color changes are no longer discernible to the naked eye. Accordingly, in order to insure uniform cloring, the aqueous solution should be allowed to stand until its color appears to have become constant. In general, it has been found that full development of the purple color will occur over a period of from about 5 to 20 minutes.

The quantitation of the iron in the sample can be carried out by any conventional colorimetric method utilizing standard spectrophotometers, such as a Beckman spectrophotometer, Coleman Junior spectrophotometer, etc.

The complex which is produced in the practice of the invention has its own spectral characteristics. Color was developed using a sample containing 6.0 μg. of iron. The absorbance of the mixture was then measured at wave lengths ranging from 450 to 700 mμ in a Beckman spectrophotometer, Model DBG, using a 1 cm. cuvette. With increasing wave lengths above 450 mμ there was a progressive rise in absorbance which reached a maximum at about 580 mμ. The absorbance diminished at higher wave lengths.

In order to determine the speed and stability of the colorimeter response, a sample containing 6.0 μg. of iron was prepared. This sample was transferred to a 1 cm. cuvette immediately after the benzodiazepine salt color reagent had been added thereto. For a period of about one hour, absorbance measurements were made continuously at 580 mμ. It was found that the absorbance increased rapidly reaching a maximum value in about seven minutes and, further, that the measurement changed little thereafter.

The quantitative determination of iron in the sample is carried out as follows: the absorbance of the purple colored sample is measured against a reagent blank at 580 mμ using a standard spectrophotometer, e.g., a Coleman Junior spectrophotometer, employing a cuvette with 10 mm. light path. The quantity of iron in the specimen is determined in the conventional manner from the absorbance of the specimen with reference to a standard. The iron content in a sample is calculated according to the following formula:

$$\text{Iron content (mcg./100 ml.)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{concentration of standard (mcg./100 ml.)}$$

As indicated heretofore, the present invention provides an extremely important diagnostic and analytical tool. The described method can be used to determine the iron in various materials rapidly and accurately. In addition to being a rapid and accurate method for making the determination, the results obtained by the test method are characterized by a high degree of reproducibility.

For a fuller understanding of the nature and objects of this invention, reference may be had to the following examples which are given merely as further illustrations of the invention and are not to be construed in a limiting sense.

Example 1

In this example absorbance measurements were made using a Beckman Model D.U. spectrophotometer. Measurements of the pH were made using a Corning Model 12 pH meter.

A one millimolar solution of 7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one (labelled Compound A solution) was prepared by weighing 36 mg. of this benzodiazepin-2-one into a 100 ml. volumetric flask containing 50 ml. of methanol, and diluting it to concentration by the addition of water.

A 10 millimolar solution of 7-bromo-1,3-dihydro-1-(3-dimethylaminopropyl) - 5 - (2 - pyridyl) - 2H - 1,4 - benzodiazepin-2-one dihydrochloride (labelled Compound B solution) was prepared by dissolving 474.3 mg. of this compound in sufficient water to make 100 ml. of solution.

A 1 millimolar solution of ferrous sulfate was prepared by adding 27.8 mg. of ferrous sulfate heptahydrate to a flask and thereafter dissolving this compound in sufficient water to make 100 ml. of solution. Further dilutions were made of this 1 millimolar solution in order to obtain the required concentrations utilized throughout this example.

A buffer solution was prepared by first adding 50 ml. of 0.1 molar potassium acid phthalate and 22.6 ml. of 0.1 molar sodium hydroxide to a sufficient amount of water to prepare a 100 ml. of buffer solution.

The various solutions to be tested were prepared by taking 1 ml. of the ferrous sulfate solution diluted to the desired concentration, 1 ml. of either Compound A solution or Compound B solution and 8 ml. of the buffer solution. By means of this method, a series of test solutions varying in ferrous ion concentration from 1×10^{-5} molar to 5×10^{-5} molar were prepared. The concentration of either Compound A or B in these test solutions was maintained at 1×10^{-3} molar. Furthermore, the pH of the solutions was maintained at 5. Blanks were prepared by replacing the ferrous sulfate in the test solution with an equal volume of water. The absorption of each of the test solutions was measured at 580 mμ. The observed absorption at 580 mμ was plotted against the ferrous ion concentration in moles per liter $\times 10^5$. The resulting graph was a straight line for both the test solutions of Compound A and Compound B. The results of this determination is given in Tables I and II which follow.

TABLE I

Absorbance of iron containing solutions treated with Compound A

Iron concentration (moles/liters $\times$ 10^5):	Absorbance at 580 mμ
0	0
1	0.177
2	0.355
3	0.540
4	0.730
5	0.920

TABLE II

Absorbance of iron containing solutions treated with Compound B

Iron concentration (moles/liters $\times$ 10^{-5}):	Absorbance at 580 mμ
0	0
1	0.195
2	0.365
3	0.560
4	0.733
5	0.930

An unknown solution containing ferrous ions was subjected to analysis as to its iron content. This was done by preparing a solution composed of 1 ml. of the aqueous solution containing an undetermined ferrous ion content, 1 ml. of either solution A or B and 8 ml. of the buffer solution. This solution was subjected to optical absroption measurements at 580 mμ. From the absorbance measurement, the concentrations of the ferrous ion in the original solution was also determined. This determination was easily made due to the fact that the concentration of ferrous ion varied in a linear relationship with the optical absorption.

The linear relationship between ferrous ion concentration and absorbance was found to exist when dichloromethane rather than water was utilized as a solvent. Similar results in iron determination were found to exist when solutions of the following compounds were substituted for either the 7-bromo-1,3-dihydro - 1 - (3-dimethylaminopropyl) - 5 - (2-pyridyl)-2H-1,4-benzodiazepin-2-one or 7-bromo-1,3-dihydro - 5 - (2-pyridyl)-2H-1,4-benzodiazepin-2-one: 7-bromo-1,3-dihydro - 1 - (4-[4-methyl-1-piperazinyl]-butyl)-5-(2-pyridyl) - 2H - 1,4-benzodiazepin - 2 - one; 7-amino-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin - 2 - one; 1-methyl-1-[3-(7-bromo-5-(2-pyridyl)-1,3-dihydro-2-oxo-2H-1,4 - benzodiazepin - 1-yl)propyl]urea: 7-bromo - 1,3 - dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepine; 7-amino - 1,3 - dihydro-1-methyl-5-(2-pyridyl)-2H-1,4-benzodiazepine; 7-bromo - 1,3 - dihydro - 5 - (2-pyridyl)-2H-1,4-benzodiazepin-2-one 4-oxide; 7-bromo-1,3-dihydro - 1 - (β-hydroxypropyl)-5-(2-pyridyl)-2H-1,4-benzodiazepin - 2 - one; 7-bromo-5-(2-pyridyl)-1,3-dihydro - 1 - [3-(N-cyano-methylamino)-propyl]-2H-1,4-benzodiazepin-2-one.

Example 2

To a stirred solution of 19 g. (0.06 mole) of 7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin - 2 - one in 200 ml. of dry N,N-dimethylformamide was added portion wise 3 g. (0.063 mole) of 50 percent sodium hydride. The resultant solution was then cooled with an ice-water bath while 39 g. (0.18 mole) of 1,4-dibromobutane was added dropwise during 25 min. After stirring overnight at room temperature, the solution was poured into 600 ml. of water and extracted with methylene chloride. The organic layer was separated, washed with brine, dried over sodium sulfate and concentrated. The resultant oil was dissolved in ether and then filtered from a small amount of precipitated solid (discarded). This filtrate was then put on silica gel and the silica gel washed with ether. The first 150–200 ml. of eluant was separated while the subsequent fractions (2 l. total) were concentrated to give 8 g. of 7-bromo-1-(4-bromobutyl)-5-(2 - pyridyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one a tan colored solid.

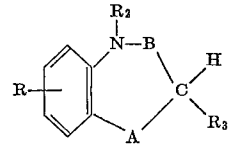
Example 3

A mixture of 9 g. (0.02 mole) of 7-bromo-1-(4-bromobutyl) - 5 - (2 - pyridyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one 3 g. (0.03 mole) of N-methylpiperazine, 3 g. (0.02 mole) of sodium iodide and 100 ml. of methylethyl ketone was stirred and refluxed for 22.5 hr. Solvents were removed at reduced pressure and the residue partitioned between methylene chloride and water. The methylene chloride layer was washed with brine, dried over sodium sulfate and concentrated. Upon the addition of ether to the resultant oil a solid separated. The suspension was filtered and the solid discarded. The ether filtrate was concentrated and the residual oil was converted to the dihydrochloride by the addition of the theoretical amount of methanolic hydrogen chloride followed by ether. The resultant highly hygroscopic solid was recrystallized from ethanol-ether to give 7-bromo-1,3-dihydro [4-(4-methyl-1-piperazinyl)butyl] - 5 - (2-pyridyl)-2H-1,4-benzodiazepin-2-one dihydrochloride as white needles.

We claim:

1. A process for determining the amount of iron present in an aqueous solution wherein said iron is present in the

ferrous state comprising treating said solution with a compound selected from the group consisting of compounds of the formula:



wherein A is selected from the group consisting of



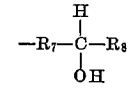
and



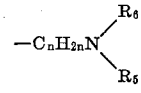
B is selected from the group consisting of



and —CH_2—; R is selected from the group consisting of halogen, hydrogen, trifluoromethyl, nitro and amino; R_2 is selected from the group consisting of



hydrogen, lower alkyl and



n is an integer from 2 to 7; R_3 is selected from the group consisting of hydrogen, hydroxy, lower alkyl, lower alkoxy and lower alkanoyloxy; R_4 is 2-pyridyl; R_5 is selected from the group consisting of lower alkyl and hydrogen; and R_6 is selected from the group consisting of lower alkyl, hydrogen



and —C≡N; and R_5 and R_6 where taken together with their attached nitrogen atom form a radical selected from the group consisting of piperazinyl, pyrrolidinyl, piperidinyl, lower alkyl substituted piperazinyl, lower alkyl substituted pyrrolidinyl and lower alkyl substituted piperidinyl; R_7 is lower alkyl; and R_8 is selected from the group consisting of lower alkyl and hydrogen;

and water soluble acid addition salts thereof to form a colored solution and thereafter colorimetrically quantitating said iron content of said solution by means of said color.

2. The process of claim **1** wherein said compound is 7-bromo-1,3-dihydro - 1 - (3-dimethylaminopropyl)-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one.

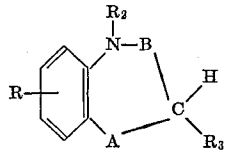
3. The process of claim **1** wherein said compound is 7-bromo-1,3-dihydro - 1 - [4 - (4 - methyl-1-piperazinyl) butyl]5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one.

4. The process of claim **1** wherein said compound is 7-bromo-1,3-dihydro - 5 - (2 - pyridyl)-2H-1,4-benzodiazepin-2-one.

5. A process for quantitating the iron present in an iron-containing material comprising

(a) extracting the iron in a ferrous state from the sample into an aqueous solution;

(b) treating said solution with a compound selected from the group consisting of compounds of the formula:



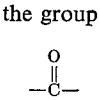
wherein A is selected from the group consisting of



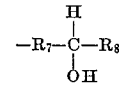
and



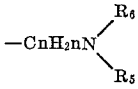
B is selected from the group consisting of



and —CH_2—; R is selected from the group consisting of halogen, hydrogen, trifluoromethyl, nitro and amino; R_2 is selected from the group consisting of



hydrogen, lower alkyl and



n is an integer from 2 to 7; R_3 is selected from the group consisting of hydrogen, hydroxy, lower alkyl, lower alkoxy and lower alkanoyloxy; R_4 is 2-pyridyl; R_5 is selected from the group consisting of lower alkyl and hydrogen; and R_6 is selected from the group consisting of lower alkyl, hydrogen



and —C≡N; and R_5 and R_6 where taken together with their attached nitrogen atom form a radical selected from the group consisting of piperazinyl, pyrrolidinyl, piperidinyl, lower alkyl substituted piperazinyl, lower alkyl substituted pyrrolidinyl, and lower alkyl substituted piperidinyl; R_7 is lower alkyl; and R_8 is selected from the group consisting of lower alkyl and hydrogen;

and acid addition salts thereof to form a colored solution; and

(c) thereafter colorimetrically quantitating the iron content of said solution by means of said color.

6. The process of claim **5** wherein said compound is 7-bromo-1,3-dihydro - 1 - (3-dimethylaminopropyl)-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one.

7. The process of claim **5** wherein said compound is 7-bromo-1,3-dihydro - 1 - [4 - (4 - methyl-1-piperazinyl) butyl]-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one.

8. The process of claim **5** wherein said compound is 7-bromo - 1,3 - dihydro - 5 - (2-pyridyl)-2H-1,4-benzodiazepin-2-one.

References Cited

UNITED STATES PATENTS

3,100,770	8/1963	Fryer et al.	260—239.3
3,182,066	5/1965	Fryer et al.	260—239.3
3,236,838	2/1966	Archer et al.	260—239.3
3,299,053	1/1967	Archer et al.	260—239.3

OTHER REFERENCES

Welcher, F. J., ed., Standard Methods of Chemical Analysis, vol. I, pp. 529–55 (1963).

MORRIS O. WOLK, Primary Examiner

E. A. KATZ, Assistant Examiner

U.S Cl. X.R.

252—408; 260—239.3