STABLE SOLUTIONS CONTAINING VITAMIN B₁₂

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6 Claims. (Cl. 167—81)

This application is a continuation of applicants' pending application, Serial No. 630,041, filed December 24, 1956.

This invention relates to aqueous solutions of vitamin B₁₂ having improved stability, and shelf-life and, more particularly, to such a vitamin B₁₂ composition wherein the vitamin B₁₂ is stabilized by a two-component system, namely, an iron compound and ethylenediaminetetraacetic acid and its water-soluble, non-toxic salts.

An object of the invention is to provide a composition of matter containing vitamin B₁₂, in which undue loss of vitamin B₁₂ and darkening of color is substantially obviated.

Another object of the invention is the provision of compositions containing vitamin B₁₂ either alone or in multi-vitamin compositions, in which the vitamin B₁₂ content is stabilized to give a shelf-life of at least one year, with substantial stabilization of the vitamin B₁₂ activity and without undue darkening of said solution.

It is well known that vitamin B₁₂ is unstable in aqueous solutions containing substances which may act as oxidizing or reducing agents, ascorbic acid (vitamin C) being one such substance (Bartlucchi and Foss, J. Am. Pharm. Assoc. 43, 159 (1954)). It is also known that certain vitamins in the B-complex group, such as thiamine, which in solution may undergo decomposition, causes vitamin B₁₂ instability. (Blitz, Eigen and Gunberg, J. Am. Pharm. Assoc. 43, 651 (1954).) Other substances commonly used in multi-vitamin solutions, such as sugars, which have a reducing action, will also cause instability of vitamin B₁₂ in varying degree.

Attempts at protecting vitamin B₁₂ under varying conditions, including those just referred to, have been described, e.g., liver extracts and certain fractions thereof (Shenoy and Ramasarma, Arch. Biochem. and Biophys. 55, 293 (1955)); certain iron compounds (Steggs, U.S. Patent No. 2,584,627), and metallic chlorophyllines with cooperating stabilizers (Winsten, U.S. Patent No. 2,662,048), have been suggested as means of stabilizing vitamin B₁₂ in solutions containing vitamin B₁₂ alone or those substances which by their chemical nature, or because of products of decomposition, are contributory to instability.

Newmark, in U.S. Patent No. 2,823,167, which issued after applicants' original filing date, describes and claims stabilized vitamin B₁₂ compositions containing iron compounds or salts. Newmark, however, shows that in order to obtain satisfactory stabilization it is necessary to use massive amounts of iron; and in referring to his results with various iron compounds, particularly with iron pep- tonate in Table 5, he asserts that the "stabilization effect increases as the amount of iron increases within the range of concentrations tested in this experiment," and he states further that "this is true regardless of the type of compound used.

Further, Newmark's Table 5 shows that with 16 mcg. of iron per cc. (in the form of iron peptonate, for example) he obtains zero stabilization; that with 160 mcg. of iron per cc. he obtains only about 35% stabilization; and that only when he used 1600 mcg. of Fe per cc. did he obtain adequate stabilization. Although the above results suggest that chelating agents, such as ethylenediaminetetraacetic acid and its salts can be used with iron compounds he still suggests using iron in large quantities in his compositions, and he indicates surprise that the iron compound is effective in its presence.

The use of large amounts of iron compounds as taught by Newmark is not a satisfactory way of stabilizing vitamin B₁₂ because: (a) iron compounds, even when complexed, tend to combine with some vitamins, and with ascorbic acid, for example, forms iron ascorbate; (b) dark-colored solutions and, in some instances, precipitates result; and (c) it is a physiological fact that the less the amount of iron that is administered the better, because of possible disadvantageous side effects particularly in infants and young children.

Applicants have discovered that very small amounts of iron compounds per part of vitamin B₁₂ are capable of effectively stabilizing vitamin B₁₂ compositions if they are synergized by ethylenediaminetetraacetic acid and its water-soluble, non-toxic salts, and that applicants' compositions containing this combination of components can have a shelf-life of at least one year without undue darkening of the solution and without undue loss of vitamin B₁₂ activity.

In accordance with applicants' invention, it has been determined that in the presence of from 13.3 to 1,000 parts of ethylenediaminetetraacetic acid per 1,000 parts of vitamin B₁₂ the amount of iron required for stabilization need not exceed 50 parts for each part of vitamin B₁₂ and can range to as low as 1.5 parts. Preferably, the proportion of iron is the minimum necessary for stabilization for the time required and is within the range from 1.33 to 50 parts for each part of vitamin B₁₂ present. It will be perceived that the proportion of ethylenediaminetetraacetic acid compound is within the range from 10 to 20 times the amount of iron present.

The compositions of the invention will ordinarily be in the form of aqueous solutions of vitamin B₁₂ either alone or in conjunction with other vitamins, such as ascorbic acid, thiamine, vitamins A and D, riboflavin, niacinamide and pyridoxine. In the examples described, our preferred range of vitamin B₁₂ is from 4 to 6 mcg. per ml. of solution, but the concentration of vitamin B₁₂ and the other vitamins in the solution is not critical; the vitamin B₁₂ ranges from 1 mcg. to a probable maximum of 100 mcg. per ml. The concentrations of iron compound and of ethylenediaminetetraacetic acid compound are, however, critical within the range from 0.0008% to 0.02% calculated for elemental iron and 10 to 20 times the amount of iron for the ethylenediaminetetraacetic acid compound, but will be taken within the proportions of iron to vitamin B₁₂ and ethylenediaminetetraacetic acid compound to vitamin B₁₂ given above.

The aqueous solutions are readily prepared by dissolving the stabilizing ingredients in water with the aid of heat if desired up to a maximum temperature of about 50° C., then cooling and then adding the vitamin B₁₂ thereto and dissolving it therein, and then adding this solution to previously prepared vitamin solution.

Any compound which contains iron and is soluble in aqueous solution, and which is non-toxic, can be employed in applicants' combination in the amounts necessary to stabilize vitamin B₁₂ when the solutions are used orally or parenterally and sufficiently soluble in water to remain in solution under all conditions to which the composition may be subjected before use. For example, the compound may be selected from the group consisting of iron peptonate, iron ammonium citrate, iron gluconate.
and the like, the iron being either in ferrous or ferric form; and in applicants' combination will stabilize aqueous solutions of vitamin B₁₂, even when the latter is compounded together with, for example, ascorbic acid, thiamine hydrochloride or mononitrate, nicin and other substances above referred to as contributory to instability of vitamin B₁₂.

The concentration of ethylenediamine tetra acetic acid compound will be within the range from about 0.008 to about 0.4% (i.e., 10 to 20 times the iron); and this term is employed herein generically, to refer both to the acid and to its water-soluble, non-toxic salts such as any of the soluble salts, for example, sodium, potassium and magnesium salts.

While the mechanism of the synergistic action discovered by applicants is not well understood, nevertheless its effects are unexpected and result in the production of a stabilized vitamin B₁₂ containing composition without impairing its commercial utility.

The stability of our vitamin B₁₂ in the solutions may vary depending on conditions such as temperature and time, but in general after an ageing test comprising subjecting the solutions to a temperature of 45° C. for a period of three weeks, at least 75% of the original vitamin B₁₂ is retained. This test is exact and simulates a period of six months to one year at room temperature. It is also significant that a retention of 75% or more of the vitamin B₁₂ over a calculated period of six months to one year is of practical importance, particularly since it is customary to utilize a calculated excess in compounding vitamin B₁₂ products, to allow for a reasonable loss of potency of this relatively labile vitamin.

In the practice of the invention, we may use either crystalline vitamin B₁₂ (cyancobalamnin) or solutions thereof, or concentrates of vitamin B₁₂ obtained commercially from extracts of mammalian liver or from fermentation liquors in which the vitamin may be present in impure form, or an other than cyancobalamnin and sometimes referred to as B₁₂(a) and B₁₂(b).

As an illustrative embodiment of a manner in which the invention may be practiced, the following examples are presented.

Example 1

210 mg. iron pentonate, N.F. and 700 mg. of the di-sodium salt of ethylenediamine tetra acetic acid were dissolved in 25 ml. water with gentle warming, i.e. about 35° C. After cooling 3340 mcg. crystalline vitamin B₁₂ was added and dissolved by stirring. This solution was then added to 475 ml. of a multi-vitamin solution containing vitamins A and D, ascorbic acid, thiamine, riboflavin, nicinamidie and pyridoxine, among others, to give a total volume of 500 ml. Upon the addition of the multi-vitamin solution, the color of the multi-vitamin solution darkened momentarily, but after 30-60 seconds agitation, the darkening disappeared and the color was the same as it was originally. On standing, the solution remained clear and light in color, and no colloidal precipitate was observed. In the accelerated ageing test, described above, 93% of the initial vitamin B₁₂ content was retained after 21 days at 45° C.

Example 2

2225 mg. vitamin B₁₂ were dissolved in 25 ml. water. To this solution were added 75 mg. iron pentonate and 250 mg. of the di-sodium salt of ethylenediamine tetra acetic acid and dissolved by stirring. This solution was then added to 475 ml. of a multi-vitamin solution similar to that used in Example 1, to give a total volume of 500 ml. The color of the multi-vitamin solution darkened momentarily, and after about one minute agitation, the solution returned to its original color and remained so without precipitation. This solution contains 4.45 mcg. vitamin B₁₂, 0.025 mg. iron and 0.5 mg. disodium ethylenediamine tetra acetic acid per ml. In the accelerated ageing test, 98% of the initial vitamin B₁₂ content was retained after 21 days at 45° C.

Example 3

25 mg. iron pentonate, N.F. and 83.25 mg. of the di-sodium salt of ethylenediamine tetra acetic acid were dissolved in 25 ml. water with gentle warming, i.e., about 35° C. After cooling 3340 mcg. crystalline vitamin B₁₂ was added and dissolved by stirring. This solution was then added to 475 ml. of a multi-vitamin solution containing vitamins A and D, ascorbic acid, thiamine, riboflavin, nicinamidie and pyridoxine, among others, to give a total volume of 500 ml. solution containing 6.0 mcg. B₁₂, 8.0 mcg. elemental iron and about 160 mcg. ethylenediamine tetra acetic acid per ml. Upon the addition of the multi-vitamin solution, the color of the multi-vitamin solution darkened momentarily, but after 30-60 seconds agitation, the darkening disappeared and the color was the same as it was originally. On standing, the solution remained clear and light in color, and no colloidal precipitate was observed. In the accelerated ageing test, described above, 93% of the initial vitamin B₁₂ content was retained after 21 days at 45° C.

Example 4

This example refers to a B-complex solution with ascorbic acid to which vitamin B₁₂ was added to give 5 mcg. per ml. 500 mg. iron ammonium citrate (green) containing approximately 80 mg. of iron were dissolved in 50 ml. of water, to which were added 1.5 grams of the disodium salt of ethylenediamine tetra acetic acid. This solution was then added to 450 ml. of a B-complex solution containing 25 grams of ascorbic acid and 2.5 mcg. vitamin B₁₂, crystalline. A momentary dark color developed which became lighter on standing, and returned to its original color after a few minutes. This solution on ageing for 21 days at 45° C., retained over 85.3% of the original vitamin B₁₂.

However, a like solution, prepared in the same manner, but omitting the ethylenediamine tetra acetic acid, remained dark and became much darker after the ageing test as described above.

The solution of Example 4 contains approximately 32 parts of elemental iron and 600 parts of disodium ethylenediamine tetra acetic acid to 1 of vitamin B₁₂. By increasing the amount of vitamin B₁₂ to twice that stipulated above, namely, 10 mcg. of vitamin B₁₂ per ml., the ratio of iron and disodium ethylenediamine tetra acetic acid becomes 16 and 300 to 1 respectively. The vitamin B₁₂ concentration of the solution thus prepared can thus be increased up to 100 mcg. of B₁₂ per ml. or 20 times the concentration given originally in the example, making the ratio of elemental iron and disodium ethylenediamine tetra acetic acid 1.6 and 30 to 1 respectively, these ratios coming well within the ratios of iron and ethylenediamine tetra acetic acid described in the specification of this application.

Example 5

This example refers to the stabilization of vitamin B₁₂ in a solution which can be suitably used for parenteral
injection. The use of multi-vitamin solution parenterally is well known. Stability of vitamin B\textsubscript{12} in this type of solution, particularly one containing ascorbic acid, is as much of a problem as in solutions for oral use. Since parenteral solutions have to undergo sterilization, usually by heating for a specified period at elevated temperatures, means of assuring the stability of vitamin B\textsubscript{12} under the added conditions of heat, as well as the utility of the product, are important.

Accordingly, the following Example 5 will demonstrate the formulation and method of preparing such a solution with respect to the instant invention:

100 mg. of iron ammonium citrate (equal to about 15 mg. iron) are dissolved in about 50 ml. double distilled water, in which solution are then dissolved 300 mg. of disodium ethylenediamine tetra acetic acid. To this solution is then added about 900 ml. of a multi-vitamin solution containing in solution the vitamins listed below. The fat-soluble vitamins in this solution are dissolved by means of a "TWEEN," and the solution is preserved by addition of benzyl alcohol.

This solution is allowed to stand for a short period, filtered, and to the filtered solution is added a solution of 5 mg. crystalline vitamin B\textsubscript{12} dissolved in a small amount of double distilled water, and the whole made up to a volume of one liter.

Such a solution will contain per ml. the following vitamin potencies: vitamin A—5,000 I.U.; vitamin D—500 I.U.; vitamin E—1 mg.; vitamin C—25 mg.; thiamine—5 mg.; riboflavin—2 mg.; niacinamide—10 mg.; pyridoxine—1.5 mg.; panthenol—2.5 mg.; B\textsubscript{12}—5 mg.; plus the usual overages normally used in compounding such solutions without the benefit of this invention, and 0.015 mg. iron and 0.5 mg. disodium ethylenediamine tetra acetic acid.

The solution of Example 5 contains the ratio of iron and ethylenediamine tetra acetic acid to vitamin B\textsubscript{12} of 3 and 60 to 1 respectively. By increasing the vitamin B\textsubscript{12} concentration to 10 mcg. per ml. the ratio of iron and di- sodium ethylenediamine tetra acetic acid become 1.5 and 30 to 1 respectively, which ratios are well within the ratios described in the specification of this application.

Since stability of the vitamin B\textsubscript{12} is a function of temperature and time, the following table gives in detail the results, expressed in percent of initial values, of the effects of stabilization of a multi-vitamin solution at a temperature of 45° C. for 3, 6, and 12 week periods, and at room temperature (20° C.) for 3 months:

<table>
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<tr>
<th>No.</th>
<th>8</th>
<th>6</th>
<th>12</th>
<th>2 months</th>
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EDTA = ethylenediamine tetra acetic acid.

Having described our invention, what we claim as new and desire to secure by Letters Patent is:

1. An aqueous vitamin B\textsubscript{12} solution of improved stability and resistant to darkening in color, comprising vitamin B\textsubscript{12}, an iron compound selected from the group consisting of iron pentionate, iron ammonium citrate, iron glyceroxophosphate and iron gluconate to lessen the deterioration of vitamin B\textsubscript{12}, and an ethylenediamine tetraacetic acid compound to enhance the stabilizing action and lessen the darkening action of the iron compound, the iron compound being in an amount to supply iron in an amount within the range from about 1 to 50 parts per part of vitamin B\textsubscript{12}, and the ethylene diamine tetraacetic acid compound being present in an amount within the range from about 13 to 1000 parts per part of vitamin B\textsubscript{12}, the ratio of the ethylenediamine tetraacetic acid compound to iron being from 10 to 20 parts to 1.

2. An aqueous vitamin B\textsubscript{12} solution of improved stability and resistant to darkening in color comprising vitamin B\textsubscript{12}, a vitamin B\textsubscript{12}-destroying substance, an iron compound selected from the group consisting of iron pentionate, iron ammonium citrate, iron glyceroxophosphate and iron gluconate to lessen the deterioration of vitamin B\textsubscript{12}, and an ethylenediamine tetraacetic acid compound to enhance the stabilizing action and lessen the darkening action of the iron compound, the iron compound being in an amount to supply iron in an amount within the range from about 1 to 50 parts per part of vitamin B\textsubscript{12}, and the ethylene diamine tetraacetic acid compound being present in an amount within the range from about 13 to 1000 parts per part of vitamin B\textsubscript{12}, the ratio of the ethylenediamine tetraacetic acid compound to iron being from 10 to 20 parts to 1.

3. A composition in accordance with claim 2 in which the vitamin B\textsubscript{12}-destroying substance is ascorbic acid, and the ethylenediamine tetraacetic acid compound is the disodium salt of ethylene diamine tetraacetic acid, and said composition also contains at least one of the group comprising riboflavin, niacinamide, pyridoxine and vitamins A and D.

4. An aqueous vitamin B\textsubscript{12} solution of improved stability and resistant to darkening in color, comprising vitamin B\textsubscript{12}, iron pentionate to lessen the deterioration of vitamin B\textsubscript{12}, and an ethylenediamine tetraacetic acid compound to enhance the stabilizing action and lessen the darkening action of the iron pentionate, the iron pentionate being in an amount to supply iron in an amount within the range from about 1 to 50 parts per part of vitamin B\textsubscript{12}, and the ethylene diamine tetraacetic acid compound being present in an amount within the range from about 13 to 1000 parts per part of vitamin B\textsubscript{12}, the ratio of the ethylenediamine tetraacetic acid compound to iron being from 10 to 20 parts to 1.

5. An aqueous vitamin B\textsubscript{12} solution of improved stability and resistant to darkening in color, comprising vitamin B\textsubscript{12}, ascorbic acid, iron pentionate to lessen the deterioration of vitamin B\textsubscript{12}, and an ethylenediamine tetraacetic acid compound to enhance the stabilizing action and lessen the darkening action of the iron pentionate, the iron pentionate being in an amount to supply iron in an amount within the range from about 1 to 50 parts per part of vitamin B\textsubscript{12}, and the ethylene diamine tetraacetic acid compound being present in an amount within the range from about 13 to 1000 parts per part of vitamin B\textsubscript{12}, the ratio of the ethylenediamine tetraacetic acid compound to iron being from 10 to 20 parts to 1.

6. A composition in accordance with claim 5, also containing at least one of the group comprising riboflavin, niacinamide, pyridoxine and vitamins A and D.

References Cited in the file of this patent

UNITED STATES PATENTS 2,823,167 Newmark February 11, 1958