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STABLE SOLUTIONS CONTAINING VITAMIN B_{12}

Louis Freedman, Bronxville, Milton Blitz, Bronx, David B. Sabine, Yonkers, and Edward Eigen, Long Island City, N.Y., assignors to U.S. Vitamin and Pharmaceutical Corporation, a corporation of Delaware

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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 B_{12} having improved stability, and shelf-life and, more particularly, to such a vitamin B_{12} composition wherein the vitamin B_{12} is stabilized by a two-component system, namely, an iron compound and ethylenediamine tetra acetic acid and its water-soluble, non-toxic salts.

An object of the invention is to provide a composition of matter containing vitamin B_{12}, in which undue loss of vitamin B_{12} and darkening of color is, substantially, obviated.

Another object of the invention is the provision of compositions containing vitamin B_{12} either alone or in multi-vitamin compositions, in which the vitamin B_{12} content is stabilized to give a shelf-life of at least a year, with substantial stabilization of the vitamin B_{12} activity and without undue darkening of said solution.

It is well known that vitamin B_{12} is unstable in aqueous solutions containing substances which may act as oxidizing or reducing agents, ascorbic acid (vitamin C) being one such substance (Bartilucci 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 B_{12} instability. (Blitz, Eigen and Gunsberg, 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 B_{12} in varying degree.

Attempts at protecting vitamin B_{12} under varying conditions, including those just referred to, have been described, e.g., liver extracts and certain fractions thereof (Shemoy and Ramasarma, Arch. Biochem. and Biophys. 55, 293 (1955)); certain iron compounds (Skeggs, 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 B_{12} in solutions containing vitamin B_{12} 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 B_{12} 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 peptonate 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 iron 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 33% stabilization; and that only when he used 1600 mcg. of Fe per cc. did he obtain adequate stabilization. Although Newmark suggests that chelating agents, such as ethylenediamine tetra acetic 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 B_{12} 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 adminstered 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 B_{12} are capable of effectively stabilizing vitamin B_{12} compositions if they are synergized by ethylenediamine tetra acetic 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 B_{12} activity.

In accordance with applicants' invention, it has been determined that in the presence of from 13.3 to 1,000 parts of ethylenediamine tetra acetic acid compound for each part of vitamin B_{12} the amount of iron required for stabilization need not exceed 50 parts for each part of vitamin B_{12} and can range to as low as 1.33 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 B_{12} present. It will be perceived that the proportion of ethylenediamine tetra acetic 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 B_{12} 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 B_{12} is from 4 to 6 mcg. per ml. of solution, but the concentration of vitamin B_{12} and the other vitamins in the solution is not critical; the vitamin B_{12} ranges from 1. mcg. to a probable maximum of 100 mcg. per ml. The concentrations of iron compound and of ethylenediamine tetra acetic 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 ethylenediamine tetra acetic acid compound, but will be taken within the proportions of iron to vitamin B_{12} and ethylenediamine tetra acetic acid compound to vitamin B_{12} 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 B_{12} 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 B_{12} 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 B_{12}, even when the latter is compounded together with, for example, ascorbic acid, thiamine hydrochloride or mononitrate, niacin and other substances above referred to as contributory to instability of vitamin B_{12}.

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 B_{12} containing composition without impairing its commercial utility.

The stability of our vitamin B_{12} in the solutions may vary somewhat 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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12} (cyanocobalamin) or solutions thereof, or concentrates of vitamin B_{12} obtained commercially from extracts of mammalian liver or from fermentation liquors in which the vitamin may be present in an impure form, or an other than cyanocobalamin and sometimes referred to as $B_{12}(a)$ and $B_{12}(b)$.

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

Example 1

210 mg. iron peptonate, N.F. and 700 mg. of the disodium 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 B_{12} 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, niacinamide 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 B_{12} content was retained after 21 days at 45° C.

By contrast, the same solution prepared in the same manner, but omitting the disodium salt of ethylenediamine tetra acetic acid turned dark and remained dark on standing, eventually forming a fine colloidal precipitate.

Example 2

2225. mcg. vitamin B_{12} were dissolved in 25 ml. water. To this solution were added 75. mg. iron peptonate and 250 mg. of the disodium 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 B_{12}, 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 B_{12} content was retained after 21 days at 45° C.

A similar solution but without a content of the disodium salt of ethylenediamine tetra acetic acid under like ageing conditions turned dark and so remained.

Example 3

25 mg. iron peptonate, N.F. and 83.25 mg. of the disodium 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 B_{12} 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, niacinamide and pyridoxine, among others, to give a total volume of 500 ml. solution containing 6.0 mcg. B_{12}, 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 B_{12} content was retained after 21 days at 45° C.

The effects of iron compounds with and without the stabilizing effect of ethylenediamine tetra acetic acid or its salts was determined in the following manner: a solution was prepared exactly as that described in the above example, but omitting the disodium salt of ethylenediamine tetra acetic acid. The solution turned dark and instead of turning light in color, remained dark and became darker on standing at room temperature

Example 4

This example refers to a B-complex solution with ascorbic acid to which vitamin B_{12} 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 ascorbic acid and 2.5 mg. vitamin B_{12}, crystalline. A momentary dark color developed which become 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.% of the original vitamin B_{12}.

However, a like solution, prepared in the same manner, but omitting the ethylenediamine tetra acetic salt, 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 B_{12}. By increasing the amount of vitamin B_{12} to twice that stipulated above, namely, 10 mcg. of vitamin B_{12} per ml., the ratio of iron and disodium ethylenediamine tetra acetic acid becomes 16 and 300 to 1 respectively. The B_{12} concentration of the solution of the example can thus be increased up to 100 mcg. of B_{12} 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 B_{12} 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_{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_{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 viamins 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_{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_{12}—5 mcg., plus the usual overages normally used in compounding such solutions without the benefit of this invention, and 0.015 mg. iron and 0.3 mg. disodium ethylenediamine tetra acetic acid.

The solution of Example 5 contains the ratio of iron and ethylenediamine tetra acetic acid to vitamin B_{12} of 3 and 60 to 1 respectively. By increasing the vitamin B_{12} concentration to 10 mcg. per ml. the ratio of iron and disodium 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_{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:

No.	3	6	12	3 months at RT	Stabilizer
	Percent	*Percent*	*Percent*	*Percent*	
A	54	6	0.5	32	None.
C	85	79	81	97	Fe Peptonate plus Na_2 salt of EDTA.
D	91	80	79	102	
E	83	82	78	101	

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_{12} solution of improved stability and resistant to darkening in color, comprising vitamin B_{12}, an iron compound selected from the group consisting of iron peptonate, iron ammonium citrate, iron gylcerophosphate and iron gulconate to lessen the deterioration of vitamin B_{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_{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_{12}, the ratio of the ethylenediamine tetraacetic acid compound to iron being from 10 to 20 parts to 1.

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

5. An aqueous vitamin B_{12} solution of improved stability and resistant to darkening in color, comprising vitamin B_{12}, ascorbic acid, iron peptonate to lessen the deterioration of vitamin B_{12}, and an ethylenediamine tetraacetic acid compound to enhance the stabilizing action and lessen the darkening action of the iron peptonate, the iron peptonate being in an amount to supply iron in an amount within the range from about 1 to 50 parts per part of vitamin B_{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_{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 ---------- Feb. 11, 1958