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(54) Title: CATECHIN COATED ASCORBIC ACID AND METHOD

(57) Abstract

Coating of certain pharmaceuticals such as ascorbic acid with a heavy-metal-chelating substance, such as catechin, to prevent the development of mutagens like "ascorbate-free-radical" in the body, due to the action of cupric ions on ascorbic acid. In a preferred embodiment, ascorbic acid tablets or core particles are coated with d-catechin in order to produce dosage structures which shield the ascorbic acid from oxidation catalyzed by heavy metal ions. As the catechin coating is released in the stomach, it binds the heavy metal ions by chelation and prevents or minimizes the interaction of copper and ascorbic acid.

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CATECHIN COATED ASCORBIC ACID AND METHOD

Background of the Invention

Field of the Invention

This invention relates to the coating of pharmaceuticals with a heavy-metal-chelating substance to prevent, or at least minimize the development of mutagenic activity in the body due to "free-radical" formation resulting from oxidation of the pharmaceuticals after ingestion. More particularly, the invention relates to the coating of ascorbic acid (Vitamin C) with d-catechin to produce a layered dosage structure which protects the Vitamin C from cupric ions and other heavy metal ions which act as oxidation catalysts, by binding and inactivating the heavy metal catalysts before they can react with the vitamin.

It is known that certain beneficial and non-toxic medications and nutritional supplements become mutagenic by the release of free-radicals in the presence of heavy metal ions which catalyze oxidation of the medications and supplements. One such substance is ascorbic acid, which has been shown by Stich, et al (1976), infra, to become mutagenic in the presence of cupric ions and oxygen, when tested by the method of Ames, et al (1973), infra, and by other appropriate tests.

It is documented that copper is present to the extent of at least two parts per million in the first water drawn in the morning from copper pipes in many homes, especially in regions where the water is "soft" and acidic, and much of this copper is present in the water as cupric ions. Research by Schroeder (1960, 1966) has also shown that the death rates from cardiovascular disease are significantly higher in regions of the United States where the water is "soft", than in regions where the water is "hard". Similar findings have been reported from many areas of Japan and the United Kingdom where the subject has been studied. It has also been suggested by Schroeder that the primary noxious factor in the water may be copper from the water pipes which reaches consumers in "soft" water areas. Presumably, the copper in household water pipes carrying "hard" water is isolated from the water by a chemical lining or crust which coats the inside surface of the pipes.

The demonstration of mutagenicity by oxidation of ascorbic acid in the presence of cupric ions and oxygen does not prove that this chemical contamination causes cancer or damage to human tissues. However, research indicates that the resulting "ascorbate-free-radical" or mono-dehydroascorbic acid is a highly potent substance which is released during X-ray irradiation of mammalian tissues (Vaughan, et al, 1973), infra. Furthermore, cell damage during such irradiation is known to be proportional to the

copper content of the tissue. Other research by Van der Schans (1978), infra, has shown that single-strand and double-strand breaks in DNA can be produced either by gamma radiation or by the action of ascorbic acid in the presence of cupric ions and oxygen. Moreover, a problem has been reported with many fish and hatching birds showing congenital abnormalities in a lake district in the Northeastern United States where acid rain having a ph of 4.1 is leaching copper from the tailings of an old copper mine.

Pure ascorbic acid is normally a safe and valuable substance for human consumption, since ascorbic acid oxidation is virtually arrested by the acid in the stomach during digestion. Furthermore, the heavy metals present in drinking water are usually chelated by food proteins and amino-acids before reaching the alkaline medium of the jejumum. However, about 10% of the population has no hydrochloric acid in the stomach, a condition known as achlorhydria, and these people are most likely to be adversely affected by ascorbic acid when taken with copper-containing tap water on an empty stomach. Indeed, there is a strong association between achlorhydria and cancer of the stomach.

Description of the Prior Art

Considerable effort has been devoted to the problem of heavy-metal contamination of water, the mutagenicity of

various bioflavonoids and related organic compounds, and to a lesser extent, to the antioxidant characteristics of bioflavonoid compounds. A summary of these efforts is documented as follows:

Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D. (1973) Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. Proc. Natl. Acad. Sci. U.S.A. 70 No. 8. 2281-2283.

Brown, J.P. (1980) A Review of the Genetic Effects of Naturally Occurring Flavonoids, Anthroquinones and Related Compounds. Mutation Research 75. 243-277.

Brown, J.P. and Dietrich, P.S. (1979) Mutagenicity of Plant Polyphenols in the Salmonella/Mammalian Microsome Test. Activation of flavonol glycosides by mixed glycosidases from rat fecal bacteria and other sources. Mutation Research 66. 223-240.

Brown, J.P., Dietrich, P.S. and Brown, R.J. (1977) Frameshift Mutagenicity of Certain Naturally Occurring Phenolic Compounds in the Salmonella/Microsome Test: Activation of anthraquinone and flavonol glycosides by gut bacterial enzymes. Biochemical Society-Transactions 5. 1489-1492.

Clemetson, C.A.B. (1967) I bioflavonoidi quali antiossidanti per l'acido ascorbico. pp 584-593 in Bioflavonoidi, Ed Zambotti, V. Published by Scuole Grafiche Artigianelli Pavoniani, Milano, being the transactions of a Symposium sui Bioflavonoidi, held at Stresa on Lago Maggiore in Italy, April 23-25, 1966.

Clemetson, C.A.B. and Andersen, L. (1966) Plant polyphenols as antioxidants for ascorbic acid. Annals of the New York Academy of Sciences. 136. Art. 14. Pages 339-378.

Hughes, R.E. (1956) The use of homocysteine in the estimation of dehydroascorbic acid. Biochem. J. 64. 203-208.

Schroeder, H.A. (1960) Relations between hardness of water and death rates from certain chronic and

degenerative diseases in the United States. J. Chron. Dis. 12. 586-591.

Schroeder, H.A. (1966) Municipal drinking water and cardiovascular death rates. J. Amer. Med. Assoc. 195. 125-129.

Stich, H.F., Karim, J., Koropatnick, J. and Lo, L. (1976) The mutagenic action of ascorbic acid. Nature 260. 722-724.

Tamura, G., Gold, C., Ferro-Luzzi, A. and Ames, B.N. (1980) Fecalase: a model for activation of dietary glycosides to mutagens by intestinal flora. Proc. Natl. Acad. Sci. U.S.A. 77. No. 8. pp. 4961-4965.

Van der Schans, G.P. (1978) Gamma-ray induced double-strand breaks in DNA resulting from randomly-inflicted single-strand breaks: temporal local denaturation, a new radiation phenomenon? International Journal of Radiation Biology 33. 105-120.

Vaughan, W.N., Henry, J.I. and Commoner, B. (1973) Radiosensitivity and the ascorbic acid electron spin resonance doublet. Biochem. Biophys. Acta. 329. 159-162.

I have demonstrated that bioflavonoids (C_6-C_3-C_6) compounds having a 3'-4' catechol couplet in the B-ring or a 3-hydroxyl, 4-carbonyl couplet in the gamma pyrone ring, act as indirect antioxidants for ascorbic acid by chelating heavy-metal catalysts. Moreover, suspensions of several bioflavonoids were found to be more effective as antioxidants for ascorbic acid, than were the small amounts of the bioflavonoids that would dissolve in aqueous media. Through experimentation, it became clear that traces of heavy metal catalysts in the salts from which phosphate buffers had been prepared were being attached to the surface

of the chelating bioflavonoid particles. It was also found that the bioflavonoid-metal complex could be filtered off and the filtrates still showed the same antioxidant effect as though the bioflavonoid were still present. However, the choice of bioflavonoids for use in the invention has been found to be somewhat limited, since research by Brown, Dietrich and Brown (1977), Brown & Dietrich (1979) and Brown (1980) has shown that bioflavonoid compounds having the 3-hydroxyl, 4-carbonyl couplet in the gamma pyrone ring, such as quercetin, are mutagenic in the Ames test (Ames, et al, 1973). Moreover, Tamura, et al (1980) have demonstrated that rutin, the L-rhamno d-glucoside of quercetin becomes mutagenic after incubation with faeces.

The bioflavonoids of plants are desirable materials from which to select protective coatings for ascorbic acid since these materials are natural, non-toxic constituents of vegetable foods. Although the materials are relatively insoluble in aqueous media, many of these plant polyphenols possess excellent indirect antioxidant activity by virtue of chelating heavy metals. Of these bioflavonoids, rutin, quercetin and catechin have been found to be particularly effective as chelating agents. However, as noted above, both rutin and quercetin are known to become mutagenic under certain conditions. In contrast, d-catechin has been found to be a chelating antioxidant for ascorbic acid by virtue of its 3', 4' catechol couplet in the B-ring and it lacks the

mutagenic 3-hydroxyl, 4-carbonyl couplet in the gamma pyrone ring. Brown and Dietrich (1979) have shown that d-catechin is non-mutagenic.

Accordingly, it is an object of this invention to provide coatings for ascorbic acid tablets and other medicaments which are adversely affected by oxidation catalyzed by heavy metal ions, which coatings are characterized by a relatively insoluble, non-toxic, non-mutagenic heavy-metal chelating agent such as d-catechin (+ catechin) and other catechins, tannins and fibers which will chelate and inactivate or precipitate copper or other heavy metal catalysts present in drinking water, before the water can gain access to the vitamin or other medicament core.

Another object of this invention is to use the bioflavonoids of plants as natural, nontoxic constituents of vegetable foods, to coat ascorbic acid tablets and other vitamins and medicaments in layered dosage structures in order to chelate heavy metals and prevent the heavy-metals from gaining access to and catalyzing oxidation of the vitamins or other medicaments.

Another object of this invention is to provide new and improved d-catechin coated ascorbic acid tablets and other medicaments for consumption by individuals needing Vitamin C.

Still another object of this invention is to provide ascorbic acid tablets and other medicaments which are coated

by d-catechin in layered dosage structures in order to facilitate chelation and inactivation of heavy-metal catalyst ions such as the Cu^{++} ion in drinking water before the ions of such metals reach and catalyze oxidation of the ascorbic acid or other medicament in the core of the coated tablet.

Yet another object of the invention is to provide a sugar coated, d-catechin encapsulated ascorbic acid tablet, vitamin or medicament with an inner layer of gelatin located between the d-catechin outer layer and the inner ascorbic acid core in dosage structures containing the medicament.

A still further object of this invention is to provide dosage structures which are characterized by catechin-coated vitamins, pills, tablets, granules, capsules and other non-toxic, non-mutagenic, bioflavonoid, tannin and catechin-coated formulations, medications and/or medicaments and dosage structures, in order to protect the oxidation-vulnerable vitamin, mediation or medicament core from oxidation which is catalyzed by heavy metal ions to form mutagenic compounds.

Another object of the invention is to provide a method of protecting ascorbic acid from contact with heavy metal ions which includes coating the ascorbic acid with at least one layer of a non-toxic, non-mutagenic bioflavonoid, catechin, tannin or other chelating fiber such as d-catechin.

Summary of the Invention

These and other objects of the invention are provided in non-toxic, non-mutagenic catechin, bioflavonoid, tannin or other chelating fiber-coated vitamins, pills, tablets, granules, capsules and/or formulations, medications and medicaments and particularly, d-catechin coated pills, tablets, granules, capsules and/or formulations, medications, medicaments and other dosage structures of ascorbic acid. The invention also includes a method of protecting ascorbic acid medicaments from heavy metal catalyzed oxidation, which method includes coating the ascorbic acid with d-catechin.

Brief Description of the Drawing

The invention will be better understood by reference to the accompanying drawing, wherein:

FIGURE 1 is a plan view, partially in section, of a dosage structure containing an ascorbic acid core and a d-catechin coating;

FIGURE 2 is a plan view, partially in section, of a dosage structure containing an ascorbic acid core, a gelatin layer and a d-catechin coating;

FIGURE 3 is a sectional view of a dosage structure containing an ascorbic acid core, an inner layer of gelatin, a first layer of d-catechin, an intermediate layer of gelatin, a second layer of d-catechin and a sugar coating;

FIGURE 4 is a plan view, partially in section, of a dosage structure containing an ascorbic acid core, a layer of d-catechin and a sugar coating;

FIGURE 5 is a plan view, partially in section, of a dosage structure containing an ascorbic acid core, a layer of gelatin, a layer of d-catechin and a sugar coating;

FIGURE 6 is a representation of the chemical formula for d-catechin;

FIGURE 7 is a generic formula for a family of flavones, flavanones, flavonols, flavanonols and flavanes, wherein the respective "R" and number combinations represent certain elements or compounds attached to the basic ring structure and delineated, along with d-catechin, in the Table below to define the respective members of the family;

FIGURE 8 consists of four graphs, A-D, of the concentrations of ascorbic acid and dehydroascorbic acid plotted versus the time of heavy metal-catalyzed oxidation and hydrolysis, respectively, of these compounds.

FIGURE 9 is a representation of the chemical changes that occur when ascorbic acid comes in contact with copper in the presence of oxygen; and

FIGURE 10 is a representation of the chelation or binding and inactivation of copper by d-catechin, which is used to coat ascorbic acid in this invention.

Description of the Preferred Embodiments

Referring to FIGURE 1 of the drawing, a first dosage structure is illustrated by reference numeral 1. The first dosage structure 1 is characterized by an ascorbic acid core 2 of selected dosage with a catechin coating 3 encapsulating the ascorbic acid core 2, as illustrated. FIGURE 2 illustrates a second dosage structure 4 which is likewise provided with an ascorbic acid core 2 of selected dosage and further including a gelatin core layer 5 of desired thickness which contains the ascorbic acid core 2 and an outer catechin coating 3 of selected thickness. FIGURE 3 illustrates a third dosage structure 6 which includes an ascorbic acid core 2 of selected dosage; a gelatin core layer 5 encapsulating the ascorbic acid core 2; a catechin inner layer 7 of selected thickness covering the gelatin core layer 5; a gelatin outer layer 8 coating the catechin inner layer 7; a catechin outer layer 9 encapsulating the gelatin outer layer 8 and a sugar coating 10, provided as an outer covering for the third dosage structure 6. FIGURE 4 illustrates a fourth dosage structure 11 which is characterized by an ascorbic acid core 2 of selected dosage, a catechin outer layer 9 of selected thickness and a sugar coating 10 which encapsulates both the catechin outer layer 9 and the ascorbic acid core 2. FIGURE 5 represents a fifth dosage structure 12 having an ascorbic acid core 2 of selected dosage, a gelatin core layer 5 covering the

ascorbic core 2, a catechin outer layer 9 of selected thickness encapsulating the gelatin core layer 5 and an outer sugar coating 10 which covers the catechin outer layer 9.

It will be appreciated by those skilled in the art that while the first dosage structure 1, second dosage structure 4, third dosage structure 6, fourth dosage structure 11 and fifth dosage structure 12 are illustrated in spherical configuration, dosage structures having alternative shapes and selected sizes are also applicable in the invention. Furthermore, the catechin coated dosage structures illustrated in FIGURES 1-5 are not all inclusive of the possible combinations for dosage structures utilizing catechin coated ascorbic acid, but are illustrative only, and it is understood that other combinations and alternative pharmaceutical cores may also be utilized in combination with d-catechin according to the teaching of this invention, in order to minimize undesirable catalyzing of the pharmaceutical core by heavy metal ions.

In a preferred embodiment of the invention, the d-catechin coating 3 and the ascorbic acid core 2 contain about 200 mg each of d-catechin and ascorbic acid. Furthermore, a suitable dosage in the range of from about 50 to about 500 mg of d-catechin coating 3 may be used to coat each ascorbic core 2, which may also contain 50 to 500 mg of ascorbic acid, in each of the first dosage structure 1,

second dosage structure 4, fourth dosage structure 11 and fifth dosage structure 12. Regarding the third dosage structure 6 illustrated in FIGURE 3, both the catechin inner layer 7 and the catechin outer layer 9 most preferably contain about 100 mg of d-catechin, while the ascorbic acid core 2 contains about 200 mg of ascorbic acid. However, it is understood that a thicker coating or coatings of d-catechin can be used under circumstances where heavy metal ions are known to be present in drinking water or other ingested material in above average concentrations.

It will be further appreciated by those skilled in the art that the ascorbic acid core 2 can be characterized as a pill, tablet, granule, capsule or other ascorbic acid structure, rather than a spherical core of ascorbic acid as illustrated in the drawing, the drawing representation being illustrative of the dosage structure. Furthermore, as above described, the first dosage structure 1, second dosage structure 4, third dosage structure 6, fourth dosage structure 11 and the fifth dosage structure 12 are not all inclusive of the possible combinations for dosage structures utilizing catechin-coated ascorbic acid according to the teaching of this invention. For example, the use of the gelatin core layer 5 in the second dosage structure 4 and fifth dosage structure 12 and the gelatin core layer 5 and outer layer 8 in the third dosage structure 6 serves to prolong dissolving of the respective dosage structures to

facilitate better interaction between the catechin in the dosage structures and any heavy metal ions which may be located in drinking water or in the stomach of the person ingesting the dosage structures. The provision of a sugar coating 10 in the third dosage structure 6, fourth dosage structure 11 and the fifth dosage structure 12 serves to act as a container for the catechin, to prevent crumbling of the dosage structure prior to consumption and to make the dosage structure more palatable.

Referring now to FIGURE 6 of the drawing, a representation of the formula of d-catechin is illustrated, using conventional nomenclature. It is understood that both d-catechin as well as isomers of d-catechin and tannins which are catechin polymers, as well as alternative forms of non-toxic and non-mutagenic chelating fibers and other chelating antioxidant coatings can be used according to the teaching of this invention to chelate heavy metal ions and reduce the oxidation of ascorbic acid in dosage structures which are designed according to the teaching of this invention.

As illustrated in FIGURE 7, various flavones, flavanones, flavonols, flavanonols and flavanes are represented by the illustrated 3-ring structure 14, where the "R" and number combinations represent various element and compound bonds to the illustrated points in the rings to define the respective compounds set forth in the following

table. The following Example I and table illustrate the antioxidant activity of d-catechin and other bioflavonoid compounds in a sodium phosphate buffer containing traces of iron, copper and tin, where the antioxidant activity is expressed as:

$$\frac{(a-b)X100}{a},$$

where "a" is the ascorbic acid lost in a given interval of time in the buffer alone and "b" represents the ascorbic acid lost in the same time interval in the presence of a suspension of the chosen bioflavonoid. The initial ascorbic acid concentration was 19.6 mcg/100 ml and the analytical results were obtained by the dichloroindophenol photometric method. The couplets which are underlined in the table are responsible for the chelation of the heavy metals.

EXAMPLE I

A one-tenth molar phosphate buffer of pH 7.4 was prepared from reagent grade Na_2HPO_4 and NaH_2PO_4, using glass distilled water. One hundred ml of this buffer was warmed to 37°C in a water bath and 2 ml of a fresh solution of ascorbic acid (1 mg/ml) at about 5°C was added to give an initial ascorbic acid concentration of 19.6 mcg/ml. Samples (4 ml each) were removed and added to cold 3 percent HPO_3 (6 ml) at five-minute intervals for 30 minutes to arrest

oxidation. Each of the resulting solutions was analyzed in triplicate for reduced ascorbic acid by a buffered 2.6-dichlorodoindophenol photometric method, using half and one-minute optical density readings to extrapolate for 0 time.

The time required for loss of 80 percent of the reduced ascorbic acid under these conditions, or (t), was obtained from the curve of these results. This varied from 20 to 30 minutes with different lots of buffer, but was relatively constant for buffer solutions made from the same salts.

The pH 7.4 phosphate buffer selected for subsequent experiments was found, on emission spectroscopy of its component salts, to contain iron, tin, magnesium and traces of copper; its total heavy metal content as Pb from the listed analysis of the lots of monobasic and dibasic sodium phosphate was approximately 13 mcg/100 ml; it gave a time (t) of 20 minutes.

Weighed amounts of each test substance, such as 1, 3, 10, 30, 100, 300 and 1000 mg, were placed in flasks and made up to 100 ml with the same buffer. These suspensions of flavonoids, catechins, or related substances were allowed to stand for one hour; their pH was checked and re-adjusted to 7.4 (7.38-7.42) when necessary. They were then warmed to 37°C before adding ascorbic acid for comparative experiments. Two controls of ascorbic acid in buffer alone were used with each set of tests.

Test blanks consisting of test substance in phosphate buffer without ascorbic acid were run for the higher concentrations of each test substance; when these differed appreciably from the results obtained for a plain buffer blank, it was necessary to run test blanks for lower concentrations and to discard the results obtained with higher concentrations which interfered with the method. Some interference by color was observed with cyanidin chloride, and interference by reduction of indophenol was observed with dihydrorobinetin, which has a pyrogallol configuration of three adjacent phenolic groups in the B-ring, so these substances are not included in the table. Most flavonoids caused no such interference.

Experiments were also performed to ensure that the highest concentration of each test substance did not adsorb ascorbic acid. This was achieved by analyzing samples obtained immediately after adding ascorbic acid. No appreciable adsorption of ascorbic acid by flavonoids was observed.

Ascorbic acid was added to the flasks at two-minute intervals and its oxidation was arrested at time (t), for each, by acidification of an aliquot with metaphosphoric acid. The suspensions were filtered to obtain clear solutions for analysis. Minor corrections for color or turbidity of the solutions and for variations of cuvette density were made by adding a few grains of ascorbic acid to

the mixture of indophenol and test solution to decolorize the indophenol after each determination, so that the optical density due to the test substance and cuvette variation could be subtracted from the original readings.

The results of testing the antioxidant activity of 0.001 molar suspensions of several bioflavonoids (flavones, flavanones, flavonols, flavanonols and flavanes) including d-catechin, according to the procedure outlined in the above example, are set forth in the following Table. It should be noted in the Table that the rate of oxidation of ascorbic acid was reduced by 51 percent in the presence of an 0.001 M suspension of d-catechin. However, in another experiment, the rate of oxidation of ascorbic acid was reduced by 80 percent in the presence of a 0.01 M suspension d-catechin. Furthermore, the filtrate of a 0.01 M suspension of d-catechin gave the same excellent antioxidant activity for ascorbic acid as shown by comparing the graphs A and B in FIGURE 8. Moreover, d-catechin treatment did not affect the rate of hydrolysis of dehydroascorbic acid, as shown by comparing graphs C and D in FIGURE 8.

TABLE

Percentage Reduction of the Oxidation of Ascorbic Acid by 10^{-3}M Concentrations of Various Bioflavonoids

Positions of chemical groups. (Ref.: Fig. 7)	7	5	4	3	3'	4'	Percentage Reduction in Rate of Oxidation	Mutagenicity
Flavones								
Apigenin	OH	OH	O	H	H	OH	7	0
Acacetin	OH	OH	O	H	H	OCH_3	6	-
Luteolin	OH	OH	O	H	OH	OH	36	0
Chrysoeriol	OH	OH	O	H	OCH_3	OH	0	-
Cosmetin	O-glucose	OH	O	H	H	OH	22	-
Flanavones								
Eriodictyol	OH	OH	O	H_2	OH	OH	26	0
Hesperetin	OH	OH	O	H_2	OH	OCH_3	7	0
Naringenin	OH	OH	O	H_2	H	OH	3	0
Hesperidin (puriss)	O-rutinose	OH	O	H_2	OH	OCH_3	-2	0
Herperidin (commercial)							35	-
Naringin	O-neohesperidose	OH	O	H_2	H	OH	14	0
Neohesperidin	O-neohesperidose	OH	O	H_2	OH	OCH_3	0	-

TABLE CONTINUED

Percentage Reduction of the Oxidation of Ascorbic Acid by 10^{-3}M Concentrations of Various Bioflavonoids							Percentage Reduction in Rate of Oxidation	Mutagenicity
Flavonols								
Quercetin	OH	OH	O	OH	OH	OH	96	***
Rhamnetin	OCH_3	OH	O	OH	OH	OH	83	***
Fisetin	OH	H	O	OH	OH	OH	54	***
Kaempferol	OH	OH	O	OH	H	OH	82	***
3-Hydroxyflavone	H	H	O	OH	H	H	15	0
Rutin	OH	OH	O	O-rutinose	OH	OH	95	**
Quercitrin	OH	OH	O	O-rhamnose	OH	OH	80	**
Hyperosid	OH	OH	O	O-galactose	OH	OH	84	*
Robinin	O-rhamnose	OH	O	O-robinobiose	H	OH	23	**
Flavanonols								
Dihydroquercetin	OH	OH	O	H.OH	OH	OH	66	-
Dihydrofisetin	OH	H	O	OH.H	OH	OH	46	-
Flavanes								
d-Catechin	OH	OH	H_2	H.OH	OH	OH	51	0
l-Epicatechin	OH	OH	H_2	OH.H	OH	OH	36	-

*** indicates that the compound is mutagenic.
** indicates that the compound is a pro-mutagen (can be converted into a mutagen in the bowel).
* indicates that the compound is probably a pro-mutagen.
0 indicates that the compound is non-mutagenic.
- indicates that no data are available concerning the mutagenicity or non-mutagenicity of this compound.

EXAMPLE II

A procedure similar to that set forth in Example I was followed in another study of the oxidation of L-ascorbic acid in 0.10 molar sodium phosphate buffer containing traces of heavy metal impurities at a pH of 7.4 at a lower temperature of (23°C); the Hughes (1956) homocysteine method was used so as to analyze both for the reduced form of ascorbic acid (AA) and for the oxidized form- dehydro-ascorbic acid (DHA). The results of this study are shown in the time and concentration graphs A-D in FIGURE 8. Each of the graphs A and B illustrates the loss of AA by heavy metal catalyzed oxidation to DHA, wherein the "ascorbic-free-radical" intermediate is formed, and also spontaneous hydrolysis of DHA, wherein vitamin C (AA & DHA) is lost.

Graph A illustrates a study of ascorbic acid in phosphate buffer alone and graph B shows a study conducted using the same buffer after treatment with d-catechin; the catechin was added to the phosphate buffer to form a suspension, which was shaken well for one minute; all of the undissolved catechin was then removed by filtration (along with the chelated heavy metals) before addition of ascorbic acid. The difference in the rate of oxidation of ascorbic acid as a result of catechin treatment is clearly shown by contrasting graphs A and B. Similar results are obtained

when d-catechin is not removed by filtration before adding the ascorbic acid.

The rates of spontaneous hydrolysis of dehydroascorbic acid in 0.10 molar phosphate buffer alone and in the same buffer after treatment with 0.01 molar d-catechin are illustrated in graphs C and D of FIGURE 8. It is evident that treatment with d-catechin does not significantly affect the rate of hydrolysis of dehydroascorbic acid.

As illustrated in FIGURE 9, cupric ions catalyze the conversion of ascorbic acid 15 to dehydroascorbic acid 17 by a two-stage oxidation. A highly reactive, short-lived compound, monodehydroascorbic acid or "ascorbate-free-radical" 16 is released; it is the release of this intermediate "ascorbate-free-radical" 16 which causes the ascorbic acid 15 to become mutagenic in the presence of copper. Dehydroascorbic acid 17 is an unstable compound, with a short half-life, and undergoes spontaneous hydrolysis, as illustrated to form diketogulonic acid 18, with loss of vitamin C activity, as illustrated.

Referring to FIGURE 10, binding of copper by d-catechin molecule is illustrated.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

Having described my invention with the particularity set forth above, what is claimed is:

CLAIMS:

1. An ascorbic acid dosage structure comprising an ascorbic acid core and at least one layer of a non-toxic, non-mutagenic chelating agent having an affinity for heavy metals encapsulating said ascorbic acid core.

2. The ascorbic acid dosage structure of Claim 1 wherein said chelating agent is a plant polyphenol selected from the group, bioflavonoids, catechins and tannins.

3. The ascorbic acid dosage structure of Claim 2 wherein said plant polyphenol is catechin.

4. The ascorbic acid dosage structure of Claim 1 further comprising a sugar coating substantially covering said chelating agent.

5. The ascorbic acid dosage structure of Claim 2 wherein said plant polyphenol is catechin and further comprising a sugar coating substantially covering said catechin.

6. The ascorbic acid dosage structure of Claim 1 further comprising at least one layer of gelatin located

between said chelating agent and said ascorbic acid core, said gelatin substantially encapsulating said ascorbic acid core.

7. The ascorbic acid dosage structure of Claim 6 further comprising a sugar coating substantially covering said chelating agent.

8. The ascorbic acid dosage structure of Claim 6 wherein said at least one layer of gelatin is a first layer of gelatin substantially encapsulating said ascorbic acid core and a second layer of gelatin substantially coating said chelating agent.

9. The ascorbic acid dosage structure of Claim 8 wherein said at least one layer of chelating agent is a first layer of plant polyphenol disposed between said first layer of gelatin and said second layer of gelatin and a second layer of plant polyphenol substantially coating said second layer of gelatin.

10. The ascorbic acid dosage structure of Claim 9 wherein said first layer of plant polyphenol and said second layer of plant polyphenol are both catechin.

11. The ascorbic acid dosage structure of Claim 9 further comprising a sugar coating substantially covering said second layer of plant polyphenol.

12. The ascorbic acid dosage structure of Claim 9 wherein said first layer of plant polyphenol is a first layer of catechin and said second layer of plant polyphenol is a second layer of catechin and further comprising a sugar coating substantially covering said second layer of catechin.

13. The ascorbic acid dosage structure of Claim 1 wherein said ascorbic acid core and said at least one layer of chelating agent are present in said dosage structure in substantially equal proportions by weight.

14. The ascorbic acid dosage structure of Claim 2 wherein said plant polyphenol is catechin, said ascorbic acid core and said catechin are present in said dosage structure in substantially equal proportions by weight and further comprising a sugar coating substantially covering said catechin.

15. The ascorbic acid dosage structure of Claim 2 further comprising at least one layer of gelatin located between said plant polyphenol and said ascorbic acid core,

said gelatin substantially encapsulating said ascorbic acid core and wherein said ascorbic acid core and said plant polyphenol are present in said dosage structure in substantially equal proportions by weight.

16. A method of protecting ascorbic acid from heavy metal ions and preventing the development of mutagens, which method comprises the step of coating the ascorbic acid with a non-toxic, non-mutagenic chelating agent, whereby the metal catalyst binds to the chelating agent before the heavy metal ions can reach the ascorbic acid.

17. The method according to Claim 16 wherein said chelating agent is a plant polyphenol selected from the group, bioflavonoids, catechins and tannins.

18. The method according to Claim 17 further comprising the step of covering the plant polyphenol with a sugar coating.

19. A method of protecting ascorbic acid from contact with a metal catalyst in a solution containing the metal catalyst, said method comprising the steps of providing an ascorbic acid core of selected dosage and coating said ascorbic acid core with a layer of a non-toxic, non-mutagenic plant polyphenol of selected thickness, whereby

the metal catalyst binds to the plant polyphenol as the plant polyphenol is released from the ascorbic acid core.

20. The method according to Claim 19 wherein said plant polyphenol is d-catechin.

21. A method for treating a person requiring an ascorbic acid supplement while protecting said ascorbic acid from heavy metal ions and preventing the development of mutagens which comprises the administration of a therapeutic dosage to the person of a unit dosage structure comprising an ascorbic acid core and at least one layer of a non-toxic, non-mutagenic chelating agent having an affinity for heavy metals encapsulating said ascorbic acid core.

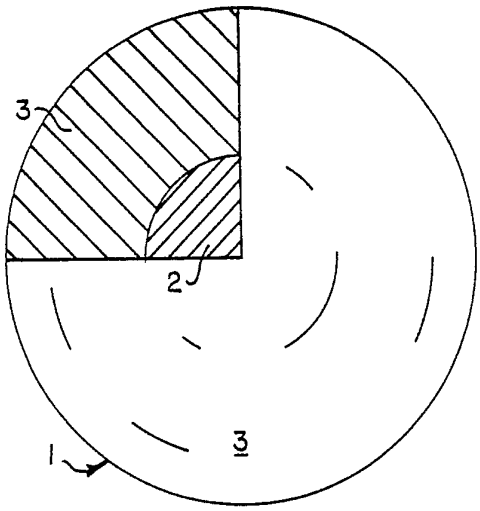


FIG. 1

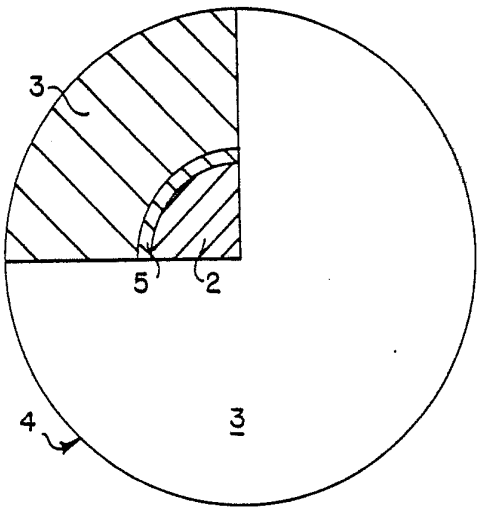


FIG. 2

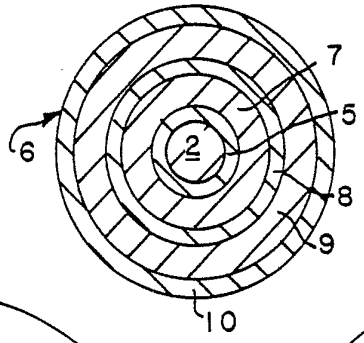


FIG. 3

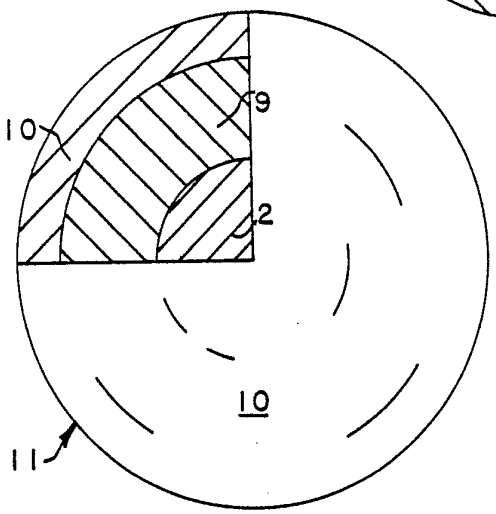


FIG. 4

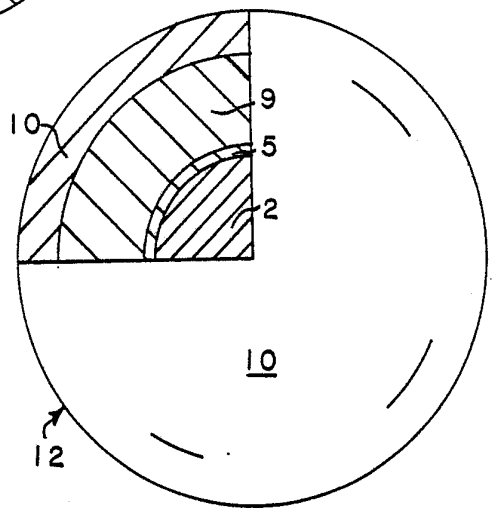


FIG. 5

RO/US 26 MAR 1987

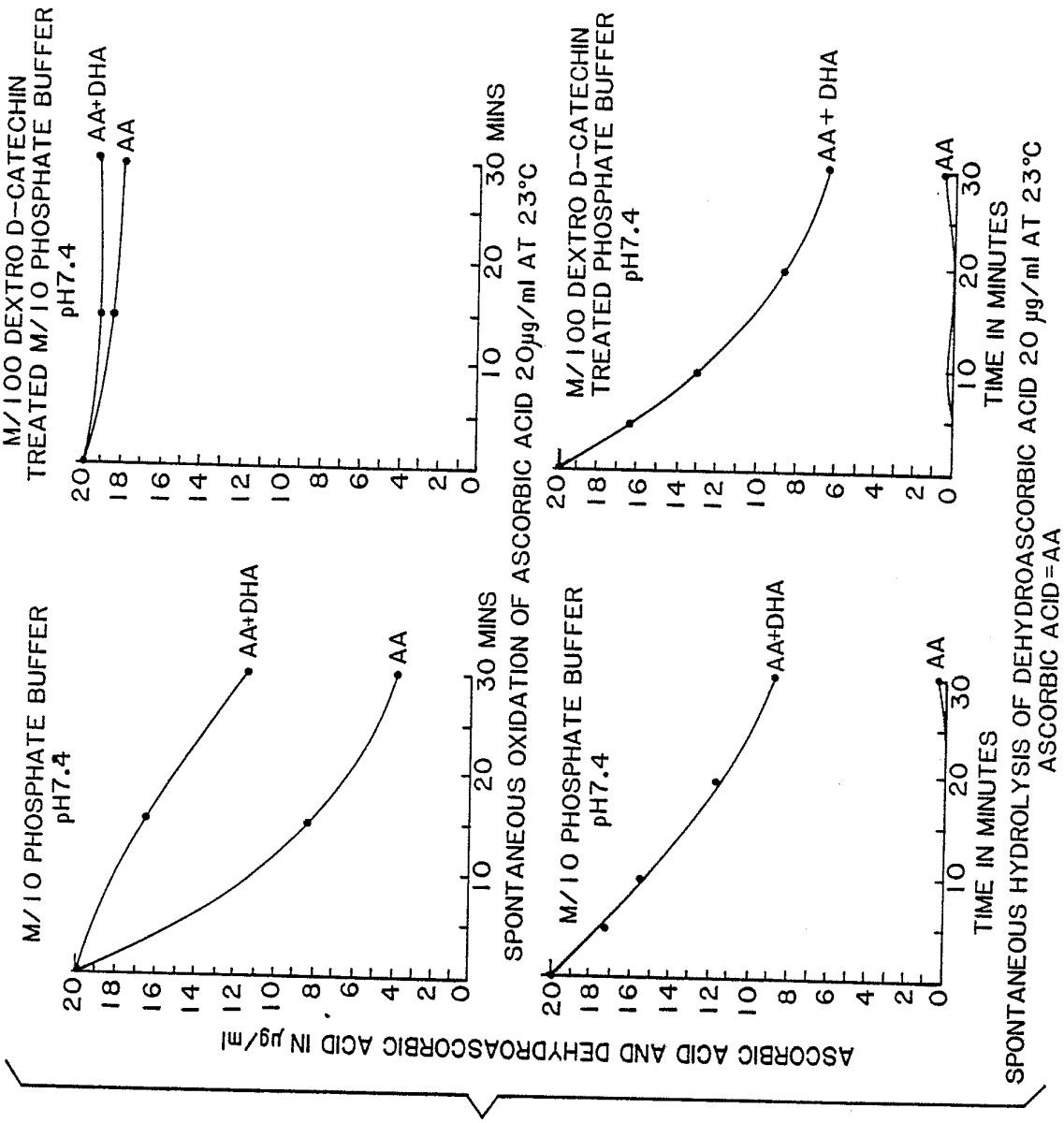


FIG. 8

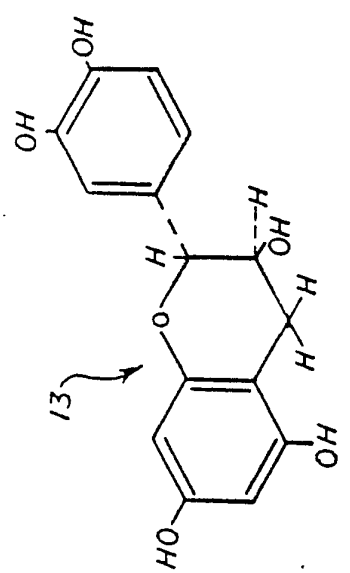


FIG. 6

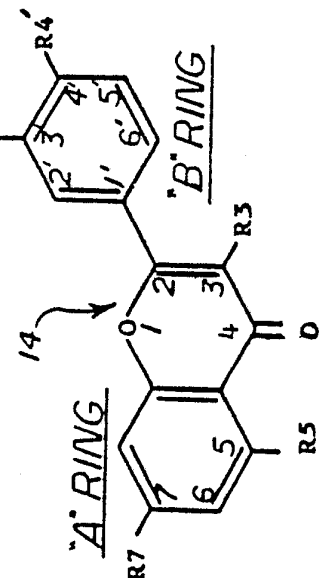


FIG. 7

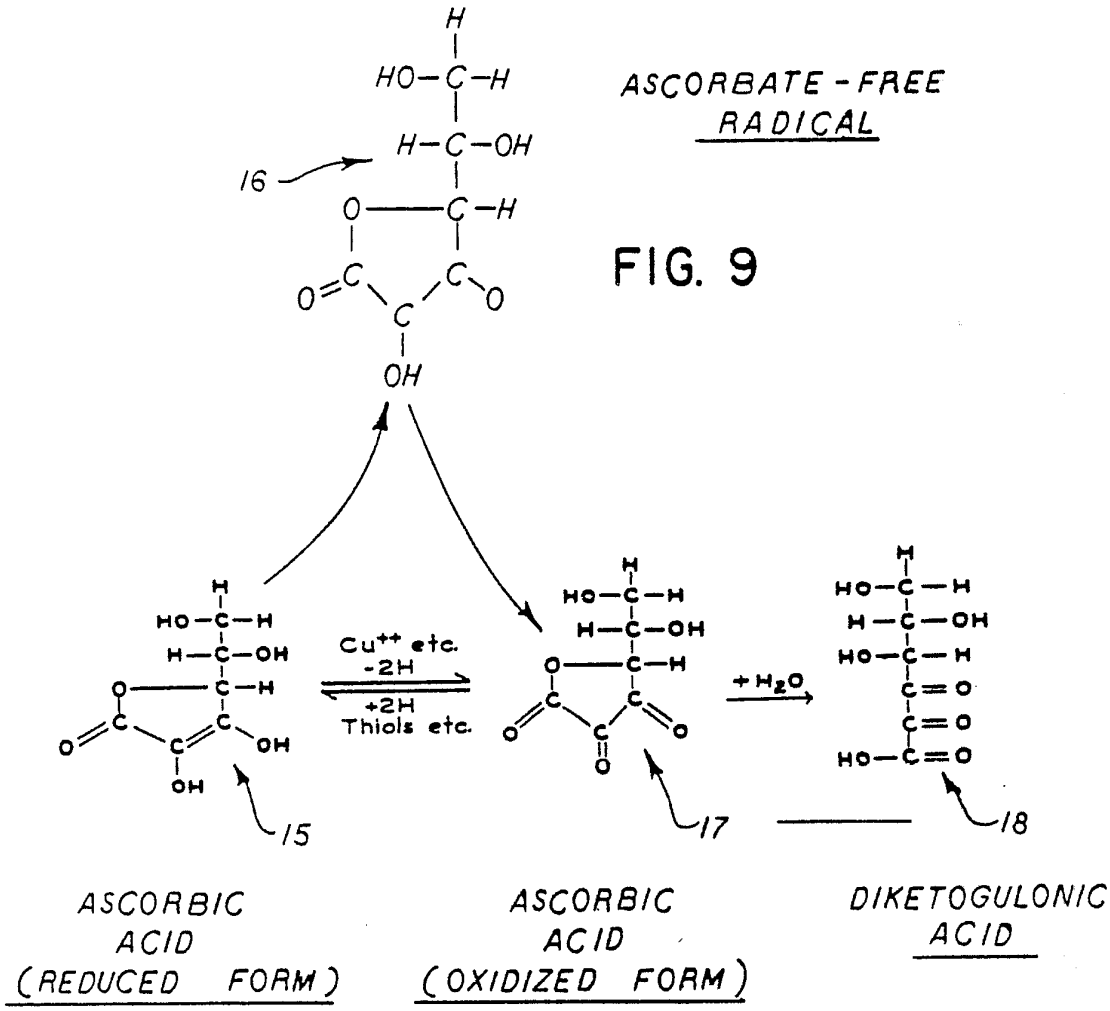
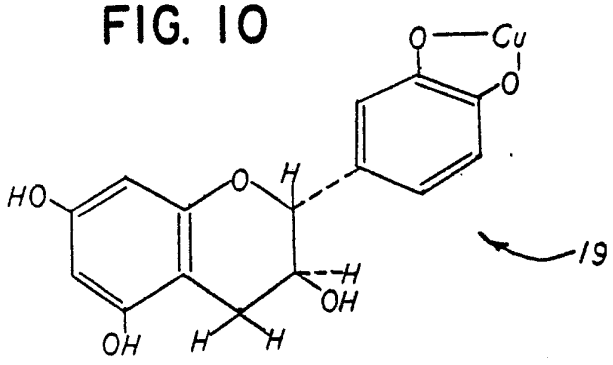

ASCORBATE - FREE RADICAL
16
Cu++ etc.
-2H
+2H
Thiols etc.
+H2O
15
17
18
ASCORBIC ACID (REDUCED FORM)
ASCORBIC ACID (OXIDIZED FORM)
DIKETOGULONIC ACID

FIG. 9

FIG. 10


19

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/00403

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 9/24; 9/36; 9/40; 31/375

US CL : 424/472 424/478 424/3 514/456, 514/457

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols			
U.S.	424/471 514/456	424/478 514/457	424/479	427/3

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US,A, 2,348,503 (TAYLOR) 9 May 1944 (See the entire document)	1,16,19, 21
A	US,A, 2,410,110 (TAYLOR) 29 Oct 1946 (See the entire document)	1,16,19, 21
A	US,A, 2,811,483 (ATERNO) 29 Oct 1957 (See the entire document)	1,16,19 21
A	US,A, 3,125,491 (ELOWE ET AL) 17 March 1964 (See the entire document)	1,16,19, 21
A	US,A, 3,247,064 (MEKAWA ET AL) 19 April 1966 (See the entire document)	1,16,19, 21

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
1 May 1987	19 MAY 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Shep K. Rose

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	US,A, 4,203,997 (KUPPERS ET AL) 20 May 1980 (Col. 4, lines 20-24)	1,16,19, 21
A	US,A, 4,495,177 (TARACATAC ET AL) 22 January 1985 (Col. 3, lines 1 to 8 (Col. 6, lines 27 to 30)	1,16,19, 21

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1 to 3, 13 and 21.

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	US,A, 207,013 (CATER) 13 Aug 1878 (See the entire document)	4,5,7,11, 12,14,18
A	US,A, 462,990 (OPPENHEIMER) 10 Nov. 1891 (Fig. 19)	1 to 21
A	US,A, 2,853,421 (ADAMS ET AL) 23 Sept. 1958 (See the entire document)	6,8,9,15
A	US,A, 3,275,519 (GLASSMAN) 27 Sept. 1966 (See the entire document)	6,8,9,15
A	US,A, 3,887,705 (SERRE ET AL) 3 June 1975 (Ex. 111) (col. 2, lines 39-45)	1 to 21
A	US,A, 3,888,990 (COURBAT ET AL) 10 June 1975 (See the entire document)	2,3,17,20
A	US,A, 4,166,861 (BONATI ET AL) 4 Sept. 1979 (See the entire document)	2,3,17,20
A	US,A, 4,268,517 (NIEBES ET AL) 19 May 1981 (See the entire document)	2,3,17,20
A	US,A, 4,285,964 (NIEBES ET AL) 25 Aug. 1981 (Col. 5, lines 35 to 40)	2,3,17,20
A	US,A, 4,507,314 (NIEBES ET AL) 26 March 1985 (Col. 5, lines 15 to 24)	1,3,17,20
A	US,A, 4,515,804 (MARTI ET AL) 7 May 1985 (See the entire document)	2,3,17,20

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

(ATTACHMENT)

I. ASCORBIC ACID LAYERED CORE AND THERAPY, claims 1 to 3, 13 and 21, class 424, subclass 472;

II. GELATIN CONTAINING COATING, claims 6 to 12 and 15, class 424, subclasses 460, 478, 492;

III. SUGAR CONTAINING COATING, claims 4, 5, and 14, class 424, subclasses 461, 479, 493;

IV. COATING PROCESSES, claims 16 to 20, class 427, subclass 3.