

PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Tetracycline Metallo Salt Complex

We, BRISTOL LABORATORIES INC., a Corporation organised and existing under the Laws of the State of New York, United States of America, of Thompson Road, East Syracuse, New York, State of New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new antibiotic compounds, more particularly, to complexes of tetracycline and sodium or potassium hexametaphosphate. These new tetracycline complexes are prepared by reacting tetracycline or acid addition salts of tetracycline with sodium or potassium hexametaphosphate or alternatively by reacting sodium or potassium salts of tetracycline with hexametaphosphoric acid, and if necessary purifying the reaction product.

The new complexes of the tetracycline are preferably made by mixing aqueous acid solutions of tetracycline with aqueous solutions of sodium or potassium hexametaphosphate, and collecting by filtration under acid conditions the precipitated complexes.

According to a preferred embodiment of this invention an aqueous solution of a tetracycline salt such as the hydrochloride is mixed with an aqueous solution of sodium hexametaphosphate and the precipitated crystalline tetracycline sodium hexametaphosphate is collected by filtration under acid conditions, i.e. pH below 2.0.

The ratios by weight of tetracycline to hexametaphosphate to be used can vary widely; ratios of 1:2 to 1:0.05 are effective and about 1:0.25 or 1:0.33 is preferred.

Preferably the acidity of the tetracycline solution is kept sufficient to maintain the tetracycline reagent in solution. A pH of less than about 2.0 is preferable.

The insolubility of the new complexes makes the concentration used of little importance; reasonably concentrated solutions are, of course, more practical.

The new complexes of tetracycline and a hexametaphosphate are non-toxic, very stable, and provide higher blood levels in dogs and in man upon oral administration than do the forms of tetracycline now in use.

The preferred embodiment of the tetracycline sodium hexametaphosphate complex contains on an anhydrous basis the following elements: carbon, hydrogen, nitrogen, oxygen, sodium and phosphorus in a ratio substantially in accord with the empirical formula

$5C_{22}H_{24}N_2O_8 \cdot NaPO_3 \cdot 5HPO_3$, which may also be described as therapeutically active, stable, non-toxic tetracycline sodium hexametaphosphate complex characterized in purified form on an anhydrous basis by the presence of 0.7 to 1.2% sodium, 5.8 to 7.8% phosphorus, a potency in the range of 760 to 940 micrograms of tetracycline hydrochloride equivalents per milligram by bio-assay, and a solubility in water at room temperature of about 3.2 mg./ml. and further characterized by the production at the end of one and four hours after oral administration to man of a concentration in the blood stream substantially greater than that produced by the administration of an equivalent amount of tetracycline hydrochloride.

Analyses expressed herein as mcg./ml. or mcg./mg. refer, according to the usual custom, to micrograms of tetracycline hydrochloride equivalents. Pure tetracycline hydrochloride has a potency of 1000 mcg./mg. and pure tetracycline base of 1080 mcg./mg., etc.

Average analytical values for nine to twelve separate preparations of tetracycline sodium hexametaphosphate complex were found to be as follows:

Moisture by vacuum-oven technique:	9.7% (range 6.6—15.2%).
Moisture by Karl Fischer:	10.9% (range 8.4—16.7%).
Other values as corrected for moisture by Karl Fischer:	
Phosphorus:	6.8% (range 5.5—7.8%).
Sodium:	1.0% (range 0.8—1.1%).
Potency in mcg./mg. by bio-assay:	843 (range 760—940).
Potency in mcg./mg. by ultraviolet absorption:	843 (range 800—893).

In the preparation of these samples, the ratio of the weights of tetracycline hydrochloride to sodium hexametaphosphate used as reagents varied from 1:2 to 1:0.1 and was 1:0.33 in about half the cases. The difference between the moisture content as determined by the vacuum-oven and Karl Fischer techniques indicates the presence of water of crystallization, probably one molecule per atom of sodium.

These results indicate that anhydrous tetracycline sodium hexametaphosphate complex has the empirical, and probably the molecular, formula $5C_{22}H_{24}N_2O_8 \cdot NaPO_3 \cdot 5HPO_3$, for which the theoretical values are 0.85% sodium, 6.8% phosphorus and 880 mcg./mg. potency, but this is purely a matter of theory and is not essential to the present invention nor is the present invention restricted thereto. This empirical expression is so written for convenience and ease of comprehension and is particularly not intended to imply the existence in this product of any PO_3 ions, as the product is a complex hexametaphosphate.

The tetracycline sodium hexametaphosphate complex is very stable and can be used for oral application in powdered form, as tablets or in capsules, but may also be used in suspensions in aqueous liquids or in anhydrous, edible oils, such as peanut oil, sesame oil or a modified coconut oil with a setting point below 60° F. The present invention also provides a therapeutic composition comprising a tetracycline sodium hexametaphosphate complex and one or more inert pharmaceutical solid carriers.

When desired for specific purposes and rendered pharmaceutically compatible, there may be admixed with a complex of the present invention various other additional medicaments, such as antihistamines, sulpha drugs (e.g. sulphadiazine, sulphabenzamide, sulphacetamide, sulphanilamide, sulphapyridine, sulphathiazole, sulphapyrazine, sulphaguandine, sulphathalidine, sulphasuxidine, sulphisoxazole, sulphamylon, phthalylsulphacetamide, N¹-3,4-dimethylbenzoylsulphanilamide, benzylsulphanilamide and N¹-2-(2-quinoxalyl)sulphanilamide), lipotropic agents (particularly

methionine, choline, inositol and beta-sitosterol and mixtures thereof), stimulants of the central nervous system (e.g. caffeine, amphetamines), local anesthetics, analgesics (e.g. aspirin, salicylamide, sodium gentisate, p-acetylamino-phenol, phenacetin, codeine), laxatives (e.g. phenolphthalein), sedatives (e.g. barbiturates, bromides), salts of penicillin (e.g. potassium penicillin G, procaine penicillin G, 1-phenylamine penicillin G, dibenzylamine penicillin G, other salts disclosed by U.S. Patent 2,627,491; these combinations are particularly useful to enable variation of the pattern of blood levels obtained), phenoxymethylpenicillin and salts thereof, other antibiotic agents (e.g. streptomycin, dihydrostreptomycin, bacitracin, polymyxin, tyrothricin, erythromycin, Aureomycin, oxytetracycline, oleandomycin, chloramphenicol, carbomycin, novobiocin, cycloserine; in some cases such combinations attack a wider range of organisms or show synergistic efficacy or provide decreased toxicity with equal efficacy), vitamins (e.g. Vitamins A, A₁, B₁, B₂, B₆, B₁₂, and members of that family, folic acid and members of that family, Vitamins C, D₂, D₃ and E), hormones (e.g. cortisone, hydrocortisone, 9 α -fluorocortisone, 9 α -fluorohydrocortisone, prednisone and prednisolone), anabolic agents (e.g. 11,17-dihydroxy-9 α -fluoro-17 α -methyl-4-androsten-3-one; 17 α -ethyl-19-nortestosterone) and antifungal agents (e.g. mycostatin, i.e. nystatin).

Following is a description by way of example of methods of carrying the invention into effect.

EXAMPLE I

Five gram lots of tetracycline hydrochloride in aqueous solution at 50 mg./ml. were mixed with aqueous solutions of sodium hexametaphosphate adjusted to pH 1.5 with hydrochloric acid and containing 2.5 g., 1.66 g., 1.25 g. and 0.50 g. respectively of sodium hexametaphosphate. An immediate precipitate of tetracycline sodium hexametaphosphate complex precipitated and was collected by filtration, washed with methanol and dried over phosphorus pentoxide with these results:

Weight Ratios of Sodium Hexametaphosphate to Tetracycline Hydrochloride	Yield in grams	Bio-assay mcg./mg.
1:2	0.5	—
1:3	2.4	630
1:4	0.5	—
1:10	0.9	730

5 This product gave blood levels in dogs of 1.29 and 1.80 mcg./ml. respectively one and four hours after oral administration of a single dose of 12.5 mg. tetracycline hydrochloride equivalents/kg. Under the same conditions tetracycline hydrochloride levels are 0.77—0.92 mcg./ml. after one hour and 0.75—0.76 mcg./ml. after four hours.

10 This product had a solubility in water of about 3.2 mg./ml. at room temperature. Therefore it is clear that the minimum quantity of tetracycline which can be used in this process is 3 mg./ml.; a smaller concentration of tetracycline would not give enough of the complex to exceed the solubility limit.

EXAMPLE II

20 Eighty grams of sodium hexametaphosphate ("Calgon"; Registered Trade Mark) was dissolved in about 1600 ml. water and the pH was adjusted to 1.5 with hydrochloric acid; this solution was then added with stirring to a solution of 240 g. tetracycline hydrochloride in 2.4 liters water (100 mg./ml.). After some tetracycline sodium hexametaphosphate complex formed as a gum, the solution was decanted and seeded to produce crystalline tetra-

cycline sodium hexametaphosphate complex (178 g.) after stirring two hours, collecting by filtration and washing thoroughly with water and then methanol. An additional 21 g. crystalline product was obtained from the gum by stirring it in water with seed crystals. The product gave the following analyses after correction for the presence of 8.4% water (Karl Fischer): Tetracycline hydrochloride equivalents in mcg./mg.: 763 (bio-assay); 815 (ultraviolet assay); sodium, 1.0%; phosphorus, 6.1%. The presence of one, or perhaps two, molecules of water of hydration was indicated by the value of 7.3% moisture found by the vacuum-oven technique.

Theory for [5 tetracycline . NaPO₃ . 5HPO₃] is potency 880, 0.85% sodium and 6.8% phosphorus.

45 The complex gave blood levels in dogs of 1.87 and 1.08 mcg./ml. respectively one and four hours after oral administration of a single dose of 12.5 mg. tetracycline hydrochloride equivalents/kg.

50 In a cross-over test in seven patients, the following blood levels were obtained by single oral doses in amounts in capsules equivalent to 250 mg. tetracycline hydrochloride:

Compound	Blood Levels in mcg./ml. Hours after Administration				
	1	2	3	8	24
Tetracycline Sodium Hexametaphosphate Complex	1.58	2.68	2.61	1.35	0.29
Tetracycline Hydrochloride	0.83	1.37	1.84	1.10	0.22

55 EXAMPLE III

In duplicate runs, a solution of 1200 g. tetracycline hydrochloride in 12.1 water, having pH 1.7, was added to a solution prepared by dissolving 400 g. sodium hexametaphosphate ("Calgon") in 4 l. water, adjusting the pH to 1.7 with concentrated hydrochloric acid and filtering. The first half of the solution was added rapidly; the mixture then grew turbid and the second half of the solution was added slowly over 30 minutes with seeding and stirring. The yellow crystalline tetracycline sodium hexametaphosphate complex which precipitated was collected by filtration after stirring for three hours, washed with 1.5 l. water and then 1 l. methanol, dried 16 hours at 55° C. and found to weigh 1063 g. and 998 g. respectively. The two yields were combined and found to contain 10.7% moisture by Karl

Fischer and, as corrected for moisture, 5.75% phosphorus and 1.1% sodium and to assay 803 and 860 mcgs. tetracycline hydrochloride equivalents/mg. by bio-assay and ultraviolet absorption assay respectively.

80 This produce gave blood levels in dogs of 1.74 and 1.30 mcg./ml. respectively one and four hours after oral administration of a single dose of 12.5 mg. tetracycline hydrochloride equivalents/kg.

EXAMPLE IV

85 Five lots of tetracycline sodium hexametaphosphate complex were prepared by mixing aqueous solutions (adjusted to pH 1.5 with hydrochloric acid) of 25 g. tetracycline hydrochloride in 250 ml. water and sodium hexametaphosphate in the indicated amounts in 125 ml. water and collecting product having the following, tabulated properties:

Weight of Sodium hexameta-phosphate in g.	Yield in grams	Moisture by Karl Fischer	Product Potency*		Na* in %	P* in %
			By Bio-assay	By Ultra-violet Assay		
50.—	7.—	12.5%	940	893	0.9	5.5
25.—	15.5	12.7%	846	800	—	7.7
12.5	17.5	10.3%	894	836	0.9	7.8
8.33	17.5	10.9%	840	838	0.8	7.7
2.5	7.—	10.7%	880	853	0.8	7.0

* Corrected for moisture.

EXAMPLE V

5 In duplicate runs 70 g. sodium hexameta-phosphate (Victor) was dissolved in 1400 ml. water with the addition of a small amount of sodium hydroxide. The pH was then adjusted to 1.5 with concentrated hydrochloric acid. To this solution there was added a solution at

100 mg./ml. of 210 g. tetracycline hydro-chloride adjusted to pH 1.5. After stirring two hours, the precipitated, crystalline tetracycline sodium hexametaphosphate complex was col-lected by filtration, washed with pH 1.5 water and with methanol, air-dried and found to have the following properties:

Analyses (corrected for moisture)

Run No.	Yield in Grams	Moisture by Karl Fischer	Potency		Na in %	P in %
			By Bio-Assay	By Ultra-violet Assay		
1	182	16.7%	855	850	1.1	6.7
2	170	13.6%	855	843	1.0	7.0

EXAMPLE VI

20 Five gram lots of tetracycline hydrochloride in aqueous solution at 50 mg./ml. were mixed with aqueous solutions of sodium hexameta-phosphate ("Calgon") adjusted to pH 1.5 with hydrochloric acid and containing 2.5 g., 1.66 g., 1.25 g., and 0.50 g. respectively of sodium hexametaphosphate. An immediate precipitate of tetracycline sodium hexameta-phosphate complex precipitated either as crystals or as a gum which gradually crystal-lized. The yields after methanol-washing and drying over P₂O₅ were 3.25 g., 3.25 g., (bio-assay 660 mcg./mg.), 2.75 g. and 1.0 g. respec-tively. The product had a solubility in water of about 3.1 mg./ml., gave blood levels in dogs of 2.59 and 1.54 mcg./ml. respectively one and four hours after oral administration of a single dose of 12.5 mg. tetracycline hydro-chloride equivalents/kg., and was found by analysis to contain 7.0% phosphorus and

2.60% moisture.

EXAMPLE VII

40 Capsules containing the tetracycline sodium hexametaphosphate complex of the present invention were prepared by mixing the salt with 5% magnesium stearate (200 mesh) and as much lactose as needed for the size of the capsule to be used, screening the mixture and filling into hard gelatin capsules. The amount of complex in each capsule was that equivalent to 250 mgs. tetracycline hydrochloride, e.g. 340 mgs. for a sample of complex having a potency by bio-assay of 730 mcg./mg.

50 The blood levels produced in man upon oral administration of a single capsule containing the equivalent of 250 mgs. of tetracycline hydrochloride were determined for the above capsules and for two brands of tetracycline hydrochloride capsules used as controls with the following results:

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Form of Tetracycline	No. of Patients	Blood Levels in Man					
		Mcgs. of Tetracycline per ml. of Plasma at the indicated number of hours after administration					
		Hours					
		1	2	3	4	8	24
Brand A of tetracycline hydrochloride	5—21	0.55	1.21	0.87	—	0.70	0.16
Brand B of tetracycline hydrochloride	10—22	0.41	0.92	0.99	—	0.63	0.20
Tetracycline Sodium Hexameta- phosphate Complex	9	0.94	—	—	1.75	1.22	0.21

- 5 It is obvious by inspection that the tetracycline sodium hexametaphosphate complex gave much higher blood levels without loss of duration. An additional quantitative measure of this improvement is furnished in the usual way by plotting these blood levels and measuring (integrating) the areas under the curves. It is thus found that tetracycline sodium hexametaphosphate complex is absorbed nearly twice as efficiently as tetracycline hydrochloride. 10

EXAMPLE VIII

Tablets containing the following ingredients:

15	Tetracycline Sodium Hexameta- phosphate Complex (60 mesh)	0.400 g.
	Calcium Phosphate Monobasic, anhydrous (60 mesh)	0.025 g.
	Calcium Phosphate Dibasic, anhydrous (60 mesh)	0.025 g.
20	Lactose U.S.P., powdered (60 mesh)	0.050 g.
	Starch U.S.P. (60 mesh)	0.057 g.
	Magnesium Stearate (200 mesh)	0.003 g.

- 25 are prepared by mixing all of the ingredients with the exception of one-half of the magnesium stearate. The mixture is then slugged and granulated into 20 to 60 mesh granules. The remaining magnesium stearate is added to the granules, which are thoroughly mixed and finally tabletted. 30

~~EXAMPLE IX~~~~Aqueous oral suspensions are prepared by using the following formulations:~~

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Aqueous oral suspensions are prepared by using the following formulations:

	A	B
Kelmar (potassium aliginate)	3.75 g.	—
Sucrose (granulated)	400 g.	647 g.
Methyl Paraben U.S.P. (200 mesh)	0.80 g.	0.80 g.
Propyl Paraben U.S.P. (200 mesh)	0.20 g.	0.20 g.
Cherry Flavor	1.97 cc.	—
Sodium Bisulphite, reagent	1.40 g.	—
Sodium Citrate U.S.P.	2.00 g.	—
Sodium Saccharin U.S.P. (200 mesh)	0.665 g.	—
Sodium Sucaryl (200 mesh)	16.66 g.	—
"Tween 85" (Registered Trade Mark)	—	0.10 cc.
Sodium Metabisulphite	—	1.00 g.
Gum Tragacanth	—	2.00 g.
Oil of Chocolate (cocoa butter; Theobroma oil)	—	0.75 cc.
Oil of Peppermint	—	0.075 cc.
Sodium Chloride	—	3.50 g.
Distilled Water — q.s. to	1000 cc.	1000 cc.

To 500 cc. of distilled water the Methyl Paraben, Propyl Paraben, sodium bisulphite or sodium metabisulphite, sodium chloride, sodium citrate, sodium saccharin and sodium sucaryl are added and agitated until solution is obtained. The sucrose and Kelmar or the sucrose and gum tragacanth are dry mixed and slowly added to the solution with mixing. Then the "Tween 85" and flavours are added under agitation and distilled water added until the total volume is about 950 cc. Finally 40 g. of tetracycline sodium hexametaphosphate complex (200 mesh) is added under agitation, the volume made up to 1000 cc. with distilled water and the product filled into bottles.

WHAT WE CLAIM IS: —

1. Tetracycline metallo-hexametaphosphate complex wherein the metallo-hexametaphosphate is sodium hexametaphosphate or potassium hexametaphosphate.

2. Therapeutically active, stable, non-toxic tetracycline sodium hexametaphosphate complex characterised in purified form on an anhydrous basis by the presence of 0.7 to 1.2%

sodium, 5.8 to 7.8% phosphorus, a potency in the range of 760 to 940 micrograms of tetracycline hydrochloride equivalents per milligram by bio-assay, and a solubility in water at room temperature of about 3.2 mg/ml. and further characterised by the production at the end of one and four hours after oral administration to man of a concentration in the blood stream substantially greater than that produced by the administration of an equivalent amount of tetracycline hydrochloride.

3. A process of preparing a tetracycline sodium hexametaphosphate complex which comprises mixing an aqueous solution containing at least 3 mg./ml. of tetracycline and an aqueous solution containing sodium hexametaphosphate in an amount, by weight, of from one-twentieth to twice the weight of tetracycline in the tetracycline solution, each of the solutions having a pH below 2.0.

4. A process of preparing a tetracycline sodium hexametaphosphate complex substantially as described with reference to any one

EXAMPLE IX

Aqueous oral suspensions are prepared by using the following formulations:

	A	B
Kelmar (potassium aliginate)	3.75 g.	—
Sucrose (granulated)	400 g.	647 g.
Methyl Paraben U.S.P. (200 mesh)	0.80 g.	0.80 g.
Propyl Paraben U.S.P. (200 mesh)	0.20 g.	0.20 g.
Cherry Flavor	1.97 cc.	—
Sodium Bisulphite, reagent	1.40 g.	—
Sodium Citrate U.S.P.	2.00 g.	—
Sodium Saccharin U.S.P. (200 mesh)	0.665 g.	—
Sodium Sucaryl (200 mesh)	16.66 g.	—
"Tween 85" (Registered Trade Mark)	—	0.10 cc.
Sodium Metabisulphite	—	1.00 g.
Gum Tragacanth	—	2.00 g.
Oil of Chocolate (cocoa butter; Theobroma oil)	—	0.75 cc.
Oil of Peppermint	—	0.075 cc.
Sodium Chloride	—	3.50 g.
Distilled Water — q.s. to	1000 cc.	1000 cc.

- To 500 cc. of distilled water the Methyl Paraben, Propyl Paraben, sodium bisulphite or sodium metabisulphite, sodium chloride, sodium citrate, sodium saccharin and sodium sucaryl are added and agitated until solution is obtained. The sucrose and Kelmar or the sucrose and gum tragacanth are dry mixed and slowly added to the solution with mixing. Then the "Tween 85" and flavours are added under agitation and distilled water added until the total volume is about 950 cc. Finally 40 g. of tetracycline sodium hexametaphosphate complex (200 mesh) is added under agitation, the volume made up to 1000 cc. with distilled water and the product filled into bottles.

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2. Therapeutically active, stable, non-toxic tetracycline sodium hexametaphosphate complex characterised in purified form on an anhydrous basis by the presence of 0.7 to 1.2%

sodium, 5.8 to 7.8% phosphorus, a potency in the range of 760 to 940 micrograms of tetracycline hydrochloride equivalents per milligram by bio-assay, and a solubility in water at room temperature of about 3.2 mg/ml. and further characterised by the production at the end of one and four hours after oral administration to man of a concentration in the blood stream substantially greater than that produced by the administration of an equivalent amount of tetracycline hydrochloride.

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4. A process of preparing a tetracycline sodium hexametaphosphate complex substantially as described with reference to any one



of Examples I to VI hereinbefore set forth.

- 5 5. A therapeutic composition comprising a tetracycline sodium hexametaphosphate complex and one or more inert pharmaceutical solid carriers.

6. A process for preparing a therapeutic

composition as claimed in claim 5 substantially as described with reference to Examples 7 or 8 hereinbefore set forth.

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