

DESCRIPTION

CONCOMITANT TREATMENT WITH ORAL-TOPICAL DOSAGE FORMS
OF BISMUTH COMPOUNDS, ANTIBACTERIALS/ANTIBIOTICS TO
ERADICATE H. PYLORI FROM THE ORAL CAVITY IN COMBINATION
WITH PERORAL BISMUTH DOSAGE FORMS TO HEAL AND PREVENT
PEPTIC ULCERS, GASTRITIS AND GASTRIC CANCER

BACKGROUND OF THE INVENTION

Until recently, excessive gastric acidity and mental stress were
thought to be major pathophysiological reasons for occurrence of peptic ulcers. In
the early 1980, Marshall and Warren (Warren, *Lancet*, 1:1273-1275, 1983 and
5 Marshall et al., *Lancet*, 2:1311-1315, 1984) first reported an unidentified curved
bacilli in the stomach of patients with gastritis and peptic ulcers. These bacilli,
which later were identified as a gram negative spiral bacterium and named
Helicobacter pylori (Goodwin et al., *Int. J. Syst. Bacteriol.* 39:397-405, 1989),
have been demonstrated to be associated with gastritis and peptic ulcers (Buck et
10 al., *J. Infect. Dis.* 153:664-669, 1986 and Graham, *Gastroenterology* 96:615-
625, 1989), and are thought to be transmitted by person-to-person contact.

Recent clinical investigations have shown a definitive presence of
H. pylori in the dental plaque (Nguyen et al., *Journal of Clinical Microbiology*
31(4):783-787, 1993; Desai et al., *Scandinavian Journal of Gastroenterology*
15 26:1205-1208, 1991; and Lambert et al., *Lancet* 341(8850):957, 1993), and have
also shown that standard oral hygiene practice does not help reduce *H. pylori*
presence in the oral cavity (Nguyen et al., *Journal of Clinical Microbiology*
31(4):783-787, 1993). As a result of these recent discoveries associating bacterial
infection in the causation of peptic ulcer disease, questions regarding the
20 previously established paradigms of ulcer treatment and healing processes have
been raised.

H₂ receptor blockers which suppress acid secretion, such as

cimetidine (Tagamet®) and ranitidine (Zantac®), have been used to treat and heal duodenal ulcers (Jones et al., *Gut* 28:1120-1127, 1987; McIsaac et al., *Aliment. Pharmacol. Therap.* 1:369-381, 1987; and Boyed et al., *Amsterdam:Excerpta Medica*, 14-42, 1984). Recently, however, a number of clinical investigations

5 have demonstrated that 70-80% of healed duodenal ulcers reoccur within the next year (Goodwin et al., *Int. J. Syst. Bacteriol* 39:397-407, 1989), and that these drugs do not reverse the tendency for ulcers to form (Wormsley, *British Medical Journal* 293:1501, 1986; Gudman et al., *British Medical Journal* i:1095-1097, 1978; and Bardhan et al., *British Medical Journal* 284:621-623, 1982).

10 For many years, bismuth compounds have been used for treating ulcers. Clinical investigations comparing the efficacy of CBS (also known as tripotassium dicitrato bismuthate (TDB)) with placebo (Lambert, *Scandinavian Journal of Gastroenterology* 26(Supplement 185):13-21, 1991), cimetidine (Bianchi, et al., *Lancet* 2:698, 1984), and ranitidine (Bianchi et al., *Gut* 25:565,

15 1984; Lee et al., *Lancet* 1:1299-1301, 1985; and Dobrilla et al., *Gut* 29:181-187, 1988) in initial healing and relapse rates of duodenal ulcers, have shown significantly lower relapse rates in patients treated with CBS. The therapeutic efficacy of CBS (and other bismuth compounds), in healing duodenal ulcers and lowering relapse rates, is attributed to its specific antibacterial activity against *H. pylori* (McNulty et al., *Antimicrobial Agents Chemotherapy* 28:837-838, 1985;

20 Lambert et al., *Antimicrob. Agents Chemotherapy* 3:510-511, 1986; and Goodwin et al., *J. of Antimicrobial Agents Chemotherapy* 17:309-314, 1986). The minimum inhibitory concentration (MIC) for CBS against *H. pylori* is reported to be 8 mg/L (Lambert et al., *Antimicrob. Agents Chemotherapy* 3:510-511) and the

25 range is 4-32 mg/L (Lambert et al., *Antimicrob. Agents Chemotherapy* 3:510-511).

In addition to its bacteriocidal activity, CBS has been demonstrated to enhance mucus glycoprotein secretion, strengthen viscoelastic gel properties of mucus, cause increased concentration of epithelial growth factor (EGF) in ulcer

30 tissue, and stimulate prostaglandin synthesis in the gastric antral mucosa (Lee, *Scandinavian Journal of Gastroenterology* 26(Supplement 185):1-6, 1991). These

gastroprotective properties of CBS may contribute to the initial healing of duodenal ulcers and the observed lower rates of relapse by returning the gastric mucosal cells to normal physiologic function. The gastroprotective effects of CBS in prevention of gastric lesions induced by various ulcerogenic agents and the
 5 mechanism of ulcer healing have been demonstrated in animal studies (Konturek et al., *Digestion* 37(Supplement 2):8–15, 1987 and Konturek et al., *Scandinavian Journal of Gastroenterology* 21(Supplement 122):6–10, 1986).

Because of the finding that bismuth is an effective antibacterial agent against *H. pylori*, concomitant dosages of bismuth-containing compounds
 10 with other antiulcer drugs have been increasingly applied in many clinical cases for treatment of peptic ulcers. The most commonly used regimens include double or triple therapy with bismuth; meanwhile, some recent reports regarding quadruple therapy (wherein a proton pump inhibitor is added to triple therapy) have shown eradication rates of over 90%, but also cause severe side effects such
 15 as vomiting and diarrhea.

Additionally, while antibacterial therapy (bismuth and amoxicillin or doxycycline) was shown to be effective in eliminating *H. pylori* from the gastric mucosa of duodenal ulcer patients, this therapy had no effect on the *H. pylori* colonies in their dental plaque (Desai et al., *Scandinavian Journal of*
 20 *Gastroenterology* 26:1205–1208, 1991, Nguyen et al., *Journal of Clinical Microbiology* 31(4):783–787, 1993). The continued presence of *H. pylori* in the dental plaque raises the question of whether the relapse of duodenal ulcers is inevitable (Desai et al., *Scandinavian Journal of Gastroenterology* 26:1205–1208, 1991 and Abraham et al., *Indian Journal of Gastroenterology* 9(4):265–6,
 25 Editorial, 1990).

Triple therapy, consisting of an antibiotic (amoxicillin, tetracycline or erythromycin), metronidazole, and bismuth compounds, has been reported to result in more than a 95% eradication rate for *H. pylori*, and reduced ulcer relapse rate to less than 10% during a 12-month follow-up period (Graham et al.,
 30 *Gastroenterology* 102:493–496, 1992 and Borody et al., *Gastroenterology* 102:A44, 1992). It is interesting to note that metronidazole as a single agent has only

5% eradication rate for *H. pylori*, but as a component of triple therapy, it increases the eradication rate to as high as 95%. When metronidazole-resistant strains of *H. pylori* are encountered (about 25% of the *H. pylori* strains are resistant), the eradication rate falls to about 50% (Logan et al., *Lancet* 338:1249-1252, 1991).

One possible explanation for this observed clinical efficacy of metronidazole in combination therapy is that metronidazole is actively secreted in the saliva (Mustofa et al., *International Journal of Clinical Pharmacology, Therapy, and Toxicology* 29(12):474-478, 1991) where it might be exerting its antimicrobial action against dental plaque-bound *H. pylori* colonies. The typical steady state saliva represent 10 to 20 times the MIC for *H. pylori*. Another antibiotic, Clarithromycin, a new-generation macrolide, which has shown a 40 to 60% cure rate as a single agent, is also secreted in the saliva. Therefore, it is reasonable to believe that in order to achieve nearly complete eradication of *H. pylori*, and prevent peptic ulcer relapse, eradication of this organism from the oral cavity is essential. Colloidal bismuth subcitrate (CBS), the most effective single agent against *H. pylori*, is however not absorbed significantly from the GI, and therefore, produces no salivary concentrations. But as a single agent, it is about 6 to 8 times more effective in eradicating *H. pylori* than metronidazole. The present invention therefore is related to development of a therapeutic modality to effectively eradicate *H. pylori* reservoir from the oral site, as well as the gastric mucosal wall.

Furthermore, recent clinical studies have implicated this insidious organism in gastric cancer (Parsonnet, *Gastroenterology Clinics of America, Helicobacter pylori Infection*, Dooley CP, Cohen, H. Guest Editors, Volume 22, No. 1, pp. 89-104, March 1993). A progression of gastric pathology from gastritis and ulcers to cancer involving *H. pylori* has been described (Recavarren-Arie et al., *Scandinavian Journal of Gastroenterology* 26(Supplement 181):51-57, 1991). In addition to *H. pylori* infection, low concentration levels of ascorbic acid in the gastric mucosa has been shown to be a risk factor for gastric cancer (Schorah et al., *American Journal of Clinical Nutrition* 53(Supplement

1):287S-293S, 1991 and Reed et al., Iarc Scientific Publications, 105:139-142, 1991). In patients suffering from dyspepsia, chronic gastritis, hypochlorhydria, and duodenal cancer, the intragastric concentrations of vitamin C were significantly lower (Sobala et al., Gastroenterology 97(2):357-363, 1989 and
 5 O'Conner et al., Gut 30(4):436-442, 1989). The present invention therefore also relates to therapies involving both bismuth compounds and ascorbic acid.

SUMMARY OF THE INVENTION

The invention relates to concomitant treatment with bismuth compounds and/or with other antibacterial compounds and/or with antibiotics in
 10 topical oral and peroral dosage forms to eradicate H. pylori from its niches both in the dental plaque and in the gastric mucosa in order to improve the ulcer cure rate and prevent ulcer relapse. The invention also provides oral topical dosage forms with pharmaceutically usable bismuth compounds and/or antibacterial compounds and/or antibiotics that eradicate or reduce H. pylori in dental plaque. The
 15 invention further provides for treatment with bismuth compounds and/or antibacterial compounds and/or antibiotics which are effective against Campylobacter rectus and Treponema denticola which are responsible for causing halitosis. The invention also provides bismuth compounds which have applications in wound healing, particularly in ocular and dermal wound healing.

20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a generalized reaction diagram for the synthesis of bismuth sulfates.

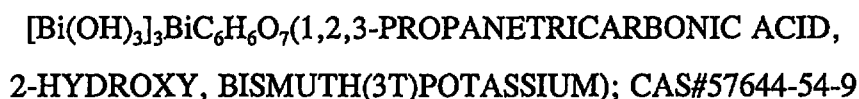
Figure 2 is a graph of human saliva concentration versus time which shows the release of bismuth from CBS chewing gum.

25 DETAILED DESCRIPTION OF THE INVENTION

Therefore, bismuth compound used in this invention should be a pharmaceutically acceptable antimicrobial agent against H. pylori, such as colloidal bismuth subcitrate (CBS), bismuth subcitrate, bismuth citrate, bismuth

salicylate, bismuth subsalicylate, bismuth subnitrate, bismuth subcarbonate, bismuth tartrate, bismuth subgallate, tripotassium dicitrato bismuthate and bismuth aluminate. Preferably, colloidal bismuth subcitrate (CBS), tripotassium dicitrato bismuthate, bismuth subcitrate, bismuth subsalicylate and their combination are
 5 chosen. More preferably, CBS and tripotassium dicitrato bismuthate are chosen. And further selection is made for CBS.

The structural formula of CBS is:

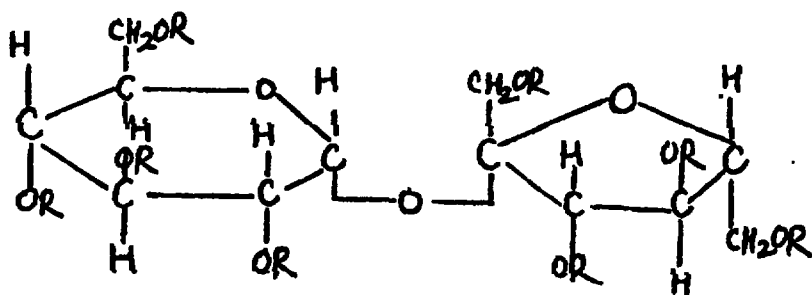


10 Other novel bismuth-containing compounds which are useful in the present invention are those described in Bos et al., U.S. Patent No. 4,801,608 and in Serfortein, U.S. Patent No. 4,153,685, both of which are expressly incorporated herein by reference. Other bismuth compounds, namely complexes of polysulfates, of polyhydroxy compounds such as sugars, sugar alcohols, and
 15 ascorbic acid and its derivatives, as well as alpha-D-glucopyranoside bismuth complex, beta-D-fructofuranosyl-oktakakis (hydrogen sulfate) bismuth complex, and L-dihydro ascorbyl-tetrakis (hydrogen sulfate) bismuth complex are part of the present invention. A generalized reaction diagram for the synthesis of bismuth sulfates is shown in Figure 1. These novel compounds will deliver bismuth more
 20 effectively and will have less side effects in treating H. pylori positive gastro duodenal diseases. The compounds will lend themselves to controlled release oral dosage forms and oral topical dosage forms for eradication of H. pylori in dental plaque.

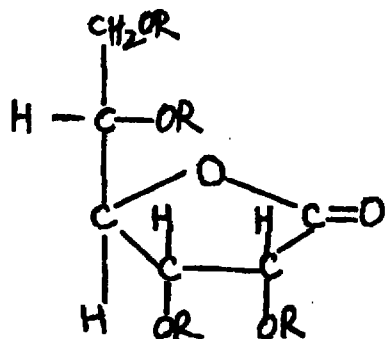
Chemical structures of the compounds conceived in this invention
 25 are complexes of poly-sulfates and of poly-hydroxy compounds such as sugars, sugar alcohols, and ascorbic acid and its derivatives. These novel compounds will deliver bismuth more effectively and will have less side effects in treating H. Pylori positive duodenal ulcers and gastritis. These compounds moreover lend themselves to controlled release oral dosage forms and oral topical dosage forms

for eradication of H. Pylori in dental plaque.

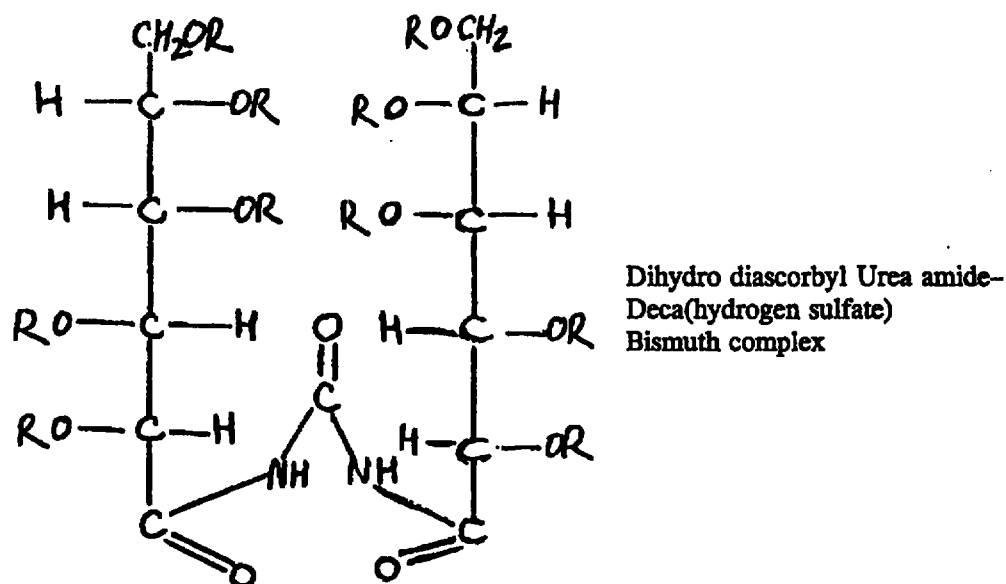
Chemical structures of the compounds conceived in this invention are illustrated below:



α -D-Glucopyranoside, β -D-Fructofuranosyl
Octakis (hydrogen sulfate) Bismuth complex



Dihydro-Ascorbyl-Tetra-
(hydrogen sulfate) Bismuth complex



In all cases $R = \text{SO}_3[\text{Bi}_2(\text{OH})_3 \cdot (\text{H}_2\text{O})_2]$

The ascorbic acid-derived molecules are synthesized in a manner completely analogous to the reaction diagram for synthesis of bismuth sulfates set forth above. These compounds can also be used in embodiments relating to wound healing as described below.

- 5 In addition to antibacterial bismuth-compounds and antibiotics to the oral cavity for reduction/elimination of Helicobacter pylori in the oral cavity as a means of treatment and prevention of gastrointestinal diseases including peptic ulcers, recurring gastritis, non-ulcer dyspepsia and gastric cancer. Antibiotics useful herein include, but are not limited to, Tetracycline, Amoxicillin,
- 10 Ampicillin, Doxycycline, Erythromycin, Clarithromycin, Metronidazole, Tinidazole, Ciproflaxacin, Ofloxacin, Norflaxacin, Furazolidine, Nitrofurantoin. Antibacterials useful herein include, but are not limited to naturally occurring peptides and synthetic peptide antibacterials such as Lanthocins, and particularly, Nicin and related peptides, Proton pump inhibitors such as Omeprazole and
- 15 Lansoprazole, Sanguinaria and other antibacterials obtained from plant sources, as well as bismuth-containing compounds.

The present methods utilize topical oral dosage forms to deliver bismuth compounds, antibiotics, and/or antibacterials directly to the oral cavity in concentrations sufficient to reduce or eliminate *H. pylori* in the oral cavity.

Each oral-topical dosage form of this invention is the one which can
5 make it possible to release bismuth compound and/or antibacterial compounds and/or antibiotics into oral cavity in a predictable manner and result in appropriate antibacterial concentration. Those examples of such forms include a chewing-gum form, a chewable form including chewable tablets, lozenges, dental paints, viscous gels, dental implants, polymer film adhesives, a troche form, a toothpaste form, a
10 gargling-gel form and mouth-rinse form. Preferably, a chewing-gum form and a troche form are chosen. Further, a chewing-gum form is most preferred due to its easy-to-use characteristic, predictable drug release and increased drug contact with dental surface. The chewing gum delivery system especially enables sustained contact of the antibacterial agents with the entire oral cavity and therefore,
15 enhance bactericidal/bacteriostatic efficacy. We have already demonstrated that chewing gum formulation containing an antibacterial agent, colloidal bismuth subcitrate, releases the drug in a precise and reproducible fashion during a 15 minute chewing time. Chewable tablets, Viscous gel formulations and dental paint formulations also will be able to provide sustained concentration of antibacterial
20 agents in the oral cavity.

Oral-topical dosage forms containing bismuth in this invention must release enough bismuth, antibiotic, and/or antibacterial into saliva for eradication of *H. pylori* in the oral cavity. The minimum inhibitory concentration (MIC) of bismuth for *H. pylori* varies in each bismuth compound. For instance, it is
25 reported that the MIC of CBS for *H. pylori* is 8 mg/L and its range is 4 to 32 mg/L.

Therefore, the dosage form is required to release bismuth into saliva up to at least two times the MIC, preferably a minimum of 2 to 10 times, most preferably 2 to 250 times. In order to achieve this level of release rate, the
30 bismuth content per dosage form should be about 50 mg to 200 mg, preferably a minimum of 10 mg to 50 mg, most preferably 25 mg to 50 mg. For instance,

each piece of CBS-containing chewing gum should contain approximately 50 mg to 200 mg of CBS, preferably a minimum of 10 mg to 50 mg, most preferably 25 mg to 50 mg.

Time release of each dosage form in this invention must be long enough to eradicate *H. pylori*. Although the duration of time release varies in each bismuth compound and in each dosage form, it is required at least 75% of the dose is released within 2 minutes, preferably within 2 to 15 minutes, most preferably within 10 to 15 minutes.

In other preferred embodiments of the invention presented herein, a chewing gum drug delivery system is utilized to provide sustained concentration of bismuth compounds, antibiotics, and/or antibacterial compounds which are proven antibacterial agents against *H. pylori* and anti-plaque agents to help these compounds penetrate the dental plaques to reach the site of *H. pylori* infection. The chewing gum delivery system enables sustained contact of the antibacterial agents with the entire oral cavity and therefore, enhances bacteriocidal efficacy.

Where antibiotic and/or antibacterial agents other than bismuth are to be used, oral-topical dosage form in this invention must release enough antibiotic/antibacterial into saliva for eradication of *H. pylori* from the oral cavity. The minimum inhibitory concentration (MIC) varies for each antibiotic/antibacterial agent. However, for most of the antibiotics listed in this invention, the MIC values are between less than 1 to 10 mcg/mL, or 1 to 10 mg/L.

Therefore, the topical-oral dosage form is required to release the antibacterial agent into saliva up to at least 2 times the MIC, preferably a minimum of 2 to 10 times, most preferably 2 to 100 times. In order to achieve this level of release, the antibacterial content per unit of dosage form should be about 10 to 100 mg, preferably a minimum of 5 to 50 mg, most preferably 10 to 25 mg. For instance, each piece of chewing gum should contain approximately 10 to 100 mg. of the antibiotic or antibacterial agent. Preferably a minimum of 5 to 50 mg, most preferably 10 to 25 mg.

The topical-oral dosage forms of this invention must release the

antibiotic/antibacterial over an extended time. The duration of release must be at least 5 minutes, preferably 10 minutes, most preferably 15 minutes. Further 75% of the antibiotics/antibacterial content is required to be released within 5 minutes, preferably within 2 to 15 minutes, most preferably within 10 to 15 minutes.

- 5 In other preferred embodiment of this invention, a chewing gum delivery system is utilized to provide sustained concentration of antibiotic/antibacterial agent several times above its MIC for H. pylori over at least 10 times.

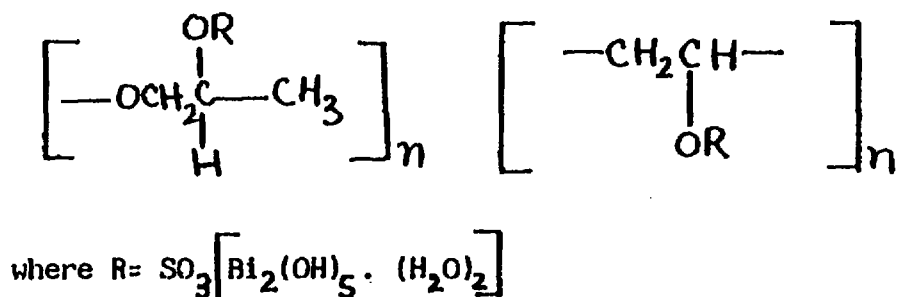
 The anti-plaque agents further contribute to improved efficacy by breaking down the plaque and exposing the bacterial colonies to the antibacterial agents.

- 10 The chewing gum formulation containing CBS, antibiotic, and/or antibacterial releases the drug in a precise and reproducible fashion during a 15-minute chewing time. Anti-plaque agents include, but are not limited to, glucanase anhydroglucosidase, glucose oxidase, silicon oil, sanguinarine, and the like. Chewing gum formulations may optionally include crystalline sorbitol, sorbitol
15 solution, mannitol, Nova-base™, or any other gum base, dextrans, cellulose derivatives, buffer salts, sweeteners, flavors, and the like.

 Optionally, metronidazole can be added to CBS chewing gum to broaden the antimicrobial activity against *H. pylori*.

- Bismuth compounds embodied in these inventions also have been
20 found to stimulate cellular production of growth factors, and therefore have applications in wound healing, specifically in ocular and dermal wound healing. Therefore, the present invention also contemplates use of novel bismuth complexes with sulfated polyhydroxy hydrophilic film-forming polymers to accelerate wound healing in ulcerative diseases of the eye, skin, and other mucosal tissues. For
25 these embodiments, the invention involves synthesis of unique complexes of bismuth with partially sulfated hydrophilic film-forming polymers such as hydroxyprobal cellulose, hydroxyethyl cellulose, hydroxypropyl-methyl cellulose, carboxymethyl cellulose, and polyvinyl alcohol. These compounds are formulated in unique film-forming solutions, aerosols, and gels for treatment of corneal
30 ulcers, skin ulcers, gastric ulcers, and other wounds of the skin and mucous membranes. The general structures of this class of complexes are represented

below:



Example 1 — Preparation of Therapeutic Substance

To an aqueous solution of ammonia are added bismuth citrate, citric acid, and caustic potash in specific stoichiometric proportions, and at specific temperatures. The solution is examined for turbidity and, if required, additional volume of ammonia solution is added to render the solution clear. The solution is then filtered on a carbon bed and spray dried to obtain free-flowing powder material. The product is packaged in an air and moisture proof glass container.

Example 2 — Preparation of Topical Dosage Form

Brief general description of the preferred topical dosage form, chewing gum, is set forth as follows. Fully melt the gum base (at approximately 90°C) in Brabender mixer, a jacketed mixer with sigma blades. Remove the hot water from the mixer jacket, allow to cool, and add lecithin and mix well. Cool further to approximately 50°C, and add liquid flavor and mannitol. Mix until uniform. Dry blend colloidal bismuth subcitrate in sorbitol, and blend sodium citrate in sorbo syrup. Add sorbitol and sorbo syrup blends to the gum base. Cool the product to 35°C, add flavor and sweetener and mix until smooth. Remove the product from the mixing kettle, roll to form a sheet of uniform thickness and score to produce chewing gum sticks weighing 2.5 g each. Wrap individual gum sticks in aluminum foil and place in plastic bags. Where the gum

is to include antibiotic or antibacterial compounds, the agent is coated with a polymeric substance to mask any untoward taste or odor, and to further regulate its release in the saliva.

Example 3 — Composition of CBS-Containing Gum

- 5 Two variations of the 50 mg CBS gum (Table 1) were used. Both formulations used were identical with the exception that Formula-2 contained sodium citrate to impart a firmer texture, while Formula-1 did not.

Table 1
FORMULATIONS OF THE GUM (APPROX. 2.5 gm A PIECE)

10	<u>Formula-1</u>	<u>Formula-2</u>
	CBS 50.0mg	CBS 50.0mg
	Crystalline Sorbitol 910.0	Crystalline Sorbitol 910.0
	Gum Base 575.0	Gum Base 575.0
	Sorbitol Solution 500.0	Sorbitol Solution 500.0
15	Mannitol 400.0	Mannitol 400.0
	Peppermint Oil 25.0	Peppermint Oil 25.0
	Spray Dried Peppermint . . . 12.5	Spray Dried Peppermint . . . 12.5
	Grade t Lecithin 10.0	Grade t Lecithin 10.0
	Aspartame 10.0	Aspartame 10.0
20	Sodium Citrate 10.0	
	2502.5mg	Total: 2492.5mg

- Colloidal Bismuth Subcitrate (CBS) and other bismuth compounds, including bismuth subcitrate, bismuth citrate, bismuth salicylate, bismuth subsalicylate, bismuth subnitrate, bismuth subcarbonate, bismuth tartrate, bismuth
 25 subgallate, tripotassium dicitrato bismuthate and bismuth aluminate. Preferably, colloidal bismuth subcitrate (CBS), tripotassium dicitrato bismuthate, bismuth subcitrate, bismuth subsalicylate are coated with the following coating agent to

regulate their dissolution and salivary release: bee's wax, carnauba wax, shellac, cellulose acetate phthalate, methyl cellulose, propyl cellulose, hydroxy propyl-cellulose, ethyl cellulose, hydroxy propylmethylcellulose, ethylcellulose, polymethyl methacrylate, and Eudragit® polymers, polyvinyl pyrohidone, polyvinyl alcohol,
 5 etc.

Moderately water soluble bismuth compounds such as bismuth ascorbyl sulfate, bismuth sucrose sulfate, bismuth subascorbate, cyclodextrin bismuth sulfate are used in the chewing gum dosage form to produce sustained concentration in the saliva.

10 Synthetic and natural latex-based chewing gum bases are used to tightly enclose bismuth compounds and other antibacterial/antibiotic compounds to cause their gradual release in the saliva.

These formulation/composition modifications are designed to:

- (1) provide control release of antibacterial/antibiotic compounds to
 15 increase their bactericidal efficacy against oral cavity/dental plaque bound *H. pylori*; and
- (2) avoid/minimize oral cavity discoloration/blackening caused by quick or instant release of bismuth compounds in the saliva.

Example 4 — Measurement of Release Rate of Bismuth into Saliva

20 Among six healthy human subjects, who gave informed consent, three chewed the CBS-containing gum with sodium citrate, and the other three chewed CBS-containing gum without sodium citrate. The subjects chewed the gum samples for a total of 15 minutes. Saliva samples were collected at time interval of 0, 1, 5, 10, and 15 minutes of chewing. The saliva samples were then
 25 submitted to an analytical laboratory for bismuth analysis. Results are shown in Table 2.

Table 2

IN VIVO SALIVARY CONCENTRATION OF CBS FROM THE CHEWING GUM						
5	Formula	chewing time (min.)	saliva vol. (mL)	conc of Bi (ppm)	conc of active CBS (u/mL)	X MIC
10	formula-1	0	4.4(±0.5)			
		1	3.3(±1.4)	900.7(±239.1)	1270.3(±334.7)	148.7(±42.0)
		5	5.4(±1.5)	257.7(±112.3)	363.3(±158.9)	45.0(±19.9)
		10	4.9(±1.3)	28.0 (±5.0)	40.0 (±6.6)	5.0 (±1.0)
		15	5.2(±2.1)	15.8 (±17.8)	25.7 (±23.0)	3.1 (±2.7)
15	formula-2	0	7.2(±0.5)			
		1	4.8(±1.9)	888.3(±329.5)	1257.0(±464.5)	156.3(±58.0)
		5	8.5(±1.7)	326.0(±113.3)	572.7(±159.7)	63.7(±19.9)
		10	7.5(±3.4)	30.0 (±9.5)	42.3 (±13.6)	5.0 (±1.7)
		15	7.7(±3.8)	10.7 (±6.7)	14.7 (±9.2)	1.8 (±1.2)

Saliva samples were analyzed for elemental bismuth in ppm units. The results were then converted to mg of active CBS per mL of saliva and also expressed as a multiple of minimum inhibitory concentration (MIC) of CBS for *H. pylori*. As can be seen from the results (formula-2 of Table-3), the salivary concentrations of CBS are 156, 64, 5, and 1.8 times the MIC at 1, 5, 10 and 15 minutes, respectively. The constant bathing of the oral cavity from saliva containing sufficient concentration of CBS (2 to 5 times the MIC) for up to 15 minutes can be expected to further reduce the viable cells of *H. pylori*. These results are plotted in Figure 2 which shows a graph of human saliva concentration versus time.

Example 5 — Sensory Analysis of Chewing Gum

Sensory characteristics of the chewing gum were evaluated by the subjects during the 15 minutes of chewing. Again, three subjects chewed the CBS gum containing sodium citrate and three subjects chewed the CBS gum without sodium citrate. A nine point rating scale was used to evaluate each category (Tables 3 and 4).

Table 3

RESULTS OF SENSORY ANALYSIS RATING OF CBS GUM
WITHOUT SODIUM CITRATE
 (Formula-1)

5	<u>SENSORY CHARACTERISTICS</u>	<u>CHEWING TIME</u>			
		<u>1 MIN</u>	<u>5 MIN</u>	<u>10 MIN</u>	<u>15 MIN</u>
	Overall Flavor (0 = dislike extremely, 8 = like extremely)	6.3 (± 1.2)	6.0 (± 1.0)	5.3 (± 1.5)	5.0 (± 1.0)
10	Flavor Intensity (0 = none, 8 = very strong)	5.7 (± 1.5)	4.7 (± 1.2)	3.7 (± 0.6)	3.0 (± 1.0)
	Chew Qualities (0 = dislike extremely, 8 = like extremely)	6.0 (± 1.0)	6.0 (± 1.0)	5.3 (± 1.5)	5.0 (± 1.0)
15	Unpleasant Aftertaste (0 = none, 8 = very strong)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)
	Overall Qualities (0 = dislike extremely, 8 = like extremely)	6.3 (± 1.2)	6.0 (± 1.0)	5.7 (± 1.2)	5.3 (± 1.5)

Table 4

RESULTS OF SENSORY ANALYSIS RATING OF CBS GUM
WITH SODIUM CITRATE
 (Formula-2)

5	<u>SENSORY CHARACTERISTICS</u>	<u>CHEWING TIME</u>			
		<u>1 MIN</u>	<u>5 MIN</u>	<u>10 MIN</u>	<u>15 MIN</u>
	Overall Flavor (0 = dislike extremely, 8 = like extremely)	6.7 (±0.6)	5.7 (±1.5)	4.7 (±1.2)	4.7 (±1.2)
10	Flavor Intensity (0 = none, 8 = very strong)	6.7 (±0.6)	6.0 (±0.0)	5.0 (±1.0)	3.7 (±1.5)
	Chew Qualities (0 = dislike extremely, 8 = like extremely)	4.7 (±2.1)	5.0 (±2.0)	4.3 (±1.5)	4.3 (±0.6)
15	Unpleasant Aftertaste (0 = none, 8 = very strong)	0.7 (±1.2)	1.7 (±2.1)	1.7 (±2.1)	2.0 (±2.0)
	Overall Qualities (0 = dislike extremely, 8 = like extremely)	6.3 (±0.6)	5.7 (±1.2)	4.7 (±1.2)	4.0 (±1.0)
20	In general, there were no dramatic differences in the sensory analysis between the two formulas. The sensory panel clearly shows that both chewing gum formulations have a desirable level of flavor and taste, and cause a minimal unpleasant aftertaste after chewing.				

Example 6 — Topical Safety

25 Topical safety was evaluated in the six volunteers for up to 60 minutes after administration of the gum. The subjects were asked to report any adverse effects such as discomfort or irritation in the oral cavity.

There were no reports of any discomfort or irritation in the oral cavity by any of the subjects at either the 15 or 60 minute post administration time

30 periods.

Example 7 — Storage Stability Study

Samples of CBS-containing gum (50mg) were wrapped individually in foil wrappers. The sticks of gum were then placed in foil laminate bags, sealed, and placed in storage. Storage conditions include 40°C and room temperature (RT). The duration of the stability testing was 90 days. The results are shown in Tables 5–8 below.

Table 5. THREE MONTH STABILITY DATA

**IN VIVO SALIVARY CONCENTRATIONS IN HUMAN SUBJECTS
OF CBS FROM THE 50 MG CBS CHEWING GUM
(Mfg. August 1993, Batch No. CBS-50CG-0002)**

TIME/CONDITION	CHEWING TIME (min)	SALIVA VOLUME (ml)	CONC OF BI (ppm)	CONC OF BI (pg/ml)	CONC OF ACTIVE CBS (pg/ml)	X NIC
ZERO TIME	0	4.2 (±1.6)	NA	NA	NA	NA
	1	4.9 (±4.5)	1937.3 (±753.5)	1937.3 (±753.5)	2729.0 (±1060.2)	341.0 (±132.7)
	5	6.4 (±3.1)	437.0 (±152.1)	437.0 (±152.1)	615.7 (±214.5)	77.0 (±26.9)
	10	3.9 (±0.1)	36.0 (±20.6)	36.0 (±20.6)	50.7 (±40.5)	6.4 (±5.0)
	15	4.5 (±1.3)	5.0 (±4.6)	5.0 (±4.6)	7.0 (±6.6)	0.9 (±0.8)
3 MONTHS/ 40 °C	0	5.6 (±1.4)	NA	NA	NA	NA
	1	2.9 (±1.8)	1922.3 (±511.0)	1922.3 (±511.0)	2710.0 (±791.9)	330.6 (±90.3)
	5	5.6 (±1.7)	399.3 (±270.1)	363.7 (±113.3)	563.0 (±329.3)	70.3 (±49.1)
	10	5.3 (±1.4)	25.7 (±11.4)	30.0 (±9.5)	362.0 (±160.5)	45.4 (±20.1)
	15	4.9 (±0.4)	7.9 (±4.9)	10.7 (±6.7)	10.0 (±6.0)	1.4 (±0.9)
3 MONTHS/ ROOM TEMP.	0	5.1 (±1.3)	NA	NA	NA	NA
	1	4.1 (±1.5)	1240.0 (±450.7)	1240.0 (±450.7)	1740.0 (±646.6)	210.0 (±80.6)
	5	7.2 (±2.3)	510.7 (±110.7)	510.7 (±110.7)	731.3 (±167.6)	91.0 (±21.0)
	10	6.0 (±2.2)	12.5 (±10.6)	12.5 (±10.6)	17.7 (±14.6)	2.1 (±1.0)
	15	5.6 (±1.6)	4.5 (±2.2)	4.5 (±2.2)	6.0 (±2.6)	0.7 (±0.3)

n = 3 for each group

Table 6. THREE MONTH STABILITY DATA

RESULTS OF SENSORY ANALYSIS RATING OF 50 MG CBS GUM
(Mfg. August 1993, Batch No. CBS-50CG-0002)

		<u>CHewing TIME</u>			
<u>SENSORY CHARACTERISTIC</u>		<u>1 MIN</u>	<u>5 MIN</u>	<u>10 MIN</u>	<u>15 MIN</u>
ZERO TIME	OVERALL FLAVOR	6.7 (±0.6)	6.3 (±0.6)	5.3 (±0.6)	5.3 (±0.6)
	FLAVOR INTENSITY	6.3 (±1.2)	5.3 (±1.2)	4.0 (±1.0)	4.0 (±1.0)
	CHEW QUALITIES	6.7 (±0.6)	6.3 (±0.6)	5.7 (±0.6)	5.3 (±0.6)
	UNPLEASANT AFTERTASTE	0.0 (±0.0)	0.0 (±0.0)	0.0 (±2.1)	0.0 (±0.0)
	OVERALL QUALITIES	6.7 (±0.6)	6.3 (±0.6)	5.7 (±0.6)	5.3 (±1.2)
3 MONTHS/ 40°C	OVERALL FLAVOR	6.0 (±0.0)	4.7 (±0.6)	2.7 (±1.2)	2.7 (±1.2)
	FLAVOR INTENSITY	5.3 (±1.2)	3.0 (±0.0)	2.3 (±0.6)	2.0 (±1.0)
	CHEW QUALITIES	5.7 (±0.6)	5.0 (±1.0)	4.3 (±0.6)	4.3 (±0.6)
	UNPLEASANT AFTERTASTE	0.3 (±0.6)	0.3 (±0.6)	0.0 (±0.0)	0.0 (±0.0)
	OVERALL QUALITIES	6.0 (±0.0)	4.3 (±0.6)	2.7 (±0.6)	2.3 (±0.6)
3 MONTHS/ ROOM TEMP.	OVERALL FLAVOR	6.3 (±0.6)	6.3 (±0.6)	5.3 (±0.6)	4.3 (±0.6)
	FLAVOR INTENSITY	5.7 (±1.5)	5.3 (±1.5)	4.3 (±1.5)	4.0 (±1.7)
	CHEW QUALITIES	6.0 (±1.0)	6.0 (±1.0)	5.3 (±0.6)	4.3 (±0.6)
	UNPLEASANT AFTERTASTE	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)
	OVERALL QUALITIES	6.3 (±0.6)	6.3 (±0.6)	5.3 (±0.6)	4.7 (±1.2)

Note: n = 3 for each analysis

Rating Scale:

0=dislike extremely, 8=like extremely for: Overall Flavor, Chew Quality, Overall Quality
0=none, 8=like extremely for: Flavor Intensity
0=none, 8= very strong for: Unpleasant Aftertaste

TABLE 7.

**EXPONENTIAL REGRESSION DATA OF TIME VS SALIVARY CONCENTRATIONS
EXPRESSED AS X MIC**

	Initial Test Lot CBS-5005-0001	Stability Lot CBS-5005-0002	Clinical Lot CBS-5005-0003
	Zero Time 3 mo./RT 3 mo./40°C		
A (intercept)	240.0	575.7	422.3
			446.5
b (slope)	-0.339	-0.432	-0.448
			-0.426
r (correlation coefficient)	0.992	0.998	0.971
			0.959
K (pseudo first order rate constant)	-0.339	-0.432	-0.448
			-0.426
t _{0.5} (min.)	2.04	1.60	1.92
			1.55

Mean t_{0.5} = 1.748 (± 0.218)

TABLE 8.

RELEASE OF CBS FROM THE CHEWING GUM AFTER
15 MINUTES OF CHEWING BY HUMAN SUBJECTS

	Stability Lot # CBS-50CG-0002			Clinical Lot # CBS-50CG-0003
	Zero Time	3 mo./RT	3 mo./40°C	
Mg CBS/2.5 g gum Before chewing (%)	45.6 (100)	44.5 (100)	46.1 (100)	46.2 (100)
Mg CBS/2.5 g gum After 15 min chewing (%)	3.5 (7.6)	4.0 (9.0)	4.5 (9.8)	3.8 (100)

Mean % of CBS Remaining in the gum after 15 min of chewing = 8.6 ($\pm$ 1.0)

Each stick of the gums used for the stability study (1 for zero-time, 2 for three month, total 3 sticks) was from the same lot number. The results show that bismuth concentration remains stable over the tested time period.

Example 8 — Denture Material Exposure Study

- 5 An evaluation of CBS salivary concentration on various denture materials was conducted in order to test any potential staining effect of the CBS on denture materials. Artificial saliva was used (Table 9).

Table 9

THE COMPOSITION OF ARTIFICIAL SALIVA

10	<u>Ingredients</u>	<u>Concentration per Liter</u>
	Sodium Bicarbonate	0.50 g
	Sodium Phosphate, Dibasic, Dihydrate	0.85 g
	Calcium Chloride	0.44 g
	Magnesium Chloride	0.06 g
15	Potassium Chloride	1.40 g
	Sodium Carboxyl Methyl Cellulose	2.00 g
	Phosphoric Acid to adjust pH to 6.4 Distilled Water	QS

- The test saliva was prepared by dissolving 0.500 g of colloidal bismuth subcitrate in 100 mL of the above artificial saliva. 500 mL of Artificial Saliva (RT) was
- 20 placed in one of two identical glass jars with lids. In the other jar was placed 500 mL of the Artificial Saliva (RT) containing 0.50% of CBS. In each of the jars the denture material block and a magnetic stirrer was placed. The jars were then placed on the magnetic platform and set to agitate at a minimum rate. The denture materials that were exposed to artificial saliva containing CBS or placebo included
- 25 (Table 10).

Table 10

DENTURE MATERIALS

- 1) Natural tooth with silver amalgam filling
- 2) Composite resin (used on anterior teeth for filling)
- 5 3) Denture base acrylic resin
- 4) Porcelain fused to metal
- 5) Partial denture metal frame
- 6) Acrylic tooth (artificial)
- 7) Natural tooth

- 10 The four hour exposure of natural tooth and other denture materials to 0.5% CBS in artificial saliva with mild agitation did not cause any staining, discoloration, or changes in texture.

Example 9 — Clinical Efficacy Data

- An open label, placebo-controlled pilot clinical study in ten patients
- 15 with initial positive response for H. pylori in the dental plaque has been initiated. Data from six patients (four patients treated with CBS 50 mg chewing gum six times-a-day and two patients treated with placebo chewing gum six times-a-day for fifteen days) has been obtained. The dental plaque samples from the patients were collected before treatment, day 7 and day 15 after treatment, and tested by
- 20 microbiological culture and CLO test. The results are set forth in Table 11 below:

Table 11

TREATED GROUP (n = 4)

		CLO	DUR POSITIVE (HRS:MINS)	CULTURE	SIDE EFFECTS (Stain/Odor)
5	Pt 1 Day 0	+	1:00	+	NE
	30/M Day 7	+	1:45	-ve	—
	Day 15	+	1:30	-ve	—
	Pt 2 Day 0	+	2:15	+	NE
	42/M Day 7	+	1:30	NA	—
10	Day 15	+	4:00	-ve	—
	Pt 3 Day 0	+	2:30	+	NIL
	31/M Day 7	+	4:30	NA	NIL
	Day 15	+	5:30	NA	NIL
	Pt 4 Day 0	+	2:30	NA	NIL
	29/F Day 7	+	4:00	NA	NIL
	Day 15	+	5:30	NA	NIL

Mean CLO response time after 15 days = 4.125 HR

PLACEBO (n = 2)

		CLO	DUR POSITIVE (HRS:MINS)	CULTURE	SIDE EFFECTS (Stain/Odor)
15	Pt 1 Day 0	+	1:00	NA	NIL
	26/M Day 7	+	1:30	NA	NIL
	Day 15	+	1:30	NA	NIL
	Pt 2 Day 0	+	1:15	NA	NIL
	28/M Day 7	²⁸ +	2:00	NA	NIL
	Day 15	+	2:30	NA	NIL

Mean CLO response time after 15 days = 2.0 HR

NA = Not available

NE = Not evaluated (before chewing)

The data show that for patients treated with CBS 50 mg chewing gum and placebo chewing gum on day 15 the mean CLO response times are 4.125 hours and 2.0 hours, respectively. The longer CLO test response time for CBS 50 mg chewing gum group compared to the placebo chewing gum group is indicative of

5 substantial reduction in *H. pylori* density in the oral cavity of the active treatment group.

Example 10 — Toxicology

A number of animal toxicity studies and human clinical investigations have demonstrated safety of bismuth compounds, especially CBS, in

10 therapeutic dose ranges. No toxicity has been reported in chronic daily administration of high doses of CBS (160, 320, and 640 mg/kg body weight representing 2, 4, and 8 times the human therapeutic dose respectively) in rats treated for three months or dogs treated for six months. See Wieriks et al., *Journal of Gastroenterology* 17(Supplement 80):11-16 (1982), incorporated herein

15 by reference.

Long term safety of CBS and treatment of peptic ulcers at a standard dose of 480 mg (expressed as bismuthtrioxide) in four daily divided doses has been examined by Bader, *Digestion* 37(Supplement 2):53-59 (1987), incorporated herein by reference. CBS was first introduced in Europe in 1971 and

20 since that time 1.5 million treatments have been dispensed. During eight years of use of CBS tablets [De-Nol^(R)] in Europe between 1978 and 1986 under a more comprehensive adverse reaction monitoring system, only 13 adverse reaction forms were completed. Five of these adverse reactions were ascribed to CBS: one case of headache, one case of stomach pain, one case of diarrhea, and two

25 cases of allergy (mainly in the form of skin rashes). A high degree of safety of CBS in therapeutic applications for the treatment of peptic ulcers is reported in a recent review of pharmacology of bismuth-containing compounds by Lambert, *Review of Infectious Diseases* 13(Supplement 8):691-695 (1991), incorporated herein by reference. In reviewing safety and pharmacokinetics of CBS, Bennet,

30 *Scandinavian Journal of Gastroenterology* 26(Supplement 185):29-35 (1991),

incorporated herein by reference, has calculated the systemic bioavailability of bismuth after oral dosing of CBS to be in the range of 0.16 to 0.28% of the administered dose, and concluded that steady-state blood levels of 50–100 mg/mL are unlikely to cause any neurotoxicity.

5 **Example 11 - Composition of Antibiotic or Antibacterial-Containing Gum**

The chewing gum formulation comprises antibiotic or antibacterial agents in concentration ranges from 10 to 50 mg per piece of gum. The chewing gum-base consists of Crystalline Sorbitol, Gum Base, Sorbitol Solution, Mannitol, Peppermint Oil, Spray Dried Peppermint, Grade t Lecithin, Aspartame, and
 10 Sodium Citrate, as set forth in Table 1 above. The formulation may also contain Glucanase, Anhydroglucosidase, Glucose oxidase, Silicon oil, Sanguinarine and related compounds as anti-plaque agents. Caboxy methyl cellulose, Hydroxy propyl methyl cellulose, Polyethylene glycol, Poly methyl methacrylates, Acrylic acid copolymers and other polymers as coating agents.

15 Saliva samples are analyzed for antibiotic or antibacterial agents in ppm units. The results are then converted to mg of active agent per mL of saliva and also expressed as a multiple of minimum inhibitory concentration (MIC) of the agent for *H. pylori*. The salivary concentrations of the agent are 156, 64, 5, and 1.8 times the MIC at 1, 5, 10 and 15 minutes, respectively. The constant bathing
 20 of the oral cavity from saliva containing sufficient concentration of the agent (2 to 5 times the MIC) for up to 15 minutes can be expected to further reduce the viable cells of *H. pylori*. These results are plotted to show a graph of human saliva concentration versus time.

Sensory characteristics of the chewing gum are evaluated by the subjects
 25 during the 15 minutes of chewing. Again, three subjects chews the gum containing sodium citrate and three subjects chewed the gum without sodium citrate. A nine point rating scale is used to evaluate each category.

In general, there are no dramatic differences in the sensory analysis between the two formulas. The sensory panel shows that both chewing gum
 30 formulations have a desirable level of flavor and taste, and cause a minimal

unpleasant aftertaste after chewing.

Topical safety is evaluated in the six volunteers for up to 60 minutes after administration of the gum. The subjects are asked to report any adverse effects such as discomfort or irritation in the oral cavity.

- 5 There are no reports of any discomfort or irritation in the oral cavity by any of the subjects at either the 15 or 60 minute post administration time periods.

Samples of the agent-containing gum (50mg) are wrapped individually in foil wrappers. The sticks of gum are then placed in foil laminate bags, sealed, and placed in storage. Storage conditions include 40°C and room temperature (RT).

- 10 The duration of the stability testing is 90 days.

Each stick of the gums used for the stability study (1 for zero-time, 2 for three month, total 3 sticks) is from the same lot number. The results show that bismuth concentration remains stable over the tested time period.

- 15 An evaluation of salivary concentration of the agent on various denture materials is conducted in order to test any potential staining effect of the CBS on denture materials. Artificial saliva is used (Table 9).

- 20 The test saliva is prepared by dissolving 0.500 g of the antibiotic or antibacterial agent in 100 mL of the above artificial saliva. 500 mL of Artificial Saliva (RT) is placed in one of two identical glass jars with lids. In the other jar is placed 500 mL of the Artificial Saliva (RT) containing 0.50% of the agent. In each of the jars the denture material block and a magnetic stirrer is placed. The jars are then placed on the magnetic platform and set to agitate at a minimum rate. The denture materials that are were exposed to artificial saliva containing the agent or placebo are included. The four hour exposure of natural tooth and other
25 denture materials to 0.5% of the agent in artificial saliva with mild agitation does not cause any staining, discoloration, or changes in texture.

- 30 To assess clinical efficacy, patients with positive response for the presence of H. pylori in the dental plaque/oral cavity are divided into two treatment groups. Group I is given placebo chewing gum to be chewed 2 or 6 times a day for 2 or 4 weeks. Group II is given chewing gum containing antibiotic/antibacterial agent to be chewed 2 or 6 times a day for 2 or 4 weeks. Patient's dental plaque/saliva

samples are collected at time 0 (Pre-treatment) on days 7, 14, 28, and tested for H. pylori presence and density. The incidence of H. pylori presence in the placebo group and the active treatment group is compared. The group receiving the chewing gum containing antibiotic/antibacterial shows significantly lower incidence of H. pylori presence in the dental plaque/saliva compared to placebo chewing gum group after 2 and 4 weeks of treatment.

Example 12 — Antibacterial Efficacy for Treatment of Halitosis

Campylobacter rectus, Helicobacter pylori, and Treponema denticola have been demonstrated to be associated with Halitosis (bad breath). The compounds and methods of the present invention, including CBS as well as ascorbyl bismuth derivative, have demonstrated in vitro activity against all three bacteria, as indicated by their minimum effective concentrations (MICs) presented in Table 12 below.

Table 12

Test Organisms	Bismuth Ascorbyl Sulfate ($\mu\text{g/ml}$)	Bismuth Sucrose Sulfate ($\mu\text{g/ml}$)	CBS ($\mu\text{g/ml}$)
Campylobacter rectus	256	> 256	256
Helicobacter pylori	8	16	2
Treponema denticola	16	32	32

Example 13 — In Vitro Mesentery Culture Model

Colloidal bismuth subcitrate and other bismuth compounds are known to accelerate wound healing by increasing the concentration of epithelial growth factor (EGF) and fibroblast growth factor (FGF) in the wounded tissue.

Utilizing a rat mesentery culture model (Wu et al., *Annals of Plastic Surgery* 33(2):155-161 (1994), incorporated herein by reference) and a medium containing 2% fetal calf serum, wound closure rates are measured. This tissue culture model is useful for gaining insights into growth factor interactions and wound healing. CBS or bismuth ascorbyl sulfate or glucose (placebo) are added to the medium in concentration ranges from 10 mcg/mL to 1,000 mcg/mL, and the wound closure is assessed at 24-hour, 48-hour and 72-hour intervals. Significantly higher concentrations of growth factors EGF and FGF are observed. Moreover, a significantly faster wound closure rate and complete closure is seen in the culture to which CBS or bismuth ascorbyl sulfate are added, compared to the placebo.

Example 14 — Diabetic Mouse Wound Healing Model

Further, wound healing potential of the above bismuth compounds is evaluated using a diabetic mouse wound healing model (Matuszewska et al., *Pharmaceutical Research* 11(1):65-71 (1994), incorporated herein by reference). Wounds treated with CBS and bismuth ascorbyl sulfate formulations at concentration ranges of 10 mcg/mL to 1,000 mcg/mL heal faster compared to placebo treated wound. The wound healing rates produced by the bismuth compounds are comparable to the wound healing rate produced by 0.5 to 5 mcg/mL concentration of basic fibroblast growth factor (bFGF).

Example 15 — Freeze-injured Skin Graft Model

The efficacy of CBS and bismuth ascorbyl sulfate at enhancing wound healing is also studied in a freeze-injured skin graft model for quantitative evaluation of promoters of wound healing (Lees et al., *British Journal of Plastic Surgery* 47(5):349-359 (1994), incorporated herein by reference). Application of CBS or bismuth ascorbyl sulfate stimulates wound healing in cryoinjured grafts in a dose-related fashion. Doses of 10 to 1000 mcg/mL produce significant increase in wound healing rates compared to placebo.

Thus, while several embodiments have been shown and described, various modifications may be made, without departing from the spirit and scope of the present invention.



What is claimed is:

1. In a method of treating *Helicobacter Pylori* infection by administering tablets of a bismuth compound, the improvement comprising concomitant administration of a bismuth compound in a topical dosage form.
- 5 2. A method of Claim 1, wherein said bismuth compound is selected from the group consisting of colloidal bismuth subcitrate, bismuth subcitrate, bismuth citrate, bismuth salicylate, bismuth subsalicylate, bismuth subnitrate, bismuth subcarbonate, bismuth tartrate, bismuth subgallate, tripotassium dicitrate
10 bismuthate, bismuth aluminate, bismuth polysulfates, bismuth polyhydroxy compounds, alpha-D-glucopyranoside bismuth complex, beta-D-fructofuranosyl-
 oktakakis (hydrogen sulfate) bismuth complex, L-dihydro ascorbyl-tetrakis (hydrogen sulfate) bismuth complex, and bismuth ascorbyl sulfate.
3. A method of Claim 1, wherein said topical dosage form is selected from the group consisting of a chewing gum, a chewable tablet, a troche, a
15 toothpaste, a gargling gel, and a mouth rinse.
4. A method of Claim 1, wherein said bismuth compound is colloidal bismuth subcitrate and wherein said dosage form is a chewing gum.
5. The method of Claim 3, wherein said tablets are administered in a peroral dosage form.
- 20 6. A topical dosage form for treatment of *Helicobacter Pylori* in oral cavity, which contains a pharmaceutically acceptable bismuth compound in a form which allows controlled release.
7. A dosage form according to Claim 6, wherein said bismuth compound is selected from the group consisting of colloidal bismuth subcitrate,
25 bismuth subcitrate, bismuth citrate, bismuth salicylate, bismuth subsalicylate,



bismuth subnitrate, bismuth subcarbonate, bismuth tartrate, bismuth subgallate, tripotassium dicitrato bismuthate, bismuth aluminate, bismuth polysulfates, bismuth polyhydroxy compounds, alpha-D-glucopyranoside bismuth complex, beta-D-fructofuranosyloktakis (hydrogen sulfate) bismuth complex, L-dihydro
5 ascorbyl-tetrakis (hydrogen sulfate) bismuth complex, and bismuth ascorbyl sulfate.

8. A dosage form according to Claim 6, which is selected from the group consisting of a chewing gum, a chewable tablet, a troche, a toothpaste, a gargling gel, and a mouth rinse.

10 9. A dosage form according to Claim 6, wherein said bismuth compound is colloidal bismuth subcitrate and wherein said dosage form is a chewing gum.

10. In a method of treating Helicobacter Pylori infection by administering tablets of an antibacterial compound, the improvement comprising
15 concomitant administration of an antibacterial compound in a topical dosage form.

11. A method of Claim 10, wherein said antibacterial compound is selected from the group consisting of naturally occurring peptides, synthetic peptides, proton pump inhibitors, and plant-derived antibacterials.

12. The method of Claim 11, wherein the synthetic peptide is derived
20 from the Lanthocins.

13. The method of Claim 12, wherein the Lanthocin is selected from the group consisting of Nicin peptides and Nicin-related peptides.

14. The method of Claim 11, wherein the proton pump inhibitor is selected from the group consisting of Omeprazole and Lansoprazole.



15. The method of Claim 11, wherein the plant-derived antibacterial is *Sanguinaria*.
16. The method of Claim 10, wherein said antibacterial compound is an antibiotic.
- 5 17. The method of Claim 16, wherein the antibiotic is selected from the group consisting of Tetracycline, Amoxicillin, Ampicillin.
18. A method of Claim 10, wherein said topical dosage form is selected from the group consisting of a chewing gum, a chewable tablet, a troche, a toothpaste, a gargling gel, and a mouth rinse.
- 10 19. A method of Claim 10, further comprising the step of administering a bismuth compound in a topical dosage form.
20. The method of Claim 18, wherein said tablets are administered in a peroral dosage form.
21. A topical dosage form for treatment of *Helicobacter Pylori* in oral
15 cavity, which contains a pharmaceutically acceptable antibacterial compound.
22. A dosage form according to Claim 21, wherein said antibacterial compound is selected from the group consisting of naturally occurring peptides, synthetic peptides, proton pump inhibitors, and plant-derived antibacterials.
23. The method of Claim 22, wherein the synthetic peptide is derived
20 from the Lanthocins.
24. The method of Claim 23, wherein the Lanthocin is selected from the group consisting of Nicin peptides and Nicin-related peptides.



25. The method of Claim 22, wherein the proton pump inhibitor is selected from the group consisting of Omeprazole and Lansoprazole.

26. The method of Claim 22, wherein the plant derived antibacterial is *Sanguinaria*.

5 27. The method of Claim 21, wherein said antibacterial compound is an antibiotic.

28. The method of Claim 27, wherein the antibiotic is selected from the group consisting of Tetracycline, Amoxicillin, Ampicillin.

29. A method of stimulating cellular production of growth factors,
10 comprising the step of administering to dermal or corneal cells a preparation comprising a bismuth compound.

30. A method of Claim 29, wherein said bismuth compound is selected from the group consisting of colloidal bismuth subcitrate, bismuth subcitrate, bismuth citrate, bismuth salicylate, bismuth subsalicylate, bismuth subnitrate,
15 bismuth subcarbonate, bismuth tartrate, bismuth subgallate, tripotassium dicitrate bismuthate, bismuth aluminate, bismuth polysulfates, bismuth polyhydroxy compounds, alpha-D-glucopyranoside bismuth complex, beta-D-fructofuranosyl-octakis (hydrogen sulfate) bismuth complex, L-dihydro ascorbyl-tetrakis (hydrogen sulfate) bismuth complex, and bismuth ascorbyl sulfate.