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☐ Patente de Modelo de Utilidad.

☒ Capítulo I.

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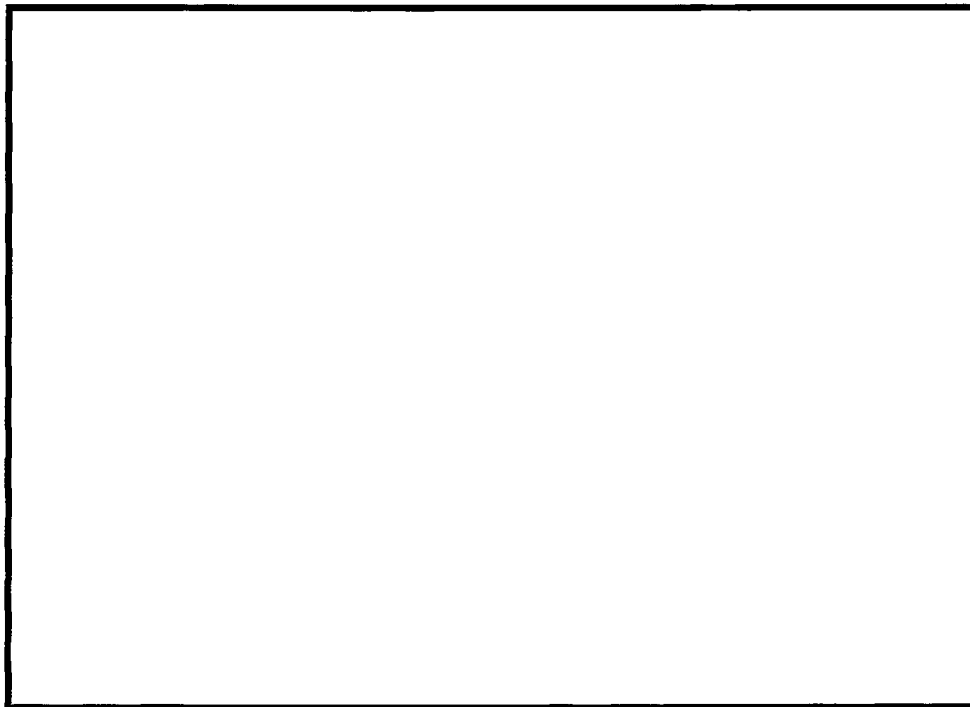
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ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud. \$443.000
- ☒ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Poderes, si fuere el caso.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☐ Copia del examen preliminar efectuado por la IPEA.
- ☐ Traducción del examen preliminar efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico.
- ☐ Arte final 12x12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☒ Otros: Tarjeta de propietarios.

11 FIGURA CARACTERÍSTICA:



12 Solicito la concesión de la patente.

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(54) Title: ENZYME CONTAINING ORAL COMPOSITION HAVING ENHANCED STABILITY

(57) Abstract: An oral care composition having enhanced oral hygiene and antiplaque properties which comprises an orally acceptable vehicle containing a combination of an enzyme, cetylpyridinium chloride and a reducing agent.

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**ENZYME CONTAINING ORAL COMPOSITION HAVING ENHANCED STABILITY****BACKGROUND OF THE INVENTION**5 1. Field of the Invention

This invention relates generally to oral compositions for enhancing oral hygiene, and more particularly, to enzyme containing oral compositions having enhanced stability and antiplaque effectiveness.

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2. The Prior Art

Oral compositions such as toothpastes, gels and mouth washes are designed to loosen and remove plaque in conjunction with a regular toothbrushing regimen. Dental plaque is present to some degree, in the form of a film, on virtually all dental surfaces. It is a byproduct of microbial growth, and comprises a dense microbial layer consisting of a mass of microorganisms embedded in a polysaccharide matrix. Plaque itself adheres firmly to dental surfaces and is removed only with difficulty even through a rigorous brushing regimen. Moreover, plaque rapidly reforms on the tooth surface after it is removed.

Plaque may form on any part of the tooth surface, and is found particularly at the gingival margin, in cracks in the enamel, and on the surface of dental calculus. The danger associated with the formation of plaque on the teeth lies in the tendency of plaque to build up and eventually produce gingivitis, periodontitis and other types of periodontal disease, as well as dental caries and dental calculus.

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It is known to the art to incorporate antimicrobial agents in oral compositions wherein these agents destroy or inhibit oral bacteria. Other agents are also incorporated in the oral composition to enhance the efficacy of the antimicrobial agents. For example, it is known to incorporate enzymes such as proteases in oral compositions, which enzymes disrupt or interfere with plaque formation and bacterial adhesion to tooth surfaces.

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A problem encountered with commercially processed enzymes such as proteases is that they often contain a broad spectrum of undesirable by-products or impurities that are difficult to remove during manufacture. One such enzyme by-product is cellulase, an enzyme that catabolizes cellulose to simple sugars by hydrolysis of  $\beta$  (1-4) linkages.

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Thickening agents conventionally used in oral compositions such as carboxymethyl cellulose, undergo degradation in the presence of cellulase enzymes which detrimentally affects the rheology of the dentifrice product. Thus, a means to inhibit the degradation of these thickeners by cellulase is a critical to obtaining stable enzyme containing oral care formulations.

Typical methods employed by the art for cellulase inhibition or isolation are not practical for use in the oral composition field. Treatments for cellulase inhibition such as salting out, heat treatment, or pH adjustment also compromise the activity of the enzymes.

Classical methods of enzyme separation based on enzyme size, charge, solubility, and binding site are cost prohibitive and ineffective due to the similarities between certain cellulases and enzymes such as amylases. Inhibition of cellulase activity by treatment with heavy metals or heavy metal complexes such as mercury, silver and palladium chloride that bind to the enzyme active site are also unacceptable as these materials are toxic to humans and certainly cannot be used in an oral care product.

#### SUMMARY OF THE INVENTION

In accordance with the present invention it has been unexpectedly determined that a small but effective amount of cetyl pyridinium chloride and a reducing agent can provide enhanced stability and antiplaque efficacy in enzyme containing oral compositions.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

##### 25 Cetyl Pyridinium Chloride

Cetyl pyridinium chloride is incorporated in containing oral care compositions of the present invention at a concentration of about 0.005 to about 1.0% by weight and preferably about 0.50 to about 1.0% by weight of the oral care composition.

##### 30 Enzymes

The enzymes useful in the practice of the present invention include carbohydrases such as glucoamylase and enzymes extracted from natural fruit products such as proteases which breakdown or hydrolyze proteins.

Glucoamylase is a saccharifying glucoamylase of *Aspergillus niger* origin cultivated by fermentation. This enzyme can hydrolyze both the alpha-D-1,6 glucosidic branch points and the alpha-1,4 glucosidic bonds of glucosyl oligosaccharides. Additional carbohydrases useful in accordance with this invention are alpha and beta-amylase, dextranase and mutanase. Glucoamylase is a preferred enzyme and is incorporated in the oral composition of the present invention at a concentration of about 0.001 to 2% by weight and preferably about 0.01 to 0.55% by weight.

Protease enzymes useful in the practice of the present invention include those extracted from natural fruit products. The proteolytic enzymes are obtained from natural sources or by the action of microorganisms having a nitrogen source and a carbon source. Examples of proteolytic enzymes useful in the practice of the present invention include the naturally occurring enzymes papain (from papaya), bromelain (from pineapple), as well as serine proteases such as chymotrypsin. Additional enzymes include ficin and alcalase. Papain is a protease enzyme preferred for use in the practice of the present invention, the papain having an activity of 150 to 939 MCU per milligram as determined by the Milk Clot Assay Test of the Biddle Sawyer Group (see J. Biol. Chem., vol. 121, pages 737-745). The protease enzymes are included in the compositions of the present invention at a concentration of about 0.1 to about 3% by weight and preferably about 0.2 to about 2% by weight.

Enzymes which may beneficially be used in combination with the proteolytic enzymes and glucoamylase enzymes include carbohydrases such as, alpha-amylase, beta-amylase, , tannase and lipases such as plant lipase, gastric lipase and pancreatic lipase.

The lipase enzyme is derived from a select strain of *Aspergillus niger*, exhibiting random cleaving of the 1,3 positions of fats and oils. The enzyme has maximum lipolytic activity at pH 5.0 to 7.0 when assayed with olive oil. The enzyme has a measured activity of 120,000 lipase units per gram. The lipase may be included in the dentifrice composition at a concentration of about 0.010 to about 5.0% by weight and preferably about 0.02 to about 0.10 % by weight.

The presence of tannase enzyme can be further beneficial in facilitating the breakdown of extrinsic stain. Tannase enzymes have been purified from *Aspergillus niger* and

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*Aspergillus allianceus* and are useful in the hydrolysis of tannins, known to discolor the tooth surface.

Other suitable enzymes which can comprise the present invention include lysozyme, derived from egg white, which contains a single polypeptide chain crosslinked by four disulfide bonds having a molecular weight of 14,600 daltons. The enzyme can exhibit antibacterial properties by facilitating the hydrolysis of bacterial cell walls cleaving the glycosidic bond between carbon number 1 of N-acetylmuramic acid and carbon number 4 of N-acetyl-D-glucosamine, which in vivo, these two carbohydrates are polymerized to form the cell wall polysaccharide. Additionally, pectinase, an enzyme that is present in most plants facilitates the hydrolysis of the polysaccharide pectin into sugars and galacturonic acid. Finally, glucanase, which may be utilized to catalyze the breakdown of complex carbohydrates to glucans and the hydrolysis of beta glucan to glucose.

#### 15 Enzyme Stabilizing Agents

Enzyme stabilizing agents which protect the enzyme from inactivation by chelating metal impurities present in the oral composition include ethylene diamine tetraacetic acid (EDTA) and sodium gluconate at concentrations between 0.01 and 1% by weight, preferably between 0.1 and 0.5% by weight. Agents stabilizing the enzyme against oxidation include reducing agents such as sodium bisulfite, metal gallates, potassium stannate, sodium stannate, ammonium sulfate, 3,5-di-tert-butyl-4-hydroxytoluene (BHT), Vitamin E ( $\alpha$ ,  $\beta$ ,  $\gamma$ , forms)/Vitamin E acetate and ascorbic acid. Potassium stannate is an enzyme stabilizing agent preferred for use in the practice of the present invention. The reducing agent is present in the oral composition of the present invention at a concentration between about 0.05 to about 1.5% by weight, preferably between about 0.1 and about 0.75% by weight.

#### Premix

When incorporating cetyl pyridinium chloride in the oral care composition of the present invention it is advantageous that the cetyl pyridinium chloride and any enzyme to be added to the oral composition be first premixed in an aqueous humectant solution so that the cetyl pyridinium chloride is interacted with and binds to and inactivates any impurity present in the enzyme added to the oral composition.

The premix product will normally be comprised of four components which are (1) cetyl pyridinium chloride (2) one or more enzymes to be incorporated in the oral composition (3) a reducing agent, (4) the balance, water and a humectant.

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Generally the concentration of cetyl pyridinium chloride present in the premix will range from about 0.15 to about 1.0% by weight of the premix and preferably about 0.2 to about 0.6% by weight. The enzyme from about 0.5 to about 10.0% by weight of the premix and preferably about 0.75 to about 8.0% by weight. When a combination of enzymes such as glucoamylase and papain is included in the premix, the concentration of papain will range from about 2 to about 10% by weight and the concentration of glucoamylase will range from about 0.5 to about 2% by weight.

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A reducing agent such as potassium stannate is also preferably included in the premix solution at a concentration of about 0.5 to about 40% by weight, and preferably about 1.0 to about 3% by weight, the presence of the reducing agent serving to further enhance the cetyl pyridinium chloride inhibition of enzyme impurity activity when subsequently incorporated in the oral composition vehicle.

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Water and humectant are further components of the premix. Concentrations will range from about 85 to 95% by weight.

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To prepare the premix, a reactor is charged first with a mixture of water and humectant followed by the addition of an effective amount of cetyl pyridinium chloride which is mixed and dissolved therein and thereafter the enzymes and reducing agent are added to the solution. These ingredients are mixed together at a temperature no higher than about 40°C and preferably about 30°C. The pH is adjusted to between about 1.5 and about 8.0 and preferably from about 6.0 to about 7.5. The so prepared premix is then sealed in suitable containers waiting for blending with the vehicle and other ingredients of the oral care composition.

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### Oral Care Composition

In the preparation of an oral composition, the premix is mixed with the ingredients of the oral care composition vehicle to prepare a product in which the enzymes remain effectively stabilized and which will exhibit antiplaque and antigingivitis activity.

5 **Dentifrice Vehicle**

Orally-acceptable vehicles used to prepare dentifrice compositions of the present invention include a water-phase, containing a humectant therein. The humectant is preferably glycerin, sorbitol, xylitol, and/or propylene glycol of molecular weight in the range of 200 to 1,000; but, other humectants and mixtures thereof may also be employed. The  
10 humectant concentration typically totals about 5 to about 70% by weight of the oral composition and preferably about 30 to about 60% by weight. Water present in the dentifrice will range from about 10 to about 30% by weight and preferably about 15 to about 25% by weight.

15 **Abrasives**

In the preparation of a dentifrice composition, abrasives which may be used in the practice of the present invention include silica abrasives such as precipitated silicas having a mean particle size of up to about 20 microns, such as Zeodent 115, marketed by J.M. Huber. Other useful dentifrice abrasives include sodium metaphosphate, potassium  
20 metaphosphate, tricalcium phosphate, dihydrated dicalcium phosphate, aluminum silicate, calcined alumina, bentonite or other siliceous materials, or combinations thereof.

Preferred abrasive materials useful in the practice of the preparation of dentifrice compositions in accordance with the present invention include silica gels and precipitated  
25 amorphous silica having an oil absorption value of less than 100cc/100g silica and preferably in the range of from about 45cc/100g to less than about 70cc/100g silica. Oil absorption values are measured using the ASTA Rub-Out Method D281. These silicas are colloidal particles having an average particle size ranging from about 3 microns to about 12 microns, and more preferably between about 5 to about 10 microns and a pH range from  
30 4 to 10 preferably 6 to 9 when measured as a 5% by weight slurry.

Low oil absorption silica abrasives particularly useful in the practice of the present invention are marketed under the trade designation Sylodent XWA by Davison Chemical Division of W.R. Grace & Co., Baltimore, MD 21203. Sylodent 650 XWA, a silica

hydrogel composed of particles of colloidal silica having a water content of 29% by weight averaging from about 7 to about 10 microns in diameter, and an oil absorption of less than 70cc/100g of silica is a preferred example of a low oil absorption silica abrasive useful in the practice of the present invention. The abrasive is present in the dentifrice composition of the present invention at a concentration of about 10 to about 40% by weight and preferably about 15 to about 30% by weight.

### Thickening Agents

Thickeners used in the dentifrice compositions of the present invention include natural and synthetic gums and colloids include cellulose thickeners such as carboxymethyl cellulose, hydroxyalkyl celluloses such as hydroxypropyl cellulose hydroxyethyl cellulose, gums such as xanthan gum, polyglycols of varying molecular weights sold under the tradename Polyox and polyethylene glycol. Inorganic thickeners which may be used in the practice of the present invention include amorphous silica compounds such as colloidal silicas compounds available under the trade designation Cab-o-sil manufactured by Cabot Corporation and distributed by Lenape Chemical, Bound Brook, New Jersey; Zeodent 165 from J.M. Huber Chemicals Division, Havre de Grace, Maryland 21078; and Sylodent 15, available from Davison Chemical Division of W.R. Grace Corporation, Baltimore, MD 21203. Other inorganic thickeners include natural and synthetic clays, lithium magnesium silicate (Laponite) and magnesium aluminum silicate (Veegum).

The thickening agent is present in the dentifrice composition in amounts of about 0.1 to about 10% by weight, preferably about 0.5 to about 4.0% by weight.

### Surfactants

Surfactants are used in the oral compositions of the present invention to achieve increased prophylactic action and render the compositions more cosmetically acceptable. The surfactant is preferably a deterative material which imparts to the composition deterative and foaming properties.

Anionic surfactants such as higher alkyl sulfates such as sodium lauryl sulfate are effective surfactants in oral compositions but are normally not compatible with enzymes and promote denaturation of the enzyme and loss in activity. However, in accordance with the practice of the present invention, premixing concentrated amounts of enzymes such as

glucoamylase with cetyl pyridinium chloride, allows the cetyl pyridinium chloride to interact and bind to any cellulase impurity present in the glucoamylase thereby preventing interaction of the cellulase with any cellulose based thickener that may be used in preparing the dentifrice as well as inhibiting interaction with the anionic surfactant.

- 5 Surfactants that are compatible with enzymes may also be used to provide the requisite foaming characteristics in the oral composition. Examples of enzyme compatible surfactants include nonionic polyoxyethylene surfactants such as Polyoxamer 407, Pluronic 127, Polysorbate 20, and amphoteric surfactants such as tauranol, cocamidopropyl betaine (tegobetaine) and cocamidopropyl betaine lauryl glucoside. Surfactants are
- 10 included in the oral composition of the present invention at a concentration of about 2 to about 10% by weight and preferably between about 0.5 to about 5.0% by weight.

#### Fluoride and Other Active Agents

- The oral composition of the present invention may also contain a source of fluoride ions or
- 15 fluorine-providing ingredient, as anticaries agent in amounts sufficient to supply about 25 ppm to 5,000 ppm of fluoride ions and include inorganic fluoride salts, such as soluble alkali metal salts. Preferred fluoride sources which are compatible with enzymes include sodium fluoride, potassium fluoride, sodium fluorosilicate, sodium monfluorophosphate (MFP), ammonium fluorosilicate, as well as tin fluorides, such as stannous fluoride and
- 20 stannous chloride. Sodium fluoride or MFP is preferred.

- In addition to fluoride compounds, there may also be included in the oral compositions of the present inventions antitartar agents such as pyrophosphate salts including dialkali or tetraalkali metal pyrophosphate salts such as  $\text{Na}_4\text{P}_2\text{O}_7$ ,  $\text{K}_4\text{P}_2\text{O}_7$ ,  $\text{Na}_2\text{K}_2\text{P}_2\text{O}_7$ ,
- 25  $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$  and  $\text{K}_2\text{H}_2\text{P}_2\text{O}_7$ , polyphosphates such as sodium tripolyphosphate, sodium hexametaphosphate and cyclic phosphates such as sodium tripolyphosphate sodium trimetaphosphate. These antitartar agents are included in the dentifrice composition at a concentration of about 1.0 to about 5.0% by weight.

- 30 Another active agent useful in dentifrice compositions of the present invention are antibacterial agents, which can be from 0.2 to 1.0% by weight of the oral composition. Such useful antibacterial agents include non-cationic antibacterial agents which are based on phenolic or bisphenolic compounds, such as halogenated diphenyl ethers such as Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether).

**Anionic Polycarboxylate**

Synthetic anionic polycarboxylates may also be used in the oral compositions of the present invention as an efficacy enhancing agent for any antibacterial, antitartar or other active agent within the dentifrice composition. Such anionic polycarboxylates are generally employed in the form of their free acids or preferably partially or more preferably fully neutralized water soluble alkali metal (e.g. potassium and preferably sodium) or ammonium salts. Preferred are 1:4 to 4:1 copolymers of maleic anhydride or acid with another polymerizable ethylenically unsaturated monomer, preferably methylvinylether/maleic anhydride having a molecular weight (M.W.) of about 30,000 to about 1,800,000 most preferably about 30,000 to about 700,000. Examples of these copolymers are available from GAF Corporation under the tradename Gantrez, e.g. AN 139 (M.W. 500,000), AN 119 (M.W. 250,000); S-97 Pharmaceutical Grade (M.W. 700,000), AN 169 (M.W. 1,200,000-1,800,000), and AN 179 (M.W. above 1,800,000); wherein the preferred copolymer is S-97 Pharmaceutical Grade (M.W. 700,000).

When present, the anionic polycarboxylates is employed in amounts effective to achieve the desired enhancement of the efficacy of any antibacterial, antitartar or other active agent within the oral composition. Generally, the anionic polycarboxylates is present within the oral composition from about 0.05% to about 4% by weight, preferably from about 0.5% to about 2.5% by weight.

**Flavor**

The oral composition of the present invention may also contain a flavoring agent. Flavoring agents which are used in the practice of the present invention include essential oils as well as various flavoring aldehydes, esters, alcohols, and similar materials. Examples of the essential oils include oils of spearmint, peppermint, wintergreen, sassafras, clove, sage, eucalyptus, marjoram, cinnamon, lemon, lime, grapefruit, and orange. Also useful are such chemicals as menthol, carvone, and anethole. Of these, the most commonly employed are the oils of peppermint and spearmint.

The flavoring agent is incorporated in the oral composition at a concentration of about 0.1 to about 5% by weight and preferably about 0.5 to about 1.5% by weight.

**Other Ingredients**

Various other materials may be incorporated in the oral compositions of this invention, including desensitizers, such as potassium nitrate; whitening agents, such as hydrogen peroxide, calcium peroxide and urea peroxide; preservatives; silicones; and chlorophyll compounds. These additives, when present, are incorporated in the oral compositions of the present invention in amounts which do not substantially adversely affect the properties and characteristics desired.

**Preparation of Dentifrice Compositions**

To prepare a dentifrice composition of the present invention, generally the humectants such as glycerin, sorbitol are dispersed in the water in a conventional mixer under agitation. Into the dispersion are added organic thickeners, such as carboxymethyl cellulose; antitartar agents such as tetrasodium pyrophosphate, sodium tripolyphosphate and any sweeteners. The resultant mixture is agitated until a homogeneous gel phase is formed. Into the gel phase are added a pigment such as  $\text{TiO}_2$ , and any acid or base required to adjust the pH in the range of 6.4 to 7.3. These ingredients are mixed until a homogenous phase is obtained. Thereafter a premix of cetyl pyridinium chloride, enzyme and a reducing agent such as potassium stannate in an aqueous humectant solution is added and admixed with the homogeneous gel phase. The resultant mixture is then transferred to a high speed/vacuum mixer; wherein, the thickener, and surfactant ingredients are added to the mixture. Thereafter the abrasive is added. Any water insoluble antibacterial agent, such as Triclosan, is solubilized in the flavor oils to be included in the composition and the solution is added along with the surfactants to the mixture, which is then mixed at high speed for from 5 to 30 minutes, under vacuum of from about 20 to 50 mm of Hg, preferably about 30 mm Hg. The resultant product is in each case a homogeneous, semi-solid, extrudable paste or gel product.

### Preparation of Liquid Oral Compositions

In the aspect of the present invention wherein the oral composition is substantially liquid in character such as a mouthwash or rinse, the vehicle is typically a water, humectant, alcohol mixture. The alcohol is a non-toxic alcohol such as ethanol or isopropanol. A humectant such as glycerine, sorbitol or an alkylene glycol such as polyethylene glycol or propylene glycol may be present in an amount of about 10 to 30% by weight, the oral rinse containing greater than about 45% by weight water and preferably about 50 to 85% by weight water, about 0 to 20% by weight of a non-toxic alcohol and about 10 to 40% by weight of the humectant. A thickener such as a Pluronic may be present at a concentration of about 1.0 to about 3.0% by weight, cetyl pyridinium chloride at a concentration of about 0.02 to about 1.0% by weight, a reducing agent such potassium stannate or ammonium sulfate at a concentration of about 0.05 to 1.0% by weight, an enzyme at a concentration of about 0.02 to about 0.2 % by weight and a flavor ingredient at a concentration of about 0.3 to about 1.0 % by weight.

In the preparation of a oral rinse, an enzyme premix comprised of cetyl pyridinium chloride, reducing agent, water, humectant and enzyme is dispersed in a mixture of mouthwash ingredients, for example, alcohol, humectants, surfactants, and flavor are then added and mixed. The ingredients are then mixed under vacuum for about 15-30 minutes. The resulting oral rinse product is then packaged.

A rinse is an advantageous vehicle for delivering actives to the oral cavity, due to its ability to get into hard-to-reach areas of the mouth, such as the interproximal regions and the crevices of the tongue. The challenge in incorporating enzymes into a rinse is maintaining enzymatic stability and activity at water levels above 50%, conventionally not suitable for enzyme containing compositions. In the present invention, the stability of enzyme activity is found to be acceptable and is optimized unexpectedly when the water content of the rinse is maintained above 45% by weight of a mixture thereof, and preferably about 50 to about 85% by weight enzyme activity as an antiplaque agent is found to increase as will hereinafter be demonstrated.

The following examples further describe and demonstrate preferred embodiments within the scope of the present invention. The examples are given solely for illustration, and are

not to be construed as a limitation of this invention as many variations thereof are possible without departing from its spirit and scope.

### Example I

5 An enzyme (papain, glucoamylase) containing dentifrice composition containing a small but effective amount of cetyl pyridinium chloride and reducing agents (potassium stannate, ammonium sulfate) added as enzyme premix and thickened with a cellulose thickener (carboxymethyl cellulose) was prepared. The ingredients of this composition designated, "Composition A", are listed in Table I below.

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Dentifrice compositions designated Compositions "B, C and D" were prepared following the procedure of Example I for the purpose of comparison. All the compositions contained the same enzymes as Composition A namely, glucoamylase and papain. Compositions B and C did not contain cetyl pyridinium chloride.

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Composition B contained the reducing agent ammonium sulfate and the metal chelating agent EDTA. Composition C contained the reducing agent potassium stannate and the preservative Nipastat. Composition D contained cetyl pyridinium chloride in the dentifrice composition base and did not contain a reducing agent and Laponite was substituted for  
20 sodium carboxymethyl cellulose as a thickening agent.

Commercial dental cream that did not contain cetyl pyridinium chloride or enzymes designated "Composition E" was used as a control.

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| TABLE I                        |         |         |         |         |
|--------------------------------|---------|---------|---------|---------|
| Composition                    | A Wt. % | B Wt. % | C Wt. % | D Wt. % |
| <b>Ingredients</b>             |         |         |         |         |
| Water                          | 18.25   | 17.75   | 18.25   | 18.25   |
| Pluronic F127                  | 1.5     | 1.5     | 1.5     | 0.75    |
| Glycerin                       | 20.0    | 20.0    | 20.0    | 22.0    |
| Tetrasodium pyrophosphate      | 2.0     | 2.0     | 2.0     | —       |
| Sodium tripolyphosphate        | 3.0     | 3.0     | 3.0     | —       |
| Monosodium phosphate           | 0.026   | 0.026   | 0.026   | —       |
| Disodium phosphate             | 0.079   | 0.079   | 0.079   | —       |
| Sodium carboxymethyl cellulose | 0.65    | 0.65    | 0.65    | —       |
| Laponite                       | —       | —       | —       | 0.7     |
| Xanthan gum                    | 0.55    | 0.55    | 0.55    | 0.6     |
| Sodium monofluoro phosphate    | 0.76    | 0.76    | 0.76    | 0.76    |
| Sodium saccharin               | 0.50    | 0.50    | 0.50    | 0.40    |
| Sorbitol (70% solution)        | 18.9434 | 19.6999 | 19.2024 | 22.8049 |
| Silica XWA650                  | 20.0    | 20.0    | 20.0    | 20.0    |
| Zeodent 115                    | 5.0     | 5.0     | 5.0     | 5.0     |
| Zeodent 165                    | 2.25    | 2.0     | 2.0     | 2.25    |
| Titanium dioxide               | 0.40    | 0.40    | 0.40    | 0.50    |
| Sodium lauryl sulfate          | 0.50    | 0.50    | 0.50    | —       |
| Tauranol                       | —       | —       | —       | 1.2     |
| Betaine                        | 1.0     | 1.0     | 1.0     | —       |
| PEG 600                        | 3.0     | 3.0     | 3.0     | 3.0     |
| Polyoxomer 407                 | 0.10    | 0.10    | 0.10    | 0.10    |
| Flavor                         | 1.10    | 1.10    | 1.10    | 1.10    |
| Papain                         | 0.2266  | 0.2266  | 0.2266  | 0.2266  |
| Glucoamylase                   | 0.044   | 0.044   | 0.044   | 0.044   |
| Potassium stannate             | 0.10    | —       | 0.10    | —       |
| Ammonium sulfate               | —       | 0.10    | —       | —       |
| Cetyl pyridinium chloride      | 0.015   | —       | —       | 0.3     |
| EDTA, disodium dihydrate       | 0.006   | 0.006   | 0.006   | 0.006   |
| Nipastat                       | —       | —       | 0.006   | —       |
| Sodium benzoate                | —       | 0.0025  | —       | 0.0025  |
| Potassium sorbate              | —       | 0.006   | —       | 0.006   |

Composition A, was prepared by premixing the reducing agent potassium stannate and a preservative (EDTA) with cetyl pyridinium chloride 23°C. The ingredients of the premix

5 designated "Premix A1" are recorded in Table II below at 23°C.

Premixes designated B1, C1 and D1 were prepared following the procedure used for Premix A1 and added to respective dentifrice Compositions B, C and D. The ingredients used in the preparation of the premix compositions for Compositions A, B, C, D are listed in Table II below.

| TABLE II                  |           |           |           |           |
|---------------------------|-----------|-----------|-----------|-----------|
| Premix Ingredients        |           |           |           |           |
| Composition               | A1 (Wt.%) | B1 (Wt.%) | C1 (Wt.%) | D1 (Wt.%) |
| Ingredients               |           |           |           |           |
| Sorbitol                  | 67.168    | 76.848    | 67.348    | 76.848    |
| Water                     | 25        | 15        | 25        | 15        |
| Papain                    | 4.532     | 4.532     | 4.532     | 4.532     |
| Glucoamylase              | 0.88      | 0.88      | 0.88      | 0.88      |
| Potassium stannate        | 2.0       | —         | 2.0       | 2.0       |
| Ammonium sulfate          | —         | 2.0       | —         | —         |
| cetyl pyridinium chloride | 0.3       | —         | —         | —         |
| EDTA, disodium dihydrate  | 0.12      | 0.12      | 0.12      | 0.12      |
| Nipastat                  | —         | —         | 0.12      | —         |
| Sodium benzoate           | —         | 0.5       | —         | 0.5       |
| Potassium sorbate         | —         | 0.12      | —         | 0.12      |

Premix compositions A1, B1, C1 and D1 were separately added to a non-enzyme dental cream containing the ingredients listed in Table I in a 1:4 ratio to observe the effects on cellulase activity. Cellulase activity was monitored via both viscosity and sugar level changes in Dentifrice Compositions A, B, C and D over time.

In viscosity measurements, greater cellulase activity was discernible by a more rapid reduction in toothpaste viscosity as the cellulase catabolized the carboxymethyl cellulose thickener used to prepare the dentifrice compositions. Brookfield model RVDTV-11 viscometer with spindle #95 at 5 rpm measured viscosity in centipose per second<sub>2</sub> (CPS). Measurements were taken daily of each sample until an 80% loss in viscosity was observed.

The sugar byproduct concentration was monitored using an Accucheck blood sugar digital analyzer. As cellulase acts upon cellulose, sugar is produced as a byproduct. The greater

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the amount of cellulase activity, the greater the amount of sugar produced. Both the sugar level and viscosity methods were used to compare the effects of cetyl pyridinium chloride, the reducing agents ammonium sulfate and potassium stannate and preservative agents sodium benzoate, and parabens on cellulase activity. The viscosity and sugar concentration results are recorded in Table III below.

| TABLE III                  |                            |                          |                                     |                                          |
|----------------------------|----------------------------|--------------------------|-------------------------------------|------------------------------------------|
| Composition<br>Ingredients | Initial Viscosity<br>(CPS) | Final Viscosity<br>(CPS) | % Viscosity Loss<br>(After 14 Days) | Sugar Conc.<br>(MG/DL)<br>(After 7 Days) |
| A                          | 25                         | 17                       | 32                                  | 28                                       |
| B                          | 20                         | 10                       | 50                                  | 62                                       |
| C                          | 20                         | 5                        | 75                                  | 62                                       |
| D                          | 30                         | 15                       | 50                                  | 62                                       |
| E                          | 28                         | 32                       | 0                                   | 0                                        |

The results in Table III show that the trend in sugar production is consistent with the viscosity loss results. Both tests indicate that Composition A formulated with a potassium stannate/cetyl pyridinium chloride/enzyme premix solution was most effective in delaying cellulase activity upon the dentifrice composition.

#### Tongue Microflora Reduction

Several compositions were tested for tongue microflora reduction, focussing on those species responsible for the generation of oral malodor. Patients swabbed the tongue for bacterial collection at baseline and four hours post treatment. These samples were plated onto agar media and incubated anaerobically. After four days, colony forming units of malodor causing bacteria and also total tongue bacteria were enumerated. The average colony forming unit results were used to calculate percent reduction from baseline.

Table IV and V represents *in-vivo* tongue microflora study with bacterial sampling at baseline and at four hours post-brushing with Composition D. For purposes of comparison Compositions F and G were also evaluated for oral malodor and tongue microflora reduction. Composition F was a commercial dentifrice which contained cetyl pyridinium chloride but no enzymes and compositions G was a commercial fluoride toothpaste which did not contain cetyl pyridinium chloride enzymes. Table V shows the increased reduction

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of oral malodor causing bacteria by the combination of enzymes with cetyl pyridinium chloride present in Composition D. Table VI demonstrates that this stabilized enzyme premix when incorporated into toothpaste systems provides an in-vivo antibacterial effect against all types of bacteria that exist on the tongue, including the malodor causing species. After four hours post treatment Formulation D, containing cetyl pyridinium chloride and enzymes significantly reduced more bacteria than the comparative dentifrices F and G.

TABLE IV

Reduction of malodor tongue bacteria 4 hours post treatment

| Composition | Baseline<br>Mean colonies | After 4 hours<br>Mean colonies | % Reduction of<br>Malodor Bacteria<br>Colonies |
|-------------|---------------------------|--------------------------------|------------------------------------------------|
| D           | $3.8 \times 10^5$         | $1.4 \times 10^5$              | 63                                             |
| F           | $2.3 \times 10^5$         | $2.3 \times 10^5$              | 0                                              |
| G           | $2.0 \times 10^5$         | $2.3 \times 10^5$              | 0                                              |

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TABLE V

Reduction of total tongue bacteria 4 hours post treatment

| Composition | Baseline<br>Mean colonies | After 4 hours<br>Mean colonies | % Reduction of<br>Malodor Bacteria<br>Colonies |
|-------------|---------------------------|--------------------------------|------------------------------------------------|
| D           | $5.2 \times 10^5$         | $2.4 \times 10^5$              | 53                                             |
| F           | $5.0 \times 10^5$         | $3.1 \times 10^5$              | 38                                             |
| G           | $4.3 \times 10^5$         | $3.9 \times 10^5$              | 10                                             |

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**Example II**

Oral rinses designated "Compositions K and L" were prepared having the ingredients listed in Table VI below.

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| TABLE VI                   |          |          |
|----------------------------|----------|----------|
| Composition<br>Ingredients | K (Wt.%) | L (Wt.%) |
| Water                      | 57.8     | 70.3     |
| Glycerin                   | 16.25    | 10.0     |
| Sorbitol                   | 16.25    | 10.0     |
| Ethanol                    | 5.00     | 5.00     |
| Gantrez                    | 1.92     | 1.92     |
| Pluronic F127              | 0.75     | 0.75     |
| Pluronic F108              | 0.75     | 0.75     |
| Tetrasodium pyrophosphate  | 0.45     | 0.45     |
| Flavor                     | 0.30     | 0.30     |
| Papain                     | 0.21     | 0.21     |
| Potassium stannate         | 0.05     | 0.05     |
| Sodium saccharin           | 0.070    | 0.070    |
| Cetylpyridinium chloride   | 0.050    | 0.050    |
| Nipastat                   | 0.050    | 0.050    |
| Monosodium phosphate       | 0.035    | 0.035    |
| Butyl hydroxy toluene      | 0.015    | 0.015    |
| Disodium phosphate         | 0.010    | 0.010    |
| Phosphoric acid            | 0.04     | 0.04     |
| Total                      | 100.0    | 100.0    |

**Papain Stability**

The papain activity of the rinse was monitored using the PanVera Protease Activity Kit.

The activity kit quantifies protease activity using a fluorescein thiocarbamoyl (FTC)-casein substrate. FTC-casein is attacked by the protease, breaking down casein into TC-peptides.

The amount of protease activity is determined by measuring the fluorescence of the FTC peptides generated. Table VII below compares the percent activity and fluorescence of Compositions K and L, and a placebo designated "Composition N" which is identical to Composition K, but does not contain enzymes. Fluorescence values are expressed in relative fluorescence units, RFU's. Percent activity is determined from the fluorescence,

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relative to the placebo. From activity measurements above 70% of the enzyme activity was maintained after 4 weeks at room temperature.

| TABLE VII<br>PROTEASE ACTIVITY |                             |                                    |             |
|--------------------------------|-----------------------------|------------------------------------|-------------|
| Oral Rinse                     | Initial<br>Florescence(RFU) | After 4 Weeks<br>Fluorescence(RFU) | % Reduction |
| K                              | 25983                       | 21518                              | 74%         |
| L                              | 26548                       | 21297                              | 72%         |
| N                              | 10360                       | 11563                              | 0           |

### 5 Plaque Removal Efficacy

To evaluate the plaque removal ability of the papain enzyme rinse Compositions K and L, a salivary flow cell study was performed in which plaque was grown onto a model surface, followed by treating the surface with a rinse and measuring the reduction of plaque. In the study, a plaque biofilm was first formed onto germanium prisms by continuously pumping bacteria growth media over the prisms for approximately 18 hours. Next, the prisms were treated with a rinse composition, pumping rinse over the prisms for several minutes, in order to facilitate plaque removal. A plaque index was calculated, using Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR FTIR) absorption band intensities at 3300, 1650, 1545 and 1080  $\text{cm}^{-1}$  from the infrared spectrum as follows:

$$\text{Plaque Score} = \text{abs}_{3300} + \text{abs}_{1650} + \text{abs}_{1545} + \text{abs}_{1080}$$

where *abs* is the maximum absorbance at the various wave numbers. The wave numbers selected represent the infrared adsorption by salivary components and bacteria on the infrared-transparent germanium prisms. The plaque index of the rinse treated prisms were then compared to a baseline (prism rinsed with saline only), and reported as percent plaque reduction.

The results recorded in Table IX below, compares the plaque reduction performance of oral rinse Compositions K and L of the present invention versus a commercial rinse designated Composition M which contained 0.05% by weight cetyl pyridinium chloride but no enzymes and the placebo designated "Composition N". The percent plaque reduction of the high water containing rinse, rinse L was 17% better than rinse K. The results indicate that there is greater improvement in the delivery of actives to the plaque surface with the high water, less viscous formula although both rinses were effective to

reduce plaque to a greater degree than the commercial rinse Composition M when compared to the placebo Composition N.

The results are recorded in Table IX below.

| TABLE IX                         |              |                    |
|----------------------------------|--------------|--------------------|
| Composition                      | Plaque Index | % Plaque Reduction |
| K                                | 2.320        | 20                 |
| L                                | 1.832        | 37                 |
| M                                | 2.864        | 1                  |
| N                                | 2.529        | 13                 |
| Rinsed with saline solution only | 2.901        | --                 |

#### Inhibition of Plaque Formation

The ability of the enzyme rinse to inhibit plaque formation was evaluated using the salivary flow cell wherein the study was performed in a similar manner as described above for plaque removal, except that the germanium prisms are pretreated with the rinse for several minutes, followed by an 18 hour post treatment with bacterial growth media to facilitate growth of a plaque biofilm onto the prism surface. The amount of plaque formed (plaque index) on the prisms was again determined by measuring the total amide absorption by ATR FTIR. The plaque index of the rinse treated prisms is compared to a placebo, Composition N and expressed as percent plaque inhibition.

Table X compares the percent plaque inhibition of rinses K and L relative to the commercial rinse M containing cetyl pyridinium chloride but no enzymes, and the placebo N which was identical to Composition K except it did not contain enzymes. The results recorded in Table X indicate that the lower water, more viscous rinse Composition K is more effective in coating the plaque free surface, forming a greater protective barrier for inhibiting plaque formation than Composition L.

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| TABLE X    |              |                     |
|------------|--------------|---------------------|
| Oral Rinse | Plaque Index | % Plaque Inhibition |
| K          | 0.683        | 35                  |
| L          | 0.801        | 24                  |
| M          | 0.968        | 8                   |
| N          | 1.053        | --                  |

#### Intradental Plaque Removal

The propensity of the enzyme rinse to remove intradental plaque was demonstrated using an in vitro cleaning model. The model involved pretreating dentures with the rinse, growing plaque onto the denture surface, followed by post-treatment of the dentures with the rinse. In this model, removable dentures were first pumiced, rinsed with water, and sterilized with ethanol for 10 minutes in a trough of a brushing machine, in order to provide a clean denture surface. Next the dentures are submersed in a rinse solution, and incubated overnight at room temperature. Following overnight treatment, the dentures are rinsed with water and submersed into bacterial growth media for 6 hours at 37°C. Finally, the models were rinsed briefly with water, followed by brief rinsing with mouth rinse formulas. The dentures were then disclosed with disclosing solution for 30 seconds, rinsed briefly with water, and then rated with a plaque score determined using the Quigley Hein Plaque method.

The high water containing rinse, Composition L was tested in the in vitro cleaning model. Results recorded in Table XI indicate, that in comparison the commercial rinse M containing cetyl pyridinium chloride but no enzymes and the placebo rinse N, the enzyme rinse L significantly reduced both total and interproximal plaque by 67% demonstrating Composition L not only effectively reduces approximal plaque, but is also effective in cleaning in interproximal regions, demonstrating a "floss type" action.

| TABLE XI<br>INTRADENTAL PLAQUE |                                 |                         |                             |                                     |
|--------------------------------|---------------------------------|-------------------------|-----------------------------|-------------------------------------|
| Oral Rinse                     | Plaque Index<br>(Interproximal) | Plaque Index<br>(Total) | % Total Plaque<br>Reduction | % Interproximal<br>Plaque Reduction |
| L                              | 1.00                            | 1.50                    | 67                          | 67                                  |
| M                              | 3.00                            | 4.58                    | —                           | —                                   |
| N                              | 3.00                            | 4.58                    | —                           | —                                   |

CLAIMS

What is claimed is:

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1. An oral care composition having enhanced stability and antiplaque effectiveness which comprises an orally acceptable vehicle containing a combination of an enzyme, cetyl pyridinium chloride and a reducing agent.
- 10 2. The composition of claim 1 wherein the enzyme is a proteolytic enzyme.
3. The composition of claim 2 wherein the proteolytic enzyme is papain.
- 15 4. The composition of claim 3 wherein the papain is present in the composition at a concentration of about 0.1 to about 5.0% by weight of the composition.
5. The composition of claim 1 wherein the enzyme is glucoamylase.
- 20 6. The composition of claim 1 wherein the glucoamylase is present in the oral composition at a concentration of about 0.1 to 5.0% by weight.
7. The composition of claim 2 wherein the cetyl pyridinium chloride is present in the composition at a concentration of about 0.005 to about 2% by weight.
- 25 8. The composition of claim 1 wherein the reducing agent is potassium stannate.
9. The composition of claim 2 wherein the reducing agent is ammonium sulfate.
- 30 10. The composition of claim 1 wherein the reducing agent is present in the composition at a concentration of about 0.05 to about 2.0% by weight of the composition.
11. The composition of claim 1 wherein the composition is a dentifrice.
12. The composition of claim 1 wherein the composition is a mouth rinse.
- 35 13. The composition of claim 1 wherein the composition is a dentifrice thickened with a cellulose polymer.

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14. The composition of claim 13 wherein the cellulose polymer is carboxymethyl cellulose.
- 5 15. The composition of claim 12 wherein the mouthrinse has a water concentration greater than about 45% by weight water.
16. The composition of claim 15 wherein the water concentration is about 50 to about 85% by weight.
- 10 17. A method for effecting enhanced stability in an oral composition containing enzymes which comprises preparing a composition comprised of an orally acceptable vehicle containing a combination of an enzyme, cetyl pyridinium chloride and a reducing agent and then applying the composition to the oral cavity.
- 15 18. The method of claim 17 wherein the enzyme is a proteolytic enzyme.
19. The method of claim 17 wherein proteolytic enzyme is papain
- 20 20. The method of claim 19 wherein the papain is present in the composition at a concentration of about 0.1 to about 5.0% by weight of the composition.
21. The method of claim 17 wherein the enzyme is glucoamylase.
- 25 22. The method of claim 21 wherein the glucoamylase is present in the oral composition at a concentration of 0.1 to 5.0% by weight.
23. The method of claim 17 wherein the enzyme is incorporated in the composition as an aqueous dispersion of a papain and glucoamylase.
- 30 24. The method of claim 17 wherein the reducing agent is potassium stannate
25. The method of claim 17 wherein the reducing agent is ammonium sulfate
- 35 26. The method of claim 17 wherein the reducing agent is present in the composition at a concentration of about 0.05 to about 2.0% by weight of the composition.
27. The method of claim 17 wherein the composition is a dentifrice thickened with a cellulose polymer.

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28. The method of claim 17 wherein the cellulose polymer is carboxymethyl cellulose.

5 29. The method of claim 17 wherein the cetyl pyridinium chloride is present in the composition at a concentration of about 0.005 to about 2% by weight.

30. The method of claim 17 wherein the composition is a mouth rinse.

10 31. The method of claim 30 wherein the mouthrinse has a water concentration greater than about 45% by weight.

32. The method of claim 31 wherein the mouthrinse has a water concentration of about 50 to about 85% by weight.

15 33. A method for the preparation of an aqueous oral care composition containing a stable enzyme compound which comprises first dissolving the enzyme in an aqueous solution of cetyl pyridinium chloride and a reducing agent or to prepare a premix and then adding the premix to the vehicle of the oral care composition.

20 34. The method of claim 33 wherein the enzyme is present in the premix at a concentration of about 0.1 to about 10% by weight.

35. The method of claim 33 wherein the enzyme is glucoamylase.

25 36. The method of claim 33 wherein the reducing agent is present in the oral care composition at a concentration of about 0.05 to about 3% by weight.

37. The method of claim 33 wherein the enzyme is papain.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US2004/003842

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K7/16 A61K7/22 A61K7/28

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages             | Relevant to claim No. |
|------------|------------------------------------------------------------------------------------------------|-----------------------|
| X          | US 5 711 937 A (GOMI TETSUO ET AL)<br>27 January 1998 (1998-01-27)<br>example 29               | 1-37                  |
| X          | GB 1 255 284 A (BLENBEX-WERKE R. SCHNEIDER<br>& CO) 1 December 1971 (1971-12-01)<br>example 10 | 1-37                  |
| X          | US 3 681 197 A (SMITH CLARENCE T)<br>1 August 1972 (1972-08-01)<br>the whole document          | 1-37                  |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*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 when the document is taken alone
- \*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
- \*Z\* document member of the same patent family

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**FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210**

Continuation of Box II.2

Claims Nos.: -

Present claims 1-37 relate to an oral composition comprising "a reducing agent" which is a vague term, for example an enzyme could be interpreted as a reducing agent. As a result the claims contain so many options, variables and possible permutations that a lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out in light of the interpretation of "reducing agent" from dependent claims 2-16, the examples and the passage on page 4 of the description (line 15-26) i.e. reducing agent is understood to be potassium stannate, ammonium sulfate or those compounds listed on page 4.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2004/003842

## Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.:  
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, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ 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 claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US2004/003842

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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**Declaraciones bajo la Regla 4.17:**

— en cuanto al derecho del solicitante para solicitar y que le se conceda una patente (Regla 4.17(iii)) para todas las designaciones.

— en cuanto al derecho del solicitante para reivindicar la prioridad de la publicación más temprana (Regla 4.17 (iii)) para todas las designaciones.

**Publicado:**

— con el informe de búsqueda internacional

— antes del vencimiento del límite de tiempo para corregir las reivindicaciones y a ser vuelto a publicar en el evento de recibir las correcciones

Para códigos de dos letras y otras abreviaciones, referirse a las "Notas de guía sobre Códigos y Abreviaturas" que aparecen al comienzo de cada edición de la Gaceta del PCT

(54) Título: COMPOSICION ORAL QUE CONTIENE ENZIMAS CON ESTABILIDAD MEJORADA

(57) Resumen: Una composición oral que tiene propiedades de higiene oral y antiplaca mejoradas la cual comprende un vehículo oralmente aceptable que contiene una enzima, cloruro de cetilpiridinio y un agente reductor.

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# **COMPOSICIÓN ORAL QUE CONTIENE ENZIMAS CON** **ESTABILIDAD MEJORADA**

## **RESUMEN**

Una composición para el cuidado oral que cuenta con propiedades mejoradas en términos de higiene oral y contra la placa y que incluye un vehículo oralmente aceptable que está conformado por una combinación de una enzima, cloruro de cetilpiridinio y un agente reductor.

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# **COMPOSICIÓN ORAL QUE CONTIENE ENZIMAS CON** **ESTABILIDAD MEJORADA**

## **ANTECEDENTES DE LA INVENCION**

### **1. Campo de la Invención**

La presente invención se relaciona de manera general con composiciones orales que permiten mejorar la higiene bucal y, más particularmente, con composiciones orales que contienen enzimas y que tienen mayor estabilidad y efectividad contra la placa.

### **2. Arte Previo**

Las composiciones orales tales como las cremas dentales, los gels y los enjuagues bucales están diseñados a los efectos de desprender y remover la placa, conjuntamente con un régimen regular de cepillado dental. La placa dental está en cierto grado presente sobre virtualmente todas las superficies dentales en la forma de una película. La misma es un subproducto del crecimiento microbiano y consta de una densa capa microbiana que está conformada por una masa de microorganismos que están embutidos en una matriz de polisacáridos. La placa en sí se adhiere firmemente a las superficies dentales y es únicamente removida con dificultad, inclusive a través de un riguroso régimen de cepillado. Por otra parte, después de ser removida, la placa se forma nuevamente y con rapidez sobre la superficie de los dientes. La placa puede formarse sobre cualquier parte de la superficie de los dientes y en particular se ubica en el margen gingival, en las grietas presentes en el esmalte y sobre la superficie del cálculo dental. El riesgo asociado con la formación de placa sobre los dientes tiene como origen la tendencia que tiene la placa a acumularse y a eventualmente producir gingivitis, periodontitis y otros tipos de enfermedades periodontales, al igual que caries dentales y cálculo dental.

En el arte se conoce la incorporación de agentes antimicrobianos a las composiciones orales, agentes estos que destruyen o inhiben el crecimiento de las bacterias orales. También se incorporan otros agentes a la composición oral, a los efectos de mejorar la eficacia de estos agentes antimicrobianos. Por ejemplo, se han incorporado enzimas tales como proteasas, a las

composiciones orales, las cuales detienen o interfieren con la formación de placa y con la adhesión de las bacterias a la superficie de los dientes.

Un problema asociado con las enzimas procesadas a nivel comercial, por ejemplo, las proteasas, es que con frecuencia contienen un amplio espectro de subproductos o impurezas indeseados que son difíciles de remover durante el proceso de fabricación. La celulasa es uno de estos subproductos enzimáticos y es una enzima que cataboliza la celulosa y la convierte en azúcares simples por medio de la hidrólisis de los enlaces  $\beta$  (1-4).

Los agentes de espesamiento convencionalmente utilizados en las composiciones orales, por ejemplo, carboximetilcelulosa, son sometidos a degradación en la presencia de las enzimas celulasas, lo que afecta la reología del producto dentífrico de forma perjudicial. En consecuencia, el disponer de un medio para impedir que las celulasas degraden estos agentes de espesamiento es un factor crítico para la obtención de composiciones estables de cuidado oral contentivas de enzimas.

Los métodos típicamente utilizados en el arte a los fines de inhibir o aislar las celulasas no son prácticos para ser utilizados en el área de las composiciones orales. Los tratamientos para la inhibición de las celulasas tales como la precipitación salina, el tratamiento térmico o el ajuste del pH, también comprometen la actividad de las enzimas. Los métodos clásicos para la separación de enzimas que se basan en el tamaño, carga, solubilidad y sitio de enlace de las enzimas son prohibitivos en términos de costos e inefectivos debido a las similitudes entre ciertas celulasas y las enzimas tales como las amilasas. La inhibición de la actividad de las celulasas por medio del tratamiento con metales pesados o con complejos a base de metales pesados tales como cloruro de mercurio, plata y paladio, que se unen al sitio activo de la enzima son igualmente inaceptables ya que estos materiales son tóxicos para los seres humanos y obviamente no pueden ser utilizados en un producto para el cuidado oral.

### **RESUMEN DE LA INVENCION**

De acuerdo con la presente invención, se ha inesperadamente determinado que una cantidad menor pero efectiva de cloruro de cetilpiridinio y un agente reductor pueden proporcionarle mayor estabilidad y eficacia contra la placa a las composiciones orales que contienen

enzimas.

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## **DESCRIPCIÓN DE LAS REPRESENTACIONES PREFERENTES**

### **Cloruro de Cetilpiridinio**

El cloruro de cetilpiridinio es incorporado en las composiciones para el cuidado oral de la presente invención a una concentración de entre aproximadamente 0,005 y alrededor de 1,0% en peso, preferiblemente de entre aproximadamente 0,50 y alrededor de 1,0% en peso de dicha composición para el cuidado oral.

### **Enzimas**

Las enzimas que son de utilidad en la puesta en práctica de la presente invención incluyen las carbohidrasas tales como la glucoamilasa y las enzimas que son extraídas de los productos a base de frutas naturales tales como las proteasas, las cuales descomponen o hidrolizan las proteínas.

La glucoamilasa es una glucoamilasa sacarificante de origen *Aspergillus niger* que es cultivada por fermentación. Esta enzima puede hidrolizar tanto los puntos de la rama glucosídica alfa-D-1,6, como los enlaces glucosídicos alfa-1,4 de los oligosacáridos glucosílicos. Otras carbohidrasas que son de utilidad de acuerdo con la presente invención son la alfa y beta-amilasa, la dextranasa y la mutanasa. La glucoamilasa es una enzima preferente y ésta es incorporada en la composición oral de la presente invención a una concentración de entre aproximadamente 0,001 y 2% en peso, preferiblemente de entre aproximadamente 0,01 y 0,55% en peso.

Las enzimas proteasas que son de utilidad en la puesta en práctica de la presente invención incluyen aquellas extraídas de los productos a base de frutas naturales. Las enzimas proteolíticas son obtenidas a partir de fuentes naturales o por medio de la acción de los microorganismos que cuentan con una fuente de nitrógeno y con una fuente de carbono. Algunos ejemplos de enzimas proteolíticas que son de utilidad en la puesta en práctica de la presente invención incluyen las enzimas de aparición natural papaína (extraída de la papaya) y bromelaína (extraída de la piña), al igual que las serina-proteasas tales como la

quimotripsina. Otras enzimas incluyen la ficina y la alcalasa. La papaína es la enzima proteasa que se prefiere utilizar en la puesta en práctica de la presente invención, enzima ésta que tiene una actividad de entre 150 y 939 MCU por miligramo, determinada de conformidad con la Prueba de Coagulación de la Leche del Grupo Biddle Sawyer (véase el texto T. Biol. Chem., tomo 121, páginas 737-745). Las enzimas proteasas son incluidas en las composiciones de la presente invención a una concentración de entre aproximadamente 0,1 y alrededor de 3% en peso, preferiblemente de entre aproximadamente 0,2 y alrededor de 2% en peso.

Las enzimas que pueden ser beneficiosamente utilizadas en combinación con las enzimas proteolíticas y con las enzimas glucoamilasas incluyen las carbohidrasas tales como la alfa-amilasa, la beta-amilasa y la tanasa, así como las lipasas tales como la lipasa vegetal, la lipasa gástrica y la lipasa pancreática.

La enzima lipasa se deriva de una familia selecta de *Aspergillus niger* que presenta escisión aleatoria de las posiciones 1,3 de las grasas y aceites. La enzima tiene su máxima actividad lipolítica a un pH de entre 5,0 y 7,0 al ser evaluada con aceite de oliva. La enzima tiene una actividad medida de 120.000 unidades de lipasa por gramo. La lipasa puede ser incluida en la composición dentífrica a una concentración de entre aproximadamente 0,010 y alrededor de 5,0% en peso, preferiblemente de entre aproximadamente 0,02 y alrededor de 0,10 % en peso.

La presencia de la enzima tanasa también puede ser beneficiosa para facilitar la descomposición de la mancha extrínseca. Las enzimas tanasas han sido purificadas utilizando *Aspergillus niger* y *Aspergillus allianceus* y son de utilidad en la hidrólisis de las taninas, las cuales se sabe decoloran la superficie de los dientes.

Otras enzimas adecuadas que pueden ser incluidas en la presente invención incluyen la lisozima, derivada de la clara de huevo, la cual contiene una única cadena de polipéptidos entrecruzada por cuatro enlaces de disulfuro que tienen un peso molecular de 14.600 Daltons. La enzima puede presentar propiedades antibacterianas al facilitar la hidrólisis de las paredes de las células bacterianas mediante la escisión del enlace glicosídico entre el carbono número 1 del ácido N-acetilmurámico y el carbono número 4 de la N-acetil-D-glucosamina; estos dos carbohidratos son polimerizados in vivo con el objeto de formar el polisacárido de la pared celular. Adicionalmente, la pectinasa, una enzima que está presente en la mayoría de las

plantas, facilita la hidrólisis de la pectina polisacárida en azúcares y ácido galacturónico. Finalmente, la glucanasa puede ser utilizada a los efectos de catalizar la descomposición de los carbohidratos complejos en glucanos y la hidrólisis del beta glucano en glucosa.

### **Agentes Estabilizadores de Enzimas**

Los agentes estabilizadores de enzimas que protegen a la enzima de la inactivación mediante la quelación de las impurezas metálicas presentes en la composición oral incluyen el ácido etilendiamina tetraacético (EDTA) y el gluconato de sodio a concentraciones de entre 0,01 y 1% en peso, preferiblemente de entre 0,1 y 0,5% en peso. Los agentes que estabilizan la enzima contra la oxidación incluyen los agentes reductores tales como el bisulfito de sodio, los galatos metálicos, el estanato de potasio, el estanato de sodio, el sulfato de amonio, el 3,5-di-terc-butil-4-hidroxitolueno (BHT), la Vitamina E (formas  $\alpha$ ,  $\beta$  y  $\gamma$ )/acetato de Vitamina E y el ácido ascórbico. El estanato de potasio es el agente estabilizador de enzimas que se prefiere utilizar en la puesta en práctica de la presente invención. El agente reductor está presente en la composición oral de la presente invención a una concentración de entre aproximadamente 0,05 y alrededor de 1,5% en peso, preferiblemente de entre aproximadamente 0,1 y alrededor de 0,75% en peso.

### **Premezclado**

Al incorporar el cloruro de cetilpiridinio en la composición para el cuidado oral de la presente invención, es preferible que el mismo, así como cualquier enzima que deba ser agregada a la composición oral, sea primero premezclado en una solución humectante acuosa, de forma tal que el cloruro de cetilpiridinio interactúe, se una e inactive cualquier impureza presente en la enzima que fue agregada a la composición oral.

El producto del premezclado normalmente estará conformado por los siguientes cuatro componentes: (1) cloruro de cetilpiridinio, (2) las una o más enzimas que deben ser incorporadas a la composición oral, (3) un agente reductor y el resto es (4) agua y un humectante.

La concentración de cloruro de cetilpiridinio presente en la premezcla por lo general oscilará entre aproximadamente 0,15 y alrededor de 1,0% en peso, preferiblemente entre

aproximadamente 0,2 y alrededor de 0,6% en peso. La enzima estará presente entre aproximadamente 0,5 y alrededor de 10,0% en peso de la premezcla, preferiblemente entre aproximadamente 0,75 y alrededor de 8,0% en peso. En aquellos casos donde se incluya una combinación de enzimas tal como glucoamilasa y papaína en la premezcla, la concentración de papaína oscilará entre aproximadamente 2 y alrededor de 10% en peso y la concentración de glucoamilasa oscilará entre aproximadamente 0,5 y alrededor de 2% en peso.

Un agente reductor tal como estanato de potasio es igual y preferiblemente incluido en la solución de la premezcla a una concentración de entre aproximadamente 0,5 y alrededor de 40% en peso, preferiblemente de entre aproximadamente 1,0 y alrededor de 3% en peso. La presencia del agente reductor tiene por objeto mejorar aún más la capacidad que tiene el cloruro de cetilpiridinio de inhibir la actividad de las impurezas enzimáticas al ser posteriormente incorporado en el vehículo de la composición oral.

El agua y el humectante son componentes adicionales de la premezcla. Las concentraciones oscilarán entre aproximadamente 85 y 95% en peso.

Para preparar la premezcla, primero se introduce una mezcla de agua y humectante en un reactor, seguida por la adición de una cantidad efectiva de cloruro de cetilpiridinio que es mezclada y disuelta en dicha mezcla. Posteriormente se agregan las enzimas y el agente reductor a la solución. Estos ingredientes son mezclados entre sí a una temperatura no superior a aproximadamente 40°C, preferiblemente de alrededor de 30°C. Se ajusta el pH a entre aproximadamente 1,5 y alrededor de 8,0, preferiblemente a entre aproximadamente 6,0 y alrededor de 7,5. La premezcla así preparada es posteriormente sellada en recipientes adecuados a la espera de ser mezclada con el vehículo y con los demás ingredientes de la composición para el cuidado oral.

#### **Composición para el Cuidado Oral**

Para preparar una composición oral, se combina la premezcla con los ingredientes del vehículo de la composición para el cuidado oral a los fines de preparar un producto en el cual las enzimas permanecen efectivamente estabilizadas y que tendrá actividad contra la placa y la gingivitis.

### **Vehículo del Dentífrico**

Los vehículos oralmente aceptables que son utilizados con el objeto de preparar las composiciones dentífricas de la presente invención incluyen una fase acuosa que contiene un humectante. El humectante preferiblemente es glicerina, sorbitol, xilitol y/o propilenglicol con un peso molecular que se ubica en el rango comprendido entre 200 y 1.000; sin embargo, también se pueden utilizar otros humectantes y mezclas de los mismos. La concentración total del humectante típicamente se ubica entre aproximadamente 5 y alrededor de 70% en peso de la composición oral, preferiblemente entre aproximadamente 30 y alrededor de 60% en peso. El contenido de agua presente en el dentífrico oscilará entre aproximadamente 10 y alrededor de 30% en peso, preferiblemente entre aproximadamente 15 y alrededor de 25% en peso.

### **Abrasivos**

Los abrasivos que pueden ser utilizados en la puesta en práctica de la presente invención para preparar una composición dentífrica incluyen los abrasivos a base de sílice, por ejemplo, los sílices precipitados que tienen un tamaño promedio de las partículas de hasta aproximadamente 20 micrones tales como el Zeodent 115, comercializado por J. M. Huber. Otros abrasivos de dentífricos que son de utilidad incluyen el metafosfato de sodio, el metafosfato de potasio, el fosfato de tricalcio, el fosfato de dicalcio deshidratado, el silicato de aluminio, la alúmina calcinada, la bentonita u otros materiales silíceos y las combinaciones de los mismos.

Los materiales abrasivos que se prefiere utilizar en la puesta en práctica de la preparación de las composiciones dentífricas de acuerdo con la presente invención incluyen los gels de sílice y el sílice amorfo precipitado que tiene un valor de absorción de aceite inferior a 100 cc/100 gramos de sílice, preferiblemente comprendido entre alrededor de 45 cc/100 gramos y menos de aproximadamente 70 cc/100 gramos de sílice. Los valores de absorción de aceite son medidos utilizando el Método de Espátula o Rub-Out D281 de la ASTA. Estos sílices son partículas coloidales que tienen un tamaño promedio que oscila entre aproximadamente 3 micrones y alrededor de 12 micrones, más preferiblemente entre aproximadamente 5 y alrededor de 10 micrones, y un rango de pH de entre 4 y 10, preferiblemente de entre 6 y 9, medido como una suspensión al 5% en peso.

Los abrasivos a base de sílices de baja absorción de aceite que son particularmente útiles en la puesta en práctica de la presente invención son comercializados bajo la denominación comercial de Sylodent XWA de la Davison Chemical Division de W. R. Grace & Co., Baltimore, MD 21203. El Sylodent 650 XWA, un hidrogel de sílice conformado por partículas de sílice coloidal que tienen un contenido de agua de 29%, un diámetro que oscila entre aproximadamente 7 y alrededor de 10 micrones y una absorción de aceite inferior a 70 cc/100 gramos de sílice, es un ejemplo preferente de un abrasivo de sílice de baja absorción de aceite que es de utilidad en la puesta en práctica de la presente invención. El abrasivo está presente en la composición dentífrica de la presente invención a una concentración de entre aproximadamente 10 y alrededor de 40% en peso, preferiblemente de entre aproximadamente 15 y alrededor de 30% en peso.

### **Agentes de Espesamiento**

Los agentes de espesamiento que son utilizados en las composiciones dentífricas de la presente invención incluyen las gomas naturales y sintéticas y los coloides que incluyen agentes de espesamiento a base de celulosa tales como carboximetilcelulosa, las hidroxialquil celulosas tales como hidroxipropilcelulosa e hidroxietilcelulosa, las gomas tales como goma de xantano, los poliglicoles de diversos pesos moleculares comercializados bajo la denominación comercial Polyox y el polietilenglicol. Los agentes de espesamiento inorgánicos que pueden ser utilizados en la puesta en práctica de la presente invención incluyen los compuestos a base de sílice amorfo, por ejemplo, los compuestos de sílices coloidales comercializados bajo la denominación comercial Cab-o-sil, fabricados por Cabot Corporation y distribuidos por Lenape Chemical, Bound Brook, New Jersey, el Zeodent 165 de J. M. Huber Chemicals Division, Havre de Grace, Maryland 21078, y el Sylodent 15, comercializado por la Davison Chemical Division de W. R. Grace Corporation, Baltimore, MD 21203. Otros agentes de espesamiento inorgánicos incluyen las arcillas naturales y sintéticas, el silicato de litio y magnesio (Laponite) y el silicato de aluminio y magnesio (Veegum).

El agente de espesamiento está presente en la composición dentífrica en cantidades que oscilan entre aproximadamente 0,1 y alrededor de 10% en peso, preferiblemente entre

aproximadamente 0,5 y alrededor de 4,0% en peso.

### **Surfactantes**

Se utilizan surfactantes en las composiciones orales de la presente invención a los efectos de lograr mayor acción profiláctica y de hacerlas más aceptables desde el punto de vista cosmético. El surfactante preferiblemente es un material detergente que le imparte propiedades detergentes y espumantes a la composición.

Los surfactantes aniónicos tales como los sulfatos de alquilo superiores, por ejemplo, laurilsulfato de sodio, actúan como surfactantes efectivos en las composiciones orales, pero normalmente no son compatibles con las enzimas y promueven la desnaturalización de la enzima y la pérdida de actividad. Sin embargo, de acuerdo con la puesta en práctica de la presente invención, el premezclado de cantidades concentradas de enzimas tales como glucoamilasa, con cloruro de cetilpiridinio, permite que el cloruro de cetilpiridinio interactúe y se una a cualquier impureza de celulasa presente en la glucoamilasa y de este modo impide la interacción de la celulasa con cualquier agente de espesamiento a base de celulosa que pudiese ser utilizado en la preparación del dentífrico e inhibe la interacción con el surfactante aniónico. También se pueden utilizar surfactantes que son compatibles con las enzimas a los fines de lograr las características espumantes requeridas en la composición oral. Algunos ejemplos de surfactantes compatibles con las enzimas incluyen los surfactantes no iónicos a base de polioxietileno tales como Poloxámero 407, Pluronic 127 y Polisorbato 20, así como los surfactantes anfotéricos tales como tauranol, cocamidopropil betaína (tegobetaína) y lauril-glucósido de cocamidopropil betaína. Los surfactantes son incluidos en la composición oral de la presente invención a una concentración de entre aproximadamente 2 y alrededor de 10% en peso, preferiblemente de entre aproximadamente 0,5 y alrededor de 5,0% en peso.

### **Fluoruro y Otros Agentes Activos**

La composición oral de la presente invención puede igualmente contener una fuente de iones de fluoruro, o un ingrediente generador de flúor, como agente contra la caries, el cual estará presente en cantidades suficientes como para suministrar entre aproximadamente 25 ppm y 5.000 ppm de iones de fluoruro. Lo anterior incluye las sales inorgánicas de fluoruro, por

ejemplo, las sales solubles a base de metales alcalinos. Las fuentes de fluoruro que son compatibles con las enzimas y que se prefiere utilizar incluyen el fluoruro de sodio, el fluoruro de potasio, el fluorosilicato de sodio, el monofluorofosfato de sodio (MFP) y el fluorosilicato de amonio, al igual que los fluoruros de estaño tales como el fluoruro estañoso y el cloruro estañoso. Se prefiere utilizar fluoruro de sodio o MFP.

Además de los compuestos a base de fluoruro, las composiciones orales de la presente invención también pueden incluir agentes anti-sarro tales como sales de pirofosfato, lo que incluye las sales de pirofosfato a base de metales alcalinos o tetra-alcalinos tales como  $\text{Na}_4\text{P}_2\text{O}_7$ ,  $\text{K}_4\text{P}_2\text{O}_7$ ,  $\text{Na}_2\text{K}_2\text{P}_2\text{O}_7$ ,  $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$  y  $\text{K}_2\text{H}_2\text{P}_2\text{O}_7$ , los polifosfatos tales como tripolifosfato de sodio y hexametafosfato de sodio y los fosfatos cíclicos tales como tripolifosfato de sodio y trimetafosfato de sodio. Estos agentes anti-sarro son incluidos en la composición dentífrica a una concentración de entre aproximadamente 1,0 y alrededor de 5,0% en peso.

Los agentes antibacterianos constituyen otro agente activo que es de utilidad en las composiciones dentífricas de la presente invención, agentes éstos que están presentes en una cantidad que oscila entre 0,2 y 1,0% en peso de la composición oral. Estos agentes antibacterianos que son de utilidad incluyen los agentes antibacterianos no catiónicos que se basan en compuestos fenólicos o bisfenólicos tales como los éteres bifenílicos halogenados, por ejemplo, Triclosan (2,4,4'-triclora-2'-hidroxidifenil éter).

#### **Policarboxilato Aniónico**

También se pueden utilizar policarboxilatos aniónicos sintéticos en las composiciones orales de la presente invención como agente mejorador de la eficacia de cualquier agente antibacteriano, anti-sarro u otro agente activo presente en la composición dentífrica. Estos policarboxilatos aniónicos por lo general son utilizados en la forma de sus ácidos libres o preferiblemente en la forma de las sales de metales alcalinos (por ejemplo, potasio y preferiblemente sodio) o de amonio parcialmente o, más preferiblemente, totalmente neutralizadas y solubles en agua. Se prefiere utilizar los copolímeros 1:4 a 4:1 de anhídrido o ácido maleico con otro monómero polimerizable y etilénicamente insaturado, preferiblemente éter metilvinílico/anhídrido maleico con un peso molecular (M. W.) de entre

aproximadamente 30.000 y alrededor de 1.800.000, más preferiblemente de entre aproximadamente 30.000 y alrededor de 700.000. Algunos ejemplos de estos copolímeros son comercializados por GAF Corporation bajo la denominación comercial Gantrez, por ejemplo, AN 139 (M. W. 500.000), AN 119 (M. W. 250.000); S-97 de Grado Farmacéutico (M. W. 700.000), AN 169 (M. W. 1.200.000-1.800.000) y AN 179 (M. W. superior a 1.800.000). El copolímero preferente es el S-97 de Grado Farmacéutico (M. W. 700.000).

De estar presentes, los policarboxilatos aniónicos son utilizados en cantidades efectivas que permitan lograr el mejoramiento deseado de la eficacia de cualquier agente antibacteriano, anti-sarro u otro agente activo presente en la composición oral. Los policarboxilatos aniónicos por lo general están presentes en la composición oral en una cantidad que oscila entre 0,05% y alrededor de 4% en peso, preferiblemente entre aproximadamente 0,5% y alrededor de 2,5% en peso.

### **Sabor**

La composición oral de la presente invención también puede contener un agente saborizante. Los agentes saborizantes que son utilizados en la puesta en práctica de la presente invención incluyen los aceites esenciales, al igual que diversos aldehídos, ésteres y alcoholes saborizantes y materiales similares. Algunos ejemplos de aceites esenciales incluyen los aceites de menta verde, de menta, de gaulteria, de sasafrás, de clavo, de salvia, de eucalipto, de mejorana, de canela, de limón, de lima, de toronja y de naranja. Las sustancias químicas tales como mentol, carvone y anetol también son de utilidad. De los anteriores, los más comúnmente utilizados son los aceites de menta y de menta verde.

El agente saborizante es incorporado en la composición oral a una concentración de entre aproximadamente 0,1 y alrededor de 5% en peso, preferiblemente de entre aproximadamente 0,5 y alrededor de 1,5% en peso.

### **Otros Ingredientes**

Se pueden incorporar diversos materiales adicionales en las composiciones orales de la presente invención, lo que incluye desensibilizantes tales como nitrato de potasio, agentes blanqueadores tales como peróxido de hidrógeno, peróxido de calcio y peróxido de urea,

productos de conservación, siliconas y compuestos de clorofila. De estar presentes, estos aditivos son incorporados en las composiciones orales de la presente invención en cantidades que no afectan sustancial y adversamente las propiedades y características deseadas.

#### **Preparación de las Composiciones Dentífricas**

Para preparar una composición dentífrica de la presente invención, por lo general se utiliza un mezclador convencional para dispersar los humectantes tales como glicerina y sorbitol en el agua, bajo agitación. A la dispersión se le agregan los agentes orgánicos de espesamiento tales como carboximetilcelulosa, los agentes anti-sarro tales como pirofosfato de tetrasodio y tripolifosfato de sodio y cualquier edulcorante. La mezcla resultante es agitada hasta formarse una fase de gel homogénea. A la fase de gel se le agrega un pigmento tal como  $\text{TiO}_2$  y cualquier ácido o base requerido para ajustar el pH dentro del rango comprendido entre 6,4 y 7,3. Estos ingredientes son mezclados hasta obtenerse una fase homogénea. Posteriormente se agrega una premezcla de cloruro de cetilpiridinio, la enzima y un agente reductor tal como estanato de potasio, en una solución humectante acuosa, y ésta es mezclada con la fase de gel homogénea. La mezcla resultante es luego transferida a un mezclador al vacío de alta velocidad, donde se agrega el agente de espesamiento y los ingredientes surfactantes a la mezcla. Luego se agrega el abrasivo. Cualquier agente antibacteriano insoluble en agua, por ejemplo, Triclosan, es disuelto en los aceites saborizantes que van a ser incluidos en la composición y la solución es agregada a la mezcla, conjuntamente con los surfactantes. Esta es luego mezclada a alta velocidad durante un período de entre 5 y 30 minutos, bajo un vacío de entre aproximadamente 20 y 50 mm de Hg, preferiblemente de aproximadamente 30 mm de Hg. En todo caso, el producto resultante es una pasta o gel, semisólido, homogéneo y extruible.

#### **Preparación de las Composiciones Orales en Estado Líquido**

En el aspecto de la presente invención en el cual la composición oral es de naturaleza sustancialmente líquida, por ejemplo, un enjuague bucal, el vehículo típicamente es una mezcla de agua, humectante y alcohol. El alcohol es un alcohol no tóxico tal como etanol o isopropanol. Un humectante tal como glicerina o sorbitol o un alquilenglicol tal como polietilenglicol o propilenglicol puede estar presente en una cantidad que oscila entre

aproximadamente 10 y 30% en peso y el enjuague oral contendrá más de aproximadamente 45% en peso de agua, preferiblemente entre aproximadamente 50 y 85% en peso de agua, entre aproximadamente 0 y 20% en peso de un alcohol no tóxico y entre aproximadamente 10 y 40% en peso del humectante. Un agente de espesamiento tal como un Pluronic puede estar presente a una concentración de entre aproximadamente 1,0 y alrededor de 3,0% en peso y la composición contendrá cloruro de cetilpiridinio a una concentración de entre aproximadamente 0,02 y alrededor de 1,0% en peso, un agente reductor tal como estanato de potasio o sulfato de amonio a una concentración de entre aproximadamente 0,05 y 1,0% en peso, una enzima a una concentración de entre aproximadamente 0,02 y alrededor de 0,2 % en peso y un ingrediente saborizante a una concentración de entre aproximadamente 0,3 y alrededor de 1,0 % en peso.

Para preparar un enjuague oral, se dispersa una premezcla de la enzima que está conformada por cloruro de cetilpiridinio, el agente reductor, agua, el humectante y la enzima, en una mezcla de los ingredientes del enjuague bucal, por ejemplo, el alcohol, los humectantes y los surfactantes, y luego se agregan y mezclan los agentes saborizantes. Los ingredientes son posteriormente mezclados al vacío durante un período de aproximadamente 15-30 minutos. Luego se envasa el enjuague oral resultante.

Un enjuague constituye un vehículo conveniente para administrar los componentes activos en la cavidad oral, debido a la capacidad que tiene para llegar a las áreas difíciles de alcanzar de la boca, por ejemplo, las regiones interproximales y las hendiduras de la lengua. El reto que afronta la incorporación de enzimas a un enjuague consiste en mantener la estabilidad y la actividad enzimática a niveles de agua por encima de 50%, niveles éstos que tradicionalmente no son apropiados para las composiciones que contienen enzimas. En la presente invención, se determinó que la estabilidad de la actividad enzimática es aceptable y que se ve optimizada de forma inesperada cuando el contenido de agua del enjuague se mantiene por encima de 45% en peso de una mezcla del mismo, preferiblemente entre aproximadamente 50 y alrededor de 85% en peso. Se determinó que la actividad enzimática como agente contra la placa incrementa, conforme se demostrará más adelante.

Los siguientes ejemplos describen e ilustran las representaciones preferentes que están

incluidas dentro del alcance de la presente invención. Los ejemplos son incluidos para fines meramente ilustrativos y no deberán ser interpretados como una limitación de la presente invención ya que se pueden realizar numerosas variaciones a la misma sin alejarse de su espíritu y alcance.

**Ejemplo I**

Se preparó una composición dentífrica que contiene enzimas (papaína, glucoamilasa) y una cantidad menor pero efectiva de cloruro de cetilpiridinio, donde los agentes reductores (estanoato de potasio, sulfato de amonio) fueron agregados en la forma de una premezcla enzimática y la cual fue espesada utilizando un agente de espesamiento a base de celulosa (carboximetilcelulosa). Los ingredientes de esta composición, identificada como “Composición A”, se encuentran enumerados en la siguiente Tabla I.

Para fines comparativos, las composiciones dentífricas identificadas como Composiciones “B, C y D” fueron preparadas siguiendo el procedimiento del Ejemplo I. Todas las composiciones contenían las mismas enzimas de la Composición A, a saber: glucoamilasa y papaína. Las Composiciones B y C no contenían cloruro de cetilpiridinio.

La Composición B contenía sulfato de amonio como agente reductor y EDTA como agente quelante de metales. La Composición C contenía estanoato de potasio como agente reductor y Nipastat como agente de conservación. La Composición D contenía cloruro de cetilpiridinio en la composición dentífrica base, no contenía un agente reductor y el Laponite fue sustituido por carboximetilcelulosa de sodio como agente de espesamiento.

La crema dental comercial que no contenía cloruro de cetilpiridinio o enzimas, identificada como “Composición E”, fue utilizada como control.

| TABLA I                        |                  |                  |                  |                  |
|--------------------------------|------------------|------------------|------------------|------------------|
| Ingredientes de la Composición | A<br>(% en peso) | B<br>(% en peso) | C<br>(% en peso) | D<br>(% en peso) |
| Agua                           | 18,25            | 17,75            | 18,25            | 18,25            |

| TABLA I                           |             |             |             |             |
|-----------------------------------|-------------|-------------|-------------|-------------|
| Ingredientes de<br>la Composición | A           | B           | C           | D           |
|                                   | (% en peso) | (% en peso) | (% en peso) | (% en peso) |
| Pluronic F127                     | 1,5         | 1,5         | 1,5         | 0,75        |
| Glicerina                         | 20,0        | 20,0        | 20,0        | 22,0        |
| Pirofosfato de tetrasodio         | 2,0         | 2,0         | 2,0         | --          |
| Tripolifosfato de sodio           | 3,0         | 3,0         | 3,0         | --          |
| Fosfato monosódico                | 0,026       | 0,026       | 0,026       | --          |
| Fosfato disódico                  | 0,079       | 0,079       | 0,079       | --          |
| Carboximetilcelulosa de sodio     | 0,65        | 0,65        | 0,65        | --          |
| Laponite                          | --          | --          | --          | 0,7         |
| Goma de xantano                   | 0,55        | 0,55        | 0,55        | 0,6         |
| Monofluorofosfato de sodio        | 0,76        | 0,76        | 0,76        | 0,76        |
| Sacarina de sodio                 | 0,50        | 0,50        | 0,50        | 0,40        |
| Sorbitol (solución al 70%)        | 18,9434     | 19,6999     | 19,2024     | 22,8049     |
| Sílice XWA650                     | 20,0        | 20,0        | 20,0        | 20,0        |
| Zeodent 115                       | 5,0         | 5,0         | 5,0         | 5,0         |
| Zeodent 165                       | 2,25        | 2,0         | 2,0         | 2,25        |
| Dióxido de titanio                | 0,40        | 0,40        | 0,40        | 0,50        |
| Laurilsulfato de sodio            | 0,50        | 0,50        | 0,50        | --          |
| Tauranol                          | --          | --          | --          | 1,2         |
| Betaína                           | 1,0         | 1,0         | 1,0         | --          |

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| TABLA I                           |             |             |             |             |
|-----------------------------------|-------------|-------------|-------------|-------------|
| Ingredientes de<br>la Composición | A           | B           | C           | D           |
|                                   | (% en peso) | (% en peso) | (% en peso) | (% en peso) |
| PEG 600                           | 3,0         | 3,0         | 3,0         | 3,0         |
| Poloxámero 407                    | 0,10        | 0,10        | 0,10        | 0,10        |
| Sabor                             | 1,10        | 1,10        | 1,10        | 1,10        |
| Papaína ✓                         | 0,2266      | 0,2266      | 0,2266      | 0,2266 ✓    |
| Glucoamilasa ✓                    | 0,044       | 0,044       | 0,044       | 0,044 ✓     |
| Estanato de potasio ✓             | 0,10 ✓      | --          | 0,10 ✓      | --          |
| Sulfato de amonio ✓               | --          | 0,10 ✓      | --          | --          |
| Cloruro de cetilpiridinio ✓       | 0,015 ✓     | --          | --          | 0,3 ✓       |
| EDTA, disodio dihidrato           | 0,006       | 0,006       | 0,006       | 0,006       |
| Nipastat                          | --          | --          | 0,006       | --          |
| Benzoato de sodio                 | --          | 0,0025      | --          | 0,0025      |
| Sorbato de potasio                | --          | 0,006       | --          | 0,006       |

La Composición A fue preparada mediante el premezclado del agente reductor estanato de potasio y de un agente de conservación (EDTA), con cloruro de cetilpiridinio a 23°C. Los ingredientes de la premezcla identificada como "Premezcla A1" están registrados en la siguiente Tabla II a 23°C.

Las Premezclas identificadas como B1, C1 y D1 fueron preparadas siguiendo el procedimiento utilizado en la Premezcla A1 y fueron agregadas a las respectivas composiciones dentífricas B, C y D. Los ingredientes utilizados en la preparación de las composiciones de las premezclas correspondientes a las Composiciones A, B, C y D están enumerados en la siguiente Tabla II.

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| <div> <div>TABLA II</div> <div>Ingredientes de la Premezcla</div> </div> |             |             |             |             |
|--------------------------------------------------------------------------|-------------|-------------|-------------|-------------|
| Ingredientes de la Composición                                           | A1          | B1          | C1          | D1          |
|                                                                          | (% en peso) | (% en peso) | (% en peso) | (% en peso) |
| Sorbitol                                                                 | 67,168      | 76,848      | 67,348      | 76,848      |
| Agua                                                                     | 25          | 15          | 25          | 15          |
| Papaína                                                                  | 4,532       | 4,532       | 4,532       | 4,532       |
| Glucoamilasa                                                             | 0,88        | 0,88        | 0,88        | 0,88        |
| Estanato de potasio                                                      | 2,0         | --          | 2,0         | 2,0         |
| Sulfato de amonio                                                        | --          | 2,0         | --          | --          |
| Cloruro de cetilpiridinio                                                | 0,3         | --          | --          | --          |
| EDTA, disodio dihidrato                                                  | 0,12        | 0,12        | 0,12        | 0,12        |
| Nipastat                                                                 | --          | --          | 0,12        | --          |
| Benzoato de sodio                                                        | --          | 0,5         | --          | 0,5         |
| Sorbato de potasio                                                       | --          | 0,12        | --          | 0,12        |

Las composiciones de las Premezclas A1, B1, C1 y D1 fueron agregadas de forma independiente a una crema dental no enzimática que contenía los ingredientes enumerados en la Tabla I en una proporción de 1:4, con el objeto de observar los efectos sobre la actividad de las celulasas. La actividad de las celulasas fue monitoreada a través de los cambios en la viscosidad y en los niveles de azúcar de las Composiciones Dentífricas A, B, C y D con el tiempo.

En el caso de las mediciones de la viscosidad, la mayor actividad de las celulasas fue determinada por una reducción más rápida en la viscosidad de la crema dental a medida que la celulasa catabolizaba el agente de espesamiento carboximetilcelulosa utilizado para preparar las composiciones dentífricas. Se utilizó un viscosímetro Brookfield, modelo RVDTV-11,

provisto de un eje #95, a 5 rpm, para medir la viscosidad en centipoises por segundo<sub>2</sub> (CPS). Se realizaron mediciones diarias de cada muestra hasta observarse una pérdida del 80% de la viscosidad.

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La concentración del azúcar generado como subproducto fue monitoreada utilizando un medidor digital de los niveles de azúcar presentes en la sangre Accucheck. Dado que la celulasa actúa sobre la celulosa, se produce azúcar como subproducto. Mientras mayor sea la actividad de las celulasas, mayor será la cantidad de azúcar producida. Se utilizó tanto el método de determinación de los niveles de azúcar como el de viscosidad a los fines de comparar los efectos que tienen el cloruro de cetilpiridinio, los agentes reductores sulfato de amonio y estanato de potasio, el agente de conservación benzoato de sodio y los parabenos, sobre la actividad de las celulasas. Los resultados correspondientes a la viscosidad y a la concentración de azúcar están registrados en la siguiente Tabla III.

| TABLA III                      |                          |                        |                                                 |                                             |
|--------------------------------|--------------------------|------------------------|-------------------------------------------------|---------------------------------------------|
| Ingredientes de la Composición | Viscosidad Inicial (CPS) | Viscosidad Final (CPS) | % de Pérdida de Viscosidad (Después de 14 Días) | Conc. de Azúcar (MG/DL) (Después de 7 Días) |
| A                              | 25                       | 17                     | 32                                              | 28                                          |
| B                              | 20                       | 10                     | 50                                              | 62                                          |
| C                              | 20                       | 5                      | 75                                              | 62                                          |
| D                              | 30                       | 15                     | 50                                              | 62                                          |
| E                              | 28                       | 32                     | 0                                               | 0                                           |

Los resultados incluidos en la Tabla III demuestran que la tendencia de la producción de azúcar guarda consistencia con los resultados de pérdida de viscosidad. Ambos estudios indican que la Composición A, formulada utilizando una solución de una premezcla de estanato de potasio/cloruro de cetilpiridinio/enzima, fue más efectiva en retardar la actividad de las celulasas sobre la composición dentífrica.

### Disminución de la Microflora de la Lengua

Se evaluaron diversas composiciones con el objeto de determinar la disminución en la microflora de la lengua, concentrándose en aquellas especies responsables de la generación del mal olor bucal. Los pacientes se limpiaron la lengua a los efectos de recolectar las bacterias en la línea de referencia y cuatro horas después del tratamiento. Estas muestras fueron aplicadas a medio agar e incubadas de forma anaeróbica. Después de transcurridos cuatro días, se contaron las unidades formadoras de colonias de bacterias generadoras de mal olor, así como el total de bacterias de la lengua. Se utilizaron los resultados promedio de las unidades formadoras de colonias a los fines de calcular el porcentaje de reducción con respecto a la línea de referencia.

Las Tablas IV y V representan los estudios *in-vivo* de la microflora de la lengua tomando muestras de las bacterias en la línea de referencia y cuatro horas después de cepillarse utilizando la Composición D. Para fines comparativos, también se evaluó el mal olor bucal y la reducción en la microflora de la lengua utilizando las Composiciones F y G. La Composición F era un dentífrico comercial que contenía cloruro de cetilpiridinio más no enzimas y la Composición G era una crema dental comercial a base de fluoruro que no contenía cloruro de cetilpiridinio o enzimas. La Tabla V ilustra el incremento en la reducción de las bacterias causantes de mal olor bucal producto de la combinación de enzimas con cloruro de cetilpiridinio presente en la Composición D. La Tabla VI demuestra que, al ser incorporada en los sistemas de cremas dentales, esta premezcla enzimática estabilizada genera un efecto antibacteriano *in-vivo* contra todos los tipos de bacterias que existen en la lengua, lo que incluye las especies causantes del mal olor. Después de trascurridas cuatro horas post tratamiento, la Composición D que contenía cloruro de cetilpiridinio y enzimas redujo significativamente más las bacterias que los dentífricos comparativos F y G.

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| TABLA IV                                                                                                           |                                                |                                         |                                                                   |
|--------------------------------------------------------------------------------------------------------------------|------------------------------------------------|-----------------------------------------|-------------------------------------------------------------------|
| Reducción de las bacteria causantes de mal olor presentes en la lengua 4 horas después de efectuado el tratamiento |                                                |                                         |                                                                   |
| Composición                                                                                                        | Promedio de colonias en la Línea de Referencia | Promedio de colonias después de 4 horas | % de Reducción de las Colonias de Bacterias que Producen Mal Olor |
| D                                                                                                                  | 3,8*10 <sup>5</sup>                            | 1,4*10 <sup>5</sup>                     | 63                                                                |
| F                                                                                                                  | 2,3*10 <sup>5</sup>                            | 2,3*10 <sup>5</sup>                     | 0                                                                 |
| G                                                                                                                  | 2,0*10 <sup>5</sup>                            | 2,3*10 <sup>5</sup>                     | 0                                                                 |

| TABLA V                                                                                 |                                                |                                         |                                                                   |
|-----------------------------------------------------------------------------------------|------------------------------------------------|-----------------------------------------|-------------------------------------------------------------------|
| Reducción del total de bacterias presentes en la lengua 4 horas después del tratamiento |                                                |                                         |                                                                   |
| Composición                                                                             | Promedio de colonias en la Línea de Referencia | Promedio de colonias después de 4 horas | % de Reducción de las Colonias de Bacterias que Producen Mal Olor |
| D                                                                                       | 5,2*10 <sup>5</sup>                            | 2,4*10 <sup>5</sup>                     | 53                                                                |
| F                                                                                       | 5,0*10 <sup>5</sup>                            | 3,1*10 <sup>5</sup>                     | 38                                                                |
| G                                                                                       | 4,3*10 <sup>5</sup>                            | 3,9*10 <sup>5</sup>                     | 10                                                                |

**Ejemplo II**

Se prepararon los enjuagues orales identificados como "Composiciones K y L", utilizando los ingredientes enumerados en la siguiente Tabla VI.

| TABLA VI                       |               |               |
|--------------------------------|---------------|---------------|
| Ingredientes de la Composición | K (% en peso) | L (% en peso) |
| Agua                           | 57,8          | 70,3          |
| Glicerina                      | 16,25         | 10,0          |
| Sorbitol                       | 16,25         | 10,0          |
| Etanol                         | 5,00          | 5,00          |
| Gantrez                        | 1,92          | 1,92          |
| Pluronic F127                  | 0,75          | 0,75          |
| Pluronic F108                  | 0,75          | 0,75          |
| Pirofosfato de tetrasodio      | 0,45          | 0,45          |
| Sabor                          | 0,30          | 0,30          |
| Papaína                        | 0,21          | 0,21          |
| Estanato de potasio            | 0,05          | 0,05          |
| Sacarina de sodio              | 0,070         | 0,070         |
| Cloruro de cetilpiridinio      | 0,050         | 0,050         |
| Nipastat                       | 0,050         | 0,050         |
| Fosfato monosódico             | 0,035         | 0,035         |
| Butil hidroxitolueno           | 0,015         | 0,015         |
| Fosfato disódico               | 0,010         | 0,010         |

| TABLA VI                       |               |               |
|--------------------------------|---------------|---------------|
| Ingredientes de la Composición | K (% en peso) | L (% en peso) |
| Ácido fosfórico                | 0,04          | 0,04          |
| Total                          | 100,0         | 100,0         |

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**Estabilidad de la Papaína**

Se monitoreo la actividad de la papaína presente en el enjuague utilizando el Kit PanVera Protease Activity. El kit para la determinación de la actividad cuantifica la actividad de la proteasa utilizando un sustrato de fluoresceína tiocarbamoilo (FTC)-caseína. El sustrato FTC-caseína es atacado por la proteasa y la caseína se descompone en TC-péptidos. La actividad de la proteasa es determinada mediante la medición de la fluorescencia de los péptidos FTC generados. La siguiente Tabla VII compara el porcentaje de actividad y de fluorescencia de las Composiciones K y L y de un placebo identificado como “Composición N” que es idéntico a la Composición K, pero que no contiene enzimas. Los valores de fluorescencia están expresados en unidades de fluorescencia relativa o RFU. El porcentaje de actividad se determina a partir de la fluorescencia con respecto al placebo. En el caso de las mediciones de la actividad superiores al 70%, la actividad enzimática se mantuvo después de transcurridas 4 semanas a temperatura ambiente.

| TABLA VII                |                             |                                          |                |
|--------------------------|-----------------------------|------------------------------------------|----------------|
| ACTIVIDAD DE LA PROTEASA |                             |                                          |                |
| Enjuague Oral            | Fluorescencia Inicial (RFU) | Fluorescencia Después de 4 Semanas (RFU) | % de Reducción |
| K                        | 25.983                      | 21.518                                   | 74%            |
| L                        | 26.548                      | 21.297                                   | 72%            |
| N                        | 10.360                      | 11.563                                   | 0              |

**Eficacia de la Remoción de la Placa**

Para evaluar la capacidad de remoción de la placa que tienen las Composiciones de enjuague a base de enzima papaína K y L, se llevó a cabo un estudio del flujo salival celular en el cual la placa creció sobre una superficie modelo; la superficie fue posteriormente tratada con un enjuague y se midió la reducción de la placa. En el estudio, primero se formó una biopelícula de placa sobre prismas de germanio mediante el bombeo continuo de medio de crecimiento bacteriano sobre los prismas durante un período de aproximadamente 18 horas. Luego se trataron los prismas utilizando una composición de enjuague, bombeando el enjuague sobre los prismas durante varios minutos, a los efectos de facilitar la remoción de la placa. Se empleó la siguiente ecuación para calcular un índice de placa utilizando la espectroscopía Infrarroja por Transformada de Fourier con Reflexión Totalmente Atenuada (ATR FTIR) e intensidades de las bandas de absorción a 3.300, 1.650, 1.545 y 1.080  $\text{cm}^{-1}$  del espectro infrarrojo:

$$\text{Índice de Placa} = \text{abs}_{3.300} + \text{abs}_{1.650} + \text{abs}_{1.545} + \text{abs}_{1.080}$$

Donde *abs* es la máxima absorbancia correspondiente a los diversos números de ondas. Los números de ondas seleccionados representan la absorción infrarroja de los componentes salivales y de las bacterias presentes sobre los prismas de germanio transparentes a la luz infrarroja. Los índices de placa de los prismas tratados con enjuague fueron posteriormente comparados con una línea de referencia (prisma lavado únicamente con solución salina) y fueron reportados como porcentaje de reducción de la placa.

Los resultados registrados en la siguiente Tabla IX comparan el desempeño en términos de reducción de la placa de las Composiciones de enjuague oral K y L de la presente invención versus un enjuague comercial identificado como Composición M que contenía 0,05% en peso de cloruro de cetilpiridinio más no enzimas y el placebo, identificado como "Composición N". El porcentaje de reducción de la placa del enjuague con un alto contenido de agua o enjuague L fue 17% mejor que en el caso del enjuague K. Los resultados indican que se observa una mejora mayor en la administración de los componentes activos sobre la superficie de la placa utilizando la fórmula menos viscosa y de alto contenido de agua, aún cuando ambos enjuagues redujeron efectivamente la placa en mayor grado que el enjuague comercial o Composición M al ser comparados con el placebo o Composición N.

Los resultados están registrados en la siguiente Tabla IX.

| TABLA IX                              |                 |                            |
|---------------------------------------|-----------------|----------------------------|
| Composición                           | Índice de Placa | % de Reducción de la Placa |
| K                                     | 2,320           | 20                         |
| L                                     | 1,832           | 37                         |
| M                                     | 2,864           | 1                          |
| N                                     | 2,529           | 13                         |
| Lavada únicamente con solución salina | 2,901           | --                         |

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Inhibición de la Formación de Placa

Se evaluó la capacidad que tiene el enjuague enzimático de inhibir la formación de placa utilizando el estudio de flujo salival celular, estudio éste que fue llevado a cabo de forma similar a la descrita anteriormente en el caso de la remoción de la placa, salvo que los prismas de germanio fueron previamente tratados con el enjuague durante varios minutos y fueron luego sometidos a un post tratamiento de 18 horas con medio de crecimiento bacteriano a los fines de facilitar el crecimiento de una biopelícula de placa sobre la superficie de los mismos. Nuevamente se determinó la cantidad de placa formada (índice de placa) sobre los prismas mediante la medición de la absorción total de amidas por ATR FTIR. El índice de placa de los prismas tratados con enjuague fue comparado con un placebo, la Composición N, y expresado como porcentaje de inhibición de la placa.

La Tabla X compara el porcentaje de inhibición de la placa de los enjuagues K y L con el enjuague comercial M que contenía cloruro de cetilpiridinio más no enzimas y con el placebo N que era idéntico a la Composición K salvo que no contenía enzimas. Los resultados registrados en la Tabla X indican que la Composición K más viscosa y de menor contenido de agua es más efectiva en términos del revestimiento de la superficie libre de placa y que forma una barrera más protectora para la inhibición de la formación de placa que la Composición L.

| TABLA X       |                 |                             |
|---------------|-----------------|-----------------------------|
| Enjuague Oral | Índice de Placa | % de Inhibición de la Placa |
| K             | 0,683           | 35                          |
| L             | 0,801           | 24                          |
| M             | 0,968           | 8                           |
| N             | 1,053           | --                          |

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**Remoción de la Placa Interdental**

Se demostró la tendencia que tiene el enjuague enzimático a remover la placa interdental utilizando un modelo de limpieza in vitro. El modelo involucró el pretratamiento de las dentaduras con el enjuague, el crecimiento de placa sobre la superficie de las mismas y el posterior tratamiento de las dentaduras con el enjuague. En este modelo, las dentaduras removibles fueron primero pulidas con piedra pómez, lavadas con agua y esterilizadas con etanol durante un período de 10 minutos en el recipiente de una máquina de cepillado, a los fines de disponer de una superficie dental limpia. Las dentaduras fueron posteriormente sumergidas en una solución del enjuague e incubadas durante toda la noche a temperatura ambiente. Luego del tratamiento durante toda la noche, las dentaduras fueron lavadas con agua y sumergidas en medio de crecimiento bacteriano durante un período de 6 horas a una temperatura de 37°C. Los modelos fueron final y brevemente lavados con agua y posterior y brevemente lavados con las fórmulas de enjuague bucal. Las dentaduras fueron luego expuestas con solución de exposición durante un período de 30 segundos, lavadas brevemente con agua y luego evaluadas empleando un índice de placa que fue determinado utilizando el método Quigley Hein.

El enjuague con alto contenido de agua o Composición L fue evaluado en el modelo de limpieza in vitro. Los resultados registrados en la Tabla XI indican que, en comparación con el enjuague comercial M que contenía cloruro de cetilpiridinio más no enzimas y el placebo o enjuague N, el enjuague enzimático L redujo significativamente tanto la placa total como la interproximal en un 67%, lo que demuestra que la Composición L no sólo reduce

efectivamente la placa cercana, sino que también sirve para limpiar las regiones interproximales, demostrando tener una acción “tipo hilo dental”.

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| TABLA XI          |                                    |                            |                                        |                                                |
|-------------------|------------------------------------|----------------------------|----------------------------------------|------------------------------------------------|
| PLACA INTERDENTAL |                                    |                            |                                        |                                                |
| Enjuague Oral     | Índice de Placa<br>(Interproximal) | Índice de Placa<br>(Total) | % Total de<br>Reducción de la<br>Placa | % de Reducción<br>de la Placa<br>Interproximal |
| L                 | 1,00                               | 1,50                       | 67                                     | 67                                             |
| M                 | 3,00                               | 4,58                       | --                                     | --                                             |
| N                 | 3,00                               | 4,58                       | --                                     | --                                             |

## **REIVINDICACIONES:**

1. Se reivindica una composición para el cuidado oral con estabilidad mejorada y efectividad contra la placa que incluye un vehículo oralmente aceptable que contiene una combinación de una enzima, cloruro de cetilpiridinio y un agente reductor.
2. La composición de la reivindicación 1, donde la enzima es una enzima proteolítica.
3. La composición de la reivindicación 2, donde la enzima proteolítica es papaína.
4. La composición de la reivindicación 3, donde la papaína está presente en la composición a una concentración de entre aproximadamente 0,1 y alrededor de 5,0% en peso.
5. La composición de la reivindicación 1, donde la enzima es glucoamilasa.
6. La composición de la reivindicación 1, donde la glucoamilasa está presente en la composición oral a una concentración de entre aproximadamente 0,1 y 5,0% en peso.
7. La composición de la reivindicación 2, donde el cloruro de cetilpiridinio está presente en la composición a una concentración de entre aproximadamente 0,005 y alrededor de 2% en peso.
8. La composición de la reivindicación 1, donde el agente reductor es estanato de potasio.
9. La composición de la reivindicación 2, donde el agente reductor es sulfato de amonio.
10. La composición de la reivindicación 1, donde el agente reductor está presente en la composición a una concentración de entre aproximadamente 0,05 y alrededor de 2,0% en peso.
11. La composición de la reivindicación 1, donde la composición es un dentífrico.
12. La composición de la reivindicación 1, donde la composición es un enjuague bucal.
13. La composición de la reivindicación 1, donde la composición es un dentífrico espesado con un polímero de celulosa.

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14. La composición de la reivindicación 13, donde el polímero de celulosa es carboximetilcelulosa.
15. La composición de la reivindicación 12, donde el enjuague bucal tiene una concentración de agua superior a aproximadamente 45% en peso.
16. La composición de la reivindicación 15, donde la concentración de agua oscila entre aproximadamente 50 y alrededor de 85% en peso.
17. Un método para lograr una mejor estabilidad de una composición oral que contiene enzimas, el cual consiste en preparar una composición conformada por un vehículo oralmente aceptable que contiene una combinación de una enzima, cloruro de cetilpiridinio y un agente reductor y luego aplicar la composición a la cavidad bucal.
18. El método de la reivindicación 17, donde la enzima es una enzima proteolítica.
19. El método de la reivindicación 17, donde la enzima proteolítica es papaína.
20. El método de la reivindicación 19, donde la papaína está presente en la composición a una concentración de entre aproximadamente 0,1 y alrededor de 5,0% en peso.
21. El método de la reivindicación 17, donde la enzima es glucoamilasa.
22. El método de la reivindicación 21, donde la glucoamilasa está presente en la composición oral a una concentración de entre 0,1 y 5,0% en peso.
23. El método de la reivindicación 17, donde la enzima es incorporada en la composición en la forma de una dispersión acuosa de papaína y glucoamilasa.
24. El método de la reivindicación 17, donde el agente reductor es estanato de potasio.
25. El método de la reivindicación 17, donde el agente reductor es sulfato de amonio.
26. El método de la reivindicación 17, donde el agente reductor está presente en la composición a una concentración de entre aproximadamente 0,05 y alrededor de 2,0% en peso.
27. El método de la reivindicación 17, donde la composición es un dentífrico espesado

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con un polímero de celulosa.

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28. El método de la reivindicación 17, donde el polímero de celulosa es carboximetilcelulosa.
29. El método de la reivindicación 17, donde el cloruro de cetilpiridinio está presente en la composición a una concentración de entre aproximadamente 0,005 y alrededor de 2% en peso.
30. El método de la reivindicación 17, donde la composición es un enjuague bucal.
31. El método de la reivindicación 30, donde el enjuague bucal tiene una concentración de agua superior a aproximadamente 45% en peso.
32. El método de la reivindicación 31, donde el enjuague bucal tiene una concentración de agua de entre aproximadamente 50 y alrededor de 85% en peso.
33. Un método para la preparación de una composición acuosa para el cuidado oral que contiene un compuesto enzimático estable, el cual consiste en primero disolver la enzima en una solución acuosa de cloruro de cetilpiridinio y un agente reductor o en preparar una premezcla y luego agregar la mencionada premezcla al vehículo de la composición para el cuidado oral.
34. El método de la reivindicación 33, donde la enzima está presente en la premezcla a una concentración de entre aproximadamente 0,1 y alrededor de 10% en peso.
35. El método de la reivindicación 33, donde la enzima es glucoamilasa.
36. El método de la reivindicación 33, donde el agente reductor está presente en la composición para el cuidado oral a una concentración de entre aproximadamente 0,05 y alrededor de 3% en peso.
37. El método de la reivindicación 33, donde la enzima es papaína.

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# TRANSMITTAL LETTER TO THE UNITED STATES RECEIVING OFFICE

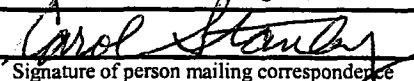
|                               |                  |
|-------------------------------|------------------|
| Date                          | 11 February 2004 |
| International Application No. |                  |
| Attorney Docket No.           | IR 7122-00       |

## I. Certification under 37 CFR 1.10 (if applicable)

|                             |
|-----------------------------|
| EV 360774329 US             |
| Express Mail mailing number |

|                  |
|------------------|
| 11 February 2004 |
| Date of Deposit  |

I hereby certify that the application/correspondence attached hereto is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

|                                                                                   |
|-----------------------------------------------------------------------------------|
|  |
| Signature of person mailing correspondence                                        |

|                                                        |
|--------------------------------------------------------|
| Carol Stanley                                          |
| Typed or printed name of person mailing correspondence |

## II. ☒ New International Application

|       |                                                              |
|-------|--------------------------------------------------------------|
| TITLE | ENZYME CONTAINING ORAL COMPOSITION HAVING ENHANCED STABILITY |
|-------|--------------------------------------------------------------|

|                                            |
|--------------------------------------------|
| Earliest priority date<br>(Day/Month/Year) |
| 11 February 2003                           |

**SCREENING DISCLOSURE INFORMATION:** In order to assist in screening the accompanying international application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied. (Note: check as many boxes as apply):

- A. ☐ The invention disclosed was **not** made in the United States.
- B. ☐ There is no prior U.S. application relating to this invention.
- C. ☒ The following prior U.S. application(s) contain subject matter which is related to the invention disclosed in the attached international application. (NOTE: priority to these applications may or may not be claimed on form PCT/RO/101 (Request) and this listing does not constitute a claim for priority).

|                 |                 |          |                  |
|-----------------|-----------------|----------|------------------|
| application no. | USSN 10/364,241 | filed on | 11 February 2003 |
| application no. |                 | filed on |                  |

- D. ☐ The present international application ☐ is identical ☐ contains less subject matter than that found in the prior U.S. application(s) identified in paragraph C.
- E. ☐ The present international application ☐ contains additional subject matter not found in the prior U.S. application(s) identified in paragraph C. above. The additional subject matter is found on pages  and ☐ DOES NOT ALTER ☐ MIGHT BE CONSIDERED TO ALTER the general nature of the invention in a manner which would require the U.S. application to have been made available for inspection by the appropriate defense agencies under 35 U.S.C. 181 and 37 CFR 5.1. See 37 CFR 5.15

## III. ☐ A Response to an Invitation from the RO/US. The following document(s) is (are) enclosed:

- A. ☐ A Request for An Extension of Time to File a Response
- B. ☐ A Power of Attorney (General or Regular)
- C. ☐ Replacement pages:

|       |  |                             |       |  |                 |
|-------|--|-----------------------------|-------|--|-----------------|
| pages |  | of the request (PCT/RO/101) | pages |  | of the figures  |
| pages |  | of the description          | pages |  | of the abstract |
| pages |  | of the claims               |       |  |                 |

- D. ☐ Submission of Priority Documents

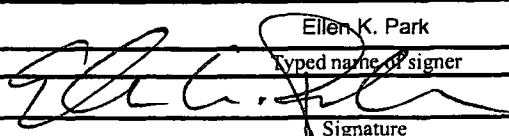
|                   |  |                   |  |
|-------------------|--|-------------------|--|
| Priority document |  | Priority document |  |
|-------------------|--|-------------------|--|

- E. ☐ Fees as specified on attached Fee Calculation sheet form PCT/RO/101 annex

## IV. ☐ A Request for Rectification under PCT 91 ☐ A Petition ☐ A Sequence Listing Diskette

## V. ☐ Other (please specify):

The person signing this form is the:

|                                                                         |                                                                                      |
|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <input type="checkbox"/> Applicant                                      |                                                                                      |
| <input checked="" type="checkbox"/> Attorney/Agent (Reg. No.)<br>34,055 |  |
| <input type="checkbox"/> Common Representative                          |                                                                                      |

Ellen K. Park  
Typed name of signer  
Signature

EV360774329US

65 ~~66~~

## PCT REQUEST

Original (for SUBMISSION)

|                |                                                                                                                                                |                                                                                 |
|----------------|------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| <b>0</b>       | <b>For receiving Office use only</b>                                                                                                           |                                                                                 |
| <b>0-1</b>     | International Application No.                                                                                                                  |                                                                                 |
| <b>0-2</b>     | International Filing Date                                                                                                                      |                                                                                 |
| <b>0-3</b>     | Name of receiving Office and "PCT International Application"                                                                                   |                                                                                 |
| <b>0-4</b>     | <b>Form - PCT/RO/101 PCT Request</b>                                                                                                           |                                                                                 |
| <b>0-4-1</b>   | Prepared Using                                                                                                                                 | PCT-SAFE [EASY mode]<br>Version 3.50 (Build 0002.153)                           |
| <b>0-5</b>     | <b>Petition</b><br>The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty |                                                                                 |
| <b>0-6</b>     | <b>Receiving Office (specified by the applicant)</b>                                                                                           | United States Patent and Trademark Office (USPTO) (RO/US)                       |
| <b>0-7</b>     | <b>Applicant's or agent's file reference</b>                                                                                                   | 7122-00                                                                         |
| <b>I</b>       | <b>Title of Invention</b>                                                                                                                      | ENZYME CONTAINING ORAL COMPOSITION<br>HAVING ENHANCED STABILITY                 |
| <b>II</b>      | <b>Applicant</b>                                                                                                                               |                                                                                 |
| <b>II-1</b>    | This person is:                                                                                                                                | applicant only                                                                  |
| <b>II-2</b>    | Applicant for                                                                                                                                  | all designated States except US                                                 |
| <b>II-4</b>    | Name:                                                                                                                                          | COLGATE-PALMOLIVE COMPANY                                                       |
| <b>II-5</b>    | Address:                                                                                                                                       | 300 Park Avenue<br>New York, New York 10022<br>United States of America         |
| <b>II-6</b>    | State of nationality                                                                                                                           | US                                                                              |
| <b>II-7</b>    | State of residence                                                                                                                             | US                                                                              |
| <b>II-8</b>    | Telephone No.                                                                                                                                  | 732-878-7157                                                                    |
| <b>II-9</b>    | Facsimile No.                                                                                                                                  | 732-878-7853                                                                    |
| <b>II-10</b>   | e-mail                                                                                                                                         | ellen_park@colpal.com                                                           |
| <b>III-1</b>   | <b>Applicant and/or inventor</b>                                                                                                               |                                                                                 |
| <b>III-1-1</b> | This person is:                                                                                                                                | applicant and inventor                                                          |
| <b>III-1-2</b> | Applicant for                                                                                                                                  | US only                                                                         |
| <b>III-1-4</b> | Name (LAST, First)                                                                                                                             | MORGAN, Andre, M.                                                               |
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|--------------|----------------------------------|----------------------------------------------------------------------------------|
| <b>III-2</b> | <b>Applicant and/or inventor</b> |                                                                                  |
| III-2-1      | This person is:                  | applicant and inventor                                                           |
| III-2-2      | Applicant for                    | US only                                                                          |
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| III-2-6      | State of nationality             | US                                                                               |
| III-2-7      | State of residence               | US                                                                               |
| <b>III-3</b> | <b>Applicant and/or inventor</b> |                                                                                  |
| III-3-1      | This person is:                  | applicant and inventor                                                           |
| III-3-2      | Applicant for                    | US only                                                                          |
| III-3-4      | Name (LAST, First)               | WILLIAMS, Malcolm                                                                |
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| III-3-6      | State of nationality             | US                                                                               |
| III-3-7      | State of residence               | US                                                                               |
| <b>III-4</b> | <b>Applicant and/or inventor</b> |                                                                                  |
| III-4-1      | This person is:                  | applicant and inventor                                                           |
| III-4-2      | Applicant for                    | US only                                                                          |
| III-4-4      | Name (LAST, First)               | HERLES, Susan, M.                                                                |
| III-4-5      | Address:                         | 139 Old Clinton Road<br>Flemington, New Jersey 08822<br>United States of America |
| III-4-6      | State of nationality             | US                                                                               |
| III-4-7      | State of residence               | US                                                                               |
| <b>III-5</b> | <b>Applicant and/or inventor</b> |                                                                                  |
| III-5-1      | This person is:                  | applicant and inventor                                                           |
| III-5-2      | Applicant for                    | US only                                                                          |
| III-5-4      | Name (LAST, First)               | MACIAS, Chanda, L.                                                               |
| III-5-5      | Address:                         | 59 Victoria Drive<br>Somerset, New Jersey 08873<br>United States of America      |
| III-5-6      | State of nationality             | US                                                                               |
| III-5-7      | State of residence               | US                                                                               |

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|         |                                  |                                                                                   |
|---------|----------------------------------|-----------------------------------------------------------------------------------|
| III-6   | <b>Applicant and/or inventor</b> |                                                                                   |
| III-6-1 | This person is:                  | applicant and inventor                                                            |
| III-6-2 | Applicant for                    | US only                                                                           |
| III-6-4 | Name (LAST, First)               | D'AMBROGIO, Robert                                                                |
| III-6-5 | Address:                         | 615 Betsy Ross Place<br>Bound Brook, New Jersey 08805<br>United States of America |
| III-6-6 | State of nationality             | US                                                                                |
| III-6-7 | State of residence               | US                                                                                |
| III-7   | <b>Applicant and/or inventor</b> |                                                                                   |
| III-7-1 | This person is:                  | applicant and inventor                                                            |
| III-7-2 | Applicant for                    | US only                                                                           |
| III-7-4 | Name (LAST, First)               | BOYD, Thomas, J.                                                                  |
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| III-7-6 | State of nationality             | US                                                                                |
| III-7-7 | State of residence               | US                                                                                |
| III-8   | <b>Applicant and/or inventor</b> |                                                                                   |
| III-8-1 | This person is:                  | applicant and inventor                                                            |
| III-8-2 | Applicant for                    | US only                                                                           |
| III-8-4 | Name (LAST, First)               | MASTERS, James, G.                                                                |
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| III-8-6 | State of nationality             | US                                                                                |
| III-8-7 | State of residence               | US                                                                                |

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|               |                                                                                                                                                                                                                                                                                               |                                                                                                                                     |
|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| <b>IV-1</b>   | <b>Agent or common representative; or address for correspondence</b><br>The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:                                                                     | <b>agent</b>                                                                                                                        |
| <b>IV-1-1</b> | <b>Name (LAST, First)</b>                                                                                                                                                                                                                                                                     | <b>PARK, Ellen, K.</b>                                                                                                              |
| <b>IV-1-2</b> | <b>Address:</b>                                                                                                                                                                                                                                                                               | <b>Colgate-Palmolive Company<br/>909 River Road<br/>P.O. Box 1343<br/>Piscataway, New Jersey 08855<br/>United States of America</b> |
| <b>IV-1-3</b> | <b>Telephone No.</b>                                                                                                                                                                                                                                                                          | <b>732-878-7157</b>                                                                                                                 |
| <b>IV-1-4</b> | <b>Facsimile No.</b>                                                                                                                                                                                                                                                                          | <b>732-878-7853</b>                                                                                                                 |
| <b>IV-1-5</b> | <b>e-mail</b>                                                                                                                                                                                                                                                                                 | <b>ellen_park@colpal.com</b>                                                                                                        |
| <b>IV-1-6</b> | <b>Agent's registration No.</b>                                                                                                                                                                                                                                                               | <b>34055</b>                                                                                                                        |
| <b>V</b>      | <b>DESIGNATIONS</b>                                                                                                                                                                                                                                                                           |                                                                                                                                     |
| <b>V-1</b>    | <b>The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.</b> |                                                                                                                                     |
| <b>VI-1</b>   | <b>Priority claim of earlier national application</b>                                                                                                                                                                                                                                         |                                                                                                                                     |
| <b>VI-1-1</b> | <b>Filing date</b>                                                                                                                                                                                                                                                                            | <b>11 February 2003 (11.02.2003)</b>                                                                                                |
| <b>VI-1-2</b> | <b>Number</b>                                                                                                                                                                                                                                                                                 | <b>10/364,241</b>                                                                                                                   |
| <b>VI-1-3</b> | <b>Country</b>                                                                                                                                                                                                                                                                                | <b>US</b>                                                                                                                           |
| <b>VI-2</b>   | <b>Priority document request</b><br>The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):                                                                                         | <b>VI-1</b>                                                                                                                         |
| <b>VII-1</b>  | <b>International Searching Authority Chosen</b>                                                                                                                                                                                                                                               | <b>European Patent Office (EPO) (ISA/EP)</b>                                                                                        |

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| <b>VIII</b> | <b>Declarations</b>                                                                                                                  | <b>Number of declarations</b> |  |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|--|
| VIII-1      | Declaration as to the identity of the inventor                                                                                       | -                             |  |
| VIII-2      | Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent             | 1                             |  |
| VIII-3      | Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application | 1                             |  |
| VIII-4      | Declaration of inventorship (only for the purposes of the designation of the United States of America)                               | -                             |  |
| VIII-5      | Declaration as to non-prejudicial disclosures or exceptions to lack of novelty                                                       | -                             |  |

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|              |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                           |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| VIII-2-1     | <b>Declaration: Entitlement to apply for and be granted a patent</b><br>Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51bis.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate:<br>Name (LAST, First) | <b>in relation to this international application</b><br><br><b>COLGATE-PALMOLIVE COMPANY is entitled to apply for and be granted a patent by virtue of the following:</b> |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from MORGAN Andre, M. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                     |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from SZELES Lori, H. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                      |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from WILLIAMS Malcolm to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                     |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from HERLES Susan, M. to COLGATE-PALMOLIVE COMPANY, dated 10 February 2003 (10.02.2003)                                                                     |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from MACIAS Chanda, L. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                    |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from D'AMBROGIO Robert to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                    |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from BOYD Thomas, J. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                      |
| VIII-2-1(iv) |                                                                                                                                                                                                                                                                                                                                     | an assignment from MASTERS James, G. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                    |
| VIII-2-1(ix) | This declaration is made for the purposes of:                                                                                                                                                                                                                                                                                       | all designations                                                                                                                                                          |

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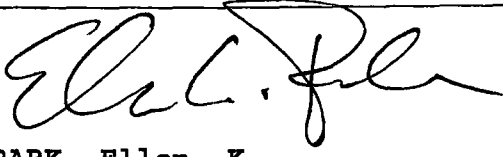
Original (for SUBMISSION)

|              |                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                   |
|--------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| VIII-3-1     | <b>Declaration: Entitlement to claim priority</b><br>Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application specified below, where the applicant is not the applicant who filed the earlier application or where the applicant's name has changed since the filing of the earlier application (Rules 4.17(iii) and 51bis.1(a)(iii))<br>Name: | in relation to this international application<br><br>COLGATE-PALMOLIVE COMPANY<br>is entitled to claim priority of earlier application No. 10/364,241 by virtue of the following: |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from MORGAN Andre, M. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                             |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from SZELES Lori, H. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                              |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from WILLIAMS Malcolm to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                             |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from HERLES Susan, M. to COLGATE-PALMOLIVE COMPANY, dated 10 February 2003 (10.02.2003)                                                                             |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from MACIAS Chanda, L. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                            |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from D'AMBROGIO Robert to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                            |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from BOYD Thomas, J. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                              |
| VIII-3-1(iv) |                                                                                                                                                                                                                                                                                                                                                                                                                            | an assignment from MASTERS James, G. to COLGATE-PALMOLIVE COMPANY, dated 11 February 2003 (11.02.2003)                                                                            |
| VIII-3-1(ix) | This declaration is made for the purposes of:                                                                                                                                                                                                                                                                                                                                                                              | all designations                                                                                                                                                                  |

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| IX    | Check list                                                 | number of sheets                                                                   | electronic file(s) attached        |
|-------|------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------|
| IX-1  | Request (including declaration sheets)                     | 8                                                                                  | -                                  |
| IX-2  | Description                                                | 21                                                                                 | -                                  |
| IX-3  | Claims                                                     | 3                                                                                  | -                                  |
| IX-4  | Abstract                                                   | 1                                                                                  | ✓                                  |
| IX-5  | Drawings                                                   | 0                                                                                  | -                                  |
| IX-7  | TOTAL                                                      | 33                                                                                 |                                    |
|       | <b>Accompanying Items</b>                                  | <b>paper document(s) attached</b>                                                  | <b>electronic file(s) attached</b> |
| IX-8  | Fee calculation sheet                                      | ✓                                                                                  | -                                  |
| IX-17 | PCT-SAFE physical media                                    | -                                                                                  | ✓                                  |
| IX-19 | Figure of the drawings which should accompany the abstract |                                                                                    |                                    |
| IX-20 | Language of filing of the international application        | English                                                                            |                                    |
| X-1   | Signature of applicant, agent or common representative     |  |                                    |
| X-1-1 | Name:                                                      | PARK, Ellen, K.                                                                    |                                    |
| X-1-2 | Name of signatory                                          |                                                                                    |                                    |
| X-1-3 | Capacity                                                   |                                                                                    |                                    |

## FOR RECEIVING OFFICE USE ONLY

|        |                                                                                                                                         |        |
|--------|-----------------------------------------------------------------------------------------------------------------------------------------|--------|
| 10-1   | Date of actual receipt of the purported international application                                                                       |        |
| 10-2   | Drawings:                                                                                                                               |        |
| 10-2-1 | Received                                                                                                                                |        |
| 10-2-2 | Not received                                                                                                                            |        |
| 10-3   | Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application |        |
| 10-4   | Date of timely receipt of the required corrections under PCT Article 11(2)                                                              |        |
| 10-5   | International Searching Authority                                                                                                       | ISA/EP |
| 10-6   | Transmittal of search copy delayed until search fee is paid                                                                             |        |

## FOR INTERNATIONAL BUREAU USE ONLY

|      |                                                                |  |
|------|----------------------------------------------------------------|--|
| 11-1 | Date of receipt of the record copy by the International Bureau |  |
|------|----------------------------------------------------------------|--|

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**ENZYME CONTAINING ORAL COMPOSITION HAVING ENHANCED STABILITY****BACKGROUND OF THE INVENTION**5 1. Field of the Invention

This invention relates generally to oral compositions for enhancing oral hygiene, and more particularly, to enzyme containing oral compositions having enhanced stability and antiplaque effectiveness.

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2. The Prior Art

Oral compositions such as toothpastes, gels and mouth washes are designed to loosen and remove plaque in conjunction with a regular toothbrushing regimen. Dental plaque is present to some degree, in the form of a film, on virtually all dental surfaces. It is a byproduct of microbial growth, and comprises a dense microbial layer consisting of a mass of microorganisms embedded in a polysaccharide matrix. Plaque itself adheres firmly to dental surfaces and is removed only with difficulty even through a rigorous brushing regimen. Moreover, plaque rapidly reforms on the tooth surface after it is removed. Plaque may form on any part of the tooth surface, and is found particularly at the gingival margin, in cracks in the enamel, and on the surface of dental calculus. The danger associated with the formation of plaque on the teeth lies in the tendency of plaque to build up and eventually produce gingivitis, periodontitis and other types of periodontal disease, as well as dental caries and dental calculus.

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It is known to the art to incorporate antimicrobial agents in oral compositions wherein these agents destroy or inhibit oral bacteria. Other agents are also incorporated in the oral composition to enhance the efficacy of the antimicrobial agents. For example, it is known to incorporate enzymes such as proteases in oral compositions, which enzymes disrupt or interfere with plaque formation and bacterial adhesion to tooth surfaces.

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A problem encountered with commercially processed enzymes such as proteases is that they often contain a broad spectrum of undesirable by-products or impurities that are difficult to remove during manufacture. One such enzyme by-product is cellulase, an enzyme that catabolizes cellulose to simple sugars by hydrolysis of  $\beta$  (1-4) linkages.

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Thickening agents conventionally used in oral compositions such as carboxymethyl cellulose, undergo degradation in the presence of cellulase enzymes which detrimentally affects the rheology of the dentifrice product. Thus, a means to inhibit the degradation of these thickeners by cellulase is a critical to obtaining stable enzyme containing oral care formulations.

Typical methods employed by the art for cellulase inhibition or isolation are not practical for use in the oral composition field. Treatments for cellulase inhibition such as salting out, heat treatment, or pH adjustment also compromise the activity of the enzymes.

Classical methods of enzyme separation based on enzyme size, charge, solubility, and binding site are cost prohibitive and ineffective due to the similarities between certain cellulases and enzymes such as amylases. Inhibition of cellulase activity by treatment with heavy metals or heavy metal complexes such as mercury, silver and palladium chloride that bind to the enzyme active site are also unacceptable as these materials are toxic to humans and certainly cannot be used in an oral care product.

#### SUMMARY OF THE INVENTION

In accordance with the present invention it has been unexpectedly determined that a small but effective amount of cetyl pyridinium chloride and a reducing agent can provide enhanced stability and antiplaque efficacy in enzyme containing oral compositions.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

##### 25 Cetyl Pyridinium Chloride

Cetyl pyridinium chloride is incorporated in containing oral care compositions of the present invention at a concentration of about 0.005 to about 1.0% by weight and preferably about 0.50 to about 1.0% by weight of the oral care composition.

##### 30 Enzymes

The enzymes useful in the practice of the present invention include carbohydrases such as glucoamylase and enzymes extracted from natural fruit products such as proteases which breakdown or hydrolyze proteins.

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Glucoamylase is a saccharifying glucoamylase of *Aspergillus niger* origin cultivated by fermentation. This enzyme can hydrolyze both the alpha-D-1,6 glucosidic branch points and the alpha-1,4 glucosidic bonds of glucosyl oligosaccharides. Additional carbohydrases useful in accordance with this invention are alpha and beta-amylase, dextranase and mutanase. Glucoamylase is a preferred enzyme and is incorporated in the oral composition of the present invention at a concentration of about 0.001 to 2% by weight and preferably about 0.01 to 0.55% by weight.

Protease enzymes useful in the practice of the present invention include those extracted from natural fruit products. The proteolytic enzymes are obtained from natural sources or by the action of microorganisms having a nitrogen source and a carbon source. Examples of proteolytic enzymes useful in the practice of the present invention include the naturally occurring enzymes papain (from papaya), bromelain (from pineapple), as well as serine proteases such as chymotrypsin. Additional enzymes include ficin and alcalase. Papain is a protease enzyme preferred for use in the practice of the present invention, the papain having an activity of 150 to 939 MCU per milligram as determined by the Milk Clot Assay Test of the Biddle Sawyer Group (see J. Biol. Chem., vol. 121, pages 737-745). The protease enzymes are included in the compositions of the present invention at a concentration of about 0.1 to about 3% by weight and preferably about 0.2 to about 2% by weight.

Enzymes which may beneficially be used in combination with the proteolytic enzymes and glucoamylase enzymes include carbohydrases such as, alpha-amylase, beta-amylase, , tannase and lipases such as plant lipase, gastric lipase and pancreatic lipase.

The lipase enzyme is derived from a select strain of *Aspergillus niger*, exhibiting random cleaving of the 1,3 positions of fats and oils. The enzyme has maximum lipolytic activity at pH 5.0 to 7.0 when assayed with olive oil. The enzyme has a measured activity of 120,000 lipase units per gram. The lipase may be included in the dentifrice composition at a concentration of about 0.010 to about 5.0% by weight and preferably about 0.02 to about 0.10 % by weight.

The presence of tannase enzyme can be further beneficial in facilitating the breakdown of extrinsic stain. Tannase enzymes have been purified from *Aspergillus niger* and

*Aspergillus allianceus* and are useful in the hydrolysis of tannins, known to discolor the tooth surface.

Other suitable enzymes which can comprise the present invention include lysozyme, derived from egg white, which contains a single polypeptide chain crosslinked by four disulfide bonds having a molecular weight of 14,600 daltons. The enzyme can exhibit antibacterial properties by facilitating the hydrolysis of bacterial cell walls cleaving the glycosidic bond between carbon number 1 of N-acetylmuramic acid and carbon number 4 of N-acetyl-D-glucosamine, which in vivo, these two carbohydrates are polymerized to form the cell wall polysaccharide. Additionally, pectinase, an enzyme that is present in most plants facilitates the hydrolysis of the polysaccharide pectin into sugars and galacturonic acid. Finally, glucanase, which may be utilized to catalyze the breakdown of complex carbohydrates to glucans and the hydrolysis of beta glucan to glucose.

#### 15 Enzyme Stabilizing Agents

Enzyme stabilizing agents which protect the enzyme from inactivation by chelating metal impurities present in the oral composition include ethylene diamine tetraacetic acid (EDTA) and sodium gluconate at concentrations between 0.01 and 1% by weight, preferably between 0.1 and 0.5% by weight. Agents stabilizing the enzyme against oxidation include reducing agents such as sodium bisulfite, metal gallates, potassium stannate, sodium stannate, ammonium sulfate, 3,5,-di-tert-butyl-4-hydroxytoluene (BHT), Vitamin E ( $\alpha$ ,  $\beta$ ,  $\gamma$ , forms)/Vitamin E acetate and ascorbic acid. Potassium stannate is an enzyme stabilizing agent preferred for use in the practice of the present invention. The reducing agent is present in the oral composition of the present invention at a concentration between about 0.05 to about 1.5% by weight, preferably between about 0.1 and about 0.75% by weight.

#### Premix

When incorporating cetyl pyridinium chloride in the oral care composition of the present invention it is advantageous that the cetyl pyridinium chloride and any enzyme to be added to the oral composition be first premixed in an aqueous humectant solution so that the cetyl pyridinium chloride is interacted with and binds to and inactivates any impurity present in the enzyme added to the oral composition.

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The premix product will normally be comprised of four components which are (1) cetyl pyridinium chloride (2) one or more enzymes to be incorporated in the oral composition (3) a reducing agent, (4) the balance, water and a humectant.

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Generally the concentration of cetyl pyridinium chloride present in the premix will range from about 0.15 to about 1.0% by weight of the premix and preferably about 0.2 to about 0.6% by weight. The enzyme from about 0.5 to about 10.0% by weight of the premix and preferably about 0.75 to about 8.0% by weight. When a combination of enzymes such as glucoamylase and papain is included in the premix, the concentration of papain will range from about 2 to about 10% by weight and the concentration of glucoamylase will range from about 0.5 to about 2% by weight.

A reducing agent such as potassium stannate is also preferably included in the premix solution at a concentration of about 0.5 to about 40% by weight, and preferably about 1.0 to about 3% by weight, the presence of the reducing agent serving to further enhance the cetyl pyridinium chloride inhibition of enzyme impurity activity when subsequently incorporated in the oral composition vehicle.

Water and humectant are further components of the premix. Concentrations will range from about 85 to 95% by weight.

To prepare the premix, a reactor is charged first with a mixture of water and humectant followed by the addition of an effective amount of cetyl pyridinium chloride which is mixed and dissolved therein and thereafter the enzymes and reducing agent are added to the solution. These ingredients are mixed together at a temperature no higher than about 40°C and preferably about 30°C. The pH is adjusted to between about 1.5 and about 8.0 and preferably from about 6.0 to about 7.5. The so prepared premix is then sealed in suitable containers waiting for blending with the vehicle and other ingredients of the oral care composition.

### Oral Care Composition

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In the preparation of an oral composition, the premix is mixed with the ingredients of the oral care composition vehicle to prepare a product in which the enzymes remain effectively stabilized and which will exhibit antiplaque and antigingivitis activity.

5 **Dentifrice Vehicle**

Orally-acceptable vehicles used to prepare dentifrice compositions of the present invention include a water-phase, containing a humectant therein. The humectant is preferably glycerin, sorbitol, xylitol, and/or propylene glycol of molecular weight in the range of 200 to 1,000; but, other humectants and mixtures thereof may also be employed. The  
10 humectant concentration typically totals about 5 to about 70% by weight of the oral composition and preferably about 30 to about 60% by weight. Water present in the dentifrice will range from about 10 to about 30% by weight and preferably about 15 to about 25% by weight.

15 **Abrasives**

In the preparation of a dentifrice composition, abrasives which may be used in the practice of the present invention include silica abrasives such as precipitated silicas having a mean particle size of up to about 20 microns, such as Zeodent 115, marketed by J.M. Huber. Other useful dentifrice abrasives include sodium metaphosphate, potassium  
20 metaphosphate, tricalcium phosphate, dihydrated dicalcium phosphate, aluminum silicate, calcined alumina, bentonite or other siliceous materials, or combinations thereof.

Preferred abrasive materials useful in the practice of the preparation of dentifrice compositions in accordance with the present invention include silica gels and precipitated  
25 amorphous silica having an oil absorption value of less than 100cc/100g silica and preferably in the range of from about 45cc/100g to less than about 70cc/100g silica. Oil absorption values are measured using the ASTA Rub-Out Method D281. These silicas are colloidal particles having an average particle size ranging from about 3 microns to about 12 microns, and more preferably between about 5 to about 10 microns and a pH range from  
30 4 to 10 preferably 6 to 9 when measured as a 5% by weight slurry.

Low oil absorption silica abrasives particularly useful in the practice of the present invention are marketed under the trade designation Sylodent XWA by Davison Chemical Division of W.R. Grace & Co., Baltimore, MD 21203. Sylodent 650 XWA, a silica

hydrogel composed of particles of colloidal silica having a water content of 29% by weight averaging from about 7 to about 10 microns in diameter, and an oil absorption of less than 70cc/100g of silica is a preferred example of a low oil absorption silica abrasive useful in the practice of the present invention. The abrasive is present in the dentifrice composition of the present invention at a concentration of about 10 to about 40% by weight and preferably about 15 to about 30% by weight.

**Thickening Agents**

Thickeners used in the dentifrice compositions of the present invention include natural and synthetic gums and colloids include cellulose thickeners such as carboxymethyl cellulose, hydroxyalkyl celluloses such as hydroxypropyl cellulose hydroxyethyl cellulose, gums such as xanthan gum, polyglycols of varying molecular weights sold under the tradename Polyox and polyethylene glycol. Inorganic thickeners which may be used in the practice of the present invention include amorphous silica compounds such as colloidal silicas compounds available under the trade designation Cab-o-sil manufactured by Cabot Corporation and distributed by Lenape Chemical, Bound Brook, New Jersey; Zeodent 165 from J.M. Huber Chemicals Division, Havre de Grace, Maryland 21078; and Sylodent 15, available from Davison Chemical Division of W.R. Grace Corporation, Baltimore, MD 21203. Other inorganic thickeners include natural and synthetic clays, lithium magnesium silicate (Laponite) and magnesium aluminum silicate (Veegum).

The thickening agent is present in the dentifrice composition in amounts of about 0.1 to about 10% by weight, preferably about 0.5 to about 4.0% by weight.

**Surfactants**

Surfactants are used in the oral compositions of the present invention to achieve increased prophylactic action and render the compositions more cosmetically acceptable. The surfactant is preferably a deterative material which imparts to the composition deterative and foaming properties.

Anionic surfactants such as higher alkyl sulfates such as sodium lauryl sulfate are effective surfactants in oral compositions but are normally not compatible with enzymes and promote denaturation of the enzyme and loss in activity. However, in accordance with the practice of the present invention, premixing concentrated amounts of enzymes such as

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glucoamylase with cetyl pyridinium chloride, allows the cetyl pyridinium chloride to interact and bind to any cellulase impurity present in the glucoamylase thereby preventing interaction of the cellulase with any cellulose based thickener that may be used in preparing the dentifrice as well as inhibiting interaction with the anionic surfactant.

- 5 Surfactants that are compatible with enzymes may also be used to provide the requisite foaming characteristics in the oral composition. Examples of enzyme compatible surfactants include nonionic polyoxyethylene surfactants such as Polyoxamer 407, Pluronic 127, Polysorbate 20, and amphoteric surfactants such as tauranol, cocamidopropyl betaine (tegobetaine) and cocamidopropyl betaine lauryl glucoside. Surfactants are
- 10 included in the oral composition of the present invention at a concentration of about 2 to about 10% by weight and preferably between about 0.5 to about 5.0% by weight.

#### Fluoride and Other Active Agents

- The oral composition of the present invention may also contain a source of fluoride ions or
- 15 fluorine-providing ingredient, as anticaries agent in amounts sufficient to supply about 25 ppm to 5,000 ppm of fluoride ions and include inorganic fluoride salts, such as soluble alkali metal salts. Preferred fluoride sources which are compatible with enzymes include sodium fluoride, potassium fluoride, sodium fluorosilicate, sodium monfluorophosphate (MFP), ammonium fluorosilicate, as well as tin fluorides, such as stannous fluoride and
- 20 stannous chloride. Sodium fluoride or MFP is preferred.

- In addition to fluoride compounds, there may also be included in the oral compositions of the present inventions antitartar agents such as pyrophosphate salts including dialkali or tetraalkali metal pyrophosphate salts such as  $\text{Na}_4\text{P}_2\text{O}_7$ ,  $\text{K}_4\text{P}_2\text{O}_7$ ,  $\text{Na}_2\text{K}_2\text{P}_2\text{O}_7$ ,
- 25  $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$  and  $\text{K}_2\text{H}_2\text{P}_2\text{O}_7$ , polyphosphates such as sodium tripolyphosphate, sodium hexametaphosphate and cyclic phosphates such as sodium tripolyphosphate sodium trimetaphosphate. These antitartar agents are included in the dentifrice composition at a concentration of about 1.0 to about 5.0% by weight.

- 30 Another active agent useful in dentifrice compositions of the present invention are antibacterial agents, which can be from 0.2 to 1.0% by weight of the oral composition. Such useful antibacterial agents include non-cationic antibacterial agents which are based on phenolic or bisphenolic compounds, such as halogenated diphenyl ethers such as Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether).

### Anionic Polycarboxylate

Synthetic anionic polycarboxylates may also be used in the oral compositions of the present invention as an efficacy enhancing agent for any antibacterial, antitartar or other active agent within the dentifrice composition. Such anionic polycarboxylates are generally employed in the form of their free acids or preferably partially or more preferably fully neutralized water soluble alkali metal (e.g. potassium and preferably sodium) or ammonium salts. Preferred are 1:4 to 4:1 copolymers of maleic anhydride or acid with another polymerizable ethylenically unsaturated monomer, preferably methylvinylether/maleic anhydride having a molecular weight (M.W.) of about 30,000 to about 1,800,000 most preferably about 30,000 to about 700,000. Examples of these copolymers are available from GAF Corporation under the tradename Gantrez, e.g. AN 139 (M.W. 500,000), AN 119 (M.W. 250,000); S-97 Pharmaceutical Grade (M.W. 700,000), AN 169 (M.W. 1,200,000-1,800,000), and AN 179 (M.W. above 1,800,000); wherein the preferred copolymer is S-97 Pharmaceutical Grade (M.W. 700,000).

When present, the anionic polycarboxylates is employed in amounts effective to achieve the desired enhancement of the efficacy of any antibacterial, antitartar or other active agent within the oral composition. Generally, the anionic polycarboxylates is present within the oral composition from about 0.05% to about 4% by weight, preferably from about 0.5% to about 2.5% by weight.

### Flavor

The oral composition of the present invention may also contain a flavoring agent. Flavoring agents which are used in the practice of the present invention include essential oils as well as various flavoring aldehydes, esters, alcohols, and similar materials. Examples of the essential oils include oils of spearmint, peppermint, wintergreen, sassafras, clove, sage, eucalyptus, marjoram, cinnamon, lemon, lime, grapefruit, and orange. Also useful are such chemicals as menthol, carvone, and anethole. Of these, the most commonly employed are the oils of peppermint and spearmint.

The flavoring agent is incorporated in the oral composition at a concentration of about 0.1 to about 5% by weight and preferably about 0.5 to about 1.5% by weight.

**Other Ingredients**

Various other materials may be incorporated in the oral compositions of this invention, including desensitizers, such as potassium nitrate; whitening agents, such as hydrogen peroxide, calcium peroxide and urea peroxide; preservatives; silicones; and chlorophyll compounds. These additives, when present, are incorporated in the oral compositions of the present invention in amounts which do not substantially adversely affect the properties and characteristics desired.

**Preparation of Dentifrice Compositions**

To prepare a dentifrice composition of the present invention, generally the humectants such as glycerin, sorbitol are dispersed in the water in a conventional mixer under agitation. Into the dispersion are added organic thickeners, such as carboxymethyl cellulose; antitartar agents such as tetrasodium pyrophosphate, sodium tripolyphosphate and any sweeteners. The resultant mixture is agitated until a homogeneous gel phase is formed. Into the gel phase are added a pigment such as  $\text{TiO}_2$ , and any acid or base required to adjust the pH in the range of 6.4 to 7.3. These ingredients are mixed until a homogenous phase is obtained. Thereafter a premix of cetyl pyridinium chloride, enzyme and a reducing agent such as potassium stannate in an aqueous humectant solution is added and admixed with the homogeneous gel phase. The resultant mixture is then transferred to a high speed/vacuum mixer; wherein, the thickener, and surfactant ingredients are added to the mixture. Thereafter the abrasive is added. Any water insoluble antibacterial agent, such as Triclosan, is solubilized in the flavor oils to be included in the composition and the solution is added along with the surfactants to the mixture, which is then mixed at high speed for from 5 to 30 minutes, under vacuum of from about 20 to 50 mm of Hg, preferably about 30 mm Hg. The resultant product is in each case a homogeneous, semi-solid, extrudable paste or gel product.

### Preparation of Liquid Oral Compositions

In the aspect of the present invention wherein the oral composition is substantially liquid in character such as a mouthwash or rinse, the vehicle is typically a water, humectant, alcohol mixture. The alcohol is a non-toxic alcohol such as ethanol or isopropanol. A humectant such as glycerine, sorbitol or an alkylene glycol such as polyethylene glycol or propylene glycol may be present in an amount of about 10 to 30% by weight, the oral rinse containing greater than about 45% by weight water and preferably about 50 to 85% by weight water, about 0 to 20% by weight of a non-toxic alcohol and about 10 to 40% by weight of the humectant. A thickener such as a Pluronic may be present at a concentration of about 1.0 to about 3.0% by weight, cetyl pyridinium chloride at a concentration of about 0.02 to about 1.0% by weight, a reducing agent such potassium stannate or ammonium sulfate at a concentration of about 0.05 to 1.0% by weight, an enzyme at a concentration of about 0.02 to about 0.2 % by weight and a flavor ingredient at a concentration of about 0.3 to about 1.0 % by weight.

In the preparation of a oral rinse, an enzyme premix comprised of cetyl pyridinium chloride, reducing agent, water, humectant and enzyme is dispersed in a mixture of mouthwash ingredients, for example, alcohol, humectants, surfactants, and flavor are then added and mixed. The ingredients are then mixed under vacuum for about 15-30 minutes. The resulting oral rinse product is then packaged.

A rinse is an advantageous vehicle for delivering actives to the oral cavity, due to its ability to get into hard-to-reach areas of the mouth, such as the interproximal regions and the crevices of the tongue. The challenge in incorporating enzymes into a rinse is maintaining enzymatic stability and activity at water levels above 50%, conventionally not suitable for enzyme containing compositions. In the present invention, the stability of enzyme activity is found to be acceptable and is optimized unexpectedly when the water content of the rinse is maintained above 45% by weight of a mixture thereof, and preferably about 50 to about 85% by weight enzyme activity as an antiplaque agent is found to increase as will hereinafter be demonstrated.

The following examples further describe and demonstrate preferred embodiments within the scope of the present invention. The examples are given solely for illustration, and are

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not to be construed as a limitation of this invention as many variations thereof are possible without departing from its spirit and scope.

**Example I**

5 An enzyme (papain, glucoamylase) containing dentifrice composition containing a small but effective amount of cetyl pyridinium chloride and reducing agents (potassium stannate, ammonium sulfate) added as enzyme premix and thickened with a cellulose thickener (carboxymethyl cellulose) was prepared. The ingredients of this composition designated, "Composition A", are listed in Table I below.

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Dentifrice compositions designated Compositions "B, C and D" were prepared following the procedure of Example I for the purpose of comparison. All the compositions contained the same enzymes as Composition A namely, glucoamylase and papain. Compositions B and C did not contain cetyl pyridinium chloride.

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Composition B contained the reducing agent ammonium sulfate and the metal chelating agent EDTA. Composition C contained the reducing agent potassium stannate and the preservative Nipastat. Composition D contained cetyl pyridinium chloride in the dentifrice composition base and did not contain a reducing agent and Laponite was substituted for sodium carboxymethyl cellulose as a thickening agent.

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Commercial dental cream that did not contain cetyl pyridinium chloride or enzymes designated "Composition E" was used as a control.

| TABLE I                        |         |         |         |         |
|--------------------------------|---------|---------|---------|---------|
| Composition                    | A Wt. % | B Wt. % | C Wt. % | D Wt. % |
| Ingredients                    |         |         |         |         |
| Water                          | 18.25   | 17.75   | 18.25   | 18.25   |
| Pluronic F127                  | 1.5     | 1.5     | 1.5     | 0.75    |
| Glycerin                       | 20.0    | 20.0    | 20.0    | 22.0    |
| Tetrasodium pyrophosphate      | 2.0     | 2.0     | 2.0     | --      |
| Sodium tripolyphosphate        | 3.0     | 3.0     | 3.0     | --      |
| Monosodium phosphate           | 0.026   | 0.026   | 0.026   | --      |
| Disodium phosphate             | 0.079   | 0.079   | 0.079   | --      |
| Sodium carboxymethyl cellulose | 0.65    | 0.65    | 0.65    | --      |
| Laponite                       | --      | --      | --      | 0.7     |
| Xanthan gum                    | 0.55    | 0.55    | 0.55    | 0.6     |
| Sodium monofluoro phosphate    | 0.76    | 0.76    | 0.76    | 0.76    |
| Sodium saccharin               | 0.50    | 0.50    | 0.50    | 0.40    |
| Sorbitol (70% solution)        | 18.9434 | 19.6999 | 19.2024 | 22.8049 |
| Silica XWA650                  | 20.0    | 20.0    | 20.0    | 20.0    |
| Zeodent 115                    | 5.0     | 5.0     | 5.0     | 5.0     |
| Zeodent 165                    | 2.25    | 2.0     | 2.0     | 2.25    |
| Titanium dioxide               | 0.40    | 0.40    | 0.40    | 0.50    |
| Sodium lauryl sulfate          | 0.50    | 0.50    | 0.50    | --      |
| Tauranol                       | --      | --      | --      | 1.2     |
| Betaine                        | 1.0     | 1.0     | 1.0     | --      |
| PEG 600                        | 3.0     | 3.0     | 3.0     | 3.0     |
| Polyoxomer 407                 | 0.10    | 0.10    | 0.10    | 0.10    |
| Flavor                         | 1.10    | 1.10    | 1.10    | 1.10    |
| Papain                         | 0.2266  | 0.2266  | 0.2266  | 0.2266  |
| Glucoamylase                   | 0.044   | 0.044   | 0.044   | 0.044   |
| Potassium stannate             | 0.10    | --      | 0.10    | --      |
| Ammonium sulfate               | --      | 0.10    | --      | --      |
| Cetyl pyridinium chloride      | 0.015   | --      | --      | 0.3     |
| EDTA, disodium dihydrate       | 0.006   | 0.006   | 0.006   | 0.006   |
| Nipastat                       | --      | --      | 0.006   | --      |
| Sodium benzoate                | --      | 0.0025  | --      | 0.0025  |
| Potassium sorbate              | --      | 0.006   | --      | 0.006   |

Composition A, was prepared by premixing the reducing agent potassium stannate and a preservative (EDTA) with cetyl pyridinium chloride 23°C. The ingredients of the premix

5 designated "Premix A1" are recorded in Table II below at 23°C.

Premixes designated B1, C1 and D1 were prepared following the procedure used for Premix A1 and added to respective dentifrice Compositions B, C and D. The ingredients used in the preparation of the premix compositions for Compositions A, B, C, D are listed in Table II below.

| TABLE II                  |           |           |           |           |
|---------------------------|-----------|-----------|-----------|-----------|
| Premix Ingredients        |           |           |           |           |
| Composition               | A1 (Wt.%) | B1 (Wt.%) | C1 (Wt.%) | D1 (Wt.%) |
| Ingredients               |           |           |           |           |
| Sorbitol                  | 67.168    | 76.848    | 67.348    | 76.848    |
| Water                     | 25        | 15        | 25        | 15        |
| Papain                    | 4.532     | 4.532     | 4.532     | 4.532     |
| Glucoamylase              | 0.88      | 0.88      | 0.88      | 0.88      |
| Potassium stannate        | 2.0       | --        | 2.0       | 2.0       |
| Ammonium sulfate          | --        | 2.0       | --        | --        |
| cetyl pyridinium chloride | 0.3       | --        | --        | --        |
| EDTA, disodium dihydrate  | 0.12      | 0.12      | 0.12      | 0.12      |
| Nipastat                  | --        | --        | 0.12      | --        |
| Sodium benzoate           | --        | 0.5       | --        | 0.5       |
| Potassium sorbate         | --        | 0.12      | --        | 0.12      |

Premix compositions A1, B1, C1 and D1 were separately added to a non-enzyme dental cream containing the ingredients listed in Table I in a 1:4 ratio to observe the effects on cellulase activity. Cellulase activity was monitored via both viscosity and sugar level changes in Dentifrice Compositions A, B, C and D over time.

In viscosity measurements, greater cellulase activity was discernible by a more rapid reduction in toothpaste viscosity as the cellulase catabolized the carboxymethyl cellulose thickener used to prepare the dentifrice compositions. Brookfield model RVDTV-11 viscometer with spindle #95 at 5 rpm measured viscosity in centipose per second<sub>2</sub> (CPS). Measurements were taken daily of each sample until an 80% loss in viscosity was observed.

The sugar byproduct concentration was monitored using an Accucheck blood sugar digital analyzer. As cellulase acts upon cellulose, sugar is produced as a byproduct. The greater

the amount of cellulase activity, the greater the amount of sugar produced. Both the sugar level and viscosity methods were used to compare the effects of cetyl pyridinium chloride, the reducing agents ammonium sulfate and potassium stannate and preservative agents sodium benzoate, and parabens on cellulase activity. The viscosity and sugar concentration results are recorded in Table III below.

| TABLE III                  |                            |                          |                                     |                                          |
|----------------------------|----------------------------|--------------------------|-------------------------------------|------------------------------------------|
| Composition<br>Ingredients | Initial Viscosity<br>(CPS) | Final Viscosity<br>(CPS) | % Viscosity Loss<br>(After 14 Days) | Sugar Conc.<br>(MG/DL)<br>(After 7 Days) |
| A                          | 25                         | 17                       | 32                                  | 28                                       |
| B                          | 20                         | 10                       | 50                                  | 62                                       |
| C                          | 20                         | 5                        | 75                                  | 62                                       |
| D                          | 30                         | 15                       | 50                                  | 62                                       |
| E                          | 28                         | 32                       | 0                                   | 0                                        |

The results in Table III show that the trend in sugar production is consistent with the viscosity loss results. Both tests indicate that Composition A formulated with a potassium stannate/cetyl pyridinium chloride/enzyme premix solution was most effective in delaying cellulase activity upon the dentifrice composition.

### Tongue Microflora Reduction

Several compositions were tested for tongue microflora reduction, focussing on those species responsible for the generation of oral malodor. Patients swabbed the tongue for bacterial collection at baseline and four hours post treatment. These samples were plated onto agar media and incubated anaerobically. After four days, colony forming units of malodor causing bacteria and also total tongue bacteria were enumerated. The average colony forming unit results were used to calculate percent reduction from baseline.

Table IV and V represents *in-vivo* tongue microflora study with bacterial sampling at baseline and at four hours post-brushing with Composition D. For purposes of comparison Compositions F and G were also evaluated for oral malodor and tongue microflora reduction. Composition F was a commercial dentifrice which contained cetyl pyridinium chloride but no enzymes and compositions G was a commercial fluoride toothpaste which did not contain cetyl pyridinium chloride enzymes. Table V shows the increased reduction

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of oral malodor causing bacteria by the combination of enzymes with cetyl pyridinium chloride present in Composition D. Table VI demonstrates that this stabilized enzyme premix when incorporated into toothpaste systems provides an in-vivo antibacterial effect against all types of bacteria that exist on the tongue, including the malodor causing species. After four hours post treatment Formulation D, containing cetyl pyridinium chloride and enzymes significantly reduced more bacteria than the comparative dentifrices F and G.

| TABLE IV                                                    |                           |                                |                                                |
|-------------------------------------------------------------|---------------------------|--------------------------------|------------------------------------------------|
| Reduction of malodor tongue bacteria 4 hours post treatment |                           |                                |                                                |
| Composition                                                 | Baseline<br>Mean colonies | After 4 hours<br>Mean colonies | % Reduction of<br>Malodor Bacteria<br>Colonies |
| D                                                           | $3.8 \times 10^5$         | $1.4 \times 10^5$              | 63                                             |
| F                                                           | $2.3 \times 10^5$         | $2.3 \times 10^5$              | 0                                              |
| G                                                           | $2.0 \times 10^5$         | $2.3 \times 10^5$              | 0                                              |

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| TABLE V                                                   |                           |                                |                                                |
|-----------------------------------------------------------|---------------------------|--------------------------------|------------------------------------------------|
| Reduction of total tongue bacteria 4 hours post treatment |                           |                                |                                                |
| Composition                                               | Baseline<br>Mean colonies | After 4 hours<br>Mean colonies | % Reduction of<br>Malodor Bacteria<br>Colonies |
| D                                                         | $5.2 \times 10^5$         | $2.4 \times 10^5$              | 53                                             |
| F                                                         | $5.0 \times 10^5$         | $3.1 \times 10^5$              | 38                                             |
| G                                                         | $4.3 \times 10^5$         | $3.9 \times 10^5$              | 10                                             |

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## Example II

Oral rinses designated "Compositions K and L" were prepared having the ingredients listed in Table VI below.

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| TABLE VI                   |          |          |
|----------------------------|----------|----------|
| Composition<br>Ingredients | K (Wt.%) | L (Wt.%) |
| Water                      | 57.8     | 70.3     |
| Glycerin                   | 16.25    | 10.0     |
| Sorbitol                   | 16.25    | 10.0     |
| Ethanol                    | 5.00     | 5.00     |
| Gantrez                    | 1.92     | 1.92     |
| Pluronic F127              | 0.75     | 0.75     |
| Pluronic F108              | 0.75     | 0.75     |
| Tetrasodium pyrophosphate  | 0.45     | 0.45     |
| Flavor                     | 0.30     | 0.30     |
| Papain                     | 0.21     | 0.21     |
| Potassium stannate         | 0.05     | 0.05     |
| Sodium saccharin           | 0.070    | 0.070    |
| Cetylpyridinium chloride   | 0.050    | 0.050    |
| Nipastat                   | 0.050    | 0.050    |
| Monosodium phosphate       | 0.035    | 0.035    |
| Butyl hydroxy toluene      | 0.015    | 0.015    |
| Disodium phosphate         | 0.010    | 0.010    |
| Phosphoric acid            | 0.04     | 0.04     |
| Total                      | 100.0    | 100.0    |

## Papain Stability

The papain activity of the rinse was monitored using the PanVera Protease Activity Kit. The activity kit quantifies protease activity using a fluorescein thiocarbamoyl (FTC)-casein substrate. FTC-casein is attacked by the protease, breaking down casein into TC-peptides. The amount of protease activity is determined by measuring the fluorescence of the FTC peptides generated. Table VII below compares the percent activity and fluorescence of Compositions K and L, and a placebo designated "Composition N" which is identical to Composition K, but does not contain enzymes. Fluorescence values are expressed in relative fluorescence units, RFU's. Percent activity is determined from the fluorescence,

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relative to the placebo. From activity measurements above 70% of the enzyme activity was maintained after 4 weeks at room temperature.

| TABLE VII<br>PROTEASE ACTIVITY |                             |                                    |             |
|--------------------------------|-----------------------------|------------------------------------|-------------|
| Oral Rinse                     | Initial<br>Florescence(RFU) | After 4 Weeks<br>Fluorescence(RFU) | % Reduction |
| K                              | 25983                       | 21518                              | 74%         |
| L                              | 26548                       | 21297                              | 72%         |
| N                              | 10360                       | 11563                              | 0           |

### 5 Plaque Removal Efficacy

To evaluate the plaque removal ability of the papain enzyme rinse Compositions K and L, a salivary flow cell study was performed in which plaque was grown onto a model surface, followed by treating the surface with a rinse and measuring the reduction of plaque. In the study, a plaque biofilm was first formed onto germanium prisms by continuously pumping bacteria growth media over the prisms for approximately 18 hours. Next, the prisms were treated with a rinse composition, pumping rinse over the prisms for several minutes, in order to facilitate plaque removal. A plaque index was calculated, using Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR FTIR) absorption band intensities at 3300, 1650, 1545 and 1080  $\text{cm}^{-1}$  from the infrared spectrum as follows:

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$$\text{Plaque Score} = \text{abs}_{3300} + \text{abs}_{1650} + \text{abs}_{1545} + \text{abs}_{1080}$$

where *abs* is the maximum absorbance at the various wave numbers. The wave numbers selected represent the infrared adsorption by salivary components and bacteria on the infrared-transparent germanium prisms. The plaque index of the rinse treated prisms were then compared to a baseline (prism rinsed with saline only), and reported as percent plaque reduction.

The results recorded in Table IX below, compares the plaque reduction performance of oral rinse Compositions K and L of the present invention versus a commercial rinse designated Composition M which contained 0.05% by weight cetyl pyridinium chloride but no enzymes and the placebo designated "Composition N". The percent plaque reduction of the high water containing rinse, rinse L was 17% better than rinse K. The results indicate that there is greater improvement in the delivery of actives to the plaque surface with the high water, less viscous formula although both rinses were effective to

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reduce plaque to a greater degree than the commercial rinse Composition M when compared to the placebo Composition N.

The results are recorded in Table IX below.

5

| TABLE IX                         |              |                    |
|----------------------------------|--------------|--------------------|
| Composition                      | Plaque Index | % Plaque Reduction |
| K                                | 2.320        | 20                 |
| L                                | 1.832        | 37                 |
| M                                | 2.864        | 1                  |
| N                                | 2.529        | 13                 |
| Rinsed with saline solution only | 2.901        | --                 |

#### Inhibition of Plaque Formation

10 The ability of the enzyme rinse to inhibit plaque formation was evaluated using the salivary flow cell wherein the study was performed in a similar manner as described above for plaque removal, except that the germanium prisms are pretreated with the rinse for several minutes, followed by an 18 hour post treatment with bacterial growth media to facilitate growth of a plaque biofilm onto the prism surface. The amount of plaque formed (plaque index) on the prisms was again determined by measuring the total amide absorption by ATR FTIR. The plaque index of the rinse treated prisms is compared to a placebo, Composition N and expressed as percent plaque inhibition.

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Table X compares the percent plaque inhibition of rinses K and L relative to the commercial rinse M containing cetyl pyridinium chloride but no enzymes, and the placebo N which was identical to Composition K except it did not contain enzymes. The results recorded in Table X indicate that the lower water, more viscous rinse Composition K is more effective in coating the plaque free surface, forming a greater protective barrier for inhibiting plaque formation than Composition L.

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| TABLE X    |              |                     |
|------------|--------------|---------------------|
| Oral Rinse | Plaque Index | % Plaque Inhibition |
| K          | 0.683        | 35                  |
| L          | 0.801        | 24                  |
| M          | 0.968        | 8                   |
| N          | 1.053        | --                  |

### Intradental Plaque Removal

5 The propensity of the enzyme rinse to remove intradental plaque was demonstrated using an in vitro cleaning model. The model involved pretreating dentures with the rinse, growing plaque onto the denture surface, followed by post-treatment of the dentures with the rinse. In this model, removable dentures were first pumiced, rinsed with water, and sterilized with ethanol for 10 minutes in a trough of a brushing machine, in order to provide a clean denture surface. Next the dentures are submersed in a rinse solution, and

10 incubated overnight at room temperature. Following overnight treatment, the dentures are rinsed with water and submersed into bacterial growth media for 6 hours at 37°C. Finally, the models were rinsed briefly with water, followed by brief rinsing with mouth rinse formulas. The dentures were then disclosed with disclosing solution for 30 seconds, rinsed briefly with water, and then rated with a plaque score determined using the Quigley Hein

15 Plaque method.

The high water containing rinse, Composition L was tested in the in vitro cleaning model. Results recorded in Table XI indicate, that in comparison the commercial rinse M containing cetyl pyridinium chloride but no enzymes and the placebo rinse N, the enzyme

20 rinse L significantly reduced both total and interproximal plaque by 67% demonstrating Composition L not only effectively reduces approximal plaque, but is also effective in cleaning in interproximal regions, demonstrating a "floss type" action.

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TABLE XI  
INTRADENTAL PLAQUE

| Oral Rinse | Plaque Index<br>(Interproximal) | Plaque Index<br>(Total) | % Total Plaque<br>Reduction | % Interproximal<br>Plaque Reduction |
|------------|---------------------------------|-------------------------|-----------------------------|-------------------------------------|
| L          | 1.00                            | 1.50                    | 67                          | 67                                  |
| M          | 3.00                            | 4.58                    | --                          | --                                  |
| N          | 3.00                            | 4.58                    | --                          | --                                  |

94 95

## CLAIMS

What is claimed is:

5

1. An oral care composition having enhanced stability and antiplaque effectiveness which comprises an orally acceptable vehicle containing a combination of an enzyme, cetyl pyridinium chloride and a reducing agent.

10 2. The composition of claim 1 wherein the enzyme is a proteolytic enzyme.

3. The composition of claim 2 wherein the proteolytic enzyme is papain.

15 4. The composition of claim 3 wherein the papain is present in the composition at a concentration of about 0.1 to about 5.0% by weight of the composition.

5. The composition of claim 1 wherein the enzyme is glucoamylase.

20 6. The composition of claim 1 wherein the glucoamylase is present in the oral composition at a concentration of about 0.1 to 5.0% by weight.

7. The composition of claim 2 wherein the cetyl pyridinium chloride is present in the composition at a concentration of about 0.005 to about 2% by weight.

25 8. The composition of claim 1 wherein the reducing agent is potassium stannate.

9. The composition of claim 2 wherein the reducing agent is ammonium sulfate.

30 10. The composition of claim 1 wherein the reducing agent is present in the composition at a concentration of about 0.05 to about 2.0% by weight of the composition.

11. The composition of claim 1 wherein the composition is a dentifrice.

35 12. The composition of claim 1 wherein the composition is a mouth rinse.

13. The composition of claim 1 wherein the composition is a dentifrice thickened with a cellulose polymer.

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14. The composition of claim 13 wherein the cellulose polymer is carboxymethyl cellulose.
15. The composition of claim 12 wherein the mouthrinse has a water concentration greater than about 45% by weight water.
16. The composition of claim 15 wherein the water concentration is about 50 to about 85% by weight.
17. A method for effecting enhanced stability in an oral composition containing enzymes which comprises preparing a composition comprised of an orally acceptable vehicle containing a combination of an enzyme, cetyl pyridinium chloride and a reducing agent and then applying the composition to the oral cavity.
18. The method of claim 17 wherein the enzyme is a proteolytic enzyme.
19. The method of claim 17 wherein proteolytic enzyme is papain
20. The method of claim 19 wherein the papain is present in the composition at a concentration of about 0.1 to about 5.0% by weight of the composition.
21. The method of claim 17 wherein the enzyme is glucoamylase.
22. The method of claim 21 wherein the glucoamylase is present in the oral composition at a concentration of 0.1 to 5.0% by weight.
23. The method of claim 17 wherein the enzyme is incorporated in the composition as an aqueous dispersion of a papain and glucoamylase.
24. The method of claim 17 wherein the reducing agent is potassium stannate
25. The method of claim 17 wherein the reducing agent is ammonium sulfate
26. The method of claim 17 wherein the reducing agent is present in the composition at a concentration of about 0.05 to about 2.0% by weight of the composition.
27. The method of claim 17 wherein the composition is a dentifrice thickened with a cellulose polymer.

96 GP

28. The method of claim 17 wherein the cellulose polymer is carboxymethyl cellulose.
29. The method of claim 17 wherein the cetyl pyridinium chloride is present in the  
5 composition at a concentration of about 0.005 to about 2% by weight.
30. The method of claim 17 wherein the composition is a mouth rinse.
31. The method of claim 30 wherein the mouthrinse has a water concentration greater than  
10 about 45% by weight.
32. The method of claim 31 wherein the mouthrinse has a water concentration of about 50  
to about 85% by weight.
- 15 33. A method for the preparation of an aqueous oral care composition containing a stable  
enzyme compound which comprises first dissolving the enzyme in an aqueous solution  
of cetyl pyridinium chloride and a reducing agent or to prepare a premix and then  
adding the premix to the vehicle of the oral care composition.
- 20 34. The method of claim 33 wherein the enzyme is present in the premix at a  
concentration of about 0.1 to about 10% by weight.
35. The method of claim 33 wherein the enzyme is glucoamylase.
- 25 36. The method of claim 33 wherein the reducing agent is present in the oral care  
composition at a concentration of about 0.05 to about 3% by weight.
37. The method of claim 33 wherein the enzyme is papain.
- 30

97 98

ABSTRACT

5 An oral care composition having enhanced oral hygiene and antiplaque properties which comprises an orally acceptable vehicle containing a combination of an enzyme, cetylpyridinium chloride and a reducing agent.

## GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s):

(Family name followed by given name; for a full legal entity, full official designation. The address must include postal code and name of country.)

COLGATE-PALMOLIVE COMPANY  
300 Park Avenue  
New York, New York 10022  
United States of America

hereby appoint(s) the following person as:



agent



common representative

Name and address

(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Martin B. Barancik, Henry S. Goldfine, Bernard Lieberman, Michael J. McGreal, Rosemary M. Miano, Richard E. Nanfeldt, Ellen Park and Clifford Wilkins of

COLGATE-PALMOLIVE COMPANY  
909 River Road  
P.O. Box 1343  
Piscataway, New Jersey 08855-1343  
United States of America

to represent the undersigned before



all the competent International Authorities



the International Searching Authority only



the International Preliminary Examining Authority only

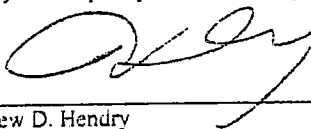
in connection with any and all international applications filed by the undersigned with the following Office

RO/US

as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) (where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power):

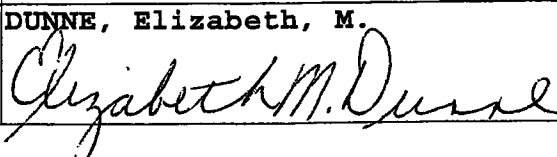


Andrew D. Hendry  
Senior Vice President, General Counsel and  
Secretary

Date: October 16, 2003

**PCT (ANNEX - FEE CALCULATION SHEET)**Original (for **SUBMISSION**)

(This sheet is not part of and does not count as a sheet of the international application)

|              |                                                                                                           |                                                                                                                     |                            |
|--------------|-----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|----------------------------|
| <b>0</b>     | <b>For receiving Office use only</b>                                                                      |                                                                                                                     |                            |
| <b>0-1</b>   | International Application No.                                                                             |                                                                                                                     |                            |
| <b>0-2</b>   | Date stamp of the receiving Office                                                                        |                                                                                                                     |                            |
| <b>0-4</b>   | <b>Form PCT/RO/101 (Annex)</b>                                                                            |                                                                                                                     |                            |
| <b>0-4-1</b> | <b>PCT Fee Calculation Sheet</b><br>Prepared Using                                                        | <b>PCT-SAFE [EASY mode]</b><br><b>Version 3.50 (Build 0002.153)</b>                                                 |                            |
| <b>0-9</b>   | <b>Applicant's or agent's file reference</b>                                                              | <b>7122-00</b>                                                                                                      |                            |
| <b>2</b>     | <b>Applicant</b>                                                                                          | <b>COLGATE-PALMOLIVE COMPANY</b>                                                                                    |                            |
| <b>12</b>    | <b>Calculation of prescribed fees</b>                                                                     | <b>fee amount/multiplier</b>                                                                                        | <b>Total amounts (USD)</b> |
| 12-1         | Transmittal fee <b>T</b>                                                                                  | ⇒                                                                                                                   | <b>300</b>                 |
| 12-2-1       | Search fee <b>S</b>                                                                                       | ⇒                                                                                                                   | <b>1818</b>                |
| 12-2-2       | International search to be carried out by <b>EP</b>                                                       |                                                                                                                     |                            |
| 12-3         | International filing fee<br>(first 30 sheets) <b>I1</b>                                                   | <b>1035</b>                                                                                                         |                            |
| 12-4         | Remaining sheets <b>3</b>                                                                                 |                                                                                                                     |                            |
| 12-5         | Additional amount <b>(X) 11</b>                                                                           |                                                                                                                     |                            |
| 12-6         | Total additional amount <b>I2</b>                                                                         | <b>33</b>                                                                                                           |                            |
| 12-7         | <b>I1 + I2 = I</b>                                                                                        | <b>1068</b>                                                                                                         |                            |
| 12-12        | EASY Filing reduction <b>R</b>                                                                            | <b>-74</b>                                                                                                          |                            |
| 12-13        | Total International filing fee (I-R) <b>I</b>                                                             | ⇒                                                                                                                   | <b>994</b>                 |
| 12-14        | Fee for priority document<br>Number of priority documents<br>requested <b>1</b>                           |                                                                                                                     |                            |
| 12-15        | Fee per document <b>(X) 20</b>                                                                            |                                                                                                                     |                            |
| 12-16        | Total priority document fee: <b>P</b>                                                                     | ⇒                                                                                                                   | <b>20</b>                  |
| 12-17        | <b>TOTAL FEES PAYABLE (T+S+I+P)</b>                                                                       | ⇒                                                                                                                   | <b>3132</b>                |
| 12-19        | <b>Mode of payment</b>                                                                                    | <b>authorization to charge deposit account</b>                                                                      |                            |
| 12-20        | <b>Deposit account instructions</b>                                                                       |                                                                                                                     |                            |
|              | The receiving Office                                                                                      | <b>United States Patent and Trademark<br/>Office (USPTO) (RO/US)</b>                                                |                            |
| 12-20-1      | Authorization to charge the total fees<br>indicated above.                                                | ✓                                                                                                                   |                            |
| 12-20-2      | Authorization to charge any deficiency or<br>credit any overpayment in the total fees<br>indicated above. | ✓                                                                                                                   |                            |
| 12-20-3      | Authorization to charge the fee for<br>priority document.                                                 | ✓                                                                                                                   |                            |
| 12-21        | Deposit account No.                                                                                       | <b>03-2457</b>                                                                                                      |                            |
| 12-22        | Date                                                                                                      | <b>11 February 2004 (11.02.2004)</b>                                                                                |                            |
| 12-23        | Name and signature                                                                                        | <b>DUNNE, Elizabeth, M.</b><br> |                            |

# PATENT COOPERATION TREATY

100 10/

From the  
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

## WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing  
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference  
see form PCT/ISA/220

**FOR FURTHER ACTION**  
See paragraph 2 below

International application No.  
PCT/US2004/003842

International filing date (day/month/year)  
11.02.2004

Priority date (day/month/year)  
11.02.2003

International Patent Classification (IPC) or both national classification and IPC  
A61K7/16, A61K7/22, A61K7/28

Applicant  
COLGATE-PALMOLIVE COMPANY

### 1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

### 2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

### 3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office  
D-80298 Munich  
Tel. +49 89 2399 - 0 Tx: 523656 epmu d  
Fax: +49 89 2399 - 4465

Authorized Officer

Mitchell, G

Telephone No. +49 89 2399-8678



**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

101 16h  
International application No.  
PCT/US2004/003842

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**Box No. I Basis of the opinion**

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1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.  
☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
  - a. type of material:  
☐ a sequence listing  
☐ table(s) related to the sequence listing
  - b. format of material:  
☐ in written format  
☐ in computer readable form
  - c. time of filing/furnishing:  
☐ contained in the international application as filed.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

102 107  
International application No.  
PCT/US2004/003842

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**Box No. II    Priority**

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1. ☒ The following document has not been furnished:

☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

103 10A  
International application No.  
PCT/US2004/003842

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. -

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. -

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form

☐ has not been furnished

☐ does not comply with the standard

the computer readable form

☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

104 105  
International application No.  
PCT/US2004/003842

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**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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1. Statement

|                               |             |      |
|-------------------------------|-------------|------|
| Novelty (N)                   | Yes: Claims |      |
|                               | No: Claims  | 1-37 |
| Inventive step (IS)           | Yes: Claims |      |
|                               | No: Claims  | 1-37 |
| Industrial applicability (IA) | Yes: Claims | 1-37 |
|                               | No: Claims  |      |

2. Citations and explanations

**see separate sheet**

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**Box No. VI Certain documents cited**

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1. Certain published documents (Rules 43bis.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43bis.1 and 70.9)

**see form 210**

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING  
AUTHORITY (SEPARATE SHEET)**

105 106  
International application No.

PCT/US2004/003842

**Re Item V.**

The following documents are referred to in this communication:

- D1: US-A-5 711 937 (GOMI TETSUO ET AL) 27 January 1998 (1998-01-27)
- D2: GB-A-1 255 284 (BLENBEX-WERKE R. SCHNEIDER & CO) 1 December 1971 (1971-12-01)
- D3: US-A-3 681 197 (SMITH CLARENCE T) 1 August 1972 (1972-08-01)

The present application is for an oral care composition which comprises an orally acceptable vehicle containing a combination of an enzyme, cetyl pyridinium chloride and a reducing agent; the method for effecting enhanced stability in an oral composition which comprises the preparation of said composition and applying it to the oral cavity; and a method for the preparation of said oral care composition.

As pointed out on the International Search report the term "reducing agent" is vague and encompasses literally thousands of possibilities including enzymes which are already present in the composition.

D1 discloses oral compositions with improved antibody stability . The specific embodiment disclosed in example 29 consists of a mouthwash comprising dextranase and cetyl pyridinium chloride.

D2 relates to cosmetic preparation with particular attention to teeth and mouth. Example 10 discloses an ointment for the mucous membrane of the mouth comprising cetyl pyridinium chloride and trypsin.

D3 discloses methods and processes for controlling and stopping the denaturing of enzymes by utilising cetyl pyridinium chloride ( example I).

Given the current formulation (and that the intended use is not a characterising feature), D1, D2 and D3 are considered to destroy the novelty and inventive step of claims 1-16 and 33-37 (Art. 33(2)-(3) PCT).

Moreover, even if novelty is installed over the cited prior art, the question of inventive step would arise since the enhanced stability of the enzymes using cetyl pyridinium chloride is known for over 30 years (from D3) and as such cannot be considered to constitute a surprising technical effect.

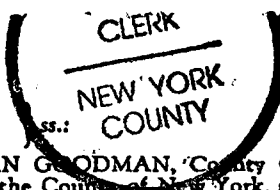
**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING  
AUTHORITY (SEPARATE SHEET)**

106 107  
International application No.

PCT/US2004/003842

The subject-matter of claims 17-32 concerns a method for effecting enhanced stability in an oral composition. The technical problem of the present application is the provision of an oral composition comprising an enzyme for enhancing oral hygiene and having enhanced stability and antiplaque effects. Thus a method for "effecting enhanced stability" is a result-to-be-achieved i.e. a desired technical effect, rather than an entity or activity. By including such an element in a claim, the Applicant effectively claims the result-to-be-achieved which amounts to claiming the underlying technical problem and leads to a lack of clarity of the application (Art. 6 PCT)

107 100



252373 Form 1

City of New York  
County of New York, ss.:  
I, NORMAN GOODMAN, County Clerk and Clerk of the Supreme Court of the State of New York, in and for the County of New York, a Court of Record, having by law a seal, DO HEREBY CERTIFY pursuant to the Executive Law of the State of New York, that

Rosie S. Dizon

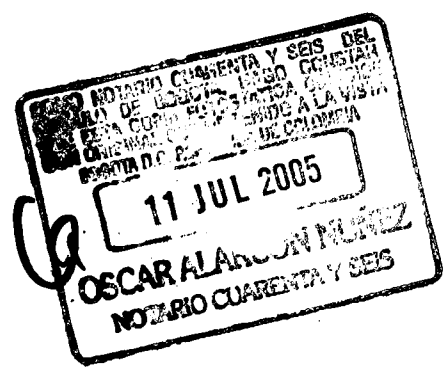
whose name is subscribed to the annexed affidavit, deposition, certificate of acknowledgment or proof, was at the time of taking the same a NOTARY PUBLIC in and for the State of New York duly commissioned, sworn and qualified to act as such; that pursuant to law, a commission or a certificate of his official character, with his autograph signature has been filed in my office; that at the time of taking such proof, acknowledgment or oath, he was duly authorized to take the same; that I am well acquainted with the handwriting of such NOTARY PUBLIC or have compared the signature on the annexed instrument with his autograph signature deposited in my office, and I believe that such signature is genuine.

IN WITNESS WHEREOF, I have hereunto set my hand affixed my official seal this

OCT 30 2001

FEE PAID \$3.00

*Norman Goodman*  
County Clerk and Clerk of the Supreme Court, New York County



# Apostille

108 109

(Convention de La Haye du 5 Octobre 1961)

1. Country: **United States of America**  
This public document
2. has been signed by **Norman Goodman**
3. acting in the capacity of **County Clerk**
4. bears the seal/stamp of the county of **New York**

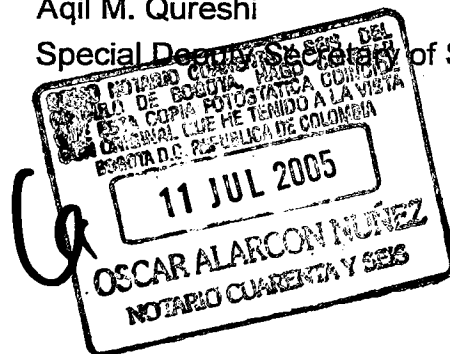
*Certified*

5. At New York, New York
6. the 30th day of October 2001
7. by Special Deputy Secretary of State, State of New York
8. No. NYC-9786540B
9. Seal/Stamp
10. Signature



*Aqil M. Qureshi*

Aqil M. Qureshi  
Special Deputy Secretary of State



**Brigard & Castro**

SANTA FE DE BOGOTA - COLOMBIA

**PODER****POWER OF ATTORNEY**

Yo (nosotros)

domiciliado(s) en

Nombro(amos) a: RAMIRO CASTRO DUQUE y/o JUAN PABLO CADENA SARMIENTO y/o BEATRIZ JARAMILLO S.

de Santafé de Bogotá, Colombia, apoderados verdaderos y legales con poder especial, amplio y suficiente, para recabar de las oficinas y autoridades colombianas administrativas, judiciales y de policía, en cualquier instancia o recurso, la obtención de patentes de invención, registros de marcas, diseños industriales, modelos de utilidad, depósitos de nombres comerciales y enseñanzas, registros de derechos de autor y contratos sobre los mismos, obtención de certificados de obtentor de variedades vegetales, así como sus prórrogas, renovaciones, anotaciones de traspaso, cambios de nombre y/o domicilio; elaborar, tramitar y registrar contratos de licencia y licencias de cualesquiera clases; intervenir como demandante o como demandado en cualquier instancia y recurso ante cualquier juez, corporación, funcionario público o autoridad en acciones judiciales, administrativas y de policía tales como: oposiciones al registro de marcas; acciones de caducidad, cancelación y nulidad; acciones reivindicatorias, acciones derivadas de la obtención o explotación de licencias; acciones de competencia desleal; demandas penales; acciones de indemnización de perjuicios, reclamos y observaciones a solicitudes de patentes, diseños industriales, modelos de utilidad, en el ejercicio de medidas cautelares y en otras acciones o medidas similares.

A este efecto, lo(s) faculto(amos) para elevar solicitudes, formular descripciones, enmiendas, protestas, declaraciones, oposiciones, cancelaciones, nulidades, apelaciones y reclamos; pagar impuestos, cuotas y gravámenes prescritos por la ley; renunciar o desistir a derechos concedidos o en curso de concesión; recibir toda clase de documentos y valores, dando el descargo respectivo, y, en general, para dar ante dichas autoridades todos los pasos necesarios al efecto indicado y tomar todas las medidas que creyeran conducentes al resguardo de mis (nuestros) intereses, con todas las demás facultades legales necesarias.

Este poder comprende la facultad de ratificar o confirmar en mi (nuestro) nombre lo actuado así como la facultad de desistir, transigir, comprometer y la de sustituirlo en todo o en parte por revocar sustituciones. Asimismo nombro(amos) a RAMIRO CASTRO DUQUE y/o JUAN PABLO CADENA SARMIENTO y/o BEATRIZ JARAMILLO S. domiciliado(s) en la Carrera 7 No. 16-56, Piso 9, mis (nuestros) apoderados en Colombia, con facultades para recibir notificaciones y designar apoderados judiciales o extrajudiciales.

Otorgado y firmado en

hoy de

Firma

Nombre

I (We)

**COLGATE-PALMOLIVE COMPANY**, a Delaware corporation, of 300 Park Avenue, New York, New York 10022, U. S. A.

Hereby appoint RAMIRO CASTRO DUQUE and/or JUAN PABLO CADENA SARMIENTO and/or BEATRIZ JARAMILLO S.

of Santafé de Bogotá, Colombia, my (our) true and lawful attorneys with special, ample and sufficient power to obtain from the Colombian administrative, judicial and police offices and authorities, in any instance, privileges of patents of invention, registration of trademarks, industrial designs and utility models; registration of commercial names and emblems, registration of copyright and contracts related thereto, certificates of obtainers of vegetables varieties, as well as extensions, renewals and recordal of assignments and changes of name and domicile related to the above; to prepare, prosecute and register licenses of any kind; to act as plaintiff or defendant in any instance or recourse, and before any judge, corporation, public official or authority in judicial, administrative and police actions, such as: oppositions to the registration of trademarks; caducity, cancellation and nullity actions; claim actions derived from the obtaining or exploitation of licenses, action of unfair competition; penal demands, actions of indemnities for damages; claims or observations on patent applications, models, industrial designs and utility models; in the exercise of preventive measures; and in other actions or similar proceedings.

To that effect they are hereby empowered to file applications, formulate descriptions, amendments, protests, declarations, oppositions, cancellations, nullities, appeals and claims; to pay taxes, quotas and assessments prescribed by law, renounce or waive the rights granted or in the process of granting; to receive all kind of documents and valuables, giving the respective release of receipt, and, in general, to take before said authorities the necessary steps to such effect, and any measures that they may deem pertinent to the safeguard of my (our) interest(s) with all the other necessary legal powers.

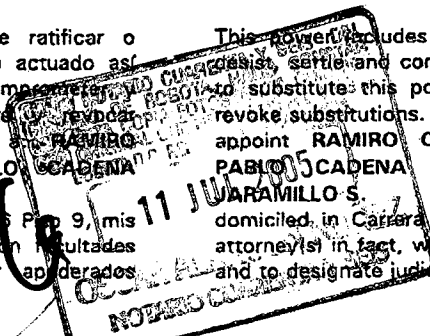
This power includes the faculty to ratify or confirm, desist, settle and compromise on my (our) behalf, and to substitute this power in whole or in part and to revoke substitutions. At the same time I (We) hereby appoint RAMIRO CASTRO DUQUE and/or JUAN PABLO CADENA SARMIENTO and/or BEATRIZ JARAMILLO S. domiciled in Carrera 7 No. 16-56, Piso 9, my (our) attorney(s) in fact, with powers to receive notifications and to designate judicial and extrajudicial attorneys.

Granted and signed in **New York, N.Y.**this **OCT 29 2001** day of 200

Signature

Name

**VICE PRESIDENT &  
ASSOCIATE GENERAL COUNSEL  
TRADEMARK**



## CERTIFICADO NOTARIAL (SOCIEDAD)

## NOTARIAL CERTIFICATE (CORPORATION)

Hoy de de  
personalmente compareció ante mí

a quien conozco, y de quien me consta que firmó el  
anterior documento, debidamente juramentado declaró  
y dijo que él es

de

Sociedad legalmente constituida para un periodo de  
duración que aún no ha terminado; que el declarante  
está autorizado por dicha compañía para conferir este  
poder; que el sello estampado por él al pie de tal poder,  
en nombre y representación de la expresada compañía,  
es el sello oficial de ella; y que el objeto para el cual se  
confiere este instrumento está comprendido dentro del  
de la sociedad.

El suscrito Notario hace constar que tuvo a la vista las  
pruebas de la existencia de la sociedad.

Estado de  
con fecha

y que quien confiere el presente poder es su  
representante.

Notario Público (sello)

CERTIFICADO NOTARIAL  
PERSONA NATURAL

COUNTY

Hoy de de , compareció(eron)  
ante mí

a quien (es) conozco y me consta que es(son) la(s)  
persona(s) firmante(s) del documento que precede y  
quien(es) declara(n) que otorgan el mismo para los  
fines y propósitos que en él se expresan.

Notario Público (sello)

On this OCT 29 2001 day of of  
personally appeared before me

SCOTT E THOMPSON

to me known, and known to me to be the person who  
signed the foregoing instrument who being by me duly  
sworn did depose and say that he (she) is the

VICE PRESIDENT &  
ASSOCIATE GENERAL COUNSEL  
TRADEMARK

COLGATE-PALMOLIVE COMPANY

Which is legally constituted for a term not yet expired,  
that deponent is authorized by said Company to confer  
this Power of Attorney: that the seal affixed hereunto  
by the deponent in the name and on behalf of said  
Corporation is the corporate seal thereof; and that the  
purpose for which this instrument is granted is  
included within the corporate purpose of company.

The undersigned Notary Public attests that he has seen  
the documents that prove the existence of the  
company.

State of Delaware  
under date of May 13, 1997

and that he (she) who grants the present power of  
attorney is its Representative.

Notary Public (Seal)

ROSIE S. DIZON

Notary Public, State of New York

No. 31-494117

Qualified in New York County

Commission Expires Aug. 15, 2002

On this OCT 29 2001 day of of  
personally appeared before me

SCOTT E THOMPSON

who (are) known to me and who I attest, executed  
the foregoing document and he (they) declared he  
(they) grant(s) the document for the uses and purposes  
therein expressed.

Notary Public (Seal)

ROSIE S. DIZON

Notary Public, State of New York

No. 31-494117

Qualified in New York County

Commission Expires Aug. 15, 2002

LEGALIZACION POR APOSTILLA ES REQUERIDA

LEGALIZATION BY

Notary Public, State of New York

No. 31-494117

Qualified in New York County

Commission Expires Aug. 15, 2002

111  
112

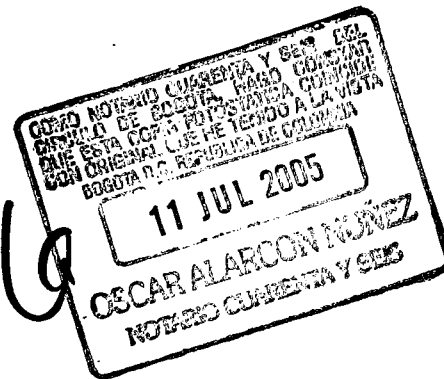
## APOSTILLE

(Convención de La Haya del 5 de Octubre de 1961)

1. País: ESTADOS UNIDOS DE AMERICA  
Este documento público
2. ha sido firmado por **Norman Goodman**
3. actuando en capacidad de **Oficial del Condado**
4. lleva el sello/timbre del condado de **New York**

## CERTIFICADO

5. en New York, New York
6. el 30 de Octubre de 2001
7. por Secretario de Estado Encargado Especial, Estado de New York
8. No. NYC-9786540B
9. Sello/Timbre
10. Firma  
(Firma)  
**Aquil M. Qureshi**  
**Secretario de Estado Encargado Especial**



112 112

**Estado de New York**               )  
                                                            ) ss.  
**Condado de New York**          )

Yo, NORMAN GOODMAN, Oficial del Condado y Oficial de la Corte Suprema del Estado de New York, en y para el Condado de New York, un Tribunal de Registros, que tiene un sello por ley.

## Rosie S. Dizon

cuyo nombre está suscrito al acta notarial, deposición, certificado de reconocimiento o prueba, era al momento de tomar el mismo NOTARIO PÚBLICO en y para el Estado de New York debidamente comisionado, juramentado y calificado para actuar como tal; que de acuerdo con la ley, una comisión o un certificado de este carácter oficial, con su firma autógrafa ha sido presentada en mi oficina; que al momento de tomar tal prueba, reconocimiento o juramento, estaba debidamente autorizado para tomar el mismo; que estoy bien familiarizado con la escritura de tal NOTARIO PÚBLICO o he comparado la firma en el instrumento anexo con la firma autógrafa depositada en mi oficina, y creo que tal firma es genuina.

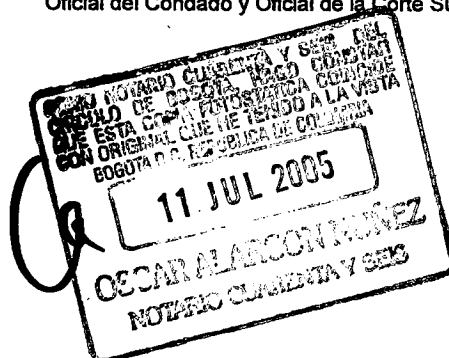
COMO TESTIGO DE LO MISMO, he fijado al presente mi firma t mi sello  
oficial este

**30 de Octubre de 2001**

**TARIFA PAGADA \$3.00**

(Firma)

Oficial del Condado y Oficial de la Corte Suprema, Condado de New York





ORGANIZACION ELECTORAL  
REGISTRADURÍA NACIONAL DEL ESTADO CIVIL

GAVIRIA

REGISTRO CIVIL DE DEFUNCIÓN

Indicativo  
Serial

5562241

|                                                                                                  |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
|--------------------------------------------------------------------------------------------------|---|--------------------|---------|------|-----------|---------------------------------------------|------------------|--------|------|---|------------------------------------------|----|----------|
| <b>Datos de la oficina de registro</b>                                                           |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Clase de oficina:                                                                                |   | Registraduría      | Notaria | X    | Consulado | Corregimiento                               | Insp. de Policía | Código | 9795 |   |                                          |    |          |
| País - Departamento - Municipio - Corregimiento a/o Inspección de Policía                        |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| COLOMBIA CUNDINAMARCA BOGOTA D.C.                                                                |   |                    |         |      |           | NOTARIA 32.                                 |                  |        |      |   |                                          |    |          |
| <b>Datos del inscrito</b>                                                                        |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Apellidos y nombres completos                                                                    |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| CASTRO DUQUE RAMIRO                                                                              |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Documento de Identificación (Clase y número)                                                     |   |                    |         |      |           | Sexo (en Letras)                            |                  |        |      |   |                                          |    |          |
| C.C.No. 170322 DE BOGOTA                                                                         |   |                    |         |      |           | MASCULINO                                   |                  |        |      |   |                                          |    |          |
| <b>Datos de la defunción</b>                                                                     |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Lugar de la Defunción: País - Departamento - Municipio - Corregimiento a/o Inspección de Policía |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| COLOMBIA CUNDINAMARCA BOGOTA D.C.                                                                |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Fecha de la defunción                                                                            |   |                    |         | Hora |           | Número de certificado de defunción          |                  |        |      |   |                                          |    |          |
| Año                                                                                              | 2 | 0                  | 0       | 4    | Mes       | 1                                           | 0                | Día    | 3    | 0 | 04:10 PM                                 | A- | 2094375. |
| Presunción de muerte                                                                             |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Juzgado que profiere la sentencia                                                                |   |                    |         |      |           | Fecha de la sentencia                       |                  |        |      |   |                                          |    |          |
| -                                                                                                |   |                    |         |      |           | Año Mes Día                                 |                  |        |      |   |                                          |    |          |
| Documento presentado                                                                             |   |                    |         |      |           | Nombre y cargo del funcionario              |                  |        |      |   |                                          |    |          |
| Autorización judicial                                                                            |   | Certificado Médico |         | X    |           | ANDRES DIAZ CAMPOS.-<br>R. M. No. 79982662  |                  |        |      |   |                                          |    |          |
| <b>Datos del denunciante</b>                                                                     |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Apellidos y nombres completos                                                                    |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| EDGAR DAVID REGINO II.                                                                           |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Documentos de Identificación (Clase y número)                                                    |   |                    |         |      |           | Firma                                       |                  |        |      |   |                                          |    |          |
| C.C.No. 15.030.154 DE LORICA/ CORDOBA                                                            |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| <b>Primer testigo</b>                                                                            |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Apellidos y nombres completos                                                                    |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| -                                                                                                |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Documentos de Identificación (Clase y número)                                                    |   |                    |         |      |           | Firma                                       |                  |        |      |   |                                          |    |          |
| -                                                                                                |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| <b>Segundo testigo</b>                                                                           |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Apellidos y nombres completos                                                                    |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| -                                                                                                |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Documentos de Identificación (Clase y número)                                                    |   |                    |         |      |           | Firma                                       |                  |        |      |   |                                          |    |          |
| -                                                                                                |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| Fecha de inscripción                                                                             |   |                    |         |      |           | Nombre y firma del funcionario que autoriza |                  |        |      |   |                                          |    |          |
| Año                                                                                              | 2 | 0                  | 0       | 4    | Mes       | 1                                           | 1                | Día    | 0    | 2 | BLANCA REGINA GARCIA RESTrepo<br>NOTARIA |    |          |
| PADRES: ALFON SO CASTRO<br>MERCEDES DUQUE.                                                       |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |
| ESPACIO PARA NOTAS                                                                               |   |                    |         |      |           |                                             |                  |        |      |   |                                          |    |          |

ORIGINAL PARA LA OFICINA DE REGISTRO

# NOTARIA 32

DEL CIRCULO DE SANTA FE DE BOGOTA, D.C.

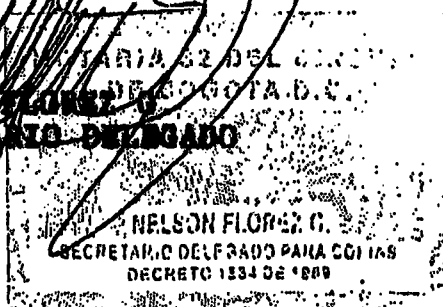
Germán Eleázar Moreno L.  
Notario

COMO SECRETARIO DELEGADO DE LA NOTARIA TREINTA Y DOS (32) DEL  
CIRCULO DE BOGOTA

## CERTIFICO QUE:

La presente acta es fiel copia del original tomada del registro  
civil de defunciones serial indicativo número: 5562241  
que expido en la ciudad de Bogotá D.C. hoy: 05 NOV. 2004

NELSON FLOREZ G.  
SECRETARIO DELEGADO



114#15

SUPERINDUSTRIA Y COMERCIO  
Radicacon : 05079289 00000000 Folios: 114  
Fecha (AMD): 2025-08-10 10:48:03  
Tramite : 011 PCT-PATENT 378 FASE NACT 411 PRESENTAC  
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL  
Δ TRAMITE

|                                                                                                              | USUARIO        | RADICADOR |
|--------------------------------------------------------------------------------------------------------------|----------------|-----------|
| PATENTE DE INVENCION / MODELO DE UTILIDAD                                                                    | ( )            | ( / )     |
| - Indicación de que se solicita la concesión de una patente.                                                 | ( )            | ( / )     |
| - Datos de identificación del solicitante o de la persona que se presenta la solicitud.                      | ( )            | ( / )     |
| - Descripción de la invención.                                                                               | ( )            | ( / )     |
| - Dibujos de ser estos pertinentes.                                                                          | ( )            | ( / )     |
| - Comprobante de Pago de las tasas establecidas.                                                             |                |           |
| COMPLETA ( / )                                                                                               | INCOMPLETA ( ) | ( )       |
| DISEÑO INDUSTRIAL ( )                                                                                        |                |           |
| - Indicación de que se solicita el registro de Diseño Industrial                                             | ( )            | ( )       |
| - Datos de identificación del solicitante o de la persona que presenta la solicitud.                         | ( )            | ( )       |
| - Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño. | ( )            | ( )       |
| - Comprobante de pago de las tasas establecidas                                                              | ( )            | ( )       |
| COMPLETA ( )                                                                                                 | INCOMPLETA ( ) | ( )       |
| ESQUEMA DE TRAZADO ( )                                                                                       |                |           |
| - Indicación de que solicita el registro de un Esquema de trazado.                                           | ( )            | ( )       |
| - Datos de identificación del solicitante o de la persona que presenta La solicitud.                         | ( )            | ( )       |
| - Representación gráfica del esquema de trazado                                                              | ( )            | ( )       |
| - Comprobante de pago de las tasas establecidas.                                                             | ( )            | ( )       |
| COMPLETA ( )                                                                                                 | INCOMPLETA ( ) | ( )       |

Firma Responsable   
Fecha:

115 

**REPUBLICA DE COLOMBIA**  
**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

Bogotá,  
2020

Doctor(a)  
CADENA SARMIENTO JUAN PABLO  
Apoderado(a) y/o Representante de  
COLGATE PALMOLIVE COMPANY



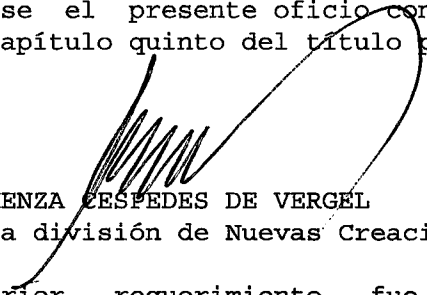
|            |                            |               |
|------------|----------------------------|---------------|
| REFERENCIA | Radicación No.             | 05 79289      |
|            | Solicitud internacional    | US2004/003842 |
|            | Fecha inicio fase nacional | 11/09/2005    |
|            | Trámite                    | 11            |
|            | Evento                     | 378           |
|            | Actuación                  | 430           |
|            | Oficio No.                 | 8728          |

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

  
ALIX CARMENZA CESPEDES DE VERGEL  
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de  
fecha 23 SET. 2005  
adpal