

Compositions and methods for treating halitosis

Related application

[0001] This application claims the benefit of priority from Australian provisional application no. 2023903602 filed 9 November 2023, the entire disclosure of which is incorporated herein by reference.

Field of the invention

[0002] The present invention relates to improved stabilised amorphous calcium phosphate and/or amorphous calcium fluoride phosphate complexes and compositions containing those complexes. Methods of making the complexes of the invention and of treating or preventing oral conditions are also provided.

Background of the invention

[0003] Halitosis, oral malodour or bad breath is a prevalent problem which impacts the quality of life. Various epidemiological studies have reported that around 25–37% of the adult population suffer from halitosis (Miyazaki et al. 1997, Ueno et al. 2007, Liu et al. 2006). About 90% of halitosis cases have intra-oral origin and only 10% have extra-oral origin (Ayers & Colquhoun 1998). Tongue coating, various forms of periodontal disease and other dental problems, as well as reduced salivary flow can cause halitosis with an intra-oral origin (oral malodour) (Tangerman 2002, Papaioannou & Dereka 2009). It is now widely recognised that microbes play an essential role in the etiopathogenesis of halitosis. The predominant source of the microbes associated with halitosis are the tongue dorsum and dental plaque (Quirynen et al. 2009). Indeed, there is a correlation between the amount of microbes on the tongue dorsum and the intensity of halitosis (Hartley et al. 1996). The crypts of the tongue and dental plaque can be sites for elevated numbers of Gram-negative anaerobic microbes with proteolytic activity involved in the production of volatile sulphur compounds (VSCs) from the release of sulphur containing amino acids. VSCs are the major odorous substances associated with halitosis (Tonzetich 1977, Persson et al. 1990, Miyazaki et al. 1995). It has been shown that inhibition of the VSC-producing enzymes and reduction in VSC levels directly reduces halitosis (Takeuchi et al. 2010, Tsai et al. 2008).

[0004] Halitosis is a substantial social problem and there is a need to provide new or improved methods for reducing the level of this condition. Zinc ions are widely used in mouthwash, chewing gum and toothpaste formulations to reduce halitosis, but zinc ions are easily sequestered by phosphate and other molecules in saliva and plaque and therefore have limited efficacy in inhibition of the VSC-producing enzymes/bacteria.

[0005] There is a need for new and/or improved treatments for halitosis.

[0006] Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Summary of the invention

[0007] In one aspect, the present invention provides a method for treating or preventing halitosis in an individual in need thereof, the method comprising administering stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) to the oral cavity of the individual, thereby treating or preventing halitosis.

[0008] In any aspect or embodiment, the method further comprises the step of identifying an individual having halitosis. The individual may have moderate or severe halitosis. For example, the individual may have an intra-oral volatile sulphur compound (VSC) level of, or about, 150 ppb (parts per billion) or greater. The individual may be identified as being 1, 2, 3, 4 or 5 on the Rosenberg scale for halitosis, preferably the individual is identified as 3, 4 or 5 on the Rosenberg scale.

[0009] In any aspect or embodiment, the individual has not been diagnosed with periodontal disease.

[0010] In any aspect or embodiment, the individual does not have periodontal disease.

[0011] In any aspect or embodiment, the individual has not been diagnosed with periodontitis or severe gingivitis.

[0012] In any aspect or embodiment, the individual does not have periodontitis or severe gingivitis.

[0013] In any aspect or embodiment, the individual has not been diagnosed with gingivitis.

[0014] In any aspect or embodiment, the individual does not have gingivitis.

[0015] In another aspect, the present invention provides a method of reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual in need thereof, the method comprising administering stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) to the oral cavity of the individual, thereby reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of the individual.

[0016] In any aspect or embodiment, the method further comprises the step of determining the intra-oral volatile sulphur compound (VSC) levels in the individual.

[0017] In any aspect or embodiment, the individual may have an intra-oral volatile sulphur compound (VSC) level of at least 150 ppb (parts per billion). Preferably, the individual has an intra-oral VSC level of at least 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 ppb.

[0018] In another aspect, the present invention provides a method of inhibiting the activity of enzymes, preferably proteases, from oral bacteria, the method comprising administering stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) to the oral cavity of the individual, thereby inhibiting the activity of enzymes, preferably proteases, from oral bacteria. Preferably the oral bacteria are those involved in the production of VSCs. Preferably the oral bacteria are Gram-negative oral bacterium associated with halitosis.

[0019] In another aspect, the present invention provides use of a stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) in the manufacture of a medicament for treating or preventing halitosis in an individual in need thereof.

[0020] In another aspect, the present invention provides a stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) for use in the treatment or prevention of halitosis in an individual in need thereof.

[0021] In another aspect, the present invention provides use of a stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) in the manufacture of a medicament for reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual in need thereof.

[0022] In another aspect, the present invention provides a stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) for use in reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual in need thereof.

[0023] In another aspect, the present invention provides use of a stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) in the manufacture of a medicament for inhibiting the activity of enzymes, preferably proteases, from oral bacteria. Preferably, the medicament is formulated for or adapted for administration to the oral cavity. Preferably the oral bacteria are Gram-negative oral bacterium associated with halitosis.

[0024] In another aspect, the present invention provides a stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) for use in inhibiting the activity of enzymes, preferably proteases, from oral bacteria. Preferably, the stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) is administered to the oral cavity. Preferably the oral bacteria are Gram-negative oral bacterium associated with halitosis.

[0025] In another aspect, the present invention provides a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex.

[0026] In any aspect or embodiment, the zinc-associated stabilized ACP or ACFP complex has a zinc ion content of, or at least, 0.5 mole of zinc per mole of phosphopeptide. Preferably, the zinc-associated stabilized ACP or ACFP complex has a zinc ion content of, or at least, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 moles of zinc per mole of

phosphopeptide. Even more preferably, the zinc ion content is in the range of about 0.5 to about 100, about 0.5 to about 50, about 0.5 to about 20 or about 0.5 to about 10 moles of zinc per mole of phosphopeptide in the water-soluble complex. The zinc ion content may be in the range of 0.5 to 100, 0.5 to 50, 0.5 to 20 or 0.5 to 10 moles of zinc per mole of phosphopeptide.

[0027] In any embodiment, the zinc ion content above may be the zinc ion content tightly-bound to the water-soluble complex (as described herein). In assessing the zinc ion content, the tightly-bound zinc ion content is measured by the methods described herein, in particular, in Example 4.

[0028] In any embodiment, the zinc-associated stabilized ACP and/or ACFP complex comprises zinc ions that remain associated with the complex after centrifugation in a 1000 molecular weight cut off filter at about 3000g for 1 hour at room temperature.

[0029] In any embodiment, the zinc-associated stabilized ACP or ACFP complex has at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of the zinc associated with the complex as tightly bound as determined by the method in Example 4.

[0030] In any embodiment, the zinc-associated stabilized ACP or ACFP complex has at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of the zinc used in preparing the complexes incorporated into the complexes. The complex may be prepared as outlined in Example 1.

[0031] In another aspect, the present invention also provides a composition comprising, consisting essentially of or consisting of, zinc-associated stabilized ACP and/or ACFP complexes as described herein. Preferably, the composition further includes a pharmaceutically acceptable carrier, diluent or excipient.

[0032] In one aspect, the present invention provides a method for treating or preventing halitosis in an individual in need thereof, the method comprising administering to the individual a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex to the oral cavity of the individual, thereby treating or preventing halitosis.

[0033] In another aspect, the present invention provides a method of reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual, the method comprising administering a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex to the oral cavity of the individual, thereby reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual.

[0034] In another aspect, the present invention provides a method of inhibiting the activity of enzymes, preferably proteases, from oral bacteria, the method comprising administering a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex to the oral cavity of the individual, thereby inhibiting the activity of enzymes, preferably proteases, from oral bacteria. Preferably the oral bacteria are those involved in the production of VSCs. Preferably the oral bacteria are Gram-negative oral bacterium associated with halitosis.

[0035] In another aspect, the present invention provides use of a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex in the manufacture of a medicament for treating or preventing halitosis in an individual in need thereof.

[0036] In another aspect, the present invention provides a zinc-associated stabilized amorphous calcium phosphate (ACP) complex and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex for use in the treatment or prevention of halitosis in an individual in need thereof.

[0037] In another aspect, the present invention provides use of a zinc-associated stabilized amorphous calcium phosphate (ACP) complex and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex in the manufacture of a medicament for reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual in need thereof.

[0038] In another aspect, the present invention provides a zinc-associated stabilized amorphous calcium phosphate (ACP) complex and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex for use in reducing of the

production of volatile sulphur compounds (VSCs) in the oral cavity of an individual in need thereof.

[0039] In another aspect, the present invention provides use of a zinc-associated stabilized amorphous calcium phosphate (ACP) complex and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex in the manufacture of a medicament for inhibiting the activity of enzymes, preferably proteases, from oral bacteria. Preferably, the medicament is formulated for or adapted for administration to the oral cavity. Preferably the oral bacteria are Gram-negative oral bacterium associated with halitosis.

[0040] In another aspect, the present invention provides a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex for use in inhibiting the activity of enzymes, preferably proteases, from oral bacteria. Preferably, the zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex is administered to the oral cavity. Preferably the oral bacteria are Gram-negative oral bacterium associated with halitosis.

[0041] In any embodiment, the method or use of the invention results in a reduction of intra-oral VSCs level of at least 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 ppb. Alternatively, or in addition, the method or use of the invention results in a reduction in the individual's Rosenberg scale measurement by at least 1. VSCs may be measured using any method described herein, including in the Examples, and may be measured at least 30 minutes, 1 hour, 2 hours, 3 hours or 4 hours after application of stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP); a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex; or a composition comprising stabilized ACP and/or ACP, or zinc-associated stabilized ACP and/or zinc-associated stabilized ACFP. In one embodiment, the reduction is observed after 14 days of treatment.

[0042] In any embodiment, the stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) is phosphopeptide stabilized. Preferably, the phosphopeptide (as defined below) is a casein phosphopeptide.

[0043] In any embodiment, the zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated amorphous calcium fluoride phosphate (ACFP) complex is phosphopeptide stabilized. Preferably, the phosphopeptide (as defined below) is a casein phosphopeptide.

[0044] In any method or use of the invention, the stabilised ACP or ACFP complex may be administered to the individual for 5 to 60 minutes, 10 to 45 minutes, 10 to 30 minutes or 20 minutes. Further, the stabilised ACP or ACFP complex may be administered 4, 5 or 6 times per day, or per 24 hour period. Preferably, the stabilised ACP or ACFP complex is administered for a 1 to 2 week period.

[0045] In any method or use of the invention, the zinc-associated stabilised ACP or ACFP complex may be administered to the individual for 5 to 60 minutes, 10 to 45 minutes, 10 to 30 minutes or 20 minutes. Further, the zinc-associated stabilised ACP or ACFP complex may be administered 4, 5 or 6 times per day, or per 24 hour period. Preferably, the zinc-associated stabilised ACP or ACFP complex is administered for a 1 to 2 week period.

[0046] In any method or use of the invention, the stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) may be administered to the oral cavity in a composition, for example a composition as described herein.

[0047] In any method or use of the invention, the zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex may be administered to the oral cavity in a composition, for example a composition as described herein.

[0048] In any aspect, the composition may be prepared and used in various forms applicable to the mouth such as dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, mouthrinses, mouth sprays, varnish, dental cement, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs including yoghurt. Preferably, the composition is a mouthwash. Preferably, the composition is a mint or chewing gum, preferably a sugar-free mint or sugar-free chewing gum.

[0049] In any aspect or embodiment, the calcium ion content of the stabilised ACP or ACFP complex is greater than about 30 moles per mole of PP. Preferably, the calcium ion content is in the range of about 30 to 100 moles of calcium per mole of PP. More preferably, the calcium ion content is in the range of about 30 to about 50 moles of calcium per mole of PP.

[0050] In any aspect or embodiment, the calcium ion content of the zinc-associated stabilised ACP or ACFP complex is greater than about 30 moles per mole of PP. Preferably, the calcium ion content is in the range of about 30 to 100 moles of calcium per mole of PP. More preferably, the calcium ion content is in the range of about 30 to about 50 moles of calcium per mole of PP.

[0051] In any aspect, the stabilised ACP complex is a stannous-associated phosphopeptide (PP) stabilized amorphous calcium phosphate (ACP) complex, and the stabilised ACFP complex is a stannous-associated phosphopeptide (PP) stabilized amorphous calcium fluoride phosphate (ACFP) complex.

[0052] In any aspect, the stabilized ACP complex is a non-stannous-associated stabilized ACP complex, and the stabilized ACFP complex is a non-stannous-associated stabilized ACFP complex.

[0053] In any aspect or embodiment, the phase of the ACP is primarily (i.e. >50%) a basic phase, wherein the ACP comprises predominantly the species Ca^{2+} , PO_4^{3-} and OH^- . The basic phase of ACP may have the general formula $[\text{Ca}_3(\text{PO}_4)_2]_x[\text{Ca}_2(\text{PO}_4)(\text{OH})]$ where $x \geq 1$. Preferably $x = 1-5$. More preferably, $x = 1$, i.e. the two components of the formula are present in equal proportions. Accordingly, in one embodiment, the basic phase of ACP has the formula $\text{Ca}_3(\text{PO}_4)_2\text{Ca}_2(\text{PO}_4)(\text{OH})$.

[0054] In any aspect or embodiment, the phase of the ACFP is a primarily (i.e. >50%) basic phase, wherein the ACFP comprises predominantly the species Ca^{2+} , PO_4^{3-} and F^- . The basic phase of ACFP may have the general formula $[\text{Ca}_3(\text{PO}_4)_2]_x[\text{Ca}_2(\text{PO}_4)\text{F}]_y$ where $x \geq 1$ when $y = 1$ or where $y \geq 1$ when $x = 1$. Preferably, $y = 1$ and $x = 1-3$. More preferably, $y = 1$ and $x = 1$, i.e. the two components of the formula are present in equal proportions. Accordingly, in one embodiment, the basic phase of ACFP has the formula $\text{Ca}_3(\text{PO}_4)_2\text{Ca}_2(\text{PO}_4)\text{F}$.

[0055] In one embodiment, the ACP complex consists essentially of phosphopeptides, calcium, phosphate and hydroxide ions and water. Preferably, the complex further includes zinc ions.

[0056] In one embodiment, the ACFP complex consists essentially of phosphopeptides, calcium, phosphate, fluoride and hydroxide ions and water. Preferably, the complex further zinc stannous ions.

[0057] In another aspect, the invention also relates to a kit for use in a method or use of the invention, the kit comprising:

- (a) a composition as described herein, or
- (b) a stabilized ACP or ACFP complex as described herein.

[0058] In another aspect, the invention also relates to a kit for use in a method or use of the invention, the kit comprising:

- (a) a composition as described herein, or
- (b) a zinc-associated stabilized ACP or stabilized ACFP complex as described herein.

[0059] Preferably the kit further includes instructions for using the kit in a method or use of the invention.

[0060] In another aspect, the present invention provides a method or process for forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex, the method or process comprising or consisting of:

- (i) obtaining a solution comprising at least one phosphopeptide and;
- (ii) admixing solutions comprising phosphopeptides, calcium ions, phosphate ions, hydroxide ions and optionally fluoride ions, while maintaining the pH at about 7 or above, preferably about 9, to form a solution comprising phosphopeptide stabilized ACP or ACFP; and

(iii) admixing a water-soluble zinc salt with the solution comprising phosphopeptide stabilized ACP or ACFP at a pH of 5.5 to 7.5 with slow addition, mixing and maintaining the solution pH,

thereby forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex.

[0061] In one embodiment, the solutions are added slowly at a rate of less than about 1% volume addition per minute with adequate mixing.

[0062] In another aspect, the present invention provides a method or process for forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex, the method or process comprising or consisting of:

- (i) providing a solution of phosphopeptide stabilized ACP or ACFP; and
- (ii) admixing a water-soluble zinc salt with the solution,

thereby forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex.

[0063] In any aspect or embodiment, the water-soluble zinc salt is in the form of zinc acetate, zinc lactate, zinc sulphate, zinc nitrate, zinc gluconate, zinc chloride, zinc citrate or any other water-soluble zinc salt.

[0064] As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

[0065] Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

Detailed description of the embodiments

[0066] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual

features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

[0067] Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

[0068] Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

[0069] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[0070] All of the patents and publications referred to herein are incorporated by reference in their entirety.

[0071] For purposes of interpreting this specification, terms used in the singular will also include the plural and vice versa.

[0072] As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps. As used herein, except where the context requires otherwise, "comprise" and "include" can be used interchangeably.

Halitosis

[0073] The terms "halitosis", "bad breath", "oral malodour", "breath malodour", "feter oris", or "fege bosta" can be used interchangeably and refer to a disorder in which a noticeably unpleasant odour is present on the exhaled breath.

[0074] The vast majority of oral malodor originates with the anaerobic bacterial degradation of sulphur containing amino acids within the oral cavity, resulting in the emission of hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3), collectively referred to as volatile sulphur compounds (VSC). In any aspect or embodiment of the present invention, the VSC may be one or more of hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3).

[0075] Halitosis (bad breath) can be measured in a number of ways, including studies using organoleptic intensity and organoleptic hedonic indices and instruments that quantitate the amount of volatile sulphur compounds or bacterial enzymes that contribute to the production of odiferous compounds.

[0076] Organoleptic measurements or the use of one's nose to smell and rank the intensity of odors are considered the criterion standard for the measurement of malodor. Either a 5-point or a 10-point scale can be used and, usually, 2 separate judges evaluate the degree of halitosis (bad breath). The intensity of halitosis (bad breath) is based on the Rosenberg scale for halitosis (Rosenberg et al., *J Periodontol*, 1991; 62:487-489), which rates odor intensity and is as follows:

- 0 - Odor cannot be detected
- 1 - Questionable malodor, barely detectable
- 2 - Slight malodor, exceeds the threshold of malodor recognition
- 3 - Malodor is definitely detected
- 4 - Strong malodor
- 5 - Very strong malodor

[0077] Intra-oral volatile sulphur compounds (VSCs) can be measured using electrochemical sensors (e.g., Halimeter[®]) and portable gas chromatographs (e.g., OralChroma[®], TwinBreather[®]), which are well known in the art. The concentration of VSCs is typically measured in parts per billion (ppb).

[0078] An individual may be determined to have, or be at risk of suffering from, halitosis if they are determined to contain elevated numbers of Gram-negative proteolytic bacteria on the dorsum of the tongue or in dental plaque. The elevated

numbers may be compared to numbers of the same bacterial species in individuals known to be not suffering from halitosis (i.e. healthy controls).

Zinc-associated stabilized amorphous calcium phosphate (ACP) or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex

[0079] As described herein, the present invention provides a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex.

[0080] In any aspect or embodiment, the zinc-associated stabilized ACP or ACFP complex has a zinc ion content of, or at least, 0.5 mole of zinc per mole of phosphopeptide. Preferably, the zinc-associated stabilized ACP or ACFP complex has a zinc ion content of, or at least, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 moles of zinc per mole of phosphopeptide. Even more preferably, the zinc ion content is in the range of about 0.5 to about 100, about 0.5 to about 50, about 0.5 to about 20 or about 0.5 to about 10 moles of zinc per mole of phosphopeptide. The zinc ion content may be in the range of 0.5 to 100, 0.5 to 50, 0.5 to 20 or 0.5 to 10 moles of zinc per mole of phosphopeptide.

[0081] In any embodiment, the zinc ion content above may be the zinc ion content tightly-bound to the complex (as described herein). In assessing the zinc ion content, the tightly-bound zinc ion content is measured by the methods described herein, in particular, in Example 4.

[0082] In any embodiment, the zinc-associated stabilized ACP and/or ACFP complex comprises zinc ions that remain associated with the complex after centrifugation in a 1000 molecular weight cut off filter at about 3000g for 1 hour at room temperature.

[0083] In any embodiment, the zinc-associated stabilized ACP or ACFP complex has at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of the zinc associated with the complex as tightly bound, for example as determined by the method in Example 4.

[0084] In any embodiment, the zinc-associated stabilized ACP or ACFP complex has at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of the zinc used in preparing the complexes incorporated into the complexes. The complex may be prepared as outlined in Example 1.

[0085] In another aspect, the present invention also provides a composition comprising, consisting essentially of or consisting of, zinc-associated stabilized ACP and/or ACFP complexes as described herein.

[0086] In any aspect or embodiment, the zinc ion is Zn^{2+} . Typically, during production of the zinc-associated stabilized ACP and/or ACFP complexes the zinc ion is provided by $\text{Zn}(\text{NO}_3)_2$ or any other water soluble zinc salt, including those described herein.

Stabilized-ACP or ACFP

[0087] A stabilized amorphous calcium phosphate (ACP) and a stabilized amorphous calcium fluoride phosphate (ACFP) may also be referred to herein as a stabilized ACP complex and a stabilized ACFP complex respectively.

[0088] A stabilized-ACP or ACFP complex as referred to herein includes a stabilized-ACP or ACFP complex as described in PCT/AU2005/001781 the contents of which are incorporated by reference.

[0089] In a preferred embodiment, the phosphopeptide stabilised amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) complex has tightly bound and loosely bound calcium, wherein the bound calcium in the complex is less than the tightly bound calcium in an ACP or ACFP complex formed at a pH of 7.0. Optionally, the ACP or ACFP is predominantly in a basic form.

[0090] A stabilized-ACP or ACFP complex as referred to herein include a stabilized-ACP or ACFP complex formed at a pH of below 7.0. Preferably the complex is formed at a pH in the range of about 5.0 up to but below 7.0. More preferably the complex is formed at a pH range of about 5.0 to about 6.0. In a preferred embodiment, the complex is formed at a pH of about 5.5. Preferably, the ACP or ACFP in the complex is predominantly in a basic form.

[0091] A stabilized-ACP may be produced by a method comprising the steps of:

- (i) obtaining a solution comprising at least one phosphopeptide and;
- (ii) admixing solutions comprising calcium ions, phosphate ions and hydroxide ions, while maintaining the pH at about 7.0 or below.

[0092] A stabilised ACFP may be produced by a method comprising the steps of:

- (i) obtaining a solution comprising at least one phosphopeptide and;
- (ii) admixing solutions comprising calcium ions, phosphate ions, hydroxide ions and fluoride ions, while maintaining the pH at about 7.0 or below.

[0093] A phosphopeptide stabilised amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) complex may also include wherein the ACP in the complex has tightly bound and loosely calcium, wherein the tightly bound calcium in the complex is less than the tightly bound calcium in an ACP or ACFP complex formed at a pH of 7.0 and the ACP or ACFP is predominantly in a basic form, obtainable or obtained by a method comprising:

admixing a first solution comprising calcium ions, a second solution comprising phosphate ions, and optionally a third solution comprising fluoride ions, to a solution comprising phosphopeptides and a solvent with a pH of from about 5 up to but below 7; and maintaining the pH of the solution at about 5.0 up to but below 7.0 during the admixing by adding hydroxide ions.

[0094] “Tightly” and “loosely” bound calcium and phosphate in ACP or ACFP can be determined using analytical ultrafiltration. Briefly, the solution of phosphopeptide, calcium, phosphate and optionally fluoride admixed while maintaining the pH at about 7.0 or below can be first filtered through a 0.1 micron filter to remove free calcium and phosphate that is not associated with the complexes. This free calcium and phosphate is present in the filtrate and discarded. Any free calcium or phosphate that is not associated in any way with the complexes would not be bioavailable, i.e. delivered by the phosphopeptide to the tooth. The retentate from the 0.1 micron filtration can be further analyzed by centrifugation through a 3000 mw cutoff filter at 1,000 g for 15 min. The resulting filtrate contains calcium and phosphate that is loosely bound or associated with the complexes. At this centrifugal force calcium and phosphate that is not tightly bound to the complexes are released and move to into the filtrate. The Ca and Pi that is tightly bound in the complexes is retained in the retentate. The amount of tightly bound Ca and Pi in the retentate can then be determined by subtracting the amount of Ca and Pi in the filtrate from the total amount of Ca and Pi in the retentate of the 0.1 micron filtration.

[0095] A stabilized-ACP or ACFP complex as referred to herein include a stabilized-ACP or ACFP complex as described in PCT/AU2006/000885 the contents of which are incorporated by reference.

[0096] A “superloaded” phosphopeptide or phosphoprotein (PP) stabilized-amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) complex. The complex may be formed at any pH (eg 3-10). Preferably the phosphopeptide includes the sequence -A-B-C-, where A is a phosphoamino acid, preferably phosphoserine, B is any amino acid including a phosphoamino acid and C is glutamic acid, aspartic acid or a phosphoamino acid. The phosphoamino acid may be phosphoserine. The PP is superloaded with calcium and phosphate ions. The calcium ions may be in the range 30-1000 mol Ca per mole of PP, or in the range of 30-100 or 30-50 mole Ca per mole of PP. In another embodiment, the mol Ca per mol of PP is at least 25, 30, 35, 40, 45 or 50.

[0097] The phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate or amorphous calcium fluoride phosphate complex may have a calcium ion content greater than about 30 moles of calcium per mole of PP. In a preferred embodiment, the calcium ion content is in the range of about 30 to 100 moles of calcium per mole of PP. More preferably, the calcium ion content is in the range of about 30 to about 50 moles of calcium per mole of PP.

[0098] The phosphopeptide or phosphoprotein (PP) stabilized-amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) complex may be produced by a method comprising the steps of:

- (i) obtaining solutions comprising calcium, inorganic phosphate and fluoride (optional); and
- (ii) admixing (i) with a solution comprising PP-ACP.

[0099] In a preferred embodiment, the PP is casein phosphopeptide (CPP).

[0100] The PP stabilized ACP and/or ACFP complex may further include at least an equal amount by weight of calcium phosphate. Preferably the calcium phosphate is CaHPO_4 . Preferably, the calcium phosphate (e.g. CaHPO_4) is dry blended with the PP stabilized ACP and/or ACFP complex. In a preferred embodiment, the PP-ACP and/or

PP-ACFP complex: calcium phosphate ratio is about 1:1-50. more preferably about 1: 1-25, more preferably about 1:5-15. In one embodiment, the PP-ACP and/or PP-ACFP complex: calcium phosphate ratio is about 1:10.

[0101] The oral care formulation that includes a phosphopeptide or phosphoprotein (PP) stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex having a calcium ion content greater than about 30 moles of calcium per mole of PP when used in the oral cavity may be produced by a method including the steps of:

- (i) obtaining a powder including a PP-ACP and/or PP-ACFP complex;
- (ii) dry blending with an effective amount of calcium phosphate; and
- (iii) formulating the dry blended PP-ACP and/or PP-ACFP and calcium phosphate mixture into an oral care formulation.

[0102] Preferably, the form of calcium phosphate for dry blending is any soluble calcium phosphate including, but not limited to, CaHPO_4 , Ca_2HPO_4 and calcium lactate.

[0103] A composition as described herein may further include free fluoride ions. The fluoride ions may be from any suitable source. A source of fluoride ions may include free fluoride ions or fluoride salts. Examples of sources of fluoride ions include, but are not limited to the following: sodium fluoride, sodium monofluorophosphate, stannous fluoride, sodium silicofluoride and amine fluoride. These may be provided in solution (typically an aqueous solution), or a suspension.

[0104] The fluoride ions are preferably present in the composition in an amount greater than 1ppm. More preferably, the amount is more than 3 ppm. In another embodiment, it is preferably more than 10 ppm. In typical embodiments described below, the amount may be several hundred or thousand ppm. The fluoride content is typically measured as a ppm in oral compositions in the manner commonly used in the art. Where the fluoride is provided from a source with the stabilized ACP, the ppm refers to the concentration of the fluoride in that source, typically a solution or suspension of bioavailable fluoride.

[0105] A stannous-associated ACP or ACFP complex as referred to herein include any described in PCT/AU2014/050447, the entire contents of which are incorporated by reference in its entirety.

[0106] A composition as described herein for use in a method of use of the invention may include a stannous-associated ACP or ACFP complex. The composition may include 2% CPP-ACP and 290ppm fluoride with 220ppm fluoride as stannous fluoride and 70ppm as sodium fluoride.

[0107] "Phosphopeptide" in the context of the description of this invention means an amino acid sequence in which at least one amino acid is phosphorylated. Preferably, the phosphopeptide includes one or more of the amino acid sequence -A-B-C-, where A is a phosphoamino residue, B is any amino acyl residue including a phosphoamino residue and C is selected from a glutamyl, aspartyl or phosphoamino residue. Any of the phosphoamino residues may independently be a phosphoserine residue. B is desirably a residue the side-chain of which is neither relatively large nor hydrophobic. It may be Gly, Ala, Val, Met, Leu, Ile, Ser, Thr, Cys, Asp, Glu, Asn, Gln or Lys.

[0108] In another embodiment, at least two of the phosphoamino acids in the sequence are preferably contiguous. Preferably the phosphopeptide includes the sequence A-B-C-D-E, where A, B, C, D and E are independently phosphoserine, phosphothreonine, phosphotyrosine, phosphohistidine, glutamic acid or aspartic acid, and at least two, preferably three, of the A, B, C, D and E are a phosphoamino acid. In a preferred embodiment, the phosphoamino acid residues are phosphoserine, most preferably three contiguous phosphoserine residues. It is also preferred that D and E are independently glutamic or aspartic acid.

[0109] In one embodiment, the ACP or ACFP is stabilized by a casein phosphopeptide (CPP), which is in the form of intact casein or fragment of the casein, and the complex formed preferably has the formula $[CPP(ACP)_8]_n$ or $[(CPP)(ACFP)_8]_n$ where n is equal to or greater than 1, for example 6. The complex formed may be a colloidal complex, where the core particles aggregate to form large (eg 100 nm) colloidal particles suspended in water. Thus, the PP can be a casein protein or a phosphopeptide.

[0110] The PP may be from any source; it may be present in the context of a larger polypeptide, including a full length casein polypeptide, or it may be isolated by tryptic or other enzymatic or chemical digestion of casein, or other phosphoamino acid rich proteins such as phosvitin, osteopontin or by chemical or recombinant synthesis, provided that it comprises the sequence -A-B-C- or A-B-C-D-E as described above. The sequence flanking this core sequence may be any sequence. However, those flanking sequences in α_{s1} (59-79), β (1-25), α_{s2} (46-70) and α_{s2} (1-21) are preferred. The flanking sequences may optionally be modified by deletion, addition or conservative substitution of one or more residues. The amino acid composition and sequence of the flanking region are not critical. Preferably, the PP is obtained from a food source, e.g phosvitin from eggs, osteopontin from milk, or any chemical or recombinantly synthesised PP comprising the sequence -A-B-C-.

[0111] In one embodiment, the ACP or ACFP is stabilized by a casein phosphopeptide (CPP), osteopontin phosphopeptide (OPN-PP), phosvitin phosphopeptide (PPP) or a chemical or recombinantly synthesised PP comprising the sequence -A-B-C-. Preferably the ACP or ACFP is stabilized by casein phosphopeptide (CPP).

[0112] Examples of conservative substitutions are shown in Table A below.

[0113] Table A

Original Residue	Exemplary Conservative Substitution	Preferred Conservative Substitution
Ala	Val, Leu, Ile	Val
Asn	Gln Lys His Phe	Gln
Gln	Asn	Asn
Gly	Pro	Pro
Ile	Leu, Val, Met, Ala, Phe	Leu
Leu	Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Val	Ile, Leu, Met, Phe, Ala	Leu
Asp	Glu	Glu
Thr	Ser	Ser
Trp	Tyr	Tyr
Tyr	Trp Phe Thr Ser	Phe

[0114] The flanking sequences may also include non-naturally occurring amino acid residues. Commonly encountered amino acids which are not encoded by the genetic code, include:

2-amino-4-phosphonobutyric acid for Phosphoserine [Ser(P)];

2-amino adipic acid (Aad) for Glu and Asp;

2-aminopimelic acid (Apm) for Glu and Asp;

2-aminobutyric (Abu) acid for Met, Leu, and other aliphatic amino acids;

2-aminoheptanoic acid (Ahe) for Met, Leu and other aliphatic amino acids;

2-aminoisobutyric acid (Aib) for Gly;

cyclohexylalanine (Cha) for Val, and Leu and Ile;

homoarginine (Har) for Arg and Lys;

2, 3-diaminopropionic acid (Dpr) for Lys, Arg and His;

N-ethylglycine (EtGly) for Gly, Pro, and Ala;

N-ethylasparagine (EtAsn) for Asn, and Gln;

Hydroxyllysine (Hyl) for Lys;

allohydroxyllysine (AHyl) for Lys;

3-(and 4) hydroxyproline (3Hyp, 4Hyp) for Pro, Ser, and Thr;

alloisoleucine (Alle) for Ile, Leu, and Val;

p-amidinophenylalanine for Ala;

N-methylglycine (MeGly, sarcosine) for Gly, Pro, Ala.

N-methylisoleucine (Melle) for Ile;

Norvaline (Nva) for Met and other aliphatic amino acids;

Norleucine (Nle) for Met and other aliphatic amino acids;

Ornithine (Orn) for Lys, Arg and His;

Citrulline (Cit) and methionine sulfoxide (MSO) for Thr, Asn and Gln;

N-methylphenylalanine (MePhe), trimethylphenylalanine, halo (F, Cl, Br and I) phenylalanine, triflourylphenylalanine, for Phe.

[0115] In one embodiment, the PP is one or more phosphopeptides selected from the group consisting of $\alpha_{s1}(59-79)$ [1], $\beta(1-25)$ [2], $\alpha_{s2}(46-70)$ [3] and $\alpha_{s2}(1-21)$ [4]:

[1] Gln⁵⁹-Met-Glu-Ala-Glu-Ser(P)-Ile-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ile-Val-Pro-Asn-Ser(P)-Val-Glu-Gln-Lys⁷⁹ $\alpha_{s1}(59-79)$ (SEQ ID NO: 1)

[2] Arg¹-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(P)-Leu-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr-Arg²⁵ $\beta(1-25)$ (SEQ ID NO: 2)

[3] Asn⁴⁶-Ala-Asn-Glu-Glu-Glu-Tyr-Ser-Ile-Gly-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser(P)-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys⁷⁰ $\alpha_{s2}(46-70)$ (SEQ ID NO: 3)

[4] Lys¹-Asn-Thr-Met-Glu-His-Val-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Ile-Ser(P)-Gln-Glu-Thr-Tyr-Lys²¹ $\alpha_{s2}(1-21)$ (SEQ ID NO: 4).

Oral compositions

[0116] In another embodiment of the invention, the stabilized ACP and/or stabilized ACFP complex is incorporated into oral compositions such as toothpaste, mouth washes or formulations for the mouth to aid in the prevention and/or treatment of gingivitis or periodontitis. The oral compositions comprising an amount of stabilized ACP and/or ACFP sufficient to form a layer on a dental surface, preferably, the layer has a calcium : phosphate ratio equivalent to normal apatite, for example the ratio is about 2:1. The layer may contain an amount of calcium that is about 20 wt%. The stabilized ACP and/or ACFP complexes may comprise 0.01 to 50% by weight of the composition, preferably 1.0 to 50%, preferably 1.0 to 30%, preferably 1.0 to 20%, preferably 1.0 to 10%, preferably 2 to 10% by weight of the composition. In a particularly preferred embodiment, the oral composition of the present invention contains about 2% stabilized ACP or ACFP complexes or a mixture of both. The oral composition of this invention which contains the above-mentioned agents may be prepared and used in various forms applicable to the mouth such as dentifrice including toothpastes,

toothpowders and liquid dentifrices, mouthwashes, mouthrinses, mouth sprays, varnish, dental cement, troches, chewing gums, mints, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs, including yogurt and confectionery. The oral composition according to this invention may further include additional well known ingredients depending on the type and form of a particular oral composition. Certain compositions of the invention such as toothpastes, toothpowders and liquid dentifrices, mouthwashes, mouthrinses and mouth sprays have relatively low viscosity and have a positive effect on treatment or prevention without significant residence time in the oral cavity.

[0117] In certain preferred forms of the invention an oral composition may be substantially liquid in character, such as a mouthwash, rinse or spray. In such a preparation the vehicle is typically a water-alcohol mixture desirably including a humectant as described below. Generally, the weight ratio of water to alcohol is in the range of from about 1:1 to about 20:1. The total amount of water-alcohol mixture in this type of preparation is typically in the range of from about 70 to about 99.9% by weight of the preparation. The alcohol is typically ethanol or isopropanol. Ethanol is preferred.

[0118] In other desirable forms of this invention, the composition may be substantially solid or pasty in character, such as toothpowder, a dental tablet or a toothpaste (dental cream) or gel dentifrice. The vehicle of such solid or pasty oral preparations generally contains dentally acceptable polishing material. Examples of polishing materials are water-insoluble sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated calcium phosphate, anhydrous dicalcium phosphate, calcium pyrophosphate, magnesium orthophosphate, trimagnesium phosphate, calcium carbonate, hydrated alumina, calcined alumina, aluminium silicate, zirconium silicate, silica, bentonite, and mixtures thereof. Other suitable polishing material include the particulate thermosetting resins such as melamine-, phenolic, and urea-formaldehydes, and cross-linked polyepoxides and polyesters. Preferred polishing materials include crystalline silica having particle sizes of up to about 5 microns, a mean particle size of up to about 1.1 microns, and a surface area of up to about 50,000 cm²/g., silica gel or colloidal silica, and complex amorphous alkali metal aluminosilicate.

[0119] When visually clear gels are employed, a polishing agent of colloidal silica, such as those sold under the trademark SYLOID as Syloid 72 and Syloid 74 or under the trademark SANTOCEL as Santocel 100, alkali metal aluminosilicate complexes are

particularly useful since they have refractive indices close to the refractive indices of gelling agent-liquid (including water and/or humectant) systems commonly used in dentifrices.

[0120] Many of the so-called "water insoluble" polishing materials are anionic in character and also include small amounts of soluble material. Thus, insoluble sodium metaphosphate may be formed in any suitable manner, for example as illustrated by Thorpe's Dictionary of Applied Chemistry, Volume 9, 4th Edition, pp. 510-511. The forms of insoluble sodium metaphosphate known as Madrell's salt and Kurrol's salt are further examples of suitable materials. These metaphosphate salts exhibit only a minute solubility in water, and therefore are commonly referred to as insoluble metaphosphates (IMP). There is present therein a minor amount of soluble phosphate material as impurities, usually a few percent such as up to 4% by weight. The amount of soluble phosphate material, which is believed to include a soluble sodium trimetaphosphate in the case of insoluble metaphosphate, may be reduced or eliminated by washing with water if desired. The insoluble alkali metal metaphosphate is typically employed in powder form of a particle size such that no more than 1% of the material is larger than 37 microns.

[0121] The polishing material is generally present in the solid or pasty compositions in weight concentrations of about 10% to about 99%. Preferably, it is present in amounts from about 10% to about 75% in toothpaste, and from about 70% to about 99% in toothpowder. In toothpastes, when the polishing material is silicious in nature, it is generally present in an amount of about 10-30% by weight. Other polishing materials are typically present in amount of about 30-75% by weight.

[0122] In a toothpaste, the liquid vehicle may comprise water and humectant typically in an amount ranging from about 10% to about 80% by weight of the preparation. Glycerine, propylene glycol, sorbitol and polypropylene glycol exemplify suitable humectants/carriers. Also advantageous are liquid mixtures of water, glycerine and sorbitol. In clear gels where the refractive index is an important consideration, about 2.5 - 30% w/w of water, 0 to about 70% w/w of glycerine and about 20-80% w/w of sorbitol are preferably employed.

[0123] Toothpaste, creams and gels typically contain a natural or synthetic thickener or gelling agent in proportions of about 0.1 to about 10, preferably about 0.5 to about

5% w/w. A suitable thickener is synthetic hectorite, a synthetic colloidal magnesium alkali metal silicate complex clay available for example as Laponite (e.g. CP, SP 2002, D) marketed by Laporte Industries Limited. Laponite D is, approximately by weight 58.00% SiO₂, 25.40% MgO, 3.05% Na₂O, 0.98% Li₂O, and some water and trace metals. Its true specific gravity is 2.53 and it has an apparent bulk density of 1.0 g/ml at 8% moisture.

[0124] Other suitable thickeners include Irish moss, iota carrageenan, gum tragacanth, starch, polyvinylpyrrolidone, hydroxyethylpropylcellulose, hydroxybutyl methyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose (e.g. available as Natrosol), sodium carboxymethyl cellulose, and colloidal silica such as finely ground Syloid (e.g. 244). Solubilizing agents may also be included such as humectant polyols such propylene glycol, dipropylene glycol and hexylene glycol, cellosolves such as methyl cellosolve and ethyl cellosolve, vegetable oils and waxes containing at least about 12 carbons in a straight chain such as olive oil, castor oil and petrolatum and esters such as amyl acetate, ethyl acetate and benzyl benzoate.

[0125] It will be understood that, as is conventional, the oral preparations will usually be sold or otherwise distributed in suitable labelled packages. Thus, a jar of mouth rinse will have a label describing it, in substance, as a mouth rinse or mouthwash and having directions for its use; and a toothpaste, cream or gel will usually be in a collapsible tube, typically aluminium, lined lead or plastic, or other squeeze, pump or pressurized dispenser for metering out the contents, having a label describing it, in substance, as a toothpaste, gel or dental cream.

[0126] Organic surface-active agents may be used in the compositions of the present invention to achieve increased prophylactic action, assist in achieving thorough and complete dispersion of the active agent throughout the oral cavity, and render the instant compositions more cosmetically acceptable. The organic surface-active material is preferably anionic, non-ionic or ampholytic in nature and preferably does not interact with the active agent. It is preferred to employ as the surface-active agent a detergent material which imparts to the composition detergent and foaming properties. Suitable examples of anionic surfactants are water-soluble salts of higher fatty acid monoglyceride monosulfates, such as the sodium salt of the monosulfated monoglyceride of hydrogenated coconut oil fatty acids, higher alkyl sulfates such as sodium lauryl sulfate, alkyl aryl sulfonates such as sodium dodecyl benzene sulfonate,

higher alkylsulfo-acetates, higher fatty acid esters of 1,2-dihydroxy propane sulfonate, and the substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds, such as those having 12 to 16 carbons in the fatty acid, alkyl or acyl radicals, and the like. Examples of the last mentioned amides are N-lauroyl sarcosine, and the sodium, potassium, and ethanolamine salts of N-lauroyl, N-myristoyl, or N-palmitoyl sarcosine which should be substantially free from soap or similar higher fatty acid material. The use of these sarconite compounds in the oral compositions of the present invention is particularly advantageous since these materials exhibit a prolonged marked effect in the inhibition of acid formation in the oral cavity due to carbohydrates breakdown in addition to exerting some reduction in the solubility of tooth enamel in acid solutions. Examples of water-soluble non-ionic surfactants suitable for use are condensation products of ethylene oxide with various reactive hydrogen-containing compounds reactive therewith having long hydrophobic chains (e.g. aliphatic chains of about 12 to 20 carbon atoms), which condensation products ("ethoxamers") contain hydrophilic polyoxyethylene moieties, such as condensation products of poly (ethylene oxide) with fatty acids, fatty alcohols, fatty amides, polyhydric alcohols (e.g. sorbitan monostearate) and polypropyleneoxide (e.g. Pluronic materials).

[0127] The surface active agent is typically present in amount of about 0.1-5% by weight. It is noteworthy, that the surface active agent may assist in the dissolving of the active agent of the invention and thereby diminish the amount of solubilizing humectant needed.

[0128] Various other materials may be incorporated in the oral preparations of this invention such as whitening agents, preservatives, silicones, chlorophyll compounds and/or ammoniated material such as urea, diammonium phosphate, and mixtures thereof. These adjuvants, where present, are incorporated in the preparations in amounts which do not substantially adversely affect the properties and characteristics desired.

[0129] Any suitable flavouring or sweetening material may also be employed. Examples of suitable flavouring constituents are flavouring oils, e.g. oil of spearmint, peppermint, wintergreen, sassafras, clove, sage, eucalyptus, marjoram, cinnamon, lemon, and orange, and methyl salicylate. Suitable sweetening agents include sucrose, lactose, maltose, sorbitol, xylitol, sodium cyclamate, perillartine, AMP (aspartyl phenyl

alanine, methyl ester), saccharine, and the like. Suitably, flavour and sweetening agents may each or together comprise from about 0.1% to 5% more of the preparation.

[0130] The compositions of this invention can also be incorporated in lozenges, or in chewing gum or other products such as breath-freshening mints, e.g. by stirring into a warm gum base or coating the outer surface of a gum base, illustrative of which are jelutong, rubber latex, vinylite resins, etc., desirably with conventional plasticizers or softeners, sugar or other sweeteners or such as glucose, sorbitol and the like. The composition of the invention may be a dual phase composition wherein each phase permits release of components over different time periods.

[0131] An alternative composition may be one that provides stabilized ACP or ACFP and a stannous or zinc salt that then *in situ*, such as the oral cavity, forms stannous-associated stabilized ACP or ACFP, or zinc-associated stabilized ACP or ACFP complexes. An exemplary composition may be a chewing gum that contains stabilized ACP or ACFP in the pellet and a water-soluble stannous or zinc salt in the centre chew.

[0132] In a further aspect, the invention provides compositions including pharmaceutical compositions comprising stabilized ACP or ACFP complexes as described above, such as zinc-associated stabilized ACP or ACFP complexes, together with a compound capable of increasing or maintaining the pH of a solution and a pharmaceutically-acceptable carrier. Such compositions may be selected from the group consisting of dental, anticariogenic compositions and therapeutic compositions. Dental compositions or therapeutic compositions may be in the form of a gel, liquid, solid, powder, cream or lozenge. Therapeutic compositions may also be in the form of tablets or capsules. In one embodiment, the stabilized ACP or ACFP complexes are substantially the only active components of such a composition. For example, a crème formulation may be employed containing: water; glycerol; CPP-ACP/SnF₂; D-sorbitol; silicon dioxide; sodium carboxymethylcellulose (CMC-Na); propylene glycol; titanium dioxide; xylitol; phosphoric acid; guar gum; sodium saccharin; ethyl p-hydroxybenzoate; magnesium oxide; butyl p hydroxybenzoate and propyl p-hydroxybenzoate.

[0133] The invention further includes a formulation described above provided together with instructions for its use to treat or prevent any one or more of dental caries or tooth decay, dental erosion and fluorosis, dentinal hypersensitivity, dental plaque, gingivitis or periodontitis.

[0134] In another embodiment, the compositions of the invention as described herein do not include a phosphate buffer and/or a calcium chelator. For example, any dentifrice described herein may not include a phosphate buffer and/or a calcium chelator.

[0135] In an embodiment of the present invention there is provided a composition, wherein the composition does not include a phosphate buffer and/or calcium chelator.

[0136] In another embodiment, the compositions of the invention as described herein do not include a viscosity regulator, or a viscosity regulator at 0.5 to 50%.

[0137] In another embodiment, the compositions of the invention as described herein do not include sodium carboxymethylcellulose, or 0.01 to 10% sodium carboxymethylcellulose having the esterification degree of 0.7 to 1.0.

[0138] In one embodiment, the active components of the composition consist essentially of the stabilized ACP or ACFP complexes.

[0139] In one embodiment, the active components of the composition consist essentially of the zinc-associated stabilized ACP or ACFP complexes.

[0140] It will be clearly understood that, although this specification refers specifically to applications in humans, the invention is also useful for veterinary purposes. Thus in all aspects the invention is useful for domestic animals such as cattle, sheep, horses and poultry; for companion animals such as cats and dogs; and for zoo animals.

[0141] The invention also provides a kit comprising stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) said kit being adapted for use in the above described methods.

[0142] The kit may include:

- a container holding a composition comprising stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP);
- a label or package insert with instructions for use.

[0143] The invention also provides a kit comprising zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous

calcium fluoride phosphate (ACFP) complexes said kit being adapted for use in the above described methods.

[0144] The kit may include:

- a container holding a composition comprising zinc-associated stabilized amorphous calcium phosphate (ACP) and/or zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complexes;

- a label or package insert with instructions for use.

[0145] In certain embodiments the kit may contain one or more further active principles or ingredients for treatment of a disease or condition.

[0146] The kit may comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a therapeutic composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the therapeutic composition is used for treating the condition of choice. In one embodiment, the label or package insert includes instructions for use and indicates that the therapeutic composition can be used for treatment of the given disease or condition.

[0147] The kit may comprise (a) a therapeutic composition; and (b) a second container with a second active principle or ingredient contained therein. The kit in this embodiment of the invention may further comprise a package insert indicating that the composition and other active principle can be used to treat a disorder or condition as described herein or prevent a complication stemming from dysbiosis. Alternatively, or additionally, the kit may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0148] The invention will now be further described with reference to the following non-limiting examples.

[0149] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

Examples

Example 1 - Preparation of CPP-ACP and Zn-CPP-ACP complexes

[0150] Stock solutions of 3.25M CaCl_2 and 1.25 M NaH_2PO_4 (pH 5.5) were added in approximately thirty aliquots to a 10 - 15 % w/v tryptic digest of casein until just before precipitation or gelation (usually producing a final concentration of approximately 78 mM to 124 mM Ca^{2+} and 48 mM to 76 mM inorganic phosphate). The solutions were added slowly (that is, less than approximately 1% volume addition per minute) with adequate mixing. An aliquot of the phosphate solution was added first, followed by an aliquot of the calcium solution. The bulk solution pH was maintained at 9.0 using 1 to 10 M NaOH with thorough mixing. The sodium hydroxide solution was added automatically by a pH stat with the addition of the hydroxide ions usually being after each addition of the calcium ions. After completion of the addition of the calcium ions, phosphate ions and hydroxide ions the solution was filtered through a 0.1 micron filter to concentrate 1-2 fold. The retentate was then washed with 1-2 volumes of water to remove salts and inactive (and bitter tasting) peptides. The CPP-ACP solutions prepared were then spray dried or freeze dried to produce a white powder. This dried powder was then added to water to form a final concentration of 10% w/v CPP-ACP solution at pH in the range 5.5 to 7.5 after the addition of zinc. Zinc(II) in the form of water-soluble zinc salts, e.g. zinc acetate, zinc lactate, zinc sulphate, zinc nitrate, zinc gluconate, zinc chloride, zinc citrate, or any other water-soluble zinc salt was added to the CPP-ACP solution such that the ratio of Zn mol to CPP mol was between 0.5 to 10. The Zn(II) salt was added slowly with mixing with constant pH adjustment to allow the formation of specific Zn-CPP-ACP complexes or Zn-CPP-ACFP complexes, where sodium fluoride (NaF) or sodium monofluorophosphate (NaMFP) has been incorporated into the complexes. For example, a solution prepared with zinc nitrate hexahydrate containing 10% CPP-ACP with 15 mM CPP and 2.23% zinc nitrate hexahydrate with 75 mM Zn had a Zn/CPP

molar ratio of 5. For a mouthwash with a Zn level of 0.1% (15 mM Zn) this was formulated with 2% CPP-ACP and 0.45% zinc nitrate hexahydrate to give a Zn/CPP molar ratio of 5. For toothpaste with CPP-ACP at 10% and a Zn level of 1.0% this would be equivalent to 4.5% zinc nitrate hexahydrate with a Zn/CPP molar ratio of 10.

Example 2 - Inhibition of *P. gingivalis* VSC-related enzymes (proteases) by CPP-ACP and Zn-CPP-ACP complexes

[0151] *P. gingivalis* whole cells (W50 and ATCC33277) were grown and harvested and used in the assay at OD₆₅₀ 0.006. Purified *P. gingivalis* proteases (rKgp and rRgpB) were also used at 5.0 µg/mL in a reaction volume of 100 µL. The buffer used was 50 mM Tris, 150 mM NaCl, 5 mM CaCl₂, pH 6.5 or 7.0 containing Cysteine 10 mM, Gly-Gly 20 mM, substrates [N-(p-tosyl)-Gly-Pro-Lys 4-nitroanilide acetate (GPK-NA), or α-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA)] at 1 mM and inhibitors Zn²⁺ (as Zn(NO₃)₂) 0 - 0.5 mM or Zn-CPP-ACP complexes 0 -0.5 mM total Zn(II). The substrates were added after all other reagents were mixed and incubated at 37 °C for 1 hour. Absorbance changes at 405 nm (A₄₀₅) were immediately monitored at 37 °C after addition of the substrates.

[0152] Zn(II) as zinc nitrate at 0.5 mM has some inhibitory activity against whole *P. gingivalis* cells and purified proteases (Table 1), but at the same total zinc concentration of 0.5 mM the Zn-CPP-ACP complexes were superior in inhibiting both whole cells and purified proteases (Table 2). These results clearly show the superiority of Zn-CPP-ACP complexes in inhibiting the enzymatic processes involved in the formation of volatile sulphur compounds (VSCs) by a common Gram-negative oral bacterium associated with halitosis.

[0153] **Table 1. Inhibition of VSC-related enzymes with Zn²⁺ as Zn(NO₃)₂.** Relative enzyme activities of *P. gingivalis* whole cells (W50 and ATCC 33277) and purified rKgp and rRgpB in the presence and absence of different concentrations of Zn(II). Relative activity: Ratios to that without Zn(II) at pH 7.0 and pH 6.5.

Zn		Relative enzyme activity pH 7.0		
[Zn] mM	W50	ATCC 33277	rKgp	rRgpB

0.000	1.000	1.000	1.000	1.000
0.010	1.000	0.818	0.897	1.000
0.100	0.941	0.818	0.897	0.857
0.500	0.941	0.636	0.667	0.576

Relative enzyme activity pH 6.5				
Zn				
[Zn] mM	W50	ATCC	rKgp	rRgpB
		33277		
0.000	1.000	1.000	1.000	1.000
0.010	1.000	1.000	0.682	0.972
0.100	0.909	1.000	0.477	0.783
0.500	0.909	0.667	0.205	0.265

[0154] Table 2. Inhibition of VSC-related enzymes with Zn(II)-CPP-ACP complexes as prepared using CPP-ACP and Zn(NO₃)₂. Relative enzyme activities of *P. gingivalis* whole cells and purified rKgp and rRgpB proteases in the presence and absence of different concentrations of Zn(II)-CPP-ACP complexes. Relative activity: Ratios to that without Zn(II)-CPP-ACP at pH 7.0 and pH 6.5.

Relative enzyme activity pH 7.0				
Zn ^(II) CPP-ACP				
[Zn] mM	W50	ATCC	rKgp	rRgpB
		33277		
0.000	1.000	1.000	1.000	1.000
0.010	0.889	0.900	1.275	1.000
0.100	0.333	0.500	0.050	0.062
0.200	0.000	0.000	0.001	0.001

0.500	0.000	0.000	0.000	0.000
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Zn ^(II) CPP-ACP				
Relative enzyme activity pH 6.5				
[Zn] mM	ATCC			
	W50	33277	rKgp	rRgpB
0.000	1.000	1.000	1.000	1.000
0.010	0.736	0.778	0.824	1.000
0.100	0.036	0.020	0.035	0.062
0.200	0.000	0.000	0.000	0.000
0.500	0.000	0.000	0.000	0.000

Example 3 - Halitosis clinical trial with CPP-ACP and Zn-CPP-ACP complexes mouthwash

Clinical Protocol

- Three-treatment, double blind, randomized, cross-over design, 20 participants
- Randomised mouthwash treatments (i) Zn 15 mM, (ii) 2% CPP-ACP, and (iii) 2% Zn-CPP-ACP complexes (Zn 15 mM)
- Mouthwash (10 ml) used three times daily for 60 s after normal oral hygiene procedures for 14 consecutive days;
- Volatile Sulphur Compounds (VSC) measured using an Halimeter at baseline and end of each treatment
- The selection criteria included a consistent baseline intra-oral VSC level of 150 ppb or greater.
- Over 80 participants were screened for the study.
- The change in VSC from baseline to end of treatment was determined for each participant and averaged for each group.

Oral Hygiene

[0155] During the treatment period, each participant continued with oral hygiene practices consisting of brushing twice a day with a fluoride toothpaste (supplied) and toothbrush (supplied). All participants were requested not to use antibiotics, antimicrobials (e.g. rinses or antimicrobial toothpastes) or consume antimicrobial mints, lozenges, or chewing gums during the treatment period. At the beginning of each leg, participants were issued with a package of mouthwash bottles containing their allocated treatment for that period, a tube of fluoride toothpaste and a toothbrush.

Participant Selection

[0156] Twenty participants, aged 18 to 65 years old, were recruited from 83 volunteers as they had intra-oral VSC levels of 150 ppb or greater on pre-screening and at baseline. Individuals were excluded if they were allergic to milk protein or had a sensitivity to zinc; had been treated with antibiotics in the month prior to starting the study; had a history of conditions requiring antibiotic treatment. Power calculations using a 10% attrition rate, indicated that 20 participants would allow for a power of 90%.

Randomization and Blinding

[0157] A randomization schedule was generated which ensured that all six treatment combinations (ABC, ACB, BAC, BCA, CAB, CBA) were equally likely. The study was examiner and participant blinded. Throughout the study, the clinical examiner/recorder was unaware of which treatment sequence a participant had been allocated. The participants were also unaware of which mouthwash had been allocated, as they were provided in identical coded packages (Mouthwash A, Mouthwash B and Mouthwash C). Personnel dispensing the test material or supervising their use did not participate in the VSC measurements of the participants to minimize potential bias. Participants were assigned a participant number. Participant numbers were recorded on their case report forms. Participants were randomly assigned to one of the three treatments. Randomization was determined from a standard randomisation table for the number of treatments in the cross-over study. Each of the three treatment periods was for 14 consecutive days. The treatment periods were separated by 14-day washout periods. Following completion of the screening examination all participants were assessed for eligibility against the inclusion/exclusion criteria. The primary analysis set consists of all

participants who completed the trial without significant protocol violations (per protocol analysis). No participants were excluded for significant protocol variations during the blind review of the data, which was performed prior to the treatment code being broken and before any analyses so Intention-To-Treat (ITT) and Per Protocol (PP) populations for analysis were equivalent.

VSC Measurement Protocol

[0158] Volatile Sulphur Compounds (VSC) were measured using an Halimeter (Interscan, CA, USA) at baseline and end of each treatment by an experienced clinician (examiner/recorder). The meter was calibrated to zero on ambient air immediately prior to each measurement. Each participant kept their mouth closed for one minute prior to measurement when a disposable straw connected to the meter was placed at the dorsal posterior midpoint of the tongue and kept in place until the maximum VSC reading in ppb was obtained. Three consecutive readings were taken and then averaged.

Statistical Analyses

[0159] Descriptive statistics (mean, standard deviation and range) were calculated for all continuous variables and frequencies for all ordinal variables. All analyses were conducted using Stata (StataCorp LP, College Station, TX, USA) or R statistical software, with a pre-specified level of significance of $P < 0.05$. The mean VSC level per participant was determined at baseline and at the 14 day visit. The differences between baseline and 14-day VSC readings were calculated and these difference between treatment groups were then analysed using the Mann-Whitney test. The Cochran-Mantel-Haenszel test was used to compare between the treatment groups where necessary.

Results

[0160] The results of the clinical trial showed that the use of the three mouthwash formulations reduced VSC levels in breath of the participants with the Zn-CPP-ACP complexes mouthwash treatment reducing VSC levels significantly better than the CPP-ACP or Zn alone mouthwash formulations. (Table 3).

[0161] **Table 3. Relative change in Volatile Sulphur Compounds by CPP-ACP and Zn-CPP-ACP complexes**

Treatment	Change in VSC ppb
Zn	-49 ± 28
CPP-ACP	-85 ± 41^a
Zn-CPP-ACP complexes	$-145 \pm 73^{a,b}$

a. Significantly different to Zn control ($p < 0.01$).

b. Significantly different to other values in column ($p < 0.01$)

Example 4 – ion analysis in complexes

[0162] The following is a protocol for PP-ACP or Zn-PP-ACP complexes solution ion analysis. Total (tightly & loosely-bound) and loosely-bound samples were prepared as follows:

Total (tightly and loosely-bound): One ml of any solution containing either PP-ACP (e.g. CPP-ACP) or Zn-PP-ACP (e.g. Zn-CPP-ACP complexes) can be taken and placed into 19 ml of 1M HNO₃ and incubated at room temperature with constant slow end over end mixing for 24 hrs (20 rpm). The mixture is centrifuged at 1000g for 15 minutes at room temperature. The supernatant is analyzed for calcium, zinc, phosphate and fluoride.

[0163] Loosely-bound: A sample of the same solution as for the 'total' analysis immediately above is taken and placed in a centricon with a 1000 MWCO filter and centrifuged at 3000g for 1 hour at room temperature to produce enough filtrate (<10% of total sample to not affect equilibrium) for analysis by atomic absorption spectrophotometry (AAS) and ion chromatography (IC). The filtrates are then measured to give loosely-bound ions.

[0164] The total and loosely bound calcium, zinc, phosphate and fluoride in the solution are determined by ion chromatography (for fluoride and phosphate) and Atomic Absorption Spectrometry (for calcium and zinc).

[0165] PP tightly-bound (colloidal retentate) ions are calculated from the difference between Total and loosely-bound (as explained above).

Example 5**[0166]** Mouth Spray

2% Zn-CPP-ACP complexes with 15 mM zinc

Flavouring (1.5%)

Preservative (e.g. sodium benzoate)

Water

Example 6**[0167]** Sugar-free mint

Sorbitol/Xylitol (95%)

Zn-CPP-ACP complexes (2.0%)

natural flavourings (1.98%),

steviol glycosides (0.48%),

anticaking agent (0.59%),

Example 7**[0168]** Mouthwash

Zn-CPP-ACP complexes with 15 mM zinc (2%)

Flavouring (1.5%)

Preservative (e.g sodium benzoate)

Water

Example 8**[0169]** Sugar-free chewing gum

Gum Base (33.4%)

Sorbitol (41%)

Mannitol (12%)

Glycerin (10%)

Zn-CPP-ACP complexes (2.0%)

Flavour (1.5%)

Aspartame (0.1%)

Example 9

[0170] Toothpaste

Water

Sorbitol

Hydrated silica

Zn-CPP-ACP complexes (10%)

Sodium Lauryl Sulphate

Caragenan

Flavour

Xanthan gum

Cocamidopropyl betaine

Stannous Fluoride

Titanium dioxide

Example 10

[0171] Toothpaste

Water

Sorbitol

Hydrated silica

Zn-CPP-ACFP complexes (10%)

Sodium Lauryl Sulphate

Caragenan

Flavour

Xanthan gum

Cocamidopropyl betaine

Sodium Fluoride

Titanium dioxide

Total Fluoride level 1450 ppm

[0172] References

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CLAIMS

1. A method for treating or preventing halitosis in an individual in need thereof, the method comprising administering stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) to the oral cavity of the individual, thereby treating or preventing halitosis.
2. The method according to claim 1, further comprising the step of identifying an individual having halitosis.
3. The method according to claim 1 or 2, wherein the individual has moderate halitosis.
4. The method according to claim 1 or 2, wherein the individual has severe halitosis.
5. The method according to any one of claims 1 to 4, wherein the individual has an intra-oral volatile sulphur compound (VSC) level of, or about, 150 ppb (parts per billion) or greater.
6. The method according to any one of claims 1 to 5, wherein the individual is identified as 3 on the Rosenberg scale.
7. The method according to any one of claims 1 to 5, wherein the individual is identified as 4 on the Rosenberg scale.
8. The method according to any one of claims 1 to 5, wherein the individual is identified as 5 on the Rosenberg scale.
9. The method according to any one of claims 1 to 8, wherein the individual has not been diagnosed with periodontal disease.
10. The method according to any one of claims 1 to 9, wherein the individual does not have periodontal disease.
11. The method according to any one of claims 1 to 10, wherein the individual has not been diagnosed with periodontitis or severe gingivitis.
12. The method according to any one of claims 1 to 11, wherein the individual does not have periodontitis or severe gingivitis.

13. The method according to any one of claims 1 to 12, wherein the individual has not been diagnosed with gingivitis.
14. The method according to any one of claims 1 to 13, wherein the individual does not have gingivitis.
15. A method of reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual, the method comprising administering stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) to the oral cavity of the individual, thereby reducing the production of volatile sulphur compounds (VSCs) in the oral cavity of an individual.
16. A method of inhibiting the activity of enzymes from oral bacteria, the method comprising administering stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) to the oral cavity of the individual, thereby inhibiting the activity of enzymes from oral bacteria.
17. The method according to claim 16, wherein the enzymes are proteases.
18. The method according to claim 16 or 17, wherein the oral bacteria are those involved in the production of VSCs.
19. The method according to any one of claims 16 to 18, wherein the oral bacteria are Gram-negative oral bacteria associated with halitosis.
20. The method of any one of claims 15 to 19, further comprising the step of determining the intra-oral volatile sulphur compound (VSC) levels in the individual.
21. The method of any one of claims 15 to 20, wherein the individual has not been diagnosed, or does not have, gingivitis or periodontal disease.
22. The method according to any one of claims 1 to 21, wherein the stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) is phosphopeptide stabilized.
23. The method according to claim 22, wherein the phosphopeptide is selected from casein phosphopeptide, phosvitin phosphopeptide, osteoponin phosphopeptide and a chemical or recombinantly synthesised phosphopeptide comprising the sequence –A-B-C–.

24. The method according to claim 23, wherein the phosphopeptide is a casein phosphopeptide.
25. The method according to any one of claims 1 to 24, wherein the stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) is a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex.
26. The method according to claim 25, wherein the zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex is phosphopeptide stabilized.
27. The method according to claim 26, wherein the zinc-associated stabilized ACP or ACFP complexes has a zinc ion content of about, or at least, 0.5 mole of zinc per mole of phosphopeptide.
28. The method according to claim 27, wherein the zinc ion content is in the range of about 0.5 moles to about 100 moles per mole of phosphopeptide.
29. The method according to claim 27, wherein the zinc ion content is in the range of about 0.5 moles to about 50 moles per mole of phosphopeptide.
30. The method according to claim 27, wherein the zinc ion content is in the range of about 0.5 moles to about 20 moles per mole of phosphopeptide.
31. The method according to claim 27, wherein the zinc ion content is in the range of about 0.5 moles to about 10 moles per mole of phosphopeptide.
32. The method according to any one of claims 27 to 31, wherein the zinc-associated stabilized ACP or ACFP complexes has a zinc ion content of about, or at least, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 moles of zinc per mole of phosphopeptide.
33. The method according to any one of claims 1 to 32, wherein the stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) is administered to the oral cavity in the form of a composition comprising the stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP).

34. The method according to any one of claims 33, wherein the composition is a dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, mouthrinses, mouth sprays, varnish, dental cement, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs including yoghurt.
35. Use of stabilized amorphous calcium phosphate (ACP) and/or stabilized amorphous calcium fluoride phosphate (ACFP) in the manufacture of a medicament for the treatment or prevention of halitosis.
36. Use of a zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex in the manufacture of a medicament for the treatment or prevention of halitosis.
37. The method or use of any one of claims 1 to 36, wherein the method or use of the results in a reduction of intra-oral VSCs level of at least 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 ppb.
38. The method or use of any one of claims 1 to 36, wherein the method or use of the results in a reduction in the individual's Rosenberg scale measurement by at least 1.
39. A zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex.
40. The complex according to claim 39, wherein the zinc-associated stabilized amorphous calcium phosphate (ACP) and/or a zinc-associated stabilized amorphous calcium fluoride phosphate (ACFP) complex is phosphopeptide stabilized.
41. The complex according to claim 40, wherein the phosphopeptide is a casein phosphopeptide.
42. The complex according to claim 41, wherein the zinc-associated stabilized ACP or ACFP has a zinc ion content of about, or at least, 0.5 mole of zinc per mole of phosphopeptide.
43. The complex according to claim 42, wherein the zinc ion content is in the range of about 0.5 moles to about 100 moles per mole of phosphopeptide.

44. The complex according to claim 42, wherein the zinc ion content is in the range of about 0.5 moles to about 50 moles per mole of phosphopeptide.
45. The complex according to claim 42, wherein the zinc ion content is in the range of about 0.5 moles to about 20 moles per mole of phosphopeptide.
46. The complex according to claim 42, wherein the zinc ion content is in the range of about 0.5 moles to about 10 moles per mole of phosphopeptide.
47. The complex according to one of claims 39 to 46, wherein the zinc-associated stabilized ACP or ACFP has a zinc ion content of about, or at least, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 moles of zinc per mole of phosphopeptide.
48. The complex according to any one of claims 39 to 46, wherein the zinc-associated stabilized ACP and/or ACFP complex comprises zinc ions that remain associated with the complex after centrifugation in a 1000 molecular weight cut off filter at about 3000g for 1 hour at room temperature.
49. A composition comprising, consisting essentially of or consisting of, zinc-associated stabilized ACP and/or ACFP complexes according to any one of claims 39 to 48.
50. A composition according to claim 49, wherein the composition is a dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, mouthrinses, mouth sprays, varnish, dental cement, troches, chewing gums, mints, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs including yoghurt.
51. A composition according to claim 50, wherein the composition is a mint or chewing gum, preferably a sugar-free mint or sugar-free chewing gum.
52. A kit comprising:
- (a) a composition according to claim 49 or 50, or
 - (b) a zinc-associated stabilized ACP or ACFP complex according to any one of claims 39 to 48.

53. The kit according to claim 49, wherein the kit further includes instructions for using the kit in a method according to any one of claims 1 to 38.
54. A method or process for forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex, the method or process comprising or consisting of:
- (i) obtaining a solution comprising at least one phosphopeptide and;
 - (ii) admixing solutions comprising phosphopeptides, calcium ions, phosphate ions, hydroxide ions and optionally fluoride ions, while maintaining the pH at about 7 or above, preferably about 9, to form a solution comprising phosphopeptide stabilized ACP or ACFP; and
 - (iii) admixing a water-soluble zinc salt with the solutions comprising phosphopeptide stabilized ACP or ACFP,
- thereby forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex.
55. The method or process of claim 54, wherein the solutions are added slowly at a rate of less than about 1% volume addition per minute with adequate mixing.
56. A method or process for forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex, the method or process comprising or consisting of:
- (i) providing a solution of phosphopeptide stabilized ACP or ACFP; and
 - (ii) admixing a water-soluble zinc salt with the solution,
- thereby forming a zinc-associated phosphopeptide stabilized ACP or ACFP complex.
57. The method or process of any one of claims 54 to 56, wherein the water-soluble zinc salt is in the form of zinc acetate, zinc lactate, zinc sulphate, zinc nitrate, zinc gluconate, zinc chloride, zinc citrate, or any other water-soluble zinc salt.