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(57) **Abrégé/Abstract:**

The present invention relates to compositions and methods for mineralizing a dental surface or subsurface including providing a composition including stabilized amorphous calcium phosphate (ACP) and a source of fluoride ions.



ABSTRACT

The present invention relates to compositions and methods for mineralizing a dental surface or subsurface including providing a composition including stabilized amorphous calcium phosphate (ACP) and a source of fluoride ions.

Compositions for Dental Mineralization and Uses Thereof

The present invention relates to a composition for mineralizing a dental surface, in particular tooth enamel. Methods of mineralizing hypomineralized lesions (including subsurface lesions) in the tooth enamel caused by dental caries, dental corrosion, erosion and fluorosis are also provided.

Background

Dental caries is initiated by the demineralization of hard tissue of the teeth usually by organic acids produced from fermentation of dietary sugar by dental plaque odontopathogenic bacteria. Dental caries is still a major public health problem. Further, restored tooth surfaces can be susceptible to further dental caries around the margins of the restoration. Even though the prevalence of dental caries has decreased through the use of fluoride in most developed countries, the disease remains a major public health problem. Dental erosion or corrosion is the loss of tooth mineral by dietary or regurgitated acids. Dental hypersensitivity is due to exposed dentinal tubules through loss of the protective mineralized layer, cementum. Dental calculus is the unwanted accretion of calcium phosphate minerals on the tooth surface. All these conditions, dental caries, dental corrosion, dental hypersensitivity and dental calculus are therefore imbalances in the level of calcium phosphates.

Fluoride-containing dentifrices and mouthrinses have been demonstrated to significantly reduce caries experience in randomized, controlled clinical trials (Biesbrock *et al.*, 1998; Biesbrock *et al.*, 2001; Curnow *et al.*, 2002; Davies *et al.*, 2002; Biesbrock *et al.*, 2003). The efficacy of these oral care products in reducing caries activity has been attributed to their ability to incorporate fluoride ions into plaque as several investigators have suggested an inverse relationship between plaque fluoride levels and caries incidence (Duckworth *et al.*, 1992, Duckworth and Stewart, 1994, Hartshorne *et al.*, 1994; Skold-Larsson *et al.*, 2000; Lynch *et al.*, 2004).

Fluoride ions in plaque immediately promote the formation of fluorhydroxyapatite in the presence of calcium and phosphate ions produced during demineralization of tooth enamel by plaque bacterial organic acids (ten Cate, 1999). This is now believed to be

the major mechanism of fluoride ion's action in preventing enamel demineralization (ten Cate, 1999; Lynch *et al.*, 2004). However, fluoride ions can also promote the remineralization of previously demineralized enamel if enough salivary or plaque calcium and phosphate ions are available when the fluoride is applied. For every two
 5 fluoride ions, ten calcium ions and six phosphate ions are required to form one unit cell of fluorapatite. Hence on topical application of fluoride ions, the availability of calcium and phosphate ions can be rate limiting for net enamel remineralization to occur. This is highly exacerbated under xerostomic (dry mouth) conditions. Furthermore, as fluoride treatments can lead to fluorosis, particularly in children, it would be advantageous to
 10 produce dental treatment compositions having the highest efficacy for the amount of fluoride present, to reduce the overall quantity of fluoride necessary to achieve the mineralization effect.

WO 98/40406 in the name of The University of Melbourne describes casein phosphopeptide-amorphous calcium phosphate complexes (CPP-ACP) and CPP-
 15 stabilised amorphous calcium fluoride phosphate complexes (CPP-ACFP) which have been produced at alkaline pH. CPP-ACP (available commercially as Recaldent™) has been shown to remineralize enamel subsurface lesions *in vitro* and *in situ* (Reynolds, 1998; Shen *et al.*, 2001; Reynolds *et al.*, 2003).

The CPP which are active in forming the complexes do so whether or not they are part
 20 of a full-length casein protein. Examples of active (CPP) that can be isolated after tryptic digestion of full length casein have been specified in US Patent No. 5,015,628 and include peptides Bos α_{s1} -casein X-5P (f59-79) [1], Bos β -casein X-4P (f1-25) [2], Bos α_{s2} -casein X-4P (f46-70) [3] and Bos α_{s2} -casein X-4P (f1-21) [4] as follows:

[1] Gln⁵⁹-Met-Glu-Ala-Glu-Ser(P)-Ile-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ile-Val-Pro-Asn-
 25 Ser(P)-Val-Glu-Gln-Lys⁷⁹ α_{s1} (59-79)

[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 β (1-25)

[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 α_{s2} (46-70)

[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 α_{s2} (1-21)

International patent application numbers WO 03/059303 and WO 03/059304 in the
5 name of the Procter & Gamble Company identify difficulties in maintaining measurable
fluoride ions levels in oral compositions containing CPP-ACP complexes and fluoride
and propose including additional components to maintain measurable fluoride levels.

It would be useful augment the remineralization activity of CPP-ACP complexes or
fluoride compositions to better treat conditions such as dental caries.

10 It is accordingly an object of the present invention to overcome, or at least alleviate, one
or more of the difficulties and/or deficiencies related to the prior art.

Summary of the invention

In one aspect, the present invention provides a composition for dental mineralization
including stabilized amorphous calcium phosphate (ACP) and a source of fluoride ions.

15 The ACP may also contain some fluoride ions, and these fluoride ions may be part of a
stabilised amorphous calcium fluoride phosphate (ACFP) complex.

The composition may include any suitable oral composition, such as a composition for
maintaining oral/dental health used by the patient and/or a treatment composition for use
by the dental practitioner. Such compositions may include toothpastes, tooth gels, tooth
20 powders, dental crèmes, liquid dentifrices, mouthwashes, troches, chewing gums,
gingival massage crèmes, gargle tablets and dental restoratives.

In a further aspect of the present invention there is provided a method of mineralizing a
dental surface or subsurface including providing a composition including stabilized ACP
and a source of fluoride ions. In a preferred embodiment the dental surface is enamel.

25 In a further aspect of the present invention there is provided a method for treating

and/or preventing dental caries including contacting a caries lesion in tooth enamel with a composition including stabilized ACP and a source of fluoride ions.

It has now been found that the dental remineralization efficacy of an oral composition including a source of fluoride ions can be substantially enhanced by the addition of
5 stabilized ACP to the composition. Furthermore, the uptake of fluoride ions into dental enamel from an oral composition containing a source of fluoride ions can be enhanced by the inclusion of stabilized ACP into the composition.

Accordingly, in a further aspect of the present invention there is provided a method of increasing the remineralisation efficacy of an oral composition having a source of
10 fluoride ions, including the incorporation of stabilized ACP into the oral composition.

In a further aspect of the present invention there is provided a method of increasing the uptake of fluoride ions into a dental surface or subsurface after treatment with an oral composition having a source of fluoride ions, including the incorporation of stabilized ACP into the oral composition.

15 Preferably, the dental surface or subsurface is tooth enamel.

In a further aspect, the present invention provides a method for increasing the uptake of fluoride ions into dental plaque after treatment with an oral composition having a source of fluoride ions, including the incorporation of stabilized ACP into the oral composition.

Typically in these methods of the invention, the fluoride ions are supplied separately but
20 substantially simultaneously with the ACP.

The ACP is preferably a basic, soluble form of ACP.

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
25 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.

The fluoride ions may be from any suitable source. A source of fluoride ions may
5 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.

Preferably the ACP is phosphopeptide (PP)-stabilized. Preferably, the phosphopeptide
10 (as defined below) is a casein phosphopeptide. In a preferred embodiment the ACP is in the form of a casein phosphopeptide stabilized ACP complex.

In a further aspect of the present invention there is provided a method for mineralizing a tooth surface or subsurface comprising applying an ACP complex and a source of fluoride ions to a tooth surface or subsurface. Preferably the tooth surface or
15 subsurface is tooth enamel. In a preferred embodiment, the tooth surface is tooth enamel containing a lesion selected from the group consisting of one or more of a caries lesion; a lesion caused by tooth erosion, a white spot lesion, or a fluorotic lesion.

In one embodiment, the dental surface is in need of such treatment. The invention also includes a method of treating a subject suffering dental caries, dentinal hypersensitivity,
20 fluorosis or dental calculus.

It has been surprisingly found that the inclusion of stabilized ACP into an oral composition including a source of fluoride ions, increases extent to which remineralization occurs throughout the body of the enamel lesion, covering the surface and subsurface of the lesion, when compared with the oral composition without the
25 stabilized ACP.

Accordingly, in a further aspect of the present invention there is provided a method for remineralizing a subsurface enamel lesion including contacting a subsurface enamel lesion with a composition including stabilized ACP and a source of fluoride ions.

In a further aspect there is provided the use of stabilized ACP and a source of fluoride ions in the manufacture of a medicament for the mineralization of a tooth surface or subsurface.

5 In a further aspect of the present invention there is provided the use of stabilized ACP and a source of fluoride ions in the manufacture of a composition for increasing the remineralization efficacy of an oral composition having a source of fluoride ions.

10 In a further aspect of the present invention there is provided the use of stabilized ACP and a source of fluoride ions in the manufacture of an oral composition for increasing the uptake of fluoride ions into a dental surface or subsurface after treatment with an oral composition having a source of fluoride ions.

In a further aspect of the present invention there is provided the use of stabilized ACP and a source of fluoride ions in the manufacture of an oral composition for increasing the uptake of fluoride ions into a dental plaque after treatment with an oral composition having a source of fluoride ions.

15 In a further aspect of the present invention there is provided the use of stabilized ACP and a source of fluoride ions in the manufacture of a composition for remineralizing a subsurface enamel lesion.

20 Without being bound by any theory or mode of action it is believed that the fluoride ions interact with the ACP to form fluorapatite on contact with the tooth surface, which is more resistant to acid challenge than normal tooth enamel. This may result in tooth enamel with superior caries resistant properties. Another part of the mechanism may involve the fluoride ions forming a PP stabilized amorphous calcium fluoride phosphate complex (which may include a mixture of basic amorphous calcium phosphate and fluoride ions, in situ during use). The use of amorphous calcium fluoride phosphate
25 includes the use of a mixture of ACP with amorphous calcium fluoride phosphate.

“Phosphopeptide” or “PP” 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 phosphoseryl residue. B is desirably a residue the side-chain of which is neither relatively large nor hydrophobic. It
5 may be Gly, Ala, Val, Met, Leu, Ile, Ser, Thr, Cys, Asp, Glu, Asn, Gln or Lys.

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,
10 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.

It will also be understood that the term "comprises" (or its grammatical variants) as used
15 in this specification is equivalent to the term "includes" and may be used interchangeably and should not be taken as excluding the presence of other elements or features.

The ACP may also include ACFP, or ACFP may be included in the compositions and methods of the present invention in the place of ACP.

20 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
25 water. Thus, the PP can be a casein protein or a polyphosphopeptide.

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 phosphitin, 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) [1], β (1-25) [2], α_{s2} (46-70) [3] and α_{s2} (1-21) [4] 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. Examples of conservative substitutions are shown in Table 1 below.

TABLE 1

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

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 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;

5 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;

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

Hydroxylysine (Hyl) for Lys;

allohydroxylysine (AHyl) for Lys;

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

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

15 ρ -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;

20 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, trifluorylphenylalanine, for Phe.

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].

- 5 In preferred embodiments, the compositions of the present invention do not include a phosphate buffer and/or a calcium chelator.

In an embodiment of the present invention there is provided a composition for dental mineralization including stabilized amorphous calcium phosphate (ACP) and a source of fluoride ions, wherein the composition does not include a phosphate buffer and/or
10 calcium chelator.

In another embodiment of the invention, the stabilised ACP complex is incorporated into an oral composition containing a source of fluoride ions such as toothpaste, mouth washes or formulations for the mouth to aid in the prevention and/or treatment of dental caries, tooth decay, dental erosion and/or fluorosis. The ACP complex may comprise
15 0.01-50% by weight of the composition, preferably 1.0-50%. For oral compositions, it is preferred that the amount of the ACP complex administered is 0.01 - 50% by weight, preferably 1.0% - 50% by weight of the composition. In a particularly preferred embodiment, the oral composition of the present invention contains about 2% CPP-ACP. The fluoride ions may be present in the oral composition at a concentration in the
20 range of about 200 ppm to 3000 ppm. In a preferred embodiment, the fluoride ions are at a concentration in the range of about 400 ppm to about 1500 ppm. In a further preferred embodiment, the fluoride ions in the oral composition are at a concentration of about 900 ppm.

An oral composition of this invention which contains the above-mentioned agents may
25 be prepared and used in various forms applicable to the mouth such as dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs. An oral composition according to this invention may further include

additional well known ingredients depending on the type and form of a particular oral composition.

- In certain preferred forms of the invention the oral composition may be substantially liquid in character, such as a mouthwash or rinse. 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.
- 10 The pH of such liquid and other preparations of the invention is generally in the range of from about 5 to about 9 and typically from about 5.0 to 7.0. The pH can be controlled with acid (e.g. citric acid or benzoic acid) or base (e.g. sodium hydroxide) or buffered (as with sodium citrate, benzoate, carbonate, or bicarbonate, disodium hydrogen phosphate, sodium dihydrogen phosphate, etc).
- 15 In other desirable forms of this invention, the stabilised ACP 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.
- 25

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.

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.

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.

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.

- 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
- 5 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. LaponiteTM 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.
- 10 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 NatrosolTM), sodium carboxymethyl cellulose, and colloidal silica such as finely ground SyloidTM (e.g. 244). Solubilizing agents may also be included such as humectant polyols such
- 15 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.

- It will be understood that, as is conventional, the oral preparations will usually be sold or
- 20 otherwise distributed in suitable labelled packages. Thus, a bottle 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
- 25 toothpaste, gel or dental cream.

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).

25 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.

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.

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.

The compositions of this invention can also be incorporated in lozenges, or in chewing gum or other products, 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.

In a further aspect, the invention provides compositions including pharmaceutical compositions comprising the ACP complex described above and a source of fluoride ions together with 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. For example, a crème formulation may be employed containing: water; glycerol; CPP-ACP; sodium fluoride, D-sorbitol; silicon dioxide; sodium carboxymethylcellulose (CMC-Na); propylene glycol; titanium dioxide; xylitol; phosphoric acid; guar gum; zinc oxide; sodium saccharin; ethyl p-hydroxybenzoate; magnesium oxide; butyl p-hydroxybenzoate and propyl p-hydroxybenzoate.

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 corrosion and fluorosis.

In a further aspect, the present invention provides a kit of parts including (a) a source of fluoride ions and (b) a CPP-ACP complex in a pharmaceutically acceptable carrier. Desirably, the kit further includes instructions for their use for the mineralization of a dental surface in a patient in need of such treatment. In one embodiment, the agent and
5 the complex are present in suitable amounts for treatment of a patient. The invention also provides a system for improved remineralization including (a) a source of fluoride ions and (b) a CPP-ACP complex in a pharmaceutically acceptable carrier for combining with the source of fluoride before application to a dental surface.

In a further aspect, the present invention provides a method for enhancing the
10 remineralization effect of fluoride ions in an oral care composition including addition of stabilised ACP to the oral care composition.

In a further aspect, the present invention provides a method of improving fluoride incorporation into an enamel lesion including contacting a lesion in tooth enamel with a composition including stabilized ACP and a source of fluoride ions.

15 In a further aspect of the present invention there is provided the use of stabilized ACP and a source of fluoride ions in the manufacture of a medicament for the mineralization of a dental surface or subsurface.

In a further aspect of the present invention there is provided the use of stabilized ACP and a source of fluoride ions in the manufacture of a medicament for the treatment
20 and/or prevention of dental caries.

In a further aspect of the present invention there is provided a method for treating and/or preventing dental erosion comprising contacting a lesion in tooth enamel caused by erosion with a composition including stabilized ACP and a source of fluoride ions.

In a further aspect of the present invention there is provided the use of stabilized ACP
25 and a source of fluoride ions in the manufacture of a medicament for the treatment and/or prevention of dental erosion.

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.

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

Example 1

A plaque fluoride study was conducted as a randomized, double-blind three-way cross-over design involving three coded mouthrinses. The three mouthrinses were (i) 2% w/v
10 CPP-ACP (Recaldent™) as supplied by Recaldent Pty Ltd (Melbourne, Australia) and 450 ppm F as NaF in deionized water, (ii) 450 ppm F as NaF in deionized water, (iii) a placebo control rinse as deionized water. The CPP-ACP mouthrinse was adjusted to pH 7.0 with 1M HCl. Subjects were supplied with the coded rinses in opaque plastic tubes and used 15 ml of each rinse for 60 s three times a day, after breakfast, after
15 lunch and at night before retiring, for four days and kept a diary of mouthrinse use. On the fifth day the rinse was used after breakfast and supragingival plaque was collected 2-3 hr later. Subjects refrained from all oral hygiene procedures while using the rinses. Each subject crossed over to use each mouthrinse with a four week washout period between treatments. Supragingival plaque was collected using a Gracey 7/8 curette
20 from the buccal and lingual surfaces of all teeth. Plaque was collected into a preweighed microcentrifuge tube, re-weighed and then stored at -70°C. After thawing of the plaque samples they were centrifuged for 5 min at 20,000 g, dried in a Jouan RC10.10 rotary evaporator and then re-weighed to determine dry weights. The dry samples were then extracted with 200 µl of 1M HCl by mixing in a vortex mixer for 1 min
25 and then treated in ice water in a Branson™ 12 ultrasonic bath (Consolidated Ultrasonic, Melbourne, Australia) for 8 h. After centrifugation (20,000 g, 5 min) fluoride ion concentrations in the supernatant were determined as described previously (Silva and Reynolds, 1996). The plaque fluoride levels were statistically analyzed using a non-

parametric Friedmans test with Wilcoxon Signed-Ranks tests (Norusis, 1993).

Both fluoride rinses produced an increase in plaque fluoride levels with the 450 ppm fluoride rinse nearly doubling the fluoride level obtained with the placebo control rinse (Table 2). The addition of 2% CPP-ACP to the 450 ppm fluoride rinse significantly
5 increased the incorporation of fluoride ions into plaque where the plaque fluoride level was over double that obtained with the fluoride rinse. No significant difference was observed in the dry weights of plaque for the three rinses, however the dry weight of the plaque obtained with the 2% CPP-ACP plus 450 ppm fluoride rinse exhibited a tendency to be greater than that obtained with the other two rinses.

10

Table 2

Fluoride levels in supragingival plaque after treatment with various mouthrinses

Mouthrinse	Plaque Fluoride Level (nmol/mg dry wt)	Dry Weight of Plaque (mg)
Placebo Control	$7.4 \pm 4.7^{a, b}$	4.3 ± 2.5^a
Fluoride (450 ppm)	$14.4 \pm 6.7^{a, b}$	3.9 ± 1.9^a
2% CPP-ACP plus 450ppm F	$33.0 \pm 17.6^{a, b}$	5.0 ± 2.1^a

^a Mean \pm SD (n = 14).

^b Significantly different from all values in same column (P < 0.001).

Example 2

15 A remineralization study was conducted as a randomized, double-blind, 5-way crossover remineralization study with five toothpaste slurries using an *in situ* model previously described (Shen *et al.*, 2001; Reynolds *et al.*, 2003). Palatal appliances containing six human enamel half-slabs with subsurface demineralized lesions were prepared as described by Shen *et al.* (2001). Toothpastes were prepared as coded
20 products and the base of the product consisted of sorbitol, silica, sodium lauryl sulphate, flavour, sodium carboxymethyl cellulose, titanium dioxide, xanthan gum, sodium saccharin and water. The pH of the formulation was adjusted to 7.0 with phosphoric acid. Five toothpaste formulations were prepared: (i) placebo, (ii) 1100 ppm fluoride as

sodium fluoride, (iii) 2800 ppm fluoride as sodium fluoride, (iv) 2% CPP-ACP and (v) 2% CPP-ACP plus 1100 ppm fluoride as sodium fluoride. Toothpaste slurries were prepared by adding 1 g of paste to 4 ml deionized water and vortex mixing for 60 s. Subjects rinsed with the slurries for 60 s four times per day for 14 days at the following
5 times: 10.00 am, 11.30 am, 2.00 pm and 3.30 pm. Subjects kept diaries of toothpaste slurry use and were instructed not to eat, drink or perform oral hygiene procedures while wearing the appliances. When the appliances were not in the mouth they were stored in a sealed moist plastic bag at room temperature. Subjects were instructed to rinse their appliances using deionized water. After the completion of each treatment the enamel
10 half-slabs were removed from the appliances and prepared for acid challenge.

For the acid challenge of the remineralized lesions, the test enamel blocks were covered with acid-resistant nail varnish to leave only half of each remineralized window (1 x 3 mm²) exposed. The slabs were mounted onto the end of 3-4 cm sticks of dental wax and immersed in 40 ml of unagitated lactic/Carbopol™ demineralization buffer
15 (Reynolds, 1997) for 8 hours at 37°C. After completion of this acid challenge the enamel slabs were rinsed with deionized water and sectioned through the midline of both windows to produce two blocks. These two enamel blocks containing remineralized lesions and acid challenged remineralized lesions were paired with their control block containing the original demineralized lesions and embedded, sectioned
20 and microradiographed as described previously (Shen *et al.*, 2001). Images of the lesions and the neighbouring sound enamel were scanned and the percent mineral profile of each lesion determined as described by Iijima *et al.* (2004). The difference between the areas under the densitometric profile of the original demineralized lesion and sound enamel, calculated by trapezoidal integration, is represented by ΔZ_d . The
25 difference between the areas under the densitometric profile of the remineralized lesion and sound enamel, calculated by trapezoidal integration, is represented by ΔZ_r . Percentage remineralization (%R) represents the percentage change in ΔZ values eg

$$\%R = \frac{\Delta Z_d - \Delta Z_r}{\Delta Z_d} \times 100$$
 (Iijima *et al.*, 2004). Data were statistically analyzed using a repeated measures ANOVA with post hoc Scheffe test (Norusis, 1993).

30 All toothpaste formulations replaced mineral in the enamel subsurface lesions in the *in*

- situ* study (Table 3). Fluoride produced a dose-response remineralization with the 2800 ppm replacing significantly more mineral than the 1100 ppm formulation which replaced significantly more than the placebo control. The toothpaste with 2% CPP-ACP produced a level of remineralization similar to the 2800 ppm fluoride formulation and the paste
- 5 with 2% CPP-ACP plus 1100 ppm fluoride was superior to all other formulations including the 2800 ppm fluoride paste. Microradiography of the lesions after remineralization revealed that fluoride ion alone tended to promote remineralization of the surface layer whereas CPP-ACP promoted remineralization, even in the presence of fluoride, throughout the body of the lesion.
- 10 Acid challenge *in vitro* of the *in situ* remineralized enamel slabs resulted in substantial loss of mineral from the placebo-treated enamel slabs. A smaller amount of mineral was lost from the lesions remineralized with 2% CPP-ACP upon acid challenge. Although there was a tendency to lose a small amount of mineral from the enamel treated with the fluoride formulations the loss was not statistically significant. The
- 15 residual remineralization after acid challenge was significantly greater for the paste containing 2% CPP-ACP plus 1100 ppm fluoride when compared with the residual remineralization obtained with all other pastes including the paste containing 2800 ppm fluoride. Microradiography of the remineralized lesions after acid challenge revealed that the acid removed mineral predominantly from underneath the remineralized zone.
- 20 This *in situ* study showed a clear dose response in enamel subsurface lesion remineralization by fluoride, with $8.2 \pm 0.2\%$ remineralization by the toothpaste containing 1100 ppm F- and $15.5 \pm 2.4\%$ by that containing 2800 ppm F-. It also revealed that the paste containing 2% CPP-ACP was superior in remineralizing enamel subsurface lesions when compared with the paste containing 1100 ppm F- and was not
- 25 significantly different to the paste containing 2800 pm F. The paste containing 2% CPP-ACP plus 1100 ppm F- produced greater remineralization than the paste containing 2800 ppm F-. Addition of 2% CPP-ACP to 1100 ppm F- increased enamel subsurface remineralization by 156% relative to the 1100 ppm F- paste.

The casein phosphopeptides (CPP) have been shown to not only stabilize amorphous

30 calcium phosphate (ACP), but also to deliver and localize ACP at the tooth surface

(Reynolds, 1998; Reynolds et al., 1999; Reynolds et al., 2003). CPP-ACP in a mouthwash significantly increased the level of calcium and inorganic phosphate ions in supragingival plaque with the CPP bound to salivary pellicle and to the surface of bacteria in the supragingival plaque biofilm (Reynolds et al., 2003). This clinical trial
5 demonstrated that CPP-ACP can also promote the uptake of fluoride ions into plaque. Therefore CPP-ACP should promote the uptake of calcium, phosphate and fluoride ions into supragingival plaque when added to a fluoride-containing toothpaste formulation. The present *in situ* study demonstrated that CPP-ACP delivered in a toothpaste
10 formulation was very effective in enamel subsurface remineralization, and that the mineral formed was more resistant to acid than natural enamel apatite. Enamel remineralized by CPP-ACP in the presence of fluoride showed greater resistance to acid challenge relative to natural enamel or enamel remineralized by CPP-ACP. This suggests that CPP-ACP in the presence of F⁻ ions promotes remineralization with acid-resistant fluorapatite. These results demonstrate that addition of CPP-ACP, to a
15 toothpaste formulation significantly enhances the ability of fluoride to remineralize enamel subsurface lesions with acid resistant fluorapatite.

Table 3
Percentage remineralization of enamel subsurface lesions by various toothpaste formulations followed by acid challenge

	Placebo control	1100 ppm fluoride	2800 ppm fluoride	2% CPP-ACP	2% CPP-ACP plus 1100 ppm fluoride
Initial lesion depth (µm)	105 ± 7 ^a	106 ± 9	102 ± 7	107 ± 8	104 ± 8
ΔZd (vol % min. µm)	4,489 ± 2,465	3,544 ± 1,432	3,704 ± 1,278	4,287 ± 2,282	4,382 ± 1,714
ΔZd-ΔZr	138 ± 122 ^d	290 ± 136 ^d	576 ± 222	580 ± 311	919 ± 462 ^d
%R ^b	3.1 ± 1.6 ^e	8.2 ± 2.0 ^e	15.5 ± 2.4	13.5 ± 1.5	21.0 ± 5.9 ^e
%R ^c _{AC}	-4.1 ± 1.6 ^{f,g}	7.1 ± 1.3 ^f	13.2 ± 1.1 ^f	8.7 ± 1.5 ^{f,g}	17.4 ± 1.2 ^f

22

^a Mean ± SD (n = 14).
^b %R = ΔZd-ΔZr/ΔZd x 100 (Shen *et al.*, 2001).
^c R_{AC} = %R after acid challenge.
^d significantly different from all other values in row (P < 0.01).
^e significantly different from all other values in row (P < 0.01).
^f significantly different from all other values in row (P < 0.01).
^g significantly different from %R value in same column (P < 0.01).

Example 3

Electron microprobe wavelength dispersive spectrometry was used to measure fluoride levels in remineralized lesions as follows.

Enamel sections were embedded in epoxy resin on a one inch specimen holder. The resin was flat polished to expose the enamel sections using 2400 grit abrasive paper. To achieve optical smoothness 3 μm and 1 μm diamond polishing pastes were used on a cloth pad with final finishing accomplished with a 0.25 μm aluminium oxide paste. All samples and standards were coated with 20nm of carbon using a DynavacTM 300. The electron probe (8900R SuperProbeTM JEOL, Japan) was operated at a 15 kV accelerating voltage, 12 nA specimen current, 40° take-off angle. Dwell times of 10 seconds for the peak and 10 seconds for the background per point were used. The detection limit for F was 800 ppm. The beam diameter employed during collection of standards was a 10 μm spot whereas the diameter for analysis of lesions was 2 μm . Calcium, phosphorous, fluoride, and chloride X-ray intensities were measured simultaneously using four spectrometers with filter crystals of Pentaerythritol, Pentaerythritol, W/Si layered synthetic, Pentaerythritol, respectively. The standard was analysed using a 10 μm (defocused) and 2 μm (focused) diameter beam to calibrate the X ray count intensity. The standard was synthetic fluorapatite with a calcium to phosphorous ratio of 1.667 and a fluoride content of 3.70 wt%. Elemental maps and quantitative line scans for calcium, phosphorous, fluoride, oxygen and chlorine were collected across the lesions starting from the base of the lesion to the surface layer. Data was corrected using a Phi(RhoZ)-Parabolic method correction procedure implemented in STRATA (Thin Film Analysis Package).

Microradiography of the remineralized lesions after acid challenge revealed that the acid removed mineral predominantly from underneath the remineralized zone. The fluoride level of the remineralized lesions for the placebo, 1100 ppmF and 2% CPP-ACP plus 1100 ppmF pastes was determined using electron microprobe wavelength dispersive spectrometry (Table 4). The fluoride incorporated into the lesion was significantly higher for the 2% CPP-ACP plus 1100 ppmF paste when compared with the 1100 ppmF paste (Table 4). Further, the measured fluoride levels for the 2% CPP-ACP plus 1100 ppmF

paste was close to that predicted assuming the remineralized mineral was fluorapatite (Table 4).

Table 4

Predicted and measured fluoride levels in the remineralized lesions

Toothpaste	Remineralization level (vol % min)	Predicted ^a F (wt %)	Measured ^b F (wt %)
Placebo	1.31	0.05	0.05 ± 0.05 ^c
1100 ppmF	2.74	0.10	0.23 ± 0.09 ^c
CPP-ACP 2% plus 1100 ppmF	8.84	0.33	0.30 ± 0.13 ^c

5

^a Predicted F level based on remineralized mineral being fluorapatite (3.768 wt % F)

^b Measured using electron microprobe wavelength dispersive spectrometry with a JEOL 8900 SuperProbe microprobe. Mean level of fluoride measured by line scans from the base of the lesion to the surface layer.

10 ^c Significantly different from other values in same column (p < 0.01).

Example 4

A topical crème may be produced in accordance with the present invention having the following ingredients:

15 Water
glycerol
CPP-ACP complexes
D-sorbitol
sodium carboxymethylcellulose (CMC-Na)
propylene glycol
20 silicon dioxide
titanium dioxide
xylitol
phosphoric acid
sodium fluoride
25 flavouring

25

sodium saccharin
ethyl p-hydroxybenzoate
propyl p-hydroxybenzoate
butyl p-hydroxybenzoate

5

Example 5

A mouthrinse formulation be produced in accordance with the present invention having the following composition:

10 Water
 Alcohol
 Poloxamer 407
 Sodium Lauryl Sulphate
 CPP-ACP complexes
 Sodium Fluoride
 Flavours
15 Sodium Saccharin
 Ethyl p-hydroxybenzoate
 Propyl p-hydroxybenzoate
 Butyl p-hydroxybenzoate

Example 6

20 A sugar-free chewing gum formulation be produced in accordance with the present invention having the following composition:

25 Crystalline sorbitol/mannitol/xylitol
 Gum base
 Calcium carbonate
 Glycerine
 CPP-ACP complexes
 Sodium Fluoride
 Flavour oil
 Water

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. All of these different combinations constitute various alternative aspects of the invention.

5

REFERENCES

- Biesbrock AR, Faller RV, Bartizek RD, Court LK, McClanahan SF (1998). Reversal of incipient and radiographic caries through the use of sodium and stannous fluoride dentifrices in a clinical trial. *J Clin Dent* 9:5-10.
- Biesbrock AR, Gerlach RW, Bollmer BW, Faller RV, Jacobs SA, Bartizek RD (2001).
10 Relative anti-caries efficacy of 1100, 1700, 2200, and 2800 ppm fluoride ion in a sodium fluoride dentifrice over 1 year. *Community Dent Oral Epidemiol* 29:382-389.
- Biesbrock AR, Bartizek RD, Gerlach RW, Jacobs SA, Archila L (2003). Dose response efficacy of sodium fluoride dentifrice at 9 and 21 months with supervised brushing. *Am J Dent* 16:305-312.
- 15 Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC (2005). Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. *J Biol Chem* 280:15362-15369.
- Curnow MM, Pine CM, Burnside G, Nicholson JA, Chesters RK, Huntington E (2002). A
20 randomised controlled trial of the efficacy of supervised toothbrushing in high-caries-risk children. *Caries Res* 36:294-300.
- Davies GM, Worthington HV, Ellwood RP, Bentley EM, Blinkhorn AS, Taylor GO, et al. (2002). A randomised controlled trial of the effectiveness of providing free fluoride toothpaste from the age of 12 months on reducing caries in 5-6 year old children. *Community Dent Health* 19:131-136.
- 25 Duckworth RM, Morgan SN, Gilbert RJ (1992). Oral fluoride measurements for estimation of the anti-caries efficacy of fluoride treatments. *J Dent Res* 71 Spec No:836-

840.

Duckworth RM, Stewart D (1994). Effect of mouthwashes of variable NaF concentration but constant NaF content on oral fluoride retention. *Caries Res* 28:43-47.

5 Hartshorne JE, Grobler SR, Louw AJ, Carstens IL, Laubscher JA (1994). The relationship between plaque index scores, fluoride content of plaque, plaque pH, dental caries experience and fluoride concentration in drinking water in a group of primary school children. *J Dent Assoc S Afr* 49:5-10.

10 Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC (2004). Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *Caries Res* 38:551-6.

Lynch RJ, Navada R, Walia R (2004). Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. *Int Dent J*:304-309.

15 Norusis M (1993). SPSS for Windows: Base System User's Guide, Release 6.0 Illinois, USA: SPSS INC.

Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PF, Johnson IH, et al. (1995). Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. *J Dent Res* 74:1272-1279.

20 Reynolds EC (1997). Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *J Dent Res* 76:1587-1595.

Reynolds EC (1998). Anticariogenic complexes of amorphous calcium phosphate stabilised by casein phosphopeptides. Invited review. *Spec Care Dentist* 18:8-16.

25 Reynolds EC, Black CL, Cai F, Cross KJ, Eakins D, Huq NL, et al. (1999). Advances in enamel remineralization: casein phosphopeptide-amorphous calcium phosphate. *J Clin Dent* X:86-88.

Reynolds EC, Cai F, Shen P, Walker GD (2003). Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res 82:206-211.

5 Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC (2001). Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 80:2066-2070.

Silva M, Reynolds EC (1996). Fluoride content of infant formula in Australia. Aust Dent J 41:37-42.

10 Skold-Larsson K, Modeer T, Twetman S (2000). Fluoride concentration in plaque in adolescents after topical application of different fluoride varnishes. Clin Oral Investig 4:31-34.

ten Cate JM (1999). Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand 57:325-329.

CLAIMS

1. A dentifrice formulation including phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) and free fluoride ions, the formulation further containing a dentally acceptable polishing material and a surfactant, wherein the formulation does not contain a calcium chelator or a phosphate buffer.
2. The dentifrice formulation according to claim 1, wherein the free fluoride ions are provided from a compound selected from the group consisting of sodium fluoride, stannous fluoride, amine fluoride and combinations thereof.
3. The dentifrice formulation according to claim 1 or 2, formulated as a toothpaste.
4. The dentifrice formulation according to any one of claims 1 to 3, wherein said phosphopeptide is a casein phosphopeptide.
5. The dentifrice formulation according to any one of claims 1 to 4, wherein said phosphopeptide includes an amino acid sequence selected from the group consisting of:
 - [1] $\text{Gln}^{59}\text{-Met-Glu-Ala-Glu-Ser(P)-Ile-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ile-Val-Pro-Asn-Ser(P)-Val-Glu-Gln-Lys}^{79} \quad \alpha_{s1}(59-79)$
 - [2] $\text{Arg}^1\text{-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(P)-Leu-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr-Arg}^{25} \quad \beta(1-25)$
 - [3] $\text{Asn}^{46}\text{-Ala-Asn-Glu-Glu-Glu-Tyr-Ser-Ile-Gly-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser(P)-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys}^{70} \quad \alpha_{s2}(46-70)$ and

[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 α₂(1-21).

6. The dentifrice formulation according to any one of claims 1 to 5, wherein the
free fluoride ions are present in the formulation in an amount of about 200 ppm to
5 about 3000 ppm.

7. The dentifrice formulation according to any one of claims 1 to 5, wherein the
free fluoride ions are present in the formulation in an amount of about 400 ppm to
about 1500 ppm.

8. The dentifrice formulation according to any one of claims 1 to 5, wherein the
10 free fluoride ions are present in the formulation in an amount of about 900 ppm or of
about 1100 ppm.

9. The dentifrice formulation according to any one of claims 1 to 8, wherein the
phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-
stabilized amorphous calcium fluoride phosphate (ACFP) is present in the dentifrice
15 at 0.01 to 50% by weight.

10. The dentifrice formulation according to any one of claims 1 to 8, wherein the
phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-
stabilized amorphous calcium fluoride phosphate (ACFP) is present in the dentifrice
at 1.0 to 50% by weight.

20 11. The dentifrice formulation according to any one of claims 1 to 8, wherein the
phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-
stabilized amorphous calcium fluoride phosphate (ACFP) is present in the dentifrice
at 2% by weight.

12. The dentifrice formulation according to any one of claims 1 to 11, wherein the polishing material is selected from the group consisting of water-insoluble sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated calcium phosphate, anhydrous dicalcium phosphate, calcium pyrophosphate, magnesium
5 orthophosphate, trimagnesium phosphate, hydrated alumina, calcined alumina, aluminium silicate, zirconium silicate, silica, bentonite, alkali metal aluminosilicate, particulate thermosetting resins and mixtures thereof.

13. The dentifrice formulation according to any one of claims 1 to 12, wherein the surfactant is selected from the group consisting of water-soluble salts of higher fatty
10 acid monoglyceride monosulfates, higher alkyl sulfates, alkyl aryl sulfonates, higher alkylsulfo-acetates, higher fatty acid esters of 1,2-dihydroxy propane sulfonate, substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds and condensation products of ethylene oxide with various reactive
15 hydrogen containing compounds reactive therewith having aliphatic chains of about 12 to 20 carbon atoms.

14. The dentifrice formulation according to claim 13, wherein the water-soluble salts of higher fatty acid monoglyceride monosulfate is the sodium salt of the monosulfated monoglyceride of hydrogenated coconut oil fatty acids, the higher alkyl sulfates is sodium lauryl sulphate, the alkyl aryl sulfonates is sodium dodecyl benzene sulfonate,
20 the substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds has 12 to 16 carbons in the fatty acid, alkyl or acyl radicals and the condensation products of ethylene oxide contain hydrophilic polyoxyethylene moieties.

15. The dentifrice formulation according to claim 14, wherein the substantially
25 saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds include N-lauroyl sarcosine, sodium, potassium, and ethanolamine salts of N-lauroyl, N-myristoyl, or N-palmitoyl sarcosine and the condensation products of ethylene oxide containing hydrophilic polyoxyethylene moieties include poly (ethylene

oxide) with fatty acids, fatty alcohols, fatty amides, polyhydric alcohols and polypropyleneoxide.

16. The dentifrice formulation according to any one of claims 1 to 15, for use in humans, domestic animals, companion animals or zoo animals.

5 17. The dentifrice formulation according to claim 16, for use in humans.

18. The dentifrice formulation according to claim 16, wherein the domestic animals are selected from the group consisting of cattle, sheep and horses.

19. The dentifrice formulation according to claim 16, wherein the companion animals are selected from cats and dogs.

10 20. A mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character comprising a phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) and free fluoride ions, wherein the composition does not contain a calcium chelator or a phosphate buffer.

15 21. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to claim 20, wherein the free fluoride ions are provided from a compound selected from the group consisting of sodium fluoride, stannous fluoride, amine fluoride and combinations thereof.

20 22. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to claim 20 or 21, wherein said phosphopeptide is a casein phosphopeptide.

23. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 22, wherein said

phosphopeptide includes an amino acid sequence selected from the group consisting of:

- [1] Gln⁶⁹-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 $\alpha_{61}(59-79)$
- 5 [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 $\beta(1-25)$
- [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 $\alpha_{62}(46-70)$ and
- 10 [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 $\alpha_{62}(1-21)$.

24. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 23, wherein the fluoride ions are present in the mouthwash, mouthrinse, tooth gel or other oral composition in an amount of about 200 ppm to about 3000 ppm.

- 15 25. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 23, wherein the fluoride ions are present in the mouthwash, mouthrinse, tooth gel or other oral composition in an amount of about 400 ppm to about 1500 ppm.

- 20 26. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 25, wherein the fluoride ions are present in the mouthwash, mouthrinse, tooth gel or other oral composition in an amount of about 900ppm or of about 1100 ppm.

27. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 26, wherein the phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the
5 mouthwash, mouthrinse, tooth gel or other oral composition at 0.01 to 50% by weight.

28. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 26, wherein the phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the
10 mouthwash, mouthrinse, tooth gel or other oral composition at 1.0 to 50% by weight.

29. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 26, wherein the phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the
15 mouthwash, mouthrinse, tooth gel or other oral composition at 2% by weight.

30. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to any one of claims 20 to 29, for use in humans, domestic animals, companion animals or zoo animals.

31. The mouthwash, mouthrinse or tooth gel composition or other oral composition
20 substantially liquid in character according to claim 30, wherein the domestic animals are selected from the group consisting of cattle, sheep and horses.

32. The mouthwash, mouthrinse or tooth gel composition or other oral composition substantially liquid in character according to claim 30, wherein the companion animals are selected from cats and dogs.

33. Use of a phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) and free fluoride ions in the manufacture of a dentifrice formulation for dental mineralization of a dental surface or subsurface, the formulation further including a dentally acceptable polishing material and a surfactant, wherein the formulation does not contain a calcium chelator or a phosphate buffer.

34. The use according to claim 33, wherein the free fluoride ions are provided from a compound selected from the group consisting of sodium fluoride, stannous fluoride, amine fluoride and combinations thereof.

35. The use according to claim 33 or 34, wherein the dental surface or subsurface is tooth enamel.

36. The use according to any one of claims 33 to 35, wherein mineralizing a dental surface or subsurface is for preventing or treating dental caries, dental hypersensitivity, fluorosis or dental calculus.

37. The use according to any one of claims 33 to 36, wherein said phosphopeptide is a casein phosphopeptide.

38. The use according to any one of claims 33 to 37, wherein said phosphopeptide includes an amino acid sequence selected from the group consisting of:

[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 $\alpha_{s1}(59-79)$

[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 $\beta(1-25)$

[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 α_{82} (46-70) and

[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 α_{82} (1-21).

5 39. The use according to any one of claims 33 to 38, wherein the free fluoride ions are present in the dentifrice in an amount of about 200 ppm to about 3000 ppm.

40. The use according to any one of claims 33 to 38, wherein the free fluoride ions are present in the dentifrice in an amount of about 400 ppm to about 1500 ppm.

10 41. The use according to any one of claims 33 to 38, wherein the free fluoride ions are present in the dentifrice in an amount of about 900ppm or of about 1100 ppm.

42. The use according to any one of claims 33 to 41, wherein the phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the dentifrice at 0.01 to 50% by weight.

15 43. The use according to any one of claims 33 to 41, wherein the phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the dentifrice at 1.0 to 50% by weight.

20 44. The use according to any one of claims 33 to 41, wherein the phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the dentifrice at 2% by weight.

45. The use according to any one of claims 33 to 44, wherein the polishing material is selected from the group consisting of water-insoluble sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated calcium phosphate, anhydrous dicalcium phosphate, calcium pyrophosphate, magnesium
5 orthophosphate, trimagnesium phosphate, hydrated alumina, calcined alumina, aluminium silicate, zirconium silicate, silica, bentonite, alkali metal aluminosilicate, particulate thermosetting resins and mixtures thereof.

46. The use according to any one of claims 33 to 45, wherein the surfactant is selected from the group consisting of water-soluble salts of higher fatty acid
10 monoglyceride monosulfates, higher alkyl sulfates, alkyl aryl sulfonates, higher alkylsulfo-acetates, higher fatty acid esters of 1,2-dihydroxy propane sulfonate, substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds and condensation products of ethylene oxide with various reactive hydrogen containing compounds reactive therewith having aliphatic chains of about
15 12 to 20 carbon atoms.

47. The use according to claim 46, wherein the water-soluble salts of higher fatty acid monoglyceride monosulfate is the sodium salt of the monosulfated monoglyceride of hydrogenated coconut oil fatty acids, the higher alkyl sulfates is sodium lauryl sulphate, the alkyl aryl sulfonates is sodium dodecyl benzene sulfonate,
20 the substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds has 12 to 16 carbons in the fatty acid, alkyl or acyl radicals and the condensation products of ethylene oxide contain hydrophilic polyoxyethylene moieties.

48. The use according to claim 46, wherein the substantially saturated higher
25 aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds include N-lauroyl sarcosine, sodium, potassium, and ethanolamine salts of N-lauroyl, N-myristoyl, or N-palmitoyl sarcosine and the condensation products of ethylene oxide

containing hydrophilic polyoxyethylene moieties include poly (ethylene oxide) with fatty acids, fatty alcohols, fatty amides, polyhydric alcohols and polypropyleneoxide.

49. The use according to any one of claims 33 to 48, wherein the dentifrice formulation is a toothpaste.

5 50. The use according to any one of claims 33 to 49, wherein the dental mineralization is of a dental surface or subsurface of a human, domestic animal, companion animal or zoo animal.

51. The use according to claim 50, wherein the domestic animal is selected from the group consisting of cattle, sheep and horses.

10 52. The use according to claim 50, wherein the companion animal is selected from cats and dogs.

53. Use of a phosphopeptide-stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) and free fluoride ions in the manufacture of a mouthwash, mouthrinse or tooth gel composition
15 or other oral composition substantially liquid in character for dental mineralization of a dental surface or subsurface, wherein the composition does not contain a calcium chelator or a phosphate buffer.

54. The use according to claim 53, wherein the free fluoride ions are provided from a compound selected from the group consisting of sodium fluoride, stannous fluoride,
20 amine fluoride and combinations thereof.

55. The use according to claim 53 or 54, wherein the dental surface or subsurface is tooth enamel.

56. The use according to any one of claims 53 to 55, wherein mineralizing a dental surface or subsurface is for preventing or treating dental caries, dental hypersensitivity, fluorosis or dental calculus.

57. The use according to any one of claims 53 to 56, wherein said phosphopeptide
5 is a casein phosphopeptide.

58. The use according to any one of claims 53 to 57, wherein said phosphopeptide includes an amino acid sequence selected from the group consisting of:

- [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 $\alpha_{s1}(59-79)$
- 10 [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 $\beta(1-25)$
- [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 $\alpha_{s2}(46-70)$ and
- 15 [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 $\alpha_{s2}(1-21)$.

59. The use according to any one of claims 53 to 58, wherein the free fluoride ions are present in the mouthwash, mouthrinse, oral composition substantially liquid in character or tooth gel in an amount of about 200 ppm to about 3000 ppm.

60. The use according to any one of claims 53 to 58, wherein the free fluoride ions
20 are present in the mouthwash, mouthrinse, oral composition substantially liquid in character or tooth gel in an amount of about 400 ppm to about 1500 ppm.

61. The use according to any one of claims 53 to 58, wherein the free fluoride ions are present in the mouthwash, mouthrinse, oral composition substantially liquid in character or tooth gel in an amount of about 900ppm or of about 1100 ppm.

62. The use according to any one of claims 53 to 61, wherein the phosphopeptide-
5 stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the mouthwash, mouthrinse, oral composition substantially liquid in character or tooth gel at 0.01 to 50% by weight.

63. The use according to any one of claims 53 to 61, wherein the phosphopeptide-
10 stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the mouthwash, mouthrinse, oral composition substantially liquid in character or tooth gel at 1.0 to 50% by weight.

64. The use according to any one of claims 53 to 61, wherein the phosphopeptide-
15 stabilized amorphous calcium phosphate (ACP) or phosphopeptide-stabilized amorphous calcium fluoride phosphate (ACFP) is present in the mouthwash, mouthrinse, oral composition substantially liquid in character or tooth gel at 2% by weight.

65. The use according to any one of claims 53 to 64, wherein the dental
20 mineralization is of a dental surface or subsurface of a human, domestic animal, companion animal or zoo animal.

66. The use according to claim 65, wherein the domestic animal is selected from the group consisting of cattle, sheep and horses.

67. The use according to claim 65, wherein the companion animal is selected from
25 cats and dogs.