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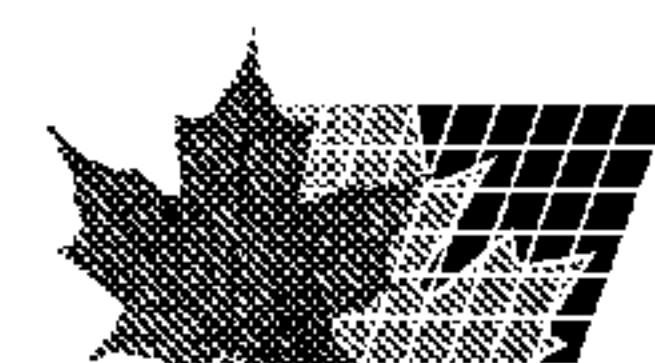
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(54) Title: DENTAL MINERALIZATION

(57) **Abrégé/Abstract:**

A method is provided for mineralizing a dental surface or subsurface including contacting the dental surface with a protein disrupting agent and stabilized amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP).



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(54) Title: DENTAL MINERALIZATION

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## DENTAL MINERALIZATION

The present invention relates to a method of 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 erosion and fluorosis are also provided.

### Background

Common causes of hypomineralized lesions are caries and fluorosis.

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 erosion, dental hypersensitivity and dental calculus are therefore imbalances in the level of calcium phosphates.

Enamel fluorosis (mottling) has been recognized for nearly a century, however, the aetiological role of fluoride was not identified until 1942 (Black and McKay, 1916). The characteristic appearance of fluorosis may be differentiated from other enamel disturbances (Fejerskov *et al.*, 1991). The clinical features of fluorotic lesions of enamel (FLE) represent a continuum ranging from fine opaque lines following the perikymata, to chalky, white enamel (Fejerskov *et al.*, 1990; Giambro *et al.*, 1995). The presence of a comparatively highly mineralized enamel outer surface and a hypomineralized subsurface in the fluorotic lesion simulates the incipient enamel "white spot" carious lesion (Fejerskov *et al.*, 1990). With increasing severity, both the depth of enamel involved in the lesion and the degree of hypomineralization increases (Fejerskov *et al.*,

1990, Giambro *et al.*, 1995). The development of fluorosis is highly dependent on the dose, duration and timing of fluoride exposure (Fejerskov *et al.*, 1990, Fejerskov *et al.*, 1996; Aoba and Fejerskov, 2002) and is believed to be related to elevated serum fluoride concentrations. Chalky “white spot” lesions may also form on developing teeth  
 5 in children such as after treatment with antibiotics or fever. Such lesions indicate areas of hypomineralization of the tooth enamel.

Depending on lesion severity, fluorosis has been managed clinically by restorative replacement or micro-abrasion of the outer enamel (Den Besten and Thariani, 1992; Fejerskov *et al.*, 1996). These treatments are unsatisfactory because they involve  
 10 restorations or removal of tooth tissue. What is desired is a treatment that will mineralize the hypomineralized enamel to produce a natural appearance and structure.

Specific complexes of casein phosphopeptides and amorphous calcium phosphate (“CPP-ACP”, available commercially as Recaldent™) have been shown to remineralize enamel subsurface lesions *in vitro* and *in situ* (Reynolds, 1998; Shen *et al.*, 2001;  
 15 Reynolds *et al.*, 2003).

WO 98/40406 in the name of The University of Melbourne (the contents of which are herein incorporated fully by reference) describes casein phosphopeptide-amorphous calcium phosphate complexes (CPP-ACP) and CPP-stabilised amorphous calcium fluoride phosphate complexes (CPP-ACFP) which have been produced at alkaline pH.  
 20 Such complexes have been shown to prevent enamel demineralization and promote remineralization of enamel subsurface lesions in animal and human *in situ* caries models (Reynolds, 1998).

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

[1] Gln<sup>59</sup>-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<sup>79</sup>  $\alpha_{s1}$ (59-79)

- [2] Arg<sup>1</sup>-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<sup>25</sup>  $\beta$ (1-25)
- [3] Asn<sup>46</sup>-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<sup>70</sup>  $\alpha_{s2}$ (46-70)
- 5 [4] Lys<sup>1</sup>-Asn-Thr-Met-Glu-His-Val-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Ile-Ser(P)-Gln-Glu-Thr-Tyr-Lys<sup>21</sup>  $\alpha_{s2}$ (1-21)

The access of mineralizing ions to the tooth enamel in many cases can be limited by the layer of salivary proteins that forms over the surface of the enamel, termed the pellicle. The proteins of the pellicle can also accumulate in sub-surface enamel lesions, thereby  
 10 inhibiting the mineralization of these lesions. Such accumulations of proteins can discolour over time, leaving unsightly patches on the tooth. Accordingly, there is a need to remove these proteins to remove discolouration and avoid limitations of access to the enamel by remineralizing ions. To overcome these and other limitations of known treatments, research to this end has been conducted.

## 15 Summary of the invention

In one aspect, the present invention provides a method of mineralizing a dental surface or sub-surface including contacting the dental surface with a protein disrupting agent, and contacting the dental surface with stabilized amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP). The dental surface is preferably  
 20 dental enamel. In one embodiment the dental surface is a lesion in the enamel, such as a lesion caused by caries, dental erosion or fluorosis.

Mineralization of dental surfaces can be significantly enhanced by the disruption of pellicle proteins from the dental surface prior to the application of a remineralizing material, such as stabilised ACP and/or ACFP. In particular, it has been found that the  
 25 mineralization of enamel by stabilized soluble forms of ACP (CPP-ACP) and ACFP (CPP-ACFP) is enhanced by pre-treatment of the enamel surface with a protein disrupting agent such as alkaline bleach.

Preferably the ACP and/or ACFP is phosphopeptide (PP)-stabilized. Preferably, the

phosphopeptide (as defined below) is a casein phosphopeptide.

In a preferred embodiment the ACP and/or ACFP is in the form of a casein phosphopeptide stabilized ACP and/or ACFP complex.

Preferably, the phase of the ACP is predominantly a basic phase, wherein the ACP  
 5 comprises predominantly the species  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$  and  $\text{OH}^-$ . 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,  $x = 1$ . Preferably the two components of the formula are present in equal proportions. Accordingly, in one embodiment, the basic phase of ACP has the formula  $\text{Ca}_3(\text{PO}_4)_2\text{Ca}_2(\text{PO}_4)(\text{OH})$ .

10 Preferably, the phase of the ACFP is predominantly a basic phase, wherein the ACFP comprises predominantly the species  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$  and  $\text{F}^-$ . 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  $x = 1$ . Preferably the two components of the formula are present in equal proportions.  
 15 Accordingly, in one embodiment, the basic phase of ACFP has the formula  $\text{Ca}_3(\text{PO}_4)_2\text{Ca}_2(\text{PO}_4)\text{F}$ .

In one embodiment, the ACP complex consists essentially of phosphopeptides, calcium, phosphate and hydroxide ions and water.

In one embodiment, the ACFP complex consists essentially of phosphopeptides,  
 20 calcium, phosphate, fluoride and hydroxide ions and water.

### Detailed description of the invention

Any suitable protein disrupting agent can be used in the method of the present invention. The agent is required to reduce the proteinaceous barrier formed over the surface to be treated, such as the pellicle over teeth. Examples of suitable agents  
 25 include bleach, detergent, chaotropic agents such as urea, high phosphate concentrations, cocktails of proteases (e.g. endopeptidases, proteinases and exopeptidases) and any other protein solubilizing, disrupting or hydrolysing agent.

Examples of suitable bleaches include sodium hypochlorite (NaOCl), and carbamide peroxide bleaches. In a preferred embodiment, the bleach is an alkaline bleach. In a further preferred embodiment the alkaline bleach is NaOCl. The protein disrupting agent acts to solubilize and partially or wholly remove proteins from the dental surface, particularly proteins of the pellicle.

In a further aspect of the present invention there is provided a method of mineralizing a dental surface comprising providing a protein disrupting agent and a source of ACP or ACFP. In a preferred embodiment the dental surface is enamel.

In a further aspect of the present invention there is provided a method for treating fluorosis comprising contacting a fluorotic lesion in tooth enamel with a protein disrupting agent and stabilized ACP and/or ACFP.

In a further aspect of the present invention there is provided a method for treating dental caries comprising contacting a caries lesion in tooth enamel with a protein disrupting agent and stabilized ACP and/or ACFP.

In a further aspect of the present invention there is provided a method for treating dental erosion comprising contacting a lesion in tooth enamel caused by erosion with a protein disrupting agent and stabilized ACP and/or ACFP.

In a further aspect of the present invention there is provided a method for reducing white spot lesions on the tooth enamel comprising contacting a white spot lesion with a protein disrupting agent and stabilized ACP and/or ACFP.

In a further aspect of the present invention there is provided a method for remineralizing a lesion in tooth enamel comprising contacting the lesion with a protein disrupting agent and stabilized ACP and/or ACFP.

Preferably the ACP and/or ACFP is stabilized by a phosphopeptide. In a preferred embodiment the phosphopeptide is a casein phosphopeptides. Preferably, the ACP or ACFP is in the form of a casein phosphopeptide stabilized ACP or ACFP complex.

In one embodiment, the protein disrupting agent is NaOCl. A concentration of about 1 to 20% NaOCl may be used. Alternatively, the concentration of NaOCl is 1 to 10%. In a preferred embodiment, about 5% NaOCl is used.

5 The protein disrupting agent may be contacted with the dental surface for a period of about 1 to 60 minutes, or for about 1 to 30 minutes. In one embodiment, the protein disrupting agent is contacted with the dental surface for about 20 minutes.

Preferably the stabilized ACP and/or ACFP is contacted with the dental surface for a period of about 1 minute to 2 hours, or 5 minutes to 60 minutes or about 10 minutes. The stabilized ACP and/or ACFP may be repeatedly applied to the dental surface over a  
10 period of 1 day to several months.

In one embodiment, the stabilized ACP and/or ACFP is contacted with the dental surface after the dental surface has been contacted with the protein disrupting agent.

In a preferred embodiment, the protein disrupting agent is contacted with the dental surface 1 to 60 minutes, or 1 to 30 minutes, or 1 to 5 minutes prior to contacting the  
15 dental surface with the stabilized ACP and/or ACFP.

In a further aspect of the present invention there is provided a method for mineralizing a tooth surface comprising applying an ACP and/or ACFP complex to a tooth surface that has been pre-treated with a protein disrupting agent. Preferably the tooth surface is tooth enamel. In a preferred embodiment, the tooth surface is tooth enamel containing  
20 a lesion selected from the group consisting of one or more of a white spot lesion; a fluorotic lesion; a caries lesion; or a lesion caused by tooth erosion. In a further preferred embodiment the protein disrupting agent is a bleach.

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

Without being bound by any theory or mode of action it is understood that pre-conditioning tooth enamel with a protein disrupting agent results in partial or

complete enamel de-proteinization, enhancing the diffusion of calcium and phosphate into subsurface enamel.

It is further understood that treatment of tooth enamel with stabilised ACFP produces fluorapatite, which is more resistant to acid challenge than normal tooth enamel. This  
5 may result in tooth enamel with superior caries resistant properties. Accordingly, in a preferred embodiment the method of the present invention includes stabilised ACFP.

“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  
10 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.

15 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  
20 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 in this specification is equivalent to the term “includes” and may be used  
25 interchangeably and should not be taken as excluding the presence of other elements or features.

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  $[\text{CPP}(\text{ACP})_8]_n$  or  $[(\text{CPP})(\text{ACFP})_8]_n$  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 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  $\alpha_{s1}$ (59-79) [1],  $\beta$ (1-25) [2],  $\alpha_{s2}$ (46-70) [3] and  $\alpha_{s2}$ (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                                 |

| Original Residue | Exemplary Conservative Substitution | Preferred Conservative Substitution |
|------------------|-------------------------------------|-------------------------------------|
| 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;

5 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;

10 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;

15 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;

5 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.

10 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].

15 In another embodiment of the invention, the stabilised ACFP or ACP 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 dental caries, tooth decay, dental erosion or fluorosis. The ACFP or ACP complex may comprise 0.01-50% by weight of the composition, preferably 1.0-50%. For oral compositions, it is preferred that the amount of the CPP-ACP and/or CPP-ACFP 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, CPP-ACFP or a mixture of both. The oral composition of this invention which  
20 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, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs. The oral composition according to this invention may further include additional well known  
25 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  
5 typically ethanol or isopropanol. Ethanol is preferred.

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. phosphoric acid, citric acid or benzoic acid) or base (e.g. sodium hydroxide) or buffered (as with sodium citrate, benzoate, carbonate, or bicarbonate,  
10 disodium hydrogen phosphate, sodium dihydrogen phosphate, etc).

In other desirable forms of this invention, the stabilised ACP or ACFP 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  
15 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  
20 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 cm<sup>2</sup>/g., silica gel or colloidal silica, and complex amorphous  
25 alkali metal aluminosilicate.

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  
30 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 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% SiO<sub>2</sub>,

25.40% MgO, 3.05% Na<sub>2</sub>O, 0.98% Li<sub>2</sub>O, 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.

Other suitable thickeners include Irish moss, iota carrageenan, gum tragacanth, starch, polyvinylpyrrolidone, hydroxyethylpropylcellulose, hydroxybutyl methyl cellulose, 5 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 10 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 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 15 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.

Organic surface-active agents may be used in the compositions of the present invention 20 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 deterative 25 material which imparts to the composition deterative 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, 30 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).

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 invention also provides an ACP or ACFP composition as described above further including a protein disrupting agent. In one embodiment, the protein disrupting agent is a bleach. In a preferred embodiment the bleach is NaOCl.

5 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.

10 In a further aspect, the invention provides compositions including pharmaceutical compositions comprising any of the ACFP and/or ACP complexes as described above together with a protein disrupting agent 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.

15 Therapeutic compositions may also be in the form of tablets or capsules. In one embodiment, the ACP and/or ACFP complexes are substantially the only remineralizing active components of such a composition. For example, a crème formulation may be employed containing: water; glycerol; CPP-ACP; D-sorbitol; silicon dioxide; sodium carboxymethylcellulose (CMC-Na); propylene glycol; titanium dioxide; xylitol; phosphoric

20 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 erosion and fluorosis.

25 In one embodiment, the active components of the composition consist essentially of the protein disrupting agent and stabilised ACP and/or ACFP. It is believed, without being bound by any theory or mode of action, that the stabilised ACP and/or ACFP and the protein disrupting agent are central to the therapeutic or preventative effect of the above embodiments of the invention, and thus embodiments consisting essentially of those

30 components (with carriers, excipients and the like as required) are included within the

scope of the invention.

The invention also relates to a kit for the treatment or prevention of one or more of dental caries, fluorosis and dental erosion including (a) a protein disrupting agent and (b) a CPP-ACP or CPP-ACFP complex in a pharmaceutically acceptable carrier.

5 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 the complex are present in suitable amounts for treatment of a patient.

In a further aspect, there is provided a method of treating or preventing one or more of each of dental caries, tooth decay, dental erosion and fluorosis, comprising the steps of  
10 administering a protein disrupting agent to the teeth of a subject followed by administering an ACP or ACFP complex or composition. Topical administration of the complex is preferred. The method preferably includes the administration of the complex in a formulation as described above.

In a further aspect there is provided the use of a protein disrupting agent in the  
15 manufacture of a first composition and use of stabilized amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) in a manufacture of a second composition, the first and second compositions being used for the treatment and/or prevention of one or more of dental caries, tooth decay, dental erosion and fluorosis, wherein the first composition is applied to a dental surface prior to the second  
20 composition.

In a further aspect there is provided a first composition including a protein disrupting agent and a second composition including stabilized amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) for the treatment and/or prevention of one or more of dental caries, tooth decay, dental erosion and fluorosis,  
25 wherein the first composition is applied to a dental surface prior to the second composition.

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 examples.

One example of a mineralizing composition is a composition comprising the following (in  
5 decreasing order of proportion):

water  
glycerol  
CPP-ACP  
D-sorbitol  
10 silicon dioxide  
sodium carboxymethylcellulose (CMC-Na)  
propylene glycol  
titanium dioxide  
xylitol  
15 phosphoric acid  
guar gum  
zinc oxide  
sodium saccharin  
ethyl p-hydroxybenzoate  
20 magnesium oxide  
butyl p-hydroxybenzoate  
propyl p-hydroxybenzoate

Such a composition is available from GC corporation under the name Tooth Mousse™.  
This is suitable for use after a protein disrupting agent, and is in the form of a paste or  
25 crème to facilitate its retention on teeth for a suitable period. Alternatively, this  
mineralizing composition may contain a protein disrupting agent, such as sodium  
hypochlorite.

The effectiveness of the invention may be demonstrated as follows.

Seven premolar teeth with FLE (Thylstrup Fejerskov Index, TF = 3) were selected from teeth extracted for orthodontic reasons from healthy patients aged 10-28 years from the Royal Dental Hospital of Melbourne, Australia. Informed patient consent was obtained for the extracted teeth and the study protocol was approved by the Human Research  
5 Ethics Committee of The University of Melbourne. All specimens were debrided of adherent soft tissue and stored in 18 % w/v formalin acetate solution at room temperature.

The teeth were cleaned with a rotating rubber cup and pumice and rinsed in double de-ionized water (DDW) (Fejerskov et al., 1988). The anatomical crowns were sectioned  
10 from the roots using a water-cooled diamond blade. Each crown was sectioned to provide a pair of enamel blocks each containing a FLE. A 4x4 mm<sup>2</sup> window was created over each lesion by placing a rectangular piece of Parafilm® ( American National Can, Chicago, Ill., USA.) over the lesion and covering the surrounding enamel with nail varnish (Revlon™, New York, USA). The parafilm was then carefully removed to reveal  
15 the enamel lesion window which was divided into halves as control and test windows. The control window was covered with nail varnish. The two lesions of each specimen were randomly assigned to one of two remineralization groups; Group I – treatment with 5% w/v CPP-ACFP and Group II – treatment with 5% w/v CPP-ACFP immediately following pre-conditioning with 5.25 % NaOCl.

20 CPP-ACFP was obtained from Recaldent Pty Ltd (Melbourne, Australia) and contained 47.6% w/w CPP, 15.7% w/w Ca<sup>2+</sup>, 22.9% w/w PO<sub>4</sub><sup>3-</sup> and 1.2% w/w F<sup>-</sup>. The CPP-ACFP was dissolved in distilled and deionized water at 5% w/v and adjusted to pH 7.0 with HCl. For the first group, each specimen was placed in 2ml of 5 % w/v, CPP-ACFP in a 5ml plastic vial at 37°C. The CPP-ACFP solution was changed daily for 10 days. For  
25 the second group, each specimen was placed in a 5.25 % NaOCl solution for 20mins, rinsed and then placed in 2ml of 5 % w/v CPP-ACFP in a 5ml plastic vial at 37°C. The CPP-ACFP solution was changed daily for 10 days.

A Chroma Meter (Minolta ChromaMeter CR241, Minolta, Japan) was used to record surface reflectance. Surface reflectance measurement was established in L\*a\*b\* color  
30 space by the Commission de L'Eclairage in 1978, and measurements relate to human colour perception in three colour dimensions (Commision Internationale de L'Eclaige,

1978). The L\* values represent colour gradients from white to black, a\* values represent colour gradients from green to red, and b\* values represent colour gradients from blue to yellow (Commision Internationale de L'Eclaige, 1978). Only L\* value measurements were used in this study with whiter colours having a higher reading, and darker colours a lower reading. To ensure a reproducible position of specimens in the Chroma Meter, a wax mold for each sample was prepared and stored. All samples were air-dried with a dental triplex syringe for 60s before each measurement. Individual specimens were repositioned ten times both before and after treatment, and colour reflectance L\* values were recorded.

Each specimen was removed from the mineralizing solution and rinsed in DDW for 60s and blotted dry with blotting paper. The nail varnish on the control window was removed gently with acetone. The control and test windows were then separated by cutting through the midline between the windows. The two half-slabs were then placed with the lesion windows parallel and embedded in cold curing methacrylate resin (Paladur, Heraus Kulzer, Germany). The two paired enamel half-slabs were then sectioned, and subjected to microradiography and microdensitometric image analysis to determine mineral content exactly as described by Shen et al. (2001).

An area free of defects close to the midline of each microradiographic image of each lesion (control and test) was chosen and scanned six times (Shen et al., 2001). Each scan comprised 200 readings, taken from the enamel surface to the mid-enamel region to include the total fluorotic lesion. The test (CPP-ACFP-treated) lesion was scanned to exactly the same depth as the control (untreated) lesion. The gray values obtained from each scan were converted to the equivalent thickness of aluminium (tA) using the image of the aluminium stepwedge included with each section (Shen et al., 2001). Using the formula of Angmar et al. (1963), the percentage volume of mineral was obtained for each reading as follows:  $V = (52.77(tA) - 4.54) / tS$ . Where: V = volume of mineral as a percentage; tA= the relative thickness of aluminium obtained from the gray value scanned; and tS = section thickness (80  $\mu$ m).

From the densitometric profile of [(vol % min versus lesion depth (mm))] for each lesion DZ values were calculated using trapezoidal integration (Reynolds, 1997). The difference between the area under the profile of the untreated fluorotic enamel in the

control window with adjacent normal enamel was designated DZf, and the difference between the area under the CPP-ACFP-treated fluorotic enamel in the test window and adjacent normal enamel was designated DZr. Percentage mineralization (%M) of the fluorotic lesion was therefore  $(1 - DZr/(DZf) \times 100$  (Reynolds, 1997).

- 5 Following the microradiography the sections containing both control and mineralized FLE were subjected to Energy Dispersive X-ray Analysis (EDAX) as described previously (Reynolds, 1997).

Mean  $L^*$  values were compared using a one way classification analysis of variance (ANOVA) with a Scheffe multiple comparison. The mean %M values were also  
10 compared using a one-way ANOVA. Overall mean  $L^*$  and %M values were analysed using a paired data Student's t-test.

The  $L^*$  values of the untreated fluorotic enamel lesions ranged from 79.1 to 87.8 with a mean value of  $83.6 \pm 3.6$  (Table 1). Treatment with 5% CPP-ACFP significantly reduced the  $L^*$  value to  $74.6 \pm 4.1$ , which was not significantly different to normal enamel (Table  
15 1). Pre-conditioning with NaOCl followed by 5% CPP-ACFP treatment significantly reduced the  $L^*$  value to  $72.6 \pm 5.6$ , which was also not significantly different to normal enamel (Table 1). There was no significant difference in  $L^*$  values for the two post-treatment (CPP-ACFP and NaOCl/CPP-ACFP) groups. The appearance of the surface enamel of both treatment groups had substantially improved with both exhibiting the  
20 appearance of normal, translucent enamel.

The difference between the mineral content of sound enamel and that of the pre-treatment lesions (DZf) varied from 426 to 12,048 vol % min. mm (Table 2). No correlation was found between surface reflectance ( $L^*$ ) and DZf of the untreated FLE. Treatment with 5% CPP-ACFP alone substantially increased the mineral content of the  
25 fluorotic lesions to restore 32.7% to 55.5% of the missing mineral, with a mean value of  $44.8 \pm 10.6$  % (Table 2). Restoring 100% of the missing mineral would convert the entire lesion to sound enamel with respect to mineral content. Pre-conditioning of the enamel with NaOCl before CPP-ACFP treatment increased mineral uptake from 73.6% to 92.8% of the missing mineral with a mean value of  $80.1 \pm 7.8$  % (Table 2). Energy  
30 dispersive X-ray analysis of the mineralized lesion of the transverse sections confirmed

the mineral formed by the CPP-ACFP treatment was a fluoride-containing apatite.

Table 1

Effect of 5% CPP-ACFP with and without NaOCl pre-conditioning on colour reflectance (L\*) of fluorotic enamel specimens

| Fluorotic enamel specimens    | Colour Reflectance (L*) Values |            |            |            |            |            |            |
|-------------------------------|--------------------------------|------------|------------|------------|------------|------------|------------|
|                               | I                              | II         | III        | IV         | V          | VI         | VII        |
| Pre-treatment                 | 82.9 ± 0.9 <sup>a</sup>        | 85.5 ± 1.8 | 84.3 ± 0.4 | 82.5 ± 1.3 | 87.8 ± 0.6 | 79.1 ± 0.9 | 83.0 ± 0.6 |
| Post-CPP-ACFP treatment       | 74.1 ± 0.7 <sup>b</sup>        | 72.0 ± 0.5 | 78.2 ± 0.4 | 76.1 ± 0.7 | 79.5 ± 0.8 | 69.7 ± 1.5 | 72.3 ± 1.7 |
| Post-NaOCl/CPP-ACFP treatment | 69.2 ± 1.0 <sup>b</sup>        | 72.3 ± 1.1 | 76.9 ± 1.4 | 72.2 ± 1.3 | 78.5 ± 1.4 | 61.6 ± 1.2 | 77.4 ± 1.0 |

Overall Mean 83.6 ± 3.6 74.6 ± 4.1° 72.6 ± 5.6°

a n = 20

5 b n = 10

c Post-treatment mean value is significantly different from pre-treatment mean value (paired Student's t-test, p < 0.01) but not significantly different from normal enamel 71.6 ± 3.1.

Table 2

Effect of 5% CPP-ACFP with and without NaOCl pre-conditioning on mineral content of fluorotic enamel

| Treatment                | Specimens                                                         |                             |                             |                             |                             |                             |                              | Overall Mean    |
|--------------------------|-------------------------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|-----------------|
|                          | I                                                                 | II                          | III                         | IV                          | V                           | VI                          | VII                          |                 |
| Natural fluorotic lesion | $\Delta Zf$<br>(vol%min. $\mu m$ )<br>2331 $\pm$ 352 <sup>a</sup> | - <sup>c</sup>              | 3869 $\pm$ 70 <sup>a</sup>  | 2468 $\pm$ 323 <sup>a</sup> | 2706 $\pm$ 103 <sup>a</sup> | 3238 $\pm$ 194 <sup>a</sup> | - <sup>c</sup>               |                 |
| CPP-ACFP treated         | $\Delta Zr$<br>(vol%min. $\mu m$ )<br>1203 $\pm$ 241 <sup>a</sup> | - <sup>c</sup>              | 1723 $\pm$ 262 <sup>a</sup> | 1618 $\pm$ 427 <sup>a</sup> | 1270 $\pm$ 596 <sup>a</sup> | 2178 $\pm$ 216 <sup>a</sup> | - <sup>c</sup>               |                 |
|                          | %M <sup>b</sup><br>48.4                                           | - <sup>c</sup>              | 55.5                        | 34.5                        | 53.1                        | 32.7                        | - <sup>c</sup>               | 44.8 $\pm$ 10.6 |
| Natural fluorotic lesion | $\Delta Zf$<br>(vol%min. $\mu m$ )<br>2199 $\pm$ 266 <sup>a</sup> | 6501 $\pm$ 441 <sup>a</sup> | - <sup>c</sup>              | 1181 $\pm$ 261 <sup>a</sup> | 2461 $\pm$ 213 <sup>a</sup> | - <sup>c</sup>              | 12048 $\pm$ 512 <sup>a</sup> |                 |
| NaOCl / CPP-ACFP treated | $\Delta Zr$<br>(vol%min. $\mu m$ )<br>581 $\pm$ 230 <sup>a</sup>  | 471 $\pm$ 285 <sup>a</sup>  | - <sup>c</sup>              | 211 $\pm$ 137 <sup>a</sup>  | 552 $\pm$ 203 <sup>a</sup>  | - <sup>c</sup>              | 3087 $\pm$ 723 <sup>a</sup>  |                 |
|                          | %M <sup>b</sup><br>73.6                                           | 92.8                        | - <sup>c</sup>              | 82.1                        | 77.6                        | - <sup>c</sup>              | 74.4                         | 80.1 $\pm$ 7.8  |

a Mean  $\pm$  SD (n=6)

b %M = percentage mineralization (1- $\Delta Zr/\Delta Zf$ ) x 100

5 c Sample lost during processing

In the clinic, as an example of a patient in need of remineralizing treatment of the tooth enamel, the patient is treated using the steps of:

1. Pretreating an enamel area in need of treatment, isolated using a rubber dam, with a 5% solution of NaOCl for 5 minutes.
- 5     2. Removing the NaOCl solution from the area with a moist cotton bud.
3. Applying the CPP-ACP-containing topical crème Tooth Mousse™ (GC Corporation) to the enamel surface immediately for 5 minutes and then the patient further applies the Tooth Mousse™ nightly without rinsing for four weeks.

10 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.

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**CLAIMS**

1. A method of mineralizing a dental surface or subsurface including contacting the dental surface or subsurface with a protein disrupting agent and stabilized amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP).
- 5 2. A method according to Claim 1, wherein the dental surface is dental enamel.
3. A method according to Claim 1, wherein the dental surface is a lesion in the dental enamel.
4. A method according to Claim 3, wherein the lesion is caused by dental caries, dental erosion or fluorosis.
- 10 5. A method according to Claim 1, wherein the ACP and/or ACFP is phosphopeptide-stabilised.
6. A method according to Claim 5, wherein the phosphopeptide is a casein phosphopeptide.
7. A method according to Claim 1, wherein the ACP or ACFP is in a basic phase.
- 15 8. A method according to Claim 1, wherein the protein disrupting agent is selected from one or more of the group consisting of a bleach, a detergent, a chaotropic agent, a protease and a mixture of proteases.
9. A method according to Claim 8, wherein the bleach is sodium hypochlorite or a carbamide peroxide bleach.
- 20 10. A method for remineralizing a lesion in tooth enamel comprising contacting the lesion with a protein disrupting agent and stabilized ACP and/or ACFP.
11. A method for treating fluorosis comprising contacting a fluorotic lesion in tooth enamel with a protein disrupting agent and stabilized ACP and/or ACFP.

12. A method for treating dental caries comprising contacting a caries lesion in tooth enamel with a protein disrupting agent and stabilized ACP and/or ACFP.
13. A method for treating dental erosion comprising contacting a lesion in tooth enamel caused by erosion with a protein disrupting agent and stabilized ACP and/or  
5 ACFP.
14. A method for reducing white spot lesions on the tooth enamel comprising contacting a white spot lesion with a protein disrupting agent and stabilized ACP and/or ACFP.
15. A kit for the treatment or prevention of one or more of dental caries, fluorosis and  
10 dental erosion including (a) a protein disrupting agent and (b) a CPP-ACP or CPP-ACFP complex in a pharmaceutically acceptable carrier.