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(56) Related Art
FAHAD, A.H. ET AL., JOURNAL OF BAGHDAD COLLEGE OF DENTISTRY, (2012), vol. 24, no. 4, pages 114 - 120
EP 2301513 A2
"MI Paste™ and MI Paste Plus™ with RECALDENT™ (CPP-ACP)", INSIDE DENTISTRY, vol. 8, no. 10, (201210), URL: www.dentalaegis.com/id/2012/10/mi-paste-and-mi-paste-plus-with-recaldent-cpp-acp, (2014-10-21)
"MI Paste™ and MI Paste Plus™", (2013-12-23), URL: <http://web.archive.org/web/2013-12-23044114/>
http://www.gcamerica.com/products/preventive/MI_Paste, (2014-10-21)
"MI Paste™ and MI Paste Plus™", (2013-12-04), URL: <http://web.archive.org/web/20140701070616/> <http://www.mipaste.com/about.php>, (2015-02-16)
COMAR, L.P. ET AL., "Effect of NaF, SnF₂, and TiF₄ Toothpastes on Bovine Enamel and Dentin Erosion-Abrasion In Vitro". INTERNATIONAL JOURNAL OF DENTISTRY, (2012), vol. 2012, pages 1 - 6
BAIG, A. ET AL., "HAP Dissolution Study II: SnF₂ vs. NaF Dentifrice Study"., 87TH SESSION OF THE IADR, (2009-04-01), URL: dentalcare.com/media/en-US/research_db/pdf/Page24_725.pdf, (2014-10-21)
GISSELSSON, H. ET AL., "Effect of professional flossing with NaF or SnF₂ gel on approximal caries in 13-16-year-old school children"., ACTA ODONTOLOGICA SCANDINAVICA, (1999), vol. 57, no. 2, pages 121 - 125
WO 9400146 A1
EP 1525878 A1
US 20090022672 A1

US 20030165442 A1
US 20120129135 A1
JP 2005-14595 A



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(54) Title: STABILIZED STANNOUS COMPOSITIONS

(57) Abstract: The present invention relates to improved complexes of amorphous calcium phosphate and/or amorphous calcium fluoride phosphate stabilised by phosphopeptides/phosphoproteins by addition of stannous ions. These complexes have anticariogenic properties useful to protect tooth structures as they remineralize (repair) early stages of dental caries and have other dental/medical applications (including anti- calculus, anti-erosion/corrosion and anti-dentinal hypersensitivity). Methods of making the complexes of the invention and of treatment or prevention of various dental conditions including dental caries, dental calculus, dental erosion/corrosion and dental hypersensitivity are also provided.



Stabilized Stannous Compositions

Field of the invention

The present invention relates to improved complexes of amorphous calcium phosphate and/or amorphous calcium fluoride phosphate stabilised by phosphopeptides/phosphoproteins by addition of stannous ions. These complexes have anticariogenic properties useful to protect tooth structures as they remineralize (repair) early stages of dental caries and have other dental/medical applications (including anti-calculus, anti-erosion/corrosion and anti-dentinal hypersensitivity). Methods of making the complexes of the invention and of treatment or prevention of various dental conditions including dental caries, dental calculus, dental erosion/corrosion and dental hypersensitivity are also provided.

This application claims priority to Australian patent application nos. 2013905081, 2014901202 and 2014903815, these applications are incorporated by reference in their entirety.

Background of the invention

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 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 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; 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. 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 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]. Moreover, improvements on these compositions are disclosed in WO 2006/056013 and WO 2007/090242 and specific uses (the contents of which are herein incorporated fully by reference).

Stannous fluoride (SnF_2) is one of a number of metal fluoride salts that has been proposed as an anticaries and antiplaque agent however there are difficulties in delivering this agent in toothpaste, mouthwash and other oral care products due to the instability of the stannous ions. In the presence of hydroxide ions and phosphate ions stannous can precipitate as stannous hydroxide and stannous phosphate complexes. This precipitate is significantly less biologically available and can be more easily removed from the oral cavity by swallowing. Furthermore the stannous ions can be oxidized to Stannic ions (SnIV) which are even more reactive to produce poorly soluble forms with very low activity. New toothpaste formulations with SnF_2 rely on being non-aqueous or use polyphosphate to stabilize the stannous ions. However, these formulations have low acceptance particularly by people with dry mouth. There is a need to provide improved or alternative treatments for hypomineralized lesions.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

Summary of the invention

In one aspect, the invention provides a stannous-associated stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex.

The stannous may be bound to the stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) as determined using the experimental protocol in Example 2. In one embodiment, stannous-associated stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex are produced by the method as described herein, including but not limited to the method described in Example 1.

Without being bound by any theory or mode of action, it is believed that phosphopeptides, such as casein phosphopeptides, can stabilize a stannous compound in an aqueous environment and in the presence of stabilized amorphous calcium

phosphate (ACP) and stabilized amorphous calcium fluoride phosphate (ACFP) these complexes are superior to other forms of fluoride and stabilized ACP or ACFP in remineralizing enamel subsurface lesions. Mineralization of dental surfaces can be significantly enhanced by providing a stannous compound during the process of mineralization by stabilized ACP and/or stabilized ACFP. In particular, it has been found that the mineralization of enamel by stabilized soluble forms of stannous-associated ACP complexes and stannous-associated ACFP complexes is enhanced compared with stabilized ACP and fluoride without associated stannous. In other words, the stannous ions complex with CPP-ACP and/or CPP-ACFP complexes, and these Sn-associated CPP-AC(F)P complexes then deliver superior properties. Various compositions incorporating these complexes for administration are useful. Where the fluoride stannous salt is used, additional fluoride ions are available in compositions of the stannous-associated ACP/ACFP complexes. Additional fluoride ions may also be provided by inclusion of NaF in the composition.

The invention also provides a stannous-associated stabilized ACP or ACFP having a stannous ion content of at least 1 mole of stannous per mole of phosphopeptide. Preferably, the stannous-associated stabilized ACP or ACFP has a stannous ion content of at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 moles of stannous per mole of phosphopeptide. Even more preferably, the stannous ion content is in the range of 1 to 100, 1 to 50, 1 to 20 or 1 to 10 moles of stannous per mole of phosphopeptide. The present invention also provides a composition comprising, or consisting of, stannous-associated stabilized ACP and/or ACFP complexes as described herein.

In any embodiment, the stannous ion content above may be the stannous ion content tightly-bound to the complex (as described herein). In assessing the stannous ion content, the tightly-bound stannous ion content is measured by the methods described herein, in particular, in Example 6.

The invention also provides a stannous-associated stabilized ACP and/or ACFP complex comprising stannous ions that remain associated with the complex after centrifugation in a 1000 molecular weight cut off filter at about 3000g for 1 hour at room temperature.

The invention also provides a stannous-associated stabilized ACP or ACFP having at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of the

stannous associated with the complex as tightly bound as determined by the method in Example 6.

The invention also provides a stannous-associated stabilized ACP or ACFP having at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of the stannous used in preparing the complexes incorporated into the complexes. The complex may be prepared as outlined in Example 1.

In one embodiment the invention provides a stannous fluoride associated stabilized amorphous calcium phosphate (ACP) and/or a stannous fluoride associated amorphous calcium fluoride phosphate (ACFP) complex. In another aspect, invention provides a composition including a stannous compound and stabilized ACP or ACFP. In either embodiment, the stannous compound is preferably stannous fluoride and/or stannous chloride. Optionally, NaF is also included.

In one aspect, the present invention provides a method of mineralizing a dental surface or sub-surface comprising contacting the dental surface or subsurface with (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

The stannous containing compound can be any soluble stannous containing compound suitable for oral use. Preferably, stannous containing compound is a stannous salt. The stannous salt may contain fluoride. A stannous salt includes, but not limited to, stannous fluoride, stannous chloride, potassium stannous fluoride, sodium stannous fluorozirconate, stannous chloride fluoride, stannous acetate, sodium stannous fluoride, stannous hexafluorozirconate, stannous sulfate, stannous tartrate, stannous gluconate, disodium monostannous citrate. Preferred stannous salts include stannous fluoride and stannous chloride

The dental surface is preferably dental enamel or dentine. In one embodiment the dental surface is a lesion in the enamel, such as a lesion caused by caries, dental erosion or fluorosis. In another embodiment the surface is exposed dentine causing dentinal hypersensitivity.

Preferably, the stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) is phosphopeptide stabilized. Preferably, the phosphopeptide (as defined below) is a casein phosphopeptide.

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

5 Optionally, the ACP or ACFP is predominantly in a basic form.

In another aspect the invention also provides a composition including stannous-associated stabilized ACP or ACFP, further comprising fluoride, wherein fluoride is provided as stannous fluoride and sodium fluoride. Preferably, the composition includes 2% w/v stabilized ACP or ACFP, about 1100ppm fluoride as stannous fluoride and
10 about 350ppm fluoride as sodium fluoride, or the composition includes 0.4% w/v stabilized ACP or ACFP, about 220ppm fluoride as stannous fluoride and about 70ppm fluoride as sodium fluoride. Preferably, the composition is a toothpaste.

In a preferred embodiment, the calcium ion content of the stabilised ACP or ACFP complex is in the range of about 30 to 100 moles of calcium per mole of PP. More
15 preferably, the calcium ion content is in the range of about 30 to about 50 moles of calcium per mole of PP.

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

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

- 5 In a further aspect of the present invention there is provided a method of mineralizing a dental surface comprising providing (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP. In a preferred embodiment the dental surface is enamel.

- 10 In a further aspect of the present invention there is provided a method of forming a layer on the surface of a tooth comprising providing (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP. Preferably the tooth is in a subject identified as being susceptible to, or suffering from, any one or more of dental erosion, demineralization, caries or dentinal hypersensitivity. Preferably, the stabilized amorphous calcium phosphate (ACP) and/or
15 amorphous calcium fluoride phosphate (ACFP) is phosphopeptide stabilized. Preferably, the phosphopeptide (as defined below) is a casein phosphopeptide.

Such a layer may be characterised has a calcium : phosphate ratio equivalent to normal apatite, preferably where the ratio is about 2:1. The layer ideally contains an amount of calcium that is about 20 wt%.

- 20 Preferably, the layer has about 3 to 12 fold more stannous ions than sound enamel.

- Preferably, the layer contains carbon, oxygen, fluoride, phosphate, calcium and stannous. The layer may exhibit about an elemental analysis of 20-30wt% carbon, 35-45wt% oxygen, 0.1-1wt% fluoride, 8 to 12wt% phosphate, 16 to 24wt% calcium and/or 0.5-2wt% stannous. Alternatively, the layer may exhibit an elemental analysis of any
25 one of the elements, such as calcium, phosphate, fluoride, carbon and/or stannous, as shown in any one of Table 3 or 4.

- In a further aspect of the present invention there is provided a process of forming a layer having a calcium : phosphate ratio at, or near, that of normal apatite on a dental surface, the process comprising contacting the dental surface with (a) a composition
30 including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

A method of protecting a tooth surface comprising providing (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP. Typically the tooth surface may be one that has been identified as benefiting from a surface layer, for example, due to an increased likelihood of demineralization. The tooth surface may be an enamel surface or dentine surface.

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) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP 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) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP 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) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

In a further aspect of the present invention there is provided a method for reducing dentinal hypersensitivity comprising contacting exposed dentine with (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

In a further aspect of the present invention there is provided a method for remineralizing a lesion in tooth enamel or dentine comprising contacting the lesion with (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

Preferably the stannous-associated stabilized ACP or ACFP are stabilized by a phosphopeptide (PP). 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 any aspect or embodiments as described herein, the stabilized ACP and/or ACFP or stannous-associated stabilized ACP or ACFP may be in a formulation with additional

calcium phosphate. Typically, the formulation includes a PP stabilized ACP and/or ACFP complex together with at least an equal amount by weight of calcium phosphate.

In a further aspect of the invention there is provided a method for remineralizing a lesion in tooth enamel comprising contacting the lesion with (a) a composition including a
5 stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP followed by administering a composition containing sodium bicarbonate or urea. Preferably, the composition is a mouthrinse or mouthwash containing sodium bicarbonate or urea.

In any aspect or embodiment of the invention described herein, a compound which is
10 capable of increasing or maintaining the pH of a solution may be administered concurrently with, as a pre-treatment to, or as a post-treatment to (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

In any aspect or embodiment of the invention described herein, (a) a composition
15 including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP may be applied to the mouth, tooth or lesion by the subject in need of treatment or by a dental health care professional.

A composition including a stannous compound and stabilized ACP or ACFP may be contacted with the dental surface for a period of about 1 to 60 minutes, or for about 1 to
20 30 minutes. In one embodiment, a composition including a stannous compound and stabilized ACP / ACFP is contacted with the dental surface for about 20 minutes.

Preferably a composition including a stannous compound and stabilized ACP 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 composition including a stannous
25 compound and stabilized ACP / ACFP may be repeatedly applied to the dental surface over a period of 1 day to several months.

A stannous-associated stabilized ACP or ACFP 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, a stannous-associated stabilized ACP / ACFP is contacted with the dental
30 surface for about 20 minutes.

Preferably a stannous-associated stabilized ACP 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 stannous-associated stabilized ACP or ACFP may be repeatedly applied to the dental surface over a period of 1 day to several months.

In one embodiment, (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP, is contacted with
5 the dental surface 1 to 60 minutes, or 1 to 30 minutes, or 1 to 5 minutes.

In one embodiment, the dental surface is in need of such treatment. Therefore the invention includes in addition to the steps of any method described herein a step of identifying a subject suffering fluorosis, dental caries, dentinal hypersensitivity or dental calculus, a white spot lesion; a fluorotic lesion; a caries lesion; or a lesion caused by
10 tooth erosion, or dental plaque or gingivitis or periodontitis.

The present invention provides a composition for mineralizing a dental surface or sub-surface that is also capable of reducing plaque, gingivitis and periodontitis comprising (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

15 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 administering (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP, thereby treating or preventing one or more of each of dental caries, tooth decay, dental erosion and
20 fluorosis. Topical administration of the complex is preferred. The method preferably includes the administration of the complex in a formulation as described above.

The present invention also provides a composition containing a stannous-associated stabilized ACP or ACFP. Preferably, the composition further includes a pharmaceutically acceptable carrier, diluent or excipient.

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

30 In another preferred embodiment, the calcium ion content of the stabilised ACP or ACFP complex in the composition is in the range of about 30 to 100 moles of calcium

per mole of PP. More preferably, the calcium ion content is in the range of about 30 to about 50 moles of calcium per mole of PP.

In any embodiment as described herein, the ACP and/or ACFP in the composition can be in the form of a casein phosphopeptide stabilized ACP and/or ACFP complex.

- 5 The invention also relates to a kit for the treatment or prevention of one or more of dental caries, fluorosis, dental hypersensitivity and dental erosion including:
- (a) a compound capable of increasing or maintaining the pH of a solution, and
 - (b) a composition including a stannous compound and stabilized ACP or ACFP, or
 - (c) a stannous-associated stabilized ACP or ACFP.
- 10 Preferably, the stannous-associated stabilized ACP or ACFP is 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. The instructions may describe the use of the kit to treat or prevent one or more of each of dental caries, tooth decay, dental erosion, dental hypersensitivity and fluorosis. In one embodiment,
- 15 the agent and the complex are present in suitable amounts for treatment of a patient.

The composition or kit of the invention may further include a source of fluoride ions. The fluoride ions may be from any suitable source. A source of fluoride ions may include free fluoride ions or fluoride salts. Examples of sources of fluoride ions include, but are not limited to the following: sodium fluoride, sodium monofluorophosphate, stannous

20 fluoride, sodium silicofluoride and amine fluoride. These may be provided in solution (typically an aqueous solution), or a suspension.

Brief description of the drawings

Figure 1: Representative SEM images of enamel subsurface lesions before (A) and after (B) treatment with stabilized SnF_2 /ACP also demonstrating the surface layer.

- 25 **Figure 2:** Representative SEM images of enamel subsurface lesions before (A) and after (B) treatment with stabilized NaF/ACP [Note no formation of a novel surface layer].

Figure 3: Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) of the mineralised surface layer resulting from treatment with stabilized SnF_2 /ACP.

- Figure 4:** Representative SEM image of elemental analysis by SEM-EDS of the
- 30 mineralised surface layer resulting from treatment with stabilized SnF_2 /ACP.

Figure 5: Representative SEM image of elemental analysis by SEM-EDS of the mineralised surface layer resulting from treatment with stabilized SnF₂/ACP.

Figure 6: Remineralization of enamel subsurface lesions by stabilized SnF₂/ACP versus stabilized NaF/ACP. The numerical values of this Figure are shown in Table 2.

- 5 **Figure 7:** *In situ* remineralization of enamel subsurface lesions by CPP-ACP SnF₂/NaF compared with CPP-ACP, SnF₂ and NaF, NaF with or without CPP-ACP.

Detailed description of the embodiments

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

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

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

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

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

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

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

The present invention is based on the finding that casein phosphopeptides can stabilize a stannous compound, such as SnF_2 , in an aqueous environment and in the presence of stabilized amorphous calcium phosphate (ACP) and stabilized amorphous calcium fluoride phosphate (ACFP) these formulations are superior to other forms of fluoride and stabilized ACP or ACFP in remineralizing enamel subsurface lesions. Mineralization of dental surfaces can be significantly enhanced by providing a stannous compound during the process of mineralization by stabilized ACP and/or stabilized ACFP. In particular, it has been found that the mineralization of enamel by stabilized soluble forms of stannous-associated ACP complexes and stannous-associated ACFP complexes is enhanced compared with stabilized ACP and fluoride without associated stannous.

The stannous ion in the presence of stabilized ACP and/or stabilized ACFP can produce a protective surface layer on the enamel/dentine to help prevent dental caries, dentinal hypersensitivity, dental plaque and periodontal disease. The layer which includes stannous ions and stabilized ACP and/or stabilized ACFP can provide a reservoir for calcium, phosphate, and fluoride for mineralization of the tooth surface or subsurface. These formulations have been designated stannous-associated stabilized ACP and stannous-associated stabilized ACFP.

Without being bound by any theory or mode of action it is believed that the stannous ions are stabilized by the presence of the phosphopeptides, particularly casein phosphopeptides. Therefore, it is believed that the phosphopeptides deliver stannous and fluoride ions together with the calcium and phosphate ions to promote remineralization. Remineralization may be a result of formation of calcium and stannous fluorapatite. It is also believed that the stannous ions cross-link the phosphopeptide stabilized ACP or stabilized ACFP at the tooth surface to form a layer that can protect the tooth surface from demineralization. The presence of the stannous in the surface layer would render it hard and resistant to degradation due to normal wear and tear or other processes such as erosion. A further advantage of the invention is that the stannous ions may assist to kill oral bacteria that produce acid and other metabolic products which promote demineralization.

Stannous is known to form precipitates with hydroxide ions and phosphate ions thereby reducing the bioavailability and activity of the stannous ion. The resulting promotion of stabilized-ACP/stabilized-ACFP driven remineralization by stannous ions is surprising

as it would be expected that the hydroxide ions (eg OH^-) and phosphate ions (eg PO_4^{3-}) provided by the stabilized-ACP or stabilized-ACFP would cause precipitation of the stannous to stannous hydroxide and stannous phosphate, thereby severely decreasing any activity of the stannous containing compound. Further, it would be expected that formation of a precipitate of stannous ions with hydroxide ions would not only reduce the bioavailability of the stannous ions but also remove hydroxide ions from the environment thereby reducing the pH. A reduction in pH would promote demineralization and hinder mineralization.

The present invention provides stannous-associated stabilized ACP or ACFP complexes and methods of mineralizing a dental surface or sub-surface comprising contacting the dental surface or subsurface with a stannous-associated stabilized ACP or ACFP complexes. A dental subsurface is typically a hypomineralized lesion such that the stannous-associated stabilized ACP or ACFP complexes contacted to the dental surface migrate through any surface layer, i.e. pellicle and/or plaque, through the porous dental surface to the region requiring mineralization. These stannous-associated stabilized ACP or ACFP complexes also generate a protective surface layer on the enamel/dentine to help prevent dental caries, dentinal hypersensitivity, dental plaque and periodontal disease. The dental surface is preferably dental enamel or dentine. The dental surface may be a lesion in the enamel, such as a lesion caused by caries, dental erosion or fluorosis.

In one aspect the invention provides, a stannous-associated stabilized amorphous calcium phosphate (ACP) or stannous-associated amorphous calcium fluoride phosphate (ACFP). The stannous may be bound to the stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) as determined using the experimental protocol in Example 2. In one embodiment, the stannous-associated stabilized ACP or stannous-associated stabilized ACFP are produced by a method as described herein, including but not limited to, the method described in Example 1.

The stannous is present in the stannous-associated stabilized amorphous calcium phosphate (ACP) or stannous-associated amorphous calcium fluoride phosphate (ACFP) complex by binding to or being incorporated in the complex. This complex associated stannous, wherein the stannous is bound to or incorporated in the complex, can be determined using filtration and atomic absorption spectrophotometry. Tightly

associated complexed stannous is measured as the difference between total stannous less loosely-bound stannous. To determine the total stannous (both tightly & loosely bound) present in a solution of stannous-associated complexes, a solution containing the complexes was taken and placed into HNO₃ and incubated at room temperature
5 with constant slow end over end mixing for 24 hrs. The mixture can then be centrifuged at about 1000g, preferably for 15 minutes at room temperature. The supernatant when analysed for stannous ion content, preferably by atomic absorption spectrophotometry (AAS), will provide the value for the total stannous present (whether that be bound/associated with the complex or free in solution). The level of loosely-bound
10 stannous in the solution can then be determined by taking a sample of a solution of stannous-associated complexes, placing it in a centricon with a 1000 MWCO filter and centrifuging at about 3000g, preferably for 1 hour at room temperature, to produce enough filtrate (<10% of total sample to not affect equilibrium) for analysis by AAS. The filtrates contain loosely-bound stannous ions. Tightly CPP-bound (colloidal retentate)
15 stannous ions can then be calculated from the difference between total and loosely-bound stannous ions. 'Loosely-bound' stannous is stannous that is separable from a complex only by a method such as centrifugation as described above. The loosely-bound stannous is still associated with the complexes but is more easily dissociable than tightly-bound stannous ions by the less stringent conditions explained above.

20 In one embodiment, the stannous-associated stabilized amorphous calcium phosphate (ACP) or stannous-associated amorphous calcium fluoride phosphate (ACFP) complex including stannous that cannot be separated from the complex by centrifugation at about 3000g, preferably for 1 hour at room temperature.

In a further aspect of the present invention there is provided a method of mineralizing a
25 dental surface comprising providing (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized 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) a composition
30 including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP 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) a composition

including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP 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) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

In a further aspect of the present invention there is provided a method for inhibiting the progression of dental erosion comprising contacting a surface exhibiting erosion with (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP, thereby forming a surface layer that inhibits the progression of dental erosion.

In a further aspect of the present invention there is provided a method for reducing dentinal hypersensitivity on exposed dentine comprising contacting the dentine with (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP 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) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP.

In a further aspect of the present invention there is provided a method of increasing the remineralization efficacy of a composition having stabilized ACP and/or ACFP the method including the step of adding a stannous compound to the composition. Preferably, the stannous compound is added to the composition when it is in aqueous state, such as during manufacture.

Preferably the stannous-associated stabilized ACP or ACFP is stabilized by a phosphopeptide. In a preferred embodiment the phosphopeptide is a casein phosphopeptide. Preferably, the ACP or ACFP is in the form of a casein phosphopeptide stabilized ACP or ACFP complex.

In any aspect or embodiments as described herein, the stabilized ACP and/or ACFP may be in a formulation with additional calcium phosphate. Typically, the formulation includes a PP stabilized ACP and/or ACFP complex together with at least an equal amount by weight of calcium phosphate.

A composition including a stannous compound and stabilized ACP or ACFP 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 composition including a stannous compound and stabilized ACP or ACFP is contacted with the dental surface for about 20 minutes. An example of how this is achieved is formulating stannous compound and stabilized ACP or ACFP into an oral composition, such as a paste, and then contacting or applying the composition to the dental surface. The oral composition, such as a paste, has sufficient viscosity to be retained on the tooth for the required time period.

Preferably a composition including a stannous compound and stabilized ACP 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 a composition including a stannous compound and stabilized ACP / ACFP may be repeatedly applied to the dental surface over a period of 1 day to several months.

A stannous-associated stabilized ACP or ACFP 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 stannous-associated stabilized ACP or ACFP is contacted with the dental surface for about 20 minutes. An example of how this is achieved is formulating the stannous-associated stabilized ACP or ACFP into an oral composition, such as a paste, and then contacting or applying the composition to the dental surface. The oral composition, such as a paste, has sufficient viscosity to be retained on the tooth for the required time period.

Preferably a stannous-associated stabilized ACP 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 stannous-associated stabilized ACP or ACFP may be repeatedly applied to the dental surface over a period of 1 day to several months.

In one embodiment, the dental surface is in need of such treatment. Therefore, in another aspect, the invention includes in addition to the steps of any method described herein a step of identifying a subject suffering fluorosis, dental caries, dentinal hypersensitivity or dental calculus, a white spot lesion; a fluorotic lesion; a caries lesion; or a lesion caused by tooth erosion, dental plaque, gingivitis or periodontitis.

The present invention provides (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP for use

in mineralizing a dental surface or sub-surface and reducing the viability of bacteria on that dental surface.

In any method of the invention, the stannous compound and stabilized ACP or ACFP are applied to the dental surface sequentially or concurrently. In any embodiment, the stannous compound is added prior to the stabilized ACP or ACFP. In any embodiment, the stannous compound is added after the stabilized ACP or ACFP.

A stannous-associated stabilized ACP or ACFP complex as referred to herein include a stannous-associated stabilized-ACP or ACFP complex formed at a pH of below 7.0. Preferably the complex is formed at a pH in the range of about 5.0 up to but below 7.0.

More preferably the complex is formed at a pH range of about 4.0 to 6.5, or 5.0 to about 6.0. In one embodiment, the pH during formation is maintained at pH 6.5 or below. In a preferred embodiment, the complex is formed at a pH of about 5.5. Preferably, the ACP or ACFP in the complex is predominantly in a basic form. The stannous-associated stabilized ACP or ACFP complex when produced on an industrial scale is produced in a bulk solution that has a pH greater than about 7.0, preferably about 9.0, however the local pH at formation of the complexes is below about 7.0, preferably about 4.0 to 6.5, preferably about 5.5.

When stannous-associated stabilized ACP or ACFP, or stabilized ACP or ACFP, is produced in the laboratory, in smaller quantities than commercial production the pH of the entire solution may be maintained at a given pH, i.e. if the CPP-ACP was prepared at pH 5.5, then the entire solution during CPP-ACP formation was maintained at pH 5.5. However, it may be neither necessary nor desirable to reduce the pH of the entire bulk solution in commercial manufacture to 5.5 as the only part of the bulk solution required to have the acidic pH is where the complexes are forming and the bulk solution can have, and does have, localised fluctuations in pH. The pH fluctuations arise particularly from protons provided by the phosphate compound, for example dihydrogen phosphate, as it is added and the protons liberated from acidic phosphate ions when they convert into the basic form, PO_4^{3-} . Therefore, while the overall pH of the bulk solution may be at above 7.0, for example about pH 9, the localised pH at which the CPP-ACP is formed is lower, typically below 7.0 or 6.5, preferably about 4.0 to 6.5, more preferably about 5.5. These fluctuations are localised due to the size of the bulk solution.

One method for forming a stannous-associated stabilized ACP of the invention is a method comprising the steps of:

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

5 or

- (i) providing a solution of stabilized ACP; and
- (ii) admixing a stannous compound.

One method for forming a stannous-associated stabilised ACFP is a method comprising the steps of:

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

or

- 15 (i) providing a solution of stabilized ACFP; and
- (ii) admixing a stannous compound.

The hydroxide ions may be titrated into the solution to maintain the phosphopeptide solution at an essentially constant pH. The calcium and phosphate ions may be titrated into the phosphopeptide solution with constant mixing and at a rate that avoids the
20 formation of a calcium phosphate precipitate in the phosphopeptide solution.

A stannous-associated stabilized ACP may be produced by a method comprising the step of admixing CPP-ACP and a stannous compound in an aqueous solution, while maintaining the pH at about 6.5 or below.

A stannous-associated stabilized ACFP may be produced by a method comprising the
25 step of admixing CPP-ACFP and a stannous compound in an aqueous solution, while maintaining the pH at about 6.5 or below.

A stannous-associated stabilized ACP may be produced by a method comprising the steps of:

- (i) obtaining a solution comprising CPP-ACP; and;

- (ii) admixing a stannous compound, while maintaining the pH at about 6.5 or below.

A stannous stabilised ACFP may be produced by a method comprising the steps of:

- (i) obtaining a solution comprising CPP-ACFP; and;
 5 (ii) admixing a stannous compound, while maintaining the pH at about 6.5 or below.

Preferably, the stannous compound is stannous fluoride. Optionally, the methods of producing stannous-associated stabilized ACP or stannous-associated stabilized ACFP further comprise admixing sodium fluoride in step (ii).

- 10 Preferably the pH is maintained with an acid, such as HCl.

Preferably the solution comprising CPP-ACP or CPP-ACFP is prepared by adding CPP-ACP or CPP-ACFP to distilled or deionised water.

- A stannous-associated stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex may be formed by mixing
 15 stabilized stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex with stannous fluoride.

A stabilized-ACP or ACFP complex as described in the current specification may be the "closed" complexes are shown in Figure 2 of Cross *et al.*, 2007.

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

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

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

at a pH of about 5.5. Preferably, the ACP or ACFP in the complex is predominantly in a basic form.

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

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

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

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

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

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

"Tightly" and "loosely" bound calcium and phosphate in ACP or ACFP can be determined using analytical ultrafiltration. Briefly, the solution of phosphopeptide, calcium, phosphate and optionally fluoride admixed while maintaining the pH at about
25 7.0 or below can be first filtered through a 0.1 micron filter to remove free calcium and phosphate that is not associated with the complexes. This free calcium and phosphate is present in the filtrate and discarded. Any free calcium or phosphate that is not associated in any way with the complexes would not be bioavailable, i.e. delivered by
30 the phosphopeptide to the tooth. The retentate from the 0.1 micron filtration can be further analyzed by centrifugation through a 3000 mw cutoff filter at 1,000 g for 15 min.

The resulting filtrate contains calcium and phosphate that is loosely bound or associated with the complexes. At this centrifugal force calcium and phosphate that is not tightly bound to the complexes are released and move to into the filtrate. The Ca and Pi that is tightly bound in the complexes is retained in the retentate. The amount of tightly bound
5 Ca and Pi in the retentate can then be determined by subtracting the amount of Ca and Pi in the filtrate from the total amount of Ca and Pi in the retentate of the 0.1 micron filtration.

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

A "superloaded" phosphopeptide or phosphoprotein (PP) stabilized-amorphous calcium phosphate (ACP) or amorphous calcium fluoride phosphate (ACFP) complex. The complex may be formed at any pH (eg 3-10). Preferably the phosphopeptide includes the sequence -A-B-C-, where A is a phosphoamino acid, preferably phosphoserine, B is
15 any amino acid including a phosphoamino acid and C is glutamic acid, aspartic acid or a phosphoamino acid. The phosphoamino acid may be phosphoserine. The PP is superloaded with calcium and phosphate ions. The calcium ions may be in the range 30-1000 mol Ca per mole of PP, or in the range of 30-100 or 30-50 mole Ca per mole of PP. In another embodiment, the mol Ca per mol of PP is at least 25, 30, 35, 40, 45 or
20 50.

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

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

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

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

The PP stabilized ACP and/or ACFP complex may further include at least an equal amount by weight of calcium phosphate. Preferably the calcium phosphate is CaHPO_4 . Preferably, the calcium phosphate (e.g. CaHPO_4) is dry blended with the PP stabilized
5 ACP and/or ACFP complex. In a preferred embodiment, the PP-ACP and/or PP-ACFP complex: calcium phosphate ratio is about 1:1-50, more preferably about 1: 1-25, more preferably about 1:5-15. In one embodiment, the PP-ACP and/or PP-ACFP complex: calcium phosphate ratio is about 1:10.

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

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

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

20 The present invention also provides a method of mineralizing a dental surface or sub-surface including the steps of:

- (i) contacting the dental surface with a protein disrupting agent, and
- (ii) contacting the dental surface with (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized
25 ACP or ACFP.

The dental surface is preferably 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. 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
30 surface to be treated, such as the pellicle over teeth. Examples of suitable agents 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 cabamide 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.

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

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

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

In another embodiment, at least two of the phosphoamino acids in the sequence are preferably contiguous. Preferably the phosphopeptide includes the sequence A-B-C-D-E, where A, B, C, D and E are independently phosphoserine, phosphothreonine, phosphotyrosine, phosphohistidine, glutamic acid or aspartic acid, and at least two,

preferably three, of the A, B, C, D and E are a phosphoamino acid. In a preferred embodiment, the phosphoamino acid residues are phosphoserine, most preferably three contiguous phosphoserine residues. It is also preferred that D and E are independently glutamic or aspartic acid.

- 5 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
10 water. Thus, the PP can be a casein protein or a phosphopeptide.

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
15 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
20 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

Original Residue	Exemplary Conservative Substitution	Preferred Conservative Substitution
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;

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;

Hydroxylysine (Hyl) for Lys;

15 allohydroxylysine (AHyl) for Lys;

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

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

p-amidinophenylalanine for Ala;

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

N-methylisoleucine (Melle) for Ile;

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

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

Ornithine (Orn) for Lys, Arg and His;

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

N-methylphenylalanine (MePhe), trimethylphenylalanine, halo (F, Cl, Br and I)

10 phenylalanine, triflourylphenylalanine, 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]:

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

15 [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 $\alpha_{s2}(46-70)$

[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 $\alpha_{s2}(1-21)$.
20

In another embodiment of the invention, the stannous-associated stabilized ACP and/or stannous-associated stabilized ACFP complex is incorporated into oral compositions such as toothpaste, mouth washes or formulations for the mouth to aid in the prevention and/or treatment of dental caries, tooth decay, dental erosion or fluorosis, dentinal hypersensitivity, dental plaque, gingivitis or periodontitis. The oral compositions comprising an amount of stannous-associated stabilized ACP and/or ACFP sufficient to form a layer on a dental surface, preferably, the layer has a calcium : phosphate ratio equivalent to normal apatite, for example the ratio is about 2:1. The layer may contain an amount of calcium that is about 20 wt%. Preferably, the layer may exhibit about an elemental analysis of any one of the elements, such as calcium, phosphate, fluoride,
30

carbon and/or stannous, as shown in any one of Table 3 or 4. The stannous-associated stabilized ACP and/or ACFP complexes 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 stannous-associated stabilized ACP or ACFP complexes 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% stannous-associated stabilized ACP or ACFP complexes or a mixture of both. The oral composition of this invention which contains the above-mentioned agents may be prepared and used in various forms applicable to the mouth such as dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, mouthrinses, mouth sprays, varnish, dental cement, troches, chewing gums, 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 ingredients depending on the type and form of a particular oral composition. Certain compositions of the invention such as toothpastes, toothpowders and liquid dentifrices, mouthwashes, mouthrinses and mouth sprays have relatively low viscosity and have a remineralizing effect without significant residence time in the oral cavity.

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

In other desirable forms of this invention, the composition may be substantially solid or pasty in character, such as toothpowder, a dental tablet or a toothpaste (dental cream) or gel dentifrice. The vehicle of such solid or pasty oral preparations generally contains dentally acceptable polishing material. Examples of polishing materials are water-insoluble sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated calcium phosphate, anhydrous dicalcium phosphate, calcium pyrophosphate, magnesium orthophosphate, trimagnesium phosphate, calcium carbonate, hydrated alumina, calcined alumina, aluminium silicate, zirconium silicate, silica, bentonite, and mixtures thereof. Other suitable polishing material include the particulate thermosetting resins such as melamine-, phenolic, and urea-formaldehydes,

and cross-linked polyepoxides and polyesters. Preferred polishing materials include crystalline silica having particle sizes of up to about 5 microns, a mean particle size of up to about 1.1 microns, and a surface area of up to about 50,000 cm²/g., silica gel or colloidal silica, and complex amorphous alkali metal aluminosilicate.

- 5 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
10 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
15 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,
20 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
25 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.

- 30 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.

5 Toothpaste, creams and gels typically contain a natural or synthetic thickener or gelling agent in proportions of about 0.1 to about 10, preferably about 0.5 to about 5% w/w. A suitable thickener is synthetic hectorite, a synthetic colloidal magnesium alkali metal silicate complex clay available for example as Laponite (e.g. CP, SP 2002, D) marketed by Laporte Industries Limited. Laponite D is, approximately by weight 58.00% SiO₂, 25.40% MgO, 3.05% Na₂O, 0.98% Li₂O, and some water and trace metals. Its true
10 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, hydroxypropyl methyl cellulose, hydroxyethyl cellulose (e.g. available as Natrosol), sodium carboxymethyl cellulose, and colloidal silica such as finely ground Syloid (e.g.
15 244). Solubilizing agents may also be included such as humectant polyols such as 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.

20 It will be understood that, as is conventional, the oral preparations will usually be sold or otherwise distributed in suitable labelled packages. Thus, a jar of mouth rinse will have a label describing it, in substance, as a mouth rinse or mouthwash and having directions for its use; and a toothpaste, cream or gel will usually be in a collapsible tube, typically aluminium, lined lead or plastic, or other squeeze, pump or pressurized dispenser for
25 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 to achieve increased prophylactic action, assist in achieving thorough and complete dispersion of the active agent throughout the oral cavity, and render the instant
30 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, 5 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, 10 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 15 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. 20 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.

25 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.

30 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. The composition of the invention may be a dual phase composition wherein each phase permits release of components over different time periods. For example, in use a dual phase composition may release stannous-associated stabilized ACP and/or stannous-associated stabilized ACFP, preferably CPP-ACP/SnF₂ and/or CPP-ACFP/SnF₂, from a first phase at a faster rate than a compound that is capable of increasing or maintaining the pH of a solution from a second phase. Preferably, the dual phase composition is a dual phase chewing gum.
- 15 An alternative composition may be one that provides stabilized ACP or ACFP and a stannous compound that then *in situ*, such as the oral cavity, forms stannous-associated stabilized ACP or ACFP. An exemplary composition may be a chewing gum that contains stabilized ACP or ACFP in the pellet and a stannous compound in the centre chew.
- 20 In a further aspect, the invention provides compositions including pharmaceutical compositions comprising any of the (a) compositions including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP complexes as described above together with a compound capable of increasing or maintaining the pH of a solution and a pharmaceutically-acceptable carrier. Such compositions may be selected from the group consisting of dental, anticariogenic compositions and therapeutic compositions. Dental compositions or therapeutic compositions may be in the form of a gel, liquid, solid, powder, cream or lozenge. Therapeutic compositions may also be in the form of tablets or capsules. In one embodiment, the stannous-associated stabilized ACP or ACFP complexes are substantially the only remineralizing active components of such a composition. For example, a cr me formulation may be employed containing: water; glycerol; CPP-ACP/SnF₂; D-sorbitol; silicon dioxide; sodium carboxymethylcellulose (CMC-Na); propylene glycol; titanium dioxide; xylitol; phosphoric acid; guar gum; zinc oxide; sodium
- 25
- 30

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, dentinal hypersensitivity, dental plaque, gingivitis or periodontitis.

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

- 10 In an embodiment of the present invention there is provided a composition for dental mineralization including stannous-associated stabilized ACP or ACFP complexes, wherein the composition does not include a phosphate buffer and/or calcium chelator.

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

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

In one embodiment, the active components of the composition consist essentially of the stannous-associated stabilized ACP or ACFP complexes.

- 20 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, dentinal hypersensitivity, dental plaque, gingivitis and periodontitis comprising the steps of administering (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP complexes, to the teeth of
25 a subject. 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) compositions including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP complexes in a manufacture of a composition for the treatment and/or prevention
30 of one or more of dental caries, tooth decay, dental erosion and fluorosis, dental hypersensitivity, dental plaque, gingivitis and periodontitis.

According to a further aspect of the invention there is provided a composition for dental restoration, including a dental restorative material to which has been added (a) a composition including a stannous compound and stabilized ACP or ACFP, or (b) a stannous-associated stabilized ACP or ACFP complexes. The base of the dental restorative material can be a glass ionomer cement, a composite material or any other restorative material which is compatible. A glass ionomer cement is preferred. It is preferred that the amount of stabilised stannous-associated stabilized ACP or ACFP complexes, preferably CPP-ACP/SnF₂ complex or CPP-ACFP/SnF₂ complex, included in the dental restorative material is 0.01-80% by weight, preferably 0.5-10% and more preferably 1-5% by weight. The dental restorative material of this invention which contains the above mentioned agents may be prepared and used in various forms applicable to dental practice. The dental restorative material according to this embodiment may further include other ions, eg. antibacterial ions Zn²⁺, Ag⁺, etc or other additional ingredients depending on the type and form of a particular dental restorative material. It is preferable that the pH of dental restorative material according to this embodiment be between 2-10, more preferably 5-9 and even more preferably 5-7. It is preferable that the pH of the dental restorative material containing a stannous-associated stabilized ACP or ACFP complex be in the range of about 2 to 10, more preferably in the range of about 5 to 9 and even more preferably in the range of about 5 to 7.

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.

One example of a mineralizing composition comprises the following (in decreasing order of proportion):

water

glycerol

CPP-ACP/SnF₂

D-sorbitol

silicon dioxide

sodium carboxymethylcellulose (CMC-Na)

propylene glycol

titanium dioxide

xylitol

phosphoric acid

5 guar gum

zinc oxide

sodium saccharin

ethyl p-hydroxybenzoate

magnesium oxide

10 butyl p-hydroxybenzoate

propyl p-hydroxybenzoate

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

Example 1

- 15 Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) was acquired from Cadbury Enterprises Pte Ltd under the trademark name Recaldent™. A solution was prepared using CPP-ACP, SnF_2 and NaF to produce at 0.4% w/v CPP-ACP, 220 ppm F as SnF_2 and 70 ppm F as NaF, pH 5.6. Specifically, the CPP-ACP/ SnF_2 complexes were prepared by adding CPP-ACP to distilled/deionised water and then SnF_2 (solid)
- 20 and NaF added with addition of 1 M HCl to maintain the pH between 4.0 – 6.5. The pH was not allowed to go above 6.5. The total volume of acid added was less than 1% of the CPP-ACP/ SnF_2 solution volume. This solution was designated stabilized SnF_2 /ACP. While NaF was added in this method it is a minor component and the majority of the fluoride derives from the SnF_2 . The method could be performed using SnF_2 only
- 25 (without NaF). Another solution was prepared using CPP-ACP and NaF to produce 0.4% w/v CPP-ACP, and 290 ppm F as NaF, pH 5.6. This solution was designated stabilized NaF/ACP.

Both solutions were stable at room temperature (20°C) for many months with no precipitate. Both solutions were tested for their ability to remineralize enamel

30 subsurface lesions. Human tooth enamel demineralized subsurface lesions were

prepared in third molar enamel blocks using the method of Reynolds, (1997). J Dent Res 76:1587-1595. Half the blocks were remineralized by suspending them individually in the stabilized SnF₂/ACP solution and the other half in the stabilized NaF/ACP solution for 7 days at 37°C. After remineralization the enamel blocks were embedded, sectioned and subjected to transverse microradiography and densitometric image analysis as previously described by Reynolds (1997) to determine percent mineral content gain (%Remineralization) shown in Table 2. A one way analysis of variance with differences in means determined using a Tukey HSD *post hoc* comparison showed that the treatment with stabilized SnF₂/ACP solution significantly increased the level of remineralization by 32%.

TABLE 2. Remineralization of enamel subsurface lesions by stabilized SnF₂/ACP versus stabilized NaF/ACP.

Solution	% Remineralization
Stabilized SnF₂/ACP [0.4% w/v CPP-ACP 220 ppm F as SnF ₂ 70 ppm F as NaF]	46.1 ± 5.7*
Stabilized NaF/ACP [0.4% w/v CPP-ACP 290 ppm F as NaF]	35.0 ± 5.4

*significantly different $p < 0.01$

Analysis of the enamel lesions using scanning electron microscopy (Figures 1 and 2) revealed that the lesions treated with the stabilized SnF₂/ACP further had a surface layer. Surprisingly the stabilized SnF₂/ACP formulation produced significantly greater enamel subsurface lesion remineralization (Table 2) and appeared also to form a protective surface layer demonstrating the unexpected superiority of the stabilized SnF₂/ACP formulation. An experiment using scanning electron microscopy – energy dispersive spectroscopy (SEM-EDS) of the surface layer (Figure 3 and Table 3) confirmed that it was apatite. The surface layer EDS analysis showed it contained 10.33 wt% P and 21.18 wt% Ca giving a typical apatite Ca:P wt% ratio of 2.05. The

sound enamel contained 15.26 wt% P and 30.99 wt% Ca giving the same typical apatite Ca:P wt% ratio of 2.03.

These data show that the surface layer is composed of apatite, however the surface layer had higher Sn (1.23 wt% versus 0 wt% for sound enamel) and fluoride (0.54 wt% versus 0.11% for sound enamel). This of course is consistent with the CPP-stabilised SnF₂/ACP solution containing Sn and F. CPP was also involved in the formation of the surface layer as the C was 26.53 wt% in the surface layer but only around 10 wt% in the sound enamel (Table 4). This supports the mechanism whereby the stannous ions can cross-link the phosphopeptide stabilized ACP or stabilized ACFP at the tooth surface to form a layer that can protect the tooth surface from demineralization.

These results indicate that the CPP-stabilised SnF₂/ACP treatment has not only produced substantially better subsurface remineralisation but it has also produced a uniform, protective surface layer.

The retention of stannous-associated stabilized ACP or ACFP in a cross-linked surface layer would (i) protect from tooth sensitivity as it would seal dentinal tubules, (ii) protect from plaque formation as it contains the antibacterial ion Sn and (iii) protect from dental erosion and dental caries. It would also promote subsurface remineralization. Without being bound by any theory or mechanism of action it appears that stannous ions help to promote the surface layer by cross-linking the CPP-ACP or CPP-ACFP at the surface of the enamel.

TABLE 3. Elemental analysis by SEM-EDS of the mineralised surface layer resulting from treatment with stabilized SnF₂/ACP.

Element	SnF ₂ /ACP Surface Layer (wt%)	Normal Sound Enamel (wt%)
C	26.53	12.28
O	39.59	40.59
F	0.54	0.11
Na	0.31	0.47
P	10.33	15.26
Cl	0.29	0.30

Ca	21.18	30.99
Sn	1.23	0
TOTAL	100	100

A more detailed scanning electron microscopy – energy dispersive spectroscopy (SEM-EDS) analysis of the various layers wherein the embedding resin is excluded is shown in Table 4. This shows that the high carbon content in the sound enamel in Table 3 is including the resin. When the resin was excluded, the carbon content of sound enamel was in the range of 6 to 10wt%. The carbon content of the surface layer produced by the CPP-stabilised SnF₂/ACP treatment was almost 3-fold greater than sound enamel indicating that the phosphopeptides are present in the layer. The protective surface layer formed by the stannous-associated stabilized ACP complexes provides a source of fluoride as the level of fluoride in the mineralised surface layer is 5 fold greater than the level of fluoride in sound enamel. Further, the level of fluoride present is greater than that present in the remineralized lesion. The level of stannous present in the surface layer was significantly higher, 12 fold, than the remineralized lesion.

The level of fluoride in the lesion remineralized with the stannous associated ACP is greater than CPP-ACP and sodium fluoride. Further, there was an increase in calcium present in the lesion remineralized by the stannous-associated stabilized ACP compared to the CPP-ACP and sodium fluoride. This level of calcium is approaching the level of calcium present in sound enamel.

TABLE 4. Detailed elemental analysis by SEM-EDS of the mineralised surface layer resulting from treatment with CPP stabilized SnF₂/ACP.

Element wt%	Sound Enamel	Demineralised Lesion	CPP-ACP/NaF Remineralised Lesion	CPP-ACP/SnF ₂ Re-mineralised Lesion	CPP-ACP/SnF ₂ Mineralised Surface Layer
C	6 – 10 ^a	ND ^b	ND	17.49 ± 0.59	26.53 ± 0.44
O	40.59 ± 0.63	38.14 ± 0.32	37.94 ± 10.44	37.93 ± 0.49	39.59 ± 0.38

F	0.11 ± 0.31	0.12 ± 0.12	0.21 ± 0.16	0.34 ± 0.23 (3.1 fold increase)	0.54 ± 0.19 (5 fold increase)
P	15.26 ± 0.26	13.64 ± 0.13	14.05 ± 0.18	14.02 ± 0.19	10.33 ± 0.13
Ca	30.99 ± 0.44	27.97 ± 0.21	28.49 ± 0.29	29.25 ± 0.33	21.18 ± 0.21
Sn	< 0.1	0.13 ± 0.13	0.11 ± 0.17	0.24 ± 0.2 (2.4 fold increase)	1.23 ± 0.16 (12.3 fold increase)
Ca:P	2.031 ^c	2.051	2.028	2.086	2.050

a. Sabel et al. Scientific World Journal (2012)

b. ND = not yet determined

c. Ca:P wt% ratio for HA = 2.157 and for ACP = 1.941

Example 2

- 5 This example describes the experimental protocol for the measurement of CPP-bound (tightly bound), loosely bound and free ions in solution

A sample of each solution prepared in Example 1 was taken and less than 10% collected as a filtrate using a 3000 molecular weight cut-off Centriprep 3 ultrafiltration membrane. The Centripreps containing the samples were centrifuged at 1,000 g for 15 min in a Beckman J2-21 centrifuge using a JA 10.5 rotor. The original sample before Centriprep centrifugation and a sample of the filtrate after Centriprep centrifugation were taken for analysis of calcium, phosphate fluoride and stannous concentrations. The analysis of the original sample gave total calcium, phosphate, fluoride and stannous ion concentrations and the analysis of the filtrate gave free (unbound) calcium, phosphate and fluoride concentrations. The difference between the total and unbound concentrations is the bound concentration of Ca, Pi, F and Sn by the CPP.

Example 3

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

Water

glycerol

Stabilised SnF₂/ACP and/or SnF₂/ACFP

D-sorbitol

5 sodium carboxymethylcellulose (CMC-Na)

propylene glycol

silicon dioxide

titanium dioxide

xylitol

10 phosphoric acid

sodium fluoride

flavouring

sodium saccharin

ethyl p-hydroxybenzoate

15 propyl p-hydroxybenzoate

butyl p-hydroxybenzoate

Example 4

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

20 Water

Alcohol

Poloxamer 407

Sodium Lauryl Sulphate

Stabilised SnF₂/ACP and/or SnF₂/ACFP

25 Sodium Fluoride

Flavours

Sodium Saccharin

Ethyl p-hydroxybenzoate

Propyl p-hydroxybenzoate

Butyl p-hydroxybenzoate

Example 5

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

Crystalline sorbitol/mannitol/xylitol

Gum base

Calcium carbonate

- 10 Glycerine

Stabilised SnF_2/ACP and/or SnF_2/ACFP

Sodium Fluoride

Flavour oil

Water

- 15 **Example 6**

The following is a protocol for CPP stabilized ACP/ SnF_2 solution ion analysis. Total (tightly & loosely-bound) and loosely-bound samples were prepared as follows:

- Total (tightly and loosely-bound): One ml of 0.4% CPP-ACP/220ppm F(as SnF_2)/70ppm F(as NaF) solution was taken and placed into 19 ml of 1M HNO_3 and incubated at room temperature with constant slow end over end mixing for 24 hrs (20 rpm). The mixture was centrifuged at 1000g for 15 minutes at room temperature. The supernatant was analyzed for calcium, stannous, phosphate and fluoride.

- Loosely-bound: A sample of the 0.4% CPP-ACP/220ppm F(as SnF_2)/70ppm F(as NaF) solution was taken and placed in a centricon with a 1000 MWCO filter and centrifuged at 3000g for 1hour at room temperature to produce enough filtrate (<10% of total sample to not affect equilibrium) for analysis by atomic absorption spectrophotometry (AAS) and ion chromatography (IC). The filtrates were then measured to give loosely-bound ions.

The total and loosely bound calcium, stannous, phosphate and fluoride in the solution were determined by ion chromatography (for fluoride and phosphate) and Atomic Absorption Spectrometry (for calcium and stannous).

CPP tightly-bound (colloidal retentate) ions were calculated from the difference between

- 5 Total and loosely-bound (as explained above). Based on this example, the stannous-associated ACP complex had a stannous ion content of about 6 to 8 mole per mole of phosphopeptide.

Table 5. Ion analysis of 0.4%CPP-ACP/220ppm F as SnF_2 /70ppm F as NaF solution at pH 5.5 and pH 4.0 by Atomic Absorption Spectrometry (for stannous and calcium) and

- 10 Ion Chromatography (for fluoride and phosphate).

	pH	Ca μM	Pi μM	Sn μM	F ppm
Total	5.5	16160.0 \pm 83.1	8380.0 \pm 385.5)	5582.2 \pm 140.3	292.5 \pm 1.7
	4.0	17045.3 \pm 92.4	8670.0 \pm 157.4	5672.1 \pm 118.3	289.5 \pm 12.0
Tightly-bound	5.5	15389 (95.2%)	7688 (91.7%)	5545 (99.3%)	120.2 (41.1%)
	4.0	13788 (80.9%)	5499 (63.4%)	5150 (90.8%)	164.2 (56.7%)
Loosely-bound	5.5	770.7 \pm 6.5 (4.8%)	692.0 \pm 193.5 (8.3%)	37.1 \pm 3.4 (0.7%)	172.3 \pm 2.7 (58.9%)
	4.0	3257.1 \pm 16.4 (19.1%)	3170.7 \pm 55.1 (36.6%)	522.3 \pm 13.4 (9.2%)	125.3 \pm 1.1 (43.3%)

Example 7

This *in situ* study was designed to compare remineralisation using an established intra-oral remineralisation model promoted by five solutions:

- 15 1) 0.4% (w/v) calcium phosphopeptide stabilised-amorphous calcium phosphate (CPP-ACP), 220 ppm stannous fluoride (SnF_2) and 70 ppm sodium fluoride (NaF) (shown in Figure 7 as 2%CPP-ACP+ SnF_2);
- 2) 0.4% (w/v) CPP-ACP and 290 ppm NaF;

3) 0.4% (w/v) CPP-ACP;

4) 220 ppm SnF₂ and 70 ppm NaF without CPP-ACP (shown in Figure 7 as SnF₂); and

5) 290 ppm NaF.

In this model subjects wear a palatal appliance and rinse 4 times a day with 5 mL of solution for five 14 consecutive-day treatment periods (including weekends) and rinsing with a different solution during each treatment period.

This randomised controlled study used a double-blind, five-way crossover design to assess the effects of five solutions to enhance enamel remineralisation using an intra-oral remineralisation model. Each solution contained the same amount of 290 ppm F (equivalent to 1450 ppm F in a toothpaste diluted 1 in 5) [see above]. Subjects were randomly allocated to one of the five solutions for the first treatment period and, after a one-week washout rest period, crossed over to another solution for the second treatment period. This was repeated for the five treatments. Each subject wore a custom made palatal appliance containing four pre-sterilised enamel slabs containing artificially-created subsurface lesions and four times a day for 14 consecutive days including weekends (treatment period) rinsed for one minute with 5 mL of their allocated solution then expectorated all the solution and accumulated saliva and continued to wear their appliance. The four rinses per day were performed (i) after breakfast, (ii) after lunch, (iii) after dinner and (iv) before retiring at night. After the first rinse per day of each treatment period subjects on three different days expectorated the solution and accumulated saliva into a clean tube for ion analysis. Subjects kept a diary of times and duration they rinsed with their solutions. Subjects were instructed to maintain their normal diet and oral hygiene procedures for the duration of the treatment periods. Appliances were removed during subjects normal oral hygiene procedures during the study period. After removing the appliances for oral hygiene procedures, subjects cleaned their appliances as instructed with a toothbrush and fluoride-free denture paste (both supplied) avoiding the trough areas and gently rinsed their appliances with DDW before replacing the appliances. When out of the mouth the appliances were stored in sealed humid containers. All subjects brushed their teeth with standard fluoride toothpaste provided by the sponsor for the duration of the study. The subjects returned to the clinical site with their appliances and diary at the conclusion of each 14-day treatment period.

The researchers did not know which treatment had been administered nor the subject. Neither the researchers, nor the recorder had access to the treatment code. Personnel dispensing the test solutions or supervising their use did not participate in the examination of the enamel half-slabs in order to minimize potential bias. Following the baseline examination, subjects were assigned a subject number. Subject numbers were recorded on their case report forms. Subjects were randomly assigned to use one of the solutions during the first treatment period then crossed over to another solution for the second to fifth treatment period. Randomisation was effected using a standard randomisation table for the number of treatments in the crossover study. Results of the *in situ* study are shown in Figure 7. These results show that in an *in situ* model a solution including stannous-associated stabilized complexes (as 2% CPP-ACP, with stannous fluoride and sodium fluoride, far right column, also referred to herein as stabilized SnF_2/ACP) provide a significantly greater level of enamel subsurface remineralization compared to sodium fluoride alone, stannous fluoride & sodium fluoride, CPP-ACP alone or CPP-ACP with sodium fluoride. The two far right columns labelled 2% CPP-ACP+NaF and 2% CPP-ACP+ SnF_2 were significantly different ($p<0.01$). This clearly demonstrates that in vivo in the oral cavity the stannous-associated stabilized complexes of the invention have a superior capacity to remineralize lesions.

A palatal appliance for each subject was prepared by taking alginate impressions of upper and lower dental arches from which study models were produced and articulated. Removable palatal acrylic appliances covering the first premolars to the last tooth in the arch were fabricated for each subject. The appliance consisted of a palatal plate that was retained in the mouth by four stainless steel clasps. Troughs on each side of the appliance adjacent to the palatal surface of maxillary premolar/molar teeth accommodated two enamel half-slabs containing demineralised subsurface lesions. Therefore, each appliance contained four enamel half-slabs. The appliances was designed to ensure its surfaces were smooth and comfortable for the subjects.

Extracted third molars were obtained from the Royal Dental Hospital of Melbourne and oral surgeons and general practitioners in private practice. Any attached soft tissues was removed and the teeth washed in distilled deionized water (DDW). All teeth in this study were sterilised by storage in a 10% (v/v) neutral buffered formalin solution for at least two weeks at room temperature. After storage in formalin the teeth were thoroughly washed in DDW and stored in DDW until required. Sound relatively planar

buccal and lingual surfaces of sterilised enamel with minimal cracking, staining and fluorosis (as viewed under a dissecting microscope) were selected and thoroughly rinsed with DDW again. The outer enamel surface including superficial cracks was removed then polished wet to a mirror finish using Soflex™ (3M) polishing discs on a
5 slow speed contra-angle dental handpiece. Each polished surface was then sawn from the tooth as an approximately 8 x 4 mm slab, using a water-cooled diamond blade saw and the whole slab covered with acid-resistant nail varnish except for two (occlusal and gingival) mesiodistal windows (approximately 1 x 7 mm) separated from each other by about 1 mm. Lesions were created in the enamel windows by mounting each slab onto
10 the end of a 3 - 4 cm stick of yellow dental sticky wax and immersing in 40 mL of unagitated demineralisation buffer, consisting of 80 mL/L Noverite K-702 (polycacrylate, Lubrizol Corporation, Wickliffe, OH; White, 1987), 500 mg/L hydroxyapatite (Bio-Gel® HTP, Bio Rad Laboratories, Richmond CL), and 0.1 mol/L lactic acid (Ajax Chemicals, Auburn NSW) pH 4.8, for four days at 37°C. A change of solution was made after two
15 days at which time the slabs were removed from the solution, rinsed thrice with DDW, blotted dry and placed into fresh demineralisation buffer. The slabs were similarly rinsed and dried after four days of demineralisation. This demineralisation procedure produces consistent subsurface lesions 80 to 110 µm deep with intact surface layers, as evaluated by microradiography of sections of the artificial lesions. After
20 demineralisation, each enamel slab was sawn through the midline of each window into two 4 x 4 mm slabs and the cut surface of each slab covered with nail varnish. One half of each slab was retained as the demineralisation control (control half-slab) and stored in a labeled 0.5 mL microcentrifuge tube together with a drop of DDW, thereby creating a humidified environment. The other half of the enamel slab was inset into the intraoral
25 appliance using dental wax for the remineralisation protocol (test half-slab). Care was taken not to cover the artificial lesions with wax. At the end of an in situ treatment period the enamel half-slabs were removed and replaced with new pre-sterilised enamel half-slabs for the beginning of a new test period. After each treatment period each test half-slab was paired with its control half-slab and embedded, sectioned and analysed to
30 determine mineral changes.

Each pair of enamel half-slabs (remineralisation half-slab paired with its demineralisation control half-slab) had the nail varnish carefully removed and was rinsed thoroughly with distilled deionised water (DDW). The pair of enamel half-slabs (test remineralisation half-slab paired with its demineralisation control half-slab) was placed

onto their cut sides into a small plastic vial with the lesion windows parallel and transparent cold curing methacrylate resin (Paladur, Heraeus Kulzer, Germany) poured over the half-slabs and allowed to set at room temperature. In order to identify the test and control half-slabs, a permanent identifying mark was drawn on the side of the methacrylate block next to the test enamel half-slab. Sections 200-300 μm thick were cut from the embedded half-slabs perpendicular to the lesion surface through the 5
midline of both half-lesions using an internal annulus saw microtome (Leitz 1600, Ernst Leitz Wetzlar, Germany). The sections were then lapped down to $80 \pm 5 \mu\text{m}$ using a RotoPol-21/RotoForce4 lapping instrument (Struers, Denmark) with 1200 grit lapping 10
paper. The lapped sections were removed from the lapping instrument and rinsed in DDW, blotted dry and stored on soft tissue between glass slides. Each section, which contains the remineralised half-lesion and the paired demineralised control half lesion from the same enamel slab, were radiographed along with an aluminium stepwedge of $10 \times 37.5 \mu\text{m}$ thick increments using Microchrome High Resolution glass plates (3" x 3" 15
 $\times 0.06$ "; Microchrome, USA) and nickel filtered copper K α radiation at 20 kV and 30 mA for 8 minutes using an XMR microradiography system (Diffraction Technologies Pty Ltd) with a PANalytical fine focus glass XRD tube with a copper target (PW2213/20)) powered by a DF3 generator (Spellman High Voltage Electronics Corporation). Each glass plate was developed in Microchrome Developer D5 (1:4 dilution, Microchrome, 20
USA) for five minutes, placed into glacial acetic acid stop bath for 30 seconds and fixed in Microchrome Developer F4 (1:4 dilution, Microchrome, USA) for five minutes. The temperature of all the photochemicals was maintained at 20°C.

Radiographic images of the lesions were viewed via transmitted light through a Dialux 20 microscope (Ernst Leitz Wetzlar, Germany). The images were acquired by a digital 25
camera (Insight™) under the control of imaging software (Image Pro Plus version 7) running on a PC (Pentium III). Images of the lesions and the neighbouring areas of sound enamel were scanned using the program's line luminance function that gives readings in gray values between 0 and 256. An area free of artifacts or cracks was selected for analysis. Each scan comprised 200 readings taken from the tooth surface 30
to sound enamel. The stepwedge image on each slide was scanned and the averaged step gray value readings plotted against aluminium thickness. The readings of the tooth section images were within the linear portion of the stepwedge curve and linear regression was used to convert the gray value data into values of equivalent thicknesses of aluminium. The section thicknesses were measured and the % mineral

data computed using the equation of Angmar et al. (1963) and the linear absorption coefficients of aluminium, organic matter plus water and apatitic mineral (131.5, 11.3, and 260.5 respectively). The image of the median strip between the two lesions was scanned six times and averaged to give a control sound-enamel densitometric profile.

- 5 The lesion images (remineralisation windows and demineralisation control windows) to the gingival and occlusal side of the median strip were similarly scanned, as close as possible to the median strip but avoiding any irregularities commonly found at the lesion edges, and the % mineral profiles computed.

Example 8

- 10 In this example, a number of formulations are provided to exemplify the ways in which complexes of the invention may be formulated for different purpose compositions as described more generally above. These are only examples of the type of formulations that may be provided using various embodiments of the invention.

Toothpaste formulations containing stannous-associated stabilized ACP or ACFP

15 Formulation 1

Ingredient	% w/v		
	A	B	C
Sorbitol	53.0	53.0	53.0
Silica (Zeodent 119)	20.0	20.0	20.0
Purified water	balance	balance	balance
Sodium lauryl sulphate	4.0	4.0	4.0
stannous-associated stabilized ACP or ACFP	1.2	1.2	2.0
Sodium monofluorophosphate	0.3	-	-
Flavour	1.0	1.0	1.0
Sodium carboxymethyl cellulose	0.75	0.75	0.75
Titanium dioxide	0.525	0.525	0.525
Xanthan gum	0.475	0.475	0.475
Sodium saccharin	0.350	0.350	0.350

pH adjusted to 7.0 with phosphoric acid

Formulation 2

Ingredient	% w/v	% w/v	% w/v
Sorbitol	22.0	22.0	22.0
Irish Moss	1.0	1.0	1.0
Gantrez	19.0	19.0	19.0
Purified water	balance	balance	balance
Sodium monofluorophosphate	-	-	0.76
Sodium saccharine	0.3	0.3	0.3
Pyrophosphate	2.0	2.0	2.0
Hydrated alumina	47.0	47.0	47.0
Flavour	0.95	0.95	0.95
stannous-associated stabilized ACP or ACFP	1.0	2.0	2.0
Sodium lauryl sulphate	2.0	2.0	2.0
pH adjusted to 5-7 with NaOH			

Formulation 3

Ingredient	% w/v
Dicalcium phosphate dihydrate	45.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Sodium lauryl sulphate	1.5
Sodium lauryl sarconisate	0.5

Flavour	1.0
Sodium saccharine	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Dextranase	0.01
stannous-associated stabilized ACP or ACFP	5.0
Purified water	balance
pH adjusted to 5-7 with phosphoric acid	

Formulation 4

Ingredient	% w/v
Sorbitol	22.0
Irish moss	1.0
Gantrez	19.0
Purified water	balance
Sodium saccharin	0.3
Pyrophosphate	2.0
Hydrated alumina	43.0
Sodium monofluorophosphate	0.3
Flavour	0.95
stannous-associated stabilized ACP or ACFP	5.0
Sodium lauryl sulphate	2.0
pH adjusted to 5.5 with NaOH	

Formulation 5

Ingredient	% w/v
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Dicalcium phosphate dihydrate	45.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Sodium lauryl sulphate	1.5
Sodium lauryl sarconisate	0.5
Flavour	1.0
Sodium saccharine	0.1
Chlorhexidine gluconate	0.01
Dextranase	0.01
Sodium monofluorophosphate	0.3
stannous-associated stabilized ACP or ACFP	5.0
Purified water	balance
pH adjusted to 5.5 with phosphoric acid	

Formulation 6

Ingredient	% w/v	
	1	2
Sorbitol	53.0	53.0
Silica (Zeodent 119)	20.0	20.0
Purified water	balance	balance
Sodium lauryl sulphate	4.0	4.0
stannous-associated stabilized ACP or ACFP	5.0	5.0
Sodium monofluorophosphate	-	0.3
Sodium dihydrogen phosphate	1.45	1.45
Flavour	1.0	1.0

Sodium carboxymethyl cellulose	0.75	0.75
Titanium dioxide (Rutile)	0.525	0.525
Xanthan gum	0.475	0.475
Sodium saccharin	0.350	0.350
Sodium fluoride	0.243	-
pH adjusted to 5-7 with phosphoric acid/NaOH		

Formulation 7

Ingredient	% w/v	
	1	2
Sorbitol (70% solution)	31.0	31.0
Purified water	balance	balance
Silica	17.0	17.0
Glycerol	8.0	8.0
Sodium lauryl sulphate	4.0	4.0
Polyethylene glycol 300	1.0	1.0
Sodium fluoride	0.243	-
Titanium dioxide (Rutile)	0.525	0.525
Xanthan gum	0.475	0.475
Sodium carboxymethyl cellulose	0.5	0.5
Sodium saccharine	0.286	0.286
Sodium acid pyrophosphate	2.4	2.4
Tetra sodium pyrophosphate	2.2	2.2
Flavour	1.0	1.0
stannous-associated stabilized ACP or ACFP	5.0	5.0
Sodium monofluorophosphate	-	0.3

pH adjusted to 5-7 with phosphoric acid/NaOH

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
5 constitute various alternative aspects of the invention.

REFERENCES

- Aoba T, Fejerskov O (2002). Dental fluorosis: chemistry and biology. Crit Rev Oral Biol Med 13:155-70.
- 10 • Den Besten PK, Thariani H (1992). Biological mechanisms of fluorosis and level and timing of systemic exposure to fluoride with respect to fluorosis. J Dent Res 71:1238-43.
- Fejerskov O, Manji F, Baelum V (1990). The nature and mechanisms of dental fluorosis in man. J Dent Res 69 Spec No:692-700; discussion 721.
- 15 • Fejerskov O, Yanagisawa T, Tohda H, Larsen MJ, Josephsen K, Mosha HJ (1991). Post-eruptive changes in human dental fluorosis--a histological and ultrastructural study. Proc Finn Dent Soc 87:607-19.
- Giambro NJ, Probst K, Den Besten PK (1995). Characterization of fluorosed human enamel by color reflectance, ultrastructure, and elemental composition.
20 Caries Res 29:251-7.
- Reynolds EC (1998). Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist 18:8-16.
- 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
25 sugar-free chewing gum. J Dent Res 82:206-11.
- 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 80:2066-70.

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CLAIMS

1. A stannous-associated casein phosphopeptide stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex having a stannous ion content of at least 1 mole of stannous per mole of casein phosphopeptide.
2. A stannous-associated casein phosphopeptide stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex according to claim 1, wherein the complex has a stannous ion content of at least 5 moles, 6 moles, 7 moles or about 8 moles of stannous per mole of casein phosphopeptide.
3. A stannous-associated casein phosphopeptide stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex according to claim 1 or 2, wherein the stannous is provided by a compound selected from the group consisting of stannous fluoride, stannous chloride, potassium stannous fluoride, sodium stannous fluorozirconate, stannous chloride fluoride, stannous acetate, sodium stannous fluoride, stannous hexafluorozirconate, stannous sulfate, stannous tartrate, stannous gluconate, disodium monostannous citrate.
4. A stannous-associated casein phosphopeptide stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex according to claim 3, wherein the compound is stannous fluoride.
5. A method of producing a stannous-associated casein phosphopeptide stabilized amorphous calcium phosphate (ACP) or stannous-associated casein phosphopeptide stabilized amorphous calcium fluoride phosphate (ACFP) comprising the step of admixing CPP-ACP or CPP-ACFP, and a stannous compound in an aqueous solution, while maintaining the pH at about 6.5 or below.
6. An oral care formulation comprising a stannous-associated casein phosphopeptide stabilized amorphous calcium phosphate (ACP) and/or amorphous calcium fluoride phosphate (ACFP) complex according to any one of claims 1 to 4.

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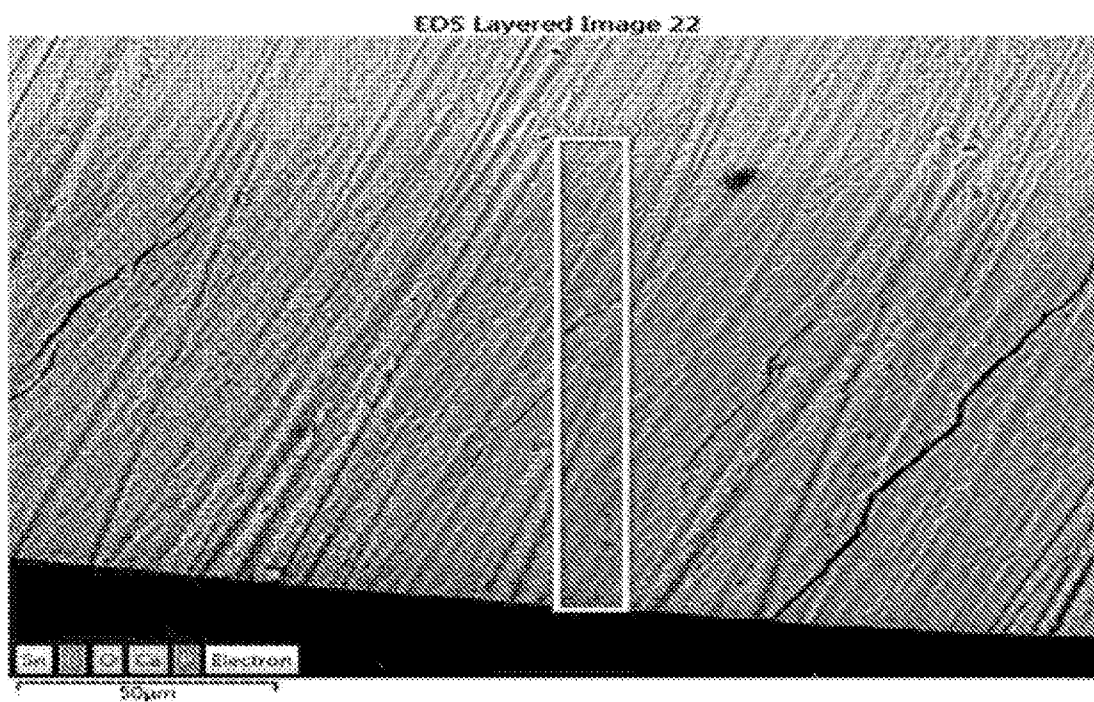
- 5 7. An oral care formulation according to claim 6, wherein the composition is in the form applicable to the mouth selected from the group consisting of dentifrice including toothpaste, toothpowder and liquid dentifrice, mouthwash, mouthrinse, mouth spray, varnish, dental cement, troche, chewing gum, dental paste, gingival massage cream, gargle tablet, dairy product and other foodstuffs.
8. An oral care formulation according to claim 7, formulated as a dentifrice.
9. An oral care formulation according to claim 8, wherein the dentifrice is a toothpaste.
- 10 10. A method of mineralizing a dental surface or sub-surface comprising contacting the dental surface or subsurface with a stannous-associated casein phosphopeptide stabilized ACP and/or ACFP according to any one of claims 1 to 4.
11. A method according to claim 10, wherein the dental surface is a lesion in the enamel caused by caries, dental erosion or fluorosis.
- 15 12. A method according to claims 10 or 11, wherein the dental surface is exposed dentine causing dentinal hypersensitivity.
13. A method of forming a protective layer bonded to the surface of the tooth, wherein the layer comprises calcium and phosphate, the method comprising contacting the dental surface or subsurface with a stannous-associated casein phosphopeptide stabilized ACP or ACFP according to any one of claims 1 to 4.
- 20 14. A method according to claim 13, wherein the layer has a calcium : phosphate ratio equivalent to normal apatite.
15. A method according to claim 13 or 14, wherein the layer contains peptides from casein phosphopeptides that stabilize the ACP or ACFP.
- 25 16. A method according to any one of claims 13 to 15, wherein the layer contains carbon, oxygen, fluoride, phosphate, calcium and stannous.
17. A method according to claim 16, wherein the layer contains 20 to 30wt% carbon, 35 to 45wt% oxygen, 0.1 to 1wt% fluoride, 8 to 12wt% phosphate, 16 to 24wt% calcium and 0.5 to 2wt% stannous.

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- 5
18. Use of a stannous-associated casein phosphopeptide stabilized ACP and/or ACFP according to any one of claims 1 to 4 in the manufacture of a medicament for mineralizing a dental surface or sub-surface.
19. The use according to claim 18, wherein the dental surface is a lesion in the enamel caused by caries, dental erosion or fluorosis.
20. The use according to claims 18 or 19, wherein the dental surface is exposed dentine causing dentinal hypersensitivity.
- 10
21. Use of a stannous-associated casein phosphopeptide stabilized ACP and/or ACFP according to any one of claims 1 to 4 in the manufacture of a medicament for forming a protective layer bonded to the surface of the tooth, wherein the layer comprises calcium and phosphate.
22. The use according to claim 21, wherein the layer has a calcium : phosphate ratio equivalent to normal apatite.
- 15
23. The use according to claims 21 or 22, wherein the layer contains peptides from casein phosphopeptides that stabilize the ACP or ACFP.
24. The use according to any one of claims 21 to 23, wherein the layer contains carbon, oxygen, fluoride, phosphate, calcium and stannous.
- 20
25. The use according to claim 24, wherein the layer contains 20 to 30wt% carbon, 35 to 45wt% oxygen, 0.1 to 1wt% fluoride, 8 to 12wt% phosphate, 16 to 24wt% calcium and 0.5 to 2wt% stannous.

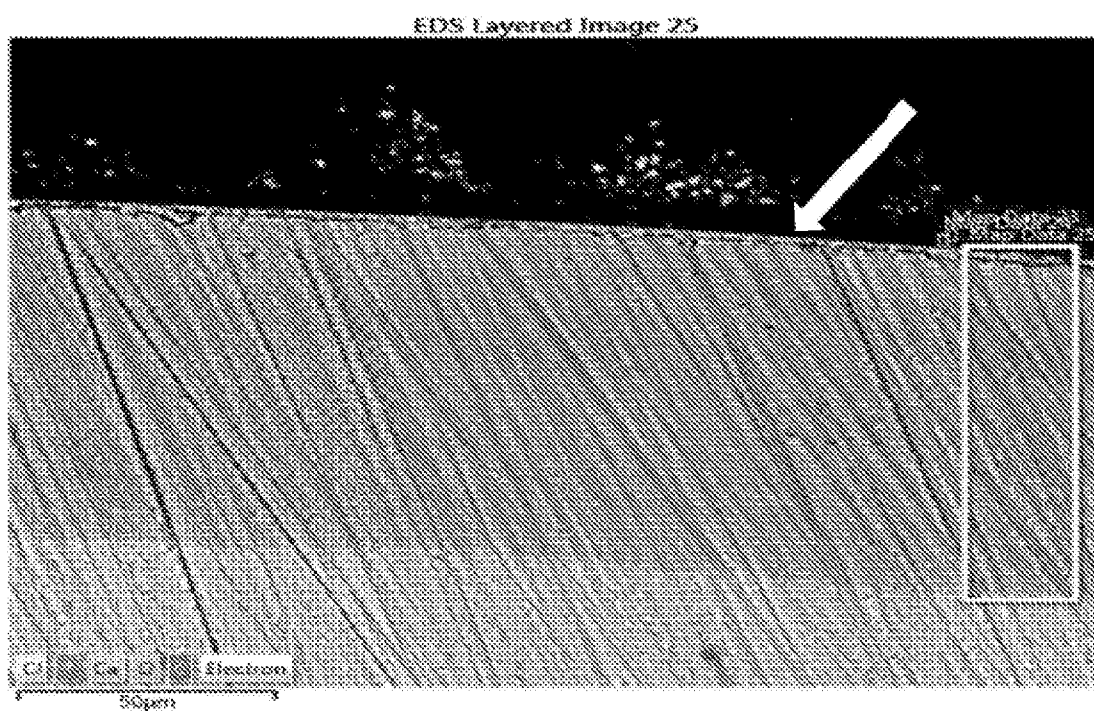
(A)

Before treatment with stabilized SnF_2/ACP



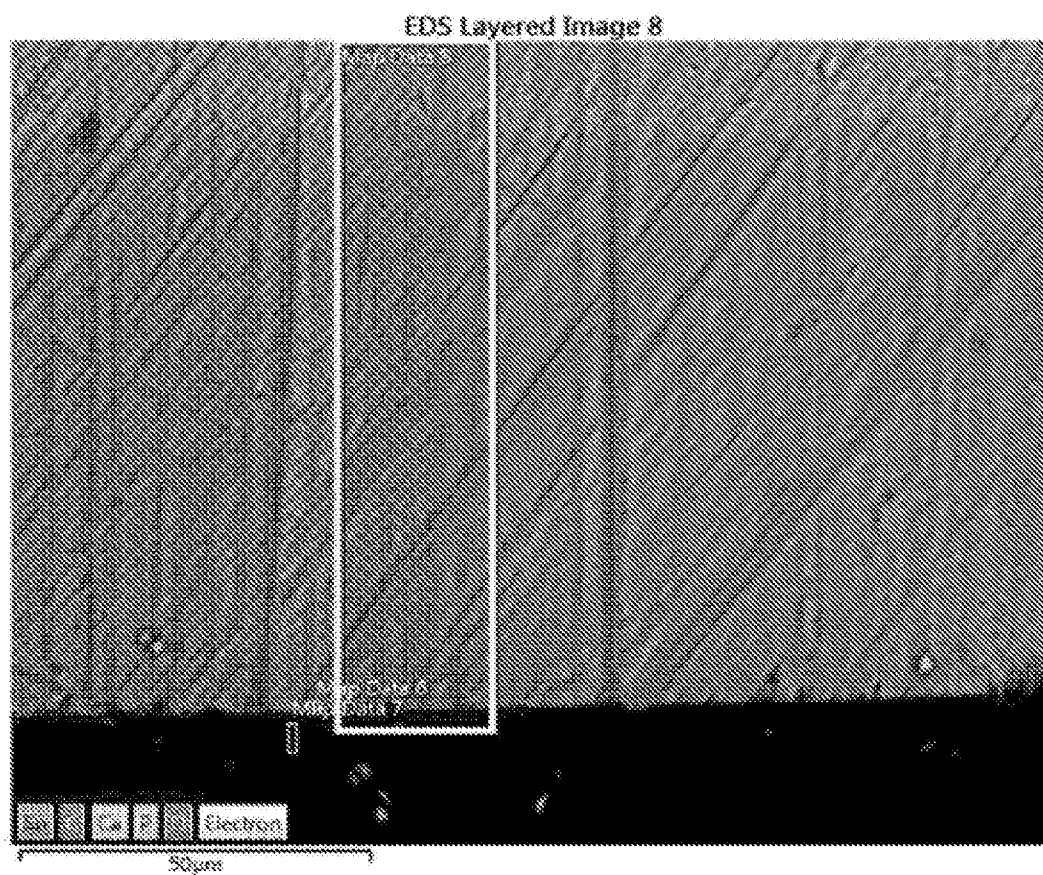
(B)

After treatment with stabilized SnF_2/ACP showing the novel surface layer (arrow)



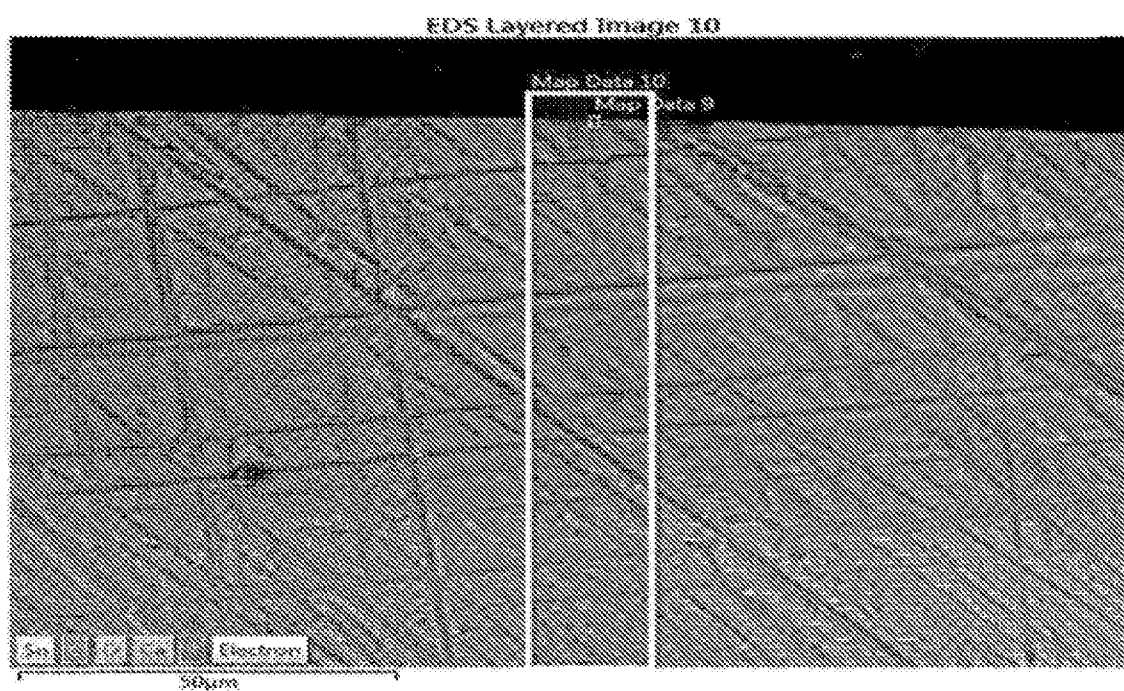
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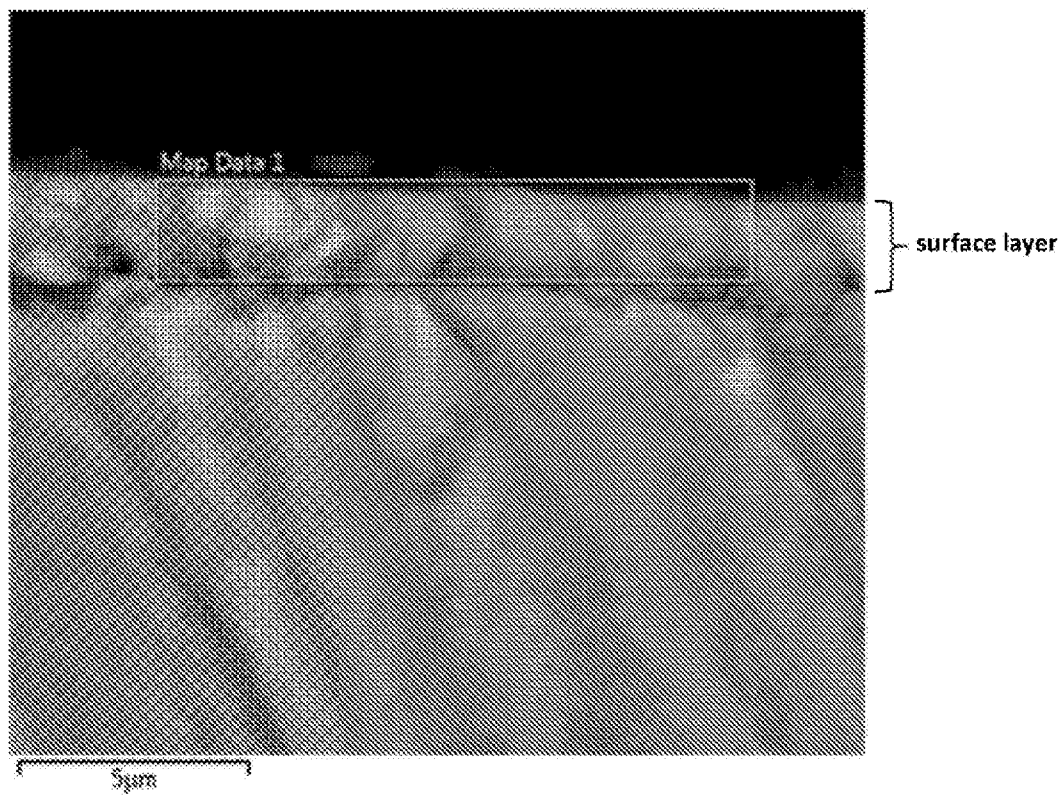
Before treatment with stabilized NaF/ACP



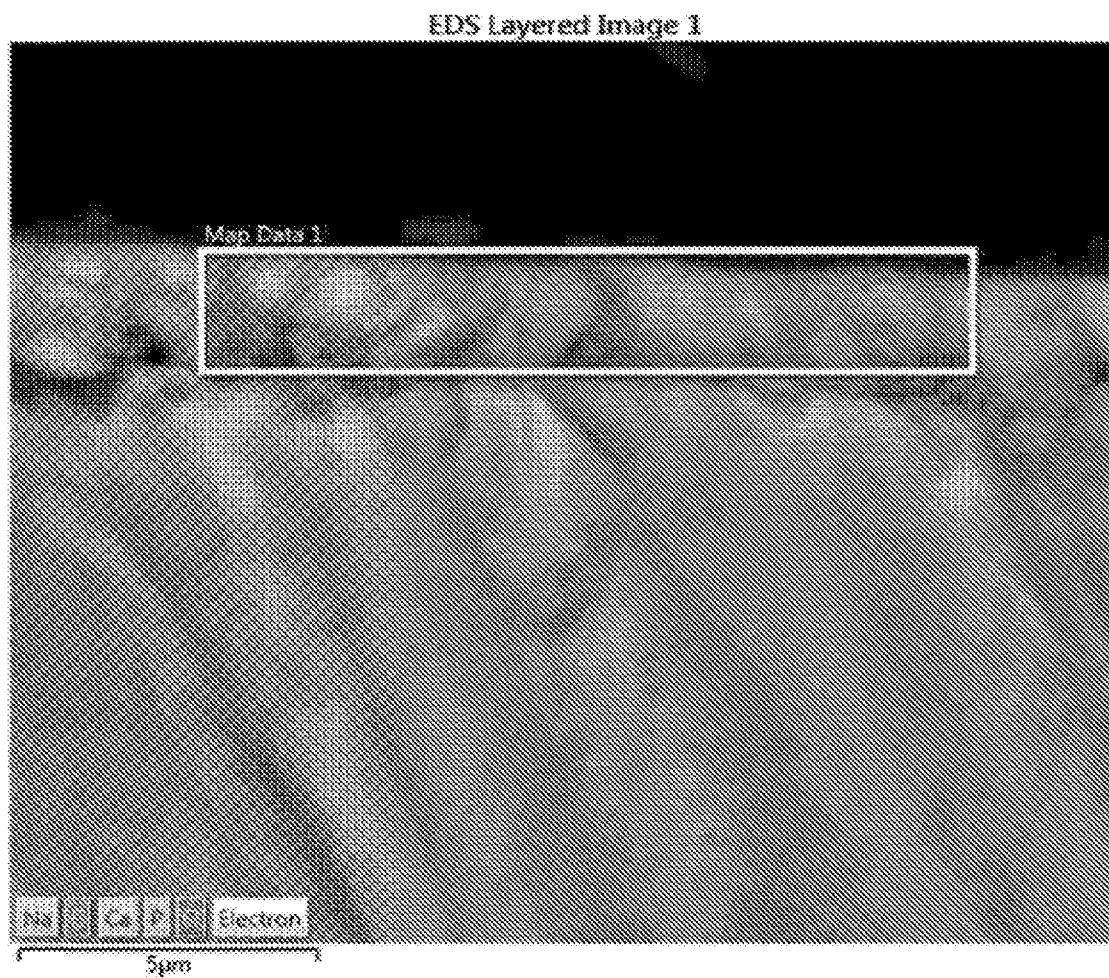
5 (B)

After treatment with stabilized NaF/ACP









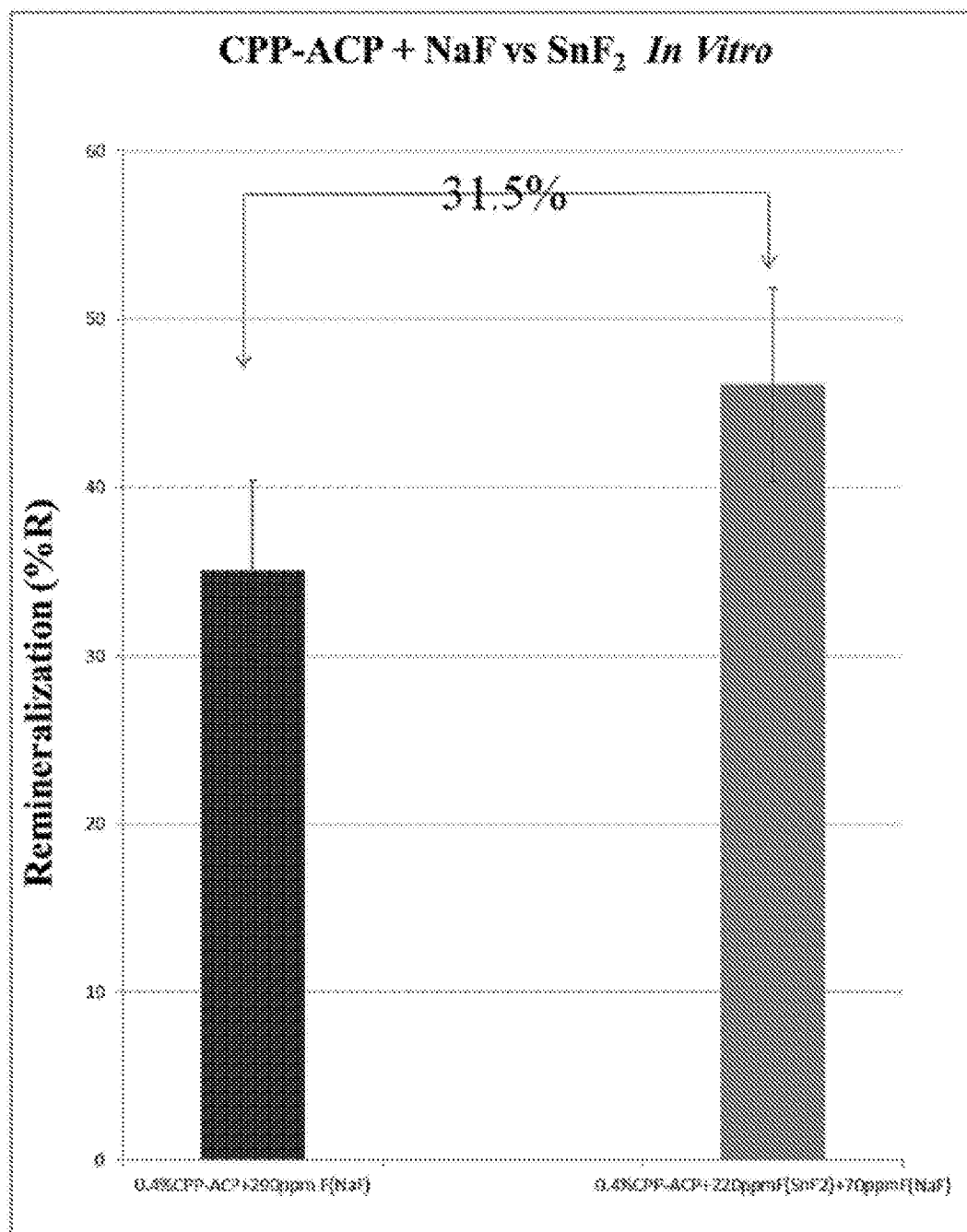


FIGURE 7

