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(54) **Title**: ORAL CARE COMPOSITION FOR ORAL BIOFILM REMOVAL

(57) **Abstract**: The invention relates to an oral care composition comprising by weight of the oral care composition: a) 0.0001 to 5 wt. % of polystyrene sulfonate having a molecular weight between 0.5 kDa and 2,000 kDa; and, b) 0.1 to 3 wt. % of a mono-substituted pyridine compound that carries the substituent in one of the ring positions 2, 3 or 4, said substituent being selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation. The invention also relates to the use of a combination of polystyrene sulfonate and said pyridine compound to inhibit formation of and/or to reduce oral biofilm in the oral cavity.

ORAL CARE COMPOSITION FOR ORAL BIOFILM REMOVAL

Field of the invention

The present invention relates to an oral care composition, such as toothpaste or a
5 mouthwash composition, which comprises polystyrene sulfonate and a pyridine
compound. The oral care composition of the present invention inhibits oral biofilm
formation in the oral cavity and/or reduces oral biofilms in the oral cavity.

Background of the invention

10 An oral biofilm is a biofilm or mass of bacteria that grows on surfaces within the oral
cavity. Oral biofilm is a sticky colourless deposit. Oral biofilm is also known as dental
plaque, microbial plaque, oral biofilm, dental plaque biofilm or bacterial plaque biofilm.
The formation of an oral biofilm is a natural process.

15 In dentistry, calculus or tartar is a form of hardened oral biofilm. It is caused by
precipitation of minerals from saliva and gingival crevicular fluid (GCF) in the oral biofilm
on the teeth. This process of precipitation kills the bacterial cells within the oral biofilm,
but the rough and hardened surface that is formed provides an ideal surface for further
plaque formation. This leads to calculus build-up, which compromises the health of the
20 gingiva (gums) and the teeth.

Calculus formation is associated with a number of clinical manifestations, including bad
breath, receding gums and chronically inflamed gingiva. Brushing and flossing can
remove plaque from which calculus forms; however, once formed, it is too hard and firmly
25 attached to be removed with a toothbrush and can only be removed by professional
cleaning.

Oral biofilm can also give rise to dental caries. The cause of dental caries is the
breakdown of the hard tissues of the teeth (enamel, dentin and cementum). Bacteria in
30 the oral cavity produce acid from food debris or simple sugars present on the tooth
surface, which causes demineralization of the teeth.

Therefore it is important to disrupt the oral biofilm and to remove it daily. Oral biofilm control and removal is achieved with correct tooth brushing with toothpaste and the use of interdental aids such as dental floss and interdental brushes. Mouthwashes, also called mouth rinsers, may additionally contribute to inhibiting oral biofilm formation.

5

Toothpaste is a paste or gel dentifrice used with a toothbrush as an accessory to clean and maintain the aesthetics and health of teeth. It aids in removing the oral biofilm and food from the teeth, helps to eliminate or mask halitosis, and delivers active ingredients such as fluoride or xylitol in order to prevent tooth and gum disease (gingivitis). Most of the cleaning is done by the mechanical use of the toothbrush.

10

Fluoride in various forms is the most popular active ingredient in toothpaste. It is included in toothpastes in order to prevent caries. Sodium fluoride is the most common form of fluoride used in toothpastes.

15

In addition to fluoride, the other fundamental ingredient in most toothpastes is an abrasive. Abrasives in toothpaste reduce the time needed to remove plaque from the teeth. Representative abrasives include particles of aluminium hydroxide ($\text{Al}(\text{OH})_3$), calcium carbonate (CaCO_3), various calcium hydrogen phosphates, abrasive silica and zeolites, and hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$).

20

Many, though not all, toothpastes contain anionic surfactants, such as sodium lauryl sulfate. Sodium lauryl sulfate is a foaming agent, although it also acts as a powerful antimicrobial.

25

Mouthwash is a product used to enhance oral hygiene. Antiseptic and anti-plaque mouth rinse claims to reduce oral biofilms, which cause dental caries, gingivitis, and bad breath. Anti-cavity mouth rinse uses fluoride to protect against tooth decay. However, the use of mouthwash does not eliminate the need for both brushing and flossing.

30

EP 2,700,395 A2 (Henkel, 2014) describes a mouth and tooth care cleaning agent comprising 0.001 to 5 wt.% of niacinamide and/or niacin, 1 to 70 wt.% of sorbitol and/or glycerol and/or 1,2-propylene glycol, and 0.01 to 5 wt.% of a fluorine compound. Further,

EP 2,700,395 describes the use of niacinamide and/or niacin for increasing lactoperoxidase activity in an oral cavity, which contributes to the improvement of the antibacterial defence system in the oral cavity.

- 5 WO 2012/001337 A1 (Lalvani, Tej) describes a composition in the form of a toothpaste or mouthwash for dental and oral hygiene, infections and inflammations, in which the active ingredients are *Pelargonium sidoides* extract, vitamin A, vitamin C, vitamin E, folic acid, niacinamide, zinc, coenzyme Q10, arginine, calcium carbonate, fluoride, strontium chloride, and papain combined with standard constituents of toothpastes or
10 mouthwashes. WO 2012/001337 describes that niacinamide appears to have an influence on gingival health via local influence and leads to a significant improvement in gingival inflammation.

- Polystyrene sulfonate is a salt of a polymer with 4-styrenesulfonic acid as monomer.
15 These polymers exist as linear polymers and as cross-linked polymers. Polystyrene sulfonates are anionic polyelectrolytes and widely used to remove ions such as potassium, calcium and sodium from solutions in both technical applications, such as water softening; and in medical applications, such as in the treatment of hyperkalaemia.
- 20 US 4,375,461 A (J&J, 1983) describes that a number of hydrophilic sulfonic acid and sulfonic acid salt derivatives of certain vinylaromatic homopolymers and copolymers have been synthesized and were found to inhibit the deposition of dental plaque onto human teeth.
- 25 Sorbic acid is a natural organic compound used as a food preservative. Sorbic acid and its salts, such as sodium sorbate, potassium sorbate, and calcium sorbate, are antimicrobial agents often used as preservatives in food and drinks to prevent the growth of molds or yeasts. Potassium sorbate is used in personal care products to inhibit the development of microbial growth in these products to enhance shelf stability.

Summary of the invention

The inventors of the present invention have found that the use of polystyrene sulfonate in combination with a pyridine compound is particularly effective in inhibiting oral biofilm formation in the oral cavity.

5 The present invention thus relates to an oral care composition comprising by weight of the oral care composition:

- a. at least 0.0001 wt.% of polystyrene sulfonate having a molecular weight between 0.5 kDa and 2,000 kDa;
- b. at least 0.1 wt. % of a mono-substituted pyridine compound that carries
10 the substituent in one of the ring positions 2, 3 or 4, said substituent being selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation.

The inventors have further found that the addition of at least 0.05 wt.% of a sorbic acid compound to the combination of polystyrene sulfonate and a pyridine compound even
15 leads to better prevention of oral biofilm formation and reduction of oral biofilms in the oral cavity.

Without wishing to be bound by theory, the inventors of the present invention believe that the polystyrene sulfonate and the pyridine compound are capable of forming deposits on
20 the tooth surface, which allows these compounds to affect the oral flora for a prolonged period of time, thereby enabling effective inhibition of biofilm formation and reduction of formed biofilms in the oral cavity, and especially on tooth enamel.

The invention additionally pertains to embodiments wherein the oral care composition is
25 a toothpaste or a mouthwash.

The present invention further relates to the use of a combination of polystyrene sulfonate and a pyridine compound to inhibit formation of and/or to reduce oral biofilm in the oral
cavity.

30

Detailed description of the invention

A first aspect of the invention relates to an oral care composition comprising by weight of the oral care composition:

- (a) at least 0.0001 wt.% of polystyrene sulfonate having a molecular weight between 0.5 kDa and 2,000 kDa;
- (b) at least 0.1 wt. % of a mono-substituted pyridine compound that carries the substituent in one of the ring positions 2, 3 or 4, said substituent being selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation.

The term "polystyrene sulfonate" as used herein refers to polymer salts with the linear molecular formula of $(\text{C}_8\text{H}_7\text{XO}_3\text{S})_n$, wherein X stands for a cation selected from Na^+ , Ca^{2+} , K^+ , Mg^{2+} and combinations thereof. When these polymer salts are present in water the cations may be dissociated from the anionic counterpart.

The term "sorbic acid compound" as used herein refers to sorbic acid, i.e. (2E,4E)-hexa-2,4-dienoic acid, and salts thereof.

The term "pyridine compound" as used herein refers to a mono-substituted pyridine compound that carries the substituent in one of the ring positions 2, 3 or 4, said substituent being selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation. Suitable monovalent cations are selected from H^+ , Na^+ or K^+ .

Polystyrene sulfonate

The oral care composition according to the present invention preferably comprises 0.0005 to 5 wt. % of the polystyrene sulfonate by weight of the oral care composition. More preferably, the oral care composition comprises 0.001 to 2.5 wt. %, even more preferably the oral care composition comprises 0.001 to 1, most preferably 0.001 to 0.5 wt. % of the polystyrene sulfonate by weight of the oral care composition. In a particularly preferred embodiment the oral care composition comprises less than 0.05 wt. % of polystyrene sulfonate, by weight of the oral care composition.

The polystyrene sulfonate according to the present invention preferably has a molecular weight between 10 kDa and 1000 kDa, more preferably between 25 kDa and 500 kDa and most preferably between 50 kDa and 250 kDa.

The polystyrene sulfonate is preferably water-soluble. The term "water soluble" within this context is defined as that at least 2 gram of polystyrene sulfonate is dissolved per liter of demineralized water at 20 °C.

- 5 The cation of the polystyrene sulfonate is preferably selected from Na⁺, K⁺ and combinations thereof. More preferably the cation is the polystyrene sulfonate Na⁺.

Pyridine compound

- 10 The oral care composition according to the present invention preferably comprises 0.1 to 3 wt. %, more preferably 0.3 to 2 wt. %, and most preferably 0.5 to 1 wt. % of the pyridine compound by weight of the oral care composition.

- 15 The pyridine compound of the present invention is preferably a mono-substituted pyridine compound that carries the substituent in the ring position 3, said substituent being selected from -COO⁻ X⁺ and -CONH₂, wherein X⁺ represents a monovalent cation.

- 20 More preferably, the X⁺ represents H⁺ and therefore the pyridine compound is more preferably a mono-substituted pyridine compound that carries the substituent in the ring position 3, said substituent being selected from -COOH and -CONH₂. Formulated differently, the pyridine compound is more preferably selected from niacin, niacinamide and combinations thereof. Most preferably the pyridine compound is niacinamide.

- 25 The weight ratio of the pyridine compound to the polystyrene sulfonate is preferably at least 1:1, more preferably at least 10:1, even more preferably said weight ratio lies between 100:1 and 1000:1. Most preferably the weight ratio of the pyridine compound to the polystyrene sulfonate lies between 400:1 and 800:1.

Sorbic acid compound

- 30 The oral care composition according to the present invention preferably further comprises at least 0.05 wt. % of a sorbic acid compound. More preferably the oral care composition comprises 0.1 to 1 wt. % and most preferably 0.5 to 0.8 wt. %, of the sorbic acid compound, by weight of the oral care composition.

The sorbic acid compound according to the present invention is preferably a salt of sorbic acid. More preferably, the salt of sorbic acid is selected from calcium sorbate, potassium sorbate, sodium sorbate and combinations thereof. Even more preferably the salt of sorbic acid is selected from potassium sorbate, sodium sorbate and combinations thereof. Most preferably the salt of sorbic acid is potassium sorbate.

The weight ratio of the sorbic acid compound to the polystyrene sulfonate is preferably at least 5:1, more preferably at least 10:1, even more preferably, said weight ratio lies between 100:1 and 1000:1. Most preferably the weight ratio of the sorbic acid compound to the polystyrene sulfonate lies between 400:1 and 800:1.

The oral care composition of the present invention preferably comprises at least 0.5 wt. % of the combination of the pyridine compound and the sorbic acid compound. More preferably the oral care composition comprises 0.75 to 4 wt. %, most preferably 1 to 3 wt. % of the combination of the pyridine compound and the sorbic acid compound, by weight of the oral care composition.

The weight ratio of the pyridine compound to the sorbic acid compound is preferably between 1:30 and 30:1. More preferably the weight ratio of the pyridine compound to the sorbic acid compound is between 1:20 and 20:1 and most preferably between 1:10 and 10:1.

The oral care composition according to the present invention preferably comprises 0.5 to 5 wt. %, by weight of the oral care composition, of the combination of polystyrene sulfonate, the pyridine compound and the sorbic acid compound. More preferably the oral care composition comprises 0.75 to 4 wt. %, most preferably 1 to 3 wt. %, by weight of the oral care composition, of the combination of polystyrene sulfonate, the pyridine compound and the sorbic acid compound.

The oral care composition according to the present invention is preferably a paste, a gel or a liquid.

The oral care composition of the present invention is preferably a toothpaste composition or a mouthwash composition. The toothpaste composition of the present invention is preferably a paste or a gel. The mouthwash composition of the present invention is preferably a liquid.

5

Toothpaste composition

In one preferred embodiment the oral care composition is a tooth paste composition and further comprises by weight of the tooth paste composition:

- (a) at least 5 wt.% of abrasives;
- 10 (b) at least 5 wt.% of water; and
- (c) fluoride compounds in an amount to provide 500 to 2500 ppm fluoride by weight of the tooth paste composition.

The abrasives in the tooth paste composition are preferably selected from aluminium hydroxide, calcium carbonate, calcium hydrogen phosphates, abrasive silica, zeolites,
15 hydroxyapatite and combinations thereof. More preferably the abrasives are selected from calcium carbonates, abrasive silica and combinations thereof. When calcium carbonate is the abrasive, the toothpaste is in the opaque paste format. When abrasive silica is used, the toothpaste is usually a transparent gel.

20 The tooth paste composition more preferably comprises 10 to 70 wt. % of abrasives, most preferably comprises 15 to 60 wt. % of abrasives, by weight of the tooth paste composition.

The tooth paste composition more preferably comprises 7.5 to 40 wt. % of water, most
25 preferably comprises 10 to 30 wt. % of water, by weight of the tooth paste composition.

The tooth paste composition more preferably comprises fluoride compounds in an amount to provide 1000 to 2000 ppm fluoride by weight of the tooth paste composition.

30 The fluoride compounds are preferably selected from sodium fluoride, stannous fluoride, olaflur, sodium monofluorophosphate and combinations thereof. More preferably the fluoride compounds are selected from sodium fluoride, sodium monofluorophosphate and combinations thereof.

The tooth paste composition preferably further comprises at least 1 wt. % of surfactant by weight of the tooth paste composition. More preferably the tooth paste composition comprises 2 to 15 wt. % of surfactant and most preferably the tooth paste composition comprises 2.5 to 10 wt. % of surfactant by weight of the tooth paste composition.

5

The surfactant is preferably an anionic or amphoteric surfactant. A preferred anionic surfactant is an alkali metal sulfate, more preferably the anionic surfactant is sodium lauryl sulfate. The amphoteric surfactant is preferably a betaine, more preferably an alkylamidopropyl betaine (wherein the alkyl group is a linear C₁₀ about C₁₈ chain), and most preferably is cocoamidopropyl betaine (CAPB).

10

The tooth paste composition preferably further comprises a humectant. Said humectant is preferably selected from xylitol, glycerol, sorbitol and combinations thereof. Preferably, the tooth paste composition comprises 0.1 to 80 wt.% of humectant.

15

Mouth wash composition

In another preferred embodiment the oral care composition is a mouth wash composition and further comprises by weight of the mouth wash composition:

20

- a. at least 20 wt. of liquid medium selected from water, alcohol and combinations thereof;
- b. at least 0.01 wt.% of sweeteners;

25

The mouth wash composition more preferably comprises 20 to 90 wt.%, most preferably 40 to 80 wt.%, by weight of the mouth wash composition, of liquid medium selected from water, alcohol and combinations thereof. Preferably the mouth wash comprises less than 0.5 wt.% by weight of the mouth wash composition of alcohol, more preferably less than 0.1 wt.%.

30

The mouth wash composition more preferably comprises 0.1-20 wt.%, most preferably 1-10 wt.%, by weight of the mouth wash composition, of sweeteners.

The sweeteners in the mouth wash composition are preferably sugar substitutes. More preferably the sugar substitutes are selected from stevia, aspartame, sucralose, neotame, acesulfame potassium, saccharin, advantame, sorbitol, lactitol, xylitol and

combinations thereof. Even more preferably, the sugar substitutes in the mouth wash composition are selected from sucralose, saccharin, sorbitol, xylitol and combinations thereof. Most preferably the sugar substitutes are selected from sorbitol, xylitol and combinations thereof.

5

The mouthwash composition of the present invention preferably comprises one or more preservatives. The preservatives are preferably selected from sodium benzoate, methyl paraben, zinc and combinations thereof.

10 Use

A second aspect of the invention relates to the use of a combination of polystyrene sulfonate and a pyridine compound to inhibit formation of and/or to reduce oral biofilm in the oral cavity, wherein the pyridine compound is a mono-substituted pyridine compound that carries the substituent in one of the ring positions 2, 3 or 4, said substituent being
15 selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation.

In a preferred use, the combination further comprises a sorbic acid compound.

The polystyrene sulfonate in the use of the present invention preferably has a molecular
20 weight between 0.5 kDa and 2,000 kDa, more preferably between 10 kDa and 1000 kDa, even more preferably between 25 kDa and 500 kDa and most preferably between 50 kDa and 250 kDa.

The pyridine compound in the use of the present invention is preferably a mono-
25 substituted pyridine compound that carries the substituent in the ring position 3, said substituent being selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation.

More preferably, the X^+ represents H^+ and therefore the pyridine compound in the use of
30 the present invention is more preferably a mono-substituted pyridine compound that carries the substituent in the ring position 3, said substituent being selected from -COOH and -CONH_2 . Formulated differently, the pyridine compound in the use of the present

invention is more preferably selected from niacin, niacinamide and combinations thereof. Most preferably the pyridine compound is niacinamide.

The oral cavity in the use of the present invention is preferably a human oral cavity.

5

The biofilm in the use of the present invention is preferably formed by the oral microflora present in the oral cavity. The oral microflora preferably comprises *Streptococcus*.

10 The combination of compounds in the use of the invention is preferably applied at least once a day in the oral cavity. More preferably the combination of compounds is applied between 1 and 4 times per day in the oral cavity, most preferably the combination of compounds is applied 2 or 3 times per day in the oral cavity.

15 The combination of compounds in the use of the invention is preferably applied on the tooth surface, gingiva and combinations thereof.

20 The combination of compounds in the use of the invention is preferably applied in the oral cavity using a toothbrush. Alternatively the combination of compounds in the use of the invention are preferably applied in the oral cavity by ingesting said combination and keeping the combination in the mouth for at least 10 seconds. More preferably the combination is kept in the mouth for 15 to 120 seconds, and most preferably the combination is kept in the mouth for 20 to 60 seconds.

25 The combination of compounds in the use of the invention is preferably removed from the oral cavity after at least 30 seconds after application of the combination in the oral cavity, wherein at least 80wt. % of the combination is removed from the oral cavity. More preferably at least 90 wt. % of the combination of compounds is removed from the oral cavity. Most preferably at least 95 wt. % of the combination of compounds is removed from the oral cavity. The removal of the combination of compounds from the oral cavity
30 typically involves spitting out, optionally after washing the oral cavity with rinse water.

The invention is further illustrated by the following non-limiting examples.

Examples

Example 1

An oral biofilm assay was performed for assessing the ability of compounds to prevent oral biofilm formation. Table 1 shows the compounds that were tested for their oral biofilm formation prevention activity and the tested concentrations of each compound respectively.

Table 1

Active	Concentration (wt.%) by weight of growth media added to each well
Polystyrene sulfonate (PSS)	0.001%
Niacinamide	0.75%
K-sorbate	0.75%

An overnight culture of *S. mutans* UA159 was grown in fresh TYB growth medium to mid-exponential phase (OD 620 of 0.5). About 10^7 of the bacteria were re-suspended in 1 ml of fresh TYB. 100µl of this suspension was added to 1.9 ml of respective sugar medium (1 wt. % glucose and 1 wt.% sucrose) to obtain a 2 ml suspension, which was added per well in a 24 wells plate. Subsequently the concentration of compound(s), as indicated in Table 1, was added to the culture medium containing *S. mutans*. The 24 well plate was incubated for 24 hours in a 5% CO₂ incubator.

After 24 hours of incubation, the formed biofilm in each well was measured. First the growth media was removed, followed by carefully rinsing the wells 3 times with 1ml distilled water. Subsequently 500 µl of 1% crystal violet in distilled water was added per well. The wells comprising the crystal violet solution were incubated for 5 minutes at room temperature. Subsequently, the crystal violet solution was removed from the wells and the wells were rinsed 3 times with 1ml distilled water. The biofilm bound crystal violet was eluted in 1ml of 33 wt.% of glacial acetic acid in distilled water per well. The biofilm bound crystal violet per well was quantified with optical density measured at 540nm in an IEMS reader.

The results of the oral biofilm assay are shown in Table 2.

Table 2

		% of biofilm-bound dye of control
A	Control (no actives)	100
B	Niacinamide	82.9
C	PSS	41.1
D	K-sorbate	46.3
1	PSS + Niacinamide + K-sorbate	8.7

5 Example 2

A similar experiment as described for example 1 was performed, except that the effect of the compounds in Table 3 were tested for their ability to disrupt a pre-formed biofilm that had been formed during 24 hours of incubation.

- 10 Thus, at the start of incubation no compounds were added to the growth medium. After 24 hour incubation of the bacteria in the 24 wells plates, the overnight growth medium was carefully aspirated and replaced with fresh growth medium containing both the sugars and the compounds in the concentration as indicated in Table 5.

15 Table 3

Active	Concentration (wt.%) by weight of growth media added to each well
Polystyrene sulfonate (PSS)	0.001%
Niacinamide	0.75%
K-sorbate	0.75%

The results of this experiment are shown in Table 4.

20 Table 4

		% of biofilm-bound dye of control
E	Control (no actives)	100
F	Niacinamide	105.5
G	PSS	106
H	K-sorbate	75.4
2	PSS + Niacinamide + K-sorbate	67.4

Example 3

An oral biofilm assay on surrogate tooth surface (Hydroxy-apatite disc) was performed, wherein the actives were added to tooth paste bases.

5 A. Preparation of toothpaste slurry with actives

3.3 g of tooth paste base, either of the chalk based or the gel based tooth paste (see Table 5) was weighed and 10ml of demi water was added to arrive at a 33 wt. % tooth paste slurry. This tooth paste slurry was vortexed well. Various actives (single or in combination) were added in required concentrations to the tooth paste slurry (see Table 6).

Table 5

Ingredients	Chalk based tooth paste base (wt. %)	Gel based tooth paste base (wt. %)
Liquid base	Up to 50	up to 80
Fillers & abrasive	20-50	15-25
Rheology modifiers	0.5 - 2.0	
Surfactants	0.5 - 2.5	
Flavour	0.5 - 2.0	
Sweetener	0.2	
Coloring	0.1	
Preservative	0.2	
Fluoride compounds	0.05 - 0.5	

15 Table 6

Active	Concentration (wt.%) by weight of tooth paste base
Polystyrene sulfonate (PSS)	0.001%
Niacinamide	0.75%

B. Preparation of hydroxy-apatite disc (HA disc)

2 ml of the tooth paste slurry plus active(s) are added to a 100 ml sample container, comprising the HA disc. The HA disc was brushed for 2 minutes and subsequently dip washed in de-ionized water. The HA disc was transferred to a 24 well plate and 1 ml of salivary protein (such as salivary amylase) was added on the HA disc. The 24 well plates were incubated at 37 °C for 2 to 4 hours.

C. Preparation and addition of oral microflora culture comprising S. mutans UA159

An overnight culture of *S. mutans* UA159 was grown in fresh TYB cell medium to mid-exponential phase (OD 620 of 0.5). This overnight culture was added to filtered TYB cell media and adjusted to an OD 620 of 0.2. Sucrose was added to provide a sucrose
5 concentration of 2 wt. %.

The salivary protein-treated HA discs were transferred to a new 12-well plate. 2 ml of the above bacterial culture suspension was added to each HA-disc. The 12-well plate comprising the HA-discs were incubated overnight in a 5% CO₂ chamber.
10

D. Staining of biofilm and colorimetric estimation of biofilm bound dye on HA discs

The incubated HA discs were transferred to a 24 well plate and dip-washed three times with 2 ml of distilled water in the 24-well plate. The discs were then blotted on tissue paper and transferred into 2 ml plaque staining solution (Plaque Check, Vishal Dentocare
15 Pvt Ltd, India) in a 24 well plate. The discs were incubated for 5 minutes in a staining solution.

After 5 minutes, the discs were dip-washed three times in 2 ml of distilled water in 3 wells for each disc. The discs were then blotted on tissue paper and air-dried for 30 minutes.
20

After the discs were dried up, biofilm bound dye was eluted in 1 ml of 0.1M NaOH (freshly prepared). 10ul of the solution and 90 ul of 1:10 diluted in 0.1 M NaOH was used for measuring absorbance. Absorbance was measured at 540 nm in IEMS reader.

25 The results of the oral biofilm assay are shown in Table 7.

Table 7

		% of biofilm-bound dye of control
		Chalk based tooth paste
I	Control (no actives)	100
J	Niacinamide	82.1
K	PSS	105.3
3	PSS + Niacinamide	39.8
		Gel based tooth paste
L	Control (no actives)	100
M	Niacinamide	121.5
N	PSS	92.5
4	PSS + Niacinamide	61.6

Claims

1. An oral care composition comprising by weight of the oral care composition:
 - (a) 0.0001 to 5 wt.% of polystyrene sulfonate having a molecular weight between 0.5 kDa and 2,000 kDa; and,
 - (b) 0.1 to 3 wt. % of a mono-substituted pyridine compound that carries the substituent in one of the ring positions 2, 3 or 4, said substituent being selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation.
2. The oral care composition according to claim 1, wherein the weight ratio of the pyridine compound to the polystyrene sulfonate is at least 1:1.
3. The oral care composition according to claims 1 or 2, wherein the oral care composition further comprises at least 0.05 wt.% of a sorbic acid compound by weight of the oral care composition.
4. The oral care composition according to claim 3, wherein the weight ratio of the sorbic acid compound to the polystyrene sulfonate is at least 5:1.
5. The oral care composition according to claim 3 or 4, wherein the oral care composition comprises at least 0.5 wt. % of the combination of the sorbic acid compound and the pyridine compound.
6. The oral care composition according to any one of claims 3 to 5, wherein the weight ratio of the pyridine compound to the sorbic acid compound is between 1:30 and 30:1.
7. The oral care composition according to any one of claims 3 to 6, wherein the oral care composition comprises 0.5 to 10 wt. % of the combination of polystyrene sulfonate, the pyridine compound and the sorbic acid compound.
8. The oral care composition according to any one of the preceding claims, wherein the oral care composition is a paste, a gel, or a liquid.

9. The oral care composition according any one of the preceding claims, wherein the oral care composition is a tooth paste composition or a mouth wash composition.
10. The oral care composition according to claim 9, wherein the oral care composition is a tooth paste composition and further comprises by weight of the tooth paste composition:
 - (a) at least 5 wt.% of abrasives;
 - (b) at least 5 wt.% of water; and
 - (c) fluoride compounds in an amount to provide 500 to 2500 ppm fluoride by weight of the tooth paste composition.
11. Oral care composition according to claim 9, wherein the oral care composition is a mouth wash composition and further comprises by weight of the mouth wash composition:
 - (a) at least 20 wt. % of liquid medium selected from water, alcohol and combinations thereof;
 - (b) at least 0.01 wt.% of sweeteners;
12. Use of a combination of polystyrene sulfonate and a pyridine compound to inhibit formation of and/or to reduce oral biofilm in the oral cavity, wherein the pyridine compound is mono-substituted pyridine compound that carries the substituent in one of the ring positions 2, 3 or 4, said substituent being selected from $\text{-COO}^- \text{X}^+$ and -CONH_2 , wherein X^+ represents a monovalent cation.
13. Use according to claim 12, wherein the combination further comprises a sorbic acid compound.
14. Use according to claim 12 or 13, wherein the polystyrene sulfonate has a molecular weight between 0.5 kDa and 2,000 kDa.
15. Use according to any one of claims 12 to 14, wherein the combination is applied at least once a day in the oral cavity.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/078335

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K8/67 A61Q11/00 A61K8/81
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61Q

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 375 461 A (GANDER ROBERT J ET AL) 1 March 1983 (1983-03-01) cited in the application page 1 example 1 examples A-F	1-15
Y	EP 2 700 395 A2 (HENKEL AG & CO KGAA [DE]) 26 February 2014 (2014-02-26) cited in the application page 4 the whole document	1-15
A	WO 02/15874 A2 (PROCTER & GAMBLE [US]) 28 February 2002 (2002-02-28) page 1 page 33; example 6	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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