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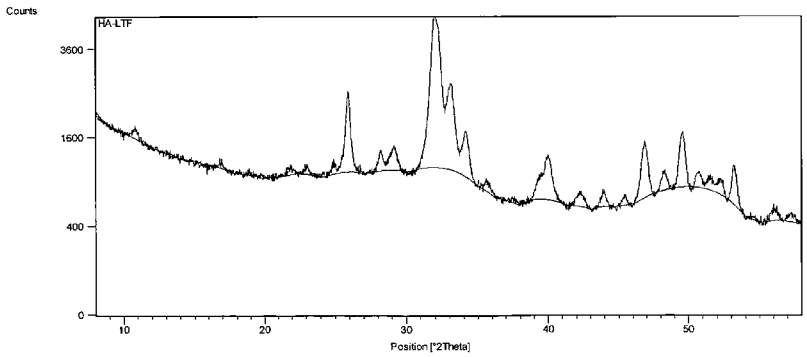

FIG. 2

(57) Abstract: A dental care product comprising carbonate- substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface, wherein said particles have the formula: $Ca_{(10-x)}$ Sr_x $(PO_4)_{(6-y)}$ $(CO_3)_y$ $(OH)_2$ wherein x is a number comprised between 0.0010 and 0.015; and y is a number comprised between 0.0010 and 0.010; and wherein said carbonate-substituted strontium-hydroxyapatite particles have a crystallinity degree CD comprised between 55 and 85%. The carbonate-substituted strontium-hydroxyapatite particles are biomimetic for composition, structure and morphology allowing a strong chemical affinity with natural enamel and dentine hydroxyapatite crystals and inducing the formation of a rimineralizing and protective surface coating on enamel and dentine.

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DENTAL CARE PRODUCTS CONTAINING BIOMIMETIC HYDROXYAPATITE PARTICLES HAVING A LACTOFERRIN-FUNCTIONALIZED SURFACE

DESCRIPTION

Field of the invention

The invention relates to dental care products containing biomimetic carbonate-substituted strontium-hydroxyapatite particles having a protein-functionalized surface, and the process for their preparation.

More specifically, the invention relates to dental care products for dental hygiene containing biomimetic carbonate-substituted strontium-hydroxyapatite particles having a protein-functionalized surface such as, for example, solutions, suspensions, oils, gels, pastes, dentifrices, or other solid products.

According to other aspects, the invention relates to a process for preparing a suspension for dental hygiene, to a process for manufacturing a dental care product containing the aforementioned biomimetic carbonate-substituted strontium-hydroxyapatite particles having a protein-functionalized surface, as well as to a method of providing at the teeth outer surface a source of Sr^{2+} ions and antibacterial proteins which may be locally released at an acidic pH by means of the biomimetic carbonate-substituted strontium-hydroxyapatite particles having a protein-functionalized surface.

Background of the invention

Enamel is the hardest material in vertebrates and is the most highly mineralized skeletal tissue present in the body. Mature enamel, considered the most resistant and tough material in the biological world, is composed of carbonate hydroxyapatite (CHA) (95-97% wt) and less, about 1% wt, of organic material. Unlike other biomineralized tissues, such as bone and dentin, mature enamel does not contain cells and therefore cannot be regenerate itself and therefore cannot be biologically remodelled.

Consequently, enamel regeneration cannot take place *in vivo*. There is no biological process that can repair degraded or damaged enamel, evidencing the need for synthetic enamel biocompatible materials able to repair teeth decay.

Enamel makes up the outermost layer of the tooth crown having a thickness of about 1-2 mm and containing a high mineral content which imparts to the enamel a high modulus, but also making it susceptible to cracking. Dentine lies below the enamel and is tougher, forming the bulk of the tooth and absorbing stresses from enamel, preventing its fracture.

The mechanisms involved in the damage of dental hard tissue are related to the acid attacks on the outer few micrometers of the enamel, with the consequent demineralization and dissolution of the minerals.

The oral cavity contains a numerous amount of microorganisms that adhere to the surface of teeth for long periods of time. These multiple species of bacteria become dental biofilm, more commonly referred to as dental plaque, which is composed of bacteria that take part in the complex ecosystems of the mouth. The natural, non-frequent regulation of tooth shedding plays a large role in making dental biofilm the most diverse biofilm in the human body despite the relatively small size of the teeth. The biofilm is usually a pale yellow that develops naturally on the teeth. Initially, the biofilm is soft enough to come off easily. However, it starts to harden within 48 hours, and in about 10 days the plaque becomes dental calculus (tartar), rock-hard and difficult to remove without the professional intervention with ultrasonic tools and specialized sharp instruments.

Moreover, dental plaque and the repeated intake of fermentable sugars in the diet can give rise to dental caries (tooth decay), the localized destruction of the tissues of the tooth by acid produced from the bacterial degradation of fermentable sugars and to periodontal problems such as gingivitis and chronic periodontitis.

Lactoferrin (formerly known as lactotransferrin) is a glycoprotein with a molecular weight of about 80 kDa, and a member of a transferrin family, thus belonging to those proteins capable of binding and transferring Fe^{3+} ions.

Lactoferrin is comprised of a single polypeptide chain containing 703 amino acids folded into two globular lobes. These lobes, also called C – (carboxy) and N – (amino) terminal regions, are connected with a α-helix.

Lactoferrin was found in mucosal secretions, including tears, saliva, vaginal fluids, semen, nasal and bronchial secretions, bile, gastrointestinal fluids, urine, amniotic fluid and at higher concentrations in milk and colostrums, which makes it the second most abundant protein in milk, after caseins. In blood plasma, neutrophils after degranulation were observed to be the main source of lactoferrin.

Lactoferrin is involved in several physiological functions, many of which do not appear to be connected with its iron binding ability: regulation of iron absorption in the bowel, immune response, antioxidant, cariostatic, anticarcinogenic and anti-inflammatory properties and protection against microbial infection. The antimicrobial activity of lactoferrin is mostly due to its iron-binding properties, which deprive the bacterial flora from an element necessary for its growth.

A lack of iron inhibits the growth of iron-dependent bacteria such as E. coli and the ability of lactoferrin to bind free iron is responsible for its bacteriostatic effect. In contrast, lactoferrin may serve as iron donor, and in this manner support the growth of some bacteria with lower iron demands such as Lactobacillus sp. or Bifidobacterium sp., generally considered as beneficial.

Moreover, lactoferrin has also a bactericidal activity due to its direct interaction with the infectious agent.

This bactericidal activity is not iron-dependent and may be mediated through more than one pathway such as the binding of lactoferrin to specific receptors on the surface of some microorganisms which induces cell-death in Gram-negative bacteria due to a disruption in the cell wall. The subsequent release of lipopolysacharide (LPS) leads to

impaired permeability and a higher sensitivity to lysozyme and other antimicrobial agents. Instead the bactericidal activity affecting Gram-positive bacteria is mediated by electrostatic interactions between the negatively charged lipid layer and the positively charged lactoferrin surface that cause changes in the permeability of the membrane.

Related art

In recent times and based on the fact that the teeth bone tissue is primarily constituted by non-stoichiometric hydroxyapatite containing specific substituting ions at both the cationic and anionic reticular sites, the use of products comprising hydroxyapatite has been proposed for the treatment of bone defects in the fields of reconstructive bone surgery, surgical stomatology, traumatology, orthopedics and dentistry.

During the past decade, it has become also evident that oral administration of lactoferrin exerts several beneficial effects on the health of humans and animals, including anti-infective, anticancer, and anti-inflammatory effects. This has enlarged the potential application of lactoferrin as a food additive and for oral care applications.

International Patent Application WO 00/03747 discloses nanocrystalline materials based on apatite having an average size of the crystallites comprised between 0.5 and 200 nm, particularly for use in the fields of dentistry and dental hygiene in order to induce enamel and dentine remineralisation.

More specifically, this reference discloses an apatite-based nanostructured material obtained by lattice destabilisation treatment under high energy.

International Patent Application WO 2007/137606 in the name of the same Applicant discloses biologically active nanoparticles of a carbonate-substituted hydroxyapatite, particularly for use in oral or dental hygiene in order to improve teeth desensitization and remineralisation.

European Patent Application EP 2039342 discloses an agent for maintaining hardness of tooth substances comprising lactoferrin and iron-lactoferrin, particularly for use in food or drink.

German Patent Application DE 102010063720 discloses dental care composition

comprising silver particles, at least one calcium phosphate compound, and lactoferrin.

International Patent Application PCT/EP2011/002606 in the name of the same Applicant discloses dental care products comprising carbonate-substituted fluoro-hydroxyapatite particles and a process for their preparation..

Iafisco et al., Dalton Trans. Jan 28 (2001); 40(4): 820-7 discloses the adsorption and spectroscopic characterization of lactoferrin on hydroxyapatite nanocrystals at two different pH values (7.4 and 9.0).

Summary of the invention

The carbonate-substituted strontium-hydroxyapatite particles of the invention are biomimetic for composition, structure and morphology allowing a strong chemical affinity with natural enamel and dentine hydroxyapatite crystals and inducing the formation of a rimineralizing and protective surface coating on enamel and dentine.

The Applicant has observed that carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface show an increased antibacterial effect with respect to the lactoferrin alone of the aforementioned prior art.

The Applicant has also surprisingly discovered that said strontium hydroxyapatite functionalized by lactoferrin presents a greater degree of crystallinity with respect to a zinc carbonate-substituted hydroxyapatite without strontium and lactoferrin.

For the purposes of the present description and of the claims which follow, the expression: having a protein-functionalized surface or having a lactoferrin-functionalized surface, is used to indicate that the carbonate-substituted strontium-hydroxyapatite surface is functionalised by lactoferrin which is bonded to the surface itself.

In this connection, the Applicant deems, without however wishing to be bound by any interpretative theory, that such a bond may derive from several kinds of interactions which can take place between the lactoferrin and the carbonate-substituted strontium-hydroxyapatite surface, for example covalent or electrostatic chemical bonds, hydrogen and van der Waals bonds, and hydrophobic and hydrophilic interactions.

Accordingly, the present invention provides improved products for dental hygiene having both remineralization and cariostatic effects, comprising carbonate-substituted strontium-hydroxyapatite particles capable to convey, directly to the surface of the enamel, Sr^{2+} ions only when this is necessary, i.e. when the pH of oral cavity becomes acidic, by means of a hydroxyapatite-type carrier substantially insoluble under conditions of normal pH of the oral cavity (6.3-7.3) which becomes soluble when the pH becomes acidic (pH<5) locally releasing Sr^{2+} ions in proximity to enamel and dentine.

The Applicant has also observed that products for dental hygiene comprising said substantially insoluble hydroxyapatite-type carrier is capable of forming a thin film on the outer surface of the enamel even in the limited time available during the normal routine of dental hygiene. Said film solubilizes when the pH of the oral cavity becomes acidic (pH<5) locally releasing strontium ions and lactoferrin, only when this is necessary, effectively improving teeth remineralisation and showing a cariostatic and antibacterial effect.

Dental care products containing biomimetic carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface according to the invention are defined in attached claim 1.

More specifically, the carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface of the invention have the formula:

$$Ca_{(10-x)}\,Sr_x\,(PO_4)_{(6-y)}\,(CO_3)_y\,(OH)_2$$

wherein x is a number comprised between 0.0010 and 0.015; and y is a number comprised between 0.0010 and 0.010; and wherein said carbonate-substituted strontium-hydroxyapatite particles have a crystallinity degree CD comprised between 55 and 85%.

The crystallinity degree being defined as

$$CD = (1-X/Y) \bullet 100$$

wherein:

Y= height of the diffraction maximum at 2θ = 33°, X = height of the diffraction background at 2θ = 33° of the particles X-ray diffraction pattern.

For the purposes of the present description and of the claims which follow, the expression: particles, is intended to indicate nanoparticles or microparticles.

When particles ere considered of nanometric size, their dimension range from few nanometers up to some hundreds of nanometers, but for biomedical applications the dimension range is usually reduced from 30 nm to 200 nm because only nanoparticles of these dimensions can go across the cellular membrane. In order to avoid any possible consequence related to the presence of nanoparticles inside the cells it is preferred to use nanoparticles larger than 100 nm for biomedical applications which do not require an intracellular action. On the other hand nanoparticles aggregated in a cluster can realize particles of micrometric dimension.

For the purposes of the present description and of the claims which follow, the term nanoparticle, is used to indicate a particle having a size generally below 0.1 μm, preferably between 0.01 μm and 0.1 μm.

For the purposes of the present description and of the claims which follow, the term microparticle, is used to indicate aggregates or "clusters" of inorganic nanoparticles mentioned above and having a size comprised between 0.2 μm and 10 μm, preferably between 0.5 μm and 2 μm.

Single nanoparticles can have quite different morphology, but preferably, the nanoparticles of the invention have a flat and round shape mimicking the morphology of bone hydroxyapatite nanoparticles. The Applicant considers the nanoparticles biomimetic morphology the most adapted to interact with the dentine and enamel surface.

For the purposes of the present description and of the claims which follow, the expression: crystallinity degree, is intended to indicate the percentage of the hydroxyapatite compound present in the crystalline state.

For the purposes of the invention, the crystallinity degree can be measured according to known methods, such as, for example, by using x-ray diffraction analysis.

Within the framework of the definition given above, the crystallinity degree CD is measured according to the method described in: Landi, E., Tampieri, A., Celotti, G., Sprio, S., *"Densification behaviour and mechanisms of synthetic hydroxyapatites", J. Eur. Ceram. Soc.*, 2000, 20, 2377-2387 (hereinafter in short: the Landi et al. method).

For the purposes of the present description and of the claims which follow, the expression: lower than, as used before any numerical value, is meant to exclude such a numerical value and used to encompass only a range of lower values.

For the purposes of the present description and of the claims which follow, except where otherwise indicated, all numerical values expressing parameters such as amounts, weights, temperatures, percentages, and so forth, are to be understood as being modified in all instances by the term "about". Also, all ranges include any combination of the maximum and minimum points disclosed and include any intermediate ranges therein, which may or may not be specifically enumerated herein.

Advantageously, the aforementioned lactoferrin, which is incorporated in the apatite structure, effectively exploit an antibacterial activity capable of preventing generation of carious tooth and periodontal diseases such as alveolar blennorrhoea and reducing halitosis phenomena. Therefore, the dental composition of the invention is also advantageously capable of effectively exploiting an antibacterial effect and, accordingly, effectively treating teeth and gums disorders and in general increasing oral hygiene even in the limited time available during the normal routine of dental hygiene.

As mentioned above, the particles of the invention are carbonate-substituted hydroxyapatite particles having a lactoferrin-functionalized surface which incorporate carbonate ions in the apatite structure.

This feature advantageously enhances the biological activity of the particles of the invention, since the carbonate ion is also found in the structure of natural hydroxyapatite. In this regard, it is to be observed that the carbonate ion can occupy in

two different sites in the natural hydroxyapatite structure: namely, it can partially substitute the OH-ion (site A) and/or the PO_4^{3-} ion (site B). Both the total carbonate content (in the range of 3-8 wt. %) and the relative quantities of type A and type B carbonation (A/B in the range of 0.7-0. 9) found in the natural carbonate-substituted hydroxyapatite depend on the age of the individual and on the biological localization of the calcified tissue.

In a preferred embodiment of the invention, the carbonation preferably takes place at site B.

In a preferred embodiment of the invention, the hydroxyapatite particles comprise from 1 to 15% by weight and, more preferably, from 1 to 10% by weight based on the total weight of the particles of carbonate substituted into the hydroxyapatite structure.

In this way, the biological activity of the particles of the invention is advantageously enhanced, since their structure more closely resembles the structure of the natural apatite present in the teeth tissues.

According to a preferred embodiment of the invention, the ratio A/B between the carbonate substitution at the hydroxyl site (A) and the carbonate substitution at the phosphate site (B) of the hydroxyapatite is comprised between 0.05 and 0.5 and, still more preferably, comprised between 0.18 and 0.33.

According to another preferred embodiment of the invention, the carbonate substitution at the phosphate site (B) of the hydroxyapatite is greater than or equal to 65% by weight and, still more preferably, comprised between 90% and 100% by weight, of the total carbonate present in the hydroxyapatite.

These preferred patterns of carbonate substitution in the hydroxyapatite structure advantageously allow to increase the solubility of the particles in a biological environment. Additionally, the carbonate substitution at the phosphate site (B) advantageously induces a higher affinity of the hydroxyapatite particles for the osteoblast cell, increasing cellular adhesion and collagen production.

Preferably, the total amount of lactoferrin in the carbonate-substituted strontium-hydroxyapatite particles is comprised between 0.01% and 5.0% by weight based on the total weight of the particles.

In one preferred embodiment, dental care products of the invention further comprise an effective amount of a metal M ion.

Preferably, the metal M is selected from the group comprising: Mg, Se, K and mixtures thereof.

More preferably, dental care products of the invention comprise from 0.1% to 20% by weight with respect of the total Ca content of a metal M ion substituted into the hydroxyapatite structure.

Preferably, the dental care product including the particles of the invention may be in any physical form suitable for oral hygiene such as suspension, oil, gel or other solid product.

According to a preferred embodiment of the invention, the dental care product is in the form of a suspension including from 1% to 40% by weight, more preferably from 10% to 20% by weight, of carbonate-substituted hydroxyapatite particles.

In a preferred embodiment of the invention, the suspension has pH comprised between 6 and 13.

In this way, the suspension may be advantageously directly used as such or mixed with other ingredients in the formulation of effective dental care products.

Most advantageously, this suspension may be produced by means of a quite simple and economic method, as will be described in more detail hereinbelow, and may be directly used, for example as a gargle or mouthwash, to treat the teeth and gums or may be mixed with other ingredients when formulating a solid or liquid product such as a toothpaste or a mouthwash.

In either case and in a preferred embodiment, it has proved advantageous to add suitable preserving agents, such as parabens or other orally acceptable preservatives known to

those in the art, in order to prolong the shelf-life of the suspension and avoid the possibility of mold or bacterial contamination.

The inventors have surprisingly observed that the suspension of the invention is stable for an extended period of time even if no stabilizing agents are added thereto.

In particular, it has been observed that the suspension of the invention is stable for at least 30 days and, more generally, for about two-three months, without using any stabilizing agent.

According to a preferred embodiment of the invention, the dental care product is selected from the group consisting of: toothpaste, tooth powder, chewing gum for oral and dental hygiene, ointment for the gums, mouthwash and mouth bath concentrate and gargle.

The dental care products of this invention will, of course, also preferably contain other ingredients commonly used and known in the art to formulate such products, depending on the form of the oral product.

For instance, in the case of an oral product in the form of a dentifrice cream or paste, the product will preferably comprise a particulate abrasive agent, a humectant-containing liquid phase and a binder or thickener which acts to maintain the abrasive agent in stable suspension in the liquid phase. A surfactant and a flavoring agent are also usual preferred ingredients of commercially acceptable dentifrices.

For the purposes of the invention, a suitable particulate abrasive agent is preferably selected from the group comprising: silica, alumina, hydrated alumina, calcium carbonate, anhydrous dicalcium phosphate, dicalcium phosphate dihydrate and water-insoluble sodium metaphosphate. The amount of particulate abrasive agent will generally range from 0.5% to 40% by weight of the toothpaste.

Humectants of preferred use are glycerol and sorbitol syrup (usually comprising an approximately 70% solution). However, other humectants are known to those in the art including propylene glycol, lactitol, and hydrogenated corn syrup. The amount of humectant will generally range from 10% to 85% by weight of the toothpaste. The

liquid phase can be aqueous or nonaqueous.

Likewise, numerous binding or thickening agents have been indicated for use in dentifrices, preferred ones being sodium carboxymethylcellulose and xanthan gum. Others include natural gum binders such as gum tragacanth, gum karaya and gum arabic, alginates and carrageenans. Silica thickening agents include the silica aerogels and various precipitated silicas. Mixtures of binders may be used. The amount of binder included in a dentifrice is generally between 0.1% and 5% by weight.

It is usual and preferred to include a surfactant in a dentifrice and again the literature discloses a wide variety of suitable materials. Surfactants which have found wide use in practice are sodium lauryl sulfate and sodium lauroylsarcosinate. Other anionic surfactants may be used as well as other types such cationic, amphoteric and non-ionic surfactants. Surfactants are usually present in an amount comprised between 0.5% and 5% by weight of the dentifrice.

Flavors of possible use are those usually used in dentifrices, for example those based on oils of spearmint and peppermint. Examples of other flavoring materials which may be used are menthol, clove, wintergreen, eucalyptus and aniseed. An amount comprised between 0.1% and 5% by weight is a suitable amount of flavor to incorporate in a dentifrice.

The dental care products of the invention may include a wide variety of other optional ingredients.

In the case of an oral product in the form of a toothpaste, these optional ingredients may include an anti-plaque agent such as moss extract, an anti-tartar ingredient, such as a condensed phosphate, e.g. an alkali metal pyrophosphate, hexametaphosphate or polyphosphate; a sweetening agent, such as saccharine and salts thereof; an opacifying agent, such as titanium dioxide; a preservative, such as formalin; a coloring agent; a pH controlling agent, such as an acid, base or buffer, such as citric acid. Suitable amounts of these optional ingredients may be easily selectable by those skilled in the art as a function of the specific characteristics to be imparted to the toothpaste.

In the case of an oral product in the form of a chewing gum, the composition will comprise in addition to the ingredients mentioned above a suitable gum base which may be easily selectable by those skilled in the art.

In the case of an oral product in the form of a mouthwash or gargle, the composition will comprise suitable ingredients in liquid or soluble form easily selectable by those skilled in the art, such as sorbitol, glycerol, oils and flavoring materials, solubilizing agents such as hydrogenated and ethoxylated ricin oil, surfactants, such as sodium lauryl sulfate and sodium lauroylsarcosinate, preserving agents, viscosity regulators and other suitable ingredients which may be easily selectable by those skilled in the art.

For a fuller discussion of the formulation of oral compositions reference is made to Harry's Cosmeticology, Seventh Edition, 1982, Edited by J.B. Wilkinson and R.J. Moore.

According to another aspect thereof, the present invention relates to dental care compositions comprising the aforementioned carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface.

According to another aspect thereof, the present invention relates to the aforementioned carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface.

According to another aspect thereof, the present invention relates to an improved process for manufacturing a dental care product comprising carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface which requires low investment and operating costs.

A first process for manufacturing a dental care product selected from the group consisting of: toothpaste, tooth powder, chewing gum, ointment for the gums, mouthwash and mouth bath concentrate and gargle, according to the invention is defined in attached claim 12 and comprises the steps of:

a) providing an aqueous suspension including particles as herein described; and

b) mixing said aqueous suspension with other ingredients of the dental care product.

As already noted above, this process advantageously allows to readily incorporate the particles in the dental care product in a quite simple and convenient manner exploiting the useful properties, in particular stability and pH characteristics, of the suspension of particles produced in accordance with the invention.

Quite advantageously, the process for manufacturing a dental care product of the invention does not require any separation or drying of the particles, with a notable reduction of the manufacturing plant complexity, of the related investment and operating costs, of product losses during the manufacture and of production rejects.

In addition, the mixing step of the aqueous suspension of particles with other ingredients of the dental care product may be carried out with a better temperature control since the aqueous suspension reduces the friction and helps in removing the heat generated in the mixing apparatus.

An alternative second process for manufacturing a dental care product selected from the group consisting of: toothpaste, tooth powder, chewing gum, ointment for the gums, mouthwash and mouth bath concentrate and gargle, according to the invention is defined in attached claim 13 and comprises the steps of:

a') providing solid particles as herein described; and

b') mixing the solid particles with other ingredients of the dental care product.

This alternative process allows to manufacture a dental care product in all those instances in which the use of the above-described suspension of particles may not be desirable for logistic or other reasons.

According to a preferred embodiment of the invention, the aforementioned step a) comprises the steps of:

a_1) preparing an aqueous solution or suspension comprising a Ca compound, Ca carbonate;

b_1) adding a Sr compound;

c_1) forming an aqueous suspension of solid particles of a carbonate-substituted strontium-hydroxyapatite by adding PO_4^{3-} ions to the aqueous solution or suspension of step b_1), while simultaneously agitating the same over a time comprised between 30 minutes and 7 hours while maintaining said suspension at a temperature comprised between 10 and 90°C; and

d_1) adding lactoferrin to the aqueous suspension of step c_1);

e_1) agitating a suspension of particles obtained from step d_1) over a time comprised between 1 and 48 hours at a temperature comprised between 10°C and 60°C.

Most advantageously, these steps allow to prepare in a fairly quick and economical way a suspension of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface, which may be readily used as such as a composition for oral hygiene or used in admixture with other ingredients to yield dental care products for oral hygiene.

Most advantageously, furthermore, these steps allow to prepare a suspension of carbonate-substituted strontium-hydroxyapatite particles which is stable for an extended period of time even if no stabilizing agents are added thereto.

As indicated above, it has been observed that the suspension thus prepared is stable for at least 30 days and, more generally, for about two-three months, without using any stabilizing agent.

In this preferred embodiment, step c_1) is preferably carried out in order to achieve an aqueous suspension having a pH comprised between 6 and 13.

For the purposes of the invention, the aforementioned step a_1) of preparing an aqueous solution or suspension comprising a Ca compound may be carried out in any conventional manner, such as by dissolving or suspending the Ca compound in water.

According to a preferred embodiment of the invention, the Ca compound is a calcium salt selected from the group comprising: calcium hydroxide, calcium carbonate, calcium acetate, calcium oxalate, calcium nitrate, and mixtures thereof.

In this way, the cost of the process may advantageously be reduced since these Ca compounds are commodities readily available from the marked at a very low cost.

Additionally, these Ca compounds are easily workable and stockable to the advantage of the manufacturing operations.

According to a preferred embodiment of the invention, the aforementioned Sr compound is a strontium salt selected from the group comprising: strontium carbonate, strontium oxide, and strontium hydroxide, and mixtures thereof.

According to a preferred embodiment of the invention, the aqueous solution or suspension of step b_1) may further comprise a metal M compound.

According to a preferred embodiment of the invention, the aforementioned M compound is a metal salt selected from the group comprising: Mg compound, Se compound, K compound and mixtures thereof.

According to a preferred embodiment of the invention, the aforementioned Mg compound is a magnesium salt selected from the group comprising: magnesium carbonate, magnesium oxide, and magnesium hydroxide, magnesium acetate, and mixtures thereof.

According to a preferred embodiment of the invention, the aforementioned Se compound is a selenium salt selected from the group comprising: selenium dioxide, selenium trioxide, selenious acid, selenic acid, and mixtures thereof.

According to a preferred embodiment of the invention, the aforementioned K compound is a potassium salt selected from the group comprising: potassium carbonate, potassium oxide, potassium hydroxide, potassium nitrate, potassium hydrogen carbonate, potassium acetate, and mixtures thereof.

In a preferred embodiment, the carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface are formed in step c_1) by adding PO_4^{3-} agitating this solution or suspension in order to capture the carbon dioxide present in the atmosphere and achieve the desired carbonate substitution at the phosphate site (B) of the hydroxyapatite compound being formed.

In this way, the carbonate substitution may be advantageously carried out by simply agitating the solution or suspension for example by means of a mechanical stirrer.

In an alternative embodiment, the required agitation of the solution or suspension may be achieved by bubbling air, a CO_2 containing gas or a mixture thereof into the liquid phase or by combining a mechanical stirring with a gas bubbling.

According to a preferred embodiment of the invention, step c_1) is carried out by adding, preferably dropwise, an aqueous solution including PO_4^{3-} ions to the aqueous solution or suspension of step a_1) and b_1).

According to an alternative preferred embodiment of the invention, the aqueous solution including PO_4^{3-} ions added in step c_1) may further comprise HCO_3^- ions.

In this way, it may be possible to adjust to the proper extent the desired carbonate substitution at the phosphate site (B) of the hydroxyapatite compound being formed.

Within the framework of this preferred embodiment, the aforementioned aqueous solution including HCO_3^-, PO_4^{3-} ions may be prepared by bubbling air, CO_2 or a mixture thereof through water to obtain a solution of carbonic acid and then adding H_3PO_4.

According to another alternative preferred embodiment of the invention, step c_1) may be carried out by simultaneously adding a first solution containing CO_3^{2-} ions and a second solution containing PO_4^{3-} ions to the aqueous solution or suspension of step a_1) and b_1).

According to an alternative preferred embodiment of the invention, lactoferrin can be added during the step d_1) after the formation of hydroxyapatite nanocrystals. In this way is possible to obtained a hydroxyapatite nanocrystals superficial functionalization by milk protein.

In an alternative embodiment lactoferrin can be in the aqueous suspension comprising a Ca compound, Ca carbonate and Sr compound. In this way, it may be possible to obtain to the desired functionalization of the hydroxyapatite compound formed.

According to a preferred embodiment of the invention, the aforementioned step a) comprises the steps of:

a_2) preparing an aqueous solution or suspension comprising a Ca compound, Ca carbonate;

b_2) adding a Sr compound and lactoferrin to the aqueous solution or suspension of step a_2);

c_2) forming an aqueous suspension of solid particles of a carbonate-substituted strontium-hydroxyapatite by adding PO_4^{3-} ions to the aqueous solution or suspension of step b_2), while simultaneously agitating the same over a time comprised between 30 minutes and 7 hours while maintaining said suspension at a temperature comprised between 10 and 90°C; and

d_2) agitating a suspension of particles obtained from step c_2) over a time comprised between 1 and 48 hours at a temperature comprised between 10°C and 60°C.

According to a preferred embodiment of the invention, the aqueous solution or suspension of step b_2) may further comprise a metal M compound.

Suitable amounts of the ingredients may be easily selected by those skilled in the art so as to obtain the desired chemical constitution of carbonate-substituted strontium-hydroxyapatite solid particles having a lactoferrin-functionalized surface.

According to a preferred embodiment of the invention, the aforementioned step a') comprises the steps of:

a_3) preparing an aqueous suspension of carbonate-substituted strontium-hydroxyapatite solid particles having a lactoferrin-functionalized surface by means of the aforementioned step a);

b_3) separating the solid particles from the suspension obtained from step a_3);

c_3) drying the wet solid particles thus obtained.

In a preferred embodiment, the separation step b_3) is carried out by decantation, centrifugation or filtration using apparatuses and techniques well known to those skilled in the art.

In a preferred embodiment, the drying step c_3) is carried out by freezing the wet solid

particles at a temperature lower than 0°C until reaching a constant weight.

Within the framework of this preferred embodiment, the drying step c_3) is preferably carried out by freeze-drying the wet solid particles at a temperature comprised between -20° and -50°C, most preferably at about -40°C.

In a preferred embodiment, the process may also comprise the additional step d_3) of washing the separated solid particles with water or a basic solution prior to effecting the drying step c_3).

Advantageously, this additional washing step d_3) serves the useful function of removing any acid residues possibly absorbed or trapped by the particles.

In a preferred embodiment of the dental care product manufacturing processes described above, the mixing step b) and b') is carried out in a mixing apparatus maintained under a predetermined vacuum degree, easily selectable by those skilled in the art in order to obtain a uniform mixture of ingredients, reached by using conventional vacuum pumps.

In a preferred embodiment of the first manufacturing process, the mixing step b) is carried out by

b^1) mixing the aqueous suspension of step a) with other ingredients of the toothpaste except for any surfactant;

b^2) incorporating at least one surfactant into the mixture thus obtained.

In this way, the formation of foam during the mixing operation may be minimized.

Within the framework of this embodiment, the incorporation step b^2) is preferably carried out under vacuum using a conventional equipment in order to minimize the undesired formation of foam.

According to another aspect thereof, the present invention relates to a method of providing at a teeth outer surface a source of Sr^{++} ions and lactoferrin which may be locally released at an acidic pH by means of hydroxyapatite-type carrier particles, the method comprising contacting the teeth with a dental care product as described herein so as to form on the teeth outer surface a film including said carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface.

Most advantageously and thanks to the characteristics of the carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface, described above, such a method allows to effectively remineralise the teeth, improving the enamel crystallinity and preventing caries even in the limited time available during the normal routine of dental hygiene.

The contacting step may be carried out in a number of ways depending upon the dental care product. For example, if the dental care product is a toothpaste, the contacting step may be simply carried out by washing the teeth, while if the dental care product is a mouthwash, the contacting step is carried out by maintaining the mouthwash in the oral cavity for a suitable time, for example few minutes.

According to the invention and as will be shown in greater detail below, the carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface of the invention have a triple effect on tooth surface.

Firstly, the dental care products of the invention are advantageously capable of restoring the enamel surface because the particles of the invention bind to the enamel surfaces, mimicking the natural bone hidroxyapatite, at any erosion area due for example, to the acid foods and drinks as will be shown in greater detail in the Examples below.

Secondly, the dental care products of the invention comprising strontium ions into the structure of hydroxyapatite functionalized by protein, advantageously form a thin biomimetic coating on the outer surface of the enamel even during the usual tooth brushing. The hydroxyapatite functionalized by lactoferrin forming the thin coating present on the outer surface of the enamel is capable of effectively exploiting an antibacterial effect and therefore, this protein protects the tooth surface from attack by bacteria and plaque.

According to the invention and as will be shown in greater detail below, the Applicant has surprisingly and unexpectedly found that said strontium hydroxyapatite functionalized by lactoferrin shows an increased antibacterial effect with respect to the lactoferrin alone.

After the formation of this biomimetic coating, the plaque, the acid food and drink partially dissolve this coating, releasing strontium ions that only in this moment are free in the mouth and are present in the physiologic percentage in which these ions are present in the tooth hydroxyapatite.

Said free ions, showing a cariostatic effect, are capable of binding with the tooth hydroxyapatite forming in situ a strontium hydroxyapatite.

According to the invention and as will be shown in greater detail below, said strontium hydroxyapatite functionalized by lactoferrin presents a greater degree of crystallinity with respect to a zinc carbonate-substituted hydroxyapatite without strontium and lactoferrin. In this way, this hydroxyapatite, having higher degree of crystallinity, is more resistant to the plaque formation and to the action of food and acid drinks.

Thirdly, the dissolution of the hydroxyapatite biomimetic coating in acid conditions, determine the release of lactoferrin protein capable of effectively exploiting also an antibacterial effect and, accordingly, of effectively treating teeth and gums disorders and in general increasing oral hygiene even in the limited time available during the normal routine of dental hygiene.

The Applicant has also observed that lactoferrin is capable of exploiting its antibacterial effect for a long time after the normal routine of dental hygiene.

Brief description of the drawings

Additional features and advantages of the present invention will be more readily apparent by the following Examples of some preferred embodiments of the various aspects of the present invention given hereinbelow by way of illustration and not of limitation, which aspects will be better understood with reference to the attached drawings.

In these drawings:

- Fig. 1 shows an X-Ray diffraction pattern of comparative zinc carbonate-substituted hydroxyapatite particles (Zn-CHA), without strontium substitution and lactoferrin functionalization, which displays a cristallinity degree CD of 60%;

- Fig. 2 shows an X-Ray diffraction pattern of one example of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface (LF-SrCHA) according to the invention (Example 2), with molar % of strontium substitution comprised between 0.0010 and 0.015, which displays a cristallinity degree CD of 67%;

- Fig. 3 shows an X-Ray diffraction pattern of one example of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface (LF-SrCHA) according to the invention (Example 3), with the aforementioned molar % of strontium, which displays a cristallinity degree CD of 66% ;

- Fig. 4 shows a FTIR spectrum of one example of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface, according to the invention, which displays an apatitic phase;

- Figs. 5a and 5b show TEM and SEM images of some examples of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface according to the invention which display the micrometric size and a flat and round shape of the single nanoparticles;

- Figs. 6a and 6b show respectively SEM image (6a) and EDX spectrum (6b) of enamel brushed with a toothpaste containing LF-SrCHA according to the invention (in vitro test).

- Figs. 7a and 7b show respectively SEM image and EDX spectrum of enamel brushed with a common toothpaste.

In the following Examples, percentages and parts are by weight unless otherwise indicated.

EXAMPLE 1

(Comparative)

(Preparation of an aqueous suspension of Zn-CHA)

An aqueous suspension of comparative zinc carbonate-substituted hydroxyapatite particles (Zn-CHA) was prepared according to the following method.

In a first step, 550 g of water was mixed with 55 g $CaCO_3$, , 22g of $ZnCO_3$ and 175 g $Ca(OH)_2$.

During this step, the pH was maintained between 3 and 8, more preferably between 3 and 5.

The resulting aqueous suspension was maintained under agitation between 0.5 and 9 hours, more preferably between 1.5 and 4.5 hours.

During this step, the resulting suspension was brought between a temperature of 23° and 45°C, more preferably between 27° and 38°C.

Successively, 232 mL of acid solution (constituted by a mixture of 90 mL of H_3PO_4 at 75% and 160 mL of distilled water) was added at the aqueous suspension previously prepared. The time of addition was included between 30 minutes and 6 hours, more preferably between 1 and 4 hours.

After this step, the suspension was maintained under agitation between 1 and 12 hours, more preferably between 3 and 7 hours.

The final pH of suspension is maintained between 6 and 13, more preferably between 7 and 12.

The X-Ray diffraction pattern of comparative Zn-CHA is showed in **Figure 1**.

EXAMPLE 2

(Preparation of an aqueous suspension of Sr-CHA functionalized by lactoferrin)

An aqueous suspension of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface according to the invention was prepared according to the following method.

In a first step, 550 g of water was mixed with 77 g $CaCO_3$, 10 g $SrCO_3$ and 175 g $Ca(OH)_2$.

During this step, the pH was maintained between 3 and 8, more preferably between 3 and 5.

The resulting aqueous suspension was maintained under agitation between 0.5 and 9 hours, more preferably between 1.5 and 4.5 hours.

During this step, the resulting suspension was brought between a temperature of 23° and 45°C, more preferably between 27° and 38°C.

Successively, 232 mL of acid solution (constituted by a mixture of 90 mL of H_3PO_4 at 75% and 160 mL of distilled water) was added to the aqueous suspension previously prepared. The time of addition was included between 30 minutes and 6 hours, more preferably between 1 and 4 hours.

At this point, 2.5 g of an inorganic suspension of lactoferrin was added to the aqueous suspension.

After this step, the suspension was maintained under agitation between 1 and 11 hours, more preferably between 2 and 7 hours.

The final pH of suspension is maintained between 6 and 13, more preferably between 7 and 12.

The X-Ray diffraction pattern of Sr-CHA functionalized by lactoferrin according to the invention is showed in **Figure 2**.

EXAMPLE 3

(Preparation of an aqueous suspension of Sr-CHA functionalized by lactoferrin)

An aqueous suspension of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface according to the invention was prepared according to the following method.

In a first step, 540 g of water was mixed with 70 g $CaCO_3$, 5 g $SrCO_3$ and 168 g $Ca(OH)_2$.

During this step, the pH was maintained between 3 and 8, more preferably between 3 and 5.

At this point, 3 g of an inorganic suspension of lactoferrin was added to the aqueous suspension.

The resulting aqueous suspension was maintained under agitation between 0.5 and 8 hours, more preferably between 1 and 4 hours.

During this step, the resulting suspension was brought between a temperature of 23°

and 40°C, more preferably between 27° and 35°C.

Successively, 225 mL of acid solution (constituted by a mixture of 85 mL of H_3PO_4 at 75% and 140 mL of distilled water) was added to the aqueous suspension previously prepared. The time of addition was included between 30 minutes and 6 hours, more preferably between 1 and 4 hours.

After this step, the suspension was maintained under agitation between 1 and 12 hours, more preferably between 3 and 9 hours.

The final pH of suspension is maintained between 6 and 13, more preferably between 7 and 12.

The X-Ray diffraction pattern of Sr-CHA functionalized by lactoferrin according to the invention is showed in **Figure 3**.

With reference to figures 2 and 3, the presence of both strontium substitution and lactoferrin functionalization into substituted hydroxyapatite advantageously increases the degree of crystallinity (+7 and +6 %) with respect to the comparative Zn-CHA which displays a cristallinity degree CD of 60%.

Therefore, this hydroxyapatite, having higher degree of crystallinity, is more resistant to the plaque formation and to the action of food and acid drinks.

EXAMPLE 4

(Toothpaste)

A toothpaste including carbonate-substituted hydroxyapatite particles having a lactoferrin-functionalized surface according to the invention was prepared according to the following method and from the following ingredients.

In a first step, an aqueous suspension including carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface (LF-SrCHA) (total solid content: 30% by weight) was prepared in the same manner and using the same ingredients and quantities described in Example 2.

The aqueous suspension thus obtained, was then mixed with the other ingredients of the toothpaste as shown in the table below except for the surfactant.

The mixing was carried out in a conventional mixing apparatus maintained under a suitable vacuum degree selected among the usual values known to those skilled in the art.

Once a homogeneous mixture was obtained, the surfactant was incorporated in the mixing apparatus while maintaining a predetermined vacuum degree selected among the usual values known to those skilled in the art.

In this way, a toothpaste was obtained having the composition reported in the following Table 1.

TABLE 1

Ingredient	Amount [%]
Sodium carboxymethylcellulose	1.0
LF-SrCHA particles	15.0
Sorbitol syrup	15.0
Glycerine	15.0
Sodium saccharine	0.25
Hydroglycolic moss extract titrated in 2% usnic acid	0.5
Thickening silica	1.0
Abrasive silica	18.0
Tetrapotassium pyrophosphate	3.0
Titanium dioxide	0.9
Sodium lauryl sulfate	0.5
Mint flavor	1.3
Citric acid	0.25
Water	balance

EXAMPLE 5

(Evaluation of the coating formation on the surface of the enamel *in vitro*)

Slabs of enamel (3x3mm) were obtained from interproximal surfaces of premolars extracted for orthodontic reasons. After the extraction, the teeth were cut with diamond disks and the slabs obtained were sonicated for 10 min in 50% by weight of ethanol in order to removed any debris. The slabs were etched with 37% by weight of

orthophosphoric acid for 1 min, then repeatedly washed with distilled water using an electric toothbrush and air dried. The test was then carried out by treating various slabs of enamel with a common toothpaste and an toothpaste containing 10% wt of the active agent LF-SrCHA according to the present invention.

There have been five tests in parallel for greater repetition of the experiment and better statistical data.

The protocol used was as follows:

Each enamel slab was brushed three times a day for a period of 21 days. The intervals between brushing sessions were more than 5 hours. Any washing process has been performed for 30 sec using an electric toothbrush submitted at constant pressure and using a bean sized aliquot of toothpaste wetted with tap water, closely resembling the in vivo usual tooth-brushing procedure. After every treatment, the single enamel slab was washed with tap water using a cleaned tooth-brush in order to remove residual tooth-paste. Toothbrushes were repeatedly washed with tap water after every utilization.

After the brushing treatment 21 days long each enamel slab have been characterized by X-Ray diffraction technique (DRX) , Scanning Electro microscopy (SEM) with EDX probe and Infrared Fourier Transformed Spectroscopy (FTIR).

The analysis made by SEM-EDX probe, shows that the enamel surfaces treated with common toothpaste have a Ca / P molar ratio of 1.9 (Fig. 7b). The Ca / P molar ratio of 1.9 is the characteristic value of natural enamel.

EDX analysis performed on the enamel brushed with toothpaste containing LF-SrCHA, according to the invention, shows that the surface of the enamel has a ratio of Ca / P of 1.5-1.7 which is the same as that of the Sr-CHA microcrystals functionalized by lactoferrin contained in the toothpaste (Fig. 6b).

Figures 6a and 7a show the presence of a crack on the enamel surface normally due to acid foods and drinks.

In the enamel treated with toothpaste containing LF-SrCHA, according to the invention, of Figure 6a, it was observed that the crack is filled with microclusters of LF-SrCHA.

Instead, the enamel treated with a common toothpaste shows an empty crack on the enamel surface (Fig. 7a).

Advantageously, the dental care products of the invention are capable of restoring the enamel surface because the particles of the invention bind to the enamel surfaces, mimicking the natural bone hydroxyapatite, at any erosion area.

EXAMPLE 6

(Mouthwash)

A mouthwash including LF-SrCHA particles according to the invention was prepared by mixing a suspension produced in accordance with the preceding Example 2 in a conventional way with conventional ingredients.

A mouthwash was obtained having the composition reported in the following Table 2.

TABLE 2

Ingredient	Amount [%]
LF-SrCHA particles	5
Sorbitol syrup	3
Glycerine	3
Sodium saccharine	0.25
Hydroglycolic moss extract titrated in 2% usnic acid	0.5
Tetrapotassium pyrophosphate	1
Sodium lauryl sulfate	0.2
Mint flavor	0.5
Citric acid	0.1
Water	balance

EXAMPLE 7

(Chewing gum for tooth cleaning)

A chewing gum including LF-SrCHA particles according to the invention was prepared by mixing a suspension produced in accordance with the preceding Example 2 in a conventional way with conventional ingredients.

A chewing gum was obtained having the composition reported in the following Table 3.

TABLE 3

Ingredient	Amount [%]
Chewing gum base	91.65
LF-SrCHA particles	4
Glycerine	3
Sodium saccharine	0.025
Hydroglycolic moss extract titrated in 2% usnic acid	0.1
Mint flavor	1

EXAMPLE 8

(Evaluation of the antimicrobial activity against oral pathogens *in vitro*)

In order to demonstrate the antimicrobial activity of carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface (LF-SrCHA particles) according to the present invention the following tests were carried out:

Test products

- lactoferrin (LF) 60 mg/ml (comparative);
- LF-SrCHA 60 mg/ml (invention);

Microorganisms

- Streptococcus Mutans ATCC 35668

Streptococcus Mutans is considered one of the most important cariogenic species of the human oral microbial flora.

Antimicrobial activity was determined by the agar diffusion method and the zones of growth inhibition were measured.

1.8 ml of the test product were inoculated with 200 µl of *Streptococcus Mutans* bacterial suspension of known concentration ($2x10^5$ CFU, colony-forming units/ml).

Streptococcus Mutans ATCC 35668 was also inoculated in equal volume of sterile water as a control.

After the contact time of 1 hour, an appropriately diluted aliquot was taken from each treated sample and then sown on the steril agar plates to determine the number of live microorganisms expressed as CFU/ml.

The inoculated plates were incubated at 37 °C for 24-48 h and then bacterial colonies were counted.

TABLE 4

TEST PRODUCTS	**Initial concentration (CFU/ml)**	**Final concentration (CFU/ml)**	**Microorganisms reduction (%)**
LF (60 mg/ml) (comparative)	$2x10^5$	$13x10^4$	35%
LF-SrCHA (60 mg/ml) (invention)	$2x10^5$	$3x10^4$	85%

Table 4 shows that lactoferrin functionalization into substituted hydroxyapatite advantageously increases the antibacterial activity (85% of microorganisms reduction) with respect to the comparative lactoferrin which displays a microorganisms reduction of 35%.

These results therefore show that LF-SrCHA particles according to the present invention having an increased antibacterial activity (over twice), with respect to the lactoferrin alone, advantageously prevent generation of carious tooth and periodontal diseases.

CLAIMS

1. A dental care product comprising carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface, wherein said particles have the formula:

$$Ca_{(10-x)}\,Sr_x\,(PO_4)_{(6-y)}\,(CO_3)_y\,(OH)_2$$

wherein x is a number comprised between 0.0010 and 0.015; and y is a number comprised between 0.0010 and 0.010; and

wherein said carbonate-substituted strontium-hydroxyapatite particles have a crystallinity degree CD comprised between 55 and 85%.

2. A product according to claim 1, wherein the total amount of lactoferrin in the carbonate-substituted strontium-hydroxyapatite particles is comprised between 0.01% and 5.0% by weight based on the total weight of the particles.

3. A product according to claim 1 or 2, further comprising an effective amount of a metal M ion.

4. A product according to claim 3, comprising from 0.1% to 20% by weight with respect to the total Ca content of a metal M ion substituted into the hydroxyapatite structure.

5. A product according to claim 4, wherein said metal M is selected from the group comprising Mg, Se, K and mixtures thereof.

6. A product according to any one of claims 1-5, in the form of suspension, oil, gel or solid.

7. A product according to claim 6, in the form of a suspension including from 1% to 40% by weight of said particles.

8. A product according to claim 7, having a pH comprised between 6 and 13.

9. A product according to claim 6, selected from the group consisting of: toothpaste, tooth powder, chewing gum for oral and dental hygiene, ointment for the gums, mouthwash and mouth bath concentrate and gargle.

10. A dental care composition comprising carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface according to any

one of claims 1-5.

11. Carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface according to any one of claims 1-5.

12. A process for manufacturing a dental care product selected from the group consisting of: toothpaste, tooth powder, chewing gum, ointment for the gums, mouthwash and mouth bath concentrate and gargle, comprising the steps of:

a) providing an aqueous suspension including particles according to any one of claims 1-5; and

b) mixing said aqueous suspension with other ingredients of the dental care product.

13. A process for manufacturing a dental care product selected from the group consisting of: toothpaste, tooth powder, chewing gum, ointment for the gums, mouthwash and mouth bath concentrate and gargle, comprising the steps of:

a') providing solid particles according to any one of claims 1-5; and

b') mixing the solid particles with other ingredients of the dental care product.

14. A process according to claim 12 , wherein said step a) comprises the steps of:

a_1) preparing an aqueous solution or suspension comprising a Ca compound, Ca carbonate;

b_1) adding a Sr compound;

c_1) forming an aqueous suspension of solid particles of a carbonate-substituted strontium-hydroxyapatite by adding PO_4^{3-} ions to the aqueous solution or suspension of step b_1), while simultaneously agitating the same over a time comprised between 30 minutes and 7 hours while maintaining said suspension at a temperature comprised between 10 and 90°C; and

d_1) adding lactoferrin to the aqueous suspension of step c_1);

e_1) agitating a suspension of particles obtained from step d_1) over a time comprised between 1 and 48 hours at a temperature comprised between 10°C and 60°C.

15. A process according to claim 14, wherein the aqueous suspension obtained from step c_1) has a pH comprised between 6 and 13.

16. A process according to claim 14, wherein said Ca compound is a calcium salt selected from the group comprising: calcium hydroxide, calcium carbonate, calcium acetate, calcium oxalate, calcium nitrate, and mixtures thereof.

17. A process according to claim 14, wherein said Sr compound is a strontium salt selected from the group comprising: strontium carbonate, strontium oxide, and strontium hydroxide, and mixtures thereof.

18. A process according to claim 14, wherein the aqueous solution or suspension of step b_1) further comprises a metal M compound selected from the group comprising Mg compound, Se compound, K compound and mixtures thereof.

19. A process according to claim 14, wherein step c_1) is carried out while bubbling air, a CO_2-containing gas or a mixture thereof through the aqueous solution or suspension of step a_1) and b_1).

20. A process according to claim 14, wherein step c_1) is carried out by adding an aqueous solution including PO_4^{3-} ions to the aqueous solution or suspension of step a_1) and b_1).

21. A process according to claim 14, wherein said aqueous solution including PO_4^{3-} ions further comprises HCO_3^- ions.

22. A process according to claim 21, wherein said aqueous solution including HCO_3^- and PO_4^{3-} ions is prepared by bubbling air, CO_2 or a mixture thereof through water to obtain a solution of carbonic acid and then adding H_3PO_4 thereto.

23. A process according to claim 20, wherein step c_1) is carried out by simultaneously adding a first solution including CO_3^{2-} ions and a second solution containing PO_4^{3-} ions to the aqueous solution or suspension of a_1) and b_1).

24. A process according to claim 12 , wherein said step a) comprises the steps of:

a_2) preparing an aqueous solution or suspension comprising a Ca compound, Ca carbonate;

b_2) adding a Sr compound and lactoferrin to the aqueous solution or suspension of step a_2);

c_2) forming an aqueous suspension of solid particles of a carbonate-substituted strontium-hydroxyapatite by adding PO_4^{3-} ions to the aqueous solution or suspension of step b_2), while simultaneously agitating the same over a time comprised between 30 minutes and 7 hours while maintaining said suspension at a temperature comprised between 10 and 90°C; and

d_2) agitating a suspension of particles obtained from step c_2) over a time comprised between 1 and 48 hours at a temperature comprised between 10°C and 60°C.

25. A process according to claim 24, wherein the aqueous solution or suspension of step b_2) further comprises a metal M compound selected from the group comprising Mg compound, Se compound, K compound and mixtures thereof.

26. A process according to claim 13 , wherein said step a') comprises the steps of:

a_3) preparing an aqueous suspension of carbonate-substituted strontium-hydroxyapatite solid particles having a lactoferrin-functionalized surface by a process according to any one of claims 14-25;

b_3) separating the solid particles from the suspension obtained from step a_3);

c_3) drying the wet solid particles thus obtained.

27. A process according to claim 26, wherein said separation step b_3) is carried out by decantation, centrifugation or filtration.

28. A process according to claim 26, wherein said drying step c_3) is carried out by freeze-drying the wet solid particles at a temperature lower than 0°C until reaching a constant weight.

29. A process according to claim 26, further comprising the step of

d_3) washing the separated solid particles with water or a basic solution prior to effecting said drying step c_3).

30. A process according to claim 12 or 13, wherein mixing step b) and b') is carried out

in a mixing apparatus maintained under a predetermined vacuum degree.

31. A process according to claim 12, wherein mixing step b) is carried out by

b^1) mixing the aqueous suspension of step a) with other ingredients of the dental care product except for any surfactant;

b^2) incorporating at least one surfactant into the mixture thus obtained.

32. A method of providing at a teeth outer surface a source of Sr^{++} ions and lactoferrin which may be locally released at an acidic pH by means of hydroxyapatite-type carrier particles, the method comprising contacting the teeth with a dental care product according to any one of claims 1-9 so as to form on the teeth outer surface a film including said carbonate-substituted strontium-hydroxyapatite particles having a lactoferrin-functionalized surface.

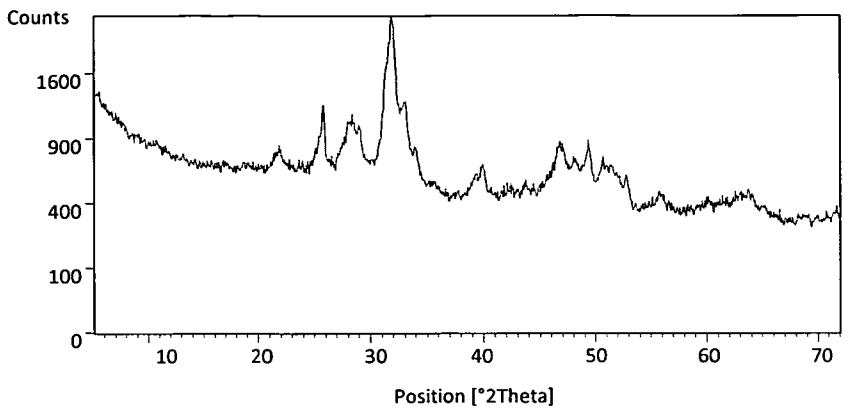
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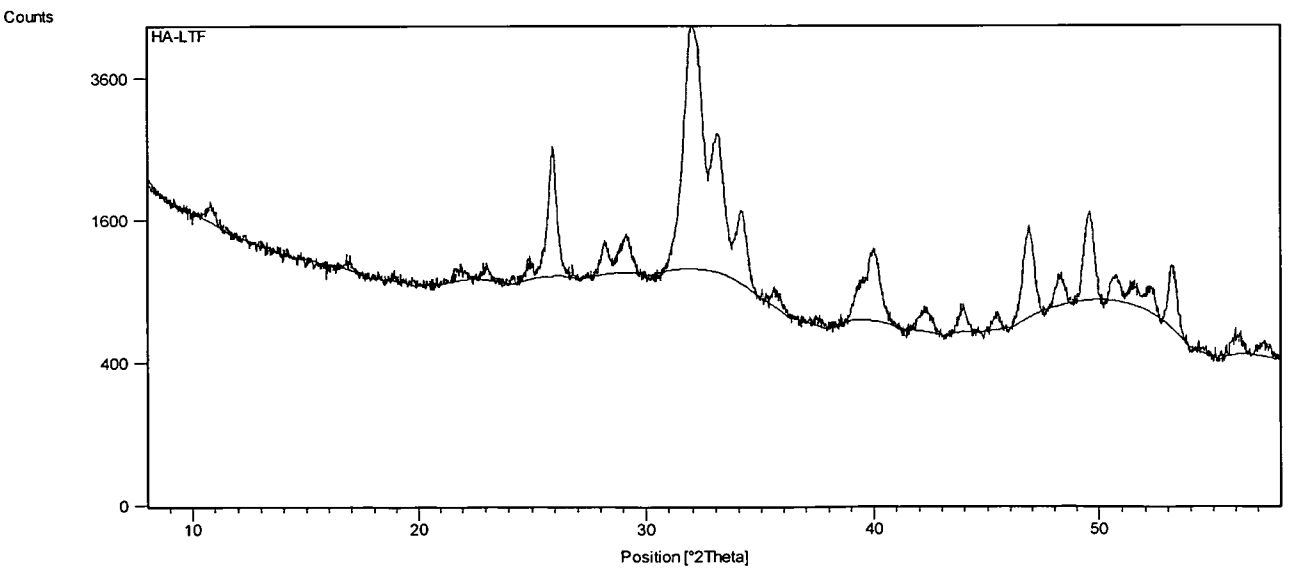
FIG. 1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Position [°2Theta]

FIG. 2

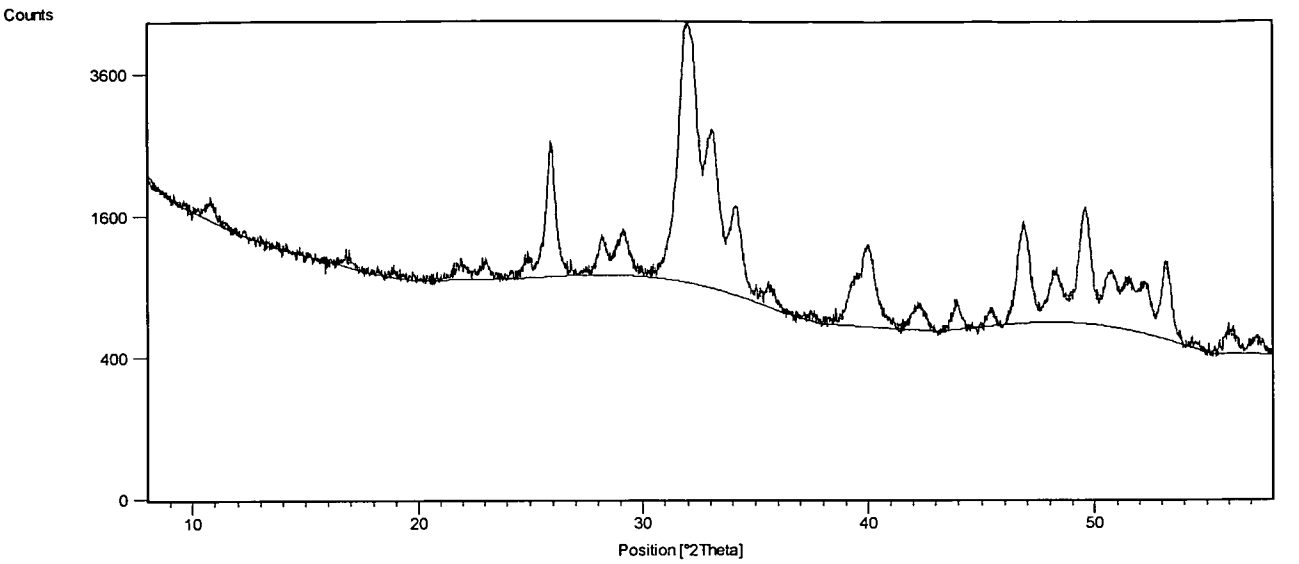
Counts
3600
1600
400
0
10
20
30
40
50
Position [°2Theta]

FIG. 3

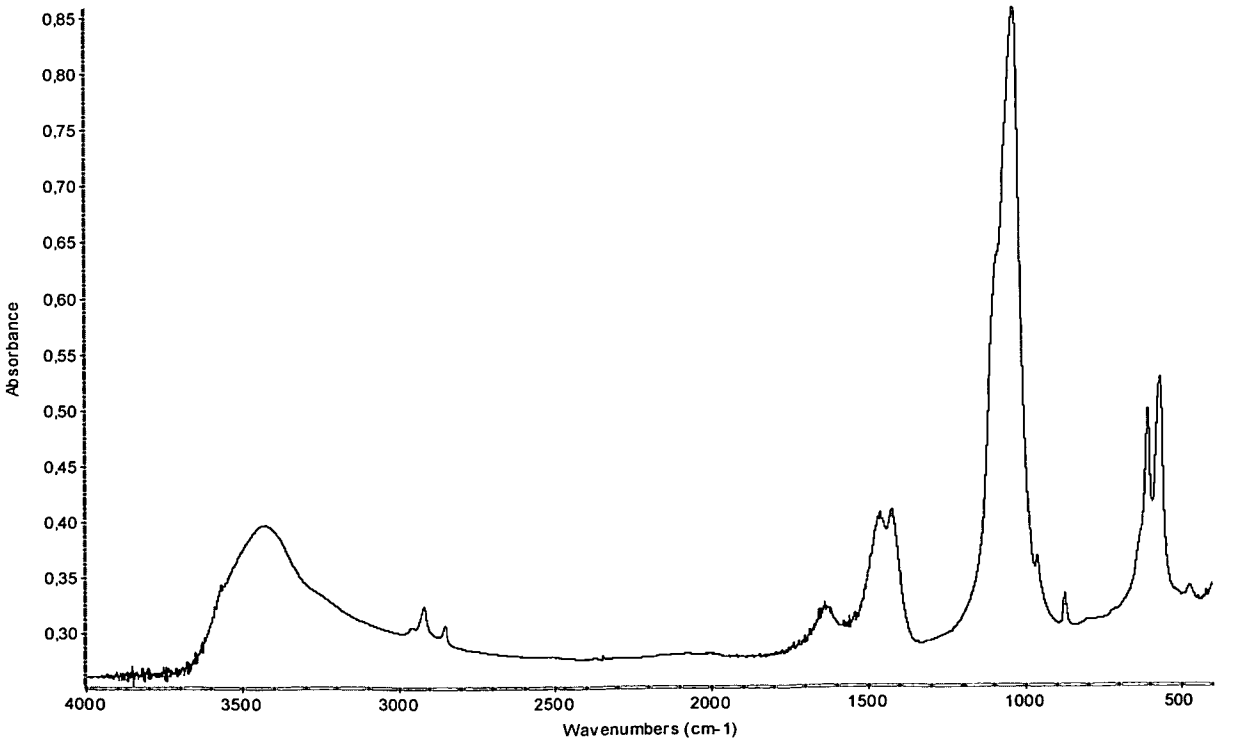
Absorbance
0,85
0,80
0,75
0,70
0,65
0,60
0,55
0,50
0,45
0,40
0,35
0,30
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

FIG. 4


100 nm

FIG. 5a

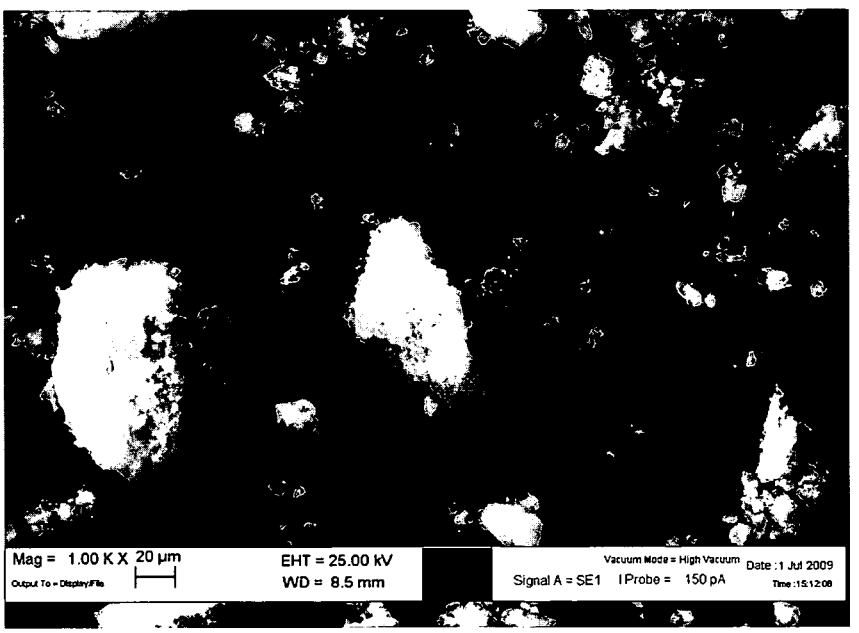
Mag = 1.00 K X 20 µm
Output To = Display/File
EHT = 25.00 kV
WD = 8.5 mm
Vacuum Mode = High Vacuum
Signal A = SE1
I Probe = 150 pA
Date :1 Jul 2009
Time :15:12:08

FIG. 5b

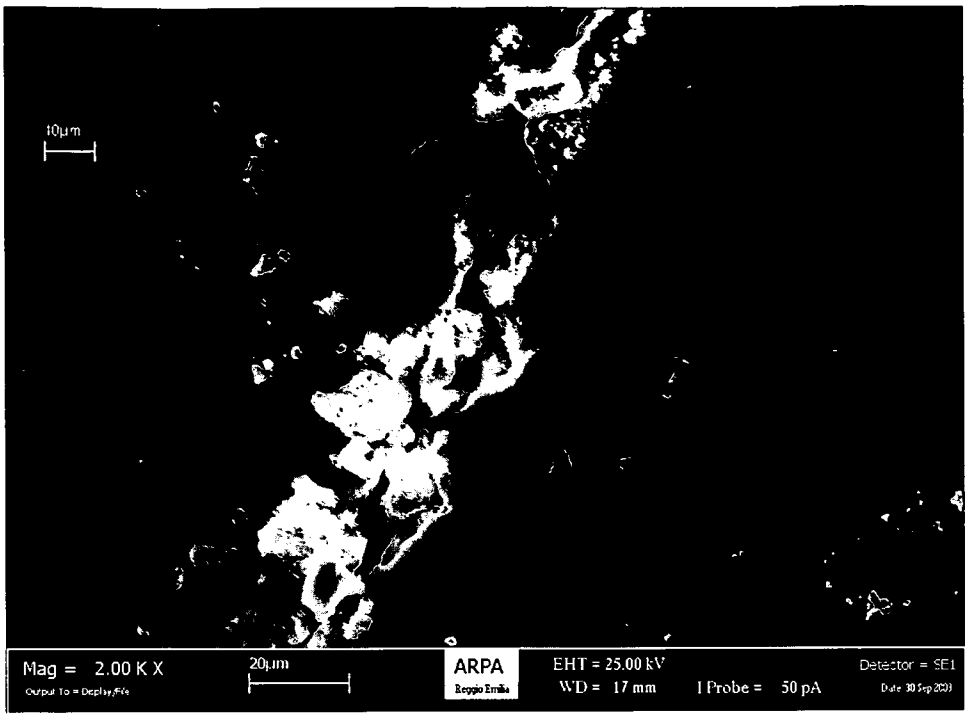
10µm
Mag = 2.00 K X
20µm
ARPA
Reggio Emilia
EHT = 25.00 kV
WD = 17 mm
I Probe = 50 pA
Detector = SE1

FIG. 6a

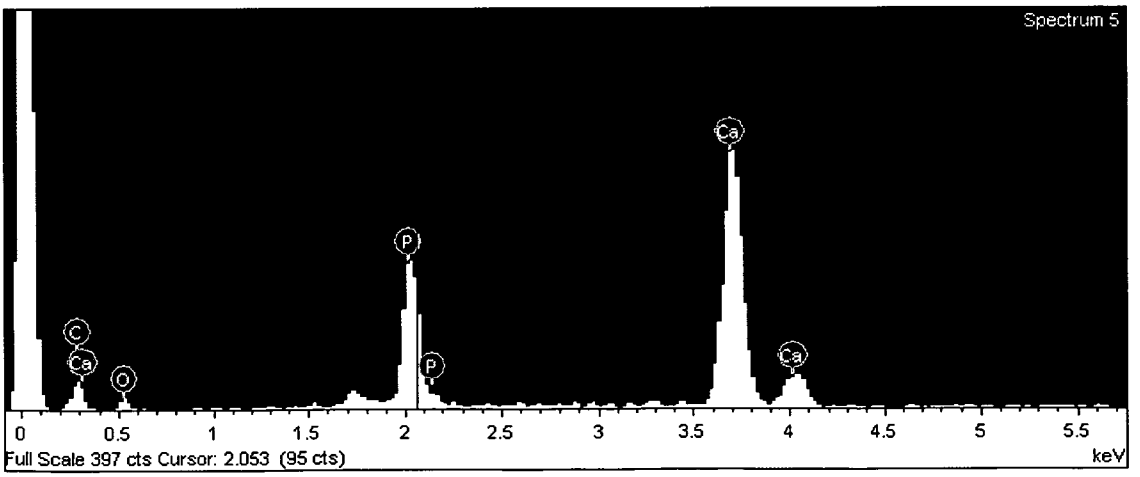
Spectrum 5
C
Ca
O
P
P
Ca
Ca
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
Full Scale 397 cts Cursor: 2.053 (95 cts)
keV

FIG. 6b

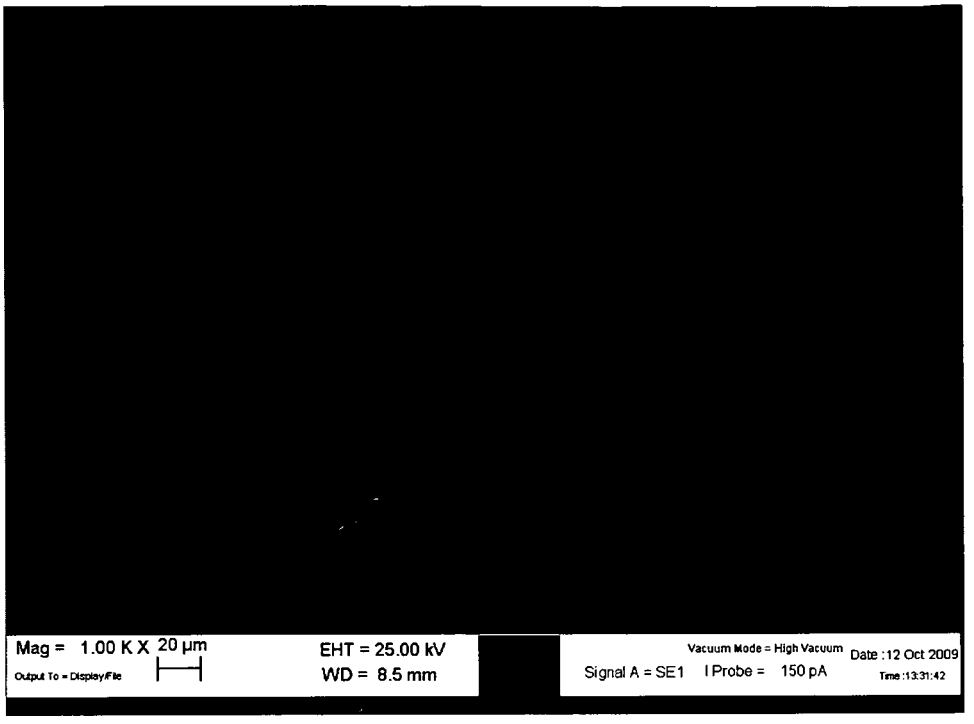
Mag = 1.00 K X 20 µm
Output To = Display/File
EHT = 25.00 kV
WD = 8.5 mm
Vacuum Mode = High Vacuum
Signal A = SE1
I Probe = 150 pA
Date :12 Oct 2009
Time :13:31:42

FIG. 7a

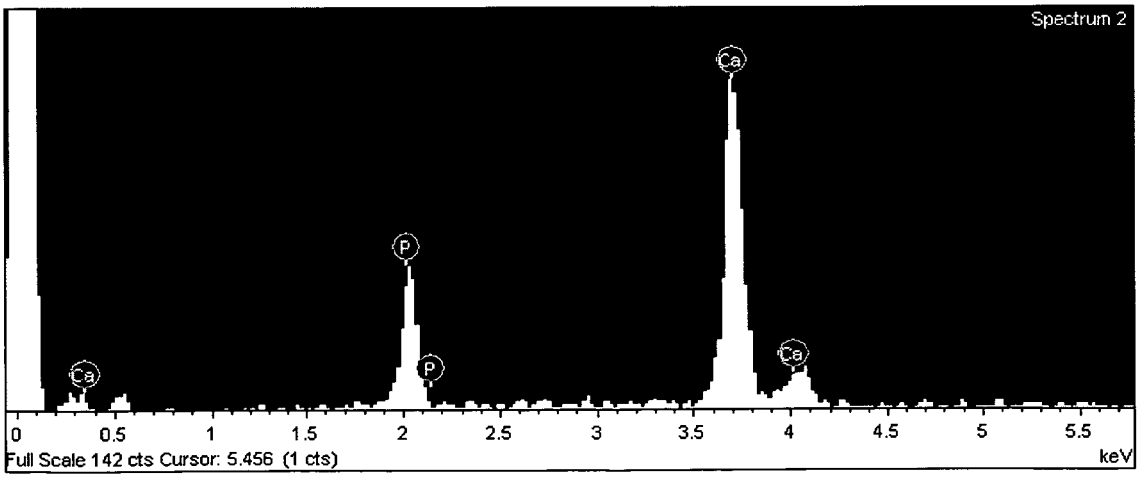
Spectrum 2
Ca
P
P
Ca
Ca
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
Full Scale 142 cts Cursor: 5.456 (1 cts)
keV

FIG. 7b

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/005601

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K33/42 A61Q11/00 C01B25/32
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 C01B

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, CHEM ABS Data, 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	MICHELE IAFISCO ET AL: "Adsorption and spectroscopic characterization of lactoferrin on hydroxyapatite nanocrystals", DALTON TRANSACTIONS, vol. 40, no. 4, 1 January 2011 (2011-01-01), page 820, XP55035146, ISSN: 1477-9226, DOI: 10.1039/c0dt00714e cited in the application abstract pages 820-821, paragraph "Introduction" ----- -/--	1-32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 August 2012	22/08/2012

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LANDI E ET AL: "Development of Sr and CO3 co-substituted hydroxyapatites for biomedical applications", ACTA BIOMATERIALIA, ELSEVIER, AMSTERDAM, NL, vol. 4, no. 3, 1 May 2008 (2008-05-01), pages 656-663, XP022588845, ISSN: 1742-7061, DOI: 10.1016/J.ACTBIO.2007.10.010 [retrieved on 2007-11-01] abstract pages 656-657, paragraph "1. Introduction" pages 658-659, paragraph "3.1. SrCHA powder"	1-32
A	Q LUO: "Cooperative Adsorption of Proteins onto Hydroxyapatite", JOURNAL OF COLLOID AND INTERFACE SCIENCE, vol. 200, no. 1, 1 April 1998 (1998-04-01), pages 104-113, XP55035162, ISSN: 0021-9797, DOI: 10.1006/jcis.1997.5364 the whole document	1-32