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(54) **Karbonát-szubsztituált hidroxipatit biológiaiilag aktív nanorészecskéi, eljárás előállításukra és azokat tartalmazó kompozíciók**

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(54) **BIOLOGICALLY ACTIVE NANOPARTICLES OF A CARBONATE-SUBSTITUTED
HYDROXYAPATITE, PROCESS FOR THEIR PREPARATION AND COMPOSITIONS
INCORPORATING THE SAME**

BIOLOGISCH AKTIVE NANOPARTIKEL AUS CARBONATSUBSTITUIERTEM HYDROXYAPATIT,
HERSTELLUNGSVERFAHREN DAFÜR UND ZUSAMMENSETZUNGEN DAMIT

NANOPARTICULES BIOLOGIQUEMENT ACTIVES D'UNE HYDROXYAPATITE SUBSTITUÉE PAR
DES IONS CARBONATE, PROCÉDÉ POUR LA PRÉPARATION DE CELLES-CI ET COMPOSITIONS
INCORPORANT CELLES-CI

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- **MAYER I ET AL: "Dissolution studies of
Zn-containing carbonated hydroxyapatites"**
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EP 2 029 480 B1

DescriptionField of the invention

[0001] The invention relates to biologically active nanoparticles of a carbonate-substituted hydroxyapatite, to a process for their preparation and to compositions incorporating the same.

[0002] More specifically, the invention relates to biologically active nanoparticles which find a preferred although not exclusive use in oral or dental hygiene applications, as well as to compositions for oral or dental hygiene which include the same such as, for example, solutions, suspensions, oils, gels or other solid products.

[0003] According to other aspects, the invention relates to a process for preparing a suspension for oral or dental hygiene, to a process for manufacturing a toothpaste comprising the aforementioned biologically active nanoparticles, as well as to a method of locally remineralizing the teeth comprising contacting the teeth with the nanoparticles.

Background of the invention

[0004] It is known that a high proportion of the population, estimated by the dental profession to be in the region of 20 to 25% of the total population, suffers from hypersensitivity or hyperaesthesia of the teeth, in other words pain in response to mechanical, chemical and thermal stimuli, without any visible dental diseases such as caries which would require dental treatment.

[0005] The hypersensitivity of the teeth occurs mainly at the neck of the teeth when the soft dentine becomes exposed between the boundary of the enamel and the gum line due to recession of the gums. The action of lactic acid forming lactobacilli or the mechanical action of braces (even in children) or the engaging parts of dentures during chewing may also damage the protective enamel so that the dentinal canaliculi which are then exposed transmit every mechanical, chemical or thermal irritation as a painful stimulus to the tooth pulp.

Related art

[0006] It is known that repeated treatment with inorganic salts, particularly sodium bicarbonate, sodium chloride or strontium chloride at high concentrations in the presence of glycerine desensitizes the sensitive parts of the teeth but that this desensitization is reversible so that pain is again felt soon after therapy has stopped.

[0007] In order to treat in a more permanent way these painful conditions in the course of the normal routine of dental hygiene of a suffering individual, it has then been proposed in the art to provide oral or dental preparations capable to improve the desensitization effect by providing a long lasting remineralisation of the teeth.

[0008] Thus, for example, Canadian patent CA 999 238 discloses an oral or dental preparation containing 5 to 90% by weight of finely divided hydroxyapatite having an average particle size of less than 10 μm , in particular in the region of about 6 to 8 μm . According to this reference, if this finely divided hydroxyapatite is repeatedly brought into contact with sensitive teeth over a prolonged period of time, for example when cleaning the teeth or when chewing a chewing gum, a long lasting remineralisation of the teeth may be achieved thanks to the diffusion of the hydroxyapatite into the exposed dentinal canaliculi by virtue of its very slight solubility in water and saliva due to hydrolysis. Accordingly, this sparingly soluble hydroxyapatite becomes deposited in the microscopically fine cavities left in the hydroxyapatite structure which has been formed in the organic matrix, so that the dentinal canaliculi are gradually sealed and permanent relief from pain is obtained.

[0009] Apart from the above-identified problem of hypersensitivity or hyperaesthesia of the teeth, it is also known that many people suffer from chronic bleeding of the gums, a consequence of gingivitis, deposits of secretions from the gingival crevices and lesions in the bed of the teeth, which in advanced stages result in loosening or loss of teeth and in loss of the tooth bed. Anaerobic putrefactive bacteria which cause halitosis are able to multiply rapidly in the protective environment of the inflamed and secretion-depositing tissue so that they easily give rise to inflammation of the buccal mucous membrane and even irritation of the pharyngeal cavity.

[0010] In order to treat in some way these teeth and gums disorders and in general to increase oral hygiene in the course of the normal routine of dental washing, it has been proposed in the art to provide oral or dental preparations capable to exploit an antibacterial effect. Thus, for example, European patent application EP 0 539 651 discloses a dentifrice (tooth paste or powder) in which an antibacterial material (metal-ion agent) is stably and firmly carried or supported by a carrier as a component of the dentifrice.

[0011] More specifically, this reference discloses a dentifrice containing a calcium compound, such as hydroxyapatite, in the form of a powder having an antibacterial metal carried by the calcium compound in a highly stable way so as to prevent the toxicity due to the ion form of the metal while contributing to a medical treatment of teeth and further providing the dentifrice with a good preservability for a long period of time.

[0012] 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 nanoparticles of a hydroxyapatite compound has been proposed for the treatment of bone defects in the fields of reconstructive bone surgery, surgical stomatology, traumatology, orthopedics and dentistry.

[0013] Thus, for example, European patent application EP 0 664 133 discloses a preparation for stimulating growth in bone tissue based on hydroxyapatite, wherein the hydroxyapatite is taken in the form of an aqueous pasta with a concentration of 18-36% by weight with a particle size of 15 to 60 nm.

[0014] According to this reference, such a preparation when applied and left to interact with the bone tissue to be repaired for an extended period of time, acts as a stimulator of the proliferative and biosynthetic activity of the osteoblasts.

[0015] International patent application WO 2005/082780, on the other hand, discloses magnesium-containing carbonated hydroxyapatite (MgCHA) suitable for creating a compound material in the form of a freeze-dried granulate suitable for bone implants, particularly in the field of dentistry, consisting of said modified hydroxyapatite and an organic polymer, preferably an alginate.

[0016] According to this reference, a compound material including such a modified hydroxyapatite is capable of efficiently repairing bone defects following application and interaction with the bone tissue for an extended period of time of the necessary amount of product at the level of the existing cavity in the bone.

[0017] The present inventors have observed that the hydroxyapatite compounds and compositions of the prior art have a limited effect in the treatment of teeth hypersensitivity in the course of the normal routine of dental hygiene.

[0018] The cleaning operations of the teeth by means of a toothpaste or the rinsing operations of teeth and mouth by means of a mouthwash or gargle, even if prolonged, may extend over a period of time which is quite limited and insufficient for the known hydroxyapatite compounds and for its optional additional components to fully exert their desensitizing and optionally antimicrobial effects.

Summary of the invention

[0019] According to one aspect thereof, the present invention therefore provides a biologically active hydroxyapatite compound capable of effectively improving teeth desensitization and remineralisation even in the limited time available during the normal routine of dental hygiene.

[0020] Biologically active nanoparticles of a carbonate-substituted hydroxyapatite according to the invention are defined in attached claim 1.

[0021] More specifically, the biologically active nanoparticles of the invention are nanoparticles of a carbonate-substituted non-stoichiometric hydroxyapatite, having:

a) a crystallinity degree CD lower than 40%, the crystallinity degree being defined as

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

wherein:

Y = height of the diffraction maximum at $2\theta = 33^\circ$, X = height of the diffraction background at $2\theta = 33^\circ$ of the nanoparticles X-ray diffraction pattern;

b) a length L ranging from 20 to 200 nm and a width W ranging from 5 to 30 nm; and

c) an aspect ratio AR comprised between 2 and 40, the aspect ratio being defined as

$$AR = L/W.$$

[0022] The inventors have observed that the nanoparticles of carbonate-substituted hydroxyapatite having the aforementioned features possess a high affinity to the mineral components of teeth dentine and enamel and are capable of "mimicking" the structure of these components to the extent that the nanoparticles are capable of effectively interact with the dentine and enamel surface.

[0023] The inventors have also observed that the nanoparticles of carbonate-substituted hydroxyapatite of the invention achieve at the same time an enhanced reactivity with the dentine and enamel surface which is effectively exploited even in the limited time available during the normal routine of dental hygiene.

[0024] Although the inventors do not wish to be bound by any theory, it is believed that this enhanced activity is due to a combination of factors which mimic biogenic hydroxyapatite nanoparticles, such as a suitable non-stoichiometric

composition of the hydroxyapatite, an extremely reduced size (nanoscale) and a consequent high surface area of the non-stoichiometric hydroxyapatite compound, a low crystallinity degree of the same and an elongated shape (as defined by their aspect ratio), which promote both an increased solubility and an enhanced affinity to the mineral components of teeth dentine and enamel.

[0025] More specifically, it is believed that a major role in enhancing the reactivity of the nanoparticles of the invention is played by the elevated surface disorder of their outer surface at which the ions stoichiometry of the bulk portion of the particles is no longer maintained.

[0026] 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 1 μm ; preferably, the nanoparticles of the invention have a flattened acicular shape which is most adapted to interact with the dentine and enamel surface.

[0027] For the purposes of the present description and of the claims which follow, the expression: biologically active, is used to indicate the capability of a substance or composition of interacting with the teeth dentine and/or enamel and/or gums and/or the other oral tissues.

[0028] 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.

[0029] 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.

[0030] 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).

[0031] The crystallinity degree of the nanoparticles can be also be evaluated according to the alternative method reported by Z. E. Erkmen "The effect of heat treatment on the morphology of D-Gun Sprayed Hydroxyapatite coatings", J. Biomed Mater Res (Appl Biomaterial) 48; 861-868, 1999 (hereinafter in short: the Erkmen method).

[0032] According to this alternative method, the crystallinity degree CD' may be defined as

$$CD' = (X/Y) \bullet 100$$

wherein:

Y= diffracted peaks net area + background area, X = diffracted peaks net area of the nanoparticles X-ray diffraction pattern.

[0033] The numerical values of the crystallinity degree CD' measured according to Erkmen method differ from those of the Landi et al. method; in this instance, in fact, the crystallinity degree CD' of the nanoparticles of the invention is lower than 70%.

[0034] 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.

[0035] For the purposes of the present description and of the claims which follow, the term: length L of the nanoparticles, is intended to mean the dimension of the nanoparticle as measured along the major axis thereof, while the term: width W of the nanoparticles, is intended to mean the dimension of the nanoparticle as measured along the minor axis thereof.

[0036] For the purposes of the invention, the length L and the width W of the nanoparticles can be measured according to known methods, such as, for example, by using transmission electron microscopy (TEM).

[0037] 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.

[0038] As mentioned above, the nanoparticles of the invention are nanoparticles of carbonate-substituted non-stoichiometric hydroxyapatite which incorporate carbonate ions in the apatite structure.

[0039] This feature advantageously enhances the biological activity of the nanoparticles, 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.

[0040] In the nanoparticles of the invention, carbonation preferably takes place at site B, as this results in a reduction of the crystallinity and an increase of the solubility of the apatite phase.

[0041] In a preferred embodiment of the invention, the hydroxyapatite nanoparticles comprise from 1 to 15% by weight and, more preferably, from 1 to 10% by weight based on the total weight of the nanoparticles of carbonate substituted into the hydroxyapatite structure.

[0042] In this way, the biological activity of the nanoparticles is advantageously enhanced, since their structure more closely resembles the structure of the natural apatite present in the teeth tissues.

[0043] 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.

[0044] 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.

[0045] These preferred patterns of carbonate substitution in the hydroxyapatite structure advantageously allow to increase the solubility of the nanoparticles in a biological environment. Additionally, the carbonate substitution at the phosphate site (B) advantageously induces a higher affinity of the hydroxyapatite nanoparticles for the osteoblast cell, increasing cellular adhesion and collagen production.

[0046] According to a preferred embodiment of the invention, the nanoparticles have a crystallinity degree CD comprised between 25% and 35% as measured according to the Landi et al. method which corresponds to a crystallinity degree CD' comprised between 40% and 60% as measured according to the Erkmén method.

[0047] In this way, the biological activity of the nanoparticles may be advantageously enhanced, since their low crystallinity helps in promoting both an increased solubility and an enhanced affinity to the mineral components of teeth dentine and enamel. According to a preferred embodiment of the invention, the nanoparticles have a surface area comprised between 30 and 60 m²/g.

[0048] In this way, the biological activity of the nanoparticles in terms of reactivity with the mineral components of teeth dentine and enamel may be advantageously enhanced.

[0049] For the purposes of the invention, the surface area of the hydroxyapatite compound can be measured according to known methods, such as, for example, by the BET method.

[0050] In connection with this parameter, the inventors have observed that the average values of the surface area of the nanoparticles may vary within the aforementioned range as a function of the total amount of nanoparticles synthesized per each production batch, the higher values being more easily reached the smaller is the entity of the production batch. As indicated above, the nanoparticles of the invention have a reduced size (a length L ranging from 20 to 200 nm and a width W ranging from 5 to 20 nm) as measured by the TEM technique, and an elongated shape as defined by an aspect ratio AR ranging from 2 to 40.

[0051] According to a preferred embodiment of the invention, the nanoparticles possess a substantially acicular or platelet shape having a length L comprised between 50 and 150 nm and a width W comprised between 5 and 20 nm.

[0052] Preferably, furthermore, the nanoparticles have a thickness T as measured by the TEM technique ranging from 2 to 15 nm.

[0053] The aspect ratio AR of the nanoparticles is preferably comprised between 2 and 16 and, still more preferably, between 5 and 10.

[0054] In this way, the biological activity of the nanoparticles may be advantageously enhanced, since the combination of reduced shape and elongate size helps in effectively sealing the dentinal canaliculi and achieving an effective permanent relief from pain even in the limited time available during the normal routine of dental hygiene.

[0055] According to a preferred embodiment thereof, the present invention also provides a biologically active hydroxyapatite compound capable of effectively exploiting also 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.

[0056] According to this preferred embodiment, the nanoparticles further comprise an effective amount of an antibacterial ion.

[0057] More preferably, the nanoparticles of the invention comprise from 0.1% to 20% by weight with respect of the total Ca content of an antibacterial metal M ion substituted into the hydroxyapatite structure.

[0058] In this way, the antibacterial effect of the metal M ion may advantageously be optimized.

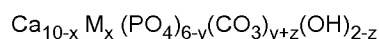
[0059] Preferably, the antibacterial metal M is selected from the group comprising Zn, Cu, Ag, and mixtures thereof.

[0060] Most advantageously, the aforementioned metal ions effectively exploit an antibacterial activity capable of preventing generation of carious tooth and periodontal diseases such as alveolar blennorrhoea and reducing halitosis phenomena.

[0061] Within the framework of this preferred embodiment, the nanoparticles have a molar ratio (Ca + M)/P greater than 1.7 and, more preferably, comprised between 1.7 and 1.8. In this way, it may be ensured that the carbonate

substitution in the hydroxyapatite structure has taken place mainly at site B, since a molar ratio (Ca + M)/P of non-substituted hydroxyapatites is of about 1.67.

[0062] Within the framework of this preferred embodiment, the nanoparticles of the invention can be represented for purely descriptive purposes by the following formula:



wherein x is a number comprised between 0.0055 and 0.6, y is a number comprised between 0.065 and 0.9 and z is a number comprised between 0 and 0.32.

[0063] According to another aspect thereof, the present invention relates to a composition comprising the biologically active nanoparticles described herein.

[0064] In one preferred embodiment, such a composition is in a form suitable for oral hygiene and is advantageously capable of effectively improving teeth desensitization and remineralisation even in the limited time available during the normal routine of dental hygiene.

[0065] If the nanoparticles also include an antibacterial metal ion, the composition 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.

[0066] In particular, it has been observed that the antibacterial effect of the metal ions may be directly obtained *in situ* at the dentine and enamel surface during the resolubilization of the nanoparticles deposited thereon, thus achieving a prolonged release of metal ions, even after dentifrice removal.

[0067] For the purposes of the invention, the composition including the nanoparticles of the invention may be in any physical form suitable for oral hygiene such as suspension, oil, gel or other solid product.

[0068] According to a preferred embodiment of the invention, the composition is in the form of a suspension including from 1% to 40% by weight, more preferably from 10% to 20% by weight, of biologically active nanoparticles.

[0069] 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.

[0070] In a preferred embodiment of the invention, the suspension has pH comprised between 7 and 8 and, more preferably between 7 and 7.4.

[0071] In this way, the suspension may be advantageously directly used as such or mixed with other ingredients in the formulation of effective oral hygiene compositions.

[0072] 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.

[0073] 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.

[0074] 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.

[0075] According to an alternative preferred embodiment of the invention, the composition may be in the form of toothpaste, tooth powder, chewing gum for oral and dental hygiene, ointment for the gums, mouthwash and mouth bath concentrate and gargle.

[0076] According to a preferred embodiment of the invention, the composition may comprise a combination of biologically active nanoparticles comprising an effective amount of an antibacterial ion and biologically active nanoparticles without said ion.

[0077] In this way, the entity of the antibacterial effect of the metal ion carried out at the dentine and enamel surface may be adjusted according to the most varied requirements. According to another preferred embodiment, in the oral composition of the invention the amount of biologically active nanoparticles will generally range from 3% to 30% by weight of the composition.

[0078] If the oral composition comprises a combination of biologically active nanoparticles comprising an effective amount of an antibacterial ion and biologically active nanoparticles without said ion, the amount of each of these two kinds of nanoparticles will generally range from 1.5% to 15% by weight of the composition.

[0079] The oral compositions of this invention will, of course, also contain other ingredients commonly used and known in the art to formulate such products, depending on the form of the oral product.

[0080] 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.

[0081] For the purposes of the invention, a suitable particulate abrasive agent may be 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.

[0082] Humectants commonly used 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.

[0083] 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.

[0084] It is usual 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 as cationic, amphoteric and non-ionic surfactants. Surfactants are usually present in an amount comprised between 0.5% and 5% by weight of the dentifrice.

[0085] Flavors that are usually used in dentifrices are those based on oils of spearmint and peppermint. Examples of other flavoring materials 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.

[0086] The oral composition of the invention may include a wide variety of other optional ingredients.

[0087] 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.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] According to another aspect thereof, the present invention relates to an improved process for producing an aqueous suspension including a biologically active hydroxyapatite compound which requires low investment and operating costs.

[0092] A process for preparing an aqueous suspension including biologically active nanoparticles of a carbonate-substituted hydroxyapatite according to the invention is defined in attached claim 20.

[0093] Most advantageously, this process allows to prepare in a fairly quick and economical way a suspension of biologically active nanoparticles which may be readily used as such as a composition for oral hygiene or used in admixture with other ingredients to yield compositions for oral hygiene.

[0094] Most advantageously, furthermore, this process allows to prepare a suspension of biologically active nanoparticles which is stable for an extended period of time even if no stabilizing agents are added thereto.

[0095] 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.

[0096] For the purposes of the invention, the aforementioned step a) of preparing an aqueous solution or suspension of 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.

[0097] In this way, the cost of the process may advantageously be reduced since these Ca compounds are commodities readily available from the market at a very low cost. Additionally, these Ca compounds are easily workable and stockable to the advantage of the manufacturing operations.

[0098] In the process of the invention, step a) is preferably carried out in order to achieve a suspension of nanoparticles having a basic pH. Preferably, the aqueous solution or suspension of step a) has a pH comprised between 8 and 12.

[0099] According to a preferred embodiment of the invention, the aforementioned step a) is carried out in substantial absence of orally non-acceptable anions.

[0100] In this way, a suspension of nanoparticles may be produced which does not require any additional treatment to remove undesired substances and which may directly used as such, save for an optional pH adjustment by means of a suitable agent, such as citric acid.

[0101] In the process of the invention, the nanoparticles of carbonate-substituted hydroxyapatite are formed in step b) by adding PO_4^{3-} ions to the aqueous solution or suspension of step a) and by simultaneously 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.

[0102] 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.

[0103] 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.

[0104] For the purposes of the invention, the PO_4^{3-} ions are added to the aqueous solution or suspension of step a) over a time which generally depends on the amount of the used phosphoric solution with respect to the amount of the basic, calcium solution or suspension, and which may be selected by those skilled in the art.

[0105] Preferably, step b) is carried out over a time comprised between 30 minutes and 2 hours in order to keep the reaction time and the operating costs as low as possible.

[0106] According to the invention, step b) is carried out while maintaining said solution or suspension at a temperature lower than or equal to 60°C .

[0107] The inventors have observed that in this way the crystallinity degree CD of the nanoparticles may be kept below the aforementioned maximum value of 40% (70% in the case of CD').

[0108] In a preferred embodiment of the invention, step b) is carried out while maintaining said solution or suspension at a temperature lower than or equal to 40°C and more preferably comprised between 25° and 40°C .

[0109] In this way, the crystallinity degree CD of the nanoparticles may be kept within the aforementioned preferred range of values (CD = 25-35%; CD' = 40-60%).

[0110] According to a preferred embodiment of the invention, step b) is carried out by adding, preferably dropwise, an aqueous solution including PO_4^{3-} ions to the aqueous solution or suspension of step a).

[0111] According to an alternative preferred embodiment of the invention, the aqueous solution including PO_4^{3-} ions added in step b) may further comprise HCO_3^- ions.

[0112] 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.

[0113] Within the framework of this preferred embodiment, the aforementioned aqueous solution including HCO_3^- and 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 thereto.

[0114] According to another alternative preferred embodiment of the invention, step b) 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).

[0115] In a preferred embodiment of the invention, the process is carried out such that the suspension of nanoparticles obtained from step c) has a pH comprised between 7 and 8 and, more preferably, between 7 and 7.4.

[0116] In this way and as already outlined above, the process of the invention allows to produce a suspension which may be advantageously directly used as such or mixed with other ingredients in the formulation of effective oral hygiene compositions with a remarkable simplification of the manufacturing operations of the compositions and a remarkable cost reduction.

[0117] As noted above, the suspension of nanoparticles produced in this way also shows remarkable stability characteristics and has a shelf life of at least 30 days and, more generally, of about two-three months, even if no stabilizing agents are added thereto. According to a preferred embodiment of the invention, the aqueous solution or suspension of step a) may further comprise an oxide or a salt of an antibacterial metal M.

[0118] Preferably, the antibacterial metal M is selected from the group comprising Zn, Cu, Ag, and mixtures thereof.

[0119] In this way, a suspension of nanoparticles may be produced which also exhibit an antibacterial effect enhancing the oral hygiene characteristics of the suspension or of the other products (liquid or solid) in which the suspension is incorporated.

[0120] In a preferred embodiment, the aforementioned metal salt is an orally acceptable organic or inorganic salt selected from the group comprising: lactates, gluconates, citrates, acetates and hydroxides.

[0121] In this way, a suspension of nanoparticles may be produced which does not require any additional treatment to remove undesired substances and which may directly used as indicated above.

[0122] In a preferred embodiment, step b) is carried out such that the ratio of the Ca and M ions contained in the

solution or suspension of step a) and the PO_4^{3-} ions added thereto is greater than 1.7.

[0123] In this way, it may be ensured that the carbonate substitution in the hydroxyapatite structure has taken place mainly at phosphate site B and a suspension of nanoparticles may be produced which advantageously exploits an antibacterial activity capable of preventing generation of carious tooth and periodontal diseases such as alveolar blennorrhoea and reducing halitosis phenomena.

[0124] In the process of the invention, the growth of the nanoparticles of a carbonate-substituted hydroxyapatite is accomplished in step c) by agitating the suspension obtained from step b) (during which mainly a nucleation of the nanoparticles is taking place) over a time of at least two hours at a temperature lower than or equal to 60°C . Preferably, step c) is carried out over a time comprised between 2 and 24 hours and more preferably between 2 and 12 hours, as required by the circumstances in order to have a growing time of the nanoparticles sufficient to reach the desired size and in order to obtain a single phase.

[0125] In a preferred embodiment of the invention, step c) is carried out while maintaining the suspension of nanoparticles at a temperature comprised between 25° and 40°C .

[0126] In a preferred embodiment of the invention, step c) is carried out while maintaining the suspension of nanoparticles at the same temperature of step b).

[0127] In this way, the process may be advantageously carried out with a simpler control and at a lower cost.

[0128] According to another aspect thereof, the present invention relates to an improved process for preparing a biologically active hydroxyapatite compound which requires low investment and operating costs.

[0129] A process for preparing biologically active nanoparticles of a carbonate-substituted hydroxyapatite according to the invention is defined in attached claim 35.

[0130] More specifically, the process of the invention comprises the steps of:

- a) preparing an aqueous suspension including said nanoparticles by means of a process as herein described;
- b) separating solid nanoparticles from the suspension obtained from step a);
- c) drying the wet solid nanoparticles thus obtained.

[0131] In a preferred embodiment, the separation step b) is carried out by decantation, centrifugation or filtration using apparatuses and techniques well known to those skilled in the art.

[0132] In a preferred embodiment, the drying step c) is carried out by freezing the wet solid nanoparticles at a temperature lower than 0°C until reaching a constant weight.

[0133] Within the framework of this preferred embodiment, the drying step c) is preferably carried out by freeze-drying the wet solid nanoparticles at a temperature comprised between -20° and -50°C , most preferably at about -40°C .

[0134] In a preferred embodiment, the process may also comprise the additional step d) of washing the separated solid nanoparticles with water or a basic solution prior to effecting the drying step c).

[0135] Advantageously, this additional washing step d) serves the useful function of removing any acid residues possibly absorbed or trapped by the nanoparticles.

[0136] According to another aspect thereof, the present invention relates to an improved process for manufacturing a toothpaste comprising a biologically active hydroxyapatite compound which requires low investment and operating costs.

[0137] A first process for manufacturing a toothpaste comprising biologically active nanoparticles of a carbonate-substituted hydroxyapatite according to the invention is defined in attached claim 39 and comprises the steps of:

- a) preparing an aqueous suspension including said nanoparticles by means of a process as herein described;
- b) mixing said aqueous suspension with other ingredients of the toothpaste.

[0138] As already noted above, this process advantageously allows to readily incorporate the nanoparticles in the toothpaste in a quite simple and convenient manner exploiting the useful properties, in particular stability and pH characteristics, of the suspension of nanoparticles produced in accordance with the invention.

[0139] Quite advantageously, the process for manufacturing a toothpaste of the invention does not require any separation or drying of the nanoparticles, 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.

[0140] In addition, the mixing step of the aqueous suspension of nanoparticles with other ingredients of the toothpaste 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.

[0141] An alternative second process for manufacturing a toothpaste comprising biologically active nanoparticles of a carbonate-substituted hydroxyapatite according to the invention is defined in attached claim 40 and comprises the steps of:

- a) preparing solid nanoparticles by means of a process as herein described;
- b) mixing the solid nanoparticles with other ingredients of the toothpaste.

[0142] This alternative process allows to manufacture a toothpaste in all those instances in which the use of the above-described suspension of nanoparticles may not be desirable for logistic or other reasons.

[0143] In a preferred embodiment of the manufacturing processes described above, the mixing step 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.

[0144] In a preferred embodiment of the first manufacturing process, the mixing step b) is carried out by

- b1) mixing the aqueous suspension of step a) with other ingredients of the toothpaste except for any surfactant;
- b2) incorporating at least one surfactant into the mixture thus obtained.

[0145] In this way, the formation of foam during the mixing operation may be minimized. Within the framework of this embodiment, the incorporation step b2) is preferably carried out under vacuum using a conventional equipment in order to minimize the undesired formation of foam.

[0146] According to another aspect thereof, the present invention relates to a method of locally remineralising the teeth comprising contacting the teeth with a composition comprising biologically active nanoparticles as described herein.

[0147] Most advantageously and thanks to the characteristics of the nanoparticles described above, such a method allows to effectively remineralise the teeth even in the limited time available during the normal routine of dental hygiene.

[0148] The contacting step of the remineralising method may be carried out in a number of ways depending upon the form of the nanoparticles-containing composition. For example, if the composition is a toothpaste, the contacting step may be simply carried out by washing the teeth, while if the composition 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.

[0149] According to the invention and as will be shown in greater detail below, in fact, the nanoparticles of the invention possess such a high remineralising activity that its effect may be detected even by contacting the nanoparticles with the teeth for a limited period of time.

Brief description of the drawings

[0150] 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.

[0151] In these drawings:

- Fig. 1 shows an X-Ray diffraction pattern of one example of biologically active nanoparticles according to the invention;
- Fig. 2 shows the X-Ray diffraction patterns of one example of biologically active nanoparticles according to the invention (a) and of natural (dentine) apatite nanoparticles (b);
- Figs. 3a, 3b, 3c and 3d show TEM images of some examples of biologically active nanoparticles according to the invention which display the nanometric size of the particles;
- Fig. 4 shows a thermogravimetric plot of one example of biologically active nanoparticles according to the invention;
- Fig. 5 shows a FTIR spectrum of one example of biologically active nanoparticles according to the invention;
- Figs. 6a, 6b and 6c show respective SEM images of a slab radicular dentine of fresh bovine teeth in a demineralized condition (Fig. 6a), after a 1-minute contact (Fig. 6b) and after a 10-minutes contact (Fig. 6c) with a suspension of biologically active nanoparticles according to the invention.

[0152] In the following Examples, percentages and parts are by weight unless otherwise indicated.

EXAMPLE 1

(Preparation of an aqueous suspension of nanoparticles)

[0153] 2184 g of an aqueous suspension of biologically active nanoparticles according to the invention was prepared according to the following method.

[0154] In a first step, an aqueous suspension comprising 356,2 g of $\text{Ca}(\text{OH})_2$, 48,8 g of ZnO and 45 g of $\text{Ca}(\text{CO}_3)$ in 1060 g of H_2O was prepared in a conventional reaction vessel while agitating the ingredients with a mechanical stirrer.

[0155] During this step, the resulting suspension was brought to a temperature of $40^\circ \pm 2^\circ \text{C}$ by means of an electrical

resistance or by any other suitable heating element such as for example a thermostated jacket in which a heating fluid, such as oil or vapor, is circulated.

[0156] Once the desired temperature was reached, nanoparticles of a carbonate-substituted hydroxyapatite were formed by adding dropwise PO_4^{3-} ions to the aqueous suspension of the preceding step while simultaneously agitating the same. In this case, 674 g of an acid solution constituted by a mixture 70/30 of H_3PO_4 (75%)/ H_2O , were added with a dripping speed of $22 \text{ g} \cdot \text{min}^{-1}$ ($0,4 \text{ g} \cdot \text{sec}^{-1}$), while continuously stirring and maintaining constant the temperature of the reaction vessel.

[0157] After about 30' and 30" a suspension of nanoparticles was obtained which was subsequently agitated over a period of time of about two hours at a temperature of $40^\circ \pm 2^\circ\text{C}$, after which a suspension having a total content of about 30-31% by weight of nanoparticles and having a pH of about 7 ± 0.2 was obtained.

[0158] The suspension of nanoparticles was readily useable as such or as an active ingredient for the subsequent preparation of a toothpaste, mouthwash or other oral or dental composition according to the invention.

EXAMPLE 2

(Preparation of solid nanoparticles)

[0159] 670 g of biologically active nanoparticles according to the invention were prepared by first preparing 2184 g of an aqueous suspension according to the method and using the same ingredients of preceding Example 1 and then by carrying out the following additional steps.

[0160] Firstly, the solid nanoparticles were separated from the liquid by filtering on a Millipore paper with a pore diameter of $45 \mu\text{m}$ and then were repeatedly washed with a diluted water solution of CaCO_3 to remove any acid residues.

[0161] The wet solid nanoparticles thus obtained were then freeze-dried at -40°C until they reached a constant weight, sieved at ($\phi=120$ -20 microns) and stored at a temperature of 0 - 4°C .

[0162] The nanoparticles thus obtained, which had the aspect of a white powder, were then characterized as follows.

1) X-ray diffraction (XRD)

[0163] X-ray powder patterns were collected using a Philips PW 1710 powder diffractometer equipped with a secondary graphite monochromator using $\text{Cu K}\alpha$ radiation generated at 40 kV and 40 mA. The instrument was configured with a 1° divergence and 0.2 mm receiving slits. The samples were prepared using the front loading of standard aluminium sample holders which are 1 mm deep, 20 mm high and 15 mm wide. The 2θ range was from 5° to 60° with a step size (2θ) of 0.05° and a counting time (s) of 3.

[0164] In Fig. 1 the XRD pattern of the nanoparticles, which allows to determine the crystallinity degree of the nanoparticles, is shown.

[0165] In Fig. 1, the line intensity is related to its intensity percentage (arbitrary units), considering the highest line equal to 100.

[0166] The crystallinity degree, was evaluated according to the aforementioned Landi et al. method and Erkmen method.

[0167] According to the Landi et al. method, the crystallinity degree of the nanoparticles evaluated from the XRD pattern reported in Fig 1 was of about 30%.

[0168] According to the Erkmen method, the crystallinity degree of the nanoparticles evaluated from the XRD pattern reported in Fig 1 was of about 52%.

[0169] As to nature of the carbonate-substituted hydroxyapatite nanoparticles and as may also be gathered from the patterns reported in Fig. 2, in which curve a is the pattern of the biologically active hydroxyapatite nanoparticles according to the invention and curve b is the pattern of natural hydroxyapatite nanoparticles, it may be observed the similarity between natural (dentine) hydroxyapatite nanoparticles and the hydroxyapatite nanoparticles according to the invention.

2) Morphological characterisation by Transmission Electron Microscopy (TEM)

[0170] Transmission Electron Microscopy (TEM) observations were carried out using a Philips CM 100. The powdered samples were ultrasonically dispersed in ultrapure water and then a few droplets were dropped on holey-carbon foils supported on conventional copper microgrids. TEM images of the biologically active nanoparticles according to the invention are reported in fig 3a, 3b, 3c and 3d respectively where it is possible to appreciate the elongate shape of the nanoparticles with acicular and platelet shape morphology very close to those observed for bone apatite crystals. The nanoparticles had an average length L of about 100 nm, an average width W of about 10 nm and an average thickness T of about 5 nm.

[0171] The average aspect ration A/R was 10.

3) Thermal analysis (TGA-DSC)

[0172] Thermogravimetric investigations were carried out on the nanoparticles using a Thermal Analysis SDT Q 600. Heating was performed in nitrogen flow (100 ml/min) using an alumina sample holder at a rate of 10°C/min up to 1000°C. The weight of the samples was around 10 mg.

[0173] Fig. 4 reports the results of a thermogravimetric analysis of the nanoparticles showing the weight decreases relating to the decomposition of the inorganic phase. Line a corresponds to percentage weight loss as a function of the treatment temperature and line b represents the derivative of the percentage weight loss respect to the temperature. Line c corresponds to heat flow involved into the phases transitions which are not present in the samples of the nanoparticles.

[0174] Line b shows a broad peak between 150°C and 300°C due to the loss of the physically absorbed water (weight loss of $2.2 \pm 0.5\%$), a broad peak between 400°C and 600°C due both to the loss of the chemically absorbed water and to the decarbonation process (weight loss of $0.7 \pm 0.3\%$). The peak between 800°C and 1000°C can be attributed to the dehydroxylation process (weight loss of $1.5 \pm 0.5\%$). The peak broadness is partially due to the low crystallinity degree of the nanoparticles.

4) Surface area analysis (BET)

[0175] The specific surface area of the nanoparticles was evaluated by the Brunauer, Emmet, Teller method [S. Brunauer, P.H. Emmet, E. Teller, Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60 (1938) 309- 319], [S.J. Greg, K.S. Sing (Eds.), Adsorption, Surface Area and Porosity, Academic Press, 1997 O. Gauthir, J.M. Boiler, E. Aguado, P. Piletand, G. Daculsi] carried out by means of a Carlo-Erba Sorpty 1750 instrument and using N₂ as adsorption gas.

[0176] Analyses were performed on 300 mg of samples. Before gas absorption, the samples were dried under vacuum (2 mbar) while increasing the temperature, at a speed of 10°C/min, from 25°C to 100°C. N₂ adsorption was then carried out keeping the sample in liquid N₂. Each surface area measurement supplied by the instrument corresponds to the mean of three values.

[0177] The average surface area of the nanoparticles was of about 30 m²/g.

5) Chemical composition: Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) analysis

[0178] The amount of calcium, zinc and phosphorous in the samples of nanoparticles was obtained using and Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) technique. The ICP-OES measurements were carried out with a Perkin Elmer Optima 4200 DV instrument. The samples had been previously dissolved in ultrapure nitric acid 1% to obtain a concentration of the elements between 1 and 8 ppm.

[0179] The nanoparticles exhibited a bulk (Ca+M)/P molar ratio of about 1.98 and a Zinc content of about 18% by weight with respect of the total Ca content.

6) Spectroscopic characterization by Fourier Transform Infrared (FTIR) analysis

[0180] The infrared spectra were registered from 4000 to 400 cm⁻¹ at 2 cm⁻¹ resolution by using a Bruker IFS 66v/S spectrometer. Pellets (KBr) were obtained under vacuum by using powdered samples (1 mg) carefully mixed with infrared grade KBr (200 mg).

[0181] The FTIR spectrum of the nanoparticles is shown in fig. 5. The spectrum shows the signals related to the groups PO₄³⁻ (1037 cm⁻¹), HPO₄²⁻ (955 cm⁻¹), OH⁻ (3444 cm⁻¹ and 1630 cm⁻¹), CO₃²⁻ (870 cm⁻¹). A comparison between the peak area at 870 cm⁻¹ of the nanoparticles and the peak area at 870 cm⁻¹ of a CaCO₃ reference standard allowed to evaluate a CO₃²⁻ amount of about 3% by weight based on the total weight of the nanoparticles.

[0182] The band at 870 cm⁻¹ provides information about the apatite carbonation type.

[0183] The deconvolution profile of the carbonate peak at 870 cm⁻¹ allows to deduce that the hydroxyapatite carbonation is predominantly of type B (A/B ratio of approximately 0.05).

[0184] The relevant characterization data of the nanoparticles are summarized in the following Table 1.

EXAMPLE 3

[0185] 2124 g of biologically active nanoparticles according to the invention were prepared by first preparing 1449.5 g of an aqueous suspension according to the method and using the same ingredients of preceding Example 1 save for the fact that the amount of water was of about 1000 g, the amount of ZnO was of 4.5 g, the amount of CaCO₃ was of 22.5 g, the amount of Ca(OH)₂ was 422.5g.

[0186] During this step, the resulting suspension was brought to a temperature of $40^{\circ} \pm 2^{\circ} \text{C}$ by the same method of the preceding Example 1.

[0187] Once the desired temperature was reached, nanoparticles of a carbonate-substituted hydroxyapatite were formed by adding dropwise PO_4^{3-} ions in the same way and in the same amounts as described in preceding Example 1.

[0188] After about 30' and 30" a suspension of nanoparticles was obtained which was subsequently treated in the same manner described in preceding Example 1.

[0189] The nanoparticles were then separated from the aqueous suspension thus obtained according to the separation method described in the preceding Example 2.

[0190] The nanoparticles thus obtained were then characterized according to the procedures and methods described in Example 2. The relevant characterization data of the nanoparticles are reported in the following Table 1.

EXAMPLE 4

[0191] 2184 g of biologically active nanoparticles according to the invention were prepared by first preparing 1510 g of an aqueous suspension according to the method and using the same ingredients of preceding Example 1 save for the fact that the amount of $\text{Ca}(\text{OH})_2$ was of about 405g and that no metal ion ingredient was added to the suspension.

[0192] During this step, the resulting suspension was brought to a temperature of $40^{\circ} \pm 2^{\circ} \text{C}$ by the same method of the preceding Example 1.

[0193] Once the desired temperature was reached, nanoparticles of a carbonate-substituted hydroxyapatite were formed by adding dropwise PO_4^{3-} ions in the same way and in the same amounts as described in preceding Example 1.

[0194] After about 30' and 30" a suspension of nanoparticles was obtained which was subsequently treated in the same manner described in preceding Example 1.

[0195] The nanoparticles were then separated from the aqueous suspension thus obtained according to the separation method described in the preceding Example 2.

[0196] The nanoparticles thus obtained were then characterized according to the procedures and methods described in Example 2. The relevant characterization data of the nanoparticles are reported in the following Table 1.

TABLE 1

Parameter	Example 2	Example 3	Example 4
Crystallinity degree CD [%]	30	35	30
Crystallinity degree CD' [%]	52	60	60
Length L [nm]	100	150	150
Width W [nm]	10	20	15
Aspect Ratio AR	10	7.5	10
Thickness T [nm]	5	5	10
Surface area [m^2/g]	30	40	30
(Ca+M)/P molar ratio	1,98	1,8	1,98***
Metal content [wt %]*	18	1,5	0
CO_3^{2-} content [wt %]**	4%	2%	4%
A/B ratio	0.05	0,1	0.05
* = % by weight with respect to the total Ca content. ** = % by weight with respect to the total weight of the nanoparticles. *** = in this case only Ca was present			

EXAMPLE 5

(Evaluation of the teeth remineralisation activity of the aqueous suspension of nanoparticles)

[0197] In order to evaluate the teeth remineralisation activity of the aqueous suspension of nanoparticles prepared in accordance with the preceding Example 1 the following test was carried out.

[0198] Fresh bovine teeth were obtained and a slab of radicular dentine was obtained by cutting the teeth with a

diamond saw. The periodontal ligament was removed using a metal curette and the root cementum were removed using a diamond bur under water cooling.

[0199] The dentine was etched with ortophosphoric acid for 1 minute in order to remove the smear layer and expose the dentinal tubules. The acid was washed away with a water spray for 1 minute and the specimens were left just wet.

[0200] Then an aliquot of the suspension of nanoparticles according to the preceding Example 1 was applied on the dentine slabs using a brush. The slabs were left wet in an incubation chamber at 37° C for an application time of about 1 minute after which time the specimens were rinsed with air-water spray for 1 minute and processed for Scanning Electron Microscopy (SEM).

[0201] Figs. 6a, 6b and 6c show the features of the dentine at baseline (Fig. 6a-demineralized) and of remineralised specimens after application of the nanoparticles for 1 minute (Fig. 6b) and for 10 minutes (Fig. 6c).

[0202] Fig. 6b shows a remarkable crystals formation and consequent obliteration of dentinal patent tubules even with a contact for a very limited time (1 minute) between the dentine and the suspension of nanoparticles. Fig. 6c shows an enhanced deposition of the nanoparticle on dentine substrate as a function of contact time.

EXAMPLE 6

(Toothpaste)

[0203] A toothpaste including biologically active nanoparticles according to the invention was prepared according to the following method and from the following ingredients.

[0204] In a first step, an aqueous suspension including biologically active nanoparticles (total solid content: 30% by weight) was prepared in the same manner and using the same ingredients and quantities described in Example 1.

[0205] 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.

[0206] 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.

[0207] 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.

[0208] In this way, a toothpaste was obtained having the composition reported in the following Table 2.

TABLE 2

Ingredient	Amount [%]
Sodium carboxymethylcellulose	1.0
HA-Zn nanoparticles*	7.5
HA nanoparticles**	7.5
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

(continued)

Ingredient	Amount [%]
Water	balance
* = Biologically active nanoparticles of a carbonate-substituted hydroxyapatite including Zn ions prepared in accordance with Example 1. ** = Biologically active nanoparticles of a carbonate-substituted hydroxyapatite prepared in accordance with the procedure of Example 1 except for the fact that no Zn ions were used.	

EXAMPLE 7

(Mouthwash)

[0209] A mouthwash including biologically active nanoparticles according to the invention was prepared by mixing a suspension produced in accordance with the preceding Example 1 in a conventional way with conventional ingredients.

[0210] A mouthwash was obtained having the composition reported in the following Table 3.

TABLE 3

Ingredient	Amount [%]
HA-Zn nanoparticles*	2.5
HA nanoparticles**	2.5
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 8

(Chewing gum for tooth cleaning)

[0211] A chewing gum including biologically active nanoparticles according to the invention was prepared by mixing solid dried nanoparticles produced in accordance with the preceding Example 2 in a conventional way with conventional ingredients.

[0212] A chewing gum was obtained having the composition reported in the following Table 4.

TABLE 4

Ingredient	Amount [%]
Chewing gum base	91.65
HA-Zn nanoparticles*	2
HA nanoparticles**	2
Glycerine	3
Sodium saccharine	0.025

(continued)

Ingredient	Amount [%]
Hydroglycolic moss extract titrated in 2% usnic acid	0.1
Mint flavor	1

Claims

1. Biologically active nanoparticles of a carbonate-substituted non-stoichiometric hydroxyapatite, having:

a) a crystallinity degree CD lower than 40%, the crystallinity degree being defined as

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

wherein:

Y = height of the diffraction maximum at $2\theta = 33^\circ$, X = height of the diffraction background at $2\theta = 33^\circ$ of the nanoparticles X-ray diffraction pattern;

b) a length L ranging from 20 to 200 nm and a width W ranging from 5 to 30 nm; and

c) an aspect ratio AR comprised between 2 and 40, the aspect ratio being defined as

$$AR = L/W.$$

2. Biologically active nanoparticles according to claim 1, comprising from 1% to 15% by weight of carbonate substituted into the hydroxyapatite structure.

3. Biologically active nanoparticles according to claim 1, wherein a ratio A/B between a carbonate substitution at the hydroxyl site (A) and a carbonate substitution at the phosphate site (B) of the hydroxyapatite is comprised between 0.05 and 0.5.

4. Biologically active nanoparticles according to claim 1 or 3, wherein the carbonate substitution at the phosphate site (B) of the hydroxyapatite is greater than or equal to 65% by weight of the total carbonate present in the hydroxyapatite.

5. Biologically active nanoparticles according to claim 1, having a crystallinity degree CD comprised between 25% and 35%.

6. Biologically active nanoparticles according to claim 1, having a surface area comprised between 30 and 60 m²/g.

7. Biologically active nanoparticles according to claim 1, having a substantially acicular or platelet shape having a length L comprised between 50 and 150 nm and a width W comprised between 5 and 20 nm.

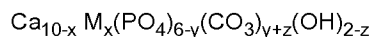
8. Biologically active nanoparticles according to anyone of claims 1-7, further comprising an effective amount of an antibacterial ion.

9. Biologically active nanoparticles according to claim 8, comprising from 0.1 to 20% by weight with respect of the total Ca content of an antibacterial metal M ion substituted into the hydroxyapatite structure.

10. Biologically active nanoparticles according to claim 9, wherein said metal M is selected from the group comprising Zn, Cu, Ag, and mixtures thereof.

11. Biologically active nanoparticles according to claim 9, having a molar ratio (Ca + M)/P greater than 1.7.

12. Biologically active nanoparticles according to claim 9, having the formula:



wherein x is a number comprised between 0.0055 and 0.6, y is a number comprised between 0.065 and 0.9 and z is a number comprised between 0 and 0.32.

13. A composition comprising biologically active nanoparticles according to anyone of the preceding claims.
14. A composition according to claim 13, in a form suitable for oral hygiene.
15. A composition according to claim 13 or 14, in the form of suspension, oil, gel or solid.
16. A composition according to claim 15, in the form of a suspension including from 1% to 40% by weight of biologically active nanoparticles.
17. A composition according to claim 15, having a pH comprised between 7 and 8.
18. A composition according to claim 15, in the form of toothpaste, tooth powder, chewing gum for oral and dental hygiene, ointment for the gums, mouthwash and mouth bath concentrate and gargle.
19. A composition according to claim 14, comprising a combination of biologically active nanoparticles comprising an effective amount of an antibacterial ion and biologically active nanoparticles without said ion.
20. A process for preparing an aqueous suspension including biologically active nanoparticles according to claim 1, comprising the steps of:
 - a) preparing an aqueous solution or suspension of a Ca compound;
 - b) forming nanoparticles of a carbonate-substituted hydroxyapatite by adding PO_4^{3-} ions to the aqueous solution or suspension of step a) while simultaneously agitating the same over a time comprised between 30 minutes and 8 hours while maintaining said solution or suspension at a temperature lower than or equal to 60°C;
 - c) agitating the suspension of nanoparticles obtained from step b) over a time of at least two hours at a temperature lower than or equal to 60°C;
 wherein a carbonate substitution at the phosphate site (B) of the hydroxyapatite compound being formed said step b) is achieved by any one of:
 - i) agitating said solution or suspension of step a) in such a way as to capture the carbon dioxide present in the atmosphere,
 - ii) bubbling air, a CO_2 -containing gas or a mixture thereof into the aqueous solution or suspension of step a)
 - iii) combining a mechanical stirring of the solution or suspension of step a) with bubbling air, a CO_2 -containing gas or a mixture thereof into said solution or suspension; or
 - iv) adding a solution containing CO_3^{2-} ions to the aqueous solution or suspension of step a).
21. A process according to claim 20, wherein step a) is carried out in substantial absence of orally non-acceptable anions.
22. A process according to claim 20, wherein step b) is carried out by adding an aqueous solution including PO_4^{3-} ions, optionally further comprising HCO_3^- ions, to the aqueous solution or suspension of step a) or 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 step a) or while maintaining said solution or suspension at a temperature comprised between 25°C and 40°C.
23. A process according to claim 20, wherein the suspension obtained from step c) has a pH comprised between 7 and 8.
24. A process according to anyone of claims 20-23, wherein the aqueous solution or suspension of step a) further comprises an oxide or a salt of an antibacterial metal M.
25. A process according to claim 24, wherein said metal salt is an orally acceptable organic or inorganic salt selected from the group comprising: lactates, gluconates, citrates, acetates and hydroxides.

26. A process according to anyone of claims 24 or 25, wherein step b) is carried out such that the ratio of the Ca and metal M ions contained in the solution or suspension of step a) and the PO_4^{3-} ions added thereto is greater than 1.7.

27. A process for preparing biologically active nanoparticles according to anyone of claims 1-12, comprising the steps of:

- a) preparing an aqueous suspension including said nanoparticles by a process according to anyone of claims 20-26;
- b) separating solid nanoparticles from the suspension obtained from step a);
- c) drying the wet solid nanoparticles thus obtained.

28. A Process according to claim 27, further comprising the step of

- d) washing the separated solid nanoparticles with water or a basic solution prior to effecting said drying step c).

29. A process for manufacturing a toothpaste comprising biologically active nanoparticles according to anyone of claims 1-12, comprising the steps of:

- a) preparing an aqueous suspension including said nanoparticles by a process according to anyone of claims 20-26;
- b) mixing said aqueous suspension with other ingredients of the toothpaste.

30. A process for manufacturing a toothpaste comprising biologically active nanoparticles according to anyone of claims 1-12, comprising the steps of:

- a) preparing solid nanoparticles by a process according to anyone of claims 27 or 28;
- b) mixing the solid nanoparticles with other ingredients of the toothpaste.

31. A composition according to anyone of claims 14-19 for use in locally remineralizing the teeth.

Patentansprüche

1. Biologisch aktive Nanopartikel aus carbonatsubstituiertem nichtstöchiometrischem Hydroxyapatit, umfassend:

- a) einen Kristallinitätsgrad (engl. cristallinity degree, CD) von weniger als 40 %, wobei dieser Kristallinitätsgrad definiert ist als

$$\text{CD} = (1 - X/Y) \cdot 100$$

wobei:

Y = Höhe des Beugungsmaximums bei $2\theta = 33^\circ$, X = Höhe des Beugungshintergrunds bei $2\theta = 33^\circ$ des Röntgenbeugungsmusters der Nanopartikel;

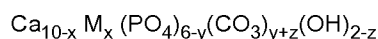
- b) eine Länge L von 20 bis 200 nm und eine Breite W von 5 bis 30 nm; und
- c) ein Aspektverhältnis AR zwischen 2 und 40, wobei das Aspektverhältnis definiert ist als

$$\text{AR} = L/W.$$

2. Biologisch aktive Nanopartikel nach Anspruch 1, die zwischen 1 und 15 Gew.-% von in die Hydroxyapatitstruktur substituiertem Carbonat umfassen.

3. Biologisch aktive Nanopartikel nach Anspruch 1, wobei ein Verhältnis A/B zwischen einer Carbonatsubstitution an der Hydroxylstelle (A) und einer Carbonatsubstitution an der Phosphatstelle (B) des Hydroxyapatits zwischen 0,05 und 0,5 beträgt.

4. Biologisch aktive Nanopartikel nach Anspruch 1 oder 3, wobei die Carbonatsubstitution an der Phosphatstelle (B) des Hydroxyapatits größer als oder gleich 65 Gew.-% des gesamten, im Hydroxyapatit vorhandenen Carbonats ist.
5. Biologisch aktive Nanopartikel nach Anspruch 1, deren Kristallinitätsgrad CD zwischen 25 % und 35 % beträgt.
6. Biologisch aktive Nanopartikel nach Anspruch 1 mit einer Oberfläche zwischen 30 und 60 m²/g.
7. Biologisch aktive Nanopartikel nach Anspruch 1, die eine im Wesentlichen nadelförmige oder plättchenförmige Gestalt haben und eine Länge L zwischen 50 und 150 nm und eine Breite W zwischen 5 und 20 nm aufweisen.
8. Biologisch aktive Nanopartikel nach einem der Ansprüche 1-7, die weiterhin eine wirksame Menge eines antibakteriellen Ions umfassen.
9. Biologisch aktive Nanopartikel nach Anspruch 8, die in Bezug auf den gesamten Ca-Gehalt zwischen 0,1 und 20 Gew.-% eines in die Hydroxyapatitstruktur substituierten antibakteriellen Metall M-Ions umfassen.
10. Biologisch aktive Nanopartikel nach Anspruch 9, wobei das Metall M ausgewählt ist aus der Gruppe umfassend Zn, Cu, Ag und Gemische davon.
11. Biologisch aktive Nanopartikel nach Anspruch 9 mit einem Molverhältnis (Ca + M)/P größer als 1,7.
12. Biologisch aktive Nanopartikel nach Anspruch 9 mit der Formel:



wobei x eine Zahl zwischen 0,0055 und 0,6, y eine Zahl zwischen 0,065 und 0,9 und z eine Zahl zwischen 0 und 0,32 ist.

13. Zusammensetzung, die biologisch aktive Nanopartikel nach einem der vorangehenden Ansprüche umfasst.
14. Zusammensetzung nach Anspruch 13 in einer für die Mundhygiene geeigneten Form.
15. Zusammensetzung nach Anspruch 13 oder 14 in Form einer Suspension, eines Öls, eines Gels oder eines Feststoffes.
16. Zusammensetzung nach Anspruch 15 in Form einer Suspension, die 1 bis 40 Gew.-% von biologisch aktiven Nanopartikeln enthält.
17. Zusammensetzung nach Anspruch 15, deren pH zwischen 7 und 8 beträgt.
18. Zusammensetzung nach Anspruch 15 in der Form von Zahnpasta, Zahnpulver, Kaugummi für die Mund- und Zahnhygiene, Salbe für das Zahnfleisch, Mundspülung, Mundbad-Konzentrat und Gurgelwasser.
19. Zusammensetzung nach Anspruch 14, die eine Kombination aus biologisch aktiven Nanopartikeln umfasst, die eine wirksame Menge eines antibakteriellen Ions und biologisch aktive Nanopartikel ohne dieses Ion umfassen.
20. Verfahren für die Herstellung einer wässrigen Suspension, die biologisch aktive Nanopartikel nach Anspruch 1 umfasst, welches die folgenden Schritte umfasst:

- a) Herstellung einer wässrigen Lösung oder Suspension einer Ca-Verbindung;
 - b) Bildung von Nanopartikeln aus carbonatsubstituiertem Hydroxyapatit durch Zugabe von PO₄³⁻-Ionen zu der wässrigen Lösung oder Suspension aus Schritt a), wobei diese gleichzeitig über einen Zeitraum von zwischen 30 Minuten bis 8 Stunden gemischt wird, während die Temperatur der Lösung oder Suspension bei 60 °C oder darunter gehalten wird.
 - c) Mischen der Nanopartikel-Suspension aus Schritt b) über einen Zeitraum von mindestens zwei Stunden bei einer Temperatur von 60 °C oder darunter;
- wobei eine Carbonatsubstitution an der Phosphatstelle (B) der in Schritt b) gebildeten Hydroxyapatitverbindung auf eine der folgenden Weisen erzielt wird:

- i) Mischen der Lösung oder Suspension aus Schritt a) auf eine Art und Weise, die das in der Atmosphäre befindliche Kohlendioxid bindet,
 ii) Einperlen von Luft, einem CO_2 -haltigen Gas oder einer Mischung davon in die wässrige Lösung oder Suspension aus Schritt a);
 5 iii) Kombination aus mechanischem Rühren der Lösung oder Suspension aus Schritt a) und Einperlen von Luft, einem CO_2 -haltigen Gas oder einer Mischung davon in diese wässrige Lösung oder Suspension; oder
 iv) Zugabe einer CO_3^{2-} -Ionen umfassenden Lösung in die wässrige Lösung oder Suspension aus Schritt a).
21. Verfahren nach Anspruch 20, wobei Schritt a) im Wesentlichen in Abwesenheit von oral nicht verträglichen Anionen
 10 ausgeführt wird.
22. Verfahren nach Anspruch 20, wobei Schritt b) ausgeführt wird, indem zu der wässrigen Lösung oder Suspension
 aus Schritt a) eine wässrige Lösung, die PO_4^{3-} -Ionen und wahlweise des Weiteren HCO_3^- -Ionen umfasst, hinzuge-
 15 geben wird oder indem gleichzeitig eine erste Lösung, die CO_3^{2-} -Ionen umfasst, und eine zweite Lösung, die PO_4^{3-} -
 Ionen umfasst, zu der wässrigen Lösung oder Suspension aus Schritt a) hinzugegeben wird oder unter Aufrecht-
 erhaltung einer Temperatur der Lösung oder Suspension zwischen 25 °C und 40 °C.
23. Verfahren nach Anspruch 20, wobei die in Schritt c) gewonnene Suspension einen pH-Wert zwischen 7 und 8
 20 aufweist.
24. Verfahren nach einem der Ansprüche 20-23, wobei die wässrige Lösung oder Suspension aus Schritt a) des Weiteren
 ein Oxid oder ein Salz eines antibakteriellen Metalls M umfasst.
25. Verfahren nach Anspruch 24, wobei dieses Metallsatz ein oral verträgliches organisches oder anorganisches Salz,
 25 ausgewählt aus der Gruppe umfassend Lactate, Gluconate, Citrate, Acetate und Hydroxide, ist.
26. Verfahren nach einem der Ansprüche 24 oder 25, wobei Schritt b) so ausgeführt wird, dass das Verhältnis zwischen
 dem Ca und den Metall M-Ionen, die in der Lösung oder Suspension aus Schritt a) enthalten sind, und den hinzu-
 30 gegebenen PO_4^{3-} -Ionen größer als 1,7 ist.
27. Verfahren für die Herstellung biologisch aktiver Nanopartikel nach einem der Ansprüche 1-12, welches die folgenden
 Schritte umfasst:
- 35 a) Herstellung einer wässrigen Suspension, die diese Nanopartikel umfasst, mithilfe eines Verfahrens nach
 einem der Ansprüche 20-26;
 b) Trennung der festen Nanopartikel aus der in Schritt a) gewonnenen Suspension;
 c) Trocknung der so gewonnenen nassen, festen Nanopartikel.
28. Verfahren nach Anspruch 27, welches weiterhin den folgenden Schritt umfasst:
- 40 d) Waschen der abgetrennten, festen Nanopartikel mit Wasser oder einer basischen Lösung vor Beginn des
 Trocknungsschritts c).
29. Verfahren zur Herstellung einer Zahnpasta, die biologisch aktive Nanopartikel nach einem der Ansprüche 1-12
 45 umfasst, welches die folgenden Schritte umfasst:
- a) Herstellung einer wässrigen Suspension, die diese Nanopartikel umfasst, mithilfe eines Verfahrens nach
 einem der Ansprüche 20-26;
 50 b) Mischen dieser wässrigen Suspension mit anderen Inhaltsstoffen der Zahnpasta.
30. Verfahren für die Herstellung einer Zahnpasta, die biologisch aktive Nanopartikel nach einem der Ansprüche 1-12
 umfasst, welches die folgenden Schritte umfasst:
- 55 a) Herstellung fester Nanopartikel mithilfe eines Verfahrens nach einem der Ansprüche 27 oder 28;
 b) Mischen dieser festen Nanopartikel mit anderen Inhaltsstoffen der Zahnpasta.
31. Zusammensetzung nach einem der Ansprüche 14-19 zur Verwendung für die lokale Remineralisierung der Zähne.

Revendications

1. Nanoparticules biologiquement actives d'une hydroxyapatite non stoechiométrique substituée par un carbonate, ayant :

a) un degré de cristallinité CD inférieur à 40 %, le degré de cristallinité étant défini par

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

dans laquelle :

Y = maximum d'intensité de diffraction à $2\theta = 33^\circ$, X = intensité de la diffraction de fond à $2\theta = 33^\circ$ du diagramme de diffraction des rayons X des nanoparticules ;

b) une longueur L allant de 20 à 200 nm et une largeur W allant de 5 à 30 nm ; et
c) un rapport d'aspect AR compris entre 2 et 40, le rapport d'aspect étant défini par

$$AR = L/W.$$

2. Nanoparticules biologiquement actives selon la revendication 1, comprenant de 1 % à 15 % en poids de carbonate substitué dans la structure d'hydroxyapatite.

3. Nanoparticules biologiquement actives selon la revendication 1, dans lesquelles un rapport A/B entre une substitution de carbonate au niveau du site hydroxyle (A) et une substitution de carbonate au niveau du site phosphate (B) de l'hydroxyapatite est compris entre 0,05 et 0,5.

4. Nanoparticules biologiquement actives selon la revendication 1 ou 3, dans lesquelles la substitution de carbonate au niveau du site phosphate (B) de l'hydroxyapatite est supérieure ou égale à 65 % en poids du carbonate total présent dans l'hydroxyapatite.

5. Nanoparticules biologiquement actives selon la revendication 1, ayant un degré de cristallinité CD compris entre 25 % et 35 %.

6. Nanoparticules biologiquement actives selon la revendication 1, ayant une surface comprise entre 30 et 60 m²/g.

7. Nanoparticules biologiquement actives selon la revendication 1, ayant une forme sensiblement aciculaire ou plaquettaire ayant une longueur L comprise entre 50 et 150 nm et une largeur W comprise entre 5 et 20 nm.

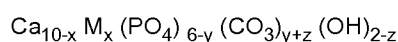
8. Nanoparticules biologiquement actives selon l'une quelconque des revendications 1 à 7, comprenant en outre une quantité efficace d'un ion antibactérien.

9. Nanoparticules biologiquement actives selon la revendication 8, comprenant de 0,1 à 20 % en poids par rapport à la teneur totale en Ca d'un ion M métallique antibactérien substitué dans la structure d'hydroxyapatite.

10. Nanoparticules biologiquement actives selon la revendication 9, dans lesquelles ledit métal M est choisi dans le groupe comprenant le Zn, le Cu, l'Ag et les mélanges de ceux-ci.

11. Nanoparticules biologiquement actives selon la revendication 9, ayant un rapport molaire (Ca + M)/P supérieur à 1,7.

12. Nanoparticules biologiquement actives selon la revendication 9, ayant la formule :



dans laquelle x est un nombre compris entre 0,0055 et 0,6, y est un nombre compris entre 0,065 et 0,9 et z est un nombre compris entre 0 et 0,32.

13. Composition comprenant des nanoparticules biologiquement actives selon l'une quelconque des revendications précédentes.

14. Composition selon la revendication 13, sous une forme appropriée pour l'hygiène buccale.

15. Composition selon la revendication 13 ou 14, sous la forme de suspension, d'huile, de gel ou de solide.

16. Composition selon la revendication 15, sous la forme d'une suspension comprenant de 1 % à 40 % en poids de nanoparticules biologiquement actives.

17. Composition selon la revendication 15, ayant un pH compris entre 7 et 8.

18. Composition selon la revendication 15, sous la forme de pâte dentifrice, de poudre dentaire, de gomme à mâcher pour l'hygiène bucco-dentaire, d'onguent pour les gencives, de bain de bouche et de concentré pour bain de bouche et de gargarisme.

19. Composition selon la revendication 14, comprenant une combinaison de nanoparticules biologiquement actives comprenant une quantité efficace d'un ion antibactérien et de nanoparticules biologiquement actives sans ledit ion.

20. Procédé de préparation d'une suspension aqueuse comprenant des nanoparticules biologiquement actives selon la revendication 1, comprenant les étapes de :

a) préparation d'une solution ou d'une suspension aqueuse d'un composé de Ca ;

b) formation de nanoparticules d'une hydroxyapatite substituée par un carbonate par l'ajout d'ions PO_4^{3-} à la solution ou la suspension aqueuse de l'étape a) tout en agitant simultanément celle-ci sur une durée comprise entre 30 minutes et 8 heures tout en maintenant ladite solution ou suspension à une température inférieure ou égale à 60 °C ;

c) agitation de la suspension de nanoparticules obtenue à l'étape b) sur une durée d'au moins deux heures à une température inférieure ou égale à 60 °C ;

dans lequel une substitution de carbonate au niveau du site phosphate (B) du composé d'hydroxyapatite formé à ladite étape b) est obtenue par l'une quelconque de :

i) agitation de ladite solution ou suspension de l'étape a) de manière à capturer le dioxyde de carbone présent dans l'atmosphère,

ii) barbotage d'air, d'un gaz contenant du CO_2 ou d'un mélange de ceux-ci dans la solution ou la suspension aqueuse de l'étape a)

iii) combinaison d'une agitation mécanique de la solution ou la suspension de l'étape a) avec un barbotage d'air, d'un gaz contenant du CO_2 ou d'un mélange de ceux-ci dans ladite solution ou suspension ; ou

iv) ajout d'une solution contenant des ions CO_3^{2-} à la solution ou la suspension aqueuse de l'étape a).

21. Procédé selon la revendication 20, dans lequel l'étape a) est réalisée en absence substantielle d'anions non acceptables par voie orale.

22. Procédé selon la revendication 20, dans lequel l'étape b) est réalisée par l'ajout d'une solution aqueuse comprenant des ions PO_4^{3-} , comprenant éventuellement en outre des ions HCO_3^- , à la solution ou la suspension aqueuse de l'étape a) ou par l'ajout simultané d'une première solution comprenant des ions CO_3^{2-} et d'une deuxième solution contenant des ions PO_4^{3-} à la solution ou la suspension aqueuse de l'étape a) ou tout en maintenant ladite solution ou suspension à une température comprise entre 25 °C et 40 °C.

23. Procédé selon la revendication 20, dans lequel la suspension obtenue à l'étape c) a un pH compris entre 7 et 8.

24. Procédé selon l'une quelconque des revendications 20 à 23, dans lequel la solution ou la suspension aqueuse de l'étape a) comprend en outre un oxyde ou un sel d'un métal antibactérien M.

25. Procédé selon la revendication 24, dans lequel ledit sel métallique est un sel organique ou inorganique acceptable par voie orale choisi dans le groupe comprenant :

les lactates, les gluconates, les citrates, les acétates et les hydroxydes.

26. Procédé selon l'une quelconque des revendications 24 ou 25, dans lequel l'étape b) est réalisée de telle sorte que le rapport des ions Ca et des ions de métal M contenus dans la solution ou la suspension de l'étape a) et les ions PO_4^{3-} ajoutés à celle-ci est supérieur à 1,7.

27. Procédé de préparation de nanoparticules biologiquement actives selon l'une quelconque des revendications 1 à 12, comprenant les étapes de :

- a) préparation d'une suspension aqueuse comprenant lesdites nanoparticules par un procédé selon l'une quelconque des revendications 20 à 26 ;
- b) séparation des nanoparticules solides à partir de la suspension obtenue à l'étape a) ;
- c) séchage des nanoparticules solides humides ainsi obtenues.

28. Procédé selon la revendication 27, comprenant en outre l'étape de

- d) lavage des nanoparticules solides séparées avec de l'eau ou une solution basique avant d'effectuer ladite étape de séchage c).

29. Procédé de fabrication d'une pâte dentifrice comprenant des nanoparticules biologiquement actives selon l'une quelconque des revendications 1 à 12, comprenant les étapes de :

- a) préparation d'une suspension aqueuse comprenant lesdites nanoparticules par un procédé selon l'une quelconque des revendications 20 à 26 ;
- b) mélange de ladite suspension aqueuse avec d'autres ingrédients de la pâte dentifrice.

30. Procédé de fabrication d'une pâte dentifrice comprenant des nanoparticules biologiquement actives selon l'une quelconque des revendications 1 à 12, comprenant les étapes de :

- a) préparation de nanoparticules solides par un procédé selon l'une quelconque des revendications 27 ou 28 ;
- b) mélange des nanoparticules solides avec d'autres ingrédients de la pâte dentifrice.

31. Composition selon l'une quelconque des revendications 14 à 19 pour une utilisation dans la reminéralisation locale des dents.

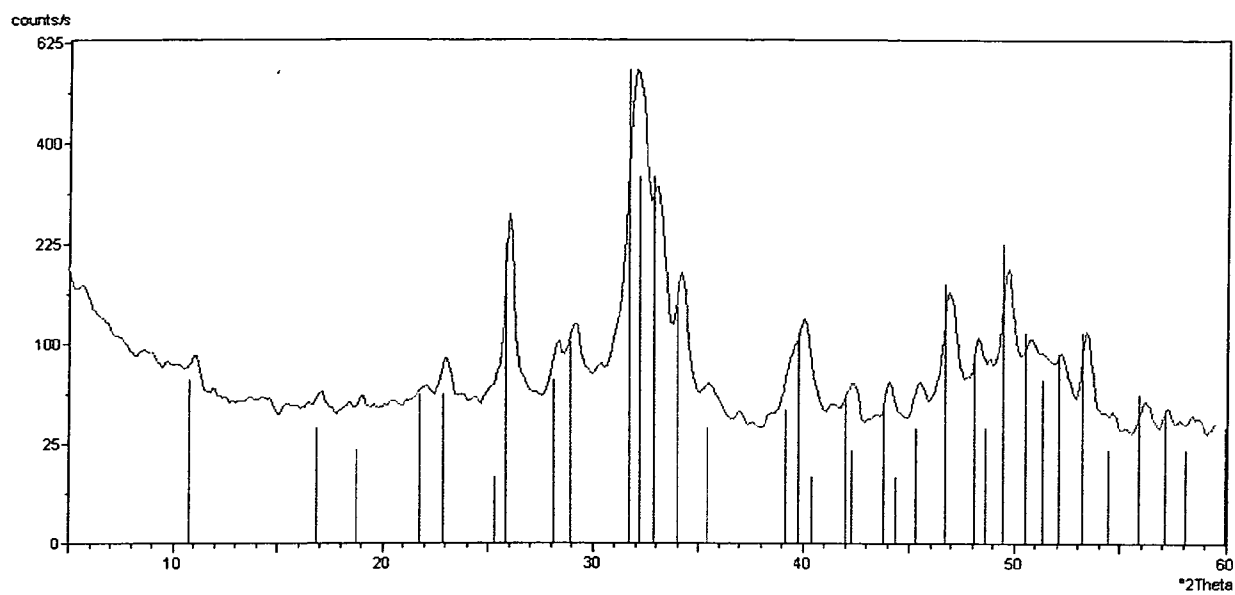


Fig. 1

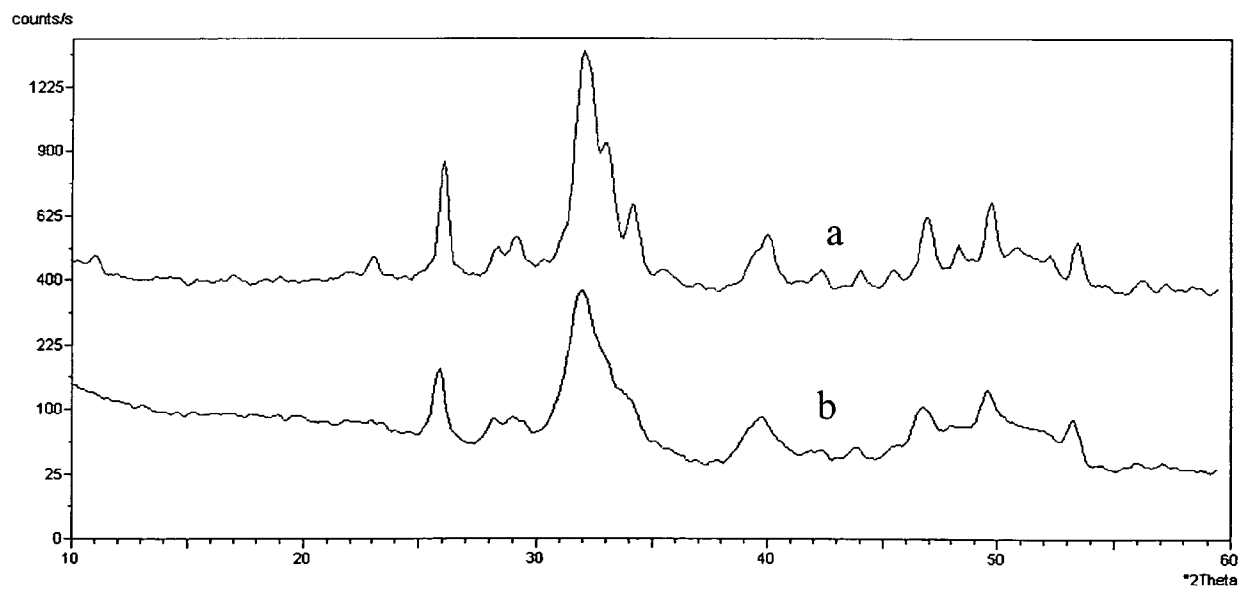


Fig. 2

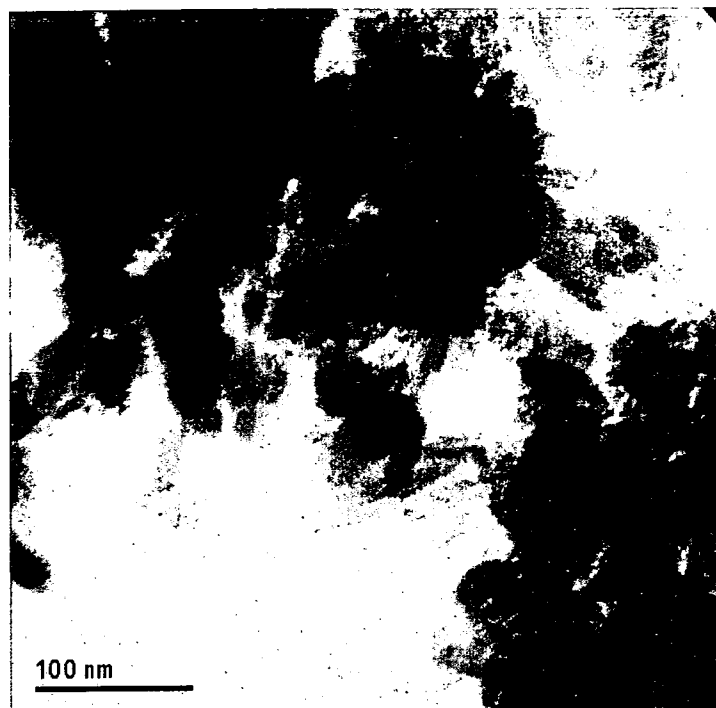


Fig. 3a



Fig. 3b

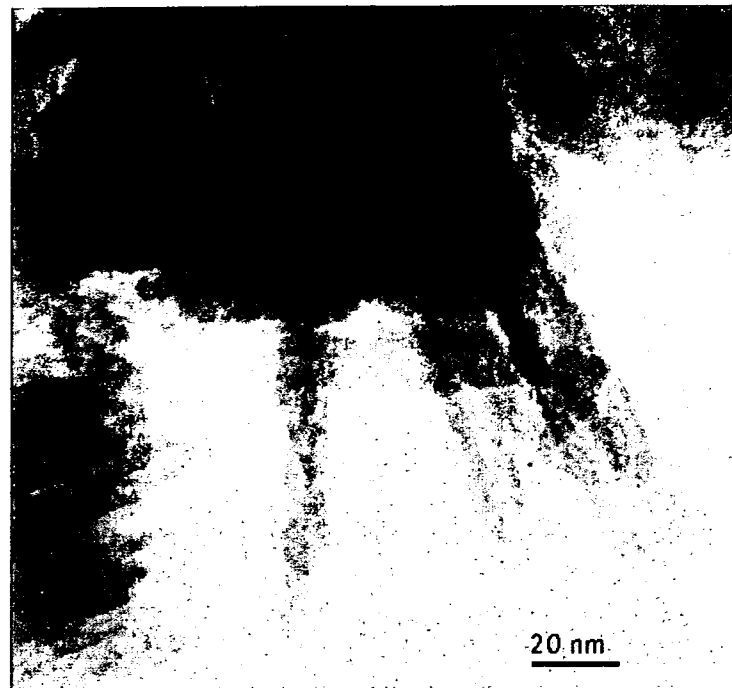


Fig. 3c

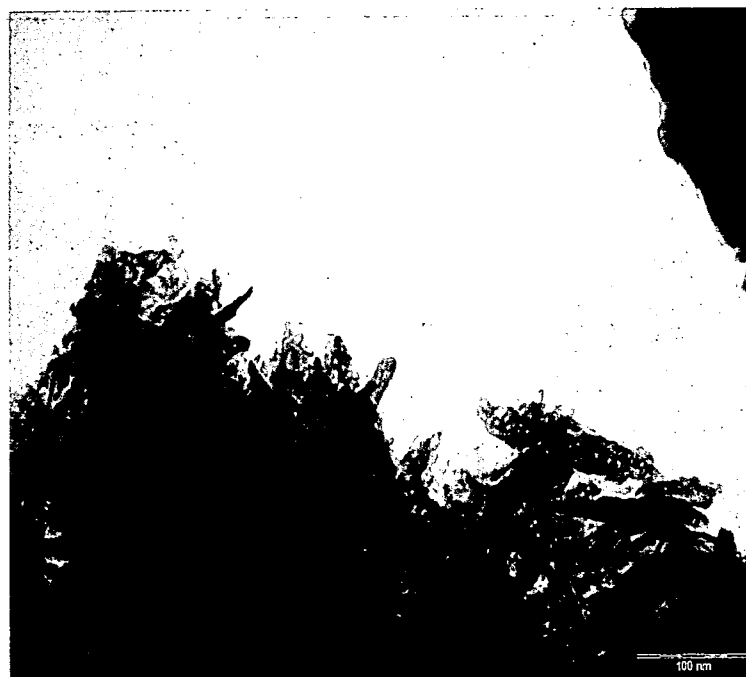


Fig. 3d

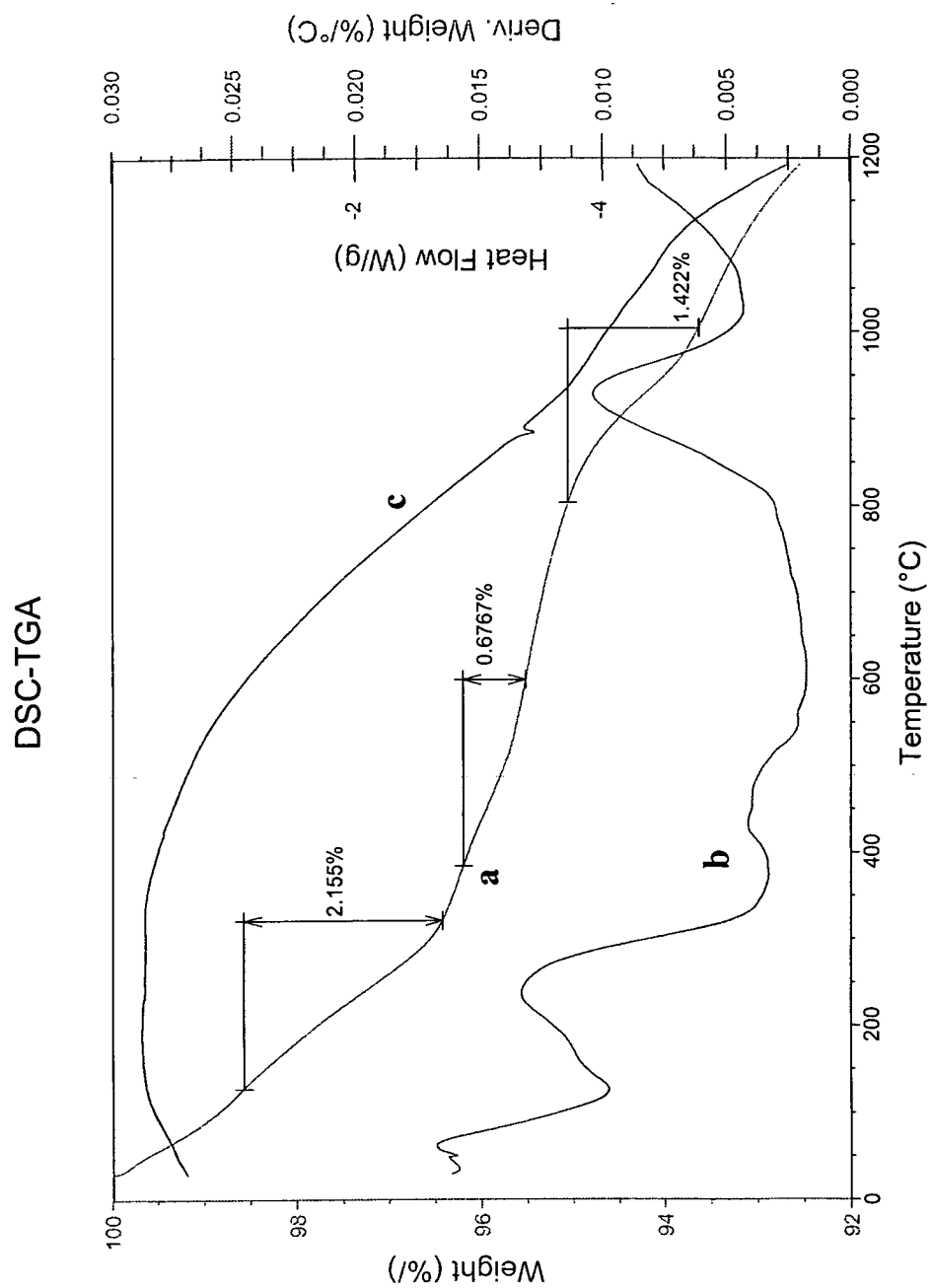


Fig. 4

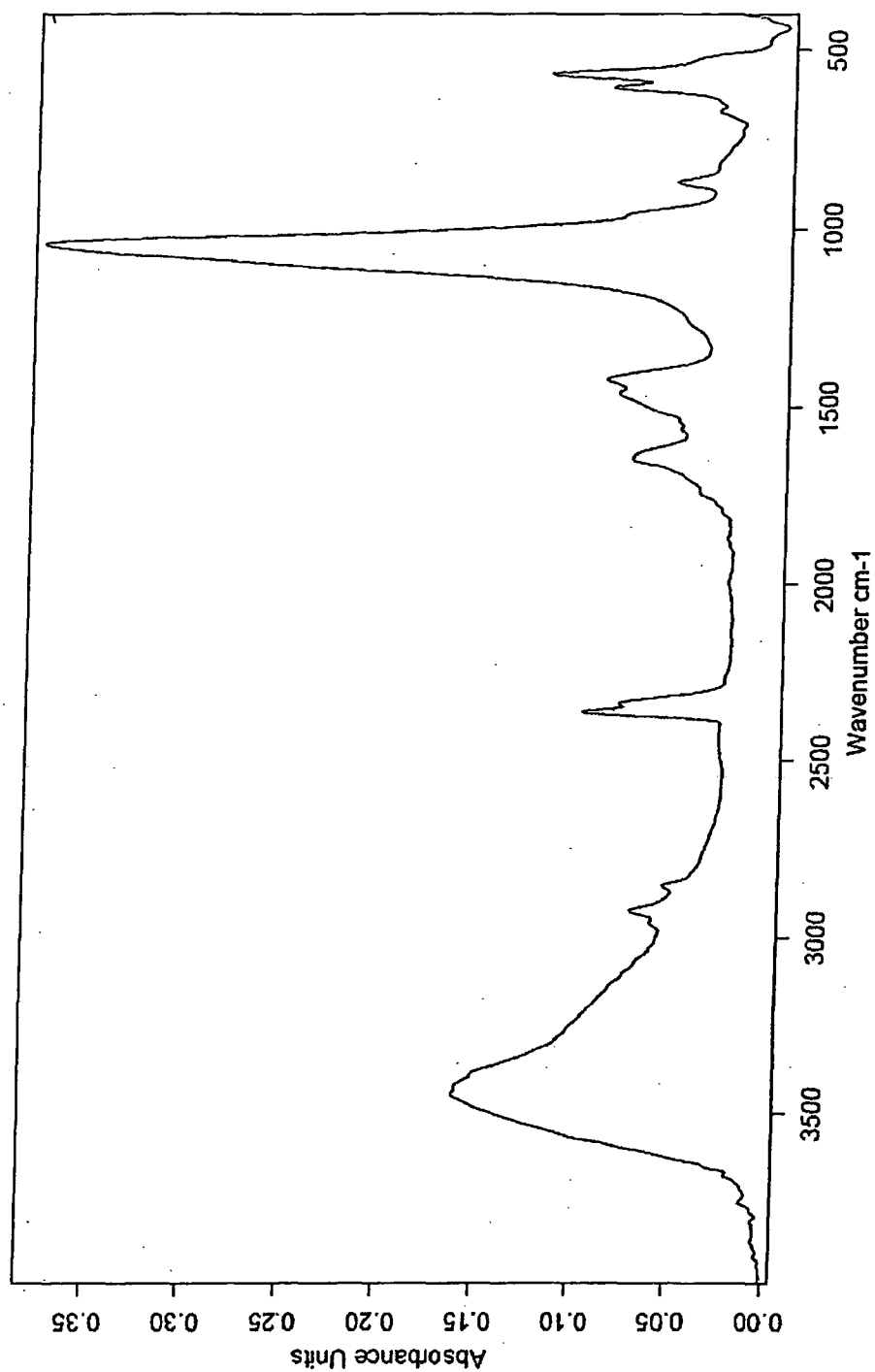


Fig. 5

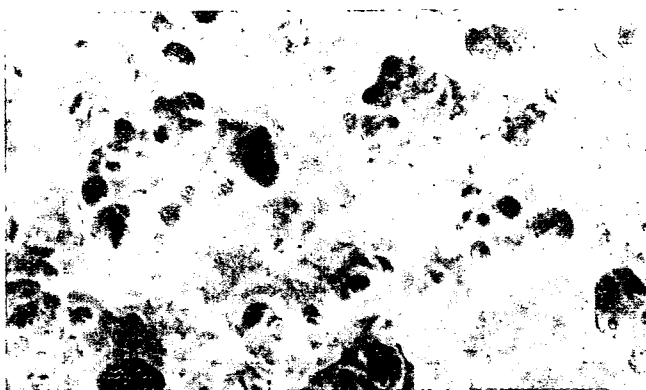


Fig. 6a



Fig. 6b

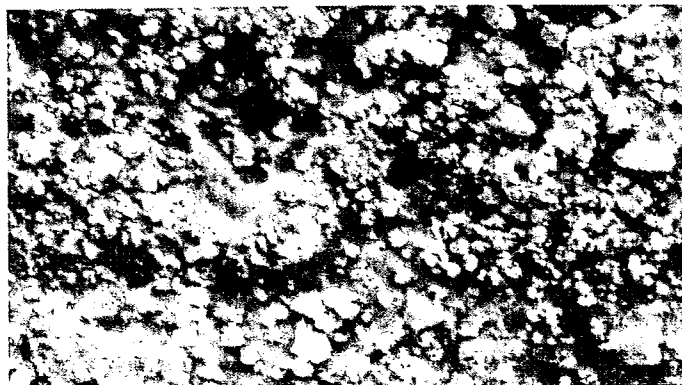


Fig. 6c

REFERENCES CITED IN THE DESCRIPTION

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Szabadalmi igénypontok

1. Karbonát-szubsztituált nem sztöchiometrikus hidroxipatit biológiailag aktív nanorészecskéi, amelyek

a) kristályossági foka (CD) 40%-nál alacsonyabb, a kristályossági fok az alábbi képlet szerinti

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

ahol:

Y jelentése a diffrakciós maximum magassága $2\theta = 33^\circ$ értéknél, X jelentése a diffrakciós háttér magassága $2\theta = 33^\circ$ értéknél a nanorészecskék röntgendiffrakciós mintázatán;

b) hossza (L) 20-200 nm és szélessége (W) 5-30 nm; és

c) oldalaránya (AR) 2 és 40 közötti, az oldalarány az alábbi képlet szerinti:

$$AR = L/W.$$

2. Az 1. igénypont szerinti biológiailag aktív nanorészecskék, amelyek 1-15 tömeg% karbonátot tartalmaznak a hidroxipatit szerkezetbe szubsztituálva.

3. Az 1. igénypont szerinti biológiailag aktív nanorészecskék, ahol a hidroxipatit hidroxil helyén történő karbonátszubsztitúció (A) és a foszfát helyén történő karbonátszubsztitúció (B) közötti arány (A/B) 0,05 és 0,5 közötti.

4. Az 1. vagy 3. igénypont szerinti biológiailag aktív nanorészecskék, ahol a karbonátszubsztitúció a hidroxipatit foszfát helyén (B) 65 tömeg% vagy annál nagyobb a hidroxipatitban jelenlévő teljes karbonátra számítva.

5. Az 1. igénypont szerinti biológiailag aktív nanorészecskék, amelyek kristályossági foka (CD) 25% és 35% közötti.

6. Az 1. igénypont szerinti biológiailag aktív nanorészecskék, amelyek felülete 30 és 60 m²/g közötti.

7. Az 1. igénypont szerinti biológiailag aktív nanorészecskék, amelyek lényegében tükrisztályos vagy lemezke alakúak 50 és 150 nm közötti hosszal (L) és 5 és 20 nm közötti szélességgel (W).

8. Az 1-7. igénypontok bármelyike szerinti biológiailag aktív nanorészecskék, amelyek tartalmazzák továbbá egy antibakteriális ion hatásos mennyiségét.

9. A 8. igénypont szerinti biológiailag aktív nanorészecskék, amelyek a teljes Ca-tartalomra számítva 0,1-20 tömeg% antibakteriális fémiont (M) tartalmaznak a hidroxipatit szerkezetbe szubsztituálva.

10. A 9. igénypont szerinti biológiailag aktív nanorészecskék, ahol a fém (M) Zn, Cu, Ag és ezek keverékei köréből választott.

11. A 9. igénypont szerinti biológiailag aktív nanorészecskék, amelyek (Ca+M)/P molaránya magasabb mint 1,7.

12. A 9. igénypont szerinti biológiailag aktív nanorészecskék, az alábbi képlet szerint:



ahol x jelentése egy 0,0055 és 0,6 közötti szám, y jelentése egy 0,065 és 0,9 közötti szám és z jelentése egy 0 és 0,32 közötti szám.

13. Egy az előző igénypontok bármelyike szerinti biológiailag aktív nanorészecskéket tartalmazó kompozíció.

14. Egy 13. igénypont szerinti kompozíció, szájhygiénára alkalmas alakban.

15. Egy 13. vagy 14. igénypont szerinti kompozíció, szuszpenzió, olaj, gél vagy szilárd alakban.

16. Egy 15. igénypont szerinti kompozíció szuszpenzió alakban, amely 1-40 tömeg% biológiailag aktív nanorészecskéket tartalmaz.

17. Egy 15. igénypont szerinti kompozíció, amelynek pH értéke 7 és 8 közötti.

18. Egy 15. igénypont szerinti kompozíció az alábbi alakban: fogkrém, fogpor, rágógumi száj- és foghygiénához, fogínykenőcs, szájvíz és szájöblítő koncentrátum és gargalizáló oldat.

19. Egy 14. igénypont szerinti kompozíció, amely egy antibakteriális ion hatásos mennyiségét tartalmazó biológiailag aktív nanorészecskék és az iont nem tartalmazó biológiailag aktív nanorészecskék kombinációját tartalmazza.

20. Eljárás egy az 1. igénypont szerinti biológiailag aktív nanorészecskéket tartalmazó vizes szuszpenzió előállítására, amely az alábbi lépéseket tartalmazza:

a) egy Ca vegyület vizes oldatának vagy szuszpenziójának előállítása;

b) karbonát-szubsztituált hidroxipatit nanorészecskék kialakítása PO_4^{3-} -ionok hozzáadásával az a) lépésben kapott vizes oldathoz vagy szuszpenzióhoz, mialatt azt egyidejűleg 30 perc és 8 óra közötti időtartamig keverjük, amely alatt az oldatot vagy szuszpenziót 60°C vagy annál alacsonyabb hőmérsékleten tartjuk;

c) a b) lépésben kapott nanorészecske szuszpenzió keverése legalább 2 óráig 60°C vagy annál alacsonyabb hőmérsékleten;

ahol a karbonátszubsztitúció a b) lépésben kialakított hidroxipatit vegyület foszfát helyén (B) az alábbiak köréből választott bármely módon valósul meg:

i) az a) lépésben kapott oldat vagy szuszpenzió keverése oly módon, hogy a levegőben lévő széndioxidot elnyelje;

ii) levegő, CO_2 -tartalmú gáz vagy ezek keverékének buborékoltatása az a) lépésben kapott vizes oldatba vagy szuszpenzióba;

iii) az a) lépésben kapott oldat vagy szuszpenzió mechanikai keverésének kombinálása levegő, CO_2 -tartalmú gáz vagy ezek keverékének buborékoltatásával az oldatba vagy szuszpenzióba; vagy

iv) egy CO_3^{2-} -ionokat tartalmazó oldat hozzáadása az a) lépésben kapott vizes oldathoz vagy szuszpenzióhoz.

21. Egy 20. igénypont szerinti eljárás, ahol az a) lépést lényegében orálisan nem elfogadható anionok jelenléte nélkül hajtjuk végre.

22. Egy 20. igénypont szerinti eljárás, ahol a b) lépést úgy hajtjuk végre, hogy egy PO_4^{3-} -ionokat tartalmazó, adott esetben továbbá HCO_3^- -ionokat tartalmazó vizes oldatot adunk az a) lépésben kapott vizes oldathoz vagy szuszpenzióhoz, vagy egyidejűleg egy CO_3^{2-} -ionokat tartalmazó első oldatot és egy PO_4^{3-} -ionokat tartalmazó második oldatot adunk az a) lépésben kapott vizes oldathoz vagy szuszpenzióhoz, vagy mielőtt az oldatot vagy szuszpenziót 25 és 40°C közötti hőmérsékleten tartjuk.

23. Egy 20. igénypont szerinti eljárás, ahol a c) lépésben kapott szuszpenzió pH értéke 7 és 8 közötti.

24. Egy 20-23. igénypontok bármelyike szerinti eljárás, ahol az a) lépésben kapott vizes oldat vagy szuszpenzió tartalmazza továbbá egy antibakteriális fém (M) egy oxidját vagy sóját.

25. Egy 24. igénypont szerinti eljárás, ahol a fém só egy orálisan elfogadható szerves vagy szervetlen só az alábbiak köréből választva: laktátok, glukonátok, citrátok, acetátok és hidroxidok.

26. Egy 24. vagy 25. igénypontok bármelyike szerinti eljárás, ahol a b) lépést úgy hajtjuk végre, hogy az a) lépésben kapott oldatban vagy szuszpenzióban lévő Ca- és (M) fémionok és az ehhez hozzáadott PO_4^{3-} -ionok aránya magasabb mint 1,7.

27. Eljárás az 1-12. igénypontok bármelyike szerinti biológiailag aktív nanorészecskék előállítására, amely az alábbi lépéseket tartalmazza:

- a) egy a nanorészecskéket tartalmazó vizes szuszpenzió előállítása egy 20-26. igénypontok bármelyike szerinti eljárással;
- b) szilárd nanorészecskék elválasztása az a) lépésben kapott szuszpenziótól;
- c) az így kapott nedves szilárd nanorészecskék szárítása.

28. Egy 27. igénypont szerinti eljárás, amely tartalmazza továbbá az alábbi lépést:

- d) az elválasztott szilárd nanorészecskék mosása vízzel vagy egy lúgos oldattal a c) szárítási lépés végrehajtása előtt.

29. Eljárás az 1-12. igénypontok bármelyike szerinti biológiailag aktív nanorészecskéket tartalmazó fogkrém előállítására, amely az alábbi lépéseket tartalmazza:

- a) egy a nanorészecskéket tartalmazó vizes szuszpenzió előállítása egy 20-26. igénypontok bármelyike szerinti eljárással;
- b) a vizes szuszpenzió vegyítése a fogkrém egyéb összetevőivel.

30. Eljárás az 1-12. igénypontok bármelyike szerinti biológiailag aktív nanorészecskéket tartalmazó fogkrém előállítására, amely az alábbi lépéseket tartalmazza:

- a) szilárd nanorészecskék előállítása egy 27. vagy 28. igénypontok bármelyike szerinti eljárással;
- b) a szilárd nanorészecskék vegyítése a fogkrém egyéb összetevőivel.

31. Egy 14-19. igénypontok bármelyike szerinti kompozíció a fogak helyi remineralizációjában való alkalmazásra.