An Experimental Method of Producing Hydroxyapatite

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The main factor in maintaining oral hygiene is first and foremost the correct dental brushing and the use of proper sanitizing means: toothbrush, toothpaste, dental floss, mouthwash. Toothpastes containing hydroxyapatite have an increased efficiency due to the ability to re-mineralize dental enamel. In view of the appearance of the secondary reaction products, the most acceptable variant for HA production is the reaction between calcium hydroxide and phosphoric acid, because it does not result any undesirable side products at a pH > 9 units. The purpose of this study was to produce and then analyse the HA properties produced experimentally in the SETICO laboratories, using a device derived from the already patented: "Process and device for the production of nano-gels and micro-gels based on alkaline-earthy silicates" issued by OSIM Bucharest on 02.26.2016 No. 128 480.For the analysis of the HA emulsion the following devices were used: The zeta potential analyzer: WALLIS and the VASCO particle size analyser. The contribution of this method is that it provides information and results for the situation where pure hydroxyapatite is used without secondary reaction products. At pH = 7.21 there are particles of ~ 170 nm, very stable (Zeta-potential -133mV) and at pH = 12.64 there are particles of ~ 148nm and more stable (Zeta-potential + 359mV).

Keywords: hydroxyapatite, nano-hydroxyapatite remineralization, Zeta-potential, sub-surface lesions

There is a generally valid principle according to which *to prevent is easier than to treat* and on the basis of which various studies have been carried in the United States of America as regards the prevention of various diseases. Establishing the doctor-patient relationship is very important, because this is the way by which patients may overcome fear, as well as preventing any medical emergencies. A good communication between the patient and the dentist is very helpful in the awareness of the importance of oral hygiene, helping to maintain oral health and the health of the entire body.

The oral cavity health is not due only to balanced nutrition but also to a correctly performed oral hygiene. Cleaning the teeth must begin before the first temporary teeth, each age requiring certain dental care features, in order to support the structural and functional evolution of the teeth.

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Toothpastes containing hydroxyapatite have an increased efficiency due to the ability to re-mineralize dental enamel. Hydroxyapatite (HA) is one of the biomaterials representative for resorbable materials, with a calcium phosphate composition. The use of calcium phosphate biomaterials for dental applications is due to the absence of toxic compounds and their resemblance to the mineral component of the human skeleton. HA is the main human skeletal crystalline component that was first synthetically produced around 1970 and used since 1980 as bioactive material.

HA is considered to be a biomaterial with a chemical structure very similar to that of the human bone, because the main form of calcium in this biomaterial is found in bone tissue, its adherence being alleviated due to this chemical composition resemblance. Hydroxyapatite (HA), is a natural occurring mineral of calcium apatite with the formula $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities [1]. Compared to enamel, the dentin contains only half of the amount of hydroxyapatite, having smaller crystals and containing less carbon. Thus due to the structure less mineralized. The dentin is softer than enamel, which allows a more rapid evolution of decay [2].

Dental cavities are a chronic, complex and continuous destructive process that evolves through the alternation of demineralization and remineralisation processes that take place at the level of dental rigid structures. Demineralisation phenomena occur under the influence of acids resulting from metabolism of the bacterial plaque that decreases oral ph, but also in the presence of acidic foods. Remineralisation processes are produced by re-precipitation of mineral salts accumulated at the dental plaque-tooth interface: calcium ions, phosphorus and fluoride ions coming from demineralised dental structures or from inorganic natural salivary components. The process of re-demineralization is governed by the degree of mineral saturation of oral fluids (saliva and plaque). Due to positive changes in conditions, remineralisation can become the predominant process leading to the healing of injuries. Such treatment can be also model for targeted dental lessions [3-7].

To improve remineralisation, it is necessary to increase the concentration of calcium and fluoride in oral fluids [8,9]. For this purpose, fluorides have traditionally been used in varied forms and, at the same time, the cariostatic mechanism can be explained by the increase in the strength of fluorapatites. Tooth remineralisation can be performed in dental practice using desensitizing gel or varnish applications, or at home, through a rigorous dental hygiene and the use of fluorinated toothpaste or special gels [10].

Significant degradation of carious processes in highly industrialized countries can be attributed to the widespread use of fluoride. This preventive effect is mainly due to calcium fluoride formation in the form of precipitation

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limiting demineralisation, while the level of fluoride needed for remineralisation is assumed to be higher than necessary to prevent lesion formation.

Nano-hydroxyapatite is considered one of the most biocompatible and bioactive materials that has gained acceptance in recent years in both general medicine and dental medicine [11]. While previous attempts to clinically use hydroxyapatite failed, the synthesis of hydroxyapatite with zinc carbonate proved to be an important and highly affective process.

Nano-HA particles synthesized in the laboratory are similar as morphology and structure with the enamel crystals of the tooth enamel. Recent studies have shown that nano-hydroxyapatites have the potential to stabilize lesions on the enamel surface (12). Also, nano-HA based products for the remineralisation of sub-surface lesions have been synthesized (13).

The purpose of this study was to produce and then analyse the HA properties produced experimentally in the SETICO laboratories, using a device derived from the already patented: *Process and device for the production of nano-gels and micro-gels based on alkaline-earthy silicates* issued by OSIM Bucharest on 02.26.2016 No. 128 480. For this, I studied the most common variants used for the production of hydroxyapatite quoted in the literature and I chose the easiest to use(14).

Experimental part

The main synthesis procedures of hydroxyapatite quoted in the literature are as follows:

The reaction between calcium hydroxide in suspension and phosphoric acid has the advantage that the reaction between calcium hydroxide and phosphoric acid does not result in undesirable side products at a *p*H > 9 units:

10 Ca(OH)2 + 6H3PO4 --- Ca10(PO4)6(OH)2 + H2O

If the *p*H is strongly alkaline >12 units, secondary reactions occur with the formation of tricalcium phosphate. 3Ca(OH)2 + 2H3PO4 --- 2Ca3(PO4)2 tricalcium phosphate + 6H2O

The reaction between triammonium phosphate and calcium nitrate at a pH of about 10 units results in a toxic by-product: ammonium nitrate, which requires further purification operations

6(NH4)3PO4 + 10 Ca(NO3)2 + 4H2O + 2NH3--- Ca10(PO4)6(OH)2 + 20NH4NO3

The reaction of monoacid diammonium phosphate with calcium nitrate, at a ph of 10-12 units, has as a result a toxic by-product, the ammonium nitrate which requires further purification

6(NH4)2HPO4+10Ca(NO3)2+8NH3 --- Ca10 (PO4)6 (OH)2 + 20 NH4NO3

Ther reaction between phosphoric acid, calcium hydrogen carbonate and sodium hydroxide, with sodium hydrogen carbonate as a by-product

H3PO4 + Ca(HCOO)2+ NaOH--- Ca10(PO4)6(OH)2 + HCOONa

In view of the appearance of the secondary reaction products, the most acceptable variant for HA production is the first reaction.

The reagents were analytical pure: calcium hydroxide from Appli Chem (Germany), phosphoric acid from MERCK (Germany) and bi-distilled water.

Description of the plant for HA production

The compressor (1) refutes compressed air with a 3 bar pressure at the entrance to the ejector type mixer at position no.(4). The piston dispensing pump from the (3) position sucks the 5% concentration of phosphoric acid solution in the measuring vessel number (2) and pushes it tangentially into the ejector type mixer (4), where, in contact with the compressed air jet, a gas-liquid type emulsion results. This gas-liquid type emulsion continuously feeds the mono-tubular reactor (5) equipped with aerodynamic, lenticular, convex/concave static mixers. The fluid jet runs at high speed in a laminar thin layer, the convex surface of the first static mixer where it meets the suspension of Ca $(OH)_2$, the chemical reaction being initiated on its surface and finished on the surface of the second static mixer with the same profile . The aqueous calcium hydroxide suspension is pumped from the spherical reservoir (10) by means of the membrane metering pump (9) at the reactor inlet (5). The reaction product: HA suspended in the emulsified air phase is strained into the gas / liquid / solid separator (6). The air is discharged through the separator side connection (6). The nano-scale HA suspension is collected in the Berzelius vessel (7). The collected product is immersed in the platinum electrode of a high precision ph-meter (8). The value of the *p*H is measured continuously, and its stabilization indicates a stably functioning of the HA synthesis plant. To keep the calcium hydroxide in suspension in the spherical vessel (10), it is subjected to continuous mixing with an anchor type stirrer, driven by a variable speed micro-motor (11).

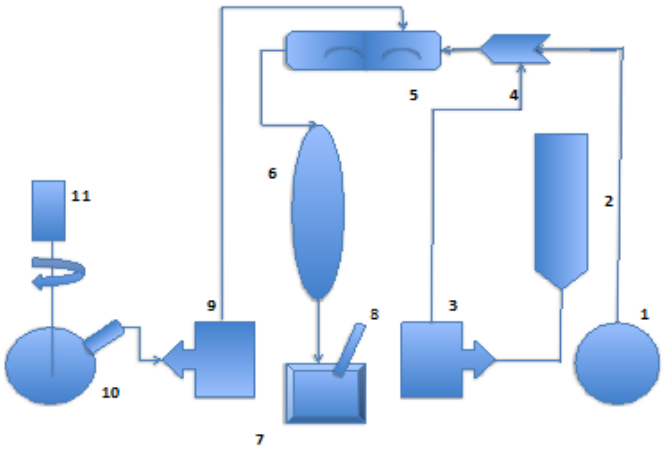

Fig. 1. Description of the plant for HA production

From the resulting emulsion several samples were used, which were considered more meaningful, namely: emulsion at *p*H = 7.2 and emulsion at *p*H = 12.64.

For the analysis of the HA emulsion the following devices were used: The zeta potential analyzer: WALLIS and the VASCO particle size analyser.

WALLIS is an innovative zeta potential analyzer dedicated to the characterization of nano-particle suspensions. It is based on a revisited and modern version of the Laser Doppler Electrophoresis (LDE) technique offering a unique and unequalled measurement resolution. It is complementary to the Cordouan's VASCO particle size analyzer to study colloidal solution stability and properties [12].

Based on a patented innovative technology developed by the French Petroleum Institute (IFP), the VASCO particle size analyser is a unique laboratory instrument for nano to micro particle size measurements in liquid. The instrument principle relies on Photon Correlation Spectroscopy (PCS) also called dynamic light scattering (DLS). Compared to conventional particle size analysers, the VASCO original optical design allows quasi-instantaneous measurements in dark and concentrated solutions without sample dilution or filtration. Powerful data collection and analysis is provided by the proprietary software nanoQ™ [13].

We performed the size analysis, respectively the stability (expressed as Zeta-potential) for the two selected HA samples, with the two measuring instruments described above. According to the specialty data, it is considered that a Zeta-potential between -30 and +30 mV indicates unstable particles [14].

Results and discussions

Report 1. The particle size of the hydroxyapatite sample at a *p*H = 7.21

I did 5 scans for a more effective evaluation. In this case, none of the acquisitions was corrected or rejected. I worked with a laser power: 27.5% (required by sample appearance), solvent: water, scan temperature: 25°C, the DTC position (mirror reflection): below. Particles of 3 sizes were obtained (I always report to the signal strength): 123nm (19% share), 170nm (20% share), 178nm (61% share).

Report Conclusion: average size / master curve 169.87nm.

Report 2. The particle size of the hydroxyapatite sample at a *p*H = 12.64

I did 5 scans for a more effective statistical evaluation (3 software acquisitions were rejected), with laser power: 27.5% (required by sample appearance), solvent: water, scan temperature: 25°C, the DTC position (mirror reflection). Perfectly homogeneous particles were obtained (I always report to the signal strength): 148nm (100% share).

Report Conclusion: average size / master curve 147.95nm.

Report 3. Stability of particles in the hydroxyapatite sample *p*H 7.21

I did 3 scans for a statistically efficient evaluation (none of the acquisitions was corrected or rejected) with laser power: 45%, solvent: water temperature, scanning: 25° C, average resolution, Henry function: Smoluchowski (recommended for aqueous). In this case, Zeta-potential was obtained: -137, -129 and -14mV respectively; so the device displays an average of -93.66mV. Personally, I recommend the exclusion of the last purchase.

Report conclusion: Average Zeta-potential -133.33mV

Report 4. Stability of particles in the hydroxyapatite sample *p*H 12.64

I did 3 scans for a statistically efficient evaluation (none of the acquisitions was corrected or rejected) with laser power: 45%, solvent: water temperature, scanning: 25°C, average resolution, Henry function: Smoluchowski (recommended for the aqueous) and Zeta-potentials have been obtained: +301, +416 and -36mV respectively; so the device displays an average of + 227mV. I personally recommend excluding the last purchase.

Report conclusion: Average Zeta-potential +358.71mV

Conclusions

The contribution of this method is that it provides information and results for the situation where pure hydroxyapatite is used without secondary reaction products. At *p*H = 7.21 there are particles of ~ 170 nm, very stable (Zeta-potential -133mV) and at *p*H = 12.64 there are particles of ~ 148nm and more stable (Zeta-potential + 359mV) [15].

Considering the stability of the samples obtained, they will be used in later studies to stabilize dental enamel lesions.

Acknowledgement: We would like to thank the support and interest of Dipl.Ing. Dr. Serban Florita, Senior Scientific Researcher at Setico S.R.L. for his support and encouragement in this study, and for the permission to use his laboratory.

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Manuscript received: 10.07.2017