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DEVELOPMENT, CHARACTERIZATION AND STABILITY STUDY OF NANOMETRIC SYSTEMS CONTAINING HYDROGEN PEROXIDE FOR DENTAL BLEACHING

Mahala Correia Cláudio[*1], Lorena Alves de Oliveira Silva [1], Juliano Geraldo Amaral [1] and Mateus Freire Leite [1, 2]

Instituto Multidisciplinar em Saúde [1], Universidade Federal da Bahia, Bahia, Brasil.
Faculdade de Ciências Farmacêuticas [2], Universidade Federal de Alfenas, Minas Gerais, Brasil.

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Correspondence to Author:
Mahala Correia Cláudio

Programa de Pós-graduação em Biociências, Universidade Federal da Bahia, Campus Anísio Teixeira, Rua Rio de Contas, Qd 17 - 58, Candeias Vitória da Conquista - BA.

E-mail: mahalacorreia@hotmail.com

ABSTRACT: The appreciation of appearance is increasingly widespread in the media and involves a smile-related beauty standard. Any change in teeth compromises the appearance and may reflect on the psychological and social behaviour of the human being. Consequently, there has been technological advance in aesthetic and technical restorative materials, such as dental bleaching, as well as the development of more effective drug delivery techniques, such as micro-emulsions and liquid crystals. In this work, dispersed systems containing hydrogen peroxide were developed, characterized and evaluated for dental bleaching agents, with permeation potential in the dentinal tubules in order to optimize the bleaching process. Two pseudo-ternary diagrams were developed. Two formulations of the second diagram were selected and characterized (optical microscopy, pH, particle size, polydispersity index, zeta potential), and then, preliminary and accelerated stability study. In these formulations, the hydrogen peroxide was incorporated in concentrations 6 and 14%. The photomicrographs of the formulations revealed dark field, characteristic of the cubic phases. The product containing 6% hydrogen peroxide obtained pH values of 6.06 ± 0.06; Zeta potential of -6.36 ± 0.6 mV; Droplet size of 9.63 ± 0.01 mV and Polydispersity index of 0.15 ± 0.002. The product containing 14% hydrogen peroxide obtained pH values of 5.93 ± 0.06; Zeta potential of -4.99 ± 0.3 mV; Droplet size of 9.62 ± 0.006 nm and polydispersity index of 0.16 ± 0.007. The rheological profile of the analyzed samples presented with thixotropic behaviour. The results suggest that the nanodisperse systems obtained are liquid crystals of cubic phase.

INTRODUCTION: The appearance has always been determinant in the relations among the individuals and constitutes a relevant factor in the interpersonal relationship [1 - 3]. There is also a beauty standard that involves the smile, which can be translated into clear, well-contoured and correctly aligned teeth [4 - 6].

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Being, therefore, increasing the search for the representation of perfect white smiles represented by the media [6, 7]. Unlike all other living beings, humans have the ability to smile, what make them so unique and special.

Smiling is a manifestation of a positive attitude towards the others, a symbol of joy and a weapon of seduction. For this reason, several people feel inhibited in emitting such facial expression due to their teeth are in bad shape [8]. Thus, any colour alteration of vital and non-vital teeth compromises this appearance and may reflect on the psychological and social behaviour of human being [9].

As a consequence, there has been a great technological advancement in aesthetic restorative materials field, as well as the emergence and the adoption of classical, simple and low-cost techniques, such as tooth whitening [10, 11, 12,] Therefore, a smile that exhibits healthy teeth accentuates facial expression and indicates self-esteem [13, 14]. Teeth darkening varies according to several factors, such as appearance, location, severity and adhesion to the dental structure of the darkening pigment. Moreover, the incorporation of pigments in teeth can occur intrinsically or extrinsically [10, 12, 15 - 17]. Extrinsic stains are acquired from the medium by ingestion of substances containing colorants, such as coffee, tobacco, use of some medications, like tetracycline [18] and plate accumulation, being sort of easy removal.

On the other hand, intrinsic stains can occur as in the case of imperfect dentinogenesis, fetal erytroblastosis [18] or acquired from dental trauma, mortification and fluorosis, and require more elaborate procedures for their removal [16, 20]. As an alternative to improve the mouth appearance and reduce tooth discoloration, tooth whitening emerges arousing interest in the population as well as in the scientific community, which led to increased related scientific technique and researches [20, 10, 21, 22]. With this in mind, when thinking about tooth whitening, it must be considered that the dental structure is permeable to bleaching agents, able to diffuse freely through the tooth promoting the desired effect [6, 12]. Haywood and Heymann detailed home or supervised dental whitening technique in 1989. Several works were performed in vitro and in situ to analyzing product effects on the tooth structure. Thus, it was found either home whitening therapy or supervised by a dentist, if it is conducted correctly, it does not harm dental tissues and structures, brings satisfactory results [9, 10, 23, 24, 25].

The difference between those therapies is related to product concentration and time of use [25]. Regarding the mechanism of action, these systems act on the chromogens within the dentin, thus reducing tooth body color and are often used in conjunction with an activating agent, such as light or heat. Complex molecules of organic pigments, by means of an oxidation-reduction reaction are cleaved into simpler molecules and water-soluble in which easily leave the dental structure [14, 27, 28, 29]. Home bleaching technique is preferably used on all teeth and is indicated in case teeth have become dark by the by diet or cigarette pigments, by age, trauma and fluorosis, and may also be performed before or after orthodontic braces use [6, 10, 21, 30].

This technique has been notable with several publications attesting its bleaching efficacy and biological safety [13, 27, 14]. In this case, carbamide peroxide or hydrogen peroxide is used [29, 30]. When that desired color is reached, the treatment can be interrupted or continued for another week, the most recommended to stabilizing the color [14]. Hydrogen peroxide can diffuse through the tooth enamel to reach the junction of the dentin and regions of the dentin itself [30, 31]. This substance is considered a potent oxidizing agent, since it presents a great concentration of liberated oxygen, facilitating its penetration through interprismatic spaces and dentinal tubules, promoting the bleaching effect [27].

Allied to the aesthetic exigency from patients, Dental Science was driven to seek a continuous improvement of the knowledge in search of new techniques and materials [32]. Among the used structures and materials for product placement we can highlight nanotechnology scale dimensions. Today, nanotechnology is one of the main focuses of research, development and innovation activities in industrialized countries. Investments in this industry surpass every year and its development has been touted as a technological revolution. It is known, therefore, that material properties contained in this atomic or subatomic level can differ significantly from properties of same materials in a larger size. Hence, nanotechnology emerges as an alternative that guarantees to products greater stability and efficacy [33, 34].

Nanometric systems have high contact surface and large number of particles per unit weight. Due to very small size, these active substance-containing systems are used with the intention of improving their functionality, availability or stability when compared to the same material in molecular form [35 - 38]. Therefore, the number of pharmaceuticals based on new drug delivery systems has increased significantly, and design and development of new molecule transport systems in order to increase

product efficacy is a process that will continue to increase in the pharmaceutical industry [35, 37].

MATERIAL AND METHODS:

Determination of Hydrogen Peroxide: Hydrogen peroxide dosage was measured by collecting 1 mL of hydrogen peroxide concentrate in a 100 mL volumetric flask, and diluted in water to make up to volume, with subsequent mixing of the contents. Then, a 20 mL aliquot was transferred to an erlenmeyer flask containing 20 mL of 2M sulfuric acid and titrated 0.1M potassium permanganate. Each 1 mL of 0.1 M potassium permanganate is equivalent to 1.701 mg of hydrogen peroxide [38].

Preparation of Nanodispersed Systems:

Pseudoternary Phase Diagram Construction: There were constructed two-phase diagrams by slow titration of series of oil / surfactant in aqueous phase at room temperature. The first diagram was constructed with the following composition: oil phase composed of Isopropyl Myristate, aqueous phase composed of water, surfactants and co-surfactants mixture composed of ethoxylated hydrogenated castor oil (PEG 40) and Glyceryl monooleate (Span 80) (2: 1). Additionally, the second diagram was composed of oil phase isopropyl myristate, aqueous phase composed of water, a surfactant and cosurfactant mixture containing glyceryl monooleate (Span 80), ethoxylated sorbitan monolaurate (Tween 20), and ethoxylated hydrogenated castor oil (PEG 40) (1: 1: 1).

Selection of Nanodispersed Systems: The nanodispersed systems were selected in order to verify the influence of both (i) percentage of oil and (ii) mixture of surfactants in the hydrogen peroxide carrier. Both diagrams obtained indicated the exact amounts of oil, surfactants and aqueous phase used to obtain those systems. Hydrogen peroxide was incorporated into one phase of the system, according to solubility test, during the preparation of the selected formulations. All formulations with the hydrogen peroxide were characterized as described in the following item.

Physicochemical Characterization of Nanodisperse Systems:

Polarized Light Microscopy: Samples from certain regions of the phase diagrams were observed under polarized light microscope. Samples that showed transparency (possible micro-emulsions) were examined, but also more or less translucent samples that were formed. All samples were placed on glass slides covered by cover slips and then observed under a microscope (Olympus BX-50).

Droplet Size and Polydispersity Index: Nanodisperse systems containing hydrogen peroxide had the droplet size determined by dynamic light scattering using a Zetaser Nano ZS model ZEN3601 (Malvern Instruments Ltd., UK), using a fixed angle of 173°, at a temperature of 25°C. All analyzes were carried out in triplicate. Data were collected and analyzed in Zetasizer Software (version 6.12).

pH: All systems containing hydrogen peroxide had their pH values determined by an MS-Tecnopom Instrumentação® pH meter, mpA-210 model.

Zeta Potential: All Hydrogen peroxide systems had their droplet size determined by dynamic light scattering using a Zetasiser Nano ZS model ZEN3601 (Malvern Instruments Ltd., UK) at 25°C. All analyzes were performed in triplicate. Data were collected and analyzed using Zetasizer Software (v.6.12).

Hydrogen Peroxide Determination in Nano-disperse Systems: From the hydrogen peroxide system, 2 mL aliquot was transferred to a flask containing 20 mL of water, and then 20 mL of 2M sulfuric acid and titrated with 0.1M potassium permanganate were added. Each 1 mL of 0.1 M potassium permanganate is equivalent to 1.701 mg of hydrogen peroxide [38].

Rheology: The rheological profile of that selected nanodispersed system was evaluated by a Brookfield Rheometer, model DV-III Ultra, coupled to a computer with Rheocalc Software v.3.2 Brookfield Engineering Laboratories, using spindle from SC4-25 to 25 °C. All measurements were made at progressively higher speeds (4 to 24 rpm intervals) in order to obtain the downward curve to 6% hydrogen peroxide formulation. On the other hand, 14% hydrogen peroxide formulation was performed at progressively higher speeds (3 to 16 rpm intervals) to obtain the ascending curve and at progressively smaller speeds (16 to 3 rpm

interval) to obtain the downward curve. Measurements were done in triplicate.

Stability Evaluation:

Preliminary Stability Test - Centrifugation: Hydrogen peroxide systems were centrifuged immediately after preparation at 3000 rpm (revolutions per minute) for 30 minutes at room temperature in order to determine their stability as a single-phase isotropic system [39].

Thermal Stress: All selected samples were submitted to heating (greenhouse), 40 to 80 ºC ± 5ºC range, for 30 minutes cycles, with increments of 5ºC after each cycle. At the end, there were evaluated macroscopic appearance, pH, conductivity, zeta potential and droplet size, keeping all records in each cycle, which has been submitted in order to evaluate its stability [39, 40].

Accelerated Stability Evaluation: For a period of 90 days all selected samples were submitted to different temperatures (4 ± 2 ºC, 25 ± 2 ºC, 40 ± 2ºC) [37]. Possible changes in macroscopic aspects, content, pH variation, conductivity, zeta potential and droplet size were measured at 7 time points: at baseline (time 1), one day (time 2), 7 days (time 3), 15th days (time 4), 30 days (time 5), 60 days (time 6), and 90 days (time 7).

RESULTS AND DISCUSSION:

Determination of Hydrogen Peroxide: According to United States Pharmacopeia [38], hydrogen peroxide concentrate must contain not less than 29% and not more than 32% by substance weight. Hydrogen peroxide concentrate mean value was 31.76% ± 0.75, thus fulfilling the test, being suitable for use as raw material in dental bleach formulation.

Obtaining Nanodisperse Systems from the Phase Diagrams: The pseudoternary phase diagram showed in **Fig. 1** and **2** represents graphically the phase equilibrium system in all preparations containing three or more components. Diagram construction constitutes a fundamental tool to characterize what experimental condition is the best to nanodisperse systems, if they do exist and what components proportions or other structures may be present [40]. The structure of this formed system will be influenced by the physicochemical properties of components used in product formulation, so microemulsions and emulsions can be formed. If a large amount of surfactants is present, the system may also form anisotropic structures either lamellar liquid crystals and hexagonal phases or isotropic cubic phases, as consequence of surfactants increase [41, 42]. From these data, formulations are selected from the region of the diagram that represents the most appropriate condition of made product.

These diagrams are graphically represented in a triangular form. At each vertex from the figure, 100% of a particular component (aqueous, oily and surfactant / co-surfactant phase) are represented [43]. Upper vertex corresponds to the surfactant, left vertex the oily phase and the right vertex the aqueous phase. Phase diagrams were plotted using the software Sigma Plot 10, as shown in the following figures:

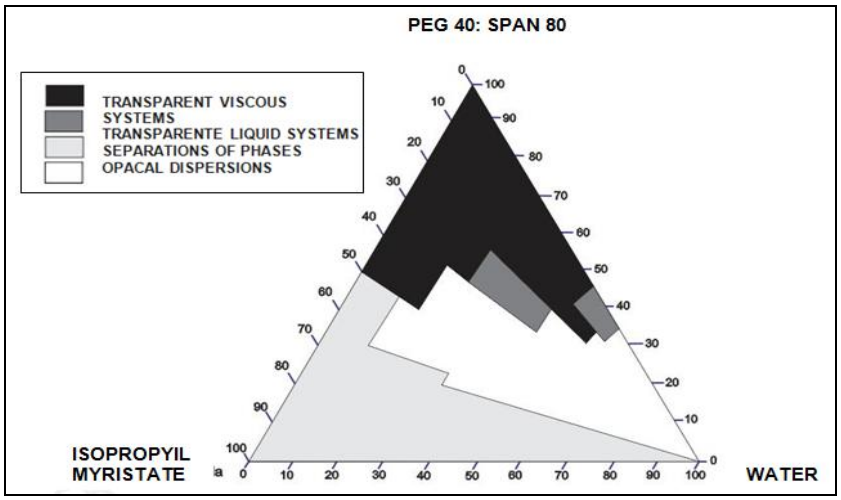

FIG. 1: PSEUDO-TERNARY PHASE DIAGRAM 1, FORMED BY PEG: 40: SPAN 80 (1:1), ISOPROPYL MYRISTATE (8:2) AND WATER

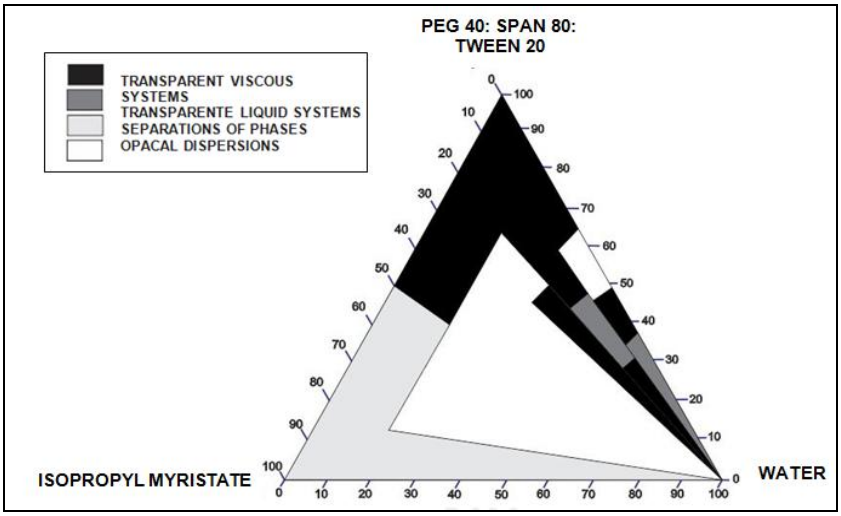

FIG. 2: PSEUDO-TERNARY PHASE DIAGRAM 2, CONSISTING OF PEG 40: SPAN 80: TWEEN 20 (1:1:1), ISOPROPYL MIRISTATE (8:2) AND WATER

Both diagrams (**Fig. 1** and **2**) showed regions with phase separation, opaque dispersions, clear liquid systems and transparent viscous systems. There were used two surfactants (ratio 2: 1) in the first diagram and three surfactants (ratio 1: 1: 1) to the second. In diagram 1, there were identified transparent nanodisperse systems regions (possible microemulsions and liquid crystals). However, they did not present PDI values less than 0.2. An ideal polydispersity index is about 0.3 or less 44; to this methodology, we have established as ideal a value less than 0.2, in order to guarantee greater system stability. For this reason, no formulations were selected from the first diagram because of surfactants have failed to stabilize the oil phase droplets in such a small size.

On the contrary, at phase diagram 2 a broad region of transparent nanodisperse systems was also identified, being suggestive of microemulsion systems and liquid crystals. Its PDI value shows as lower than 0.2 and characteristic droplet sizes for this type of system. Thus, we have selected two formulations (**Table 1**) followed by hydrogen peroxide incorporation into two different concentrations: 6 and 14%.

Use of hydrogen peroxide-containing products for dental bleaching with concentrations of 3 to 38% has been described in the literature [10, 45 - 48] and they are available on the market to consumers and dentistry professionals. Home teeth whitening agents are typically up to 16% hydrogen peroxide concentration, which is the lowest concentration, while dentists use teeth whitening with higher concentrations. Both are used under dentist supervision [45].

According to the different states of aggregation present in equilibrium, these diagrams reveal many possibilities. Solubility domains can be related to regions such as those of liquid crystal structures and present a broad spectrum of possibilities by simply changing the relative proportion of system components [49].

Thus, surfactants composition plays an important role in this system due to their affinity with oils, fats, surfaces of solutions with solids, liquids or gases, as well as water, so they may belong to two media. Therefore, surfactants are used as immiscible phase's conciliators with many uses in cosmetic products. They reduce interfacial tension and/or avoid the coalescence of the globules by guaranteeing a greater stability in the system [32, 50]. The oil phase used in both diagrams consisted of isopropyl myristate, which is a mixture of isopropyl ester and myristic acid applied in cosmetic preparations. This oil has been described as dispersing and emollient properties [51].

The surfactant components differential of Diagram 2 was due to the presence of Tween 20 that drove to a better result. In order to obtain pseudoternary systems, it is necessary to use a co-surfactant whose molecule increases system solubility and its stability. This type of surfactant provides a better formulation once the interaction between the surfactant's heads in the micelle is smaller thanks to the fact it does not have ions [52].

Formulation Selection: Two formulations were selected **Table 1** through their macroscopic characteristics, for example, translucency, to verify the influence of oil percentage and surfactants mixture to carry through the active substance **Table 2**.

TABLE 1: SELECTED FORMULATIONS SURFACTANT PROPORTION IN OIL AND WATER PHASES, OIL AND SURFACTANT

Proportion T/FO	Denomination	Water (%)	Oil (%) Isopropyl miristate	PEG 40, Span 80, Tween 20 (1:1:1) Surfactant (%)
8:2	1	28.57	14.29	57.14
8:2	2	35.49	12.90	51.61

Physicochemical Characterization: In **Table 2**, there are specified physicochemical characterizations of two evaluated nanodisperse systems.

TABLE 2: PHYSICOCHEMICAL CHARACTERIZATION OF SELECTED FORMULATIONS (NO ACTIVE ADDITION)

Physicochemical characterization	Formulation 1	Formulation 2
pH	6,04±0,06	5,93±0,06
Zeta Potential (mV)	-6,36±0,6	-4,99±0,3
Droplets size (nm)	9,6300,01	9,62±0,006
Polydispersity Index	0,15±0,002	0,16±0,007

Droplets Size: Formulation 1 had the mean droplet size of 9.63 ± 0.01nm while formulation 2 showed 9.62 ± 0.006nm. Nanotechnology products have a size of up to 100 nanometers [53], then more important the surface effects will become in a smaller size sample [54]. The target of nanodisperse system developed is the permeation inside dentin tubules, which have a variable size, with micro branches of at least 25 nm [55].

Polydispersity Index: Polydispersity Index (PDI) values obtained from formulations 1 and 2 were 0.15 ± 0.002 e 0.16 ± 0.007, respectively. PDI values allow analyzing the uniformity among droplets in a formulation so greater index values suggests less droplets uniformity [56]. PDI also reveals dispersion quality. An ideal value is found when measures are less than 0.2 because it indicates a small distribution size [57, 58].

When there is homogeneity of droplets size in nanodisperse system, it is called monodisperse; otherwise, the system is polydisperse [57].

Polarized Light Microscopy: Anisotropic samples are substances capable of deflecting the plane of incident light and isotropic if it does not to do so. Lamellar and hexagonal liquid crystals are anisotropic while the cubic liquid crystals are isotropic. Microemulsions also have isotropy. When analyzed under a polarized light microscope, it does not cause birefringence and, therefore, it isn't visible in objective lens [42, 49]. Thus, both formulations in our study are isotropic so they are classified as microemulsion or cubic liquid crystals.

FIG. 3: MICROSCOPY OF POLARIZED LIGHT OF THE NANODISPERSED SYSTEM 1 AND 2, RESPECTIVELY

pH: To determine pH, the following criteria should be considered: formulation stability, product efficacy, and safety [39].

According to Marson *et al*., [11], bleaching agents that retain the basic pH or nearby neutrality can be used on tooth structure for up to 45 minutes, without any change in the structure of the tooth enamel. Bleaching substances used on vital and non-vital teeth, it is normally established pH value should not be less than 5.5 for enamel and 6.0 for dentin. If these values have exceeded, there can occur demineralization, enamel erosion, and root reabsorption [58]. Taking it into account, our formulations have presented satisfactory values,

5.93 ± 0.06 and 6.04 ± 0.06 **Table 2**. Very acid or basic substances used on tooth structure have to attend such requirements time of exposure and frequency of use. Consequently, a pH that is below the range of 5.2 to 5.8 is sufficient to initiate an enamel demineralization [59]. As a result, those formulations pH values do not put teeth at risk being suitable for using in this product.

Zeta Potential: Looking at Zeta Potential values (-6.36 ± 0.6 e -4.99 ± 0.3), it has noticed a predominance of negative charges whose values are far from zero value favouring great stability formulation. A high zeta potential is important to physicochemical stability of solution [60]. Considering both zeta potential values obtained, it is possible the repulsion phenomena among the dispersed droplets in the system are more prominent than attraction. In consequence, aggregation probability between particles is decreased [61].

Rheology: It is well known that viscosity is the flowability of a fluid, which can reflect continually deformation when under force action. The more viscous the mass is the harder is to drain, and higher its viscosity coefficient. Thus, the rheology comprises the study of deformations behaviour of a fluid under certain temperature conditions and determined time period [62, 63].

Depending on fluid behaviour, it can be classified as Newtonian or non-Newtonian.

Newtonian types are fluids or materials that do not depend on shear force, time and temperature, which means the viscosity, does not change. Unlike Newtonian fluids, the non-Newtonian fluids have viscosity values regulated by applied parameters. Non-Newtonian fluids can be called viscoelastic, time dependent or independent like those two formulations in the present study **Fig. 4** [62, 63].

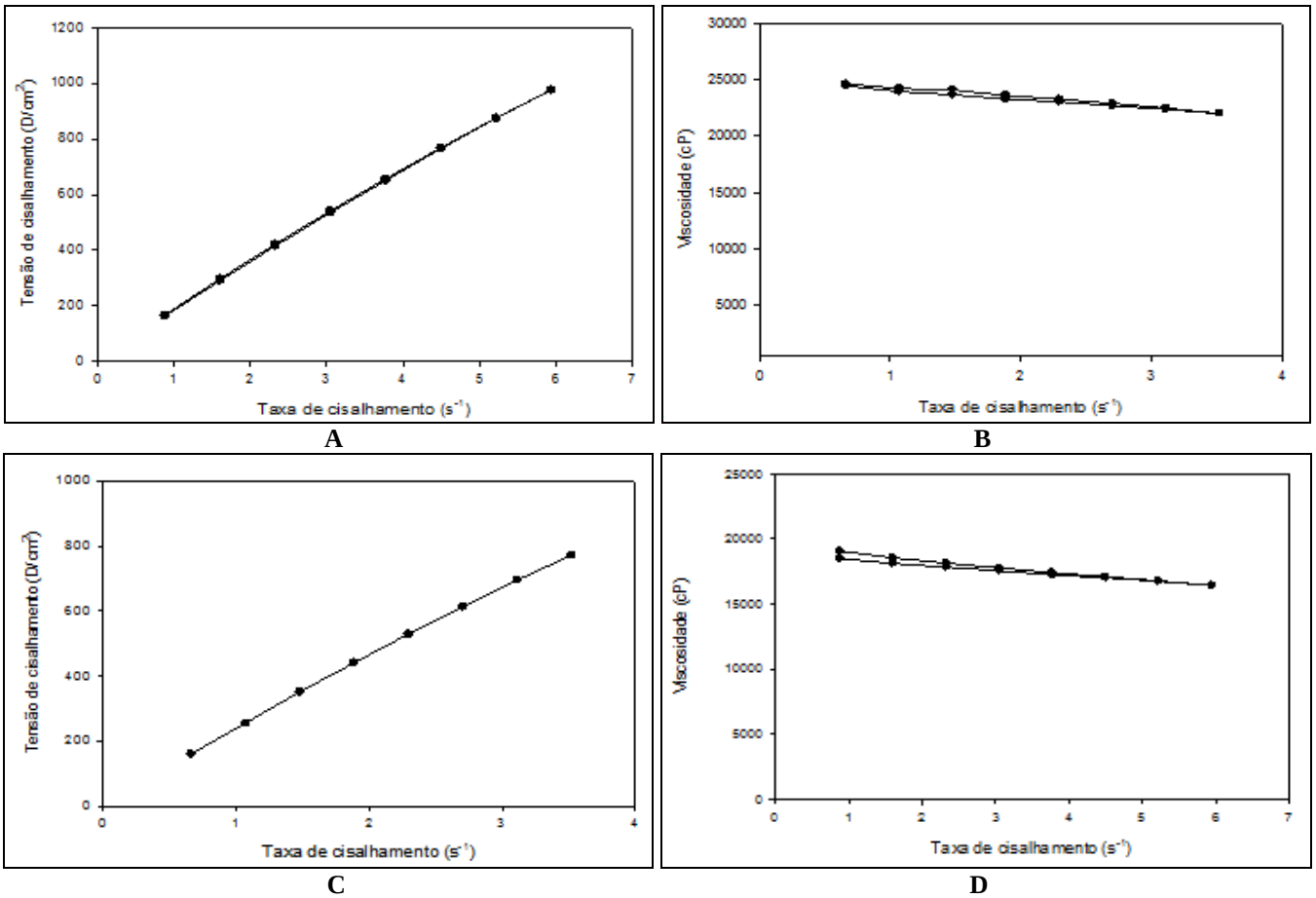

FIG. 4: (A) SHEAR STRESS AS A FUNCTION OF SHEAR RATE OF FORMULATION CONTAINING 6% HYDROGEN PEROXIDE; (B) VISCOSITY AS A FUNCTION OF THE SHEAR RATE OF FORMULATION CONTAINING 6% HYDROGEN PEROXIDE. (C) SHEAR STRESS AS A FUNCTION OF THE SHEAR RATE OF THE FORMULATION CONTAINING 14% HYDROGEN PEROXIDE AND (D) VISCOSITY AS A FUNCTION OF SHEAR RATE OF FORMULATION CONTAINING 14% HYDROGEN PEROXIDE

For that type of formulation analyzed, it is known that rheology is one of the most important properties in practical terms, since it determines characteristics like appearance, sensation and mode of product spreading [49].

Thus, as shown in the graphs **Fig. 4**, viscosity values decreased with the shear rate and with shear rate decrease, these values returned to higher values of viscosity, similar to the initial values, with the presence of thixotropy in our two analyzed samples. In the thixotropic behaviour, there is a viscosity change as a function of deformation time or shear rate, and as it can be seen from graphs above, this phenomenon is reversible, returning its initial viscosity some time later the applied deformation ceases. However, it does not recover its initial state after a rest state [64]. Shear stress or shear force is an amount of force when applied to a certain area of a fluid may or may not generate a deformation, a flow [62]. Microemulsions usually behave as Newtonian fluids and their viscosity is compared to water's, even at high droplet concentrations, most likely due to the reversible coalescence of the droplets [65]. Liquid crystals in cubic phases are recognized by their high viscosity [58, 59, 60] and their non-Newtonian rheological behaviour [66].

The selected nanodisperse systems, characterized as isotropic, with high viscosity and non-Newtonian rheological behaviour are suggestive of cubic phase liquid crystals. However, the small-angle X-ray scattering technique (SAXS) can be used to characterize microemulsions and liquid-crystalline phases, which are located in regions of high concentration of surfactants in diagrams, to better clarify structural modifications that occur in the various phases of the diagrams [36]. According to Atkins [66], the SAXS technique can be used to identify liquid-crystalline phases because they have a wavelength compatible to the scattering between the crystal forming groups. To formulations 1 and 2 described in **Table 1**, hydrogen peroxide was incorporated as 6 and 14% concentrations, respectively. Subsequently, these formulations were submitted to the stability study.

Stability Evaluation:

Preliminary Stability Test - Centrifugation and Thermal Stress: The preliminary stability study consists of performing the test in initial phase during product development, using different laboratory formulations combined to a reduced duration. It uses extreme temperature conditions in order to accelerate possible reactions between its components and appearance signals that must be observed and analyzed according to specific characteristics of each type of product. Due to those conditions mentioned before, this study is not intended to estimate the product useful life, but rather to aid in formulation screening [39].

The centrifugation test is important for assessing the preliminary stability since it provides rapid information about formulation properties. It produces a stress condition in the sample with an increase on gravity force and particles' mobility, thus simulating instable conditions. Some phenomena such as cream formation, sedimentation and phase separation that can be detected under normal storage conditions are evaluated in a short time period and may be accelerated by centrifugation [39].

After both formulations submission to a centrifugation process, they behaved as a single-phase dispersed system. In other words, there was no phase separation or precipitation was observed. On the other hand, thermal stress test **Fig. 5** is based on temperature as a stress condition on the formulation. According to Aulton [67], an increase in temperature can cause a cream increase rate, given the fact that occurs a decrease in the viscosity of continuous phase. Besides that, it favours coalescence by increasing kinetic motility.

From thermal stress test results, nanodisperse systems showed an increase in PDI mean values, but not enough to change mean droplet size, which remained stable during the heating ramp. On the other hand, the zeta potential was the parameter that presented the highest oscillations, occurring nonlinear variation according to temperatures submitted. Zeta potential values were far from zero, which causes repulsion between the droplets being characteristic of a stable system. Systems' macroscopic aspect also did not change significantly. Therefore, both systems, when subjected to thermal stress test, showed no signs of macroscopic instability.

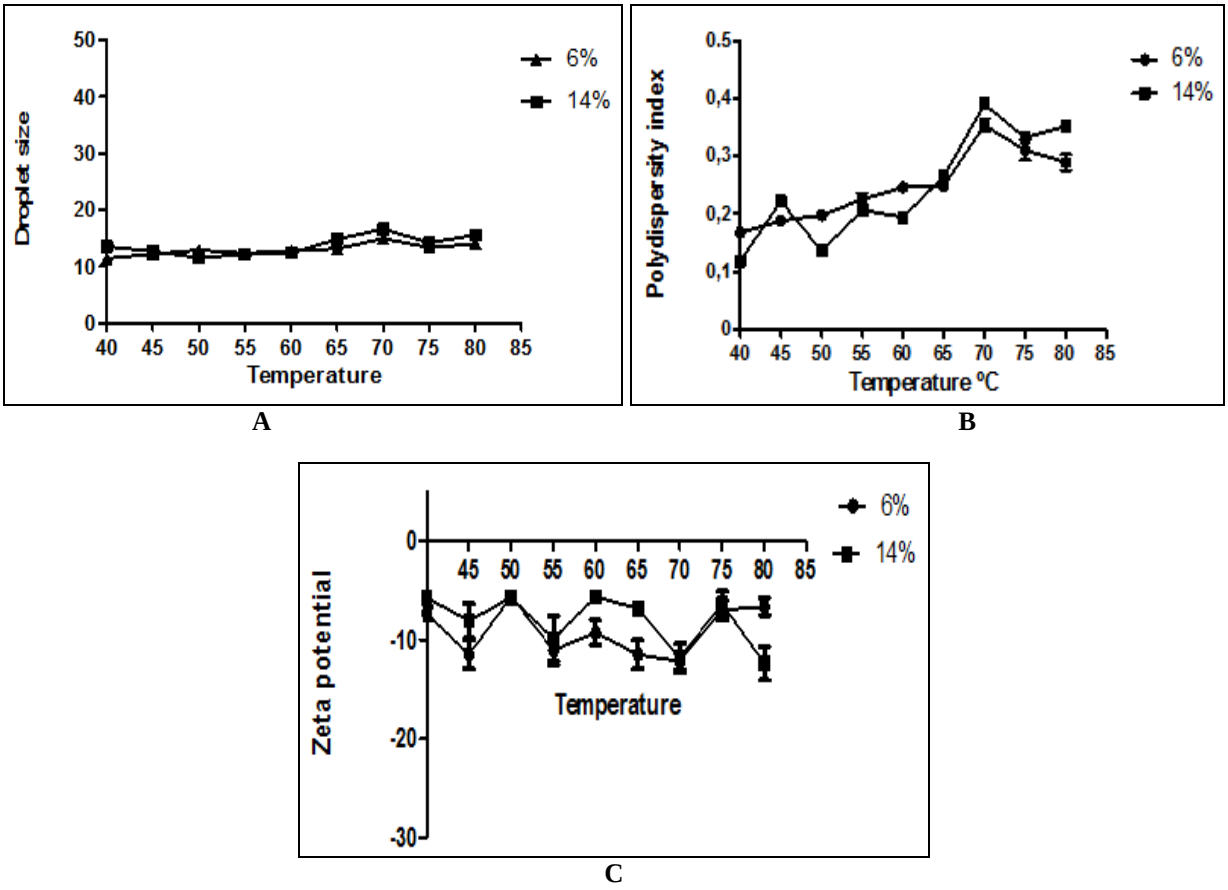

FIG. 5: DIFFERENT PHYSICOCHEMICAL CHARACTERISTICS EVALUATION OF EMULSIFIED NANOMETRIC SYSTEMS CONTAINING 6% AND 14% HYDROGEN PEROXIDE DURING THERMAL STRESS. (A) DROPLET SIZE, (B) POLYDISPERSITY INDEX, (C) ZETA POTENTIAL

Values represent the mean ± standard deviation (n = 3).

Accelerated Stability Evaluation: The accelerated stability study of cosmetic products provides insights about the formulation behaviour over a given time period, in face to various environmental conditions that can be submitted from manufacturing to expiration date. This study also aims to bring information that guides formulation development, packaging material, and pharmaceuticals / cosmetics safety. This kind of test exertless extreme conditions than the previous test and assists in product stability. Moreover, it is a predictive study that can be used to estimate a product expiration date, which should be confirmed concomitantly with shelf life [37].

Our formulated systems were evaluated under high temperature conditions (40 ± 2.0 °C), ambient temperature (25 ± 2.0 °C), and low temperature (4 ± 2.0 °C). No changes were observed to macroscopic aspect from two formulations in all concentrations during 90 days in all analyzes of droplet size, PDI, zeta potential, and pH.

The particle size determined is based on light scattering analysis of moving particles [68]. Our selected systems had ananometric property, presenting average droplet size within the specified according to classification [69]. All values remained stable during the whole testing time at three different temperatures as shown in **Fig. 6**.

pH: One of the most important properties of a cosmetic product is pH, which should be as close as possible to the region where it will be applied [70]. As can be seen in **Fig. 7**, the pH during the stability evaluation suffered a slight drop in all three temperatures over time for the 6% concentration.

In 14% formulation, there were discrete drops in both ambient and refrigeration temperatures. At 40°C, it suffered a greater fall, from day 60th reaching values that are not tolerable for use as a bleaching product. Bleaching substances designed for vital and non-vital teeth should not have their pH values less than 5.5 in enamel and 6.0 in dentin.

If these values have exceeded, demineralization, enamel erosion, and root reabsorption may occur [58]. In this regard, the formulations also presented favorable values, 5.93 ± 0.06 and 6.04 ± 0.06 (**Table 2**). Very acid or basic substances used on tooth structure have to attend such requirements time of exposure and frequency of use. Consequently, a pH that is below the range of 5.2 to 5.8 is sufficient to initiate an enamel demineralization [59].

Dental bleaching products containing hydrogen peroxide in different concentrations have different pH values range from 3.08 to 7.29. It is known that the critical pH for the enamel is 5.5, and for the dentin it is 6.058. However, according to Shannon [71], the salivary pH increases in the first 15 minutes after placing the tray and remains higher until two hours, even with the use of lower pH products.

This parameter can also be adjusted in a cosmetic formulation using chemical buffering. This type of pH adjustment is very important to ensure the formulation conservation [63]. Hence, the pH of formulation might be adjusted to that pH region where the product will be applied as long as buffering system does not influence the product's therapeutic properties [39].

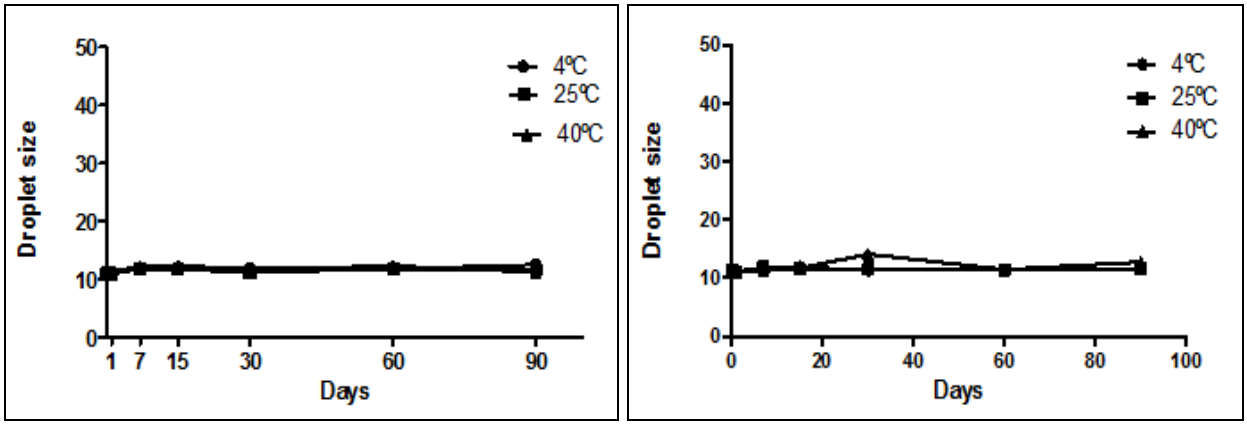

FIG. 6: DROPLET SIZE ANALYSIS FOR ACCELERATED STABILITY TEST AT TEMPERATURES 4 ºC ± 2, 25 ºC ± 2 AND 40 ºC ± 2 FOR (A) 6% HYDROGEN PEROXIDE AND (B) 14% HYDROGEN PEROXIDE

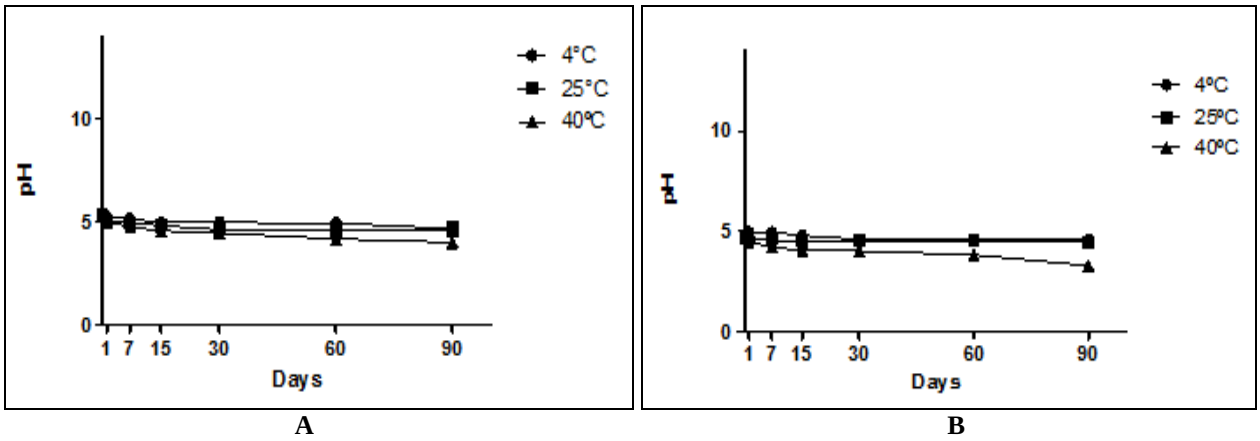

FIG. 7: pH ANALYSIS DATA DURING ACCELERATED STABILITY TEST AT TEMPERATURES OF 4 ºC ± 2, 25 ºC ± 2 AND 40 ºC ± 2 ANALYZED IN (A) 6% HYDROGEN PEROXIDE AND (B) 14% HYDROGEN PEROXIDE

Hydrogen Peroxide Determination in Micro-emulsion: Fig. 8 shows dosing values of two formulations containing hydrogen peroxide at temperatures 4 °C, 25 °C and 40 °C during the accelerated stability study. According to Brazilian Pharmacopoeia [72], the dosage of formulation's active is a very important item to meet the requirements and ensure that the content is within the limits specified for the product. Both formulations in our study presented stable hydrogen peroxide concentration values for the analyzes carried out during the stability study and close to what is established as reference for dental bleaching (6% and 14%) over the 90 days of study.

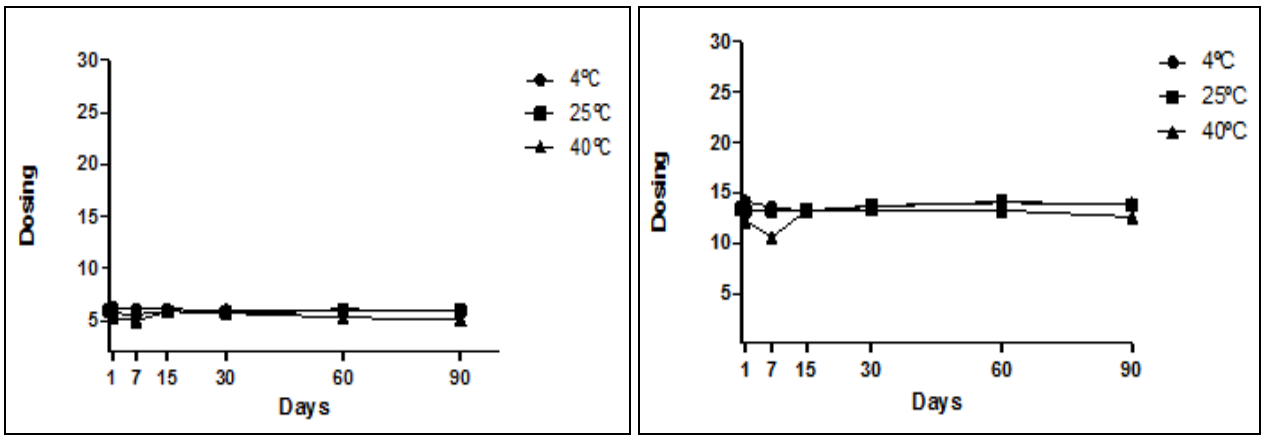

FIG. 8: ASSAY ANALYSIS DATA DURING ACCELERATED STABILITY TESTING AT TEMPERATURES 4 °C ± 2.25 °C ± 2 AND 40 °C ± 2 IN (A) 6% HYDROGEN PEROXIDE AND (B) 14% HYDROGEN PEROXIDE

Zeta Potential: The zeta potential measurement **Fig. 9** represents an useful tool in monitoring system stability over time. The most widely used method for measuring zeta potential is through electrophoretic mobility of dispersed particles in a charged electric field [58]. If zeta potential value is close to zero, there is an attraction probability between the particles and droplets. On the contrary, if zeta potential is different from zero, like those ones presented by formulations in this study, there will be an electrostatic repulsion between the particles, which does not allow particles' flocculation, providing greater stability to the developed systems [73].

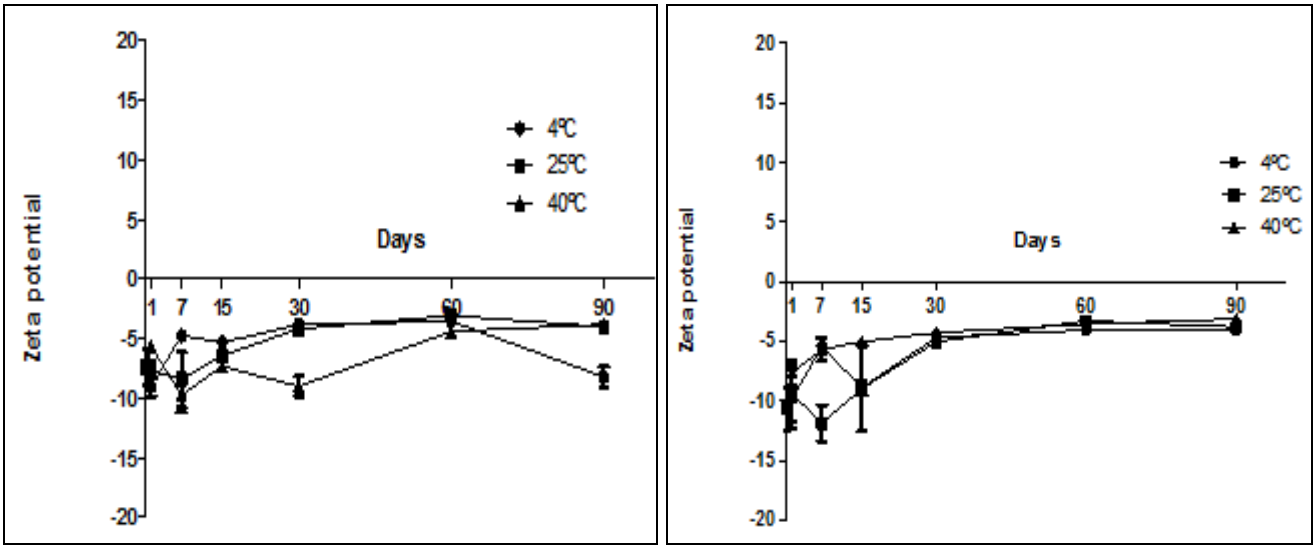

FIG. 9: ZETA POTENTIAL ANALYSIS DATA DURING ACCELERATED STABILITY TESTING AT TEMPERATURES 4 ºC ± 2, 25 ºC ± 2 AND 40 ºC ± 2 IN (A) 6% HYDROGEN PEROXIDE AND (B) 14% HYDROGEN PEROXIDE

Although the zeta potential values for the two formulations oscillated over the 90 days, they maintained a stabilizing tendency, remaining far from zero during the stability study period. It is a characteristic reduces the aggregation probability of particles since in this condition the repulsive forces prevail. This parameter, therefore, is used to investigate the nanodispersed systems stability, which is based on the particle surface charge under analysis and reflects the effective charge on the particles. They correlate to electrostatic repulsion between themselves, and with system stability [74, 60].

CONCLUSION: We have developed satisfactorily nanodispersed systems for 6% and 14% hydrogen peroxide delivery to using as dental bleach. Both formulations were characterized as cubic phase liquid crystal nanodispersed systems remaining

stable during the stability test; however, it is necessary to confirm this information by small Angle X-ray Scattering (SAXs). Our results suggest brings an innovative nanotechnological product with high value, stable and capable of replacing the conventional bleaching agents maintaining the objective of bleaching. Their main differential is that nanometric particles could lead to a greater product permeation in dentinal tubules increasing the whitening effectiveness with prospective of increased safety, either by reducing the dose or reducing the time of exposure.

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