



Physicochemical evaluation of hydrogen peroxide bleaching gels containing titanium dioxide catalytic agent, and their influence on dental color change associated with violet LED

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ARTICLE INFO

Keywords:

Bleaching
Hydrogen peroxide
Titanium dioxide
Violet LED

ABSTRACT

Background: The purpose of this study was: 1) to analyze the physical-chemical properties of hydrogen peroxide (HP) agents at 7.5% (HP7) and 35% (HP35), and the association with or without TiO₂ nanotubes; 2) to evaluate dental bleaching effectiveness by using HP7 and HP35 together with or without TiO₂ nanotubes, and applied with or without violet LED (VL). Methodology: 80 bovine incisors were treated according to groups ($n = 10$): HP35; HP35 + VL; HP35T (HP35 + TiO₂); HP35T + VL; HP7; HP7 + VL; HP7T (HP7 + TiO₂); HP7T + VL. Bleaching effectiveness was measured at 4 time points according to the Vita Classical, CIEL*a*b*, CIEDE2000, and WI_D parameters. HP35, HP35T, HP7, and HP7T were evaluated for mass change, pH, mean particle size (P), polydispersity (PDI), and zeta potential (ZP), over 6 months of storage.

Results: The pH of HP35 thickener was higher when associated to TiO₂. At baseline, both of the bleaching gels containing TiO₂ had lower P, PDI, and PZ ($p < 0.05$). All groups showed a significant decrease in Vita Classical color scores ($p = 0.0037$). There was a higher L* value, and lower b* values for HP7 when associated to VL after the 3rd session. ($p < 0.05$). HP35T showed higher color change (ΔE_{ab} , ΔE_{00}), and lower a* value in the presence of VL ($p < 0.05$). ΔWI_D presented lower values for both gels, when TiO₂ was incorporated ($p \leq 0.05$).

Conclusion: The incorporation of TiO₂ to the bleaching gel showed good stability with minimal variations in physical-chemical properties. The color change in HP35 was more effective than in HP7, but the VL boosted the bleaching effectiveness of HP7, whereas TiO₂ did not increase bleaching effectiveness.

1. Introduction

The in-office bleaching technique is usually performed with high concentrations of hydrogen peroxide, which promote fast color change [1–3]. Although this procedure is well-accepted by patients and dentists alike, the technique can present side effects, such as gingival irritation, if the gums are not protected, and dental sensitivity [1,4,5].

Hydrogen peroxide releases free radicals [6] that oxidize the organic matrix and provide a whitening effect [7]. The cytotoxicity effects of free radicals appear to be proportional to the hydrogen peroxide

concentration, and the contact time with the dental surface [8,9]. This suggests that lower concentrations of hydrogen peroxide and shorter application time may be viable alternatives to decrease or avoid sensitivity problems associated with in-office bleaching, but the issue of effectiveness under these conditions must be researched.

Some 6%–15% of the hydrogen peroxide agents used at lower concentrations in the in-office technique [10–14] have reduced tooth color up to 5 shades when applying the Vita Classical scale [15]. In these studies, bleaching gels containing catalytic agents achieved the same color change with or without a light source [10,11,13,14,16] as that

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<https://doi.org/10.1016/j.pdpdt.2022.103254>

Received 13 October 2022; Received in revised form 14 December 2022; Accepted 21 December 2022

Available online 23 December 2022

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provided by a high-concentration hydrogen peroxide gel.

Titanium dioxide (TiO₂) acts as a catalyst and photocatalyst semiconductor, absorbing ultraviolet light (wavelength 388 nm) [17,18] and visible light in the blue-violet range [19,20]. When activated by light, it promotes electron excitation and generates oxygen ions to ultimately form superoxide [21,22].

The action of TiO₂ could be related to the hydrogen peroxide concentration of the bleaching agent, the pH of the gel, and the particle shape and size and substrate area of the TiO₂ [21,23]. Most studies have used TiO₂ particles in a spherical nanostructure shape [4,13,24,25]. However, particles in a tubular and nanometric shape would provide more benefits, since they would increase the proportion of surface area to particle volume, and thus improve the catalytic reaction [21], and benefit the color change results [16]. On the hand, the addition of a catalyst agent to the bleaching gels could change the pH values, the mass, the particle size and the colloidal stability, thereby compromising bleaching effectiveness and negatively influencing gel storage.

The use of a light source associated with in-office bleaching agents increases the temperature of the hydrogen peroxide, and leads to a higher free radical release rate to improve oxidation of the complex organic molecules [26,27]. Violet LED (VL) has a wavelength between 405 nm +/- 10 nm, corresponding to the range of violet light [28]. VL may act on the same absorption peak of pigmented molecules, breaking them down into smaller molecules [28].

Although some studies have shown that high-concentration hydrogen peroxide (35% up to 40%) bleaching agents are effective for in-office bleaching, an alternative with promising consequences would be to associate VL with lower concentrations of hydrogen peroxide agents, aiming to reduce dental sensitivity [2,29,30], especially when photocatalysts are incorporated into the bleaching gels. Therefore, the purpose of this study was: 1) to analyze the physical-chemical properties of the tested hydrogen peroxide agents (7.5% and 35%), and the association with or without TiO₂ nanotubes; 2) to evaluate dental bleaching effectiveness by using hydrogen peroxide agents (7.5% and 35%) together with or without TiO₂ nanotubes, and applied with or without VL. The null hypotheses to be tested in this study were that TiO₂ incorporation into the hydrogen peroxide bleaching agents, associated with or else without VL, would not influence: H1) the physicochemical properties (pH, mass change, particle size, polydispersity and zeta potential) of the agents; or H2) the bleaching effectiveness of the dental enamel.

2. Materials and methods

2.1. Bleaching agents and incorporation of the TiO₂ nanotubes

This study used bleaching agents containing 35% hydrogen peroxide (HP35) (Whiteness HP, FGM, Joinville-SC, Brazil; composition according to the manufacturer - 35% hydrogen peroxide, thickeners, dye mixture, glycol, inorganic filler and deionized water, lot number: 050919, 040620, and 261120), and 7.5% hydrogen peroxide (HP7) (White Class 7.5%, FGM, Joinville-SC, Brazil, composition according to the manufacturer - 7.5% hydrogen peroxide, neutralized Carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, humectant, deionized water, lot number 04052 and 180121). The 35% hydrogen peroxide bleaching gel was prepared prior to each application with one drop of the thickening agent mixed with three drops of the hydrogen peroxide for 15 s.

TiO₂ nanotubes were obtained as described by Arruda et al. [31]. The TiO₂ was vortexed for 1 min to make the particles detach, and was then incorporated into the bleaching agents immediately before each application. The TiO₂ nanotubes were weighed on a precision balance (Shimadzu do Brasil, ATY 224, São Paulo, SP, Brazil), and then manually added to the bleaching agents at a concentration of 1% (HP35T and HP7T) [16,32,33]. The 1% TiO₂ was incorporated into HP35 by first weighing the thickener and the hydrogen peroxide separately on a

precision balance (in a 1:3 ratio), then adding the TiO₂ to the thickening agent, and mixing the resulting solution with hydrogen peroxide to obtain the total weight of the bleaching gel.

2.2. Physicochemical properties of bleaching agents

The pH and mass change of the bleaching gels was measured ($n = 10$) and monitored at different time points: baseline, after 7 days, 1 month, 3 months and 6 months of storage. The evaluations were carried out directly on the HP7 and HP7T bleaching agents, whereas the measurements for HP35 and HP35T were made before adding the thickener (with and without TiO₂) and the hydrogen peroxide gel, to evaluate the stability across the storage time. The pH was obtained only at baseline, after the thickener (or no thickener) was mixed with the hydrogen peroxide of the HP35 and HP35T agents. The pH evaluations were performed with a pH meter (PHS-3B, MS Tecnoyon Digital Instrumentation, Piracicaba, Brazil). Mass change was determined by weighing 0.5 g of each bleaching agent in a microtube on a high-precision analytical scale (Shimadzu do Brasil, São Paulo, Brasil). The samples were stored in an environment with a controlled temperature of 23 °C, without exposure to light.

The average particle size, the polydispersity (PDI) (molecular heterogeneity or size of the particles in the solution) and the zeta potential (ZP) (colloidal stability) of all bleaching gels were evaluated by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) (Monteiro et al., 2020). ZP was evaluated in order to measure colloidal stability using the Helmholtz-Smoluchowski model [34], by measuring the electrophoretic mobility of the particles dispersed in the applied electric field.

ZP analysis was performed by laser electrophoresis with 30 made-to-measure runs at 25 °C. The ZP was automatically calculated from the electrophoretic mobility, using the Smoluchowski approximation: $UE = 2 * \epsilon * z * f(ka) / 3 * \eta \rightarrow z \approx UE * \eta / \epsilon$, where UE is the electrophoretic mobility, ϵ is the dielectric constant, z is the ZP, $f(ka)$ is the function of Henry, and η is the viscosity. Particle size analysis of PDI and ZP was also performed at baseline, and after 7 days, 1 month, 3 months and 6 months of storage.

2.2.1. Specimen preparation

Eighty fresh bovine incisors were cleaned and stored at 18 °C for the experimental phase. The teeth were thawed at room temperature, after which prophylaxis was performed with pumice and water using a Robinson brush. A cut was made on the apex of the roots with a diamond disk (KG Sorensen, São Paulo, Brazil) attached to a handpiece to remove the pulp content with an endodontic file.

The coronary portion remained intact, and the enamel surface of the buccal face was smoothed with water and 400- and 600-grit sandpaper (3 M ESPE, Sumaré, SP, Brasil) in a manual polisher (Aropol 2 V, Arotec São Paulo, SP, Brazil) under water cooling, to perform color reading. Naturally darkened bovine teeth were selected to standardize the baseline color of the groups by using colors from the Vita Classical shades that were as dark as or darker than A3 (colors A3, D3, B3, A3.5, B4, C3, A4 or C4). A marking was made on the buccal surface to specify where the color measurements had to be performed, so that the color readings would always be made in the same place. Then, the roots were fixed with polyester resin (Maxi Rubber, Campinas, SP, Brazil) in a PVC matrix, up to the cemento-enamel level, keeping the long axis of the tooth perpendicular to the horizontal plane. The PVC molds were removed after resin polymerization. The teeth were stored in artificial saliva (5 mM Ca, 0.9 mM P, 150 mM KCl 0.05 µg F/mL in 0.1 mol/L Tris buffer) at 37 °C throughout the study [35].

2.3. VL specification and protocol of use

The VL unit (Bright Max Whitening, MMOptics, São Carlos, SP, Brazil) used in this experiment had a wavelength of 405–410 nm,

irradiance of 112 mW/cm², target area of 10.7 cm² and total energy/session of 1440 J [36]. The device was positioned 8 mm away from the dental surface, following the manufacturer’s protocol of 20 cycles of 60 s of light with an interval of 30 s of rest (no light), for a total session time of 30 min.

2.4. Bleaching treatment

The teeth were removed from the artificial saliva solution and dried. Both of the bleaching agents containing TiO₂ (HP35T and HP7T), and those not containing TiO₂ (HP35 and HP7) were applied to the buccal surface in a 2-mm-thick layer, and remained in contact for 30 min. VL was applied (✓) or not applied (x), according to the groups. At the end of the time period, the gel was removed with gauze, and the tooth was washed with water for 10 s, and then returned to the artificial saliva solution, which was changed twice a week. The bleaching treatment was performed in 3 sessions lasting 30 min each at an interval of 7 days between each session.

2.5. Color evaluation

Color analysis was performed by an operator using a spectrophotometer (VITA Easyshade® Advance, Vita, Germany) and a box with a white background to standardize the lighting. The measurements were taken in duplicate to improve accuracy. The tooth color was checked using the Vita Classical shade guide, and parameters L*, a*, and b* from the CIEL*a*b*. The measurements were performed at the following time points: baseline, 24 h after the 1st session, 24 h after the 2nd session, and 24 h after the 3rd session.

Color change was evaluated using the Vita Classical scale (converted to a numerical scale from 1 (shade B1) to 16 (shade C4) [37,38]. The coordinates of the CIEL*a*b* system were obtained, after which the ΔE_{ab} value was calculated using a mathematical formula (CIE, 2004) [39]. The limits of perceptibility and acceptability considered for ΔE_{ab} were 1.2 and 2.7, respectively [39,40]. The color change was also evaluated by CIEDE2000 (ΔE₀₀), which uses h (hue) and C (chroma) values [41]. ΔE₀₀ values of 0.8 and 1.8 were adopted as the perceptibility and limits of acceptability [40]. Monitoring of the tooth whitening process was performed according to the Whiteness Index for Dentistry (WI_D), where L*, a* and b* parameters were used in the following equation [42]: WI_D = 0.511L* - 2.324a* - 1.100b*. Differences in WI_D between the initial and final evaluations were evaluated (ΔWI_D) using the ΔWI_D threshold values of 0.72 for perceptibility and 2.60 for acceptability [42].

2.6. Statistical analysis

Descriptive and exploratory tests were applied initially. P, PDI, ZP, pH, mass, color data from the Vita Classical scale, and L* and b* parameters were analyzed by mixed generalized linear models for repeated measures in time. The Tukey-Kramer test was also applied for pH and mass analyses. ΔE_{ab}, ΔE₀₀ and ΔWI_D data were analyzed by generalized linear models. The a* and WI_D data were analyzed by nonparametric Mann-Whitney tests for comparisons made among the associations with

VL and the incorporation of TiO₂ and the bleaching gels, whereas the Friedman and Nemenyi non-parametric tests were used for comparisons among the time points. The analyses were performed using the SAS [43] and R [44] programs, with a significance level of 5%.

3. Results

The incorporation of TiO₂ significantly increased the pH of HP7 and HP35 thickeners at most of the evaluated time points (p < 0.0001) (Table 1). However, HP35T presented a decrease in pH after 7 days of storage (p < 0.0001). The mean pH values (standard deviation) after mixing the thickener and the hydrogen peroxide at baseline were: HP35 = 6.34 (0.12); HP35T = 6.44 (0.15).

The mass for HP7 and HP35 thickener was significantly lower when TiO₂ was added (p < 0.0001) (Table 2). The mass increased significantly for HP7 and HP7T after 3 months of storage, whereas the mass decreased significantly for HP35 and HP35T thickeners, when comparing the baseline to 6 months of storage (p < 0.0001).

At baseline, particle size, PDI and ZP were significantly lower for both agents (HP7T and HP35T) when TiO₂ was incorporated, compared with when it was not (p < 0.05) (Table 3). After 7 days, the particle size was significantly higher for HP7 incorporated versus not incorporated with TiO₂ (p < 0.05). HP35 presented minor particle size at 3 months, when TiO₂ was incorporated, but had a greater particle size at 6 months (p < 0.05). PDI was significantly lower for TiO₂-incorporated HP7 after 1 month storage. PDI was also significantly lower for TiO₂-incorporated HP35 during up to 3 months, but was significantly higher at 6 months (p < 0.05). ZP was significantly more negative up to 3 months storage time for HP7 not incorporated versus incorporated with TiO₂ (p < 0.05). ZP was significantly more negative for HP35 in the absence of TiO₂ (p < 0.05). Overall, HP35 differed significantly from HP7 in particle size, PDI and ZP, whether in the absence or presence of TiO₂.

There was a significant decrease in the color scores of all the groups, based on the Vita Classical scale (p = 0.0037) (Table 4). After the 3rd bleaching session, the HP35 groups exhibited lower scores than the HP7 groups (p < 0.05), regardless of VL irradiation or presence of TiO₂. After the 1st session, the HP35 groups had lower scores than the HP7 groups (p < 0.05), regardless of VL and TiO₂. As for HP35T and HP7T, the scores were lower after the 3rd session with rather than without VL (p < 0.05).

After the 3rd session, HP35 had a higher L* value than HP7, regardless of VL or TiO₂ (p < 0.05). As for HP35 after the 3rd session, the groups with TiO₂ had a higher L* value than the groups without it, and the groups with VL (p < 0.05). After the 3rd session, HP35 with VL had higher WID than the groups without VL (p < 0.05). As for HP7 after the 3rd session, higher L* was observed with VL and without TiO₂ (p < 0.05).

In general, the b* value was significantly lower in HP35 than HP7 (p < 0.05), with a significant decrease at all the time points, and in all the groups (p < 0.05). VL presence caused HP35 to have lower b* values throughout the bleaching process, regardless of the presence or absence of TiO₂. HP7 showed lower b* values both with and without VL after the 3rd session.

The HP35 group had a lower a* value than HP7 in the presence of VL

Table 1 Mean (standard deviation) pH value according to bleaching agent and storage time.

Time	Bleaching agent		HP35 (Thickener)	HP35T (Thickener)	HP35 (Hydrogen peroxide)
	HP7	HP7T			
Baseline	5.39 (0.03) Bb	5.44 (0.03) Abc	9.28 (0.16) Bab	9.45 (0.07) Aab	2.11 (0.03) a
7 days	5.38 (0.01) Ab	5.41 (0.02) Ac	9.35 (0.05) Aab	9.39 (0.06) Aab	1.97 (0.05) b
30 days	5.38 (0.02) Bb	5.44 (0.02) Ac	9.23 (0.08) Bb	9.51 (0.07) Aa	1.97 (0.05) b
3 months	5.46 (0.02) Ba	5.72 (0.07) Aa	9.38 (0.08) Aa	9.41 (0.11) Aab	1.95 (0.02) b
6 months	5.27 (0.03) Bc	5.50 (0.03) Ab	8.93 (0.11) Bc	9.33 (0.13) Ab	1.60 (0.09) c
	p(HP)<0.0001; p(time)<0.0001; p(HP vs. time)<0.0001.		p(HP)<0.0001; p(time)<0.0001; p(HP vs. time)<0.0001.		p < 0.0001

Different letters (uppercase horizontally and lowercase vertically for each hydrogen peroxide concentration) indicate statistically significant differences (p ≤ 0.05).

Table 2
Mean (standard deviation) mass value (in g) according to bleaching agent and storage time.

	HP7	HP7T	Multiple Comparisons	HP35 (Thickener)	HP35T (Thickener)	Multiple Comparisons	HP35 (Hydrogen Peroxide)
Baseline	1.2957 (0.0135)	1.2250 (0.0295)	a	1.2960 (0.0196)	1.2382 (0.0178)	b	1.2973 (0.0123) a
7 days	1.2939 (0.0130)	1.2208 (0.0298)	a	1.2982 (0.0123)	1.2381 (0.0178)	ab	1.2933 (0.0211) ab
30 days	1.2886 (0.0133)	1.2136 (0.0308)	a	1.2998 (0.0124)	1.2387 (0.0178)	ab	1.2873 (0.0239) ab
3 months	1.2740 (0.0185)	1.1920 (0.0355)	b	1.3039 (0.0129)	1.2401 (0.0177)	ab	1.2805 (0.0265) ab
6 months	1.2710 (0.0447)	1.1778 (0.0392)	b	1.3086 (0.0134)	1.2411 (0.0173)	a	1.2740 (0.0298) b
Multiple Comparisons	A	B		A	B		–
	p(HP)<0.0001; p(time)=0.0221; p(HP vs. time)=0.2304.			p(HP)<0.0001; p(time)=0.0221; p(HP vs. time)=0.3762			

Different letters (uppercase horizontally and lowercase vertically for each hydrogen peroxide concentration) indicate statistically significant differences ($p \leq 0.05$).

after the 1st session (Table 5) ($p < 0.05$). The HP35T group also presented a lower a^* value than the HP7T group in the presence of VL after the 1st and 3rd sessions ($p < 0.05$). HP7 presented higher WID values than HP7T in the absence of VL, in the 2nd session ($p < 0.05$). WID was significantly higher for HP35 in the presence of VL and absence of TiO_2 . HP7T showed lower WID values than HP7 in the absence of VL.

ΔE_{ab} (Table 6) was higher for HP35 than for HP7 at all time points ($p < 0.05$). HP7 and HP35T had higher ΔE_{ab} values in the presence versus absence of VL in the 3rd session ($p < 0.05$). ΔE_{00} was higher in the HP35 and HP35T groups than in the HP7 and HP7T groups, in the presence of VL, in the time interval between the 1st session and baseline ($p < 0.05$). ΔE_{00} was higher in the HP35 group than the HP7 group in the time interval between after the 2nd session and baseline, regardless of VL and TiO_2 . ΔE_{00} was higher in the presence of VL in the HP35T group ($p < 0.05$). ΔWID was higher in the HP35 than the HP7 group when VL was applied, and also in the time interval between the 2nd session and baseline ($p < 0.05$). In the interval between the baseline and after the 3rd session, the HP35, HP35T, HP35+VL and HP35T+VL groups had higher ΔE_{ab} , ΔE_{00} , ΔWID than the HP7 group ($p < 0.05$).

4. Discussion

The addition of a new component to the bleaching gel can change its properties and the stability of the product, and hence also affect its bleaching potential. Nanoscale particle stability can pose problems related to storage over time, such as sedimentation, agglomeration, and crystal growth [45]. When TiO_2 was incorporated into the bleaching agents, the pH of HP7 and HP35 thickeners tended to increase, pointing out that HP35T thickener recorded the highest pH values (over 9.33). However, right after mixing the thickener and the hydrogen peroxide, the pH values for the mixtures, whether with (6.32) or without TiO_2 (6.33), were similar, despite the higher pH value of the thickener. The pH of the HP7 was higher in the presence of TiO_2 , although the pH for both HP7 and HP7T was acidic. The formation of bubbles resulting from the incorporation of the particle to the bleaching agent HP7 was visually observed, a characteristic that could change its mass. The release of by-products can lead to mass loss and compromise the final volume of the material, especially when mixing the components in the highest concentration agent. Although some differences were found in the mass analysis of all the agents, the numerical differences were very small, owing to the accuracy of the high-precision analytical scale. Therefore, it is possible to conclude that TiO_2 did not change the mass of bleaching gels, maintaining storage stability.

At baseline, the bleaching gels incorporated with TiO_2 showed a reduction in average particle size and lower PDI, the latter of which decreased the agglomeration rate [46,47]. PDI is a measure of the heterogeneity of a sample based on agglomeration or aggregation of the sample during isolation or analysis. The incorporation of TiO_2 showed

no trend toward particle agglomeration, as seen by ZP values within the limits of good stability. For particles that are small enough, a high ZP will confer stability, and the gel will resist aggregation. When the potential is small, attractive forces may exceed this repulsion and the gel may break and flocculate. In this respect, ZP values above (+/-) 40 mV indicate molecules with good stability in suspension, since the surface charge prevents particle aggregation [48]. Incorporation of a catalyst agent into the bleaching gel, performed before each application, seems to have provided a homogeneous mixture following particle dispersion after agitation during manipulation.

At other time points, a significant increase in the average particle size was observed for HP7 when TiO_2 was incorporated, although it didn't have a sizable impact on the properties of PDI or ZP, since the values obtained were greater than -38 mV, indicating good colloidal stability [47,49]. However, smaller average particle size and PDI were observed for HP35, possibly because TiO_2 was incorporated into the thickener and stored. In this respect, the possibility of incorporating TiO_2 into the thickening agent (instead of allowing direct contact of TiO_2 with hydrogen peroxide) seems to have favored the storage stability of the material (up to 6 months). Therefore, homogeneous mixtures can favor the best performance of TiO_2 .

The color analysis using the Vita Classical scale showed that all bleaching treatments were effective, by providing 2.5 to 10 shades of color change after the end of the three bleaching sessions. Although some manufacturers advocate the reapplication of gel in the same session, it has been recognized that a single application of bleaching gel provides enough bleaching efficacy without requiring a second or third application in the same session [50]. Performing a single 30-minute application instead of three 15-minute applications makes it possible to avoid a higher concentration of hydrogen peroxide in the pulp chamber [28,51], thus decreasing the clinical risk of the patient's experiencing higher sensitivity during bleaching.

HP35 had the lowest scores, regardless of TiO_2 addition or VL application, indicating that the success of the bleaching treatment may be associated with the number of sessions performed, and the hydrogen peroxide concentration [11,52,53]. The bleaching efficacy of the HP35 group was established by the significant increase in the L^* and WID values, and the reduction in the b^* values throughout the treatment. Both L^* and b^* parameters are of great importance in determining bleaching treatment efficacy [54]. Another consideration is that the ΔE_{ab} values were above the threshold values of perceptibility and acceptability right after the 1st bleaching session (1.2 and 2.7, respectively) [40].

On the other hand, the bleaching treatment with HP7 used lower hydrogen peroxide concentrations, owing to its application with a tray, and required daily application to obtain a satisfactory result. This led to smaller changes in shades among the treatments [2,4,11,52,53,55]. Thus, the addition of a reaction catalyst, whether physical agents (such

Table 3

Mean (standard deviation) particle size value (in nm), PDI and ZP according to treatment, TiO₂ and time.

Parameter	Treatment	Time	TiO ₂ x	✓	
Particle size	HP7	Baseline	6840.67 (868.04)	477.03 (46.23) Be	
		7 days	863.60 (37.35) Bd	5900.67 (748.49) Ac	
		1 month	1253.00 (183.67) Bc	8799.00 (346.62) Aa	
		3 months	4675.33 (448.72) Bb	8200.33 (32.19) Ab	
		6 months	1362.00 (175.01) Bc	2880.00 (473.52) Ad	
		HP35	Baseline	*900.37 (14.20) Aa	430.93 (57.26) Ba
	7 days	*1146.67 (59.14) Aa	*456.90 (5.66) Ba		
	1 month	*830.13 (76.54) Ac	*418.03 (36.09) Ba		
	3 months	*752.07 (66.20) Ac	*294.77 (15.87) Bc		
	6 months	*146.33 (3.68) Bd	*334.73 (9.22) Ab		
	p-value: p(treatment)=0.0393; p(TiO ₂)=0.0243; p(treatment vs. TiO ₂)=0.0207; p(time)=0.1236; p(TiO ₂ vs. time)=0.0499; p(treatment vs. time)=0.1105; p(TiO ₂ vs. treatment vs. time)=0.1117				
	PDI	HP7	Baseline	0.96 (0.06) Aa	0.61 (0.06) Bb
			7 days	0.98 (0.04) Aa	0.98 (0.04) Aa
			1 month	1.00 (0.00) Aa	0.37 (0.05) Bc
			3 months	1.00 (0.00) Aa	0.57 (0.11) Bb
			6 months	1.00 (0.00) Aa	0.58 (0.17) Bb
			HP35	Baseline	*0.85 (0.07) Aa
		7 days	0.80 (0.18) Aa	*0.54 (0.08) Ba	
1 month		*0.75 (0.13) Aa	*0.56 (0.12) Ba		
3 months		*0.80 (0.07) Aa	*0.40 (0.02) Bb		
6 months		*0.18 (0.01) Bb	*0.39 (0.02) Ab		
p-value: p(treatment)=0.0024; p(TiO ₂)=0.0136; p(treatment vs. TiO ₂)=0.0050; p(time)=0.0444; p(TiO ₂ vs. time)=0.0474; p(treatment vs. time)=0.0594; p(TiO ₂ vs. treatment vs. time)=0.0502					
ZP		HP7	Baseline	-49.47 (1.03) Aa	-45.30 (1.81) Bb
	7 days		-39.10 (0.95) Ae	-38.73 (1.18) Ad	
	1 month		-46.27 (1.79) Ac	-42.73 (0.91) Bc	
	3 months		-43.53 (0.86) Bd	-48.87 (1.1) Aa	
	6 months		-45.70 (2.33) Ab	-47.67 (1.33) Aa	
	HP35		Baseline	*-51.47 (1.63) Abc	*-41.57 (1.85) Bbc
	7 days	*-46.07 (4.85) Ad	-39.63 (1.88) Bc		
	1 month	*-54.10 (1.4) Aa	*-49.30 (1.05) Ba		
	3 months	*-52.80 (2.45) Ab	-48.97 (2.27) Ba		
	6 months	*-50.73 (1.29) Ac	*-43.20 (1.21) Bb		
	p-value: p(treatment)=0.0035; p(TiO ₂)=0.0028; p(treatment vs. TiO ₂)=0.0031; p(time)=0.0207; p(TiO ₂ vs. time)=0.0314; p(treatment vs. time)=0.0212; p(TiO ₂ vs. treatment vs. time)=0.0262				

x: absent, ✓: present. Different letters (upper case horizontally and lower case vertically comparing treatments under VL and TiO₂ conditions) indicate statistically significant differences ($p \leq 0.05$). *Differs significantly from the HP7 group under the same conditions of time and TiO₂ incorporation ($p \leq 0.05$).

as light) or chemical agents (such as TiO₂), could benefit the effectiveness of tooth color change promoted by this agent of lower concentration. In this respect, in particular, the association of the catalyst to VL led to boosting the bleaching effect of lower concentration gels for in-office use [2,29,56].

The VL wavelength coincides with the absorbance peak of the pigment molecules, because it breaks them down into smaller molecules [2,28]. Although using VL in the absence of peroxide promotes an insubstantial bleaching effect that requires many sessions to bring it up to par [5,57], this same VL with peroxide-containing gels seemed to enhance the bleaching effect [2,29,56]. In the present study, this potentiating effect was notable after the 3rd bleaching session, when VL

was associated to a bleaching agent at a lower hydrogen peroxide concentration (7%). The result was significantly lower b* values, and higher L* and ΔE_{ab} values than when no light was used. The ΔE_{ab} values were above the limits of acceptability and perceptibility (ΔE_{ab} > 2.7) when the lowest concentration of hydrogen peroxide was used, both in the absence and presence of light. However, they were lower than those obtained when a high concentration of hydrogen peroxide was used (35%). Although use of the lower concentration provided a bleaching treatment with fewer side effects (lower sensitivity and cytotoxicity), it also produced greater color change, compared with the ΔE_{ab} values obtained by using HP35, both in the presence and absence of light. Therefore, using VL together with HP35 also favored a more effective bleaching treatment, by promoting lower b* and higher L* and W_D values after three bleaching sessions. The whiteness index showed that the value found for HP35 when combined with VL (27.19) was higher than without it (17.98) after two bleaching sessions. In addition, performing one more bleaching session increased this rate even more, resulting in a mean W_D value of 35.05 for the group with VL, and 20.79 for the alternative without VL, at the end of the third session.

On the other hand, the present study showed that the addition of TiO₂ to high- and low-concentration bleaching agents did not improve bleaching effectiveness, even when associated to VL. TiO₂ is recognized as a photosensitive agent, activated by visible light (with a wavelength in the ~405 nm range) [19,57]. As such, VL (with a wavelength of about 405 nm [28]) would boost the whitening effect. When analyzing the ΔW_{ID} parameter in particular, TiO₂ incorporated into hydrogen peroxide gel at a low concentration (HP7) led to lower color change values (mean of 14.91), compared with the gel without incorporation, in the presence of light (mean of 20.46), after the 3rd clinical session. Lower mean values were also observed for ΔE₀₀ after the 2nd session, when both bleaching agents were associated to TiO₂, compared with non-association to TiO₂ and association to light. Monteiro et al. [16] observed that addition of TiO₂ did not change the physicochemical properties (pH, P, PDI and ZP) of the gels in their study (10% carbamide and 40% hydrogen peroxide), and that TiO₂ increased the bleaching efficacy of the 40% hydrogen peroxide bleaching agent. However, the physicochemical characteristics resulting from addition of TiO₂ to the bleaching agents evaluated in the present study—which differ in composition from those used by Monteiro et al. [16]—may have influenced the bleaching response. In addition, the TiO₂ concentration used (1 wt%), or the light application protocol, may not have been sufficient to obtain greater color change effectiveness, as observed by Bortolatto et al. [4] and Cuppini et al. [13], who also showed that adding TiO₂ to lower concentration bleaching agents associated to light did not cause bleaching effectiveness to increase. Therefore, higher concentrations of TiO₂ should be evaluated with the consideration that a 5% concentration, as evaluated by Kury et al. [25], improved bleaching efficacy, even though the particles used in their study were different from those of the present study.

Although HP35 presented higher bleaching effectiveness than HP7, the use of VL boosted the bleaching treatment even with an agent of lower concentration. Hydrogen peroxide used in a lower concentration can have fewer harmful effects on the dental element, especially on the pulp, thus causing less sensitivity and providing the patient with greater comfort. The addition of TiO₂ did not boost the bleaching effect of hydrogen peroxide, even in the presence of light; however, further studies must be conducted using different concentrations and protocols for combining light sources. The P, PDI, and ZP properties of the bleaching agents showed good stability soon after the TiO₂ was incorporated into the bleaching agent. Moreover, the bleaching agents showed acceptable changes in mass and pH.

CRedit authorship contribution statement

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Table 4

Mean (standard deviation) color score value by the Vita Classical scale, and L* and b* values according to the bleaching treatment, the presence of VL and time.

Parameter	VL	Treatment	Time				p-value:
			Baseline	After 1st session	After 2nd session	After 3rd session	
Vita Classical	x	HP35	12.70 (2.00) Aa	7.80 (3.88) Ba	4.70 (3.50) Ca	2.70 (0.95) Db	p(LED)=0.0043; p(TiO ₂)=0.0005; p(HP)=0.3173; p(LED vs. TiO ₂)=0.3444; p(LED vs. HP)<0.0001; p(TiO ₂ vs. HP)=0.0168; p(LED vs. TiO ₂ vs. HP)=0.0146; p(time)=0.0037; p(LED vs. time)=0.0371; p(TiO ₂ vs. time)=0.0401; p(HP vs. time)<0.0001; p(LED vs. TiO ₂ vs. time)=0.1834; p(LED vs. HP vs. time)=0.0130; p(TiO ₂ vs. HP vs. time)=0.8087; p(LED vs. TiO ₂ vs. HP vs. time)=0.0724.
		HP7	12.10 (2.13) Aa	7.60 (3.27) Ba	5.50 (3.50) Ba	7.00 (3.53) Ba	
		HP35T	*10.10 (1.45) Ab	6.60 (3.10) Bb	5.00 (3.46) BCb	3.90 (2.85) Cb	
		HP7T	*13.30 (2.31) Aa	*10.00 (2.54) Ba	*8.90 (3.07) Ba	*9.40 (2.46) Ba	
	✓	HP35	\$9.90 (1.45) Aa	\$4.40 (3.20) Bb	\$2.00 (0.47) Cb	\$1.70 (0.48) Db	
		HP7	\$9.90 (1.45) Aa	8.40 (1.90) Ba	6.60 (3.20) BCa	4.90 (3.54) Ca	
		HP35T	9.90 (1.45) Aa	5.50 (3.69) Bb	3.30 (3.02) Cb	\$1.70 (0.48) Db	
		HP7T	\$10.20 (1.55) Aa	9.30 (0.95) Ba	7.10 (3.07) Ca	*\$7.70 (2.75) Ca	
L*	x	HP35	82.58 (5.60) Da	89.14 (4.11) Ca	90.77 (3.51) Ba	92.99 (2.81) Aa	p(LED)<0.0001; p(TiO ₂)<0.0001; p(HP) <0.0001; p(LED vs. TiO ₂) <0.0001; p(LED vs. HP)<0.0001; p(TiO ₂ vs. HP)<0.0001; p(LED vs. TiO ₂ vs. HP)<0.0001; p(time)<0.0001; p(LED vs. time)<0.0001; p(TiO ₂ vs. time)=0.1379; p(HP vs. time)<0.0001; p(LED vs. TiO ₂ vs. time)=0.6032; p(LED vs. HP vs. time)=0.00649; p(TiO ₂ vs. HP vs. time)=0.9531; p(LED vs. TiO ₂ vs. HP vs. time)=0.0484.
		HP7	84.36 (5.41) Ca	89.30 (1.94) Ba	90.21 (1.87) Aa	90.82 (1.63) Ab	
		HP35T	84.63 (2.91) Da	89.47 (1.82) Ca	91.71 (2.82) Ba	91.85 (3.68) ABa	
		HP7T	82.96 (4.68) Ca	*87.99 (2.94) Bb	*88.06 (2.51) Bb	*89.50 (2.43) Ab	
	✓	HP35	85.04 (2.73) Ca	89.17 (3.51) Ba	\$93.05 (2.54) Aa	\$94.27 (2.77) Aa	
		HP7	86.13 (4.87) Ca	88.17 (1.94) Ca	89.31 (4.33) BCb	\$92.83 (1.57) Ab	
		HP35T	84.55 (4.14) Db	89.74 (2.73) Ca	\$93.47 (2.31) Ba	\$95.40 (2.36) Aa	
		HP7T	*\$89.32 (6.05) Ba	*\$89.03 (3.99) Ba	*\$91.25 (2.22) Ab	*\$92.18 (2.93) Ab	
b*	x	HP35	35.63 (4.44) Aa	27.14 (2.17) Ba	25.00 (2.10) Cb	23.37 (1.87) Db	p(LED)<0.0001; p(TiO ₂)<0.0001; p(HP) <0.0001; p(LED vs. TiO ₂) <0.0001; p(LED vs. HP)<0.0001; p(TiO ₂ vs. HP)<0.0001; p(LED vs. TiO ₂ vs. HP)<0.0001; p(time)<0.0001; p(LED vs. time)<0.0001; p(TiO ₂ vs. time)=0.1409; p(HP vs. time)<0.0001; p(LED vs. TiO ₂ vs. time)=0.1682; p(LED vs. HP vs. time)=0.4309; p(TiO ₂ vs. HP vs. time)=0.0171; p(LED vs. TiO ₂ vs. HP vs. time)=0.0199
		HP7	33.17 (3.80) Ab	27.67 (2.68) Ba	26.76 (3.06) Ba	27.15 (2.66) Ba	
		HP35T	*32.86 (3.57) Ab	26.87 (3.55) Bb	25.24 (3.11) Cb	23.65 (3.75) CDb	
		HP7T	35.08 (4.42) Aa	*28.83 (3.07) Ba	*29.43 (2.94) Ba	*28.84 (2.82) Ba	
	✓	HP35	\$31.64 (2.68) Ab	\$22.81 (4.10) Bb	\$18.71 (3.64) Cb	\$18.14 (4.48) Cb	
		HP7	\$35.23 (4.87) Aa	\$29.61 (3.29) Ba	26.45 (3.31) Ca	\$23.80 (3.94) Da	
		HP35T	32.20 (4.54) Ab	*\$25.38 (4.44) Bb	*\$21.76 (4.47) Cb	*\$18.87 (4.16) Db	
		HP7T	*\$37.84 (5.48) Aa	*\$31.33 (2.51) Ba	*\$28.26 (2.43) Ca	*\$27.54 (1.91) CDa	

x: absent, ✓: present. Different letters (upper case horizontally and lower case vertically comparing treatments under VL and TiO₂ conditions) indicate statistically significant differences ($p \leq 0.05$). *Differs significantly from the group without TiO₂ under the same conditions as the other factors ($p \leq 0.05$). \$ differs significantly from the group without VL under the same conditions as the other factors ($p \leq 0.05$).

Table 5

Median (minimum and maximum value) of a* and WI_D according to the bleaching treatment, the presence of TiO₂, the presence of VL and time.

Parameter	VL	Treatment	Time				p-value	
			Baseline	After 1st session	After the 2nd session	After the 3rd session		
a	x	HP35	5.20 (1.30; 11.20) Aa	1.65 (-0.70; 2.70) ABa	0.70 (-1.00; 2.20) BCa	0.30 (-1.40; 1.50) Ca	<0.0001	
		HP7	3.35 (1.70; 15.10) Aa	0.95 (-0.10; 2.50) ABa	0.70 (-0.30; 2.30) Ba	0.45 (-0.50; 1.70) Ba	<0.0001	
		p-value	0.3443	1.0000	0.8498	0.2565		
		HP35T	*2.60 (1.10; 4.80) Ab	1.05 (-0.10; 1.80) ABa	0.90 (-1.00; 1.30) Ba	0.40 (-1.20; 1.70) Ba	<0.0001	
		HP7T	6.00 (1.30; 10.40) Aa	1.70 (-0.30; 5.30) ABa	1.75 (-0.30; 4.40) ABCa	1.45 (-0.50; 3.70) Ca	<0.0001	
		p-value	0.0232	0.1978	0.0636	0.0687		
		✓	HP35	\$1.40 (0.00; 3.60) Aa	1.05 (0.10; 2.40) Ab	-0.20 (-1.50; 1.00) Bb	-0.60 (-1.80; 1.20) Bb	<0.0001
			HP7	2.15 (0.40; 6.80) Aa	1.80 (1.10; 3.20) ABa	\$1.65 (0.60; 2.80) ABa	1.10 (-0.40; 1.80) Ca	<0.0001
	p-value		0.1615	0.0044	0.0004	0.0232		
	HP35T		1.25 (0.00; 4.70) Aa	1.15 (-0.40; 2.60) Ab	1.10 (-1.70; 2.30) ABa	-0.45 (-1.90; 1.60) Bb	<0.0001	
	WI _D	x	HP7T	\$2.35 (0.90; 4.40) Aa	2.00 (1.30; 3.50) Aa	1.30 (0.80; 2.20) Ba	1.30 (1.00; 2.20) ABa	0.0005
			p-value	0.1728	0.0489	0.2111	0.0018	
			HP35	-7.95 (-29.98; 5.06) Ca	10.80 (6.45; 23.74) BCa	17.98 (6.67; 26.13) ABa	20.79 (15.66; 29.75) Aa	<0.0001
			HP7	-1.58 (-41.05; 7.69) Ba	13.06 (2.87; 19.44) ABa	16.83 (8.80; 19.75) Aa	15.61 (8.19; 21.20) Ab	<0.0001
		✓	HP35T	*3.70 (-14.19; 7.83) Ca	13.42 (5.19; 23.4) BCa	16.95 (11.15; 28.86) ABa	19.74 (9.27; 30.70) ABa	<0.0001
			HP7T	-10.29 (-20.34; 9.87) Bb	9.21 (-0.46; 20.86) ABb	*8.45 (2.89; 21.63) ABb	9.36 (3.45; 23.27) Ab	<0.0001
p-value			0.0232	0.1655	0.0029	0.0089		
HP35			\$5.37 (-4.42; 15.23) Ca	18.88 (5.16; 24.89) BCa	\$27.19 (17.94; 37.38) ABa	\$32.05 (17.72; 38.74) Aa	<0.0001	
p-value	HP7	1.01 (-15.55; 10.03) Ca	6.73 (1.54; 15.73) BCb	14.69 (4.05; 19.69) ABb	18.16 (9.29; 28.98) Ab	<0.0001		
	HP35T	7.20 (-14.02; 16.56) Ca	15.91 (5.26; 26.26) BCa	20.77 (10.94; 37.18) ABa	\$29.28 (18.69; 38.63) Aa	<0.0001		
	HP7T	-1.66 (-13.19; 8.09) Ca	6.63 (-4.95; 12.56) BCb	11.70 (5.93; 19.01) ABb	*14.16 (8.10; 18.32) Ab	<0.0001		
	p-value	0.0892	0.0147	0.0011	<0.0001			

x: absent, ✓: present. Different letters (upper case horizontally and lower case vertically comparing treatments within VL and TiO₂ conditions) indicate statistically significant differences ($p \leq 0.05$). *Differs significantly from the group without TiO₂ under the same conditions as the other factors ($p \leq 0.05$). \$ differs significantly from the group without VL under the same conditions as the other factors ($p \leq 0.05$).

Table 6

Mean (standard deviation) of ΔE_{ab} , ΔE_{00} , ΔWI_D , according to bleaching treatment, presence of TiO_2 and presence of VL.

	VL	Treatment	Time		
			After 1st session - Baseline	After 2nd session - Baseline	After 3rd session - Baseline
ΔE_{ab}	x	HP35	12.53 (4.38) a	14.99 (3.80) a	17.65 (3.74) a
		HP7	9.12 (5.16) b	10.44 (4.96) b	10.54 (5.46) b
		HP35T	*8.05 (2.31) a	10.83 (2.90) a	*12.36 (3.44) a
	✓	HP7T	9.77 (2.98) a	9.68 (2.56) b	11.18 (2.93) a
		HP35	10.26 (2.70) a	15.50 (3.23) a	16.65 (2.53) a
		HP7	7.16 (3.34) b	11.30 (4.36) b	§13.92 (4.05) a
		HP35T	*8.79 (3.79) a	14.00 (4.42) a	§17.47 (4.35) a
ΔE_{00}	x	HP35	6.84 (2.94) a	8.16 (2.43) a	9.74 (2.59) a
		HP7	5.24 (3.63) a	6.08 (3.60) b	6.23 (3.83) b
		HP35T	4.34 (1.44) a	*5.94 (1.63) a	*6.69 (2.27) a
	✓	HP7T	5.42 (1.94) a	*5.47 (1.89) b	6.37 (2.25) a
		HP35	5.22 (1.51) a	8.23 (1.98) a	9.02 (1.48) a
		HP7	§3.56 (1.89) b	5.78 (2.22) b	7.00 (2.36) a
		HP35T	4.68 (2.13) a	*7.65 (2.44) a	§9.59 (2.5) a
ΔWI_D	x	HP35	22.71 (9.39) a	27.27 (8.98) a	31.49 (9.04) a
		HP7	17.08 (11.50) a	19.87 (12.32) a	19.99 (12.43) b
		HP35T	*12.92 (4.03) a	16.65 (6.36) a	*19.37 (6.22) a
	✓	HP7T	18.02 (7.70) a	17.61 (6.91) a	19.97 (6.96) a
		HP35	§13.08 (4.69) a	22.41 (6.06) a	§23.82 (5.04) a
		HP7	9.06 (8.11) a	§13.89 (7.26) b	20.46 (7.34) a
		HP35T	11.06 (6.11) a	18.39 (6.63) a	24.86 (6.11) a
		HP7T	§7.64 (4.65) a	§13.69 (5.46) b	*§14.91 (6.34) b

x:absent, ✓: present. Different vertical letters (comparing between treatments within VL and TiO_2 conditions) indicate statistically significant differences ($p \leq 0.05$). * Differs significantly from the group without TiO_2 under the same conditions as the other factors ($p \leq 0.05$). § differs significantly from the group without VL under the same conditions as the other factors ($p \leq 0.05$).

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Declarations of Competing Interest

None.

Acknowledgments

The authors acknowledge the São Paulo Research Foundation-FAPESP (2020/00710–1 and 2020/11031–8) and CNPq (308736/2021–4).

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