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RESEARCH ARTICLE

Effect of surfactant concentration in sodium ascorbate on contact angle and tensile bond strength after bleaching

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ABSTRACT

Free radical residue due to intracoronal bleaching using 35% hydrogen peroxide (HP) can be removed with 35% sodium ascorbate (SA) combined with surfactants. This study aimed to determine the effect of surfactant concentration in SA 35% on the contact angle and tensile bond strength of composite resin after intracoronal bleaching with 35% HP. The contact angle was observed in 3 groups of 35% SA: group 1 (without surfactant); group 2 (with 0.2% surfactant); group 3 (with 0.4% surfactant). Each sample was dropped on a glass slide perpendicularly, then the image was taken at the fifth minute and contact angle value was obtained using ImageJ software. Tensile bond strength in this study used 21 premolars, which were cut and fixed with acrylic resin. 35% HP (0.01 ml) was applied to tooth surface for 5 days, then washed and dried. Specimens were divided into 3 groups, each of which was applied with 0.01 ml 35% SA without surfactant (group 1), 35% SA with 0.2% surfactant (group 2), and 35% SA with 0.4% surfactant (group 3) for 5 minutes, before they were washed and dried. All specimens were filled with composite resin, and incubated in artificial saliva for 7 days inside an incubator (37^{o}C) before the specimens were tested with Universal Testing Machine (speed 0.5 mm/minute). ANOVA analysis showed the effect of surfactant concentration in 35% SA on the contact angle and tensile bond strength after intracoronal bleaching with 35% HP. In conclusion, 35% SA with 0.4% surfactant had a smaller contact angle and application of 35% SA with 0.4% surfactant after intracoronal bleaching with 35% HP had a greater tensile bond strength.

Keywords: 35% sodium ascorbate; bleaching; contact angle; surfactant; tensile bond strength

INTRODUCTION

Bleaching is a cosmetic procedure that is widely used because of its simplicity, proven clinical success, and non-invasiveness, since it is conducted without removing the tooth structure.[1] Intracoronal bleaching is a procedure performed on discolored teeth to whiten their appearance by applying chemicals to the pulp chamber after root canal treatment.[2] The active ingredients in bleaching agents are peroxide compounds, and the most commonly used ingredients are hydrogen peroxide, sodium perborate, and carbamide peroxide.[3] 35% hydrogen peroxide is a widely used bleaching active ingredient.[4] The traditional bleaching mechanism is based on the "chromophore theory," which is derived from the interaction of hydrogen peroxide with organic chromophores in the tooth structure. Organic chromophores are colored molecules that consist of conjugated pi systems, such as aromatic compounds that have electron-rich regions, or bioinorganic metal complexes, such as chelates.[5] Free radicals are released by hydrogen peroxide, namely dihydroxyl (HOO•) and onascen (O•). One of the side effects of bleaching is the presence of free radicals after the bleaching procedure, which will inhibit the polymerization of resin-based materials, thereby reducing bonding strength.[6] The application of a bleaching agent to enamel can reduce the bonding strength of enamel, which is proportional to the concentration of the bleaching agent used.[7] The greater the number of highly reactive free radicals, the greater the failure of polymerization in composite resin so that the failure rate of composite resin adhesion increases.[8]

The application of antioxidants to teeth after intracoronal bleaching that need to be restored immediately is an effort to minimize the risk of failure. Antioxidants are substances that can reduce free radicals' concentration in cells and the body.[6] Antioxidants provide hydrogen atoms, which will bind to free radicals as a way to improve their stability.[9] Some applicable antioxidants are sodium ascorbate, catalase, ethanol, and alpha-tocopherol.[10] It is recommended to apply sodium ascorbate after bleaching with the same concentration as hydrogen peroxide. Freire's research showed that application of 35% sodium ascorbate with a concentration for 5 minutes was able to neutralize free radical residues on post-bleaching teeth with 35% hydrogen peroxide.[11] Similarly, Nugraheni et al. revealed that two applications of 35% sodium ascorbate could increase the strength of composite resin shear in teeth after bleaching with 35% H_2O_2.[12] Coppla et al. also demonstrated that double application of 35% sodium ascorbate in 5-10 minute intervals could restore the bonding strength of post-bleaching enamel as well as non-bleached enamel.[7] Sodium ascorbate is a derivative of ascorbic acid which is widely used as an antioxidant, but has unstable properties, and thus to achieve effective results it is necessary to add substances that can increase the stability of sodium ascorbate, including stabilizers or preservatives, such as citric acid, boric acid or tartaric acid, synergists, and emulsifiers such as surfactants.[13]

Surfactants (surface active agents) are substances added to liquids to increase their penetrating properties by reducing the surface tension of the liquid. Surfactants have an amphipathic molecular structure, which consists of a hydrophilic and hydrophobic group that will break hydrogen molecules on tooth surface so that it can reduce surface tension. Polysorbate is one of nonionic surfactants that is often used in the food, cosmetic and pharmaceutical industries because it is non-toxic, easy to obtain, can serve as an emulsifier or a solvent. Tween®80 is often used in drug doses to control moisture, stability and the solvent properties of drugs, which are hydrophobic.[14] Moosavi et al. highlighted that 0.2% surfactant (Tween®80) in sodium ascorbate significantly reduced micro-leakage after nonvital bleaching. Moosavi et al. also declared that the function of surfactants was to reduce interface stress and contact angle as well as the cohesion between sodium ascorbate molecules. This process will stabilize substances so that antioxidants can penetrate deeper into the dentin in the dentinal tubules and free radicals can be eliminated properly.[15] Complete elimination of free radical residue will increase mechanical properties such as bond strength.[11,16]

The mechanical properties of restorative materials refer to a description of the stresses and the restorative material stress received from external forces, in the form of tensile, shear, and compressive stresses.[17] Restorative materials with high compressive strength, flexural strength and tensile strength are clinically applicable and will be more resistant to mastication forces.[18] The tensile strength test is useful to know the bonding strength of the material to the tooth structure.[19] Carissa in her study insinuated that the application of 35% sodium ascorbate and 35% sodium ascorbate with 0.2% surfactant (Tween®80) provide the same tensile bond strength of composite resin after intracoronal bleaching.[20] This situation can be caused by the residue in the dentin that has not been completely lost, thus disrupting attachment between bonding agent and tooth surface resulting in the low mechanical strength in bond formation. Therefore, it was suggested to study the contact angle formed between sodium ascorbate and several surfactant concentrations to determine the polarity and surface tension properties to know material characteristics. Further researches regarding the effect of surfactants in sodium ascorbate 35% using higher concentrations without exceeding the CMC limit on the adhesive tensile strength of composite resin are also needed.[20]

The polarity and surface tension properties can be determined by measuring the contact angle of a material. Contact angle measurement can give information on surface energy, hydrophobicity, hydrophilicity, surface roughness and surface heterogeneity.[21] Contact angle measurement is one of the appropriate methods to study and develop solvent wetting phenomenon.[22] Surfactants are substances added to liquids to increase penetration by reducing the surface tension of the liquid.[23] The surfactant works to reduce surface tension that will form micelle and is expressed as CMC (Critical Micelle Concentration), which is the concentration of saturated surfactant in an emulsion. The surface tension of surfactant solution will decrease rapidly with an increasing concentration up to the CMC point. However, after reaching the CMC point, the further increase in concentration will not decrease the surface tension.[24] Based on previous studies, the CMC Tween®80 value is about 5.9 µg/mL (0.59%)- 5.7 µg/mL (0.57%), so the surfactants will achieve maximum function at concentrations close to 0.57% -0.59%.[25] On this ground, our research was conducted to study the effect of surfactant concentration in sodium ascorbate on contact angle and tensile bond strength after tooth bleaching.

MATERIALS AND METHODS

This experimental study was conducted at the Integrated Research Laboratory, Faculty of Dentistry, Faculty of Pharmacy and Faculty of Engineering, Universitas Gadjah Mada, Yogyakarta. The ethical clearance of this research was obtained from the Ethics Committee of Faculty of Dentistry, Universitas Gadjah Mada, based on certificate number 00486/KKEP/FKG-UGM/EC/2020.

Gel of 35% sodium ascorbate without surfactant was made by mixing 0.5 grams of CMC-Na powder with 10 ml warm distilled water, then added with 3.5 grams of sodium ascorbate powder and stirred homogeneously. 35% sodium ascorbate gel with 0.2% surfactant was made by mixing 0.5 grams of CMC-Na powder with 10 ml of warm distilled water, then added with 3.5 grams of sodium ascorbate powder and 0.02 ml of surfactant (Tween®80, USA) to be stirred homogeneously. 35% sodium ascorbate gel with 0.4% surfactant was made by mixing 0.5 grams of CMC-Na powder with 10 ml warm distilled water, then added with 3.5 grams of sodium ascorbate powder and 0.04 ml of surfactant (Tween®80, USA) to be stirred homogeneously. The finished result was stored in the refrigerator at 4 °C. This study used 3 molds, namely A-mold, B-mold and C-mold. The A-mold was used to mold composite resin restorations, made of metal with a 50 mm length, 50 mm width and 4 mm height. The center of mold had a cone-shaped hole with 3 mm (base) and 6 mm (top) diameters. The A-mold can be split into 2 parts to easily open it after the composite resin hardens (Figure 1A). The B-mold was used to mold acrylic resin as a way to fix teeth. It was made of square-shaped metal (50 mm x 50 mm x 10 mm). The center of the mold was given a cylindrical-shape hole with 10 mm diameter. This mold can be split into two parts so that it can be opened easily after the acrylic resin hardens (Figure 1B). The C-mold was used to mold acrylic resin as a way to fix the composite resin, made of a square-shaped metal (50 mm x 50 mm x 100 mm). The center of this mold had a cylindrical hole with 10 mm diameter and 10 mm height for molding the acrylic resin and also a square-shaped hole (10 mm x 5 mm x 70 mm) for fixing the wire. The C-press can be split into two parts to easily open it after the acrylic resin hardens (Figure 1C).

The contact angle was observed from three groups of 35% sodium ascorbate gel (Sigma-aldrich, Louis, MO, USA), consisting of group 1 (35% sodium ascorbate gel without surfactant), group 2 (35% sodium ascorbate gel with 0.2% surfactant) and group 3 (35% sodium ascorbate gel with 0.4% surfactant). The sample was dropped perpendicularly on the glass slide and left for 5 minutes. The image was taken at the fifth minute using a camera and a block that has been set to get the same distance consistently of each sample as in Figure 2. The images were transferred to ImageJ software to line determination of interaction between the outer liquid surface and surface of the glass plate to obtain the contact angle value.

The tensile bond strength of composite resin was observed from 21 human premolar teeth that met the inclusion criteria and stored in 10% formalin buffer solution at 4 °C temperature for a maximum of 2 months until the time of the study.

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The crowns were cut at 2 mm apically from the CEJ and 4 mm coronally from the CEJ, then fixed using self-cured acrylic resin with the occlusal side facing upward parallel to the surface of the acrylic resin. All specimens were bleached using 0.01 ml of 35% hydrogen peroxide (Opalescence Endo, Ultradent, USA) then put in a closed box and stored in an incubator 37 °C for 5 days (Figure 3).

Specimens that have been incubated for 5 days were washed with 10 ml of distilled water using a disposable syringe and dried for 10 seconds with a three-way syringe, then divided into 3 groups based on the addition of surfactants: Group 1 (35% sodium ascorbate without surfactant), Group 2 (35% sodium ascorbate with 0.2% surfactant), and Group 3 (35% sodium ascorbate with 0.4% surfactant). Each group consisted of 7 specimens, and all specimens were applied with 0.01 ml of 35% sodium ascorbate gel for 5 minutes, then washed thoroughly using 10 ml of distilled water and dried for 10 seconds (Figure 4).

The specimens were then applied with 8th bonding generation (Single Bond Universal, 3M, USA) using a microbrush for 20 seconds, dried with a three-way syringe for 5 seconds and activated with light curing unit for 10 seconds (figure 5A). The specimen was fixed on the B-mold, then A-mold was placed coinciding with B-mold, with the hole in the A-mold located on the fixed dentin surface (Figure 5B). Bulkfill composite resin (Filtek Bulkfill, 3M, USA) was applied to the A-mold with 4 mm depth, then covered with celluloid tape and cured for 20 seconds (Figure 5C). The specimens were incubated in artificial saliva solution (pH = 7) in an incubator at 37 °C for 7 days.

All specimens were dried, then fixed with a composite resin on the C-mold with a wire so that the specimen could be held by the tool holder (Figure 6A). The test on the tensile bond strength of composite resin was performed by using Universal Testing Machine at 0.5 mm/minute speed (Figure 6B). The measurement result of tensile strength in Mpa was obtained by dividing the numbers shown on the Universal Testing Machine (Newton, Salter, England) using the diameter of composite resin surface (mm^2).

(A) (B) (C)

Figure 1. (A) The A-mold (B) The B-mold (C) The C-mold

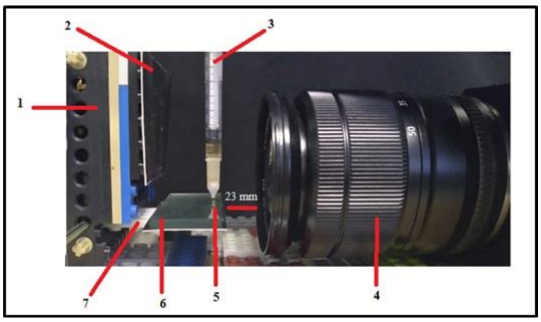

Figure 2. Camera and block setting for contact angle photography: (1) Back beam (2) Black background (3) Disposable syringe (4) Camera (5) Droplets sample (6) Glass slide (7) Base beam

(A) (B) (C)

Figure 3. Bleaching application (A) 35% hydrogen peroxide (Opalescence Endo, Ultradent) (B) Bleaching application on the dentin occlusal surface (C) Specimens were put in a closed box and stored in an incubator

(A) (B) (C)

Figure 4. Specimen group: (A) Group I (applied with 35% sodium ascorbate without surfactant) (B) Group II (applied 35% with sodium ascorbate with 0.2% surfactant) (C) Group III (applied with sodium ascorbate 35% with 0.4% surfactant)

(A) (B) (C)

Figure 5. (A) Bonding application using microbrush for 20 seconds (B) A-mold and B-mold was placed coincided (C) Specimens that have been restored with bulkfill composite resin

(A) (B)

Figure 6. (A) Specimens that have been fixed with wire (B) Test on tensile bond strength of composite resin with the Universal Testing Machine.

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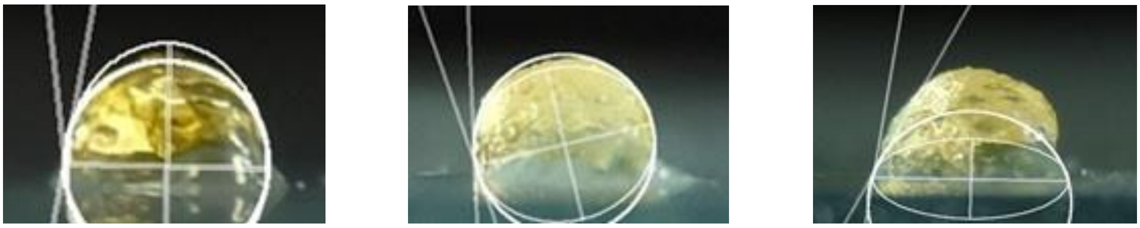
RESULTS

The contact angle of the three groups of 35% sodium ascorbate gel (Sigma-Aldrich, Louis, MO, USA), consists of group 1 (35% sodium ascorbate gel without surfactant), group 2 (35% sodium ascorbate gel with 0.2% surfactant and group 3 (35% sodium ascorbate gel with 0.4% surfactant). This study was conducted to determine the effect of surfactant concentration in 35% sodium ascorbate on the contact angle for 5 minutes and the image was taken using a digital camera at the fifth minute. The process obtained the average two image angles observed using image-J software (Figure 7).



(A) (B) (C)

Figure 7. Measurement result of contact angle using image-J software: (A) Groups I (B) group 2 (C) Group 3

Table 1. Mean value of sodium ascorbate gel (degree)

Group	Mean ± standard deviation
1	96.66 ± 4.41
2	90.70 ± 5.94
3	65.78 ± 8.35

Table 2. Result of Shapiro-wilk test

Shapiro-wilk			
Group	Statistic	df	Sig.
1	0.820	4	0.143
2	0.961	4	0.788
3	0.911	4	0.489

Table 3. Anova result of sodium ascorbate contact angle

	df	F	Sig.
Between group	2	25.866	0.000
Within group	9		
Total	11		

Table 4. Results of LSD post hoc test

(I) Group	(J) Group	Mean difference (I-J)	Sig.
1	2	5.95250	0.224
	3	30.87750	0.000*
2	1	-5.95250	0.224
	3	24.2500	0.000*
3	1	-30.87750	0.000*
	2	-24.92500	0.000*

*The mean difference is significant at the 0.05 level
Group 1: sodium ascorbate without surfactant
Group 2: sodium ascorbate with 0.2% surfactant
Group 3: sodium ascorbate with 0.4% surfactant

Data were analyzed using IBM SPSS Statistic software. The mean and standard deviation of sodium ascorbate contact angles are presented in Table 1.

The Shapiro-Wilk normality test in Table 2 indicated that the data in the three groups are normally distributed ($p > 0.05$). The results of Levene's test showed a significance of $p = 0.228$ which indicated homogenous variance of these data ($p > 0.05$). The analysis revealed that the data were normally distributed and homogenous, and thus a parametric test was done on the current study using one-way ANOVA.

One-way ANOVA (Table 3) showed that contact angle of 35% sodium ascorbate with difference surfactant concentration were

Table 5. Mean value of tensile bond strength of composite resin (MPa)

Group	Mean ± standard deviation
1	5.52 ± 0.97
2	5.58 ± 1.12
3	6.82 ± 0.41

Table 6. Result of Shapiro-wilk test

Shapiro-wilk			
Group	Statistic	df	Sig.
1	0.944	7	0.678
2	0.879	7	0.223
3	0.959	7	0.808

Table 7. Anova result of tensile bond strength of composite resin

	df	F	Sig.
Between group	2	4.722	0.022
Within group	18		
Total	20		

Table 8. Results of LSD post hoc test

I (Group)	J (Group)	Mean difference (I-J)	Sig.
1	2	-.06429	0.894
	3	-1.29714	0.014*
2	1	.06429	0.894
	3	-1.23286	0.018*
3	1	1.29714	0.014*
	2	1.23286	0.018*

*The mean difference is significant at the 0.05 level
Group1: sodium ascorbate without surfactant
Group2: sodium ascorbate with 0.2% surfactant
Group3: sodium ascorbate with 0.4% surfactant

significantly different among the groups ($p < 0.05$). Further $LSD_{0.05}$ post hoc test (Table 4) portrayed that there were significantly different contact angles between Group 1 with Group 3 and also between Group 2 and Group 3, but, there was no significant difference between the contact angles of Group 1 and Group 2.

The tensile bond strength of composite resin study used 21 first premolar teeth which were randomly divided into 3 groups after intracoronal bleaching with a total of 7 specimens in each group. Group 1 was applied with 35% sodium ascorbate without surfactant, group 2 was applied with 35% sodium ascorbate gel with 0.2% surfactant and group 3 was applied with 35% sodium ascorbate gel with 0.4% surfactant. Specimens were tested using Universal Testing Machine with 0.5 mm/minute tensile speed. The results obtained on Universal Testing Machine screen was the maximum tensile strength when the specimen and composite resin were released in Newton. The value of tensile strength was obtained by dividing the numbers on Universal Testing Machine (in Newton) by diameter of composite resin surface area used ($\pi r^2 = 3.14 \times (1.5 \text{ mm})^2 = 7.07 \text{ mm}^2$). After the tensile strength value was obtained, the results of the study were analyzed using IBM SPSS Statistic software. The mean and standard deviation of tensile bond strength of composite resin are displayed in Table 5.

The results of the Shapiro-Wilk normality test in Table 5 pinpointed that the data in the three groups are normally distributed with $p > 0.05$. The results of Levene's test showed a significance of $p = 0.104$, which means that the variance of the data were homogenous ($p > 0.05$). The analysis showed that the data were normally distributed and homogenous, and thus a parametric test was done using one-way ANOVA.

One-way ANOVA (Table 7) delineated that there was a significantly different level of tensile bond strength of composite resin that is applied to 35% sodium ascorbate with difference surfactant concentration among the groups ($p < 0.05$). Further $LSD_{0.05}$ post hoc test (Table 8) demonstrated that there was significantly different contact angles between Group 1 with Group 3 and also between Group 2 and Group 3, but, there was no significant difference between contact angles of Group 1 and those of Group 2.

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DISCUSSION

The addition of surfactant to 35% sodium ascorbate gel increases the penetrating properties of sodium ascorbate gel by reducing its surface tension. The greater surfactant concentration in the mixture, the smaller the surface tension between phases.[26] The surfactant concentration can be increased until it reaches the critical concentration (CMC). Hence, to reduce the maximum surface tension, the CMC value of Tween®80 is about 0.57%-0.59%.[25]

The results revealed that the mean value of the contact angle of the sodium ascorbate gel 35% combination of surfactant 0.4% was smaller than the sodium ascorbate gel 35% with the combination of 0.2% surfactant and without surfactant. Increasing the surfactant concentration closed to the CMC value in 35% sodium ascorbate could reduce the 35% sodium ascorbate surface tension, thereby increasing its wetting ability. The value of the contact angle was directly proportional to the surface tension of the liquid.[27] The smaller the contact angle, the lower the surface tension of the liquid and thus the greater its wetting ability.[28]

The study also showed that the average value of the tensile bond strength of composite resin was greater in the application of sodium ascorbate 35% with the combination of 0.4% surfactant compared with those in the application of sodium ascorbate 35% with the combination of 0.2% surfactant and without surfactant. Increasing the surfactant concentration in 35% sodium ascorbate also could increase the penetration of 35% sodium ascorbate on the dentin surface, the function of 35% sodium ascorbate in binding free radicals became more optimal, the dentin surface bond strength with composite resin increased and the tensile bond strength of composite resin also increased. The addition of surfactants in sodium ascorbate resulted in deeper penetration of sodium ascorbate into dentin which could bind free radicals better.[15] Sodium ascorbate was able to increase the bond strength between composite resin and intracoronal dentin. Sodium ascorbate reacted with free radicals due to the bleaching process and neutralized them in the structures where they are trapped.[29] Sodium ascorbate was easily degraded, especially in the presence of oxygen, threonic acid, glyceric acid and glyoxalic acid.[30] The gel form used in sodium ascorbate application and had a high viscosity. The higher the viscosity of a material the lower its penetrating ability.[31] Sodium ascorbate also tends to recrystallize and has a greater crystallization rate in a longer storage time.[32] The addition of surfactants was an effort to increase the stability and penetrating ability of sodium ascorbate.[13]

Surfactant concentration of 0.2% was still far from the CMC value. It was not optimal to reduce the surface tension of 35% sodium ascorbate so it had a lower penetration of 35% sodium ascorbate on the dentin surface and lower the bond between dentin surface and composite resin. The results also showed no differences in tensile bond strength of composite resin between the application of 35% sodium ascorbate without surfactant and the application of sodium ascorbate 35% with 0.2% surfactant. These results are the same asCarissa's research, which concluded that the application of 35% sodium ascorbate and 35% sodium ascorbate with 0.2% surfactant resulted the same tensile bond strength of composite resin.[20]

Carissa in her research showed that one of the low result in tensile strength was attributed to the fact that 5 ml of distilled water did not completely remove the sodium ascorbate residue on the dentin surface.[20] The author had increased the volume of distilled water to 10 ml in this study, but in fact it had not been able to clean the dentin surface completely, and thus it is necessary to have a better cleaning technique after sodium ascorbate application. Kimyai et al. and Dabas et al. in their research washed and soaked the specimens in distilled water for 10 minutes to dissolve the sodium ascorbate crystals precipitated.[33,34] Other applicable technique is pumice prophylaxis on exposed dentin before bonding procedure to clean the dentin surface.[35] The surface condition of the teeth also affects the contact angle of the sodium ascorbate gel. The contact angle changes with the surface topography, the surface tension of the liquid, the surface energy of the substrate, and the degree of interaction between liquid and solid.[36] The technique and quantity of 35% sodium ascorbate application can also affect the value of tensile bond strength of composite resin. Briso et al. stated that using 10% sodium ascorbate passively for 10 minutes failed to show a better bond between restorative material and dentin, so it is necessary to stir it to eliminate free radicals.[37] Freire et al. stated that the quantity of sodium ascorbate application is more important than the duration of contact, and that 2x1 minute application is sufficient to eliminate free radical residue from the dentinal tubules.[16] Ismail et al. in their research concluded that two applications of 35% sodium ascorbate solution with 1 minute per application were very effective in restoring the bonds damaged by the bleaching process with 35% hydrogen peroxide.[35] Coppla et al. in their research also showed that double application of 35% sodium ascorbate in 5-10 minute intervals can restore the bonding strength of post-bleaching enamel as well as non-bleached enamel.[7]

CONCLUSION

In conclusion, the study showed that the contact angle of 35% sodium ascorbate with 0.4% surfactant was smaller than that of 35% sodium ascorbate with 0.2% surfactant and without surfactant. This study also revealed that the tensile bond strength of composite resin of teeth after intracoronal bleaching with 35% hydrogen peroxide applied with 35% sodium ascorbate with 0.4% surfactant was greater than those applied with 35% sodium ascorbate with 0.2% surfactant and without surfactant.

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