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The effect of vanillin nanoparticles on antimicrobial and mechanical properties of an orthodontic adhesive

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Abstract

AIMS: To evaluate the effect of adding vanillin nanoparticles on the antimicrobial and mechanical properties of the orthodontic adhesive.

MATERIALS AND METHODS: Transbond XT orthodontic adhesive (3M Unitek, Monrovia, California, USA) was modified with 1% and 2% vanillin nanoparticles. The chemical composition and degree of chemical conversion in orthodontic adhesive before and after adding vanillin nanoparticles to orthodontic adhesive were measured using Fourier transformation infrared spectroscopy (FTIR). Mechanical properties of unmodified orthodontic adhesive (UMOA) and 1% and 2% vanillin-modified orthodontic adhesive (VMOA) were assessed in shear bond strength (SBS) and tensile bond strength (TBS). The antimicrobial properties were evaluated using a Mueller–Hinton plate swapped with streptococcus mutans. The zone of bacterial inhibition for UMOA, 1% VMOA, and 2% VMOA was measured. Descriptive statistics, multiple comparisons, one-way ANOVA, and post hoc Duncan's test were used to compare among the results.

RESULTS: FTIR showed no chemical conversion of 1% VMOA and 2% VMOA. There was significant streptococcus mutans growth inhibition in 1% VMOA and 2% VMOA compared to UMOA. No significant difference in streptococcus mutans growth inhibition in 1% VMOA and 2% VMOA. The SBS decreased significantly in 1% VMOA compared to UMOA. In addition, SBS decreased insignificantly when comparing 1% VMOA and 2% VMOA. TBS significantly reduced in 2% VMOA compared with UMOA. In addition, there was no significant difference in TBS between UMOA and 1% VMOA, and 1% VMOA and 2% VMOA, respectively.

CONCLUSIONS: The 1% VMOA has improved antimicrobial properties and kept mechanical properties of orthodontic adhesive within the acceptable level.

Keywords:

Nano particles, orthodontic adhesive, shear bond strength, vanillin

Introduction

In recent years, increased demands on aesthetic requirements put a significant challenge on general dental therapy and orthodontic treatment indefinitely. One of the major conditions affecting esthetic demands is malocclusion. High prevalence of malocclusion (54–56%) around the world in various forms, with no gender difference.^[1]

Various approaches have been developed to treat malocclusion involving fixed and removable orthodontic appliances.^[2] Orthodontic fix appliance itself facilitates accumulation and adherence of dental plaque, which participates in oral biofilm formation. Orthodontic adhesive remnant around orthodontic brackets creates a rough surface that promotes adherence to dental plaque.^[3] Dental plaque accumulation at the adhesive-enamel junction affects adhesion strength and tooth structure, leading to a zone of demineralization

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around orthodontic brackets.^[4] White spot lesions and dental caries are the main unwanted consequence of orthodontic treatment. High prevalence of white spot lesions in orthodontic patients of more than (68%).^[5]

Many attempts have been conducted to improve the antimicrobial abilities of restorative materials^[6] and orthodontic adhesives.^[7,8] Nanoparticles additives have been anticipated to improve antimicrobial effects with or without improvement in the mechanical properties.^[7,9,10] Titania, silver, graphene oxide, cationic curcumin-doped zinc oxide, C-phycoyanin, and cinnamon powder are nanoparticles additives to test their antimicrobial effects when added to an orthodontic adhesive.^[9,11-16]

Vanillin is a natural aromatic product, and the major component of vanilla is used as a flavoring agent.^[17] Vanillin is a phenolic compound with antioxidant and antimicrobial effects.^[18] Previous studies have demonstrated that vanillin has an antimicrobial effect against *Candida albicans*, preventing their adhesion to polymethyl methacrylate.^[19] A recent study demonstrated vanillin-modified methyl methacrylate's antifungal and antimicrobial effects.^[20] Several studies have shown the antimicrobial impact of orthodontic adhesives modified with nanoparticles.^[9,13,21-25] However, other studies failed to demonstrate the antimicrobial effect of orthodontic adhesive modified with nanoparticles.^[23,26]

To the best of our knowledge, this is the first research investigating the antimicrobial and mechanical effects of adding vanillin nanoparticles to orthodontic adhesive. The null hypothesis tested is that adding vanillin nanoparticles to orthodontic adhesive does not affect the antimicrobial and mechanical properties of the orthodontic adhesive.

So, the purpose of this article was to evaluate the effect of vanillin nanoparticles on an orthodontic adhesive's antimicrobial and mechanical properties.

Materials and Methods

The ethics committee of the College of Dentistry, University of Mosul approved this study (UoM.Dent/A.DM.L.84/21). Vanillin nanoparticles (Graham Chemical Corporation, Illinois, USA) were prepared by the mechanical attrition method of vanillin described by Rajput (2015).^[27] The nanoparticle's size was measured using scanning electron microscopy (VEGA3 electron microscope, Tescan, Kohoutovice, Czech Republic).

Transbond XT orthodontic adhesive (3M Unitek, Monrovia, California, USA) was used in this study. The orthodontic adhesive modification was achieved by adding vanillin nanoparticles in two concentrations

1% and 2% and was mixed using a glass slab and a spatula in a dark room to get a homogenous mixture. The tested concentration of modified orthodontic adhesive was prepared according to this formula: Vanillin wt./1 gm orthodontic adhesive $\times 100 =$ tested concentration of orthodontic adhesive. The desired weight of vanillin nanoparticles was measured using a digital scale (CENT-2, Gibertini Elettronica, MI, Italy).

Fourier transformed infrared spectroscopy (FTIR) test

The chemical composition and degree of chemical conversion in orthodontic adhesive before and after adding vanillin nanoparticles to orthodontic adhesive were measured using Fourier transformation infrared spectroscopy (FTIR) (Platinum Atr, Bruker, Germany) at FTIR spectra wavelength range 400–4000 cm^{-1} . The FTIR spectra were produced and documented.

Shear and tensile bond strength test

Sixty maxillary first premolar extracted teeth for orthodontic purposes were used in this study. Thirty teeth were used for the tensile bond strength test, and a similar number was used for the shear bond strength test. The teeth were examined thoroughly with no caries, filling, or enamel cracks. After removing the soft tissues remnants and washing them in tap water, the teeth were stored in 0.1% thymol (Caesar & Loretz GmbH, Hilden, Germany). For the shear bond strength test, the teeth were mounted in plastic rings using cold cure acrylic resin (Duracryl™ Plus, SpofaDental, Jicin, The Czech Republic) to the level of the cemento-enamel junction. The teeth were sectioned at the cemento-enamel junction using a diamond disc with a high-speed handpiece with water-cooling for the tensile bond strength test. The teeth were mounted in plastic rings using cold-cure acrylic resin. The lingual surface of each crown was embedded into the cold cure acrylic resin into plastic molds with a buccal surface facing upward. For the shear and tensile bond strength test, the teeth were randomly divided into three groups equally: Control group: Brackets were bonded on the buccal surface of each tooth using unmodified orthodontic adhesive (UMOA). Second group: Brackets were bonded on the buccal surface of each tooth using 1% vanillin-modified orthodontic adhesive (VMOA). Third group: Brackets were bonded on the buccal surface of each tooth using 2% VMOA. The buccal surfaces of the teeth were polished with non-fluoridated pumice and then washed with water and dried. The teeth's buccal surfaces were etched for 20 seconds with 37% phosphoric acid (Total etch, Ivoclar Vivadent, Liechtenstein) and then washed for 10 seconds and dried with air spray. A bonding agent (Primer, 3M Unitek, Monrovia, California, USA) was applied to the buccal surface. Excess primer was dispersed using gentle airflow, and light-cured for

20 seconds using light cure (Radium plus, SDI, Victoria, Australia). The orthodontic brackets placed at 4 mm from the tip of the buccal surface using Boone gauge (Morelli, Sorocaba, Brazil) under a static load of (200 gms) applied by a dental surveyor (DENTSPLY International, Inc., PA, USA). The brackets were adapted completely on the tooth surface, and the excess of the adhesive was removed then the brackets were cured (Radium plus, SDI, Victoria, Australia) for 40 seconds, 10 seconds for each side with a 2 mm distance from the bracket. The shear bond strength was recorded in newtons using a universal testing machine (Gester Instruments Co, Fujian, PR China) in push mode at speed of 0.5 mm/min. Tensile bond strength was measured using a universal testing machine in pull mode at a 0.5 mm/min speed. The specimen was secured with a unique clamping device so that the metal slot of the bracket would be perpendicular to the testing machine head. The four wings of the metallic bracket were connected to the head of the testing machine using ligature wire (0.012-inch, stainless-steel wire, Orthotechnology). The dimensions of the bracket base were measured using a digital caliper (Parkside, Germany). The surface area of the bracket was calculated by multiplying the bracket width and length. The debonding forces for both tests were calculated in MPa by dividing the peak failure load measured in Newtons on the bracket base measured surface area.

Antimicrobial test

Six disks specimens of each UMOA, 1% VMOA, and 2% VMOA were formed using plastic molds. The size of the disks was (3 mm in diameter and 2 mm in thickness). After the mold was filled with composite, it was covered by a celluloid matrix strip and light-cured (Radium plus, SDI, Victoria, Australia) for (20) seconds from the top of the mold and then removed from the mold after setting. Sterilization of the disks was carried out by immersing the disks into 70% alcohol for 30 minutes at room temperature. For evaluation of the sterilization method, one of the disks is incubated in broth media for twenty-four hours and showed no growth. According to the manufacturer's instructions, Mueller–Hinton medium plates were prepared by dissolving 38 grams in 1000 ml of purified/distilled water. Then cooled to 45–50°C and poured into the sterile Petri dish to the level surface about 4 mm thick. Agar was allowed to solidify at room temperature, then stored until use at 4° C. Standardization inoculation was made with a broth culture diluted to match a 0.5 McFarland turbidity standard, roughly equivalent to 150 million cells per ml. The media used in Kirby–Bauer testing with Mueller–Hinton agar at only 4 mm deep, was poured into either 100 mm or 150 mm Petri dishes. The procedure of testing the growth of streptococcus mutans involved swabbing a Mueller–Hinton plate with streptococcus mutans. The surface was allowed to dry for about 5 minutes

before placing tested disks of UMOA, 1% VMOA, and 2% VMOA on the agar surface. Using flame-sterilized forceps, gently press the test material disks to the agar ideally. Plates were incubated for 48 h at an incubation temperature of 37°C under anaerobic conditions for reading the inhibition zone after incubation. The total number of Petri dishes used was six. Each Petri dish is implanted with three disks: UMOA, 1% VMOA, and 2% VMOA. The zone of microbial inhibition around tested disks was measured in millimeters.

Statistical analysis

Statistical analyses were assessed using SPSS software version 25 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics, one-way ANOVA, and multiple comparisons Duncan's test were used to compare between shear and tensile bond strength of UMOA, 1% VMOA, and 2% VMOA. One-way ANOVA and multiple comparisons Duncan's test were used to compare microbial growth of UMOA, 1% VMOA, and 2% VMOA. The level of significance was recorded to be $P < 0.05$.

Results

The scanning electron microscopy figure showed vanillin particles within the nanoscale, as shown in Figure 1. FTIR spectra of UMOA are demonstrated in Figure 2. FTIR spectra of 1% VMOA shown in Figure 3 and 2% VMOA in Figure 4 showed the adding carbonyl compound at wave number between 2800 and 4000 cm^{-1} . FTIR spectra confirmed the absence of any chemical interaction between the UMOA and vanillin nanoparticles additives at 1% VMOA and 2% VMOA. The presence of an absorption beam at (3648 cm^{-1}) belongs to the stretching pack of the functional group's phenolic hydroxide (OH) in 1% and 2% VMOA. Carboxylic stretch (H-C = O) presents at 2960 cm^{-1} , carbonyl stretch (C = O) at 1683.94 cm^{-1} , carbonyl stretch (C-O) at 1295.46 cm^{-1} , and alkene

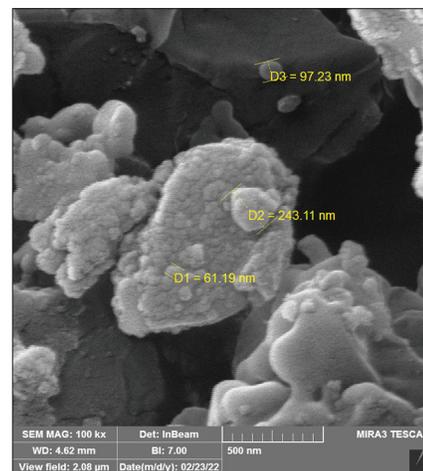


Figure 1: Scanning electron microscopy of vanillin particles showed that particle size within nanoscale

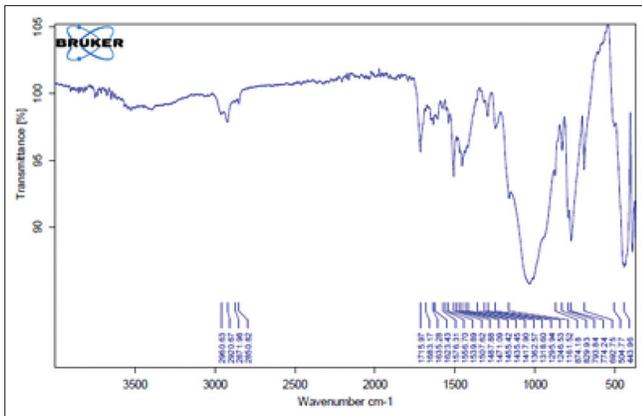


Figure 2: FTIR spectra of UMOA

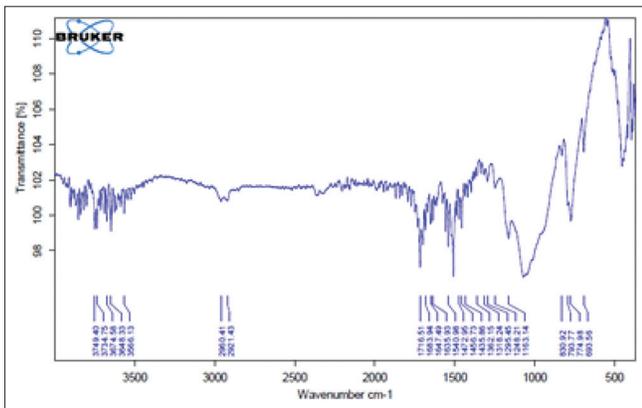


Figure 3: FTIR spectra of 1% VMOA

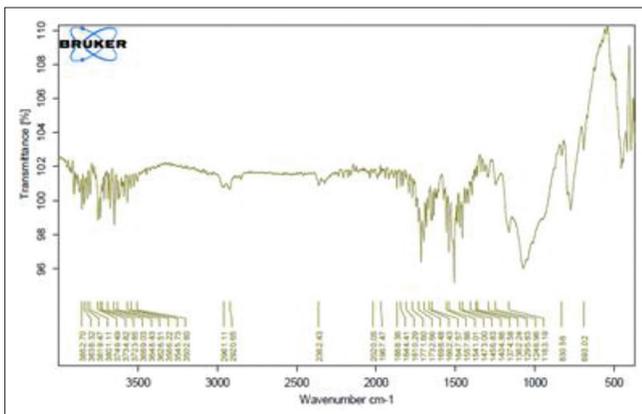


Figure 4: FTIR spectra of 2% VMOA

stretch ($C = C$) at 1635 cm^{-1} that confirm the presences of vanillin additives.

Shear bond strength decreased significantly in 1% VMOA compared to UMOA. In addition, shear bond strength decreased insignificantly when comparing 1% and 2% VMOA as shown in Table 1 and Figure 5. Tensile bond strength decreased significantly when comparing UMOA with 2% VMOA. In addition, there was no significant difference in tensile bond strength between

the UMOA and 1% VMOA and 1% VMOA and 2% VMOA, respectively, as shown in Table 2 and Figure 5.

Multiple comparisons Duncan's test showed significant streptococcus mutans growth inhibition in 1% VMOA and 2% VMOA compared to UMOA [Figure 6]. In addition, there was no significant difference in streptococcus mutans growth inhibition in 1% VMOA and 2% VMOA as shown in Table 3.

Discussion

This study tried to improve the antimicrobial and mechanical properties of an orthodontic adhesive with vanillin nanoparticles, as the vanillin nanoparticles additives had proven antimicrobial properties.^[28,29] The null hypothesis was partially rejected, as the antimicrobial properties improved while the mechanical properties were reduced. Orthodontic treatment comprises relatively a lengthy dental procedure that needs excellent patient care over the time of treatment. One of the most significant drawbacks of orthodontic treatment is the development of white spot lesions and dental caries. Many in-vitro trials were conducted to improve an orthodontic adhesive's antimicrobial activities without impacting the shear or tensile bond strength of orthodontic adhesive by combining nanoparticles.^[13] Streptococcus mutans is the causative bacterial in dental caries and periodontal diseases.^[30] Previous studies reported growth inhibition of Streptococcus mutans of modified orthodontic adhesive with various nanoparticles at different concentrations and keeping shear bond strength within the acceptable level. Poosti *et al.*^[9] stated the streptococcus growth inhibition zone by 88.1% by adding 1% titanium oxide nanoparticles. The same bacterial growth inhibition effect of titanium oxide nanoparticles of 6.67 mm was reported by Sodagar *et al.*^[24] with 1% titanium oxide nanoparticles additives. Other researchers reported that adding copper nanoparticles of 0.01% inhibits streptococcus growth by 25 mm.^[21] In comparison, other researchers reported inhibition by streptococcus growth by 8 mm.^[25] Degrazia *et al.*^[22] modified orthodontic adhesive by adding silver nanoparticles of 0.1,0.2,0.3%, and they reported Streptococcus mutans growth inhibition by 25.9%, 26.3%, and 29.4%, respectively. On the other hand, Ahn *et al.*^[26] reported no bacterial inhibition effect of silver nanoparticles of 0.025% against streptococcus mutans. In addition, 1% of curcumin nanoparticles did not affect the growth inhibition of streptococcus mutans.^[24] This study showed that incorporating 1% and 2% vanillin nanoparticles into the orthodontic adhesive produced a significant inhibition of the microbial growth of streptococcus mutans. Fitzgerald *et al.*^[31] confirmed the bacteriostatic activity of vanillin against Lactobacillus tarum. Thaweboon *et al.*^[29] demonstrated the vanillin coating prevents Candida albicans' adhesion

Table 1: Descriptive statistics and multiple comparisons Duncan's test of shear bond strength of the UMOA, 1% VMOA, and 2% VMOA

Orthodontic adhesive	n	Range	Minimum	Maximum	Mean±SD	Sig.	Duncan's test
UMOA	10	31.879	6.272	38.151	24.626±10.135	0.000	A*
1% VMOA	10	21.310	10.053	31.363	20.673±7.194		B*
2% VMOA	10	20.021	9.710	29.731	19.900±7.742		B

* Different letters mean significant differences, SD: Standard deviation

Table 2: Descriptive statistics and multiple comparisons Duncan's test of tensile bond strength of the UMOA, 1% VMOA, and 2% VMOA

Orthodontic adhesive	n	Range	Minimum	Maximum	Mean±SD	Sig.	Duncan's test
UMOA	10	4.124	3.180	7.304	4.563±1.160	0.029	A*
1% VMOA	10	2.325	3.002	5.327	4.244±0.950		AB
2% VMOA	10	1.547	2.750	4.297	3.417±0.585		B*

* Different letters mean significant differences, SD: Standard deviation

Table 3: Descriptive statistics and multiple comparisons Duncan's test of streptococcus mutans growth inhibition

Orthodontic adhesive	n	Range	Minimum	Maximum	Mean±SD	Sig.	Duncan
UMOA	6	5	15	20	18.732±2.500	0.022	A*
1% VMOA	6	9	19	28	23.750±3.686		B
2% VMOA	6	8	22	30	26.600±3.594		B

* Different letters mean significant differences, SD: Standard deviation

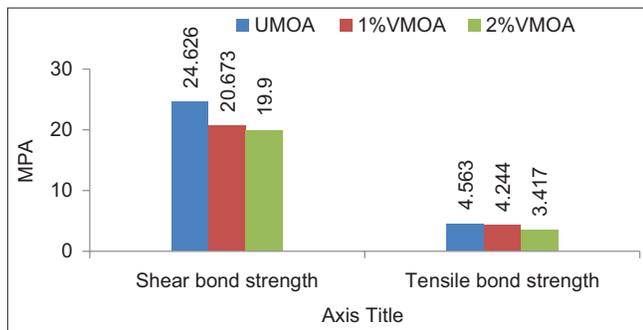


Figure 5: Mean value of shear bond strength and tensile bond strength of UMOA, 1% VMOA, and 2% VMOA

to the surface of methyl methacrylate. Similar results were obtained by Thaweboon *et al.*^[28] However, there was no significant bacterial growth inhibition zone difference between 1% and 2% VMOA. The vanillin has antimicrobial and antioxidant activities.^[32]

This study showed a significant decrease in shear bond strength between UMOA and VMOA with a greater non-significant reduction in shear bond strength with 2% VMOA. This decline in mechanical properties may be related to the aggregation of vanillin nanoparticles in VMOA.^[33,34] Previous researchers reported the decreased shear bond strength of orthodontic adhesive modified with curcumin nanoparticles of 1%, 5%, and 10%.^[23] Yaseen *et al.*^[16] investigated the effect of nano-cinnamon particles (1%) on shear bond strength, and they found an insignificant decrease in the shear bond strength of the modified orthodontic adhesive. It seems that organic nanoparticles additives may affect shear bond

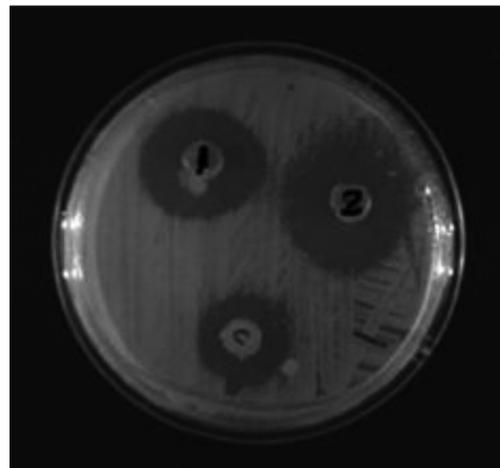


Figure 6: Culture media of streptococcus mutans bacterial shows bacterial inhibition zone around UMOA disk (c), 1% VMOA disk (1), and 2% VMOA disk (2)

strength. Similar results reported decreased shear bond strength of orthodontic adhesive modified with silver nanoparticles at various concentrations.^[22,26] However, Blöcher *et al.*^[35] found an increase in shear bond strength in orthodontic adhesive modified with 0.1% and 0.2% silver nanoparticles. Sodagar *et al.*^[24] reported that orthodontic adhesive modified with titanium oxide nanoparticles (1%, 5%, 10%) had a lower shear bond strength compared to the control group. In addition, Poosti *et al.*^[9] found no significant decrease in shear bond strength of orthodontic adhesive modified with (1%) titanium oxide nanoparticles. On the other hand, copper nanoparticles of (0.01%) can improve shear bond strength significantly.^[21]

The tensile bond strength decreased significantly in 2% VMOA, while 1% VMOA showed insignificant decreases when compared to the control group. Similar results of tensile bond strength of UMOA were recorded Reicheneder *et al.*^[36] and Chandulal *et al.*^[37] The FTIR results showed no chemical interaction between vanillin additives and orthodontic adhesive. The FTIR of VMOA showed the presence of vanillin particles at spectra 2000–4000 cm^{-1} with no chemical conversion of orthodontic adhesive. The decrease of the shear bond of VMOA within the accepted value was recorded by Reynolds.^[38] There is a need for long-term clinical investigation to investigate the appropriate concentration of vanillin nanoparticles to achieve the best antimicrobial effect against oral biofilm pathogens and the best mechanical performance.

Conclusions

The addition of vanillin nanoparticles to orthodontic adhesive can improve the antimicrobial activity against streptococcus mutans. 1% VMOA showed antimicrobial activity against streptococcus mutans and kept shear and tensile bond strength within acceptable levels. However, 2% VMOA shear and tensile bond strength decreased significantly without offering a significant increase in antimicrobial activities over 1% VMOA. Therefore, 1% VMOA showed the best overall characteristics compared to 2% VMOA.

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Conflicts of interest

There are no conflicts of interest.

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