Preparation, Characterization and Incorporation of Ascorbic Acid Coated Superparamagnetic Fe3O4 Nanoparticles into Universal Dental Adhesives to Enhance their Dentin Bond Strength and Stability

Mohammed A. Fadhil[1], Abdulla M.W. Al-Shamma[2]

[1]Department of Restorative and Esthetic Dentistry, College of Dentistry, University of Baghdad - Iraq.
[2]Assistant Professor at Department of Restorative and Esthetic Dentistry, College of Dentistry, University of Baghdad -Iraq.
Corresponding author: Mohammed A. Fadhil
Email: Mohamoa@gmail.com

ABSTRACT

Aims (Purpose): The study aims were to prepare, characterize, and incorporate colloidal suspension containing Ascorbic Acid Coated Superparamagnetic Nanoparticles (AA-SPN) into two universal adhesives to increase the dentin Shear Bond Strength (SBS) and stability by subjecting them to external magnetic field.

Settings and Design: An invitro study.

Methods and Material: A colloidal suspension of AA-SPN was prepared, characterized, and incorporated into All Bond universal (Bisco, USA) and Prime&Bond universal (Dentsply, Sirona) at 2% by mass. Sixty-four freshly extracted third-molars were divided randomly into four groups, i.e., GI: All Bond Universal (control), GII: All Bond Universal (2%-incorporated), GIII: Prime&Bond universal (control), and GIV: Prime&Bond universal (2%-incorporated). GII and GIV are subjected to 1min magnetic force before adhesive curing. The teeth were restored with Filtek Z350 composite (3M ESPE, USA). Each group was subdivided into two subgroups of 8 teeth each. The first subgroup subjected to thermocycling followed by 1-day water storage, and the second subgroup subjected to thermocycling followed by 6-months water aging before testing the SBS.

Statistical analysis used: Statistical analysis was conducted by independent variable t-test performed by using IBM-SPSS program version 22.

Results: GII and GIV showed significantly higher (p<0.05) immediate SBS after thermocycling, and maintained a significantly higher bond strength after 6 months water aging in comparison to their control groups. GIV showed significantly higher SBS in comparison to the GII. Groups I, II showed the lowest SBS after 6 months of water aging.

Conclusions: Universal adhesives containing 2% AA-SPN subjected to external magnetic field significantly improved the immediate and delayed shear bond strength values compared to their corresponding control adhesives.

Significance: The synergistic actions of superparamagnetism of the magnetite nanoparticles in response to magnetic field, and the medicinal values of vitamin C including collagen cross linking and MMPs inhibition, significantly increased and maintained the bond strength of universal adhesive to sound dentin when subjected to external magnetic field in comparison to the non-incorporated adhesives.

Keywords: Universal adhesives, Ascorbic acid coated superparamagnetic nanoparticles, magnetic force, thermocycling & water aging, Shear bond test.

Correspondence:
Mohammed A. Fadhil
Department of Restorative and Esthetic Dentistry, College of Dentistry, University of Baghdad, Iraq
E-mail: Mohamoa@gmail.com
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INTRODUCTION

Universal 'multimode' adhesives are the newest generation of dental resin. They are part of an ongoing industry trend to simplify the bonding technology that aimed to achieve satisfying bonding durability with an accelerated, simplified, and repeatable adhesion process [1].

Regardless of the advances in dental adhesives, the hybrid layer created on the variable and dynamic organic dentin surface is not perfect, and susceptible to failure over time [2]. This issue is probably related to incomplete resin infiltration into the deepest parts of collagen fibrils network exposed by the etching process. This is mainly attributed to the difficulty of monomers to displace and replace the free water trapped between interfibrillar spaces, resulting in inadequate hybridization and will affect the stability of bond [3]. Thus, different strategies have been used to develop bonding agents with special functions to overcome problems of bonding to dentin, like incorporating of matrix metalloproteinase (MMPs) inhibitors, remineralizing or antibacterial agents into the adhesives, or by enhancing resin penetration into dentin [4],[5],[6].

Superparamagnetic iron oxide nanoparticles (magnetite Fe_3O_4) are known for their excellent immediate response to external magnetic fields at nanoscale below 50nm better than other types of iron oxide family like hematite (Fe_2O_3), maghemite ($Y-Fe_2O_3$), and goethite [FeO (OH)] [7]. This phenomenon makes them unique, and utilized in various medical fields. Due to their superparamagnetic behaviour, magnetite nanoparticles under the guidance of magnetic forces transport more drugs to the target tissue than either passive diffusion or iontophoresis [8].

Ascorbate (vitamin C), is essential for the growth of human body, repair of tissues, and proper immune system function. It plays a critical role in the biosynthesis and maintenance of mature collagen fibrils in humans because it is essential ingredient for the synthesis of hydroxyproline and hydroxylysine, which are important for the intramolecular and intermolecular collagen cross-linking [9]. Also, ascorbic acid is a well-established candidate to be used as a coating agent for magnetite nanoparticles without losing their medicinal values, by acting as a surface surfactant to the particles preventing their agglomeration and aggregation, and thereby can enhance their movement and penetration in response to external magnetic field [10],[11].

Thus, the goal of the current in-vitro study is to enhance and maintain the adhesive/dentin bond of universal adhesives through the incorporation of ascorbic acid coated superparamagnetic nanoparticles into those adhesives, to utilize the synergistic actions of high magnetic saturation and immediate response of magnetite nanoparticles to magnetic field, and the medicinal values of vitamin C coating to achieve this goal.

SUBJECTS AND METHODS

2.1. Preparation of colloidal suspension

The method used in this study for coating the superparamagnetic Fe_3O_4 nanoparticles by ascorbic acid was done according to the principles of a patented protocol developed by Cave & Mundell, in 2015 under title of "COATING METAL OXIDE PARTICLES" [12]. In summary, the steps of coating procedure are:

1. Mechanical dry mixing and grinding: 100 mg of magnetite nano-powder (Us Research Nanomaterials Inc, USA) of 8 nm average particle size, and 100 mg of pure chemistry ascorbic acid powder (99% ROMIL-Pure Chemistry, UK), was weighted using electronic weight scale, and then added to agate mortar. The two powders are mixed and grinded together with the pestle in a vigorous agitation motion for 5 minutes until the mixture became homogeneous brown powder.

2. Solvent dispersion and dilution: 2ml of 60% ethanol was added to the mixture in a closed container. The mixture is shacked very using Vortex mixer (Labnique Inc., USA) for 5min, then a further 3ml of ethanol was added, the mixture is then shacked again for 5min until the colloidal suspension is homogenized.

3. Filtration: the mixture is then filtered and purified by using a sequence of filter membranes of different pores sizes ($0.45\mu m$, $0.20\mu m$, $0.10\mu m$, $0.05\mu m$, $0.025\mu m$) (MERCK; MF-Millipore™-filter membranes, USA) to allow passage of only ultrafine coated nanoparticles, and prevent the passage of aggregated particles.

By following this protocol, 15ml colloidal suspension is obtained first, then a rotary evaporator (Heidolph;HB Digital, Germany) was used to evaporate excess ethanol. Finally, a clear highly concentrated dark-brown colloidal suspension ($\approx$5ml) containing the ascorbic acid-coated magnetite nanoparticles as the main ingredients was obtained.

2.2. Characterization of the Colloidal Suspension

2.2.1. Characterization by atomic force microscopy (AFM)

The prepared colloidal suspension is characterized first by AFM (Nano-Compact AFM/ PHYWE, Germany) to identify the size and morphology of the coated nanoparticles. One drop of the colloidal suspension is first applied to a flat surface Mica glass, spread evenly, blown dry with high purity nitrogen, and then the glass slide is mounted on the AFM imaging table. The imaging procedure was carried out by using AFM in the non-contact mode operation in air. Silicon nitride nano-tips with a nominal 2nm radius, $125\mu m$ of length, force constant of 42N/m, and a resonance frequency of about 330Hz were used. The approach-retract cycle was carried out at typical z-scan rate of 0.1Hz to 1Hz, which is preferred for particles size measuring. The AFM imaging size was 0.5×0.5 μm. Grain statistics (i.e. coated particles mean-size) was determined by the AFM software.

2.2.2. Characterization by Transmission Electron Microscopy (TEM)

TEM imaging was performed to confirm the surface coating by ascorbic acid, and to confirm the size of the nanoparticles. The imaging procedure started by dispersing a single drop of the prepared colloidal solution ($10\mu m/mL$) onto carbon-coated copper TEM-grid (400-mesh). The grid is left for 30 to 45 min to dry in the room temperature, and then dried under vacuum to be ready for imaging by using (TEM, Philips CM120, USA) at 120-KeV. ImageJ Digital analysis software was used to analyze the particles size distribution from TEM micrographs.

2.2.3. Characterization by FTIR Spectroscopy

FTIR spectroscopy was used for chemical characterization of the created colloidal suspension, to confirm the chemical reaction between the magnetite nanoparticles and the ascorbic acid. This was done by comparing the FTIR charts of ascorbic acid and magnetite nanoparticles powders alone with FTIR of the colloidal suspension after being dried. The method of sample preparation done by using the classical KBr pellet method at ratio a of 1:100, where the sample powder is diluted to 1 % in the KBr powder and packed into the sample plate for FTIR measurement (Nicolet 380 FTIR, USA) (the scan range was 400-4000cm^{-1}).

2.3. Incorporation the coated nanoparticles into the universal adhesives

The prepared colloidal suspension was incorporated into the Universal adhesives at 2% concentration by mass. After incorporation, each adhesive bottle was shacked with the aid of mechanical Vortex mixing device for 2min in a spiral agitation motion at 2800rpm speed to allow even blending and dispersion of the coated nanoparticles into the universal adhesives. The 2% incorporation percentage was selected among other percentages (1%, 3%, and 4%) according to the results of preliminary pilot studies because it does not affect the adhesive's wettability, optical properties, degree of conversion, and showed the highest shear bond strength values in response to magnetic field in comparison to the other percentages.

2.4. Testing the shear bond strength

2.4.1. Sample selection and preparation

Sixty-four freshly extracted caries-free human third molars (patients age was between 18-40 years old) were collected. Teeth of comparable crown size, shape, free from cracks, and caries were chosen. All teeth were cleaned from blood and debris, then thymol solution of 0.1% was used to store the teeth for 1day. After that, the teeth stored in deionized distilled water at room temperature before being used.

The teeth were mounted in self-cured acrylic resin blocks by using polyvinyl-tubes as molds. After setting, they removed from the tubes. To expose sound mid coronal dentin surface, the occlusal enamel was removed using super-thin diamond disc mounted in electrical saw. To simulate clinical conditions, the distance from the exposed dentin to the bottom of acrylic block was 25 mm for all samples [13].

2.4.2. Adhesives application and composite insertion

After dentin exposure, each sample was polished with 600 grits silicone-carbide paper under running water for 60s to create a standardized smear layer. Each tooth was etched with 37% phosphoric-acid for 15s and rinsed with distilled water for 15s. Each adhesive was then applied following manufacturer's instructions (Table 1), and the solvent was evaporated for 10 seconds with a gentle stream of air. The 2%-incorporated adhesives was subjected to external magnetic field for 1min by using a commercial cube-shaped super-magnet (K&J Magnetics Inc., USA:surface field 0.54 Tesla; N52, maximum internal field:1.4 Tesla) placed below the sample, then cured for 20s using LED curing light (Elipar 3M ESPE; device intensity: 1200W/cm2) at fixed bond-curing tip distance of 5 mm to simulate the clinical conditions of bonding to dentin (i.e. deep cavities or class II boxes) [14].

After that, composite restoration (Filtek Z350, 3M ESPE) was applied in a single increment using a custom-designed device fabricated from transparent Teflon to standardize the manner of composite application to dentin surface. After composite insertion, a celluloid strip was placed over it, then glass slide with 200gm load is placed over the celluloid strip for 1min before light curing for 40s. The created composite cylinder was 3mm in thickness and diameter for all samples.

2.4.3. Grouping and shear bond strength testing

The sixty-four samples were divided into four main groups of sixteen sample each as following:

GI: Sixteen teeth received a composite filling bonded with the non-incorporated All Bond Universal (Control).

GII: Sixteen teeth received a composite filling bonded with the 2% AA-SPN incorporated All Bond Universal.

GIII: Sixteen teeth received a composite filling bonded with the non-incorporated Prime&Bond Universal (Control).

GIV: Sixteen teeth received a composite filling bonded with the 2% AA-SPN incorporated Prime&Bond Universal.

For groups II and IV, the incorporated adhesives were subjected to external magnetic field for 1 minute before curing. The 1min magnetic application time was selected among other application times according to the results of a preliminary pilot study; which showed no statistically significant difference in the SBS values in comparison to 2min and 3min application times.

Table 1: Chemical composition and the instructions of manufacturers for the universal adhesives used in this study.

Materials	Manufacturer	Composition	pH	Instructions for use
All Bond Universal	Bisco, USA	10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP), bisphenol-A di-glycidyl methacrylate (Bis-GMA) (10–25%), 2-hydroxyethyl methacrylate (HEMA) (10-15%), ethanol (10-25%), water (20%), initiators (1-5%).	3.2	Apply two separate coats the adhesive, agitate with a micro-brush for 10-15 seconds per each coat. Evaporate excess solvent by air-drying using an air-syringe for 10 s (there should be no visible movement of the adhesive). Cure for 20s.
Prime&Bond Universal	Dentsply, Sirona	Bisacrylamide-1 (25–50%), 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP) (10–25%), bisacrylamide-2 (2.5–10%), 4-dimethylamino benzonitrile (0.1–1%), Dipenta-erythritol pentacrylate-phosphate (PENTA), Isopropyl alcohol (10–25%), water (20%)	2.5	Etch&rinse mode: 10 s etching with 37% phosphoric acid for dentin, thorough water rinsing (> 10 s), gentle drying with air, Apply the adhesive, slight agitation motion for 20s, mild air blowing (> 5s), light cure (20s).

All samples were subjected to thermocycling of 3000rounds (at 5°C/55°C) using a custom designed automatic thermocycling device set at dwell time of 30s. Each group was then subdivided into two subgroups of eight samples each: Subgroup A, was stored for 1-day in distilled water at 37C°, and subgroup B was aged for 6-months in distilled water at 37C° before testing the SBS.

A notched type stainless-steel rod was used to test the SBS in accordance with 'ISO 29022' [15]. The dimensions of the notch are 3 mm height and width, and 0.25mm thickness of a notched protruding edge to come in close contact with the composite-bond interface. This rode is mounted on the testing machine (Instron-5500 Universal Testing Machine, China) and aligned to be parallel to the composite–dentin interface. Load was applied at cross-head speed of 0.5mm/min until the bond failed. The dentin shear bond strength was calculated using the following formula: Shear **bond strength** $= 4P/\pi\, d^2$

Where P is the load applied at failure, and d is the diameter of the bonded composite. Also, failure modes were

examined using a stereoscopic microscope, and were classified as adhesive and mixed failure modes.

RESULTS

3.1. Results of characterization tests

3.1.1. AFM characterization results

The results of 2D and 3D AFM images showed that the prepared coated nanoparticles have homogeneous shape, with an average coated-particles (grains) size mean of 18nm (Figure 1 A-C). Also, the prepared coated nanoparticles are well dispersed with no apparent aggregation.
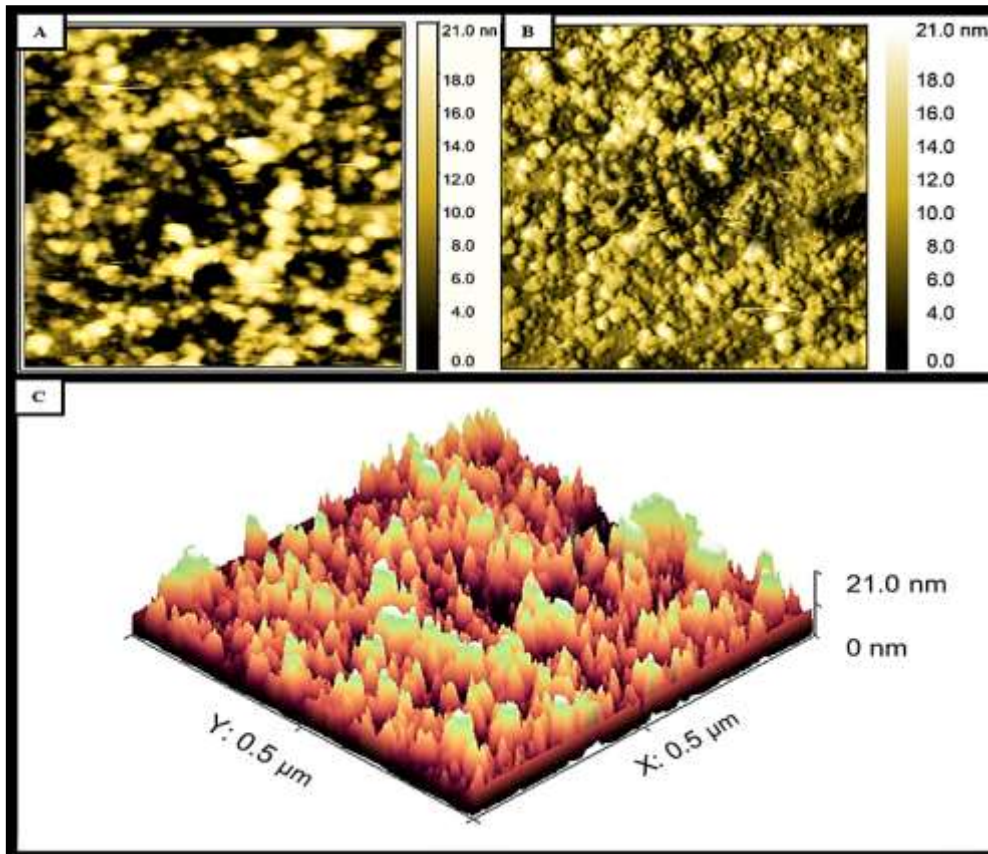

Figure 1: (A-C) 2D and 3D AFM micrographs for the nanoparticles of prepared colloidal suspension.

3.1.2. TEM characterization results

The results of the TEM micrographs shown in (Figure 2 A-D) of the ascorbic acid coated magnetite nanoparticles demonstrated some areas of agglomerated clusters as well as individually dispersed spherical shaped magnetite nanoparticles cores that are clearly coated by ascorbic acid

shell all around the nanoparticles. The results of ImageJ digital image analysis software confirmed the size of the particles to be in the range of 18.7±2.4nm (Figure 2 E). The apparent clustering of the particles in the TEM images is probably an artifact results from drying the sample.
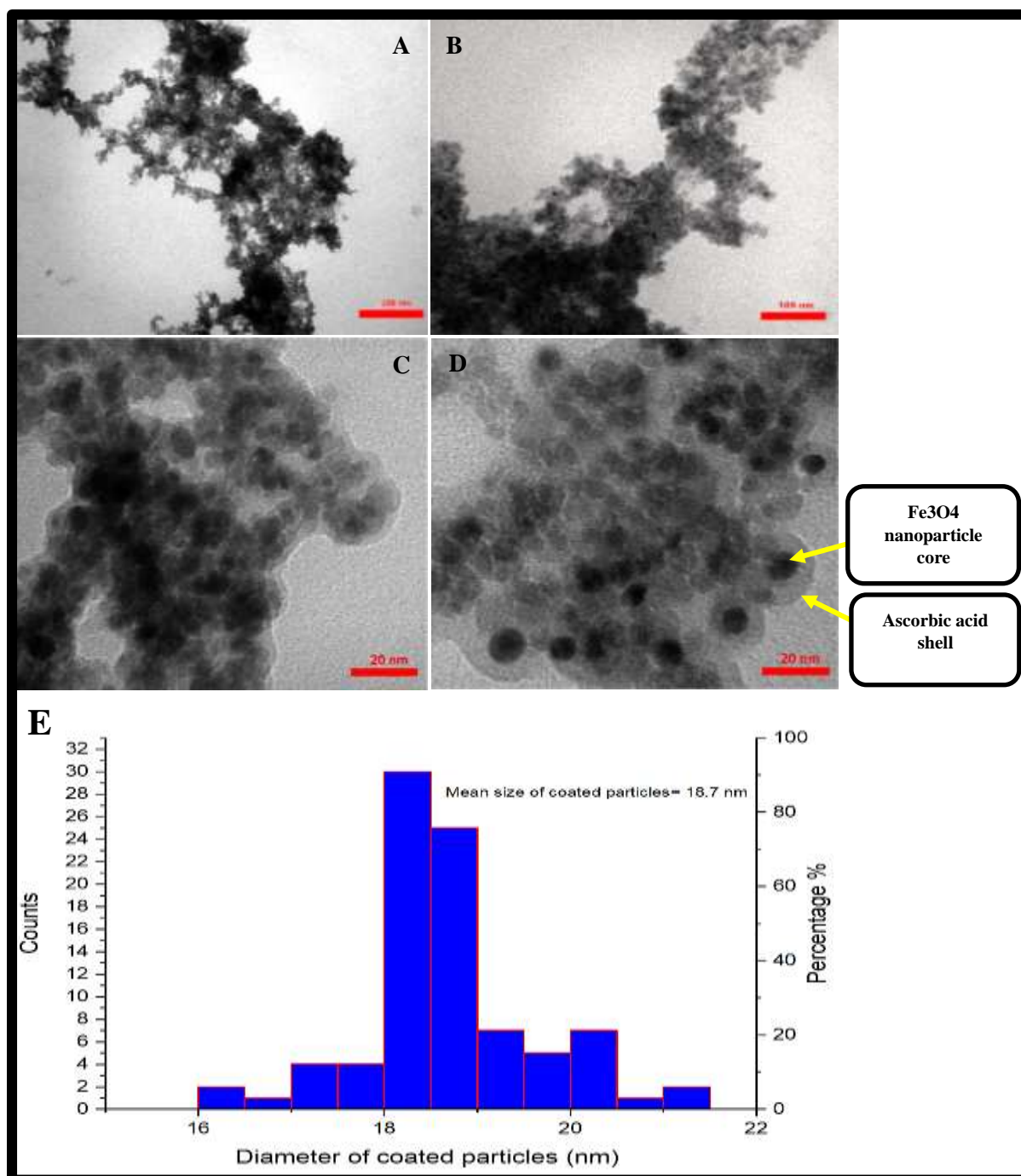
Figure 2: (A-D) Some TEM micrographs for the coated nanoparticles at different magnifications clearly showing the core of magnetite nanoparticles (black) are surrounded by a thick shell of ascorbic acid coating (Shadow around the nanoparticles), (E) Histogram showing the coated particles size distribution obtained by ImageJ digital images analysis software of TEM micrographs.

3.1.3. FTIR characterization results

The FTIR spectra (Figure 3) of magnetite nanoparticles showed the presence of characteristics transmission bands at ($3408 cm^{-1}$ O-H band, $632 cm^{-1}$, $585 cm^{-1}$ Fe-O band) which are assigned to Fe_3O_4. The FTIR spectra of ascorbic acid showed the presence of characteristics transmission bands including: ($3485 - 3313 cm^{-1}$ correspond to the hydroxyl groups O-H), ($1731 cm^{-1}$ for the carbonyl group C=O), ($1632 cm^{-1}$ for the C=C band), ($1151 cm^{-1}$ for the C-O-C band), ($1020 cm^{-1}$ for the C-O-H band), and at ($912 cm^{-1}$ for the C-C band), which are all assigned to ascorbic acid. While the FTIR spectra of the coated nanoparticles showed the disappearance of the carbonyl band (C=O) of ascorbic acid, and a shift in the C=C stretching band from 1640 to $1622 cm^{-1}$, as well as the appearance of (Fe-O) band at $632 cm^{-1}$ after coating. This probably indicates that the carbonyl-group of ascorbic acid essentially binds on the surface of the magnetite nanoparticles by the formation of electrostatic covalent bonding (through C=O⋯Fe interaction) [16].
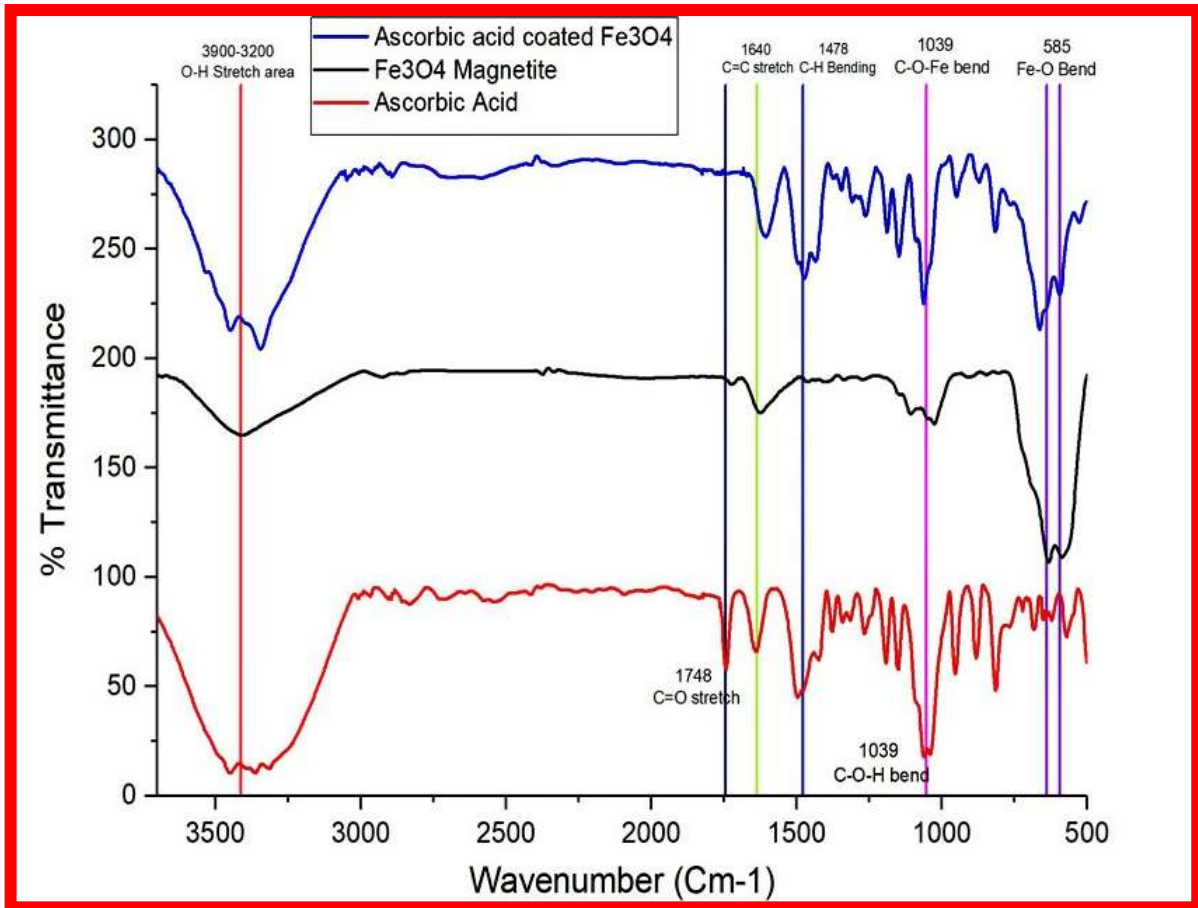
Figure 3: FTIR characterization charts for ascorbic acid, magnetite (Fe3O4), and ascorbic acid coated magnetite nanoparticles. Notice the disappearance of carbonyl 'C=O' band, and the appearance of 'Fe-O-C' bands after coating confirming the chemical reaction between ascorbic acid and Fe3O4 nanoparticles.

3.2. Shear bond test results

The descriptive statistics including standard deviation and means of SBS values for all groups are presented in (Table 2) and shown (Figure 4). Independent samples t-test showed that incorporated adhesive groups (GII, GIV) produced statistically higher immediate and 6-months SBS compared to their respective control groups. Prime&Bond Universal groups (whether alone GIII or incorporated GIV) showed significantly higher SBS values in comparison to corresponding All Bond Universal groups (GI and GII) for both aging periods

Table 2: Descriptive statistical results of shear bond strength in Megapascals (MPa) after thermocycling and water aging for all groups.

Groups	Storage period	Means	Std. Deviation	Minimum	Maximum
GIA	1-day	16.625	1.685	14.00	19.00
GIB	6-Months	12.500	1.069	11.00	14.00
GIIA	1-day	29.625	1.408	27.00	31.00
GIIB	6-Months	27.500	1.690	25.00	29.00
GIIIA	1-day	18.750	1.165	17.00	20.00
GIIIB	6-Months	15.750	1.489	14.00	18.00
GIVA	1-day	34.125	1.126	32.00	35.00
GIVB	6-Months	31.750	0.886	31.00	33.00

Shear bond strength after thermocycling and water aging

24 hours	6 months	24 hours	6 months	24 hours	6 months	24 hours	6 months
All Bond Universal (Control)	All Bond Universal (Control)	All Bond Universal (2% Incorporated)	All Bond Universal (2% Incorporated)	Prime&Bond Universal (control)	Prime&Bond Universal (control)	Prime&Bond Universal (2% Incorporated)	Prime&Bond Universal (2% Incorporated)

Values shown on bars: 16.6250, 12.5000, 29.6250, 27.5000, 18.7500, 15.7500, 34.1250, 31.7500
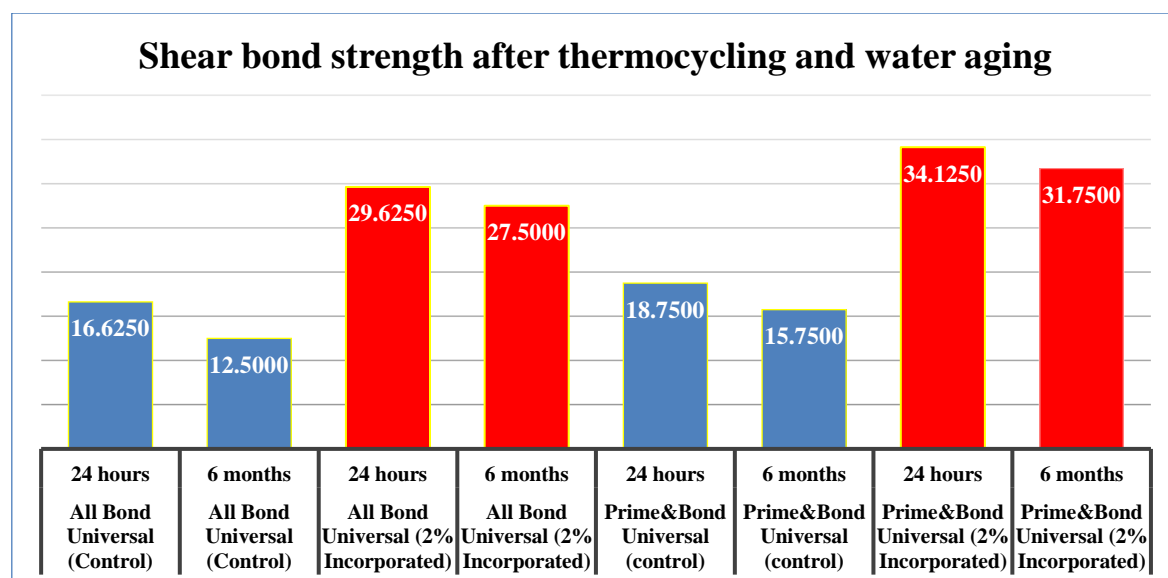
Figure 4: Graph showing the mean values of shear bond strength after thermocycling and water aging for all groups (MPa).

Results also revealed a pronounced negative effect of 6-months water aging on the SBS of the non-incorporated adhesives (GIB and GIIIB) when compared to 1-day SBS values (GIA and GIIIA). However, this aging process did not statistically affect the SBS for the 2% AA-SPN-incorporated groups (GII and GIV).

Table 3: Inferential statistical results of Independent samples t-test comparing the significance of difference between the shear bond strength mean values of the tested groups and subgroups (24h and 6 months water aging).

Groups	Mean Difference	Std. Error Difference	P-value	Significance
GIA vs GIIA (1-day)	-13.000-	0.776	.000	(HS)
GIB vs GIIB (6 months)	-15.000-	0.707	.000	(HS)
GIIIA vs GIVA (1-day)	-15.375-	0.573	.000	(HS)
GIIIB vs GIVB (6 months)	-16.500-	0.612	.000	(HS)
GIA vs GIIIA (1-day)	-2.125-	0.724	.011	(HS)
GIB vs GIIIB (6 months)	-3.250-	0.648	.000	(HS)
GIIA vs GIVA (1-day)	-4.500-	0.637	.000	(HS)
GIIB vs GIVB (6 months)	-4.750-	0.675	.000	(HS)
GIA (1-day) vs GIB (6 months)	4.125	0.706	.000	(HS)
GIIA (1-day) vs GIIB (6 months)	1.250	0.729	.108	(NS)
GIIIA (1-day) vs GIIIB (6 months)	3.000	0.668	.001	(HS)
GIVA (1-day) vs GIVB (6 months)	0.750	0.496	.152	(NS)

DISCUSSION

Different technologies have been investigated to enhance the adhesive/dentin bonding strength and durability, such as; the use of electric current [17], fluoride release/recharge by iontophoresis [18], and subpressure technique [4], to enhance resin penetration into dentin. Other methods are based on incorporation of collagen cross-linkers and MMPs inhibitors [2].

A recent approach has been studied to enhance resin penetration into dentin by using external magnetic force to steer acrylate-functionalized iron-core inorganic-silica coated magnetic nanoparticles incorporated into three steps scotchbond™ Multi-Purpose bond to pull the adhesive deeper into dentin [5],[13].

This study was conducted to enhance the adhesive/dentin bond strength, and to enhance the adhesive-dentin bond stability over time of universal adhesives applied in the etch & rinse mode. This is done by preparation and incorporation of colloidal suspension containing ascorbic acid coated superparamagnetic Fe_3O_4 nanoparticles into two types of commercially available universal adhesives. These adhesives were selected because they are based on different chemistry, and are completely free from any fillers.

Till now, there are no studies incorporated magnetite nanoparticles coated with organic 'Vitamin C' compound into universal adhesives to enhance the adhesive/dentin bond and stability in response to magnetic force.

Magnetite nanoparticles were selected because they have the property of superparamagnetism at nanoscale because of

their higher iron mineral content, and its highly crystalline structure. So, they respond immediately to external magnetic field, with the highest degree of magnetic saturation when compared to other types of iron oxide nanoparticles [19]. While the main reasons for selecting ascorbic acid as a coating agent are because of its well-established medicinal values and its affinity to form chemical bond with organophosphates,[20] like the dihydrogen phosphate end of the 10-MDP and PENTA functional monomers of the universal adhesives. In addition, it has the affinity to form ionic bond with the magnetite nanoparticles acting as surfactant that can prevent their aggregation [10],[11]. All that can enhance the chemical integration and dispersion of magnetite nanoparticles into the adhesives.

In this study, a patent method [12], was used for preparation of the coated nanoparticles. It is less technique sensitive, and does not require complex additional functionalizing steps and/or chemical ligands exchange in order to coat the nanoparticles. The grinding agitation process of the magnetite nanoparticles with the organic coating agent is the key step in this protocol. It provides energy in the form of heat from friction. This will possibly help to overcome electrostatic repulsion between particles, and break intermolecular forces between them, thus forcing the particles closer together. So, they will have more chance to interact with each other and form an electrostatic interaction [10].

This coating method proved to be successful by the results of AFM and TEM characterization tests. The results of these tests clearly showed the uniformity of particles shape and size distribution in the range of 18.7 ± 2.4 nm, and the nanoparticles were entirely coated by ascorbic acid. The FTIR analysis also proved the chemical reaction between the magnetite nanoparticles and the coating agent by the disappearance of the carbonyl band (C=O) of ascorbic acid, and a shift in the C=C stretching band from 1,640 to 1,622cm^{-1}, as well as the appearance of (Fe-O) band at 632cm-1 after coating the nanoparticles. This is in agreement with two studies [11],[16].

Thermocycling followed by long term water storage has been found to be useful in predicting the durability of adhesive/dentin bond in vivo [21]. Therefore, thermocycling and water aging was conducted to evaluate the long-term stability of adhesive/dentin bond by testing the shear bond strength of the incorporated adhesives after thermocycling at 3000-cycles followed by water storage at 1-day and 6-months in comparison to the control groups.

The results of this study showed significant increase in the immediate bond strength values of the incorporated adhesives in comparison to the control. This is probably attributed to the incorporated coated nanoparticles that can be actively steered by the use of magnetic field forces allowing them to pull the resin monomers deeper into dentin, which would probably increase the hybrid-layer thickness and resin tags penetration depth into dentinal tubules. This is in agreement with two studies [5,13].

Also, those results could be related to the medicinal values of vitamin C, which is known to be a natural collagen cross-linker, as it plays a critical role in the biosynthesis and maintenance of mature collagen fibrils in humans especially type I and III collagen [22]. Ascorbic acid is an important component in the synthesis hydroxylation of hydroxyproline and hydroxylysine which are essential to stabilize the collagen triple helix formation of inter- and intra-molecular cross-links in collagen fibrils. The collagen cross-linking action of ascorbic acid and its buffered form of sodium ascorbate is proved by many studies [23],[24].

For the control groups, the study showed a significant reduction in bond strength after 6 months water aging in comparison to the immediate (1-day) value. This is mainly related to the actions of MMPs in dentin, like collagenase (MMP-1, MMP-8) that cause collagenolysis in the presence of water, and gelatinases (MMP-2, MMP-9) can cleave intact collagen molecule at the cleavage site.[2] Second, it could be related to plasticization of adhesives resulted from water sorption by the hydrophilic resin content in the hybrid layer causing hydrolytic degradation of unreacted adhesive resin resulting in reduction in the bond strength over time [25].

While the 2%AA-SPN incorporated adhesives maintained a good shear bond strength values after 6 months of water aging. This could also be related to that magnetic force caused deeper infiltration and encapsulation of adhesives into the collagen fibrils spaces replacing the free water within the hybrid layer, thereby may prevent adhesive plasticization and activation of the MMPs within the hybrid layer. The second cause could be attributed to the MMPs inhibitory effects of vitamin C. Vitamin C can cross-link collagen molecules, stiffening them to the point that they may not be able to unwind when collagenase MMPs binds to collagen, or it can bind directly to the enzyme, or can cross-link the allosteric and active sites of the enzyme, thereby may inactivate them.[26] This is supported by another study [27].

Also, those results can be explained by the fact that both adhesives are free from any fillers; and by incorporating the coated nanoparticles, they can copolymerize with the polymer network of the adhesives enhancing their mechanical properties.[28] This could also reduce the water permeability of the polymer by decreasing the free spaces within polymer network, and thereby can reduce their water sorption and resin dissolution [29].

The 2%AA-SPN incorporated Prime&Bond Universal showed higher shear bond strength values in comparison to 2%AA-SPN incorporated All Bond Universal. This may be related to the differences in chemistry and composition of those adhesives. Prime&Bond Universal has a unique feature 'as claimed by the manufacturers' that distinguish it from All Bond Universal, which is the 'Active Guard Technology'. It actively controls moisture on the prepared dentin surface. This feature is achieved through balancing hydrophobic and hydrophilic components of the adhesive by using Bis-acrylamide monomers containing N-allyl group, which is multi-functional cross linking acrylate that cross-link the hydrophobic and hydrophilic components preventing phase separation, and at the same time helps to achieve an optimized surface tension, better dentin wetting, and improved adhesive penetration [30].

CONCLUSIONS

Universal adhesives containing 2% ascorbic acid coated superparamagnetic nanoparticles subjected to external magnetic field showed significantly higher immediate and delayed shear bond strength values than the commercial controls.

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