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Degree of Conversion of Two Universal Adhesives Incorporated with Ascorbic Acid Coated Superparamagnetic Nanoparticles Assessed by FTIR Analysis

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ABSTRACT

Aims: To prepare and characterize a nano-ferro fluid of ascorbic acid coated superparamagnetic Fe_3O_4 nanoparticles and incorporate them into two universal dental adhesives to test their effect on the immediate and 24-hours post-cure Degree of Conversion (DC) in comparison to their control non-incorporated adhesives.
Study design: An invitro study.
Methods and Material: A colloidal nano ferro fluid of ascorbic acid coated superparamagnetic nanoparticles was prepared and characterized by transmission electron microscopy and incorporated into two universal adhesives. Thirty-two adhesive samples in Potassium Bromide (KBr) discs were prepared for FTIR analysis, and distributed as following, i.e., GI: Eight adhesive samples of non-incorporated All Bond Universal (Control), GII: Eight adhesive samples of 2%-incorporated All Bond Universal, GIII: Eight adhesive samples of non-incorporated Prime&Bond Universal (Control), GIV: Eight adhesive samples of 2%-incorporated Prime&Bond Universal. Equal amount (25µL) of each adhesive was placed on KBr disc, the solvents was evaporated by a gentle steam of air, and then a second KBr disc was mounted over the first one and pressed softly to form a thin adhesive film. Finally, the KBr discs containing the adhesive were placed in the demountable cell holder and mounted into the sample chamber of the FTIR spectrometer. FTIR spectra was then recorded for each adhesive before, immediately after, and 24-hours post-curing to calculate the degree of conversion.
Statistical analysis used: Statistical analysis was conducted using independent variable t-test by the IBM-SPSS software version 22.
Results: The study results showed good DC values for the incorporated adhesives with no statistically significant differences in the degree of conversion values (i.e. Immediate and 24-hours post-cure) in comparison to control groups for both adhesives. The Prmie&Bond universal (Both control and incorporated) revealed significantly higher DC than All bond universal.
Conclusions: Incorporation of ascorbic acid coated Fe_3O_4 nanoparticles at 2% by mass into the universal adhesives resulted in good degree of polymerization with no significant difference in comparison to their commercial controls.

Keywords: Degree of conversion, universal adhesives, Superparamagnetic Fe_3O_4 nanoparticles, FTIR analysis.

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INTRODUCTION

Nanotechnology is the art and science of engineering materials in a scale of less than 100nm. It revolutionized the medical and dental fields by improving physical and mechanical properties of materials, helped introduce new diagnostic modalities, nano-delivery systems, and other biomedical applications [1, 2].

Superparamagnetic (Fe_3O_4) nanoparticles are distinguished by their excellent immediate magnetic response and high magnetic saturation to applied magnetic field better than other magnetic nanoparticles, and therefore are commonly utilized in several biomedical applications [3,4].

Ascorbic acid (vitamin C); is an excellent antioxidant that can protects the body against different harmful oxidative stresses. It plays key roles in numerous human body functions like the collagen synthesis and other several medicinal values [5]. Also, it is considered a suitable organic coating agent for Fe_3O_4 nanoparticles because it has a good chemical affinity to establish a surface ionic bonding with those particles [6]. In addition, it can act as an excellent surface surfactant to the coated particles preventing their agglomeration & aggregation, and thereby can enhance their movement and penetration in response to externally applied magnetics forces [7].

A new generation to dental adhesive systems has been immerged into the marketplace named as “multi-modal” or “universal adhesives”, because they are designed to allow the clinicians to utilize them in different adhesion strategies including the etch-and-rinse, self-etch, and selective enamel etching approaches [8].

Thus, the excellent super paramagnetism of the Fe_3O_4 nanoparticles when exposed magnetic forces, in addition to the surfactant action and medicinal values of the ascorbic acid when used as a coating agent for those nanoparticles can be utilized synergistically when incorporated into dental adhesives to enhance the penetration of the adhesive monomers deeper into demineralized dentin substrate to increase their dentin bond strength and stability.

In dental adhesives, the quantity of carbon-carbon double bonds (C=C) of the adhesive's monomers converted into carbon-carbon single bonds (C-C) during the process of photo-polymerization is described as the degree of conversion [9]. A high degree of curing is very important property to enhance the adhesives mechanical, physical, and biocompatibility properties [10].

Different methodologies have been utilized to test the DC of dental adhesives. The commonly utilized methods are based on vibrational spectroscopies (i.e. Infrared and Raman spectroscopies). Fourier Transform Infrared Spectroscopy (FTIR) is an infrared vibrational spectroscopy and is one of the most used and reliable methods to test the degree of conversion [11].

Table 1: Chemical composition of the universal adhesives used in this study.

Materials	Manufacturer	Composition	pH
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**
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**

It utilizes the electromagnetic radiation in the infrared-region to determine and identify the molecular structure, chemical functional groups, and the chemical interactions of various inorganic and organic materials [12].

Therefore, the aim of the current *in vitro* study is to evaluate the effect of incorporation of AA-SPN into two universal adhesives on the degree of polymerization of those adhesives in comparison to their commercial non-incorporated controls.

Materials and Methods

Preparation, characterization, and incorporation of ascorbic acid coated Fe_3O_4 nanoparticles into the universal adhesives

A patented protocol developed by Cave & Mundell [13], under the title "COATING METAL OXIDE PARTICLES" was utilized by this study for the preparation of a colloidal suspention of ascorbic acid coated Fe_3O_4 nanoparticles as following:

First: equal amounts (100 mg) of 99% purity Fe_3O_4 nano-powder (Us Research Nanomaterials, USA) (8 nm average size) and 99% purity powder of L-ascorbic acid (ROMIL-Pure Chemistry, UK), was weighted by an electronic weight scale, and then added to agate mortar. The two powders in the mortar was then grinded and mixed together using vigorous agitation motion with the pestle for 5min until the powder became homogeneously fine brown in color.

Second: the prepared powder mixture was added into 5ml-capacity glass container, then 2ml of ethanol solvent (60% concentration) was added to the powder mixture and the container is closed. The glass container was then shacked for 5min using mechanical mixing device (Vortex-mixer, Labnique Inc., USA). Then an additional 3ml of the solvent were added to the mixture and was shacked for another 5min until the colloidal suspension is became well dispersed and homogenous dark brown in color.

Third: the colloidal suspension was then filtered and purified by utilizing a series of filter membranes of different pore sizes (i.e. 0.45µm, 0.20µm, 0.10µm, 0.05µm, 0.025µm) (MERCK; MF-MilliporeTM filters, USA) to exclude the aggregated and agglomerated non-coated particles allowing the passage of ultrafine coated nanoparticles only.

Through the steps mentioned above, 15ml of coated nanoparticles colloidal suspension was prepared, then a rotary evaporator (Heidolph, HB-Digital, Germany) was utilized to evaporate excess solvent from the colloidal suspension. Finally, ≈5 ml of highly concentrated, dark brown, and clear colloidal suspension containing the AA-SPN was obtained. Then the prepared colloidal suspension was characterized by transmission electron microscopy (TEM) (Philips CM120, USA) at 120-KeV to determine particles size distribution by placing one drop of the colloidal suspension onto carbon-coated copper TEM-grid (400-mesh).

After preparation of the colloidal suspension, 2% by mass of AA-SPN was incorporated into two universal adhesives, i.e., All-Bond universal (Bisco, USA) and Prime&Bond universal (Dentsply, Sirona) (Table 1) , then each adhesive bottle was shacked in spiral agitation motion by a mechanical mixing device (Vortex-Mixer; Labnique Inc., USA) for 2min at speed of 2800rpm to allow excellent dispersion of the incorporated nanoparticles into the adhesives.

Grouping

Thirty-two adhesive samples between Potassium Bromide (KBr) discs were prepared to be tested by FTIR, and distributed as following:

GI: Eight adhesive samples of non-incorporated All Bond Universal (Control).

GII: Eight adhesive samples of 2% incorporated All Bond Universal.

GIII: Eight adhesive samples of non-incorporated Prime&Bond Universal (Control).

GIV: Eight adhesive samples of 2% incorporated Prime&Bond Universal.

Samples preparation and FTIR testing

To test the adhesives degree of conversion; first, equal amount (i.e. 25 µL) of each universal adhesive was placed on a KBr cell window (i.e. 25 x 4 mm dimensions) of the demountable cell holder of the FTIR spectrometer. Then with a gentle stream of air, the solvents in adhesives were evaporated for 30 sec., then a second KBr cell window is

mounted over the first one and pressed gently to form a thin film of adhesive [14]. After that, the KBr cell windows containing the adhesive are placed in the demountable cell holder, which is then mounted into the sample chamber of the FTIR spectrometer (Shimadzu, USA). The absorbance peaks of the unpolymerized adhesives were then recorded in the transmittance mode at resolution of 4 cm^{-1} with a scan range between 400-4000 cm^{-1} [9,15]. After that, the adhesives were then light–cured with an LED light–curing unit (Elipar, 3M ESPE; USA) with a light intensity of 1200 mW/cm^2 for 40s through the KBr cell windows because they are completely transparent and can allow excellent light penetration. After that, FTIR spectra was recorded immediately after curing of the adhesive. Then all KBr cell windows containing the cured adhesives were stored in dark containers in incubator at 37 ^oC, and finally the FTIR spectra was recorded again 24h Post-curing [14].

The DC% was calculated from the ratio of absorbance bands intensities of aliphatic C=C bands (peak at 1636-1638 cm^{-1}) and the internal reference of aromatic C=C bands (peak at 1606-1608 cm^{-1}) (Figure 1 A&B), that were recorded before, immediately after and 24h post-curing for each adhesive specimen according to the following equation [16]:

DC%= 1- ([C aliphatic/C aromatic]) / [U aliphatic/ U aromatic]) x100

in which '**C**' refers to cured, and '**U**' refers to uncured adhesive.

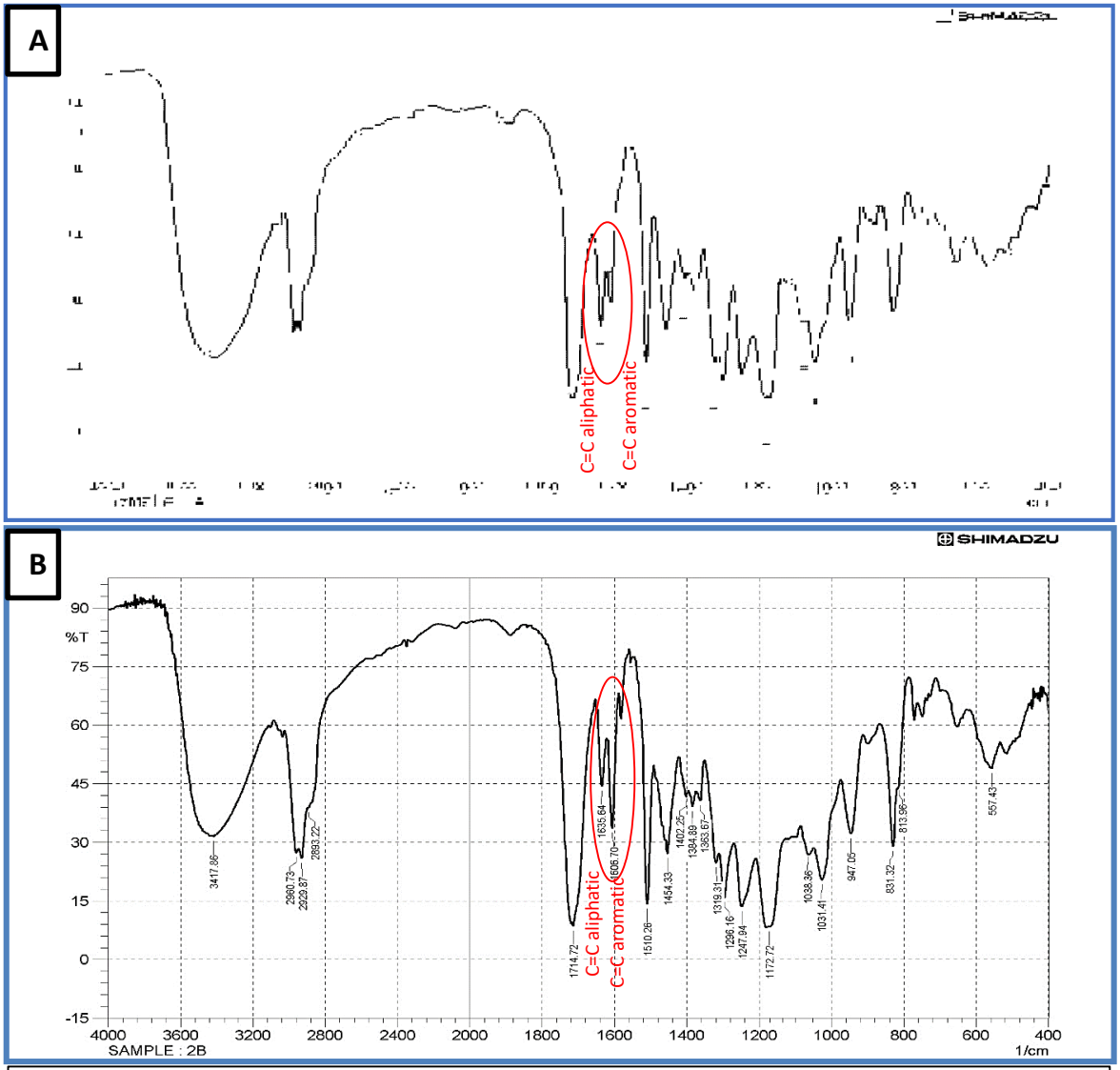

Figure 1: An example FTIR charts for GII showing the C=C band intensities (aliphatic and aromatic as demarcated by the red circles) used to calculate the degree of conversion of the dental adhesive: (A) Before curing, (B) Immediately after curing.

Results

The ascorbic acid coated superparamagnetic nanoparticles colloidal suspension was prepared successfully with an average coated particles size distribution of 18.7±2.4 nm as measured by ImageJ digital image analysis software from the micrographic images taken by TEM **(Fig. 2)**.

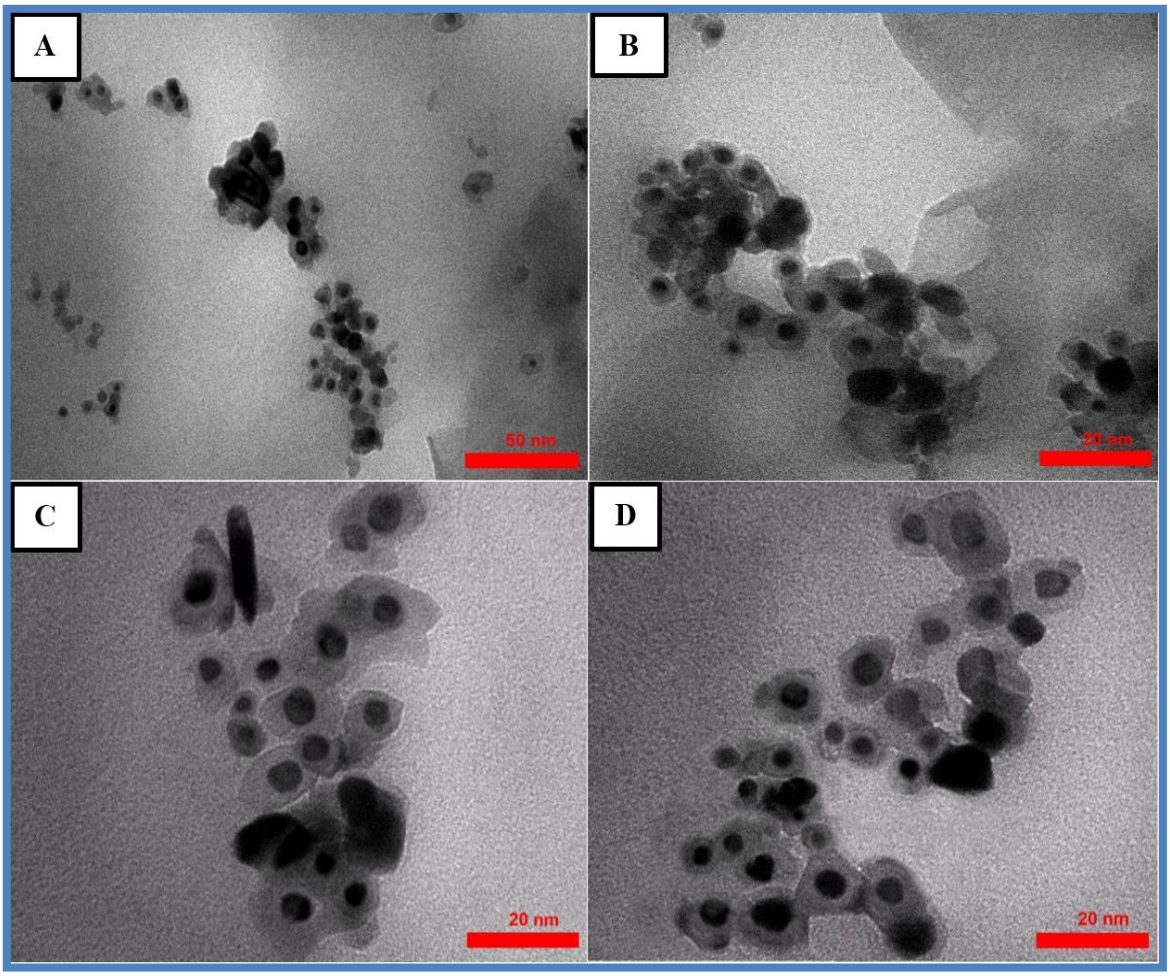

Figure 2: (A-D) Composite TEM micrographs for the prepared colloidal suspension at different magnifications clearly showing the core of Fe_3O_4 nanoparticles (i.e. black) are surrounded by a thick shell of ascorbic acid coating (i.e. shadow around the nanoparticles). The apparent clustering of the particles in some areas of the TEM images is probably an artifact results from drying the sample.

Thus, the protocol used by this study that was developed by Cave and Mundell [13], is proved to be successful for coating the Fe_3O_4 nanoparticles by ascorbic acid. This protocol was used because it is less technique sensitive, and it does not require complex additional functionalizing steps and/or chemical ligands exchange for coating the Fe_3O_4 nanoparticles. The grinding agitation process used by this protocol is the key step. It provides energy in the form of heat from friction which helps 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.

Degree of conversion results

The results of the descriptive statistics including minimum, maximum, means, and standard deviation values of the DC for all groups are presented in (Table 2) and shown in (Figure 2). Both of 2%-incorporated adhesives immediately after polymerization showed slightly lower DC values than their controls. However; 24h post-curing, the incorporated adhesives showed slightly higher DC values than the controls. The Prime & Bond universal adhesives (i.e. control and incorporated) showed greater DC values (both immediate and 24h post-cure) than All Bond Universal. The 2% incorporated All Bond Universal (immediately after curing) showed the lowest DC mean values, while the 2%-incorporated Prime&Bond (24h pos-cure) showed the highest DC mean values.

Table 2: Descriptive statistical results of the DC (%) for all groups.

Groups	Testing Time	Minimum	Maximum	Mean	Std. Deviation
GI	**Immediate**	70.60	76.00	73.33	4.70
	24 hours post-cure	70.90	78.20	73.95	1.61
GII	**Immediate**	68.50	73.70	71.05	1.83
	24 hours post-cure	69.10	77.10	74.68	1.32
GIII	**Immediate**	78.60	84.20	81.43	1.92
	24 hours post-cure	79.80	85.50	81.61	1.77
GIV	**Immediate**	76.20	81.90	78.53	1.29
	24 hours post-cure	79.50	84.10	82.13	1.05

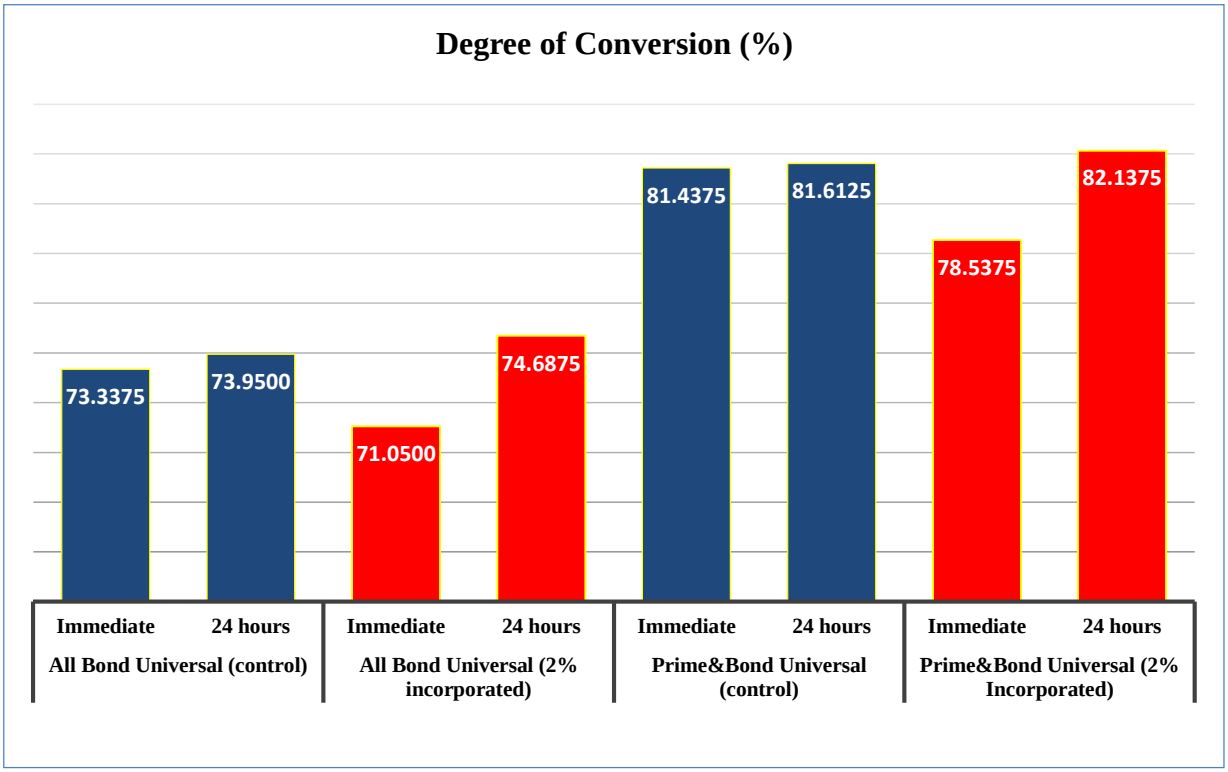

Figure 3: Graph showing the mean values (Immediate, 24 hours post-cure) of degree of conversion of all groups (%).

While the results of the inferential statistics using independent samples t-test to determine the significance of difference between the DC means of two unrelated groups are presented in (Table 3). This test showed no statistically significant differences between the control and the 2%-incorporated adhesives (Immediate and 24h post-cure), but revealed highly significant differences in DC (immediate and 24h post-cure) between the All bond universal and Prime & Bond universal groups (both for control and incorporated).

Table 3: Independent samples t-test to compare the significance of difference in degree of conversion mean values (immediate and after 24 hours) of the 2% incorporated adhesives in comparison to the control groups.

Groups	Mean Difference	Std. Error Difference	P-value	Significance
GI (Immediate) GII (Immediate)	2.473	0.968	0.083	(NS)
GI (24 hours post-cure) GII (24 hours post-cure)	0.513	1.119	0.654	(NS)
GIII (Immediate) GIV (Immediate)	1.788	0.955	0.082	(NS)
GIII (24 hours post-cure) GIV (24 hours post-cure)	-0.550-	0.820	0.513	(NS)
GI (Immediate) GIII (Immediate)	-7.750-	0.979	0.000	(HS)
GI (24 hours post-cure) GIII (24 hours post-cure)	-8.087-	1.041	0.000	(HS)
GII (Immediate) GIV (Immediate)	-8.375-	0.923	0.000	(HS)
GII (24 hours post-cure) GIV (24 hours post-cure)	-9.150-	0.917	0.000	(HS)

Discussion

Optimum infiltration and penetration of adhesive monomers deeper into the demineralized dental substrates, followed by adequate *in situ* polymerization to achieve high degree of conversion are essential factors to establish a good and long-lasting adhesion [10]. Low polymerization degree of dental adhesives is often associated with lower bond strength to dental substrates, lower mechanical properties, high degree of monomers elution, increased water permeability, resin hydrolysis, and subsequent failure of the adhesive restoration [9,17].

To solve the common issue associated with all dental adhesive systems which is their insufficient capability to completely infiltrate the deepest parts of the demineralized dentin collagen-matrix [18], ascorbic acid coated superparamagnetic Fe_3O_4 nanoparticles were incorporated into the universal adhesives to enhance the monomers penetration deeper into dentin to increase the adhesive/dentin bond strength and stability by subjecting them to external magnetic force before curing.

Therefore, this study was conducted to assess the effects of the incorporated ascorbic acid coated Fe_3O_4 nanoparticles on the degree of cure of the universal adhesives. To test that, FTIR spectra were recorded for the 2%-incorporated adhesives in comparison to their control groups before cure, immediately after cure, and 24h post-curing using the FTIR spectrometer in the transmittance mode. For All Bond universal, the FTIR analysis results showed that the immediate mean DC values for the control group of was 73.33%, while the 2%-incorporated group showed an immediate DC mean value of 71.05 %. However; 24h post-cure, the DC values was 73.95% for control group and 74.68 for the 2%-incorporated adhesives, with no statistically significant differences among them. On the other hand, the results for Prime & Bond universal showed an immediate DC mean values for the control group of 81.43%, while the 2%-incorporated group showed an immediate DC value of 78.53. While 24h post-cure, the DC values were 81.51% for control groups and 82.93% for the 2%-incorporated adhesives, with no statistically significant differences between them. Although non-significant differences, incorporation of colloidal suspension containing ascorbic acid coated Fe_3O_4 nanoparticles at 2% by mass into the adhesives initially results in a very slight reduction in the immediate degree of polymerization values when compared with the control groups. This could be related to the antioxidant properties of ascorbic acid that may interact with the generated free radicals upon photo-polymerization [19].

However, 24h post-curing, the mean DC values was very slightly increased for of the control groups, but noticeably increased for the incorporated adhesives when compared with their immediate results and that of the control groups (i.e. also there was no statistically significant differences). This increase in DC values after 24 hours could be related to the phenomenon of 'Post-polymerization or dark cure' [20], which occurs in adhesive monomers during the first 24 h post-curing, similarly to composites. This is because the free radicals and unreacted monomers remain trapped within the resin matrix shortly after the start of light curing [21]. Thus, upon ceasing of light irradiation, a slow generation of free radicals continue to occur, and this may be accelerated by the presence of ascorbic acid antioxidant in the incorporated groups, which interact and eliminate any oxygen radicals that can scavenge the free radicals. This will allow continuous free radicals generation [22],

that may increase the polymerization degree post-curing as demonstrated by the results of this study.

Those results are comparable to the results of a study made by Gotti *et al* [19], where they incorporated different antioxidants into different adhesive systems, and tested their effects of the degree of conversion and bond strength after long-term water storage. This study concluded that although the presence of antioxidants slightly decreased the immediate DC after incorporation, the incorporated adhesives showed maintained or even increased bond strength values after 6 months of water aging in comparison to the immediate results. The study attributed such findings to the fact that the incorporated antioxidants can react with reactive oxygen species released from the degradation process allowing the free unpolymerized adhesive monomers and photo-initiators to be continuously released, leading to a late polymerization process. Such continued polymerization results in the increased bond strength after 6 months of aging. Also, the study showed that despite the slight changes in the pattern of nano-leakage, there was a decrease in silver nitrate deposits for ascorbic acid incorporated adhesives, which also attributed these results to the delayed polymerization process of the antioxidant-doped adhesives [19].

Finally, the study showed that Prime&Bond universal results in significantly higher immediate and 24h post-cure DC mean values (Both control and incorporated groups) than All Bond universal groups. These findings are probably related the differences in the chemical compositions of the two adhesives. Prime&Bond universal is based on a unique chemistry as claimed by the manufacturers which is based on the new patented "Active Guard Technology" that crosslinks the adhesive monomer components (hydrophilic & hydrophobic) through the use of bis-acrylamide copolymers that can enhance the adhesive monomers crosslinking upon polymerization preventing the phase monomers separation [23, 24]. Also, this adhesive is based on different photo-initiators system including the 4-dimethylamino benzonitrile [23], that can undergo intramolecular charge transfer upon photoexcitation which may enhance the degree of polymerization. While All Bond universal is based on the conventional Bis-GMA/HEMA and 10-MDP copolymer blends and Camphorquinone/tertiary amine photopolymerization system [25].

Conclusion

Universal adhesives containing 2% ascorbic acid coated superparamagnetic nanoparticles showed no significant differences in the immediate and 24h post-cure DC values in comparison to their control groups. The Prime & Bond universal showed significantly higher immediate and 24h post-cure DC values (Both control and incorporated groups) than All Bond universal adhesive groups.

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