

Degree of Conversion and Antibacterial Activity of Total-Etch Versus Self-Etch Adhesive Systems

Hamouda IM^{1,2*}, Beyari MM³, Samra NA⁴ and Badawi MF⁴

¹Dental Biomaterials Department, Mansoura University, Mansoura, Egypt

²Conservative Dentistry Department, Umm Al-Qura University, KSA

³Faculty of Dentistry, Umm Al-Qura University, Makkah, KSA

⁴Faculty of Dentistry, Mansoura University, Egypt

Abstract

Objectives: This research was conducted to compare the degree of conversion and antibacterial activity of the total-etch (etch-and-rinse) versus self-etch adhesive systems.

Materials and methods: Degree of conversion was done using Fourier Transformation Infrared Spectroscopy. Uncured and cured specimens were prepared from each adhesive system and tested using potassium bromide disks. The antibacterial activity of the adhesives was evaluated using agar disc-diffusion test against the following bacteria: *Staphylococcus aureus*, *Streptococcus mutans* and *Lactobacillus salivarius* obtained from soft carious dentin. Paper disks were coated with the tested adhesives and placed in the suitable growth media for each microorganism. The diameters of inhibition zones were measured at three different points. Sizes of inhibition zones were calculated by subtracting the diameter of the specimen from the average of the three measurements of the halo.

Results: G-Bond showed higher degree of conversion than that of Stae and Adper Prompt L-Pop adhesives. Adper Prompt L-Pop exhibited the highest antibacterial activity against *S. mutans* and *S. aureus*, while Stae exhibited the lowest antibacterial activity against *S. mutans* and *S. aureus*. All adhesives failed to demonstrate antibacterial activity against *L. salivarius*.

Conclusions: The tested adhesives showed degree of conversion with various percentages. All adhesives showed antibacterial action against *S. aureus* and *S. mutans*. On the other hand, they were failed to inhibit the growth of *L. salivarius*.

Keywords: Adhesive systems; Antibacterial activity; Degree of conversion; Self-etch; Total-etch

Introduction

True adhesion has been the “holy grail” of dental materials for many decades. True adhesion is the interdiffusion between the dentin of the tooth structure and the applied adhesive. If true adhesion of restorative materials to tooth structure is to be achieved, three conditions must be satisfied: sound tooth structure must be conserved, optimal retention must be achieved and microleakage must be prevented [1]. The foundation for modern adhesive dentistry was laid in 1955, when Bounocore reported that acids could be used to alter the surface of enamel to render it more receptive for adhesion. Since that time, dental adhesive systems have evolved through several generations with changes in chemistries, mechanisms, number of application techniques and clinical effectiveness [2]. Modern adhesive systems can be grouped into two categories according to their etching technique: total-etch and self-etch products [3]. The first category utilizes the total-etch (etch-and-rinse) technique which relies on the removal of the smear layer and exposure of the collagen matrix by acid etching, followed by the application of a self-priming agent that combines the primer and the adhesive resin into one solution [4]. The second category utilizes the self-etch technique in which prior etching step is omitted. The self-etching products may be one-step or two-step self-etch adhesives. Two-step self-etch adhesives are characterized by utilizing a self-etching, hydrophilic primer that is followed by the application of a comparatively more hydrophobic bonding agent while the one-step self-etch adhesives contain two liquids which are applied to the tooth substrate after mixing. The latest approach in simplifying bonding to enamel and dentin are the adhesives in which all the adhesive components for etching, priming and bonding are supplied in a single bottle [5]. Degree of polymerization is one of the important factors that affect clinical

performance of dental resins. Among several methods to determine the degree of conversion (DC), Fourier transformation infrared spectroscopy (FTIR) has been proven to be a powerful technique and has been widely used as a reliable method as it detects the C=C stretching vibrations directly before and after curing of materials [6,7]. Dentin bonding systems which possess antibacterial properties would be beneficial in eliminating the harmful effect caused by bacteria. Among different methodologies used to determine the antibacterial activity of dentin bonding systems, simple direct inhibition tests such as agar-disc diffusion methods have been most frequently used [8,9]. This research was conducted to compare the degree of conversion and antibacterial activity of the total-etch (etch-and-rinse) versus self-etch adhesive systems.

Materials and Methods

The materials used in this test are listed in Table 1.

Degree of conversion test (DC)

Degree of conversion was done using Fourier Transformation

***Corresponding author:** Ibrahim M. Hamouda, Conservative Dentistry Department, Umm Al-Qura University, KSA, Tel: +966542812148; E-mail: imh100@hotmail.com

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Materials	Composition	Batch Number	Manufacture
Stae (Fifth generation), Dentine/enamel , 1-component adhesive	UDMA, Acetone fluoride stabilizer	0406165	SDI, Australia
Adper Prompt L-Pop (Sixth generation) 2-component, single-step, self-etch adhesive	Liquid 1 (red blister), Methacylated phosphoric, ester, Bis –GMA, Initiator , based on comphorquinone , Liquid 2 (yellow blister)_Water, 2-hydroxyethyl methacrylate, Polyalkenoic acid Stabilizers_droxyethyl	244893	3M ESPE, St, Paul , MN,USA
G-bond (Seventh generation) , 1-component, single-step, self-etch adhesive	4-methacryloxyethyltrimelletic, acid (4- MET), Urethane , dimethacrylate (UDMA), Phosphate monomer, Fused silica filler Photoinitiator, Acetone Stabilizer	0506141	GC Corp, Tokyo, Japan
Glacier Microfilled hybride , composite	Multifunctional methacrylic , ester, Inorganic filler	060273	SDI, Australia

Table 1: Materials used in the study.

Materials	Mean ± SD	F-value	P-value	LSD
Stae	24.7 ^b ± 8.2	28.8	< .001	24.88
Adper Prompt	23.2 ^b ± 3.9			
L-Pop				
G-Bond	49.6 ^a ± 11.1			

Means with different superscripts are significantly different

Table 2: Mean degree of conversion and standard deviations of the three adhesives used in the study.

Infrared Spectroscopy (MATTERSON 500 FTIR Spectroscopy). Uncured specimen was taken from each bonding agent (Stae, Adper Prompt L-Pop and G-bond). Each uncured specimen was smeared onto a potassium bromide disk [6]. Nine cured specimens were prepared from each adhesive as follows: the occlusal enamel of the mandibular molar teeth was removed perpendicular to the long axis of the teeth with diamond burs under water-cooling to form a flat superficial, coronal dentin surface [6]. The adhesive was applied and cured according to the manufacturer's instructions for 20 seconds. Immediately after curing, the cured adhesive was scratched by a scalpel and changed into fine powder. Fifty micrograms of the powder was mixed with 5mg potassium bromide (KBr) spectroscopic powder [10]. Each specimen was irradiated by the infrared spectrum of the FTIR Spectroscopy. The frequency of the infrared region used was between 4000 to 400cm⁻¹ wave number [6] and resolution was 4 cm⁻¹.

The percentage of DC for each specimen was calculating from the following equation:

$$(C=C / C...C) \text{ after curing}$$

$$DC \% = (1 - \frac{C=C}{C...C}) \times 100 [10, 11].$$

$$(C=C / C...C) \text{ before curing}$$

C=C is the aliphatic carbon=carbon bond; while C...C is the aromatic carbon... carbon bond.

Antibacterial activity test

Although 200 to 300 bacterial species have been found in saliva, *S. mutans* has been considered as a potent caries causing bacteria. Saliva can act as a selective medium for bacterial growth, but continuously repeated swallowing results in clearing of bacteria. *S. mutans* is a facultative anaerobe and is a Gram-positive bacterium that lives in the mouth. It can thrive in temperature ranging from 18-40 degrees Celsius. It metabolizes different kinds of carbohydrates, creating acidic environment in the mouth as a result of this process. This acidic environment in the mouth is what causes the tooth decay. It is the leading cause of dental caries (tooth decay) worldwide. *S. mutans* is considered to be the most cariogenic of all of the oral *Streptococci* [12]. *S. aureus* belongs to the family *Staphylococcaceae*. It affects all known mammalian species, including humans. The microscopic appearance of *S. aureus* is round and resembles that of a sphere (cocci). Gram-positive cocci in clusters. Because of the way the bacteria divide and

multiply, it will appear in clusters or tetrads. *L. salivarius* is a gram-positive, non-spore forming bacillus bacteria. It is a homofermentative organism (only produces one byproduct of metabolism-lactic acid) that is found occurring naturally in the human oral cavities. *L. salivarius* is a facultative anaerobe, meaning that it can grow in the presence or absence of oxygen [13].

The antibacterial activity of the adhesives was evaluated using agar disc-diffusion test against the following bacteria: *Staphylococcus aureus*, *Streptococcus mutans* and *Lactobacillus salivarius*. This study was conducted in the Medical Diagnostic and Infection Control Unit (MDICU) at the Microbiology & Immunology Department, Faculty of Medicine, Mansoura University.

Sample collection

Random samples of soft carious dentin were directly taken from carious cavities by a sterile excavator from randomly selected patients [14]. *S. aureus*, *S. mutans*, and *L. salivarius* were isolated, incubated and identified by means of the strain morphology, microscopic examination and biochemical reactions.

Preparation of the tested materials

Paper disks were used to be coated with the tested adhesives. These disks were prepared as follows: filter paper was punched using a hole punch to make small circular paper discs. These disks were wrapped in aluminum foil and sterilized in the hot air oven at 160°C for 30 min [15,16]. Twenty µl of each adhesive were impregnated into a sterile paper disk (diameter: 6 mm, thickness: 1.5 mm) and cured for 20 seconds. A 20-µl volume was chosen as the optimum for impregnation into paper disk without overflow of the tested materials [17]. The antibacterial effect was evaluated using the disk diffusion method: culture medium was Muller-Hinton agar for *S. aureus*, blood agar for *S. mutans* and rogosa agar for *L. salivarius*. The paper disks containing the tested materials were seated on the sterile Petri dish at equal distances from each other by applying firm pressure to the disks with a sterile forceps against the medium surface, 3 disks per dish. Each plate was labeled with the names of the tested materials and microorganisms, and incubated at 37°C for 24 h in the case of *S. mutans* and *S. aureus* [18] and at 35°C for 3 days in case of *L. salivarius* [19]. The diameters of inhibition zones were measured at three different points. Sizes of inhibition zones were calculated by subtracting the diameter of the specimen from the average of the three measurements of the halo [20].

Statistical analysis

One-way ANOVA test was used to analyze the data obtained by the tested adhesives. The least significant difference (LSD) statistical test was used to detect the significant differences between the tested groups.

Results

Degree of conversion (%)

Mean DC (%) and standard deviation for Stae, Adper Prompt L-Pop and G-Bond are presented in Table 2. G-Bond had the highest mean DC (%) value ($P < 0.05$), while Adper Prompt L-Pop and Stae had similar values.

Graphical presentations of the absorbance peaks for uncured and cured Stae, uncured and cured Adper Prompt L-Pop and uncured and cured G-Bond are shown in Figures 1-6: respectively. For Stae, the absorbance peaks of the infrared rays for the aliphatic C=C of the monomer was 1635 cm^{-1} and 1636 cm^{-1} for the uncured and cured resin respectively. The absorbance peaks of infrared rays for aromatic C...C of the monomer for uncured and cured resin were 1534 cm^{-1} and 1537 cm^{-1} respectively.

For Adper Prompt L-Pop, the absorbance peaks of the infrared rays for the aliphatic C=C of the monomer was 1635 cm^{-1} and 1636 cm^{-1} for the uncured and cured resin respectively. The absorbance peaks of infrared rays for aromatic C...C of the monomer for uncured and cured resin were 1512 cm^{-1} and 1512 cm^{-1} respectively.

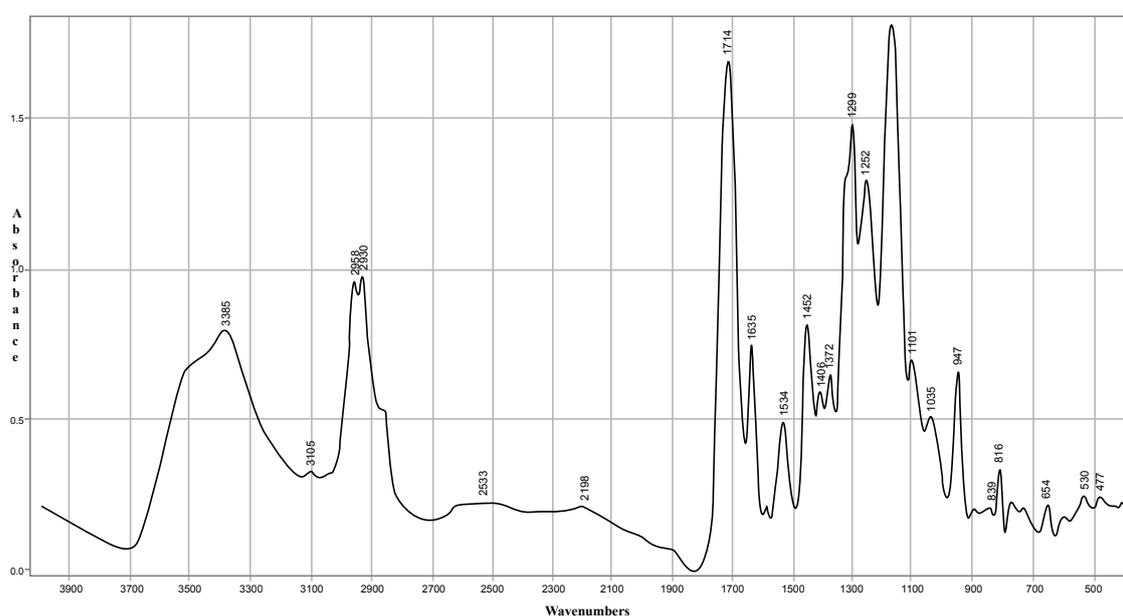


Figure 1: The absorbance peaks of the infrared rays by C=C and N...H in the monomer of the uncured adhesive resin of Stae.

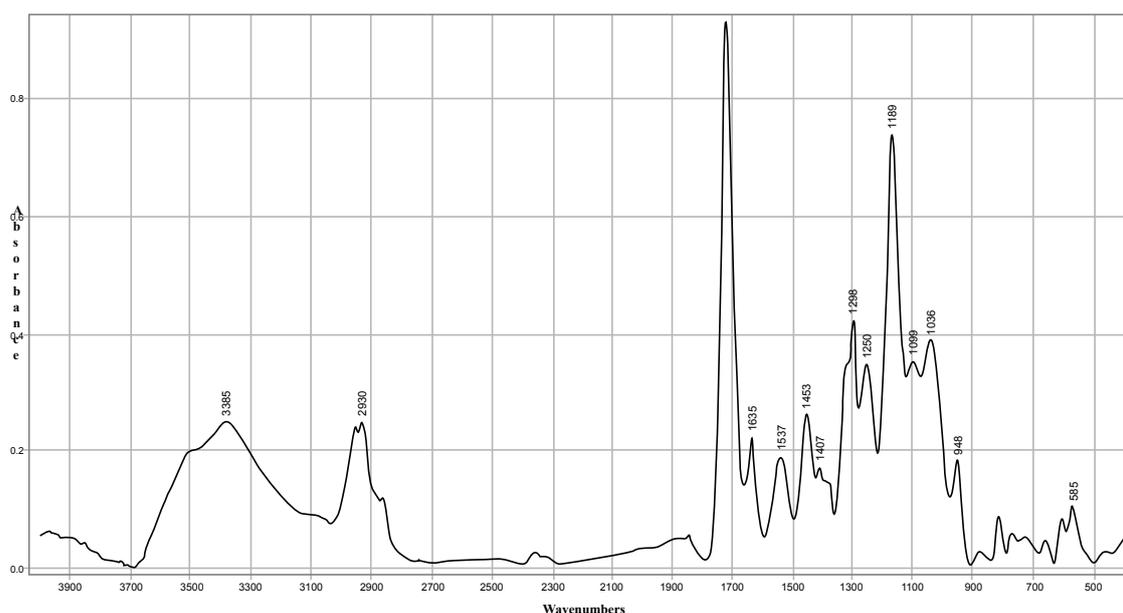


Figure 2: The absorbance peaks of the infrared rays by C=C and N...H in the monomer of the cured adhesive resin of Stae.

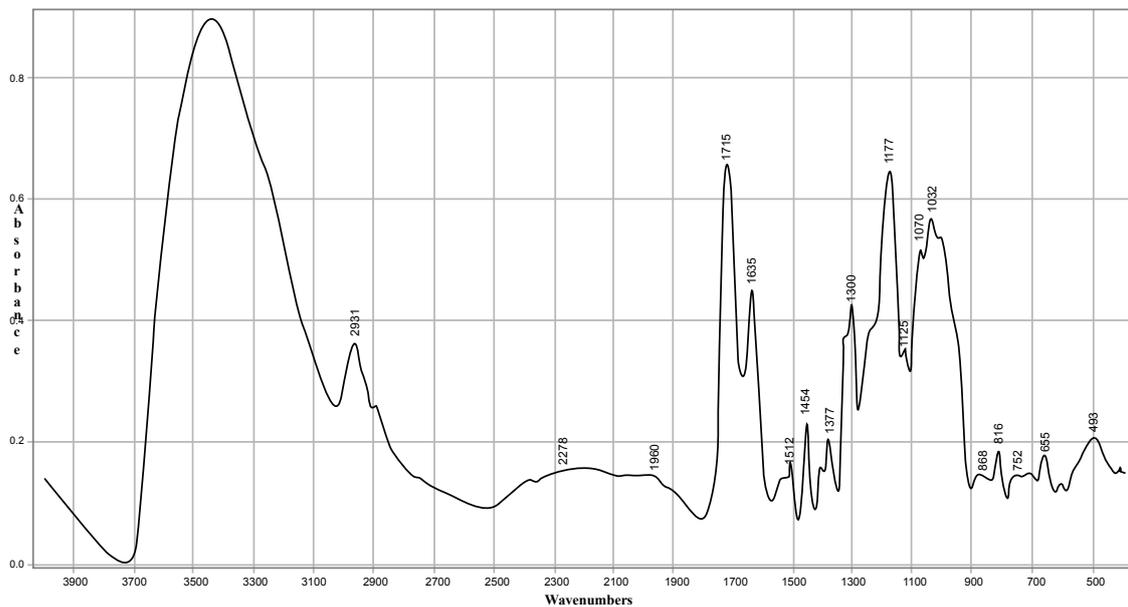


Figure 3: The absorbance peaks of the infrared rays by C=C and C...C in the monomer of the uncured adhesive resin of Adper Prompt L-Pop.

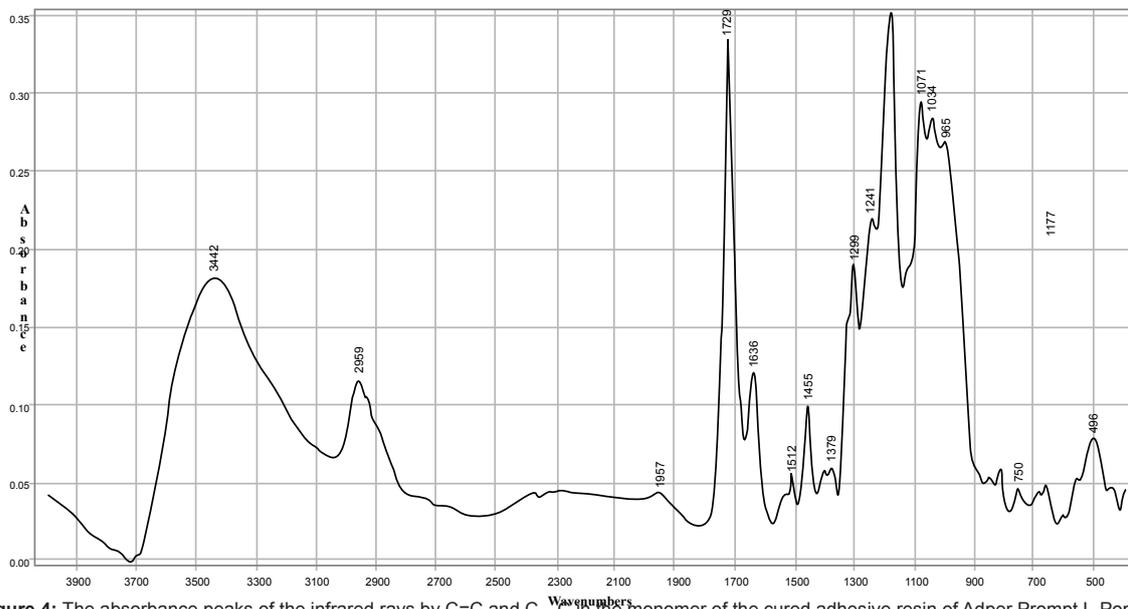


Figure 4: The absorbance peaks of the infrared rays by C=C and C...C in the monomer of the cured adhesive resin of Adper Prompt L-Pop.

For G-Bond, the absorbance peaks of the infrared rays for aliphatic C=C of the monomer was 1637 cm^{-1} and 1639 cm^{-1} . The absorbance peaks of aromatic C...C of the monomer for uncured and cured resin were 1532 cm^{-1} and 1538 cm^{-1} respectively.

Antibacterial activity test

Mean inhibitory zones and standard deviations for all adhesives are presented in Table 3. Graphical presentations of inhibitory zones are shown in Figures 7-9. Stae, Adper Prompt L-Pop and G-Bond demonstrated growth inhibition against *S. mutans* and *S. aureus* and no inhibition was observed against *L. salivarius*.

Significant differences were observed between all adhesives

($P < 0.05$), with Adper Prompt L-Pop exhibiting the highest mean inhibitory zones and Stae the lowest.

Discussion

The current interest in dentin bonding research is focused on reducing the number of application steps in the bonding procedures providing simpler and faster adhesives and reducing the technique sensitivity as well as operator variability [6]. The constant development in adhesive restorative dentistry has already caused profound changes in dental practice, and future advances such as one-step self-etching systems have been proposed as suitable agents for dentin bonding. They combine the three steps of etching, priming and bonding in a single application [21].

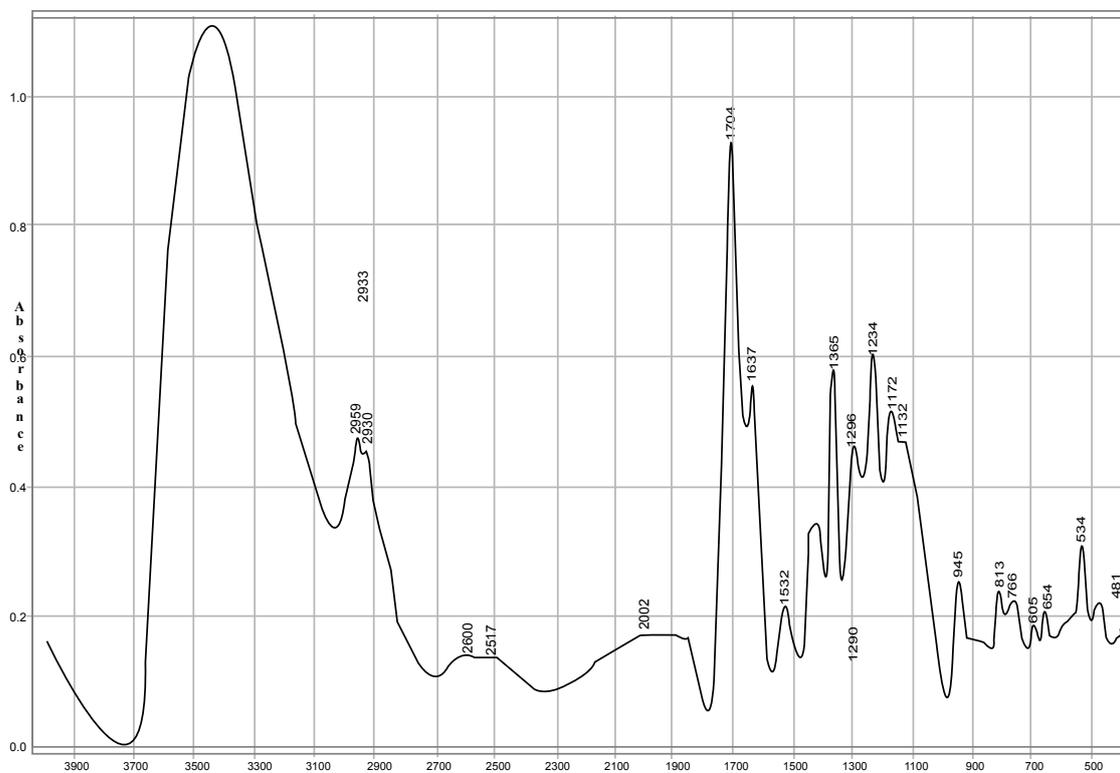


Figure 5: The absorbance peaks of the infrared rays by C=C and N-H in the monomer of the uncured of G-Bond adhesive resin.

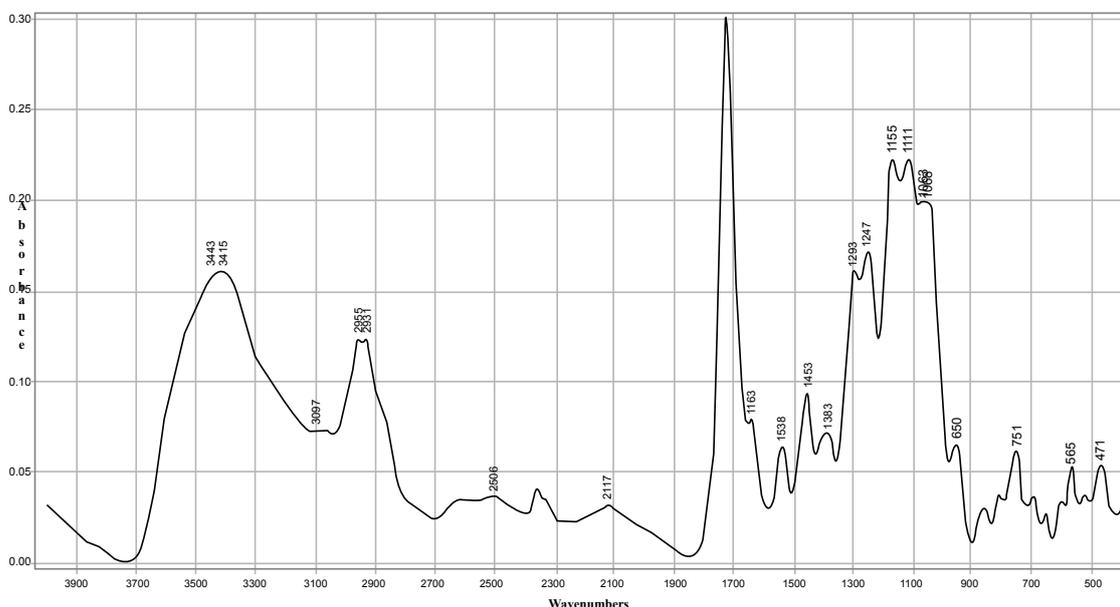


Figure 6: The absorbance peaks of the infrared rays by C=C and N...H in the monomer of the cured of G-Bond adhesive resin.

HEMA, hydrophilic primer is commonly included in the adhesive system to improve the bond strength. On the other hand HEMA lowered the vapor pressure of water when added to water, making it more difficult to remove water from the adhesive and retaining water within the adhesive layer. The results showed that G-bond had the

highest mean degree of conversion value of the adhesives used in the study and this difference was statistically significant. The high results of G-bond may be attributed to the fact that it is HEMA-free one-step self-etch adhesives.

The improved results of the Stae adhesive may be attributed to the

acetone content of the adhesive. The role of acetone in the bonding solution is to lower the viscosity of the solution, so enhancing the penetration of the bonding agents into the demineralized collagen-rich dentin matrix, and to lower the surface tension of water due to “water chasing” effect. Moreover, acetone may increase the vapor pressure of water and enhance the removal of collagen matrix water, which may then be exchanged for the acetone and ultimately for the adhesive resin [22].

Complete sealing at the bonded interface is a prerequisite for successful restorations [23]. It is well recognized that residual bacteria after removal of a carious lesion causes increased pulp sensitivity, pulp inflammation and secondary caries [15]. Additionally, microleakage of bacteria through the gap between the restoration and the cavity

Materials	<i>S. mutans</i>	<i>S. aureus</i>	<i>L. salivarius</i>
Stae	0.5 ^c ± 0.3	1.61 ± 1.08 ^c	0
Adper Prompt			
L-Pop	1.82 ^a ± 0.8	4.23 ± 1.26 ^a	0
G-Bond	0.91 ^b ± 0.7	1.81 ± 1.46 ^b	0
F-value	5.46		
P-value	0.02*		
LSD	0.91		

Means with different superscripts are significantly different

Table 3: Mean inhibitory zones (mm) and slandered deviations of the bonding agents.



Figure 7: Photographs showing the inhibitory zones of Stae (A), Adper Prompt L-Pop (B) and G-Bond (C) against streptococci.

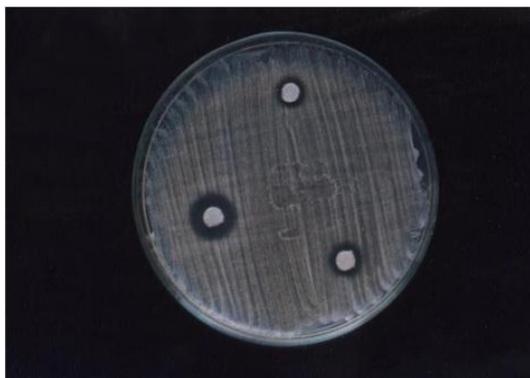


Figure 8: Photographs showing the inhibitory zones of Stae (A), Adper Prompt L-Pop (B) and G-Bond (C) against staphylococci.



Figure 9: Photographs showing the inhibitory zones of Stae (A), Adper Prompt L-Pop (B) and G-Bond (C) against lactobacilli.

wall in known to be the main cause of unpleasant symptoms, which occur after placement of restorations. However, even dentin adhesive systems which show high bond strength have been reported to be incapable of preventing the occurrence of microgaps between the tooth and the restoration in vitro [23,15]. Secondary caries, whether the result of bacterial invasion through microleakage or from residual bacteria left in the cavity preparation, has consistently been found to be the most common reason for the replacement of amalgam and composite restorations [7]. Therefore, dentin bonding systems which posses antibacterial activity even after placement in the cavity would be beneficial for eliminating the harmful effect caused by bacterial micro leakage [23]. *S. mutans* and *S. sobrinus* are associated with the initiation of human dental caries, while *L. salivarius* is associated with the progression of the established lesion [7]. Agar-disc diffusion test method is a simple direct inhibition test and it has been most frequently used [9]. Addition of acidic monomers in large amounts for self-etching adhesive systems to promote adhesion, decrease pH values of these materials enough to kill or at least inactivate the bacteria [9]. The antibacterial effect shown by dentin bonding agents in this study may be related to their pH [7]. The pH of the bonding agents used in the study are Prompt L-Pop (pH 0.7) [17], G-Bond (pH > 2.0) [24] and Stae (pH 0.5) [8]. The self-etching adhesives used in this study have demonstrated antibacterial action to varying degrees. In what regards Adper Prompt L-Pop and G-Bond, antibacterial activity was shown against *S. mutans* and *S. aureus* but failed to demonstrate antibacterial action against *L. salivarius*. These results can be explained on the basis of the previous observation which attributed this effect to low pH values of these materials. However the lower inhibitory effect against *L. salivarius* may attribute to the acid tolerance of such species [8]. As regard to total-etch systems, Stae has demonstrated antibacterial action against *S. mutans* and *S. aureus* but to lesser degrees and failed to demonstrate antibacterial action against *L. salivarius*. This effect could be compensated by the antibacterial effect of its etchant which is applied on the cavity walls before applying the stae adhesive as a part of total-etch protocol. Etching of the dentinal surface with acidic solution, such as phosphoric acid, during the bonding procedures may be effective to reduce the number of residual bacteria in the cavity [25].

Conclusions

Within the limitations of this research, the following conclusions were conducted.

1. The degree of conversion values for the 1-component self-etch adhesives (G-Bond) were significantly higher than that

of 2-component total-etch and self-etch adhesives tested (Stae& Adper Prompt L-Pop).on the other hand, there was no significant difference between 2-component total-etch and self-etch adhesives tested (Stae& Adper Prompt L-Pop).

2. Two-component self-etch adhesives (Adper Prompt L-Pop) exhibited the highest antibacterial activity against *S. mutans* and *S. aureus*, while 2-component total-etch adhesives (Stae) exhibited the lowest antibacterial activity against *S. mutans* and *S. aureus*.
3. One-component self-etch adhesives (G-Bond) exhibited antibacterial activity against *S. mutans* and *S. aureus* which was significantly different with Stae in case of *S. mutans*. G-Bond wasn't significantly different with Stae in case of *S. aureus*.
4. All the tested adhesives failed to demonstrate antibacterial activity against *L. salivarius*.

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