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Degree of Conversion of Self-etch Adhesives: *In Situ* Micro-Raman Analysis

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Clinical Relevance

The degree of conversion is a fundamental parameter with which to evaluate adhesives' clinical performance and stability in the oral environment; based on the results of this study, all of the tested self-etch adhesives showed a clinically acceptable degree of conversion.

SUMMARY

Purpose: Degree of conversion (DC) affects the physicochemical properties of dental adhesives. The aim of this study was to measure the DC within the hybrid layer of four one-step self-etch adhesives using Raman microspectroscopy. The hypothesis tested was that there was no difference among the tested adhesives.

Methods and Materials: The selected one-step self-etch adhesives (Clearfil S^3 Bond Plus, I-BOND, G-BOND, and Adper Easy Bond) were applied on human dentin disks and polymerized in accordance with the manufacturers' instructions. Specimens were transversally cut to expose the bonded interfaces to the micro-Raman beam, and Raman spectra were collected along the dentin/adhesive interface. Measurements were performed at 1-μm intervals. The relative intensities of bands associated with the C=C bond (at 1640 cm^{-1}) and an internal stable peak (1610 cm^{-1}) were determined to calculate the degree of conversion within the hybrid layer. Data were statistically analyzed with Kolmogorov-Smirnov and Bartlett tests and Kruskal-Wallis and Mann-Whitney *U*-tests.

Results: The DC ranked as follows: G-BOND (93%±6%) ≥ Adper Easy Bond (92%±6%) ≥ I-BOND (89%±7%) > Clearfil S3 Bond Plus (80%±14%) ($p<0.05$).

Conclusions: Based on the results of this study, all of the tested self-etch adhesives showed a

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DOI: 10.2341/15-165-L

clinically acceptable DC that was material dependent.

INTRODUCTION

Dentin bonding systems can be divided into etch-and-rinse or self-etch materials depending on their bonding application procedures. The current self-etch adhesives are characterized by the application of an acidic primer/adhesive mixture that can be simultaneously applied on enamel and smear layer–covered dentin: this procedure avoids the discrepancy between etched collagen network and resin infiltration and reduces the technique sensitivity that characterizes the etch-and-rinse approach.[1]

One-step self-etch adhesives combine etching, priming, and bonding into a single product. They are easy and fast to use; however, previous studies[2-5] reported that immediate bond strength to dentin of one-step self-etch adhesive systems is generally lower in comparison to that associated with multi-step systems. Additionally, significant bond strength reduction and increased interfacial nanoleakage expression were reported[6,7] for one-step self-etch adhesives in long-term studies.

A good collagen impregnation and a high degree of conversion (DC) of the adhesive monomers are fundamental for the longevity of composite restorations. It was hypothesized that the poor performance of self-etch adhesives could depend upon shallow resin tag penetration produced by the self-etching process, an inefficient curing caused by their acidic nature,[8] or solvent retention and phase separation phenomena due to the coexistence of both hydrophilic and hydrophobic moieties in the same product.[9,10] A low rate of monomer-to-polymer conversion in polymers leads to weaker polymer networks, low physicochemical properties, and, therefore, higher susceptibility to degradation processes.[11-13] Moreover, self-etch adhesives behave as permeable membranes,[13,14] presenting unreacted monomers in their hybrid layer.[15] The hydrophilicity of one-step self-etch adhesives into the hybrid layer can lead to increased water uptake, plasticization of the polymer network, and elution of unreacted monomers.[13,16,17]

Micro-Raman spectroscopy is a helpful tool for the *in situ* evaluation of DC of adhesive resins because it can directly measure the percentage of converted double-bonds within the adhesive layer in a nondestructive manner. Indeed, a spatially resolved chemical analysis of the hybrid layer can be performed through micro-Raman spectroscopy, detecting chemical changes in bands of interest, such as vinyl and phenyl C=C bonds, after minimal specimen preparation steps. Although other spectroscopy techniques, such as micro–Fourier transform infrared spectroscopy (FTIR), can also provide a high–spatial resolution map of molecular vibrational changes, micro-Raman spectroscopy is less sensitive to water and requires minimal specimen preparation.[15,18,19] Although the DC of dental adhesives was previously investigated *in vitro,* there is still a lack of knowledge of the DC *in situ* into the hybrid layer of commercial adhesives.

The aim of this study was the evaluation of the DC within the hybrid layer of four commercial one-step self-etch adhesives. The tested hypothesis was that no differences in terms of DC were present among the adhesives systems investigated in this study.

METHODS AND MATERIALS

Tooth Preparation and Bonding Procedures

Noncarious human third molars (n=12) stored in 0.5% chloramine in water at 4°C were collected after informed consent was obtained from patients under a protocol approved by the University of Trieste, Italy. To ensure that the sample size was adequate, the power of the statistical test had been previously verified (Software G*Power 3, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). A low-speed diamond saw with water cooling (Isomet 5000 Buehler Ltd, Lake Bluff, IL, USA) was used to expose middle/deep dentin, and a uniform smear layer was obtained with 180-grit silicon carbide (SiC) paper to simulate the bur's action. To ensure that any pulp was not exposed, an accurate surface inspection under a 50× light microscope (Leica, Wetzlar, Germany) was performed.

The dentin disks were then randomly and equally assigned to the tested one-step self-etch adhesives (n=3), as follows: group 1: Clearfil S^3 Bond Plus (Kuraray Medical Inc, Tokyo, Japan); group 2: I-BOND (Heraeus Kulzer, Hanau, Germany); group 3: G-BOND (GC America Inc, Alsip, IL, USA); group 4: Adper Easy Bond (3M ESPE, St Paul, MN, USA). Adhesive compositions and application modes are listed in Figures 1 through 4.

Dentin bonding systems were applied in accordance with manufacturers' instructions (Figures 1 through 4) and polymerized using a quartz-halogen lamp (Elipar 2500; 3M ESPE) with an irradiance of 600 mW mm^{-2}, as previously assayed using a 400-500-nm radiometer (100 Optilux radiometer; SDS Kerr, Danbury, CT, USA), maintaining the light tip at a distance of 1 mm from the adhesive surface. A 2-

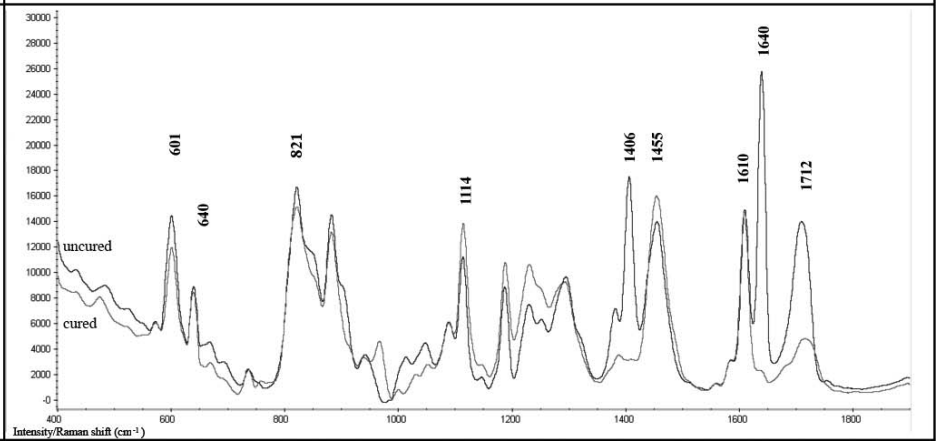
Adhesive system	Composition	Application mode	Raman spectrum
Group 1: CLEARFIL S^3 BOND PLUS	MDP, Bis-GMA, HEMA, Colloidal silica, Ethanol, Water, dl-Camphorquinone, Photo initiator, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Photo polymerization accelerator, Chemical polymerization accelerator, Sodium fluoride, Others	Apply to tooth surface for 10 s. Dry with mild air for more than 5 s until the bond does not move. Light cure for 10 s.	

Figure 1. *Composition, application mode (as recommended by the respective manufacturer), and Raman spectra of cured and uncured Clearfil S^3 Bond Plus. The decrease in the C=C peak after polymerization is visible when comparing the spectra of the uncured adhesive. Refer to Table 1 for other peaks. Abbreviations for all the figures: Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloxydecyl dihydrogen phosphate; 4-META, 4-methacryloxyethyltrimellitic acid anhydride; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.*

mm layer of resin composite (Filtek Z250; 3M ESPE) was applied on the adhesive surface and light-cured for 20 seconds. Each specimen was then cut transversally to obtain two 2-mm slabs. The two middle slabs were selected and then polished to expose the adhesive interface for micro-Raman analysis.[20] Polishing was performed for 30 seconds with wet SiC papers (up to 800 grit), followed by 6-, 1-, and 0.05-μm diamond pastes, with distilled water rinsing between each step. Slabs were then cleaned with soap, sonicated for one minute, and rubbed with a cotton swab saturated with 5% sodium hypochlorite to remove smeared proteins produced by polishing from the surface. The specimens were dry-stored in a dedicated cupboard at controlled room temperature (22°C) with silica gel to obtain a dry environment.

Micro-Raman Spectroscopy

The equipment used consisted of a computer-controlled laser Raman microprobe connected to a DM/LM optical microscope with a 100× objective (NA 0.9; N Plan EPI objective; Leica, Wetzlar, Germany) and

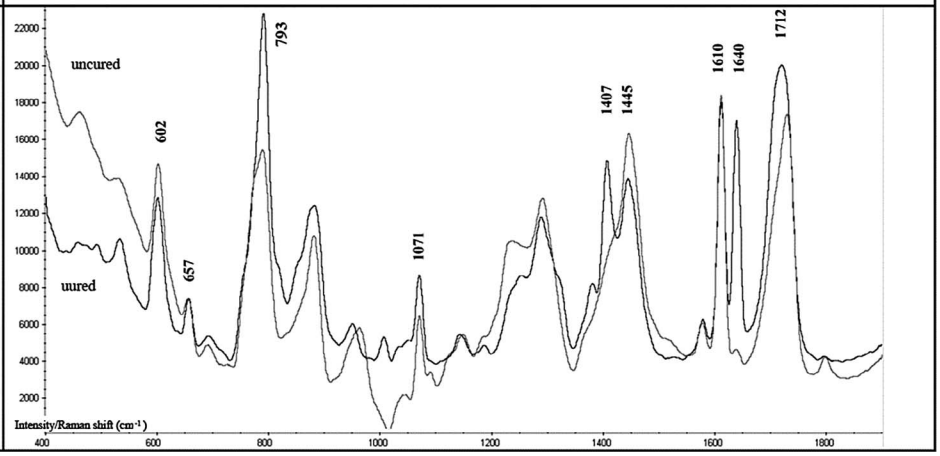
Adhesive system	Composition	Application mode	Raman spectrum
Group 2: I-BOND	Acetone, Water, Methacrylates, 4-META, UDMA, Photo initiator	Apply to dentin and agitate slightly for 20 s. Air-dry at least for 5 s. Light cure for 20 s.	

Figure 2. *Composition, application mode (as recommended by the respective manufacturer), and Raman spectra of cured and uncured I-BOND. The C=C peak decreased after polymerization. Refer to Table 1 for other peaks.*

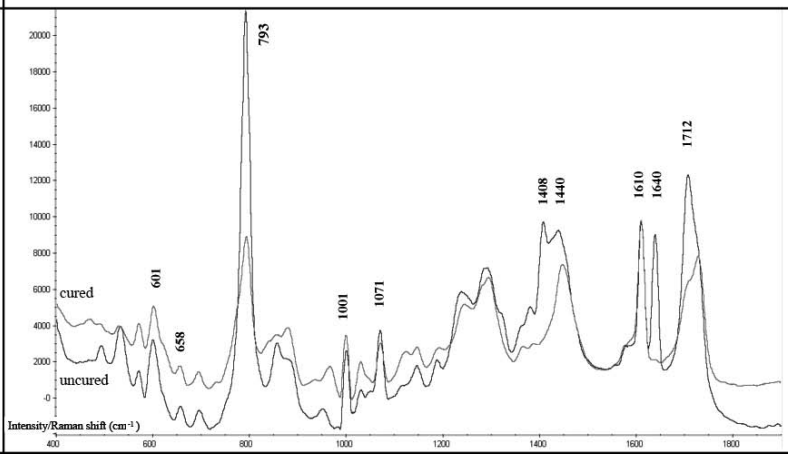
Adhesive system	Composition	Application mode	Raman spectrum
Group 3: G-BOND	Acetone, Distilled water, 4-META, UDMA, TEGDMA, Dimethacrylate component, Phosphoric ester monomer	Brush, apply one coat and wait 5 s Thin with high-pressure syringe air. Light cure for 10 s.	

Figure 3. *Composition, application mode (as recommended by the respective manufacturer), and Raman spectra of cured and uncured G-BOND. The decrease in the reaction peak is clearly visible in the spectrum of the cured adhesive. Refer to Table 1 for other peaks.*

a CCD detector attached to a modular research spectrograph (Renishaw InVia; Renishaw PLC, New Mills, Wotton-under-Edge, Gloucestershire, UK). The 100× objective increased the precision of the beam, resulting in a laser spot size of $\leq$1 μm. A monochromatic, near-infrared diode 785-nm laser was used to induce the Raman scattering effect. The spectral range of this model was from 100 to 3450 cm^{-1}, with an average spectral resolution of 5 cm^{-1}. A wavelength and intensity calibration was performed with a silicon standard using the calibration system integrated with the software (WiRE 2.0; Renishaw PLC) before each experiment following the manufacturer's specifications.

The dentin slabs were analyzed by acquiring spectra in three line-scans for each slab, starting in the sound mineralized dentin and ending in the adhesive resin layer. One Raman spectrum was collected every 1 μm along the dentin-adhesive interface in the intertubular region clearly detected by the microscope camera (laser wavelength" 785 nm; Renishaw InVia; Renishaw PLC) using a computer-controlled motorized *x-y-z* stage, with an exposure time of 40 seconds and a laser power on the

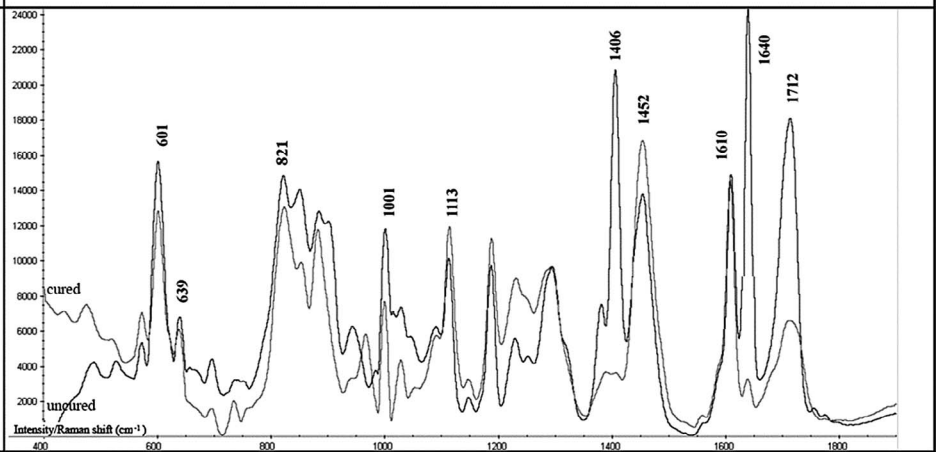
Adhesive system	Composition	Application mode	Raman spectrum
Group 4: ADPER EASY BOND	HEMA, Bis-GMA, Methacrylated phosphoric esters, 1,6 hexanediol dimethacrylate, Methacrylate functionalized, Polyalkenoic acid (VitrebondTM Copolymer), 7 nm silica filler, Ethanol, Water, Camphorquinone based initiators, Stabilizers	Apply to dentin and rub for 20 s. Gentle air-dry for 5 s. Light cure for 10 s.	

Figure 4. *Composition, application mode (as recommended by the respective manufacturer), and Raman spectra of cured and uncured Adper Easy Bond. The decrease in the C=C peak at 1640 cm^{-1} is visible in the cured adhesive. Refer to Table 1 for other peaks.*

Table 1: *Attribution of the Peaks of Interest to the Corresponding Functional Group*[21]

Functional Group	Range Signal, cm^{-1}
O-C=O bending	590-700
C-O-H bending	620-680
Mono-substituted benzene	690-800
Para-substituted benzene	800-850
P-O-C antisymmetrical stretch	915-1055
C-O-C antisymmetrical stretch	1070-1280
OH in-plane bending (acid)	1400-1440
CH_2 scissors vibration CH_3 antisymmetrical deformation	1440-1475
C=C aromatic ring stretch	1590-1615
C=C aliphatic stretch	1630-1680
C=O carbonyl ester stretch	1650-1870

specimen of ~8 mW. Data were acquired over the spectral region from 400 to 1900 cm^{-1}. They were analyzed with software developed for spectrographic analysis (Grams/AI 7.02; Thermo Galactic Industries, Salem, NH, USA).

Hybrid Layer Detection and Conversion Calculation

The spectra were acquired, starting from the dentin, and the appearance of peaks associated with the adhesive and dentin components indicated the beginning of the hybrid layer. This allowed investigation of the impregnation pattern of the adhesives into the dentin structure. To detect the hybrid layer within the adhesive interface the relative intensities of bands associated with mineral dentin components (the PO_4^{3-} functional group at 960 cm^{-1}) and adhesive (vinyl C=C at 1640 cm^{-1} and phenyl C=C at 1610 cm^{-1}) were identified (Table 1).[21] The major peak at 960 cm^{-1} is associated with the mineral PO_4^{3-} group, while the bands at 1245 (C–N), 1450 (CH_2), and 1667 cm^{-1} (C=O) are associated with the dentin organic components[19] (Table 1).[21] Because mild and ultramild self-etch adhesives demineralize dentin only superficially and create a very thin hybrid layer,[22,23] only the first two spectra (2 μm) in which the mineral and the adhesive coexisted were considered as the hybrid layer (ie Figure 5a, spectra at micron 6). Even when both of these spectra were

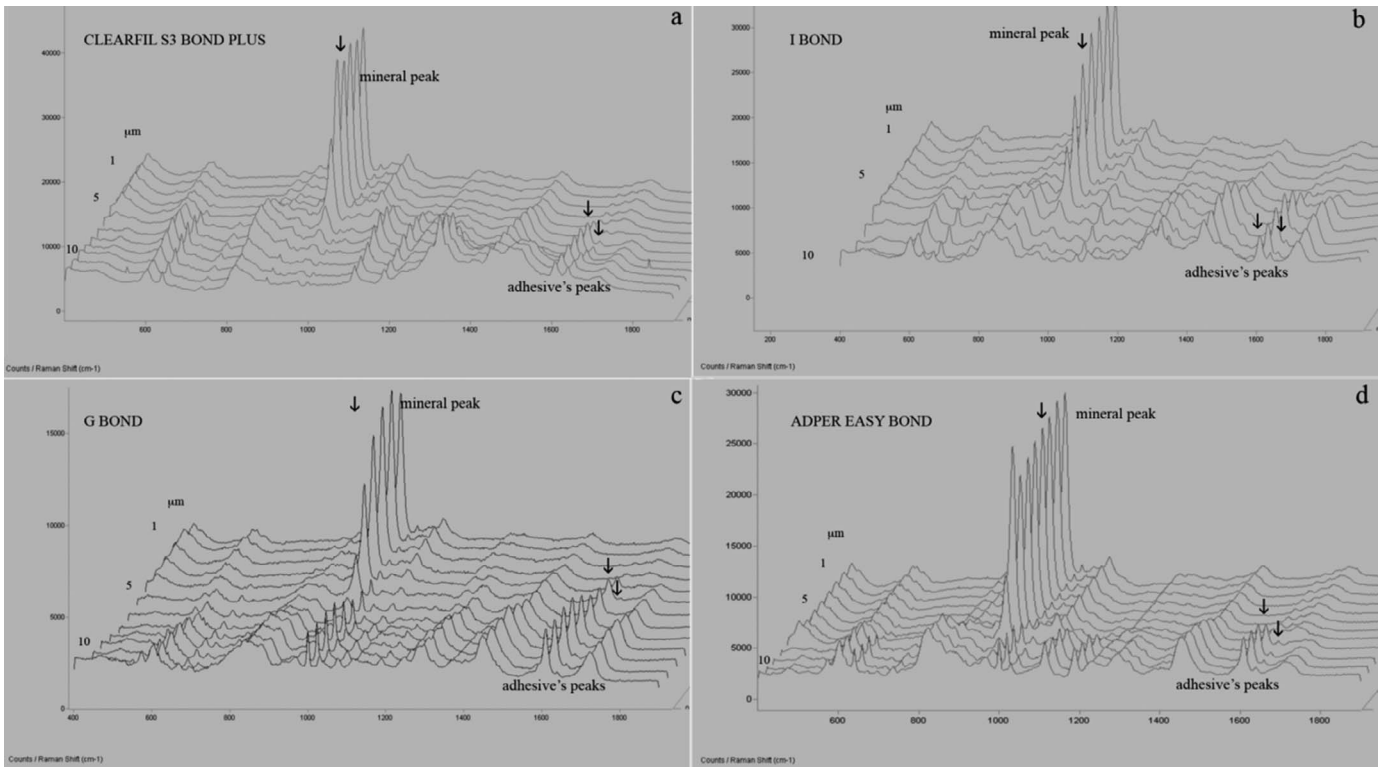


Figure 5. *(a and c) Spectra 1-5 were in mineralized dentin, spectrum 6 was taken along the hybrid layer, and spectra 7-12 were representative of the adhesive layer. (b) Spectra 1-6 were in mineralized dentin, spectrum 7 was taken along the hybrid layer, and from spectra 8 they were in the adhesive layer. (d) Spectra 1-8 were in mineralized dentin, spectrum 9 was taken along the hybrid layer, and the last two were representative of the adhesive layer.*

Table 2: *Degree of Conversion (DC) of the Adhesives Tested**

Adhesive System	DC, %
Cleafil S^3 Bond Plus	80 ± 14^{a}
I-BOND	89 ± 7^{b}
G-BOND	93 ± 6^{c}
Adper Easy Bond	92 ± 6^{bc}

*Means of DC followed by the same superscript letter indicate no statistical difference at the 95% confidence level ($p<0.05$).

still present, more superficial spectra were considered to represent the adhesive layer only, in which the demineralized buffered mineral might be dispersed. Raman spectra of each uncured adhesive were also collected to identify the reference and reaction peaks needed for conversion calculations, and these spectra were compared with the spectra of cured adhesive on dentin (Figures 1 through 4). The phenyl C=C peak at 1610 cm^{-1}, which was stable and unmodified during polymerization, was selected as a reference peak, while the vinyl C=C 1640 cm^{-1} peak was selected as a reaction peak. The DC of the adhesive within the hybrid layer was calculated using the ratio between the reaction (A_{rxn}) and the internal reference (A_{ref}) peak areas, as follows:

$$\%conversion = \left\{1 - \frac{[A_{rnx}(p)/A_{ref}(p)]}{[A_{rnx}(u)/A_{ref}(u)]}\right\} \times 100,$$

where u refers to unpolymerized and p to the polymerized adhesive system, respectively.

Statistical Analysis

The data were checked for normality and equal variances using Kolmogorov-Smirnov and Bartlett tests. As data were not normally distributed, they were analyzed using the nonparametric Kruskal-Wallis test for multiple comparisons. The Mann-Whitney U-test was used for pairwise comparison between the groups. Statistical significance was set at $p < 0.05$.

RESULTS

When comparing the uncured vs cured Raman spectra of the tested adhesives, the C=C peak clearly decreased after polymerization in each adhesive system (Figures 1 through 4). The vinyl signal, set as reference peak, was originated from the bisphenol A diglycidyl ether dimethacryalate (Bis-GMA) resin component for Clearfil S^3 Bond Plus and Adper Easy Bond, and the 4-methacryloxyethyltrimellitic acid anhydride (4-META) in G-BOND and I-BOND (Figures 1 through 4). As expected, the OH acidic bending group peak of the acidic monomers also disappeared in the polymerized specimens for all of the adhesives because it was buffered by the mineral components of dentin. The OH acidic bending group was clearly visible in the unpolymerized G-BOND and I-BOND spectra, where it was originated with the opening of the anhydride ring of the 4-META monomer because of the instability of 4-META in a wet environment. Generally, all specimens showed that the hydroxyapatite peak at 960 cm^{-1} decreased in intensity from dentin to adhesive. Representative Raman spectra acquired in line-scans across the adhesive interface are shown in Figure 5. Overlapping spectra acquired in the same region demonstrated the technique's reproducibility.

Means and standard deviations (SDs) of DC percentages and statistical differences ($p<0.05$) among adhesives are reported in Table 2.

The DC ranked as follows: G-BOND (93% ±6%) ≥ Adper Easy Bond (92%±6%) ≥ I-BOND (89%±7%) > Clearfil S^3 Bond Plus (80%±14%).

DISCUSSION

The present study based on micro-Raman spectroscopic data presented detailed information on the monomer DC at the adhesive/dentin interface of four one-step self-etch adhesives when applied on human dentin, even though a limiting factor to a complete analysis of the interaction between the chemical components of these materials and dentin is that the real composition of commercial materials is not disclosed by manufacturers.

Among different spectroscopic techniques, micro-Raman spectroscopy is the most appropriate to use to study the adhesive/dentin interface from the chemical point of view because it is not negatively influenced by the presence of water, does not require complicated specimen preparation (which does not affect the tested specimen), and offers detailed chemical information (ie, the double-bond converted percentage *in situ* within the hybrid layer).[10,20,24]

The hypothesis tested—that no differences in terms of DC would be found among the adhesives systems—was rejected because statistically significant differences were found between the DCs of the adhesives tested. Self-etching adhesives have been classified according to their pH as strong (pH≤1), intermediate (pH~1.5), and mild (pH~2).[25] I-BOND and G-BOND can be defined as mild self-etch adhesives because their reported pH is ~2,[26] as was the case with Clearfil S^3 Bond Plus, the pH of

which was measured in our lab, while Adper Easy Bond can be described as ultramild because its stated pH is slightly higher (~2.7).[27] Mild self-etch adhesives demineralize dentin only superficially and create a submicron hybrid layer,[22] as do the the ultramild self-etch adhesives[23]; on the contrary, strong self-etch adhesives demineralize dentin comparably to etch-and-rinse adhesives.[2,28] The different composition of the tested materials, as shown in Figures 1 through 4, likely influenced the results. Clearfil S^3 Bond Plus showed the lowest DC values and the highest standard deviation. It was the only adhesive tested that contains methacryloyloxydecyl dihydrogen phosphate (MDP), a monomer capable of forming strong ionic bonds with the hydroxyapatite's calcium.[29] MDP is a quite hydrophobic etching monomer, with a long carbonyl chain.[30] The steric hindrance of the carbonyl chain could hamper the conversion of the vinyl groups in the aliphatic bond. Another hypothesis is that the monomer mixture of Clearfil S^3 Bond Plus promotes phase separation between the domains rich in hydrophilic monomers (2-hydroxyethyl methacrylate [HEMA]) and those rich in hydrophobic monomers (Bis-GMA) that do not polymerize properly with camphorquinone.[31,32] Adper Easy Bond also contains Bis-GMA and HEMA and showed a higher DC. Recent studies[33] demonstrated that Adper Easy Bond showed good microtensile bond strength values when compared with other self-etch adhesives. DC is not the only factor contributing to the longevity of dental restorations: despite its inferior polymerization results (as recorded in this study), in previous investigations[34] Clearfil S^3 Bond Plus showed an improved bond strength when compared with two contemporary one-step self-etch adhesives. Other factors, such as the nature of the chemical interaction between the MDP contained in Clearfil S^3 Bond Plus and the dentin substrate, could have positively influenced its bond strength.

El-Damanhoury and Gaintantzopoulou[35] recently reported lower DC values for both I-BOND and Adper Easy Bond) than were reported for this study, while they confirmed the results contained herein for Clearfil S^3 Bond Plus. These controversial results can be attributed to the different specimen treatments and study setup. In that study, the DC of adhesives applied on dentin disks was calculated by attenuated total reflectance FTIR after removing the oxygen-inhibited layer with acetone and focusing on the adhesive surface (ie, the DC was assayed in the adhesive layer). Conversely, the micro-Raman technique used in the present investigation allowed assessment not only of the DC on the specimen surface but also the assessment of DC micron by micron across the hybrid layer, in which different measurements were obtained since the acidic monomers contained in the self-etch adhesives are neutralized by the dentin calcium ions. Indeed, it is well known that the chemical interaction between acidic monomers and dentin mineral in self-etch adhesives is crucial for an adequate polymerization, as residual acidic monomers might compromise the polymerization, and thus the DC, of the adhesive.

Both I-BOND and G-BOND contain 4-META, which is an acidic monomer with an aromatic group that is soluble in acetone.[26] 4-META is used both as an adhesion-promoting monomer,[36] because it is able to establish an ionic bond with calcium in hydroxyapatite, and as a demineralizing monomer. As the composition of these two adhesives is only partially disclosed by their manufacturers, we referred to the study of Van Landuyt and others,[26] who reported that both I-BOND and G-BOND contain urethane dimethacrylate (UDMA), while G-BOND also includes triethylene glycol dimethacrylate (TEGDMA) in its composition. It could be speculated that G-BOND contain less UDMA than I-BOND because of the presence of TEGDMA and that perhaps the long C-C chain of UDMA (PM: 470.56) interfered with the polymerization of the vinyl groups more than did TEGDMA (PM: 286.32).

A low percentage of monomer-to-polymer conversion is related to low physicochemical properties of adhesives[11,12] in resins with lower degrees of conversion, higher elution of monomers occurs,[11,37] negatively affecting the longevity of the bond.[11,12] Thus, *in situ* analysis of the DC is an important factor in predicting adhesive performance *in vivo*.

Previous studies[18,38] demonstrated that simplified adhesives such as one-step self-etch adhesives exhibit a lower DC and higher adhesive permeability than do the two-step self-etch as a result of the higher presence of hydrophilic monomers and solvents. Solvents are included to remove intrinsic water from the wet dentin matrix, promoting adhesive impregnation of the demineralized dentin.[39] If residual water and solvents are trapped within the adhesive layer, this can result in low polymerization,[40] rendering residual unreacted monomers more prone to leaching.[41,42] A previous study[43] demonstrated that high residual ethanol percentages may compromise the polymerization of dental adhesives as a result of residual solvent entrapped within the polymer network.

Clearfil S^3 Bond Plus and Adper Easy Bond contain ethanol and water as solvents, while I-BOND and G-BOND are acetone-based adhesives (Figures 1 through 4). Thus, the type of organic solvent used may also have influenced the differences in DC of the tested adhesives. Nevertheless, no correlation between solvent type and DC was found in this study, as both the adhesive with the lowest DC (Clearfil S^3 Bond Plus) and that with the highest DC (Adper Easy Bond) contained ethanol and water. The ability of Adper Easy Bond to efficiently cure confirm the previously published reports.[44,45] This might be explained by the different type of photoinitiators blended within the adhesive system, which is undisclosed by manufacturers. It has been shown that the photoinitiating system should be selected in relation to adhesive system composition, especially the presence of hydrophilic elements. Water-compatible photoinitiators are recommended in highly hydrophilic adhesive formulations to guarantee an appropriate DC of the adhesive layer and to diminish aging phenomena.[31] Recently, photoinitiators such as TPO [ethyl 4-dimethylaminobenzoate and diphenyl(2,4,6-trimethylbenzoyl)-phosphine oxide] have become popular, and their inclusion in new adhesive formulations (even if not disclosed by manufacturers) might also explain the higher DC of the adhesives tested in this study compared to previously marketed one-step self-etch adhesives.

CONCLUSIONS

Within the limitations of this study, the results showed that the DC, which is one of the factors contributing to the longevity of an adhesive, varied among the tested self-etch adhesives and was material dependent. Further studies are necessary to evaluate whether the DC of the tested materials can be correlated to their stability over time and to their bond strength performance.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Department of Medical Sciences, the University of Trieste, Italy.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 28 September 2015)

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