EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

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MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

REVIEWING PEAK SELECTION IN VIBRATIONAL SPECTROSCOPY (FT-IR AND MICRO-RAMAN SPECTROSCOPY) FOR THE POLYMERISATION KINETICS OF DENTAL ADHESIVES: A LABORATORY STUDY

Trabalho submetido por
Íris Manuela Meira Santos Guimarães Capela
para a obtenção do grau de Mestre em Medicina Dentária

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Resumo

Objetivos: Avaliar a precisão e reprodutibilidade da taxa de conversão (D_C %) de dois adesivos dentários, utilizando diferentes pares de picos na técnica de espectroscopia de refletância total atenuada, em espectroscopia de infra-vermelhos (ATR-FTIR).

Materiais e métodos: Foram utilizados dois adesivos comerciais: Scotchbond™ Universal Plus e Optibond™ Solo Plus. Cada material foi testado em três replicações. O protocolo experimental incluiu aplicação do adesivo sobre um cristal de diamante (ATR), secagem com secador (20 s), e fotopolimerização por 15 s, durante uma aquisição espectral contínua. Três pares de picos vibracionais foram comparados: 1320-1340 cm^{-1}, 1610-1640 c m^{-1} e 1635-1648 cm^{-1}. A taxa de conversão (D_C%) foi calculada com base na variação da absorbância ao longo do tempo. A análise estatística foi realizada com testes de Levene (Brown–Forsythe) e modelos lineares mistos (LMM), com significância estabelecida em $\alpha = 0,05$.

Resultados: O par de picos 1320–1340 cm^{-1} apresentou os valores mais consistentes de D_C % para ambos os adesivos 81.1% e 80.3%, com baixa variabilidade (DP ≤ 2,7%). O par 1635–1648 cm^{-1} teve desempenho semelhante. Em contrapartida, o par convencional 1610–1640 cm^{-1} resultou em valores inflacionados e errados, especialmente em Optibond™ Solo Plus (D_C > 150%), indicando que existem interferências espectrais significativas. A análise de variância (ANOVA) confirmou diferenças estatísticas significativas entre os pares de picos ($p < 0,05$), sendo o par 1320–1340 cm^{-1} o mais estável e preciso.

Conclusão: A escolha do par de picos influencia diretamente a precisão da quantificação do D_C% em adesivos dentários. O pico em 1320 cm^{-1} parece demonstrar maior robustez e menor suscetibilidade a interferências, sugerindo ser uma alternativa viável ao tradicional 1640 cm^{-1} em protocolos de análise por ATR-FTIR.

Palavras-chave: taxa de conversão, adesivos dentários, espectroscopia FTIR, metacrilatos, picos vibracionais.

Abstract

Objectives: To evaluate the accuracy and reproducibility of the degree of conversion (D_C %) of two dental adhesives using different peak pairs in the attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) technique.

Materials and Methods: Two commercial adhesives were used: Scotchbond™ Universal Plus and Optibond™ Solo Plus. Each material was tested in three replicates. The experimental protocol included application of the adhesive onto a diamond crystal (ATR), drying with an air blower (20 s), and light-curing for 15 s during continuous spectral acquisition. Three pairs of vibrational peaks were compared: 1320–1340 cm^{-1}, 1610–1640 cm^{-1}, and 1635–1648 cm^{-1}. The degree of conversion (D_C %) was calculated based on the change in absorbance over time. Statistical analysis was performed using Levene's test (Brown–Forsythe) and linear mixed models (LMM), with significance set at $\alpha = 0.05$.

Results: The 1320–1340 cm^{-1} peak pair yielded the most consistent D_C % values, 81.1 % and 80.3 %, with low variability (SD ≤ 2.7%) for both adhesives. The 1635–1648 cm^{-1} pair showed similar performance. In contrast, the conventional 1610–1640 cm^{-1} pair produced inflated and inconsistent values, particularly in Optibond™ Solo Plus (D_C > 150%), indicating significant spectral interference. ANOVA confirmed statistically significant differences between peak pairs ($p < 0.05$), with the 1320–1340 cm^{-1} pair being the most stable and precise.

Conclusion: The choice of spectral peak pair significantly affects the accuracy of D_C quantification in dental adhesives. The 1320 cm^{-1} region appears more robust and less prone to spectral interference, suggesting it as a potentially more reliable alternative to the traditional 1640 cm^{-1} region in ATR-FTIR protocols.

Keywords: degree of conversion, dental adhesives, FTIR spectroscopy, methacrylates, vibrational peaks.

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List of Abbreviations

10-MDP – 10-methacryloyloxydecyl dihydrogen phosphate

4-META – 4-methacryloxyethyl trimellitate anhydride

ATR – Attenuated Total Reflectance

ATR-FTIR – Attenuated total reflectance/Fourier transform infrared spectroscopy

Bis-GMA – Bisphenol A-glycidyl methacrylate

CV – Coefficient of variation

D_C – Degree of conversion

E&R – Etch-and-rinse

FTIR – Fourier transform infrared spectroscopy

GPDM – Glycerophosphate dimethacrylate

HEMA – 2-hydroxyethyl methacrylate

HL – Hybrid layer

IR – Infrared light

LCU – Light curing unit

LMM – Linear-mixed effects model

PPGDMA – Polypropylene glycol dimethacrylate

SD – Standard deviation

SE – Self-etch

TEGDMA – Triethylene glycol dimethacrylate

UDMA – Urethane dimethacrylate

I. INTRODUCTION

1.1. Dental adhesive systems

Understanding the interaction between mineralized tooth tissues and resin-based materials is fundamental for the advancement of dental adhesive systems. Dental adhesive systems create an interface between mineralized tooth tissues (i.e., enamel and dentin) and resin-based materials, through etching and infiltration processes that are succeeded by a free-radical addition polymerization reaction that cures the material *in situ* [1]. As the monomers react and collide, the material hardens and forms a dense polymer network [2,3]. The polymerization of dental adhesives and its quality is widely researched due to its clinical implications [1]. The efficiency of the reaction is a key determinant in bonding properties and material longevity [4]. Adequate resin polymerization is a result of adhesive bond stability and resistance to polymer degradation [5]. Although polymerization is extensively studied, the methods and techniques used to characterize its efficiency are currently varied and prone to errors [3,6]. Therefore, a clear need for a comprehensive study that critically analyzes current protocols, identifies their limitations, and proposes standardized guidelines for the accurate monitoring of polymerization efficiency in dental adhesives, in order to improve both the scientific assessment and clinical performance of these materials.

1.2. Methacrylate monomer matrices

To further understand the challenges of polymerization, it is crucial to explore the chemical composition of dental adhesives and the role of their constituent monomers. Dental adhesives are a necessary link for the bond between the resin composite and the tooth surface [5,7]. Regardless of their characterization, their composition is similar, containing a co-polymer of methacrylate monomers, solvents (water, ethanol, and acetone are commonly used to improve monomer flow and promote dentin infiltration), filler particles (most of the commercial adhesives, although not all), light initiators and stabilizers [4,7,8]. Monomers are the primary component of dental adhesives and can be classified as crosslinking monomers, diluent monomers, or specialized monomers such as acidic functional monomers (Figure 1) [9]. Bisphenol A-glycidyl methacrylate (Bis-GMA) and urethane dimethacrylate (UDMA) are widely used crosslinkers, contributing to the mechanical strength of the adhesive system by forming densely cross-linked polymer networks [3,8]. Triethylene

glycol dimethacrylate (TEGDMA) and polypropylene glycol dimethacrylate (PPGDMA) are typical diluent monomers, with lower viscosity than Bis-GMA, improving handling and promoting resin diffusion [3,8,10]. Hydroxyethyl methacrylate (HEMA), also a diluent, enhances surface wetting and stabilizes solutions by allowing the mixing of hydrophilic and hydrophobic components [11].

In contrast to monomethacrylate monomers, most crosslinking and diluent monomers are characterized by hydrophobicity, making them only marginally soluble in water and reducing water absorption after polymerization [12]. Examples of acidic functional monomers include 4-methacryloxyethyl trimellitate anhydride (4-META), glycerophosphate dimethacrylate (GPDM), and 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP). These facilitate bonding to mineralized substrates via ionic interaction with calcium in hydroxyapatite [8,9,12]. An overview of their representative chemical structures is shown in (Fig. 2), highlighting the molecular diversity that underlies their distinct physicochemical roles within the adhesive formulation [13].

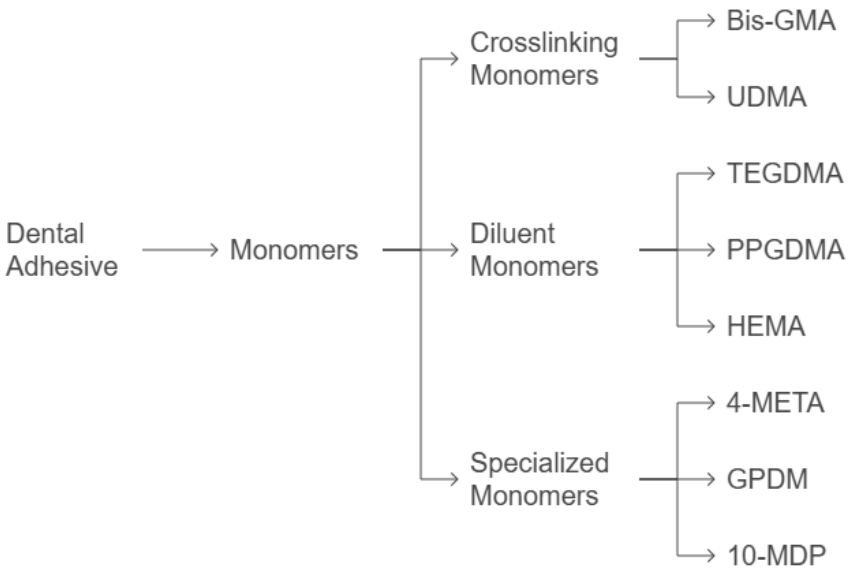

Figure 1. Schematic classification of the main types of monomers found in dental adhesive systems. Monomers are classified into crosslinking monomers, diluent monomers, and specialized functional monomers.

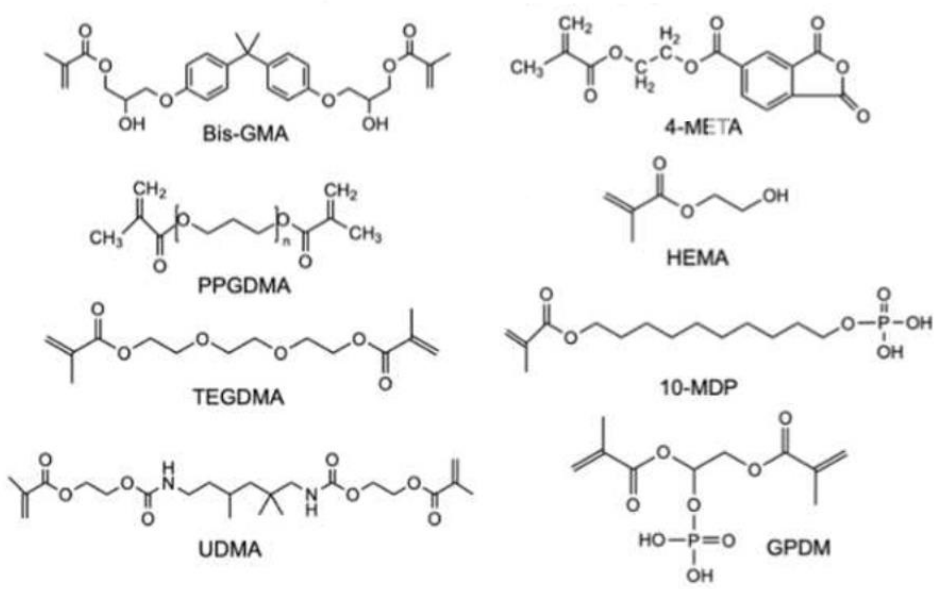

Figure 2. Chemical structures of commonly used monomers in dental adhesive formulations. Adapted from Delgado (2021).

The collision and propagation of methacrylate monomers due to free-radicals and active center formation is what causes chain-growth polymerization reaction [3]. During this process, a conversion occurs in the carbon double-bond [C=C] occurs, allowing monomers to initially link in a linear arrangement before progressing to cross-linking if they contain more than one methacrylate group (e.g., dimethacrylates or trimethacrylates). At the onset of polymerization, monomers remain very mobile, while the monomeric matrix is in a liquid phase [3]. With progression of the reaction, molecular movement becomes restricted, with the material gradually solidifying and hardening as larger molecular structures develop, eventually reaching a stage where—known as the gel point [3,14]. A well-executed polymerization reaction leads to the development of a densely cross-linked polymer network, ensuring the material attains its desired mechanical and functional characteristics [3,12].

1.3. Solvent evaporation and polymerization

Achieving effective polymerization is not solely dependent on monomer chemistry but also heavily influenced by other factors, namely solvent management during adhesive application. To fully comprehend these interactions, it is crucial to first

understand the underlying chemistry of dental adhesives. Adhesive systems can be classified as two classical strategies, either etch-and-rinse (E&R) or self-etch (SE) and, also, recently, as all-in-one systems with an acid-etching arbitrary step, named universal or multi-mode systems [7,9,15]. This classification is related to the interaction that adhesives have with the smear layer [5,7,16]. The smear layer is a layer of debris formed on dentine surfaces during mechanical instrumentation, composed of inorganic particles, collagen, saliva, microorganisms, and remnants of pulpal tissue [17–19]. Its presence can significantly influence the effectiveness of adhesive procedures in restorative dentistry [5,12,20]. In E&R, phosphoric acid is used to completely remove the smear layer, exposing the collagen matrix and facilitating monomer infiltration to form a hybrid layer (HL) [16]. In contrast, SE partially dissolve and incorporate the smear layer into the bonding interface, as their acidic monomers etch and infiltrate simultaneously (3).

The thickness and composition of the smear layer can hinder resin infiltration, particularly in self-adhesive materials, where no prior etching is performed. Although total removal is not always required, partial dissolution is recommended to enhance monomer interaction with the dentine substrate and improve bond strength [3,5]. Adhesive systems can be grouped by the number of steps involved (3-step or 2-step E&R, 2-step or 1-step SE and arbitrary universal) (Figure 3) [5,7,21]. The acid etching step removes the smear layer, conditioning the tooth surface. The primer is a blend of resin monomers dispersed in a solvent, that is usually water, acetone or ethanol or a co-mixture [4,7]. Upon application, the primer permeates the collagen network, with the solvent aiding in the displacement of water. This process enables the monomers to infiltrate the dentin while maintaining the expansion of the collagen matrix, a phenomenon known as hybridization [3]. During the polymerization process, a portion of the water within the collagen matrix is displaced, allowing monomers to occupy the vacated spaces and undergo polymerization [3,16]. In ideal conditions, the mineral loss in demineralized dentin after etching should be substituted with an equivalent amount of monomers [3].

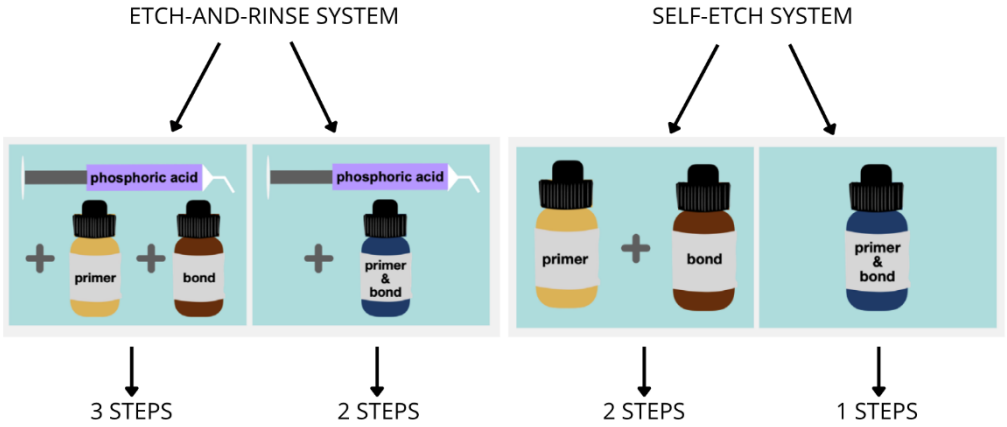

Figure 3. Classic adhesive strategies and their subdivision - etch-and-rinse can involve 3 or 2 steps, due to requirement of an acid etching step, whereas a self-etch strategy has 2 or 1 steps only, owing to the primer or primer & bond combination being acidic.

Solvents play a critical role in the formulation of adhesive systems, as they facilitate the dissolution of monomers, contributing to the low viscosity of the primer and enhancement of its wettability in dentin [12,22]. Thus, the incorporation of solvents is essential in adhesive formulations that require bonding to dentin [9,12]. The inherently moist nature of dentin permits effective wetting only when a hydrophilic bonding agent is utilized [23]. To sum up, solvents serve different purposes: they rewet and expand the demineralized collagen network and also act as a vehicle for the monomer that will come into contact with a water-rich environment, which in turn promotes hybridization [3,16]. Effectively managing moisture in dentin is highly challenging yet essential for achieving an optimal bonding strategy [16]. Excessive drying of dentin can lead to the collapse of the collagen network exposed by acidic demineralization [3,4]. Conversely, excessive moisture can lead to the solubilization of the monomeric adhesive mixture, thereby hindering polymerization efficiency and compromising the strength of the HL [3,9]. If the resin undergoes solubilization, it will evidently result in the exposure of collagen fibrils, leading to the disruption of the HL. Polymerization is thus influenced by multiple factors, one of which is the quantity of water and/or solvent retained within the collagen matrix [3].

1.4. Polymerization affects bonding outcomes

Given the interplay of these chemical and physical factors, it becomes evident that polymerization outcomes are fundamental to adhesive bond strength and long-term success. As said before, polymerization is a critical determinant in the success of adhesive bonding to tooth substrates, as it directly influences the formation and stability of the HL in dentin, which is essential for long-term bonding performance [4]. The HL, formed by the infiltration of adhesive monomers into the demineralized collagen matrix, is widely recognized as the weakest link in adhesive interfaces [6,24]. Its integrity and mechanical stability depend on a properly polymerized resin network that resists degradation over time [23]. The quality and extent of polymerization are often compromised by factors such as residual solvents, improper polymerization protocols, phase separation, and excess moisture which interfere with the polymerization kinetics [4,12,25].

Polymerization efficiency parameters, such as the degree of conversion (D_C) — the proportion of monomers that have been successfully converted into polymer chains during the polymerization process — are paramount in adhesive dentistry [26]. Expressed as a percentage, D_C% is a key parameter influencing the mechanical, chemical, and biological performance of restorative materials [7,9,27]. This comparison allows for the quantification of the percentage of reactive bonds that have been incorporated into the final polymer network [9,12,27]. A low D_C% leads to a poorly formed polymer network, making the HL more susceptible to hydrolytic degradation, nanoleakage, and eventual bond failure [28,29]. Moreover, the presence of water and solvents in adhesives, although necessary for proper dentin infiltration and collagen matrix expansion, can hinder polymerization if not adequately evaporated prior to light-curing, reducing the mechanical properties of the final polymer network [4,6,25]. Inadequate polymerization within the HL affects not only the initial bond strength but also the long-term durability of adhesive restorations, as unreacted monomers can leach out, compromising biocompatibility and mechanical stability [12,28]. Thus, standardized and reliable protocols to assess polymerization and ensure a sufficient D_C are necessary to improve the performance and durability of adhesive interfaces. Clear guidelines on how to monitor polymerization effectively would help

to reduce variability in outcomes and enhance the clinical success of adhesive restorations by ensuring stable HL formation and long-term bond integrity [3].

1.5. Vibrational spectroscopy for the study of free-radical addition polymerization

1.5.1. ATR-FTIR to monitor methacrylate polymerization

Vibrational spectroscopy techniques in analytical chemistry, such as Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy have established themselves as fundamental techniques, opening new research possibilities in the field of dental materials [4]. FTIR has been widely used for the chemical characterization of mineralized tissues in recent decades, applied in material characterization studies and in the analysis of polymerization kinetics, as it examines molecular vibrations [3,4,6,9,30,31]. Atoms within molecules are not stationary; rather, they undergo vibrations in well-defined patterns known as vibrational patterns. FTIR utilizes infrared light (IR) to interact with matter [7], inducing a net change in the dipole moment during the vibration of a molecule or its functional groups [3,6,7,30]. These functional groups absorb IR light at specific wavenumbers or frequencies, which are determined by the physicochemical properties of the corresponding molecule [3,6,7,9]. As a result, FTIR serves as a powerful tool for material characterization, particularly in the analysis of organic substances such as polymers. In the field of dentistry, this technique is recognized as the key approach for investigating the polymerization kinetics of materials used in dental treatments, especially when combined with Attenuated Total Reflectance (ATR) accessories [6,30]. ATR can be employed to obtain infrared (IR) spectra. In this method, the sample is placed in direct contact with a high-refractive-index crystal, typically diamond, due to its optical properties and durability. An IR light beam is directed towards the crystal, where it undergoes internal reflection. During this process, an evanescent wave extends beyond the crystal surface and penetrates the sample. If the sample absorbs energy within the IR region, the intensity of the reflected beam is attenuated. The modified beam is then redirected within the crystal and subsequently detected by the instrument's sensor for spectral analysis [3].

Additionally, FTIR plays a crucial role in assessing compositional changes in polymer chemistry, providing insights into how these materials interact with dental

substrates [6,32]. Using ATR-FTIR sample preparation and analytical method is a straightforward, rapid, and accessible technique that enables the acquisition of spectra at various stages of the light-curing process—prior to, during, and following polymerization. This method allows *in situ* curing without compromising the accuracy of spectral measurements [6]. It has been extensively employed to examine bonding systems, evaluate final monomer conversion rates, and assess the quality of the HL in adhesive interfaces [9].

1.5.2. Micro-Raman to monitor methacrylate polymerization

Building upon the established capabilities of FTIR, Raman spectroscopy has emerged as a complementary technique that provides unique advantages in specific applications. Although FTIR spectroscopy is traditionally used to assess the D_C%, Raman spectroscopy offers a simpler and more versatile alternative in certain experimental projects, as it requires minimal sample preparation, is not affected by water interference, and allows non-destructive, repeated measurements on the same specimen - although is more time consuming and has a bigger learning curve [4,6,7,27,33]. Raman is also widely used in the study of adhesive dentistry since it helps understand the different interactions that monomers have with dentin within the HL, in a non-destructive way, allowing multiple measurements on the same sample [5,27,30]. In addition, there are no specific requirements for processing the sample [27,29]. A laser beam is incident upon the sample, inducing molecular excitation and prompting a transition to a higher vibrational energy state. Upon relaxation to the ground state, the molecules emit photons with energy levels that differ from those of the incident photons [27]. Analogous to FTIR, the vibrational energy states of chemical bonds and distinct functional groups exhibit characteristic energy levels, corresponding to specific spectral distributions of the emitted photons [3,27,30,33]. Raman spectroscopy is based on the inelastic scattering of photons, where the energy shift in the scattered light corresponds to molecular vibrational transitions, whereas FTIR spectroscopy characterizes molecular structures by detecting the absorption of incident radiation at specific wavelengths, while both allow identification of a specific molecule in a particular spectral regions [3,27]. Both spectroscopy methods have been used to study the D_C, yielding similar results [5,27].

1.6. Research background of ATR-FTIR/Raman in dental polymerization: problems and limitations in recent studies

While both FTIR and Raman spectroscopy offer valuable insights, it is important to explore the limitations and challenges encountered in recent studies to improve the accuracy of these methods. As previously stated, distinct functional groups exhibit characteristic absorption bands within the IR spectrum, which correspond to their fundamental vibrational modes [7,30]. When evaluating the conversion rate, the co-polymerization reaction is typically assessed in dental literature using the standard absorption peak at 1640 cm^{-1}, corresponding to the vibrational mode of the carbon-carbon double bond [v(C=C)] found in monomers containing methacrylate groups [27,29,33,34]. This approach has been undertaken in several studies, as shown below in Table 1 [35–39].

Table 1. Summary of studies using the 1640 cm^{-1} peak: Material tested, FTIR and Raman peaks used, degree of conversion (D_C %) reported, and whether statistically significant differences were observed.

Study	Adhesives studied	FTIR/Raman peaks	D_C % reported	Statistical significance
Rifane et al. 2023 [36]	Ambar Universal with silanized/non-silanized 45S5 or Sr-45S5 bioglass	Micro-Raman: **1639 cm^{-1}** 1609 cm^{-1}	~55 to 60%	Yes (p < 0.05)
Yang et al. 2023 [37]	Synthesized UMP monomers	FTIR: **1638 cm^{-1}** 1608 cm^{-1}	From ~ 44 to 54%	Yes (p < 0.05)
He et al. 2023 [38]	Experimental adhesive	FTIR: **1636 cm^{-1}** 1720 cm^{-1}	~<60%	Yes (p < 0.05)
Varghese et al. 2025 [35]	Experimental composites Hybrid S-2 Glass fibre-reinforced dental resin	FTIR: **1635/1640 cm^{-1}** 1608/1610 cm^{-1}	% Not reported	No (p > 0.05)
Neves et al. 2025 [39]	Experimental adhesive with PAC-DESIGNER biopolymers	FTIR: **1635 cm^{-1}** 1740 cm^{-1}	% Not reported	Yes (p < 0.05)

Although the majority of studies rely on this peak for analysis, it presents several limitations. Addressing these drawbacks constitutes the primary focus of the current investigation [3,6,28]. On one hand, O-H bending vibrations absorb within the same spectral region, making solvent-based materials (such as adhesives) susceptible to measurement errors and leading to an underestimation of polymerization efficiency when this peak is utilized. Furthermore, the presence of carbonyl groups can interfere with the 1640 cm^{-1} peak, reinforcing the necessity of selecting alternative peaks for a more accurate analysis [6,9]. Thus, this study aims to determine an alternative peak to the 1640 cm^{-1} peak using FTIR and Raman spectrometry, evaluating associated errors and identifying/specifying the most suitable method for determining the D_C during the polymerization of methacrylates [7]. The goal is to evaluate the most reproducible and least error-sensitive band for determining the conversion of the methacrylate group in monomers present in dental adhesives.

Over time, various studies have been carried out to determine the D_C of dental co-polymers [3,6,40]. The studies found in the literature evidently focus on different dental materials, not only dental adhesives but all types of resin-based materials, including resin composites and glass ionomer cements or derivatives [6,27,29,33,34,40]. However, most of these studies focus on analyzing the conversion rate at the standard peak of 1640 [v(C=C)] cm^{-1} [27,29,33,34,41–43], which, as already mentioned, is not ideal [6,9]. This peak has been systematically selected for the ATR-FTIR technique but also in micro-Raman studies, as already seen in Table 1. Thus, current knowledge of polymerization effectiveness may be flawed.

Nevertheless, studies are beginning to validate alternative peaks, such as the 1320 cm^{-1} [v(C-O)] [3,4,6,9,28,44,45]. Delgado et al. (2021) published a study detailing the flaws of the standard method, reiterating peak selection recommendations [6]. Moreover, in the past, Young (2004) also looked into the 1630 cm^{-1} peak, confirming its unsuitability for quantifying polymerization fractions, as water present in the formulations absorbs at this wavenumber, preventing the accurate determination of the peak baseline [28].

2. Study rationale

In light of these considerations, it is evident that a more nuanced approach is necessary to refine the methods used for evaluating polymerization efficiency in dental materials. Although the use of the 1640 cm^{-1} peak remains widespread in studies assessing polymerization efficiency, only a limited number of investigations have acknowledged the significant methodological limitations associated with this approach, particularly the interference caused by water absorption at this same wavenumber [28]. The studies that do exist, have focused on dental composites rather than in dental adhesives. The scarcity of studies critically addressing these inaccuracies highlights an important gap in the literature, as this interference can lead to substantial underestimation or misinterpretation of the D_C in adhesives [3].

Therefore, given the clinical relevance of achieving optimal polymerization for the long-term performance of dental materials, there is a pressing need for a comprehensive and standardized investigation that not only elucidates the potential sources of error in current analytical methods but also proposes clear, evidence-based guidelines. This study should aim to establish a reliable and reproducible protocol for polymerization monitoring, including the selection of appropriate analytical peaks, to ensure accurate and consistent assessment of polymerization studies across different dental adhesive formulations.

This laboratory research aims to study and compare polymerization kinetic parameters using different peak selections, in comparison to the standard 1640 cm^{-1} peak, to quantify the error, deviation and reliability of the alternative peaks.

II. OBJECTIVES

The **general aim** of this study is to critically evaluate the influence of vibrational peak selection on the reduction of estimation errors of the degree of conversion measurement in dental adhesives using ATR-FTIR spectroscopy.

i) Specifically, this will be undertaken by **comparing** the **D_C % calculation** using conventional and alternative peak pairs, quantifying their associated errors and variability, to identify the most reliable peak-pair;

ii) This comparison will be further investigated by **testing the data variability among two different commercial dental adhesives.**

III. HYPOTHESES

NULL

Null Hypothesis ($H_{0.1}$)

There are no statistically significant differences in the degree of conversion mean values (D_C%) obtained using the 1320/1340 cm^{-1} peak, compared to the conventional 1635/1648 cm^{-1} peak pair and to the 1610/1640 cm^{-1} peak pair.

Null Hypothesis ($H_{0.2}$):

That the statistically significant differences found in the comparison of the different peak pairs, are regardless of the commercial adhesive tested.

ALTERNATIVE

Alternative Hypothesis ($H_{1.1}$)

There are no statistically significant differences in the degree of conversion mean values (D_C%) obtained using the 1320/1340 cm^{-1} peak, compared to the conventional 1635/1648 cm^{-1} peak pair and to the 1610/1640 cm^{-1} peak pair.

Alternative Hypothesis ($H_{1.2}$):

That the statistically significant differences found in the comparison of the different peak pairs, are dependent upon the adhesive formulation tested.

IV. MATERIALS AND METHODS

4.1 Materials

The present work was carried out at the Biomaterials Laboratory of Egas Moniz School of Health & Science, using two different commercial adhesives: Scotchbond Universal Plus (3M ESPE) and Optibond Solo Plus (Kerr) as samples, in three replications for each run, (Figure 4). The chemical composition details supplied by the manufacturers are shown in the table below, Table 2.

Table 2. Composition of the adhesive systems used in this study: organic monomers present in the matrix, type and size of fillers, filler load (vol %), manufacturer, and batch number for OptiBond™ Solo Plus, Scotchbond™ Universal Plus.

	OptiBond™ Solo Plus	**Scotchbond™ Universal Plus**
Organic Matrix	GPDM, HEMA, Bis-GMA, GDMA	10-MDP, HEMA, Bis-GMA
Filler Type and Size	Silica nanoparticles (SiO2) (0.4 µm) barium aluminosilicate, sodium hexafluorosilicate (Na2SiF6)	Silica nanoparticles (SiO2) (0.4 µm)
Filler load (vol %)	15	N/A
Manufacturer	Kerr™ (Orange, CA, USA)	3M™ ESPE™ (St. Paul, MN, USA)
Batch	10043811	10042332
Expiration date	2025-06-30	2026-05-30

GDMA: glycidyl dimethacrylate

Figure 4. Commercial dental adhesives analyzed in this study. Represented in A) Scotchbond™ Universal Plus (3M ESPE), represented in B) OptiBond™ Solo Plus (Kerr).

4.2 Equipment and sample runs

An FTIR spectrometer (FTIR Spectrum 65, Perkin-Elmer, Beaconsfield, UK) (Fig.5) coupled with a Golden Gate Attenuated Total Reflectance (ATR) accessory (Specac, Orpington, UK) (Fig. 6) was used to obtain spectra of commercial adhesive droplets, before, during and after drying and polymerization, for a total of 20 min of time. The spectra was further processed using Spectrum TimeBase (V3.1.4.0, Perkin-Elmer, MA, USA). Continuous spectral acquisition during polymerization, without disconnecting the ATR diamond, permits a continuous monitoring of exactly the same volume of material during polymerization, as described by Delgado & Young (2021).

Figure 5. FTIR spectrometer (FTIR Spectrum 65, Perkin-Elmer, Egas Moniz School of Health and Science, Monte da Caparica, Portugal).

Figure 6. Golden Gate Attenuated Total Reflectance (ATR) accessory (Specac, Egas Moniz School of Health and Science, Monte da Caparica, Portugal).

4.3 Polymerization kinetics using ATR-FTIR

In this study, two different dental adhesives were evaluated. A metal circlip of 1 mm thickness x 8 mm internal diameter was used to contain 5 μL of the adhesive (n=3)—Optibond Solo Plus (Kerr, Brea, California, USA) and Scotchbond Universal Plus, (3M ESPE, Mapplewood, Minnesota, United States). Each material was pipetted into the circlips and positioned on the ATR (Specac Ltd., UK) diamond crystal plate. Once spectral acquisition began, a 20 s delay was employed before initiating the drying process.

Afterwords, the material was dried for 20 s with a hairdryer (Infini Pro AC Motor, Rowenta, Offenbach, Germany) equipped with a power output of 2200 W, with voltage of 220-240 V. An acetate sheet was placed over the circlips, waiting 20 additional seconds. The top surface of the material was irradiated using a light curing unit (LCU) (Elipar Deep Cure-S, 3M, St. Paul, MN, USA) with a single emission peak LED light, featuring a power output range between 1.470 mW/cm^2, and a spectral emission between 430–480 nm for 15s. FTIR spectra were collected before, during, and after 20 seconds of light exposure, over a total time period of 1200 s, yielding an average of 677 spectra per repetition. These spectra were recorded over a wavenumber range of 700 to 4000 cm^{-1}, with a resolution of 4 cm^{-1}, at a temperature of 37°C. Light curing began 20 ± 2 s after material placement and after acquiring the first spectrum. The full sequence of sample preparation, solvent evaporation, light-curing, and spectral acquisition which illustrates the standardized timeline followed across all repetitions, is summarized in Fig. 7.

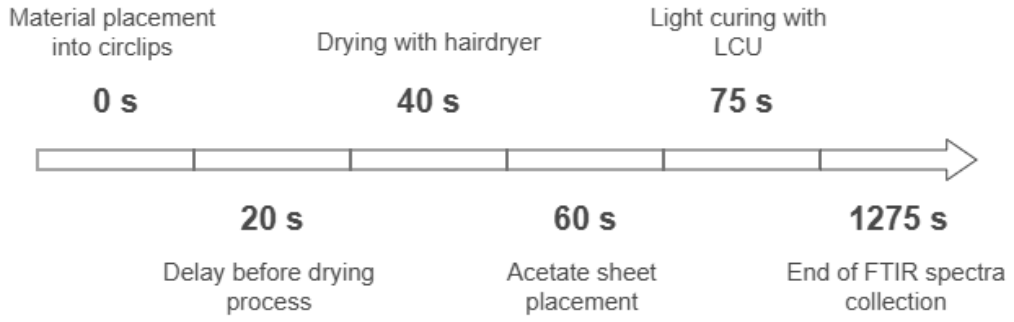

Figure 7. Schematic representation of the experimental protocol used for real-time polymerization monitoring with ATR-FTIR. The timeline includes adhesive placement, drying, pressure application, light-curing, and continuous spectral acquisition.

4.4 Peak selection and calculations

For this study, different reaction peaks and bases were selected to test their effect on determining polymerization kinetics and to analyze the variability of the data. These are shown below:

Table 3. Peaks analyzed for the comparison of error in DC% determination.

Peak-pair (cm^{-1})	Code
1320–1340	PP_1
1610–1640	PP_2
1635–1648	PP_3

To calculate the D_C (%), the following equation was applied (Equation 1), where (h_0) and (h_t) represent the height of the reaction methacrylate peak above baseline (reference peak) at the initial time and at time t, respectively, after the start of polymerization.

$$D_C = \frac{100(h_0 - h_t)}{h_0}$$

Equation 1. Where, h0 represents the initial height of the methacrylate reactive peak above the baseline (reference peak) before polymerization, and ht corresponds to the peak height at time t after the light curing reaction begins

Additionally, to assess spectral variations between the initial and final time points, the difference between the final and initial spectra was calculated and analyzed across repetitions of the three different materials. The resulting spectra were collected and analyzed using spectral treatment software— Spectrum TimeBase (v3.1.4.0, Perkin-Elmer, MA, USA). This software facilitated the calculation of the intensity ratio on the ATR diamond, both with and without the sample, followed by the conversion of the data to absorbance as a function of wavenumber (cm^{-1}).

4.5 Statistical analysis

All statistical work was performed in Python 3.11 (statsmodels 0.14), considering a significance level of 5%. Data comprised the maximum D_C calculated at 20 min (D_C %)

obtained from two commercial adhesives—*Optibond Solo Plus* and *Scotchbond Universal*—measured in triplicate, considering three ATR-FTIR peak-pair bands shown in Table 3.

Each physical specimen contributed one spectrum; all three peak-pairs were extracted from that same spectrum, so the data have a repeated-measures structure (3 peak-pairs × 2 adhesives × 3 specimens = 18 rows). To test whether the three peak-pairs differed in random error, we applied the median-based Levene (Brown–Forsythe) test and a linear-mixed effects model (LMM) of the form:

$$\mathrm{DC}_{ijk} = \beta_0 + \beta_1 \mathrm{Adh}_j + \beta_2 \mathrm{PP}_k + \beta_3 (\mathrm{Adh} \times \mathrm{PP})_{jk} + u_i + \varepsilon_{ijk},$$

Equation 2. Legend, where *Adh* denotes adhesive, *PP* the peak-pair, $u_i \sim N(0,\sigma^2_B)$ is a random intercept for specimen *i* and $\varepsilon_{ijk} \sim N(0,\sigma^2)$ the residual error. Restricted maximum likelihood (REML) was used throughout.

However, because the sample size is too small for a stable heterogeneous-residual model, overall precision was assessed in two complementary ways; the Brown–Forsythe test as mentioned above, and peak-pair-specific LMMs: the model above was refitted separately for each peak-pair, retaining adhesive as a fixed effect and specimen as a random intercept; the fitted residual variance (σ^2) from each model served as the measure of repeatability.

V. RESULTS

5.1 ATR-FTIR adhesive spectra and fingerprint regions

The spectra obtained before and after light-curing (final spectra after 20 min) of both adhesives and the isolated regions can be seen in Figure 9 and Figure 10. Peaks with the greater amount of change in Scotchbond Universal are 1245 cm^{-1} and 1270 cm^{-1} (absorbance increase), 1300 and 1320 cm^{-1} (absorbance decrease upon polymerization), 1638 cm^{-1} (absorbance decrease upon polymerization) and 1718 cm^{-1} absorbance increase, with slight shift to higher wavenumbers.

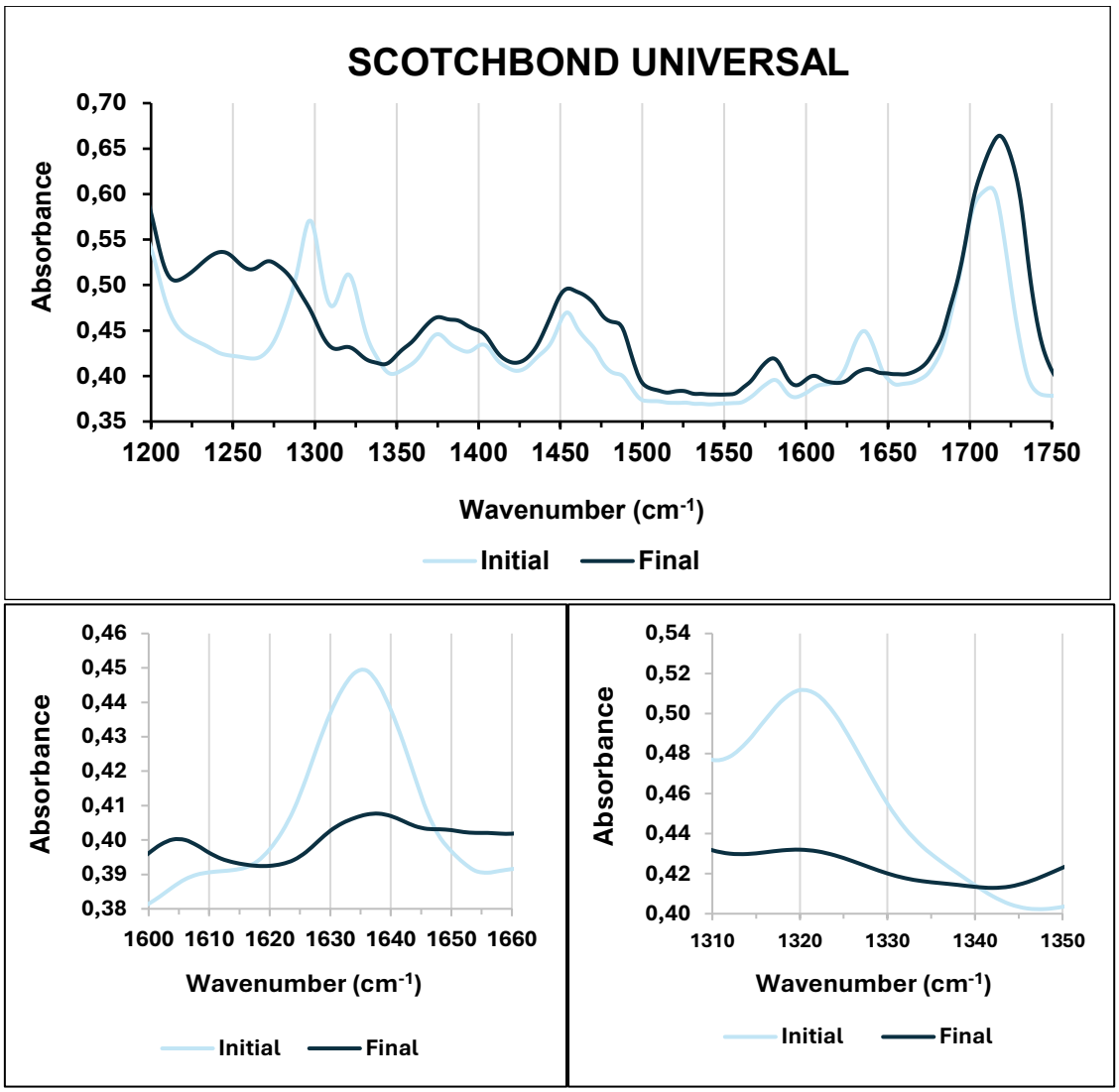

Figure 8. Average spectra of the 1200-1750 cm^{-1} fingerprint region of Scotchbond Universal, with two zoomed in regions, showing the differences upon 20 s of light-curing (initial 5 s spectrum vs. final 1200 s spectrum) in the 1635 cm^{-1} peak, 1320 cm^{-1} peak and 1605 cm^{-1}.

Peaks with the greater amount of change in Optibond Solo Plus are 1248 cm^{-1} (absorbance increase and slight shift to lower wavenumber), 1300 and 1320 cm^{-1} (absorbance decrease upon polymerization), 1638 cm^{-1} (absorbance decrease upon polymerization) and 1718 cm^{-1} absorbance increase, with slight shift to higher wavenumbers.

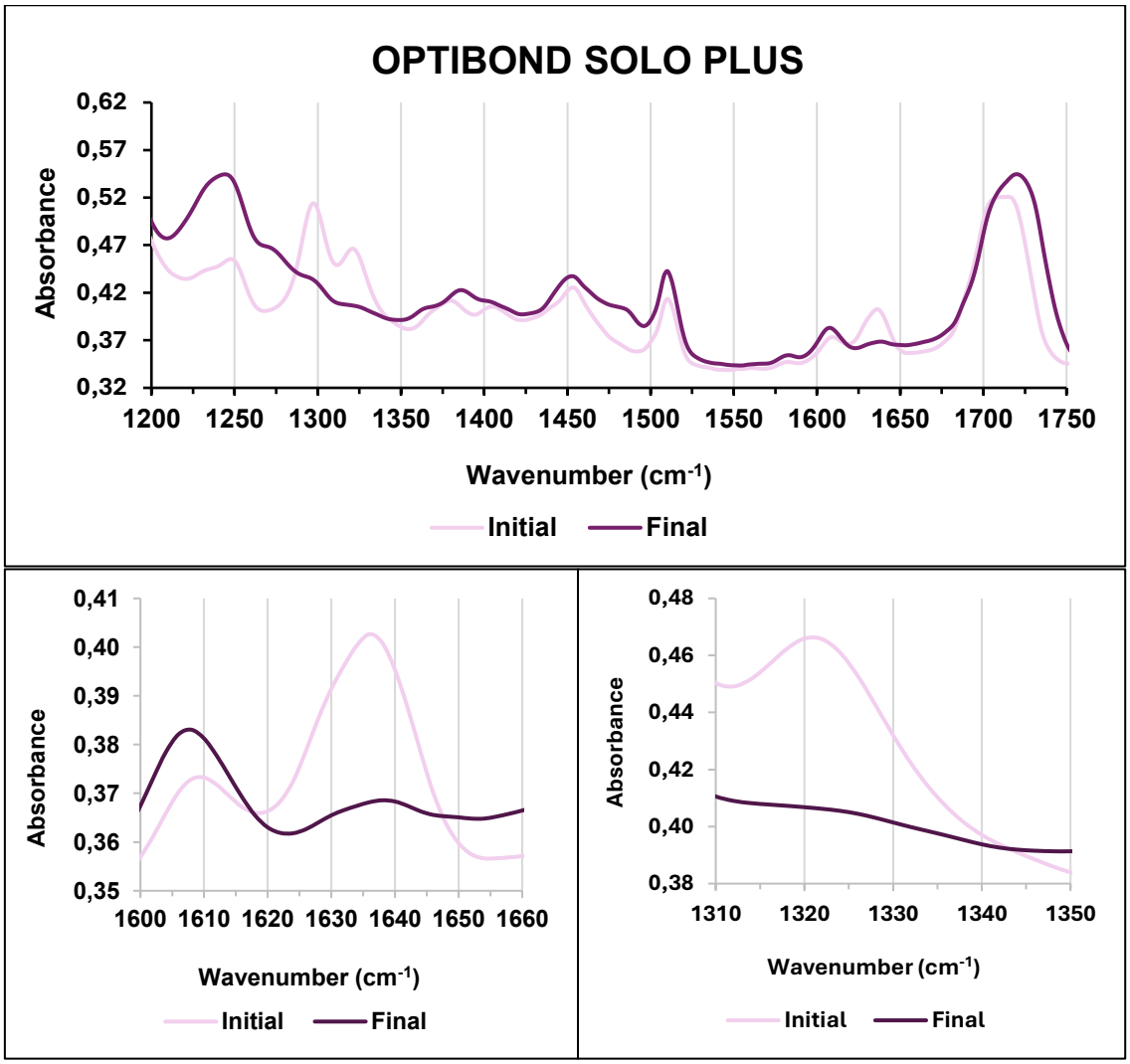

Figure 9. Average spectra of the 1200-1750 cm^{-1} fingerprint region of Optibond Solo Plus, with two zoomed in regions, showing the differences upon 20 s of light-curing (initial 5 s spectrum vs. final 1200 s spectrum) in the 1635 cm^{-1} peak, 1320 cm^{-1} peak and 1605 cm^{-1}.

In both adhesives the polymerizable aliphatic C═C at 1638 cm^{-1} sits on the flank of a broader aromatic/amide absorption centered near 1605–1610 cm^{-1}. The shoulder gives the local baseline a pronounced negative slope that varies specimen-to-specimen and adhesive-to-adhesive; small shifts in baseline placement can produce large area changes. The initial vs. final difference curve in *Optibond Solo Plus* is very subtle, in Figure 9. The

true loss of aliphatic C═C is only a fraction of the total absorbance under that composite band, so signal-to-noise is intrinsically poor.

5.2 D_C% results for peak-pairs: descriptive and inferential statistics

Descriptive statistics for D_C % results are summarized in Table 4. This table summarizes the spread of the D_C % values obtained from each adhesive–peak-pair combination. For both materials the 1320–1340 cm^{-1} and 1635–1648 cm^{-1} windows are tightly grouped (SD ≤ 3 %, CV ≤ 3 %), whereas the 1610–1640 cm^{-1} window is markedly erratic. The contrast is most striking for *Optibond Solo Plus*: the 1610–1640 peak-pair in this adhesive wrongly estimates the D_C > 100% — and inflates the within-adhesive variance.

Table 4. Descriptive statistics which include measures of variability of data – SD: standard deviation, variance and CV: coefficient of variation (calculated by dividing the SD/sample mean). Highlighted is the erroneous estimation of D_C, due to the selection of 1610-1640 cm^{-1} peak pair in Optibond Solo Plus.

Adhesive	Peak-pair (cm^{-1})	n	Mean (%)	SD (%)	Variance	CV (%)
Optibond Solo Plus	1320–1340	3	81.1	2.7	7.3	3.3
	1610–1640	3	158.6	15.7	246.6	9.9
	1635–1648	3	92.6	3.2	10.3	3.4
Scotchbond Universal Plus	1320–1340	3	80.3	1.7	2.8	2.1
	1610–1640	3	77.5	5.1	26.1	6.6
	1635–1648	3	91.1	2.1	4.3	2.3

Considering Brown-Forsythe, the *null hypothesis that* the three peak-pairs have equal variances within that adhesive was rejected, as both adhesives show a significant difference (at least one peak-pair is less precise than the others), as shown in Table 5.

Table 5. Brown-Forsythe statistics, considering an α=0.05.

Adhesive	**F (2, 6)**	***p-value***
Optibond	**20.56**	**0.002**
Scotchbond	**6.63**	**0.030**

Peak-pair-specific mixed models substantiated differences (Table 6). For the **1320–1340 cm^{-1}** ratio, the residual standard deviation was **1.65 %** (variance = 2.7), with no meaningful adhesive effect (–0.1 ± 1.1 %, p = 0.93). The **1635–1648 cm^{-1}** peak pair performed similarly (residual SD = 1.91 %, adhesive effect = –1.4 ± 1.6 %, p = 0.43). By contrast, the **1610–1640 cm^{-1}** peak pair was markedly less precise (residual SD = 8.28 %, variance = 68.5) and showed a large, adhesive-dependent bias (Scotchbond – Optibond = –81.1 ± 13.0 %, p<0.001). Thus, the random error at 1610–1640 cm^{-1} was ~5 times larger than that of the other two peak selections.

Table 6. LMM-based variance breakdown for each peak-pair (random intercept per specimen, adhesive as fixed effect, analyzed one peak-pair at a time).

Peak-pair (cm^{-1})	Adhesive effect†	p	Between-specimen σ^2	Residual σ^2	Residual SD
1320–1340	−0.12 %	0.93	2.71	2.71	1.65
1610–1640	−81.13 %	<0.001	68.50	68.50	8.28
1635–1648	−1.40 %	0.43	3.64	3.64	1.91

† Positive numbers would mean Scotchbond > Optibond; here there is essentially no adhesive difference at 1320–1340 or 1635–1648, but a huge reversal (–81 %) at 1610–1640.

VI. DISCUSSION

6.1. Importance of the study and rationale

The accurate quantification of the D_C% is fundamental for comprehending the polymerization behavior of methacrylate-based adhesives, as this process directly influences their mechanical performance, long-term stability, and clinical effectiveness in restorative dentistry [34,46]. Traditionally, the C=C stretching vibration at 1640 cm^{-1}, often paired with the 1610 cm^{-1} aromatic ring vibration, has been extensively used as the principal spectral marker for assessing D_C through infrared spectroscopy, particularly in ATR-FTIR based studies [4,9,34,41]. However, increasing evidence points to critical limitations of this region due to vulnerability to spectral interference [3,6,28]. These sources of interference collectively undermine the reliability of D_C% determination when relying solely on the 1640 cm^{-1} peak pair, especially in simplified, ethanol- or water-based adhesives [47,48]. Consequently, the present investigation was designed to systematically evaluate the suitability of this conventional spectral marker by comparing it to alternative vibrational modes, such as the methacrylate C–O stretching at ~1320 cm^{-1}, with the aim of identifying more accurate, reproducible, and interference-resistant spectral regions for real-time monitoring of adhesive polymerization [6,9] using ATR-FTIR spectroscopy.

The limitations of the 1640 cm^{-1} band have led several researchers to propose alternative vibrational modes for assessing methacrylate polymerization [4,6,28,45]. Among these, the 1320 cm^{-1} [ν(C–O)] stretching vibration has emerged as a promising candidate due to its relative insensitivity to water interference, spectral isolation, and universality across different dimethacrylate monomers [3,6,9]. Furthermore, this band exhibits strong correlation with the degree of polymer crosslinking and has shown high reproducibility in kinetic models [6]. Despite these documented advantages, the use of the 1320 cm^{-1} peak remains underrepresented in adhesive-focused research, as most validation studies have targeted restorative composites or model resins with simpler and more hydrophobic matrices [9,40,45]. In contrast, dental adhesives are chemically dynamic systems that incorporate hydrophilic monomers, solvents, and acidic functional groups, which introduce additional challenges for spectroscopic quantification [3,49]. In fact, recent findings suggest that in hydrophilic, solvent-rich adhesives, conventional FTIR peak pairs may yield systematically overestimated D_C values when based on

aromatic or aliphatic C=C bands due to nonlinear absorbance behaviour and limited baseline reproducibility [3]. Moreover, some studies suggest that interference from temperature fluctuations during curing and incomplete solvent evaporation can further affect DC estimations in these systems, complicating spectral interpretation and compromising polymerization uniformity [9,48,50]. Moreover, there is still no universal consensus on the implementation of 1320 cm^{-1}-based protocols, particularly under clinically relevant conditions, and comparative evaluations involving direct application of adhesives are scarce. Therefore, the present study addresses this critical gap by systematically assessing the performance of the 1320 cm^{-1} band alongside the conventional 1640 cm^{-1} marker, with the aim of proposing a more accurate, reproducible, and application-specific spectroscopic framework for quantifying polymerization in dental adhesives [4,9].

The experimental protocol was carefully developed to replicate clinical adhesive handling while preserving the control required for precise spectroscopic analysis. ATR-FTIR was selected in the present study due to its ability to monitor surface-level chemical changes in real time, without the need for extensive sample preparation [4,9,51]. Its non-destructive nature and minimal sample manipulation make it particularly suitable for evaluating thin adhesive films, which are heterogeneous and sensitive to environmental variation [6,52]. Additionally, its high temporal resolution makes it ideal for capturing the rapid kinetics of light-induced polymerization [9,53]. To address the spectral limitations of traditional peak choices, the study treated peak selection as an experimental variable, comparing not only the conventional 1640 cm^{-1} marker, but also the 1320 cm^{-1} methacrylate peak and the 1635 cm^{-1} carbonyl stretch, previously used in resin studies but less validated in adhesives [6,9]. This comparative approach enhances the analytical robustness of ATR-FTIR and supports the development of optimized, material-specific protocols for accurate DC quantification in dental adhesives. Additionally, the inclusion of the underexplored 1635 cm^{-1} [ν(C=O)] band provides new insight into its potential application in adhesive systems, which remain underrepresented in the literature [3,54]. Notably, comparative assessments between peak pairs revealed that the 1320–1350 cm^{-1} region exhibited the lowest coefficient of variation among all tested combinations, reinforcing its analytical robustness for in situ monitoring of polymerization processes.

6.2. D_C% results based on peak selection

The null hypothesis $H_{0.1}$, which stated that there would be no statistically significant differences between the D_C % values calculated using the peak pairs 1320–1340 cm^{-1}, 1635–1648 cm^{-1}, and 1610–1640 cm^{-1}, was rejected based on the experimental data. Specifically, the Brown–Forsythe test revealed significant differences in variance for both Optibond Solo Plus (p=0.002) and Scotchbond Universal Plus (p=0.030). These findings were further substantiated by the LMM analysis, which showed that the residual variance (σ^2) for the 1610–1640 cm^{-1} pair was 68.5, nearly five times higher than the variance of the other two peak pairs 2.71 and 3.64, respectively. This suggest that this conventional peak pair presents reduced precision in estimating polymerization.

These findings align with prior literature, which notes that the 1640 cm^{-1} peak, typically referenced to 1610 cm^{-1}, is widely used to monitor polymerization, as shown in Table 1, although its reliability may be affected by many different factors. Most importantly in dental adhesives, the spectral overlap from O–H bond vibrations in that region [9]. A consistent performance profile was noted for both adhesive materials. The peak pairs 1320–1340 cm^{-1} and 1635–1648 cm^{-1} produced more consistent and precise D_C % values, with SD for Optibond Solo Plus of 2.7 and 3.2, and for Scotchbond Universal Plus of 1.7 and 2.1, respectively. Also, there are no statistically significant differences between adhesives (p=0.93 and p=0.43, respectively). In contrast, the 1610–1640 cm^{-1} pair produced an implausible D_C % of 158.6% in Optibond Solo Plus, along with the highest within-adhesive variability (CV=9.9%), indicating that the measurement was not only imprecise but fundamentally flawed. Additionally, a large adhesive-dependent bias was observed, with a mean difference of −81.13% between adhesives for this peak pair (p<0.001), thus also leading to the rejection of hypothesis $H_{0.2}$, which assumed adhesive formulation would not influence the observed differences. Several methodological implications stem from these findings. The 1320–1340 cm^{-1} peak pair, in particular, presents notable advantages. Its use has been corroborated by several studies utilizing baselines at 1336 or 1350 cm^{-1}, establishing it as a dependable reference for assessing methacrylate concentration [6,55,56].

This study presents evidence that the selection of FTIR peak pairs can influence the accuracy and repeatability of D_C % measurements in dental adhesives. The data suggest that the 1320–1340 cm^{-1} and 1635–1648 cm^{-1} pairs may offer more consistent

performance across different adhesive formulations, while the 1610–1640 cm^{-1} pair appears less reliable, particularly in systems containing UDMA and solvents, such as the case of modern dental adhesives/primers.

Taken together, these findings do not support the validity of the null hypotheses. $H_{0.1}$ was rejected, as the results indicated statistically significant differences in precision and variance between the three peak pairs. Moreover, $H_{0.2}$ was also rejected, since a pronounced adhesive-dependent effect, particularly evident in the 1610–1640 cm^{-1} pair, suggests that the adhesive formulation influences D_C% outcomes. While these conclusions are based on a limited sample, they provide evidence that both peak pair selection and material composition may impact the reliability of FTIR-based polymerization analysis.

6.3. Differences in material composition

Both adhesives evaluated in this study, Optibond Solo Plus and Scotchbond Universal Plus, present notable differences in their chemical composition, particularly in terms of monomer types and solvent systems. Optibond Solo Plus is formulated with GPDM, HEMA, Bis-GMA, and GDMA, using ethanol as the solvent, whereas Scotchbond Universal Plus contains 10-MDP, HEMA, and Bis-GMA, and incorporates a mixed solvent system of ethanol and water. These compositional variations are essential to consider when interpreting polymerization behavior and D_C% outcomes, especially in the context of FTIR spectral analysis and solvent evaporation dynamics. In addition, the aromatic C=C peak at 1610 cm^{-1} is characteristic of Bis-GMA-based adhesives but is absent in UDMA-based formulations, which is consistent with the spectral behavior observed for Scotchbond, a UDMA-based adhesive, during the current study [57].

Differences in solvent type and volatility are known to influence adhesive performance during polymerization [48]. These solvents reduce the viscosity of the adhesive, facilitating its adaptation to the hydrophilic dental substrate [58]. Proper solvent evaporation during clinical application is essential, as insufficient or excessive evaporation can significantly influence the kinetics of monomer polymerization. Importantly, acetone and ethanol are more volatile compared to water, making them easier to eliminate during the drying phase [57]. The complete removal of water from dental adhesive systems proves to be inherently difficult, as residual moisture tends to

persist even after standard drying protocols [4,59,60]. Scotchbond Universal Plus, as already mentioned, includes water as part of its solvent system, is inherently more prone to residual solvent retention. Even with standardized drying protocols, the higher boiling point of water and hydrophilic interactions with adhesive components contribute to its incomplete evaporation. These effects seem to arise mainly from water that is not fully eliminated during the drying process, remaining entrapped within the adhesive structure and continuing to influence its behavior post-application [4,57,61]. Residual water in the adhesive can be detected in FTIR spectra via O–H bond vibrations, which interfere with the 1610–1640 cm^{-1} region. This spectral overlap is a known limitation, particularly when using this peak pair, as it may compromise the accuracy of polymerization assessments in water-containing formulations [9,57]. In contrast, Optibond Solo Plus, which does not contain water, is less susceptible to these spectral complications. Its solvent system based on ethanol facilitates more efficient solvent evaporation, minimizing plasticizing effects and baseline variability in FTIR readings. As a result, adhesives without water generally yield more accurate and consistent D_C % values [20,62], especially when analyzed with more reliable peak pairs such as 1320–1340 cm^{-1}.

These compositional differences carry implications in two principal aspects. First, they reinforce the need for adhesive-specific analytical strategies, as solvent volatility and monomer structure affect not only polymerization kinetics but also spectroscopic interpretation. Secondly, these findings highlight a key limitation of universal peak-pair approaches in FTIR analysis: spectral pairs that perform reliably in systems with low O–H content, such as ethanol-based formulations, may fail when water is present. In the case of Scotchbond, the retention of residual water within the adhesive interface introduces spectral interference, particularly in regions affected by O–H vibrations, thereby compromising the accuracy of polymerization assessment. More broadly, the presence of such residual solvents can increase the susceptibility of the adhesive to degradation and compromise the structural integrity between hydrophilic and hydrophobic phases [57].

6.4. Study Limitations

The present study was designed to ensure strong internal validity by standardizing experimental parameters such as adhesive volume, solvent evaporation procedures, and light-curing protocols. All materials were tested in triplicate with sufficient statistical power, as proven in Delgado & Young (2021). Although the protocol replicated key

aspects of clinical handling, the experimental environment cannot fully simulate the biological and physicochemical complexity of the oral cavity, where factors such as temperature variability, pulpal pressure, salivary enzymes, and substrate heterogeneity may affect adhesive behaviour. Minimal procedural deviations, particularly in air-drying or adhesive placement, may also have introduced minor inconsistencies in solvent retention or film thickness, potentially influencing D_C% outcomes.

The incomplete evaporation of solvents may also have subtly altered the local refractive index and affected spectral accuracy [3,4]. Moreover, only two commercial adhesives were tested in this study, one a conventional etch-and-rinse (Optibond Solo Plus) and the other a universal adhesive (Scotchbond Universal Plus), which may limit the generalizability of the findings across different adhesive systems and compositions. Nonetheless, despite these limitations, the results remain valid and relevant within the defined experimental framework. Careful standardization ensured reproducibility across conditions, and the observed statistically significant differences between spectral peak pairs—especially between 1320–1340 cm^{-1} and the conventional 1610–1640 cm^{-1} combination—demonstrate that the choice of spectral region substantially affects D_C% estimation. These findings directly support the central research question and suggest that the proposed FTIR peak combination is more suitable than others for quantifying polymerization in solvent-rich or hydrophilic adhesive systems.

6.5. Future Recommendations

The inclusion of a wider range of adhesive systems, beyond the two tested in this study, would be essential to assess whether the observed trends in spectral performance and conversion consistency hold across different chemical compositions and adhesive strategies. Such efforts will be essential for developing material-specific protocols for DC quantification, ultimately contributing to the standardization of polymerization analysis in restorative research. Additionally, integrating complementary spectroscopic techniques, particularly Raman spectroscopy, which is less affected by water and offers greater spatial resolution, could provide valuable depth profiling and enhance chemical discrimination within polymerizing systems [9,30,33]. Combining ATR-FTIR and Raman analysis in dual-mode studies would enable real-time, multilayer characterization of adhesive interfaces and support more accurate modelling of polymerization kinetics under clinically relevant conditions. These integrative approaches are especially

recommended when investigating innovative formulations incorporating novel solvents, multifunctional co-initiators, or structurally modified monomers. In the long term, such strategies are expected to strengthen the analytical robustness, clinical applicability, and scientific reproducibility of polymerization studies in adhesive dentistry.

VII. CONCLUSION

The findings of this study suggest that the selection of spectral peak pairs can substantially influence the accuracy and reproducibility of D_C % measurements in methacrylate-based dental adhesives using ATR-FTIR spectroscopy. Among the three peak combinations evaluated (1640 cm^{-1} with 1610 cm^{-1}, 1320 cm^{-1} with 1336 cm^{-1}, and 1635 cm^{-1} with 1648 cm^{-1}), the 1320–1336 cm^{-1} pair showed the lowest variability and the most consistent values across the materials tested. In contrast, the traditional 1640–1610 cm^{-1} combination exhibited greater susceptibility to spectral interference and variability, which may compromise the reliability of conversion assessments, particularly when dental adhesive systems, given their solvent-rich nature.

The intrinsic chemical diversity among dental adhesives, particularly in terms of monomer composition and solvent type, makes it difficult to identify vibrational peak pairs that are universally applicable. Each adhesive behaves as a unique system, and these compositional differences highlight two critical implications: the need for material-specific analytical strategies, and the limitation of universal peak-pair approaches, especially in systems where water is present. Therefore, while alternative peak regions such as 1320 cm^{-1} may improve quantification, their applicability must still be validated individually for each material. Future studies should prioritize the development of material, specific calibration protocols, explore adaptive spectral analysis methods, and consider the interaction of monomer–solvent systems to enhance both clinical reliability and standardization in adhesive characterization.

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