



MARIA MALERBA COLOMBI HUMEL

“Efeito da contaminação por primer e pelo excesso de
água, álcool ou clorexidina nas propriedades mecânicas e
cinética de conversão de sistemas adesivos
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UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA
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cinética de conversão de sistemas adesivos
convencionais”.

Orientador: Prof. Dr. Luís Alexandre Maffei Sartini Paulillo

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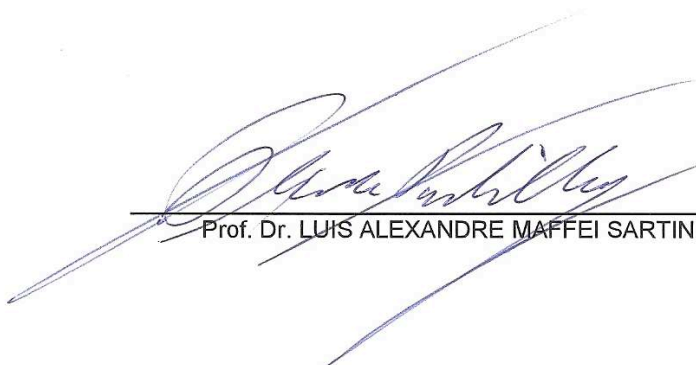
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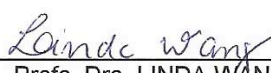
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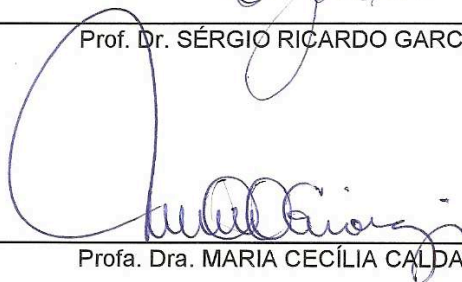
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*“Foi o tempo que dedicaste à tua rosa
que fez tua rosa tão importante.”*

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Este estudo teve como objetivo avaliar a influência de diferentes concentrações destas soluções na resistência à flexão (FS), módulo de Elasticidade (E) e grau de conversão (DC) de sistemas adesivos convencionais, e ainda, sua influência na cinética (K) e taxa de conversão (RP). Para isso foram realizados dois trabalhos experimentais, sendo que no primeiro avaliou-se as quantidades de primer nos adesivos, e no segundo trabalho avaliou-se a influência das soluções de enxague de cavidade, água, EtOH e CHX, quando em excesso, nos sistemas adesivos. Para se avaliar as diferentes concentrações do primer residual dois adesivos foram manipulados, M1 e M2 e testados com quatro concentrações de primer: 0, 5, 10 e 15%. Já para se avaliar o excesso de água, EtOH e CHX, dois adesivos foram testados: M1, adesivo manipulado, e o adesivo comercial SBMP; diluídos com 0, 2 e 9% para cada uma das soluções. Para se avaliar as propriedades mecânicas, nos dois estudos, foram preparadas amostras a partir de moldes de silicone em forma de barras (7 x 2 x 1mm) e após armazenamento por 24h, foram testadas em máquina de ensaio mecânico para o teste de FS a uma velocidade de 0,5 mm/min. DC foi mensurado utilizando Espectroscopia Transformada Infravermelha de Fourier (FTIR). Para cinética de conversão, a polimerização em cada diluição foi monitorada por FTIR pelo tempo de 60s, sendo que a conversão foi calculada a cada dois 2s de polimerização, avaliando-se também RP. No primeiro estudo, as diferentes concentrações da diluição com primer não mostrou diferença no teste de FS entre os adesivos testados, independente da concentração avaliada. Os dois adesivos testados apresentaram redução nos valores de DC com o aumento das quantidades de primer, sendo o adesivo M2 o mais susceptível às diluições. No segundo trabalho, para as diluições com água, EtOH, independente da quantidade testada, uma redução nas propriedades mecânicas e DC pôde ser observada. As diluições em água e CHX alteraram mais as propriedades mecânicas quando comparadas as diluições com EtOH. Para K e RP com o aumento da concentração de primer residual todos adesivos apresentaram redução quando se avaliou os gráficos de conversão. Para as diluições em água, o aumento de sua concentração levou a queda progressiva na K e as diluições com CHX apresentaram menor influência na K, com redução apenas para 9% de diluição. Porém, para as diluições em EtOH houve aumento na K e RP especialmente para o adesivo SBMP. Assim, para o primeiro estudo, os resultados

sugerem que as diferentes quantidades de primer usadas na diluição do adesivo não influenciam as propriedades mecânicas dos adesivos testados, porém, interferem na sua reação química de polimerização, uma vez que a velocidade reação foi reduzida (K e RP). Por outro lado, no segundo estudo, para as soluções residuais de água, EtOH e CHX, uma diminuição nas propriedades mecânicas dos adesivos ocorreu somente quando quantidades residuais de 9% foram simuladas. No entanto, pequenas quantidades de EtOH e CHX parecem não afetar as propriedades e velocidade de reação de polimerização dos adesivos.

Palavras-chave: Adesivos Dentinários, Polimerização, Materiais Dentários.

The present study evaluates the influence of these solutions in different concentrations on flexural strength (FS), elastic modulus (E) and degree of conversion (DC) of conventional adhesives evaluating also the kinetic of conversion (K) and rate of polymerization (RP). Two distinct experiments were plotted, the first one evaluating the primer amount diluted in adhesives, and the second experiment evaluating rinse solutions amounts like water, ethanol and chlorhexidine diluted in different adhesives. For different concentrations of primer solutions, two manipulated adhesives; M1 and M2 were tested at 0, 5, 10 and 15% of dilution. For different concentrations of water, ethanol (EtOH) and chlorhexidine (CHX) dilutions, two adhesives were evaluated: M1, a manipulated adhesive, and the SBMP; diluted at 0, 2 and 9% of dilutions. Both studies, for mechanical properties, specimens were obtained by silicon mold bars (7 x 2 x 1mm), and after 24h, FS were performed at Mechanical Test Machine at cross speed of 0.5 mm/min. DC were measured with a Fourier Transformed Infrared Spectroscopy (FTIR). For K test, Infrared Spectroscopy monitored each 2 seconds of conversion for 60sec of polymerization, and the RP were evaluated. The first experiment, for different concentrations of primer dilutions, the FS for all adhesives tested was not affected by primer concentrations. All adhesives tested showed a decrease for DC as increased the primer dilution, with M2 presenting as the most sensitive for primer concentration. For second experiment, for all concentrations dilution of water, EtOH and CHX, a decrease on mechanical properties and DC was noted. Water and chlorhexidine seems to affect more mechanical properties when compared to EtOH results. For K and RP results, increasing primer concentration, all adhesives presented a decrease of conversion and RP. For water dilutions, increasing water concentrations on tested adhesives, a progressive decrease could be noted on K graphics. CHX dilutions presented less influence on K, with decreasing RP and K only with 9% dilution. Although, EtOH dilutions presented an improve K as the RP, especially to SBMP adhesive. The results suggested that mechanical properties were not affected regardless of the primer amount diluted on adhesives, interfering only on chemistry of polymer reaction, once reaction speedy were affected (K and RP). However, for second experiment, a decrease on mechanical properties of adhesive solutions could be noted only when extreme amounts of residual solution where diluted.

Notwithstanding, at lowest concentrations, EtOH and CHX seems not interfere on K and RP of adhesives.

Key Word: dentinal adhesive, polymerization, dental materials.

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Encontra-se disponível no mercado odontológico uma grande variedade de sistemas adesivos que usam diferentes técnicas para a abordagem do tecido dentinário visando a união entre dentina e material restaurador (Van Landuyt *et al.*, 2007). Porém, apesar dos desenvolvimentos e aprimoramentos, os sistemas adesivos utilizados com o condicionamento ácido prévio do substrato, ainda são os mais utilizados e apresentam efetividade e estabilidade de união (De Munck *et al.*, 2005, Pashley *et al.*, 2011).

Independente da abordagem de tratamento do substrato, adesivos de condicionamento prévio de 2 ou 3 passos possuem técnica clínica crítica, sendo esta a sua principal desvantagem (De Munck *et al.*, 2005; Van Landuyt *et al.*, 2007). São vários os fatores que podem interferir na qualidade da união destes sistemas, como a quantidade de água remanescente na dentina, o tempo de volatilização do solvente e a quantidade de monômero hidrófobo usado, além disso, a qualidade do sistema adesivo depende da quantidade de solvente e água utilizados na sua composição. Assim, qualquer erro durante a aplicação clínica do sistema adesivo pode interferir nas propriedades mecânicas, no grau de conversão e na qualidade da camada híbrida formada (Reis *et al.*, 2004; Dickens *et al.*, 2005; Ito *et al.*, 2005; Guo *et al.*, 2008; Cadenaro *et al.*, 2009b; Stansbury, 2012).

Durante o condicionamento ácido do substrato metade do volume superficial mineral é removido e substituído por água (Pashley *et al.*, 2011), esta água preenche os espaços ocupados pela parte inorgânica da dentina evitando o colapso das fibrilas de colágeno, e em seguida é removida através da aplicação de uma camada de solventes e monômeros hidrófilos (De Munck *et al.*, 2005; Guo *et al.*, 2008). Uma camada híbrida ideal teria a água e solventes completamente substituídos por monômeros resinosos, criando assim uma zona de interdifusão de colágeno e resina, garantindo resistência e propriedades mecânicas adequadas para resistir aos desafios a que a restauração é submetida (Pashley *et al.*, 2011). Porém, uma completa substituição da água e solventes por monômeros resinosos é impossível de ser conseguida clinicamente (Pashley *et al.*, 2007; Pashley *et al.*, 2011), o que gera na camada híbrida regiões ricas em água e pobre em resina (Pashley *et al.*, 2011), prejudicando a estabilidade e a resistência da mesma por dificultar a conversão dos monômeros em polímeros.

A quantidade de primer aplicado na dentina após o condicionamento é, na maioria das vezes, negligenciado nos protocolos de aplicação dos sistemas adesivos (Pashley *et al.*, 2007; Pashley *et al.*, 2011). Como os componentes do primer tem a função de facilitar na penetração dos monômeros formadores da camada híbrida, esta solução não pode ser aplicada em excesso porque o polímero formado pelos monômeros hidrófilos apresenta propriedades mecânicas inferiores, muito aquém do ideal para uma camada híbrida de qualidade (De Munck *et al.*, 2005; Van Landuyt *et al.*, 2007; Ikemura *et al.*, 2010). O monômero HEMA, principal monômero contido no primer, aparece como sendo o maior responsável pelas baixas propriedades, por possuir baixo peso molecular e formar uma cadeia polimérica pequena, além de apresentar baixa resistência à flexão (Ito *et al.*, 2005; Ikemura *et al.*, 2010). O excesso deste componente, assim como o excesso de água, levam a uma separação de fases entre primer hidrófilo e os monômeros hidrófobos (Hosaka *et al.*, 2010), diminuindo o grau e a taxa de conversão de todo o sistema (Guo *et al.*, 2008). O baixo grau de conversão dos monômeros, o excesso de água, e a conseqüente diminuição das propriedades mecânicas dos sistemas adesivos aparecem como maior causa da diminuição da durabilidade da camada híbrida e falha da restauração (Reis *et al.*, 2004; Pashley *et al.*, 2007; Cadenaro *et al.*, 2009b).

Por outro lado, os estudos que avaliam as restaurações adesivas mostram que as mesmas apresentam pequena longevidade (NIDCR, 2009-2013). Somado a isto, algumas técnicas tem sido sugeridas para aumentar essa longevidade como enxague da cavidade com CHX após condicionamento ácido (Pashley *et al.*, 2004; Carrilho *et al.*, 2007b; Pashley *et al.*, 2007; Shin *et al.*, 2009). Nos espaços interfibrilares, após o condicionamento e enxague com água, proteoglicanas altamente hidratadas com cargas negativas formam um hidrogel por entre os espaços interfibrilares. Este hidrogel contém enzimas metaloproteinases que regulam o metabolismo do colágeno nos tecidos (Pashley *et al.*, 2004; Carrilho *et al.*, 2007b). Ao longo do tempo, estas metaloproteinases agem degradando o colágeno e prejudicando a rede colágeno-monômeros formada na adesão, causando diminuição acentuada na longevidade da restauração. A Clorexidina, aplicada na concentração 2% após o condicionamento ácido, provou agir como inibidora de metaloproteinase, por inibir a atividade colagenólica e gelatinolítica da dentina desmineralizada pelo condicionamento prévio (Carrilho *et al.*, 2007b) desacelerando esta resposta fisiológica e conseqüente degradação da camada híbrida.

Apesar da efetividade desta solução no que diz respeito ao aumento da resistência à união e longevidade da restauração (Carrilho *et al.*, 2007a; Pashley *et*

al., 2007), a técnica de aplicação de CHX não parece estar bem sedimentada na literatura (Carrilho *et al.*, 2007b; Pashley *et al.*, 2007). Como a CHX utilizada para o enxague da cavidade é, na sua maioria, manipulada em veículo aquoso, o remanescente desta solução na cavidade pode influenciar negativamente o grau de conversão dos monômeros, bem como as propriedades mecânicas do polímero formado, através de uma possível separação de fases entre primer e monômeros hidrófobos dificultando a polimerização do conjunto e estabilidade da cadeia polimérica, aumentando a formação de cadeias lineares (Liu *et al.*, 2011). A CHX também pode levar a uma diluição das cadeias poliméricas formadas induzindo a ciclização primária do monômeros, o que também acarretaria diminuição nas propriedades mecânicas do polímero em formação (Ye *et al.*, 2007).

Da mesma maneira que a CHX, a técnica de aplicação de etanol na cavidade, previamente à hibridização dos tecidos, foi desenvolvida com o intuito de melhorar a longevidade dos sistemas adesivos de condicionamento prévio nas restaurações em resina composta (Pashley *et al.*, 2007). O etanol é usado para desidratar quimicamente a matriz dentinária, previamente condicionada, resultando em contração lateral das fibrilas de colágeno, aumento na largura dos espaços interfibrilares e a redução da hidrofilicidade da matriz de colágeno (Pashley *et al.*, 2007; Shin *et al.*, 2009). Uma vez que os espaços interfibrilares são aumentados, uma melhor penetração dos monômeros ocorre, aumentando o entrelaçamento entre polímero e fibrilas de colágeno. Nesta técnica o colágeno exposto após a desmineralização pelo condicionamento ácido se torna menos hidrófilo e age prevenindo a separação de fases (Becker *et al.*, 2007). Somado a isto, uma vez que a maioria dos monômeros hidrófobos são solúveis em etanol, é possível se usar uma quantidade maior desses monômeros. Com o uso de mais monômeros hidrófobos uma camada híbrida menos hidrófila é criada o que acarretaria em uma menor absorção de água e maior longevidade da restauração (Shin *et al.*, 2009).

Assim como no protocolo de aplicação da clorexidina, a técnica alcoólica ainda gera dúvidas e questionamentos quanto a quantidade que pode ser aplicada na cavidade para sua efetividade (Cadenaro *et al.*, 2009a; Sauro *et al.*, 2009; Shin *et al.*, 2009). O excesso do álcool usado como enxague leva ao aumento significativo da quantidade de solvente orgânico no sistema adesivo, o que poderia alterar as propriedades coligativas das soluções (Elliott *et al.*, 2002). Assim, quando ocorre a evaporação do solvente, ocorreria também uma redução na temperatura da mistura resina/solvente acarretando em menor conversão dos monômeros em polímeros, uma vez que a temperatura tem grande influência na extensão da reação de polimerização (Pashley *et al.*, 1998; Daronch *et al.*, 2006; Holmes *et al.*,

2007).excesso de álcool também poderia levar a uma saturação de solvente na zona de interdifusão, além de aumentar a fluidez do sistema monomérico, dificultando a união e polimerização do adesivo aplicado após o enxague da cavidade, e, conseqüente redução na conversão dos monômeros, causando diminuição nas propriedades mecânicas do polímero formado (Holmes *et al.*, 2007; Stansbury, 2012).

Dentre todos os fatores envolvidos na adesão, a camada adesiva possui os menores valores de rigidez, sendo considerada o elo mais fraco do conjunto, depois da camada híbrida. No entanto, devido a sua resiliência, consegue resistir às forças da contração de polimerização dos compósitos. Assim sendo, soluções utilizadas previamente a aplicação dos monômeros adesivos, como água, clorexidina, etanol, e mesmo a quantidade de primer utilizado antes da aplicação do monômero hidrófobo, devem ser melhor investigadas uma vez que o excesso destes enxaguantes, ou até mesmo de primer pode interferir na qualidade do polímero formado na camada adesiva, o que pode diminuir a longevidade da restauração. Assim, este estudo teve como premissa avaliar a interferência destas soluções, em diferentes concentrações, nas propriedades mecânicas, grau de conversão, cinética e taxa de conversão de sistemas adesivos convencionais.

O presente trabalho é apresentado no formato alternativo de tese de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas. O artigos referente ao Capítulo Um desta tese foi submetido ao periódico J Dentistry e o artigo referente ao Capítulo Dois será submetidos ao periódico Operative Dentistry.

Influence of primer and adhesive mixture on the real-time polymerization and mechanical properties of experimental dental adhesives

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ABSTRACT

This study evaluated the influence of primer concentration on the physical and chemical properties of model adhesive systems. Two model adhesives were tested: M1 (40% BisGMA and 60% HEMA) and M2 (40% BisGMA, 40% HEMA and 20% TEGDMA). The adhesives were analysed mixed with different concentrations of the primer agent (0, 5, 10 and 15%). The properties evaluated were the flexural strength (FS), elastic modulus (E), degree of conversion (DC), the kinetics of conversion (K) and the rate of polymerisation (RP). To evaluate the mechanical properties, we prepared specimens using silicone bar moulds (7 mm x 2 mm x 1 mm), and tested them by the three-point bending test, at a crosshead speed of 0.5 mm/min. DC and the kinetics of cure were measured by Fourier Transform infrared spectroscopy (FTIR). The data were statistically analysed by a two-way ANOVA and Tukey's test ($\alpha = 0.05$). The real-time polymerisation was monitored for 120 seconds. The FS and E of all adhesives tested were not affected by different primer concentrations. All adhesives tested had decreased DC as the primer concentration increased. When the real-time conversion was analysed, the model adhesives presented decreased RP according to increases in primer concentration. The results suggested that the mechanical properties of non-solvated adhesives were not affected by the primer concentrations tested; however, the degree of conversion and rate of polymerisation are influenced by the different concentrations of the agents tested.

Keywords: Flexural strength, elastic modulus, degree of conversion, adhesive layer, hybrid layer, photocuring, HEMA.

Introduction

The adhesive techniques for dental substrate have developed significantly in recent decades.¹ A variety of adhesive systems is available on the market with different brands and approaches to the treatment of dental tissues.² Despite progress in the simplification of adhesive techniques, three-step, etch-and-rinse adhesive systems are still considered the gold-standard for dentin bonding.^{1,2} However, dentin hybridization is still not considered ideal, since the multiple steps might also generate a susceptibility to technique sensitivity.³

An important feature of the technique is the dentin priming step.^{1,4} The function of the primer is to allow monomers to penetrate into the demineralized moist dentin, but the primer might result in reduced mechanical properties in terms of hybridization quality.^{2,3,5} 2-hydroxyethyl methacrylate (HEMA) is a major component of the primer agent of the two-bottle adhesives. HEMA is a low-weight, short-chain monomer, responsible for generating high stress on polymerisation and, consequently, a low flexural strength.^{5,6} In contrast to dimethacrylate monomers, which present two reactive groups, the monovinyl HEMA is unable to generate polymer crosslinking.^{1,2} The behavior of the hydroxyl group at the extremity of the molecule is too hydrophilic, even after polymerisation.² As a result, a polymer more prone to hydrolysis in the oral environment, with a potential decrease in the mechanical properties of dental materials, is formed by resin materials with high HEMA content.^{2,7}

It is known that low degree of C=C conversion, presence of water, and excess HEMA can reduce the mechanical properties of adhesive systems, a fact that seems to be the major cause of decreased hybrid layer durability and subsequent restoration failure.^{4,8,9} Therefore, the aim of this study was to evaluate the influence of primer and adhesive mixture, testing different primer concentrations, on the conversion and mechanical properties of experimental dental adhesives. The null hypothesis tested was that different primer concentrations have no influence on the mechanical properties of adhesives, and also that, regardless of primer concentration, the kinetics and rate of polymerisation will not be influenced.

1. Materials and methods

2.1. Adhesive systems

Bisphenol-A glycidyl dimethacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), HEMA and camphorquinone (CQ) were generously

supplied by Esstech (Essington, PA, USA). 4-dimethylamine benzoate (EDAB) was purchased from Sigma-Aldrich (Milwaukee, WI, USA). All reagents were used without further purification.

Two different formulations of light-cured, model unfilled dental resins were evaluated: M1 (40 wt% Bis-GMA and 60 wt% HEMA) and M2 (40 wt% Bis-GMA, 40 wt% HEMA, and 20 wt% TEGDMA). To both blends, 1 mol% of CQ and 2 mol% of EDAB were added as photoinitiators.¹⁰ The materials were prepared by intensive motorized mixing in a dark room with controlled temperature and humidity. The primer of the commercial adhesive system Adper Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA) was used. The Scotchbond primer contains water (40-50%), HEMA (35-45%), and polyacrylic and itaconic acid copolymers (10-20%).

1.2. *Mechanical properties*

The experiment was performed according to ISO 4049/2009 standard for dental polymers, except for the dimensions of the specimens. Each adhesive tested was mixed with 0, 5, 10, or 15% of the primer (n=5). The primer/adhesive volumes were determined by several pilots studies until authors be sure of best clinical standard with a calibrate laboratory technique control. The primer/adhesive mixture was prepared, vigorously agitated to promote a homogeneous solution, then inserted into a silicone mould. Specimens were prepared according to a protocol previously described,¹¹ except for the solvent evaporation step, placing 20- μ L of each adhesive/primer solution in the silicone mold, yielding a bar-shaped specimen (7 mm $\times$ 2 mm $\times$ 1 mm).

Before light-activation, a Mylar strip was placed over the mold to obtain a flat surface and prevent the inhibition of cure by oxygen. Adhesives were irradiated for 60 s using a light-emitting diode curing unit (Elipar FreeLight2; 3M ESPE) with 850- mW/cm^2 irradiance. The prolonged photocuring was applied to evaluate primer/adhesive mixture in the best possible curing scenario. After dry storage for 24 h, the specimens were subjected to three-point bending tests at a crosshead speed of 0.5 mm/min until failure in a mechanical testing machine (model 4411; Instron Corp., Canton, MA, USA). Flexural strength (FS) and elastic modulus (E) were calculated based on the load-displacement trace.

1.3. *Degree of C=C conversion*

Degree of C=C conversion (DC) of the primer/adhesive mixtures was measured by Fourier Transform mid-infrared (FTIR) spectroscopy (Spectrum 100 Optica; Perkin-Elmer, Wellesley, MA, USA), equipped with an attenuated total

reflectance (ATR) device composed of a horizontal ZnSe crystal (Pike Technologies, Madison, WI, USA). The specimens evaluated were the same as those tested for mechanical properties ($n=5$). A preliminary reading for uncured material was taken in the absorbance mode under the following conditions: 24 scans, 1665–1580 cm^{-1} frequency range, 4 cm^{-1} resolution, and Happ-Genzel apodisation. Additional spectra were obtained immediately after light-curing. Specimens were placed on the horizontal face of the ATR cell with a constant weight of 90 bar. DC was calculated according to a baseline technique,¹² based on band ratios of 1638 cm^{-1} (methacrylate aliphatic C=C) and, as internal standard, 1608 cm^{-1} (aromatic C=C) between the polymerised and uncured samples.

1.4. *Polymerisation kinetics and rate*

Real-time polymerisation FTIR spectroscopy as previously described. A constant 5 μL volume of primer/adhesive mixture was dispensed onto the crystal and photo-activated for 120 s, during which the polymerisation reaction was monitored in real time acquiring one spectrum every 2 s. Three specimens were tested for each primer/adhesive concentration. Average conversion vs. time data were plotted, and Hill's 4-parameter non-linear regressions were used for curve-fitting. As the coefficient of determination was greater than 0.99 for all curves, the rate of polymerisation (R_p) was calculated based on these data-fitted plots.

1.5. *Statistical analysis*

The mechanical properties (FS, E), as well as the DC, were analysed by two-way ANOVA and Tukey's test, at a 0.05 level of significance, with "adhesive" and "primer concentration" as the two factors (SAS 9.1 version; The SAS Institute, Cary, NC, USA).

2. Results

Figures 1 and 2 show the results for FS and E . Irrespective of the primer concentration, M1 and M2 showed similar results. The DC, however, was influenced by the different amounts of primer, as shown in Table 1. M2 appeared to be the more sensitive to the primer concentration, showing statistically significant differences with 10% of primer on dilution. SBMP showed less sensitive behavior, with significant results only after 15% primer concentration.

Fig 1. Results for flexural strength (bars are means $\pm$ standard deviations). No statistically significant differences were observed between groups.

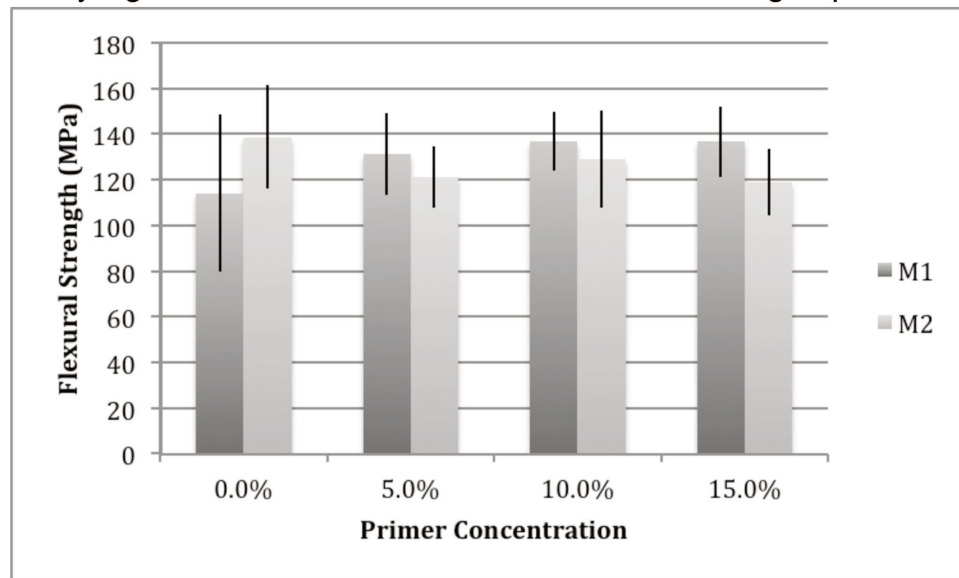


Fig 2. Results for elastic modulus (bars are means $\pm$ standard deviations). No statistically significant differences were observed between groups.

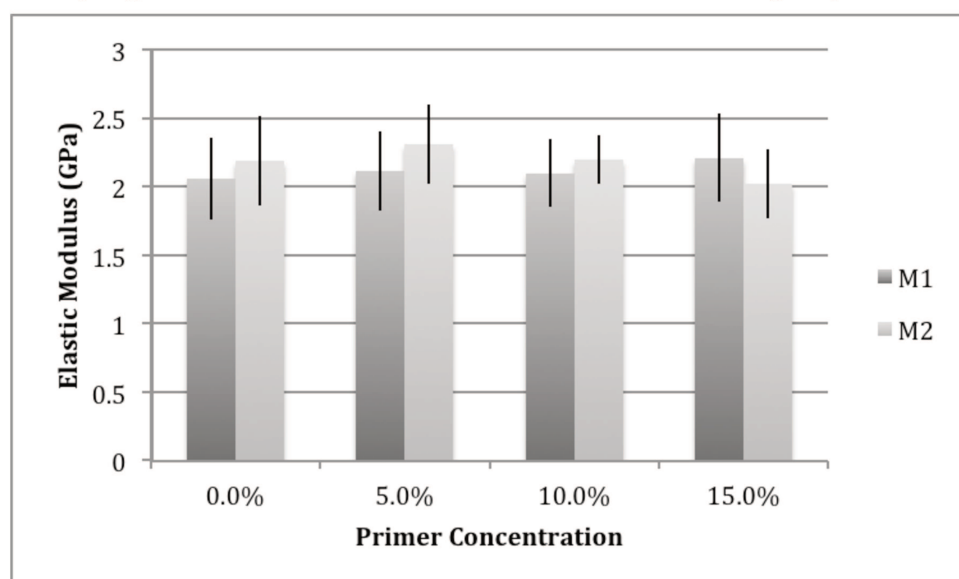


Table 1 - Means (standard deviations) for the DC (%) of the primer/adhesive mixtures

Adhesive	Primer concentration			
	0%	5%	10%	15%
M1	58.4 (1.0) A,a	47.4 (1.0) A,b	46.8 (1.7) A,b	44.2 (1.8) A,c
M2	50.6 (0.5) B,a	47.5 (1.1) A,b	40.6 (1.2) B,c	41.4 (1.3) B,c

Distinct capital letters in the same column, and lowercase letters in the line, indicate significant differences ($p < 0.05$).

The greater the primer concentration, the worse the K, and again, the M2 adhesive was shown to be the more sensitive adhesive, with final conversion decreasing as the primer concentration increased on adhesive dilution (Fig. 3). Also, with the increasing primer concentration on adhesive dilution, RP decreases were noted for manipulated adhesives (Fig. 4).

Fig 3. Polymerization kinetics of the primer/adhesive mixtures.

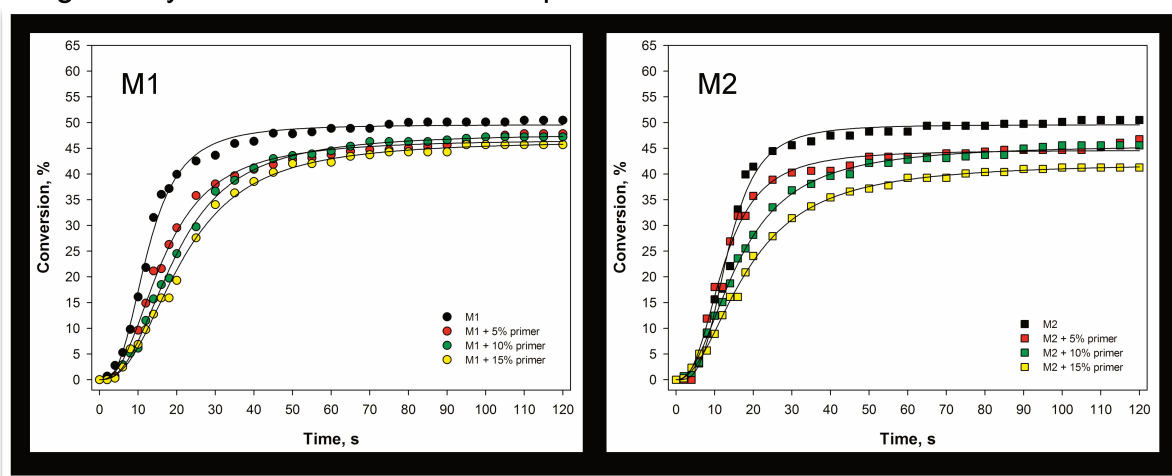
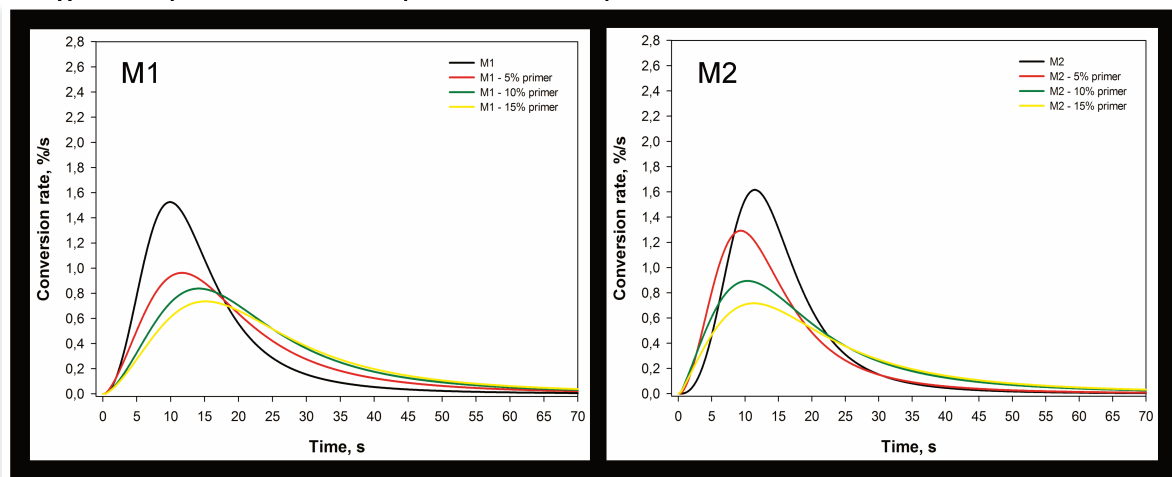


Fig 4. Polymerization rate profiles of the primer/adhesive mixtures.



The primer agent used in two-bottle dental adhesives allows for the infiltration of hydrophobic monomers into demineralised dentin, to re-expand collapsed dentin and coat the wet collagen fibrils with a hydrophilic monomer, such as HEMA.¹ However, the amount of primer could lead to excess solvent and functional monomers, resulting in a less effective polymer.^{6,13} However, in our study, the first null hypothesis was accepted, since the FS and *E* were similar in all conditions tested, independent of the primer concentration in adhesive dilutions.

The high content of HEMA and water in adhesives can cause a reduction in their mechanical properties, due to the formation of polyHEMA, a flexible and porous polymer form, jeopardising the clinical performance of the adhesives.^{2,14,15} However, in the present study, the mechanical properties were not influenced, possibly due to the high amount of Bis-GMA and TEGDMA, cross-linking monomers with a high molecular weight, used in dental adhesives to provide mechanical strength, forming densely cross-linked polymers.²

Regarding the polymerisation process, the adhesives were activated for 60 seconds. Based on the real-time conversion, it was observed that at approximately 50 seconds, the polymerisation process was almost completed for all formulations. Since the aims of the present study were to evaluate the influence of the primer content on the mechanical and chemical properties of the model adhesives, the authors evaluated the specimens at the maximum possible conversion, to guarantee that the results obtained would be due to the primer addition, and not to reduced irradiation.

Despite the fact that the addition of primer did not influence the mechanical properties of the adhesive formulations tested, the degree of conversion, kinetics and rate of polymerisation were negatively influenced by the different amounts of the hydrophilic agent. Therefore, the second null hypothesis must be rejected.

For DC, independent of the adhesive tested, with the increase of primer concentration on adhesive dilution, a decrease of DC was recorded. The M2 blend was shown to be the adhesive more sensitive to primer addition, with the degree of conversion decreasing as the primer concentration on adhesive dilution increased to reduced rates compared with those of M1. The M2 adhesive presented a composition with 20% TEGDMA. The TEGDMA monomer has a flexible aliphatic structure, with, in addition, a free fractional volume, *i.e.*, free space between chains and major lateral groups.¹⁶ These spaces appear to be crucial for molecular movement, leading the TEGDMA with its principal chain characteristic, low viscosity, since its movement also improves linear and cross-link chain formation, although, with the excess water

provided by the primer, the TEGDMA monomer, with the free space among chains^{2,17} and also its hydroxyl group,¹⁸ can easily incorporate the water present in the primer agent, compromising the cure of the adhesive.

The M1 adhesive has a high concentration of HEMA on formulation (60%). HEMA, as already mentioned, is a hydrophilic monomer, which guarantees a satisfactory degree of conversion even in aqueous environments when compared with TEGDMA, a monomer with more hydrophobic characteristics,^{2,16} explaining the high degree of conversion obtained for M1 compared with M2. However, with the hydrophilic behavior after polymerisation,^{13,18} HEMA continues to suffer hydrolysis even after polymerisation in an aqueous medium, and this characteristic can be a negative factor for adhesives containing high HEMA content compared with those formulations containing monomers such as TEGDMA.

The excess water due to the primer addition may cause difficulty for cross-link chain formation, generating a decrease in the 'jellification' phase, with a consequent decrease in the RP of adhesives^{19,20}. Also, this excess could cause a phase separation between Bis-GMA and HEMA.¹⁹, resulting in difficulty in monomer conversion, thus explaining the negative influence of the primer addition on cure characteristics of the adhesive formulations tested.

The authors remind readers that these findings were obtained under conditions far from those found clinically. The controlled temperature, standardised amounts of adhesive and primer, as well as the incorporation of primer into the adhesive as a homogeneous solution should be considered. However, even with these controlled variables, the chemical properties of the model adhesives were negatively influenced. This fact should be considered in clinical situations, since, when adhesive procedures are performed, the amount of water after acid-etching, the water present in dentinal tubules, as well as the difficulty in controlling the amount of primer content in dentin, can prejudice the quality of the polymer formed, and, consequently, the adhesive performance as well as the longevity of the bond.

Based on the results obtained, it can be concluded that the addition of primer agent did not influence the flexural strength and elastic modulus of the model adhesives tested; however, even low concentrations of primer jeopardised the degree of conversion of the adhesives, modulating the kinetics of cure, and reducing the rate of polymerisation of the dentin bonding agents.

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Effects of residual contamination by water, ethanol and chlorhexidine on mechanical properties and real-time conversion of etch and rinse adhesive

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ABSTRACT

This study evaluated the influence of 0%, 3% and 9% water, ethanol 100% (EtOH) or chlorhexidine (CHX) on two adhesive resin dilutions. A model adhesive (M1 – 40% BisGAM and 60% HEMA), and a commercially available Adper Scotchbond Multi-Purpose Adhesive (SBMP) were tested. To evaluate the mechanical properties, specimens were prepared using bar silicon molds (7 mm X 2 mm X 1 mm), and tested through the three-point bending test, at a crosshead speed of 0,5 mm/min. DC and the kinetics of cure were measured using Fourier Transform infrared spectroscopy (FTIR). The data were statistically analyzed using a two-way ANOVA and Tukey's test ($\alpha=0.05$). The real-time polymerization was monitored for 120 seconds. All solutions presented a decrease on mechanical properties, FS and E , independent of solution concentration on dilutions. For SBMP adhesive, water and chlorhexidine seems to affect more mechanical properties when compared to EtOH results. For M1 adhesive, for all solutions tested, a decrease on FS and E could be noted with increase of dilutions ($\alpha=0.05$). When DC was evaluated, a decreased on M1 values were noted as dilutions increased, independent of solution tested. And for SBMP adhesive, an increase of DC with the increase of solution was noted especially for Ethanol, but with statistical significance also for water and CHX. For K test, adding water to adhesives, a progressive decrease could be noted on graphics. CHX dilutions presented less influence on adhesives, decreasing RP and K only with 9% dilution, but EtOH solution presented an improvement on K and RP results, especially for SBMP adhesive. In conclusion, increasing CHX, water or even EtOH concentration on adhesives dilution, a loss of mechanical properties and characteristics of formed blends of adhesives is achieved. Only EtOH and CHX in low concentration seems not interfere K and RP results of adhesives tested.

Introduction

During etching proceed, half of superficial mineral volume are removed and replaced by water (Pashley *et al.*, 2011). Water fills spaces before occupied by inorganic dentin, avoiding collagen fibrils collapse and allowing monomer infiltration by solvent and hydrophilic monomers applied (De Munck *et al.*, 2005; Guo *et al.*, 2008). An ideal hybrid layer should have water and solvents completely replaced by resin monomers, creating an interdiffusion zone with collagen and resin, guaranteeing resistance and adequate mechanical properties to resist to the challenges incurred in restoration (Pashley *et al.*, 2011). However, independent of adhesive technique used, a complete water and solvent elimination are practically impossible to be achieved (Pashley *et al.*, 2007; Pashley *et al.*, 2011), and located spaces rich on water and poor of monomers are commonly identified (Pashley *et al.*, 2007), which impairs the restorative stability by hindering degree of conversion on the hybrid layer monomers (Guo *et al.*, 2008).

In order to guarantee the substrate adhesion stability, some preventives approaching techniques, as rinse cavity with Chlorhexidine or with ethanol after etching are proposed by actual literature (Pashley *et al.*, 2004; Carrilho *et al.*, 2007b; Pashley *et al.*, 2007; Shin *et al.*, 2009). Between interfibrillar spaces, after etching and rinse steps, besides water, proteoglycans highly hydrated, with negative charge forms a hydrogel through the interfibrillar spaces (Pashley *et al.*, 2004). These hydrogel contain also metalloproteinase's enzymes that regulate metabolism of collagen-based tissues (Pashley *et al.*, 2004; Carrilho *et al.*, 2007b). The application of Chlorhexidine 2%, a metalloproteinase inhibitor, acts inhibiting the collagenolytic and gelatinolytic activities in partially demineralized dentin that is treated with the etch-and-rinse adhesives (Carrilho *et al.*, 2007b) decelerating degradation. Meanwhile, the ethanol wet-bonding was developed to enhance the durability of etch-and-rinse adhesives (Pashley *et al.*, 2007) by chemically dehydrate acid-etched demineralized dentin matrices, resulting in lateral shrinkage of collagen fibrils that causes an increase in the width of their interfibrillar spaces and a reduction in the hydrophilicity of the collagen matrix (Pashley *et al.*, 2007; Shin *et al.*, 2009).

Despite the effectiveness of these solutions regarding the improvement of bonding strength and restoration longevity (Carrilho *et al.*, 2007a; Pashley *et al.*, 2007), the application techniques of both EtOH and CHX are not very known in the actual literature (Carrilho *et al.*, 2007b; Pashley *et al.*, 2007). Once the water excess generates complications on hybrid layer (Pashley *et al.*, 2007; Guo *et al.*, 2008), and the CHX used as cavity rinse are mostly manipulated as an aqueous vehicle, these

quantities may influence degree of conversion as mechanical properties of formed polymer (Elliott *et al.*, 2002). Further, the EtOH excess, causes a gained on organic solvent quantities of adhesive system and may alter the colligative properties of solutions, *i.e.*, lower the freezing point and vapor pressure, and increase the osmotic pressure and boiling point solvents (Holmes *et al.*, 2007). Also, this EtOH excess may generate a solvent saturation at interdiffusion zone, increasing fluidity of monomeric system applied defaulting crosslinking of monomer resin applied after rinse (Pashley *et al.*, 1998). As consequence, a reduction of degree of conversion may occurs causing decrease on mechanical properties of formed polymer (Holmes *et al.*, 2007; Stansbury, 2012).

Therefore, as the influence of these solutions on adhesive monomers and the consequences of its excess on quality of formed polymer should be better elucidated, this study has as objective evaluate the influence of different concentrations of solutions used as residual cavity rinse on adhesive dilution, evaluating the mechanical properties and kinetic of conversion of different adhesive systems. The first null hypothesis tested was that water, CHX or ethanol solutions have no influence over mechanical properties of adhesives tested independent of dilution concentration. And the second null hypothesis was that, increasing dilution concentration of adhesives has no influence at the kinetic of conversion as at the rate of polymerization, independent of adhesive and solution tested.

Materials and Methods

Adhesive Systems

Bisphenol-A glycidyl dimethacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), HEMA and camphorquinone (CQ) were generously supplied by Esstech (Essington, PA, USA). 4-dimethylamine benzoate (EDAB) was purchased from Sigma-Aldrich (Milwaukee, WI, USA). All reagents were used without further purification.

Two different formulations of light-cured, model unfilled dental resins were evaluated: M1 (40 wt% Bis-GMA and 60 wt% HEMA) and a commercial adhesive, (Adper Scotchbond Multi-Purpose Adhesive – 3M/ESPE, St. Paul. MN, USA).

For M1 manipulated adhesive, Bisphenol A glycidyl dimethacrylate (96% BisGMA), tri-ethyleneglycol dimethacrylate (96.5% TEGDMA), 2-hydroxyethyl methacrylate (99.5%, HEMA) and camphorquinone (97%, CQ) were gently supplied by Esstech, Inc. (Essington, PA, USA). The 4-dimethylamine benzoate (98% EDAB) were purchase from Aldrich chemical (Milwaukee, WI, USA). All monomers were

used without further purification. To blend, 1 mol% of CQ and 2 mol% of EDAB were added as photoinitiation system (Ogliari et al., 2008). The materials were prepared through intensive mixture (Magnetic Mixture, MPL Laboratory Products, Piracicaba, Brazil) in dark room with controlled temperature and humidity. All materials are listed at Table 1.

Table 1. Description of materials used and their formulations.

Description	Composition	Manufactured
Adper Scotchbond Multi-Purpose Adhesive *	- Bis-GMA (60-70%) - HEMA (30-40%)	3M ESPE Dental Products St. Paul, MN
M1	- Bis-GMA (40%) - HEMA (60%)	Manipulated adhesive

* The exact percentages of monomers are protecting by manufactures patents.

Mechanical Properties

The experiment was performed according to the ISO 4049/2009 for dental polymers, except to the sample dimensions. Each adhesive were tested mixed with 0; 3 or 9% of primer (n=6). The mix of primer and adhesive were prepared, vigorous agitated to promote a homogeneous solution and then inserted in the silicone mold. Specimens were prepared according to a previous study protocol (Gaglianone *et al.*, 2012). Except for solvent evaporation step. Twenty microliters (μL) of each adhesive/primer solution were placed into a silicon mold, to prepare a specimen with a bar shape (7 mm X 2 mm X 1 mm).

Before light-activation, a Mylar strip was placed over the mold in an attempt to obtain a flat sample surface and prevent the inhibition of cure by the oxygen. Adhesives were irradiated using a light-emitted diode source (LED Elipar FreeLight2; 3M/ESPE, St. Paul. MN, USA) for 60 seconds (850 mW/cm^2). This procedure was performed, since the aim of the study was evaluate the effect of primer concentration with the best condition of cure available.

After storage for 24 h on dry container, specimens were subjected to three-point bending test for measure the flexural strength (FS) and elastic modulus (E), at a crosshead speed of 0.5 mm/min using a universal testing machine (Instron model 4411, Instron Corp., Canton, MA, USA). Prior to the test, the dimensions of each specimen were recorded with Bluehill 2 software (Instron Corp., Canton, MA, USA), which calculated the E (GPa) and FS (MPa), according to the dimensions and tension.

Degree of conversion

Degree of Conversion (DC) of bonding agents was measured using Fourier Transform infrared spectroscopy - FTIR (Spectrum 100 Optica; PerkinElmer, MA, USA), equipped with an attenuated total reflectance (ATR) device that is composed of a horizontal ZnSe crystal 10 (Pike Technologies, Madison, WI, USA). The specimens evaluated were the same tested for mechanical properties (n=5).

A preliminary reading for uncured material was taken under the following conditions: 1665–1580 cm^{-1} frequency range, 4 cm^{-1} resolution, Happ-Genzel apodization, in absorbance mode. Additional FTIR spectra were obtained immediately after light- curing. Specimens were placed on the horizontal face of the ATR cell with a constant weight of 90bar.

DC was calculated using a baseline technique (Rueggeberg et al., 1990), based on band ratios of 1638 cm^{-1} (aliphatic carbon-to-carbon double bond) and, as internal standard, 1608 cm^{-1} (aromatic component group) between the polymerized and uncured samples (n=5).

Conversion Kinetics and Rate of Polymerization

The real-time polymerization (K) was evaluated by Fourier transform infrared spectroscopy (Spectrum 100 Optica; PerkinElmer, MA, USA) as previously described, using an attenuated total reflectance device composed of a horizontal ZnSe crystal. A constant volume (5 μL) of bonding at neat form, or at 2 or 9% dilution in Water, CHX or Ethanol solution was dispensed immediately after mixture onto the crystal and photo-activated for 120s using a Light Emitted Diode source with 850 mW/cm^2 irradiance (LED Elipar FreeLight2; 3M/ESPE, St. Paul. MN, USA). The polymerization reaction was monitored in real time for those 2 min using Happ-Genzel apodization, collecting spectra in the 1680 to 1540 cm^{-1} range, with a resolution of 8 cm^{-1} . With this setup, one spectrum (one scan) every two seconds was acquired. Three specimens were tested for each bond/primer concentration.

The DC for each scan was calculated as previously described. Average conversion vs. time data was plotted and Hill's 4-parameter non-linear regressions were used for curve fitting. As the coefficient of determination was greater than 0.99 for all curves, the rate of polymerization (RP) was calculated using these data-fitted plots.

Statistical Analyses

The mechanical properties (FS, *E*), as well as as the DC were analyzed by two-way ANOVA and Tukey's test, at a 0.05 level of significance, having "adhesive" and "solution concentration" as the two variables (SAS 9.1 version – The SAS Institute, Cary, NC, USA).

Results

Flexural Strength

Decomposition ANOVA showed significant effect only for isolated variables, adhesive and solution concentration, with no statistical significance for interactions. For M1 adhesive, Ethanol, Water and CHX solutions demonstrated a decrease of flexural strength as increasing the concentration of solutions on adhesives (Table 2). For SBMP adhesive, only the ethanol dilution showed no statistical difference independent of concentration dilution. For both water and CHX solutions, as increase the concentration of dilution a decrease of results could be noted (Table 2).

Table 2. Means (SD) for Flexural Strength (Mpa) of Adhesive Systems Testing Different Dilutions with Water, Ethanol and Chlorexidine

Adhesive	Solution	Concentration		
		0%	2%	9%
M1	Ethanol	156,37 (19,03) Aa	149,87 (15,12) Aab	121,17 (2,62) Ab
	Water	173,26 (30,53) Aa	153,70 (20,70) Aa	116,09 (15,62) Ab
	CHX	146,10 (9,78) Aa	134,27 (14,26) Aab	114,10 (7,40) Ab
SBMP	Ethanol	142,79 (13,71) Aa	141,28 (12,75) Aa	113,91 (19,42) Aa
	Water	151,20 (21,79) Aa	133,72 (7,54) Aab	103,11 (13,15) Ab
	CHX	138,18 (18,40) Aa	118,60 (10,38) Aab	90,42 (19,22) Ab

For each adhesive means followed by distinct capital letters in the same row and distinct small letters in the same column are significantly different at $p < 0.05$ - ANOVA 2-Way and Tukey test comparing means. *Indicate statistical differences between adhesives at same solution concentration.

Elastic Modulus

When elastic modulus (E) were evaluated, decomposition ANOVA presented significance effect for factors solution, adhesive and concentration and also for double interactions “solution*adhesive”, “solution*concentration” and “adhesive*concentration”, with no significance for triple interactions. For M1 adhesive, increasing dilution concentration a decrease on E could be noted independent of solution tested (Table 3). For Ethanol solution, when SBMP were evaluated, adhesives presented worse behavior at 0% and 9% concentrations dilution, and best results with statistical significance difference at 2% ($p \leq 0.05$).

When compared the solutions, for SBMP adhesive, at 0% of dilution the water solution presented best results when compared to others solutions at same concentration. For M1 adhesive, only at 2% of water dilutions the adhesive presented best results when compared to other solutions at same concentration.

For comparisons between adhesives, only for CHX at 2% and water at 9% statistical significance showed that SBMP presented best behavior when compared to M1 adhesive (Table 3).

Table 3. Means (SD) for Elastic Modulus (GPa) of Adhesive Systems Testing the Different Dilutions with Water, Ethanol and Chlorexidine

Adhesive	Solution	Concentration		
		0%	2%	9%
M1	Ethanol	2,10 ($\pm$ 0,38) Aa	1,95 ($\pm$ 0,18) ABab	1,70 ($\pm$ 0,09) Ab
	Water	2,41 ($\pm$ 0,23) Aa	2,25 ($\pm$ 0,19) Aa	1,70 ($\pm$ 0,15) Ab*
	CHX	2,20 ($\pm$ 0,06) Aa	1,88 ($\pm$ 0,16) Bab*	1,55 ($\pm$ 0,08) Ab
SBMP	Ethanol	2,04 ($\pm$ 0,08) Bab	2,31 ($\pm$ 0,08) Aa	1,95 ($\pm$ 0,26) ABb
	Water	2,53 ($\pm$ 0,20) Aa	2,36 ($\pm$ 0,10) Aab	2,21 ($\pm$ 0,21) Ab*
	CHX	2,14 ($\pm$ 0,09) Ba	2,14 ($\pm$ 0,15) Aa*	1,72 ($\pm$ 0,12) Bb

For each adhesive means followed by distinct capital letters in the same row and distinct small letters in the same column are significantly different at $p < 0.05$ - ANOVA 2-Way and Tukey test comparing means. *Indicate statistical differences between adhesives at same solution concentration.

Degree of Conversion

For degree of conversion (DC) results, only dilutions and solutions at same adhesive were compared. Comparisons between adhesives were not evaluated once for DC, comparisons between materials with different compositions were not the best form to demonstrate their best or worse behavior (Arrais *et al.*, 2009; Cadenaro *et al.*, 2009a; Aguiar *et al.*, 2010).

The M1 adhesive showed best results for CHX solutions when comparison between solutions was evaluated ($p \leq 0.05$) and worse results for water dilutions at 9% (Table 4). For SBMP adhesive, at 2% of dilution, CHX presented the worse results, with statistical significances, and at 9% of dilution CHX and water presented similar worse results ($p \leq 0.05$) when compared to Ethanol dilution (Table 4).

For comparisons at same adhesive and same solution, the M1 adhesive, for all solutions tested, presented a decrease on values as the concentration of dilution increased, with statistical significance. For SBMP adhesive, the DC increased as ethanol concentration was increased on dilution, showing statistical significant differences at each concentration dilution tested ($p \leq 0.05$). For water solution, the results were increased only with higher of water on dilution independent of water concentration, and for CHX, only with the increasing for 9% of dilution on SBMP adhesive, an increase with statistical significance difference could be noted ($p \leq 0.05$) (Table 4).

Table 4. Means and Standard Deviations (SD) for DC (%) of Adhesive Systems Testing the Different Dilutions with Water, Ethanol and Chlorhexidine

Adhesive	Solution	Concentration		
		0%	2%	9%
M1	Ethanol	68,97 ($\pm 0,38$) Aa	58,20 ($\pm 0,92$) Bb	55,61 ($\pm 0,36$) Bc
	Water	58,36 ($\pm 1,00$) Ba	57,52 ($\pm 0,61$) Ba	52,59 ($\pm 1,12$) Cb
	CHX	68,97 ($\pm 0,38$) Aa	68,74 ($\pm 0,59$) Aa	65,62 ($\pm 0,77$) Ab
SBMP	Ethanol	57,47 ($\pm 0,55$) Ac	60,22 ($\pm 0,29$) Ab	67,97 ($\pm 0,78$) Aa
	Water	57,47 ($\pm 0,55$) Ab	60,22 ($\pm 0,70$) Aa	60,35 ($\pm 1,33$) Ba
	CHX	57,47 ($\pm 0,55$) Ab	58,30 ($\pm 0,80$) Bb	61,56 ($\pm 1,67$) Ba

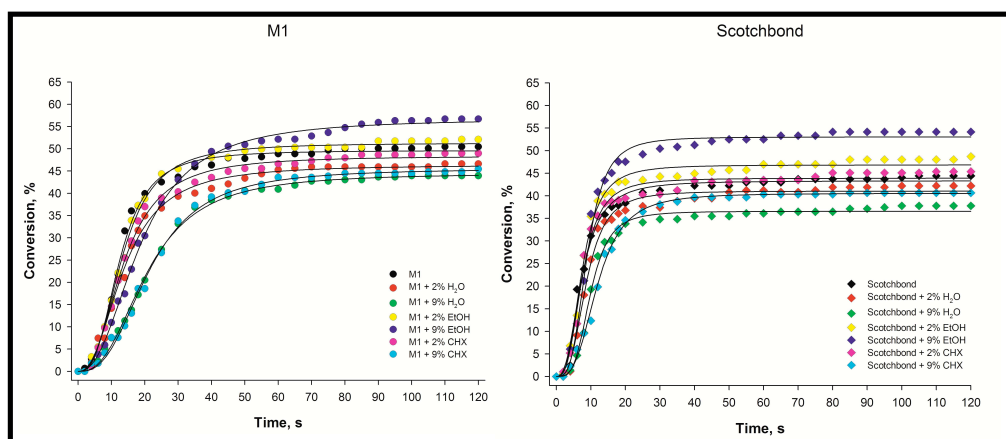
For each adhesive means followed by distinct capital letters in the same row and distinct small letters in the same column are significantly different at $p < 0.05$ - ANOVA 2-Way and Tukey test comparing means.

Kinetic of Conversion

Independent of the adhesive tested, the groups containing 9% of water or CHX on adhesive dilution, were those who had most influence on K peaks, presenting the water solution the worst results, reducing K for maximum of 40%.

Although, for all tested adhesives, the K results demonstrated an increase on peaks for ethanol solution on dilution, with the most noticeable increase for adhesives without solvent on blend, as SBMP, witch presented at neat form 44% of conversion passing to 54% with 9% of ethanol solution on its dilution.

Figure 1. Kinetic Conversion Graphic of water, Ethanol and CHX concentrations dilutions (%) versus time (s).



Rate of Polymerization

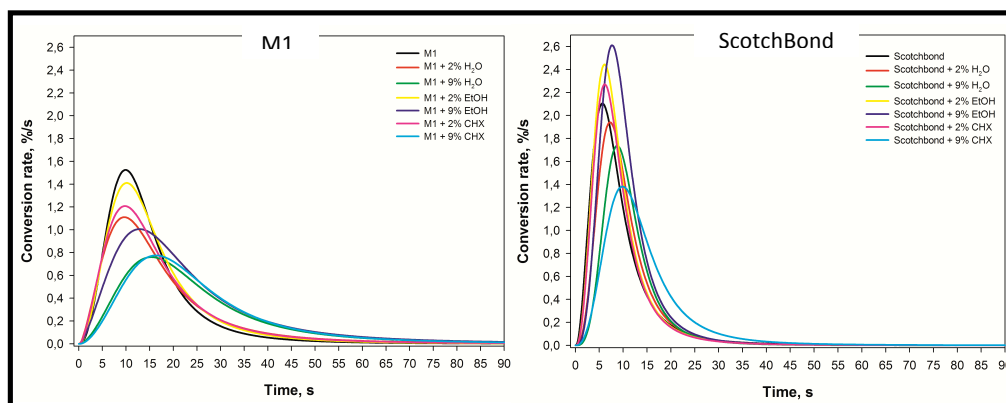
The best Rate of polymerization (RP) result were presented by SBMP adhesive, who showed an increase in RP for the 9% ethanol solution on adhesive dilutions, result even better than the 2% ethanol solution. Also, for SBMP, the 2% CHX dilution presented best results when compared to the neat form. The SBMP at neat form showed only best results when compared to 2 and 9% of dilution with water and 9% with CHX solutions (Figure 2).

After SBMP, the M1 adhesive was the adhesive how presented the best Rp, presenting however half of SBMP peaks values. The M1 adhesive presented best results at neat form and showed to be very sensitive to water, with worst results for 9% of water on dilution followed by 9% of CHX.

The adhesives with worst Rp were adhesives with solvent on formulation, M1/Acetone and M1/Ethanol, presenting best results at neat form, with 2% of ethanol

or with 2% of CHX on dilutions. And worst results with 2 and 9% of water on adhesives dilution respectively.

Figure 2. Rate of Polymerization (R_p) Graphics of water, Ethanol and CHX concentrations dilutions (%) versus time (s).



Discussion

The experimental results denied the first null hypothesis, once the increases of water, CHX or Ethanol, influenced the mechanical properties of resin polymer formed. For both adhesives, the M1 manipulated and SBMP, increasing the dilution concentration, independent of solution tested, a decrease on FS and E could be noted. For SBMP adhesive, only the ethanol dilution showed no statistical difference independent of concentration dilution. For water and CHX solutions as increase the concentration of dilution a decrease of results could be noted (Table 2). These results corroborate with some authors (Ito *et al.*, 2005; Guo *et al.*, 2008), which defending that further dilutions of the model resin separates photoactivation components and also dilutes radical concentration. Increased dilution also separates resin components from the growing polymer chain, decreasing the overall extent of conversion. As the physical space between possible reactive species of the polymeric reaction is increased by increasing solvent it leads to decreased monomer conversion, confirmed as DC also decrease with increasing solution concentration. Lastly, as solvent concentration increases, oxygen diffusion into the mixture would increase as a result of the decrease in viscosity. Increasing oxygen content may also contribute to the decline in conversion observed when solvent concentrations of higher amounts were tested (Holmes *et al.*, 2007).

To SBMP adhesive, the 2% CHX and 9% Water dilution presented best results with statistical significance when compared to M1 adhesive. Since M1 formulation contain higher HEMA concentration (60%), a higher mobility chain is predictable, and could difficult the crosslinking process, different of SBMP adhesive, a more hydrophobic characteristics adhesive, since only the covered monomer bottle was used, containing 65% of BisGMA, ensuring a higher quality of formed polymer (Van Landuyt *et al.*, 2007). However, this monomer presents worse behavior when associated with increased concentrations of water, decreasing the chain quality formed (Pashley *et al.*, 2011), explained once at 9% of water or CHX, a worse *E*, DC and FS result could be noted.

Nevertheless, for SBMP adhesive, when evaluated the DC, for all solutions, CHX, water or EtOH, an increase on results with statistical significance difference could be noted. This DC increased could be explained by the increase of resin viscosity, once with this system, as already mentioned, only the hydrophobic cover adhesive layer was used standardizing the hydrophilicity of adhesive, since the primer variable were not an objective at this study (research send for publication of this group). Increasing the adhesive viscosity, an increase of chain mobility may occurs, facilitating radicals diffusion thru chains, increasing crosslinking (Elliott *et al.*, 2002). Also, this DC increases indicates that DC increase should not be associated to mechanical properties improvements (Pashley *et al.*, 2004; Carrilho *et al.*, 2007a), since adding solvent to the reaction increases the probability of cycling, due to the diluted concentration of monomer and solvent rate of polymerization, causing the local radical on its own chain to remain longer in close proximity to pedant double bonds (Ye Q, 2006), creating more linear reactions decreasing network quality.

Different of SBMP, for M1 adhesives, increasing diluents concentration caused a decrease on DC results (Table 4). In dental adhesive formulations, hydrophobic components, such as Bis-GMA, are usually employed to enhance the mechanical properties and compatibility with restorative resin composites (Van Landuyt *et al.*, 2007; Guo *et al.*, 2008). Hydrophilic components, such as HEMA, are employed to improve water-compatibility and enhance the infiltration of adhesives into wet, demineralized dentin (Spencer *et al.*, 2002; Van Landuyt *et al.*, 2007). In the presence of water, phase separation of the uncured hydrophobic/hydrophilic components can easily develop (Spencer *et al.*, 2002). The presence of water and monomer phase separation affects the polymerization rate and degree of conversion, and induces an inhomogeneous structure of the cured adhesive, which might be a potential mechanism for degradation (Abebe *et al.*, 2005; Pashley *et al.*, 2007;

Pashley *et al.*, 2011). Therefore, the performance of photoinitiator in presence of water becomes very critical, extended by CHX, a solution with an aqueous vehicle.

The second null hypotheses were also denied, since increasing dilutions concentrations an interference at the K and RP results of all adhesives systems tested could be noted. Increasing water and CHX concentration, a decrease at peak loaded for K could be noted independent of adhesive analyzed, resulting at maximum of 35-40% conversion to all adhesives systems tested (Figures 1). The worsts results, with greatest decrease at load peak were presented by higher dilutions of water followed by CHX. Water and CHX excess, known as aqueous agents, generated a high efficacy on polymer plasticization (Shin *et al.*, 2009). Hydrogen bridges between water and polar hydroxyl, carboxylate, or phosphate group of polymer networks disrupt interchain hydrogen bonding, altering the molecular structure and increasing the segmental mobility of polymer chain segments (Z.H Ping, 2001; Ito *et al.*, 2005). These changes are reflected by reduction in the mechanical properties, and decline in the glass transition temperatures of the polymer resins difficult the final polymer formation (Nogueira P, 2001).

Meantime, when adhesives where diluted to Ethanol concentrations, all adhesives presented an increase to K loaded peak, with increasing dilutions presenting increasing peaks (Figures 2). Since, at the presented study, the K loaded peaks surfed an increase with EtOH addition, and as this solvent is a very common organic solvent used as vehicle at adhesives systems, ethanol solvent seems acts decreasing the viscosity of comonomer blends, allowing radical propagation to continue longer without the reaction being diffusion controlled (Ye *et al.*, 2007). Notwithstanding, this solvent excess, despite increases the K peaks, influenced at a negative form the final system conversion (Table 4). It may be hypothesized that the anticipated influence of the higher polarity and hydrogen bonding values of ethanol interfere with free radical formation and lead to quenching of the polymerization reaction, reducing the temperature of solvent/resin mixture, reducing the overall conversion. Solvent can also absorb heat generated during the polymeric exothermic reaction, also decreasing the overall rate and extend of cure (Lee TY, 2004).

When RP were evaluated, a significance decrease at loaded peaks could be noted when maximum dilution concentrations groups where analyzed, independent of adhesive and solution tested. We should hypothesize that adding solvent to the reaction, or at this case, water, CHX or EtOH, increases the probability of cycling, due to the diluted of monomer, slowing the all rate of polymerization causing the local radical on its own chain to remain longer in proximity to pedant double bonds (Ye Q,

2006). In addition, the dilutions concentrations during polymerization will also change the gel-point conversion of polymer because of varying degrees of cyclization, which is demonstrated by the photopolymerization kinetics presented in this study, resulting also in decrease quality of polymer formed (Elliott *et al.*, 2002), also confirmed, once FS and *E* values suffered a significant decrease with increasing concentrations dilutions of water, CHX and EtOH on adhesives tested.

Once demonstrated the effectiveness of EtOH and CHX as pre-treatment techniques in reference of longevity and adhesive strength (Carrilho *et al.*, 2007a; Carrilho *et al.*, 2007b; Pashley *et al.*, 2007), the present study demonstrated that these techniques should be used carefully, with a controlled amount of rinse and controlling the remained solution in cavity, avoiding at maximum monomers dilution which may create loss of characteristics of the formed polymer and also decreasing quality of bonding system. With all this study limitations, clinically, the authors should hypothesis that once applied CHX or EtOH to cavity before bonding procedure, if a controlled remained humidity are achieve, the clinician guarantee a very stable hybrid layer formation with formation of a polymer with high qualities and satisfactory restorative longevity.

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Conclusão

Considerando os resultados deste estudo pôde-se concluir que:

- Diferentes quantidades de primer incorporadas ao sistema adesivo não influenciaram as propriedades mecânicas de adesivos convencionais;
- Aumentando-se a diluição do sistema adesivo com o primer houve diminuição no grau de conversão, cinética de conversão, e na taxa total de polimerização, acarretando diminuição da qualidade do polímero nesta condição;
- O aumento das diluições com água e clorexidina no adesivos indicados para a técnica do condicionamento total interferiu negativamente nas propriedades mecânicas pela diminuição dos valores de resistência à flexão, módulo de elasticidade e grau de conversão, prejudicando a qualidade do polímero formado;
- Somente quando o sistema adesivo foi diluído com álcool a 9% houve influência nos resultados de propriedades mecânicas. Sendo que para adesivos mais hidrófobos, houve aumento nos valores de resistência à flexão, módulo de elasticidade e grau de conversão quando aumentou-se as diluições com álcool;
- Para os resultados de cinética e taxa de conversão, o aumento da concentração de solventes como a água e a clorexidina levou a uma redução na cinética;
- Para diluições com Álcool, um aumento na cinética foi notado; e a taxa de conversão só foi afetada, com observando-se redução nos gráficos, na concentração máxima de diluição – 9%.

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Apêndice

Materiais e Métodos

Para realização deste estudo, foram utilizados dois adesivos manipulados em laboratório: M1 e M2; e um adesivo comercial como controle, Adper Scotchbond Multi-Purpose Plus (SBMP – 3M/ESPE, St. Paul, Min, USA) (Figura 1).



Figure 1 - Sistemas adesivos após manipulação em frasco de armazenamento e adesivo comercial Scotchbond Multi-Purpose Plus (3M/ESPE, St. Paul, USA)

Os adesivos foram manipulados de maneira semelhante aos adesivos comerciais não hidrófobos sovatados para a técnica clínica de condicionamento ácido prévio a aplicação do sistema adesivo. Os monômeros utilizados foram gentilmente fornecidos pela empresa Esstech (Essington, PA) e pela empresa Aldrich Chemical (Milwaukee, WI). Todos monômeros foram utilizados sem demais purificações, sendo a adição de 1 mol% de Canforoquinona (CQ) e 4-Etildimetilaminobenzoato (EDAB) usados como iniciadores fotopolimerizáveis (Figura 2a e 2b).

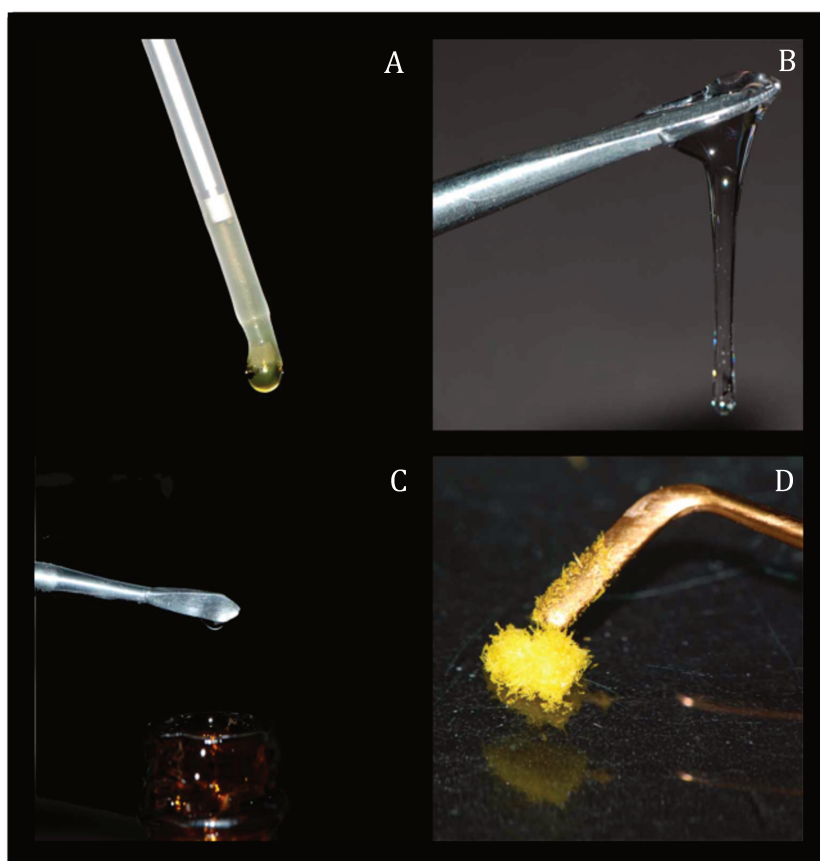


Figure 2a - Monômeros utilizados no estudo: TEGDMA (A), Bis-GMA (B) e HEMA (C) e Canforoquinona (D)

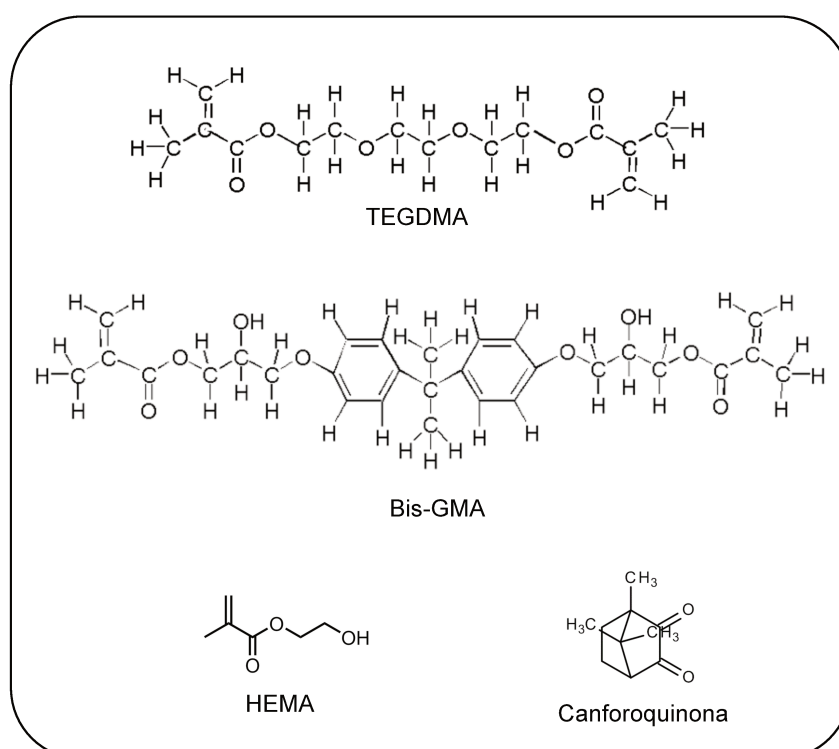


Figure 3b – Estrutura molecular de cada monômero utilizado.

Os adesivos foram preparados em sala escura com temperatura e umidade controladas. As quantidades de cada monômeros foram calculadas de acordo com a porcentagem de cada adesivo e seu peso molecular, sendo que suas quantidades finais foram obtidas por peso em balança analítica e misturadas em Misturador Magnético por 30 minutos, sob inspeção visual constante, evitando a perda de material e a não homogeneização completa dos componentes monoméricos (Figura 3).

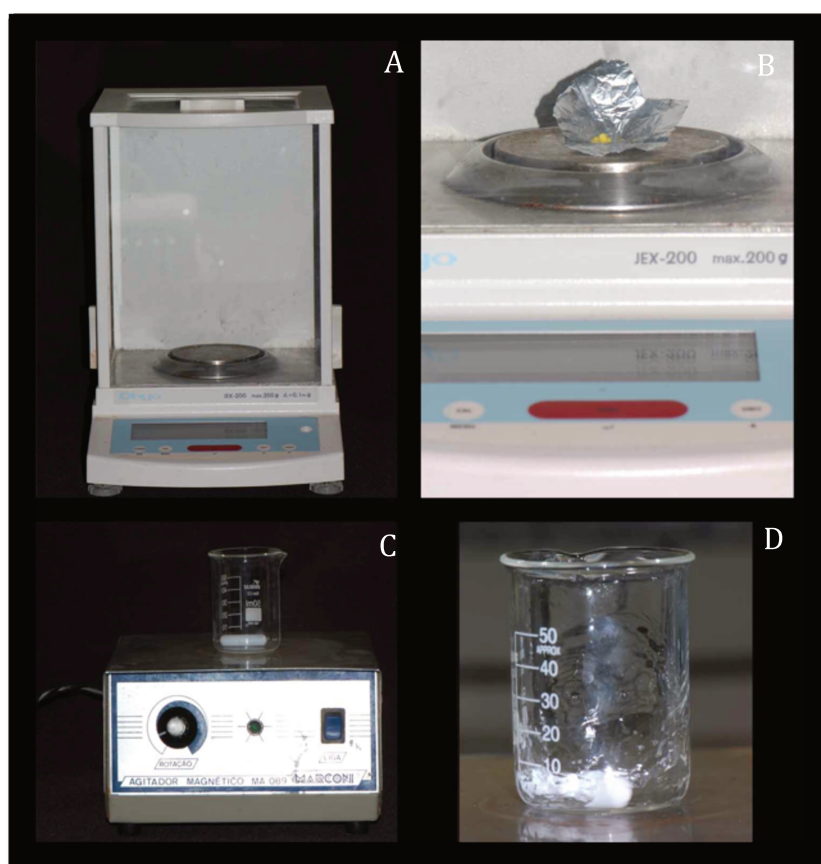


Figure 4 - Equipamento utilizado para medição e mistura dos monômeros: Balança de Precisão (A e B) e Misturador Magnético (C e D)

Os grupos foram divididos de acordo com os fatores em estudo de cada experimento. No Capítulo 1, em que foi avaliada a influência de diferentes concentrações de primer ScotchBond Multi-Purpose Plus Primer (3M/ESPE, St. Paul, USA): 0%, 5%, 10% e 15% (Figura 4) nas propriedades de dois diferentes sistemas adesivos de condicionamento prévio de três passos, M1 e M2 (n=6).

Já para o Capítulo 2, o objetivo do estudo foi avaliar a influência de diferentes concentrações (0%, 2% e 9%) de água, álcool e clorexidina (Figura 4) nas propriedades de dois sistemas adesivos de condicionamento prévio: M1 e ScotchBond Multi-Purpose Plus Adhesive (3M/ESPE, St. Paul, USA).

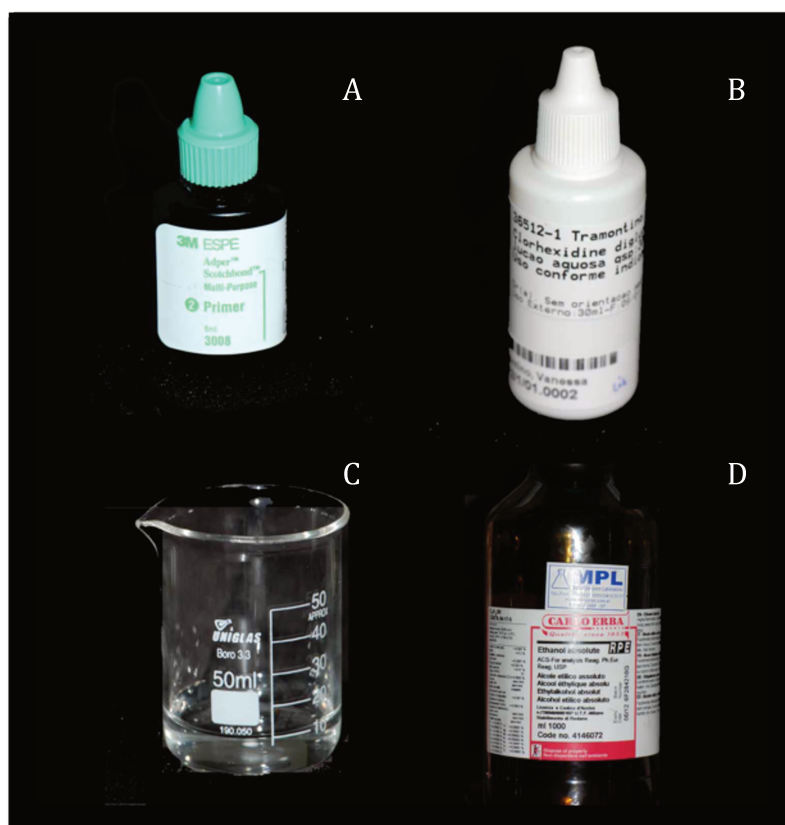


Figure 5 - Soluções utilizadas para diluição nos sistemas adesivos do Capítulo 1 e 2: Primer Scotchbond Multi-Purpose Plus (A), Clorexidina 0,2% manipulada em veículo aquoso (B), água destilada (C) e Álcool Absoluto - 100% (D)

Propriedades Mecânicas

Para os testes de resistência à flexão e módulo de elasticidade as amostras, tanto do Capítulo 1 quanto do Capítulo 2, foram preparadas de acordo com a ISO 4049/2009 – cap. 7.11, para teste de flexão de três pontos, com exceção das dimensões do corpo de prova.

Para confecção dos corpos de prova, foram feitos moldes em silicone de adição (Express, 3M/ESPE, St. Paul, USA) a partir impressão de matrizes de Teflon

com uma elevação interna central em forma de barra, medindo 2 mm de largura x 1 mm de altura x 7 mm de comprimento (Figura 5). Uma vez prontos os moldes de silicone, barras foram confeccionadas de acordo com os diferentes grupos experimentais supracitados inserindo-se o sistema adesivo/diluição no interior do molde e em seguida realizava-se a fotoativação.

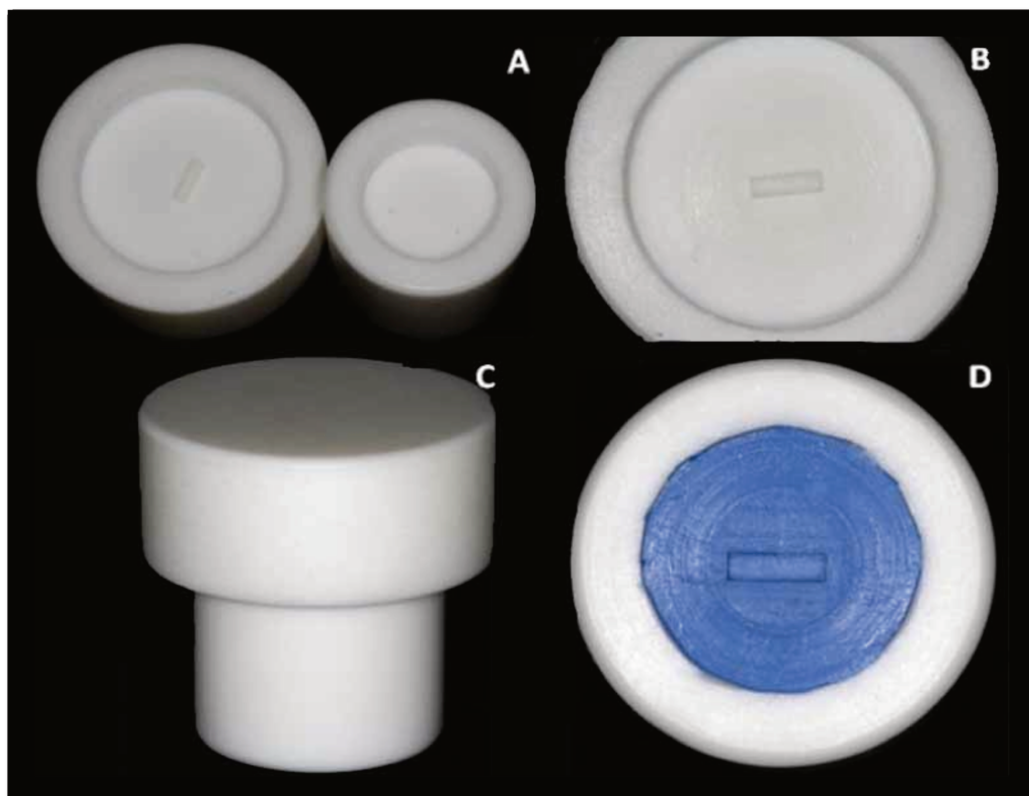


Figure 6 - Matriz de teflon. Parte superior e inferior da matriz (A); Parte superior da matriz com elevação central em forma de barra (B); matriz em posição para impressão com o material de moldagem (C); e molde em silicone de adição confeccionado a partir da matriz de teflon

Antes de serem inseridos nos moldes, os sistemas adesivos foram dispensados, em primeiro lugar, em um casulo, em seguida a quantidade de solução diluente (primer, água, álcool ou CHX) com a porcentagem determinada para cada grupo experimental também foi dispensada no mesmo casulo e vigorosamente misturadas por 30 segundos. A mistura adesivo/diluente foi inserida, com o auxílio de pipeta de precisão (Microman Classic, modelo M25, Gilson, FRA), nos moldes de silicone (Figura 6).

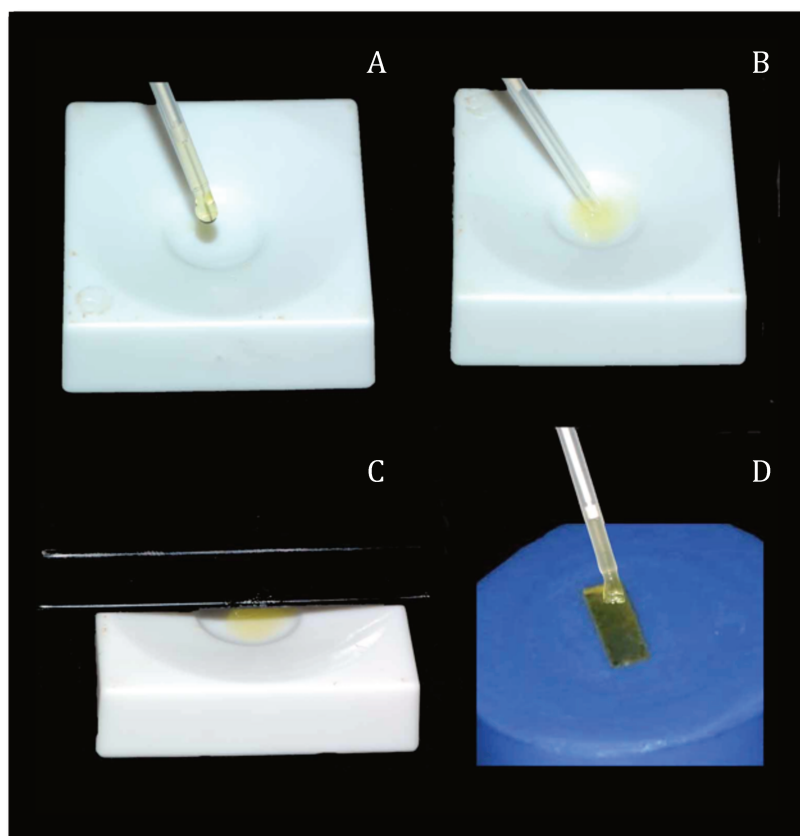


Figure 7 - Adesivo sendo inserido em casulo para diluição em solução conforme o grupo experimental (A); Adesivo e solução misturados no casulo (B); Solução protegida da luz (C); Solução inserida na matriz para confecção do corpo de prova (D).

Após a mistura, as soluções foram cobertas por uma tira de poliéster para facilitar a regularização da superfície da amostra. A fotoativação foi realizada pelo tempo de 60 segundos para cada amostra, para isto foi usado o aparelho fotoativador utilizado foi um LED de segunda geração, o Elipar FreeLight2 (3M/ESPE, St, Paul, USA) (Figura 7).



**Figure 8 - Fotoativador utilizado no estudo:
Elipar FreeLight2 (3m/ESPE, St. Paul, USA)**

Após confecção as amostras foram armazenadas em ambiente escuro com temperatura a 37°C e umidade controlada por 24 horas. As mensurações da resistência à flexão e módulo de elasticidade foram realizadas depois de decorrido o tempo da confecção das amostras através do teste de três pontos (ISO 4049/2009), conforme mostrado na figura 8. O teste foi conduzido na velocidade de 0,5 mm/min com célula de carga de 50N em Máquina de Ensaio Universal (Instron, model 4411, Buckinghamshire, England), cujos resultados foram obtidos através do programa Blue Hill II (Instron, model 4411, Buckinghamshire, England). Os valores de Resistência flexural foram calculados em Megapascal (MPa) e os de Módulo de elasticidade em Gigapascal (GPa).

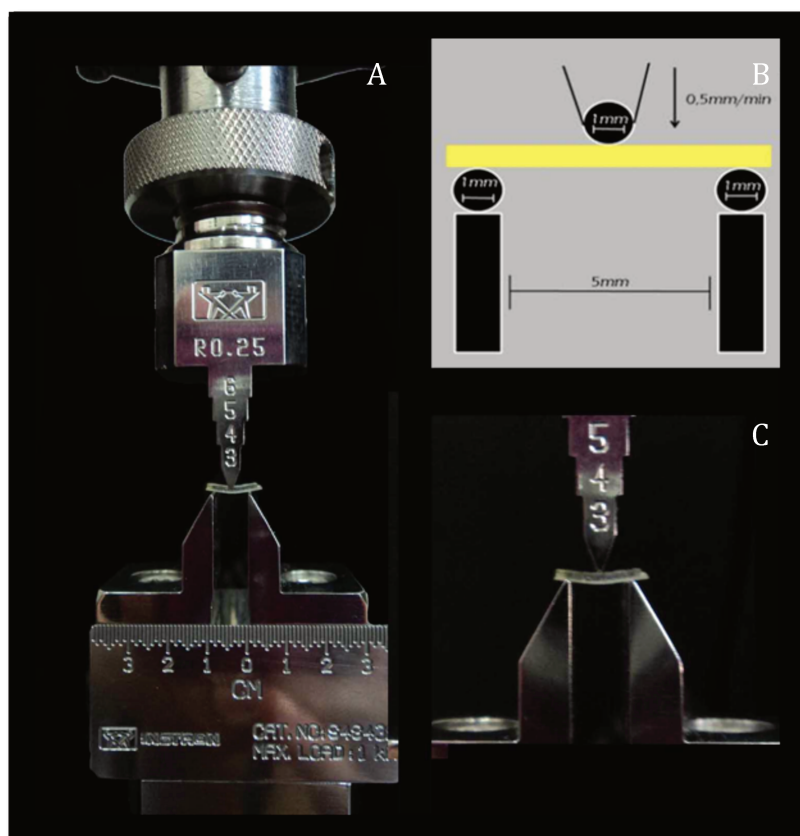


Figure 9 - Teste de resistência à flexão (A); representação esquemática do teste de resistência à flexão realizado nos estudos (B); amostra posicionada para o teste de flexão em maior aumento (C).

Grau de Conversão

Em ambos estudos foram realizadas cinco leituras do grau de conversão ($n=6$) para cada grupo experimental. As amostras foram confeccionadas da mesma maneira que para o teste de resistência à flexão, sendo as mesmas posicionadas diretamente no elemento de refletância total atenuada acoplada (ATR), do Espectrômetro de raios infra-vermelhos Transformado de Fourier – FTIR (Spectrum 100 Optica; PerkinElmer, MA, USA), o qual possui um cristal horizontal de Seleneto de Zinco (Pike Technologies, Madison, WI, USA) no centro que age como substrato ativo para os raios infra-vermelhos.

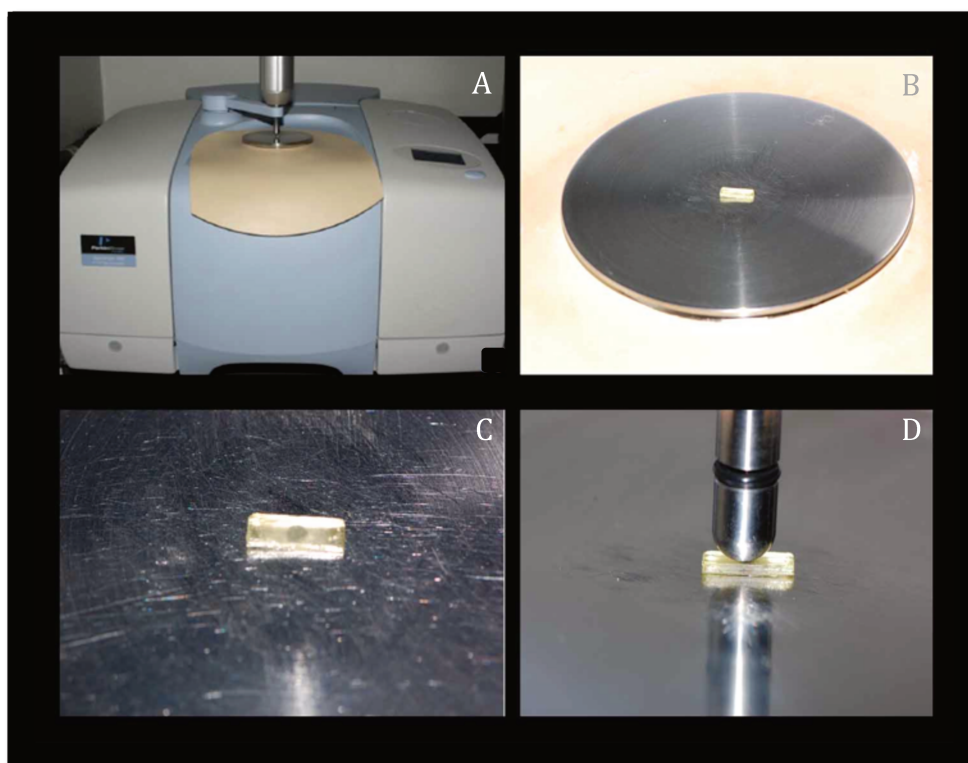


Figure 10 - Espectrômetro de Raios Infra-vermelhos Transformado de Fourier (FTIR)- (A); Plataforma de leitura com cristal de seleneto no centro e amostra posicionada sobre o cristal (B); vista aproximada da amostra posicionada sobre o cristal (C); pressão aplicada sobre a amostra para leitura (D).

Após posicionar a amostra sobre o cristal, uma pressão de 70 barr foi exercida sobre a amostra para facilitar a leitura e evitar a interferência do oxigênio e aumentar o contato da mesma com o cristal (Figura 9), padronizando-se as leituras. O ensaio foi conduzido a partir da leitura inicial de cada amostra não fotoativada, para isto, dispensou-se sobre o cristal a solução diluída, conforme grupo experimental ou estudo, na quantidade de 5µm. A leitura da solução não polimerizada foi considerada o controle para cada grupo, pois fornecia os valores das áreas correspondentes às bandas dos anéis aromáticos e alifáticos previamente a polimerização do material em estudo. Durante cada leitura foram obtidos espectros infravermelhos com o auxílio do programa Spectrum (PerkinElmer) presente em microcomputador conectado ao espectrômetro. O programa foi utilizado no modo de monitoramento da varredura usando a apodização Happ-Genzel, com redução de 4 cm^{-1} e os espectros sendo coletados no intervalo de 1665 a 1580 cm^{-1} .

Com a mesma solução utilizada para avaliação dos monômeros não convertidos, corpos de prova semelhante aos realizados para teste de resistência a flexão foram obtidos. A amostra foi posicionada sobre o cristal e leituras do

monômeros convertidos foi realizada. O cálculo do grau de conversão foi feito baseado na proporção entre as duplas ligações de carbono alifáticas (C=C) e aromáticas, usadas como controle interno por ser considerada inerente à composição do material, nos estados polimerizados e não polimerizados (Ferracane & Greener, 1985*). Durante a reação de polimerização a absorbância das duplas ligações de carbono aromáticas permanecem constantes, enquanto há a redução da quantidade de duplas ligações alifáticas. A dupla ligação de carbono alifática absorve a energia no comprimento de onda de 1638 cm^{-1} , enquanto a aromática no comprimento de 1608 cm^{-1} . Para o cálculo, foi utilizada a técnica de *baseline* (Rueggeberg et al., 1990**), traçado pelo próprio programa Spectrum (Figuras 10 e 11). A partir desta foram mensurados alguns parâmetros pelo próprio programa. A intensidade corrigida dos picos observados nos comprimentos de onda 1638 cm^{-1} e 1608 cm^{-1} foi utilizada na seguinte fórmula: $R = \text{intensidade em } 1638\text{ cm}^{-1} / \text{intensidade em } 1608\text{ cm}^{-1}$, sendo o grau de conversão (%) calculado da seguinte forma:

$$GC (\%) = \left[1 - \frac{\frac{1638\text{ cm}^{-1}}{1608\text{ cm}^{-1}} \text{ polimerizado}}{\frac{1638\text{ cm}^{-1}}{1608\text{ cm}^{-1}} \text{ não polimerizado}} \right] \times 100$$

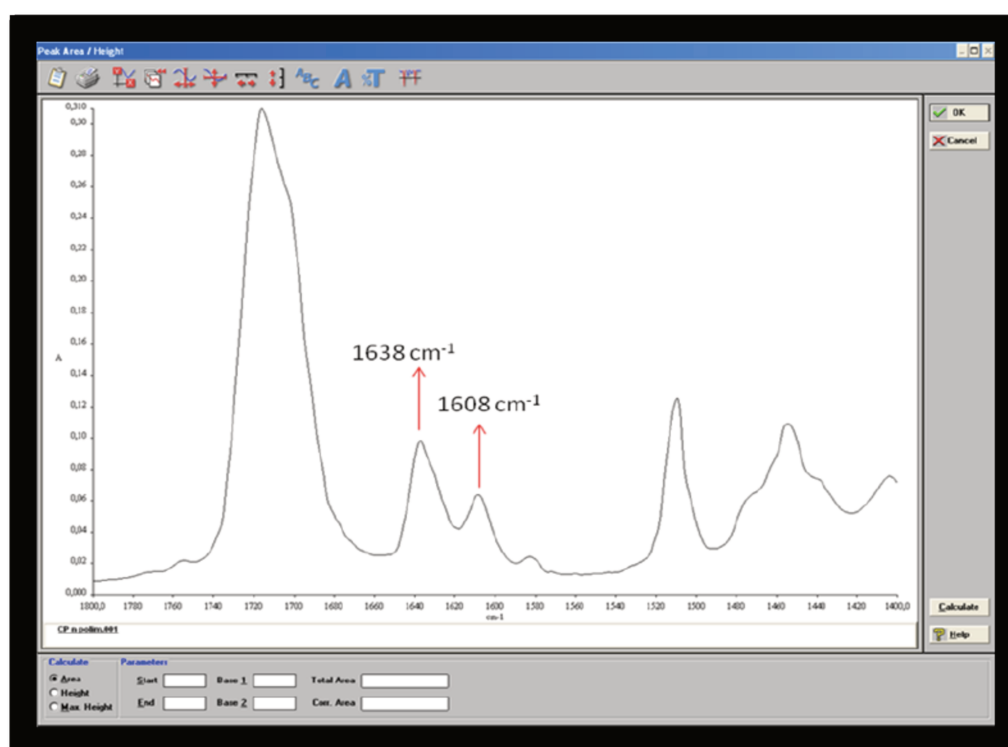


Figure 11 - Gráfico do programa Spectrum ilustrando as duplas ligações alifáticas (1638 cm^{-1}) e aromáticas (1608 cm^{-1}) de um adesivo em seu estado não polimerizado

* Ferracane, J. L., Moser, J. B., Greener, E. H. Ultraviolet light-induced yellowing of dental restorative resins. J Prosthet Dent, 1985; 54(4): 483-7

** Rueggeberg, F. A., Hashinger, D. T., Fairhurst, C. W. Calibration of FTIR conversion analysis of contemporary dental resin composites. Dent Mater 1990; 6(4): 241-9.

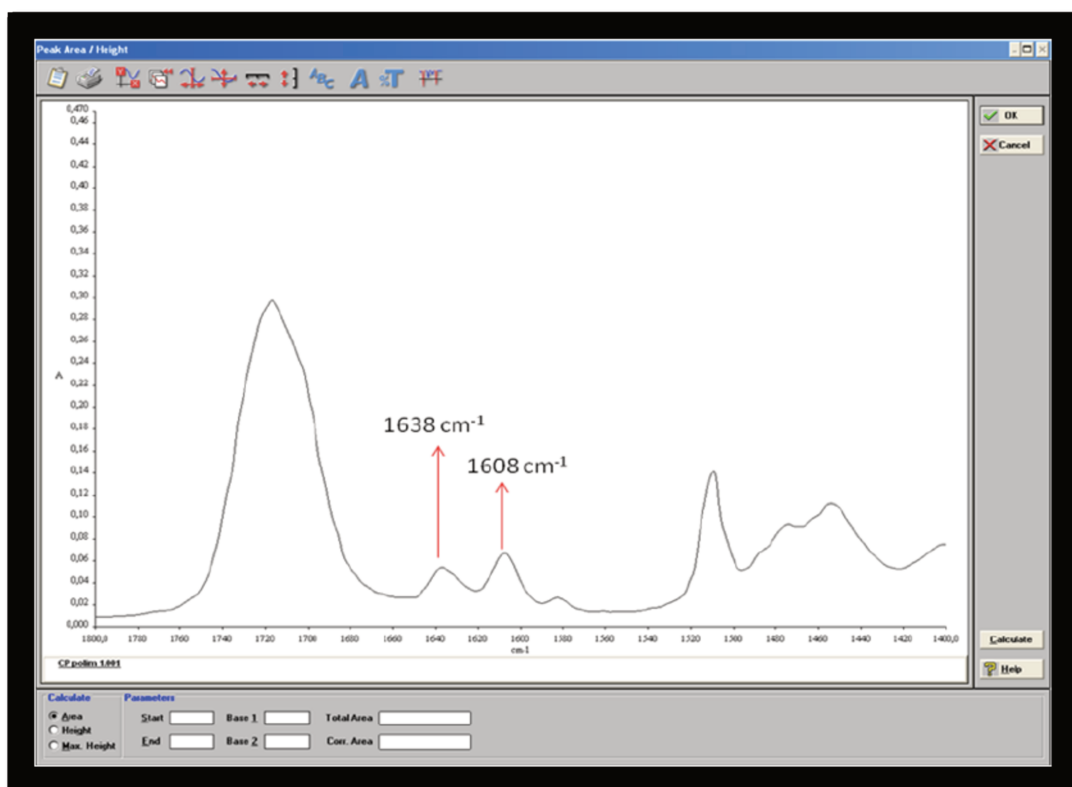


Figure 12 - Gráfico do programa Spectrum ilustrando as duplas ligações alifáticas (1638 cm^{-1}) e aromáticas (1608 cm^{-1}) do mesmo adesivo que a figura anterior, porém em estado polimerizado

Cinética de Conversão e Taxa de Polimerização

Da mesma forma que no teste de grau de conversão, para a cinética de conversão tanto para o Capítulo 1 quanto para o Capítulo 2, foi utilizado o Espectrômetro de Raios Infravermelhos Transformados de Fourier (FTIR - Spectrum 100 Optica; PerkinElmer, MA, USA). Para cada leitura, soluções respectivas de cada grupo experimental foram obtidas e misturadas em casulo. Volume constante ($5\mu\text{L}$) de cada solução foi aplicada sobre o cristal e coberta com uma tira de poliéster. Esta última etapa foi realizada para evitar a inibição da reação de polimerização pelo oxigênio.

Foram obtidos espectros infravermelhos com o auxílio do programa HyperCan2 (PerkinElmer, MA, USA) presente em microcomputador ligado ao espectrômetro. O programa foi utilizado no modo de monitoramento da varredura, usando a apodização Happ-Genzel com uma resolução de 4 cm^{-1} e os espectros sendo coletados no intervalo de 1665 a 1580 cm^{-1} . Com os parâmetros utilizados, um espectro era coletado a cada 2 segundos.

A cinética de conversão de todos os grupos foi monitorada durante dois minutos, sendo o experimento realizado em sala com temperatura ($23\pm 2\text{ }^{\circ}\text{C}$) e

umidade ($60\pm 5\%$) controladas. O primeiro espectro coletado foi considerado como da solução não-polimerizada e foi utilizado no cálculo do grau de conversão. A fotoativação foi realizada com a ponta do aparelho fotoativador a uma distância de 1 mm da tira de polyester pelo tempo de dois minutos, ou seja 120 segundos. O cálculo do grau de conversão, realizado a cada 2 segundos, foi feito baseado na proporção entre as duplas ligações de carbono alifáticas e aromáticas, usadas como padrão interno, nos estados polimerizados e não polimerizados como já explanado no teste de grau de conversão.

Os dados de grau de conversão em cada tempo foram abertos no programa SigmaPlot 9.0 (Systat Software Inc., San Jose, CA, EUA), em que foram traçados os gráficos de grau de conversão versus tempo. A partir deste gráfico foram obtidas curvas de regressão não-linear utilizando a fórmula de Hill de três parâmetros. Os dados resultantes das curvas de regressão foram utilizados para o cálculo da taxa de polimerização. A taxa de polimerização foi calculada baseada no acréscimo de conversão em cada segundo, subtraindo-se o grau de conversão do tempo t do obtido no tempo $t-1$. As curvas de taxa de polimerização também foram traçadas utilizando o programa SigmaPlot 9.0. Três leituras foram realizadas para cada grupo experimental ($n=3$) (Rueggeberg, 1990).