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Research Article

Chemical Interaction of a Self-Etch Adhesive Containing 10-MDP and HEMA with Dentin by Infrared and NMR Spectroscopies

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ABSTRACT

Objectives: Physico-chemical interactions between human dentin and a self-etching adhesive containing 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), 2-Hydroxyethyl methacrylate (HEMA), methacrylate-modified polyacrylic acid and ethanol has been investigated in order to highlight the functional monomer's actions.

Methods: *In vitro* application of this self-etch adhesive on dentin was studied using Fourier Transform Infrared and Nuclear Magnetic Resonance spectroscopies.

Results: The infrared spectra illustrate the changes that occurred when the dentin powder were demineralized and infiltrated by the studied adhesive. The carbonate peaks are replaced by sharper vibrations related to smaller organic functional groups.

1D ^{1}H NMR spectrum in the mixture adhesive + dentin exhibits different percentages than adhesive alone showing that HEMA is consumed. 1D ^{31}P spectrum of adhesive + dentin shows two wide signals. These wide signals indicate the presence of different phosphorus compounds. These are calcium salts due to the demineralization of dentin by 10-MDP. There is the formation of a mineral layer composed of 10-MDP-Ca salts absorbed on amorphous calcium phosphate or 10-MDP salts - phosphates. These salts formations are not disturbed by HEMA. The mixture adhesive + dentin, highlights that Bis-GMA is not consumed, 10-MDP partially and HEMA entirely.

Conclusion: The tested self-etching system produced different interactions with dentin. There are calcium salts due to the demineralization of dentin by 10-MDP. These salts formations are not disturbed by HEMA.

Clinical Significance: Understanding the molecular interactions between an adhesive and dentine is a major challenge to evaluate adhesive solutions that lead to the formation of an impermeable and durable resin/dentine interface. Functional monomers used in the adhesive must not interact negatively. Our results show that in the system tested 10-MDP does not inhibit the action of HEMA.

KEYWORDS

Self-etch adhesive, Chemical Bonding, Functional monomers, Calcium salt of MDP, Dentin, NMR, FT-IR.

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Introduction

Obtaining an adherent and impermeable hybrid layer is a prerequisite to promote immediate satisfactory performances and to prevent early chemical and mechanical degradation of the bonded interfaces. Hybridization process for dentin is known, but remains a difficult challenge as it depends on many factors related to dentin substrate, adhesive nature with different monomers whose association should be studied.

Functional monomers are considered as adhesion promoters. The hydrophilic property of monomers, such as 2-Hydroxyethyl methacrylate (HEMA), contributes to increase the bond strength of adhesives to dentin, and some functional monomers can chemically bond to calcium, such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) [1,2]. The literature has reported differing results on the combination of monomers such as HEMA and 10-MDP in the same adhesive system. The intended use of hydrophilic HEMA is then to infiltrate the demineralized dentin and prevent collagen collapse [3,4]. Functional monomers have already been ranked based on their chemical bonding potential and 10-MDP has been identified as being capable of establishing a very intensive and stable chemical interaction with hydroxyapatite. 10-MDP contributes to the overall bonding to dentin by forming ionic chemical bonds with surface calcium ions [5]. The unique chemical structure of 10-MDP has been proven to play a key role in both the initial bonding performance as well as the durability of the adhesive interface [6-8]. Although the use of an MDP-based self-etch adhesive is effective, chemical bonding alone is not always sufficient [9]. MDP has also been applied with co-monomers that interact cooperatively with MDP to enhance bonding properties at the adhesive-dentin interface. For instance, HEMA is often used as a co-monomer in self-etch adhesives [1]. The hydrophilicity of HEMA can penetrate wet biological tissues, such as tooth dentin, so that high retentive properties are expected. However, the hydroxyl group of its molecular structure may reduce the formation of nano-layering by MDP [10,11].

The MDP-Ca water-insoluble salts contribute to the protection of the collagen fibers. The atomic relation of the 10-MDP molecule favors the chemical interaction [12]. The intense chemical interaction established between MDP and hydroxyapatite is attributed to the superficial dissolution of hydroxyapatite induced by the adsorption of MDP, and subsequent deposition of MDP-Ca salts with lower solubility than of the salts produced by other functional monomers [12,13]. MDP-HEMA aggregates were found to compromise the MDP- collagen interaction leaving collagen fibrils unprotected by MDP and HEMA [14]. A "nanolayer" is formed, composed of two 10-MDP molecules with their methacrylate groups directed toward each other and their functional hydrogen phosphate groups directed away from each other, in which the methacrylate binds to the calcium ions, and the hydrogen phosphate group binds to the dentin [15].

One study revealed that HEMA could significantly affect the chemical interaction between 10-MDP and hydroxyapatite. When the monomer HEMA was added, MDP-Ca salt formation decreased, significantly reducing the nano-layer. Therefore, HEMA could interfere, but did not completely inhibit MDP from interacting chemically with hydroxyapatite. The findings of the study conducted by Yoshida [10] are in line with the results of a study conducted by Oliveira [2] as the adhesive that contained the interaction of these two monomers had higher mean bond strength, both with respect to the group containing only HEMA and the group whose formulation contained neither of these two functional monomers.

In order to gather more information on the physico-chemical interactions occurring between human dentin and a self-etching adhesive containing 10-MDP, HEMA, methacrylate-modified polyacrylic acid and ethanol, we investigated the use of infrared and nuclear magnetic resonance spectroscopies and hypothesized that these combined techniques should better define the mode of the action of functional monomers. The working hypothesis is that 10-MDP will not inhibit the action of HEMA in dentin.

Materials and Methods

Samples

Human non-carious, unerupted third molars extracted for prophylactic reasons from 18- to 25-year-old patients were used to make dentin particles for Fourier Transform InfraRed (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopies. Specimens were prepared immediately after extractions. To obtain dentin particles, roots were sectioned, pulp was removed and enamel eliminated with diamond bur under water cooling. The dentin blocks obtained were crushed at room temperature and high pressure using a 13 mm diameter anvil placed in a hydraulic press (P/ O/Weber, Remshalden, Germany) under pressures near 50 GP [16]. The obtained powder was sifted and collected, only fragments smaller than 100µm were kept. They were stored in small tightly closed vials to prevent dehydration.

NMR spectroscopy

Samples preparations:

- Adhesive alone: 50 µl of adhesive were transferred to a Wilmad 5 mm NMR tube before adding 0.5 ml of deuterated DMSO as solvent.
- Adhesive + dentin: 5 mg of dentin were mixed with 50 µl of adhesive in an Eppendorf. After polymerisation, 0.5 ml of deuterated DMSO was added. An ultrasonic bath was necessary to dissolve the mixture. The solution was then transferred to a Wilmad 5 mm NMR tube.

NMR experiments:

^{1}H, ^{13}C and ^{31}P NMR experiments were carried out at 298 K.
All NMR experiments (1D ^{1}H, 1D ^{13}C, 1D ^{31}P and 2D COSY ^{1}H-^{1}H, 2D HSQC ^{1}H-^{13}C, 2D HMBC ^{1}H-^{13}C, 2D HMBC ^{1}H-^{31}P, DOSY…) were acquired using BRUKER AVANCE III HD 500 and NEO 600 MHz UltraShield spectrometers equipped respectively with a 5 mm BBFO ATMA Prodigy cryoprobe and a 5 mm QCI $^{1}H\{^{13}C, ^{31}P, ^{15}N\}$ ATMA cryoprobe. Experiments were processed with

BRUKER TopSpin software, version 3.5 on the 500 and 4.2 on the 600 NMR spectrometer.

Infrared Spectroscopy

The adhesive, the composition of which is presented in Table 1, was mixed directly into the dentin powder. Typically, 80 mg of dentin powder was mixed with 20 mg of adhesive solution for 30 s. The samples were analysed by Fourier transform infrared (FTIR) using Perkin Elmer Spectrum Two UATR spectrometer with a diamond anvil reflection sample holder from SPECAC in the reflection mode. All cases spectra were acquired from 500 to 4000 cm^{-1} wave number range at room temperature (24±1°C). After each spectrum, the ATR plate was cleaned in situ with ethanol solution. The IR spectra of the dentin powder, of the dentin powder with adhesive are compared to the IR spectra or the adhesive alone. Each spectral acquisition is repeated five times.

Table 1: Composition of adhesive "Cention Primer" as given by manufacturer.

Adhesive	Manufacturer	Composition	Batch
Cention Primer	IVOCLAR VIVADENT, Schaan, Liechtenstein	2-Hydroxyethyl methacrylate (HEMA) 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP) Bis-glycidyl methacrylate (Bis-GMA) Decanediol 1-10 diméthacrylate (D3MA) methacrylate-modified polyacrylic acid camphorquinone silicon dioxide potassium hydroxide	R71805

Results

NMR assignments

NMR experiments were carried out. ^{1}H, ^{13}C and ^{31}P assignments have been achieved thanks to 1D (^{1}H, ^{13}C and ^{31}P) and 2D (COSY ^{1}H-^{1}H, HSQC ^{1}H-^{13}C, HMBC ^{1}H-^{13}C, HMBC ^{1}H-^{31}P, DOSY) NMR experiments (Table 2).

^{1}H NMR spectra assignments

In order to compare the NMR spectra of the adhesive with its mixture with dentin, the adhesive solution was analysed by ^{1}H NMR spectroscopy (Figure 1).

Signals at 0.8 and 0.99 ppm correspond to the methyl groups of camphorquinone wheras signals at 1.1 and 3.4 ppm correspond respectively to the CH_3 and the CH_2 of the ethanol molecule. Signals around 1.25 and 1.6 ppm has been assigned to long CH_2 chains. Additional signals have been assigned to: CH_2 group of 10-MDP (3.76 ppm) and of HEMA (3.62 and 4.10 ppm), water signal at 4.5 ppm [17,18]. Resonances at 6.06 and 5.99 ppm correspond respectively to ethylene $=CH_2$ of HEMA, Bis-GMA and 10-MDP. Aromatic protons of Bis-GMA resonate at 6.8 and 7.1 ppm.

Table 2: NMR spectroscopic data of Primer Cention adhesive (500 MHz, 600 MHz for ^{1}H and 125 MHz, 150 MHz for ^{13}C - δ in ppm and J in Hz).

	HEMA		10-MDP		Bis-GMA		Cam-phorquinone		Ethanol	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	18.36	1.88	18.30	1.86	18.30	1.83	17.27	0.99	18.86	1.06 (t, J=7)
2	136.36	-	136.44	-	136.22	-	20.97	0.80	56.53	3.45 (q, J=7)
3	126.02	6.06-5.64	125.75	5.99-5.61	126.30	6.06-5.64	42.57	-	-	4.50
4	167.12	-	167	-	166.92	-	58.60	-		
5	66.50	4.10	64.70	4.06	66.06	4.16	8.92	0.98		
6	59.44	3.62	28.48	1.58	67.24	4.06	29.70	1.53		
7	-	4.50	25.83	1.30	69.39	3.94	22.10	2.12		
8			25.80	1.24	156.60	-	57.80	2.62		
9					114.26	6.82	203.40	-		
10					127.80	7.08	205.42	-		
11					143.20	-				
12			25.69	1.27	41.55	-				
13			30.50	1.52	31.08	1.56				
14			65	3.76						

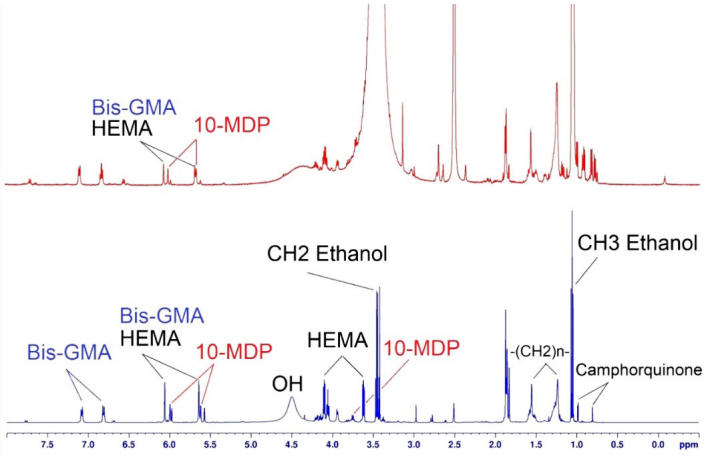

Figure 1: 1D ^{1}H NMR spectra of adhesive (Bottom - blue) and adhesive + dentin (top - red). 500.13 MHz; DMSO d6; 298 K Spectra were processed using the same Line Broadening parameter (LB=0.3).

The proton spectra of adhesive alone and dentin-adhesive show differences. First of all, a very intense peak of ethanol appears in the mixture spectrum. Secondly, it is noted that OH of the adhesive at 4.3-4.5 ppm changes shape when the adhesive is brought into contact with dentin.

The ^{1}H 2D DOSY NMR spectrum is illustrated in Figure 2 and shows the different components of the adhesive as a function of their diffusion coefficient (related to the shape and size of the molecule considered in the mixture). Thus, the most mobile components with faster movements are located at the top of the graph, whereas the heavier molecules stand at the base. It can be

seen that the smaller more mobile molecules have fewer protons than the larger molecules and that they contain several kinds of functional groups, including aliphatic and aromatic parts. Diffusion coefficients values are found between 1.7×10^{-10} m2 /s (larger molecules) and 7.6×10^{-10} m2 /s (water). Ethanol, readily identifiable just under the water signals, has a diffusion coefficient of 7.0×10^{-10} m2 /s. [19]

^{1}H NMR Quantitative Analysis

Figure 3 shows an enlargement of the 5.4-7.2 ppm spectra region corresponding to the resonance frequency of the ethylenic (5.55-6.1 ppm) and aromatic (6.7-7.2 ppm) protons of 3 constituents of the adhesive: Bis-GMA, HEMA and 10-MDP. Bis-GMA is used as an internal reference to analyse HEMA and 10-MDP. Focusing on these 3 constituents, the relative proportions found in the adhesive are the following: 40% for HEMA, 20% Bis-GMA, and 40% for 10-MDP. These percentages were calculated from the areas of the proton spectrum signals (Figure 3). On the other hand, in the mixture adhesive + dentin, the obtained percentages are different: Bis-GMA is not consumed, 10-MDP is partially consumed and HEMA is entirely consumed (Figure 3).

1D ^{31}P NMR spectra

a) The sole resonance of the ^{31}P NMR spectrum of 10-MDP (δ = -1.05 ppm; $\nu_{1/2}$ = 1.5 Hz where $\nu_{1/2}$ is the width of the signal at half-height) corresponds to the single phosphorus nucleus of the 10-MDP molecule.

b) The ^{31}P nucleus resonance of the 10-MDP in the adhesive mixture (compare to 10-MDP alone) exhibits a different chemical shift (-0.41 ppm) and a wider signal ($\nu_{1/2}$ = 5 Hz), showing interactions between 10-MDP and the other compounds of the adhesive mixture.

c) The ^{31}P spectrum of adhesive + dentin clearly shows two wide signals. The most unshielded signal has a $\nu_{1/2}$ = 8.5 Hz and the most shielded has a $\nu_{1/2}$ = 5 Hz. It should be noticed that the chemical shifts of these signals (-0.68 ppm and -0.93 ppm) do not correspond to either 10-MDP alone or 10-MDP in the adhesive mixture. These wide signals indicate the presence of different phosphorus compounds. These are calcium salts, due to the demineralization of dentin by 10-MDP.

Infrared Spectroscopy

The infrared spectra illustrated in Fig.5 illustrate the changes that occurred when the dentin particles were demineralized and infiltrated by the studied adhesive.

The IR spectrum of powdered dentin (a) shows broad water peak (labelled OH) at 3360,3370 cm^{-1}, absorption peaks attributed to the organic phase (CH stretching modes near 2900 cm^{-1} and amide peptide linkages at1700 cm^{-1}) and to the mineral species (carbonates at 1500 cm^{-1} and phosphates at 1200, and 600 cm^{-1}).

The Reference Spectra of the Adhesive (b) demonstrated the characteristic peaks of O-H at 3370 cm^{-1}, C-H near 2939 cm^{-1}, CO_3^- at 1510 cm^{-1}, C=H at 1297 cm^{-1}, HEMA showed characteristic strong absorptions near 900,1042 cm^{-1},1050/1080 cm^{-1} C–OH stretch ethanol,10-MDP showed characteristic absorptions near 940, 1100 cm^{-1} and Si-O silicon dioxide 815 cm^{-1}.

When the Adhesive Reacts with Dentin (c) spectrum shows the carbonate peaks replaced by sharper vibrations related to smaller organic functional groups. Additional peaks observed for the treated dentin can be attributed to the adhesive components.

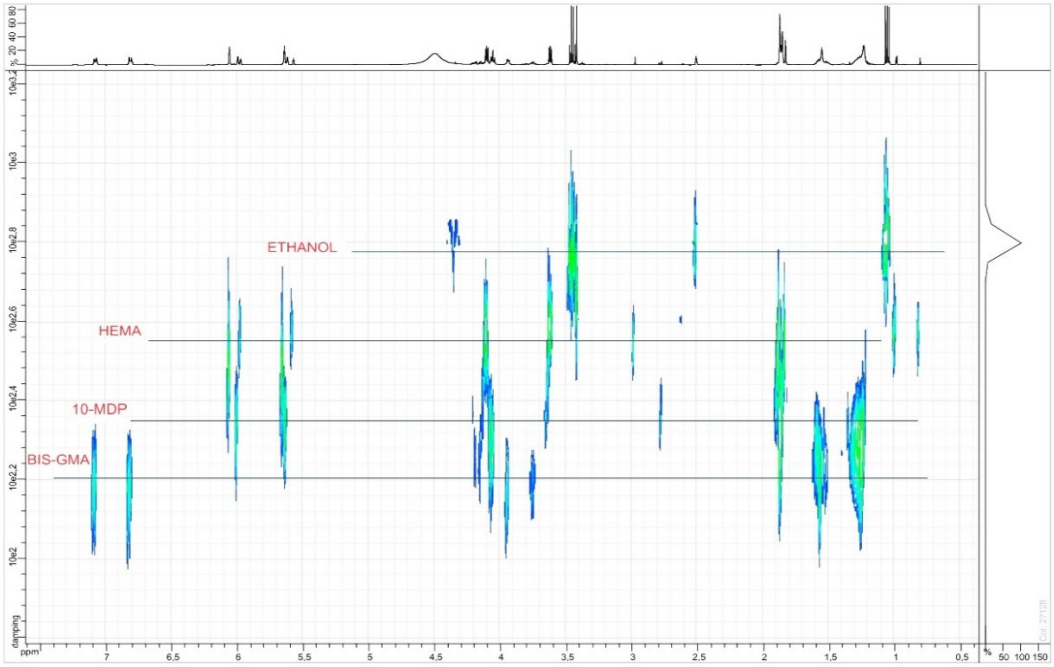

Figure 2: 2D ^{1}H NMR DOSY spectrum of the adhesive (adhesive) showing the presence of ethanol and some functional monomers including Bis-GMA, HEMA and 10-MDP. 600.13 MHz; DMSO d6; 298 K.

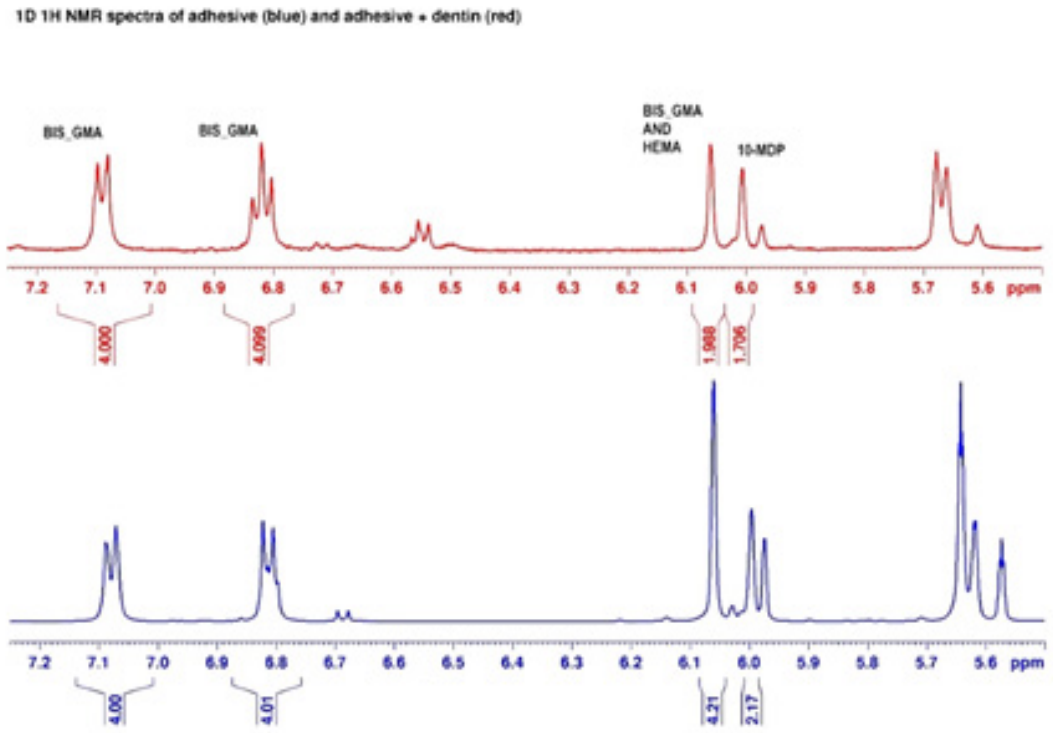

Figure 3: 5.4-7.2 ppm expanded region of the 1D ^{1}H NMR spectra of adhesive (blue) and adhesive + dentin (red).600.13 MHz; DMSO d6; 298 K Spectra were processed using the same Line Broadening parameter (LB=0.3).

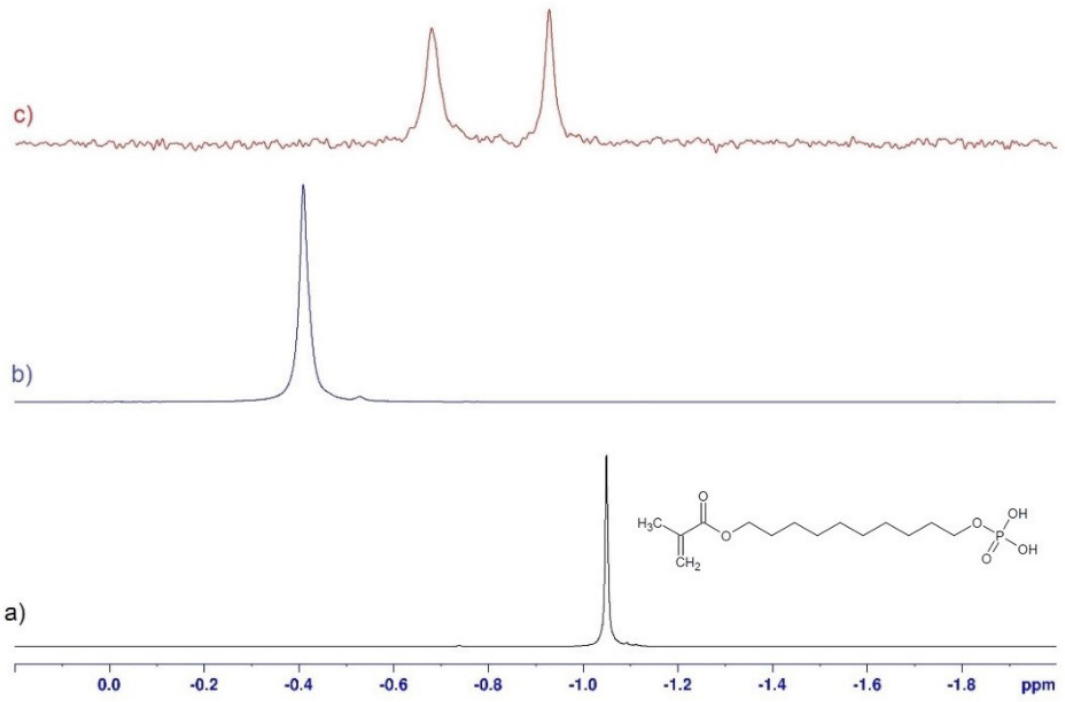

Figure 4: 1D ^{31}P NMR spectra of 10-MDP alone a), adhesive alone b) and adhesive + dentin c) 202.45 MHz; DMSO d6; 298 K; Ref. δ = 85% H_3PO_4 in H_2O.

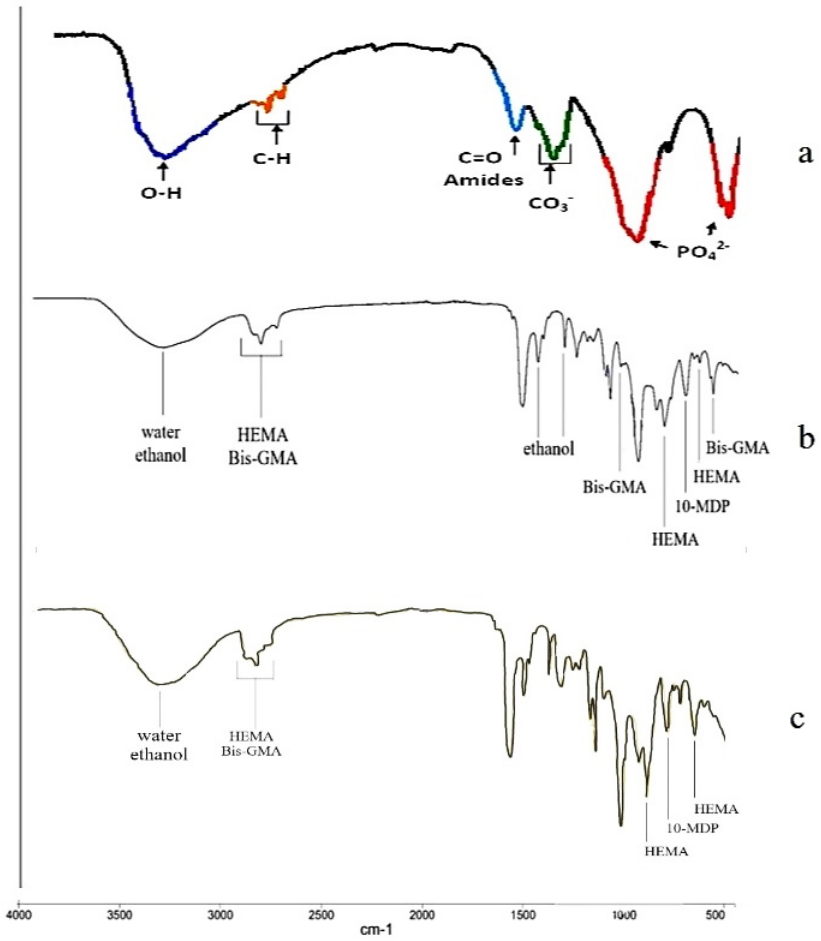

Figure 5: Infrared spectra of dentin (a), adhesive (b) and adhesive placed on dentin (c). The dentin spectrum (a) shows broad water peak, phosphate peak and carbonate due to carbonated apatite presence, and carbonyl amide peaks from collagen. The spectrum of adhesive is characterised by C\H stretching vibrations near 2900 cm^{-1} and a strong carbonyl band at 1710 cm^{-1}. On the adhesive + dentin c), we can distinguish an amplification in the phosphate / carbonate peak due to an increase of the phosphate.

Discussion

The formation of a hybrid layer depends on all the constituents of the adhesive that are placed on the dentin. It is therefore necessary to take into account a set within which there are interactions rather than 1 or 2 constituents of the formulation.

The purpose of our work is to evaluate the interaction of a commercial adhesive containing 10-MDP and HEMA mixed solvent water / ethanol with dentin by analysing whether or not their functions contradict when they are in all the components of the formula. The experimental model of dentine powder is a model that has been approved and used in many works [4-5,16-20].

FT-IR spectroscopy has excellent potential as method of detection and quantification of dentin adhesives. [21,22] According to Delgado [23] FT-IR is widely used in studies of material characterisation [24]. It can quantify compositional changes in polymer chemistry, facilitate an understanding of the nature of the polymer materials and how they may react when in contact with dental substrates [22]. FT-IR has been used extensively to investigate bonding systems and to study the quality of the hybrid layer [12,25,26]. The data obtained from Figure 5 are compatible with previous observations [4,5,20,22,23,25] and suggest a change in the components.

We show here that NMR spectroscopy enables a fine monitoring of the interactions at the molecular level between adhesive solution and dentin material and can provide important structural data on dentin and adhesive components when they are mixed together [18]; This investigative technique can resolve key atomic structural details within these materials and has emerged as a crucial tool in characterising dentin adhesives structure and properties. It is crucial to obtain such information for optimizing the composition of adhesive solution in order to improve the stability of the dentin resin hybrid layer over time. The ability of NMR to provide valuable information regarding mixture analysis has created broad applicability across chemistry, biochemistry, biology and medicine. Since adhesives can be considered as complex mixtures (composed

of many different substances or including simultaneously large and very low quantities of compounds), DOSY NMR is therefore a useful tool for studying such formulations without any prior separation.

The adhesive studied also contains Bis-GMA. Bis-GMA has reactive epoxy functions that react with amino groups of collagen. Results of a previous study clearly confirm that GMA reacts spontaneously with lysine, a ubiquitous amino acid found in collagen [4]. In contrast, HEMA was not found to react with this same amino acid, but was found to stick to collagen in partly demineralized dentin particles. Bis-GMA is acknowledged to form a solid composite material with collagen, but is too hydrophobic to penetrate the wet demineralized zone. The mixture of HEMA and Bis-GMA monomers was operational in forming the hybrid layer [27]. To prevent HEMA diffusion and water pick-up, HEMA must be completely co-polymerized by appropriate catalysis and crosslinked sufficiently to minimize water uptake [4]. The impact of HEMA is clearly different from that of reactive monomers at the resin-dentin interface [28]. Hydrophilic monomers such as HEMA are also present to help cross the smear layer and bind to the dentin substrate. The smear layer is then incorporated into the hybrid layer. A major advantage of HEMA relies on its ability to improve the miscibility between hydrophilic and hydrophobic monomers. As a consequence, the incorporation of this monomer to one step self-etch adhesive can prevent phase separation and therefore enhance the adhesive performance [3].

Focusing on ^{1}H NMR spectra (adhesive alone (x), adhesive + dentin (o), Figure 3), the integration of ethylenic and aromatic signals between 5.95 and 7.20 ppm assigned to HEMA, Bis-GMA and 10-MDP allow to compare the percentages of HEMA and 10-MDP in the adhesive alone and in the adhesive + dentin. This analysis clearly shows that HEMA interacted with the dentin.

HEMA does not interfere with HAp and does not prevent MDP from chemically interacting with it [10]. In this work, we show that HEMA is more consumed than 10-MDP when adhesive is placed on dentin.

Our results with ^{31}P NMR spectra (Figure 4) show the formation of a mineral layer composed of 10-MDP-Ca salts absorbed on amorphous calcium phosphate (ACP) or 10-MDP salts - phosphates. They are analogous to previously published findings by Fukegawa [13], establishing that the 10-MDP bonded to the hydroxyapatite surface is accompanied by the formation of an intermediary layer of 10-MDP [18]. Recent work shows the molecular species of 10-MDP-Ca salts that form a layered structure which were determined as 10-MDP dimer mono-calcium salts (MCS-MD) and di-calcium salts (DCS-MD) [29,30]. These salts formations, under the study's conditions, are not disturbed by HEMA. These observations are in line with Yoshida's study [10].

The adhesive solvent is considered as one of the important factors of the interface's stability [31]. Ethanol readily penetrates the demineralized collagen by capillary forces and sucks in the accompanying monomers [19]. Ethanol works mainly by mixing with unbound water through the rearrangement of the collagen monomers [18,32]. During the adhesive's application, interfibrillar water and ethanol evaporate together, leaving behind a monomer-rich composition that avoids collagen collapsing and can limit its hydrolytic degradation.

In this work, the OH signal of the adhesive changes when the adhesive is in the dentin. The widening of this signal seems to show an interaction between ethanol and free water molecules, which are at 4.3, 4.5 ppm. In studies performed with ethanol as solvent for adhesive [31,33,34], it has been shown that the layers of water strongly bound to collagen (that represent about 25% of total dentin water) are very difficult to remove, but that adhesive solution can permeate tightly bound water during infiltration in collagen. Those authors speculated that bound water holds intermolecular spaces between collagen molecules open for adhesive resin monomer uptake. They also demonstrated that water/ ethanol mixing in the non-bound hydration layer is enhanced through the rearrangement of the collagen monomers. The removal of free and loosely bound water is therefore facilitated with ethanol molecules that can potentially replace them. Replacement of all loosely bound or free water with water-free but water-miscible solvents are considered as a key to creation of ideal resin-dentin bonds [20].

Conclusion

Within the limitations of this study, the tested self-etching system containing 10-MDP and HEMA mixed solvent water / ethanol produced different interactions with dentin.

1D ^{1}H spectrum in the mixture adhesive + dentin exhibits different percentages than adhesive alone, clearly showing that HEMA interacted with dentin.

1D ^{31}P spectrum of adhesive + dentin clearly shows two wide signals. It should be noticed that the chemical shifts of these signals (-0.68 ppm and -0.93 ppm) do not correspond to either 10-MDP alone or 10-MDP in the adhesive mixture. These wide signals indicate the presence of different phosphorus compounds. These are calcium salts due to the demineralization of dentin by 10-MDP. There is the formation of a mineral layer composed of 10-MDP-Ca salts absorbed on amorphous calcium phosphate (ACP) or 10-MDP salts - phosphates. These salts formations are not disturbed by HEMA.

A mix of ethanol and free water molecules has been shown on 1D ^{1}H spectrum. Ethanol molecules enhance the removal of free and loosely bound water from the dentin collagen matrix while being able to replace them in their compartments within the demineralised structure, thereby helping the penetration of less hydrophilic components of the adhesive. This characteristic is considered a major factor in the stability of resin-dentin bonds.

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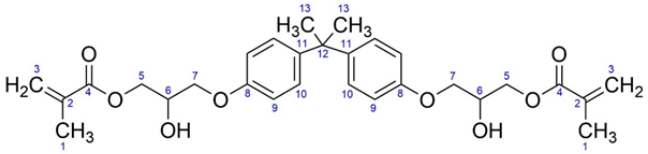
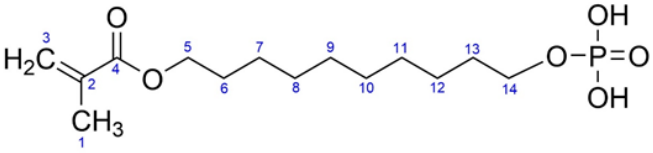
Supplement Materials

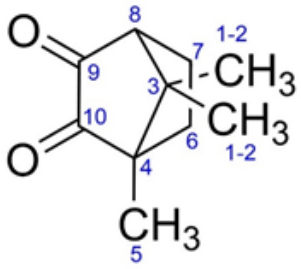
1°) Self-etch adhesive components :



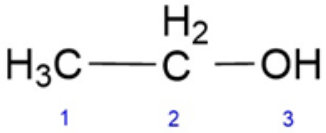
Bisphenol A diglycidylmethacrylate (Bis-GMA)



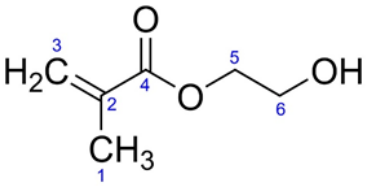
10-methacryloyloxydecyl dihydrogen phosphate (10-MDP)



Camphorquinone



Ethanol



2-Hydroxyethyl methacrylate (HEMA)

2°) Details of NMR experiments performed :

1D ^{1}H (600.13 MHz): « zg30 » BRUKER pulse program was used with a Time Delay (TD) of 64 K points, 16 scans and a relaxation delay (d1) of 3 s.

1D ^{13}C (150.90 MHz): « jmod » BRUKER pulse program was used with a Time Delay (TD) of 64 K points, 7168 scans and a relaxation delay (d1) of 2,5 s.

1D ^{31}P (202.45 MHz): « zgig30 » BRUKER pulse program was used with a Time Delay (TD) of 128 K points, 32 scans and a relaxation delay (d1) of 3 s.

2D COSY ^{1}H-^{1}H (600.13 MHz): « cosygpmfppqf » BRUKER pulse program was used with a total of 256 experiments of 8 scans each. Sweep width in F2 of 4854 Hz; size 2K in F2; zero filling to 4K in F2 and to 1K in F1 was applied.

2D HSQC ^{1}H-^{13}C (600.13 MHz): « hsqcetgpsisp » BRUKER pulse program was used with a total of 1024 experiments of 8 scans each. Sweep width in F2 of 4854 Hz and 21128 Hz in F1; size 2K in F2; no zero filling was applied in F2 and F1. The time delay (d4 = 1/4 J_{XH}) was set to 1.72 ms equivalent to a coupling constant of 145 Hz.

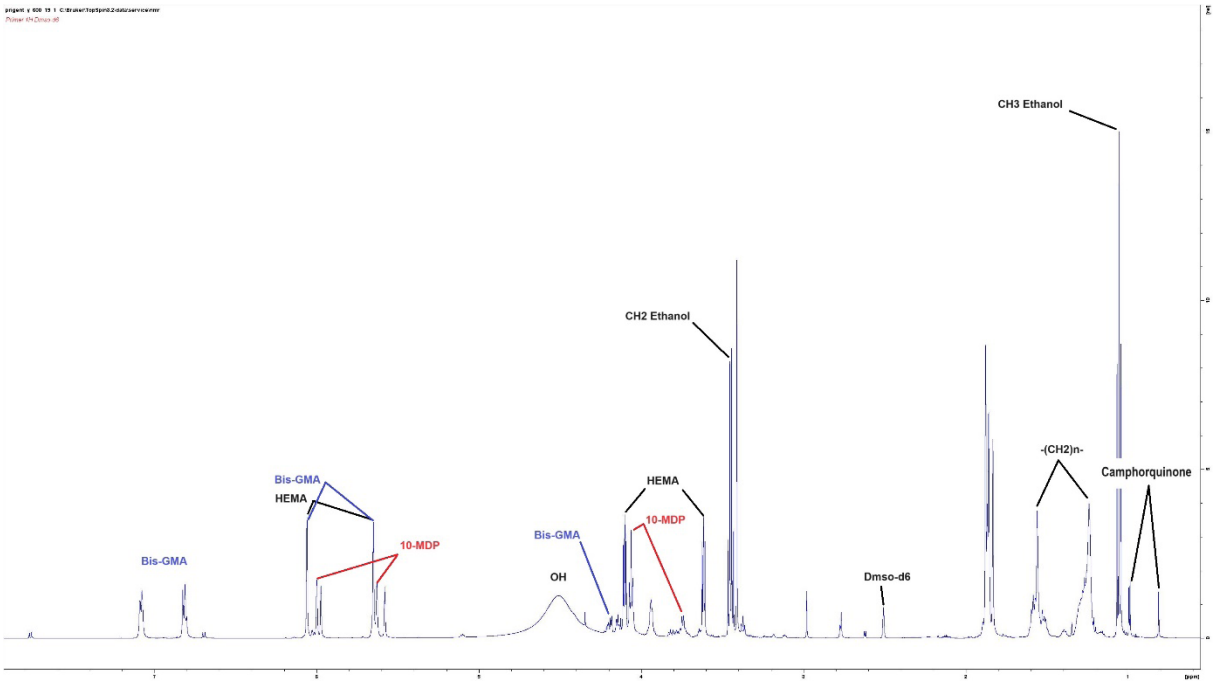
2D HMBC ^{1}H-^{13}C (600.13 MHz): « hmbcetgpl3nd » BRUKER pulse program was used with a total of 512 experiments of 16 scans each. Sweep width in F2 of 4854 Hz and 31693 Hz in F1; size 4K in F2; no zero filling in F2 and 1K in F1. The time delay (d6 = 1/2 $J_{XH\ Long\ range}$) was set to 62.5 ms equivalent to a coupling constant of 8 Hz.

2D selective HMBC ^{1}H-^{13}C (600.13 MHz): « shmbcctetgpl2nd » BRUKER pulse program was used with a total of 128 experiments of 4 scans each. Sweep width in F2 of 4854 Hz and 1509 Hz in F1; size 2K in F2; no zero filling in F2 and 1K in F1. The time delay (d6 = 1/2 $J_{XH\ Long\ range}$) was set to 62.5 ms equivalent to a coupling constant of 8 Hz.

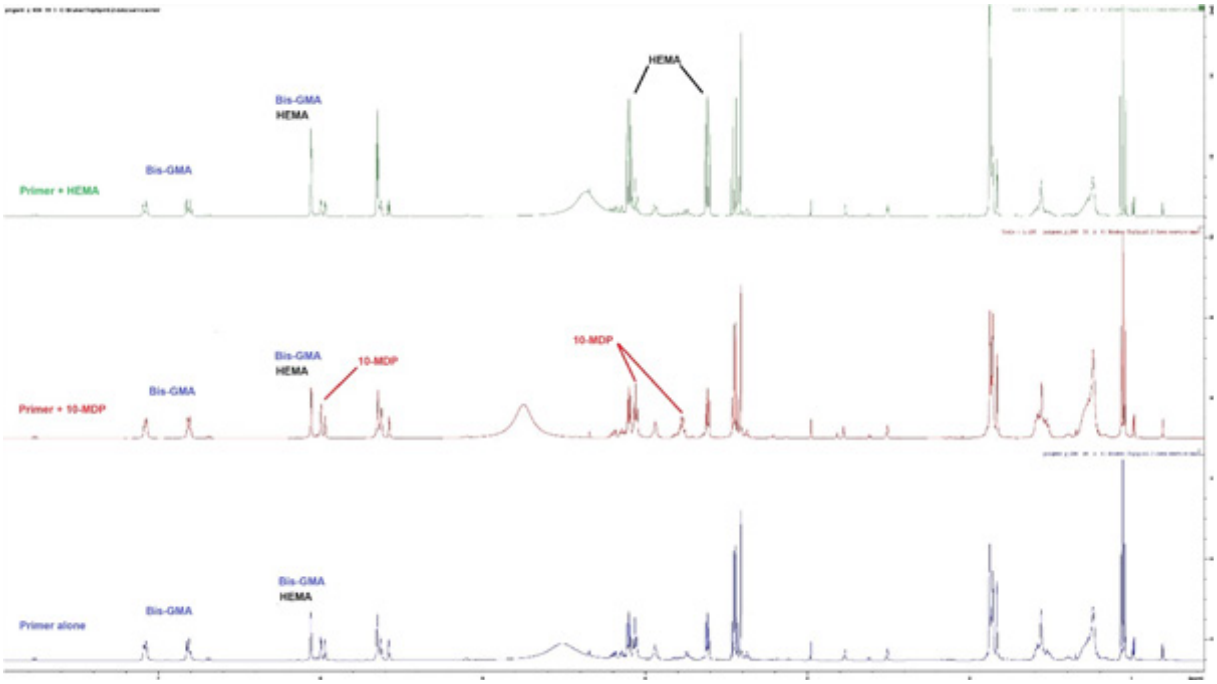
2D HMBC ^{1}H-^{31}P (500.13 MHz): « hmbcgplpndqf » BRUKER pulse program was used with a total of 512 experiments of 4 scans each. Sweep width in F2 of 5000 Hz and 6073 Hz in F1; size 2K in F2; no zero filling in F2 and 1K in F1. The time delay (d6 = 1/2 $J_{XH\ Long\ range}$) was set to 62.5 ms equivalent to a coupling constant of 8 Hz.

DOSY (600.13 MHz): Diffusion measurements were performed using the 2D sequence for diffusion measurement using stimulated echo and led (ledbpgp2s). The recycle delay was adjusted to 3 s to allow full relaxation. The shape of the gradients was Smoothed Square with a length of 0.8 ms, and the strength was varied in 8 increments (5–95%) of the gradient ramp created by the DOSY BRUKER software. The diffusion time was set to 140 ms, according to the sample.

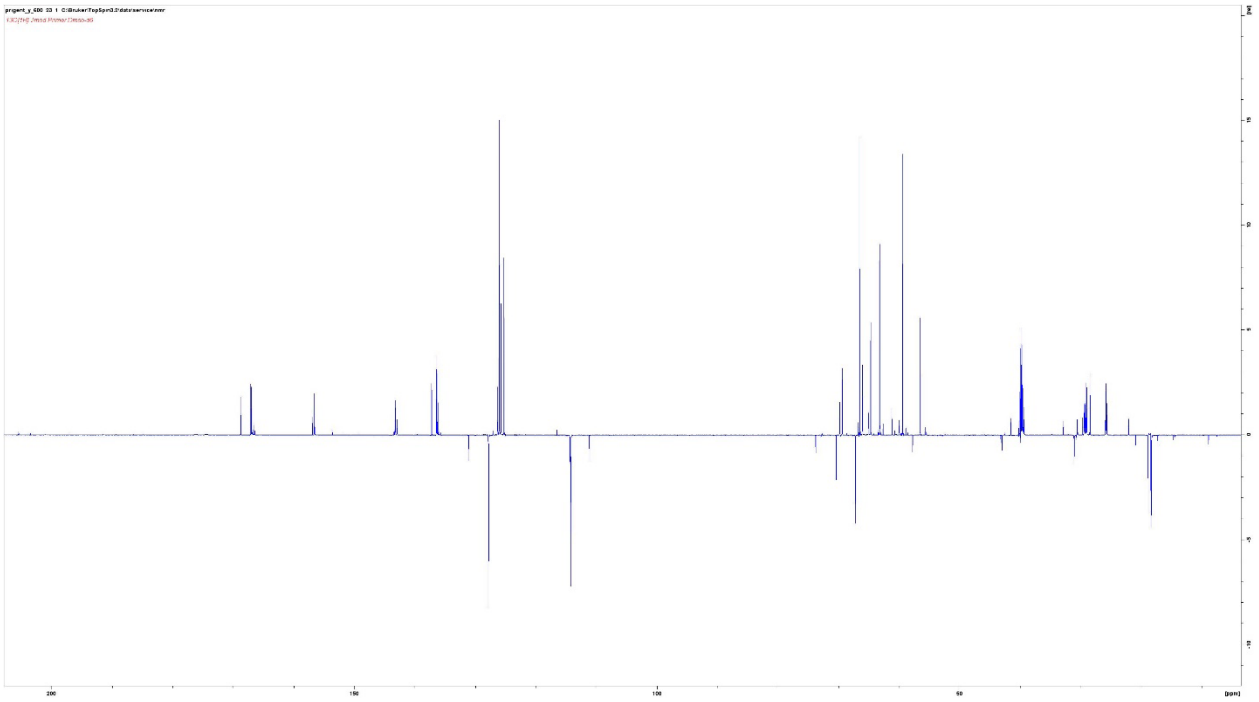
3°) Self-etch adhesive NMR spectra :



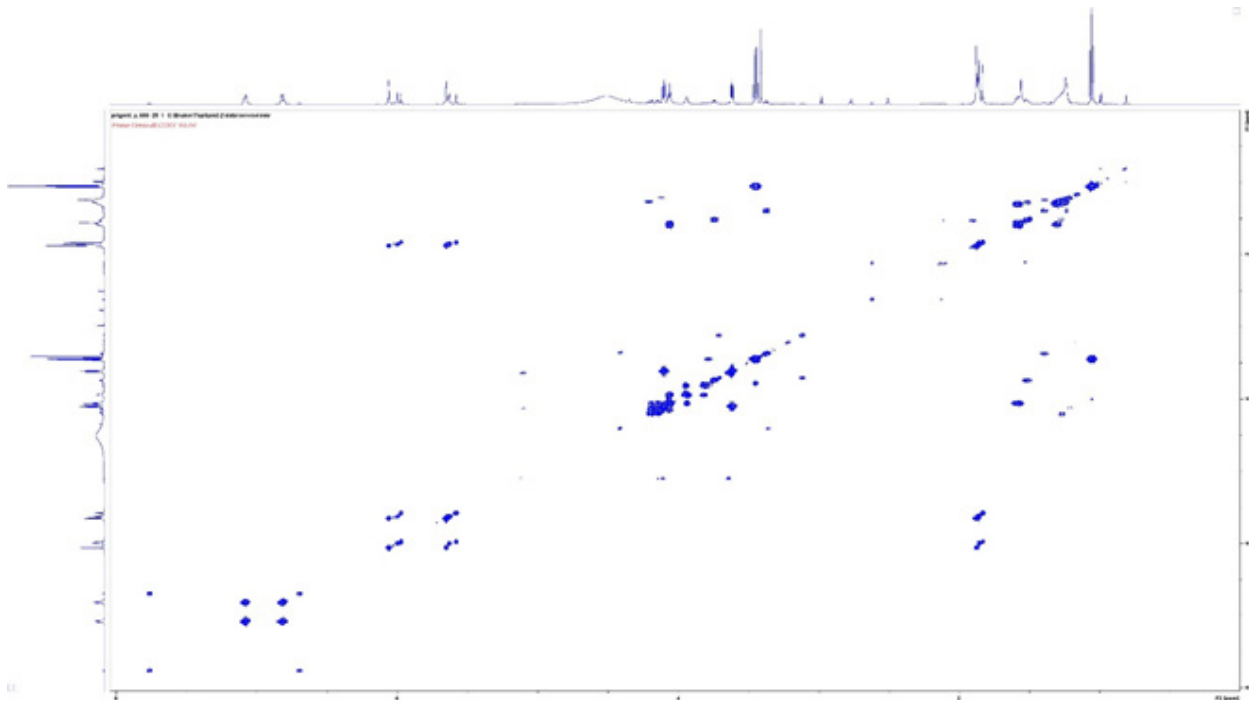
1D ^{1}H NMR



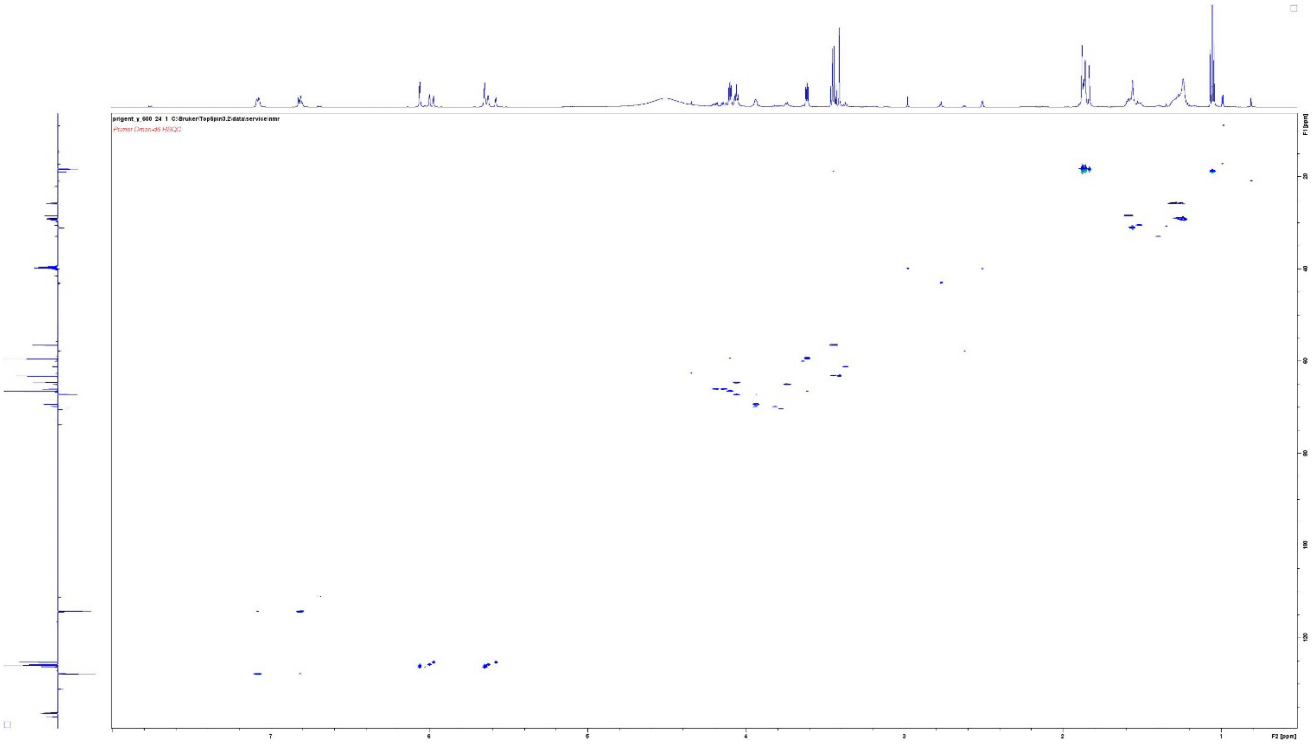
1D ^{1}H NMR spectra : Adhesive alone (blue), Adhesive with addition of 10-MDP (red), Adhesive with addition of HEMA (green)



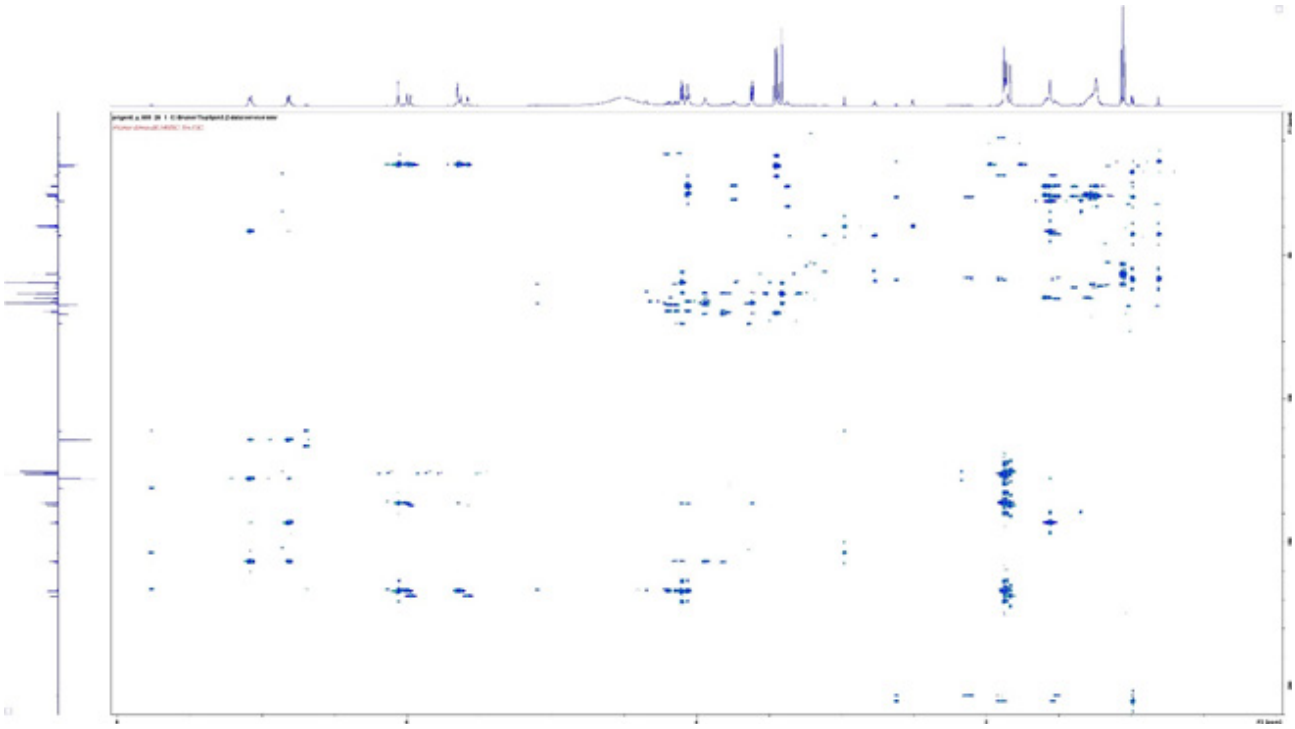
1D ^{13}C NMR



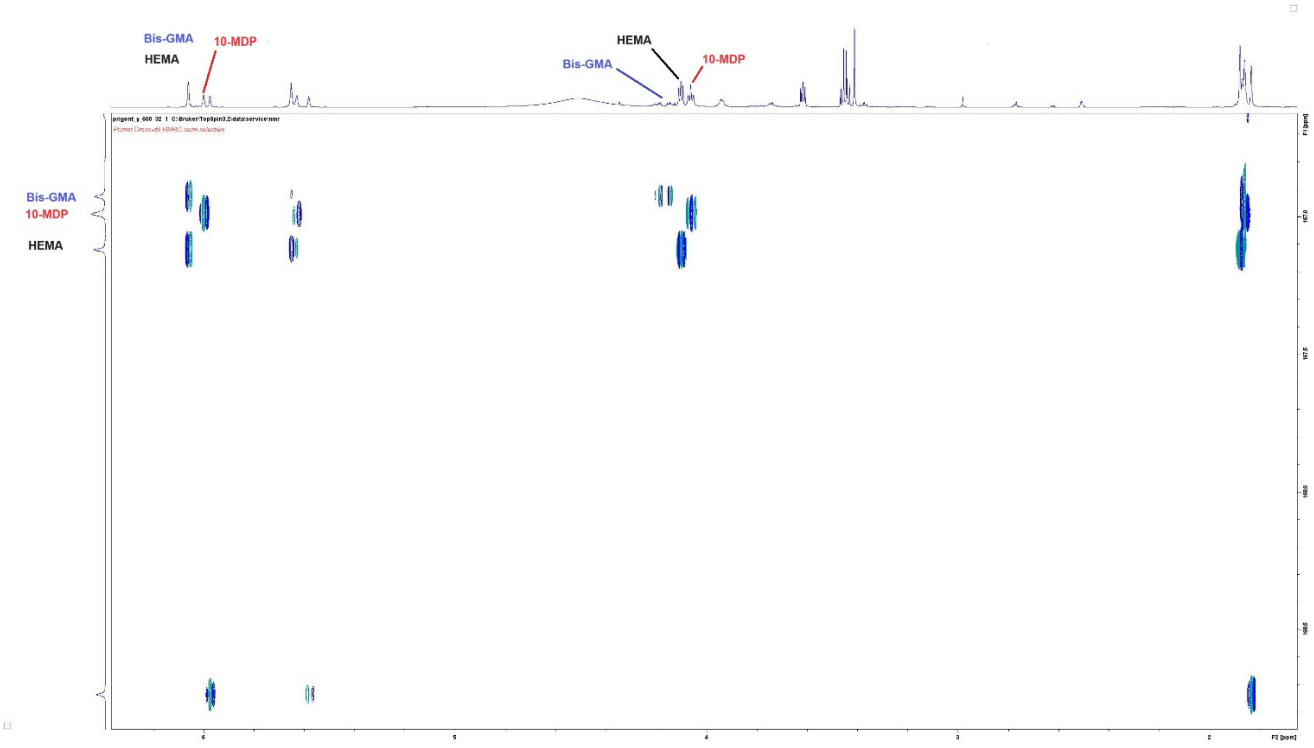
2D COSY ^{1}H-^{1}H NMR



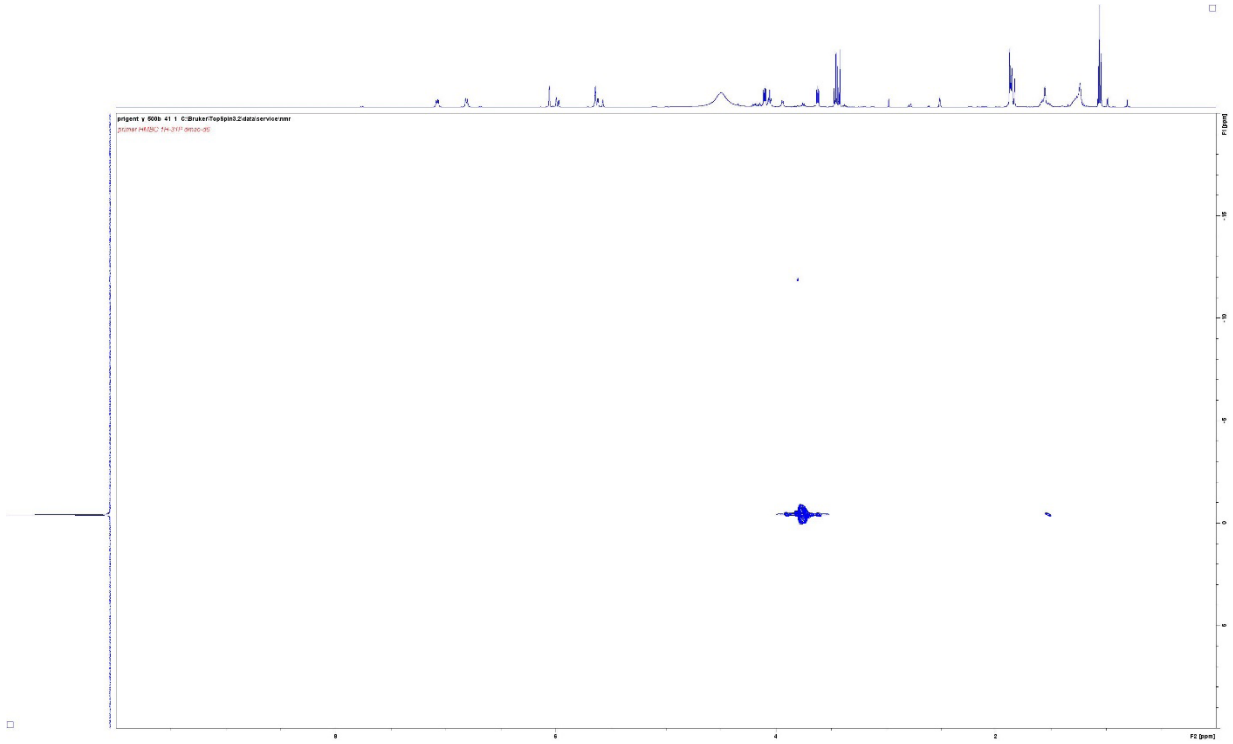
2D HSQC ^{1}H-^{13}C NMR



2D HMBC ^{1}H-^{13}C NMR



2D selective HMBC ^{1}H-^{13}C NMR



2D HMBC ^{1}H-^{31}P NMR